Growth Factors Pathways and Cancers

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Growth Factors and Receptors have played a significant role in cell homeostasis as well as malignancy. We examine some of the major ones in an attempt to place them in the context pf putative therapeutic targets.

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Contents

1 INTRODUCTION

Growth Factors abound in cells. They activate, via Growth Factor Receptors (GFR), a variety of internal pathways which in turn control the cells status. They become initiators and promoters of proliferation. The object of this note is to present the collection of growth factors into a somewhat holistic collection. There still is a looseness in these significant drivers of cell behavior. The GFs float around in a manner ranging from endocrine to paracrine, and some say even exocrine and autocrine. They can have a significant effect on the extracellular matrix as well as the mesenchymal to cell interfaces and characteristics. This is not a definitive study, but merely a benchmark to attempt a focus back on the GF elements.

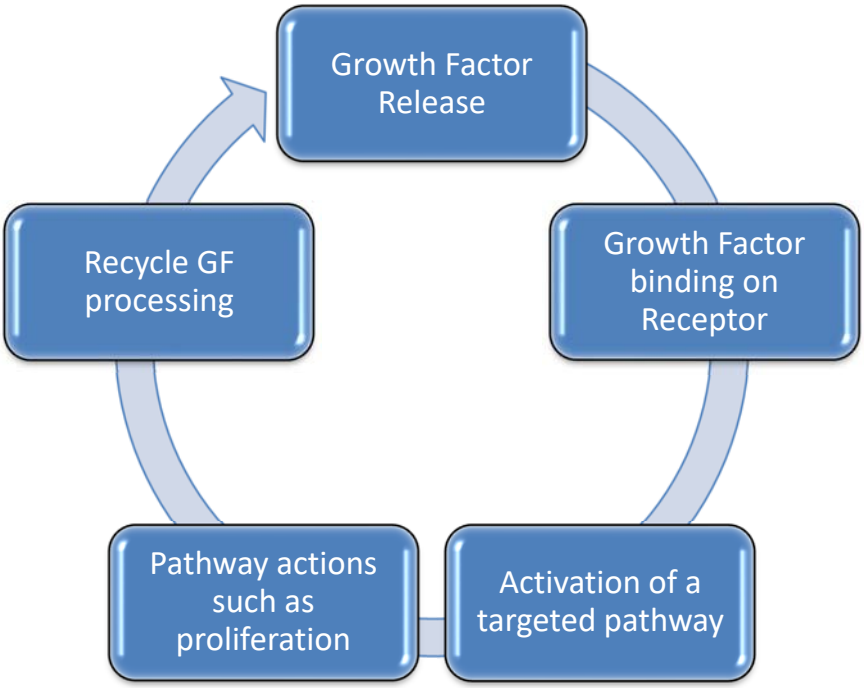
As Tekeuchi and Ito note:

The majority of growth factor receptors are composed of extracellular, transmembrane, and cytoplasmic tyrosine kinase (TK) domains. Receptor tyrosine kinase (RTK) activation regulates many key processes including cell growth and survival. However, dysregulation of RTK has been found in a wide range of cancers, and it has been shown to correlate with the development and progression of numerous cancers.

Therefore, RTK has become an attractive therapeutic target. One way to effectively block signaling from RTK is inhibition of its catalytic activity with small-molecule inhibitors. Low-molecular-weight TK inhibitors (TKIs), such as imatinib, targeting tumors with mutant c-Kit, and gefitinib, targeting non-small cell lung cancer with mutant epidermal growth factor receptor (EGFR), have received marketing approval in Japan. MET, fibroblast growth factor receptor (FGFR), and insulin-like growth factor-I receptor (IGF-IR) are frequently genetically altered in advanced cancers.

TKIs of these receptors have not yet appeared on the market, but many anticancer drug candidates are currently undergoing clinical trials. Most of these TKIs were designed to compete with ATP at the ATP-binding site within the TK domain. … Targeting agents specifically inhibiting the target kinase were previously searched for based on the hypothesis that a narrow target window might reduce unexpected side effects, but agents with multiple targets have been recently developed to overcome tumors resistant against a single-targeting agent.

This is just a simple summary of the possible options available with the multiplicity of GF. We may view the GF actions in the figure below. The end point may be proliferation, apoptosis, and/or sending out GF to other cells.



There are several key questions regarding the collection of GF. Specifically:

1. Are there GF/GFR which are specific for specific cancers and if so what are the details associated with their involvement.

2. If GF/GFR are drivers of certain malignancies what are the drivers of these GF/GFR expressions.

3. If GF/GFR can be such drivers than can we approach mitigating their effects by use of blockers such as mAbs, blocking them by Ab attachments?

4. GF/GFR are used by a variety of cells for normal homeostasis. Blocking them may result in a variety of effects that are detrimental. Can we ascertain what blockages would result in such detrimental effects.

5. GF/GFR are a complex inter-cellular signalling network. What can we say about such a network in general and then in specific cases?

These are just a few of the general questions we try to examine. This is a working paper and as such there is no attempt at completeness and no representation of innovation or therapeutic interpretation.

2 CELL CYCLE ISSUES

Cancer is basically uncontrolled cell growth, replication, and failure for cells to die off, normal apoptosis. It may also include loss of location stability and metabolic enhancement, but let us start with the key issue, replication. Then we examine two other major factors; apoptosis or cell death and cell to cell adhesion, or simply cells being where they should be. All of this examination is to be focused on the cell cycle. This section is a discussion of what is necessary to understand the importance of the cell cycle. The cycle is what often is broken in cancer cells, namely the cell reproduces again and again.

Cancer in many ways is a loss of the three factors:

1. Cell Replication: This is the normal or abnormal cell cycle.

2. Cell Death: This is normal cell death or apoptosis.

3. Cell Localization: The establishment and maintenance of a cells relative position and function.

We shall thus begin with the control of the cell cycle and then work upwards in terms of the cells control mechanism.

The following Figure presents a simple view of how cell signalling functions. There are six functions described, and not all must be present in any cell function. The steps are generally:

1. Ligand: There is some external activator that floats about and ultimately finds its home on the surface of a cell. Now the issue is not that there is one such protein floating about that eventually may find itself attached to the surface of a cell. The protein may be from afar or it may be from the very same cell. We could then consider the concentration of the protein as well, and its flow across cells themselves as well. This issue is a complex one and all too often it is treated like a simple one protein to one receptor issue. In reality it is a distributed random process.

2. Receptor: The ligand seeks and may ultimately find a receptor. The receptor is a protein on the cell surface. A cell produces the protein and the number of such receptors may be significant as well. Thus there exists a concentration in space of the ligands and they can attach to and activate receptors, proteins, on cell surfaces.

3. Adaptor: The Receptor when connected to a ligand effects a response and there may be an adaptor protein which then gets connected and starts the inter-cell communications process.

4. Transducer: The transducer, such as RAS or PI3K, converts the signal to the receptor as displayed by the adaptor into the beginning of a chain down through the cytoplasm. This is a highly controlled and redundant chain which can become unstable if certain genes are affected and the controlling proteins disabled.

5. Kinase Cascade: This is the chain of protein communicating links and effectors from the Transducer to the cell nucleus and includes the initiation of the targeted transcription factor. As

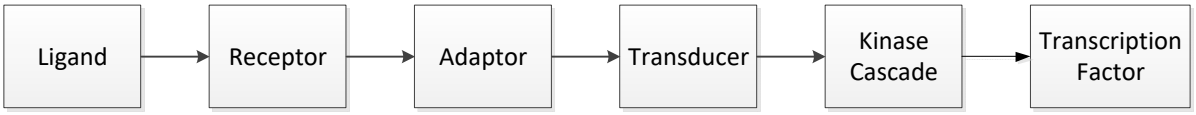
with the Transduce this kinase chain is controlled by redundant checks but if they become defective then the chain internal controls can be lost and the result become unstable.

6. Transcription Factor: This is the protein which has been activated within the nucleus which then commences transcription of the targeted sets of genes for the purpose of producing the resulting product.

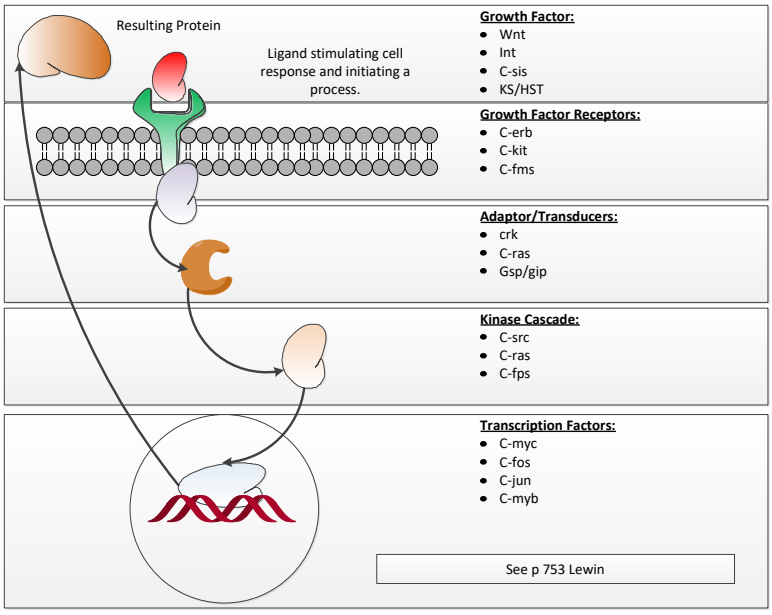
Note that this is a complex process.

Ligand	PDGF	Insulin	Growth Hormone	IL-1β	TGF-β
Receptor	PDGF Receptor	Insulin Receptor	GH Receptor	IL Receptor	TGF Receptor
Adaptor	SHP2/Grb2	IRS 1			
Transducer	SOS/Ras	PI3K	JAK	JAK	Type 1 Receptor
Kinase Cascade	MAPK	Akt			
Transcription Factor	Ternanry complex factors	FOXO	STATs	STATs	SMADs

See p 818 Lewin



The following depicts the process at several levels in a cell.



Now there are two major states a cell finds itself in; stasis and reproduction. A third, apoptosis, is natural cell death, we shall consider later. In stasis the cell is in G0 and producing proteins generally in response to external ligands or through normal internal processes. Unlike most standard biological models, we look at the proteins generally in terms of their concentrations and thus look at cell kinetics as well.

A cell in stasis is a little protein production factory, and each cell is pumping out the proteins and they then are in some extracellular balance. The cells in stasis communicate with one another via their respective ligands. In contrast when a cell reproduces it is standing out from the crowd if one will and looking out for itself.

We now examine first gene operations and then cell replication.

2.1 CELL REPLICATION

We first address cell replication. First we examine the cell cycle from a generic perspective. We then examine the details on the pathways which may result in unstable cell reproduction.

The cell replication cycle goes through 4 stages. The dormant stage, G0, is not part of this process. The stages in cell reproduction are:

G0: This is the resting phase. It is during this phase that the cell is producing proteins via normal transcription processes. G0 may be resting related to the reproductive mitotic activities but the cell is quite active as a protein generating factory.

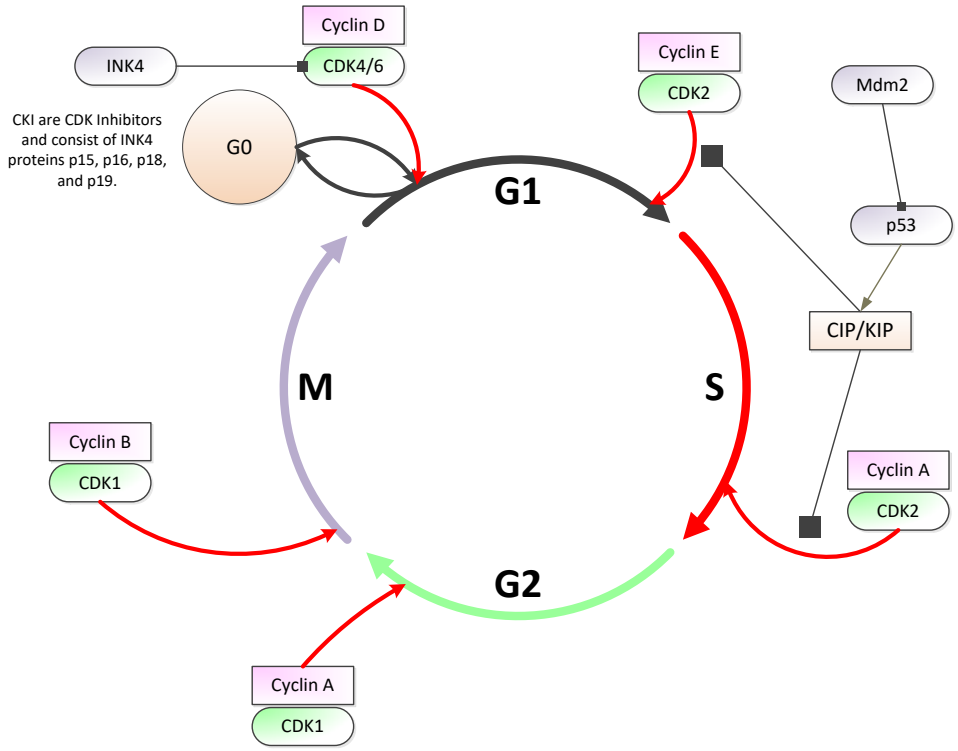
G1: Once the cell begins the G1 phase it is on its way to reproducing via mitosis.

S: The S phase is the phase where the DNA is duplicated. This is a sensitive stage; any error here can be propagated forward albeit there may still be checks available.

G2: This is the second gap phase.

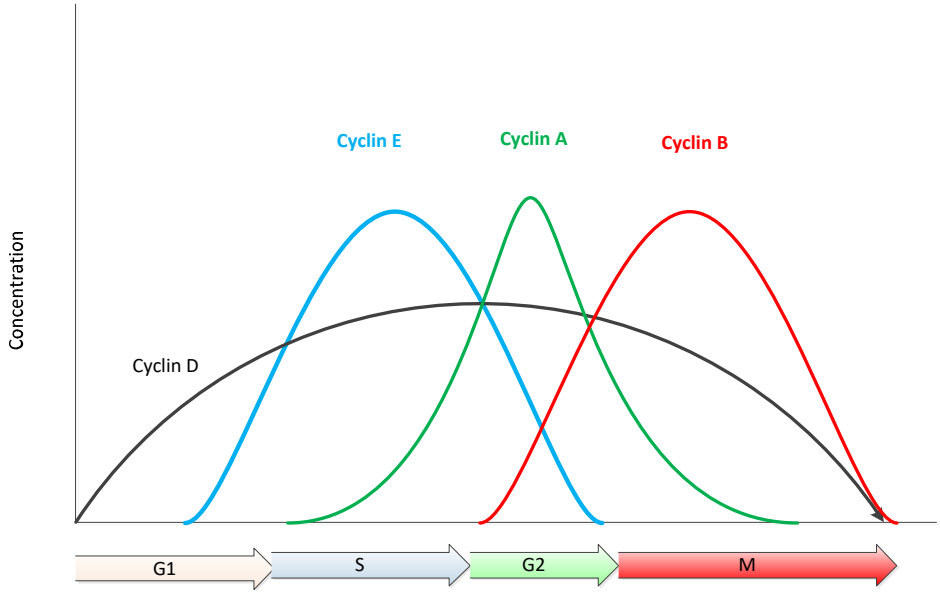
M: M phase includes mitosis and cytokinesis, namely the creation of two identical new cells.

Now the cell starts G1 by being instigated by a bound pair of a cyclin and a CDK, a cyclin dependent kinase. In this specific case we start with a binding of cyclin D and CDK4/6. This is the initiating event moving into G1 from senescence in G0. We depict these processes below (from McKinnell et al p. 169.):



The cyclins in each stage grow in concentration and as such move the cell along in each of its reproductive stages.

The following shows the phases and the relevant concentrations of cyclin bound to CDKs. Note the increase in concentration activates a change or movement along the mitotic path.



Note in the above the concentration of a specific cyclin above a level of a previous cyclin initiates the next step in mitosis. The details as to how and why this happens are detailed in Morgan (Chapter 3).

Protein[1]	***Gene***	***Function***[2]
Cyclin A (also CCN1; CCNA, CCNA2, Cyclin A2)	4q25-q31	The protein encoded by this gene belongs to the highly conserved cyclin family, whose members are characterized by a dramatic periodicity in protein abundance through the cell cycle. Cyclins function as regulators of CDK kinases. Different cyclins exhibit distinct expression and degradation patterns which contribute to the temporal coordination of each mitotic event. In contrast to cyclin A1, which is present only in germ cells, this cyclin is expressed in all tissues tested. This cyclin binds and activates CDC2 or CDK2 kinases, and thus promotes both cell cycle G1/S and G2/M transitions.
Cyclin B1 (CCNB1)	5q12	The protein encoded by this gene is a regulatory protein involved in mitosis. The gene product complexes with p34 (cdc2) to form the maturation-promoting factor (MPF). Two alternative transcripts have been found, a constitutively expressed transcript and a cell cycle-regulated transcript that is expressed predominantly during G2/M phase. The different transcripts result from the use of alternate transcription initiation sites.
Cyclin B2 (CCNB2)	15q22.2	Cyclin B2 is a member of the cyclin family, specifically the B-type cyclins. The B-type cyclins, B1 and B2, associate with p34cdc2 and are essential components of the cell cycle regulatory machinery. B1 and B2 differ in their subcellular localization. Cyclin B1 co-localizes with microtubules, whereas cyclin B2 is primarily associated with the Golgi region. Cyclin B2 also binds to transforming growth factor beta RII and thus cyclin B2/cdc2 may play a key role in transforming growth factor beta-mediated cell cycle control.
Cyclin C (CCNC)	6q21	The protein encoded by this gene is a member of the cyclin family of proteins. The encoded protein interacts with cyclin-dependent kinase 8 and induces the phophorylation of the carboxy-terminal domain of the large subunit of RNA polymerase II. The level of mRNAs for this gene peaks in the G1 phase of the cell cycle. Two transcript variants encoding different isoforms have been found for this gene.

[1] http://www.ncbi.nlm.nih.gov/gene/983

[2] From http://www.ncbi.nlm.nih.gov/gene/595 data bases as a source.

Protein[1]	*Gene*	*Function*[2]
Cyclin D (Cyclin D1)	11q13	The protein encoded by this gene belongs to the highly conserved cyclin family, whose members are characterized by a dramatic periodicity in protein abundance throughout the cell cycle. Cyclins function as regulators of CDK kinases. Different cyclins exhibit distinct expression and degradation patterns which contribute to the temporal coordination of each mitotic event. This cyclin forms a complex with and functions as a regulatory subunit of CDK4 or CDK6, whose activity is, required for cell cycle G1/S transition. This protein has been shown to interact with tumor suppressor protein Rb and the expression of this gene is regulated positively by Rb. Mutations, amplification and overexpression of this gene, which alters cell cycle progression, is observed frequently in a variety of tumors and may contribute to tumorigenesis.
Cyclin E (CCNE1)[3]	19q12	The protein encoded by this gene belongs to the highly conserved cyclin family, whose members are characterized by a dramatic periodicity in protein abundance through the cell cycle. Cyclins function as regulators of CDK kinases. Different cyclins exhibit distinct expression and degradation patterns which contribute to the temporal coordination of each mitotic event. This cyclin forms a complex with and functions as a regulatory subunit of CDK2, whose activity is, required for cell cycle G1/S transition. This protein accumulates at the G1-S phase boundary and is degraded as cells progress through S phase. Overexpression of this gene has been observed in many tumors, which results in chromosome instability, and thus may contribute to tumorigenesis. This protein was found to associate with, and be involved in, the phosphorylation of NPAT protein (nuclear protein mapped to the ATM locus), which participates in cell-cycle regulated histone gene expression and plays a critical role in promoting cell-cycle progression in the absence of pRB. Two alternatively spliced transcript variants of this gene, which encode distinct isoforms, have been described.

The CDKs involved are:

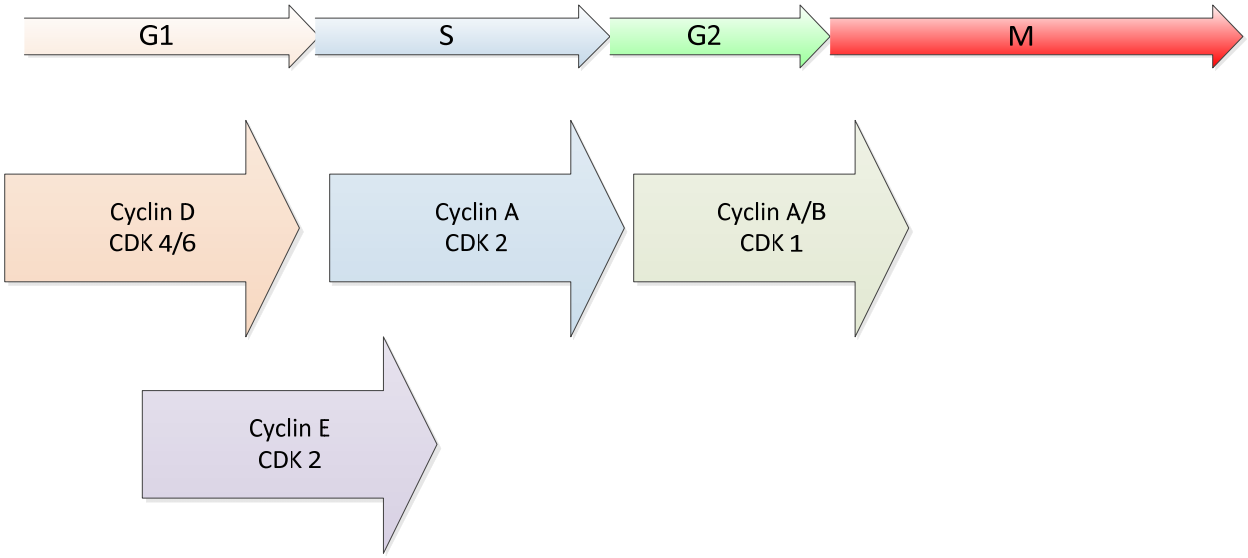
[3] http://www.ncbi.nlm.nih.gov/gene/898

Protein[4]	*Gene*	*Function*[5]
CDK 1 (also known as CDC2; CDC28A; P34CDC2)	10q21.1	This protein is a catalytic subunit of the highly conserved protein kinase complex known as M-phase promoting factor (MPF), which is essential for G1/S and G2/M phase transitions of eukaryotic cell cycle. Mitotic cyclins stably associate with this protein and function as regulatory subunits. The kinase activity of this protein is controlled by cyclin accumulation and destruction through the cell cycle. The phosphorylation and dephosphorylation of this protein also play important regulatory roles in cell cycle control.
CDK 2 (also called p33)	12q13	It is a catalytic subunit of the cyclin-dependent protein kinase complex, whose activity is restricted to the G1-S phase, and essential for cell cycle G1/S phase transition. This protein associates with and regulated by the regulatory subunits of the complex including cyclin A or E, CDK inhibitor p21Cip1 (CDKN1A) and p27Kip1 (CDKN1B). Its activity is also regulated by its protein phosphorylation.
CDK 3	17q22	This gene encodes a member of the cyclin-dependent protein kinase family. The protein promotes entry into S phase, in part by activating members of the E2F family of transcription factors. The protein also associates with cyclin C and phosphorylates the retinoblastoma 1 protein to promote exit from G0.
CDK 4 (also CMM3; PSK-J3)	12q14	This protein is a catalytic subunit of the protein kinase complex that is important for cell cycle G1 phase progression. The activity of this kinase is restricted to the G1-S phase, which is controlled by the regulatory subunits D-type cyclins and CDK inhibitor p16 (INK4a). This kinase was shown to be responsible for the phosphorylation of retinoblastoma gene product (Rb). Mutations in this gene as well as in its related proteins including D-type cyclins, p16 (INK4a) and Rb were all found to be associated with tumorigenesis of a variety of cancers.
CDK 6 (also PLSTIRE)	7q21-22	The protein encoded by this gene is a member of the cyclin-dependent protein kinase (CDK) family. CDK family members are known to be important regulators of cell cycle progression. This kinase is a catalytic subunit of the protein kinase complex that is important for cell cycle G1 phase progression and G1/S transition. The activity of this k+inase first appears in mid-G1 phase, which is controlled by the regulatory subunits including D-type cyclins and members of INK4 family of CDK inhibitors. This kinase, as well as CDK4, has been shown to phosphorylate, and thus regulate the activity of, tumor suppressor protein Rb. Expression of this gene is up-regulated in some types of cancer.

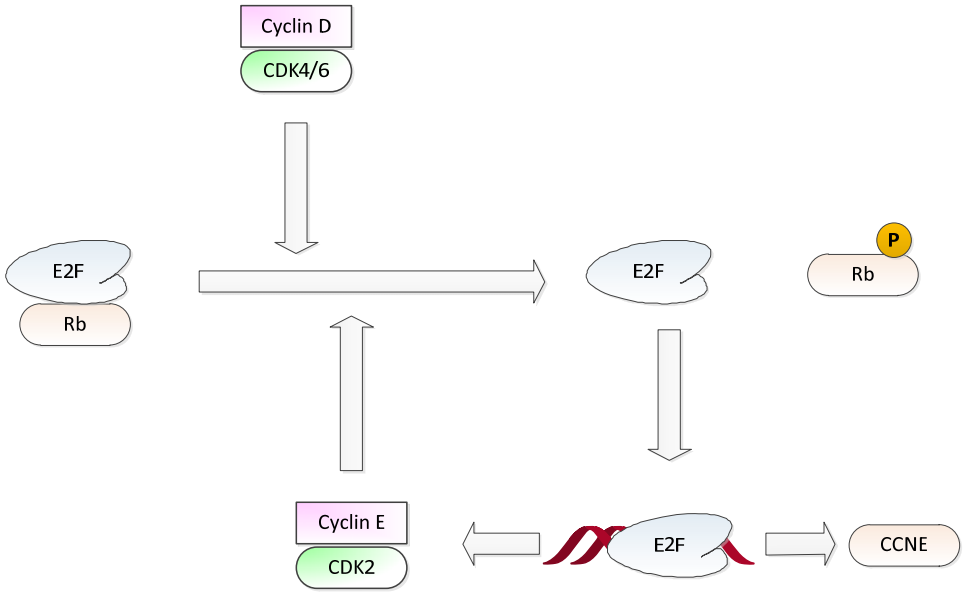
[4] http://www.ncbi.nlm.nih.gov/gene/983

[5] From http://www.ncbi.nlm.nih.gov/gene/595 data bases as a source.

Now the question is what activates these proteins, the cyclins and the CDKs, to make the cell cycle progress. This begins the creep upward in this pathway concern. We can redraw this process as follows and it will help to focus:



Now we ask what activates these proteins. We look at the activation of Cyclin E as shown by Bunz (p 219) below:



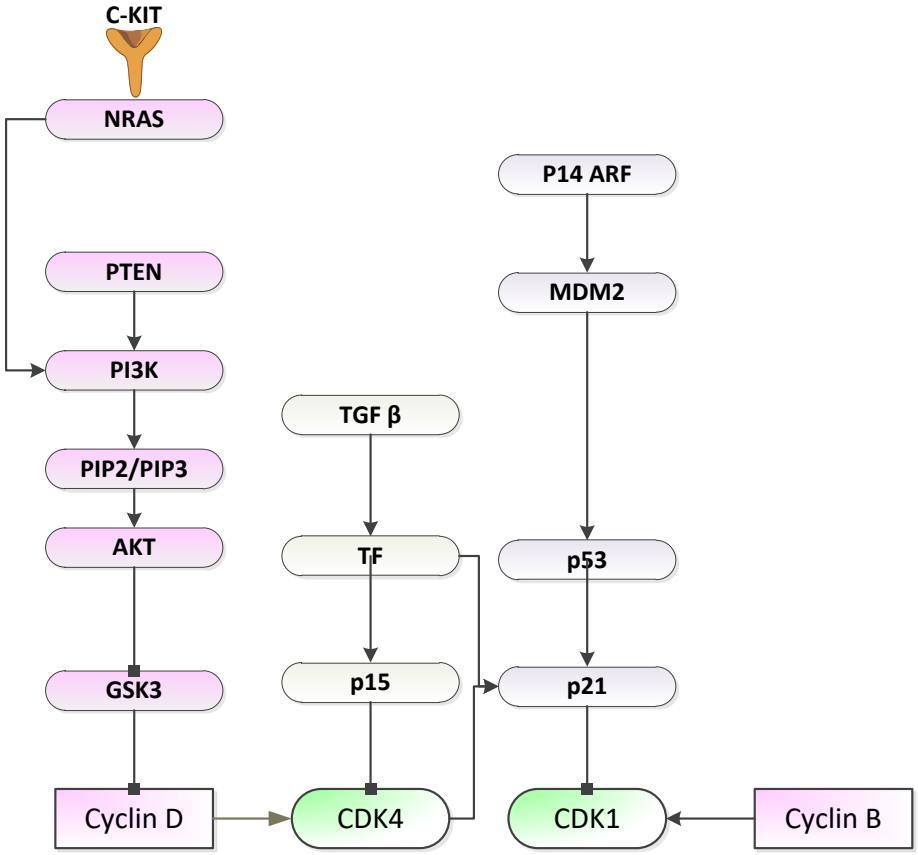
This is a feedback type reaction initiated by Rb the retinoblastoma gene protein. This feedback generates cyclin E which drives the cell through G1 and into the S cycle.

Gene	*Location*	*Function*
E2F1[6] (also RBP3; E2F-1; RBAP1; RBBP3)	20q11.2	The protein encoded by this gene is a member of the E2F family of transcription factors. The E2F family plays a crucial role in the control of cell cycle and action of tumor suppressor proteins and is also a target of the transforming proteins of small DNA tumor viruses. The E2F proteins contain several evolutionally conserved domains found in most members of the family. These domains include a DNA binding domain, a dimerization domain which determines interaction with the differentiation regulated transcription factor proteins (DP), a transactivation domain enriched in acidic amino acids, and a tumor suppressor protein association domain which is embedded within the transactivation domain. This protein and another 2 members, E2F2 and E2F3, have an additional cyclin binding domain. This protein binds preferentially to retinoblastoma protein pRB in a cell-cycle dependent manner. It can mediate both cell proliferation and p53-dependent/independent apoptosis.
RB 1[7] (also RB; pRb; OSRC; pp110; p105-Rb)	13q14.2	The protein encoded by this gene is a negative regulator of the cell cycle and was the first tumor suppressor gene found. The encoded protein also stabilizes constitutive heterochromatin to maintain the overall chromatin structure. The active, hypophosphorylated form of the protein binds transcription factor E2F1. Defects in this gene are a cause of childhood cancer retinoblastoma (RB), bladder cancer, and osteogenic sarcoma.
CCNE1[8]	19q12	The protein encoded by this gene belongs to the highly conserved cyclin family, whose members are characterized by a dramatic periodicity in protein abundance through the cell cycle. Cyclins function as regulators of CDK kinases. Different cyclins exhibit distinct expression and degradation patterns which contribute to the temporal coordination of each mitotic event. This cyclin forms a complex with and functions as a regulatory subunit of CDK2, whose activity is, required for cell cycle G1/S transition. This protein accumulates at the G1-S phase boundary and is degraded as cells progress through S phase. Overexpression of this gene has been observed in many tumors, which results in chromosome instability, and thus may contribute to tumorigenesis. This protein was found to associate with, and be involved in, the phosphorylation of NPAT protein (nuclear protein mapped to the ATM locus), which participates in cell-cycle regulated histone gene expression and plays a critical role in promoting cell-cycle progression in the absence of pRB. Two alternatively spliced transcript variants of this gene, which encode distinct isoforms, have been described.

[6] http://www.ncbi.nlm.nih.gov/gene/1869

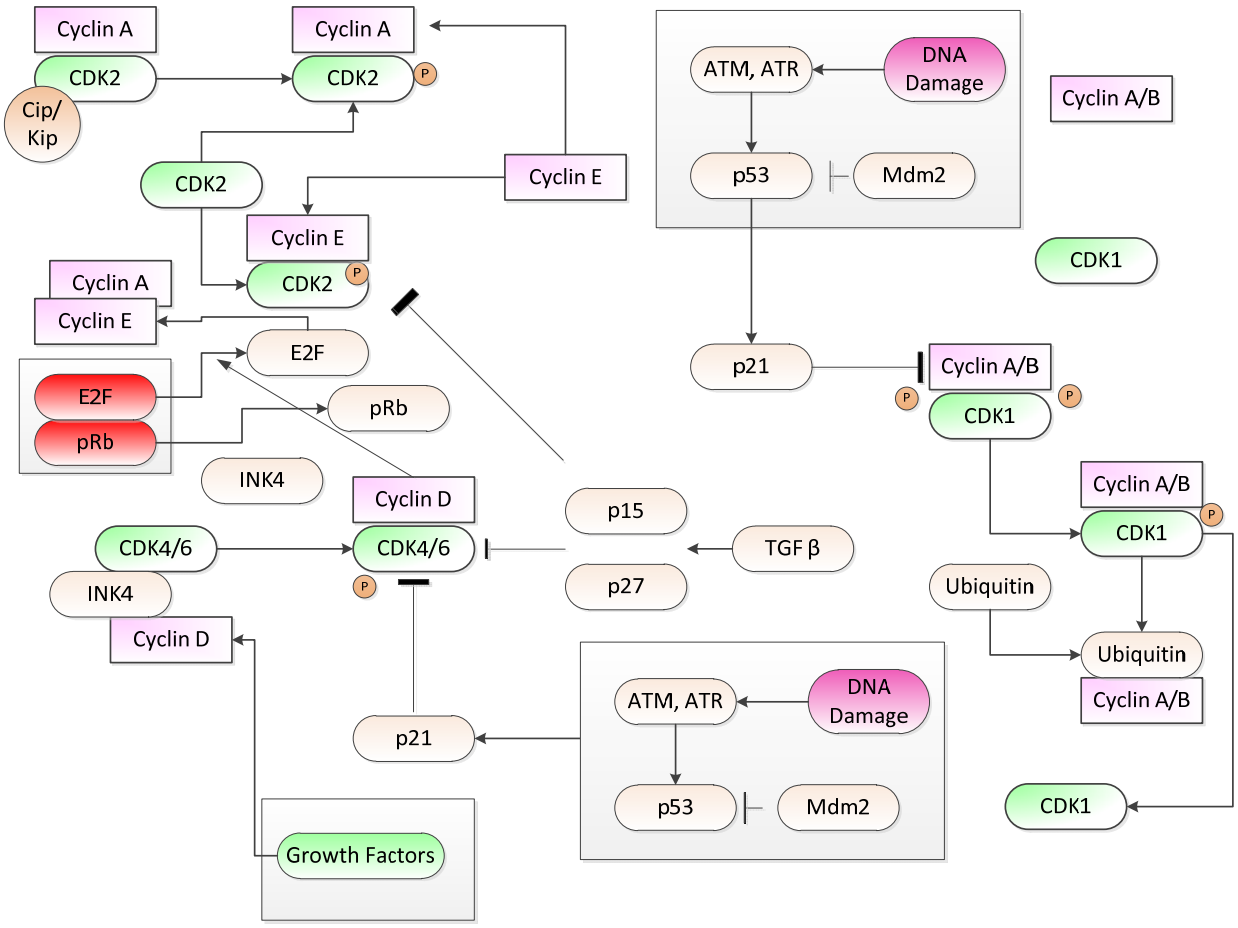
[7] http://www.ncbi.nlm.nih.gov/gene/5925

Now this establishes one base line for understanding cancer at the base of cell reproduction. Namely what can cause this process to continue unabated?



A more details analysis has been by Vermulen et al almost a decade ago. We shall use this as a baseline and then add to what we have learned in that period. The Vermulen network is shown as follows:

[8] http://www.ncbi.nlm.nih.gov/gene/898



Now in the Vermulen configuration we have the following elements:

1. CDKs: These are the cyclin dependent kinases we have been discussing.

2. Cyclins:

3. CDK Activating Enzymes:

4. CKI or CK Inhibitors

The following is a detailed list of some major CKIs or Cyclin Kinase Inhibitors. We have discussed them briefly before but they play a critical role in managing cell reproduction.

CKI Family	*Member Name*	*Alternative Name*	*Gene*	*Function*
INK4 Family	p15[9] (also P15; MTS2; TP15; CDK4I; INK4B; p15INK4b)	INK-4b	9p21	This gene lies adjacent to the tumor suppressor gene CDKN2A in a region that is frequently mutated and deleted in a wide variety of tumors. This gene encodes a cyclin-dependent kinase inhibitor, which forms a complex with CDK4 or CDK6, and prevents the activation of the CDK kinases, thus the encoded protein functions as a cell growth regulator that controls cell cycle G1 progression. The expression of this gene was found to be dramatically induced by TGF beta, which suggested its role in the TGF beta induced growth inhibition.
	p16[10] (also ARF; MLM; P14; P16; P19; CMM2; INK4; MTS1; TP16; CDK4I; CDKN2; INK4A; MTS-1; P14ARF; P19ARF; P16INK4; P16INK4A; P16-INK4A)	INK-4a	9p21	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, MDM1, 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.

[9] http://www.ncbi.nlm.nih.gov/gene/1030

[10] http://www.ncbi.nlm.nih.gov/gene/1029

CKI Family	*Member Name*	*Alternative Name*	*Gene*	*Function*
	p18[11]	INK-4c	1p32	The protein encoded by this gene is a member of the INK4 family of cyclin-dependent kinase inhibitors. This protein has been shown to interact with CDK4 or CDK6, and prevent the activation of the CDK kinases, thus function as a cell growth regulator that controls cell cycle G1 progression. Ectopic expression of this gene was shown to suppress the growth of human cells in a manner that appears to correlate with the presence of a wild-type RB1 function. Studies in the knockout mice suggested the roles of this gene in regulating spermatogenesis, as well as in suppressing tumorigenesis.
	p19[12]	INK-4d	19p13	The protein encoded by this gene is a member of the INK4 family of cyclin-dependent kinase inhibitors. This protein has been shown to form a stable complex with CDK4 or CDK6, and prevent the activation of the CDK kinases, thus function as a cell growth regulator that controls cell cycle G1 progression. The abundance of the transcript of this gene was found to oscillate in a cell-cycle dependent manner with the lowest expression at mid G1 and a maximal expression during S phase. The negative regulation of the cell cycle involved in this protein was shown to participate in repressing neuronal proliferation, as well as spermatogenesis.

[11] http://www.ncbi.nlm.nih.gov/gene/1031

[12] http://www.ncbi.nlm.nih.gov/gene/1032

CKI Family	*Member Name*	*Alternative Name*	*Gene*	*Function*
Cip-Kip Family	p21[13] also P21; CIP1; SDI1; WAF1; CAP20; CDKN1; MDA-6; p21CIP1	Waf1, Cip1	6p21.2	This gene encodes a potent cyclin-dependent kinase inhibitor. The encoded protein binds to and inhibits the activity of cyclin-CDK2 or -CDK4 complexes, and thus functions as a regulator of cell cycle progression at G1. The expression of this gene is tightly controlled by the tumor suppressor protein p53, through which this protein mediates the p53-dependent cell cycle G1 phase arrest in response to a variety of stress stimuli. This protein can interact with proliferating cell nuclear antigen (PCNA), a DNA polymerase accessory factor, and plays a regulatory role in S phase DNA replication and DNA damage repair. This protein was reported to be specifically cleaved by CASP3-like caspases, which thus leads to a dramatic activation of CDK2, and may be instrumental in the execution of apoptosis following caspase activation.
	p27[14] also p27; Rpn4	Cip2	12q24.31-q24.32	The 26S proteasome is a multicatalytic proteinase complex with a highly ordered structure composed of 2 complexes, a 20S core and a 19S regulator. The 20S core is composed of 4 rings of 28 non-identical subunits; 2 rings are composed of 7 alpha subunits and 2 rings are composed of 7 beta subunits. The 19S regulator is composed of a base, which contains 6 ATPase subunits and 2 non-ATPase subunits, and a lid, which contains up to 10 non-ATPase subunits. Proteasomes are distributed throughout eukaryotic cells at a high concentration and cleave peptides in an ATP/ubiquitin-dependent process in a non-lysosomal pathway. An essential function of a modified proteasome, the immunoproteasome, is the processing of class I MHC peptides. This gene encodes a non-ATPase subunit of the 19S regulator.

[13] http://www.ncbi.nlm.nih.gov/gene/1026

[14] http://www.ncbi.nlm.nih.gov/gene/5715

CKI Family	*Member Name*	*Alternative Name*	*Gene*	*Function*
	p57[15] also BWS; WBS; p57; BWCR; KIP2	Kip2	11p15.5	This gene is imprinted, with preferential expression of the maternal allele. The encoded protein is a tight-binding, strong inhibitor of several G1 cyclin/Cdk complexes and a negative regulator of cell proliferation. Mutations in this gene are implicated in sporadic cancers and Beckwith-Wiedemann syndrome, suggesting that this gene is a tumor suppressor candidate.

The following genes are elements of cell cycle control.

Gene	*Location*	*Function*
Jun[16]	1p32-p31	This gene is the putative transforming gene of avian sarcoma virus 17. It encodes a protein which is highly similar to the viral protein, and which interacts directly with specific target DNA sequences to regulate gene expression. This gene is intronless and is mapped to 1p32-p31, a chromosomal region involved in both translocations and deletions in human malignancies.
Fos[17]	14q24.3	The Fos gene family consists of 4 members: FOS, FOSB, FOSL1, and FOSL2. These genes encode leucine zipper proteins that can dimerize with proteins of the JUN family, thereby forming the transcription factor complex AP-1. As such, the FOS proteins have been implicated as regulators of cell proliferation, differentiation, and transformation. In some cases, expression of the FOS gene has also been associated with apoptotic cell death.

[15] http://www.ncbi.nlm.nih.gov/gene/1028

[16] http://www.ncbi.nlm.nih.gov/gene/3725

[17] http://www.ncbi.nlm.nih.gov/gene/2353

Gene	*Location*	*Function*
Myc[18]	8q24.21	The protein encoded by this gene is a multifunctional, nuclear phosphoprotein that plays a role in cell cycle progression, apoptosis and cellular transformation. It functions as a transcription factor that regulates transcription of specific target genes. Mutations, overexpression, rearrangement and translocation of this gene have been associated with a variety of hematopoietic tumors, leukemias and lymphomas, including Burkitt lymphoma. There is evidence to show that alternative translation initiations from an upstream, in-frame non-AUG (CUG) and a downstream AUG start site result in the production of two isoforms with distinct N-termini. The synthesis of non-AUG initiated protein is suppressed in Burkitt's lymphomas, suggesting its importance in the normal function of this gene

2.2 OTHER FACTORS IN THE CELL CYCLE

In a recent paper by Solimini et al the authors discuss the concepts of STOP and GO genes and carcinogenesis[19]. The paper reports on some extensive experimental results focusing on the issue of proliferation and the loss of certain sets of gene sites, the STP and GO sites.

The authors begin by discussing the current concepts of changes in oncogenes and tumor suppressor genes, some of the key pathway elements that we examine in analyzing intracellular pathway dynamics. They state:

Cancer progression is directed by alterations in oncogenes and tumor suppressor genes (TSGs) that provide a competitive advantage to increase proliferation, survival, and metastasis. The cancer genome is riddled with amplifications, deletions, rearrangements, point mutations, loss of heterozygosity (LOH), and epigenetic changes that collectively result in tumorigenesis.

How these changes contribute to the disease is a central question in cancer biology. In his "two-hit hypothesis," Knudson proposed that two mutations in the same gene are required for tumorigenesis, indicating a recessive disease. In addition, there are now several examples of haploinsufficient TSGs.

Current models do not explain the recent observation that hemizygous recurrent deletions are found in most tumors. Whether multiple genes within such regions contribute to the tumorigenic phenotype remains to be elucidated…

[18] http://www.ncbi.nlm.nih.gov/gene/4609

[19] Solimini, N., et al, Recurrent Hemizygous Deletions in Cancers May Optimize Proliferative Potential, Science, 6 JULY 2012 VOL 337, p 104.

The last sentence regarding the inability to explain the presence of hemizygous deletions under the current model is the main driver for this effort. Thus they argue and demonstrate experimentally that:

Tumors exhibit numerous recurrent hemizygous focal deletions that contain no known tumor suppressors and are poorly understood. To investigate whether these regions contribute to tumorigenesis, we searched genetically for genes with cancer-relevant properties within these hemizygous deletions.

We identified STOP and GO genes, which negatively and positively regulate proliferation, respectively.

STOP genes include many known tumor suppressors, whereas GO genes are enriched for essential genes.

Analysis of their chromosomal distribution revealed that recurring deletions preferentially over-represent STOP genes and under-represent GO genes.

*We propose a hypothesis called the **cancer gene island model**, whereby gene islands encompassing high densities of STOP genes and low densities of GO genes are hemizygously deleted to maximize proliferative fitness through cumulative haploinsufficiencies.*

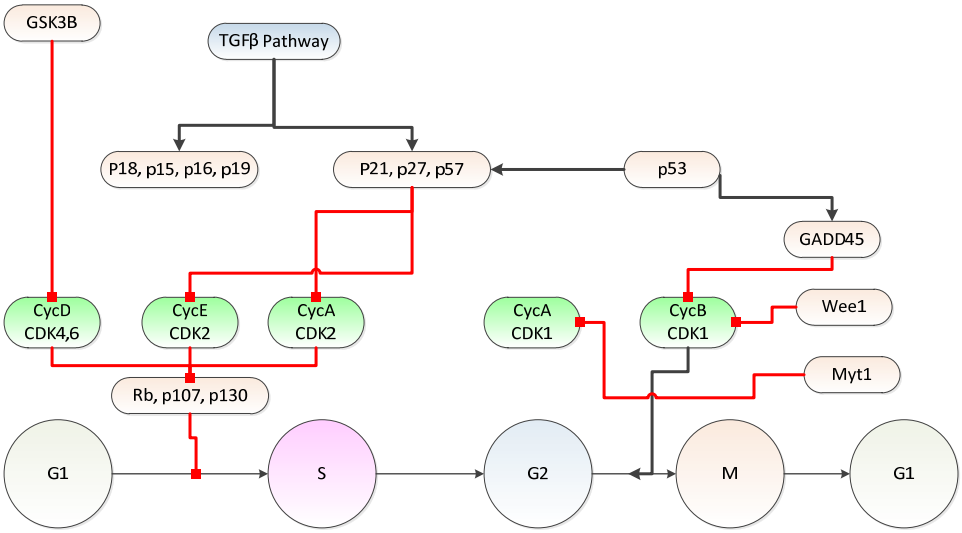
Because hundreds to thousands of genes are hemizygously deleted per tumor, this mechanism may help to drive tumorigenesis across many cancer types.

This is an intriguing hypothesis. It adds more pieces to an already complex puzzle. The Cancer Gene Island, CGI, hypothesis seems to indicate the complex changes in multiple gene sites. In particular there was a deletion of the STOP genes in preference to the GO genes. Unfortunately there did not seem to be a mechanism for these deletions, however the experimental evidence does indicate the phenomenon.

In their experimental analysis they have observed certain in vitro results which compel their hypothesis. They state:

This in silico analysis suggests that the loss of a single copy of GO genes has a negative impact on cellular fitness. To independently test this hypothesis, we turned to the other arm of our screen that identified candidate GO genes whose depletion limits proliferation and survival. Because both normal and cancer cells are dependent on these essential GO genes, we analyzed data from proliferation screens on HMECs, one normal prostate epithelial cell line, and seven breast or prostate cancer cell lines

They provide an interesting pathway model as shown below (as modified, and also not that they have short hairpin RNAs (shRNAs)).



From Solimini et al: *"Known pathways with multiple shRNAs in the validation screen. Genes that validated in the secondary screen by a factor of ≥4 with multiple shRNAs are denoted with circles corresponding to three to five shRNAs (orange) and six or more shRNAs (red)."*

They conclude as follows:

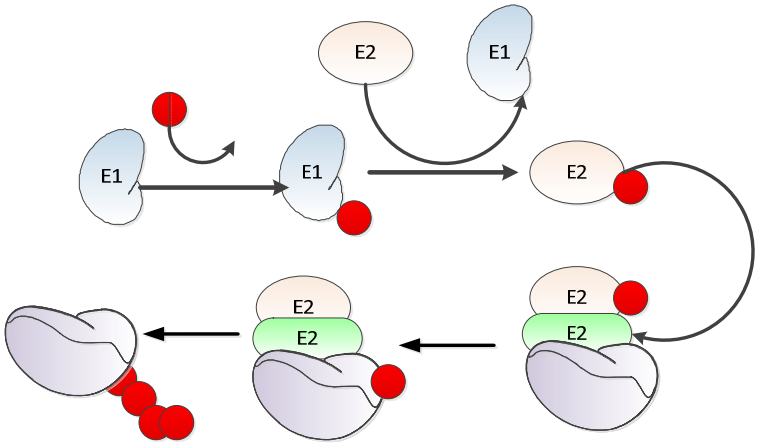
The enrichment for genes localized to deletions suggests that we have identified dozens of new TSGs in recurrent deletions. We have also likely identified more TSGs outside of these regions because the STOP gene set is (i) enriched for known TSGs, many of which are not found in recurrent deletions, and (ii) enriched for genes that undergo somatic loss-of-function mutation.

Finally, this work suggests that cells possess a substantial number of genes that restrain proliferation in vitro, which could be inactivated to promote clonal expansion during tumorigenesis in addition to the traditional driver genes currently known. Given the prevalence of multiple, large, recurring hemizygous deletions encompassing skewed distributions of growth control genes in tumors, we propose that the elimination of cancer gene islands that optimize fitness through cumulative haplo-insufficiencies may play an important role in driving tumorigenesis, with implications for the way in which we think about cancer evolution.

As with many such works this raises as many questions as it seems to answer. However the control or lack thereof of proliferation and the cell cycle is a critical issue in carcinogenesis.

2.2.1 Ubiquination

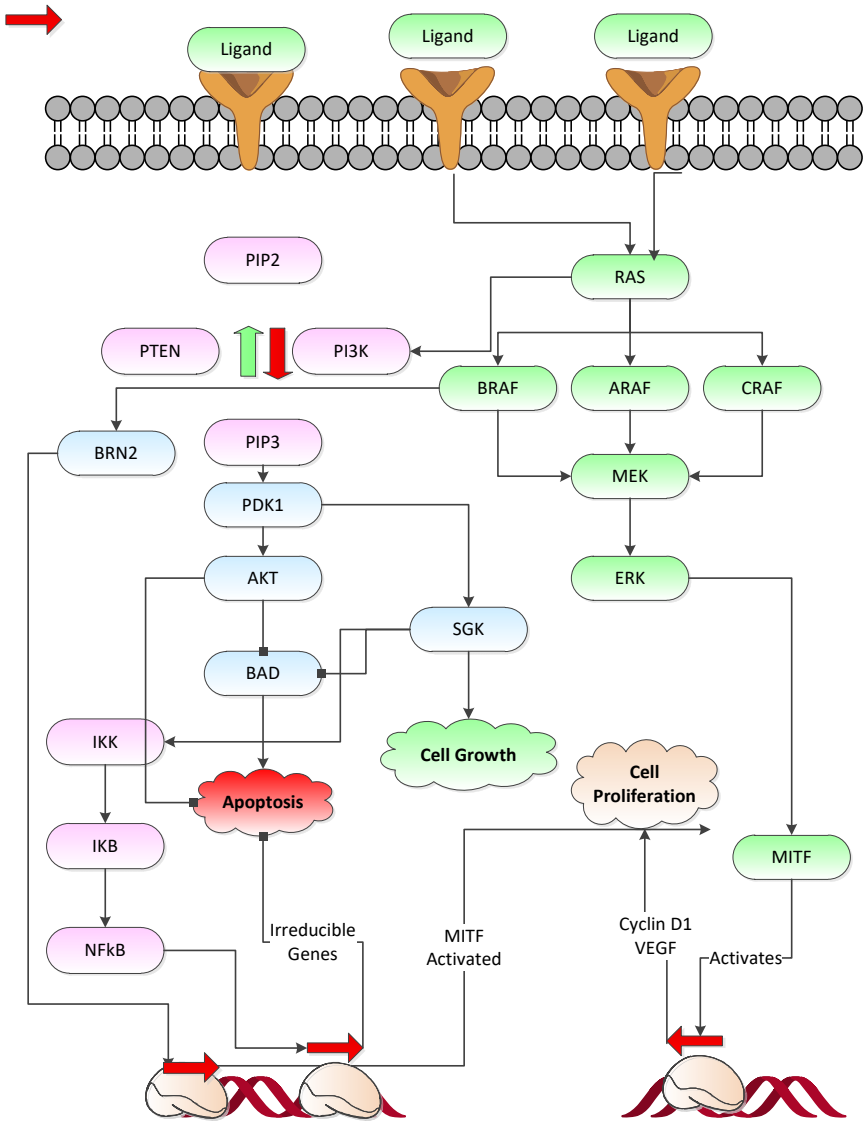
Ubiquitin is a small protein which acts with three related proteins; E1, E2, and E3. E1 is also called the ubiquitin activating enzyme, E2 the ubiquitin conjugating enzyme, and E3 ubiquitin ligase. Together they act to attach ubiquitin to a target protein and mark it for digestion and elimination. The process is shown below in general graphic form.



Ubiquination is an essential process within a cell to eliminate used or excess proteins. Although we will not discuss tis in detail, it is an essential process and the reader should refer to standard texts[20].

The following Figure depicts some of the mechanics in terms of genetic flow and control as to how Ubiquination occurs.

[20] See: Cassimeris et al p 688, Weinberg, p 242, Alberts et al, p 1065.



Simply there are three end states:

1. Cell Proliferation or Cell Cycle Mitosis

2. Cell Growth or the expansion and operations of a single cell outside of mitosis.

3. Apoptosis or cell death.

Now in the simplified model above we have several feedback loops, many driven by external ligands.

In this section we briefly review the issue of cellular growth. What makes cells reproduce? If we first examine skin cells, one of the many cells in the body which reproduce all the time, like blood cells, we can gain some insight.

Skin cells are reproducing all the time. Mostly the keratinocytes and getting sloughed off at the surface or rebuilding after a wound. The melanocytes frequently do not reproduce. They are neural crest derives and often just remain in the G0 state. They produce such products as melanosomes, and other proteins required for homeostasis. There are times when they may reproduce to a cluster state, such as found in lentigenes. This is a common response to excessive sun exposure. Namely we may see heavily pigmented areas of clustered melanocytes. Then we may have a nevus, the raised collection of melanocytes. In both cases the melanocytes tend to stay attached to the cluster, thus having functional E cadherin molecules.

Now what of prostate cells, they do not reproduce as quickly. The glands are generally stable and often reproduce after some nominal lifetime of the basal or luminal cell. However a cell is stressed, for example by some external driver as inflammation, or other external attack, and then the cells may regenerate and thus reproduce. Perhaps that is one of the mechanism which underlies indolent PCa. Melanoma for example is highly aggressive in any form, most likely driven by the aggressive growth medium. However, as is known, melanocytes alone are indolent. This is one of those "on the one hand, on the other hand" arguments.

2.2.2 Kinetics of Cell Cycles

One of the questions we may ask is related to the kinetics of these processes. For example in many cancers the cell doubling time is highly variable at different locations and at different times and with different cells. There have been a few studies regarding the kinetics, namely what facilitates and accelerates the cell cycle but there does not appear at this time to be a definitive conclusion.

2.3 SUMMARY

We have presented a high level summary of the DNA activity and the resulting cell cycle in mitotic activity. The cell cycle play an important role in cancer since inherent in any cancer is uncontrolled cell reproduction. The cyclins are at the heart of that process. It will be useful to go back to these basic ideas from time to time yet we do not consider the cell cycle as an integral part of our control model. Generally we try to take actions which prevent it from ever being entered. However it may become more critical to examine the cell cycle as a control point.

3 CANCER AND THE GF

Growth Factors have been an ongoing focus for cancer therapeutics for decades. They lack the clarity and specificity however of such immunotherapeutic targets such as CD19 in CAR-T cells. However we know they are often over or even under excited in various cancers.

As Aaronson had noted in 1991 in a paper summarizing the then know facts of growth factors in cancer:

Multicellular organisms have highly coordinated mechanisms to control cellular interactions. These complex signaling networks mediate normal embryonic development and are responsible for systemic responses to wounding and infection. The discovery of nerve growth factor (NGF) and ***epidermal growth factor (EGF)*** *has led to the identification of a wide array of factors that affect the growth of virtually all cell types. Such factors can act as positive or negative modulators of cell proliferation and influence differentiation.*

The interaction of growth factors, cytokines and hormones with specific membrane receptors triggers a cascade of intracellular biochemical signals, resulting in the activation and repression of various subsets of genes. Genetic aberrations in growth factor signaling pathways are inextricably linked to developmental abnormalities and to a variety of chronic diseases, including cancer.

Malignant cells arise as a result of a stepwise progression of genetic events that include the unregulated expression of growth factors or components of their signaling pathways.

This review focuses on normal aspects of growth factor signal transduction, as well as genetic aberrations in growth factor signaling pathways commonly implicated in human malignancy.

Growth factors cause cells in the resting or Go phase to enter and proceed through the cell cycle. The mitogenic response occurs in two parts; the quiescent cell must first be advanced into the G1 phase of the cell cycle by "competence" factors, traverse the G1 phase, and then become committed to DNA synthesis under the influence of "progression" factors. Transition through the G1 phase requires sustained growth factor stimulation over a period of several hours. If the signal is disrupted for a short period of time, the cell reverts to the Go state.

There is also a critical period in G, during which simultaneous stimulation by both factors is needed to allow progression through the cell cycle. After this restriction point, only the presence of a "progression" factor, such as insulin-like growth factor 1 (IGF-1), is needed. Cytokines such as transforming growth factor m (TGFO), interferon, or tumor necrosis factor (TNF) can antagonize the proliferative effects of growth factors. In the case of TGFOi, these effects can be observed even when cells are treated with the cytokine relatively late in G1.

In some cell types, the absence of growth factor stimulation causes the rapid onset of programmed cell death or apoptosis. Certain growth factors can also promote differentiation of a progenitor cell, while at the same time stimulating proliferation; others acting on the same cell

induce only proliferation. Thus, there must be specific biochemical signals responsible for differentiation that only certain factors can trigger.

The actions of a series of growth factors can cause a hematopoietic progenitor to move through stages to a terminally differentiated phenotype. However, at intermediate stages, in the absence of continued stimulation by the factor, this commitment is reversible. Although the differentiation program of the cell governs the diversity of phenotypic responses elicited, there are some common, highly conserved biochemical pathways for mitogenic signaling.

The above also includes the growth factors in hematopoetic cells as well as somatic cells. Our focus is on somatic cell GF/GFR. We will not consider cytokines or other exogeneous growth factors. However it is clear that the recognition of the GF/GFR complexes play an important role in many malignancies.

GF/GFR complexes act over a significant distance in the body. They also act to change the nature of cells. As Yang et al note:

To detach from the primary tumour and invade into the surrounding tissue, tumour cells have to break down cell-cell contacts, remodel cell-matrix adhesion sites, and transit from an epithelial phenotype to a mesenchymal phenotype, this transition is called ***epithelial-mesenchymal transition (EMT)****. EMT is an early stage of cell migration and invasion which occurs during tumour progression and is characterised by the disruption of intercellular junctions and the replacement of apical-basolateral polarity with front-to-back polarity.*

We have examined this effect in other cancers such as melanoma and prostate cancer. In melanoma we see the loss of E cadherin bonding and the movement of the melanocyte from a basal layer upwards and then downwards. Melanoma in situ, MIS, for example, is the movement of the melanocyte from the basal layer upwards, loosing its localization. In PCa, we see the same as there is a progression in high grade PIN, HGPIN[21].

Yang et al continue:

EMT transforms cancer cells from an epithelial morphology to a migratory and invasive phenotype which is critical for the metastasis of many carcinomas. Loss of E-cadherin function is a key step in the EMT process. E-cadherin is a major component of epithelial adheren junctions and mediates intercellular adhesion in epithelial cancers.

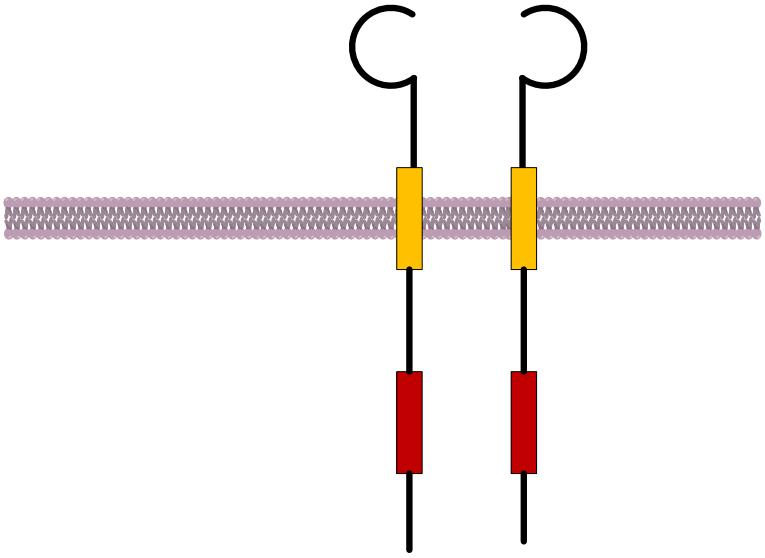
Loss of E-cadherin function not only leads to a mechanical disruption of adherens junctions, but also liberates proteins from the cytoplasmic cell adhesion complex which exert ambivalent functions depending on their subcellular localization.

Following a series of molecular processes the cellular static actin structures are reorganized, a pliable membrane protrusion forms and membrane ruffling occurs. After loss of epithelial E-cadherin, cancer cells start to express mesenchymal N-cadherin which has a specific affinity for

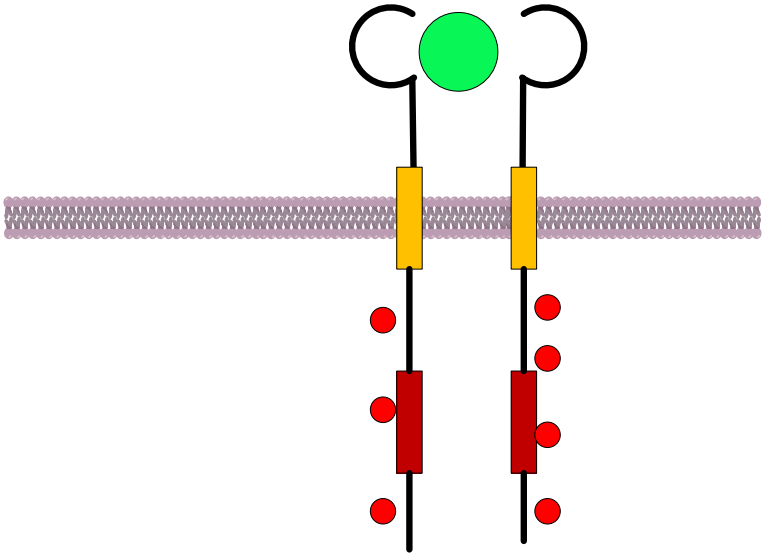
[21] See McGarty, Melanoma, a Systems Approach and Prostate Cancer, A Systems Approach, https://telmarc.com

mesenchymal cells. This cadherin switch leads to a drastic change in the adhesive properties of a cell, as it loses its affinity for its epithelial neighbours and gains affinity for mesenchymal cells, such as fibroblasts or vascular endothelial cells

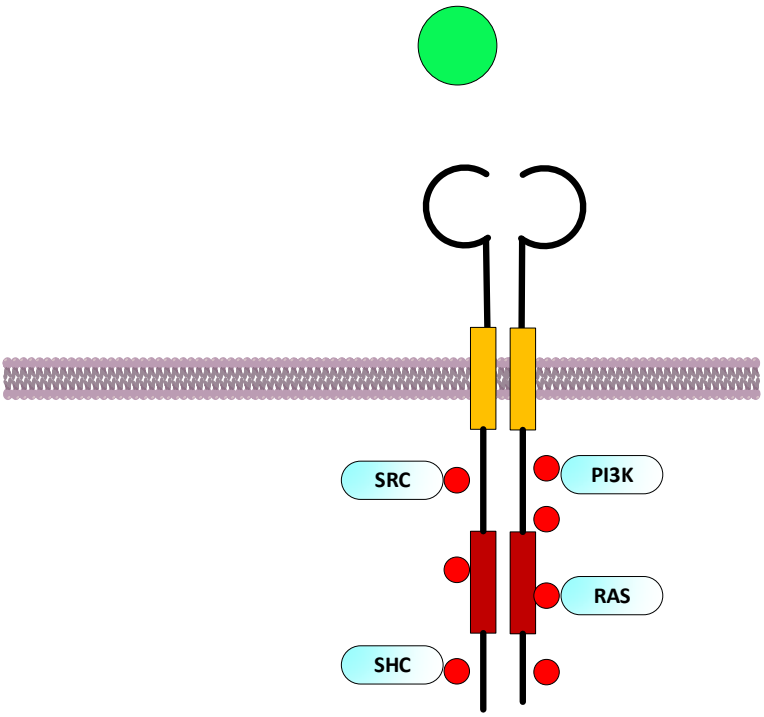
Now a typical operation of a GF/GFR complex is presented below. First we have a GFR composed of a multiplicity of proteins in a dimer fashion with an extracellular bonding region open.



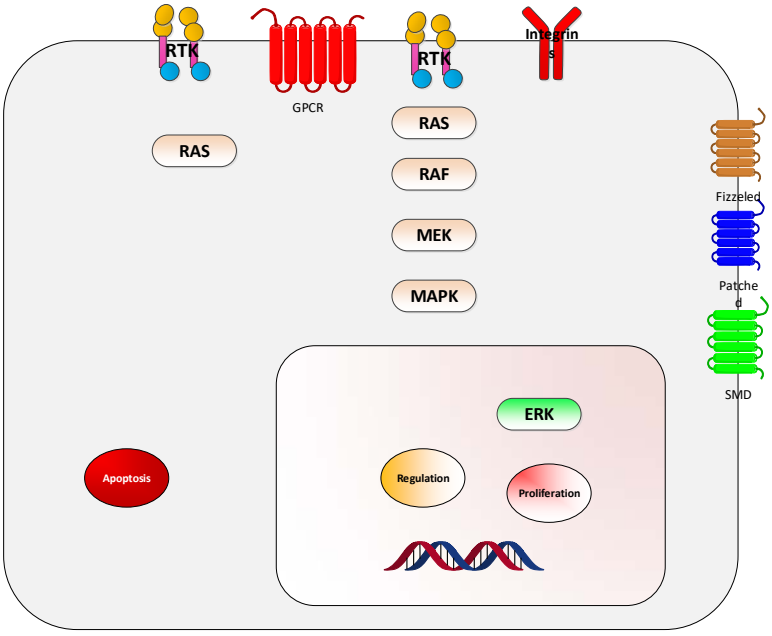
Then we see a GF approach and attach itself to the region. This causes phosphorylation of the intracellular elements which then will create an activation of internal pathways.



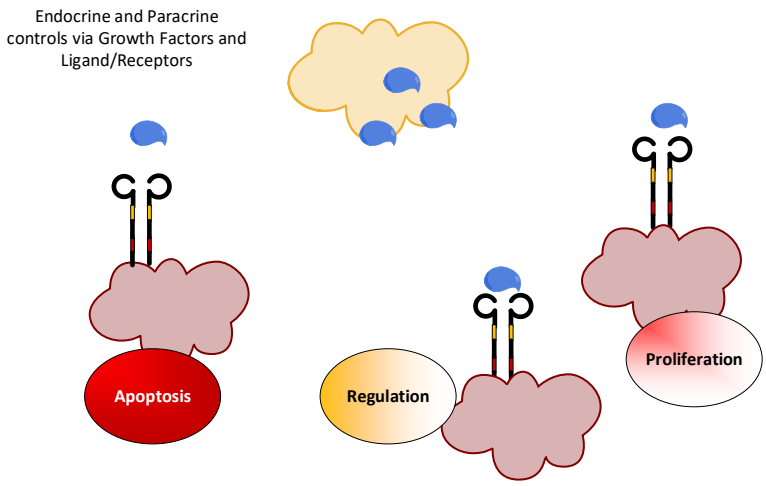
Then we see the release of the GF and the activated GFR set to initiate internal pathway processing and the resulting actins based on the activations.



Some of these activations are depicted below. The RAS/RAF/MEK path is activated and this may result in anything from apoptosis to proliferation depending on the other pathways so activated. We will discuss this in more detail latter.



The most critical factor resulting from the control or lack thereof of a growth factor is uncontrolled proliferation. Also there is a secondary factor which is loss of apoptotic control over cells as well.



Cells are very complex entities and do not operate as isolated entities. As Venere et al note:

Cells sense their microenvironment by detecting other cells through cell adhesion molecules, extracellular matrix through integins, and secreted factors through growth factor receptors. Notably, members of the receptor tyrosine kinase (RTK) family have important roles in the maintenance of the stem cell phenotype. Modulating levels of RTKs in CSCs can alter a variety of key CSC phenotypes, including the self-renewal required to maintain an undifferentiated state, tumorigenicty, and invasiveness as well as impact overall viability.

It is the actions of these RTK pathways that result in the complexity of cellular functions. As Sengupta et al note:

The blood levels of growth factors such as insulin and IGF-1 reflect the fed status of the organism. When food is plentiful, levels of these growth factors are sustained and promote anabolic cell processes such as translation, lipid biosynthesis, and nutrient storage via mTORC1. The binding of insulin to its cognate tyrosine kinase receptor recruits insulin receptor substrate 1 (IRS1) to the receptor and activates phosphoinositide 3-kinase (PI3K), which through the production of phosphatidylinositol (3,4,5)-triphosphate [PtdIns(3,4,5)P3] recruits Akt to the plasma membrane, where it becomes activated by direct phosphorylation by PDK1 and mTORC2. Akt, along with other kinases downstream of growth factor signaling, such as MAPK and p90 RSK1, phosphorylates TSC2.

TSC2 (also known as tuberin) is the GTPase activating protein (GAP) for Rheb, and together with its partner TSC1 (also known as hamartin) forms the heterodimeric tuberous sclerosis complex (TSC). Tsc1 and Tsc2, when lost, lead to the development of tuberous sclerosis complex,

a tumor syndrome characterized by the appearance in a variety of tissues of benign tumors containing large cells. When active, TSC2 inhibits mTORC1 by promoting the conversion of Rheb-GTP to Rheb-GDP. The phosphorylation of TSC2 in response to growth factors correlates with an increase in mTORC1 activation, but exactly how TSC2 phosphorylation leads to its repression is unclear.

We shall expand on these concepts as we examine a collection of significant GF/GFR complexes.

4 TGF

The Transforming Growth Factor, TGF, has several forms. They control the SMAD signalling pathway which in turn controls transcriptional actions. We will outline some of these herein. Some of these gene activations control the S phase as discussed above.

From NCBI we list the following:

TGFB1[22]*: This gene encodes a secreted ligand of the TGF-beta (transforming growth factor-beta) superfamily of proteins. Ligands of this family bind various TGF-beta receptors leading to recruitment and activation of SMAD family transcription factors that regulate gene expression. The encoded preproprotein is proteolytically processed to generate a latency-associated peptide (LAP) and a mature peptide, and is found in either a latent form composed of a mature peptide homodimer, a LAP homodimer, and a latent TGF-beta binding protein, or in an active form consisting solely of the mature peptide homodimer. The mature peptide may also form heterodimers with other TGFB family members. This encoded protein regulates cell proliferation, differentiation and growth, and can modulate expression and activation of other growth factors including interferon gamma and tumor necrosis factor alpha. This gene is frequently upregulated in tumor cells, and mutations in this gene result in Camurati-Engelmann disease*

TGFB2[23]*: he protein encoded by this gene is a transmembrane protein that has a protein kinase domain, forms a heterodimeric complex with TGF-beta receptor type-1, and binds TGF-beta. This receptor/ligand complex phosphorylates proteins, which then enter the nucleus and regulate the transcription of genes related to cell proliferation, cell cycle arrest, wound healing, immunosuppression, and tumorigenesis. Mutations in this gene have been associated with Marfan Syndrome, Loeys-Deitz Aortic Aneurysm Syndrome, and the development of various types of tumors.*

As Yang et al note:

TGF-β is a member of a growth factor family that regulates cellular proliferation, differentiation, apoptosis and extracellular matrix formation. TGF- β usually serves as a tumour suppressor in the normal tissues by inhibiting cell proliferation and inducing apoptosis, but it promotes tumour progression and invasion if the tumour cells overcome its cytostatic and apoptotic effects.

Thus TGF is a powerful GF and can effect a multiplicity of changes. It appears to be a regulator of excessive cell proliferation and suppression can result in proliferation and uncontrolled cellular activity. The author continues:

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

[23] https://www.ncbi.nlm.nih.gov/gene/7048

In fact TGF-β is one of the most potent inducers of EMT both in cultured cells and animal models. It initiates carcinogenic EMT in different systems in vitro and in vivo, inhibits the growth of epithelial cells and promotes the growth of mesenchymal cells.

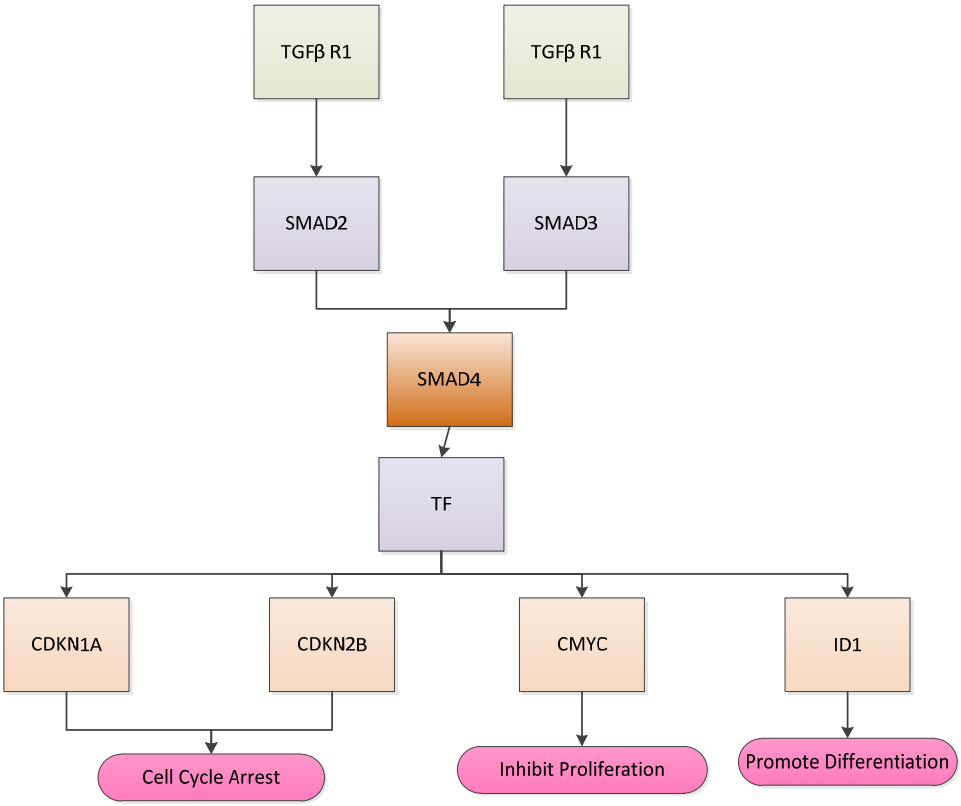
TGF-β signals through three cell surface receptors: the type I (TβRI), type II (TβRII) and type III (TβRIII) receptors. TβRIII can bind all TGF-β isoforms and presents them to TβRII. After binding with ligand, TβRII recruits and phosphorylates TβRI to activate its kinase activity. TβRI then phosphorylates and activates Smad2/3, which bind to Smad4, and the complex accumulates in the nucleus and interacts with other transcription factors to regulate the expression of a multitude of target genes

Thus TGF should be considered one of the more critical GF to be examined.

4.1 TGF, SMAD4 AND SIGNALLING

SMAD4 is an element in the TGF-β signalling chain. TGF is a cytokine, specifically a transforming growth factor cytokine. Like the Wnt-Apc pathway, the TGF pathway links defective development to cancer. The pathway is shown in part below (from Bunz p 199). Normal TGF signalling down-regulates the growth of most normal cells. Several of the genes in the TGF/SMAD pathway activation suppress growth. Specifically the genes CDKN1A and CDKN2B encode the cyclin dependent kinase inhibitors which suppress growth. Activated SMAD pathways also appear to suppress the transcription of other genes including c-Myc.

We show some of the TGF SMAD signalling below. We will elaborate this later.



SMAD4 controls the G1 to S transition. As stated in NCBI[24]:

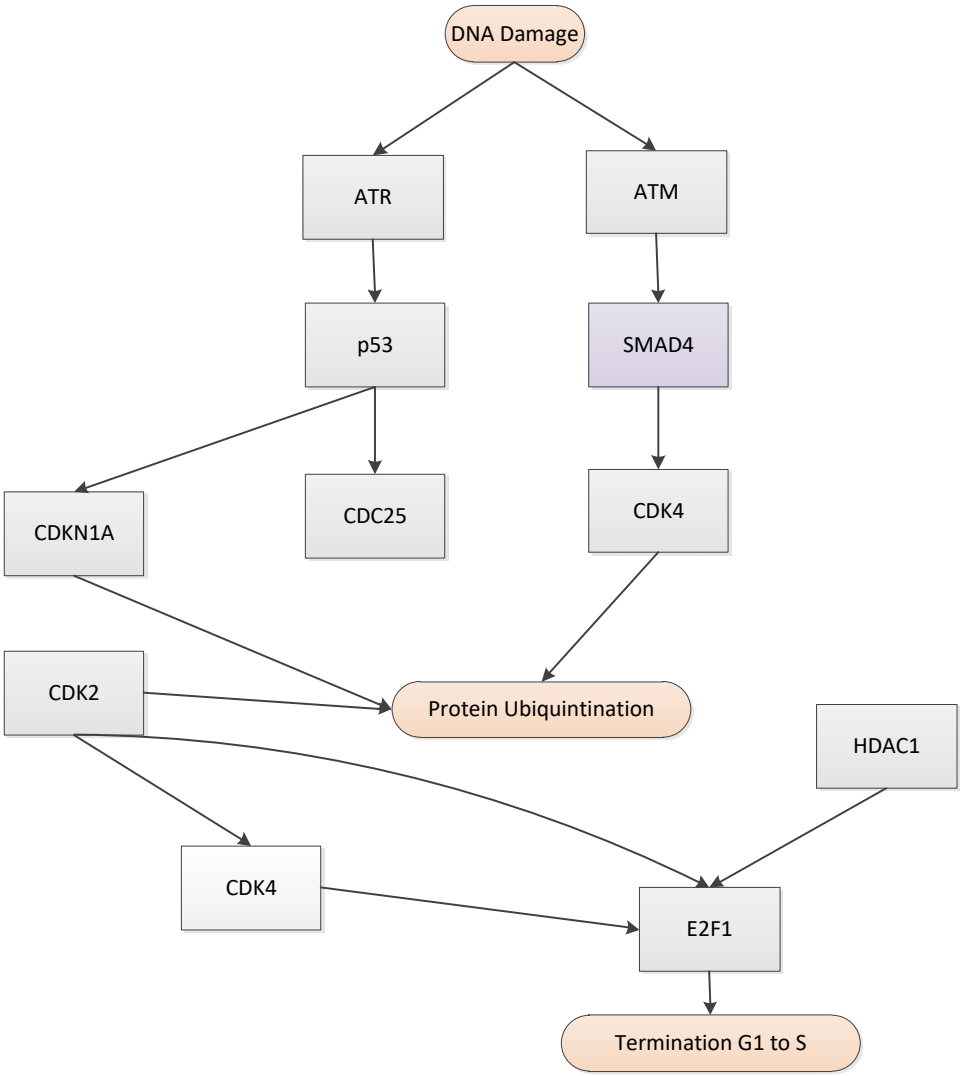
This gene encodes a member of the Smad family of signal transduction proteins. Smad proteins are phosphorylated and activated by transmembrane serine-threonine receptor kinases in response to TGF-beta signaling. The product of this gene forms homomeric complexes and heteromeric complexes with other activated Smad proteins, which then accumulate in the nucleus and regulate the transcription of target genes.

This protein binds to DNA and recognizes an 8-bp palindromic sequence (GTCTAGAC) called the Smad-binding element (SBE). The Smad proteins are subject to complex regulation by post-translational modifications. Mutations or deletions in this gene have been shown to result in pancreatic cancer, juvenile polyposis syndrome, and hereditary hemorrhagic telangiectasia syndrome.

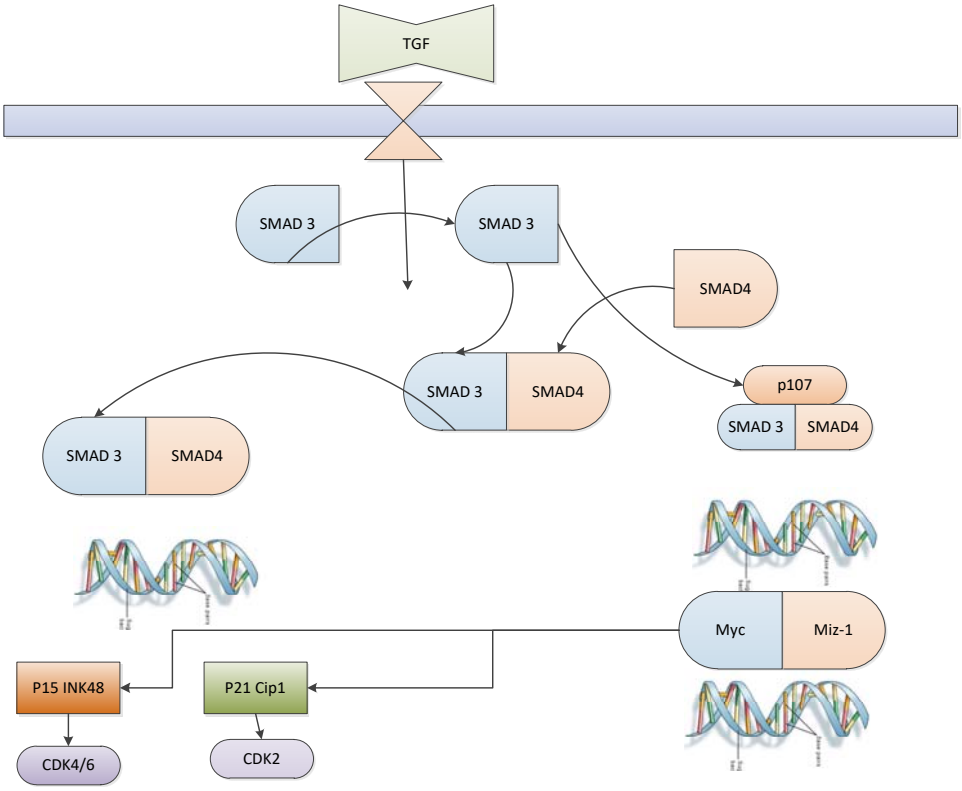
We use the NCI data set for its pathway[25]:

[24] http://www.ncbi.nlm.nih.gov/gene/4089

[25] http://pid.nci.nih.gov/search/pathway_landing.shtml?pathway_id=100160&source=BioCarta&genes_a=4089&genes_b=&what=graphic&jpg=on&ppage=1



The SMAD pathway is also detailed by NCI and one is referred to that source for further detail. From Weinberg (p 291) we also have the SMAD4 pathway showing its immediate control of the DNA transcription.



As Weinberg states (p 292):

"… Half of all pancreatic carcinomas and more than a quarter of all colon carcinomas carry mutant inactivated Smad4 proteins. Without the presence of Smad4 neither Smad2-Smad4 norr Smad3-Smad4 complexes can form. These two complexes are the chief agents dispatched by the TGF-β receptor to the nucleus with the important assignment to shut down proliferation."

This control mechanism is shown above.

4.2 TGF CANCERS

In the thesis of Schlegel, the author states:[26]

We contend that melanoma cells switch between two defined gene expression signatures, each underlying a distinct cell phenotype, which together drive disease progression. Presented in this thesis are the in vitro and in vivo experimental validations for this model, the investigation of the role of TGF-β-like signalling, predominantly its role in growth inhibition, and the identification of Id2 as a gene involved in TGF-β-induced growth inhibition response. After a literature review of genes identified to have phenotype-specific expression, we identified Wnt and TGF-β signalling as drivers of the identified transcriptional signatures. By in vitro characterization of phenotypically opposed cells, we identified the two phenotypes as proliferative and invasive. As

[26] http://e-collection.ethbib.ethz.ch/eserv/eth:30488/eth-30488-01.pdf

well as showing divergent proliferative and invasive behavior, cell types could be discriminated based on their growth susceptibility to TGF-ß and their capacity for vasculogenic mimicry.

Reduced susceptibility to the growth inhibiting effects of TGF-ß and the capacity for vasculogenic mimicry have both been associated with increased invasive and metastatic properties of melanoma cells. Our model suggests that both proliferative and invasive transcriptional signatures are important in disease progression and that each melanoma cell retains the capacity to express either signature given appropriate signalling. Our model also accounts for much observed gene expression heterogeneity in melanoma tumours.

This heterogeneity and reversibility of transcription programs were also shown in vivo using a xenograft mouse model. We also investigated the motive forces behind differential TGF-ß signalling. Smad activation was present in all melanoma cultures irrespective of the presence of a TGF-ß signature, which suggested Smad-independent TGF-ß signalling. The TGF-ß Smad-dependent pathway has long been considered as being central to TGF-ß signalling but it is now recognized that TGF-ß signals via crosstalk with alternative pathways.

We investigated alternative pathways but could identify no link between the activation status of several MAPK pathways and the TGF-ß signature. TGF-ß is a multifunctional cytokine which controls aspects of cell proliferation, differentiation, migration, apoptosis, adhesion, angiogenesis, immune surveillance, and survival. TGF-ß was initially defined as a transforming cytokine but it is now understood that TGF-ß has dual roles both as tumor suppressor and tumor promoter.

To better understand the regulation behind the expression of these opposite behaviors, we studied TGF-ß's cytostatic effect, which plays an important role in its tumor suppressing function and which is lost as melanoma cells become more invasive and metastatic. We identified the Id2 gene as differentially regulated by TGF-ß and link the loss of its regulation to acquired resistance to TGF-ß in invasive phenotype cells.

We show that TGF-ß induces cell cycle arrest through induction ofpI5fnk4b and repression of Id2. Furthermore, Id2 overexpression in proliferative phenotype cells counteracts pI5fnk4b induction and consequently protects melanoma cells from TGF-ß-mediated inhibition of proliferation.

Treating tumours comprised of cells with variably expressing transcription signatures presents a difficult challenge. This is because specific therapies have targeted factors we identify here as being subject to repeated changes in regulation. It is therefore of primary importance we recognize that the existing paradigm for melanoma progression is insufficient for the design of effective therapies.

The above work is a recent and reasonable summary of TGF status.

5 VEGF

The vascular endothelial growth factor is a critical GF associated with vascular growth and ultimately metastasis.

As NCBI notes:

VEGFA[27]: This gene is a member of the PDGF/VEGF growth factor family. It encodes a heparin-binding protein, which exists as a disulfide-linked homodimer. This growth factor induces proliferation and migration of vascular endothelial cells, and is essential for both physiological and pathological angiogenesis. Disruption of this gene in mice resulted in abnormal embryonic blood vessel formation. This gene is upregulated in many known tumors and its expression is correlated with tumor stage and progression. Elevated levels of this protein are found in patients with POEMS syndrome, also known as Crow-Fukase syndrome. Allelic variants of this gene have been associated with microvascular complications of diabetes 1 (MVCD1) and atherosclerosis. Alternatively spliced transcript variants encoding different isoforms have been described. There is also evidence for alternative translation initiation from upstream non-AUG (CUG) codons resulting in additional isoforms. A recent study showed that a C-terminally extended isoform is produced by use of an alternative in-frame translation termination codon via a stop codon readthrough mechanism, and that this isoform is antiangiogenic. Expression of some isoforms derived from the AUG start codon is regulated by a small upstream open reading frame, which is located within an internal ribosome entry site.

VEGFB[28]: his gene encodes a member of the PDGF (platelet-derived growth factor)/VEGF (vascular endothelial growth factor) family. The VEGF family members regulate the formation of blood vessels and are involved in endothelial cell physiology. This member is a ligand for VEGFR-1 (vascular endothelial growth factor receptor 1) and NRP-1 (neuropilin-1). Studies in mice showed that this gene was co-expressed with nuclear-encoded mitochondrial genes and the encoded protein specifically controlled endothelial uptake of fatty acids. Alternatively spliced transcript variants encoding distinct isoforms have been identified.

In general we would focus on the A type.

5.1 VEGF FUNCTIONING

We now consider the functions associated with VEGF. It is primarily related to vascular growth and as such becomes a key element in any cancer proliferation. As Botehlo et al note:

Vascular Endothelial Growth Factor (VEGF) is a growth factor involved in the promotion of endothelial cell proliferation, vascular permeability and angiogenesis, which are critical stages for tumor growth and development, namely prostate cancer. It is synthesized by adenocarcinoma

[27] https://www.ncbi.nlm.nih.gov/gene/7422

[28] https://www.ncbi.nlm.nih.gov/gene/7423

cells, and in prostatic cancer patients the prostatic gland contributes considerably to circulating VEGF levels. Elevated plasma VEGF levels could reflect prostatic VEGF production, making VEGF a potentially interesting tumor marker to support the decision of submitting a patient to prostatic biopsy.

Previous studies on this topic are conflicting. Some authors have found higher levels of VEGF in prostatic cancer patients, while others found no differences between subjects with benign prostatic hyperplasia (BPH) and those with malignant disease, or increased values only in patients with metastatic prostatic cancer or hormone-refractory disease. However, most previous studies evaluated relatively small samples and all suffered from limited-challenge bias, as prostatitis, which may interfere with the diagnostic value of VEGF, was not evaluated separately in any of the studies and in many studies the control group only included subjects with no suspicion of prostatic cancer.

We attempted to evaluate VEGF as a diagnostic tool for prostatic cancer, comparing its serum levels across groups of patients with suspected prostate cancer, presenting different prostatic pathologies (including BPH, prostatitis, high grade prostate intraepithelial neoplasia (HGPIN) and prostate cancer)….

VEGF levels are higher in subjects with prostatitis and prostatic cancer compared to patients at high prostate cancer risk but whose prostatic biopsy only revealed normal or hyperplasic tissue. However, in this consecutive series of patients eligible for prostatic biopsy there were no overall differences in VEGF serum levels between subjects with benign prostatic disease and prostate cancer cases….

Our results contribute to explain the heterogeneity observed in the literature on this topic. Prostatitis is an inflammatory condition associated with angiogenesis that raises VEGF levels, similar to the observed in prostate cancer, and may be highly prevalent in patients with increased tPSA levels.

Reports of prostatitis prevalence range from 10% to 63% , and was 16.1% in our series. We observed no relevant difference in VEGF circulating levels between patients with benign prostatic histology and cancer, when patients with prostatitis were also considered in the latter group. The two previous studies that evaluated participants with high risk of prostate cancer also observed no significant associations between cancer and VEGF levels….Other studies showed higher VEGF levels in patients with prostate cancer when compared with healthy controls or subjects with benign prostatic hypertrophy.

Such comparisons however, are not clinically relevant since elevated tPSA is the most frequent indication for prostatic biopsy, and reflect limited-challenge-bias. A diagnostic test must be evaluated in a clinically relevant population, preferably in a consecutive series of individuals in whom the target condition is suspected. Studies using healthy controls, not representing the whole spectrum of potential diagnosis alternative to prostate cancer which are able to generate false-positive results, namely when prostatitis is present, produce inflated estimates of diagnostic accuracy.

We have noted before two of the VEGF isoforms. It is worth noting the others as well. As Yang et al note:

VEGF is the key regulator in tumour angiogenesis and expressed in all solid tumours studied. Its expression has been regarded as a risk factor for metastases from colon and breast cancer. VEGF belongs to a family of angiogenic factors and consists of several isoforms termed as VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E and PIG-F.

Among these factors VEGF-A plays an essential role in angiogenesis which binds to two tyrosine kinase receptors, named VEGFR-1 and VEGFR- 2. VEGF-A have six isoforms with different lengths of amino acid residues resultant from alternative splicing. VEGFA is involved in every stage of vascular development due to its potential of inducing endothelial cell proliferation and survival. The capability of VEGF in promotion of vascular ***endothelial cell (EC)*** *proliferation has been well documented in in vitro and in vivo models, its pro-survival effect on EC has been suggested by a study in which the ablation of VEGF has significantly increased apoptosis of EC.*

VEGF is also a vascular permeability factor which enhances vascular leakage and permeability. Induction of vascular permeability is an essential early step in angiogenesis which results in leakage of plasma proteins, including fibrinogen and other clotting proteins. The clotting system is rapidly activated by tissue factors and results in the deposition of extravascular fibrin in tumour stroma; the fibrin can transform the antiangiogenic stroma into a provisional stroma that is strongly pro-angiogenic. Consistent with a role in the regulation of vascular permeability, VEGF also induces endothelial fenestration in some vascular beds

Recently Karaman et al have noted:

The vascular circulatory system evolved to enable the shuttling of nutrients, oxygen or waste products between various tissues, employing networks of blood vessels and lymphatic vessels that arise by the processes of angiogenesis and lymphangiogenesis, respectively.

Over the past few decades, vascular endothelial growth factors (VEGFs) and their receptors (VEGFRs) have emerged as the principal drivers of angiogenesis and lymphangiogenesis, and hence the development and maintenance of both of these vascular systems.

The field of VEGF/VEGFR signaling was established by seminal papers describing the functional role of VEGFA (which was initially named as VPF, for vascular permeability factor) and the identification of VEGFA as an endothelial growth factor These discoveries were followed by the identification of the receptor tyrosine kinases VEGFR1 (FLT1), VEGFR2 (KDR/FLK1) and VEGFR3 (FLT4), which were later shown to bind to VEGFs.

Since then, a multitude of studies has provided insights into the mechanisms and regulation of VEGF/VEGFR signaling. As organ development and function relies heavily on the parallel development and maintenance of organ-specific vascular systems, understanding the role and contribution of VEGF/VEGFR signaling to these processes is essential for furthering our understanding of development.

In addition, it is now clear that VEGF signaling is essential for the physiological function of many tissues and plays important roles in the pathogenesis of diseases such as cardiovascular disease, ***cancer*** *and ocular disease….*

During the past few decades, it has been established that angiogenesis is essential for embryonic development and homeostasis in adults, as well as for the progression of cancer and other diseases. More recently, lymphangiogenesis was also shown to be essential for embryonic development, and to be involved in many pathological processes such as lymphedema, inflammatory diseases and ***tumor metastasis****.*

Our knowledge of the circulatory system in general and of the molecular mechanisms controlling angiogenesis and lymphangiogenesis has improved considerably due to progress in the identification of regulatory molecules and markers specific to the blood vascular and lymphatic endothelium.

5.2 VEGF AND CANCERS

We have noted the impact of VEGF in PCa but it is a player in a broad base of cancers. As Fryczkowski et al note:

VEGF secreting tumors are able to grow rapidly and metastasize. Solid tumors require consistent angiogenesis together with tumor growth to supply them with nutrients and oxygen. High concentration of VEGF in plasma of patients with tumor results in poor prognosis. Hepatocyte growth factor (HGF) is another molecule inducing tumor angiogenesis. It was initially identified as a potent hepatotrophic factor responsible for liver regeneration, but now its other functions like mediating tumor-stromal interaction with morpho-, moto-, and mitogenic activities have become known.

We shall discuss HGF next but the complex interaction between various GF/GFR is an essential observation. The complexity of these interactions has not yet been fully understood. The authors continue:

Moreover, HGF intensifies the potential angiogenic activity in vascular endothelial cells [40]; it also acts as a paracrine factor responsible for morphogenesis, cell growth, and cell motility. It has been shown that cancer-associated fibroblasts promote cell scattering, epithelial-mesenchymal transition (EMT), and migration of cancer cells in an HGF-dependent manner. Both HGF and c-Met are upregulated in different types of human cancers such as breast, lung, colorectal, gastric, and oesophageal cancer

Now VEGF errors can affect a wide variety of cell aberrations. For example in retinopathy:

The increased expression of VEGF has become a focal point of current research on the pathogenesis of diabetic retinopathy, as well as other retinal and choroidal vascular diseases. The VEGFs are a family of peptides produced from a single gene by alternative splicing. VEGF isoforms are specifically mitogenic for vascular endothelial cells and also increase permeability

at blood–tissue barriers — hence the original name, vascular permeability factor. VEGF is essential for the formation of the fetal vascular system; targeted disruption (knockout) of the VEGF gene in mice leads to impaired vasculogenesis and death in utero.

Normally, VEGF expression decreases substantially after birth, but some cells constitutively secrete picomolar amounts; cells in the neural retina secrete 15 to 20 pg per milligram of protein, and cells in the combined choroid and retinal pigment epithelium secrete 50 pg per milligram of protein. Constitutive VEGF secretion from the retinal pigment epithelium is asymmetric, occurring primarily from the basal surface of these cells, and perhaps accounts in part for the richly vascular choriocapillaris, which lies opposite the basal surface of the retinal pigment epithelium.

The choriocapillary endothelium is itself asymmetrical, with a thin, fenestrated inner portion facing the retinal pigment epithelium and a thick, nonfenestrated outer portion facing the deeper layers of the choroid. In vitro experimentation has shown that VEGF appears to induce endothelial fenestrations in cultured capillary endothelial cells that are derived from bovine adrenal cortex. Endothelial fenestrations are thought to increase vascular permeability. VEGF expression is enhanced by hypoxia, which is a major stimulus for retinal neovascularization. Reduced retinal blood flow and accompanying hypoxia may be present even before the early signs of retinopathy, such as loss of capillary pericytes and endothelial cells, are identified, and these changes are likely to be accompanied by an increase in the synthesis and secretion of VEGF.

Indeed, increased VEGF protein has been demonstrated by immunocytochemical analysis of nonvascular cells in the eyes of persons with diabetes even in the absence of retinopathy, supporting the hypothesis that diabetic retinopathy begins as a disease of retinal neurons and glia and only later involves the retinal vasculature.

Thus understanding VEGF and its functions is essential to understanding not only malignancies but a variety of other cellular aberrations.

6 HGF

The hepatocyte growth factor, HCF, has already been mentioned in its interactions with VEGF.

As NCBI notes[29]:

This gene encodes a protein that binds to the hepatocyte growth factor receptor to regulate cell growth, cell motility and morphogenesis in numerous cell and tissue types. Alternative splicing results in multiple transcript variants, at least one of which encodes a preproprotein that is proteolytically processed to generate alpha and beta chains, which form the mature heterodimer. This protein is secreted by mesenchymal cells and acts as a multi-functional cytokine on cells of mainly epithelial origin. This protein also plays a role in angiogenesis, tumorogenesis, and tissue regeneration. Although the encoded protein is a member of the peptidase S1 family of serine proteases, it lacks peptidase activity. Mutations in this gene are associated with nonsyndromic hearing loss.

HGF activates the MET pathway and in turn MEK and results in proliferation. As Linehan and Ricketts note:

METencodes the cell surface receptor for the growth factor, hepatocyte growth factor (HGF). Growth factor-dependent activation of the phosphatidylinositol 3-kinase (PI3K) signaling pathway increases cell surface expression of nutrient transporters, resulting in increased uptake of glucose, amino acids and other nutrients, and increased growth and proliferation through the up regulation of the PI3K-AKT and PI3K-RAS-Erk pathways.

Additionally, nutrient-stimulated HGF/MET signaling induces phosphorylation of LKB1 on Ser428 through the RAS-Erk1/2-p90RSK pathway in a manner that results in uncoupling it with its low energy sensing partner, 5'AMP -activated protein kinase (AMPK).(38) If this occurred in an uncontrolled manner it would inhibit AMPK activation in the presence of low energy levels and implicates HGF/MET activation in deregulation of the LKB1-AMPK- mTOR nutrient and energy sensing pathway.

Therefore normal growth patterns can be controlled by the growth factor receptor activity levels and the surrounding nutrient levels, whereas the mutated constitutively active HGF/MET can drive uncontrolled growth irrelevant of the surrounding environmental conditions and overcome the negative regulation of AMPK.

A clinical trial has been conducted evaluating the role of a small molecule inhibitor of both HGF/MET and VEGFR2/KDR in patients with papillary kidney cancer either with or without germline METmutations. A partial response to therapy has been seen in patients with papillary kidney cancer, and results in patients with or without germline METmutation is currently being analyzed

[29] https://www.ncbi.nlm.nih.gov/gene/3082

6.1 HGF FUNCTIONS

As noted, HGF functions via the MET pathway. From Yang et al we have the following discussion regarding that function:

HGF is the most potent mitogen for mature hepatocytes in primary culture and acts as a trigger for liver regeneration after partial hepatectomy and liver injury. It is also implicated in the metastatic spread of tumours as a scatter factor and has been proposed as a strong and independent predictor of recurrence in human breast cancer.

Its receptor (cMET) is over-expressed in most human cancers. Colon cancer cell invasion and motility potential is significantly increased following incubation with HGF.

This suggests that HGF plays an important role in cancer metastasis initiation. Membrane ruffling is an early event in cell movement; HGF induces rapid membrane ruffling, formation of microspikes and increased cell motility in colon cancer cells indicating HGF enhances cell motility through induction of cell membrane ruffling. An investigation into the tyrosine phosphorylation and translocation of ruffling proteins in colon cancer cells has found that HGF stimulates the function of the ruffling protein (ezrin) which initiates cancer cell membrane ruffling and other early signals for cancer cells to move and invade.

The mechanisms which trigger cancer cell membrane ruffling are largely unestablished. A further study indicated that cyctosolic free Ca2+ may be involved in the mechanism (64). In addition to acting as a cancer cell motility and invasion stimulator, HGF also enhances cancer angiogenesis by increasing Vascular Endothelial Growth Factor (VEGF) promoter activity and inducing hypoxia inducible factor-1 (HIF- 1) expression. The pivotal role and the comprehensive function of HGF and its receptor (cMET) in cancer metastasis initiation and development...

The HGF impact is across a wide variety of cell types. As Peruzzi and Bottaro note:

On binding to the cell surface receptor tyrosine kinase (TK) known as c-Met, hepatocyte growth factor (HGF) stimulates mitogenesis, motogenesis, and morphogenesis in a wide range of cellular targets including, epithelial and endothelial cells, hematopoietic cells, neurons, melanocytes, and hepatocytes.

These pleiotropic actions are fundamentally important during development, homeostasis, and tissue regeneration. HGF signaling also contributes to oncogenesis and tumor progression in several human cancers and promotes aggressive cellular invasiveness that is strongly linked to tumor metastasis.

Our present understanding of c-Met oncogenic signaling supports at least three avenues of pathway selective anticancer drug development: antagonism of ligand/receptor interaction, inhibition of TK catalytic activity, and blockade of intracellular receptor/effector interactions.

Potent and selective preclinical drug candidates have been developed using all three strategies, and human clinical trials in two of the three areas are now under way.

The identification of hepatocyte growth factor (HGF) as the natural ligand for the c-Met receptor protein and the identity of scatter factor (SF) and HGF united a collection of findings showing that a single receptor transduced multiple biological activities, including motility, proliferation, survival, and morphogenesis (3–6). Both HGF/SF and c-Met proteins are processed proteolytically from single-chain precursors into mature disulfide-linked heterodimers.

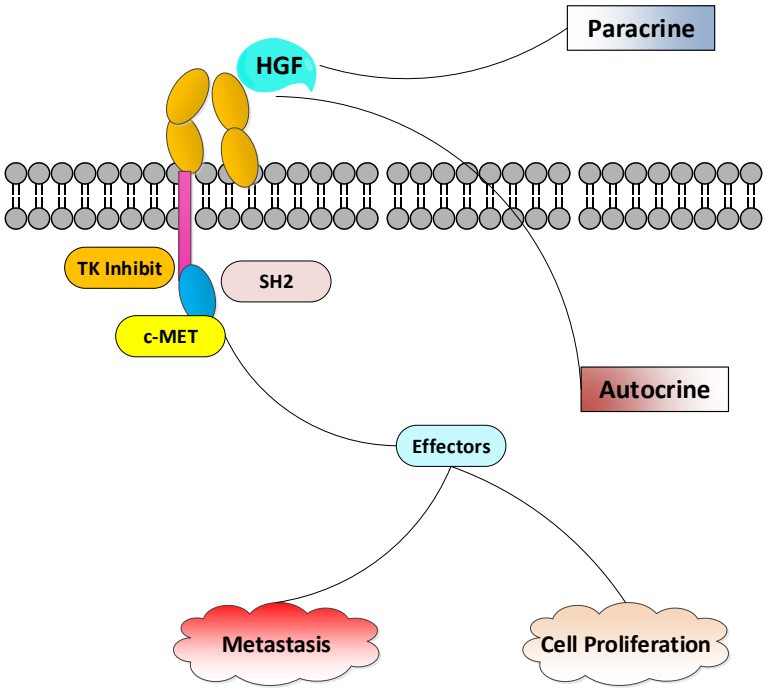
Both are widely expressed early in development; deletion of either gene causes lethal disruptions to embryogenesis; and widespread expression persists throughout adulthood (3, 4, 6).

Both MET and HGF/SF genes are up-regulated after kidney, liver, or heart injury, suggestive of a general homeostatic mechanism of protection against tissue damage and promotion of tissue repair and regeneration (7–11).

Upon HGF/SF binding, c-Met autophosphorylation occurs on two tyrosine residues (Y1234 and Y1235) within the activation loop of the TK domain, which regulate kinase activity.

Phosphorylation on two tyrosine residues near the COOH terminus (Y1349 and Y1356) forms a multifunctional docking site that recruits intracellular adapters via Src homology- 2 domains and other recognition motifs, leading to downstream signaling. An intact multifunctional docking site is required to mediate transformation and induce a metastatic phenotype.

The HGF paths are graphically shown below:



6.2 HGF AND CANCERS

As Linehan et al note in discussing Kidney Cancer:

The proto-oncogene MET (hepatocyte growth factor receptor) was identified as the gene for hereditary papillary renal carcinoma by genetic linkage analysis in families with this inherited renal cancer syndrome. MET encodes the cell surface receptor for ***hepatocyte growth factor (HGF), which is involved in mitogenesis, morphogenesis and motogenesis.***

Activating mutations in the tyrosine kinase domain of MET, have been detected in the germline of affected patients and in a subset of sporadic type 1 papillary kidney cancers.

The histological patterns of hereditary and sporadic type 1 papillary kidney tumors with MET mutations share a distinct morphological phenotype consisting of papillary or tubulo/papillary architecture with slender short papillae containing delicate fibrovascular cores lined by small cells with low grade basophilic nuclei and scant amphophilic cytoplasm.

One effect of growth factor-dependent activation of the phosphatidylinositol 3-kinase (PI3K) signaling pathway is increased cell surface expression of nutrient transporters increasing uptake of amino acids, glucose and other nutrients.(4) Nutrient-stimulated HGF/MET signaling induces phosphorylation of serine/threonine-protein kinase 11 [STK11; also referred to as LKB1, the upstream kinase of 5'AMP–activated protein kinase (AMPK)] on Ser428 through the RAS-Erk1/2-p90RSK pathway, implicating MET in the LKB1-AMPK-mTOR nutrient and energy sensing pathway. A clinical trial is currently underway to determine the effect of foretinib, a kinase inhibitor of both MET and VEGF receptors, in patients with papillary kidney cancer (hereditary and sporadic). There is early evidence of efficacy of this agent in patients with germline mutations in the tyrosine kinase domain of MET.

It is possible that there would be response to an agent which has activity against MET in tumors that are characterized by a mutation in the tyrosine kinase domain of MET; it is also possible that such an agent would have activity in tumors which have MET amplification. It is not known if an agent such as foretinib would have activity against kidney tumors that are caused by mutation of other genes such as TSC1 or TSC2.

7 PDGF

PDGF is the platelet derived growth factor. There are multiple variants which we detail as follows.

PDGF	***Description***
PDGFA[30]	This gene encodes a member of the protein family comprised of both platelet-derived growth factors (PDGF) and vascular endothelial growth factors (VEGF). The encoded preproprotein is proteolytically processed to generate platelet-derived growth factor subunit A, which can homodimerize, or alternatively, heterodimerize with the related platelet-derived growth factor subunit B. These proteins bind and activate PDGF receptor tyrosine kinases, which play a role in a wide range of developmental processes. Alternative splicing results in multiple transcript variants.
PDGFB[31]	This gene encodes a member of the protein family comprised of both platelet-derived growth factors (PDGF) and vascular endothelial growth factors (VEGF). The encoded preproprotein is proteolytically processed to generate platelet-derived growth factor subunit B, which can homodimerize, or alternatively, heterodimerize with the related platelet-derived growth factor subunit A. These proteins bind and activate PDGF receptor tyrosine kinases, which play a role in a wide range of developmental processes. Mutations in this gene are associated with meningioma. Reciprocal translocations between chromosomes 22 and 17, at sites where this gene and that for collagen type 1, alpha 1 are located, are associated with dermatofibrosarcoma protuberans, a rare skin tumor. Alternative splicing results in multiple transcript variants
PDGFC[32]	The protein encoded by this gene is a member of the platelet-derived growth factor family. The four members of this family are mitogenic factors for cells of mesenchymal origin and are characterized by a core motif of eight cysteines. This gene product appears to form only homodimers. It differs from the platelet-derived growth factor alpha and beta polypeptides in having an unusual N-terminal domain, the CUB domain. Alternatively spliced transcript variants have been found for this gene.

[30] https://www.ncbi.nlm.nih.gov/gene/5154

[31] https://www.ncbi.nlm.nih.gov/gene/5155

[32] https://www.ncbi.nlm.nih.gov/gene/56034

PDGF	*Description*
PDGFD[33]	The protein encoded by this gene is a member of the platelet-derived growth factor family. The four members of this family are mitogenic factors for cells of mesenchymal origin and are characterized by a core motif of eight cysteines, seven of which are found in this factor. This gene product only forms homodimers and, therefore, does not dimerize with the other three family members. It differs from alpha and beta members of this family in having an unusual N-terminal domain, the CUB domain. Two splice variants have been identified for this gene.

7.1 PDGF FUNCTIONS

As Pietras et al note:

Four PDGF polypeptide chains have been identified, which make up five dimeric PDGF isoforms: PDGF-AA, -AB, -BB, -CC, and -DD. The isoforms exert their cellular effects through tyrosine kinase a- and p-receptors. All PDGF isoforms, except PDGF-DD, induce PDGF a-receptor dimerization, whereas PDGF-BB and -DD activate PDGF preceptor dimers. In addition, all isoforms except PDGF-AA activate both receptor types in cells coexpressing the a- and p-receptors.

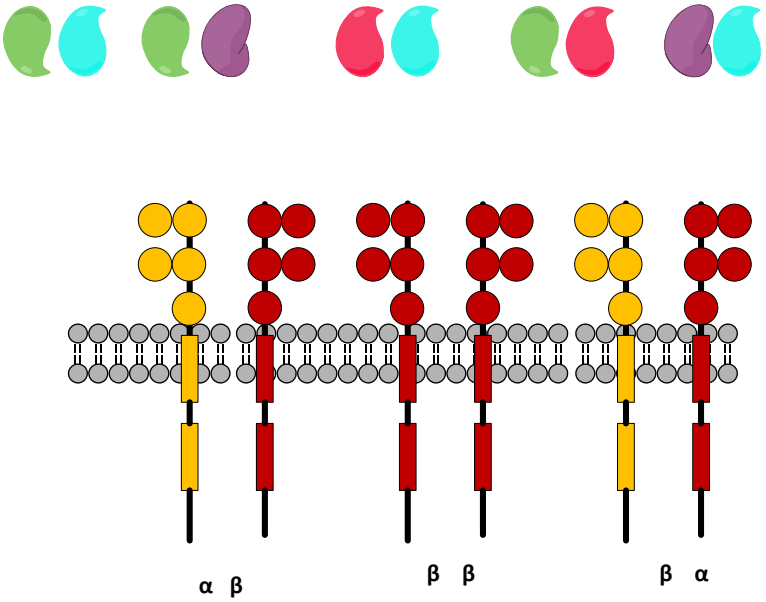
Ligand-induced receptor dimerization causes receptor autophosphorylation, whereafter intracellular signaling pathways are activated by recruitment of SH2 domain-containing signaling molecules (e.g., c-Src, phospholipase C-y, phos- phatidyl-inositol-3'-kinase and the Grb2/Sos complex) to specific phosphorylated tyrosine residues. Activation of these pathways ultimately induces various cellular responses, including cell proliferation, survival, and migration.

Targeting of the genes for PDGF-A and B chains, and for the two receptors, has provided a detailed understanding of the physiological functions of PDGF during development. The selective upregulation of PDGF receptors on endothelial cells in the mouse model of prostate cancer bone metastases should stimulate further study of expression of PDGF receptors on tumor endothelial cells. The frequent expression of PDGF receptors on perivascular cells also suggests as yet unexploited therapeutic opportunities. The well-documented effect of PDGF on pericyte recruitment points to the possibility of combining anti-endothelial agents, like vascular endothelial growth factor receptor inhibitors, with pericyte-targeting PDGF antagonists.

Finally, it has been shown that bone marrow- derived cells contribute to the angiogenic switch in tumors (Coussens et al., 2000). PDGF receptor inhibitors can possibly inhibit this process, since PDGF stimulates migration and proliferation of macrophages

[33] https://www.ncbi.nlm.nih.gov/gene/80310

From Litwak (p528) we have the following 3 PDGFR and five PDGF structures. The PDGFR gave two separate chains, an A and a B, or as shown, an α and β. There are five PDGF, A thru E, but we focus on the first 4 only as noted above.



7.2 PDGF AND CANCER

As Chen et al note:

Platelet-derived growth factor-D (PDGF-D) plays a crucial role in the progression of several cancers. However, its role in colorectal cancer (CRC) remains unclear. Our study showed that PDGF-D was highly expressed in CRC tissues and was positively associated with the clinicopathological features. Down-regulation of PDGF-D inhibited the tumor growth, migration and angiogenesis of SW480 cells in vitro and in vivo. Whereas up-regulation of PDGF-D promoted the malignant behaviors of HCT116 cells. Moreover, PDGF-D up-regulated the expression of Notch1 and Twist1 in CRC cells.

In addition, PDGF-D expression promoted Epithelial to mesenchymal transition (EMT), which was accompanied with decreased E-cadherin and increased Vimentin expression. Consistently, PDGF-D, Notch1, and Twist1 are obviously up-regulated in transforming growth factor-beta 1 (TGF-β1) treated HCT116 cells. Since Notch1 and Twist1 play an important role in EMT and tumor progression, we examined whether there is a correlation between Notch1 and Twist1 in EMT status.

Our results showed that up-regulation of Notch1 was able to rescue the effects of PDGF-D down-regulation on Twist1 expression in SW480 cells, whereas down-regulation of Notch1 reduced Twist1 expression in HCT116 cells. Furthermore, we found that Twist1 promoted EMT

and aggressiveness of CRC cells. These results suggest that PDGF-D promotes tumor growth and aggressiveness of CRC, moreover, down-regulation of PDGF-D inactivates Notch1/Twist1 axis, which could reverse EMT and prevent CRC progression.

They further make the following observations regarding PDGF-D as an example:

1. PDGF-D is highly expressed in CRC tissues and cell lines
2. PDGF-D expression promotes cell growth and colony formation in CRC cell lines
3. PDGF-D expression promotes cell cycle distribution, aggressiveness, and angiogenesis, but not apoptosis in CRC cell lines
4. PDGF-D increases the expression of Notch1 in CRC cells
5. PDGF-D induces the EMT profile in CRC cells
6. PDGF-D is significantly increased in TGF-β1 treated HCT116 cells
7. Downregulation of PDGF-D reversed EMT in TGF-β1 treated HCT116 cells
8. PDGF-D promotes cell growth, aggressiveness and EMT transformation of CRC through activation of Notch1/Twist1 pathway
9. PDGF-D promotes tumorigenesis, angiogenesis and EMT profile of CRC cells in vivo

8 IGF

The IGF and the IGFR, and its respective sub-elements, are major factors in many malignancies. For example as noted in NCBI:

IGF1[34]: 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[35]: 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.

There has been a great deal of study of the IGF and its constituents[36].

8.1 IGF OVERVIEW

The insulin growth factor is a key element in glucose control. Spravchikov et al have discussed the impact of poor glucose management on skin keratinocytes. 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

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

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

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

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.

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 (25). 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. As can be seen in Fig. 9, 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.

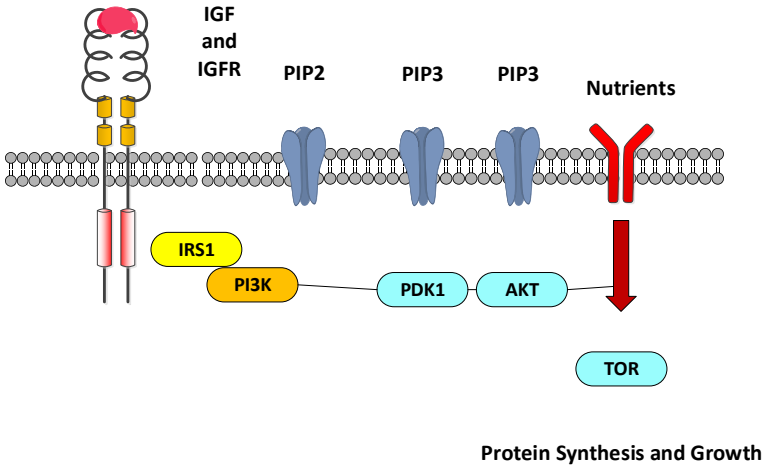
From Yang et al:

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 (69, 70). IGFs act on a variety of mammalian cells in an endocrine, paracrine and autocrine manner (71) to regulate cell proliferation, apoptosis, transformation and differentiation (72, 73). 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 (74-76). 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 (77). Blockade of the paracrine action of IGF-I can suppress liver metastases from colorectal cancer (78). 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[37]:



8.2 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.

In 3D Matrigel™ and hydrogel cultures, 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

[37] See Morgan, p 216

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 invasion2. IGF-I can act as a paracrine factor that drives malignant cell transformation through the activation of the IGF type-I receptor (IGF-IR)3. All melanocytic cells express the IGF-IR, with increased expression correlated with disease progression4,5.

In addition, growth factor interactions with the extracellular matrix (ECM) play important roles in tumor biology, facilitating tumor cell attachment, proliferation and invasion6,7, and resistance against chemotherapeutic drugs8. 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-I9,10. Previous studies have demonstrated that IGF-I interacts with VN through IGFBPs to form IGF-I:IGFBP:VN trimeric (TRI) complexes11. 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 vessels12.

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 stimulation13. Indeed, complexes of TRI have been shown to promote enhanced cell attachment and migration, as well as protein synthesis, in human keratinocytes14 and breast cancer cell lines11,15,16

9 CTGF

CTGF is the connective tissue growth factor.

As NCBI notes[38]:

*The protein encoded by this **gene is a mitogen** that is secreted by vascular endothelial cells. The encoded protein plays a role in chondrocyte proliferation and differentiation, cell adhesion in many cell types, and is related to **platelet-derived growth factor**. Certain polymorphisms in this gene have been linked with a higher incidence of systemic sclerosis.*

As with many of the GF, the mitogenic actions are critical in many if not most cancers. Reducing mitosis is reducing metastasis.

9.1 CTGF FUNCTIONS

Bao et al note that down-regulating CTGF has a significant effect on reducing proliferation. They note:

TGF-β2 promoted proliferation and inhibited apoptosis of human Tenon capsule fibroblasts in a dose-dependent manner. TGF-β2 induced down-regulation of miR-26 and up-regulation of CTGF in a dose-dependent manner. CTGF was the target gene of miR-26 and miR-26 had a negative regulatory effect on CTGF expression. miR-26 up-regulation could significantly decrease proliferation and increase apoptosis of human Tenon capsule fibroblasts after induced by TGF-β2 ($P < 0.05$).

Down-regulation of CTGF could markedly decrease proliferation and increase apoptosis of human Tenon capsule fibroblasts after induced by TGF-β2 ($P < 0.05$). miR-26 could inhibit proliferation and promote apoptosis of human Tenon capsule fibroblasts after they were induced by TGF-β2 through suppressing CTGF expression.

9.2 CTGF AND CANCER

As Zhu et al note:

In this study, we therefore analyzed the expressions of a large panel of cytokines (132 known cytokines and growth factors) in several tumor-stromal, as well as normal-stromal clinical breast cohorts. The results consistently indicated the potential importance of connective tissue growth factor (CTGF) in tumor progression.

In particular, CTGF is preferentially produced in tumor cells, and the elevated CTGF gene expression in tumor cells significantly correlates with poor clinical prognosis in breast tumors. Furthermore, in our tissue microarray analysis on 84 patient-derived xenograft models, high

[38] https://www.ncbi.nlm.nih.gov/gene/1490 also known as CCN2

protein expression of CTGF correlates remarkably with the stroma-rich tumors that have poor clinical prognosis and outcome. For breast cancer, especially the triple-negative subtype, patients with stroma-rich tumors have shown a significant higher risk of poor prognosis and worse outcome compared to those with stroma-poor tumors [9], which neither currently used clinic-pathological parameters nor molecular profiling techniques are able to categorize this set of patients with respect to prognosis [10, 11].

CTGF has previously been identified as a fibrogenic cytokine that is highly expressed in wound healing and fibrotic lesions [12].

In human cancers, the pleiotropic functions of CTGF have been investigated, including the function as an oncoprotein in glioma and melanoma, but a tumor-suppressor in lung cancer and colon cancer. In breast cancer, studies have shown that CTGF cooperates with other genes to mediate osteolytic metastasis, and high expression of CTGF mRNA in the bulk tumor correlated with advanced tumor stages, however, the mechanistic origin of CTGF has rare been explored. Whether the high level of CTGF is from tumor cells or stromal cells, and furthermore, whether CTGF mediates tumor-stroma dialogue and how CTGF regulates tumor progression in the microenvironment have not yet been clearly shown.

Our data show that CTGF in tumor epithelial cells but not stromal cells had significant clinical relevance, and through a series of bioinformatics and biological analyses, we also identified that

1) CTGF facilitated tumor growth and metastasis via promoting the deposition and orientation of collagen I fibers at the primary tumor stroma;

2) CTGF was capable to promote tumor cell migration, invasion and mammosphere formation via inducing epithelial-mesenchymal transition (EMT); and

3) the CTGF-tumor necrosis factor receptor I (TNFR1)-IκB autocrine signaling is the predominant mechanism in CTGF-mediated tumor progression.

Our data provided amble evidence that targeting the CTGF-TMFR1- IκB signaling is a promising strategy to prohibit breast tumor progression.

10 EGF

The epidermal growth factor, EGF, is another GF associated with malignancies. As NCBI notes[39]:

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.

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.

10.1 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.

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

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.

10.2 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[40]:

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.

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

11 FGF

There are about 20 fibroblast growth factors, FGF. They act both as endocrine and paracrine promoters, being secreted and then acting upon FGF receptors on target cells[41]. In addition certain FGF act in an autocrine manner as well as noted below.

Fibroblasts are cells which inhabit and promote the extracellular matrix. The FGF a named for these cells but are generated in a variety of cells throughout the body. They can stimulate various pathways in target cells initiating proliferation as well as angiogenesis.

From NCBI we have listed a few as follows:

FGF	***Description***
FGF7[42]	The protein encoded by this gene is a member of the fibroblast growth factor (FGF) family. FGF family members possess broad mitogenic and cell survival activities, and are involved in a variety of biological processes, including embryonic development, cell growth, morphogenesis, tissue repair, tumor growth and invasion. This protein is a potent epithelial cell-specific growth factor, whose mitogenic activity is predominantly exhibited in keratinocytes but not in fibroblasts and endothelial cells. Studies of mouse and rat homologs of this gene implicated roles in morphogenesis of epithelium, re-epithelialization of wounds, hair development and early lung organogenesis.
FGF19[43]	The protein encoded by this gene is a member of the fibroblast growth factor (FGF) family. FGF family members possess broad mitogenic and cell survival activities, and are involved in a variety of biological processes including embryonic development cell growth, morphogenesis, tissue repair, tumor growth and invasion. This growth factor is a high affinity, heparin dependent ligand for FGFR4. Expression of this gene was detected only in fetal but not adult brain tissue. Synergistic interaction of the chick homolog and Wnt-8c has been shown to be required for initiation of inner ear development.

[41] See Sherbet Chapter 15.
[42] https://www.ncbi.nlm.nih.gov/gene/2252
[43] https://www.ncbi.nlm.nih.gov/gene/9965

FGF	*Description*
FGF10[44]	The protein encoded by this gene is a member of the fibroblast growth factor (FGF) family. FGF family members possess broad mitogenic and cell survival activities, and are involved in a variety of biological processes, including embryonic development, cell growth, morphogenesis, tissue repair, tumor growth and invasion. This protein exhibits mitogenic activity for keratinizing epidermal cells, but essentially no activity for fibroblasts, which is similar to the biological activity of FGF7. Studies of the mouse homolog of suggested that this gene is required for embryonic epidermal morphogenesis including brain development, lung morphogenesis, and initiation of lim bud formation. This gene is also implicated to be a primary factor in the process of wound healing.
FGF4[45]	The protein encoded by this gene is a member of the fibroblast growth factor (FGF) family. FGF family members possess broad mitogenic and cell survival activities and are involved in a variety of biological processes including embryonic development, cell growth, morphogenesis, tissue repair, tumor growth and invasion. This gene was identified by its oncogenic transforming activity. This gene and FGF3, another oncogenic growth factor, are located closely on chromosome 11. Co-amplification of both genes was found in various kinds of human tumors. Studies on the mouse homolog suggested a function in bone morphogenesis and limb development through the sonic hedgehog (SHH) signaling pathway.
FGF12[46]	The protein encoded by this gene is a member of the fibroblast growth factor (FGF) family. FGF family members possess broad mitogenic and cell survival activities, and are involved in a variety of biological processes, including embryonic development, cell growth, morphogenesis, tissue repair, tumor growth, and invasion. This growth factor lacks the N-terminal signal sequence present in most of the FGF family members, but it contains clusters of basic residues that have been demonstrated to act as a nuclear localization signal. When transfected into mammalian cells, this protein accumulated in the nucleus, but was not secreted. The specific function of this gene has not yet been determined. Two alternatively spliced transcript variants encoding distinct isoforms have been reported.

[44] https://www.ncbi.nlm.nih.gov/gene/2255

[45] https://www.ncbi.nlm.nih.gov/gene/2249

[46] https://www.ncbi.nlm.nih.gov/gene/2257

FGF	*Description*
FGF3[47]	The protein encoded by this gene is a member of the fibroblast growth factor (FGF) family. FGF family members possess broad mitogenic and cell survival activities and are involved in a variety of biological processes including embryonic development, cell growth, morphogenesis, tissue repair, tumor growth and invasion. This gene was identified by its similarity with mouse fgf3/int-2, a proto-oncogene activated in virally induced mammary tumors in the mouse. Frequent amplification of this gene has been found in human tumors, which may be important for neoplastic transformation and tumor progression. Studies of the similar genes in mouse and chicken suggested the role in inner ear formation
FGF6[48]	The protein encoded by this gene is a member of the fibroblast growth factor (FGF) family. FGF family members possess broad mitogenic and cell survival activities, and are involved in a variety of biological processes, including embryonic development, cell growth, morphogenesis, tissue repair, tumor growth and invasion. This gene displayed oncogenic transforming activity when transfected into mammalian cells. The mouse homolog of this gene exhibits a restricted expression profile predominantly in the myogenic lineage, which suggested a role in muscle regeneration or differentiation
FGF22[49]	The protein encoded by this gene is a member of the fibroblast growth factor (FGF) family. FGF family members possess broad mitogenic and cell survival activities and are involved in a variety of biological processes including embryonic development, cell growth, morphogenesis, tissue repair, tumor growth and invasion. The mouse homolog of this gene was found to be preferentially expressed in the inner root sheath of the hair follicle, which suggested a role in hair development. Alternative splicing results in multiple transcript variants
FGF1[50]	The protein encoded by this gene is a member of the fibroblast growth factor (FGF) family. FGF family members possess broad mitogenic and cell survival activities, and are involved in a variety of biological processes, including embryonic development, cell growth, morphogenesis, tissue repair, tumor growth and invasion. This protein functions as a modifier of endothelial cell migration and proliferation, as well as an angiogenic factor. It acts as a mitogen for a variety of mesoderm- and neuroectoderm-derived cells in vitro, thus is thought to be involved in organogenesis. Multiple alternatively spliced variants encoding different isoforms have been described.

[47] https://www.ncbi.nlm.nih.gov/gene/2248

[48] https://www.ncbi.nlm.nih.gov/gene/2251

[49] https://www.ncbi.nlm.nih.gov/gene/27006

[50] https://www.ncbi.nlm.nih.gov/gene/2246

FGF	*Description*
FGF2[51]	The protein encoded by this gene is a member of the fibroblast growth factor (FGF) family. FGF family members bind heparin and possess broad mitogenic and angiogenic activities. This protein has been implicated in diverse biological processes, such as limb and nervous system development, wound healing, and tumor growth. The mRNA for this gene contains multiple polyadenylation sites, and is alternatively translated from non-AUG (CUG) and AUG initiation codons, resulting in five different isoforms with distinct properties. The CUG-initiated isoforms are localized in the nucleus and are responsible for the intracrine effect, whereas, the AUG-initiated form is mostly cytosolic and is responsible for the paracrine and autocrine effects of this FGF

11.1 FGF FUNCTIONS

The fibroblast growth factor is a complex set of growth factors that are also related to multiple malignancies.

As Ornitz and Itoh note:

The Fibroblast Growth Factor (FGF) family is comprised of secreted signaling proteins (secreted FGFs) that signal to receptor tyrosine kinases and intracellular non-signaling proteins (intracellular FGFs (iFGFs)) that serve as cofactors for voltage gated sodium channels and other molecules.

Additionally, secreted FGFs and iFGFs may have direct functions in the nucleus and functional interactions with other cellular proteins. Members of both branches of the FGF family are related by core sequence conservation and structure and are found in vertebrates and invertebrates.

Secreted FGFs are expressed in nearly all tissues and they serve essential roles in the earliest stages of embryonic development, during organogenesis, and in the adult, where they function as homeostatic factors that are important for tissue maintenance, repair, regeneration, and metabolism).

In general, secreted FGFs function as autocrine or paracrine factors (canonical FGFs; also called paracrine FGFs), however, three members of the secreted FGFs have evolved to function as endocrine factors (endocrine FGFs) with essential roles in the adult where they regulate phosphate, bile acid, carbohydrate and lipid metabolism in addition to the canonical FGF functions that control cell proliferation, differentiation and survival. At the cellular level, secreted FGFs regulate fundamental cellular processes that include positive and negative regulation of proliferation, survival, migration, differentiation, and metabolism.

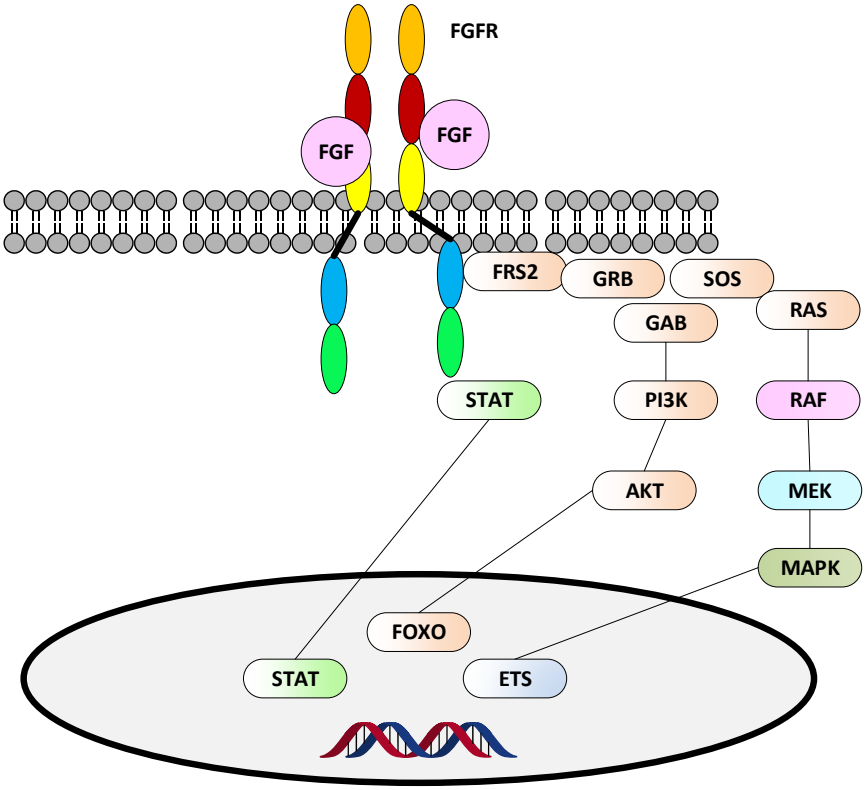
[51] https://www.ncbi.nlm.nih.gov/gene/2247

As Ornitz and Itoh (2001) noted:

Most Fgf genes are found scattered throughout the genome. In human, 22 FGF genes have been identified and the chromosomal locations of all except FGF16 are known (Table 1) [3-7]. Several human FGF genes are clustered within the genome. FGF3, FGF4 and FGF19 are located on chromosome 11q13 and are separated by only 40 and 10 kb, respectively; FGF6 and FGF23 are located within 55 kb on chromosome 12p13; and FGF17and FGF20 map to chromosome 8p21-p22.

These gene locations indicate that the FGF gene family was generated both by gene and chromosomal duplication and translocation during evolution. Interestingly, a transcriptionally active portion of human FGF7, located on chromosome 15q13-q22, has been amplified to about 16 copies, which are dispersed throughout the human genome.

For the most part FGF1 and FGF2 are predominant as regards to a focus on cancer growth. The cell pathway controls are depicted below. Note they lead to proliferation and growth.



11.2 FGF AND CANCERS

FGF are critical to many cellular proliferations. Thus they like the others are key in many cancers. As Wesche et al state:

FGFs (fibroblast growth factors) and their receptors (FGFRs) play essential roles in tightly regulating cell proliferation, survival, migration and differentiation during development and adult life. Deregulation of FGFR signalling, on the other hand, has been associated with many developmental syndromes, and with human cancer. In cancer, FGFRs have been found to become overactivated by several mechanisms, including gene amplification, chromosomal translocation and mutations. FGFR alterations are detected in a variety of human cancers, such as breast, bladder, prostate, endometrial and lung cancers, as well as haematological malignancies.

Accumulating evidence indicates that FGFs and FGFRs may act in an oncogenic fashion to promote multiple steps of cancer progression by inducing mitogenic and survival signals, as well as promoting epithelial– mesenchymal transition, invasion and tumour angiogenesis. Therapeutic strategies targeting FGFs and FGFRs in human cancer are therefore currently being explored. In the present review we will give an overview of FGF signalling, the main FGFR alterations found in human cancer to date, how they may contribute to specific cancer types and strategies for therapeutic intervention.

FGF signalling is crucial during development, and mutated FGFRs have been found to be the cause of several developmental syndromes. Prominent examples include the germline gain-of-function mutations often found in human skeletal dysplasia. For instance, in achondroplasia, a mutation in the transmembrane helix of FGFR3 (G380R) promotes dimerization and subsequent activation of the tyrosine kinase domain. In the lethal skeletal disorder thanatophoric dysplasia, single mutations generating a new cysteine residue (S249C or Y373C) in the extracellular part of the receptors cause the formation of a disulfide bond, linking two individual receptors. Thus an intermolecular bond forces dimerization in the absence of ligand, resulting in ligand-independent constitutive signalling. Interestingly, the same mutations discovered to be the cause of many developmental disorders are also found mutated in tumour cells.

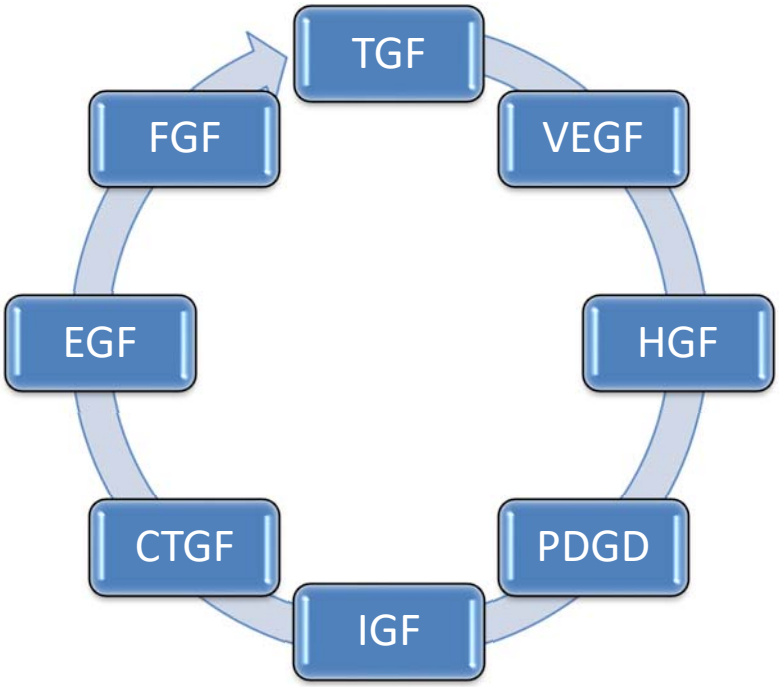
The mutations found in achondroplasia and thanatophoric dysplasia, which cause dimerization and thereby constitutive activation of FGFR3, are also frequently found in bladder cancer. Yet other mutations in FGFR2 that cause dimer formation are implicated in craniosynostosis syndromes and have also been found in endometrial cancers. A mutation that promotes dimerization is just one mechanism that can increase ligand-independent signalling from FGFRs.

Other mutations located to the kinase domain of FGFRs can change the conformation of the domain to cause permanently active kinases. Mutations in the kinase domain of FGFR4 have been found in the childhood sarcomaRMS(Rhabdomyosarcoma), and these mutations were shown to cause autophosphorylation and constitutive signalling. Some mutations in FGFRs identified in human cancer have also been shown to cause loss-of-function suggesting that, in certain circumstances, FGFRs can act as tumour suppressors. The majority of FGF ligand mutations described in human disease are germline loss-of-function mutations.

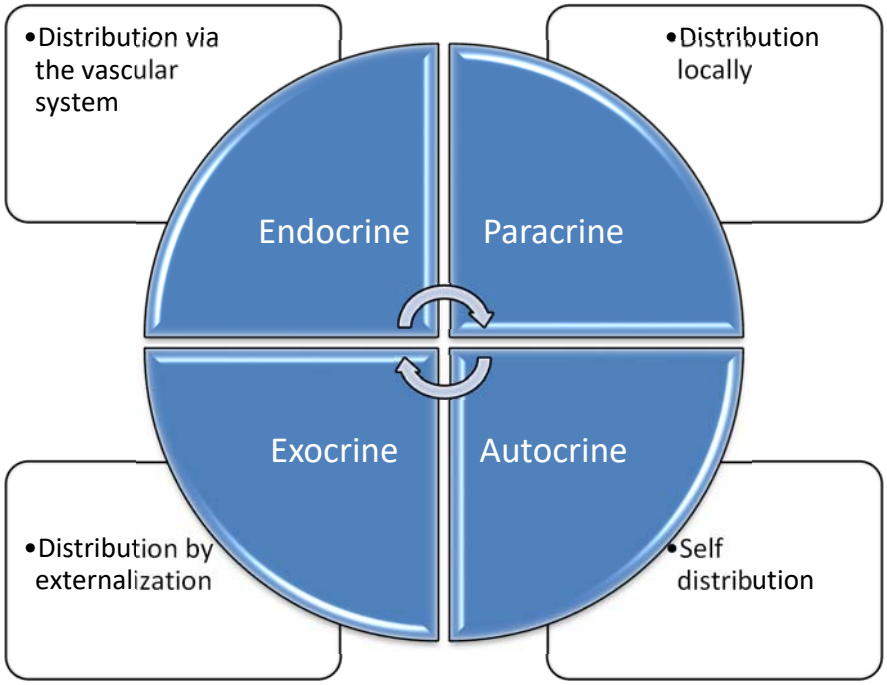
12 OBSERVATIONS

There has been considerable examination of growth factors and growth factor receptors as we have noted. However, unlike therapeutics targeting immuno-specific surface markers or aberrant pathway dynamics, the GF approach has not demonstrated significant efficacy. We have reviewed the GF space and in so doing have tried to re-energize this effort.

There are a multiplicity of GF as repeated below.



In many of these general classes are another multiplicity of sub GF so that the totality of GF are a multiplicity of these eight shown above. Their actions are as shown below:



In effect the GF can exhibit a complex and pervasive set of drivers on cell proliferation and death. For cancers, cells can be activated in such a manner as to drive other cells to proliferate and thus metastasize.

12.1 Growth Factors can exhibit both positive and negative effects.

Growth factors are essential for development and cell integrity. However when malfunctioning they become a key element in the malignancy.

12.2 Growth Factors and Receptors may be Therapeutic Targets

Blocking or in some cases activating GF and GFR may be reasonable targets for cancer therapeutics. However understanding the effects on other cells is critical because damage may occur in these cases.

12.3 Growth Factors may Interact with One Another

The GF and GFR may actually have mutual interactions and this has not been studies extensively if at all.

12.4 Growth Factors Have Complex and Yet to be Fully Understood Actions

Certain GF and GFR actions are most likely still poorly understood if at all. Thus although attractive may not be yet acceptable alternatives.

12.5 Growth Factors May be Overactive or Underactive

We do not have a well defined activity level for GF. Moreover we find that they interact and that even with a given GF there may be an interaction between paracrine and autocrine.

12.6 At Present there does not appear to be a Clear Path for GF in Cancer Therapeutics

There has been a great deal of interest in GF/GFR therapeutics but currently there does not appear to be a clear path forward.

13 REFERENCES

1. Aaronson, Growth Factors and Cancer, Science, Vol 254 Nov 1991.
2. 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
3. Botehlo et al, Vascular Endothelial Growth Factor (VEGF) and Prostate Pathology, International Braz J Urol Vol. 36 (4): 430-438, July - August, 2010
4. Carpenter and Cohen, Epidermal Growth Factor, The Journal Of Biological Chemistry, Vol. 265, No. 14, Issue of May 15, pp. 7709-7712.1990
5. 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
6. 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
7. Frank, Diabetic Retinopathy, NEJM, 2004;350:48-58
8. Green, Cell Death, CSHL Press (New York) 2018.
9. Gupta and Massagué, Cancer Metastasis: Building a Framework, Cell 127, November 17, 2006
10. Karaman et al, Vascular endothelial growth factor signaling in development and disease, Development (2018) 145
11. Linehan and Ricketts, The Metabolic Basis of Kidney Cancer, Semin Cancer Biol. 2013 February ; 23(1): 46-55.
12. Linehan et al, The genetic basis of kidney cancer: a metabolic disease, Nat Rev Urol. 2010 May ; 7(5): 277–285
13. Litwack, Human Biochemistry, Academic (New York) 2018, pp 515-543
14. Mendelsohn and Baselga, The EGF receptor family as targets for cancer therapy, Oncogene (2000) 19, 6550 - 6565
15. Morgan, Cell Cycle, Sinauer (San Francisco) 2007.
16. Murekatete et al, Targeting Insulin-Like Growth Factor-I and Extracellular Matrix Interactions in Melanoma Progression, Scientific Reports, 2018
17. 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
18. Ornitz and Ito, The Fibroblast Growth Factor signaling pathway, WIREs Dev Biol 2015, 4:215–266

19. Ornitz and Itoh, Fibroblast growth factors, Genome Biology 2001, 2(3):reviews3005.1–3005.12, March 2001
20. Peruzzi B, Bottaro DP. Targeting the c-Met signaling pathway in cancer. Clin Cancer Res 2006 Jun 15;12(12):3657–60
21. Pietras et al, PDGF receptors as cancer drug targets, CANCER CELL : MAY 2003 • VOL. 3
22. Rahim et al, Molecular Regulation of Bone Marrow Metastasis in Prostate and Breast Cancer, Bone Marrow Research, Volume 2014, Article ID 405920, 12 pages
23. Rucci and Angelucci, Prostate Cancer and Bone: The Elective Affinities, BioMed Research International, Volume 2014, Article ID 167035, 14 pages
24. Salinero and Rafii, Endothelial cell adaptation in regeneration, Science, Dec 2018
25. Sengupta et al, Regulation of the mTOR Complex 1 Pathway by Nutrients, Growth Factors, and Stress, Molecular Cell 40, October 22, 2010
26. Singh et al, EGF receptor ligands: recent advances, F1000 Research 2016.
27. Spravchikov et al, Glucose Effects on Skin Keratinocytes, Diabetes, Vol. 50, July 2001
28. Sherbet, Growth actors and Their Receptors, Elsevier (New York) 2011
29. Takeuchi and Ito, Receptor Tyrosine Kinases and Targeted Cancer Therapeutics, Biol. Pharm. Bull. 34(12) 1774—1780 (2011)
30. Venere et al, Growth Factor Receptors Define Cancer Hierarchies, Cancer Cell 23, February 11, 2013
31. Wesche et al, Fibroblast growth factors and their receptors in cancer, Biochem. J. (2011) 437, 199–213
32. Witsch et al, Roles for Growth Factors in Cancer Progression, PHYSIOLOGY 25: 85–101, 2010
33. Yang et al, Growth Factors and their receptors in cancer metastases, Frontiers in Bioscience 16, 531-538, January 1, 2011
34. Zhu et al, Epithelial derived CTGF promotes breast tumor progression via inducing EMT and collagen I fibers deposition, Oncotarget, Vol. 6, No. 28, 2015
35. Zingales et al, Metformin: A Bridge between Diabetes and Prostate Cancer, Frontiers in Oncology, October 2017, Volume 7, Article 24**3**

14 APPENDIX A: GROWTH FACTORS AND MALIGNANCY[52]

Growth Factor	Symbol	Receptor	Origin	Action	Stage
Activin A (inhibinBA+ inhibin BA)	INHBA	ACVR1, ACVR1A, ACVR1C, ACVR2A ACVR2B	CRC cells, others	proliferation, differentiation, apoptosis	primary, metastatic
Amphiregulin	AREG	EGFR	CRC cells, others	unclear; growth effects depend on cell type	primary, metastatic
Autocrine Motiliy Factor	AMF/PGI	AMFR E3	melanoma cells	mitogenic, motogenic, differentiation	primary, metastatic
Bone morphogenic protein 2	BMP2	BMPR1A, BMPR1B, BMPR2	CRC cells, others	inhibits growth, pro-apoptotic, differentiation	primary, metastatic
Bone morphogenic protein 3	BMP3	BMPR1A, BMPR1B, BMPR2	CRC cells, others	negative growth regulator	primary
Bone morphogenic protein 4	BMP4	BMPR1A, BMPR1B, BMPR2	CRC cells, others	migration and invasion	intermediate, metastatic
Bone morphogenic protein 7	BMP7	BMPR1A, BMPR1B, BMPR2	CRC cells, others	invasion	primary, intermediate, metastatic
Colony stimulating factor 2 (granulocyte macrophage)	CSF2 (GM-CSF)	CSF2RA, CSFRB	Hematopoietic cells	proliferation	primary
Colony stimulating factor 3 (granulocyte)	CSF3 (G-CSF)	CSF3R	Hematopoietic cells , CRC cells	unknown; overexpressed in CRC	metastatic
Colony stimulating factor 1 (macrophage)	CSF1 (M- CSF)	CSF1R	Hematopoietic cells	angiogenesis, tumor progression	primary, metastatic
Epidermal growth factor	EGF	EGFR	Numerous cell types	proliferation, differentiation, survival	primary, metastatic
Epiregulin	EREG	EGFR	CRC cells, others	unclear; growth effects depend on cell type	primary, metastatic
Fibroblast growth factor 1	FGF1	FGFR1	Fibroblasts, CRC cells	angiogenesis, proliferation	primary, metastatic

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Growth Factor	Symbol	Receptor	Origin	Action	Stage
Fibroblast growth factor 2	FGF2	FGFR2	Fibroblasts, CRC cells	angiogenesis, proliferation	primary
Fibroblast growth factor 7	FGF7 (KGF)	FGFR2iiib	Fibroblasts, CRC cells	angiogenesis, proliferation, differentiation, migration, adhesion	primary
Fibroblast growth factor 10	FGF10	FGFR2iiib	Mesenchymal cells	growth	primary
Growth differentiation factor 11	GDF11	ACVR1B, ACVR1C, TGFBR1	CRC cells, others	unknown; overexpression correlates with metastasis	primary
Heparin-binding EGF- like growth factor	HB-EGF	EGFR	CRC cells, others	growth	primary
Insulin-like growth factor 1	IGF-I	IGF1R	Liver, CRC cells, others	proliferation, anti-apoptotic, migration	primary, metastatic
Insulin-like growth factor 2	IGF-II	IGF2R	Liver, CRC cells, others	proliferation, migration	primary, metastatic
Interleukin 2	IL-2	IL2RA, IL2RB, IL2RG	Hematopoietic cells	growth, survival	primary, metastatic
Interleukin 3	IL-3	IL3RA, IL3RB	Hematopoietic cells	proliferation, differentiation	primary
Interleukin 4	IL-4	IL4RA, IL2RG	Hematopoietic cells	anti-apoptotic, invasion, proliferation	primary, metastatic
Interleukin 5	IL-5	IL5RA, IL3RB	Hematopoietic cells	tumor progression	intermediate, metastatic
Interleukin 6	IL-6	IL6RA, IL6B	Hematopoietic cells	growth, invasion	primary, metastatic
Interleukin 7	IL-7	IL7RA, IL2RG	Hematopoietic cells	growth, tumor progression	Primary, metastatic
Interleukin 8	IL-8	IL8RA, ILR8RB	Hematopoietic cells	tumor progression	primary, metastatic
Interleukin 10	IL-10	IL10RA, IL10RB	Hematopoietic cells	tumor progression	primary, metastatic
Interleukin 11	IL-11	IL11RA	Bone marrow stroma cells	Bone metastasis Primary osteosarcoma	primary, metastatic
Interleukin 12	IL-12	IL12RA, IL12RB1	Hematopoietic cells	anti-metastatic	intermediate, metastatic
Interleukin 13	IL-13	IL13RA1, IL4RA	Hematopoietic cells	tumor progression, growth	primary, metastatic
Interleukin 15	IL-15	IL15RA	Intraepithelial	Anti-apoptotic	primary

Growth Factor	Symbol	Receptor	Origin	Action	Stage
Interleukin 16	IL-16	CD4	Hematopoietic cells	Pro-inflammatory, additional roles unknown	primary
Interleukin 17	IL-17	IL17RA, IL17RB	Epithelium, endothelium, other	Pro-inflammatory, angiogenesis, tumor progression	primary, metastatic
Inhibin BA	INHBA	Unknown	CRC cells, others	unclear; tumor expression increases with disease progression	primary, metastatic
INF-alpha	INFA1	INFAR1, INFAR2	Hematopoietic cells	angiogenesis	primary, intermediate
INF-beta	INFB1	INFAR1, INFAR2	Hematopoietic cells	anti-growth, pro-apoptotic	primary, metastatic
INF-gamma	INFG	INFGR	Hematopoietic cells	anti-growth, apoptotic	primary, metastatic
Kit ligand	KITLG, (SCF)	c-KIT	Hematopoietic cells	proliferation, invasion	primary, metastatic
Leptin	LEP	LEPR	Adipocytes	proliferation	primary
Lymphotoxin alpha	LTA (TNF-beta)	LTBR	CRC cells, others	pro-apoptotic	primary
Nicotinamide phosphoribosyl-transferase	NAMPT (PBEF)	INSR	Beta cells, adipocytes, others	Unknown; overexpressed in CRC	primary
Platelet-derived growth factor alpha	PDGFA	PDGFRA, PDGFRB	Hematopoietic cells, others	proliferation, differentiation, angiogenesis, migration	primary, metastatic
Platelet-derived growth factor alpha/beta	PDGF-AB	PDGFRA, PDGFRB	Hematopoietic cells, others	proliferation, differentiation, angiogenesis, migration	primary
Platelet-derived growth factor beta	PDGFB	PDGFRA, PDGFRB	CRC cells, others	proliferation, differentiation, angiogenesis, migration	primary, intermediate
Resistin	RETN	TLR4	Adipocytes, macrophages, epithelial cells,	inflammation, angiogenesis, invasion, progression	primary, metastatic
Tumor necrosis factor	TNF	TNFR	CRC cells, others	pro-apoptotic	primary
Transforming growth factor, alpha	TGFA	EGFR	Macrophages, neurons, keratinocytes,	proliferation, tumor progression	primary, metastatic

Growth Factor	Symbol	Receptor	Origin	Action	Stage
Transforming growth factor, beta 1	TGFB1	TGFBR1	Leukocytes	proliferation, differentiation, apoptosis, tumor progression	primary, intermediate
Transforming growth factor, beta 2	TGFB2	TGFB2R	Fibroblasts	proliferation, invasion	metastatic
Vascular endothelial growth factor	VEGF	FLT1	CRC cells, others	angiogenesis	intermediate, metastatic
Wingless-type MMTV integration site family member 1	WNT1	FZD1	CRC cells, others	proliferation, differentiation, migration	primary, intermediate
Wingless-type MMTV integration site family member 2	WNT2	FZD4	CRC cells, others	proliferation, migration, contact-independent growth	primary
Wingless-type MMTV integration site family member 4	WNT4	FZD6	CRC cells, others	proliferation, migration, contact-independent growth	primary
Wingless-type MMTV integration site family member 5A	WNT5A	FZD5	CRC cells, others	proliferation, migration, contact-independent growth	primary, intermediate
Wingless-type MMTV integration site family member 6	WNT6	Numerous FZD receptors	CRC cells, others	proliferation, migration, contact-independent growth	primary, intermediate
Wingless-type MMTV integration site family member 7A, 7B	WNT7A WNT7B	FZD5, FZD10, FZD9	CRC cells, others	proliferation, migration, contact-independent growth	primary
Wingless-type MMTV integration site family member 8B	WNT8B	Numerous FZD receptors	CRC cells, others	proliferation, migration, contact-independent growth	primary, intermediate
Wingless-type MMTV integration site family member 10A	WNT10A	Numerous FZD receptors	CRC cells, others	proliferation, migration, contact-independent growth	primary, intermediate

Growth Factor	Symbol	Receptor	Origin	Action	Stage
Wingless-type MMTV integration site family member 11	WNT11	FZ7	CRC cells, others	proliferation, migration, cytoskeletal rearrangement, contact-independent growth	primary, intermediate