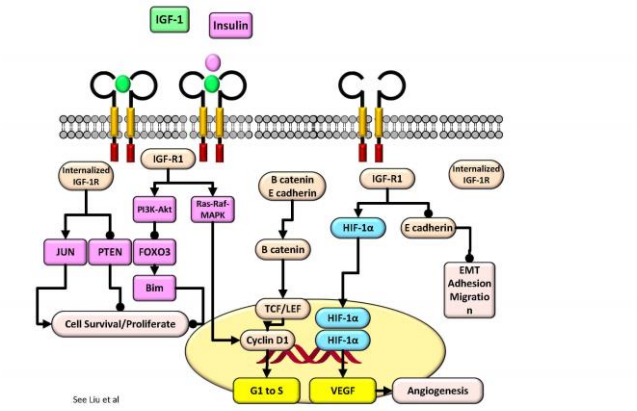

IGF-1 AND PROSTATE CANCER

December 2023

ABSTRACT
This Note examines the influence of IGF-1 on prostate cancer. The impact also of loss of insulin control and hyperglycemia is examined. We address the inter-relationship between insulin and IGF-1 and the control of pathways leading to metastatic growth. Putative therapeutics are considered.

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Contents

1 INTRODUCTION

We have examined many of the genetic and localized attributes of prostate cancer. This is a complex cancer with many varied drivers and variants. We examine another of these herein, namely the Insulin Growth Factor-1. IGF-1 has been studied for several decades and it seems to evoke multiple understandings. It finds itself in the midst of many signally pathways as well as gross physiological processes. In a simple sense IGF-1 is driven by GH, the growth hormone. It is produced primarily in the live but can be produced in multiple organs. It genetically drives several pathways that result in uncontrolled proliferation and growth. Thus its rather central role, driven by large scale physiological process such as diabetes, and small scall intracellular process such as carcinogenic pathways makes it an interesting and attractive target for diagnosis, prognosis and therapeutics. There has been a recent flurry of papers discussing these areas and we examine them herein.

1.1 HISTORY

Early observations by Mantzoros et al (1997) noted:

Blood samples were collected from 52 incident cases of histologically confirmed prostate cancer, an equal number of cases of benign prostatic hyperplasia (BPH) and an equal number of apparently healthy control subjects. The three groups were matched for age and town of residence in the greater Athens area.

Steroid hormones, sex hormone-binding globulin, and insulin-like growth factor 1 (IGF-1) were measured in duplicate by radioimmunoassay in a specialized US centre. Statistical analyses were performed using multiple logistical regression. The results for IGF-1 in relation to prostate cancer and BPH were adjusted for demographic and anthropometric factors, as well as for the other measured hormones.

There was no relation between IGF-1 and BPH, but increased values of this hormone were associated with increased risk of prostate cancer; an increment of 60 ng ml(-1) corresponded to an odds ratio of 1.91 with a 95% confidence interval of 1.00-3.73.

There was also some evidence for an interaction between high levels of testosterone and IGF-1 in relation to prostate cancer. This finding suggests that, in addition to testosterone, IGF-1 may increase the risk of prostate cancer in humans.

From and older paper by Deutsch et al (2004):

IGF1 is a potent stimulator of normal and neoplastic cell growth and exerts antiapoptotic activity on prostate epithelial cells. Insulin-like growth factor binding protein 3 (IGFBP3), the major circulating binding protein for IGF1, affects the bioavailability of IGF1 and has independent proapoptotic activity in prostate-cancer cell lines. In vitro and in vivo experiments

have shown that IGF1 increases the proliferation of both androgen-dependent and androgenindependent prostate-cancer cell lines, and that IGFBP3 can decrease the growth-stimulating effects of IGF1. It is noteworthy that circulating IGF1 and IGFBP3 concentrations are associated with the incidence of advanced-stage prostate cancer.

Also as McCarty et al noted at the same time:

Excessive activation of tyrosine kinase growth factor receptors, by stimulating the PI3K-Akt-mTOR-p70S6k and Ras-Erk1/2 pathways, works in a variety of complementary ways to promote proliferation, inhibit apoptosis, boost angiogenic capacity, and stimulate migration/invasion. Furthermore, in prostate cancers that have evolved to androgen independence, growth factor receptors may have the remarkable ability to activate androgen receptors in the absence of androgens.

The type 1 IGF receptor (IGFR1) and the receptor for epidermal growth factor (EGFR) are known to play key roles in the evolution of prostate cancer. IGFR1 is now drawing particular attention in light of prospective epidemiological evidence that relatively high serum levels of IGF-I, and relatively low serum levels of its functional antagonist IGFBP-3, are associated with increased risk for advanced prostate cancer.

Recent studies indicate that expression of IGFR1 in primary prostate cancers tends to be upregulated as compared with benign prostate epithelium. High expression of this receptor tends to be maintained in metastastic lesions, although a subset of these lesions is characterized by a decrease in IGFR1 expression coupled with a loss of phosphatase and tensin homolog (PTEN) activity; presumably, the chronic upregulation of PI3K-Akt-mTOR signaling stemming from the loss of PTEN can compensate for a reduction in IGF signaling.

Prostate cancers commonly produce IGF-II (rather than IGF-I), giving rise to an autocrine stimulation loop that helps to sustain growth even when malignant prostate cells are cultured in serum free medium.

Conversely, antisense suppression of IGFR1 expression slows proliferation and boosts apoptosis in cultured prostate cancer cells. IGF-II production is typically higher in advanced metastatic lesions and high Gleason score cancers, as opposed to primary cancers and those with low Gleason scores.

Benign prostate epithelium, as well as prostate cancers, produce the full range of IGF binding proteins, excepting IGFBP-1. Normal epithelium, however, produces the IGFBP-1-related protein, the expression of which is usually lost in cancers; this protein is induced by IGF-I in healthy epithelium and thus appears to act as a feedback mechanism for controlling prostate growth.

In advanced prostate cancers, IGFBP-2 production is usually upregulated, whereas IGFBP-3 is downregulated. Remarkably, IGFBP-2, which suppresses IGF-driven growth in healthy prostate epithelium, acts to accelerate growth in prostate cancer cells; the mechanism of this latter effect is obscure. Systemic levels of IGFBP-2 are usually elevated in advanced prostate cancer and

tend to correlate with prostate-specific antigen (PSA) levels and cancer aggressiveness. The downregulation of IGFBP-3 in prostate cancers is also of functional significance; not only does this protein oppose IGF-driven growth by binding IGFs, but it also exerts a direct growth-inhibitory effect, likely by activating transforming growth factor (TGF)-β receptors.

Although prostate cancers do not produce IGFBP-1, exogenous IGFBP-1, which has high affinity for both IGF-I and IGF-II, suppresses the growth of prostate cancer cells in vitro by intervening in the IGF-II/IGFR1 autocrine loop.

Although most prostate cancers generate their own IGF-II, systemic IGFs have the potential to contribute to IGFR1 activation in prostate cancers. Conversely, serum IGFBP-1 and IGFBP-3 have the potential to inhibit prostate cancer growth. These considerations are of importance in light of the fact that lifestyle measures can influence hepatic production of both IGF-I and IGFBP-1.

Barnard and colleagues have recently demonstrated that a very-low-fat whole-food diet, coupled with daily walking exercise—the classical "Pritikin regimen"—can markedly influence these parameters. In 14 male volunteers participating in the Pritikin regimen for 11 days, serum IGF-I fell by an average of 20%, whereas IGFBP-1 increased by 53%. In 8 subjects of comparable age who had engaged in this program for an average of 14 years, IGF-I was 55% lower and IGFBP-1 150% higher relative to initial values in the first group. The regimen did not influence serum IGFBP-3 in either group. The researchers then assessed the ability of pre- and postregimen serum from the experimental group, as well as serum from the 8 "veteran" subjects, to support the growth of androgen-sensitive LNCaP human prostate cancer cells in vitro.

1.2 OVERVIEW

There have been multiple points of attack on PCa cells and their progression. The classic approach was androgen suppression which can function for a while but is often limited. The Insulin-like growth factor, IGF, is putatively an additional target since it directly affects cell proliferation and can be generated even from metastatic cells creating a positive feedback loop. Moreover IGF has a somewhat close relationship with insulin, more than just the form of the peptide structure. Thus there is a nexus between loss of insulin control and excess glucose and PCa.

In this note we examine IGF and PCa relationships. We look at various studies in some detail and attempt to examine the genetic footprint of how IGF functions. We do not examine such factors as the epigenetics, translocations, miRNAs, the micro tumor environments etc which we have clearly discussed elsewhere[1].

We cover the following here:

1. IGF and its functions.

[1] https://www.researchgate.net/profile/Terrence-Mcgarty/research

2. IGF and related cancer interactions

3. IGF and insulin and the impact of hyperglycemia.
4. Prostate cancer fundamentals and structures

5. Basic genetics of PCa. This is a challenge. There are a multiple set of genes that are elements of PCa. The classic ones of PTEN, p53, and AR, the androgen receptor, have been considered elsewhere. We examine several others that find themselves in the controlling pathways.

6. Some basis epigenetic elements of PCa.

7. We look at several therapeutics. There are specific ones which target IGF and IGFR. To date there are no

8. We then examine metformin and its impact on PCa. Metformin basically reduces circulatory glucose and it does so by a control of the liver's release. However it does have IGF interactions and we examine some these herein. As Aguirre et al note:

However, GH can also exert metabolic actions independent from IGF-1 generation in the liver via activation of the phosphoinositide 3-kinase (PI3 K) and IRS pathways. In this way, GH and insulin act in symphony with IGF-1 to produce a coordinated response. Supported by an increasing number of studies these effects suggest the involvement of IGF-1 in metabolism coordination.

Namely the interactions between metabolism, insulin, glucose, and IGF present drivers for PCa and most likely many other cancers.

2 IGF

The insulin-like growth factor, IGF, is one of many growth factors that activate pathways in a cells and these pathways then result in such things as proliferation. We begin with some simple observations about Insulin-Like Growth Factor 1 (IGF-1)

- *What is it? A secreted and circulating protein that stimulates cell proliferation and protects cells from death (apoptosis).*
- *Binds to IGF-1 Receptors on cells.*
- *Highly variable between individuals.*
- *Men with very high levels of circulating IGF-1 have an increased risk of developing prostate cancer (RR=4.3)*

From Liu et al:

Insulin-like growth factor-1 (IGF-1) is a single-chain peptide composed of 70 amino acids and shares 50% homology with insulin . It contains three disulfide bridges, which create a tertiary structure that is critical for optimum binding to the insulin-like growth factor-1 receptor (IGF-1R).

In normal individuals, IGF-1 can not only be delivered to target tissues by insulin-like growth factor binding proteins (IGFBPs) as a circulating hormone, but also synthesized in target organs, where it exerts actions through paracrine and autocrine mechanisms . IGFBPs can bind approximately 98% of all circulating IGF-1 and form a trimeric complex with the acid-labile subunit (ALS) to serve as carrier proteins that regulate IGF-1 transport and prolong its comparatively short half-life .

Scholars have found six IGFBPs in our body, and approximately 80% of all bound IGF-1 are bound to IGFBP-3. In addition, the bioavailability of IGF-1 is negatively associated with the concentrations of specific IGFBPs in the extracellular fluids because IGFBPs have an even greater binding affinity for IGF-1 than IGF-1R . IGF-1R is widely displayed on the surface of normal tissue and solid tumor cells . IGF-1R is composed of two extracellular α-subunits that are activated upon IGF-1 binding and two β-subunits that have intracellular tyrosine kinase domains that are phosphorylated by IGF-1.

Activated IGF-1R can activate phosphatidylinositol-3 kinase (PI3K)/serinethreonine kinase (Akt)/mammalian target of rapamycin (mTOR) and Ras/Raf/mitogenactivated protein kinase (MAPK) signaling to achieve cell survival and proliferation.

It can also undergo internalization and translocate to the nucleus of cells, where it can modulate the expression of genes involved in cell cycle regulation, DNA synthesis, and damage repair. In summary, IGF-1 signaling can enhance cell growth due to its anabolic effects.

Another detailed description notes[2]:

[2] See https://www.sigmaaldrich.com/US/en/technical-documents/technical-article/research-and-disease-areas/cell-signaling/insulin-like-growth

Insulin-like growth factors (IGF-I and IGF-II) are mitogenic and anabolic peptides structurally homologous to insulin. IGF-I and -II are single polypeptide chains of approximately 7.5 kDa comprised of 70 and 67 amino acid residues, respectively. IGF-I and -II share 70% homology in amino acid sequence, while IGF-I and proinsulin share 48% homology. Both IGFs are highly conserved between species, with 100% identity among human, bovine and porcine IGFs. Unlike insulin, IGF-I and -II are primarily involved in normal growth and development. Circulating IGF is mainly secreted from the liver and acts as an endocrine to distant cells.

Many other tissues also make IGFs, where they act with autocrine and paracrine functions to regulate a number of different cellular functions.

It should be noted as part of this Note that in PCa, with bone metastasis, the bone matrix resorption releases both TGF-β and IGF-1. These proteins then promote proliferation and survival of the malignant cells. As noted in Shen and Rubib (p 315) this becomes the "vicious cycle" of PCa. The control of IGF-1 as a means to manage and mitigate PCa mets has been spotty at best. We shall examine this in some detail later. Continuing they note:

IGF-I receptor (IGF-IR) is homologous to the insulin receptor (IR) and is comprised of two 130-kDa ligand-binding a-subunits and two 95-kDa transmembrane b-subunits. IGF-IR binds IGF-I with highest affinity, IGF-II with somewhat lower affinity, and insulin with rather weak affinity. IGF-IR is a tyrosine kinase receptor with signal transduction pathways that include substrates IRS-1, IRS-2, Shc, and Grb10. IGF-IIR has anabolic functions (like IR) but also shows three distinguishing qualities concerned with growth:

1) It signals mitosis in a variety of cells.

2) It is a necessary factor in establishing and maintaining cells in a transformed phenotype.

3) It protects cells from apoptosis, both in vitro and in vivo.

This last quality is the subject of considerable interest, as it was found that IGF-I administration to cells stimulates the formation of bcl-2, a prominent anti-apoptotic intracellular messenger. While other known anti-apoptosis treatments inhibit apoptotic pathways without actually preventing cell death, IGF-I stimulation may actually decrease the probability of apoptosis initiation.

2.1 IGF OVERVIEW

The insulin-like growth factor, insulin growth factor or IGF, is a key element in glucose control[3]. Spravchikov et al have discussed the impact of poor glucose management on skin keratinocytes.

[3] Note: IGF-1 is a small peptide consisting of 70 amino acids with a molecular weight of 7649 Da. Similar to insulin, IGF-1 has an A and B chain connected by disulphide bonds. The C peptide region has 12 amino acids. See Laron. The use of insulin in the name of the growth factor is reminiscent of its structure rather than similarity in function. There is a modest amount of interaction which we shall discuss herein.

This discussion is critical in trying to understand the role of the IGF and glucose on cancer initiation and progression. Thus it is worth a mild digression to understand their findings.

They note:

Glucose is known to affect insulin action as well by regulating the expression of several genes, including the IGF-I receptor (IGFR) and insulin receptor (IR) genes, at both the transcriptional and translational levels.

Moreover, hyperglycemia was shown to inhibit insulin action.

This inhibition is thought to be a result of serine phosphorylation through a PKCmediated mechanism as well as by activation of protein tyrosine phosphatases, which deactivates the IR function. In addition to its possible involvement in the development of complications of chronic diabetes, glucose was shown to downregulate its own transport and metabolism.

As a result, high glucose levels create a vicious cycle in which even less glucose enters the cells, resulting in increased blood glucose levels, which in turn further disrupt the transport and metabolism of glucose into the cells.

It is therefore clear that glucose per se, either directly or via changes in insulin signaling, is an important factor in both the regulation of its own transport and metabolism and in the pathogenesis of chronic complications of diabetes…

Glucose inhibits the phosphorylation of the IGFR.

One of the observations we make is the interaction between IGF and insulin and blood glucose. Perhaps due to the similarity of the protein structure or perhaps for other yet specified reasons. But the phosphorylation of IGFR may actually be beneficial if it inhibits pathway activation allowing for inhibition of apoptosis and the activation of proliferation. We will see other results as we proceed.

We have shown so far that exposure of keratinocytes to high glucose concentrations, mimicking the hyperglycemic state, has effects on skin cells, resulting in inhibition of proliferation and an abnormal differentiation process. However, in diabetic patients, development of hyperglycemia also results in changes in insulin and IGF-I signaling….

As mentioned earlier, another effect of insulin and IGF-I on keratinocytes is an increase in cellular proliferation . Therefore, we evaluated the proliferation rate of keratinocytes in response to chronic insulin or IGF-I stimulation in the presence of 2 or 20 mmol/l D-glucose. … both insulin and IGF-I induced an increase in the proliferation rate of the cells (142 and 155% above control, respectively). However, in the presence of high glucose concentrations, the effects of both hormones—but mainly of IGF-I—were reduced (129 and 123% above control, respectively). Glucose effects were specific, as there was no effect on the activity of keratinocyte growth factor on glucose transport…

We have previously shown that in skin keratinocytes, IR and IGFR have different roles in skin proliferation that are mediated via distinct signaling pathways. In addition, we have shown in the present study that high glucose levels, in the absence of any additional perturbation, are associated with decreased cellular proliferation. Thus, glucose inhibits proliferation by both direct effects as well as by reducing the stimulatory effect of IGF-I on proliferation. In conclusion, the consequence of high glucose inhibition on the proliferation of skin keratinocytes and its enhancement of their differentiation is obvious.

By changing the proliferation-differentiation balance, which is one of the essential steps in the healing process, as well as by decreasing other possible local effects of IGF-I on wound healing, high glucose levels might indeed contribute to impaired wound healing in diabetes.

Hakuno and Takahashi have noted:

Insulin-like growth factors (IGFs) bind specifically to the IGF1 receptor on the cell surface of targeted tissues.

Ligand binding to the α subunit of the receptor leads to a conformational change in the β subunit, resulting in the activation of receptor tyrosine kinase activity. Activated receptor phosphorylates several substrates, including insulin receptor substrates (IRSs) and Src homology collagen (SHC).

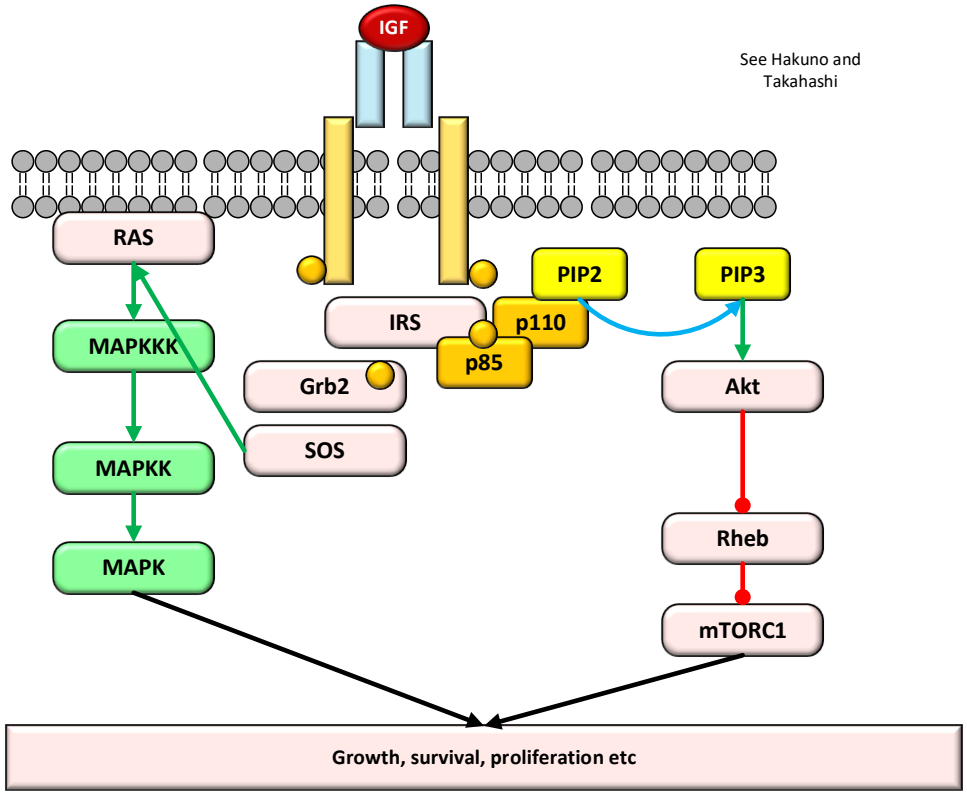
Phosphotyrosine residues in these substrates are recognized by certain Src homology 2 (SH2) domain-containing signaling molecules. These include, for example, an 85kDa regulatory subunit (p85) of phosphatidylinositol 3-kinase (PI 3-kinase), growth factor receptor-bound 2 (GRB2) and SH2-containing protein tyrosine phosphatase 2 (SHP2/Syp).

These bindings lead to the activation of downstream signaling pathways, PI 3-kinase pathway and Ras-mitogen-activated protein kinase (MAP kinase) pathway.

We shall examine these pathways shortly. But it is critical to understand what effects result from this IGF binding.

Activation of these signaling pathways is known to be required for the induction of various bioactivities of IGFs, including cell proliferation, cell differentiation and cell survival. In this review, the well-established IGF1 receptor signaling pathways required for the induction of various bioactivities of IGFs are introduced. In addition, we will discuss how IGF signals are modulated by the other extracellular stimuli or by themselves based on our studies

We show the effects of IGF-1 from the above authors.



From Yang et al:

The insulin-like growth factor system consists of two ligands (IGF-I and -II), two main receptors (IGF-IR and IGFIIR), six different IGF binding proteins (IGFBP1- 6) and four IGFBP related peptides (IGFBP Rp1-4). The IGF ligands have a short life-span unless they are bound to a binding protein which transports them in the circulation and delivers them to specific tissues. Components of the IGF system are found throughout the body in various fluids and tissues . IGFs act on a variety of mammalian cells in an endocrine, paracrine and autocrine manner to regulate cell proliferation, apoptosis, transformation and differentiation . They influence the growth of normal tissue as well as that of several cancers.

Converging data from clinical and laboratory studies clearly indicate that IGF-I is implicated in cancer cell migration and invasion.

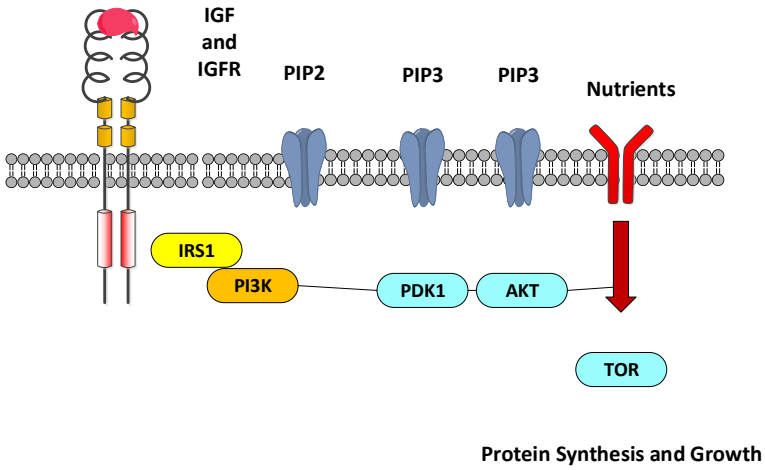
IGF-I receptor (IGF-IR) expression is correlated with colorectal cancer venous invasion and liver metastasis, and has been proposed as a predictor of liver metastasis from colorectal cancer .

Blockade of the paracrine action of IGF-I can suppress liver metastases from colorectal cancer .

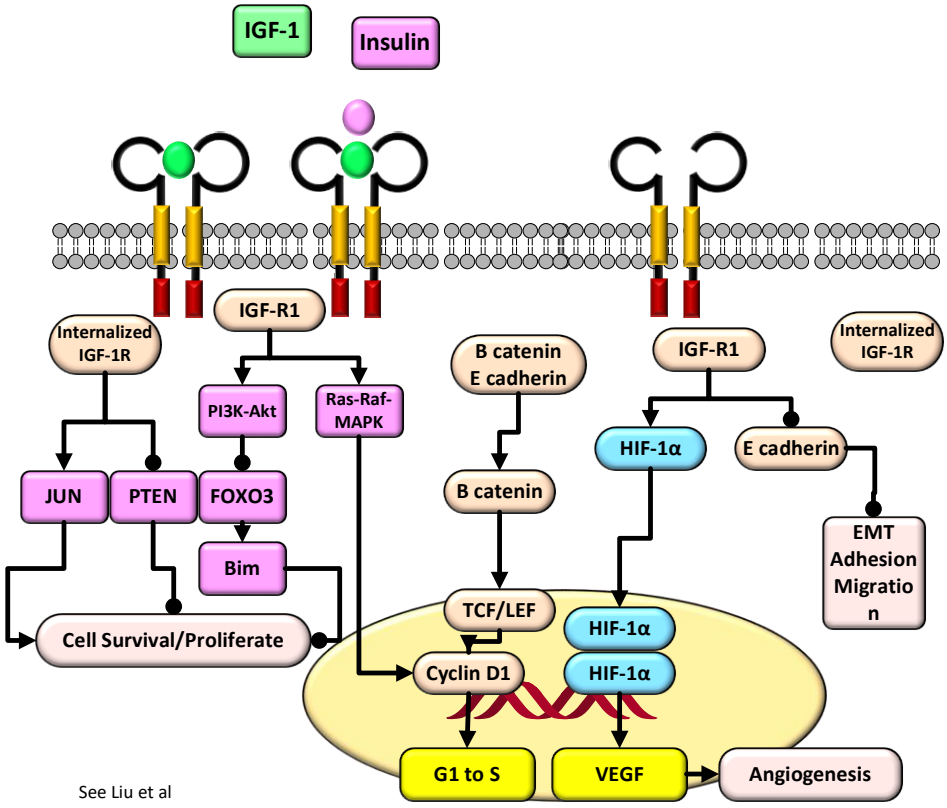
It has been established that IGF-IR and the integrins interact together to form a complex at the colon cell-cell contact sites, whilst addition of IGF-I to this complex causes integrin

redistribution within the cell-cell contact site and is associated with an increase in the migration of colorectal cancer cells.

From Morgan we have the putative interaction of IGF with the IGFR and the resulting cell reaction as shown below[4]:



Referring to Liu et al we have the following pathways and functions:



[4] See Morgan, p 216

As Dehkhoda et al note:

The effects of cellular signaling from GHR activation are responsible for a vast array of important physiological roles. Growth hormone is secreted by the anterior pituitary gland and not only has a role in increasing bone length, bone density, and muscle mass during childhood and adolescence but also importantly in the regulation of metabolism of lipids, carbohydrates, and body water throughout life .

The effects of GH are exerted by binding to the GH receptors on target cells, which in turn stimulates the production and secretion of IGF-1 from many tissues, mainly the liver .

Since 1957 when IGF-1 and IGF-2 were identified and first designated as "sulfation factors" , the interest in the study of these molecules that structurally resembled proinsulin increased, especially when IGF-1 was found to be the mediator of the anabolic and mitogenic activity of GH . IGFs were first named as somatomedins due to their concentration dependence by GH regulation.

A subsequent isolation and amino acid sequence determination of two homogeneous polypeptides from purified non-suppressible insulin-like activity factors established the current designation of these molecules as insulin-like growth factors (IGFs) 1 and 2 .

IGF-1 is a 70 amino acid peptide with a molecular weight of 7,649 Da. It has the ability to bind to the insulin receptor, although with low affinity.

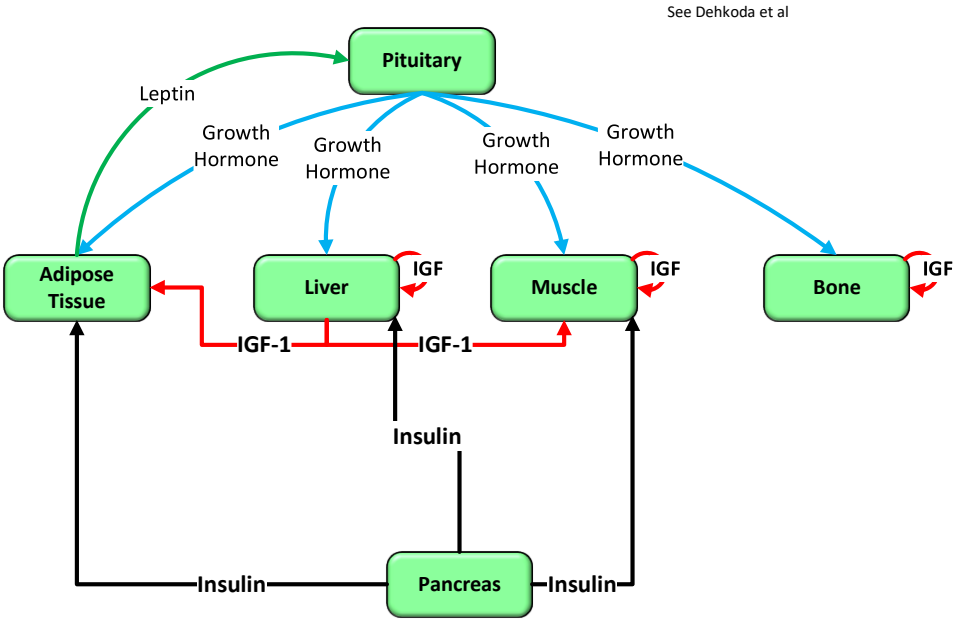
It should be noted that IGF does bind at times to the insulin receptor. Thus in patients with impaired insulin secretion we may have activation, albeit weakly, from IGF binding. This aberrant process may then effect others downstream.

Most IGF-1 is secreted by the liver and acts as an endocrine hormone, although it can be secreted by many other tissues . One of the main roles for which IGF-1 has promoted subsequent research is its involvement in growth and its relation to growth hormone. Exogenous IGF-1 was shown to stimulate growth when administered to hypophysectomized rats.

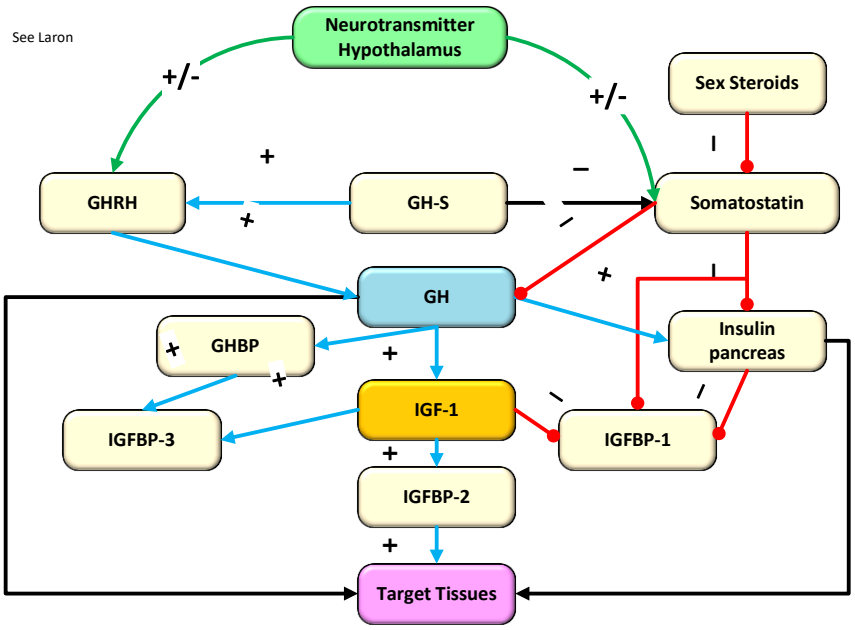
Furthermore, children with IGF-1 deficiency primary GH insensitivity or children with Laron syndrome who were treated with biosynthetic IGF-1 showed increases in their serum alkaline phosphatase and serum procollagen and IGF-binding protein-3 (IGFBP-3). This treatment was subsequently widely used in other parts of the world. In terms of efficacy, GH and IGF-1 both stimulated linear growth but some variables including the greater growth deficit in infants with Laron syndrome than those with isolated growth hormone deficiency, insufficient IGF-1 dose, or the IGF-1 dependency on the GH-linked stem cell population of prechondrocytes made GH more efficient in terms of linear growth stimulation.

However, IGF-1 was shown to be an important growth related hormone that has a GH-independent growth stimulating effects that in some cases acts synergistically with GH . On the

other hand, … GHR signaling has been shown to have growth promoting effects independent of IGF-1.



In the paper by Laron there is a more detailed flow of control as shown below:



The above are macro level flows. Also we have the intracellular micro level interactions. The macro flows depict the inter-organ interactions thus resulting in the micro elements we have also depicted.

2.2 IGF CANCERS

Excess activation of IGF has been linked to a variety of cancers. As Murekatete et al have noted regarding melanomas:

Insulin-like growth factor (IGF)-I binds to the ECM protein vitronectin (VN) through IGF binding proteins (IGFBPs) to enhance proliferation and migration of skin keratinocytes and fibroblasts. Although evidence exists for the role of individual components of the complex (IGF-I, IGFBP-3 and VN), the cellular functions stimulated by these proteins together as a complex remains un-investigated in melanoma cells. We report here that the IGF-I:IGFBP-3:VN trimeric complex stimulates a dose dependent increase in the proliferation and migration of WM35 and Sk-MEL28 melanoma cells.

... both cell lines formed primary tumor-like spheroids, which increased in size in a dose-dependent manner in response to the trimeric complex. Furthermore, we reveal IGFBP- 3:VN protein complexes in malignant melanoma and squamous cell carcinoma patient tissues, where the IGFBP-3:VN complex was seen to be predominantly tumor cell-associated. Peptide antagonists designed to target the binding of IGF-I:IGFBP-3 to VN were demonstrated to inhibit IGF-I:IGFBP- 3:VN-stimulated cell migration, invasion and 3D tumor cell growth of melanoma cells. Overall, this study provides new data on IGF:ECM interactions in skin malignancies and demonstrates the potential usefulness of a growth factor:ECM-disrupting strategy for abrogating tumor progression.

They continue:

The high mortality rate of melanoma is associated with the metastasis of malignant melanoma cells to critical organs of the body1. Insulin-like growth factor-I (IGF-I), amongst others, is known to enhance tumor growth and invasion. IGF-I can act as a paracrine factor that drives malignant cell transformation through the activation of the IGF type-I receptor (IGF-IR). All melanocytic cells express the IGF-IR, with increased expression correlated with disease progression.

In addition, growth factor interactions with the extracellular matrix (ECM) play important roles in tumor biology, facilitating tumor cell attachment, proliferation and invasion, and resistance against chemotherapeutic drugs. Proteins in the IGF system have been shown to interact with ECM proteins such as fibronectin (FN), vitronectin (VN), laminins, as well as integrins, which in turn, modulate the function of IGF-I9. Previous studies have demonstrated that IGF-I interacts with VN through IGFBPs to form IGF-I:IGFBP:VN trimeric (TRI) complexes. Further, IGFBP:VN complexes have been observed in tumor biopsies from breast cancer patients, associating with the invasive front of tumor clusters and around tumor blood vessels.

This is aligned with the concept that VN is a matricellular protein that functions as a scaffold onto which growth factors, such as IGF-I, are captured, exposing cells to concentrated foci of growth factors available for receptor stimulation. Indeed, complexes of TRI have been shown to promote enhanced cell attachment and migration, as well as protein synthesis, in human keratinocytes and breast cancer cell lines

As Siech et al have noted:

Insulin-like growth factor-1 (IGF-1) is a growth hormone and is implicated in prostate cancer progression. Most prostate cancers begin in an androgen-dependent state so that androgen deprivation therapy results in improved clinical outcome.

However, some cancerous cells may survive androgen deprivation, growing into therapy-resistant, androgen-independent prostate cancer.

The present study investigated the influence of IGF-1 on tumor growth and migration properties using androgen-dependent LNCaP and VCaP and androgen-independent PC3 and DU145 prostate cancer cells. Stimulation with IGF-1 activated growth in all cell lines. There were changes in transmembrane receptors (integrins) that bind cells to each other and changes in focal adhesion kinase that controls cell motility. Intracellular Akt/mTOR signaling, regulating cell division, was also activated.

Thus, it seems that prostate cancer progression is controlled by a fine-tuned network between IGF-1-driven integrin-FAK signaling and the Akt-mTOR pathway.

Concerted targeting of both pathways may, therefore, help prevent cancer dissemination.

2.3 HYPERGLYCEMIA

It has been observed that PCa is more common in patients with hyperglycemia, especially when poorly controlled. In contrast the use of metformin has a dual role; it reduced hyperglycemia while somehow having an anti-PCa action. We shall examine this latter. As Mansor et al have noted:

Localized prostate cancer (PCa) is a manageable disease but for most men with metastatic disease, it is often fatal.

A western diet has been linked with PCa progression and hyperglycaemia has been associated with the risk of lethal and fatal prostate cancer. Using PCa cell lines, we examined the impact of IGF-I and glucose on markers of epithelial-to-mesenchymal transition (EMT), migration and invasion. We examined the underlying mechanisms using cell lines and tumour tissue samples. IGF-I had differential effects on the process of EMT: inhibiting in normal and promoting in cancer cells,

whereas hyperglycamia alone had a stimulatory effect in both.

These effects were independent of IGF and in both cases, hyperglycaemia induced an increase IGFBP-2(tumour promoter) and FOXA1.

A positive correlation existed between levels of IGFBP-2 and FOXA1 in benign and cancerous prostate tissue samples and in vitro and in vivo data indicated that FOXA1 strongly interacted with the IGFBP-2 gene in normal prostate epithelial cells that was associated with a negative regulation of IGFBP-2, whereas in cancer cells the level of FOXA1 associating with the IGFBP-2 gene was minimal, suggesting loss of this negative regulation. IGF-I and hyperglycaemia-induced FOXA1/IGFBP-2 play important roles in EMT. …

The bioactivity of IGF-I is intricately integrated with nutritional status and energy balance; and alterations in metabolism associated with adoption of a western lifestyle have been postulated to contribute to the increased risk of developing prostate cancer . Increased expression of IGF-I has been associated with poor prognosis and more aggressive cancers that exhibit increased metabolism and increased glucose uptake. The activation of the EMT program contributes to the progression of metastatic prostate cancer, which is the principle cause of death in most of prostate cancer patients. In this study we investigated the role of IGF-I in promoting prostate cancer progression through activation of the EMT program.

With normal PNT2 cells, under euglycaemic conditions, EMT was inhibited by IGF-I, whereas opposite effects were observed in the cancer cells, which suggest that with normal epithelial prostate cells, IGF-I prevented a malignant phenotype by maintaining the normal characteristics of differentiated cells whereas in cancer cells, IGF-I promoted a more mesenchymal phenotype that could potentially promote a more aggressive phenotype.

The ability of IGF-I to induce EMT in prostate cancer cells was also consistent with studies conducted in brain and breast cancer cell lines. Similar observations were made by Graham et al (2015) where IGF-I induced EMT in ARCaP prostate cancer cells through up-regulation of Zinc finger E-box-binding homeobox (ZEB1), a transcription factor that regulated EMT activation. IGF-I induced cell proliferation in both cancer and non-cancerous cells confirming its mitogenic role in regulating cell growth and differentiation in wide variety of cells as reported in the literature.

Even though similar proliferative effects of IGF-I were observed in cancer and non-cancerous cells, its effect on the migratory ability was different. IGF-I decreased migration of normal PNT2 cells but promoted migration of the DU145 prostate cancer cells: this reflected the changes observed in the EMT markers upon IGF exposure. Increases in E-cadherin by IGF-I that was observed with PNT2 cells, tightens the cell-cell junctions thus limiting the ability of cells to move and migrate. On the other hand, with DU145 cells, IGF-I increased the cells' potential for metastasis as evidenced by increases in cell migration.

Epidemiology studies have shown a positive association between metabolic syndrome such as obesity and diabetes with cancer development and mortality. Increases in IGF-I levels appear to be one of the factors linking these different diseases .

Hyperglycaemia, a hallmark of diabetes, has been shown to promote cancer progression.

We have also shown previously that hyperglycaemia induced chemoresistance in prostate cancer cells .

In this study, we revealed for the first time that exposure to high concentrations of glucose (25 mM) alone induced EMT in both cancer and non-cancerous prostate epithelial cells compared to normal glucose (5 mM) conditions.

The transition from epithelial to mesenchymal characteristics in these cell lines was also correlated with increases in proliferation and migratory potential in both cell lines. More interestingly, with PNT2 cells, despite the ability of hyperglycaemia alone to induce EMT, the effects were still reversed by addition of IGF-I: IGF-I was able to promote an epithelial phenotype in PNT2 cells in both normal and high levels of glucose. However, with DU145 cells, addition of IGF-I in high glucose conditions was not able to augment the effect of high glucose alone on EMT. These data suggested that different concentrations of glucose do not influence the action of IGF-I and that glucose alone can independently promote tumour aggressiveness and metastasis. The involvement of hyperglycaemia in prostate cancer cell EMT, progression and metastasis may underpin why prostate cancer patients that present with hyperglycaemia have a worse prognosis.

High intake of total energy has been associated with increased risk of fatal prostate cancer .

Dietary restriction and lifestyle changes are suggested as an intervention for suppression of cancer growth. A study of calorie restriction performed in mice showed a reduction in circulating IGF-I and insulin levels and deactivation of the PI3K/AKT pathway which resulted in prostate tumour growth inhibition .

In addition, calorie restriction combined with IGF-1R blockade resulted in growth inhibition in prostate cancer xenografts .

Taken together, this evidence suggests that limiting energy consumption may reduce the risk of prostate cancer progression by improving the metabolic profile. β-catenin plays an important role in both maintaining epithelial integrity and as a co-activator of Wnt-mediated gene transcription.

The activation of Wnt/ β-Catenin signalling has been shown to promote EMT in prostate cancer.

This study revealed that IGF-I promoted destabilization of β-catenin by inducing its phosphorylation at Ser33/37/T41 in both PNT2 and DU145 cells and that this effect was not affected by levels of glucose. It is also interesting to note that despite this observation (destabilization of β-catenin), IGF-I also induced β-catenin nuclear translocation, which is indicative of Wnt/β-catenin transcriptional activity.

Likewise, increased hyperglycaemic conditions alone had the same effect on β-catenin phosphorylation and nuclear translocation in DU145 in contrast to the PNT2 cells although in

both cell lines hyperglycaemia yielded a similar effect on EMT. IGF-I exerts its biological effects through binding to its receptor, predominantly the IGF-IR, activating downstream signalling cascades. With PNT2 cells, both IGF-IR/PI3K/AKT and IGF-IR/RAS/MAPK pathways were found to be equally activated by IGF-I whereas with DU145 cells, IGF-IR/PI3K/AKT appeared to be the dominant signalling pathway in mediating IGFI-induced EMT in DU145 cells.

In contrast to IGF-I, hyperglycaemia did not induce activation of the IGF-IR or components of the IGF-IR signalling pathway (p-AKT or p-MAPK) in either cell model although all the features of EMT were enhanced by hyperglycaemia in a similar way to IGF-I in euglycaemic conditions.

This suggests that hyperglycaemia-induced EMT in PNT2 and DU145 cells was independent of IGF signalling, that was confirmed using AG1024 a tyrosine kinase inhibitor to block IGFIR activation. In euglycaemic conditions, blocking the IGF-IR reduced cell growth as expected, however in hyperglycaemic conditions, the hyperglycaemiainduced effect of increasing cell proliferation was unaffected.

This shows that a different mechanism in which hyperglycaemia activates the EMT program was involved: one of which could be through the regulation by IGFBP-2.

There is accumulating evidence that IGFBP-2 may have an important role in prostate cancer progression . We have shown previously that IGFBP-2 promotes prostate cancer growth in both an IGF-I-dependent and independent manner and that hyperglycaemia induced up-regulation of IGFBP-2 in prostate cancer cells which resulted in resistance to chemotherapy .

Apart from IGFBP-2, an increase in the level of FOXA1 by hyperglycaemia in prostate cell lines was also observed in this study. In light of these findings, we investigated if there was interplay between IGFBP-2 and FOXA1. Our in vivo study indicated that a positive correlation existed between levels of IGFBP-2 and FOXA1 in benign and cancerous prostate tissue samples. With the CHiP analysis using cell lines, we found that FOXA1 strongly interacted with the IGFBP-2 gene in normal prostate epithelial. The siRNA data showed that silencing FOXA1 in normal prostate cells resulted in a large increase in IGFBP-2. Collectively, these data may suggest that in normal prostate epithelial cells, FOXA1 binds to the IGFBP-2 gene to negatively regulate IGFBP-2 levels.

As Friedrich et al have noted:

GF-I, predominantly synthesized in the liver upon stimulation by growth hormone (GH), is usually bound to IGF-binding protein 3 (IGFBP-3) in circulation .

IGF-I has an almost 50% amino acid sequence homology with insulin and elicits nearly the same hypoglycemic response . Several studies have investigated the effect of IGF-I on insulin sensitivity and its relation to type 2 diabetes. Large longitudinal studies, including the National Health and Nutrition Examination Survey (NHANES) III, reported a higher risk of insulin resistance, metabolic syndrome (MetS), and type 2 diabetes in subjects with low IGF-I serum concentrations or low IGFI–to–IGFBP-3 ratios. A recent German study in 7,665 subjects, however, showed that low and high baseline IGF-I serum concentrations were both related to a

higher risk of developing type 2 diabetes within 5 years . This U-shaped association seems to be likely in face of a higher prevalence of MetS or type 2 diabetes in patients with GH deficiency , a state of low IGF-I levels, as well as with acromegaly , a disease characterized by high IGF-I levels, although endogenous GH secretion may confound short-term glucose homeostasis in these patients.

On the basis of these findings, we also hypothesize a U-shaped relation between IGF-I levels and insulin sensitivity as precursor to manifest type 2 diabetes.

In general, the effects of IGF-I in the control of glucose homeostasis is well known. Animal models showed that a deletion of hepatic IGF-I production, resulting in 80% reduced IGF-I levels, led to hyperinsulinemia and abnormal glucose clearance. An epidemiological study reported a negative correlation between IGF-I levels and insulin resistance measured by the homeostasis model assessment of insulin resistance (HOMA-IR) . Confirming results were found in an Italian study that investigated subjects with and without type 2 diabetes as well as with impaired glucose tolerance . IGF-I levels were positively correlated with insulin sensitivity among all three groups. …

IGF-I has structural homology with insulin, and several studies supported a positive influence of IGF-I on glucose homeostasis, which strengthened the relation between decreased IGF-I and insulin resistance.

IGF-I leads to an increase in peripheral glucose uptake and a decreased production of hepatic glucose causing better insulin sensitivity.

This again is the observation that IGF-1 drives glucose uptake.

Furthermore, low IGF-I serum concentrations were related to a higher anthropometric status, which in turn is related to insulin resistance. On the other side, adult patients with GH replacement therapy revealed a higher prevalence of insulin resistance and MetS. However, whether this is an IGF-I effect or rather a free fatty acid–mediated GH effect is questionable.

Therefore, some have argued that GH replacement therapy might be associated with the development of MetS or an acceleration of the manifestation of type 2 diabetes in patients at risk.

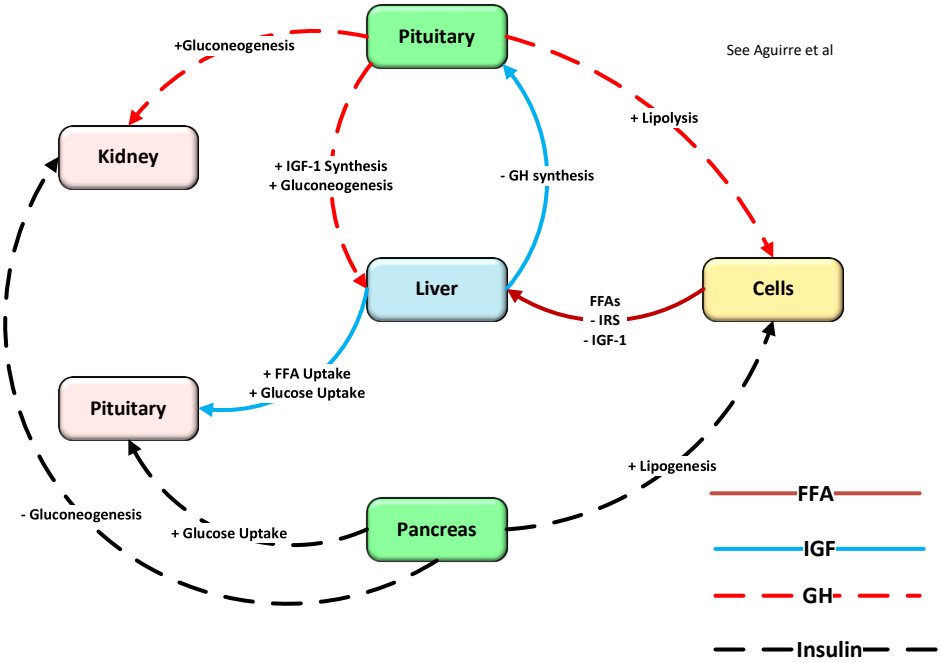
In contrast, one of the suggested benefits of GH replacement on body composition includes a decrease in abdominal fat mass , which should theoretically reduce insulin resistance, the features of MetS, and incident type 2 diabetes. The available data of observational studies regarding this issue showed no clear picture, and controlled end point studies are scarce.

A further issue arises because GH is indeed the major stimulus for IGF-I production in the liver, but both hormones can have opposing metabolic effects. As mentioned, IGF-I increases peripheral glucose uptake, whereas GH shows diabetic actions and increases glucose production.

Further differences are apparent for free fatty acid homeostasis. Although IGF-I may reduce serum free fatty acid levels, GH promotes lipolysis and ketogenesis. The effect of IGF-I in reducing serum free fatty acid levels may be important in improving insulin sensitivity related to the "lipotoxic" effects of free fatty acids.

Aguirre et al have noted:

The figure summarises schematically some of the metabolic effects that IGF-1 (blue continuous line), GH (red discontinuous line), and insulin (black dotted line) exert on kidney (upper left), brain (upper centre), skeletal muscle (left), liver (centre), adipose tissue (right), and pancreas (bottom). GH growth hormone, GHRH growth hormone releasing hormone, FFA free fatty acid, IRS insulin receptor substrate, IGF-1 insulin-like growth factor 1, IGBBP-1 insulin-like growth factor binding protein 1



Aguirre et al continue:

As a brief review of insulin signalling and its resistance molecular basis: insulin and IGF-1 receptors (IR and IGF-1R) are tyrosine kinases.

As such they attract molecules containing a Src-homolgy 2 (SH2) domain (several docking sites for phosphorylated tyrosines). The most often and potent ones attracted are the insulin receptor substrates 1/2 (IRS1/2)—although there are 6 found to date- (not to forget that Shc proteins, p60dok, Cbl, APS, and Gab-1 are also recruited to activated IRs).

These provide additional tyrosine residues to be phosphorylated by the tyrosine kinase domain of the activated receptor that will attract further molecules containing SH2 domains or plekstrin homoly (PH) domains, these last will anchor IRS to phosphoinositides on the cell membrane.

When PI3K and its regulatory proteins, p85 and p110, are recruited by IRS, they will further recruit and activate PDK1 (PIP3-dependent kinase 1), Akt (PKB), mTORC2, S6 kinases and PKC; all leading to augmented glucose transport, glycogen and protein synthesis.

Zick and colleagues have elegantly summarised recent evidence that show how IRS also possess serine residues that can be phosphorylated. When this happens tyrosine phosphorylation becomes less likely to happen. This is, in a certain way, a termination pathway to uncouple the insulin signalling. There are other mechanisms to terminate the insulin signalling that include lipid and protein phosphatases along the cascade and controlling mechanisms; long-term regulation includes transcription inhibition of the IR and proteolysis by ubiquitination. One convergent pathway activated by IGF-1R and IR is the mTORC1 and mTORC2 signalling. It is widely known they both possess serine and threonine phosphorylation capability.

However, it has been recently described that mTORC2 also possesses tyrosine phosphorylation capacity , and that it phosphorylates tyrosines on IRS and tyrosine kinases in both receptors, IGF-1 and IR, thus reinstituting the signal of the activated receptors . Whilst mTORC1 activates S6 kinase, which phosphorylates serine residues on IRS which in turn uncouples IRS from the receptor and its substrates, mTORC2 can reactivate this signalling.

Complementary to the above, it has been thought for a long time that only supraphysiological concentrations of IGF-1 are able to activate the IR, as will be further discussed in this manuscript. However, the exact mechanism by which IGF-1 improves insulin signalling has not yet be explained (other than indirect actions through lipid clearance from the bloodstream by inhibiting GH (lipolysis on adipocytes) and FFA uptake by muscles; all these mechanisms are collected below).

We now propose a feasible mechanism: Denley and colleagues have beautifully designed a study where they demonstrate how IR has a splice variant lacking exon 11 which confers the receptor affinity for IGF-1 and IGF-2.

In this way, IGF-1 gains the ability to stimulate the IR, and without activating the tyrosine kinase domain, recruits IRS-2.

Complementarily, IGF-1R preferentially activates IRS-2 as it was found that IRS-2 contains a KLRB domain that functions to block the tyrosine kinase domain in the cytoplasmic region of the IR, and that such does not happen in the IGF-1R. Thus suggesting a specificity for IGF-1R. It has been found, using specific knock out (KO) mice and cultures, different specific activities for IRS-1 and IRS-2, additional further complexity comes with tissue-specific roles. For example, in muscle, IRS-1 is more related to glucose uptake whereas IRS-2 stimulates the MAPK pathway .

In the liver, they both have metabolic regulation actions, but IRS-2 has a more profound role in lipid metabolism . Additional complexity, and in accordance with IGF-1 secretion patterns, appeared when researchers found that IRS-1 was found more active in post-prandial states contrary to IRS-2 in fasting states . Even more interesting is the fact that Shc and PLC were found to only interact with IRS-2 . Recall that Shc ultimately activates the MAPK pathway, while PLC has more metabolic effects including GLUT4 translocation. IGF-1 displays more binding

sites for SHP2 (a phosphatase related to growth) and seems more prone to recruit Cbl (an E3 ligase that targets the receptor for ubiquitination and destruction) and thus may explain a different regulatory mechanism not mediated by serine phosphorylation, and thus not so sensitive to metabolic derangements.

Intriguingly, another interesting research lead to the discovery of a differential role for IRSs in apoptosis, suggesting an antiapoptotic effect for IRS-2 which is consistent with known differential roles of IGF-1 and insulin. Taking all this data together it seems logical or appropriate to conclude that, because IGF1-R has a different signalling pathway that can maintain lipid oxidation in the liver, FFA uptake in muscle, and activates mTORC1 could reactivate IR through tyrosine kinase activity on IRS, thus displacing serine phosphorylation, reinstituting insulin signalling.

Also since most of serine inhibiting phosphorylation occurs in IRS-1, it renders IGF-1R a rescue pathway to reinstitute insulin sensitivity. Because IGF-1 is normally found at low levels in MetS and T2D, maybe because of insulin cessation to inhibit IGFBP-1 production by the liver and because of decreased liver IGF-1 secretion by insulin stimulation, as insulin resistance prevails in the liver. Consistent with the evidence presented we suggest a positive effect towards reestablishing IGF-1 levels by substitutive therapy only to physiological levels, never above them.

3 PCa

We have examined prostate cancer in various dimensions over the past fifteen years. Unlike breast cancer where there are multiple therapeutic options, prostate cancer often becomes metastatic and is then terminal. Early surgery generally can be satisfactory but even then there may be stem cells resident at distant locations in the patient and there are generally undetectable. Now we examine PCa in the context if the IGF functionality. The IGF and the IGFR, and its respective sub-elements, are major factors in many malignancies. For example as noted in NCBI:

IGF1[5]*: The protein encoded by this gene is similar to insulin in function and structure and is a member of a family of proteins involved in mediating growth and development. The encoded protein is processed from a precursor, bound by a specific receptor, and secreted. Defects in this gene are a cause of insulin-like growth factor I deficiency. Alternative splicing results in multiple transcript variants encoding different isoforms that may undergo similar processing to generate mature protein.*

IGF1R[6]*: This receptor binds insulin-like growth factor with a high affinity. It has tyrosine kinase activity. The insulin-like growth factor I receptor plays a critical role in transformation events. Cleavage of the precursor generates alpha and beta subunits. It is highly overexpressed in most malignant tissues where it functions as an anti-apoptotic agent by enhancing cell survival. Alternatively spliced transcript variants encoding distinct isoforms have been found for this gene.*

As Melmed et al note:

Insulin-like growth factor 1 (IGF1) is a polypeptide hormone secreted into the bloodstream from the liver and other tissues but is also a paracrine factor produced locally in most tissues to control cell proliferation. …

Thus, hormones and paracrine factors have several distinct strategies regulating biosynthesis, sites of action, transport, and metabolism. These differing strategies may partly explain why a hormone such as IGF1, unlike its close relative insulin, has multiple binding proteins to control its action in tissues. IGF1 exhibits a double life as both a hormone and a paracrine factor.

Presumably the IGF1 actions mandate an elaborate binding protein apparatus to enable appropriate hormone signaling. … insulin-like growth factor 1 (IGF1) is produced and secreted by the liver under the positive influence of GH and circulates to target tissues like bone, but it is also produced locally by some tissues (e.g., chondrocytes at bone growth plates) to exert effects on neighboring cells. …

[5] https://www.ncbi.nlm.nih.gov/gene/3479

[6] https://www.ncbi.nlm.nih.gov/gene/3480

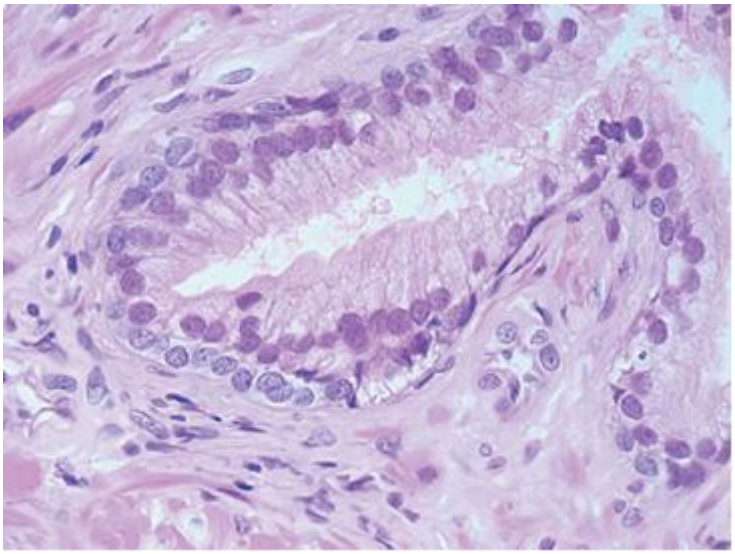
A notable exception to the general rule that peptide hormones turn over quickly and have short durations of action is provided by IGF1. Unlike most peptide hormones, IGF1 circulates in the bloodstream bound to one or more binding proteins, which has two important consequences. First, the concentration of total IGF1 in blood is much greater than that of the unbound, biologically active hormone. Second, the lifetime of IGF1 is greatly extended, such that circulating levels of the hormone change slowly over the course of hours or days. As predicted by these properties, IGF1 primarily influences phenotypes that are modified over extended periods, such as growth and differentiation, and in marked contrast to its cousin insulin, most of the cellular targets of IGF1 are transcriptional.

There has been a great deal of study of the IGF and its constituents[7].

3.1 THE NORMAL PROSTATE

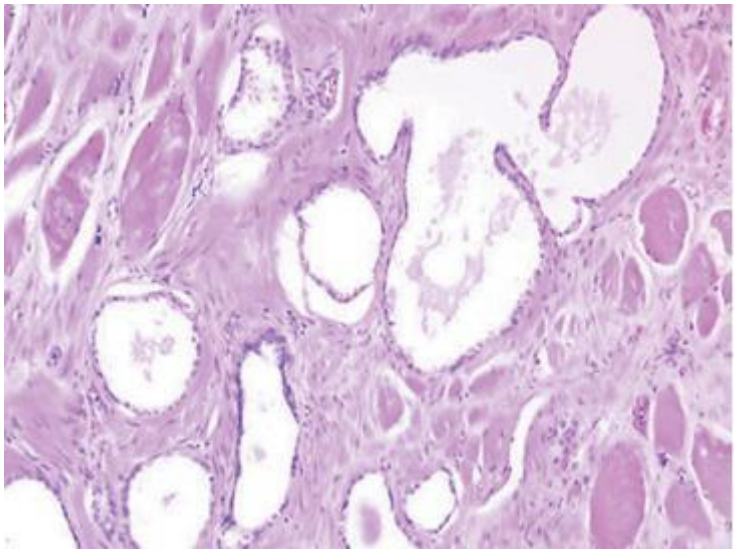
We first examine the normal prostate. The prostate is normally about 40 cc in dimension with the prostate surrounding the urethra below the bladder. The basic structure of the prostate consists of three major zones; peripheral (dominant zone), central zone which is around the urethra), and the transition zone. The cellular structure is depicted below. There are approximately 35-50 glands in the prostate, mostly in the peripheral zone and the glands have a lumen in which the prostatic secretions flow, and the glands have basal cells and luminal cells as shown below. The basal cells are dark and the luminal cells are somewhat lighter.

Between the cells is the stroma which includes the blood flow from veins and arteries, the muscle and other stroma elements. Simply stated, the prostate is a collection of the basal/luminal glands scattered about veins, arteries, muscles and nerves.



[7] https://www.sciencedirect.com/topics/neuroscience/insulin-like-growth-factor-1

The figure below depicts a second view of the prostate glands. Again this is with HE stain and under low magnification. The basal cells are clearly see with their dark stains and the luminal stand above them. The stroma is fairly well articulated in this slide.



The normal prostate then is merely a collection of glands, glands composed of basal and luminal cells, with open glandular portions, the white areas above. As we noted before these glands emit various proteins and are an integral part of the male reproductive system.

3.2 Summary of Prostate States

We now provide a high level summary of the changes in the prostate histologically as PCa is developed. We do this to lay out the various changes we will examine and to better understand what we may be looking for when developing pathways. We believe that it is essential that we always go back and forth between abstractions of pathways, and the reality of the cell histology. To understand this question, and hopefully set a path to answering it, we lay out the known elements in the path towards malignancy, look at the gene maps and dynamics, and then attempt to establish a model for examining the dynamic processes which move the cell forward to malignancy or backwards towards a benign state. We shall now examine each of these in some detail.

3.3 PCa Histology and Grading

In this sections we provide more detail on grading of PCa. The emphasis here is upon histological change and does not reflect any changes in pathways.

3.3.1 Prostate Cancer Histology

Prostate Cancer is simply the growth of abnormal glandular like structures outside of the normal prostate glands the resulting continued growth of the cells making up those structures both within and without the prostate. The PCa cells take over the stroma, pushing aside the normal stromal cells and then migrate in a metastatic fashion throughout the body.

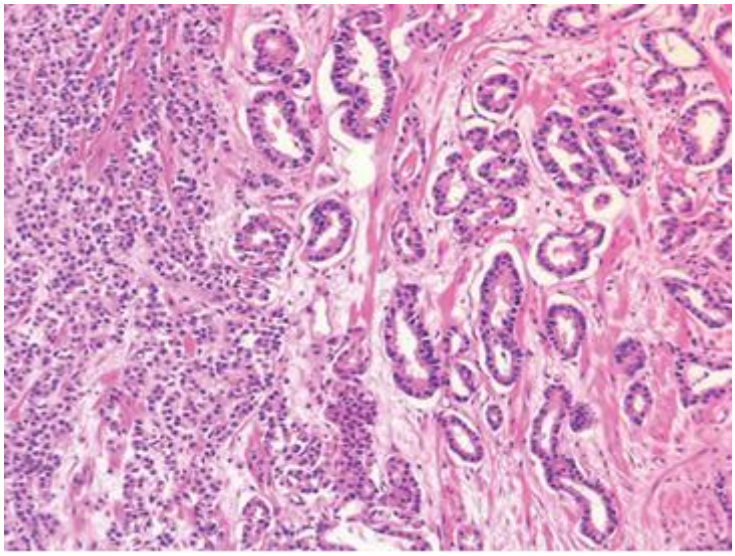
We will use the Gleason grading score as a means to characterize the level of cancer progression within the prostate.

3.3.2 Grading

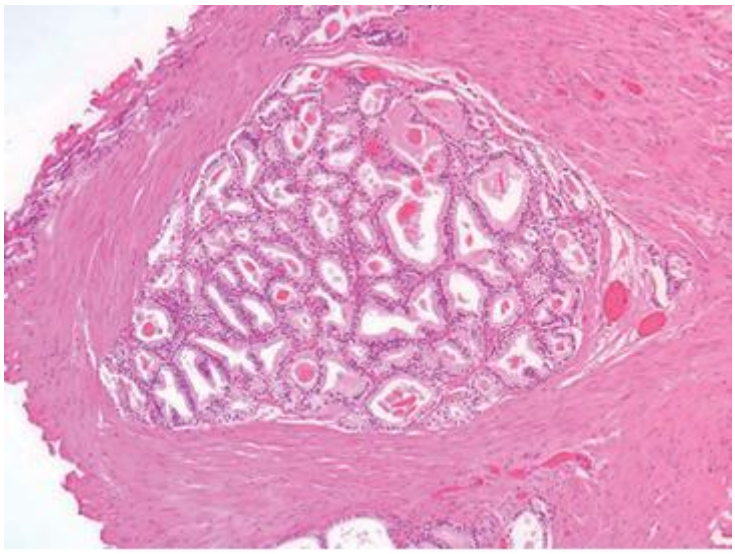
We present the grading system developed by Gleason. On the one hand this has been used as a gold standard for ascertaining future progress and yet it is still just a morphological tool. It fails to determine the pathways and regulators in a cell by cell basis.

3.3.2.1 Gleason 1

The following is a Gleason 1 grade tumor. Note that there are a proliferation of small glandular like clusters with dark basophillic stains and they are separate and have clear luminal areas. Gleason 1 is generally composed of many single and separate and closely packed glands of well circumscribed uniforms glands. One rarely sees Gleason 1 grade tumors, and they are often found as incidental findings when examining for other issues.



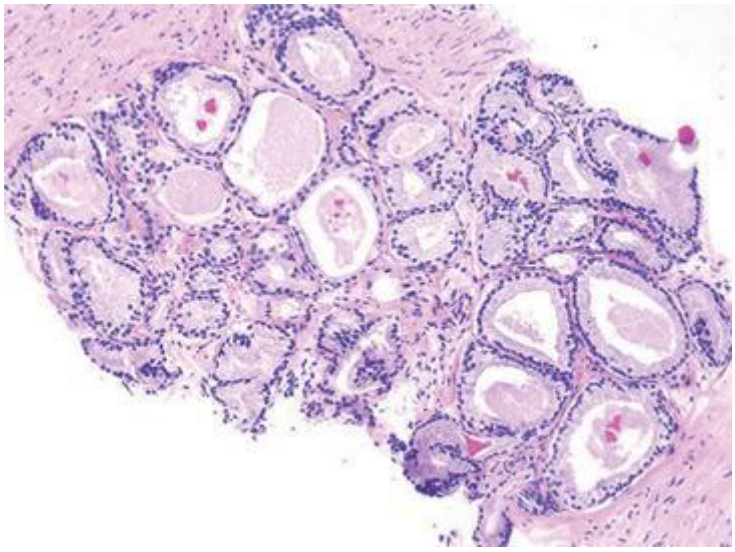
We show another view of a Gleason 1 below. This is especially descriptive of such a form because it appears almost as a single and isolated structure. The interesting question will be if this is PCa then if PCa is clonal is this cluster an aberrant outgrowth of a normal cells, if so which one, and if so is this just one cell growing. It appears that at this stage the intercellular signaling is still trying to function. However the clarity of cell form is being degraded.



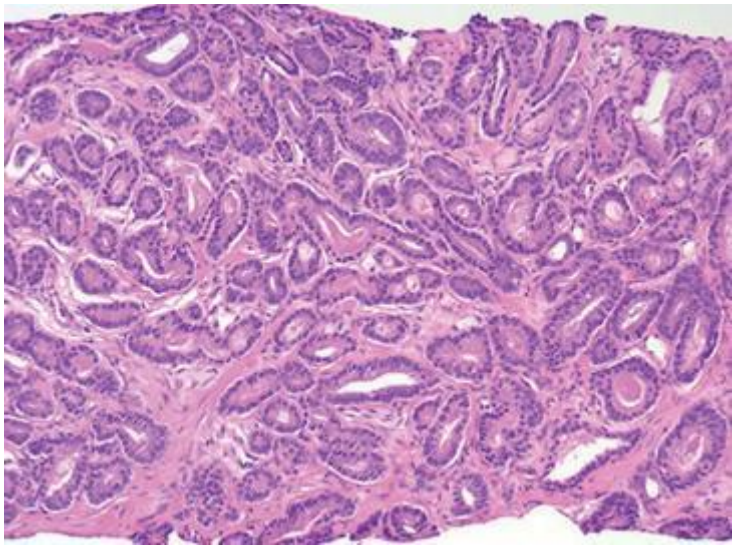
3.3.2.2 Gleason 2 and 3

Gleason 2 shows many newer glandular like cells but now of varying larger sizes. As Epstein notes: "*Grade 2 … is still fairly circumscribed, at the edge of the tumor nodule there can be minimal extension by neoplastic glands into the surrounding non-neoplastic prostate. The glands are more loosely arranged and not as uniform as Gleason 1*." We see those in the figure below which combines Gleason 2 and 3.

Gleason 3 is often composed of single glands. The Gleason 3 infiltrates in and amongst the non-neoplastic glands. Gleason 3 still can be seen as a separate gland and there are no single cells starting to proliferate. In Gleason 3 we still have some semblance of intercellular communications and coordination, albeit with uncontrolled intracellular growth. Again in the figure below we see both the smaller 2 and the larger 3 with gland structure being preserved and no separate cells proliferating.

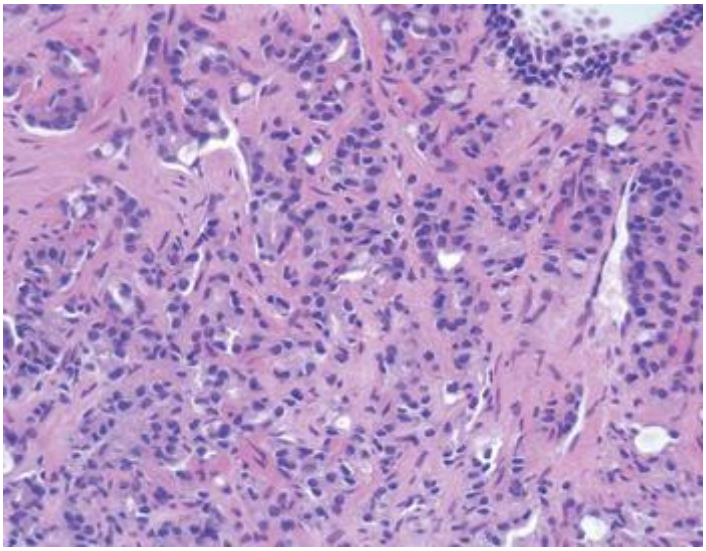


A Gleason 3 throughout is shown below.

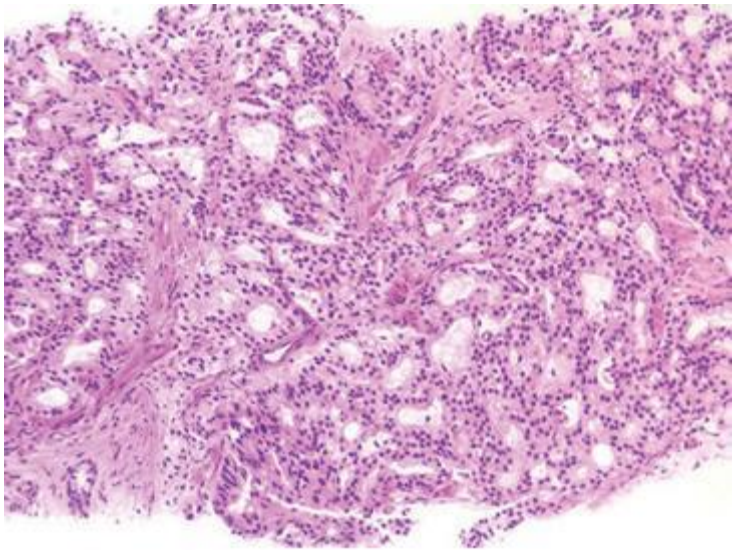


3.3.2.3 Gleason 4

Gleason 4 consists mostly of cribiform cells (perforated like a sieve) or fused and ill-defined glands with poorly formed glandular lumina. The glands appear to start to "stick" together. A Gleason 4 with a Gleason 3 is shown below. Note the sieve like structure and the closing of the glands.

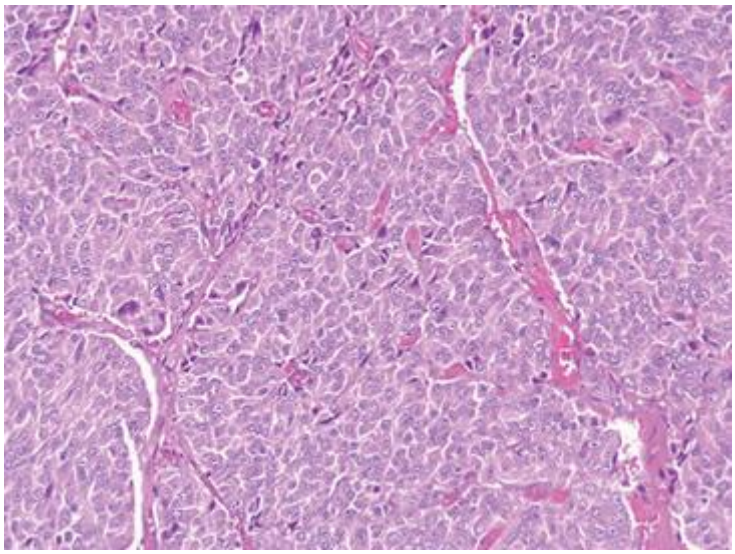


A Gleason all 4 is shown below. Note that the cells are sticking closed and the entire mass appears as a sieve like mass.



3.3.2.4 Gleason 5

Gleason 5 is a complete conversion to independent malignant cells. They have lost all intercellular coordination. As shown below it is a mass or mat or sheet of independent cancer cells and it has lost any of the sieve like structures. There may also appear to be some necrosis



3.3.3 Gleason Summary

The Gleason scores are then determined by taking the predominant type and adding it to the secondary type. Thus a 4+3 yields a Gleason combined 7 but it is 4+3 and that is more aggressive than say a 3+4 with the same total score.

We repeat the grading commentary below.

Gleason 1	Gleason 2	Gleason 3	Gleason 4	Gleason 5
Many acini with no basal layers and large nucleoli. Closely packed clumps of acini.	Many very small single separate glands (acini) with no basal layer and large nucleoli . Glands, acini, are more loosely arranged and not close packed.	Many small microglands extending throughout the stroma and out of the normal gland structure	Glands are now spread out and fused to one another throughout the stroma.	No gland structure seen, all luminal cells throughout the stroma with large nucleoli.

3.3.4 Models From Grading

In looking at the grading one may also hypothesize a possible path of progression. The steps appear to be:

1. Movement from existing benign gland to a separate but glandular like proliferation. Cells which would normally remain dormant go through a replication cycle, apoptosis and cell proliferation control seems lost. New glands appear clustered but appear separate.

2. Growth of the new glands makes them expand but remain morphologically glandular. They close packing begins to disappear and glands start to stand on their own. It is as if they are expanding and growing and the basal layer begins to disappear. Luminal like cancer cells start to be predominant.

3. Many small micro-glands start expanding and cell growth accelerates and the cells appear more cancer like but there is still some morphological glandular structure left.

4. The many glands have dramatically different shaped and start closing in one another and appear sieve like with small openings. They look as if they are losing any intercellular communications resulting is a common mat of cells.

5. Cells have lost any morphological form related to glands and appear as a mat of cancer cells replacing the stroma totally. No intercellular communications is left and cellular growth control has been eliminated totally.

These five steps are consistent with the Gleason grading but they also parallel the way the intracellular and intercellular controls are lost. We will look at these mechanism later.

3.4 PCa Genetics

There are an ever increasing set of genes and gene adjuncts that have been related to PCa. Some of the early ones were p53 and PTEN. But there are genes which are oncogenic, transcription factors, methylation and epigenetic factors, miRNAs, fusion genes, as well as the tumor micro environments and immune cells that facilitate the progression of the malignancy. Over the years we have examined a multiplicity of these targets. A key one has been the androgen receptor, AR, gene and its functioning as part of a set of transcription factors. Here we examine a list of some of the key genes that can be considered as well as understanding just where IGF-1 fits in.

As Mancarella et al have noted:

Prostate cancer (PCa) is characterized by clinical and biological heterogeneity and has differential outcomes and mortality rates. Therefore, it is necessary to identify molecular alterations to define new therapeutic strategies based on the risk of progression. In this study, the prognostic relevance of the insulin-like growth factor (IGF) system was examined in molecular subtypes defined by ***TMPRSS2-ERG (T2E) gene fusion*** *within a series of patients with primary localized PCa. …*

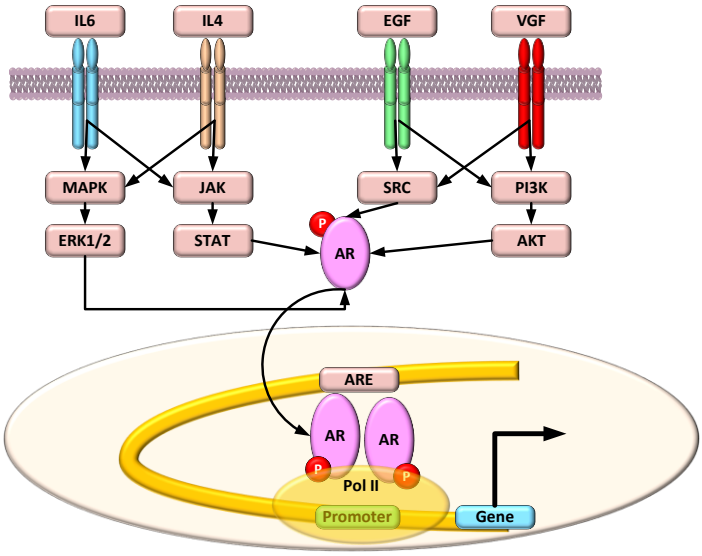
An ***association between IGF-1R overexpression and better BPFS was found in T2E-negative patients*** *(35.3% BPFS, p-value = 0.016). Multivariate analysis demonstrated that* ***IGF-1R expression constitutes an independent variable in T2E-negative patients*** *[HR: 0.41. CI 95% (0.2–0.82), p = 0.013]. These data were confirmed using immunohistochemistry of ERG as*

subrogate of T2E. High IGF-1 expression correlated with prolonged BPFS and PFS independent of the T2E status. …

IGF-1R, a reported target of T2E, constitutes an independent factor for good prognosis in T2Enegative PCa. Quantitative evaluation of IGF-1/IGF-1R expression combined with molecular assessment of T2E status or ERG protein expression represents a useful marker for tumor progression in localized PCa.

3.5 DRIVERS

Drivers are entities, usually proteins or protein segments, that activate pathways in a cells that result in proliferation or block apoptosis. The androgen receptor is a major player on PCa and normal prostate homeostasis. Thus we begin by examining what are drivers to the AR protein to act as a transcription factor. One of the first drivers is the Heat Shock protein which is initially bound to AR and replaced by DHT which initiates the process. Then there are other pathways that begin the AR chain in a variety of gene expressions. From Tang et al we start with the graphic example below:



See Tang et al

The above depicts the canonical elements we will examine as AR effects transcription.

As Pincik et al note:

Furthermore, there is need for novel targeted therapies of metastatic PCa based on a better molecular understanding of the disease. The lack of markers to stratify PCa cases into low- and high-risk groups results in overtreatment of 20–42% of patients5. STAT3, the major downstream mediator of IL-6 signalling, was shown to be related to advanced tumour growth, by tumour-autonomous mechanisms and by modulating tumour-associated stroma6. Although STAT3 activation is observed in B50% of PCa7 its functional role in tumorigenesis and metastasis has not been elucidated. Data from the majority of human PCa cancer cell lines support an oncogenic and growth promoting role of IL-6 and STAT3 in vitro8. However, metastatic LNCaP

cells were growth inhibited in vitro and in vivo in response to IL-6 treatment8. Moreover, treatment of patients with an IL-6 blocking antibody did not result in a survival advantage in patients with advanced PCa.

Thus, addressing the precise in vivo role of IL-6/STAT3 in PCa is of utmost importance to reassess diagnostic and therapeutic approaches. PTEN is one of the most frequently deleted or mutated tumour suppressors in PCa, with an estimated incidence of 70% in metastatic PCa, causing aberrant activation of the PI3K– AKT–mTOR signalling pathway. Loss of Pten leads to senescence, which is critically regulated by the ARF–p53 pathway. While the tumour suppressor ARF (p14ARF in humans; p19ARF in mice) is readily degraded in normal cells, it is stabilized to increase p53 function on loss of Pten. ARF was shown to augment p53 stability by promoting the degradation of Mdm2, a negative regulator of p53.

Concomitant inactivation of Pten and p53 leads to bypass of senescence and as a consequence to a malignant PCa phenotype. Previous studies report PTEN–STAT3 signalling crosstalk in malignant glioblastoma, but the detailed molecular mechanisms in cancer progression and metastasis remain unresolved. In this study, we show that loss of IL-6/Stat3 signalling in a Pten-deficient PCa model accelerates cancer progression leading to metastasis. Loss of IL-6/Stat3 signalling in PCa bypasses senescence via disrupting the ARF–Mdm2–p53 tumour suppressor axis.

We identify ARF as a novel direct Stat3 target.

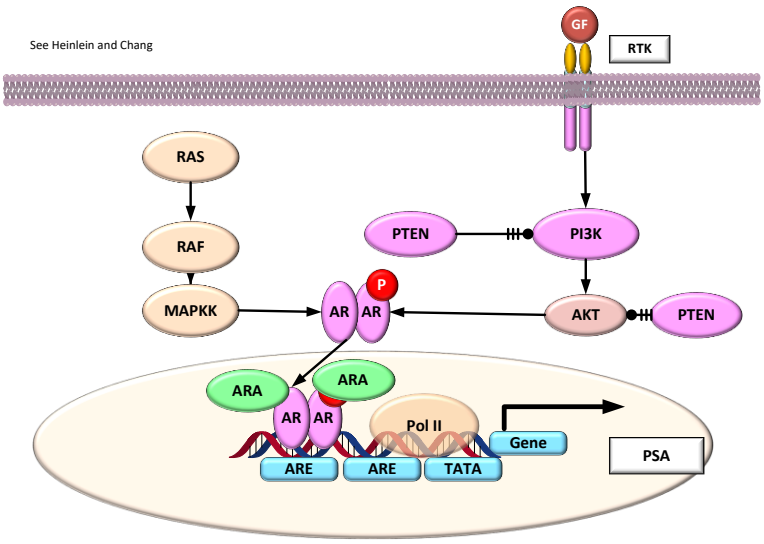
Notably, loss of STAT3 and p14ARF expression correlates with increased risk of recurrence in PCa patients.

In addition, STAT3 and p14ARF expression was lost in metastasis compared with the primary tumours. We identified STAT3 and CDKN2A mutations in primary PCa patients. Furthermore, PCa metastases show a high frequency of STAT3 and CDKN2A deletions. We propose STAT3 and ARF as prognostic markers for high versus low risk PCa patient stratification

As Heinlein and Chang have noted:

Crosstalk of MAPK and PI3K/Akt pathways with A/AR. Both MAPK and PI3K/Akt may influence the phosphorylation of AR and AR coregulators, resulting in modulation of AR activity. The tumor suppressor PTEN can modulate AR activity via PI3K/Akt pathways or by interacting directly with AR. MAPKK, MAPK kinase; A/AR, androgen/androgen receptor; RTK, receptor tyrosine kinase; APPL, adapter protein containing PH domain, PTB domain, and leucine zipper motif; P, protein phosphorylation.

And as shown below



3.5.1 Heat Shock Proteins

Heat Shock proteins are bound to AR before the AR become activated by DHT, They drive the AR to a state whereby the activation can occur. As Dubey et al note:

Heat shock proteins (HSPs) are the molecular chaperones, that are not only expressed during the normal growth process of cell cycle consecutively, but also get induced in cells during various stress conditions produced by cellular insult, environmental changes, temperature, infections, tumors etc.

According to their molecular weight and functions, HSPs are divided into five major families. HSP90, HSP70, HSP60 and HSP100 are the most studied members of the family. Experimental studies have proved that overexpression and/or inhibition of HSPs play an important role in maintaining the tolerance and cell viability under above-described stress conditions. HSP90 is found to be a promising candidate for the diagnosis, prognosis and treatment of cancer. Similarly, HSP70, HSP60 and small HSPs experimentally and clinically have potential for the treatment of neurodegenerative disease, ischemia, cell death, autoimmunity, graft rejection, etc….

The heat shock response was first described in 1962 by Ritossa and is named as heat shock proteins (HSPs) based on their increased synthesis after heat shock in house fly . Later it has been noted that HSPs exist in all the organisms from bacteria to humans, and they are among the most conserved proteins known . HSPs, are multimolecular complexes expressed constitutively (up 5-10% of the total protein) under normal growth condition in cells and act as molecular chaperones, which play a regulatory role in the folding of proteins, intracellular transport of proteins in cytosol, endoplasmic reticulum and mitochondria; repair or degradation of proteins and refolding of misfolded proteins .

In addition to being constitutively expressed, these proteins are markedly induced (up to 15%) by a range of environmental, pathological, or physiological stimuli . These proteins are also modulated by nutrient deprivation, oxidative stress, hypoxia-ischemia, apoptotic stimuli and neuronal injury in the brain, etc..

HSPs are divided into five major families, HSP100, 90, 70, 60, and the small HSP (sHSP)/α-crystallins, according to their molecular weight, structure and function. HSP synthesis results in tolerance to insult, such as thermotolerance or stress tolerance in various organisms .

In various acute and chronic cell injuries, pathogenic conditions such as malignancies, and infectious diseases overexpression of HSPs were found to be playing cytoprotective and immunoregulatory roles . Recently, HSP reactivity in autoimmune diseases and transplantation have been proven to be down-regulated in the disease process. Togetherness, induction or inhibition of HSPs provides vast area of therapeutic target for combating various diseases. Considering, the regulatory role of HSPs in physiological and pathological conditions, HSPs have emerged as potential drug candidates for drug development and can be a breakthrough in the near future. Regulation of HSPs Stress condition causes protein unfolding, misfolding or aggregation, which triggers the stress response that leads to the induction of gene transcription of proteins.

HSP gene transcription is mediated by the interaction of the heat shock factor (HSF1) with heat shock elements (HSEs) in the HSP gene promoter regions. In unstressed state, HSF1 is present in the cytoplasm as a latent monomeric molecule.

Under stress, HSF1 is hyperphosphorylated in a ras-dependent manner by members of the mitogen-activated protein kinase (MAPK) subfamilies (e.g. ERK1, JNK/SAPK, p38 protein kinase). HSF1 is then converted to phosphorylated trimers with the capacity to bind DNA and translocates from the cytoplasm to the nucleus. The generation of HSPs is transient, and the presence of HSPs negatively influences the protein homeostasis. The activity of HSF trimers is downregulated by HSPs (e.g. HSP70) and the heat shock binding protein 1 which is found in the nucleus.

As Ciocca et al have noted:

The heat shock proteins (HSP) constitute a superfamily of chaperone proteins present in all cells and in all cell compartments, operating in a complex interplay with synergistic/overlapping multiplicity of functions, even though the common effect is cell protection.

Several reasons explain the need for investigating HSP in prostate cancer:

1. these molecules function as chaperones of tumorigenesis accompanying the emergence of prostate cancer cells,

2. they appear as useful molecular markers associated with disease aggressiveness and with resistance to anticancer therapies including hormone therapy, radiotherapy, chemotherapy and hyperthermia, and

3. they can be used as targets for therapies.

The latter can be accomplished by:

(i) interrupting the interaction of HSP (mainly HSPC1) with various client proteins that are protected from degradation when chaperoned by the HSP;

(ii) using the chaperone and adjuvant capabilities of certain HSP to present antigenic peptides to the immune system, so this system can recognise the prostate tumour cells as foreign to mount an effective antitumoral response; and

(iii) using treatment planning models taking into account the HSP expression levels to obtain more effective therapies.

In summary, the study of the HSP during tumorigenesis as well as during cancer progression, and the inclusion of treatment designs targeting HSP combined with other treatment modalities, should improve prostate cancer survival in the near future…

The HSP are but one of many such targets. Examining them provides another window on using them in a therapeutic manner. They continue:

Carcinogenesis involves a cascade of molecular events that mediate the transformation of normal cells into cancer cells. Although prostate cancer is a malignancy with a high incidence, the events associated with its initiation remain poorly understood and there are still many enigmas about the pathophysiology of prostate cancer.

Early prostate tumorigenesis appears to be associated with a dysplasia that initiates with proliferative inflammatory atrophy (PIA), and progresses to prostatic intraepithelial neoplasia (PIN), which in some cases leads to carcinoma.

Existing evidence suggests that these early lesions may be initiated by inflammation that occurs with exposure to different infectious agents and/or ingestion of carcinogens.

When a premalignant lesion progresses to primary cancer, to metastatic cancer, and to androgenindependent cancer, genetic alterations continue to accumulate within the tumour cells. Moreover, normal prostate and early-stage prostate cancers cells depend on androgens for growth and survival. As the cancer advances and metastasizes, it becomes dominated by cells that proliferate and survive independently of androgens.

We have examined several cases where HGPIN. Often a precursor to PCa, and in fact termed PCa in situ, disappears. One wonders if this is a result of an immune attack, or even if it is a response to the biopsy process itself. They continue:

With a practical/didactic purpose we can identify the following entities during prostate cancer progression:

1. ***normal prostate epithelium,***
2. ***PIA,***
3. ***PIN,***
4. ***localised prostate cancer,***
5. ***metastatic prostate cancer (all of them androgen-dependent), and***
6. ***androgen-independent prostate cancer.***

Owing to their role as molecular chaperones, HSP participate in many events related to cancer, starting from the beginning of carcinogenesis . During this process, the transformed cells begin to express abnormal/elevated levels of HSP, and in some cases this induction continues during tumour progression. At present there exists an important body of evidence to support the participation of this family of proteins in the initiation and progression of prostate carcinogenesis. In accordance with the above, an interesting paper of Byun et al. has demonstrated that during prostate tumorigenesis the expression of several sets of housekeeping genes (including HSP) are differentially expressed, suggesting that the process is driven by modulation of the expression of these genes.

The expression of HSP was up-regulated during the transition of localised prostate cancer to metastatic prostate cancer, indicating that in advanced stages prostate tumour cells could be under cellular stress. Therefore, the authors suggest that during this period of cellular stress the prostate tumour may be more vulnerable and responsive to treatment….

The identification and assessment of level of these genes/proteins in the prostate tumour progression will allow the best management of prostate cancer patients and to improve the treatments that have HSP as potential targets for the therapy.

As Jin et al noted concerning the HSP variants:

The androgen receptor (AR) is a member of hormonal transcription factors. The expression of AR protein and its activation by male hormone androgen are fundamental to prostate development during pubertal and malignant transformation during later ages.

These biological/ pathological processes are determined by critical regulation of downstream molecules/pathways by the AR. AR is a DNA-binding protein that regulates a wide-range of target genes through directly binding to cis-regulatory elements. In the absence of androgen, the AR is sequestered in the cytoplasm by the ***chaperone super-complex including heat shock proteins (Hsp) 90, 70 and 56.***

Once bound by androgen, AR undergoes conformational changes to dissociate from Hsp complex, becomes phosphorylated and translocates into the nucleus.

Albany and Hahn note:

Heat shock proteins HSPs are highly conserved stress-induced factors that play an essential role as molecular chaperones by regulating protein folding, stability transport and aggregation. HSPs have cytoprotective roles and are essential for cancer cell survival. HSPs are often upregulated in cancer and this constitutive expression is necessary for cancer cells' survival. Several of these proteins have demonstrated a direct interaction with components of the cell signaling pathways. For example, the androgen receptor (AR) is a major player in PCa growth and progression and is a well-known interacting factor of HSPs.

Since AR function is very dependent on HSP activity, many emerging compounds address AR-associated HSPs as novel drug targets.

We have examined the AR in detail and it is often the dominant control mechanism in early to mid stage PCa. However in mPCa it does not play such a role.

HSPs have been classified into four families according to their molecular weight: HSP90, HSP70, HSP60 and small HSPs (15–30kDa) that include HSP27. HSPs are powerful regulators of apoptosis through an ability to interact with key components of the apoptotic signaling pathway, in particular, those involved in caspase activation. HSP90 is a molecular chaperone involved in the conformational maturation and function of a large number of 'client' proteins that have been implicated in oncogenesis.

The AR, a key driver of PCa growth and treatment resistance, is an HSP90 client and its function is dependent on HSP90 chaperone activity.

HSP27 and HSP70 are the most strongly induced chaperones during cellular stress. HSP27 is an ATP-independent, small HSP that, once phosphorylated, forms a chaperoning oligomer that regulates multiple cell survival and signaling pathways.

At the post-mitochondrial level, HSP27 binds to cytochrome C and inhibits caspase activation and apoptotic cell death. HSP27 and CLU act together to stabilize the cell against apoptotic stressors.

From Ratajczak et al we have:

Two out of three diseases of the prostate gland affect aging men worldwide. Benign prostatic hyperplasia (BPH) is a noncancerous enlargement affecting millions of men. Prostate cancer (PCa) in turn is the second leading cause of cancer death. The factors influencing the occurrence of BPH and PCa are different; however, in the course of these two diseases, the overexpression of heat shock proteins is observed.

Heat shock proteins (HSPs), chaperone proteins, are known to be one of the main proteins playing a role in maintaining cell homeostasis. HSPs take part in the process of the proper folding of newly formed proteins, and participate in the renaturation of damaged proteins. In addition, they are involved in the transport of specific proteins to the appropriate cell organelles and directing damaged proteins to proteasomes or lysosomes.

Their function is to protect the proteins against degradation factors that are produced during cellular stress. HSPs are also involved in modulating the immune response and the process of apoptosis.

One well-known factor affecting HSPs is the androgen receptor (AR)—a main player involved in the development of BPH and the progression of prostate cancer. HSPs play a cytoprotective role and determine the survival of cancer cells. These chaperones are often upregulated in malignancies and play an indispensable role in tumor progression.

Therefore, HSPs are considered as one of the therapeutic targets in anti-cancer therapies.

In this review article, we discuss the role of different HSPs in prostate diseases, and their potential as therapeutic targets…. In normal cells under physiological conditions, in a state of undisturbed homeostasis, cytoprotective mechanisms operate, thanks to which they are able to survive the stressful conditions. Cells that are not exposed to stress factors show enough HSP expression to protect their proteome and ensure cellular homeostasis (proteostasis).

A number of significant changes take place in neoplastic cells, including, at the level of activity of the transcription factors and metabolic activity, glycolysis levels, lipid metabolism or amino acid metabolism.

Cancer cells are exposed to high levels of proteotoxic stress.

They enter stress response pathways for survival and proliferation and become dependent on stress-induced HSPs. Moreover, the intracellular homeostasis of neoplastic cells is regulated by the increased expression of HSPs. In this case, the HSP-mediated cytoprotection of cancer cells takes place by inhibiting apoptosis, which is important for the proliferation, invasiveness and metastasis of tumor cells . In addition, the high level of HSP expression promotes the folding of oncoproteins, which ensures their stability and reduces the likelihood of their proteolytic degradation.

The expression of HSPs is induced in response to a variety of physiological and environmental factors, including anti-cancer chemotherapy.

Such a strategy allows the cells to survive even under lethal conditions. Importantly, in neoplastic diseases, HSP expression is usually increased, which has been confirmed in gastric cancer , breast cancer , endometrial cancer, ovarian cancer, gastrointestinal cancers , lung cancer and in prostate cancer .

Many signaling pathways play an important role in the pathogenesis of neoplastic diseases, and their incorrect regulation leads to changes in the cell phenotype and disturbances of such important processes, such as the regulation of the cell cycle, growth, death, differentiation and cell adhesion .

In eukaryotic cells, two complementary processes aimed at the degradation of native intracellular proteins can be distinguished: lysosomal degradation, including macroautophagy, and proteasomal degradation. Lysosomes mainly break down extracellular proteins that enter the cell through endocytosis, or, in the case of macroautophagy, also the intracellular proteins under strong cellular stress.

Proteasomes, in turn, are responsible for the controlled degradation of proteins with lower molecular weights, including signaling proteins with a short half-life and misfolded proteins . Current therapeutic strategies for neoplastic diseases mainly aim to induce apoptosis in these cells by genotoxic action or the inhibition of their proliferation.

Proteasome inhibitors lead to an increase in the transcription of genes encoding proteins from the HSP90, HSP70, HSP40, HSP28, HSP APG-1 and mitochondrial HSP75 families. These proteins play a significant role in the development of mechanisms of resistance to therapeutic compounds.

Cancer cells treated with proteasome inhibitors aim to compensate for the decreased activity of this protease by increasing its synthesis and the synthesis of chaperone molecules

3.5.2 SRC

Kinases are a broad class of intracellular proteins. From Kim et al:

Src family kinases (SFKs) have a critical role in cell adhesion, invasion, proliferation, survival, and angiogenesis during tumor development.

SFKs comprise nine family members that share similar structure and function. Overexpression or high activation of SFKs occurs frequently in tumor tissues and they are central mediators in multiple signaling pathways that are important in oncogenesis. SFKs can interact with tyrosine kinase receptors, such as EGFR and the VEGF receptor.

SFKs can affect cell proliferation via the Ras/ERK/MAPK pathway and can regulate gene expression via transcription factors such as STAT molecules.

SFKs can also affect cell adhesion and migration via interaction with integrins, actins, GTPase-activating proteins, scaffold proteins, such as p130CAS and paxillin, and kinases such as focal adhesion kinases.

Furthermore, SFKs can regulate angiogenesis via gene expression of angiogenic growth factors, such as fibroblast growth factor, VEGF, and interleukin 8. On the basis of these important findings, small-molecule SFK inhibitors have been developed and are undergoing early phase clinical testing. In preclinical studies these agents can suppress tumor growth and metastases. The agents seem to be safe in humans and could add to the therapeutic arsenal against subsets of cancers.

3.5.3 STAT

Signal transducer and activator of transcription (STAT) proteins are powerful controllers of gene expression. Recent work has involved them in Prostate Cancer along with the many other targets which have been identified. We examine this specific gene and its recently identified significance. The specific STAT is STAT3. Previously it has been linked to aggressive cancers. In fact attempts have been made to therapeutically target this pathway. The authors in a recent paper however contend that it is just the opposite. Namely STAT3 actually prevent metastatic behavior.

This discussion is a critical one as we examine further the targeting of genes and their behavior. The STAT3 issue seems to state that on one hand over-expression is bad, yet then on the other hand over-expression is good. This highlights the issue of cross talk between paths as well as the yet to be fully understood dynamics of pathways. Add to this is the fact that STAT3 is driven by IL-6 and this links in the immune system as well.

We begin the discussion with information in Science Daily which reports[8]:

A gene that is responsible for cancer growth plays a totally unexpected role in prostate cancer. The gene Stat3 is controlled by the immune modulator interleukin 6 and normally supports the growth of cancer cells. The international research team led by Prof. Lukas Kenner from the Medical University of Vienna, the Veterinary University of Vienna, and the Ludwig Boltzmann Institute for Cancer Research (LBI-CR) discovered a missing link for an essential role of Stat3 and IL-6 signalling in prostate cancer progression.

Interleukin 6 (IL-6) is an important cytokine that controls the cell survival and tumor growth. Hyperactive IL-6 may support cancer growth, particularly as it controls STAT3, which was shown to have an oncogenic role in most tumours. Many therapies are therefore designed to suppress IL-6 or STAT3. But the situation is different in prostate cancer. Lukas Kenner's research group has shown that, contrary to expectations; active STAT3 suppresses cell growth in prostate tumours. It activates the gene $p14^{ARF}$, which blocks cell division and thus inhibits tumour growth.

IL-6 is one of many interleukin cytokines, activating immune cells and leading to their proliferation. In a classic model for STAT3, it is activated by IL-6 and then it progresses via phosphorlyation to act as a promoter or enhancer for a multiplicity of genes whose expression leads to cancerous growth. However there is an alternative pathway, the ARF-MDM2-p53 pathway the controls and may mitigate some of these processes. This paper focuses on this crossover effect.

The article continues:

[8] http://www.sciencedaily.com/releases/2015/07/150722081410.htm

For this reason, STAT3 and p14^{ARF} are ideally suited to act as biomarkers for the prognosis of this disease. If these two factors are missing in tissue samples, the risk is massively increased that the tumour grows and forms metastases.

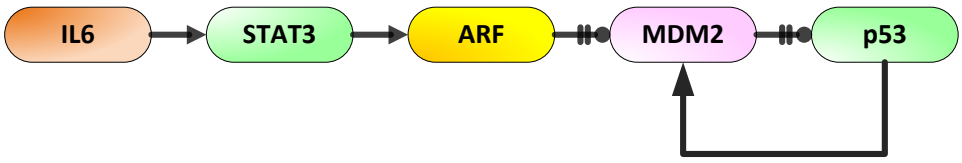
According to Lukas Kenner, this is important, as the predictive power of these proteins as biomarkers is twice as good as the previous gold standard. As only about 10 % of patients with prostate cancer die from the disease, this can help to prevent unnecessary therapeutic interventions with severe side effects such as incontinence and impotence. A non-invasive nuclear medical test based on these findings might soon be able to replace the painful removal of tissue samples to be examined.

The reversed role of interleukin 6 as an inhibitor of prostate cancer has an additional significance. Blockade of interleukin 6 is used to treat other diseases, such as rheumatoid arthritis. According to Kenner, this means that therapies that block the IL-6 pathway may enhance the growth of prostate cancer.

Thus, the drug that is used to treat inflammatory disease may exacerbate malignancies. "Applying IL-6/Stat3 blockers to clinical practice might be dangerous for patients with cancerous lesions, further studies are mandatory to assess the possibility of increased cancer risk right now," says coauthor of this study, Helmut Dolznig, also from the Medical University of Vienna. The study was financed mainly by the LBI-CR and the FWF..

The following is a generalized paradigmatic summary of Pencik et al. Namely; they observed that IL6 controls STAT3 which in turn controls the ARF-MDM2-p53 pathway, which is critical in the overall control of PCa metastasis.

1. IL6 drives increase in STAT3
2. STAT3 drives increase in ARF
3. ARF blocks MDM2
4. Low MDM2 allows increase in p53
5. p53 inhibits metastasis



Now it should also be noted that the above is not the complete presentation. For example in this pathway p53 actually drives MDM2. There are other linkages that should be considered as well. We shall discuss some of these later.

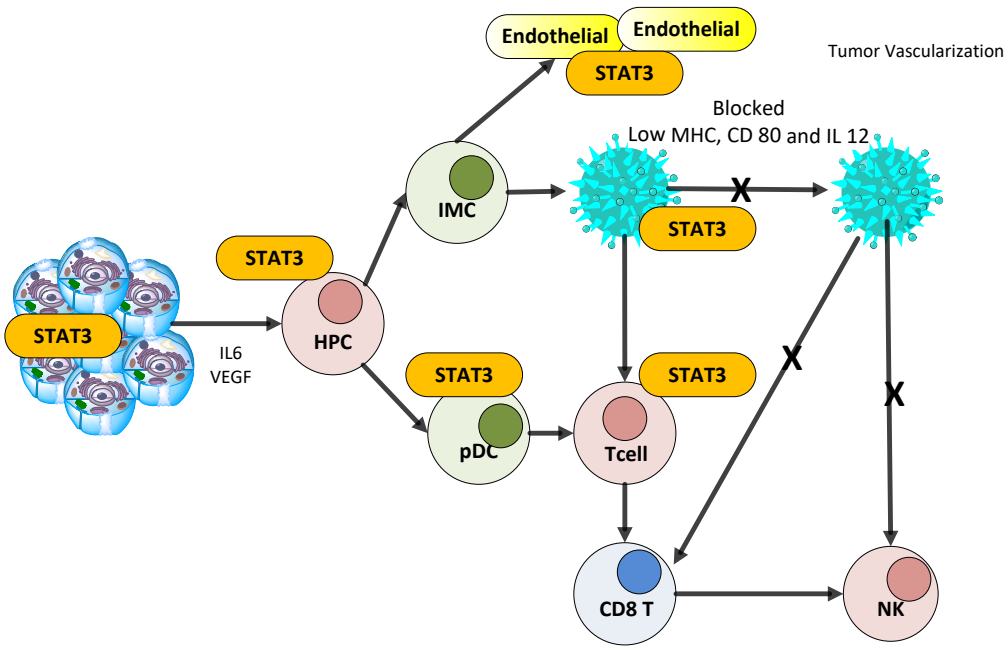
Now from the paper in question, namely Pencik et al, they conclude:

We have uncovered a paradigm shift in understanding the key function of STAT3 in tumorigenicity and metastatic progression in PCa. Therefore, our results call for cautious use of anti-IL-6- STAT3 signalling blockers in the treatment of PCa as this may turn low-grade tumours into highly malignant cancers by loss of senescence controlled by the STAT3–ARF axis. As IL-6/STAT3 signalling blockers are successful in the treatment of chronic inflammatory or autoimmune diseases, their influence on PCa development needs to be carefully evaluated in future studies.

Reactivating the IL-6/STAT3/ARF-dependent senescence pathway57 might be a promising strategy for PCa therapy via downregulation of Mdm2 (ref. 58) or p53 induction59. Alternatively, triggering ARF–p53-independent cellular senescence by a small molecule inhibitor could be beneficial for PCa patients in whom other therapies have failed.

Namely, they argue that the STAT3 control of the ARF-MDM2-p53 pathway should not be interfered with. That pathway actually enables control over metastatic behavior. We will discuss each element in some detail in what follows.

The classic understanding of STAT3 is that is acts to promote cancers. The figure below is a modification from Yu et al:



STAT3 signalling allows crosstalk between tumour cells and dendritic cells, forming an immunosuppressive network. *Tumour-associated factors such as vascular endothelial growth factor (VEGF), IL-10 and IL-6 can not only be upregulated by signal transducer and activator of transcription 3 (STAT3), but are also STAT3 activators. Increased STAT3 activity in haematopoietic progenitor cells (HPCs) promotes the generation of immature myeloid cells (iMCs) and increases the numbers of both immature dendritic cells and plasmacytoid dendritic cells (pDCs), each of which promotes the accumulation of regulatory T (TReg) cells in the tumour microenvironment. ...preventing their maturation and compromising their ability to stimulate the anti-tumour effects of CD8+ T cells and natural killer (NK) cells.*

As Yu et al state:

Immune cells in the tumour microenvironment not only fail to mount an effective anti-tumour immune response, but also interact intimately with the transformed cells to promote oncogenesis actively. Signal transducer and activator of transcription 3 (STAT3), which is a point of convergence for numerous oncogenic signalling pathways, is constitutively activated both in tumour cells and in immune cells in the tumour microenvironment.

Constitutively activated STAT3 inhibits the expression of mediators necessary for immune activation against tumour cells. Furthermore, STAT3 activity promotes the production of immunosuppressive factors that activate STAT3 in diverse immune-cell subsets, altering gene-expression programmes and, thereby, restraining anti-tumour immune responses. As such, STAT3 propagates several levels of crosstalk between tumour cells and their immunological microenvironment, leading to tumour-induced immunosuppression. Consequently, STAT3 has emerged as a promising target for cancer immunotherapy.

Thus the classic view is that STAT3 is an essential element in the pathology of tumorogenesis which as we indicated earlier is in contrast to the recent results. Thus do we block it or allow it? That is the question. Yu et al conclude:

The ability of STAT3 to broadly and profoundly affect tumour immunity strongly indicates that constitutively activated STAT3 both in tumour cells and in tumour stromal immune cells is an attractive target for cancer immunotherapy. Another unique and appealing aspect of targeting STAT3 for cancer immunotherapy is due to the crucial role of STAT3 in tumour-cell survival and tumour angiogenesis. Many experiments have shown that tumour rejection mediated by CD8+ T cells is always preceded by the inhibition of tumour-induced angiogenesis.

Because targeting STAT3 is expected to decrease the survival and angiogenic potential both of tumour cells and of the tumour stroma, targeting STAT3 could facilitate immune-cell-mediated anti-tumour effects at several levels. Although STAT3 is the first oncogenic target for cancer immunotherapy, other important onco proteins, such as MAPKs, might have similar roles. With the emergence of targeted delivery systems, and small molecule inhibitors or RNAi technology to block STAT3 and other relevant oncogenic pathways, a new era of molecular targeting for cancer immunotherapy is on the horizon.

Yu et al are focusing on hematopoietic cells not prostate cells. There is no reason why one should expect the same effect in different cells. Yet from a therapeutic perspective if such a drastically different model is functioning, the results would be problematic at best.

As Niu et al have stated:

Loss of p53 function by mutation is common in cancer. However, most natural p53 mutations occur at a late stage in tumor development, and many clinically detectable cancers have reduced p53 expression but no p53 mutations.

It remains to be fully determined what mechanisms disable p53 during malignant initiation and in cancers without mutations that directly affect p53.

We show here that oncogenic signaling pathways inhibit the p53 gene transcription rate through a mechanism involving Stat3, which binds to the p53 promoter in vitro and in vivo.

Site-specific mutation of a Stat3 DNA-binding site in the p53 promoter partially abrogates Stat3-induced inhibition. Stat3 activity also influences p53 response genes and affects UV-induced cell growth arrest in normal cells. Furthermore, blocking Stat3 in cancer cells up-regulates expression of p53, leading to p53-mediated tumor cell apoptosis. As a point of convergence for many oncogenic signaling pathways, Stat3 is constitutively activated at high frequency in a wide diversity of cancers and is a promising molecular target for cancer therapy.

Thus, repression of p53 expression by Stat3 is likely to have an important role in development of tumors, and targeting Stat3 represents a novel therapeutic approach for p53 reactivation in many cancers lacking p53 mutations.

Thus, Niu et al also present a model for Stat3 inhibiting p53, again in contrast to the paper in question. Niu et al conclude:

1. Stat3 protein interacts with the p53 promoter.

2. Stat3 inhibits p53 expression at the transcription level.
3. Stat3 binds to the p53 promoter in vitro as determined by EMSA.
4. Interaction between Stat3 protein and the p53 promoter contributes to Stat3-mediated inhibition.
5. Stat3 activity inhibits the p53-responsive element and UV-induced p53-mediated growth arrest.
6. Blocking Stat3 activates p53 expression in human cancer cells.
7. Blocking Stat3 induces p53-mediated tumor cell apoptosis and facilitates UV-induced tumor cell growth inhibition.

The results of these two studies seem fairly conclusive regarding Stat3. Namely it is oncogenic. But despite the study in question here seems to reverse that position. We will examine that in some detail.

Let us now review what is understood about the ARF-MDM2-p53 pathway. This will be necessary before linking this pathway to STAT3 and its functions.

Now this is a classic pathway whose ultimate control mechanism is p53 expression. p53 is generally understood to be a control gene, keeping the cell in some homeostasis and preventing malignancy. As we will not later this may not always be the case but that will not apply to the current discussion.

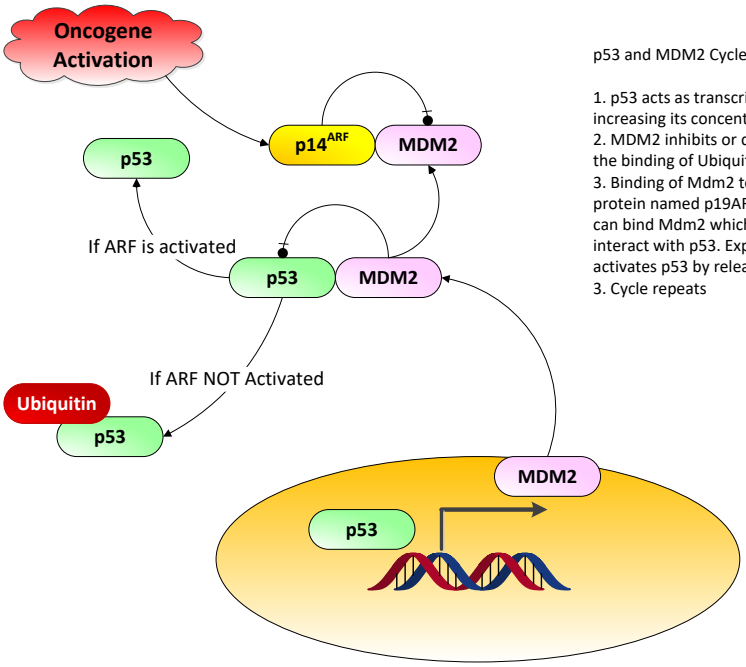
The following Figure depicts the process of the three gene control mechanism. Simply:

1. p53 activates the production of MDM2

2. MDM2 can bind to p53 and result in its dissolution via an Ubiquination

3. ARF can bind to MDM2 and allow the p53 to survive.

4. The process, albeit a bit complex, reaches a steady state for all three proteins.

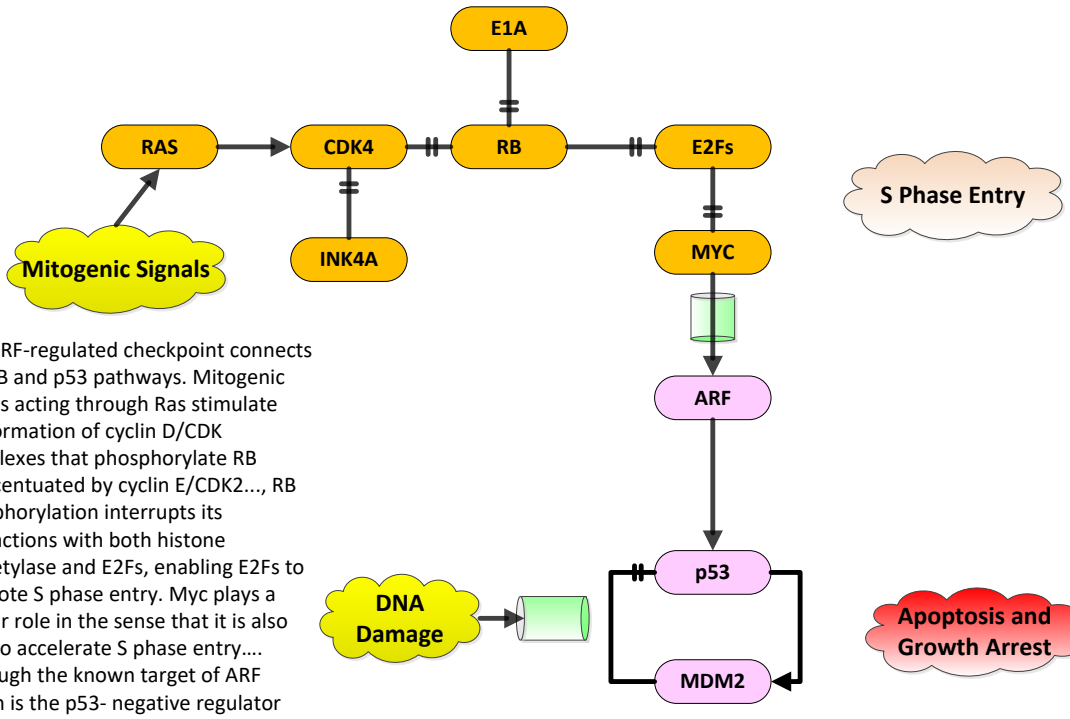


p53 and MDM2 Cycle

1. p53 acts as transcription activator for MDM2 increasing its concentration
2. MDM2 inhibits or decreases p53 by allowing the binding of Ubiquitin
3. Binding of Mdm2 to p53 can be disrupted by a protein named p19ARF (or ARF for short). ARF can bind Mdm2 which renders it unable to interact with p53. Expression of ARF therefore activates p53 by releasing Mdm2.
3. Cycle repeats

From Sherr and Weber (as modified) we have the following details are shown graphically:

From Sherr and Weber



The ARF-regulated checkpoint connects the RB and p53 pathways. Mitogenic signals acting through Ras stimulate the formation of cyclin D/CDK complexes that phosphorylate RB ….Accentuated by cyclin E/CDK2..., RB phosphorylation interrupts its interactions with both histone deacetylase and E2Fs, enabling E2Fs to promote S phase entry. Myc plays a similar role in the sense that it is also able to accelerate S phase entry…. Although the known target of ARF action is the p53- negative regulator and p53-inducible gene product Mdm2, other targets for ARF action cannot be precluded...

Note in the above we have the cyclic MDM2 and p53 control as well as the cell instigators. Now Van Maerken, T., et al notes the following regarding the details of this feedback loop:

The p53-MDM2 autoregulatory feedback loop.

(a) The p53 protein induces expression of MDM2, which negatively regulates the stability and activity of p53, providing a means to keep p53 levels and activity low in unstressed cells and to switch off p53 at the end of a stress response.

(b) The p53-mediated expression of MDM2 results from binding of p53 to response elements in the MDM2 gene and subsequent transactivation of MDM2. The domain structure of p53 is shown schematically:

i. *TAD, transactivation domain, amino acids;*
ii. *PRD, proline-rich domain, amino acids; DBD, DNA-binding domain, amino acids;*
iii. *TD, tetramerization domain, amino acids;*
iv. *CTD, C-terminal regulatory domain, amino acids.*

(c) The p53-inhibitory activity of MDM2 relies on multiple mechanisms. Binding of MDM2 to p53 conceals the TAD and consequently blocks the transcriptional activity of p53. MDM2 also recruits several corepressor proteins to p53, including HDAC1, CTBP2, YY1, and KAP1.

The E3 ubiquitin ligase activity of MDM2 results in ubiquitination of lysine residues in the CTD of p53, preventing acetylation of p53, favoring nuclear export, and promoting proteasomal degradation (see text for details). Some of these lysine residues can also be neddylated by MDM2, resulting in inhibition of the transcriptional activity of p53. Finally, MDM2 may also serve as a p53-specific transcriptional silencer by binding and monoubiquitinating histone proteins in the proximity of p53-responsive promoters. Nd, NEDD8; Ub, ubiquitin. …

They continue the discussion as follows:

The $p14^{ARF}$ protein is predominantly localized to the nucleolus, in which it is stabilized by binding to nucleophosmin within maturing pre-ribosomal particles, pointing to a function in the regulation of ribosome biogenesis.

Nucleophosmin promotes the processing of ribosomal RNA precursors and the nuclear export of ribosomal subunits, whereas overexpression of $p14^{ARF}$ or its murine homolog $p19^{ARF}$ interferes with transcription and processing of ribosomal RNA, impedes nucleocytoplasmic shuttling of nucleophosmin, and inhibits ribosome nuclear export. However, the precise biological function of the nucleophosmin– $p14^{ARF}$ complexes remains a subject of debate. Stress signals trigger the disruption of the interaction between $p14^{ARF}$ and nucleophosmin, and induce translocation of p14ARF to the nucleoplasm.

This redistribution enables $p14^{ARF}$ to interact with p53-bound MDM2 and to antagonize MDM2 function by inhibiting its E3 ubiquitin ligase activity and by blocking nucleocytoplasmic shuttling of MDM2 and p53, resulting in p53 stabilization. The p53-inhibitory activity of MDM2 may also be neutralized by $p14^{ARF}$ -mediated mobilization of MDM2 into the nucleolus, although this mechanism is not strictly required for the p53-dependent functions of $p14^{ARF}$.

This is clearly a highly complex mechanism. They continue:

Furthermore, the $p14^{ARF}$ protein is capable of inhibiting the activity of another E3 ubiquitin ligase that targets p53 for degradation, ARF-BP1/Mule, and of counteracting the p53-antagonizing NF-kappaB pathway. It should be noted that p14ARF also exerts a potent tumor suppressor activity independently of p53.

Various researchers have tried to model these systems using different techniques. One technique is the use of Petri Nets[9]. From CSML we have a Petri Net models describing the details of such a network and they state[10]:

Proteins p53, MDM2, and $p19^{ARF}$ are proteins closely related to cancer. The protein p53 is a protein which suppresses the formation of tumors, and the protein MDM2 promotes the formation of tumors by decreasing the activity of the protein p53.

Understanding of control mechanism of these proteins connects to development of an effective medicine for suppressing the tumor. It is known that protein p53 works as a transcription factor for many genes and its transcriptional activity is controlled by a complex formed with proteins MDM2 and $p19^{ARF}$.

However, it is still unclear whether protein p53 keeps its transcriptional activity in the form of the trimer with proteins p53, MDM2 and $p19^{ARF}$. …

a hybrid functional Petri net (HFPN) model which has been constructed by compiling and interpreting the information of p53-MDM2 interactions... With our HFPN model, we have simulated mutual behaviors between genes p53, MDM2, $p19^{ARF}$, and their products. Through simulation, we discussed whether the complex p53-MDM2-$p19^{ARF}$ has transcriptional activity for genes Bax and MDM2 or not.

It is worth examining these structures, namely the Petri Nets. We leave the examination to the reference. From Moll and Petrenko we have the following result:

Activation of the p53 protein protects the organism against the propagation of cells that carry damaged DNA with potentially oncogenic mutations. MDM2, a p53- specific E3 ubiquitin ligase, is the principal cellular antagonist of p53, acting to limit the p53 growthsuppressive function in

[9] See Reisig

[10] http://www.csml.org/models/csml-models/p53-arf-dependent-stabilization-pathway/

unstressed cells. In unstressed cells, MDM2 constantly monoubiquitinates p53 and thus is the critical step in mediating its degradation by nuclear and cytoplasmic proteasomes.

The interaction between p53 and MDM2 is conformation-based and is tightly regulated on multiple levels. Disruption of the p53-MDM2 complex by multiple routes is the pivotal event for p53 activation, leading to p53 induction and its biological response. Because the p53-MDM2 interaction is structurally and biologically well understood, the design of small lipophilic molecules that disrupt or prevent it has become an important target for cancer therapy.

Let us go back and re-examine the functions of STAT3 and this time in the context of the paper in study. As NCBI states[11]:

The protein encoded by this gene is a member of the STAT protein family. In response to cytokines and growth factors, STAT family members are phosphorylated by the receptor associated kinases, and then form homo- or heterodimers that translocate to the cell nucleus where they act as transcription activators.

This protein is activated through phosphorylation in response to various cytokines and growth factors including IFNs, EGF, IL5, IL6, HGF, LIF and BMP2. This protein mediates the expression of a variety of genes in response to cell stimuli, and thus plays a key role in many cellular processes such as cell growth and apoptosis. The small GTPase Rac1 has been shown to bind and regulate the activity of this protein. PIAS3 protein is a specific inhibitor of this protein.

As Niu et al have noted:

Loss of p53 function by mutation is common in cancer.

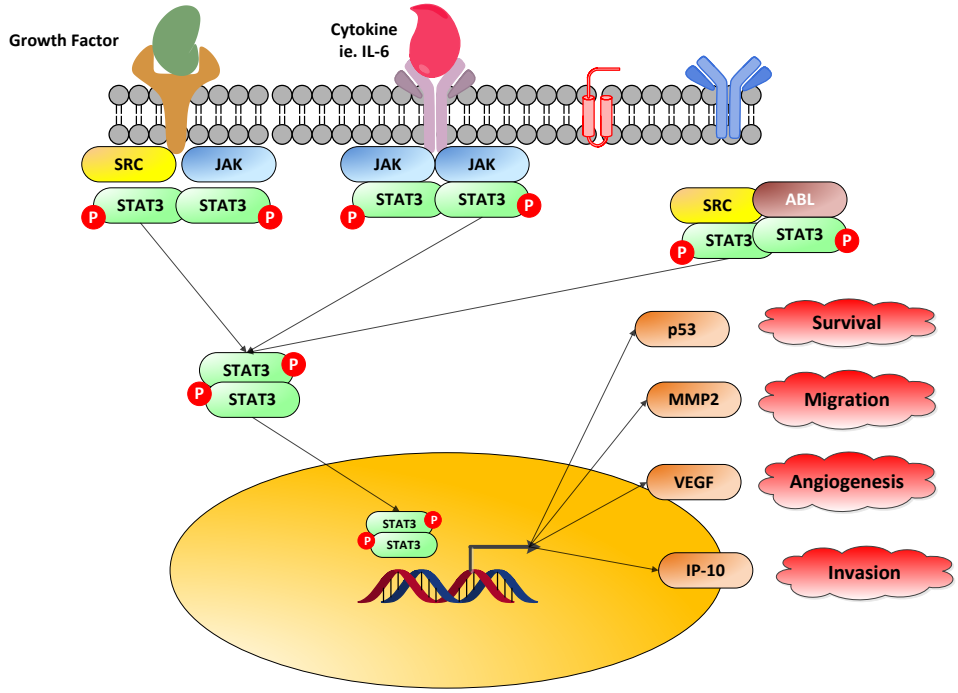
However, most natural p53 mutations occur at a late stage in tumor development, and many clinically detectable cancers have reduced p53 expression but no p53 mutations. It remains to be fully determined what mechanisms disable p53 during malignant initiation and in cancers without mutations that directly affect p53. We show here that oncogenic signaling pathways inhibit the p53 gene transcription rate through a mechanism involving Stat3, which binds to the p53 promoter in vitro and in vivo.

Site-specific mutation of a Stat3 DNA-binding site in the p53 promoter partially abrogates Stat3-induced inhibition. Stat3 activity also influences p53 response genes and affects UV-induced cell growth arrest in normal cells. Furthermore, blocking Stat3 in cancer cells up-regulates expression of p53, leading to p53-mediated tumor cell apoptosis. As a point of convergence for many oncogenic signaling pathways, Stat3 is constitutively activated at high frequency in a wide diversity of cancers and is a promising molecular target for cancer therapy.

Thus, repression of p53 expression by Stat3 is likely to have an important role in development of tumors, and targeting Stat3 represents a novel therapeutic approach for p53 reactivation in many cancers lacking p53 mutations.

[11] http://www.ncbi.nlm.nih.gov/gene/6774

Namely in many cancers the excess expression of STAT3 leads to an inactivation of p53 and thus an oncogenic state. The figure below is a depiction of this process.



However, Pencik et al have recently noted the following as regards to PCa.

Prostate cancer (PCa) is the most prevalent cancer in men.

Hyperactive STAT3 is thought to be oncogenic in PCa.

However, targeting of the IL-6/STAT3 axis in PCa patients has failed to provide therapeutic benefit. Here we show that genetic inactivation of Stat3 or IL-6 signalling in a Pten-deficient PCa mouse model accelerates cancer progression leading to metastasis. Mechanistically, we identify p19ARF as a direct Stat3 target.

Loss of Stat3 signalling disrupts the ARF–Mdm2–p53 tumour suppressor axis bypassing senescence. Strikingly, we also identify STAT3 and CDKN2A mutations in primary human PCa. STAT3 and CDKN2A deletions co-occurred with high frequency in PCa metastases. In accordance, loss of STAT3 and p14ARF expression in patient tumours correlates with increased risk of disease recurrence and metastatic PCa. Thus, STAT3 and ARF may be prognostic markers to stratify high from low risk PCa patients. Our findings challenge the current discussion on therapeutic benefit or risk of IL-6/STAT3 inhibition.

But Pencik et al further note:

PTEN is one of the most frequently deleted or mutated tumour suppressors in PCa, with an estimated incidence of 70% in metastatic PCa, causing aberrant activation of the PI3K– AKT–mTOR signalling pathway

We have examined this extensively in our analyses of PCa.

Loss of Pten leads to senescence, which is critically regulated by the ARF–p53 pathway.

PTEN is a major controller of PI3K and its pathway. Loss of PTEN is common in most PCa. On the other hand we have the ARF-MDM2-p53 dynamic which we shall discuss later.

While the tumour suppressor ARF ($p14^{ARF}$ in humans; $p19^{ARF}$ in mice) is readily degraded in normal cells, it is stabilized to increase p53 function on loss of Pten. ARF was shown to augment p53 stability by promoting the degradation of Mdm2, a negative regulator of p53.

Concomitant inactivation of Pten and p53 leads to bypass of senescence and as a consequence to a malignant PCa phenotype.

Loss of PTEN and of p53 is potentially a universally catastrophic event. It is a loss of two of the most significant stabilization elements in any cell, especially the prostate.

Previous studies report PTEN–STAT3 signalling crosstalk in malignant glioblastoma, but the detailed molecular mechanisms in cancer progression and metastasis remain unresolved.

In this study, we show that loss of IL-6/Stat3 signalling in a Pten-deficient PCa model accelerates cancer progression leading to metastasis. Loss of IL-6/Stat3 signalling in PCa bypasses senescence via disrupting the ARF–Mdm2–p53 tumour suppressor axis.

We identify ARF as a novel direct Stat3 target. Notably, loss of STAT3 and p14ARF expression correlates with increased risk of recurrence in PCa patients. In addition, STAT3 and p14ARF expression was lost in metastasis compared with the primary tumours.

This is the nexus between the STAT3 pathway and the ARF-MDM2-p53 pathways. Namely the authors seem to argue that STAT3 targets ARF and it is through this "targeting" that the latter pathway becomes defective.

We identified STAT3 and CDKN2A mutations in primary PCa patients. Furthermore, PCa metastases show a high frequency of STAT3 and CDKN2A deletions.

We propose STAT3 and ARF as prognostic markers for high versus low risk PCa patient stratification.

Pencik et al also note the following inference:

Stat3 regulates the ARF–Mdm2–p53pathway. Since loss of Pten triggers senescence thereby restricting cancer progression and metastasis, we next tested whether Stat3 exerts a tumour

suppressive function by activating senescence-inducing programmes in Ptenpc-/-PCa cells at an early stage of PCa development.

Senescence is generally characterized by upregulation of p53, cyclin-dependent kinase inhibitor 1 (Cdkn1, p21), promyelocytic leukaemia protein (PML) and elevated senescence-associated-b-galactosidase activity. Of note, Ptenpc-/-Stat3-/- tumours lacked p21 expression, displayed reduced numbers of PML nuclear bodies and decreased SA-b-Gal activity compared with Ptenpc-/- tumours, suggesting Stat3 as a novel mediator of senescence in response to loss of Pten.

Again the statement is "suggesting" and there is no definitive well defined mechanism.

Senescence associated with loss of Pten was shown to be bypassed by deletion of p53 leading to early lethality. We show here that loss of Stat3 and Pten revealed a phenotype strikingly similar to that of p53 and Pten loss. Intriguingly, Stat3 and Pten deletion resulted in downregulation of p53 expression in the prostate epithelium, which was accompanied by the loss of p19ARF

The authors make the following statement:

The p53 expression in the tumour stromal cells remained unchanged. Since $p19^{ARF}$ is a critical regulator of Mdm2 degradation, our results suggest that the tumour suppressive capacity of Stat3 in senescent tumour cells may rely on the p19ARF–Mdm2–p53 tumour suppressor axis.

The conclusion is still a bit tentative. Just what the mechanism is may not be well understood.

Now Yu et al state:

The Janus kinases (JAKs) and signal transducer and activator of transcription (STAT) proteins, particularly STAT3, are among the most promising new targets for cancer therapy. In addition to interleukin-6 (IL-6) and its family members, multiple pathways, including G-protein-coupled receptors (GPCRs), Toll-like receptors (TLRs) and microRNAs were recently identified to regulate JAK–STAT signalling in cancer.

Well known for its role in tumour cell proliferation, survival, invasion and immunosuppression, JAK–STAT3 signalling also promotes cancer through inflammation, obesity, stem cells and the pre-metastatic niche. In addition to its established role as a transcription factor in cancer, STAT3 regulates mitochondrion functions, as well as gene expression through epigenetic mechanisms. Newly identified regulators and functions of JAK–STAT3 in tumours are important targets for potential therapeutic strategies in the treatment of cancer.

Huang, et al state that STAT3 is a preferred target for cancer therapy. Specifically:

Numerous cytokines, growth factors, and oncogenic proteins activate signal transducer and activator of transcription 3 (Stat3), which has been recognized as one of the common pathways

in cancer cells. Stat3 signaling affects the expression and function of a variety of genes that are critical to cell survival, cell proliferation, invasion, angiogenesis, and immune evasion.

Evidently, the Stat3 signaling pathway regulates cancer metastasis and constitutes a potential preventive and therapeutic target for cancer metastasis. .

Furthermore Huang et al outline the reasons for this:

Contribution of Stat3 signaling pathway to cancer metastasis.

Stat3 in the cytoplasm of unstimulated cells becomes activated by recruitment to phosphotyrosine motifs within complexes of growth factor receptors (e.g., epidermal growth factor receptor), cytokine receptors (e.g., IL-6 receptor), or non-receptor tyrosine kinases (e.g., Src and BCR-ABL) through their SH2 domain. Stat3 is then phosphorylated on a tyrosine residue by activated tyrosine kinases in receptor complexes.

Phosphorylated Stat3 forms homodimers and heterodimers and translocates to the nucleus. In the nucleus, Stat3 dimers bind to specific promoter elements of target genes and regulate gene expression. The Stat3 signaling pathway regulates cancer metastasis by regulating the expression of genes that are critical to cell survival, cell proliferation, invasion, angiogenesis, and tumor immune evasion.

It would be useful if somehow these conflicting views could be brought into alignment. In addition we have the work Marcias et al, who state:

Pathways associated with Stat3 activation. Stat3 is activated downstream of receptor tyrosine kinases (e.g., EGFR), cytokine receptors via associated Janus family kinases (JAKs) (e.g., IL-6 receptor), and nonreceptor-associated tyrosine kinases (e.g., c-src). Tumor promoters such as TPA and UVB activate Stat3 in keratinocytes primarily via the EGFR.

Activation of PKCs by tumor promoters leads to the processing of membrane-bound preforms of EGFR ligands such as heparin-binding EGF (HB-EGF) by matrix metalloproteinases (MMPs). In addition, PKCs associate with and phosphorylate Stat3 at Ser727, which is necessary for maximal Stat3 transcriptional activity. Furthermore, transcriptional induction of cytokines and EGF ligands can lead to autocrine stimulation and sustained Stat3 phosphorylation.

After phosphorylation, STAT3 dimerizes and translocates to the nucleus, where Stat3 dimers directly regulate gene expression of transcriptional targets including Bcl-xL, cyclin D1, c-myc, Twist and Survivin. STAT3-mediated regulation of target gene expression is involved in various cellular functions including cell differentiation, proliferation, survival, and oncogenesis. Stat3 can also act through noncanonical signaling pathways. In this regard, unphosphorylated Stat3 (U-Stat3) can drive gene expression of a subset of genes that are different from p-Stat3 dimers in an NF-κB-dependent and independent manner.

In addition, p-Stat3 Ser727 can translocate into the mitochondria and influence mitochondrial respiratory chain activity. These noncanonical Stat3 signaling pathways have protumorigenic

roles in certain cell/tissue types; however their role in epithelial carcinogenesis has not been evaluated.

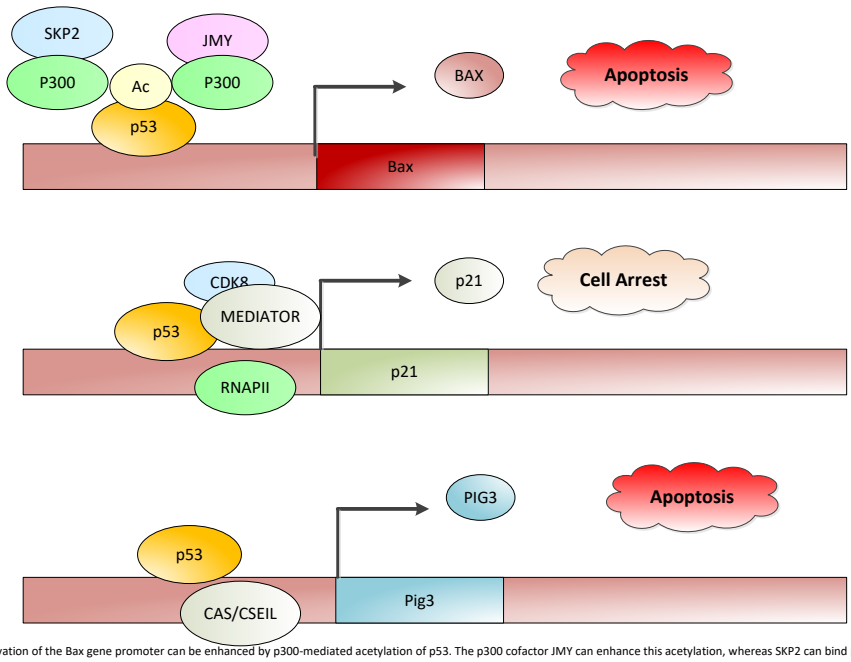
Thus the nature of STAT3 and its importance must be better investigated.

This paper by Pencik et al presents an interesting challenge to the ability to identify genetic markers for various cancers. What may at one time seem to be a problem may later be understood in a more complete fashion to be a necessary control element. To some degree we have observed this with BRAF inhibitors in melanoma, which lead to SCC and thus require a MEK inhibitor. In some sense unless a full dynamic understanding of pathways is established one may continue to see this "whack a mole" approach to therapeutics.

To reiterate the Pencik et al observations:

1. *Co-deletion of Stat3 and Pten triggers PCa*: We know that PTEN loss is found in PCa and we also know that active Stat3 is a significant factor in many malignancies. Yet the loss of both may appear as being of significance.
2. *Stat3 regulates the ARF–Mdm2–p53pathway*: This is the key observation which they articulate and stress and the main divergence from standard thought.
3. *Loss of IL-6 and Pten leads to cancer and metastasis*: We know that IL-6 drives Stat3 and that loss of IL-6 would most likely lead to a loss of Stat3 expression. As noted above loss of both Pten and Stat3 would lead to a malignant state.
4. *Loss of STAT3 and ARF in PCa is associated with metastases*: ARF is key to the ARF-MDM2 –p53 pathway. MDM2 inhibits p53. Thus the association of Stat3 being the "driver" of the ARF process is essential.

We reiterate the p53 processes as shown below. The three lead to either apoptosis or cell arrest as one would expect. In all cases p53 plays a key role but it is also clear that other proteins are required in some cases.



Activation of the Bax gene promoter can be enhanced by p300-mediated acetylation of p53. The p300 cofactor JMY can enhance this acetylation, whereas SKP2 can bind p300 and antagonize acetylation of p53. p53-dependent transactivation of the p21 gene promoter can be enhanced through the recruitment of CDK8 to the Mediator complex. Independent binding of the long-range chromatin modifier CAS/CSE1L, which is the human orthologue of yeast Cse1, to promoters, such as the Pig3 gene promoter, can also enhance p53-dependent transcription.

Pencik et al finally note:

Interestingly, loss of PTEN expression in primary human PCa did not correlate with overall survival and could not predict PCa-specific death. Moreover, heterozygous PTEN deletions far outnumber homozygous deletions in primary human PCa and we show here that PTEN is mutated or lost only in a small subset (4.7%) of a large cohort of patients with primary PCa.

However, PTEN is lost in >50% of human PCa metastases suggesting an important role for PTEN in this process. Finally, we show in our study that STAT3 is co-deleted with PTEN in 66% of human PCa metastases in two independent data sets.

Since PTEN is mutated or lost in only a minor fraction of primary PCa, other aberrations must occur (oncogene induction or loss of tumour suppressor function) to activate STAT3 and ARF to induce senescence in human cancers. Indeed, several studies indicate that different aberrations can lead to induction of senescence in human cancers

From Soissi and Wiman:

The standard classification used to define the various cancer genes confines tumor protein p53 (TP53) to the role of a tumor suppressor gene. However, it is now an indisputable fact that many p53 mutants act as oncogenic proteins.

This statement is based on multiple arguments including the mutation signature of the TP53 gene in human cancer, the various gains-of-function (GOFs) of the different p53 mutants and the heterogeneous phenotypes developed by knock-in mouse strains modeling several human TP53 mutations.

In this review, we will shatter the classical and traditional image of tumor protein p53 (TP53) as a tumor suppressor gene by emphasizing its multiple oncogenic properties that make it a potential therapeutic target that should not be underestimated.

Analysis of the data generated by the various cancer genome projects highlights the high frequency of TP53 mutations and reveals that several p53 hotspot mutants are the most common oncoprotein variants expressed in several types of tumors.

The use of Muller's classical definition of mutations based on quantitative and qualitative consequences on the protein product, such as 'amorph', 'hypomorph', 'hypermorph' 'neomorph' or 'antimorph', allows a more meaningful assessment of the consequences of cancer gene modifications, their potential clinical significance, and clearly demonstrates that the TP53 gene is an atypical cancer gene.

There is an interesting paper from CSHL on progress on cancer classification. Linnaeus some 300 years ago came up with a classification system for various species. Aristotle was driven by his desire to classify, and ever since we have people trying their best to do that task. Patients always want to know what they have, and that is a form of classification.

We classify cancers based upon organs. We may modify it based on cell types or based on cell markers such as immunological markers. I remember back in the 60s that Leukemias were simple; acute or chronic, you died now or later. Now we have a plethora of subtypes and a multiplicity of therapeutics.

But we also know genomic data. Perhaps then we should classify cancers based upon genes, not upon organs, binding proteins, or the like,

As the authors state:

Classification is an everyday instinct as well as a full-fledged scientific discipline. Throughout the history of medicine, disease classification is central to how we organize knowledge, obtain diagnosis, and assign treatment. Here we discuss the classification of cancer, the process of categorizing cancers based on their observed clinical and biological features. Traditionally, cancer nomenclature is primarily based on organ location, e.g., "lung cancer" designates a tumor originating in lung structures.

Within each organ-specific major type, further subgroups can be defined based on patient age, cell type, histological grades, and sometimes molecular markers, e.g., hormonal receptor status in breast cancer, or microsatellite instability in colorectal cancer. In the past 15+ years, high-throughput technologies have generated rich new data for somatic variations in DNA, RNA, protein, or epigenomic features for many cancers. These data, representing increasingly large tumor collections, have provided not only new insights into the biological diversity of human cancers, but also exciting opportunities for discovery of new cancer subtypes.

They continue:

An ever finer classification system has many potential benefits. It is needed to capture the full spectrum of biological diversity—the "endless forms" that Darwin spoke of. It could lead to a better recognition of patient-specific disease mechanisms, and importantly, could suggest treatment options that are more accurately matched to the patient's tumor. Precision medicine, at its very foundation, relies on valid and continuously optimized disease classification that reflects the underlying mechanisms. However, a fine-grained classification system also has many potential drawbacks. The newly proposed splits may not be technically robust. Even when the finer categories are robustly supported by statistical significance and by replication, they may still lack a clear biological meaning, or have little impact on treatment options (#3 below) if it turns out that some subtypes share the same clinical endpoint, or if treatment options are limited.

Indeed, we may find it much more powerful to have a new Linnaeus type look at classification. Classifying genomically, via genes, RNA, and epigenetic factors, may help stratify and focus on therapeutics. This article raises an interesting dialog.

Overall we can make some summary observations:

1. Perhaps one should be cautious as regards to murine and human models. All too often what we see in mouse models does not pan out in human. The reasons may very well be the complexity of the signally paths.

2. Signalling paths are complex and dynamic. What may work at one instant may not at another? The question then is: how critical are realistic repeatable and predictive models in assisting in both prognostic evaluation and therapeutic approaches?

3. Cells are not the same everywhere. Thus when we perform a prostate biopsy we may get one profile but when that cell metastasizes to other organs we get dramatically different cells. As we have discussed before the paper by Gundem et al presets a compelling picture of the complexity of gene expression in PCa. Namely each cell cluster may have complex and disparate genes expressed. If that is the case then we would also be concerned that we look at similar expression when performing biopsies.

3.5.4 ERK

As Torrealba et al note:

Prostate cancer may emerge as result of dysregulated balance between cell proliferation and death rates, increased angiogenesis and chronic.

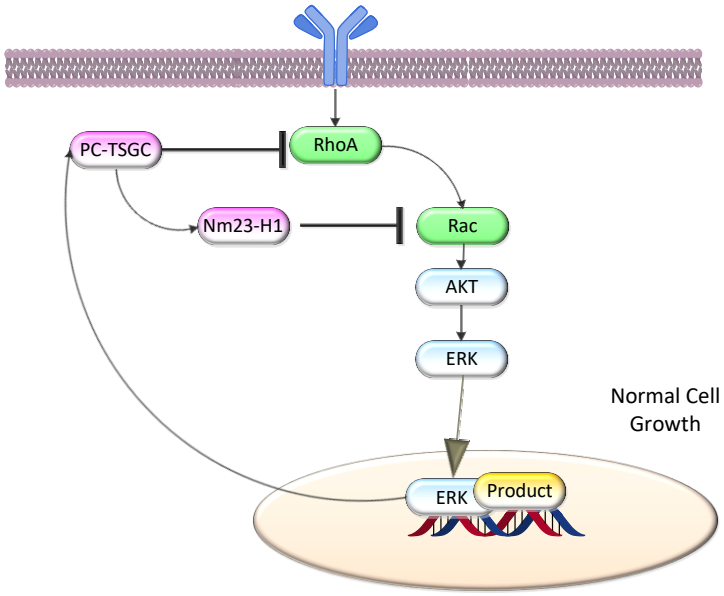
These processes are regulated by numerous signaling proteins, including the mitogen-activated protein kinases (MAPKs). JNK, p38 and extracellular signal-regulated kinase (ERK) are the three major sub-families of MAPKs. The pro-oncogenic effects of ERK isoforms (ERK1 and ERK2) lie in their aberrant activation through phosphorylation by any mutation along the pathway of receptor tyrosine kinase (RTK)-Ras-Raf-MEK-ERK1/2. Once activated, ERKs phosphorylate cytoskeletal proteins, kinases, and transcription factors.

Active ERK proteins induce strong proliferative and anti-apoptotic effects.

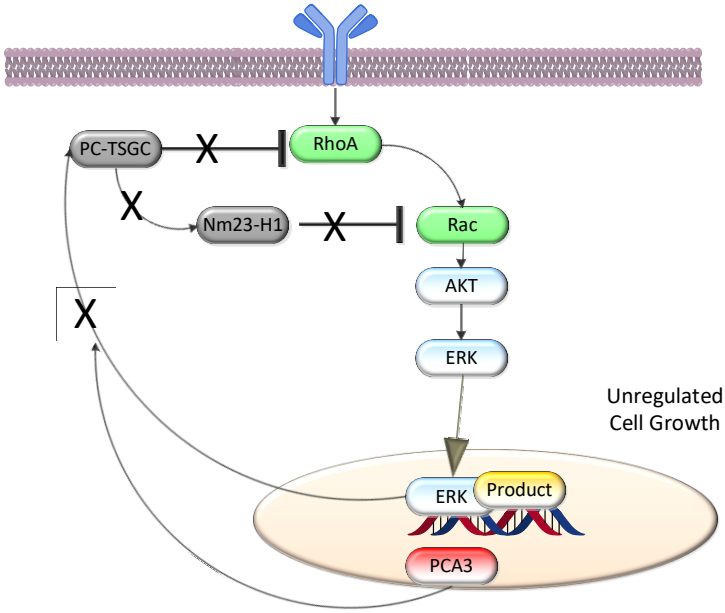
Our group has tested variations in expression, activation and localization of ERKs in human prostate. Differential ERK1/2 expression and phosphorylation status may be linked to the progression of prostate cancer. The major striking observation is that ERKs are expressed in tumors with higher proportion than normal prostate.

We believe that this is an important notion because the status (expression, localization, phosphorylation and the ERK1/ERK2 ratio) of ERK in the prostate may be developed into an important prognostic marker that predicts patient responce to the anti-cancer treatment.

We show the key paths below:



The Figure below depicts the results of path blockage resulting in unregulated growth.



3.5.5 AKT

We now consider another kinase. As Shorning et al note:

AKT isoforms 1, 2, and 3 (encoded by AKT1, AKT2, and AKT3 respectively) form a subfamily of serine/threonine protein kinases that possess both overlapping and distinct cellular functions to regulate a variety of cellular processes during normal tissue homeostasis and cell transformation.

PI3K activity elevates PIP3 levels to recruit AKT to the plasma membrane where is it activated (Figure 1). AKT is activated by multiple kinases, including PDK1 and mTORC2 that phosphorylate AKT at residues Thr308 and Ser473 respectively, triggering a wave of phosphorylation through multiple downstream targets that stimulate cell survival, proliferation, metabolism and differentiation to promote tumor growth.

AKT downstream targets include PRAS40 (a component of mTORC1), BAD, FOXOs, and MDM2 (reviewed in). AKT signaling is negatively regulated by several protein phosphatases that dephosphorylate and inactivate AKT, including protein phosphatase 2 (PP2A), and PH domain and leucine-rich repeat protein phosphatase-1 and -2 (PHLPP1 and PHLPP2).

…we outline the various genetic alterations within the AKT isoforms and their regulators that have been detected in prostate cancer, and discuss their potential to activate AKT signaling and promote prostate tumor growth.

AKT Mutation and Amplification AKT genetic aberrations that increase AKT activity have been detected in multiple malignancies and are especially common in breast cancer, where AKT3 amplification and AKT1 E17K oncogenic mutation have been reported in up to 24% and 1–8% of cases respectively.

AKT1, AKT2, and AKT3 activating mutations are rare in prostate cancer (≤0.9%, predominantly in AKT1 at E17K), whereas AKT1, AKT2, and AKT3 high-level gene amplification that can increase AKT activity is more common, particularly in advanced disease.

Moreover, AKT activation in prostate cancer has been shown to positively correlate with Gleason score and invasive progression , and over-expression of myristoylated AKT (which causes constitutive AKT activation) causes prostate neoplasia in mice . In support of an oncogenic role in prostate cancer and therapeutic resistance, conditional activation of AKT in either the LNCaP human prostate cancer cells or a transgenic mouse results in increased cell proliferation and inhibits cell

3.5.6 MAPKK

As Burotto et al note:

There are four independent MAPK pathways composed of four signaling families:

1. *the MAPK/ERK family or classical pathway,*
2. *and Big MAP kinase-1 (BMK-1),*
3. *c-Jun Nterminal kinase (JNK),*
4. *and p38 signaling families.*

These families share a basic organization composed of two serine/threonine kinases and one double specificity threonine/ tyrosine kinase.

Generically, these kinases are designated from upstream to downstream, closer to the nucleus, as MAPK kinase-kinase (MAPKKK), MAPK kinase (MAPKK) and MAPK. The canonical MAPK/ERK pathway is composed of three types of MAPKKK: A-RAF, B-RAF and RAF-1 or C-RAF kinases. BRAF is the gene most commonly mutated at this level in human cancer.

One level below are the MAPKKs, which are composed of MEK1 and MEK2. Finally, further downstream are ERK1 and ERK2, which are the final effectors of the MAPK pathway.

From Burotto et al,

The MAPK/ERK pathway is activated by upstream genomic events and/or activation of multiple signaling events where information coalesces at this important nodal pathway point. This pathway is tightly regulated under normal conditions by phosphatases and bidirectional communication with other pathways, such as the AKT/m-TOR pathway. Recent evidence

indicates that the MAPK/ERK signaling node can function as a tumor suppressor as well as the more common prooncogenic signal.

The effect that predominates depends on the intensity of the signal and the context or tissue in which the signal is aberrantly activated. Genomic profiling of tumors has revealed common mutations in MAPK/ERK pathway components, such as BRAF. Currently approved for the treatment of melanoma, inhibitors of B-RAF kinase (BRAFi) are being studied alone and in combination with inhibitors of the MAPK and other pathways to optimize treatment of many tumor types.

Therapies targeted toward MAPK/ERK components have variable response rates when used in different solid tumors, such as colorectal cancer and ovarian cancer.

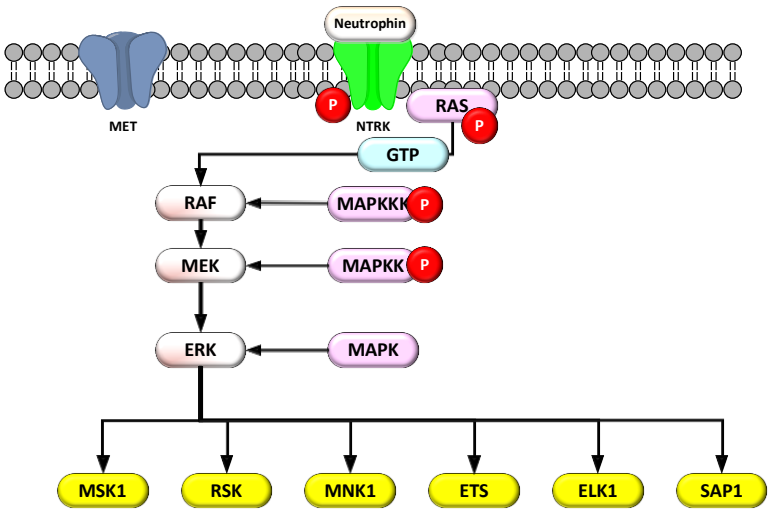
Understanding the differential nature of activation of the MAPK/ERK pathway in each tumor type is critical in developing single and combination regimens, as different tumors have unique mechanisms of primary and secondary signaling and subsequent sensitivity to drugs. …

There are four independent MAPK pathways composed of four signaling families: the MAPK/ERK family or classical pathway, and Big MAP kinase-1 (BMK-1), c-Jun Nterminal kinase (JNK), and p38 signaling families.

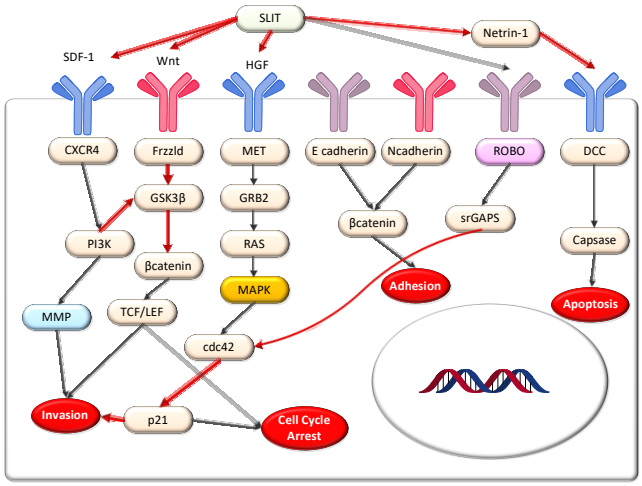
These families share a basic organization composed of two serine/threonine kinases and one double specificity threonine/ tyrosine kinase. Generically, these kinases are designated from upstream to downstream, closer to the nucleus, as MAPK kinase-kinase (MAPKKK), MAPK kinase (MAPKK) and MAPK. The canonical MAPK/ERK pathway is composed of three types of MAPKKK: A-RAF, B-RAF and RAF-1 or C-RAF kinases.

BRAF is the gene most commonly mutated at this level in human cancer. One level below are the MAPKKs, which are composed of MEK1 and MEK2. Finally, further downstream are ERK1 and ERK2, which are the final effectors of the MAPK pathway….

The figure below shows the MAPKKK element in its pathway.

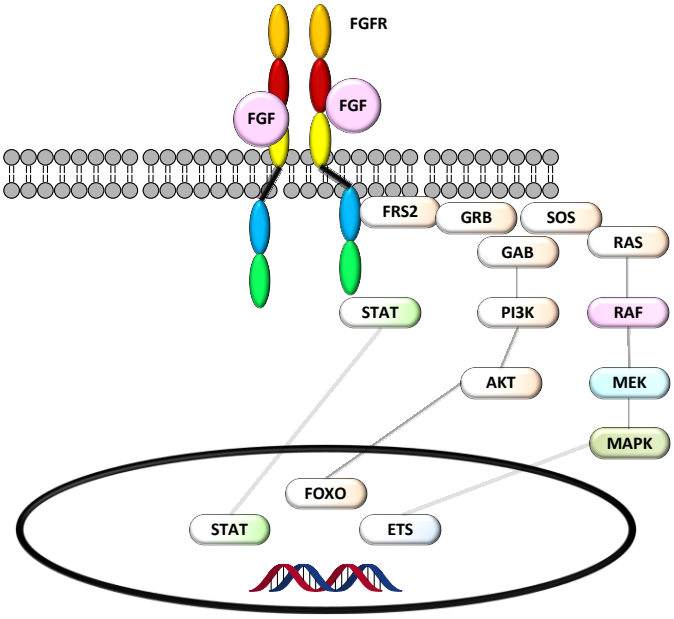


The effects of the various pathways are shown below. The insertion of MAPK and its derivatives play a significant role in invasion and cell cycle control.



From: Dickinson and Duncan

The figure below incorporates details regarding receptors, here FGFR and ligand FGF. They un turn activate MAK and derivatives.



3.5.7 IL-6

Cytokines flow from one cells to another and cytokines like many drivers activate various pathways. IL cytokines are such a broad class. As Pencik et al note the following about a specific such cytokine, IL-6:

Interleukin-6 (IL-6) is a multifunctional cytokine that is implicated in the regulation of immune responses, inflammation and cellular processes in several cancers, including cancer of the prostate.

IL-6 is detectable in stromal cells, but preferentially localised in the epithelium of prostate tissue. The IL-6 receptor displays a highly restricted expression pattern including hepatocytes, leucocyte subsets and megakaryocytes, but is also ubiquitously expressed in prostate cancer cells.

In benign prostatic tissue IL-6 expression is confined to the basal cells of the epithelium. In particular, the androgen receptor-negative human prostate cancer cell lines (DU-145 and PC3) express high levels of IL-6 . Whether this is a result of a direct mechanism involving the androgen receptor remains unknown. IL-6 expression is governed by nuclear factor kappa B, which is suppressed by treatment with androgenic hormones and may otherwise result in an aberrant activation of the androgen receptor …,

IL-6 expression levels are high in the tissue of prostate cancer patients after radical prostatectomy, as well as in sera of patients with advanced prostate cancer that is resistant to therapy. IL-6 levels are upregulated by transforming growth factor-beta (TGF-β) as well, which is an important determinant of metastatic transformation .

Thus, IL-6 signal transduction is important for regulating cellular processes in prostate cancer. Studies with primary cells also demonstrated that there is a positive growth effect of IL-6 in such a condition….

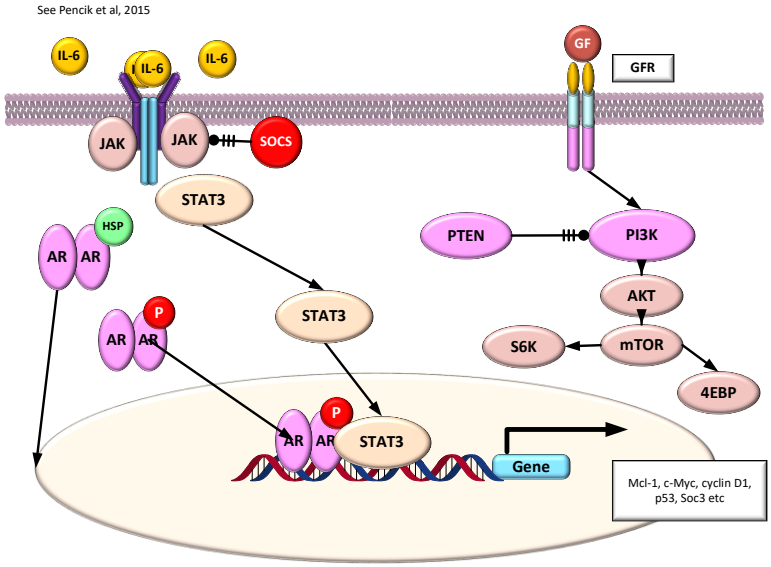
They then note the dynamics as follows:

Overview of IL-6/STAT3/ARF and PI3K/PTEN/AKT/mTOR pathways.

Activation of IL-6/STAT3 signalling leads to phosphorylation and translocation of STAT3 to the nucleus, which is associated with AR interaction regulated by HSP.

JAKs serve to phosphorylate tyrosine (Y) or serine (S) residues of STAT3 and to translocate to nucleus or mitochondrial matrix. Some of these downstream signalling events (STAT3-AR-S6K) could regulate activity or expression of prostate cancer related genes. ARF = alternative reading frame protein; IL-6 (interleukin-6); PI3K (phosphatidylinositol-3-kinase); PTEN (phosphatase and tensin homologue); STAT3 (signal transducer and activator of transcription-3)…

as shown below:



As Neuwirt et al noted:

Prostate cancer initiation and progression strongly depend on activation of the AR, but chronic inflammation of the prostate may also play an important role.

Therefore, it is not surprising that the role of the proinflammatory cytokine interleukin-6 (IL-6) in prostate carcinogenesis has received a considerable interest. IL-6 is a multifunctional cytokine that acts in a cell type-specific manner through activation of signaling pathways of

Janus kinases/signal transducer and activator of transcription factors (STAT), mitogen-activated protein kinases, and/or phosphotidylinositol 3-kinase.

In prostate cancer cells, either pro-differentiation or survival effects of IL-6 have been described. The mechanisms responsible for differential activation of IL-6 signaling pathways in prostate tumor cells are being investigated. It is assumed that various regulators of phosphorylation of STAT3, in particular suppressors of cytokine signaling ( ) and protein inhibitors of activated STAT, determine activation status of this transcription factor. The family comprises eight members, 1 through 7 and CIS.3 family members share the central Src homology 2 domain and box in the carboxy-terminal end, which plays a crucial role in proteasomal degradation of binding partners. -1 and -3 contain a kinase inhibitory region, which has a pivotal function in antagonizing activation of Janus kinases.

3.5.8 IL-17

As Kuen et al note:

IL-17 is produced by RAR-related orphan receptor gamma t (RORγt)-expressing cells including Th17 cells, subsets of γδT cells and innate lymphoid cells (ILCs).

The biologicalsignificance of IL-17-producing cells is well-studied in contexts of inflammation,autoimmunity and host defense against infection. While most of available studies in tumorimmunity mainly focused on the role of T-bet-expressing cells, including cytotoxic CD8+T cells and NK cells, and their exhaustion status, the role of IL-17-producing cells remainspoorly understood.

While IL-17-producing T-cells were shown to be anti-tumorigenic inadoptive T-cell therapy settings, mice deficient in type 17 genes suggest a protumorigenicpotential of IL-17-producing cells. This review discusses the features of IL-17-producingcells, of both lymphocytic and myeloid origins, as well as their suggested pro- and/or antitumorigenic functions in an organ-dependent context. Potential therapeutic approachestargeting these cells in the tumor microenvironment will also be discussed…

The biologicalsignificance of IL-17-producing cells is well-studied in contexts of inflammation,autoimmunity and host defense against infection. While most of available studies in tumorimmunity mainly focused on the role of T-bet-expressing cells, including cytotoxic CD8+T cells and NK cells, and their exhaustion status, the role of IL-17-producing cells remainspoorly understood.

Potential therapeutic approaches targeting these cells in the tumor microenvironment will also be discussed granulocytic in nature in squamous cervical cancers, and associated with poor survival.

In addition, IL-17-expressing cells were independently associated with poor survival in early stage of the disease . IL-17 producing mast cells in esophageal squamous cell carcinoma were found to be densely located in the muscularis propria, and were suggested to function in the

recruitment of effector CTLs and M1 macrophages to the site of tumor, thus acting as a favorable prognostic factor…

Now Zhang et al (2012) noted:

The contributions of interleukin (IL)-17 to cancer remain unclear and somewhat controversial.

We took a genetic approach to explore its role in prostate cancers by interbreeding IL-17 receptor C (IL-17RC)–deficient mice with mice that are conditionally mutant for PTEN, one established preclinical model for prostate cancer. Mice that were IL-17RC–deficient (IL-17RC) displayed prostates that were smaller than mice that maintained IL-17RC expression (IL-17RCþ).

In addition, IL-17RC mice developed a reduced number of invasive prostate adenocarcinomas with lower rates of cellular proliferation and higher apoptosis than IL-17RCþ mice. Moreover, the fibromuscular stroma surrounding prostatic glands was relatively thicker in IL-17RC mice and was associated with decreased matrix metalloproteinase (Mmp)7 expression and increased Timp1, 2, and 4 expression, whereas administration of recombinant mouse IL-17 induced prostatic expression of Mmp7.

Taken together, our results suggested that IL-17 promotes the formation and growth of prostate adenocarcinoma, and that an IL-17–MMP7 signaling axis is required for the transition of prostatic intraepithelial neoplasia to frank adenocarcinoma.

Zhang et al (2017) then noted:

Chronic inflammation has been associated with a variety of human cancers. Approximately 15% of all human cancers have been suggested to result from infection and chronic inflammation.1 Almost all surgical prostate specimens contain evidence of inflammation.

Chronic inflammation invokes proliferative inflammatory atrophy of prostate – a potential precursor lesion to prostatic intraepithelial neoplasia (PIN) and carcinoma. The cause of prostatic inflammation includes infection, urine reflux, diet, estrogen, and physical trauma. Inflammation is a complex response involving many immune cells, chemokines, and cytokines as well as matrix-degrading enzymes.

Interleukin-17 (IL-17, also named IL-17A) is a key pro-inflammatory cytokine that plays critical roles in many inflammatory and autoimmune diseases. IL-17 has been demonstrated to promote development of colon cancer, skin cancer, breast cancer, prostate cancer, lung cancer, and pancreas cancer. IL-17 is secreted by T helper 17 (TH17) cells, γδ T cells, natural killer cells, and other immune cells.

IL-17 acts on IL-17RA/ IL-17RC receptor complex to recruit nuclear factor-κB (NF-κB) activator 1 (Act1). Act1 activates tumor necrosis factor receptor-associated factor 6 (TRAF6),2and subsequently activates transforming growth factor-β-activated kinase 1 (TAK1)

and IκB kinase (IKK) complex, resulting in activation of NF-κB pathway that initiates transcription of a variety of chemokines and cytokines, such as C-X-C motif ligand 1 (CXCL1), C-C motif ligand 20 (CCL20), IL-1β, and IL-6.

These IL-17-downstream factors promote cancer formation through increased cellular proliferation, attenuated apoptosis, and sustained angiogenesis, as well as creation of an immunotolerant microenvironment. …

We have previously generated an IL-17 receptor C (Il-17rc) and prostate-specific conditional phosphatase and tensin homolog (Pten) double knockout (KO) mouse model. IL-17RCdeficient (IL17-RC− or RC−) mice display smaller prostates and develop a reduced number of invasive prostate adenocarcinomas, compared to IL-17RC-sufficient (IL17-RC+ or RC+) mice.

Further, matrix metalloproteinase 7 (MMP7) expression is increased in RC+ mice compared to RC−mice. However, whether MMP7 mediates IL-17's action and the underlying molecular mechanisms remain unknown. MMP7 (also known as putative metalloproteinase I or matrilysin) is exclusively expressed in the epithelial cells.

MMP7 is overexpressed in human prostate cancer, but not expressed in normal prostate glands. Here, we investigated the role of MMP7 in mediating IL-17's action, using an Mmp7 and Pten double KO mouse model. Our findings demonstrate that MMP7 mediates IL-17's function in promoting prostate carcinogenesis through induction of epithelial-tomesenchymal transition (EMT) …

E-cadherin interacts with a β-catenin-based complex to act on actin cytoskeleton and mediate adhesion-dependent signaling, and several proteinases including MMP7 are known to be able to cleave E-cadherin. Thus, we tested if MMP7 could cleave E-cadherin in three human prostate cancer cell lines. …

Together, these results suggested that MMP7 cleaved E-cadherin to release β-catenin from E-cadherin/β-catenin complex, leading to nuclear translocation of β-catenin and subsequently activation of downstream transcription factors Snail and Slug, hence inducing EMT

3.5.9 EGF

The epidermal growth factor, EGF, is another GF associated with malignancies. *As NCBI* notes[12]:

This gene encodes a member of the epidermal growth factor superfamily. The encoded preproprotein is proteolytically processed to generate the 53-amino acid epidermal growth factor peptide. This protein acts a potent mitogenic factor that plays an important role in the growth, proliferation and differentiation of numerous cell types. This protein acts by binding with high affinity to the cell surface receptor, epidermal growth factor receptor. Defects in this gene are the cause of hypomagnesemia type 4.

[12] https://www.ncbi.nlm.nih.gov/gene/1950

Dysregulation of this gene has been associated with the growth and progression of certain cancers. Alternative splicing results in multiple transcript variants, at least one of which encodes a preproprotein that is proteolytically processed.

3.5.9.1 EGF Functions

We begin with a simple overview of the EGF functions. As Singh et al note:

EGF is the prototypic and founding member of the EGFR ligand family, first identified from submaxillary gland extracts during nerve growth factor studies. The EGF-EGFR ligand-receptor system has greatly enhanced our understanding of receptor tyrosine kinase signaling, as evidenced by more than 70,000 publications for EGF alone. A recent review has distilled our current understanding of EGF and its actions.

More recently, a study uncovered that EGF-induced EGFR signaling enhances production of intracellular reactive oxygen species (ROS) by dual oxidase 1 (DUOX1) This nicely complements earlier studies in which ROS were shown to enhance EGFR signaling by modulating both positive and negative regulators of EGFR signaling (ADAMs and protein tyrosine phosphatases). In another recent study, urinary EGF has been shown to be an independent risk factor for progression of chronic kidney disease, substantiating earlier findings.

They then note its functioning:

Modes of signaling via epidermal growth factor receptor (EGFR) ligands.

Autocrine signaling occurs when a ligand is released from a cell and binds to EGFR on that same cell.

Paracrine signaling refers to the released ligand acting on a nearby cell, usually a different cell type.

Juxtacrine signaling occurs when a non-cleaved, transmembrane ligand binds to EGFR on an adjacent cell; this is best documented for heparin-binding epidermal growth factor-like growth factor (HBEGF). Amphiregulin (AREG), transforming growth factor-alpha (TGFA), and HBEGF, as well as EGFR, can be packaged into signaling competent exosomes. Uptake of exosomal AREG by recipient cells is, at least in part, dependent on EGFR, leading to the term exosomal targeted receptor activation (ExTRAcrine).

ExTRAcrine signaling has features of autocrine, paracrine, and juxtacrine signaling as well as possibly endocrine signaling since EGFR and AREG can be detected in human plasma exosome.

3.5.9.2 EGF and Cancer

Relationships between EGF and cancers are significant. From Yang et al we have the following:

EGF and its receptor (EGFR) have been associated with tumour cell invasion and metastasis initiation.

Dysregulation of EGFR signalling, including receptor over expression and/or activation has been shown to be a significant effector in the progression of human cancers including neoplasms of the brain, lung, breast, ovary, prostate, and pancreas.

A recent study investigated the relationship between EGFR and the adhesion molecule-integrin in human pancreatic carcinoma cells and demonstrated that the crosstalk between EGFR signalling and integrin in the cancer cell membrane is implicated in carcinoma cell invasion and metastasis. Integrins are a family of adhesion proteins that regulate cell migration.

The fact that EGF stimulated integrins-mediated carcinoma cell migration on vitronectin suggests that EGFR regulates cancer cell migration through the adhesion proteins, the integrins. EGFR inhibitors, such as erlotinib, provide clinical benefit in patients with advanced non-small cell lung cancer metastasis which suggests a critical role for EGF and its receptor in the initial steps of cancer metastasis. The mechanism of EGF activation of adhesion proteins in cancer cell remains to be elucidated.

Some studies indicate EGF induces tumour cell invasion and metastasis through de-phosphorylation and downregulation of focal adhesion kinase, while other studies suggest EGFR activates the ***Src family of kinases (SFK)****. The fact that activated Src kinase is involved in the rearrangement of the actin cytoskeleton, cell-matrix interactions, and cell-cell adhesion processes that promote cell invasion suggests a role for Src activity in tumour metastasis development.*

Added insight is provided by Mendelsohn and Baselga who note:

Human carcinomas frequently express high levels of receptors in the EGF receptor family, and overexpression of at least two of these receptors, the EGF receptor (EGFr) and closely related ErbB2, has been associated with a more aggressive clinical behavior. Further, transfection or activation of high levels of these two receptors in nonmalignant cell lines can lead to a transformed phenotype. For these reasons therapies directed at preventing the function of these receptors have the potential to be useful anti-cancer treatments. In the last two decades monoclonal antibodies (MAbs) which block activation of the EGFr and ErbB2 have been developed.

These MAbs have shown promising preclinical activity and `chimeric' and `humanized' MAbs have been produced in order to obviate the problem of host immune reactions. Clinical activity with these antibodies has been documented: trastuzumab, a humanized anti-ErbB2 MAb, is active and was recently approved in combination with paclitaxel for the therapy of patients with metastatic ErbB2-overexpressing breast cancer; IMC- C225, a chimeric anti-EGFr MAb, has shown impressive activity when combined with radiation therapy and reverses resistance to chemotherapy. In addition to antibodies, compounds that directly inhibit receptor tyrosine kinases have shown preclinical activity and early clinical activity has been reported. A series of phase III studies with these antibodies and direct tyrosine kinase inhibitors are ongoing or

planned, and will further address the role of these active anti-receptor agents in the treatment of patients with cancer.

Finally from Calderon and Prins[13]:

Epidermal growth factor (Egf), a secreted peptide, is produced by the luminal epithelial cells in the prostate, and is found at the highest concentration in human prostatic secretions compared to the rest of the body.

Epidermal growth factor exerts its effects by binding to its tyrosine kinase receptor, epidermal growth factor receptor (EgfR).

Upon binding, EgfR can homo- or heterodimerize with erbB2 receptors, causing autophosphorylation of its tyrosine residues that in turn activate the phosphatidylinositol 3'-kinase (PI3K), mitogen activated protein kinase (MAPK), or phospholipase C-γ (PLC-γ) signaling cascades. In the developing murine prostate gland, Egf has been shown to mediate its actions through the PLC-γ signaling pathway.

Furthermore, rat UGS explants treated with exogenous Egf showed stimulation of prostate bud formation in the absence of androgens, thus positively regulating prostatic budding.

3.5.10 ERG

ERG has been considered a master transcription factor[14]. As Kish et al note:

The ETS-related gene (ERG) is proto-oncogene that is classified as a member of the ETS transcription factor family, which has been found to be consistently overexpressed in about half of the patients with clinically significant prostate cancer (PCa). The overexpression of ERG can mostly be attributed to the fusion of the ERG and transmembrane serine protease 2 (TMPRSS2) genes, and this fusion is estimated to represent about 85% of all gene fusions observed in prostate cancer. Clinically, individuals with ERG gene fusion are mostly documented to have advanced tumor stages, increased mortality, and higher rates of metastasis in non-surgical cohorts.

In the current review, we elucidate ERG's molecular interaction with downstream genes and the pathways associated with PCa. Studies have documented that ERG plays a central role in PCa progression due to its ability to enhance tumor growth by promoting inflammatory and angiogenic responses.

ERG has also been implicated in the epithelial–mesenchymal transition (EMT) in PCa cells, which increases the ability of cancer cells to metastasize. In vivo, research has demonstrated

[13] https://www.sciencedirect.com/science/article/pii/B9780128126363000055

[14] https://www.researchgate.net/publication/340539918_ERG_A_Master_Transcription_Factor

that higher levels of ERG expression are involved with nuclear pleomorphism that prompts hyperplasia and the loss of cell polarity …

In prostate cancer cells, a surprisingly common occurrence involves the fusion of ERG to TMPRSS2, which forms the fusion product of TMPRSS2-ERG. The most common mechanism by which these two genes fuse involves the deletion of intronic sequences on the long arm of chromosome 21 via an intron deletion between TMPRSS2 and ERG on chromosome 21q22.2-3. This fusion mechanism has been identified as being prevalent in approximately 50% of prostate cancer patients .

The frequent occurrence of this fusion protein can be attributed to the presence of a homogenous deletion site that is present between ERG and TMPRSS2 . Moreover, this deletion site is separated into two different classifications according to various start sites. In both of the deletion products, the 50 end of the TMPRSS2 gene has been ligated to the 30 end of ERG. TMPRSS2-ERG fusion results in ERG overexpression due to the androgen responsive promoter of the TMPSS2 gene allowing for the constitutive transcription of ERG, which has been shown to be correlated with increased cell proliferation, cell invasion, angiogenesis, and invasiveness in PCa cells.

In addition, this TMPRSS2-ERG fusion enhances the transcription and activates downstream oncogenes

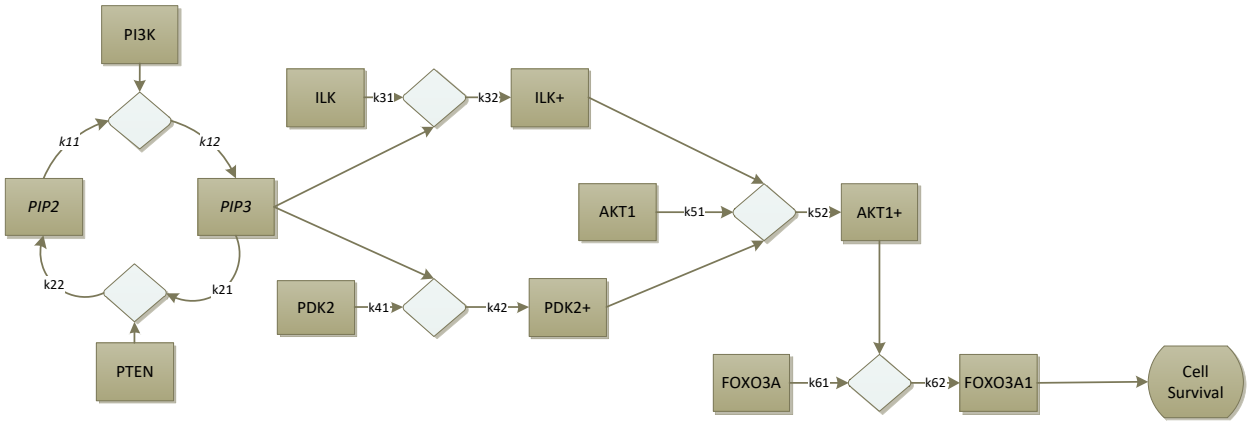
3.5.11 FOXO

The FOXO gene, specifically FOXO3a, forkedhead box zero gene, is located at 6q21 in humans and is a key nuclear transcription regulator. It has the ability to mediate cell cycle arrest, DNA repair, apoptosis and as such acts in many ways like a tumor suppressor gene. Loss of the FOXO gene activity may lead to uncontrolled cell growth. Also impairment or suppression of FOXO can result in impaired DNA repair capabilities as well. In a normal situation a reduced level of FOXO in a cell would lead to normal cell death however in cancerous cells this is no longer the case. As Lam et al state the FOXO molecule is key to the regulation of normal cell homeostasis. Although mutations in FOXO are not common it is the FOXO function controlled via PI3K and PTEN that often are of interest.

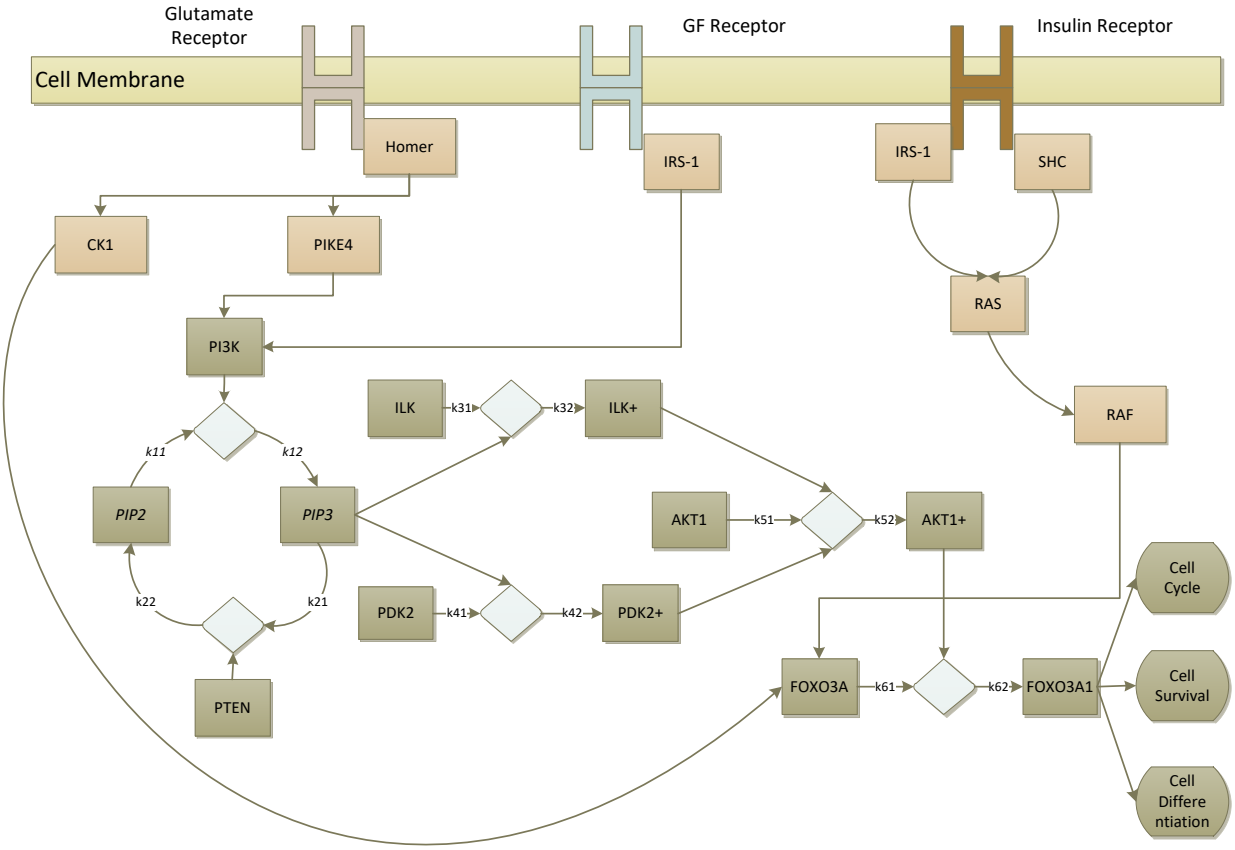
As noted by van der Heide et al, FOXO is a major player in pathways activated by Glutamate and insulin. We will depict that detail later. However the nexus to the insulin activator may also provide a connection to the role that inflammation may have in PCa and especially Type 2 Diabetes and its related hyperglycemia.

FOXO is a key element in the PI3K pathway and has its control facilitated by such elements as PTEN, growth factors, insulin and glutamate. As Essaghir et al state, in the absence of growth factors, FOXO remains in the nucleus and FOXO up-regulates genes which inhibit cell cycle such as p27 KIP1 and p21 WAF1. It also promotes apoptosis via the Fas ligand, Bim and TRAIL, and decreases oxidative stress. As a blocker of cell growth therefore FOXO is often considered as a tumor suppressor. There has been a recent interest in dealing with the FOXO gene directly as a way to control certain cancers as discussed by Yang et al (2010).

One view of the FOXO pathway is shown as follows:



However we can also add the receptors which are drivers of the internal elements. We do that as follows. This shows the multiple ligan responses, with limited detail regarding reactions. We have taken the pathway we have analyzed elsewhere and included it as a core element of the FOXO control mechanism.



FOXO is a facilitator gene, it facilitates homeostasis of the cell. However it is regulated by many genes above it which are often inhibited in their normal functions in a cancer cell.

As Lam et al state:

The PI3K signal transduction pathway critically regulates cell proliferation, differentiation and apoptosis. Perturbation in the PI3K signalling pathway is strongly implicated in the pathogenesis of many diseases, including heart and neural diseases, autoimmune/inflammatory disorders, cancer and the development of chemo- and endocrine-resistance in tumor cells.

Constitutive activation of the PI3K pathway, a hallmark of many cancers, is commonly a consequence of enhanced expression of genes that encode either class I PI3K subunits or PKB (protein kinase B) or is a result of genetic mutations that inhibit negative regulators of the pathway. For example, somatic deletions or mutations of PTEN (phosphatase and tensin homologue deleted on chromosome 10), an antagonist of the PI3K pathway, have been identified in a large proportion (12–60%) of human tumours of different tissue origins.

They continue:

In mammals, the ability of FOXO factors to mediate cell-cycle arrest, DNA repair and apoptosis makes them attractive candidates as tumor suppressors. Loss of FOXO function can lead to uncontrolled cell proliferation. Furthermore, reduced ability to repair damaged DNA due to impaired FOXO activity may also result in genomic instability and carcinogenesis. Finally, a deficiency in FOXO proteins in abnormal and damaged cells that would normally undergo programmed cell death may result in tumor development and expansion.

FOXO transcription factors control cell proliferation and survival by regulating the expression of genes involved in cell-cycle progression [e.g. $p27_{Kip1}$, p130(RB2), cyclin D1/2 and Bcl-6 (B-cell lymphocytic leukemia proto-oncogene 6)] and apoptosis [e.g. Bim, Fas ligand, TRAIL (tumor-necrosis-factor-related apoptosis inducing ligand) and Bcl-X_L. Thus one way by which PKB and the related SGK promote cell survival is by phosphorylating FOXOs, which results in their sequestration in the cytoplasm away from cell death-inducing genes. PKB phosphorylation also reduces the DNA-binding ability of FOXO and enhances its degradation.

Common FOXO target genes that mediate apoptosis include bNIP3 and BCL2L11, which encode the pro-apoptotic Bcl-2 family members, bNIP3 and Bim. Furthermore, FOXOs also indirectly down-regulate the expression of the pro-survival Bcl-2 family member Bcl-X_L by inducing the expression of the transcriptional repressor Bcl-6 . In neurons, FOXO3a triggers cell death circuitously by inducing the expression of Fas Ligand, which triggers programmed cell death through the death receptor pathway.

Thus FOXO control is a strategic part of controlling cell growth and stability.

3.5.12 SOCS1

As Neuwirt et al note:

Suppressor of cytokine signaling (SOCS) proteins play a pivotal role in the development and progression of various cancers.

We have previously shown that SOCS-3 is expressed in prostate cancer, and its expression is inversely correlated with activation of signal transducer and activator of transcription factor 3.

We hypothesized that SOCS-1, if expressed in prostate cancer cells, has a growth-regulatory role in this malignancy.

The presence of both SOCS-1 mRNA and protein was detected in all tested cell lines.

To assess SOCS-1 expression levels in vivo, we analyzed tissue microarrays and found a high percentage of positive cells in both prostate intraepithelial neoplasias and cancers. SOCS-1 expression levels decreased in samples taken from patients undergoing hormonal therapy but increased in specimens from patients who failed therapy. In LNCaP-interleukin-6 prostate cancer cells, SOCS-1 was up-regulated by interleukin-6 and in PC3-AR cells by androgens; such up-regulation was also found to significantly impair cell proliferation.

To corroborate these findings, we used a specific small interfering RNA against SOCS-1 and blocked expression of the protein. Down-regulation of SOCS-1 expression caused a potent growth stimulation of PC3, DU-145, and LNCaP-interleukin-6 cells that was associated with the increased expression levels of cyclins D1 and E as well as cyclin-dependent kinases 2 and 4. In summary, we show that SOCS-1 is expressed in prostate cancer both in vitro and in vivo and acts as a negative growth regulator. Prostate cancer is the second most common cause of tumor-related deaths in the Western world. Although localized tumors can be successfully treated with surgery or radiotherapy, clinically approved therapy for advanced prostate cancer is limited to androgen ablation, blockade of the androgen receptor (AR) or chemotherapy.

Recent modest improvements in chemotherapy have been achieved with the anti-microtubule agent docetaxel …

The role of SOCS-1 and -3 in carcinogenesis is of interest since it was shown by several groups that their expression may be altered in head and neck cancer, gastric carcinoma, chronic myeloid leukemia, melanoma, or prostate cancer.

There is an increasing evidence showing that SOCS have different functions depending on the origin of the tumor. Tannapfel and colleagues have shown that methylation-dependent silencing of the SOCS-1/3 genes in head and neck squamous cell and Barretts adenocarcinoma is associated with tumor growth in vitro and in vivo. On the other hand, it was demonstrated that SOCS-1 is constitutively expressed in patients with chronic myeloid leukemia or in human melanoma.8 Our previous studies revealed that SOCS-3 is increasingly expressed in prostate cancer and can exert inhibitory effects on induction of apoptosis by cAMP.

Other researchers have reported that SOCS-1 can also act as an inhibitor of phosphorylation of STAT In particular, IL-4 and IL-13 stimulate expression of SOCS-1 in keratinocytes, which in turn inhibits phosphorylation of STAT3. The two cytokines receptors were detected in prostate

cells. Furthermore, in breast cancer a N-Myc downstream-regulated gene can induce SOCS-1, which negatively regulates STAT3 activation. Thus, we have asked whether SOCS-1 is expressed in prostate cancer cell lines and patient samples and what impact it has on tumor cell proliferation

3.6 FACILITATORS

There are a wide variety of facilitator genes. We start with the ARF-MDM2-p53 axis.

3.6.1 ARF-MDM2-p53 Axis

Let us now review what is understood about the ARF-MDM2-p53 pathway. This will be necessary before linking this pathway to STAT3 and its functions.

Now this is a classic pathway whose ultimate control mechanism is p53 expression. p53 is generally understood to be a control gene, keeping the cell in some homeostasis and preventing malignancy. As we will not later this may not always be the case but that will not apply to the current discussion.

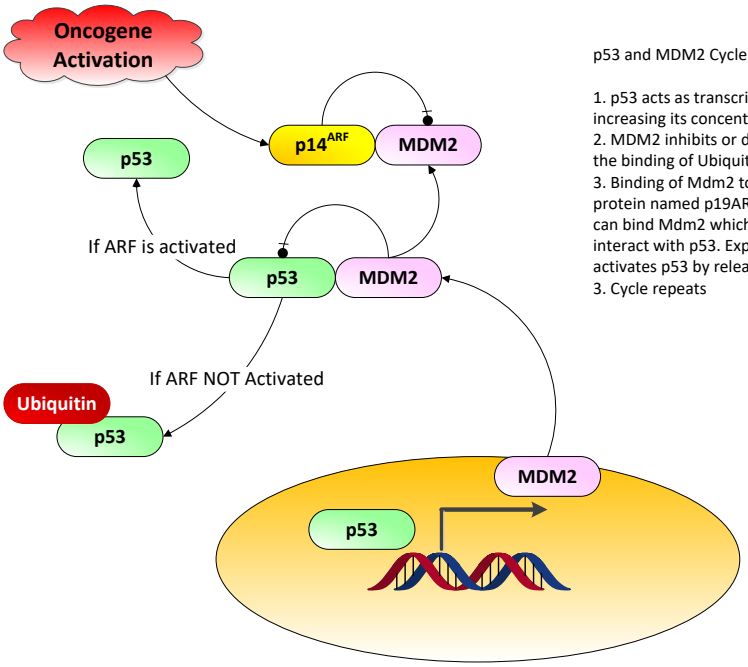
The following Figure depicts the process of the three gene control mechanism. Simply:

1. p53 activates the production of MDM2

2. MDM2 can bind to p53 and result in its dissolution via an Ubiquination

3. ARF can bind to MDM2 and allow the p53 to survive.

4. The process, albeit a bit complex, reaches a steady state for all three proteins.

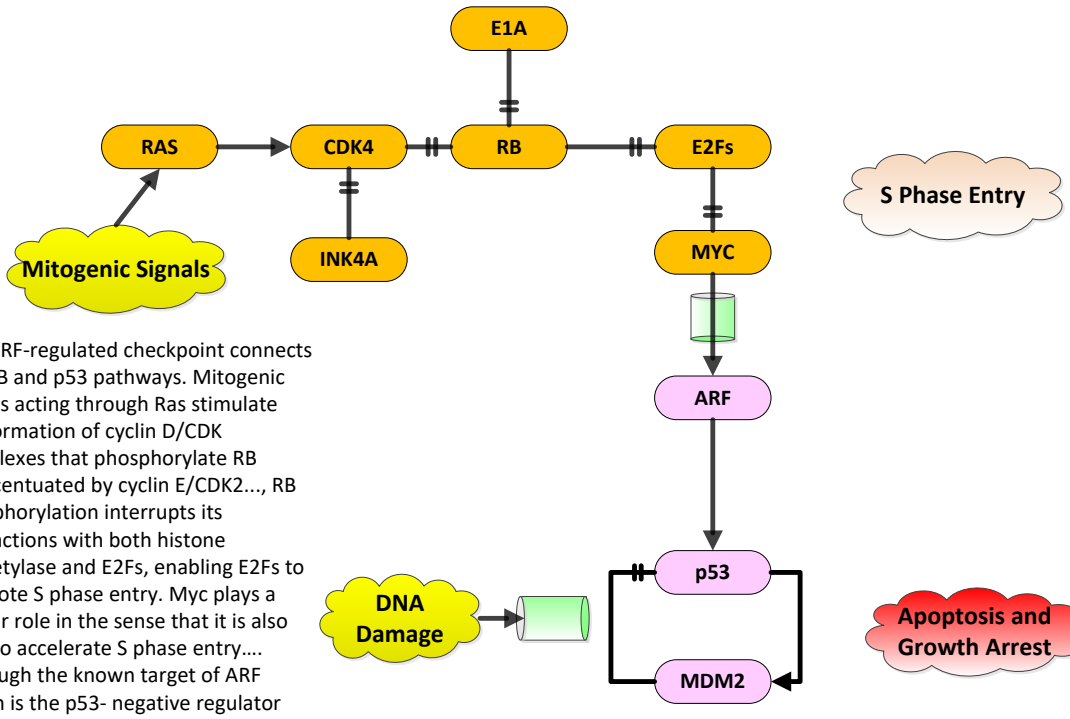


p53 and MDM2 Cycle

1. p53 acts as transcription activator for MDM2 increasing its concentration
2. MDM2 inhibits or decreases p53 by allowing the binding of Ubiquitin
3. Binding of Mdm2 to p53 can be disrupted by a protein named p19ARF (or ARF for short). ARF can bind Mdm2 which renders it unable to interact with p53. Expression of ARF therefore activates p53 by releasing Mdm2.
3. Cycle repeats

From Sherr and Weber (as modified) we have the following details as well shown graphically:

From Sherr and Weber



The ARF-regulated checkpoint connects the RB and p53 pathways. Mitogenic signals acting through Ras stimulate the formation of cyclin D/CDK complexes that phosphorylate RB ….Accentuated by cyclin E/CDK2..., RB phosphorylation interrupts its interactions with both histone deacetylase and E2Fs, enabling E2Fs to promote S phase entry. Myc plays a similar role in the sense that it is also able to accelerate S phase entry…. Although the known target of ARF action is the p53- negative regulator and p53-inducible gene product Mdm2, other targets for ARF action cannot be precluded...

Note in the above we have the cyclic MDM2 and p53 control as well as the cell instigators.

Now Van Maerken, T., et al notes the following regarding the details of this feedback loop:

The p53-MDM2 autoregulatory feedback loop.

(a) The p53 protein induces expression of MDM2, which negatively regulates the stability and activity of p53, providing a means to keep p53 levels and activity low in unstressed cells and to switch off p53 at the end of a stress response.

(b) The p53-mediated expression of MDM2 results from binding of p53 to response elements in the MDM2 gene and subsequent transactivation of MDM2. The domain structure of p53 is shown schematically:

v. TAD, transactivation domain, amino acids;
vi. PRD, proline-rich domain, amino acids; DBD, DNA-binding domain, amino acids;
vii. TD, tetramerization domain, amino acids;
viii. CTD, C-terminal regulatory domain, amino acids.

(c) The p53-inhibitory activity of MDM2 relies on multiple mechanisms. Binding of MDM2 to p53 conceals the TAD and consequently blocks the transcriptional activity of p53. MDM2 also recruits several corepressor proteins to p53, including HDAC1, CTBP2, YY1, and KAP1.

The E3 ubiquitin ligase activity of MDM2 results in ubiquitination of lysine residues in the CTD of p53, preventing acetylation of p53, favoring nuclear export, and promoting proteasomal degradation (see text for details). Some of these lysine residues can also be neddylated by MDM2, resulting in inhibition of the transcriptional activity of p53. Finally, MDM2 may also serve as a p53-specific transcriptional silencer by binding and monoubiquitinating histone proteins in the proximity of p53-responsive promoters. Nd, NEDD8; Ub, ubiquitin. …

They continue the discussion as follows:

The $p14^{ARF}$ protein is predominantly localized to the nucleolus, in which it is stabilized by binding to nucleophosmin within maturing pre-ribosomal particles, pointing to a function in the regulation of ribosome biogenesis.

Nucleophosmin promotes the processing of ribosomal RNA precursors and the nuclear export of ribosomal subunits, whereas overexpression of $p14^{ARF}$ or its murine homolog $p19^{ARF}$ interferes with transcription and processing of ribosomal RNA, impedes nucleocytoplasmic shuttling of nucleophosmin, and inhibits ribosome nuclear export. However, the precise biological function of the nucleophosmin– $p14^{ARF}$ complexes remains a subject of debate. Stress signals trigger the disruption of the interaction between $p14^{ARF}$ and nucleophosmin, and induce translocation of p14ARF to the nucleoplasm.

This redistribution enables $p14^{ARF}$ to interact with p53-bound MDM2 and to antagonize MDM2 function by inhibiting its E3 ubiquitin ligase activity and by blocking nucleocytoplasmic shuttling

of MDM2 and p53, resulting in p53 stabilization. The p53-inhibitory activity of MDM2 may also be neutralized by $p14^{ARF}$ -mediated mobilization of MDM2 into the nucleolus, although this mechanism is not strictly required for the p53-dependent functions of $p14^{ARF}$.

This is clearly a highly complex mechanism. They continue:

Furthermore, the $p14^{ARF}$ protein is capable of inhibiting the activity of another E3 ubiquitin ligase that targets p53 for degradation, ARF-BP1/Mule, and of counteracting the p53-antagonizing NF-kappaB pathway. It should be noted that p14ARF also exerts a potent tumor suppressor activity independently of p53.

Various researchers have tried to model these systems using different techniques. One technique is the use of Petri Nets[15]. From CSML we have a Petri Net models describing the details of such a network and they state[16]:

Proteins p53, MDM2, and $p19^{ARF}$ are proteins closely related to cancer. The protein p53 is a protein which suppresses the formation of tumors, and the protein MDM2 promotes the formation of tumors by decreasing the activity of the protein p53.

Understanding of control mechanism of these proteins connects to development of an effective medicine for suppressing the tumor. It is known that protein p53 works as a transcription factor for many genes and its transcriptional activity is controlled by a complex formed with proteins MDM2 and $p19^{ARF}$.

However, it is still unclear whether protein p53 keeps its transcriptional activity in the form of the trimer with proteins p53, MDM2 and $p19^{ARF}$. … a hybrid functional Petri net (HFPN) model which has been constructed by compiling and interpreting the information of p53-MDM2 interactions... With our HFPN model, we have simulated mutual behaviors between genes p53, MDM2, $p19^{ARF}$, and their products. Through simulation, we discussed whether the complex p53-MDM2-$p19^{ARF}$ has transcriptional activity for genes Bax and MDM2 or not.

It is worth examining these structures, namely the Petri Nets. We leave the examination to the reference. From Moll and Petrenko we have the following result:

Activation of the p53 protein protects the organism against the propagation of cells that carry damaged DNA with potentially oncogenic mutations. MDM2, a p53- specific E3 ubiquitin ligase, is the principal cellular antagonist of p53, acting to limit the p53 growthsuppressive function in unstressed cells. In unstressed cells, MDM2 constantly monoubiquitinates p53 and thus is the critical step in mediating its degradation by nuclear and cytoplasmic proteasomes.

The interaction between p53 and MDM2 is conformation-based and is tightly regulated on multiple levels. Disruption of the p53-MDM2 complex by multiple routes is the pivotal event for

[15] See Reisig

[16] http://www.csml.org/models/csml-models/p53-arf-dependent-stabilization-pathway/

p53 activation, leading to p53 induction and its biological response. Because the p53-MDM2 interaction is structurally and biologically well understood, the design of small lipophilic molecules that disrupt or prevent it has become an important target for cancer therapy.

3.6.2 ARF

As NCBI notes[17]:

This gene generates several transcript variants which differ in their first exons. At least three alternatively spliced variants encoding distinct proteins have been reported, two of which encode structurally related isoforms known to function as inhibitors of CDK4 kinase.

The remaining transcript includes an alternate first exon located 20 Kb upstream of the remainder of the gene; this transcript contains an ***alternate open reading frame (ARF)*** *that* ***specifies a protein which is structurally unrelated to the products of the other variants****.*

This ARF product functions as a stabilizer of the tumor suppressor protein p53 as it can interact with, and sequester, the E3 ubiquitin-protein ligase MDM2, a protein responsible for the degradation of p53*.*

In spite of the structural and functional differences, the CDK inhibitor isoforms and the ARF product encoded by this gene, through the regulatory roles of CDK4 and p53 in cell cycle G1 progression, share a common functionality in cell cycle G1 control. This gene is frequently mutated or deleted in a wide variety of tumors, and is known to be an important tumor suppressor gene.

From Casalou et al:

The ***Adenosine diphosphate-Ribosylation Factor (ARF) family belongs to the RAS superfamily of small GTPases and is involved in a wide variety of physiological processes, such as cell proliferation, motility and differentiation by regulating membrane traffic and associating with the cytoskeleton.***

Like other members of the RAS superfamily, ARF family proteins are activated by Guanine nucleotide Exchange Factors (GEFs) and inactivated by GTPase-Activating Proteins (GAPs). When active, they bind effectors, which mediate downstream functions.

Several studies have reported that cancer cells are able to subvert membrane traffic regulators to enhance migration and invasion.

Indeed, members of the ARF family, including ARF-Like (ARL) proteins have been implicated in tumorigenesis and progression of several types of cancer.

[17] https://www.ncbi.nlm.nih.gov/gene/1029

Here, we review the role of ARF family members, their GEFs/GAPs and effectors in tumorigenesis and cancer progression, highlighting the ones that can have a pro-oncogenic behavior or function as tumor suppressors.

Moreover, we propose possible mechanisms and approaches to target these proteins, toward the development of novel therapeutic strategies to impair tumor progression…

Dysregulation of expression and/or activity of ARF family proteins and/or their effectors, GEFs and GAPs has been associated with enhanced cell migration, invasion and proliferation in several types of cancer. In this section, we review the ARF family members, as well as their activity regulators and effectors that have been found overexpressed in cancer and play essential roles in cancer progression…

ARF1 plays a central role in maintaining the structure and function of the Golgi apparatus and is highly expressed in breast, prostate and ovarian cancers

In the context of cancer, ARF1 has an important function in inter- and intracellular signaling, cell cycle regulation and DNA repair, as well as necrosis and apoptosis. Moreover, ARF1 regulates breast cancer cell adhesion and proliferation, being essential for EGF-mediated phosphorylation of Focal Adhesion Kinase (FAK) and Src.

Furthermore, ARF1 sensitizes MDA-MB-231 breast cancer cells to the anti-tumor drugs actinomycin D and vinblastine through ERK and Akt signaling.

In prostate cancer, ARF1 promotes tumorigenesis by controlling MAPK activation and cell growth.

In myeloma cells, ARF1 expression promotes cell proliferation and inhibits cell adhesion, controlling proliferation- and cell adhesion-mediated drug resistance. Finally, ARF1 is upregulated in ovarian tumors, when compared with adjacent non-cancerous tissues and its overexpression is associated with ovarian cancer cell proliferation and migration through the PhosphoInositide 3-Kinase (PI3K) pathway

Lu et al note:

Androgen receptor (AR) signaling is essential for prostate cancer (PCa) development in humans. The initiation of prostate malignancy and progression to a castration-resistant stage are largely contributed by the modulation of AR activity through its coregulatory proteins.

We and others previously reported that p14 ***alternative reading frame*** *(ARF) expression is positively correlated with the disease progression and severity of PCa. Here, we provide evidence that p14ARF physically interacts with AR and functions as an AR corespressor in both an androgen-dependent and androgen-independent manner.*

Endogenous ARF (p14ARF in human and p19ARF in mouse) and AR colocalize in both human PCa cells in vitro and PCa tissues of mouse and human in vivo.

Overexpression of p14ARF in PCa cells signifiicantly attenuates the activities of androgen response region (ARR2)-probasin and prostate-specifiic antigen (PSA) promoters.

The forced expression of p14ARF in cells resulted in a suppression of PSA and NK transcription factor locus 1 (NKX3.1) expression. Conversely, knockdown of endogenous p14ARF in human PCa cells with short hairpin RNA enhanced AR transactivation activities in a dose-dependent and p53- independent manner. Furthermore, we demonstrated that p14ARF binds to both the N-terminal domain and the ligand-binding domain of AR, and the human double minute 2 (HDM2)-binding motif of p14ARF is required for the interaction of p14ARF and AR proteins. p14ARF perturbs the androgen-induced interaction between the N terminus and C terminus of AR. Most importantly, we observed that the expression of PSA is reversely correlated with p14ARF in human prostate tissues. Taken together, our results reveal a novel function of ARF in modulation of AR transactivation in PCa.

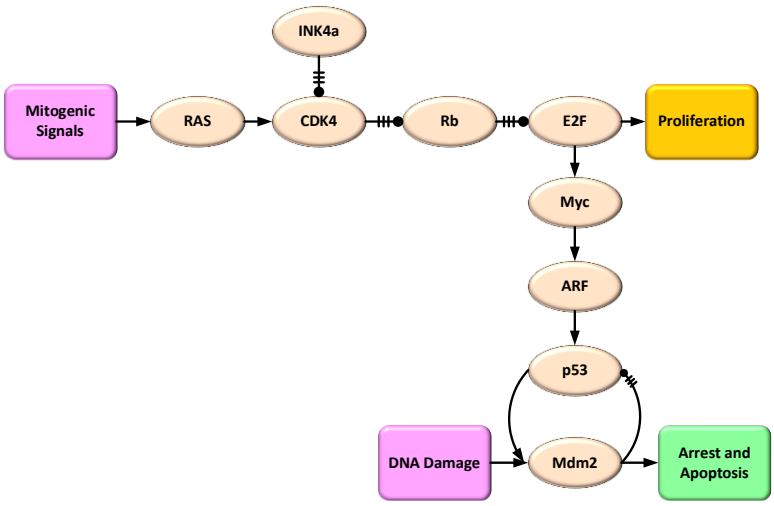
From Sherr we note:

ARF checkpoint control. ARF responds to proliferative signals that are normally required for cell proliferation. When these signals exceed a critical threshold, the ARF-dependent checkpoint (gray vertical barrel) is activated, and ARF triggers a p53-dependent response that induces growth arrest and/ or apoptosis.

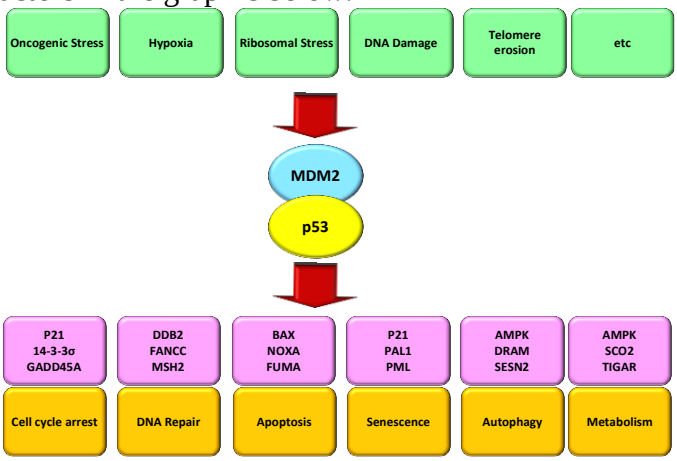
Signals now known to induce signaling via the ARF–p53 pathway include Myc, E1A, and E2F-1. In principle, 'upstream' oncoproteins, such as products of mutated Ras alleles, constitutively activated receptors, or cytoplasmic signal transducing oncoproteins, might also trigger ARF activity via the cyclin D– cdk4–Rb–E2F or Myc-dependent pathways, both of which are normally necessary for Sphase entry. In inhibiting cyclin D-dependent kinases, p16INK4a can dampen the activity of mitogenic signals.

E1A is shown to work, at least in part, by canceling Rb function, although its ability to inhibit p300 contributes to the response by interfering with mdm2 expression. Again for simplicity, Myc and E2F-1 are only shown to activate p53 via ARF. However, highly overexpressed levels of these proteins can activate p53 in ARF-negative cells, albeit with an attenuated efficiency. ARF activation of p53 likely depends on inactivation of some Mdm2-specific function (implied by the unfilled box bracketing the latter two proteins). DNA damage signals (ionizing and UV radiation, hypoxic stress, genotoxic drugs, etc.) access p53 through multiple signaling pathways shown, again for simplicity, as a single DNA damage checkpoint (gray horizontal barrel). Signals through the ARF and DNA damage pathways can synergize in activating p53.

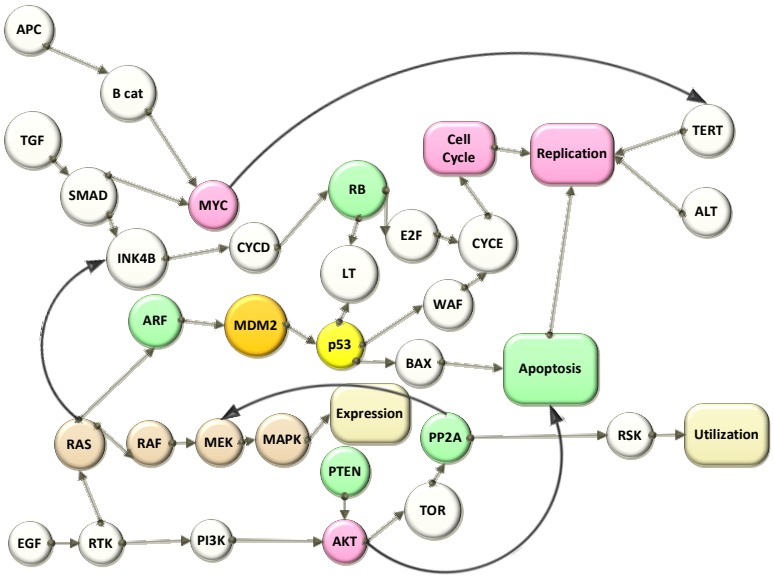
The flow shown below is a critical path involving ARF. Mitogenic signals drive RAS and in turn activates CDK4. RB plays a key role as we show herein as well for cell cycle activation. E2F drives proliferation also MYC and ultimately the MDM2-p53 loop.



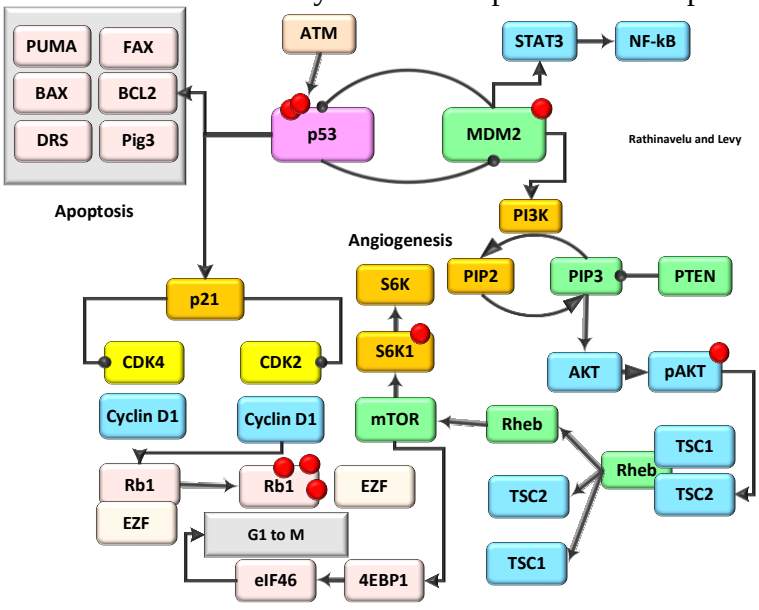
We summarize these factors in the graphic below.



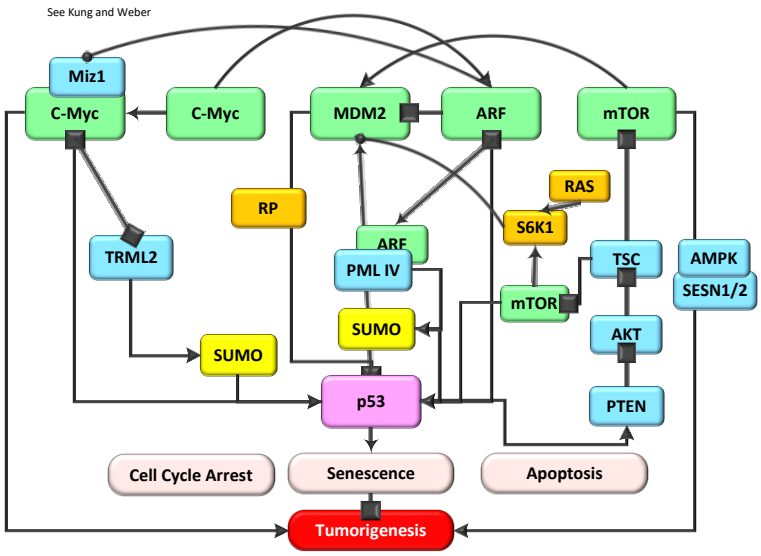
Finally the graphic below is an attempt to details all of the gene flow and control efforts in this process.



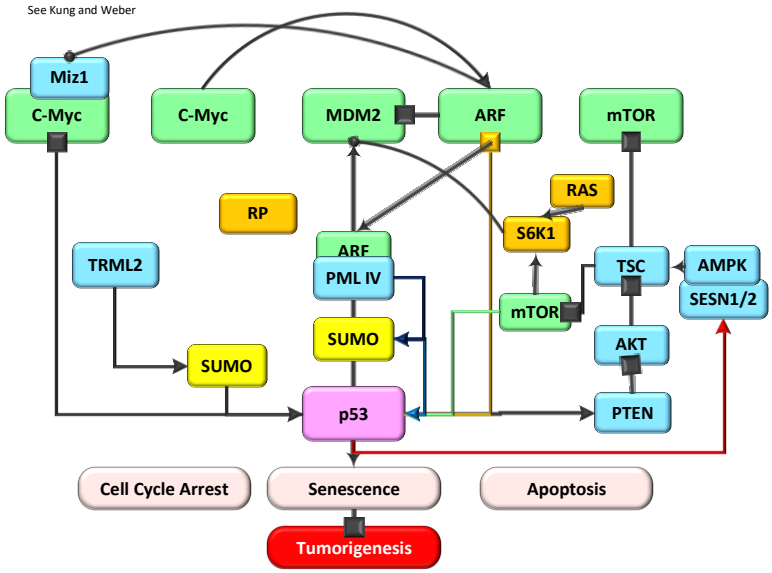
Cell apoptosis is shown below controlled by the MDM2-p53 control loop.



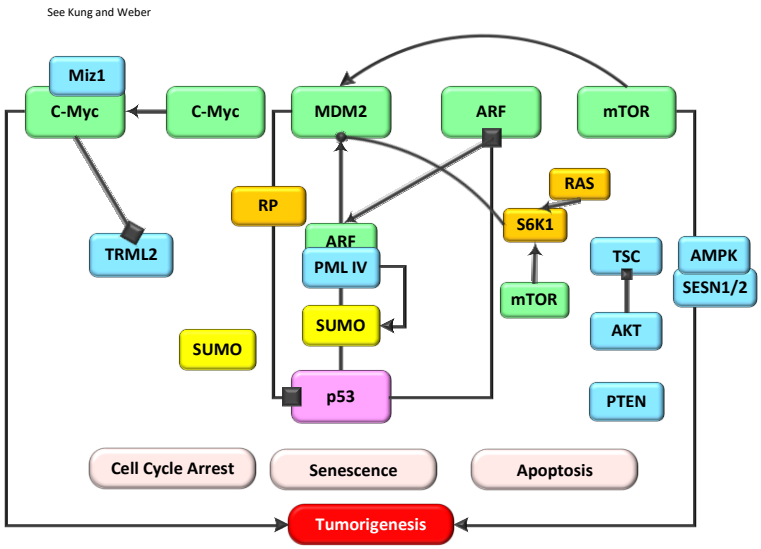
We then show in the following three graphics the flow resulting in tumor generation. The function of ARF is detailed in each of these flows.



Below we have p53 activation and tumor growth blocked.



Finally we show p53 blocked and tumor growth proceeds.

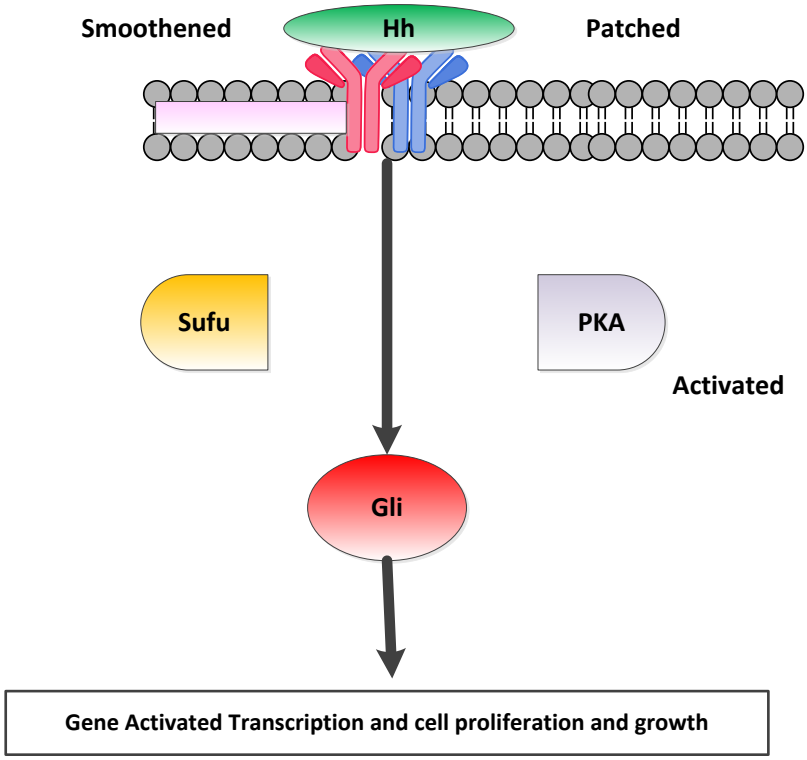


3.6.3 SPOP

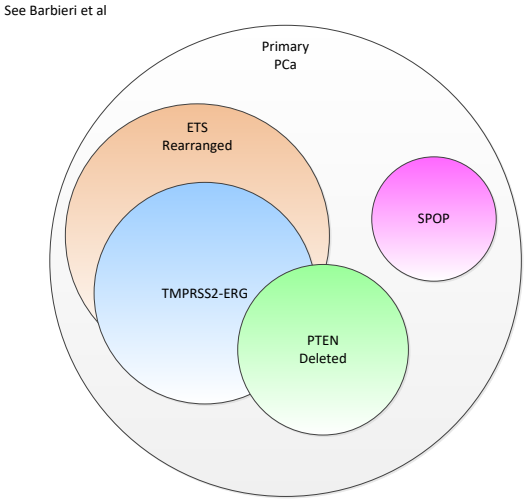
SPOP is part of the Hedgehog signalling pathway[18]. The Hedgehog signalling pathway controls amongst other factors the formation of body segments in insects and in vertebrates the development of the neural tube, limbs and left-right asymmetry. In adult tissues Hedgehog is responsible for homeostasis, equilibrium between cells loss and gain while maintaining total mass and function. With an overactive Hedgehog pathway one sees excess cell proliferation and tumor growth[19]. Thus SPOP has a controlling mechanism for cell replication. Here Hedgehog attaches to Patched and the Patched inhibition of Smoothened is eliminated allowing Smoothened to start a transcription process enabling replication.

[18] http://pid.nci.nih.gov/search/MoleculePage?molid=203488 and http://pid.nci.nih.gov/search/search_landing.shtml?atom_id=208460,208462&what=graphic&jpg=on and pathway at http://pid.nci.nih.gov/search/advanced_landing.shtml?what=graphic&svg=&jpg=true&xml=&biopax=&complex_uses=on&family_uses=on&degree=1&molecule=&pathway=hedgehog&macro_process=&source_id=5&evidence_code=NIL&evidence_code=IAE&evidence_code=IC&evidence_code=IDA&evidence_code=IFC&evidence_code=IGI&evidence_code=IMP&evidence_code=IOS&evidence_code=IPI&evidence_code=RCA&evidence_code=RGE&evidence_code=TAS&output-format=graphic&Submit=Go

[19] See Marks et al p 210-212.



Now upon the activation of Smoothened a set of processes are activated and one product is a protein called the zinc finger transcription factor Gli, which when mutually supported by SPOP allows movement to the nucleus as a transcription factor activating the DNA to transcribe[20]. From Barbieri et al we have the following putative relationships:



The authors argue that SPOP is a separate and significant marker for PCa. The pathway involved is somewhat understood and is a transcription driven pathway initiated by Hedgehog activation

[20] See Pecorino, p. 168-170.

and Patched suppression with Smothered activation. From the NCI pathway databases we have a putative requirement that SPOP is needed to activate GLI for subsequent transcription and cell reproduction.

Specifically Barbieri et al state:

As demonstrated by a subsequent analysis of significantly more genomes, there are only a few truly recurrent non-synonymous mutations in PCa. The most common recurrent non-synonymous mutation in PCa involves SPOP. The SPOP gene encodes for the substrate-recognition component of a Cullin3-based E3-ubiquitin ligase. Mutations in SPOP in PCa were reported originally in two systematic sequencing studies. We have now identified the presence of recurrent mutations in SPOP in 6–13% of human PCas in multiple independent patient cohorts.

Recurrent missense mutations are found exclusively in the structurally defined substrate-binding cleft of SPOP, and structural analysis suggests that these mutations will inactivate SPOP function by disrupting SPOP–substrate interaction.

Further, we found that loss of SPOP function in prostate cell lines resulted in increased invasion and altered gene expression; evidence of this expression signature was identified in primary tumours harbouring SPOP mutation.

Importantly, all SPOP mutations occurred in tumours that were negative for ERG rearrangement; these tumours displayed characteristic somatic copy number aberrations. Taken together, these findings support a distinct molecular class of PCa.

In a recent Nature Medicine article the same authors relate[21]:

Prostate cancer is the second most common cancer in men worldwide and causes over 250,000 deaths each year. Overtreatment of indolent disease also results in significant morbidity.

Common genetic alterations in prostate cancer include losses of NKX3.1 (8p21) and PTEN (10q23), gains of AR (the androgen receptor gene) and fusion of ETS family transcription factor genes with androgen-responsive promoters.

Recurrent somatic base-pair substitutions are believed to be less contributory in prostate tumorigenesis but have not been systematically analyzed in large cohorts. Here, we sequenced the exomes of 112 prostate tumor and normal tissue pairs.

New recurrent mutations were identified in multiple genes, including MED12 and FOXA1. SPOP was the most frequently mutated gene, with mutations involving the SPOP substrate-binding cleft in 6–15% of tumors across multiple independent cohorts.

[21] http://www.nature.com/ng/journal/vaop/ncurrent/full/ng.2279.html

Prostate cancers with mutant SPOP lacked ETS family gene rearrangements and showed a distinct pattern of genomic alterations. Thus, SPOP mutations may define a new molecular subtype of prostate cancer.

This just adds another gene in the mix for PCa. Namely they authors argue that it is a different type. We would still ask the same questions:

1. What is the issue regarding the presence or absence of a CSC stem cell in PCa.

2. When does this mutation occur?

3. What causes the mutation?

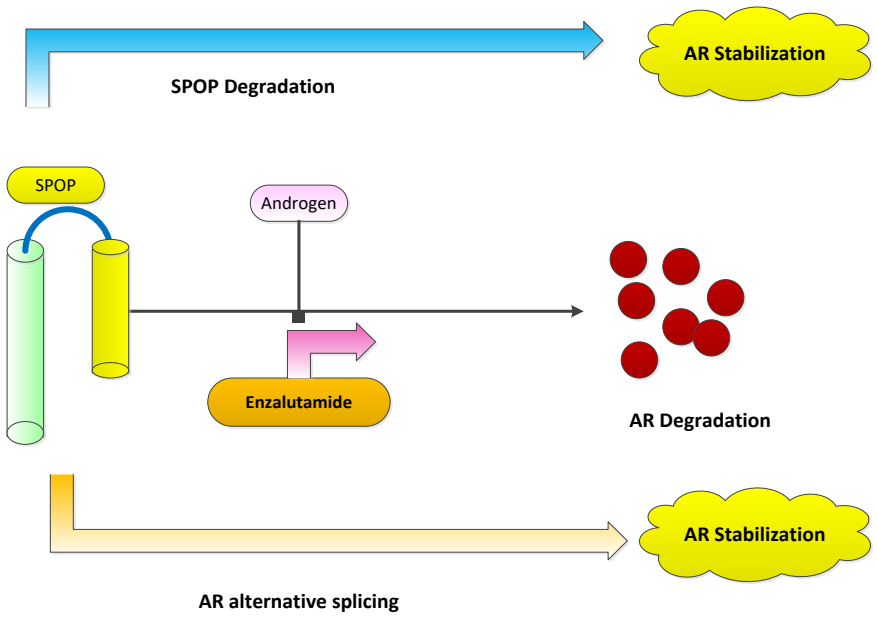
4. SPOP is not a true kinase so what type of blocking would be possible to mitigate the presence of a mutant.

The following also is noted from a Cell Reports article[22]:

The SPOP E3 ubiquitin ligase gene is frequently mutated in human prostate cancers. Here, we demonstrate that SPOP recognizes a Ser/Thr-rich degron in the hinge domain of androgen receptor (AR) and induces degradation of full-length AR and inhibition of AR-mediated gene transcription and prostate cancer cell growth. AR splicing variants, most of which lack the hinge domain, escape SPOP- mediated degradation.

Prostate-cancer-associated mutants of SPOP cannot bind to and promote AR destruction. Furthermore, androgens antagonize SPOP-mediated degradation of AR, whereas anti-androgens promote this process. This study identifies AR as a bona fide substrate of SPOP and elucidates a role of SPOP mutations in prostate cancer, thus implying the importance of this pathway in resistance to antiandrogen therapy of prostate cancer

[22] http://download.cell.com/cell-reports/pdf/PIIS2211124714000308.pdf?intermediate=true



In a discussion of some prior SPOP research it is noted[23]:

… researchers have shed light on a new mechanism by which prostate cancer develops in men. Central to development of nearly all prostate cancer cases are malfunctions in the androgen receptor — the cellular component that binds to male hormones.

The research team has shown that SPOP, a protein that is most frequently mutated in human prostate cancers, is a key regulator of androgen receptor activity that prevents uncontrolled growth of cells in the prostate and thus helps prevent cancer. The findings appear in the journal Cell Reports.

"By uncovering this new and important pathway of androgen receptor destruction, we may one day be able to develop more effective treatments for a substantial proportion of prostate cancer patients who have developed resistance to standard antiandrogen therapy,"

SPOP mutations have been detected in approximately 15 percent of prostate cancer cases. In addition, it has been shown that in about 35 percent of prostate cancers, the SPOP protein is expressed at abnormally low levels. Despite its prevalence in prostate cancer, it was not known whether or how SPOP defects contributed to tumor development. What the research team discovered is that SPOP is an enzyme that selectively destroys androgen receptor protein. Failure to do so due to alterations in SPOP results in overabundance of androgen receptor, a master regulator of prostate cancer cell growth.

The above mentioned Mayo Clinic research team made four major discoveries:

[23] http://www.healthcanal.com/cancers/prostate-cancer/47500-mayo-clinic-identifies-a-key-cellular-pathway-in-prostate-cancer.html

1. *The antiandrogen receptor is a bona fide degradation substrate of SPOP.*
2. *Androgen receptor splicing variants are resistant to SPOP-mediated degradation.*
3. *Prostate cancer-associated SPOP mutants cannot bind to and promote androgen receptor degradation.*
4. *Androgens antagonize, but antiandrogens promote SPOP-mediated degradation of androgen receptor.*

It is noted and well known that the Androgen receptor (AR) is essential for normal prostate cell growth and survival. It is also important for initiation and progression of prostate cancer. Androgen deprivation therapy, including chemical castration and/or antiandrogen therapy, is the mainstay for treating advanced/disseminated prostate cancer. However, tumors almost always reoccur two to three years after initial response and relapse into a disease called castration-resistant prostate cancer. Development of this therapy-resistant symptom is related to a persistent activation of androgen receptor.

As Medical Express states concerning the work on SPOP[24]:

The gene SPOP is mutated in up to 15 percent of all cases of prostate cancer, making it one of the most mutated genes in the disease. However, when the gene is functioning properly, it acts as a tumor suppressor. Despite what's known about SPOP, scientists have not been able to determine exactly how the gene is able to halt the progression of disease.

In a paper published in 2012, a large study analyzed mutations in prostate cancer tumors and found that the SPOP gene was the most frequently mutated among genes identified in this cohort, suggesting that tumors exhibiting a mutation of SPOP could be characterized as a specific subtype of the disease. Further studies found several proteins that interact with SPOP, but this information still failed to explain exactly how SPOP is able to suppress tumors….

The Zhang laboratory began to unravel this mystery by determining if there was a connection between SPOP and senescence. Indeed, they were able to show that SPOP was found in higher concentrations in senescent cells. Next, they compared samples of wild-type (not mutated) SPOP with their mutated counterparts, which were associated with cancer. Wild-type SPOP samples showed senescent behavior, whereas their cancer-associated mutants were impaired in their ability to induce senescence.

In this study, the research team directly linked this behavior of SPOP to an enzyme called SENP7.

The function of SENP7 is not entirely clear, but this study showed just how important it is with regard to SPOP. When SPOP is not mutated, SENP7 remains in check and senescent cells are able to keep cancer activity at bay.

[24] http://medicalxpress.com/news/2015-10-scientists-frequently-mutated-prostate-cancer.html

To test what happens when SPOP is not functioning properly, the researchers inactivated the gene and observed the effect this had on SENP7. They found that the levels of SENP7 increase enough that cells are able to overcome senescence and become cancerous. Notably, when SENP7 activity was inhibited, prostate cancer cells showed senescent behavior and stopped growing, suggesting that SENP7 might be an important therapeutic target.

As Zhu et al note[25]:

The SPOP gene, which encodes an E3 ubiquitin ligase adaptor, is frequently mutated in a number of cancer types.

However, the mechanisms by which SPOP functions as a tumor suppressor remain poorly understood. Here, we show that SPOP promotes senescence, an important tumor suppression mechanism, by targeting the SENP7 deSUMOylase for degradation. SPOP is upregulated during senescence.

This correlates with ubiquitin-mediated degradation of SENP7, which promotes senescence by increasing HP1α sumoylation and the associated epigenetic gene silencing.

Ectopic wild-type SPOP, but not its cancer-associated mutants, drives senescence. Conversely, SPOP knockdown overcomes senescence. These phenotypes correlate with ubiquitination and degradation of SENP7 and HP1α sumoylation, subcellular re-localization, and its associated gene silencing.

From NCBI we note regarding SENP7:

The reversible posttranslational modification of proteins by the addition of small ubiquitin-like SUMO proteins is required for many cellular processes. SUMO-specific proteases, such as SENP7, process SUMO precursors to generate a C-terminal diglycine motif required for the conjugation reaction. They also display isopeptidase activity for deconjugation of SUMO-conjugated substrates.

As we have shown before SUMO and SPOP all play a role in degrading via ubiquination. The degrading process is a part of normal homeostasis. The loss of such functionality is often noted in PCa. However it is not at all clear that these can or should be therapeutic targets.

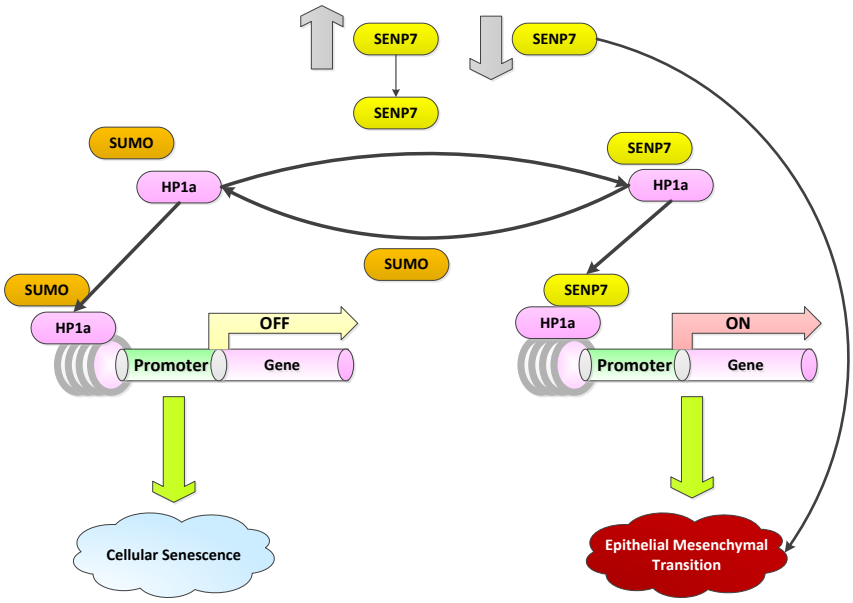
As Bawa-Khalfe et al state:

SENP7L levels dictate PCa cells' choice between senescence and EMT. Onset of cancer in breast epithelia decreases the SENP7S splice variant and increases SENP7L, which expresses an HP1α-interaction motif. Loss of SENP7LHP1α interaction causes HP1α hyper-SUMOylation, an enrichment of HP1α at E2F-responsive and mesenchymal gene promoters, silences transcription of these genes, and elicits cellular senescence. Induction of SENP7Lmaintains hypo-SUMOylated HP1α, which relieves HP1α-mediated repression of proliferation promoting E2F-

[25] http://www.cell.com/cell-reports/abstract/S2211-1247%2815%2901137-7

responsive genes as well as mesenchymal genes. SENP7L decreases epithelial gene expression via an unidentified HP1α-independent pathway, and concurrently with the HP1α-dependent pathway promotes dedifferentiation.

We demonstrate this below:



3.6.4 ARE

As Tan et al note:

In the nucleus, receptor dimers bind to androgen response elements (AREs) in the promoter regions of target genes, such as prostate specific antigen (PSA) and transmembrane protease serine 2 (TMPRSS2), etc, to which they recruit various coregulatory proteins to facilitate transcription, leading to responses such as growth and survival…

The DBD (residues 556–623) is a cysteine-rich region that is highly conserved among steroid hormone receptors. According to the crystal structure of the AR DBD, each DBD monomer has a core composed of two zinc fingers (PDB: 1R4I), each of which consists of four cysteine residues that coordinate a zinc ion. The AR functions as a dimer that, like other steroid receptors, binds to promoter DNA response elements consisting of two equal, common hexameric half-sites (5'-AGAACA-3') separated by a 3 base-pair spacer (IR3). The α-helix of the N-terminal zinc finger (the "recognition helix") interacts directly with nucleotides in the hormone response element in the DNA major groove .

Three amino acid residues at the N terminus of this α-helix, named the P(roximal) box [glycine-serine-valine] (amino acids 577–581; GSCKV), are identical in the PR, GR and MR and are responsible for the specific recognition of the DNA response element. A question that persisted was how steroid receptors achieve target specificity if the AR, PR, GR, and MR bind a common

DNA response element. Studies have identified selective androgen response elements (AREs) (eg, 5'-GGTTCT-3') that allow specific AR activation. AREs have hexameric half-sites in a direct repeat orientation. Structural studies have confirmed that selectivity is achieved by receptor dimerization in a "head-to-head" fashion through the D(istal) box region (amino acid 596–600; ASRND), which allows the AR to bind to direct repeat half-sites in its.

Because the DBD domains are highly conserved among the different steroid receptors, the reason why other steroid receptors do not recognize selective AREs is still a matter of debate. Based on crystallographic data, it was speculated that the AR contains an additional interface that stabilizes the AR dimer/ARE complex. In contrast, the dimerization strength of other steroid receptors would not be sufficient to retain stable binding to selective AREs

From Wilson et al:

Sequence motifs are short, recurring patterns in DNA that can mediate sequence-specific binding for proteins such as transcription factors or DNA modifying enzymes.

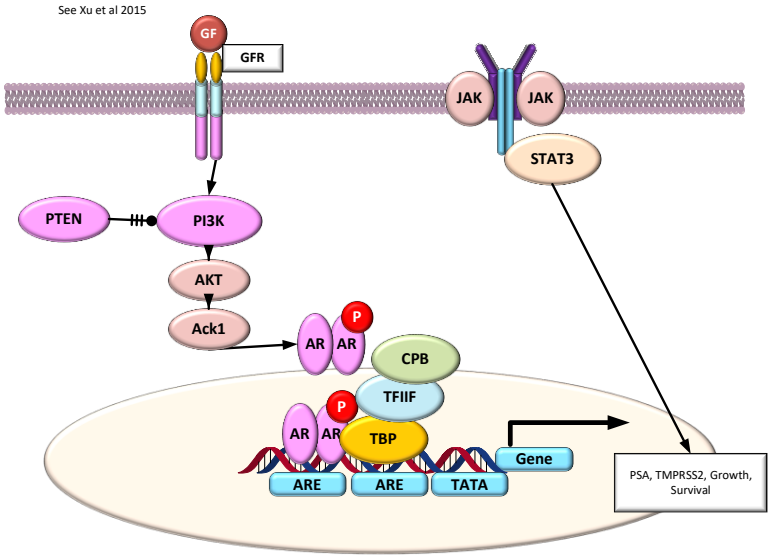
The androgen response element (ARE) is a palindromic, dihexameric motif present in promoters or enhancers of genes targeted by the androgen receptor (AR).

Using chromatin immunoprecipitation sequencing (ChIP-Seq) we refined AR-binding and AREs at a genome-scale in androgen-insensitive and androgen-responsive prostate cancer cell lines. Model-based searches identified more than 120,000 ChIP-Seq motifs allowing for expansion and refinement of the ARE. We classified AREs according to their degeneracy and their transcriptional involvement. Additionally, we quantified ARE utilization in response to somatic copy number amplifications, AR splice-variants, and steroid treatment. Although imperfect AREs make up 99.9% of the motifs, the degree of degeneracy correlates negatively with validated transcriptional outcome.

Weaker AREs, particularly ARE half sites, benefit from neighboring motifs or cooperating transcription factors in regulating gene expression. Taken together, ARE full sites generate a reliable transcriptional outcome in AR positive cells, despite their low genome-wide abundance. In contrast, the transcriptional influence of ARE half sites can be modulated by cooperating factors….

AREs are well studied but poorly defined and have been shown to contain two hexamers with a three base-pair spacer with an inverted repeat in the second hexamer. The sequence elements similar to this canonical ARE have been identified in some ChIP-Seq studies, whereas half AREs or tandem repeats of two hexamers were also found in other ChIP-Seq or ChIP-on-chip studies. In the past, studies revealed binding motifs adjacent to the AR binding sites but belonging to other transcription factor families such as the forkhead box A1 protein (FOXA1, GeneBank: 3169). Cooperative interactions facilitate chromatin binding of the AR and contribute to a promiscuous behavior of AREs23,24,25. AREs and adjacent transcription binding motifs have been well described in LNCaP cells but remain to be defined in CWR22Rv1 cells.

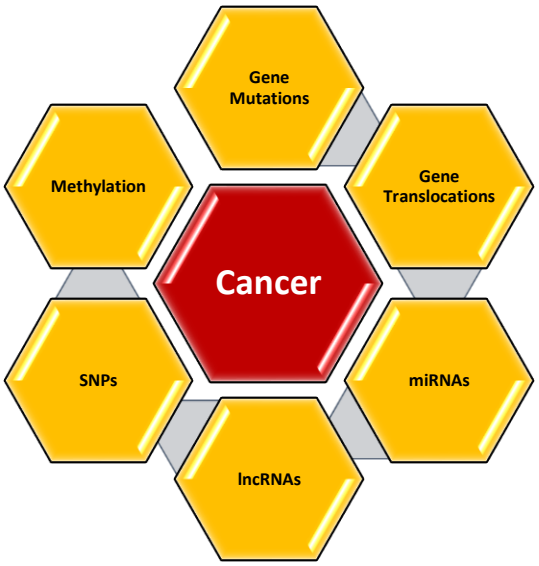
Therefore, the purpose of our AR ChIP-Seq study is to further characterize the ARE and identify cooperation with adjacent transcription binding motifs in androgen-responsive and androgen-insensitive prostate cancer cell lines.



3.7 EPIGENETICS

There seems to be a continuous flow of genes, miRNAs, epigenetic factors including methylation, SNPs and the like all both diagnostic and prognostic for various cancers. A decade or more ago one looked for a gene, some gene that somehow got broken, changed, deleted, or the like. The paradigm was the Philadelphia chromosome of a cut and paste example. With the understanding we now have of methylation we see the same occur here, and methylation can be acquired and/or genetically inherited (see imprinting examples). However methylation is still somewhat poorly understood; what causes it, why does it work positively in some cases and negatively in others?

Methylation is but one of the many facets of what we now see as causes of Cancer. We depict a short summary below.



We examine the work of Wojno et al which has received recent interest. They examine the impacts of methylation upon 3 genes and see their presence as prognostic of potential aggressive prostate cancer. Specifically they conclude:

The diagnosis of prostate cancer is dependent on histologic confirmation in biopsy core tissues. The biopsy procedure is invasive, puts the patient at risk for complications, and is subject to significant sampling errors.

An epigenetic test that uses methylation-specific polymerase chain reaction to determine the epigenetic status of the prostate cancer–associated genes GSTP1, APC, and RASSF1 has been clinically validated and is used in clinical practice to increase the negative predictive value in men with no history of prostate cancer compared with standard histopathology. Such information can help to avoid unnecessary repeat biopsies.

The repeat biopsy rate may provide preliminary clinical utility evidence in relation to this assay's potential impact on the number of unnecessary repeat prostate biopsies performed in US urology practices.

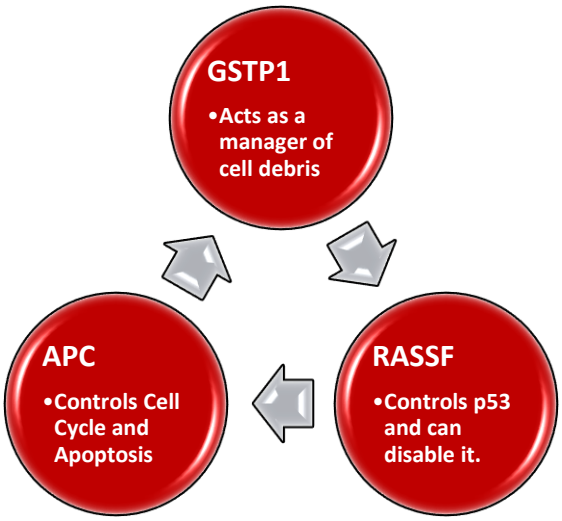
DNA methylation normally can result in the silencing of genes by interrupting the normal process of promoters. CpG islands are often hypermethylated and thus the gene which may regulate cell proliferation is silenced. This may result in uncontrolled cell growth. For example genes controlling MYC are not produced and MYC may then result in excess cell cycle proliferation. Methylation is hypermethylated in the regions of intergenic regions and in repetitive elements and this hypermethylation silences these regions and facilitates normal cell DNA transcription of the gene. Disruption of DNA, namely hypomethylation, in the intergenic and repetitive regions may result in possible loss of imprinting. This hypomethylation is also related to the production of lncRNAs which may in turn interfere with normal gene transcription.

Decitabine is a DNMT inhibitor. Namely, it inhibits the DNA methyltrasferases that facilitate methylation (such as DNMT3 which are de novo and DNMT1 which is maintenance).

Decitabine thus has then tendency on the specific hematologic cell lines in MDS to remove methylations which have caused the aberrant cell line proliferations and allow for the return of homeostasis. MDS is a quasi-malignant condition originating in the bone marrow which may in many cases result in Acute Myelogenous Leukemia. With the use of decitabine or a similar DNMTI azacitidine, demethylation of these rapidly reproducing cells may be achieved and possible a normal state of homeostasis achieved.

The use of pharmaceuticals that alter the methylation patterns of DNA can have lasting effects because those patterns may last through subsequent mitotic changes. On the one hand that may be beneficial as is the case with MDS but such broad demethylation may also alter other segments of the DNA altering essential control elements and pathways. In cell development there are two sensitive periods; germ cell development and early embryonic development. It is during these periods that methylation is cleared and reset and that a drug-like a DNMTI would pose a serious risk to the proper resetting of the marks and could result in substantial DNA expression damage.

In summary we will examine the three gene methylation proposition with this test. We summarize this below:



Let us examine what the study presents in a bit more detail. Basically it does the following:

1. It examines three gene products; *GSTP1, APC, and RASSF1*

2. It determines if the genes are methylated so that the gene products are suppressed.

3. If that is the case after a first biopsy which is deemed normal, then a second biopsy is mandated due to the high incidence of a positive result on the second biopsy for PCa.

Specifically from the paper by Partin et al on the same topic the authors' state:

The DOCUMENT multicenter trial in the United States validated the performance of an epigenetic test as an independent predictor of prostate cancer risk to guide decision making for repeat biopsy. Confirming an increased negative predictive value could help avoid unnecessary repeat biopsies. We evaluated the archived, cancer negative prostate biopsy core tissue samples of 350 subjects from a total of 5 urological centers in the United States. All subjects underwent repeat biopsy within 24 months with a negative (controls) or positive (cases) histopathological result. Centralized blinded pathology evaluation of the 2 biopsy series was performed in all available subjects from each site.

Biopsies were epigenetically profiled for GSTP1, APC and RASSF1 relative to the ACTB reference gene using quantitative methylation specific polymerase chain reaction. Predetermined analytical marker cutoffs were used to determine assay performance. Multivariate logistic regression was used to evaluate all risk factors.

The epigenetic assay resulted in a negative predictive value of 88% (95% CI 85-91). In multivariate models correcting for age, prostate specific antigen, digital rectal examination, first biopsy histopathological characteristics and race the test proved to be the most significant independent predictor of patient outcome.

The DOCUMENT study validated that the epigenetic assay was a significant, independent predictor of prostate cancer detection in a repeat biopsy collected an average of 13 months after an initial negative result. Due to its 88% negative predictive value adding this epigenetic assay to other known risk factors may help decrease unnecessary repeat prostate biopsies.

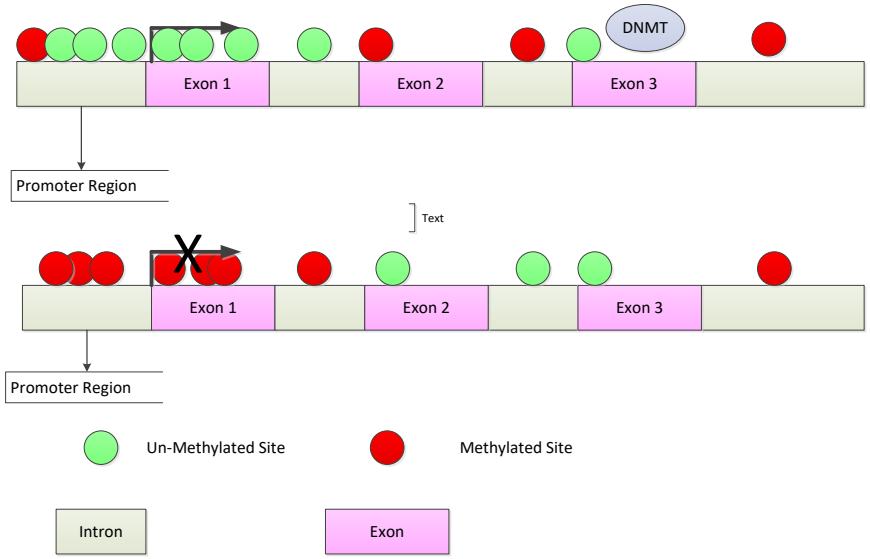
Recall that the *negative predictive value* or NPV is defined as:

$$NPV = \frac{\text{Number True Negatives}}{\text{Number True Negatives + Number False Negatives}}$$

Thus an NPV of 88% for the sample size used implies that it is fairly high in predicting True Negatives a priori but may still miss a percentage. There of course is the issue of the pathologist missing the PCa as well. This could be done by a sampling deficiency or confusion on a reading. It is not clear if for example a HGPIN is read.

Thus focusing on methylated genes, specifically just 3 of them, GSTP1, APC and RASSF1, they were able in a small sample to ascertain that there would be no need of a second biopsy if they were found to be unmethylated in the first.

Recall the effects of methylation as we show below:



From an article in Medical Express[26] as well as from an article in Eureka[27] as well as from an article in Science Codex[28] we have the following summary:

More than one million prostate biopsies are performed each year in the U.S. alone, including many repeat biopsies for fear of cancer missed. Therefore there is a need to develop diagnostic tests that will help avoid unnecessary repeat biopsies. Two independent trials have now validated the performance of an epigenetic test that could provide physicians with a better tool to help eliminate unnecessary repeat prostate biopsies, report investigators in The Journal of Urology.

In the previously reported independent MATLOC (Methylation Analysis To Locate Occult Cancer) trial, a multiplex epigenetic assay (ConfirmMDx for Prostate Cancer) profiling the APC, GSTP1 and RASSF1 genes demonstrated a negative predictive value of 90%. GSTP1 methylation is a specific biomarker for (prostate) cancer and this gene is methylated in up to

[26] http://medicalxpress.com/news/2014-07-accurate-epigenetic-unnecessary-biopsies-prostate.html

[27] http://www.eurekalert.org/pub_releases/2014-07/ehs-nae072114.php

[28] http://www.sciencecodex.com/new_accurate_epigenetic_test_could_eliminate_unnecessary_repeat_biopsies_for_prostate_cancer-137932

90% of prostate cancer cases. Additionally, APC and RASSF1 are important field effect markers and increase the diagnostic sensitivity of the assay.

A second multicenter study, DOCUMENT (Detection Of Cancer Using Methylated Events in Negative Tissue), has validated the performance of the epigenetic assay used in the MATLOC trial as an independent predictor of prostate cancer risk to guide decision making for repeat biopsy. In the DOCUMENT study patients with a negative biopsy were evaluated to identify those at low risk for harboring cancer missed, through biopsy sampling error, who could forego an unnecessary repeat biopsy. The validation study resulted in a negative predictive value of 88%.

"This epigenetic assay is a significant, independent predictor and has been shown to be the most valuable diagnostic aid of all evaluated risk factors in two independent trials," comments Alan W. Partin, MD, PhD, of the James Buchanan Brady Urological Institute, The Johns Hopkins University School of Medicine, Baltimore, Maryland. "Negative findings of this assay could be used to reduce concern over unsampled cancer and effectively avoid unnecessary repeat biopsies."

A total of 350 patients were enrolled in the DOCUMENT trial from five geographically dispersed medical centers: Cleveland Clinic, Eastern Virginia Medical School, Lahey Hospital & Medical Center, Johns Hopkins University, and University of California Los Angeles. Patients were grouped into those with two consecutive negative biopsies (controls) and those with a negative biopsy followed by a positive biopsy within 24 months. The initial archived, negative for cancer, prostate biopsy core tissue samples were evaluated. All of the men underwent a repeat biopsy on average one year after the initial biopsy.

Only biopsies with a minimum of eight cores per biopsy, collected no earlier than 2007, were included in the study, while initial biopsies with atypical cells suspicious for cancer, i.e. atypical small acinar proliferation by the sites' pathologists, were excluded, since this would have triggered a repeat biopsy based upon histopathology alone.

After correcting for age, prostate specific antigen (PSA), digital rectal exam, histopathological characteristics of the first biopsy, and race, this epigenetic test proved to be the most significant, independent, and strongest predictor of patient outcome with an odds ratio of 2.69 as well as the most valuable diagnostic aid of all evaluated risk factors. The slightly decreased sensitivity of the DOCUMENT trial compared to the MATLOC trial is most likely associated with a higher PSA screening prevalence in the DOCUMENT cohort.

It is important to note the following:

1. The genes selected have been studied for over two decades and especially as regards to their hypermethylation status.

2. The test is an early prognostic test which when combined with prostate biopsy data, especially a benign reading on the prostate biopsy.

3. The test has reasonable statistics given its small sample size but as we shall see there is substantial variability in these tests.

3.7.1 GSTP1

We start with a general cellular household maintenance gene, GSTP1. GSTP1 is a gene whose protein is involved in general housekeeping efforts in a cell. As Laborde states:

Glutathione transferases (GSTs) are enzymes that catalyze the conjugation of glutathione (GSH) to a variety of electrophilic substances. Their best known role is as cell housekeepers engaged in the detoxification of xenobiotics. Recently, GSTs have also been shown to act as modulators of signal transduction pathways that control cell proliferation and cell death. Their involvement in cancer cell growth and differentiation, and in the development of resistance to anticancer agents, has made them attractive drug targets.

This review is focused on the inhibition of GSTs, in particular GSTP1-1, as a potential therapeutic approach for the treatment of cancer and other diseases associated with aberrant cell proliferation.

GSTP1 seems to have a beneficial capability in cells and thus if it is no longer functioning there is an accumulation of toxic elements in the cells which can thus result in cell degradation.

From NCBI we have the following descriptive[29]:

Glutathione S-transferases (GSTs) are a family of enzymes that play an important role in detoxification by catalyzing the conjugation of many hydrophobic and electrophilic compounds with reduced glutathione. Based on their biochemical, immunologic, and structural properties, the soluble GSTs are categorized into 4 main classes: alpha, mu, pi, and theta. This GST family member is a polymorphic gene encoding active, functionally different GSTP1 variant proteins that are thought to function in xenobiotic metabolism and play a role in susceptibility to cancer, and other diseases.

The above is a reasonable duplication of what we generally find in the literature. Specifics as to what and how it functions are contained in the referenced literature, Now from Townsend et al we find the following[30]:

Glutathione S-transferase Pi 1 (GSTP1) *belongs to a family of phase II detoxification enzymes that catalyze the conjugation of glutathione (GSH) and electrophilic compounds, resulting in the detoxification of electrophiles. A multitude of studies in vitro and in vivo have shown that GSTP is important in xenobiotic detoxification, and its overexpression contributes to the drug-resistant phenotype. GSTP1/GSTP2-null mice have an increased susceptibility to skin tumorigenesis*

[29] http://www.ncbi.nlm.nih.gov/gene/2950

[30] http://www.signaling-gateway.org/molecule/query?afcsid=A002956

induced by chemical carcinogen. GSTP has been shown to form protein–protein interactions with several proteins and to act as an endogenous negative regulator. Among the ligand-binding partners identified so far are c-Jun N-terminal kinase (JNK), tumor necrosis factor (TNF)-receptor-associated factor 2 (TRAF2) and peroxiredoxin-1.

JNK is a mitogen-activated protein (MAP) kinase that has a pivotal role in cell survival and death pathways. Dissociation of GSTP from JNK1 in vitro and in GSTP-deficient mice shows activation of JNK activity. GSTP also has a pivotal regulatory role in the TNF-α-induced signaling cascade through protein–protein interactions with TRAF2. Dissociation of GSTP from TRAF2 leads to activation of the apoptosis signal-regulating kinase (ASK1) pathway. The protein–protein interactions with peroxiredoxin-1 mediate the S-glutathionylation of its active site cysteine, leading to enzyme activation.

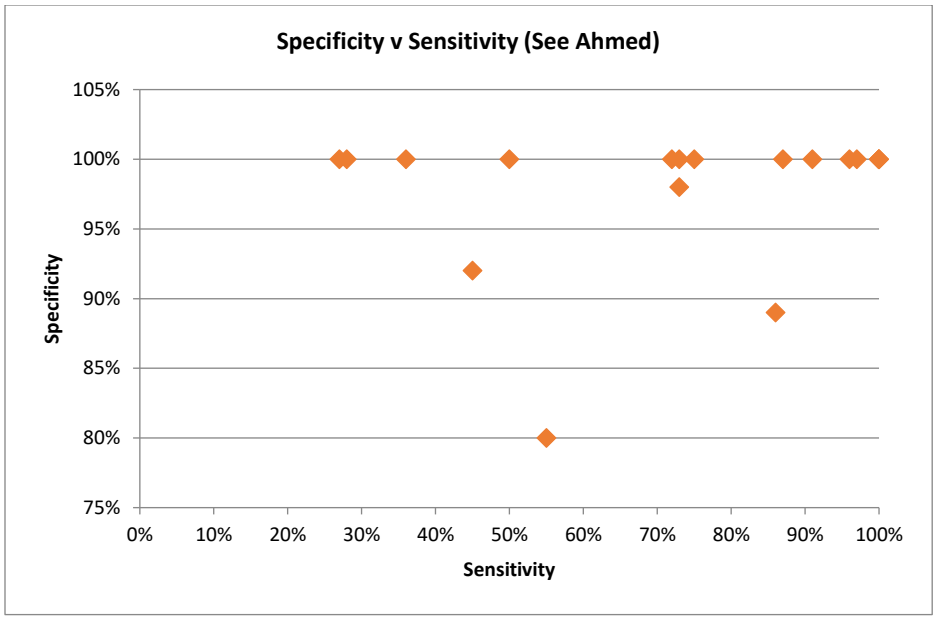
Aberrant expression of GSTP has been linked with tumor development and resistance to cancer drugs. The recent understanding of the dual functionality of GSTs has shed light on the initial confusion arising from the fact that not all drugs used to select for resistance were substrates for thioether bond formation.

This yields some detail on its functioning. It does not appear to have significant pathway functioning.

In a review by Ahmed in 2010 the author details a considerable amount of research regarding methylation and several of the genes discussed in the initial work. Specifically he presents an interesting set of tables on GTSP1. This result follows as has been modified:

Gene/Gene cohort	Specimen	Sensitivity %	Specificity %
GSTP1	Biopsy	91	100
GSTP1	Biopsy	73	100
GSTP1	Biopsy	75	100
GSTP1, RARβ2, APC, TIG1	Biopsy	97	100
GSTP1	Biopsy washing	100	100
GSTP1	ejaculate	44	NA
GSTP1	ejaculate	50	100
GSTP1	Serum	72	100
GSTP1, PTGS2, TIG1	Serum	45	92
GSTP1, RASSF1, RARβ2	Serum	28	100
GSTP1	Urine	27	100
GSTP1	Urine post massage	36	100
GSTP1	Urine post massage	73	98
GSTP1	Urine post biopsy	39	NA
GSTP1, APC, EDNRB	Urine post biopsy	71	NA
GSTP1, INK4α, ARF, MGMT	Urine	87	100
GSTP1, INK4α, ARF, MGMT, RARβ2, TIMP3, CDH1, RASSF1A, APC	Urine	100	100
GSTP1, RARβ2, APC, RASSF1A	Urine post massage	86	89
GSTP1, RASSF1A, ECDH1, APC, DAPK, MGMT, p14, p16, RARb2, TIMP3	Urine post massage	93	NA
GSTP1, RARβ2, APC	Urine	55	80
GSTP1, gal3	Biopsy	96	100
GSTP1, gal3	Serum	100	100
GSTP1, gal3	Urine	100	ND

We summarize this in the Figure below:



Thus there is a rich body of literature on these genes and PCa. In fact the papers by Brooks et al in 1998 and by Wang et al in 2001 are already focusing on GSTP1 and its methylation as an association with PCa. Therefore this provides a strong basis for using this gene and its logic as a cell maintenance product also has substantial merit.

3.7.2 RASSF1

RASSF1 is an effector gene and it drives other genes that control cell growth, proliferation and apoptosis. Richter et al have recently presented and excellent summary of RASFF and their involvement in many cancers. As noted above, the Ahmed summary on GTPS1 did include RASSF1 as well. Thus RASSF1 has been recognized as a major player in many cancers. Now methylation of that gene can result in lack of its expression and it is this suppression and its sequellae which are of import.

As Richter et al state:

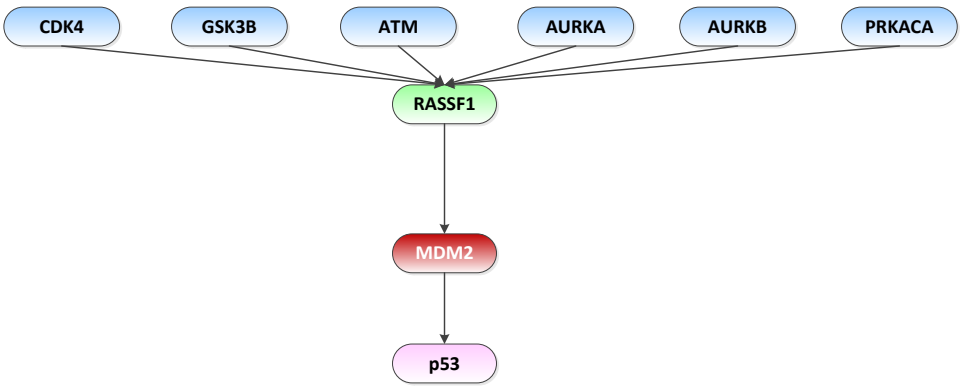
Since methylation of the RASSF1A promoter is described as an early and frequent event in tumorigenesis, RASSF1A could serve as a useful diagnostic marker in cancer screens. RASSFs are implicated in various cellular mechanisms including apoptosis, cell cycle control and microtubule stabilization, though little is known about the underlying mechanisms. Tumor suppressing functions were reported for several members. Here we review the current literature on RASSF members focusing on structural, functional and epigenetic aspects. Characterizing the cellular mechanisms that regulate the signaling pathways RASSFs are involved in, could lead to a deeper understanding of tumor development and, furthermore, to new strategies in cancer treatment.

From NCBI[31]:

This gene encodes a protein similar to the RAS effector proteins. Loss or altered expression of this gene has been associated with the pathogenesis of a variety of cancers, which suggests the tumor suppressor function of this gene. The inactivation of this gene was found to be correlated with the hypermethylation of its CpG-island promoter region. The encoded protein was found to interact with DNA repair protein XPA. The protein was also shown to inhibit the accumulation of cyclin D1, and thus induce cell cycle arrest. Several alternatively spliced transcript variants of this gene encoding distinct isoforms have been reported.

Now RASSF1 is activated by a wide collection of genes but what is important is that it activates MDM2. MDM2 is an analog of MDM4 but it also activates p53.

[31] http://www.ncbi.nlm.nih.gov/gene/11186



Richter et al also state:

The RASSF1 gene, which is located on the small arm of chromosome 3 (locus 3p21.3) codes for eight exons (1α, 1β, 2αβ, 2γ, 3, 4, 5 and 6). There are seven different RASSF1 isoforms (RASSF1A to RASSF1G) that are generated by differential usage of two promoters (distance 3.5 kb) and through alternative splicing. So far however, the biological relevance of only two isoforms, RASSF1A and RASSF1C, was demonstrated. Regarding the transcripts RASSF1B and RASSF1E there is currently not enough evidence to support a biological role, as well as for the candidates RASSF1F and RASSF1G that possibly enter the nonsense-mediated mRNA decay. The two main variants are RASSF1A and RASSF1C containing a RA domain, SARAH domain and ATM domain, whereas the C1 domain can only be found in RASSF1A.

The isoform A is being transcribed from the upstream promoter and isoform C from the downstream promoter and both promoters are located within CpG-islands. However only the upstream promoter is often hypermethylated in various tumor entities.

We briefly examine the sequellae to RASSF1 in a cell; MDM2 and p53.

3.7.3 MDM2

Now as Mancini et al state:

MDM4, formerly named MDMX, is an inhibitor of p53 with in vitro and in vivo oncogenic potential. The relevance of MDM4 regulation of p53 has been established by the Mdm4 knockout (KO) mice.

These animals show embryonic lethality, but have a normal development when simultaneously deleted for Trp53 gene.

Different models have been proposed to explain the activity of MDM4 towards p53, particularly to distinguish MDM4 from its analogue MDM2, the best characterized negative regulator of p53.

As the most evident phenotype of Mdm4-KO mice is a generalized cell cycle arrest, MDM4 has been considered as a negative regulator of p53 growth arresting function. Conversely, the

control of p53-apoptotic function has been attributed to MDM2 because of the presence of early embryonic cell death in Mdm2-KO mice. This model, therefore, attributes the control of distinct activities of p53 to different proteins.

In contrast to this, a second model is based on the evidence that MDM4 and MDM2 efficiently associate and regulate each other's function. It has been proposed that the interdependence of the two MDM proteins is the basis for the negative non-overlapping regulation of p53. The presence of apoptosis in Mdm4-KO mice in neuronal progenitors, an increased transcription of some p53 targets genes, have raised a third hypothesis: MDM4 controls the transcriptional function of p53, whereas MDM2 controls its protein levels. All these models apply mainly to the regulation of p53 in unstressed conditions and/or during the mouse development, although some data also extend them to stressing situations.

MDM4, also called Mdm4 p53 binding protein homolog, is located at 1q32. From NCI we have[32]:

This gene encodes a nuclear protein that contains a p53 binding domain at the N-terminus and a RING finger domain at the C-terminus, and shows structural similarity to p53-binding protein MDM2. Both proteins bind the p53 tumor suppressor protein and inhibit its activity, and have been shown to be overexpressed in a variety of human cancers. However, unlike MDM2 which degrades p53, this protein inhibits p53 by binding its transcriptional activation domain. This protein also interacts with MDM2 protein via the RING finger domain, and inhibits the latter's degradation. So this protein can reverse MDM2-targeted degradation of p53, while maintaining suppression of p53 transactivation and apoptotic functions.

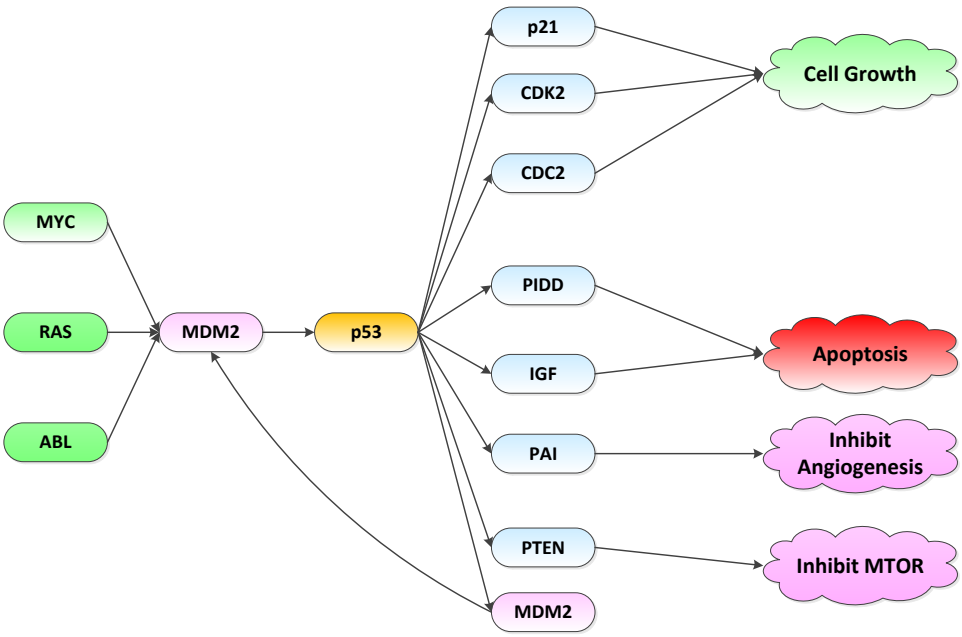
p53 data is extensive[33]. The p53 pathway can be developed based upon the detailed NCI data[34] or from the KEGG genome database we have shown below in a modified form[35]:

[32] http://www.ncbi.nlm.nih.gov/gene/4194

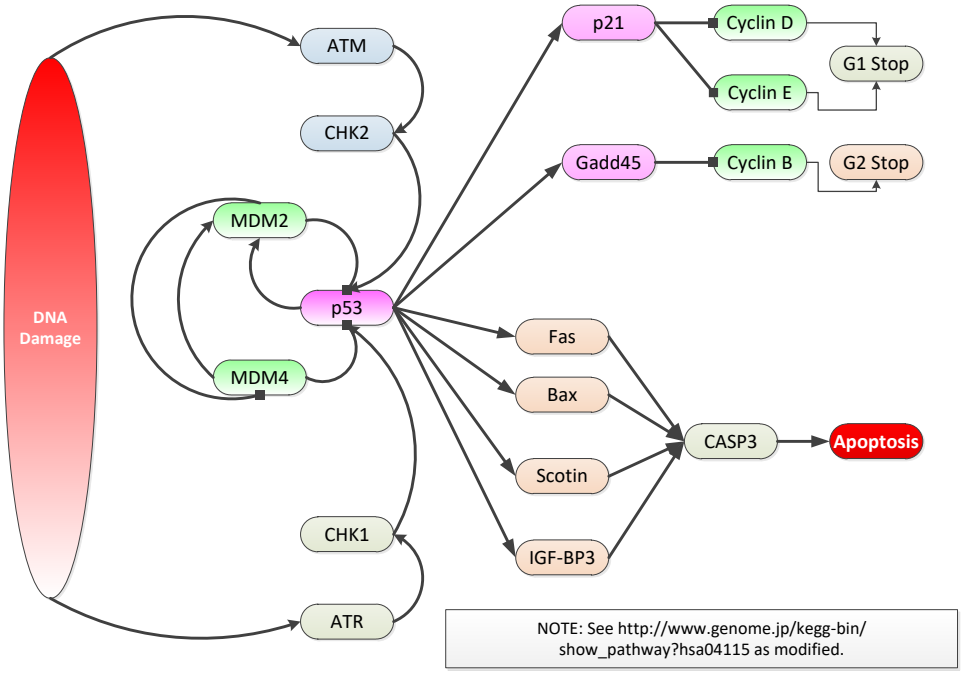
[33] http://www.mmmp.org/MMMP/public/biomap/searchBiomap.mmmp

[34] http://pid.nci.nih.gov/search/pathway_landing.shtml?pathway_id=200207&source=NATURE&genes_a=4194&genes_b=&what=graphic&jpg=on&ppage=1

[35] http://www.genome.jp/kegg-bin/show_pathway?hsa04115



We demonstrate the interactions below in a slightly different manner focusing on MDM2. Note MDM2 and MDM4 interact and it is through this that we have control over p53. Thus any blockage of p53 is a loss of apoptotic capability as well as loss of G1 and G2 stoppage.



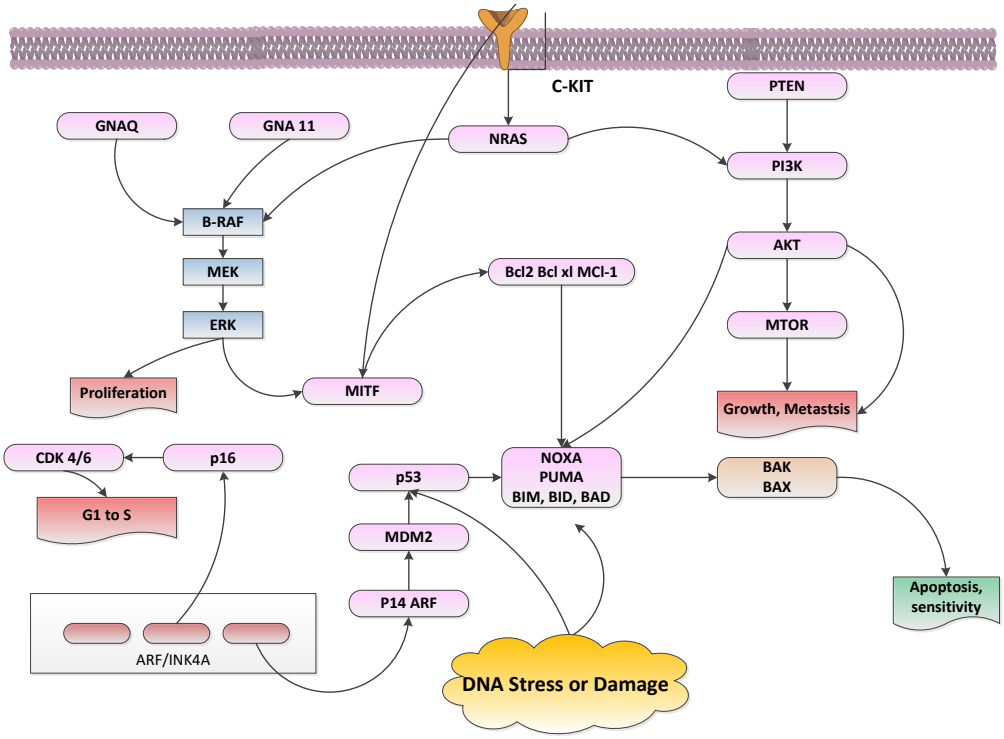
3.7.4 p53

p53 is a classic cancer related gene. Originally thought of an oncogene it was later understood to be just the opposite. It is often seen turned off in many cancers.

As Muller et al state:

In about half of all human cancers, the tumor suppressor p53 protein is either lost or mutated, frequently resulting in the expression of a transcriptionally inactive mutant p53 protein. Loss of p53 function is well known to influence cell cycle checkpoint controls and apoptosis. But it is now clear that p53 regulates other key stages of metastatic progression, such as cell migration and invasion. Moreover, recent data suggests that expression of mutant p53 is not the equivalent of p53 loss, and that mutant p53s can acquire new functions to drive cell migration, invasion, and metastasis, in part by interfering with p63 function.

The relationships between MDM2 and p53 are shown below:



Thus, turning p53 off, results in conditions favorable for metastatic growth.

3.8 TRANSCRIPTION FACTORS

Transcription factor facilitate the effecting of genes into proteins. There are multiple such factors and we briefly address two of them.

3.8.1 Androgen Receptor

AR is a transcription factor[36]. In that role it facilitate the transcription of a multiplicity of genes that are central to the development and evolution of PCa. We now discuss transcription factors and AR specifically.

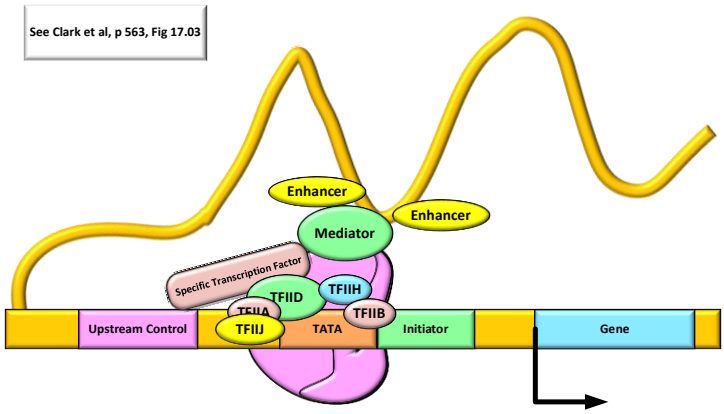
There are a multiplicity of transcription factors in the human gene set. AR is one of them. As Lambert et al note:

Transcription factors (TFs) recognize specific DNA sequences to control chromatin and transcription, forming a complex system that guides expression of the genome. Despite keen interest in understanding how TFs control gene expression, it remains challenging to determine how the precise genomic binding sites of TFs are specified and how TF binding ultimately relates to regulation of transcription.

This review considers how TFs are identified and functionally characterized, principally through the lens of a catalog of over 1,600 likely human TFs and binding motifs for two-thirds of them. Major classes of human TFs differ markedly in their evolutionary trajectories and expression patterns, underscoring distinct functions. TFs likewise underlie many different aspects of human physiology, disease, and variation, highlighting the importance of continued effort to understand TF-mediated gene regulation.

We show a simple example below.

[36] https://www.researchgate.net/publication/370125480_Androgen_Receptor_Whither_Goest_Thou



The TF are elements necessary for the expression of certain genes. Lambert et al continue:

Transcription factors (TFs) directly interpret the genome, performing the first step in decoding the DNA sequence. Many function as ‘‘master regulators’’ and ‘‘selector genes’’, exerting control over processes that specify cell types and developmental patterning and controlling specific pathways such as immune responses. In the laboratory, TFs can drive cell differentiation and even de-differentiation and trans-differentiation. Mutations in TFs and TF-binding sites underlie many human diseases. Their protein sequences, regulatory regions, and physiological roles are often deeply conserved among metazoans, suggesting that global gene regulatory ‘‘networks’’ may be similarly conserved. And yet, there is high turnover in individual regulatory sequences, and over longer timescales, TFs duplicate and diverge.

The same TF can regulate different genes in different cell types (e.g., ESR1 in breast and endometrial cell lines), indicating that regulatory networks are dynamic even within the same organism. Determining how TFs are assembled in different ways to recognize binding sites and control transcription is daunting yet paramount to understanding their physiological roles, decoding specific functional properties of genomes, and mapping how highly specific expression programs are orchestrated in complex organisms. …

today, most known and putative TFs have instead been identified by sequence homology to a previously characterized DNA-binding domain (DBD), which is also used to classify the TF. With the possible exception of the very simple AT-hook, all extant examples of DBDs are assumed to be derived from a small set of common ancestors representing the major DBD folds, with the families arising by duplication…

3.8.2 ONECUT

ONECUT is a second important transcription factor. As Qian et al noted:

ONECUT2 (OC2) is a master transcription factor that alters lineage identity by activating gene networks associated with both neuroendocrine prostate cancer and prostate adenocarcinoma[37].

A small molecule inhibitor of OC2 represses the lineage plasticity program activated by enzalutamide, suggesting OC2 inhibition as a novel therapeutic strategy to prevent emergence of treatment-resistant variants. …

Androgen receptor- (AR-) indifference is a mechanism of resistance to hormonal therapy in prostate cancer (PC). Here we demonstrate that the HOX/CUT transcription factor ONECUT2 (OC2) activates resistance through multiple drivers associated with adenocarcinoma, stem-like and neuroendocrine (NE) variants. Direct OC2 targets include the glucocorticoid receptor and the NE splicing factor SRRM4, among others. OC2 regulates gene expression by promoter binding, enhancement of chromatin accessibility, and formation of novel super-enhancers. OC2 also activates glucuronidation genes that irreversibly disable androgen, thereby evoking phenotypic heterogeneity indirectly by hormone depletion.

Pharmacologic inhibition of OC2 suppresses lineage plasticity reprogramming induced by the AR signaling inhibitor enzalutamide. These results demonstrate that OC2 activation promotes a range of drug resistance mechanisms associated with treatment-emergent lineage variation in PC.

Our findings support enhanced efforts to therapeutically target this protein as a means of suppressing treatment resistant disease. …

Prostate cancer (PC) is driven by the AR, a hormone-dependent nuclear receptor. AR-driven prostate adenocarcinoma can evolve to contain cell types with diminished luminal features, indicating lineage identity has been altered. This "lineage plasticity" is thought to play a key role in tumor heterogeneity and development of lethal disease. Treatment-resistant phenotypes documented in PC include epithelial-mesenchymal transition (EMT) , neuroendocrine (NE) differentiation , and activation of the glucocorticoid receptor (GR; NR3C1) .

While EMT and NE transcriptional programs operate outside the AR axis and give rise to distinct histologic features, the GR assumes control of certain AR-regulated genes, resulting in preservation of the luminal phenotype of adenocarcinoma.

The HOX/CUT protein ONECUT2 (OC2) is a master transcription factor that is active in roughly 60% of mCRPC. OC2 promotes NEPC features, suggesting it plays a role as a driver of lineage plasticity and the emergence of drug resistance following ARSI therapy. Notably, OC2 can be directly targeted with a small molecule inhibitor (SMI) that suppresses established PC metastases in mice . Despite these insights, OC2 activity in disease progression is not well

[37] https://www.ncbi.nlm.nih.gov/gene/9480 *This gene encodes a member of the onecut family of transcription factors, which are characterized by a cut domain and an atypical homeodomain. The protein binds to specific DNA sequences and stimulates expression of target genes, including genes involved in melanocyte and hepatocyte differentiation.*

defined. In this study, we describe a novel role for OC2 as a broadly acting lineage plasticity driver that operates across several distinct molecular pathways to promote lineage variation and drug resistance.

3.9 MULTITARGETED

As McCarty has noted:

The aberrant behavior of cancer reflects upregulation of certain oncogenic signaling pathways that promote proliferation, inhibit apoptosis, and enable the cancer to spread and evoke angiogenesis. Theoretically, it should be feasible to decrease the activity of these pathways—or increase the activity of pathways that oppose them—with noncytotoxic agents. Since multiple pathways are dysfunctional in most cancers, and cancers accumulate new oncogenic mutations as they progress, the greatest and most durable therapeutic benefit will likely be achieved with combination regimens that address several targets.

Thus, a multifocal signal modulation therapy (MSMT) of cancer is proposed.

This concept has already been documented by researchers who have shown that certain combinations of signal modulators—of limited utility when administered individually—can achieve dramatic suppression of tumor growth in rodent xenograft models. The present essay attempts to guide development of MSMTs for prostate cancer. Androgen ablation is a signal modulating measure already in standard use in the management of delocalized prostate cancer.

The additional molecular targets considered here include the type 1 insulin-like growth factor receptor, the epidermal growth factor receptor, mammalian target of rapamycin, NF-κB, hypoxia-inducible factor-1α, hsp90, cyclooxygenase-2, protein kinase A type I, vascular endothelial growth factor, 5-lipoxygenase, 12-lipoxygenase, angiotensin II receptor type 1, bradykinin receptor type 1, c-Src, interleukin-6, ras, MDM2, bcl-2/bclxL, vitamin D receptor, estrogen receptor-β, and PPAR- . Various nutrients and phytochemicals suspected to have potential utility in prostate cancer prevention and therapy, but whose key molecular targets are still unknown, might reasonably be incorporated into MSMTs for prostate cancer; these include lycopene, selenium, green tea polyphenols, genistein, and silibinin.

MSMTs can be developed systematically by testing various combinations of signal-modulating agents, in concentrations that can feasibly be achieved and maintained clinically, on human prostate cancer cell lines; combinations that appear promising can then be tested in xenograft models and, ultimately, in the clinic. Some signal modulators can increase response to cytotoxic drugs by upregulating effectors of apoptosis. When MSMTs fail to raise the spontaneous apoptosis rate sufficiently to achieve tumor stasis or regression, incorporation of appropriate cytotoxic agents into the regimen may improve the clinical outcome.

4 THERAPEUTICS

There are a multiplicity of therapeutics for PCa. However, many just prolong the inevitable, especially for metastatic disease. PCa generally can be treated surgically but there are a certain small percentage whose disease is so aggressive that in a short time it can become both metastatic and ultimately terminal. When examining therapeutics we see two distinct dimensions; stopping progression and targeting malignant cells. The former approach demands an understanding of what the progression pathways are in malignant cells. If properly targeted it just stops the proliferation without killing off the cells. The second approach demands a unique identification of the malignant cells and then uses some form of immune of chemotherapeutic attack mechanism.

4.1 CURRENT RATIONALE

Currently the general approach is to do androgen deprivation. This may be a combination of castration plus medication. Yet once the tumor becomes androgen independent this becomes a fatal step. We have examined the androgen receptor and its functioning[38].

As Macaulay et al note:

Androgen deprivation therapy is standard of care for advanced or metastatic prostate cancer .

Unfortunately, most patients will progress to a castration-resistant disease state after 2–3 years. Recognition that androgen receptor (AR) signalling is a key driver of castration-resistant prostate cancer (CRPC) led to the development of potent inhibitors of the AR pathway. Enzalutamide, a second generation androgen antagonist, targets multiple steps in the AR signalling pathway and is licensed for use in CRPC.

Nevertheless, most patients eventually develop resistance , highlighting a need for novel agents for patients with refractory disease.

The insulin-like growth factor (IGF) axis plays an important role in prostate cancer progression.

Castration leads to increased signalling via the type I IGF tyrosine kinase receptor (IGF-1R), activating the PI3K/AKT/mTOR pathway which may lead to androgen-independent AR transactivation, facilitating progression to castration-resistance.

The above is a critical observation. Androgen dependent PCa has been treated in a multiple set of ways. However as has been noted PCa evolves into an AR independent state where it is then metastasizing. The understanding of this state can lead to alternate therapeutics. They continue:

[38] https://www.researchgate.net/publication/370125480_Androgen_Receptor_Whither_Goest_Thou

IGF-1R activation and signalling has also been shown to dephosphorylate AR, enhancing AR translocation to the nucleus .

Thus, there is biological rationale for cotargeting AR and IGF signalling in patients with CRPC.

Xentuzumab is a humanised monoclonal antibody that binds to and neutralises the IGF-1 and IGF-2 ligands .

Xentuzumab has demonstrated antitumour activity in preclinical studies , including in combination with enzalutamide in prostate cancer models .

In Phase I trials undertaken in patients with advanced solid tumours, xentuzumab monotherapy has demonstrated manageable tolerability and antitumour activity . This Phase Ib/II trial evaluated xentuzumab plus enzalutamide in patients with metastatic CRPC (mCRPC). In patients who had progressed on docetaxel-based chemotherapy and abiraterone, a Phase Ib dose escalation part was conducted to determine the maximum tolerated dose (MTD), and a randomised Phase II part assessed the combination versus enzalutamide alone.

A Phase Ib expansion cohort evaluated the addition of xentuzumab to enzalutamide in docetaxel/abiraterone naïve patients experiencing prostate-specific antigen (PSA) progression on enzalutamide. …

Xentuzumab in combination with enzalutamide demonstrated a manageable safety profile across all three parts of the study.

No DLTs were reported in the first cycle during the Phase Ib dose escalation phase and the MTD/RP2D was determined to be Xe1000 + En160. The most common AEs across all parts of the study were fatigue and decreased appetite. There were no notable differences in safety profile between the xentuzumab plus enzalutamide and enzalutamide alone arms in the Phase II part. No new safety signals were observed, and the AE profile was as expected based on previous xentuzumab monotherapy trials and the known profile of enzalutamide .

The exploratory dose expansion phase suggested that xentuzumab did not have antitumour activity in combination with enzalutamide in patients with mCRPC with rising PSA levels on enzalutamide.

Addition of xentuzumab to enzalutamide in the Phase II part did not prolong PFS versus enzalutamide alone in patients with mCRPC after previous treatment with docetaxel and abiraterone. The groups were imbalanced with respect to baseline characteristics, with more adverse factors (ECOG PS, Gleason score, mutation burden) in the Xen1000 + En160 arm, although the outcomes with respect to PFS and OS were not altered after correction for PFS and Gleason score.

Thus, while there is a strong preclinical rationale for targeting of IGF in prostate cancer, this did not translate to clinical efficacy in this study. This finding is consistent with other trials of IGF-1R inhibitory drugs in mCRPC patients.

In a study of chemotherapy-naïve patients, addition of the IGF-1R inhibitory antibody, figitumumab, to docetaxel/ prednisone did not significantly improve the PSA response rate above the null value of 45%, had a detrimental effect on PFS (4.9 vs 7.9 months) and substantially increased toxicity versus docetaxel/prednisone alone . Another IGF-1R antibody, cixutumumab, had limited antitumour activity in combination with the mTORC1 inhibitor temsirolimus, with an unexpectedly high degree of toxicity and no patient having a >50% PSA decrease from baseline .

While the IGF-1R/INSR tyrosine kinase inhibitor linsitinib was well tolerated in a study of patients with chemotherapy-naïve mCRPC, there was no evidence of antitumour activity . Although the current and previous studies indicate that IGF or IGF-1R inhibition do not confer clinical benefit in unselected patients, certain patients with mCRPC appear to benefit. Therefore, there is a need to identify predictive biomarkers that might identify patients most likely to respond. As part of the current trial, there was an exploratory analysis of biomarkers to expand understanding of the disease and study treatment.

PTEN is frequently downregulated by gene deletion or mutation as prostate cancers progress to mCRPC , with evidence that PTEN gene loss correlates with reduction or loss of PTEN signal by IHC .

We tested PTEN H-score cut-offs of >130 (the median value for all tumours tested) and >80 vs 0–80, the latter reported to reflect heterozygous PTEN loss but neither were predictive. There were too few tumours with H-score 0–10 (12/90, 1.3%) to test the predictive power of very low PTEN signal consistent with biallelic loss of PTEN . In patients whose tumours expressed high PTEN (H-score >220) there was a trend towards improved OS in those on Xen1000 + En160 versus En160.

Conversely there was a trend towards lack of OS benefit in patients with low PTEN tumours. These observations provide initial clinical support for preclinical data suggesting that PTEN status may be a marker of responsiveness to xentuzumab plus enzalutamide. In PTEN proficient prostate cancer cells and xenografts, treatment with xentuzumab plus enzalutamide was growth inhibitory, but treatment resistance was induced upon PTEN depletion . …

However, primary prostate cancers show intra-tumoral heterogeneity that is poorly captured at diagnostic biopsy, and it is increasingly recognised that clones with metastatic potential are identifiable only at the genetic level.

Therefore, PTEN IHC on diagnostic biopsies cannot be used reliably to infer PTEN status of metastatic sites, and it would have been preferable to have had access to tumour tissue biopsied at trial entry to ascertain current PTEN status. As a result, we cannot exclude PTEN proficiency as a driver for response to xentuzumab plus enzalutamide. Several IGF-pathway-related genes, including IGF1, IGF1R and IGFBP5 were identified as potential predictive biomarkers in this study, with high expression associated with PFS benefit from xentuzumab plus enzalutamide.

IGFBP5 is a well-characterised transcriptional readout of IGF axis activity in multiple cell types.

Thus, IGF1 and IGFBP5 upregulation reflect high baseline IGF axis activity; it is plausible that this state could indicate IGF dependence, potentially contributing to possible benefit from xentuzumab.

Another potential biomarker of interest identified in this study was ERG gene expression. Patients appeared to be more likely to derive OS benefit from xentuzumab plus enzalutamide if tumour expression of ERG was low. This observation contrasts with an in vitro study where ERG silencing reduced the sensitivity of prostate cancer cells to IGF-1R inhibition . While these observations are of interest, patients in this trial were not preselected or stratified for PTEN status or transcriptional profiles, and further studies would be required to assess these parameters as predictive biomarkers for response to IGF inhibition in CRPC.

4.2 ADVANCED THERAPEUTICS

Targeting IGF-1 has become an attractive adjunct. As Liu has noted:

A better understanding of IGF-1/IGF-1R activity and regulation has therefore emerged as an important subject of PCa research. IGF-1/IGF-1R signaling affects diverse biological processes in cancer cells, including promoting survival and renewal, inducing migration and spread, and promoting resistance to radiation and castration. Consequently, inhibitory reagents targeting IGF-1/IGF-1R have been developed to limit cancer development.

Multiple agents targeting IGF-1/IGF-1R signaling have shown effects against tumor growth in tumor xenograft models, but further verification of their effectiveness in PCa patients in clinical trials is still needed. Combining androgen deprivation therapy or cytotoxic chemotherapeutics with IGF-1R antagonists based on reliable predictive biomarkers and developing and applying novel agents may provide more desirable outcomes. This review will summarize the contribution of IGF-1 signaling to the development of PCa and highlight the relevance of this signaling axis in potential strategies for cancer therapy

Namely, as an adjuvant one may try to block IGF-1 or its receptors. One should recall that IGF-1 can be generated not only by the liver but by metastasis in bone and elsewhere creating a positive feedback system in metastatic disease. This blocking IGF-1 may be an attractive alternative. IGF-1 has a complex set of interactions.

As Matsushita et al noted:

IGF-1 decreased miR-143 expression and increased IGF1R expression in PC-3 and DU145 cells, and made these cell lines more resistant to docetaxel treatment, suggesting that IGF-1 levels are also involved in resistance to treatment in PCa . IGF-1 is also implicated in castration-resistant PCa and has been shown to activate androgen receptor (AR) signaling in prostate cancer cells via the IGF-1R-forkhead box protein O1 (FOXO1) signaling. Elevated blood IGF-1 levels increase the future risk of PCa in healthy men . Acromegaly patients with

systemically high GH and IGF-1 levels also have significantly higher incidence of PCa and risk of PCa-related mortality (HR = 1.33 and 1.44, respectively), suggesting that IGF-1 has a positive effect on PCa development and progression, even in humans .

Several studies reported that blood IGF-1 levels in elderly men with suspected PCa on screening tests are not associated with cancer positivity. Serum IGF-1 levels in 94 men who required prostate biopsy showed no significant difference between positive and negative cancer(26.4 vs. 23.7 nmol/ L; P = 0.08) .

This discrepancy suggests that prostate epithelial cells may be at an increased risk of cancer development or progression only after prolonged exposure to high concentrations of IGF-1. Suppression of IGF-1 signaling is a potential therapeutic approach, because the IGF1R inhibitor in combination with castration inhibited PCa growth in rodent models of bone metastasis and subcutaneous xenografts.

However, in a phase 2 study, limsitinib, the most extensively evaluated IGF1R inhibitor, failed to significantly improve levels of prostate-specific antigen after 12 weeks of treatment and did not improve overall survival in men with metastatic castrate-resistant PCa . In the future, as a more potent treatment strategy, a combination of novel IGF1R inhibitors and existing prostate cancer therapies is expected to be effective.

Again as Liu et al note:

An intricate balance between cancer cell proliferation-related elements and apoptosis regulating elements is critical for preventing PCa growth. Disruption of the balance between these elements triggers evasion of apoptosis and promotes cell survival, thus contributing to cancer initiation and progression . As a factor affecting this balance, IGF-1 signaling is essential for the survival and proliferation of many normal and malignant cell types and protects these cells from programmed cell death .

Loss of IGF-1R induced by transient transfection with small-interfering RNA (siRNA) oligonucleotides or inhibition of IGF-1R activity by specific inhibitors inhibits the survival and proliferation of PCa cells.

Several mediators have been reported to participate in the regulation of cell survival and proliferation triggered by IGF-1R signaling, including forkhead box transcription factors (FOXOs), oncogenes, and tumor suppressor genes, as elaborated in the following paragraphs.

The binding of IGF-1 to its tyrosine kinase receptor results in the phosphorylation of several cellular proteins, including FOXO transcription factors, Bcl-2-associated agonist of cell death (BAD), and glycogen synthase kinase-3 (GSK3α/β), to facilitate cell survival and cell cycle entry via phosphorylation of Akt on Thr308 or Ser253 . For instance, activated IGF-1R signaling dampens FOXO3 signaling and thus reduces the expression of the proapoptotic protein Bim to inhibit tumor cell mitochondrial apoptosis by increasing the activity of the phosphorylated PI3K-Akt pathway.

As one of the most highly expressed members of the FOXO family in PCa cells, FOXO3 expression is negatively correlated with PCa progression . While FOXO3 is active inside the nucleus, nuclear exclusion and accumulation of FOXO3 in the cytoplasm are induced by AKT-induced phosphorylation . Therefore, the level of nuclear FOXO3 is decreased, which prevents FOXO3 from playing an anticancer role and mediates the promotion of adenocarcinoma growth by IGF-1 . IGF-1R can enhance the expression of cyclin D1 and the progression of the cell cycle from the G1 to the S phase through three mechanisms.

First and foremost, the Ras/MAPK pathway is one of the main downstream mediators of IGF-1 signaling and is closely connected with cell cycle transitions and cell proliferation. IGF-1 can induce tyrosine phosphorylation of β-catenin and promote its dissociation from a complex and transport it into the cytoplasm. In cancer cells, β-catenin accumulates in the cytoplasm, translocates into the nucleus, and interacts with T-cell factor/lymphoid enhancer factors (TCF/LEF) to activate cyclin D1 and promote cell cycle transitions . Finally, SUMOylated IGF-1R undergoes nuclear translocation and binds to enhancers or nuclear proteins to activate cell cycle-regulated genes and increase G1-S progression .

In addition to the mechanisms illustrated above, several studies have reported that IGF-1 signaling also mediates cell survival and proliferation by disturbing the expression of oncogenes and tumor suppressor genes. For example, internalized IGF-1R can upregulate the expression of the oncogene JUN and enhance the recruitment of RNA Pol II to JUN promoter regions, which is beneficial to cell survival .

In addition it is noted that IGF-1 inhibits PTEN, the main controller of proliferation. Thus a therapeutic employing IGF-1 suppression has substantial merit. Liu et al note:

IGF-1 signaling is frequently dysregulated in cancer development, and its overexpression plays a vital role in the malignant transformation of mammary cells, provides prostate tumors with inherent resistance to radiotherapy or ADT, and worsens the prognosis of patients. Hence, IGF-1/IGF-1R inhibitory reagents have been developed to prevent cancer development and improve survival, including a variety of human neutralizing antibodies, anti-IGF-1R monoclonal antibodies, and a few small molecules. A portion of these agents has been tested in clinical trials alone or in combination with conventional therapies in PCa before radical prostatectomy, PCa before metastasis, and even CRPC . Our review mainly covers therapies investigated in PCa patients, and other agents that have potential clinical applicability and have been tested in preclinical experiments will be minimally discussed.

Noe the authors delineate several therapeutic paths.

4.2.1 Anti-IGF-1R Mabs

Monoclonal antibodies (Mabs) have been used extensively to address many malignancies. The following target the IGF-1 receptor, blocking actions of IGF-1 on cells.

1. Cixutumumab[39] (IMC-A12)

[39] See McKian and Haluska

2. Figitumumab[40]
3. A12
4. Ganitumab[41]

4.2.2 IGF-1 Neutralizing Mabs

The following Mabs attack the IGF-1 itself, blocking its ability to attach to a cell.

1. Xentuzumab[42]
2. Dusigitumab[43]

4.2.3 IGF-1R Inhibitors

Like many of the early kinase inhibitors, IGF-1R inhibitors are also available.

1. Linsitinib
2. BMS-754807
3. Picropodophyllin

Many of these therapeutics are in early stages of evaluation.

[40] See DiMaio and Scagliotti

[41] See Yee et al

[42] See Schmid et al

[43] See Truong et al

5 METFORMIN, DIABETES, AND PCA

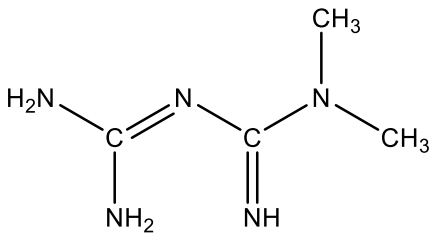
Metformin is a drug used in T2 diabetes. It basically inhibits glucose release from the liver thus allowing a limited insulin supply to deal with less glucose load. However it has been considered as a PCa therapeutic. Matsushita et al noted:

We have reported that SCFAs metabolized by intestinal bacteria contribute to PCa growth by increasing systemic and prostate local IGF-1 productions, and revealed the "gut–prostate axis" involving bacterial metabolites . Prostate-specific phosphatase and tensin homolog (PTEN)-knockout mice [PbCre+; Ptenfl/fl] were used as a PCa model. In these mice, a western-style high-fat diet (HFD) containing mainly lard accelerated PCa growth .

This diet-induced PCa growth was inhibited by oral administration of metformin or celecoxib, as well as by an antibiotic mixture (ampicillin, vancomycin, neomycin, and metronidazole).

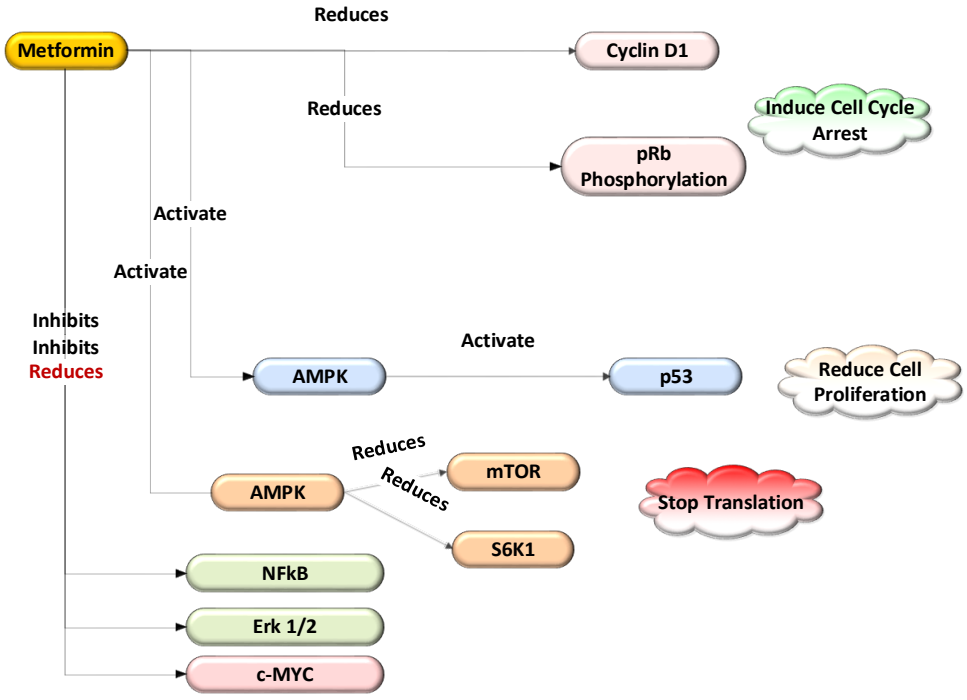
Antibiotics cause substantial changes in the composition of the gut microbiota of HFD-fed mice. Fecal SCFAs in the mice were reportedly reduced by 75%, resulting in decreased production of IGF-1 in the liver and prostate. In addition, phosphorylation of IGF-1R, ERK, and AKT was reduced in PCa cells of mice fed a HFD who received antibiotic, suggesting that decreased IGF-1 might suppress the activity of MAPK and PI3K signaling cascades.

Metformin is a classic Type 2 Diabetic control medication and has been used extensively with many patients for several decades. We demonstrate below the areas in which Metformin exercises its influence. Metformin[44] is configured as shown below:

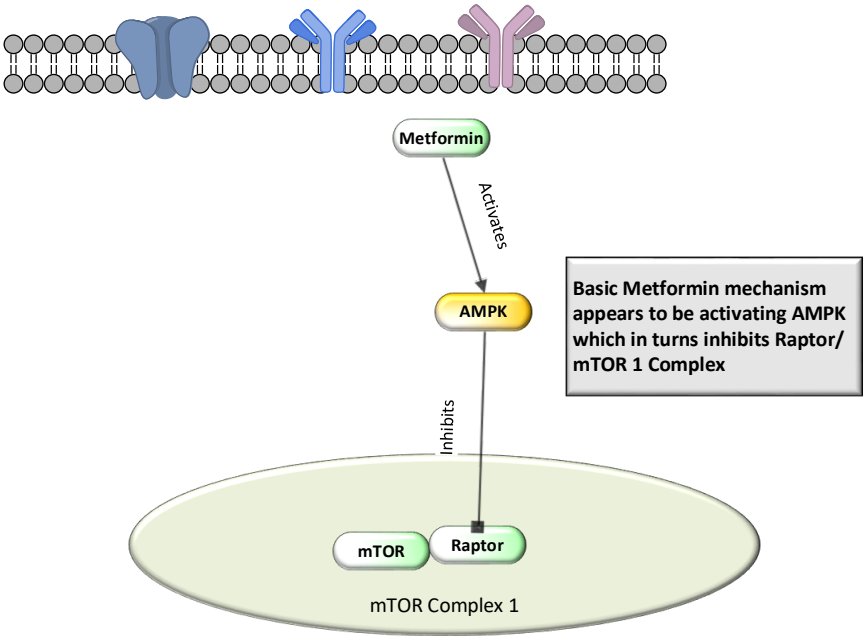


It is a simple molecule but can exert significant impact on multiple metabolic pathways. The impact of metformin on various genes and gene products is shown below

[44] https://pubchem.ncbi.nlm.nih.gov/compound/Metformin



The end state actions shown above clearly show a significant potential for metformin. We shall examine the recent work to date to better understand its potential. The basic step involves the control of AMPK and in turn mTOR. We have examined the latter (mTOR) in some detail before.



Prostate cancer has frequently been seen related to inflammatory processes. The exact connection is yet to be determined. However recent results have indicated that metformin has

shown some effect on PCa and a recent paper by Danzig et al shows significant effects with metformin and statins. Both drugs have a certain antiinflammatory role, one in glucose metabolism management and the other through lipid pathways. In this paper we examine both the Danzig et al results as well and the details regarding the specific pathways involved. Specifically, the drugs deal with metabolic related pathways, which is no surprise given the nature of Type 2 Diabetes. However, the statin usage is not directly metabolic but may very well be so.

Shao et al state[45]:

The widely used anti-diabetic drug metformin has been shown to exert strong antineoplastic actions in numerous tumor types, including prostate cancer (PCa).

In this study, we show that BI2536, a specific Plk1 inhibitor, acted synergistically with metformin in inhibiting PCa cell proliferation.

Furthermore, we also provide evidence that Plk1 inhibition makes PCa cells carrying WT p53 much more sensitive to low-dose metformin treatment. Mechanistically, we found that co-treatment with BI2536 and metformin induced p53-dependent apoptosis and further activated the p53/Redd-1 pathway.

Moreover, we also show that BI2536 treatment inhibited metformin-induced glycolysis and glutamine anaplerosis, both of which are survival responses of cells against mitochondrial poisons. Finally, we confirmed the cell-based observations using both cultured cell-derived and patient-derived xenograft studies. Collectively, our findings support another promising therapeutic strategy by combining two well tolerated drugs against PCa proliferation and the progression of androgen-dependent PCa to the castration-resistant stage.

For example, in the work of Margel et al they note:

By using fractional polynomials, we verified that the association between cumulative metformin use after PC diagnosis and PC specific mortality is linear. Onmultivariable analysis, for each additional 6 months of metformin use after PC diagnosis, there was a 24% reduction in PC-specific mortality (adjusted HR [aHR], 0.76; 95% CI, 0.64 to 0.89). Increasing durations of cumulative use of all other antidiabetic medications was not associated with PC-specific mortality.

It reduces, inhibits, and activates a variety of pathway elements all of which control cell cycles and apoptosis. It controls the metabolic cycles that relate to the pathway elements we have shown in the previous sections.

The impact of AMPK and in turn p53 is a significant pathway. AMPK is as we have seen a significant metabolic player and metformin modulates it behavior. It manages the Cyclin D1

[45] http://www.jbc.org/content/290/4/2024.abstract

which controls cell cycle growth. One may wonder why so effectively in the prostate, however. The mTOR management is via AMPK as well and then through mTOR C1.

As Mendelsohn et al state:

Metformin belongs to the biguanide class of antidiabetic drugs and activates the LKB1/AMPK axis (mediating glucose and energy homeostasis) and inhibits cancer cell viability through the inhibition of mTOR. Metformin can also downregulate mTOR and subsequent cell growth through AMPK-independent mechanisms. A recent study using mouse models of lung cancer to assess the protective effect of metformin suggested two possible mechanisms: decreased levels of circulating insulin and lowered energy stress leading to inhibition of mTOR.

Owing to the fact that studies show metformin is associated with a decreased risk of cancer incidence compared with other treatments (such as insulin) among diabetic patients, metformin is rightfully garnering interest for its role in cancer prevention and therapy and supports further testing in the clinical setting.

The Mendelsohn comment has been demonstrated in Danzig somewhat.

Birzniece et al have noted:

Androgen deprivation therapy (ADT), a principal therapy in patients with prostate cancer, is associated with the development of obesity, insulin resistance, and hyperinsulinemia. Recent evidence indicates that metformin may slow cancer progression and improves survival in prostate cancer patients, but the mechanism is not well understood. Circulating insulin-like growth factors (IGFs) are bound to high-affinity binding proteins, which not only modulate the bioavailability and signalling of IGFs but also have independent actions on cell growth and survival.

The aim of this study was to investigate whether metformin modulates IGFs, IGF-binding proteins (IGFBPs), and the pregnancy-associated plasma protein A (PAPP-A) – stanniocalcin 2 (STC2) axis[46]*.*

Design and methods: In a blinded, randomised, cross-over design, 15 patients with prostate cancer on stable ADT received metformin and placebo treatment for 6 weeks each. Glucose metabolism along with circulating IGFs and IGFBPs was assessed. Results: Metformin significantly reduced the homeostasis model assessment as an index of insulin resistance (HOMA IR) and hepatic insulin resistance. Metformin also reduced circulating IGF-2 ($P < 0.05$) and IGFBP-3 ($P < 0.01$) but increased IGF bioactivity ($P < 0.05$). At baseline, IGF-2 correlated

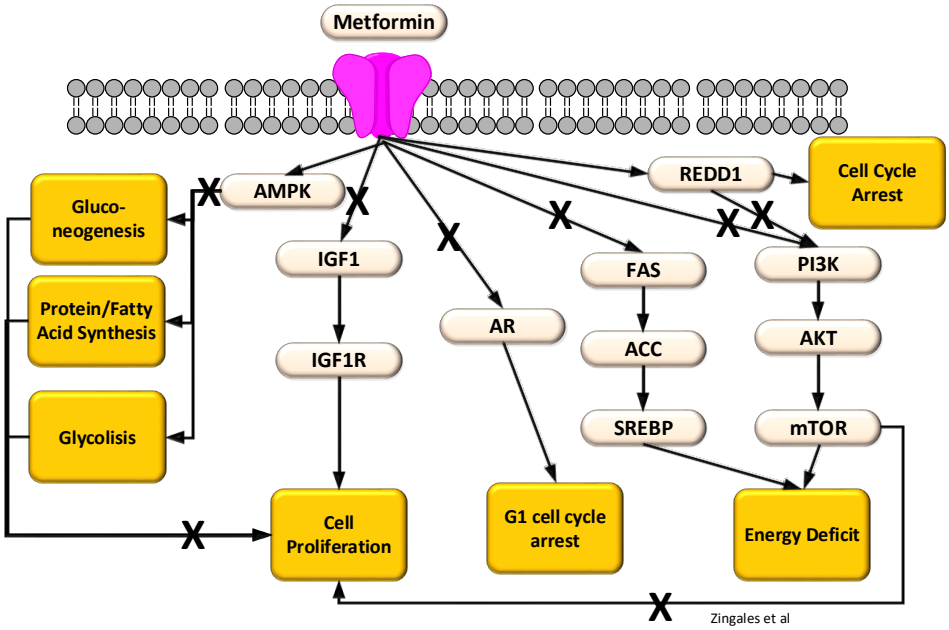
[46] Note from Aguirre et al: *IGF-1 availability is tightly regulated by the so-called* ***insulin-like growth factor binding proteins*** *(IGFBPs), which may act by* ***increasing IGF-1 half-life, from minutes to hours*** *(most commonly by forming a tertiary complex with Acid-Labile Subunit and IGFBP3), however blocking its binding to the insulin-like growth factor 1 receptor (IGF-1R).* ***IGFBPs can also act to guide IGF-1 to specific tissues****, or even to inhibit or potentiate IGF-1 actions by acting as an independent substrate for the IGF-1R and/or other specific membrane, intracellular or nuclear receptors. To date there have been described 6 high affinity IGFBPs.*

significantly with the hepatic insulin resistance (r2 = 0.28, P < 0.05). PAPP-A remained unchanged but STC2 declined significantly (P < 0.05) following metformin administration. During metformin treatment, change in HOMA IR correlated with the change in STC2 (r2 = 0.35, P < 0.05). …

Metformin administration alters many components of the circulating IGF system, either directly or indirectly via improved insulin sensitivity. Reduction in IGF-2 and STC2 may provide a novel mechanism for a potential metformin-induced antineoplastic effect.

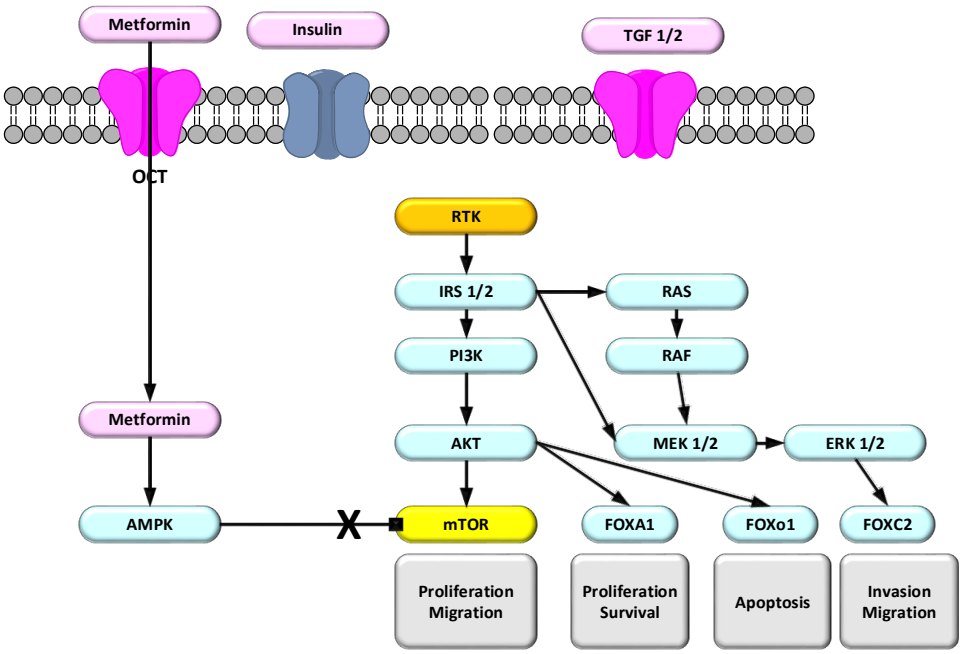
5.1 A PARADIGM

Metformin has been found to be a regulator or controller of a multiplicity of critical cellular pathways, especially those related to malignant progression. We first present a paradigm showing many of these control elements. As Zingales et al have noted:



This is a somewhat complicated and compressed chart but it demonstrates the putative effects of metformin, especially on metabolic actions. AMPK plays a significant role as does mTOR and the REDD1 gene. AMPK is a key element in the control balance of ATP and AMP.

In a similar manner Knura et al note the above in the following manner:



Knura et al follow by noting:

Metformin (Met) is the drug of first choice in type 2 diabetes mellitus. It reduces the level of circulating glucose and is particularly effective against insulin resistance and in obese patients. In the animal models, metformin inhibited proliferation of tumor cells, but not cell migration of PC . Using metformin also induces apoptosis via activation of AMPK (AMP-activated kinase) pathway in prostate cancer cells.

AMPK is a regulator sensitive to cell energy status, it controls the balance between the anabolic and catabolic processes. Through enzyme phosphorylation and regulation of gene expression, it allows cells to adapt to environmental conditions. Inhibiting proliferation is also reached by blocking the cell cycle in G0/G1. Metformin decreases cyclin D1 level, pRb phosphorylation, and increases p27kip protein expression.

Metformin also is effective in lowering IGF-1 and insulin levels. These hormones can stimulate prostate cancer proliferation through activation of the FOXO1 subunit of the androgen receptor. Metformin upregulates REDD1 (regulated in development and DNA response-1) that promotes cell cycle arrest and inhibits PI3K/AKT/mTOR. These actions lead to tumor suppression and increase apoptosis.

Met also inhibits NF-κB, leading to delay of cell aging. However, modulation of inflammatory cytokines profile leads to improved response against cancer cells . Despite the promising outcomes of the wide array of pre-clinical studies, clinical trials considering the risk of PC incidence and progression of this malignancy present with varying results upon administration

of Met. The available data present a spectrum of findings of Met having reduction of risk no effect, to even an increased risk of PC.

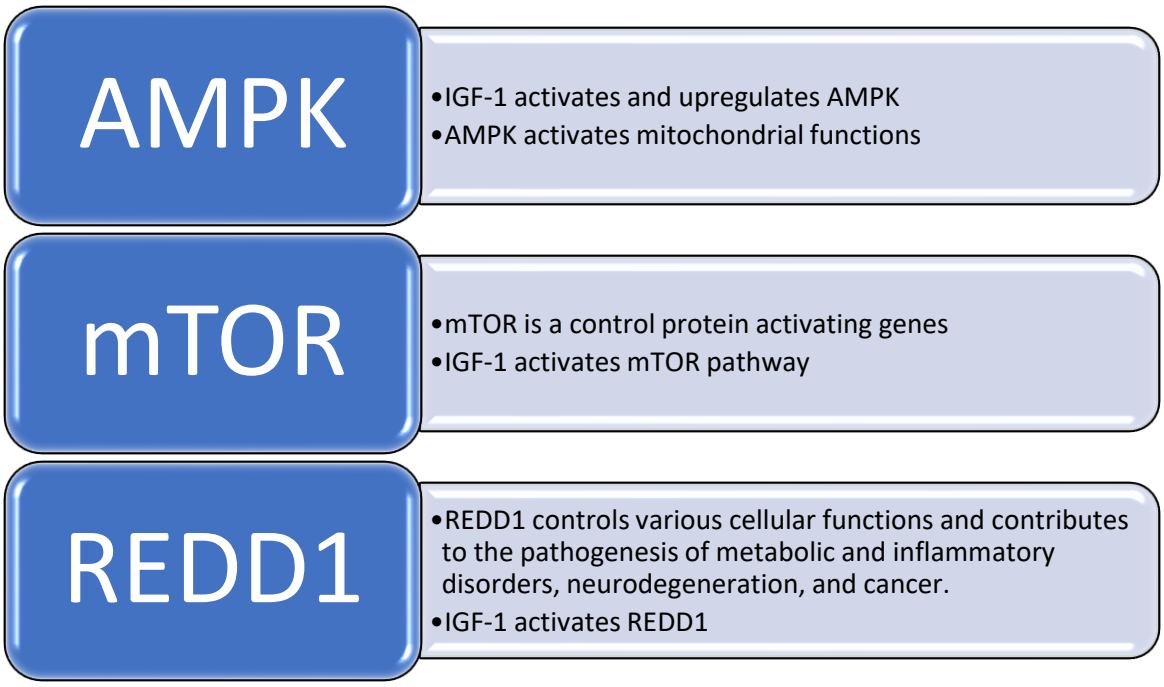
Similar discrepancy is observed in meta-analyses…statistically significant reduction of PC risk was associated with metformin therapy. These two meta-analyses…are based on older observational studies, and consequently, less patients are included….

Another aspect that should be taken into consideration in clinical studies is the impact of metformin on the progression of disease among patients with already diagnosed PC and further therapy outcomes.

Some previous research … do not support a beneficial correlation between all-cause mortality and metformin use.

As well as no association with cancer-specific mortality and metastasis, there is no supporting evidence of a positive impact on the recurrence of PC. In the results of all meta-analyses from the last 5 years, … overall survival among patients with PC treated with metformin was improved. Also, the recurrence of PC among metformin-users in the recent three large meta-analyses is supposed to be decreased. These meta-analyses included a larger patient database than older ones. The mentioned research articles use different survival analysis statistics. The reason for the discrepancy among presented studies could be confounding factors and heterogeneity between research samples.

There are three genes and their products that are a focus about the efficacy of metformin in the context of PCa. They are graphically shown below:



We now examine these three IGF-1 related genes in the context of metformin modulation.

5.2 AMPK

Cell metabolism is the process whereby a cell uses energy that is made available to it to maintain normal processes and to grow and reproduce as may be required. Normal metabolic processes in a cell allow for the control of all of the elements in a balanced manner. Excess glucose as seen in Type 2 Diabetes can result in quasi-inflammatory states and loss of homeostasis.

Let us focus briefly upon AMPK, AMP kinase, as an initial point to understand the intra-cellular metabolic processes. AMPK is also a key control element in many intracellular pathways[47].

From the paper by Mihaylova and Shaw we have[48]:

One of the central regulators of cellular and organismal metabolism in eukaryotes is AMP-activated protein kinase (AMPK), which is activated when intracellular ATP production decreases.

AMPK has critical roles in regulating growth and reprogramming metabolism, and has recently been connected to cellular processes such as autophagy and cell polarity. Here we review a number of recent breakthroughs in the mechanistic understanding of AMPK function, focusing on a number of newly identified downstream effectors of AMPK.

From the work of Shackelford and Shaw we have[49]:

In the past decade, studies of the human tumour suppressor LKB1 have uncovered a novel signalling pathway that links cell metabolism to growth control and cell polarity.

LKB1 encodes a serine–threonine kinase that directly phosphorylates and activates AMPK, a central metabolic sensor. AMPK regulates lipid, cholesterol and glucose metabolism in specialized metabolic tissues, such as liver, muscle and adipose tissue. This function has made AMPK a key therapeutic target in patients with diabetes.

The connection of AMPK with several tumour suppressors suggests that therapeutic manipulation of this pathway using established diabetes drugs warrants further investigation in patients with cancer.

In particular Shackelford and Shaw demonstrate the impact of Metformin on this pathway. As Mendelsohn et al state:

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[47] http://www.cellsignal.com/contents/science-pathway-research-cellular-metabolism/ampk-signaling-pathway/pathways-ampk This is a useful pathway description worth examining in detail.

[48] http://www.nature.com/ncb/journal/v13/n9/full/ncb2329.html

[49] http://www.nature.com/nrc/journal/v9/n8/full/nrc2676.html

While growth factor–stimulated signaling cascades promote cell growth under favorable conditions, cells have sophisticated nutrient sensing systems that serve to block growth when the internal energy supply is limiting. These regulators ensure that, during periods of intracellular nutrient depletion, metabolites are redirected from anabolic pathways and instead used to fuel catabolic pathways that will provide the energy required to survive the period of nutrient limitation. The AMP-activated protein kinase (AMPK) plays a major role coordinating cellular energy status with appropriate metabolic responses.

AMPK directly senses cellular energy levels in the form of the AMP/ATP ratio. Falling energy levels increase the cellular AMP/ATP ratio, priming AMPK for activation by the liver kinase B1 (LKB1). AMPK phosphorylates multiple targets with the cumulative effect of blocking anabolic reactions and stimulating energy-generating catabolic pathways.

For example, AMPK phosphorylates and inhibits acetyl-CoA carboxylase (ACC), with the dual effect of blocking fatty acid synthesis and activating fatty acid oxidation. AMPK also directly inhibits cell growth, both by inducing a p53-dependent cell cycle arrest and by blocking mTOR activity at multiple levels. Through these diverse activities, AMPK functions as a metabolic checkpoint, ensuring that cell growth is halted until bioenergetic conditions are favorable for growth.

AMPK is a powerful regulator of cell dynamics. It senses and manages energy via the ATP control cycle. Its impact on p53 which we have discussed earlier is also a major factor which may lead to cell oncogenesis. Thus examining how AMPK reacts to excess glucose and how it can be reset is a key observation.

As Zingales et al note:

Metformin is an insulin-sensitizing oral biguanide used by diabetic patients every day to maintain their glycemic homeostasis. Metformin is an ideal drug: it is well tolerated and inexpensive. Metformin regulates glucose homeostasis exerting an important control of metabolism. In particular, metformin reduces intestinal absorption of glucose and it increases peripheral glucose uptake and its utilization by adipose tissue and skeletal muscles leading to increased insulin sensitivity.

Through AMPK activation, metformin decreases insulin secretion, inhibits gluconeogenesis and energy consuming processes (such as protein and fatty acid synthesis), and stimulates ATP-generating processes (such as glycolysis and fatty acid oxidation). This results in a shift from anabolic to catabolic metabolism and in an inhibition of proliferation*….*

AMPK activation appears the main mechanism through which metformin inhibits cancer growth. AMPK plays a key role in the maintaining of cellular energy homeostasis. It is an important sensor of the AMP/ATP ratio. AMPK appears as a potential anticancer agent when it is highly activated, but it may not be critical as inhibitor of cancer growth when it acts at low levels.

Metformin primarily acts to directly inhibit the mitochondrial respiratory chain which then reduces the production of ATP resulting in an increase in the ratio of AMP to ATP which then

results in activation of AMPK. Under energy stress conditions, the tumor suppressor LKB1 is the major kinase involved in the AMPK activation and mTOR reduction. Through the mTOR inhibition, metformin arrests cell cycle and cell growth, because mTOR is a downstream effector of PI3K/ AKT pathway, a signaling pathway linked to cancer cell growth and proliferation. PI3K/AKT/mTOR signaling pathway leads to an abnormal cells proliferation, inhibition of apoptosis, and carcinogenesis.

*…**metformin owns an antiproliferative effect in PCa cells through the activation of pAMPK and subsequent inhibition of downstream mTOR signaling and the induction of cell cycle arrest.** In this study, metformin was used in combination with bicalutamide, a known agent used in the hormonal therapy of PCa. It acts blocking the AR and inducing a G1/S phase arrest of the cell cycle. Combining metformin with bicalutamide, the authors obtained a reduction of PCa cell survival, especially in cells expressing functional AR*

The anti-PCa effect of metformin via AMPK activation … demonstrated, in vitro and in vivo, that metformin induces apoptosis and attenuates PCa cell proliferation. Furthermore, a stronger decrease of PCa growth was achieved when metformin was combined with Exenedin-4, a glucagon-like peptide-1 receptor agonists

As Hua et al note:

mTORC1 not only senses growth factors, but also responds to cellular energy. Low cellular energy results in an increase in AMP/ATP ratio, which activates the energy sensor AMP-dependent kinase (AMPK). AMPK stimulates the GAP activity of TSC and then promotes the inhibition of RHEB by TSC, leading to the downregulation of mTORC1. In addition, the TCA cycle metabolite ketoglutarate inhibits mTORC1 through repressing ATP synthase, increasing AMP/ATP ratio and activating AMPK. Cellular energy deficiency usually leads to endoplasmic reticulum stress, which in turn induces the unfolded protein response (UPR). Ire1, ATF6, and PERK are three major mediators of the UPR.

Upon ER stress, ATF6 can induce RHEB expression, which in turn promotes mTORC1 activation and cell survival. However, overactivated mTORC1 is also harmful to cell survival under ER stress. Mutations in TSC1/2 or activation of RHEB renders cells hypersensitive to ER stress-induced apoptosis, which may be due to the downregulation of ATF4/6 by mTOR. Therefore, mTORC1 may have versatile effects on cell survival under ER stress.

As Xi et al noted:

IGF-I/insulin-like growth factor binding protein 2 (IGFBP-2) coordinately stimulate osteoblast differentiation but the mechanisms by which they function have not been determined.

AMP-activated protein kinase (AMPK) is induced during differentiation and AMPK knockout mice have reduced bone mass.

IGF-I modulates AMPK in other cell types; therefore, these studies determined whether IGF-I/IGFBP-2 stimulate AMPK activation and the mechanism by which AMPK modulates differentiation.

Calvarial osteoblasts and MC-3T3 cells expressed activated AMPK early in differentiation and AMPK inhibitors attenuated differentiation. However, expression of constitutively activated AMPK inhibited differentiation. To resolve this discrepancy we analyzed the time course of AMPK induction. AMPK activation was required early in differentiation (day 3–6) but down-regulation of AMPK after day 9 was also necessary. IGF-I/IGFBP-2 induced AMPK through their respective receptors and blocking-receptor activation blocked AMPK induction. To determine the mechanism by which AMPK functioned we analyzed components of the autophagosome. Activated AMPK stimulated ULK-1 S555 phosphorylation as well as beclin-1 and microtubule-associated protein 1A/1B light-chain phosphatidylethanolamine conjugate (LC3II) induction. Inhibition of AMPK attenuated these changes and direct inhibition of autophagy inhibited differentiation. Conversely, expression of activated AMPK was associated with persistence of these changes beyond day 9 and inhibited differentiation. Blocking AMPK activation after day 9 down-regulated these autophagosome components and rescued differentiation. This allowed induction of mechanistic target of rapamycin and AKT, which suppressed autophagy. The results show that early induction of AMPK in response to IGF-I/IGFBP-2 followed by suppression is required for osteoblast differentiation. AMPK functions through stimulation of autophagy. The findings suggest that these early catabolic changes are important for determining the energy source for osteoblast respiration and down-regulation of these components may be required for induction of glycolysis, which is required during the final anabolic stages of differentiation.

5.3 mTOR

We start with a brief overview of mTOR. As NCBI states[50]:

The protein encoded by this gene belongs to a family of phosphatidylinositol kinase-related kinases. These kinases mediate cellular responses to stresses such as DNA damage and nutrient deprivation. This protein acts as the target for the cell-cycle arrest and immunosuppressive effects of the FKBP12-rapamycin complex.

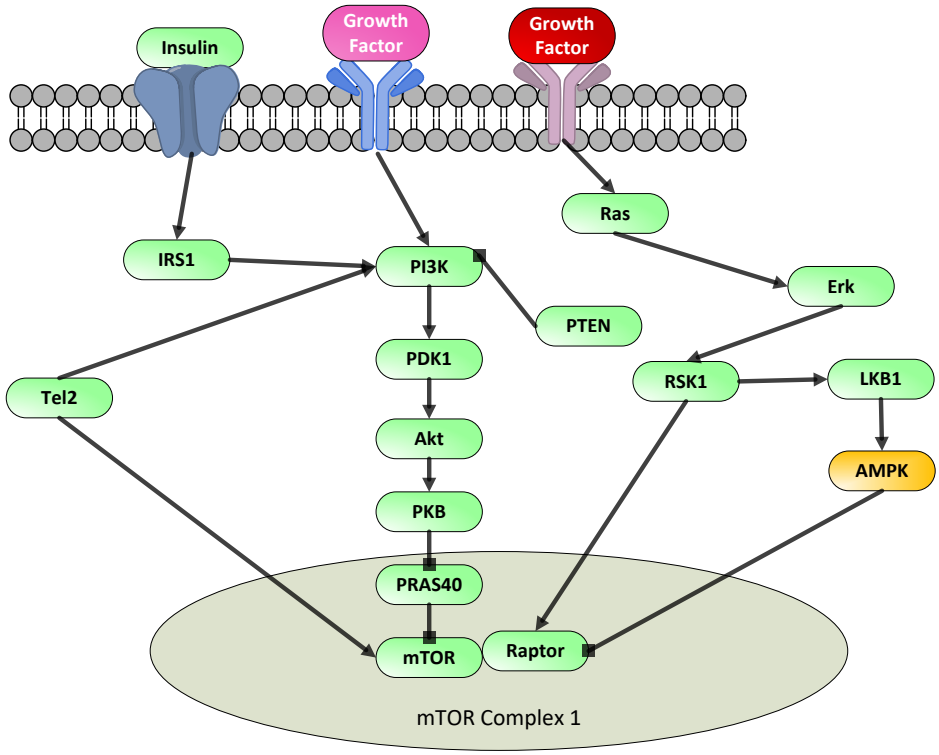
Now mTOR by itself plays a role only when conjugated with other products, namely those generating mTORC1 and mTORC2. We now briefly explain the structure of each of these two.

mTOR is a control protein that in involved in metabolic related pathways. mTOR, the mammalian target of rapamycin, is a gene product (1p36.2) is a protein which acts in a critical manner in interconnecting the genetic circuits in mammals, and especially man. It fundamentally controls glucose transport and protein synthesis. The pathway depicted below is a modification of the graphic from Weinberg (p 785) which shows mTOR in its two modes, one with Raptor

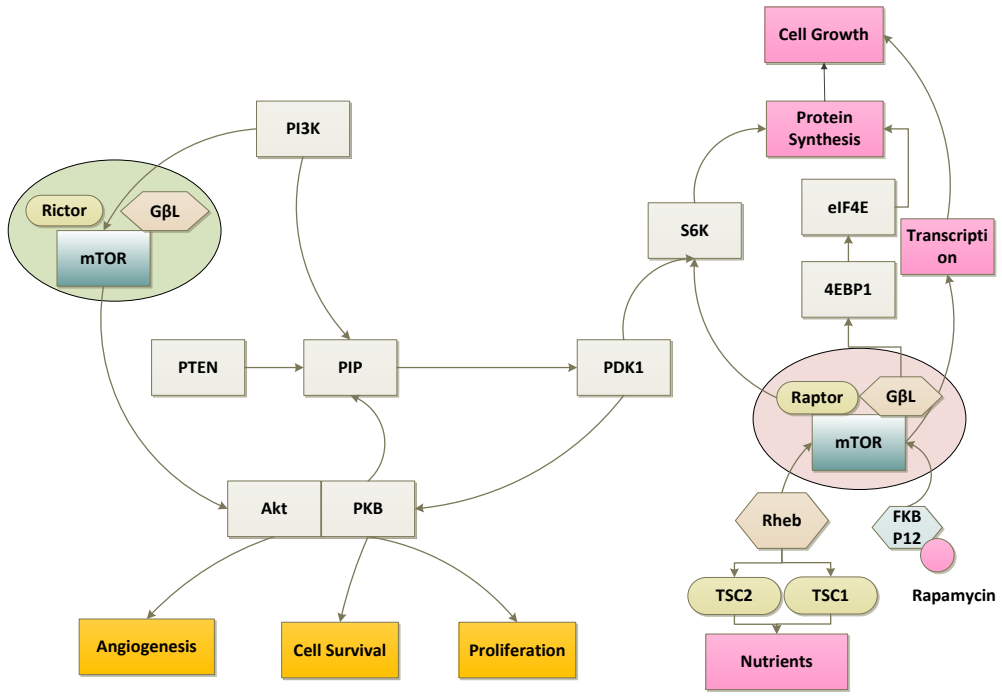
[50] https://www.ncbi.nlm.nih.gov/gene/2475

assisting and one with Rictor. The Rictor/mTOR mode activates the Akt pathway via the placement of a phosphate and this manages the protein synthesis portion. The inclusion of rapamycin will block the Raptor/mTOR path and reduce the protein synthesis and cell growth portion. The inhibitory effect on Akt/PKB by rapamycin is assumed to be the main factor in its anti-cancer effects.

We depict the mTOR C1 pathway below:



The following chart presents a more complex version of the mTOR C1 pathway (Raptor). This allows us to best understand the complex interactions. The mTOR C1 and C2 pathways are depicted in the combined chart below:



Looking at the complexity of the mTOR pathway it presents an interesting one for addressing PCa. Kinkaide et al (2008) indicate:

Among the major signaling networks that have been implicated in advanced prostate cancer are the AKT/mammalian target of rapamycin (AKT/mTOR) and MAPK pathways. Indeed, deregulated expression and/or mutations of the phosphate and tensin homolog tumor suppressor gene (PTEN) occur with high frequency in prostate cancer, leading to aberrant activation of AKT kinase activity as well as its downstream effectors, including the mTOR signaling pathway. In addition, many prostate tumors display deregulated growth factor signaling, which may result in activation of MAPK kinase 1 (MEK) kinase and ultimately ERK MAP.

Notably, previous studies have demonstrated that the AKT/mTOR and MAPK signaling pathways are alternatively and/ or coordinately expressed in advanced prostate cancer and function cooperatively to promote tumor growth and the emergence of hormone- refractory disease. These observations formed the basis for our hypothesis that targeting these signaling pathways combinatorially may be effective for inhibiting tumorigenicity and androgen independence in prostate cancer.

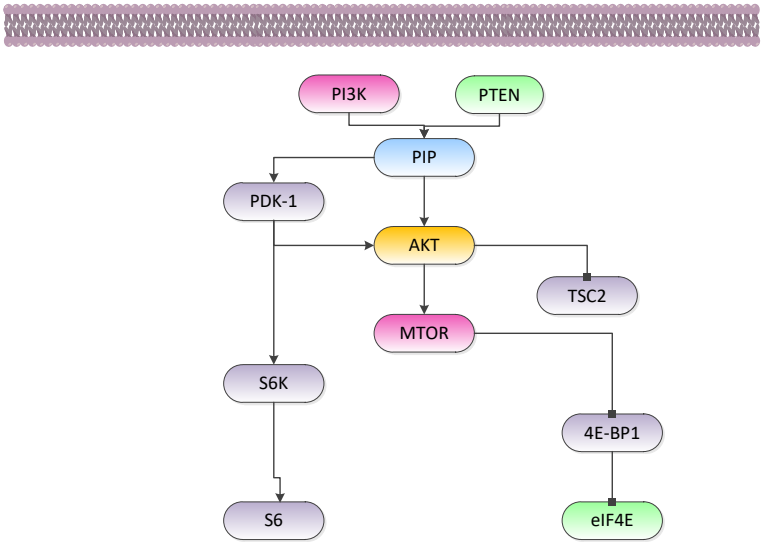
Kinkaide et al also demonstrate the creation of HGPIN via their work. This represents another pathway of HGPIN to PCa. LoPiccolo et al state:

The PI3K/Akt/mTOR pathway is a prototypic survival pathway that is constitutively activated in many types of cancer. Mechanisms for pathway activation include loss of tumor suppressor PTEN function, amplification or mutation of PI3K, amplification or mutation of Akt, activation of growth factor receptors, and exposure to carcinogens. Once activated, signaling through Akt

can be propagated to a diverse array of substrates, including mTOR, a key regulator of protein translation. This pathway is an attractive therapeutic target in cancer because it serves as a convergence point for many growth stimuli, and through its downstream substrates, controls cellular processes that contribute to the initiation and maintenance of cancer.

Moreover, activation of the Akt/mTOR pathway confers resistance to many types of cancer therapy, and is a poor prognostic factor for many types of cancers. This review will provide an update on the clinical progress of various agents that target the pathway, such as the Akt inhibitors perifosine and PX-866 and mTOR inhibitors (rapamycin, CCI-779, RAD-001) and discuss strategies to combine these pathway inhibitors with conventional chemotherapy, radiotherapy, as well as newer targeted agents. We (show) how the complex regulation of the PI3K/Akt/mTOR pathway poses practical issues concerning the design of clinical trials, potential toxicities and criteria for patient selection.

LoPiccolo et al show the more simplified pathway as follows:

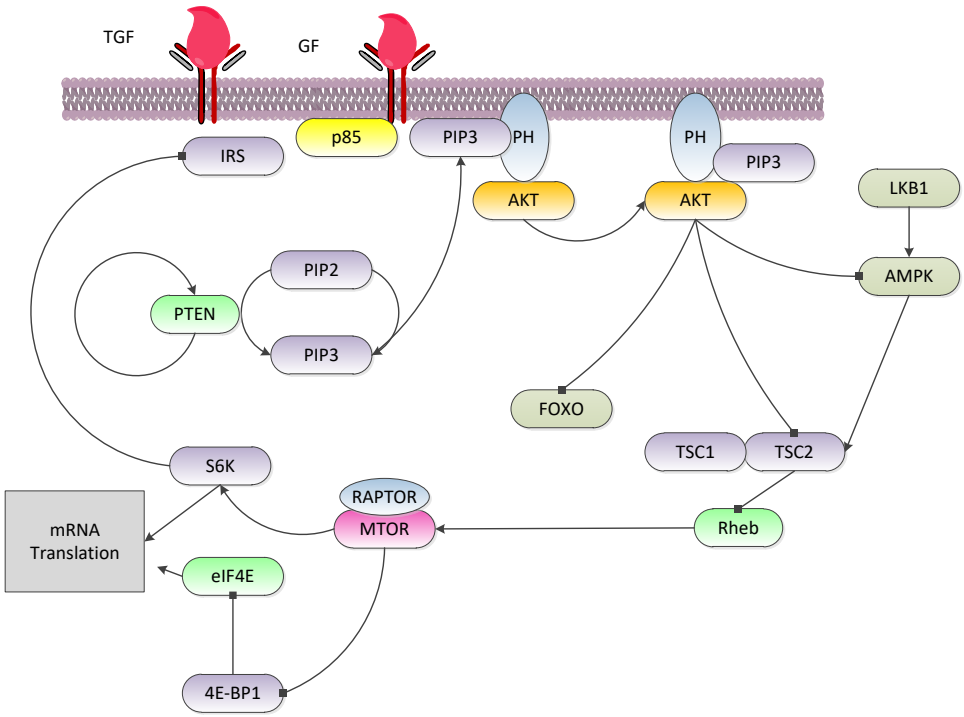


As we have shown with the more complex Weinberg model, here mTOR and PTEN play a strong role in the overall control. The authors show the points of possible control. The complexity of the pathways will be a challenge. It is less an issue of size complexity than a feedback and instability complexity. Nelson et al (2007) have demonstrated similar results as well.

Other researchers have also posited other simple models. We demonstrated the one by Hay as has been stated:

The downstream effector of PI3K, Akt, is frequently hyperactivated in human cancers. A critical downstream effector of Akt, which contributes to tumorigenesis, is mTOR. In the PI3K/Akt/mTOR pathway, Akt is flanked by two tumor suppressors: PTEN, acting as a brake upstream of Akt, and TSC1/TSC2 heterodimer, acting as a brake downstream of Akt and upstream of mTOR.

In the absence of the TSC1/TSC2 brake, mTOR activity is unleashed to inhibit Akt via an inhibitory feedback mechanism. Two recent studies used mouse genetics to assess the roles of PTEN and TSC2 in cancer, underscoring the importance of Akt mTOR interplay for cancer progression and therapy.



The Baldo et al model is quite similar to the Weinberg model shown initially. It clearly demonstrates the overall controlling influence of mTOR. As Baldo et al state:

There is a great body of evidence supporting consideration of the mTOR signaling system as an important network in cell regulation, differentiation and survival. mTOR is a sensor of mitogen, energy and nutritional levels, acting as a "switch" for cell-cycle progression from phase G1 to phase S.

The antibiotic Rapamycin, a potent mTOR inhibitor, has been known to the National Cancer Institute and recognized for its potential anticancer properties since the 1970s. The observation that cell lines from different cancer types exposed to low doses of Rapamycin underwent cell-cycle arrest in phase G1, provided the basis for considering mTOR as a target for cancer therapy.

Development of mTOR inhibitor compounds has proceeded empirically due to the lack of understanding of the precise molecular targets and the required dose of the new compounds . The development of Rapamycin analogs ("Rapalogs"), but also of other, structurally different, mTOR inhibitors, was directed at the selection of specific cancer type sensitivity and an optimization of pharmaceutical forms.

To give an example, Temsirolimus revealed clinical responses in patients with renal cell carcinoma in advanced stage. Temsirolimus was approved by the FDA on May 2007 for this therapeutic use and is being investigated in clinical trials for other cancer types (breast cancer, lymphoma, renal cancer, glioblastoma); significantly there are a considerable number of clinical studies involving mTOR inhibitors currently active worldwide…

The mTOR pathway controls cell size and cellular proliferation*.…nutrient metabolism, mRNA translation and cell survival control. Disruption of TOR leads to early embryonic death in flies and mammalian cells, indicating mTOR plays an important role in regulating cell survival. … deregulation of several mTOR components leads to modified cell proliferation patterns and, on the other, that many mTOR components are deregulated in several human cancers.*

… ***Therefore, inhibition of mTOR leads to slowing or arrest of cells in the G1 phase****. Translational control may have an important role in the balance of cell survival and death, and hence for apoptosis. Importantly, components of mTOR are deregulated in some human cancers, for example, breast and colon. Alteration of PI3-K/Akt is frequently observed in head and neck cancer .*

PTEN, a phosphatase that acts on PIP3 to convert it to PIP2, normally regulates the mTOR pathway negatively, and shows decreased activity in some tumors. A strong relation seems to exist between the sensitivity to the effect of Rapamycin and PTEN loss or deregulation. PTEN is frequently mutated in several cancers and in cancer-like syndromes like Cowden and Proteus syndromes…

Loss of PTEN function can occur in 26-80% of endometrial carcinomas*, …recent studies of human prostate cancer have shown that loss of PTEN is strongly associated with more aggressive cancers. The relationship between PTEN status and sensitivity to rapalogs has been questioned by several investigators. Some attention has recently been dedicated to the role of the mTORC2 complex in the mTOR pathway.*

In fact this complex, believed until recently to be completely insensitive to the effect of Rapamycin, after long-term exposure to Rapamycin is able to prevent mTOR-mediated Akt phosphorylation and the activation of the mTOR pathway. Another component, the TSC1/TSC2 complex located upstream of mTOR, is predicted to integrate signals derived from nutrients, cellular energy status and hypoxia into a common growth regulatory signal to the mTORC1 complex.

As Easton and Houghton state:

Proteins regulating the mammalian target of rapamycin (mTOR), as well as some of the targets of the mTOR kinase, are overexpressed or mutated in cancer. Rapamycin, the naturally occurring inhibitor of mTOR, along with a number of recently developed rapamycin analogs (rapalogs) consisting of synthetically derived compounds containing minor chemical modifications to the parent structure, inhibit the growth of cell lines derived from multiple tumor types in vitro, and tumor models in vivo.

Results from clinical trials indicate that the rapalogs may be useful for the treatment of subsets of certain types of cancer. The sporadic responses from the initial clinical trials, based on the hypothesis of general translation inhibition of cancer cells are now beginning to be understood owing to a more complete understanding of the dynamics of mTOR regulation and the function of mTOR in the tumor microenvironment. This review will summarize the preclinical and clinical data and recent discoveries of the function of mTOR in cancer and growth regulation.

The other observation here is that we often find multiple characterizations of the pathways. Namely there is no canonical form, and often a pathway is depicted to demonstrate a specific protein function. Thus we may see an emphasis on one set of proteins while others are neglected. As much as we currently attempt to unify this process we are left somewhat adrift in model development at this stage. This can be exemplified by now looking at the next section on LKB1. There we show its control over PTEN whereas in an earlier model we have it controlling AMPK. In reality there are multiple links as we have discussed. The literature can be even more confusing on this issue as well.

As Mendelsohn et al state:

It is now widely accepted that mTORC1 positively controls an array of cellular processes critical for growth, including protein synthesis, ribosome biogenesis, and metabolism, and negatively influences catabolic processes such as autophagy—all of which have roles in cancer pathogenesis. Elucidating the key downstream targets of mTORC1 driving these events is an intense area of research.

Originally, much of the study of mTOR relied on experiments in which rapamycin was used acutely to inhibit mTOR (which we now know was mTORC1) in cultured cells. This led to extensive characterization of the best known mTORC1 substrates eiF-4E-binding protein 1(4E-BP1) and S6 kinase 1 (S6K1), both of which regulate protein synthesis.3 In the unphosphorylated state, 4E-BP1 binds and inhibits the cap-binding protein and translational regulator eIF4E. When phosphorylated by mTOR, 4E-BP1 is relieved of its inhibitory duty, promoting eIF4E interaction with the eIF4F complex and the translation of capped nuclear transcribed mRNA.

Following co-regulatory phosphorylation by mTORC1 and another kinase called phosphatidylinositol 3-dependent kinase 1 (PDK1), S6K1 positively affects mRNA synthesis at multiple steps including initiation and elongation by phosphorylating several translational regulators. Although the preponderance of evidence indicates that S6K1 and 4E-BP1 are directly phosphorylated by mTOR, an unidentified phosphatase activity may also be involved in their regulation. For example, the rapamycin-sensitive phosphorylation site on S6K1 is rapidly dephosphorylated (i.e., within minutes) of exposure to the drug.

They continue:

Conditions that inhibit growth, such as decreased energy, low oxygen, and insufficient nutrients, are associated with the harsh microenvironment of poorly vascularized tumor. The ability of cancer cells to overcome these adverse conditions would promote tumor growth, putting the

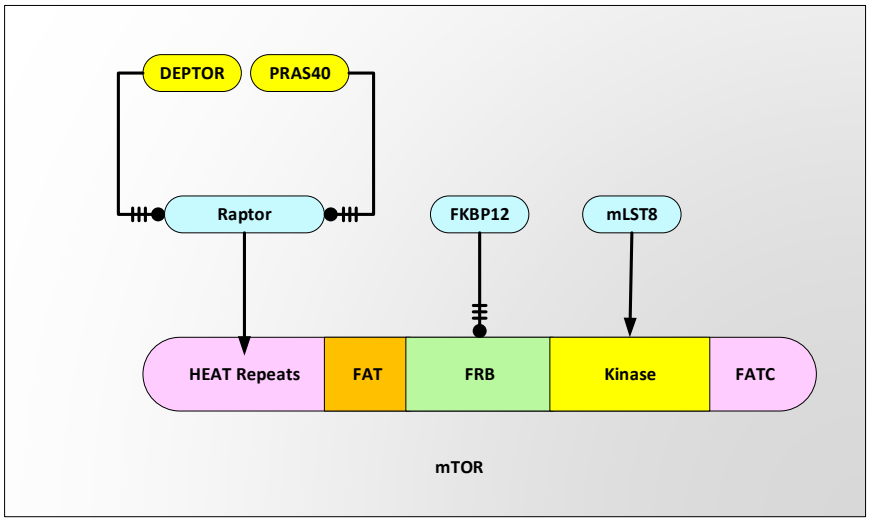
desensitization of mTORC1 signaling in the spotlight as a potential mechanism cancer cells could exploit to enhance their viability. Whether mutations in the amino acid– and glucose-sensing pathway that activates mTORC1 exist in cancer is not known. Mutations in the growth factor inputs to mTORC1 are prominent in cancer.

For example, mutations causing loss of PTEN function or oncogenic activation of PI3K or AKT are associated with many aggressive human cancers (Table 12-1).17-20 The findings that AKT promotes mTORC1 activity through TSC and PRAS40 suggest that cancers with elevated PI3K-AKT signaling may in part thrive because of an mTORC1-driven growth advantage. Activation of PI3K-AKT signaling also facilitates nutrient uptake by cells, which indirectly contributes to mTORC1 activity by localizing mTORC1 to lysosomes.

Therefore, understanding the contribution and relevance of mTORC1 signaling in the progression of cancers with aberrant PI3K-AKT signaling is an important area of research.

5.3.1 mTORC1

As we noted earlier mTORC1 has the most significant set of impacts on cell stability. Also as we noted there are upstream and downstream influences generated by this complex. We start with the structure of the mTORC1 complex as noted below:



The mTOR protein is composed of five sections, including the kinase element. The HEAT Repeats, as noted by Neuwald and Hirano are:

HEAT repeats correspond to tandemly arranged curlicue-like structures that appear to serve as flexible scaffolding on which other components can assemble. Using sensitive sequence analysis techniques we detected HEAT repeats in various chromosome-associated proteins, including four families of proteins associated with condensins and cohesins, which are nuclear complexes that contain structural maintenance of chromosome (SMC) proteins.

RAPTOR is the regulatory associated protein of mTOR[51]. RAPTOR is an mTOR binding protein. As Saxton and Sabatini have noted:

In order to grow and divide, cells must increase production of proteins, lipids, and nucleotides while also suppressing catabolic pathways such as autophagy. mTORC1 plays a central role in regulating all of these processes and therefore controls the balance between anabolism and catabolism in response to environmental conditions... the critical substrates and cellular processes downstream of mTORC1 and how they contribute to cell growth.

Most of the functions discussed here were identified and characterized in the context of mammalian cell lines, while the physiological context in which these processes are important will be discussed in greater detail below.

Protein Synthesis mTORC1 promotes protein synthesis largely through the phosphorylation of two key effectors, p70S6 Kinase 1 (S6K1) and eIF4E Binding Protein (4EBP). mTORC1 directly phosphorylates S6K1 on its hydrophobic motif site, Thr389, enabling its subsequent phosphorylation and activation by PDK1.

S6K1 phosphorylates and activates several substrates that promote mRNA translation initiation, including eIF4B, a positive regulator of the 50cap binding eIF4F complex. S6K1 also phosphorylates and promotes the degradation of PDCD4, an inhibitor of eIF4B, and enhances the translation efficiency of spliced mRNAs via its interaction with SKAR, a component of exon-junction complexes.

The mTORC1 substrate 4EBP is unrelated to S6K1 and inhibits translation by binding and sequestering eIF4E to prevent assembly of the eIF4F complex. mTORC1 phosphorylates 4EBP at multiple sites to trigger its dissociation from eIF4E, allowing 50cap-dependent mRNA translation to occur.

Although it has long been appreciated that mTORC1 signaling regulates mRNA translation, whether and how it affects specific classes of mRNA transcripts has been debated. Global ribosome footprinting analyses, however, revealed that, while acute mTOR inhibition moderately suppresses general mRNA translation, it most profoundly affects mRNAs containing pyrimidine-rich 50 TOP or ''TOP-like'' motifs, which includes most genes involved in protein synthesis

Now the upstream influencers or drivers are detailed below from Seeboeck et al:

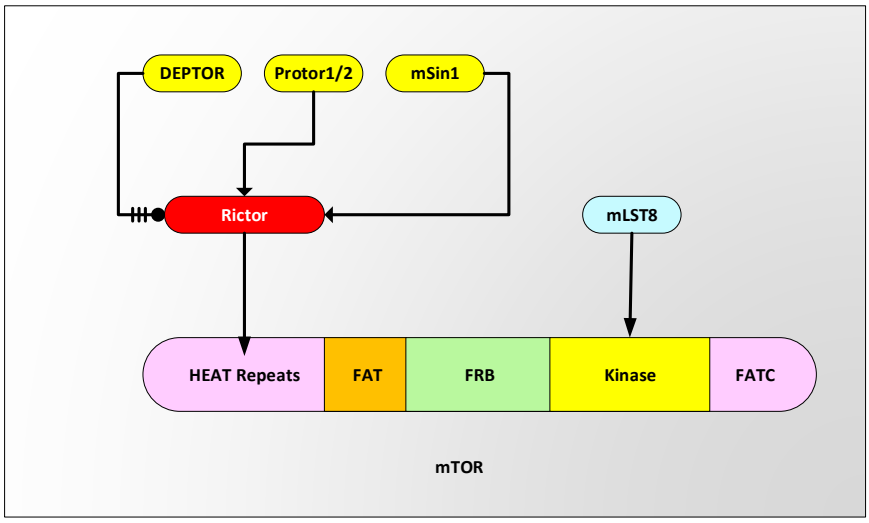
[51] https://www.ncbi.nlm.nih.gov/gene/57521

mTORC1 Upstream	
Rapamycin	rapamycin
FKBP12	FK506-binding protein 12 kDa
TSC	tuberous sclerosis complex
Rheb	Ras homolog enriched in brain
IGF-1 pathway	insulin/insulin like growth factor
AKT	AKT serine/threonine kinase
mTORC2	promotes dissociation of PRAS40 from mTORC1.
Wnt	Wnt
TNFα 1	tumor necrosis factor α
AMPK	5'-AMP-activated protein kinase
REDD1	regulated in development and DNA damage responses 1

The above each in their own manner effects the actions of mTORC1. Rapamycin is a major driver when present. Some of these are exogenous to the cell itself such as the growth factors and others are part of the cell normal pathway. Note that mTORC2 has a driving factor as well. We shall briefly explore that next.

5.3.2 mTORC2

Now we consider mTORC2. From Seeboeck et al the structure appears as below:



Rictor is akin to Raptor. We see the same underlying mTOR base elements and then the complex binding to create the multiprotein complex. Now the drivers or upstream elements are shown below. Like mTORC1, it also is a driver here.

mTORC2 Upstream	
Rapamycin	rapamycin
FKBP12	FK506-binding protein 12 kDa
PIP3	Phosphatidylinositol (3,4,5)-trisphosphate
AKT	AKT serine/threonine kinase
mTORC1	Negative feedback loop between mTORC1 and insulin/PI3K signaling

Saxton and Sabatini have noted the downstream effects of mTORC2:

While mTORC1 regulates cell growth and metabolism, mTORC2 instead controls proliferation and survival primarily by phosphorylating several members of the AGC (PKA/PKG/PKC) family of protein kinases.

The first mTORC2 substrate to be identified was PKCa, a regulator of the actin cytoskeleton. More recently, mTORC2 has also been shown to phosphorylate several other members of the PKC family, including PKCd, PKCz, as well as PKCg and PKCε, all of which regulate various aspects of cytoskeletal remodeling and cell migration.

The most important role of mTORC2, however, is likely the phosphorylation and activation of Akt, a key effector of insulin/ PI3K signaling.

Once active, Akt promotes cell survival, proliferation, and growth through the phosphorylation and inhibition of several key substrates, including the FoxO1/3a transcription factors, the metabolic regulator GSK3b, and the mTORC1 inhibitor TSC2.

However, while mTORC2- dependent phosphorylation is required for Akt to phosphorylate some substrates in vivo, such as FoxO1/3a, it is dispensable for the phosphorylation of others, including TSC2. Finally, mTORC2 also phosphorylates and activates SGK1, another AGC-kinase that regulates ion transport as well as cell survival.

The mTORC1-dependent shift toward increased anabolism should only occur in the presence of pro-growth endocrine signals as well as sufficient energy and chemical building blocks for macromolecular synthesis. In mammals, these inputs are largely dependent on diet, such that mTORC1 is activated following feeding to promote growth and energy storage in tissues such as the liver and muscle but inhibited during fasting conserve limited resources. Here, we discuss the cellular pathways upstream of mTORC1 and the mechanisms through which they control mTORC1 activation.

Many have targeted mTOR in a therapeutic sense but progress seem limited.

5.4 REDD1

REDD1 is an interesting gene in the context of PCa and IGF. From Chang et al:

*REDD1 (**REgulated in Development and DNA Damage response 1**, also known as RTP801/Dig1/DDIT4) was first identified in 2002.*

It is a stress related protein induced by hypoxia and multiple DNA damage stimuli and is expressed broadly in many human tissues . The gene is located at human chromosome 10q24.33 and is homologous to two Drosophila melanogaster genes of unknown function, Scylla and Charybde, which are designated as Hox targets in the National Institutes of Health genetic sequence database GenBank. As a potent repressor of the mechanistic target of rapamycin in complex 1 (mTORC1), REDD1 regulates cell growth, tumorigenesis, cell aging, and autophagy

Talty and Olino in examining the impact of the innate immune system also reflect on metformin. They note:

Several ongoing clinical trials focus on combining metabolism-targeted agents with immunotherapy treatments. Many of these trials exploit the use of previously approved drugs such as metformin and rosiglitazone which are used in the treatment of diabetes and alter downstream metabolic pathways. For example, metformin decreases peripheral insulin resistance by inhibiting mitochondrial respiration and activating AMPK.

Activated AMPK inhibits metabolic processes such as gluconeogenesis and lipogenesis and stimulates glucose uptake and fatty acid oxidation thus affecting additional pathways tied in with immunometabolism.

Metformin can also target mTOR, insulin-like growth factor, and mitogen-activated protein kinase (MAPK) pathways. In various preclinical studies, metformin has been shown to potentiate antitumor immunity more directly by promoting STING and Hippo signaling, PD-L1 degradation, and a reduction in tumor hypoxia. Rosiglitazone activates PPARγ and has had similar preclinical results.

Kim et al (2023) have noted:

Regulated in development and DNA damage-response 1 (REDD1) is a stress-induced protein that controls various cellular functions, including metabolism, oxidative stress, autophagy, and cell fate, and contributes to the pathogenesis of metabolic and inflammatory disorders, neurodegeneration, and cancer.

REDD1 usually exerts deleterious effects, including tumorigenesis, metabolic inflammation, neurodegeneration, and muscle dystrophy; however, it also exhibits protective functions by regulating multiple intrinsic cell activities through either an mTORC1-dependent or -independent mechanism. REDD1 typically regulates mTORC1 signaling, NF-κB activation, and cellular pro-oxidant or antioxidant activity by interacting with 14-3-3 proteins, IκBα, and thioredoxin-interacting protein or 75 kDa glucose-regulated protein, respectively.

The diverse functions of REDD1 depend on cell type, cellular context, interaction partners, and cellular localization (e.g., mitochondria, endomembrane, or cytosol). …

mTORC1 promotes anabolic metabolism and tumor progression, and rapamycin analogs inhibit the growth of several tumor derived cell lines in vitro and in vivo. In this respect, REDD1 shows antitumor activity and is likely downregulated in tumors. REDD1 expression is reduced in human breast and pancreatic cancer specimens compared to that in patient matched normal tissues, indicating that REDD1 suppresses tumor growth and metastasis.

REDD1 inhibits cancer initiation and progression, as evidenced by an increase in tumorigenesis, tumor growth, and metastasis of immortalized Redd1-deficient cells or Redd1-knockdown KrasG12D/+ pancreatic neoplasms in mouse models.

The tumorigenicity of Redd1-deficient cells is dependent on mTORC1 activation and mitochondrial ROS production. Human hepatocellular carcinomas (HCCs) with inactive Tsc2 mutations exhibit more aggressive tumor behavior in patients, and Tsc2 mutation-bearing HCCs are more sensitive to rapamycin in patient-derived tumor xenograft models. These findings suggest that REDD1 downregulation promotes tumor progression by stimulating mTORC1-mediated tumor cell proliferation or by increasing the levels of mitochondrial ROS as a regulator of HIF-1-dependent tumorigenic metabolism.

Therefore, upregulation of REDD1 by treatment with various chemotherapeutic drugs has been associated with decreased viability of breast cancer cells. In contrast, REDD1 is upregulated in various types of cancers, such as myeloid leukemia, glioblastomas, carcinomas, gastric cancers, and breast cancers, resulting in poor prognosis, aggressive malignancy, and reduced overall and disease-free survival in cancer patients.

Furthermore, a meta-analysis showed that high levels of REDD1 were associated with a worse prognosis in acute myeloid leukemia, breast cancer, glioblastoma, and colon and lung cancer but, in contrast, better prognosis in gastric cancer. These results suggest that REDD1 exhibits either oncogenic or tumor-suppressive functions, depending on the cell type and cellular context.

Redd1 deficiency reprograms lipid metabolism to drive the invasion and metastasis of Ras-mutant tumors in mice. Furthermore, decreased REDD1 levels can predict poor patient survival specifically in Ras-mutant lung and pancreatic carcinomas. The tumor microenvironment is composed of various nonmalignant cell types, including tumor-associated macrophages (TAMs) and endothelial cells (TECs), which play important roles in tumor progression. Redd1-deficient TAMs enhances glucose uptake and glycolysis via mTORC1-dependent GLUT1 upregulation, resulting in low glucose availability and quiescence in TECs; quiescent TECs maintain vascular integrity, thus inhibiting metastasis.

Treatment with low-dose doxorubicin or cisplatin elevates REDD1 expression and reduces mTORC1-dependent translation of VEGF receptor-2/3 and eNOS in TECs and endothelial progenitor cells, all of these effects are inhibited in Redd1-deficient cells, resulting in suppressed tumor angiogenesis and lymphangiogenesis, thereby inhibiting tumor growth and metastasis.

Overall, although some results are debated, REDD1 shows cell type-specific functions in inhibiting tumorigenesis, tumor progression, and metastasis.

6 OBSERVATIONS

We now make some observations based upon the results presented from the literature. Some of these issues we have previously addressed in other contexts.

6.1 MULTI THERAPEUTIC TARGETING THERAPIES MAY HAVE SUBSTANTIAL ADVANTAGES

Just targeting IGF-1 is a possible strategy but as is well known in oncology the use of multiple therapeutics often enhance the overall effect. Thus IGF-1 targeting along with other approaches has substantial merit.

6.2 STEM CELLS MAY BE PRESENT AND MAY BE MULTI-VARIANT

Cancer stem cells have been posited and examined in multiple environments including PCa. There actually may be multiple and disparate PCa stem cells and thus single targeting ma have limited merit. Thus understanding the stem cell genomic dynamics is essential.

6.3 GENETIC PROFILES OF INDIVIDUAL CELLS IS A CRITICAL STEP IN ANALYSIS

We have been observing the increased use of single cell genomic profiling. The ability to do so has become common place but the analytical tools to assess the therapeutic usefulness is still a work in progress. IGF-1 may be useful in a large selection of such cells but not necessarily in all.

6.4 THE INTERACTION OF POOR GLUCOSE CONTROL AND INCREASED PRESENCE OF PCA SEEM TO HAVE MERIT BUT NEEDS FURTHER CLINICAL RESULTS.

Diabetic patients often have earlier and more aggressive PCa as well as other malignancies. There relationship between IGF-1 and insulin and glucose presents an appealing yet unsubstantiated nexus. It may be that for example T2 diabetes patients have other significant co-morbidities that drive the process. Details clinically are essential.

6.5 THE CHOICE OF IMMUNE TARGETING OR MAB INHIBITION IS PERHAPS UNNECESSARY AND BOTH MAY BE APPLIED SIMULTANEOUSLY.

Multi-therapeutic approaches are now quite common. Thus the use of a mAb and immune targeting may be of significant merit. Namely the mAb blocks receptors while the immune approach targets other ligands specific for that malignancy.

6.6 AS MORE LIGANDS AND PATHWAYS ARE ATTACKED TO STOP PROLIFERATION, WHAT ARE THE POTENTIALS FOR ADVERSE EVENTS? NAMELY CAN THEY BE REDUCED BECAUSE OF THE MORE PRECISE ATTACKING?

Adverse event are the bane of new therapeutics. The classic case is that of CAR T cells and cytokine storms. However with multi ligand targeting using polyclonal Ab one may get highly cell specific targeting.

6.7 IGF-1 HAS BEEN ALLEGED TO BE A MARKER AS WELL AS A TARGET. CAN IGF-1 AND PSA, ALONG WITH OTHER MARKERS BECOME VIABLE DIAGNOSTIC AND/OR PROGNOSTIC TOOLS?

There is an ongoing issue of diagnostic and prognostic markers. PSA still dominates. As we noted IGF-1 is putatively another marker. However we do not have the metrics on how best to use these in tandem in a proven clinical setting.

6.8 THERE ARE CLEARLY A LARGE NUMBER OF TARGET AND TARGETABLE GENES THAT CAN BE USED FOR THERAPEUTICS. THE QUESTION IS WHICH SET AND HOW LARGE A SET CAN RESULT IN MAXIMUM EFFICACY WITH MINIMAL ADVERSE EFFECTS?

The appendix listing the genes and their products we have examined herein lists well over 100 possible targets. It would be a useful exercise to delineate each and their efficacy and what therapeutic is currently available to enhance or suppress. They then would commence to a set of combinatorial tests as well.

7 REFERENCES

1. Aaronson, Growth Factors and Cancer, Science, Vol 254 Nov 1991.
2. Abbas et al, Basic Immunology, 4th Ed, Elsevier, 2014
3. Aguirre et al, Insulin-like growth factor-1 deficiency and metabolic syndrome, J Transl Med (2016) 14:3
4. Ahmed, H, Promoter Methylation in Prostate Cancer and its Application for the Early Detection of Prostate Cancer Using Serum and Urine Samples, Biomarkers in Cancer 2010:2.
5. Ahn et al, Current Status and Application of Metformin for Prostate Cancer: A Comprehensive Review, Inter Jrl Mol Sci, 12 Nov 2020
6. Akoto and Saini, Role of Exosomes in Prostate Cancer Metastasis, Molecular Sciences, 29 March 2021
7. Albany and Hahn, Heat shock and other apoptosis-related proteins as therapeutic targets in prostate cancer, Asian Journal of Andrology (2014) 16, 359–363
8. Aghanoori et al, Insulin-like growth factor-1 activates AMPK to augment mitochondrial function and correct neuronal metabolism in sensory neurons in type 1 diabetes, Molecular Metabolism 20 (2019) 149e165
9. Alimirah et al, Expression of Androgen Receptor Is Negatively Regulated By p53, Neoplasia, Vol. 9, No. 12, December 2007, pp. 1152 – 1159
10. Alimoradi et al, How metformin affects various malignancies by means of microRNAs: a brief review, Cancer Cell Int (2021) 21:207
11. Allott, et al, Postoperative statin use and risk of biochemical recurrence following radical prostatectomy: results from the Shared Equal Access Regional Cancer Hospital (SEARCH) database, BJU International, Volume 114, Issue 5, pages 661–666, November 2014
12. Al-Samerria and Radovick, The Role of Insulin-like Growth Factor-1 (IGF-1) in the Control of Neuroendocrine Regulation of Growth, Cells, 2021
13. An, J., et al, Destruction of Full-Length Androgen Receptor by Wild-Type SPOP, but Not Prostate-Cancer-Associated Mutants, Cell Rep. 2014 February 27; 6: 657–669
14. Armstrong, L., Epigenetics, Garland (New York) 2013.
15. Azzouni et al, The 5 Alpha-Reductase Isozyme Family: A Review of Basic Biology and Their Role in Human Diseases, Advances in Urology, Volume 2012, Article ID 530121
16. Baldo, P., et al, mTOR Pathway and mTOR inhibitors as Agents for Cancer Therapy, 2008, Curr Can Drug Targets, pp 647-668.
17. Bao et al, TGF-β2 induces proliferation and inhibits apoptosis of human Tenon capsule fibroblast by miR-26 and its targeting of CTGF, Biomedicine & Pharmacotherapy, Volume 104, August 2018, Pages 558-565, open access

18. Barbieri, C. et al, Molecular genetics of prostate cancer: emerging appreciation of genetic complexity, Histopathology 2012, 60, 187–198.

19. Barbieri, C., et al, Exome Sequencing Identifies Recurrent SPOP, FOXA1 and MED12 Mutations in Prostate Cancer, Nature Genetics (2012).

20. Bass, A., et al, Complete Polarization of Single Intestinal Epithelial Cells upon Activation of LKB1 by STRAD, Cell, Vol. 116, 457–466, February 6, 2004

21. Bauer, A., C. Stratakis, The lentiginoses: cutaneous markers of systemic disease and a window to new aspects of tumourigenesis, J Med Genet 2005;42:801–810. doi: 10.1136

22. Bawa-Khalfe, T., et al, Differential expression of SUMO-specific protease 7 variants regulates epithelial–mesenchymal transition, PNAS, October 23, 2012, vol. 109, no. 43

23. Ben-Salem et al, Novel insights in cell cycle dysregulation during prostate cancer progression, https://erc.bioscientifica.com, https://doi.org/10.1530/ERC-20-0517, 2021

24. Berger, M., et al, The genomic complexity of primary human prostate cancer, Nature 470, 214–220 (10 February 2011)

25. Birzniece et al, Insulin-like growth factor role in determining the anti-cancer effect of metformin: RCT in prostate cancer patients, Endocrine Connections, (2022) 11, e210375

26. Bolton et al, Cell- and gene-specific regulation of primary target genes by the androgen receptor, Genes & Development 21:2005–2017, 2007

27. Bonollo et al, The Role of Cancer-Associated Fibroblasts in Prostate Cancer Tumorigenesis, Cancers, 13 July 2020

28. Botehlo et al, Vascular Endothelial Growth Factor (VEGF) and Prostate Pathology, International Braz J Urol Vol. 36 : 430-438, July - August, 2010

29. Brooks, Cell Cycle Commitment and the Origins of Cell Cycle Variability, Frontiers in Cell and Developmental Biology, July 2021, Volume 9, Article 698066

30. Brooks, J., et al, CG Island Methylation Changes Near the GSTP1 Gene in Prostatic Intraepithelial Neoplasia, Cancer Epidemiology, Biomarkers & Prevention , Vol. 7, 531-536, June 1998

31. Bunz, Principles of Cancer Genetics, Springer, 2022

32. Burotto et al, The MAPK pathway across different malignancies: A new perspective, Cancer. 2014 November 15; 120

33. Cantley et al, Signal Transduction, CSHL Press, 2014

34. Carpenter and Cohen, Epidermal Growth Factor, The Journal Of Biological Chemistry, Vol. 265, No. 14, Issue of May 15, pp. 7709-7712.1990

35. Casalou et al, The Role of ARF Family Proteins and Their Regulators and Effectors in Cancer Progression: A Therapeutic Perspective, Frontiers in Cell and Developmental Biology, April 2020, Volume 8, Article 217

36. Chan K, at al. The statins as anticancer agents. Clin Cancer Res 2003; 9: 10–19.\

37. Chang et al, Overexpression of the recently identified oncogene REDD1 correlates with tumor progression and is an independent unfavorable prognostic factor for ovarian carcinoma. Diagn Pathol 13, 87 (2018).

38. Chappell et al, p53 expression controls prostate cancer sensitivity to chemotherapy and the MDM2 inhibitor Nutlin-3, Cell Cycle Volume 11 Issue 24, 2012

39. Chen et al, PDGF-D promotes cell growth, aggressiveness, angiogenesis and EMT transformation of colorectal cancer by activation of Notch1/Twist1 pathway, Oncotarget, 2017, Vol. 8, (No. 6), pp: 9961-9973

40. Chen Y., J. Jiang, Decoding the phosphorylation code in Hedgehog signal transduction, Cell Research (2013) 23:186–200; doi:10.1038/cr.2013.10

41. Chesire, D., W. Isaacs, β-Catenin signaling in prostate cancer: an early perspective, Endocrine-Related Cancer (2003) 10 537–560.

42. Chopra et al, Activation of p53 and destabilization of androgen receptor by combinatorial inhibition of MDM2 and MDMX in prostate cancer cells, Oncotarget, 2018, Vol. 9, (No. 5), pp: 6270-6281

43. Ciocca et al, Heat shock proteins in prostate cancer: from tumorigenesis to the clinic, Int. J. Hyperthermia, December 2010; 26: 737–747

44. Danzig, et al, Synergism between metformin and statins in modifying the risk of biochemical recurrence following radical prostatectomy in men with diabetes, Prostate Cancer and Prostatic Disease (2015) 18, 63–68

45. Davey and Grossman, Androgen Receptor Structure, Function and Biology: From Bench to Bedside, Clin Biochem Rev 37 2016

46. Dayeh, T., et al, Genome-Wide DNA Methylation Analysis of Human Pancreatic Islets from Type 2 Diabetic and Non-Diabetic Donors Identifies Candidate Genes That Influence Insulin Secretion, March 2014, Volume 10, Issue 3.

47. Dehkhoda et al, The Growth Hormone Receptor: Mechanism of Receptor Activation, Cell Signaling, and Physiological Aspects, Frontiers in Endocrinology, Feb 2018, Vol 9

48. Delma, Three May Be Better Than Two: A Proposal for Metformin Addition to PI3K/Akt Inhibitor-antiandrogen Combination in Castration-resistant Prostate Cancer, Cureus 10, 2018

49. Deutsch et al, Environmental, genetic, and molecular features of prostate cancer, The Lancet Oncology Vol 5 May 2004

50. DiMaio and Scagliotti, The lesson learned from figitumumab clinical program and the hope for better results in squamous lung cancer, Transl Lung Cancer Res 2015;4:15-17

51. Donkena, K., et al, Oxidative Stress and DNA Methylation in Prostate Cancer, Hindawi Publishing Corporation, Obstetrics and Gynecology International, Volume 2010.

52. Dubey et al, Heat shock proteins: a therapeutic target worth to consider, Veterinary World 8: 46-51, 2015

53. Easton, J., P. Houghton, mTOR and Cancer Therapy, Oncogene, 2006, pp 6436-6446.

54. Eisermann et al, Androgen up-regulates vascular endothelial growth factor expression in prostate cancer cells via an Sp1 binding site, Molecular Cancer 2013, 12:7

55. Elmore, Apoptosis: A Review of Programmed Cell Death, Toxicol Pathol. 2007 ; 35: 495–516.

56. Epstein, Biopsy Interpretation of the Prostate, 6th Ed, Wolters Kluwer, 2021

57. Fan and Zong, The cellular decision between apoptosis and autophagy, Chin J Cancer; 2013; Vol. 32 Issue 3

58. Feldman and Feldman, The Development Of Androgen-Independent Prostate Cancer, Nature Reviews, Cancer Volume , October 2001

59. Feng and He, Androgen Receptor Signaling in the Development of Castration-Resistant Prostate Cancer, Frontiers in Oncology, September 2019, Volume 9

60. Fizazi et al, Nonmetastatic, Castration-Resistant Prostate Cancer and Survival with Darolutamide, NEJM, 2020;383:1040-9

61. Francis, J., et al, b-Catenin Is Required for Prostate Development and Cooperates with Pten Loss to Drive Invasive Carcinoma, PLOS Genetics, www.plosgenetics.org , 1 January 2013,| Volume 9, Issue 1, e1003180.

62. Friedrich et al, The Association Between IGF-I and Insulin Resistance, Diabetes Care 35:768–773, 2012

63. Fryczkowski et al, Circulating Levels of Omentin, Leptin, VEGF, and HGF and Their Clinical Relevance with PSA Marker in Prostate Cancer, Disease Markers Volume 2018, Article ID 3852401, 9 pages

64. Fujita and Nonomura, Role of Androgen Receptor in Prostate Cancer: A Review, World J Mens Health 2019 Sep 37: 288-295

65. Galbraith et al, CDK8, Transcription 2010

66. Galbraith et al, HIF1A Employs CDK8-Mediator to Stimulate RNAPII Elongation in Response to Hypoxia, Cell, 153, 1327–1339, June 6, 2013

67. Gao and Wang, Subcellular Localization of p44/WDR77 Determines Proliferation and Differentiation of Prostate Epithelial Cells, PLOS ONE, November 2012, Volume 7, Issue 11

68. Gelman et al, Molecular Oncology, Cambridge, 2014

69. Geng, C., et al, Androgen receptor is the key transcriptional mediator of the tumor suppressor SPOP in prostate cancer, Cancer Res. 2014 October 1; 74: 5631–5643

70. Glick et al, Autophagy: cellular and molecular mechanisms, J Pathol. 2010 May ; 221: 3–12

71. Gonzalgo, M., et al, Prostate Cancer Detection by GSTP1 Methylation Analysis of Post biopsy Urine Specimens, Clin Cancer Res 2003; 9, 2673-2677.

72. Goodman, Medical Cell Biology, Academic, 2022

73. Gordon, M., et al, RASSF1 Polymorphisms in Cancer, Molecular Biology International, Volume 2012, Article ID 365213, 12 pages

74. Green, Cell Death, CSHL Press, 2018

75. Gundem et al, The evolutionary history of lethal metastatic prostate cancer, Nature 2015. doi:10.1038/nature14347

76. Gupta and Massagué, Cancer Metastasis: Building a Framework, Cell 127, November 17, 2006

77. Hakuno and Takahashi, IGF1 receptor signaling pathways, Journal of Molecular Endocrinology, 2018

78. Hanna, C., G. Kelsey, The specification of imprints in mammals, Heredity (2014) 113, 176–183.

79. Hao, Q., W. Cho, Battle Against Cancer: An Everlasting Saga of p53, Int. J. Mol. Sci. 2014, 15, 22109-22127

80. Hart, J., et al, Essential role of Stat3 in PI3K-induced oncogenic transformation, PNAS,| August 9, 2011,| vol. 108,| no. 32,| 13247–13252 MEDICAL SCIENCES

81. Hartnett, L., L. Egan, Inflammation, DNA methylation and colitis-associated cancer, Carcinogenesis vol.0 no.0 pp.1–9, 2012.

82. Hay et al, An Sp1 Modulated Regulatory Region Unique to Higher Primates Regulates Human Androgen Receptor Promoter Activity in Prostate Cancer Cells, PLOS ONE, October 8, 2015

83. Hayat, Autophagy, Vol 1, Academic, 2014

84. He, T., et al, Identification of c-MYC as a Target of the APC Pathway, Science, 281, 1509 (1998).

85. Heinlein and Chang, Androgen Receptor in Prostate Cancer, Endocrine Reviews 25:276–308, 2004

86. Hotchkiss, R., L. Moldawer, Parallels between Cancer and Infectious Disease, NEJM, 371;4, July 24, 2014.

87. Hua et al, Targeting mTOR for cancer therapy, Journal of Hematology & Oncology (2019) 12:71

88. Huang and Oliff, Signaling pathways in apoptosis as potential targets for cancer therapy, Trends in Cell Biology Vol.11 No.8 August 2001

89. Huang et al, Functional mapping of androgen receptor enhancer activity, bioRxiv preprint doi: https://doi.org/10.1101/2020.08.18.255232 . this version posted August 18, 2020

90. Huang, S., Regulation of Metastases by Signal Transducer and Activator of Transcription 3 Signaling Pathway: Clinical Implications, Clin Cancer Res March 1, 2007 13; 1362.

91. Jaiswal, J., S. Naryan, p53-dependent Transcriptional Regulation of the APC Promoter in Colon Cancer Cells Treated with DNA Alkylating Agents, The Journal Of Biological Chemistry Vol. 276, No. 21, Issue of May 25, pp. 18193–18199, 2001

92. Jin et al, Androgen receptor genomic regulation, Transl Androl Urol 2013;2:158-177

93. Justice et al, A geroscience perspective on immune resilience and infectious diseases: a potential case for metformin, GeroScience, 2020

94. Kalinska et al, Kallikreins - the melting pot of activity and function, Biochimie. 2016 March ; 122: 270–282

95. Karaman et al, Vascular endothelial growth factor signaling in development and disease, Development (2018) 145

96. Katayiar et al, REDD1, an inhibitor of mTOR signalling, is regulated by the CUL4A–DDB1 ubiquitin ligase, EMBO reports Vol 10 | No 8 | 2009

97. Kim et al, Current Status and Future Perspectives of Androgen Receptor Inhibition Therapy for Prostate Cancer: A Comprehensive Review, Biomolecules, 2021, 11,492

98. Kim et al, Src kinases as therapeutic targets for cancer, Nature Reviews Clinical Oncology volume 6 , pages 587–595 (2009)

99. Kim et al, The stress-responsive protein REDD1 and its pathophysiological functions, Experimental & Molecular Medicine volume 55, pages 1933–1944 (2023

100. Kinkaide, C., et al, Targeting Akt/mTOR and ERK MAPK Signalling Inhibits Hormone Refractory Prostate Cancer in Preclinical Mouse Model, Jrl Clin Inv 2008, 99 3051-3064.

101. Kirk, P., et al, Systems biology (un)certainties, Science, 23 October 2015 • VOL 350 ISSUE 6259

102. Kish et al, The Expression of Proto-Oncogene ETS-Related Gene (ERG) Plays a Central Role in the Oncogenic Mechanism Involved in the Development and Progression of Prostate Cancer, Int. J. Mol. Sci. 2022, 23, 4772.

103. Knudsen and Knudsen, Tailoring to RB: tumour suppressor status and therapeutic response, Nat Rev Cancer. 2008 September ; 8: 714–724.

104. Knura et al, The Influence of Anti-Diabetic Drugs on Prostate Cancer, Cancers, 2021, 13, 1827

105. Kokal et al, Mechanisms of Androgen Receptor Agonist- and Antagonist-Mediated Cellular Senescence in Prostate Cancer, Cancers, 8 July 2020

106. Kuen et al, IL-17-Producing Cells in Tumor Immunity: Friends or Foes?, Immune Netw. 2020 Feb;20:e6

107. Kumar and Asthana, Autophagy and Metabolism, Academic, 2022

108. Kundu, J., Y. Surh, Inflammation: Gearing the journey to cancer, Mutation Research 659 (2008) 15–30.

109. Kung and Weber, It's Getting Complicated—A Fresh Look at p53-MDM2-ARF Triangle in Tumorigenesis and Cancer Therapy, Frontiers in Cell and Developmental Biology. January 2022, Volume 10, Article 818744

110. Kurelac et al, he multifaceted effects of metformin on tumor microenvironment, Seminars in Cell and Developmental Biology 98 (2020) 90–97

111. Kyprianou, Molecular exploitation of Apoptosis Pathways in Prostate Cancer, Imperial College Press, 2012

112. Laborde, E., Glutathione transferases as mediators of signaling pathways involved in cell proliferation and cell death, Cell Death and Differentiation (2010) 17, 1373–1380.

113. Lam, E., et al, FOXO Transcription Factors, Biochem Soc Trans 2006, pp 722-726.

114. Lambert et al, The Human Transcription Factors, Cell 172, February 8, 2018

115. Lang et al, Combined targeting of Arf1 and Ras potentiates anticancer activity for prostate cancer therapeutics, Journal of Experimental & Clinical Cancer Research (2017) 36:112

116. Laron, Insulin-like growth factor 1 (IGF-1): a growth hormone, J Clin Pathol: Mol Pathol 2001;54:311–316

117. Larsson et al, The functional interlink between AR and MMP9/VEGF signaling axis is mediated through PIP5K1α/pAKT in prostate cancer, Int. J. Cancer: 146, 1686–1699 (2020)

118. Lawrence et al, Kallikreins on Steroids: Structure, Function, and Hormonal Regulation of Prostate-Specific Antigen and the Extended Kallikrein Locus,

119. Linehan and Ricketts, The Metabolic Basis of Kidney Cancer, Semin Cancer Biol. 2013 February ; 23: 46-55.

120. Linehan et al, The genetic basis of kidney cancer: a metabolic disease, Nat Rev Urol. 2010 May ; 7: 277–285

121. Litwack, Human Biochemistry, Academic (New York) 2018, pp 515-543

122. Liu et al, A comprehensive analysis of coregulator recruitment, androgen receptor function and gene expression in prostate cancer, eLife 2017;6:e28482

123. Liu et al, The role of CDC25C in cell cycle regulation and clinical cancer therapy: a systematic review, Cancer Cell Int (2020) 20:213

124. Liu, A., et al, Correlated Alterations in Prostate Basal Cell Lauer and Basement Membrane, Int Jrl Bio Sci, V 5, 2009, pp 276-285.

125. Liu, Q., et al, Metformin Inhibits Prostate Cancer Progression by Targeting Tumor-Associated Inflammatory Infiltration, Clin Cancer Res; 24 November 15, 2018

126. LoPiccolo, J., et al, Targeting the PI3K/Akt/mTOR Pathway, Drug Res Up, 2008, pp 32-50.

127. Loubiere et al, Metformin-induced energy deficiency leads to the inhibition of lipogenesis in prostate cancer cells, Oncotarget, Vol. 6, No. 17, March 10, 2015

128. Lu et al, ARF Represses Androgen Receptor Transactivation in Prostate Cancer, Mol Endocrinol. 2013 Apr; 27: 635–648

129. Lu et al, Emerging Role of IGF-1 in Prostate Cancer: A Promising Biomarker and Therapeutic Target, Cancers 2023, 15, 1287

130. Lum and Beachy, The Hedgehog Response Network: Sensors, Switches, and Routers, Science Vol 304 18 June 2004

131. Macaulay et al, A Phase Ib/II study of IGF-neutralising antibody xentuzumab with enzalutamide in metastatic castration-resistant prostate cancer, British Journal of Cancer (2023) 129:965 – 973

132. Maitland, Resistance to Antiandrogens in Prostate Cancer: Is It Inevitable, Intrinsic or Induced?, Cancers 2021, 13, 327

133. Mancarella et al, Insulin-like growth factor 1 receptor affects the survival of primary prostate cancer patients depending on TMPRSS2-ERG status, BMC Cancer (2017) 17:367

134. Mancini et al, MDM4 (MDMX) localizes at the mitochondria and facilitates the p53-mediated intrinsic-apoptotic pathway, The EMBO Journal (2009) 28, 1926–1939.

135. Mansor et al, IGF-1 and hyperglycaemia-induced FOXA1 and IGFBP-2 affect epithelial to mesenchymal transition in prostate epithelial cells, Oncotarget, 2020, Vol. 11, (No. 26), pp: 2543-2559 Correction: Oncotarget, 2023, Vol. 14, pp: 44-46

136. Mantzoros et al, Insulin-like growth factor 1 in relation to prostate cancer and benign prostatic hyperplasia, BMJ, November 1997

137. Marcias, E., et al, Role of Stat3 in Skin Carcinogenesis: Insights Gained from Relevant Mouse Models, Journal of Skin Cancer Volume 2013 (2013), Article ID 684050, 10 pages

138. Margel, D., et al, Metformin Use and All-Cause and Prostate Cancer Specific Mortality Among Men With Diabetes, JCO Sep 1, 2013:3069-3075; DOI:10.1200/JCO.2012.46.7043.

139. Marks, F., et al, Cellular Signal Processing, 2nd Ed, Garland (New York), 2017

140. Marks, F., et al, Cellular Signal Processing, Garland (New York) 2009.

141. Martinez-Marin et al, PEDF increases the tumoricidal activity of macrophages towards prostate cancer cells in vitro, PLOS ONE, April 2017

142. Matsushita et al, Connecting the Dots Between the Gut–IGF-1–Prostate Axis: A Role of IGF-1 in Prostate Carcinogenesis, Frontiers in Endocrinology, March 2022, Volume 13, Article 852382

143. McCarty, Targeting Multiple Signaling Pathways as a Strategy for Managing Prostate Cancer: Multifocal Signal Modulation Therapy, Integrative Cancer Therapies 3; 2004 pp. 349-380

144. McKian and Haluska, Cixutumumab, Expert Opin Investig Drugs. 2009 July ; 18: 1025–1033

145. Meehan and Sadar, Androgens And Androgen Receptor In Prostate And Ovarian Malignancies, Frontiers in Bioscience 8, d780-800, May 1, 2003

146. Meirs, I., Glutathione S-Transferase pi (GSTP1), Atlas Genet Cytogenet Oncol Haematol. 2010; 14.

147. Melmed et al, Williams Textbook on Endocrinology, 14th Ed, Elsevier, 2020

148. Mendelsohn and Baselga, The EGF receptor family as targets for cancer therapy, Oncogene (2000) 19, 6550 - 6565

149. Mendelsohn, et al, The Molecular Basis of Cancer, Elsevier (New York) 2014.

150. Meng et al, The inflammation patterns of different inflammatory cells in histological structures of hyperplasic prostatic tissues, Transl Androl Urol 2020;9:1639-1649

151. Mihaylova, Maria, Reuben J. Shaw, The AMPK signalling pathway coordinates cell growth, autophagy and metabolism, Nature Cell Biology13, 1016–1023 (2011) doi:10.1038/ncb2329

152. Miller and Asim, Unravelling the Role of Kinases That Underpin Androgen Signalling in Prostate Cancer, Cells 2022, 11, 952.

153. Moll, U., O., Petrenko, The MDM2-p53 Interaction, Molecular Cancer Research , Vol. 1, 1001–1008, December 2003.

154. Mora et al, Constitutive Activation of Stat3 in Human Prostate Tumors and Cell Lines: Direct Inhibition of Stat3 Signaling Induces Apoptosis of Prostate Cancer Cells, Cancer Research 62, 6659–6666, November 15, 2002

155. Morgan, Cell Cycle, Sinauer (San Francisco) 2007.

156. Morin, P., A. Weeraratna, The APC Tumor Suppressor Pathway, El Deiry (Editor), Tumor Suppressor Genes, Humana (New York) 2003.

157. Muller et al, p53 and its mutants in tumor cell migration and invasion, J. Cell Biol. Vol. 192 No. 2 209–218.

158. Muniyappa and Sowers, Endothelial Insulin and IGF-1 Receptors: When Yes Means NO, Diabetes, Vol. 61, September 2012

159. Murekatete et al, Targeting Insulin-Like Growth Factor-I and Extracellular Matrix Interactions in Melanoma Progression, Scientific Reports, 2018

160. Murray-Zmijewski, F., et al A complex barcode underlies the heterogeneous response of p53 to stress, Nature Reviews Molecular Cell Biology 9, 702-712 (September 2008)

161. Neuwirt et al, Suppressor of Cytokine Signaling (SOCS)-1 Is Expressed in Human Prostate Cancer and Exerts Growth-Inhibitory Function through Down-Regulation of Cyclins and Cyclin-Dependent Kinases, The American Journal of Pathology, Vol. 174, No. 5, May 2009

162. Newton et al, Cell Survival and Cell Death, CSHL Press, 2020

163. Niu, et al, Role of Stat3 in Regulating p53 Expression and Function, Molecular And Cellular Biology, Sept. 2005, p. 7432–7440

164. O'Neil et al, Differential Role of Insulin/IGF-1 Receptor Signaling in Muscle Growth and Glucose Homeostasis, Cell Reports, 11, May 26, 2015

165. O'Shea, et al, JAKs and STATs in Immunity, Immunodeficiency, and Cancer, NEJM, 368;2 nejm.org January 10, 2013

166. Ohlsson et al, The Role of Liver-Derived Insulin-Like Growth Factor-I, Endocr Rev. 2009 Aug; 30: 494–535

167. Olsen et al, Fibroblast Growth Factor (FGF) Homologous Factors Share Structural but Not Functional Homology with FGFs, The Journal Of Biological Chemistry Vol. 278, No. 36, Issue of September 5, pp. 34226–34236, 2003

168. Ornitz and Ito, The Fibroblast Growth Factor signaling pathway, WIREs Dev Biol 2015, 4:215–266

169. Ornitz and Itoh, Fibroblast growth factors, Genome Biology 2001, 2:reviews3005.1–3005.12, March 2001

170. Ozturan et al, Androgen Receptor-Mediated Transcription in Prostate Cancer, Cells, 2022, 11, 898

171. Park et al, Apoptotic Death of Prostate Cancer Cells by a Gonadotropin-Releasing Hormone-II Antagonist, PLOS ONE June 2014, Volume 9, Issue 6

172. Partin, A., et al, Clinical Validation of an Epigenetic Assay to Predict Negative Histopathological Results in Repeat Prostate Biopsies, J Urol. 2014 Apr 18.

173. Parveen et al, Dual Role of p21 in the Progression of Cancer and Its Treatment, Critical Reviews in Eukaryotic Gene Expression, 26:49–62 (2016)

174. Patten, M. et al, The evolution of genomic imprinting: theories, predictions and empirical tests, Heredity (2014) 113, 119–128.

175. Pecorino, L, Molecular Biology of Cancer, Oxford (New York) 2008.

176. Pederzoli et al, "Stromal cells in prostate cancer pathobiology: friends or foes?", British Journal of Cancer (2023)

177. Pencik et al, IL-6/STAT3/ARF: the guardians of senescence, cancer progression and metastasis in prostate cancer, Swiss Med Wkly. 2015;145:w14215

178. Pencik et al, STAT3 regulated ARF expression suppresses prostate cancer metastasis, Nature Communications, 2015

179. Peruzzi B, Bottaro DP. Targeting the c-Met signaling pathway in cancer. Clin Cancer Res 2006 Jun 15;12:3657–60

180. Pestell and Nevalainen, Prostate Cancer, Humana, 2008

181. Pietras et al, PDGF receptors as cancer drug targets, CANCER CELL : MAY 2003 • VOL. 3

182. Qian et al, ONECUT2 Activates Diverse Resistance Drivers of Androgen Receptor Independent Heterogeneity in Prostate Cancer, bioRxiv preprint doi: https://doi.org/10.1101/2023.09.28.560025 ; this version posted October 1, 2023

183. Rabiau, N., et al, Methylation Analysis of BRCA1, RASSF1, GSTP1 and EPHB2 Promoters in Prostate Biopsies According to Different Degrees of Malignancy, in vivo 23: 387-392 (2009)

184. Rahim et al, Molecular Regulation of Bone Marrow Metastasis in Prostate and Breast Cancer, Bone Marrow Research, Volume 2014, Article ID 405920, 12 pages

185. Ratajczak et al, Heat Shock Proteins in Benign Prostatic Hyperplasia and Prostate Cancer, Int. J. Mol. Sci. 2022, 23, 897

186. Rathinavelu and Levy, Key Genes in Prostate Cancer Progression: Role of MDM2, PTEN, and TMPRSS2-ERG Fusions, Prostate Cancer - Leading-edge Diagnostic Procedures and Treatments, Intech, http://dx.doi.org/10.5772/64337 2016

187. Reed, Mechanisms of Apoptosis, American Journal of Pathology, Vol. 157, No. 5, November 2000

188. Rhind and Russell, Signaling Pathways that Regulate Cell Division, Cold Spring Harb Perspect Biol 2012;4:a005942

189. Richter, A., et al, The RASSF proteins in cancer; from epigenetic silencing to functional characterization, Biochimica et Biophysica Acta xxx (2009) xxx–xxx.

190. Roberts, Textbook of Cell Signalling in Cancer, Springer,2015

191. Robinson et al, Integrative Clinical Genomics of Advanced Prostate Cancer. Cell, 161, 1215–1228, May 21, 2015

192. Rucci and Angelucci, Prostate Cancer and Bone: The Elective Affinities, BioMed Research International, Volume 2014, Article ID 167035, 14 pages

193. Russo et al, Androgen Receptor Interaction with Mediator Complex is Enhanced in Castration-Resistant Prostate Cancer by CDK7 Phosphorylation of MED1, Cancer Discov. 2019 November ; 9: 1490–1492

194. Saad et al, Treatment of nonmetastatic castration-resistant prostate cancer: focus on second-generation androgen receptor inhibitors, Prostate Cancer and Prostatic Diseases, 8 Feb 2021

195. Saber et al, Exosomes are the Driving Force in Preparing the Soil for the Metastatic Seeds: Lessons from the Prostate Cancer, Cells, 28 Feb 2020

196. Salinero and Rafii, Endothelial cell adaptation in regeneration, Science, Dec 2018

197. Saxton and Sabatini, mTOR Signaling in Growth, Metabolism, and Disease, Cell, March 9, 2017

198. Schiewer et al, The AR dependent cell cycle: Mechanisms and cancer relevance, Mol Cell Endocrinol. 2012 April 16; 352: 34–45

199. Schmid et al. XENERA-1: a randomised double-blind Phase II trial of xentuzumab in combination with everolimus and exemestane versus everolimus and exemestane in patients with hormone receptor-positive/HER2-negative metastatic breast cancer and non-visceral disease. Breast Cancer Research (2023) 25:67

200. Sengupta et al, Regulation of the mTOR Complex 1 Pathway by Nutrients, Growth Factors, and Stress, Molecular Cell 40, October 22, 2010

201. Serrano and Munoz-Espi, Cellular Senescence in Disease, Academic, 2022

202. Shackelford, D., Reuben J. Shaw, The LKB1–AMPK pathway: metabolism and growth control in tumour suppression, Nature Reviews Cancer 9, 563-575 (August 2009) | doi:10.1038/nrc2676

203. Shaffer et al, Structural basis of androgen receptor binding to selective androgen response elements, PNAS, April 6, 2004, vol. 101, no. 14

204. Shao, et al, Inhibition of Polo-like Kinase 1 (Plk1) Enhances the Antineoplastic Activity of Metformin in Prostate Cancer, J. Biol. Chem. 2015 290: 2024-2033.

205. Shaw, R., The tumor suppressor LKB1 kinase directly activates AMP-activated kinase and regulates apoptosis in response to energy stress, PNAS March 9, 2004 vol. 101 no. 10 3329–3335

206. Shen and Rubin, Prostate Cancer, CSHL Press, 2019

207. Sherbet, Growth Factors and Their Receptors in Cell Differentiation Cancer and Cancer Therapy, Elsevier, 2011

208. Sherr, C., J. Weber, The ARF/p53 pathway, Current Opinion in Genetics & Development 2000, 10:94–99

209. Sherr, Tumor surveillance via the ARF–p53 pathway, Genes & Development 12:2984–2991, 1998

210. Shorning et al, The PI3K-AKT-mTOR Pathway and Prostate Cancer: At the Crossroads of AR, MAPK, and WNT Signaling, Int. J. Mol. Sci. 2020, 21, 4507

211. Shtivelman et al, Molecular pathways and targets in prostate cancer, Oncotarget, Vol. 5, No. 17, August 2014

212. Siech et al, Insulin-like Growth Factor-1 Influences Prostate Cancer Cell Growth and Invasion through an Integrin α3, α5, αV, and β1 Dependent Mechanism, Cancers 2022, 14, 363.

213. Singh et al, EGF receptor ligands: recent advances, F1000 Research 2016.

214. Sobhani et al, AR-V7 in Metastatic Prostate Cancer: A Strategy beyond Redemption, Mol. Sci. 2021, 22, 5515

215. Soussi, T., K. Wiman, TP53: an oncogene in disguise, Cell Death and Differentiation (2015) 22, 1239–1249

216. Spravchikov et al, Glucose Effects on Skin Keratinocytes, Diabetes, Vol. 50, July 2001

217. Spravchikov et al, Glucose Effects on Skin Keratinocytes, Diabetes, Vol. 50, July 2001

218. Stabiano, Prostate Cancer: Morphology to Biology, Springer, 2013

219. Takeuchi and Ito, Receptor Tyrosine Kinases and Targeted Cancer Therapeutics, Biol. Pharm. Bull. 34 1774—1780 (2011)

220. Talty and Olino, Metabolism of Innate Immune Cells in Cancer, Cancers 2021, 13, 904

221. Tan et al, Androgen receptor: Structure, role in prostate cancer and drug discovery, Article in Acta Pharmacologica Sinica · June 2014

222. Tang et al, The Role of Androgen Receptor in Cross Talk Between Stromal Cells and Prostate Cancer Epithelial Cells, Frontiers in Cell and Developmental Biology, October 2021, Volume 9

223. The Cancer Genome Atlas Research Network, The Molecular Taxonomy of Primary Prostate Cancer, Cell, Nov 2015

224. Theurillat, J., et al, Ubiquitylome analysis identifies dysregulation of effector substrates in SPOP-mutant prostate cancer, Science 3 October 2014: Vol. 346 no. 6205 pp. 85-89

225. Thornburn, Apoptosis and Autophagy: regulatory connections between two supposedly different processes, Apoptosis. 2008 January ; 13: 1–9

226. Torrealba et al, Expression of ERK1 and ERK2 in prostate cancer, MAP Kinase 2015; volume 4:5265

227. Triscott et al, PI5P4Kα supports prostate cancer metabolism and exposes a survival vulnerability during androgen receptor inhibition, Sci. Adv. 9, eade8641 (2023) 1 February 2023

228. Truong et al, Targeting the IGF/PI3K/mTOR pathway and AXL/YAP1/TAZ pathways in primary bone cancer, Journal of Bone Oncology, Volume 33, April 2022, 100419

229. Van Eden et al, Heat-Shock Proteins Induce T-Cell Regulation Of Chronic Inflammation, Nature Reviews, Immunology Volume 5, April 2005

230. Van Maerken , T., et al, Escape from p53-mediated tumor surveillance in neuroblastoma: switching off the p14ARF-MDM2-p53 axis, Cell Death and Differentiation (2009) 16, 1563–1572

231. Venere et al, Growth Factor Receptors Define Cancer Hierarchies, Cancer Cell 23, February 11, 2013

232. Wan, X., et al, Activation of β-Catenin Signaling in Androgen Receptor–Negative Prostate Cancer Cells, Clin Cancer Res, 2012.

233. Wang et al, Direct and indirect induction of apoptosis in human mesenchymal stem cells in response to titanium particles, Journal of Orthopaedic Research 21 (2003) 697-707

234. Wang et al, Function of p21 and its therapeutic effects in esophageal cancer (Review), Oncology Letters 21: 136, 2021

235. Wang et al, Metformin suppressed tumor necrosis factor-α-induced epithelial-mesenchymal transition in prostate cancer by inactivating the NF-κB signaling pathway, Transl Cancer Res 2020

236. Wang, T., et al, Glutathione S-transferase P1–1 (GSTP1–1) Inhibits c-Jun N-terminal Kinase (JNK1) Signaling through Interaction with the C Terminus, THE JOURNAL OF BIOLOGICAL CHEMISTRY Vol. 276, No. 24, Issue of June 15, pp. 20999–21003, 2001.

237. Wantanabe, M., et al, APC Gene Mutations in Human Prostate Cancer, (Jpn J Clin Oncol 26: 77-81, 1996.

238. Watt and Goel, Cellular mechanisms underlying response and resistance to CDK4/6 inhibitors in the treatment of hormone receptor-positive breast cancer, Breast Cancer Research (2022) 24:17

239. Weinberg, The Biology of Cancer, 2nd Ed, Garland, 2014

240. Wesche et al, Fibroblast growth factors and their receptors in cancer, Biochem. J. (2011) 437, 199–213

241. Whitburn et al, Metformin and Prostate Cancer: A New Role for an Old Drug, Curr Urol Rep (2017) 18: 46

242. Wilson et al, Refinement of the androgen response element based on ChIP-Seq in androgen-insensitive and androgen-responsive prostate cancer cell lines, Sci Rep. 2016; 6: 32611

243. Winman, The retinoblastoma gene: role in cell cycle control and cell differentiation, FASEB Journal, 01 July 1993

244. Witsch et al, Roles for Growth Factors in Cancer Progression, Physiology 25: 85–101, 2010

245. Wojno et al, Reduced Rate of Repeated Prostate Biopsies Observed in ConfirmMDx Clinical Utility Field Study, Am Health Drug Benefits. 2014;7:129-134, www.AHDBonline.com

246. Wrigley et al, Insulin-Like Growth Factor 1: At the Crossroads of Brain Development and Aging, Frontiers in Cellular Neuroscience, February 2017, Volume 11

247. Xie et al, Metformin and Androgen Receptor-Axis-Targeted (ARAT) Agents Induce Two PARP-1-Dependent Cell Death Pathways in Androgen-Sensitive Human Prostate Cancer Cells, Cancers, 2021, 13, 633

248. Yang et al, Growth Factors and their receptors in cancer metastases, Frontiers in Bioscience 16, 531-538, January 1, 2011

249. Yee et al, Ganitumab and metformin plus standard neoadjuvant therapy in stage 2/3 breast cancer, npj Breast Cancer (2021) 131

250. Yu, H., et al, Crosstalk between cancer and immune cells: role of STAT3 in the tumour microenvironment, Nature Reviews, Immunology, Volume 7, January 2007, p| 41

251. Yu, H., et al, Revisiting STAT3 signalling in cancer: new and unexpected biological functions, Nature Reviews Cancer, 14, 736–746, 2014.

252. Xi et al, IGF-I and IGFBP-2 Stimulate AMPK Activation and Autophagy, Which Are Required for Osteoblast Differentiation, Endocrinology, Volume 157, Issue 1, 1 January 2016

253. Zamagni et al, Non-nuclear AR Signaling in Prostate Cancer. Frontiers in Chemistry, 26 Feb 2019

254. Zeng, C., et al, SPOP suppresses tumorigenesis by regulating Hedgehog/Gli2 signaling pathway in gastric cancer, Journal of Experimental & Clinical Cancer Research 2014, 33:75

255. Zhang et al, Androgen Receptor Splice Variant, AR-V7, as a Biomarker of Resistance to Androgen AxisTargeted Therapies in Advanced Prostate Cancer, Clinical Genitourinary Cancer February 2020

256. Zhang et al, Interleukin-17 promotes prostate cancer via MMP7-induced epithelial-to-mesenchymal transition, Oncogene. 2017 February 02; 36: 687–699

257. Zhang et al, Marchantin M Induces Apoptosis of Prostate Cancer Cells Through Endoplasmic Reticulum Stress, Med Sci Monit, 2015; 21: 3570-3576

258. Zhao et al, Therapeutic Repurposing of Biguanides in Cancer, Trends in Cancer, 2021

259. Zheng et al, The Crucial Role of AR-V7 in Enzalutamide-Resistance of Castration-Resistant Prostate Cancer, Cancers 2022, 14, 4877.

260. Zheng, L. et al, NF-kB Regulates Androgen Receptor Expression and Prostate Cancer Growth, Am Soc Invest Path, V 175, 2009, pp 489-499.

261. Zhu and Sun, 5α-Reductase Isozymes in the Prostate, J Med Sci. 2005 ; 25: 1–12

262. Zhu et al, Epithelial derived CTGF promotes breast tumor progression via inducing EMT and collagen I fibers deposition, Oncotarget, Vol. 6, No. 28, 2015

263. Zhu, H., et al, SPOP E3 Ubiquitin Ligase Adaptor Promotes Cellular Senescence by Degrading the SENP7 deSUMOylase, Cell Reports, October 2015

264. Zingales et al, Metformin: A Bridge between Diabetes and Prostate Cancer, Frontiers in Oncology, October 2017, Volume 7, Article 243

8 INDEX

9 TECHNICAL NOTES

See https://www.researchgate.net/profile/Terrence-Mcgarty/research

1. No 204 IGF-1 and Prostate Cancer (December 2023)
2. No 203 Sialic Acid: Another Target or Just a Red Queen? (October 2023)
3. No 201 Reprogramming and Cancer (September 2023)
4. No 200 Basal Cell Carcinoma: A Paradigm? (July 2023)
5. No 199 Androgen Receptor: Whither Goest Thou? (May 2023)
6. No 198 Extrachromosomal DNA and Cancers (March 2023)
7. N9 197 Prostate Cancer and Chromatin Conformation (February 2023)
8. No 196 Prostate Cancer: CAR-NK vs Ab (February 2023)
9. No 195 Neutrophils and Inflammation (December 2022)
10. No 193 Granulomas (December 2022)
11. No 194 Astrocytomas (November 2022)
12. No 192 Biomarkers: Targets for Cancer Diagnosis (September 2022)
13. No 191 Itch, Cancer and the Immune System (April 2022)
14. No 190 Glycans: COVID and Cancer(November 2021)
15. No 189 Memory T Cells: COVID and Cancer (August 2021)
16. No 188 PSMA: A Prostate Cancer Target (June 2021)
17. No 186 COVID-19: Variants and Vaccines (Feb 2021)
18. No 185 COVID-19: Autoantibodies (Feb 2021)
19. No 184 COVID-19: Mutations and Infectivity (Jan 2021)
20. No 183 COVID-19: Multi-Organ Sequellae (Dec 2020)
21. No 182 Poly-specific Antibodies (Nov 2020)
22. No 181 COVID-19 Vaccine: An Update and Primer (Nov 2020)
23. No 180 Correlation vs. Causation: The Perils of AI (Oct 2020)
24. No 179 Multimedia Communications (Revised) (Oct 2020)
25. No 178 AR-V7: A Driver of Prostate Metastasis? (August 2020)
26. No 177 Fibroblasts and Cancer: "The Wound That Would Not Heal" (May 2020)
27. No 176 Thyroglobulin: When is it a tumor marker? (April 2020)
28. No 175 IL-6, COVID-19, Cytokine Storms and Galen (April 2020)
29. No 174 ERG, A Master Transcription Factor? (April 2020)

30. No 173 A Primer for COVID-19 (March 2020)
31. No 172 Eikonomics, (March 2020)
32. No 171 miRNAS: REDUX (January 2020)
33. No 170 mTOR: Target of Opportunity? (January 2020)
34. No 169 Adaptive Mutability and Cancer. (January 2020)
35. No 168 Bladder Cancer: An Interesting Set of Diagnostic Options. (October 2019)
36. No 167 Tumor Associated Immune Cells: On the one hand and on the other hand? (October 2019)
37. No 166 Thyroid Cancer and Genetic Differentiation. (September 2019)
38. No 165 Immunotherapy: Possible Directions (August 2019)
39. No 164 What is Cancer? (August 2019)
40. No 163 LPCAT1 and Cancer Progression (July 2019)
41. No 162 miRNAs, Genes, and Cancer Cytology (July 2019)
42. No 161 EMT, lncRNA, TGF, SMAD and Cancer (June 2019)
43. No 160 Thyroid Cancer: Seek and Ye Shall Find (March 2019)
44. No 159 Exosomes and Cancer (February 2019)
45. No 158 NSD2, PCa, and Metastasis. (February 2019)
46. No 157 EMT and Cancers. Report number (January 2019)
47. No 156 Growth Factors, Pathways and Cancers. (December 2018)
48. No. 155 Autophagy and PCa: On the One Hand and on the Other Hand. (October 2018)
49. No. 154 Autophagy and Cancer. (September 2018)
50. No 153 Adoptive Cell Transfer (June 2018)
51. No 152 Neuroendocrine PCa: Galen, Logic, and Rationalism (June 2018)
52. No 151 Liquid Biopsy and Cancer. (May 2018)
53. No 150 Aptamers and Cancer (May 2018)
54. No. 149 Warburg and Prostate Cancer (January 2018)
55. No 148 Glucose, Warburg, Cancer and Pathways. (January 2018)
56. No 147 Inflammation, The Immune System and Cancer. (December 2017)
57. No 146 Microbiome, Immune System and Cancer. (November 2017)
58. No 145 PCA Mortality and Treatments. (July 2017)
59. No 144 Platelets and Checkpoint Inhibitors. (March 2017)
60. No 143 PARP, DNA and PCa (February 2017)
61. No 142 Immune Set Points (February 2017)

62. No 141 Gene Drive (December 2016)
63. No. 140 CAR T Cells and Cancer (November 2016)
64. No. 139 A Proposed Metric for Measuring Bacterial Growth (September 2016)
65. No. 138 NKX3.1 and PCa (July 2016)
66. No. 137 Biofilm Growth and Infiltration (May 2016)
67. No. 136 Cancer Stem Cells and Cancer Cell of Origin Redux, (May 2016)
68. No. 135 Seven Types of PCa (April 2016)
69. No. 134 CHK2, AR, and PCa (February 2016)
70. No. 133 LY6 and Prognostic Markers (February 2016)
71. No. 132 More PCa Markers (February 2016
72. No. 131 pro-NPY and PCa (February 2016)
73. No. 130 SPOP and Prostate Cancer (November 2015)
74. No. 129 Trust But Verify: The Value Of PSA (November 2015)
75. No. 128 Prostate Cancer Prognostic Tests: Pre And Post Diagnosis (October 2015)
76. No. 127 Stat3 and PCA: Of Mice And Men (August 2015)
77. No.126. Prostate Cancer Metastasis: Some Simple Cases (July 2015)
78. No. 125 CRISPR and Cancer: Revised (April 2015)
79. No. 124 CRISPR Cas9: A Genomic Tool (April 2015)
80. No. 123 Metformin, Statins and PCa (February 2015)
81. No. 122 MDS and DNMT1 Pathway Control (January 2015)
82. No. 121 Sirt1, Exosomes and Prostate Cancer (January 2015)
83. No. 120 CNVs and Prostate Cancer (December 2014)
84. No. 119 SNPs and Prostate Cancer (October 2014)
85. No. 118 Vitamin D and Prostate Cancer (October 2014)
86. No. 117 SPDEF, ETS Transcription Factors and PCa (October 2014)
87. No. 116 Methylation, Prostate Cancer, Prognostics (August 2014)
88. No. 115 Endosomes and Melanoma (July 2014)
89. No. 114 NOTCH, miR-146a and Melanoma (June 2014)
90. No. 112 Prostate Cancer: miR-34, p53, MET and Methylation (May 2014)
91. No. 111 CRISPR and Cancer (April 2014)
92. No. 110 ERG and Prostate Cancer (January 2014)
93. No. 108 Cancer Cell Dynamics (January 2014)
94. No. 107 Prostate Cancer Genetic Metrics (January 2014)

95. No. 106 Divergent Transcription (December 2013)
96. No. 104 Prostate Cancer and Blood Borne Markers (December 2013)
97. No. 103 Prostate Cancer Indolence (December 2013)
98. No. 102 MDS and Methylation (August 2013)
99. No. 101 Exosomes and Cancer (August 2013)
100. No. 100 lncRNA and Prostate Cancer (July 2013)
101. No. 99 SNPs and Cancer Prognostics (July 2013)
102. No. 98 CCP and Prostate Cancer (July 2013)
103. No. 97 ATF2 and Melanoma (July 2013)
104. No. 96 PD-1 and Melanoma Therapeutics (June 2013)
105. No. 95 MER Tyrosine Kinase Receptors and Inhibition (June 2013)
106. No. 94 Melanoma Therapeutics (May 2013)
107. No. 93 Cancer Cell Dynamics Methylation and Cancer (April 2013)
108. No. 91 Methylation and Cancer (March 2013)
109. No. 90 Telomeres and Melanoma (February 2013)
110. No. 89 miRNA and Melanoma (January 2013)
111. No. 88 Extracellular Matrix vs. Intracellular Pathways (November 2012)
112. No. 87 Prostate Cancer Prognostic Markers (October 2012)
113. No. 86 Cancer Models for Understanding, Prediction, and Control (June 2012)
114. No. 85 Prostate Cancer Stem Cells (May 2012)
115. No. 84 Epistemology of Cancer Genomics (May 2012)
116. No. 83 Prostatic Intraepithelial Neoplasia (February 2011)
117. No 82 Prostate Cancer: Metastatic Pathway Identification (February 2011)
118. No 81 Backscatter Radiation and Cancer (December 2010)
119. No 80 PSA Evaluation Methodologies (December 2010)
120. No 79 The PSA Controversy (November 2010)
121. No 77 Obesity and Type 2 Diabetes: Cause and Effect (June 2010)
122. No 63 Remediable Diseases (April 2009)
123. No. 61 Type 2 Diabetes: A Controllable Epidemic (March 2009)

10 GENES

The following is a list of the principal genes and related proteins discussed herein.

ABL	AKT1	AKT2	AKT3
AR	ARE	ARF	ASK1
ATM	BAD	BIM	BMK-1
CBL	CDK4	CDKN2A	C-MYC
Cyclin D1	E2F	EGF	EGFR
ERG	ERK	EZF	FOXA1
FOXO3	GH	GHR	GLI
GLUT4	GRB2	GST	GSTP1
HH	HIF	HSP100	HSP60
HSP70	HSP90	IGF-1	IGFBP
IGFR	IL-17	IL-6	INK4A
IR	IRS	IRS-2	JAK
JMY	JNK	JUN	LEF
MAPK	MAPKK	MAPKKK	MDM2
MED12	MEK	MITF	MMP2
mTOR	MYC	NF-kB	NKX3.1
ONECUT	P110	P14	P21
P53	P85	Patched	PDK1
PI3K	PIP2	PIP3	PKA
PTEN	RAC	RAF	RAS
RASSF	RASSF1	Rb1	REDD1
RhoA	S6K1	SENP7	SH2
SKP2	Smoothened	SOCS1	SPOP
SRC	STAT3	STAY	SUFU
SUMO	SYP	TCF	TMPRSS2
TRAF2	VEGF		