Neuroendocrine PCa: Galen, Logic and Rationalism

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Neuroendocrine cells are cells which take signals from neural cells and produce signals for other cells. Neuroendocrine cells are in the prostate, and the prostate itself is highly innervated. Neuroendocrine PCa is highly aggressive. It has been observed and now logically asserted that by blocking the signals to the neuroendocrine cells we block the release of certain growth factors essential to angiogenesis and proliferation. We examine this work in some detail.

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1 INTRODUCTION

Rationalism and Empiricism may be two ends of the same process. Empiricism is "knowing" by observing facts, and that alone leads to knowledge. Rationalism assumes inherently that the human intellect can through logic attain new knowledge. Galen in his writings and his approached to medicine espoused the amalgam of both the empirical and rational. Empirically there are observations of facts. Rationally we can then relate those facts in a logical construct and within that construct we can attempt to ascertain new understanding. Oftentimes the "facts" is an observation lacking in the interconnecting "facts" but through a logical construct and subsequent validation we can then construct a valid sequence that demonstrates how best to attract a disorder[1].

In a recent examination of PCa there is an interesting blending of both the rational and empirical. We use the brief discussion of prostate neuroendocrine functioning from the paper in NEJM by Chen and Ayala who note:

Thirty years ago, Sir James W. Black shared the Nobel Prize in Physiology or Medicine for his contribution to the development of propranolol (a beta-blocker) and cimetidine (a histamine H2 blocker). Since that time, beta-blockers have been and remain widely used as antihypertensive drugs. An interesting side effect of these drugs is a reduction in the risk of prostate cancer and associated death. Thus, there exists an epidemiologic link between a drug that affects the adrenergic nervous system and prostate tumorigenesis.

This statement provides an interesting example of examining the above mentioned interplay of rationalism and empiricism in cancer diagnosis and treatment. Namely we have the empirical relationship between beta blockers, a therapeutic that works on the neurological system's control of other cells, and the unregulated cell growth of prostate cancer.

1.1 NEUROENDOCRINE PARADIGM

Namely we look at neuroendocrine type effects and thus it requires a slightly more detailed understanding of the prostate As NCI notes[2]:

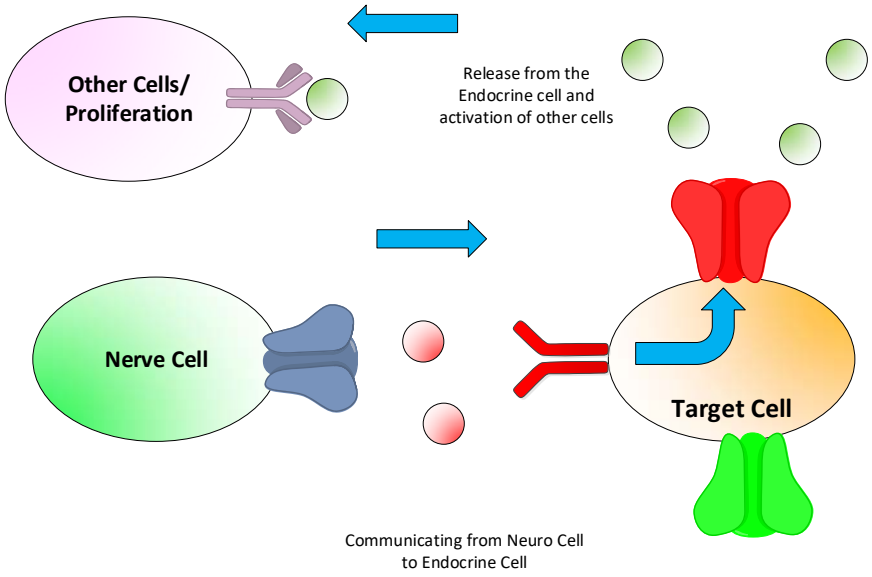
Neuroendocrine: Having to do with the interactions between the nervous system and the endocrine system. Neuroendocrine describes certain cells that release hormones into the blood in response to stimulation of the nervous system.

[1] See Mattern (2013) p 37-39 where there is a reasonable discussion of Galen and his approaches. Also one could examine the interactions between Marsilius of Padua, a Physician and Political Scientist in the 14th century with William of Ockham, the Philosopher. Both built an understanding of the blend of rationalism and empiricism.

[2] https://www.cancer.gov/publications/dictionaries/cancer-terms/def/neuroendocrine

We then, in a rationalistic manner, can try and connect the other empirical facts and see if the initial observation can also be logically correct and from that logic ascertain a new therapeutic approach.

A simplistic view of a neuroendocrine system is shown below. Basically the neuro cell activates the endocrine cell which in turn sends out signals to other collections of cells to do whatever they are supposed to do.



The above is simplistic but based upon a substantial base of validated cellular signalling factors. Namely these results are empirical in a broad sense. Now when examining various cancers we often look at the cancer cell as being the driving factor. However in a neuroendocrine environment, the cancer cell may be getting its signalling from a cancer initiating cell which in turn is being signaled by a neuro cell. The cancer initiating cell may be blocked by blocking the signalling between it and the causative neuro cell. That is the logical or rationalistic part of this exercise.

The questions now are;

(i) If the malignancy occurs in the neuroendocrine cell, then does it create an environment for proliferation of other cells?

(ii) If the malignancy occurs in the neuroendocrine cell does it send out signals that either block other homeostatic processes or does it accelerate angiogenesis in the new malignancy?

(iii) If the malignancy starts in a non-neuroendocrine cell, are there processes that effectively "turn on" the neuroendocrine cell to facilitate such effects as proliferation, angiogenesis, gene suppression or activation in other cells?

These are but a few of the questions which may be posed. Again we indicate that this is a bit simplistic but it does present the key issues related hereto.

1.2 EMPIRICISM AND RATIONALISM

The process of blending rationalism and empiricism in this specific case is accomplished as follows:

1. A set of basic facts are assembled.

2. The basic facts are assembled in some logical manner.

3. Missing links are identified

4. New facts are obtained

5. The logical process is reiterated

6. This proceeds until a conclusive result is obtained.

Let us summarize some of the Basic Facts:

1. PCa is common among men being the most significant cancer in older males.

2. The prostate is a highly enervated organ.

3. The prostate is fundamentally a glandular organ having many small glandular structures with basal cells and luminal cells.

4. However the prostate also contain a small percentage of cells activated by nerve cells via such ligands as those activated by nerve cell activating molecules.

5. The activation of these neuroendocrine cells, the prostate cells activated by neurons, then results in a variety of actions in other cells by means of an endocrine like action.

6. PCa is seen as a progressive malignancy starting in the proliferation of the basal and luminal cells and the proliferation

7. The most aggressive PCa is neuroendocrine PCa.

8. The neuroendocrine actions overcome androgen control leading to CRCP, castration resistant prostate cancer.

9. If one can disable the neuroendocrine activity then perhaps PCa can be controlled.

10. Beta blockers control neuroendocrine activity.

11. Thus beta blockers may be effective against PCa.

This supposition we explore in some detail herein.

2 NEUROENDOCRINE CELLS

We first examine neuroendocrine cells. Fundamentally as discussed above they are cells which interact with the nerves and in turn have an endocrine type function releasing molecules whose effect results in change to other cells.

From Li et al we have:

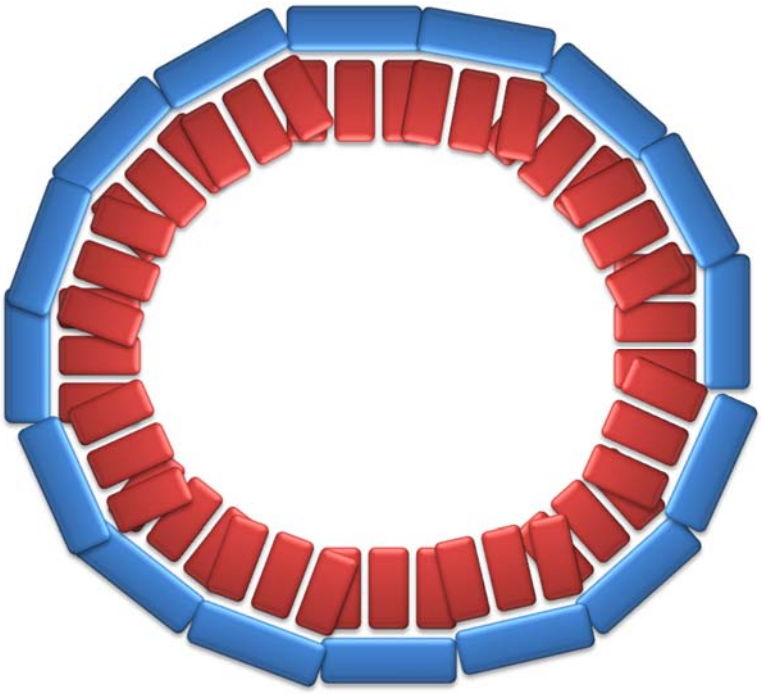
Neuroendocrine ("NE") cells are found in many tissues including normal prostate. NE cells in normal prostate, though a small subset of cells, are randomly interspersed amongst the luminal and basal cells of the prostate glands in all anatomic zones, with a slight more cells in transitional zone and peripheral zone than that in central zone.

They are not readily recognized under the light microscope using conventional hematoxylin and eosin staining, but can be easily demonstrated by immunohistochemical staining with specific markers, such as Syn, CgA and CD56 etc. Under electron microscope, there are two different morphologic types of NE cells: the open-type cells and the closed-type cells.

The open-type cells possess long surface microvilli through which the cells reach the lumen and receive luminal stimuli (pH, chemicals). The closed-type cells have lateral processes like dendritic cells through which the cells can contact the adjacent epithelial cells (luminal cells and basal cells), and receive stimuli from nerve endings, neighboring blood vessels and underlying stromal cells. The different morphologic types of NE cells are found to distribute differently in the prostate and seminal vesicles when the topography and structure of the excretory ducts of the different glands are analyzed in male rats.

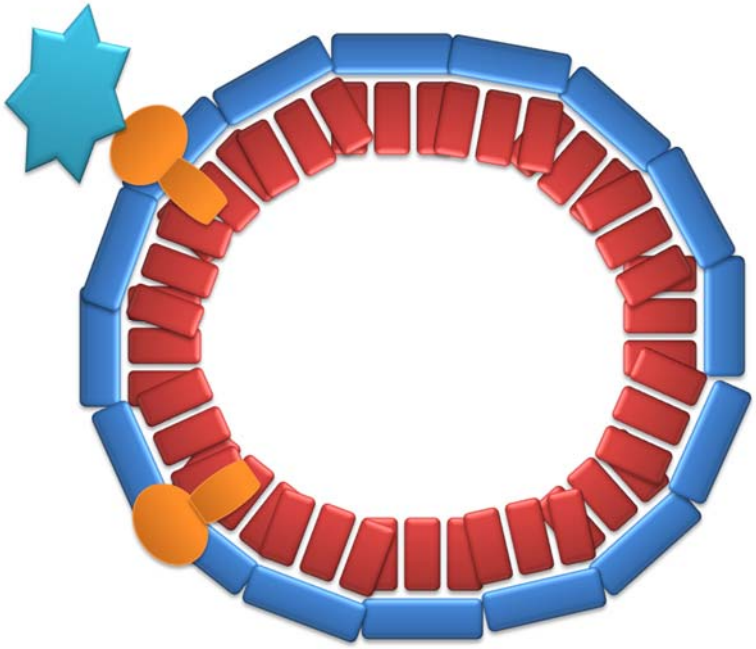
Approximately 40% of the NE cells of the ventral prostate ducts are of the open-type, whereas 14% of the seminal vesicle ducts, where most of the NE cells are of the closed-type. The finding suggests that the distribution pattern and different morphologic types of NE cells may be associated with different function

We can obtain a simplistic understanding as follows. The prostate is filled with glandular structures as shown below composed of basal cells at the base (blue cells) and luminal cells (red cells) looking inward to the gland.



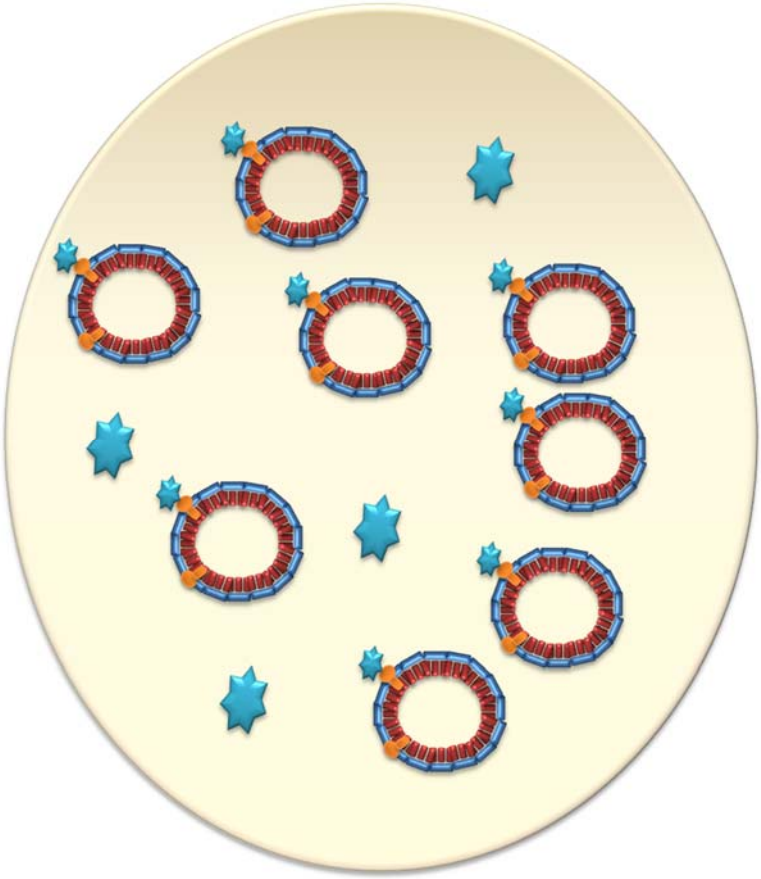
However the prostate is filled with many nerves and certain of these cells are the neuroendocrine cells, namely part of the gland but controllable by the nerve cells surrounding them. We simplistically depict this below[3]. We show the gland as previously described but the neuroendocrine cell is in orange and the neuron in light blue.

[3] See Mydlo and Godec, pp 149-153.



Note above the neuroendocrine cell may participate in the normal structure of the prostate but that it communicates via neurotransmitters with the nerves. These cells are part of the process of sending prostatic fluid out with semen and other such fluids. Identifying these cells is complex because of the need to use certain staining methods and these cells were only identified in the last few decades.

Now the entire prostate may look as follows where there are many glandular cells and many additional nerve fibers. One must remember that the prostate is highly innervated.



There are many nerves and many small glandular structures and the neuroendocrine cells participate in the overall innervation process.

As Feldman and Feldman have noted:

The main function of the prostate is to produce seminal fluid. The prostate is made up of epithelial glands and a fibromuscular stroma. The glandular epithelium, which gives rise to prostate adenocarcinoma, has three types of cells: basal, luminal secretory and neuroendocrine.

There are fewer basal cells and their function is not fully understood, although they secrete components of the basement membrane. A subset of the basal cells might be epithelial stem cells for the luminal epithelial cells. The luminal cells secrete components of prostatic fluid, express the androgen receptor and secrete prostatespecific antigen (PSA) in an androgen-dependent manner.

The stroma is composed of fibroblasts, smooth muscle cells, endothelial cells, dendritic cells, nerves and some infiltrating cells, such as mast cells and lymphocytes. Some stromal cells are androgen responsive and produce growth factors that act in a paracrine fashion on the epithelial cells. This stromal–epithelial crosstalk is an important regulator of the growth, development and hormonal responses of the prostate.

The well-organized secretory glandular structure in the normal prostate, accentuated here by immunostaining for E-cadherin, becomes disrupted in invasive prostate cancer.

3 NEUROENDOCRINE PROSTATE CANCER

Prostate cancer originates most often in the basal and luminal cells. There is an ongoing debate as to the cell of origin but we shall not discuss that here, we have elsewhere. Yet it is also possible in rare cases, some 2%, that the process begins with the neuroendocrine cell. These cancers are very virulent and have a poor prognosis. Also

Neuroendocrine tumors are defined as[4]:

A tumor that forms from cells that release hormones into the blood in response to a signal from the nervous system. Neuroendocrine tumors may make higher-than-normal amounts of hormones, which can cause many different symptoms. These tumors may be benign (not cancer) or malignant (cancer). Some examples of neuroendocrine tumors are carcinoid tumors, islet cell tumors, medullary thyroid cancer, pheochromocytomas, neuroendocrine carcinoma of the skin (Merkel cell cancer), small cell lung cancer, and large cell neuroendocrine carcinoma (a rare type of lung cancer).

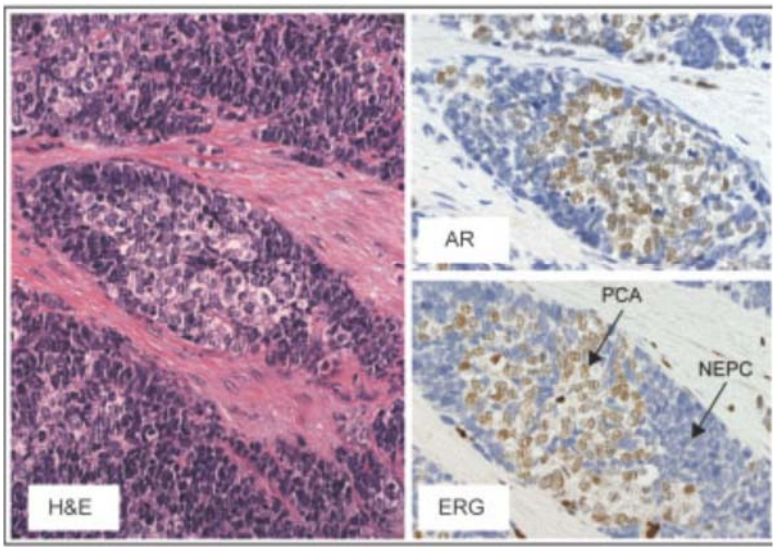
From Beltram et al:

Neuroendocrine prostate cancer (NEPC) is an aggressive subtype of prostate cancer that can arise de novo but much more commonly arises after hormonal therapy for prostate adenocarcinoma (PCA). NEPC frequently metastasizes to visceral organs, responds only transiently to chemotherapy, and most patients survive <1 year.

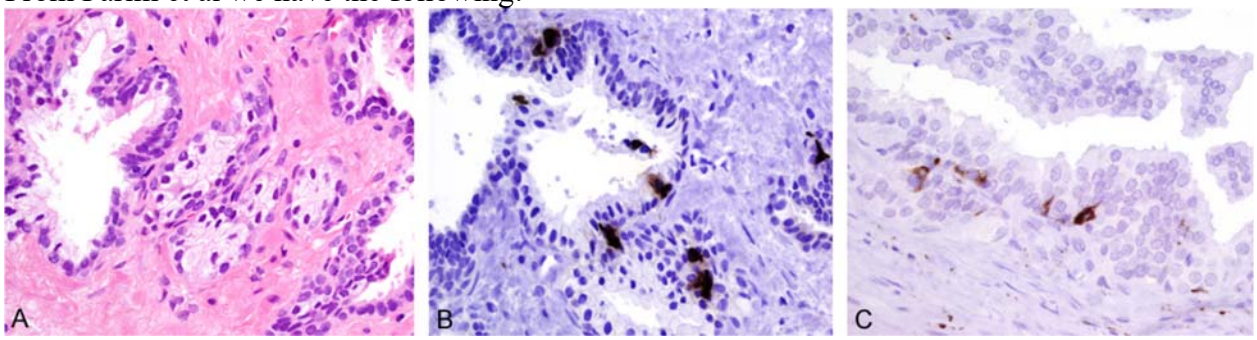
NEPC differs histologically from PCA and is characterized by the presence of small, round, blue neuroendocrine cells, which do not express androgen receptor (AR) or secrete prostate specific antigen (PSA), but usually express neuroendocrine markers such as chromogranin A, synaptophysin, and neuron- specific enolase (NSE).

The prostate cancer–specific TMPRSS2-ERG gene rearrangement has been reported in approximately 50% of NEPC cases, similar to the frequency in PCA. This suggests that NEPC is clonally derived from PCA and distinguishes NEPC from small carcinomas of other primary sites. The poor molecular characterization of NEPC accounts in part for the lack of disease-specific therapeutics……tumor with mixed features of NEPC and PCA; hematoxylin and eosin (H&E) staining, immunohistochemical analysis for AR and ERG,…

[4] https://www.cancer.gov/publications/dictionaries/cancer-terms/def/neuroendocrine-tumor



From Parim et al we have the following:



Generally, neuroendocrine cells cannot be recognized in a benign prostatic gland with routine H&E staining (A). However, the neuroendocrine immunostains such as chromogranin (B) or synaptophysin (C) can highlight the neuroendocrine cells which are typically situated in the basal cell compartment with cell processes projecting into the layer of luminal cells.

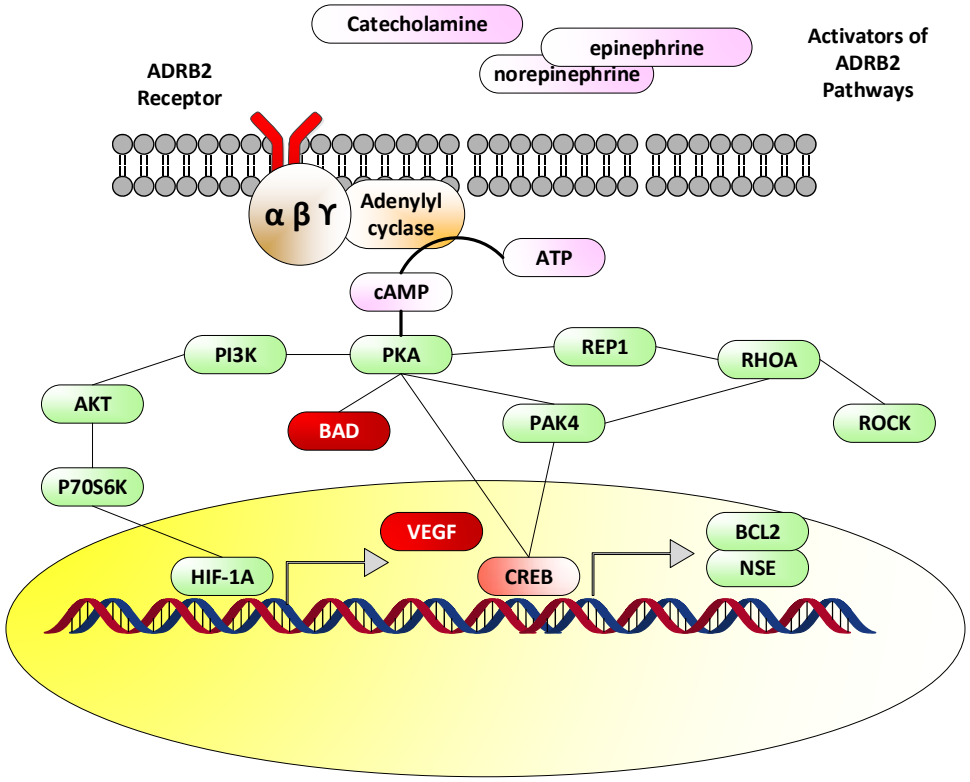
Braadland et al present the pathway activation as shown below. They focus on the gene ADRB2. This gene is defined as follows[5]:

This gene encodes beta-2-adrenergic receptor which is a member of the G protein-coupled receptor superfamily. This receptor is directly associated with one of its ultimate effectors, the class C L-type calcium channel Ca(V)1.2. This receptor-channel complex also contains a G protein, an adenylyl cyclase, cAMP-dependent kinase, and the counterbalancing phosphatase, PP2A. The assembly of the signaling complex provides a mechanism that ensures specific and rapid signaling by this G protein-coupled receptor. This gene is intronless. Different

[5] https://www.ncbi.nlm.nih.gov/gene/154

polymorphic forms, point mutations, and/or downregulation of this gene are associated with nocturnal asthma, obesity and type 2 diabetes.

Simply noted, some one of the three activators as noted activate the ADRB2 pathway ultimately releasing VEGF and other promoters.



Braadland et al comment on the above as follows:

Cyclic AMP produced in response to adrenergic stimulation binds the regulatory subunit of PKA and the activated catalytic subunit is released.

The catalytic subunit may translocate to the nucleus and phosphorylate cAMP responsive element binding protein (CREB), which induces the expression of e.g., neuron specific enolase/enolase 2 (ENO2, a neuroendocrine marker), and B-cell CLL/lymphoma 2 (BCL2, encoding an anti-apoptotic protein).

PKA-induced phosphorylation of CREB may either be direct or indirect through regulation of p21-activated protein kinase 4 (PAK4) and/or ERK activity.

Stress may also promote apoptosis-resistance through PKA-dependent phosphorylation of BCL2-associated agonist of cell death (BAD), as shown.

Furthermore, PKA may inhibit the ras homolog family member A (RhoA) – Rho-associated PKA (ROCK) pathway leading to neurite outgrowth either directly or mediated through either Rap1, a member of the RAS oncogene family, or PAK4.

Rap1 is also possibly involved in PKA-induced regulation of ERK activity.

Finally, PKA-mediated effects of adrenergic stimuli up-regulate vascular endothelial growth factor (VEGF) levels and HUVEC capillary tube formation via the PI3K/AKT/p70S6K/HIF-1α pathway.

Besides regulating the transcription factor activity of CREB and HIF-1α, the ADRB2/cAMP/PKA signaling pathway has been shown to stimulate the androgen receptor responsive gene transcription

As Zahalka et al note:

Solid tumors depend on angiogenesis to sustain their growth. The transition from hyperplasia to highly vascularized growing tumor, referred to as the "angiogenic switch," is a state in which proangiogenic factors—such as vascular endothelial growth factor (VEGF) and other secreted angiocrine factors—predominate over antiangiogenic signals. During development, peripheral nerves associate closely with growing blood vessels, organizing vascular pattern, a phenomenon that has also been described in models of wound healing.

Emerging studies suggest that nerves can also regulate tumorigenesis. Sympathetic nerve fibers deliver adrenergic signals that act via b-adrenergic receptors (bAdRs) expressed in the tumor microenvironment. However, the cellular target(s) and molecular mechanism(s) responsible for neural regulation of cancer are not known and may provide novel therapeutic avenues.

They summarize as follows:

1. Adrenergic nerves regulate angiogenesis in early tumor growth
2. Endothelial ADRB2 controls the angiogenic switch
3. ADRB2 regulates oxidative metabolism in angiogenic prostate endothelial cells
4. Increased endothelial COA6 activity mediates the shift toward oxidative phosphorylation

4 SIRT1 AND NEUROENDOCRINE CANCER

Yuan et al. have provided a review of the impact of SIRT1 on PCa of neuroendocrine differentiation. They summarize as follows:

The epigenetic factor SIRT1 can promote prostate cancer progression, but it is unclear whether SIRT1 contributes to neuroendocrine differentiation. In this study, we showed that androgen deprivation can induce reactive oxygen species production and that reactive oxygen species, in turn, activate SIRT1 expression.

The increased SIRT1 expression induces neuroendocrine differentiation of prostate cancer cells by activating the Akt pathway. In addition, the interaction between Akt and SIRT1 is independent of N-Myc and can drive the development of neuroendocrine prostate cancer when N-Myc is blocked. Furthermore, SIRT1 facilitates tumor maintenance, and targeting SIRT1 may reduce the tumor burden during androgen deprivation. Our findings suggest that SIRT1 is a potential target for therapeutic intervention….

Neuroendocrine cells are one of three types of epithelial cells in normal prostate tissue, where they constitute <1% of total epithelial cells and have an unclear physiological role.

In prostate adenocarcinoma, an increased number of neuroendocrine cells is observed, and this change is always associated with poor prognosis, including frequent metastasis, relatively low serum prostate-specific antigen (PSA) level, and resistance to androgen ablation.

Therefore, identifying novel elements involved in NED is critical for understanding the mechanism of Pca progression and developing new drug targets for the treatment.

The prevailing hypothesis is that Pca undergoes NED, especially under the selective pressure of androgen deprivation. Our results functionally demonstrated that SIRT1 can arise from ROS production in Pca cells responding to ADT. Further, SIRT1 upregulation can activate Akt expression, which, in turn, promotes NED.

Taking advantage of published gene expression from GEO, we downloaded and reanalyzed raw data from both LNCaP cells and LNCaP-NED cells. Focusing on epigenetic factors involved in NED, we discovered that multiple epigenetic factors, including SIRT1, are clustered and that their expression is upregulated during NED. Importantly, amplification of SIRT1 was evident in neuroendocrine prostate tumors from TCGA database, providing validation of our expression analysis.

From our previous work we have seen the following. The recent paper Di Sante et al states[6]:

[6] http://ajp.amjpathol.org/article/S0002-9440%2814%2900561-6/pdf

Prostatic intraepithelial neoplasia is a precursor to prostate cancer. Herein, deletion of the NAD+-dependent histone deacetylase Sirt1 induced histological features of prostatic intraepithelial neoplasia at 7 months of age; these features were associated with increased cell proliferation and enhanced mitophagy.

In reality the statement is not definitive. We have observed HGPIN actually disappearing and doing so for prolonged periods. The question is; what makes HGPIN disappear. Also there is still a lack of total clarity as to the genetic progression of PCa. One may still consider inflammation as a major cause and possible mitigation of inflammation being a reason for the reversal of HGPIN. However that also is conjecture. The problem here is the definitive statement regarding HGPIN.

In human prostate cancer, lower Sirt1 expression in the luminal epithelium was associated with poor prognosis. Genetic deletion of Sirt1 increased mitochondrial superoxide dismutase 2 (Sod2) acetylation of lysine residue 68, thereby enhancing reactive oxygen species (ROS) production and reducing SOD2 activity.

The question on the expression of Sirt1 is; is this a cause or an effect, or is it a concomitant from some related but no causal element?

The PARK2 gene, which has several features of a tumor suppressor, encodes an E3 ubiquitin ligase that participates in removal of damaged mitochondria via mitophagy. Increased ROS in Sirt1−/− cells enhanced the recruitment of Park2 to the mitochondria, inducing mitophagy. Sirt1 restoration inhibited PARK2 translocation and ROS production requiring the Sirt1 catalytic domain.

Thus, the NAD+-dependent inhibition of SOD2 activity and ROS by SIRT1 provides a gatekeeper function to reduce PARK2-mediated mitophagy and aberrant cell survival.

Sirt1 seems to be a gene whose function, if expression is reduced, could lead to malignant behavior. Now articles like this often get significant Press coverage. In Medical express we have[7]:

Prostate cancer affects more than 23,000 men this year in the USA however the individual genes that initiate prostate cancer formation are poorly understood. Finding an enzyme that regulates this process could provide excellent new prevention approaches for this common malignancy. Sirtuin enzymes have been implicated in neurodegeneration, obesity, heart disease, and cancer. Research published online Thursday in The American Journal of Pathology show the loss of one of sirtuin (SIRT1) drives the formation of early prostate cancer (prostatic intraepithelial neoplasia) in mouse models of the disease.

"Using genetic deletion we found that SIRT1 normally restrains prostatic intraepithelial neoplasia in animals. Therefore too little SIRT1 may be involved in the cellular processes that

[7] http://medicalxpress.com/news/2014-12-prostate-cancer.html

starts human prostate cancer," said Dr. Richard Pestell, M.D., Ph.D., MBA, executive Vice President of Thomas Jefferson University and Director of the Sidney Kimmel Cancer Center. "As we had shown that gene therapy based re expression of SIRT1 can block human prostate cancer tumor growth, and SIRT1 is an enzyme which can be targeted, this may be an important new target for prostate cancer prevention."

Upregulation of SIRT1 is one path and developing a therapeutic for initiating that upregulation is also critical. However there may be a multiplicity of other factors that would or could be required. The mouse studies are clearly not definitive for humans. They are suggestive at best.

The researchers led by Dr. Pestell, created a mouse model that lacked SIRT1 and noticed that these mice were more likely to develop an early form of prostate cancer called prostatic intraepithelial neoplasia (PIN).

One of our ongoing concerns is the use of mouse models. We know that they are useful for certain studies but problematic for others. In addition a knockout mouse may have more complex genetic interactions that a random human. For example, generating a specific knockout mouse model may also affect many other gene expressions which the experimenter may not have full knowledge of. In addition the human and murine models of a knockout are not comparable, because we cannot do the same in a human.

Other researchers had shown that SIRT1 can defend the cell against damage from free radicals. Pestell's group took the work further by showing that in this prostate cancer model, free radicals built up in cells lacking SIRT1. They showed that normally, SIRT1 proteins help activate a mitochondrial protein called SOD2, in turn activating those proteins to keep free-radical levels in check. When SIRT1 level are diminished, SOD2 is no longer effective at removing free radicals, allowing a dangerous build up in the cells, and leading to PIN.

Now Pestell and his group are highly respected and they have reported on Sirt1 effects before[8].

"The next step," says first author Gabriele DiSante, Ph.D., a postdoctoral fellow in the department of Cell Biology at Jefferson, "is to determine if this is also important in the development of human prostate cancer."

Overall it is known that Sirt1 does work against inflammatory tendencies. The last statement however is critical. It is clear that the determination for human cells is still problematic. This seems to be one of the major problems in murine models. The mouse prostate growth is not always the same as human. Goldstein et al some five years ago did studies in mice regarding the cell leading to HGPIN and thus PCa. Was it a basal cell or a luminal cell? Carrying this over the humans was and is not definitive in any manner.

[8] See the book by Pestell and Nevalainen pp 157-158

We now will examine Sirt1 and the family of genes from which it derives the Sirtuins. These genes have generally been examined in other venues and not PCa. However they are well examined and we shall consider them in some detail.

4.1 SIRT 1 DETAILS

We begin with the work of Guatente has recently written an extensive review paper on Sirtuins and especially Sirt1 in NEJM. It concludes as follows:

Sir2 is one of a complex of proteins that mediate transcriptional silencing at selected regions of the yeast genome. Mutations that extend the replicative life span of yeast mother cells have been shown to increase the silencing activity of Sir2 at the ribosomal DNA repeats. Although the silencing of ribosomal DNA has turned out to be an idiosyncratic feature of aging in yeast, the role of Sir2-related gene products (sirtuins) in aging appears to be universal. Sir2 orthologues slow aging in the nematode Caenorhabditis elegans, in the fruit fly Drosophila melanogaster, and in mice. The sirtuins have been shown to have NAD-dependent protein deacetylase activity, which is associated with the splitting of NAD during each deacetylation cycle…

The studies to date have been on yeasts and fruit flies and there have been some studies on humans. However the main focus on sirtuins is their beneficial effects on the aging process, and one suspects as an antioxidant and anti-inflammatory type of behavior.

Of the mammalian sirtuins, SIRT1, 2, 3, 4, 5, and 6 have been shown to have this activity. Some SIRT family members (e.g., SIRT4 and SIRT6) also have ADP-ribosyltransferase activity. In mammals, the Sir2 orthologue SIRT1 is primarily a nuclear protein in most cell types and has evolved to deacetylate transcription factors and cofactors that govern many central metabolic pathways.

Targets of SIRT1 *include transcriptional proteins that are important in energy metabolism, such as nuclear receptors, peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α), and* ***forkhead box subgroup O (FOXO)****. SIRT1 also regulates components of the circadian clock, such as BMAL1 and PER2, which underscores the interconnectedness of protein acetylation, metabolism, circadian rhythm, and aging.*

SIRT1 is also closely coupled to AMP-kinase activity in a mutually enforcing mechanism that adjusts cellular physiology for conditions of energy limitation.

Sirt1 is the gene of focus yet Sirt2-6 also play roles, none of which seem to have a role in PCa. The FOXO target is of considerable interest[9].

[9] As Brunet et al state: S*IRT1's effects on FOXO3 are reminiscent of SIRT1's effects on the tumor suppressor p53. Under conditions of cellular stress, SIRT1 deacetylation of p53 leads to an inhibition of apoptosis. Given that SIRT1 also reduces FOXO3-induced apoptosis in the presence of stress stimuli, it is possible that FOXO3 and p53 somehow function together to mediate the effects of SIRT1.*We know p53 is an oncogene and its suppression can result in metastatic behavior and thus SIRT1 has a pivotal role in many areas of cancer development and spread.

The earliest connection between SIRT1 and endothelial cells was the finding that SIRT1 deacetylates and activates endothelial nitric oxide synthase (eNOS). The activation of eNOS and repression of AT1 suggest that SIRT1 activity ought to curb high blood pressure.

SIRT1 also inhibits the senescence of endothelial cells, and its salutary effect on these cells may mitigate atherosclerosis. Interestingly, calorie restriction is known to protect against atherosclerosis,46 and many of the physiological effects of calorie restriction are blunted in eNOS−/−mice.21 These findings all indicate that SIRT1 helps facilitate the favorable effect of calorie restriction on cardiovascular function by its effects on eNOS, AT1, and perhaps other targets.

4.2 SOME OTHER GENES

It is worth examining a few other related genes. First we examine SIRT1 based upon NCBI.

From NCBI we have for SIRT1[10]:

SIRT1: This gene encodes a member of the sirtuin family of proteins, homologs to the yeast Sir2 protein. Members of the sirtuin family are characterized by a sirtuin core domain and grouped into four classes. The functions of human sirtuins have not yet been determined; however, yeast sirtuin proteins are known to regulate epigenetic gene silencing and suppress recombination of rDNA. Studies suggest that the human sirtuins may function as intracellular regulatory proteins with mono-ADP-ribosyltransferase activity. The protein encoded by this gene is included in class I of the sirtuin family. Alternative splicing results in multiple transcript variants.

The regulatory nature of SIRT1 is a key element in its functioning in PCa. We will examine how this may function shortly.

And relating to SOD2[11]:

SOD2 superoxide dismutase 2, mitochondrial: This gene is a member of the iron/manganese superoxide dismutase family. It encodes a mitochondrial protein that forms a homotetramer and binds one manganese ion per subunit. This protein binds to the superoxide byproducts of oxidative phosphorylation and converts them to hydrogen peroxide and diatomic oxygen.

Mutations in this gene have been associated with idiopathic cardiomyopathy (IDC), premature aging, sporadic motor neuron disease, and cancer. Alternate transcriptional splice variants, encoding different isoforms, have been characterized.

[10] http://www.ncbi.nlm.nih.gov/gene/23411

[11] http://www.ncbi.nlm.nih.gov/gene/6648

And for PARK2 we have[12]:

The precise function of this gene is unknown; however, the encoded protein is a component of a multiprotein E3 ubiquitin ligase complex that mediates the targeting of substrate proteins for proteasomal degradation. Mutations in this gene are known to cause Parkinson disease and autosomal recessive juvenile Parkinson disease. Alternative splicing of this gene produces multiple transcript variants encoding distinct isoforms. Additional splice variants of this gene have been described but currently lack transcript support.

From Powell et al we have as more detailed discussion of the functions of Sirt1:

The Sirtuin family of proteins (SIRT) encode a group of evolutionarily conserved, NAD-dependent histone deacetylases, involved in many biological pathways. SIRT1, the human homologue of the yeast Silent Information Regulator 2 (Sir2) gene, de-acetylates histones, p300, p53, and the androgen receptor. Autophagy is required for the degradation of damaged organelles and long-lived proteins, as well as for the development of glands such as the breast and prostate. Herein, homozygous deletion of the Sirt1 gene in mice resulted in prostatic intraepithelial neoplasia (PIN) associated with reduced autophagy.

Genome-wide gene expression analysis of Sirt1/ prostates demonstrated that endogenous Sirt1 repressed androgen responsive gene expression and induced autophagy in the prostate. Sirt1 induction of autophagy occurred at the level of autophagosome maturation and completion in cultured prostate cancer cells. These studies provide novel evidence for a checkpoint function of Sirt1 in the development of PIN and further highlight a role for SIRT1 as a tumor suppressor in the prostate.

The autophagy cleans up the cells and brings them back to a normal stasis. The recognition of Powell et al regarding the role of Sirt1 is key. They continue:

The role of SIRT1 in regulating prostate gland formation and androgen signaling in vivo was previously unknown. SIRT1is expressed in several cell types in the prostate gland including basal cells, luminal cells, and stromal cells. Given the evidence that SIRT1 functions as a tissue-specific regulator of cellular growth and that SIRT1 inhibits tumor cell line growth in nude mice, we sought to determine the role of endogenousSirt1 in regulating prostate gland development. Genome-wide expression profiling of Sirt1/ mice prostates and their littermate controls identified a molecular, genetic signature regulated by endogenous Sirt1.

The above clearly shows the understanding of the function of Sirt1. Note that the Powell work was in 2010 so that this understanding has been available for a while.

This signature highlights the ability of Sirt1 to inhibit androgen signaling and apoptosis in the prostate, while promoting autophagy. The Sirt1/ prostates demonstrated epithelial hyperplasia

[12] http://www.ncbi.nlm.nih.gov/gene/5071

and PIN suggesting that Sirt1 promotes autophagy and inhibits prostate epithelial cell proliferation in vivo.

The above demonstrates the ability of Sirt1 to control androgen signalling. This also is a key factor in controlling prostate health.

Gene expression analysis further demonstrated that loss of endogenous Sirt1 inhibited autophagy. At a higher level of resolution, our studies demonstrated that SIRT1 antagonized DHT-mediated inhibition of autophagy in the prostate. Autophagy allows for degradation of proteins and organelles and is induced by nutrient withdrawal, rapamycin (inhibition of mTOR signaling), and hormone signaling.

Our findings are consistent with prior studies demonstrating that SIRT1 induces autophagy by deacetylating ATG5, ATG7, and ATG8 and inhibits AR signaling via deacetylation of the AR. Comparisons with previously published studies identified an overlap of 12.45% between genes regulated by endogenous Sirt1 and those targeted by androgens in the prostate gland and in prostate cancer cells. These results are consistent with prior findings that Sirt1 inhibits ligand-dependent AR signaling and gene expression in vitro

Again we come back to the role of autophagy. Perhaps the buildup of protein segments may act as normal cell blockage, inhibiting normal expression and control. The autophagy allows for a return to such normality. The emphasize this issue as follows:

The role of autophagy in cancer was proposed over 20 years ago. Autophagy appears to be essential for tumor suppression as well as for cell survival. Autophagy plays a prosurvival function for cancer cells during nutrient deprivation or when apoptotic pathways are compromised, a phenotype often accompanied by inflammation.

Again we see the putative role of inflammation. This appears to be a significant factor in PCa and the suppression of genes which deal with the remnants of inflammation seem to be a key benchmark in PCa progression. They continue:

In contrast, upon disruption of tumor suppressors, autophagy adopts a pro-death role with apoptotic pathways. In prostate, breast, ovarian, and lung cancer, loss of Beclin1 or inhibition of Beclin1 by the BCL-2 family of proteins causes defective autophagy, increased DNA damage, metabolic stress, and genomic instability.

These cancers also display neoplastic changes and increased cell proliferation, unlike cells overexpressing Beclin1, which undergo apoptosis. Loss of PTEN, p53, ATG4, ATG5, and MAP1LC31 (ATG8) are linked to tumorigenesis, whereas upregulation of PI3K, AKT, BCL-2, and mTOR are associated with inhibition of autophagy and the promotion of tumorigenesis.

Prostate cancer onset and progression are correlated strongly with aging and SIRT1 function governs aging in multiple species. Further studies will be required to determine whether this checkpoint function of Sirt1 in regard to prostate growth is linked to its role in organismal aging.

From Shackelford et al we have additional insights including pathway control issues as follows:

AMPK has recently been shown to increase sirtuin 1 (SIRT1) activity by increasing cellular NAD+ levels, resulting in the regulation of many downstream SIRT1 targets, including FOXO3 and peroxisome proliferator activated receptor-γ co-activator 1 (PgC1; also known as PPARgC1A), both of which have also been proposed to be direct substrates of AMPK46,76. As SIRT1 is also implicated in tumorigenesis, this connection between AMPK and SIRT1 might further explain how nutrients control cell growth. AMPK also suppresses mTOR-dependent transcriptional regulators to inhibit cell growth and tumorigenesis.

Two mTORC1-regulated transcription factors involved in cell growth are the sterol-regulatory element-binding protein 1 (SReBP1) and hypoxiainducible factor 1a (HIF1α). SReBP1 is a sterolsensing transcription factor that drives lipogenesis in many mammalian cell types. mTORC1 signalling is required for nuclear accumulation of SReBP1 and the induction of SReBP1 target genes78, and this can be inhibited by rapamycin or AMPK agonists

From Hines et al we have an expression of Sirt1 in terms of overall cell control:

The NAD + -dependent deacetylase SIRT1 is an evolutionarily conserved metabolic sensor of the Sirtuin family that mediates homeostatic responses to certain physiological stresses such as nutrient restriction. Previous reports have implicated fluctuations in intracellular NAD + concentrations as the principal regulator of SIRT1 activity. However, here we have identified a cAMP-induced phosphorylation of a highly conserved serine (S434) located in the SIRT1 catalytic domain that rapidly enhanced intrinsic deacetylase activity independently of changes in NAD + levels.

Attenuation of SIRT1 expression or the use of a nonphosphorylatable SIRT1 mutant pre- vented cAMP-mediated stimulation of fatty acid oxidation and gene expression linked to this path- way. Overexpression of SIRT1 in mice significantly potentiated the increases in fatty acid oxidation and energy expenditure caused by either pharmacological b -adrenergic agonism or cold exposure. These studies support a mechanism of Sirtuin enzymatic control through the cAMP/PKA pathway with important implications for stress responses and maintenance of energy homeostasis

From Dominy et al we have:

From an evolutionary perspective, the nutrient-dependent control of protein acetylation through acetyltransferases and deacetylases is highly conserved and is a major mechanism for coupling metabolic activity with carbon/energy availability. The regulated acetylation of PGC-1a by GCN5 and Sirt1 is an excellent example: PGC-1a acetylation by GCN5 is favored under conditions of nutrient/energy abundance, whereas deacetylation by Sirt1 is favored under conditions of nutrient dearth and high energy demand

Finally Brooks and Gu state:

SIRT1 is a multifaceted, NAD+-dependent protein deacetylase that is involved in a wide variety of cellular processes from cancer to ageing. The function of SIRT1 in cancer is complex: SIRT1 has been shown to have oncogenic properties by down regulating p53 activity, but recent studies indicate that SIRT1 acts as a tumour suppressor in a mutated p53 background, raising intriguing questions regarding its mechanism of action.

Here we discuss the current understanding of how SIRT1 functions in light of recent discoveries and propose that the net outcome of the seemingly opposite oncogenic and tumour-suppressive effects of SIRT1 depends on the status of p53.

They clearly indicate the tumor suppressor role of Sirt1. p53 status is important but the observation above is truly intriguing if it is sustained.

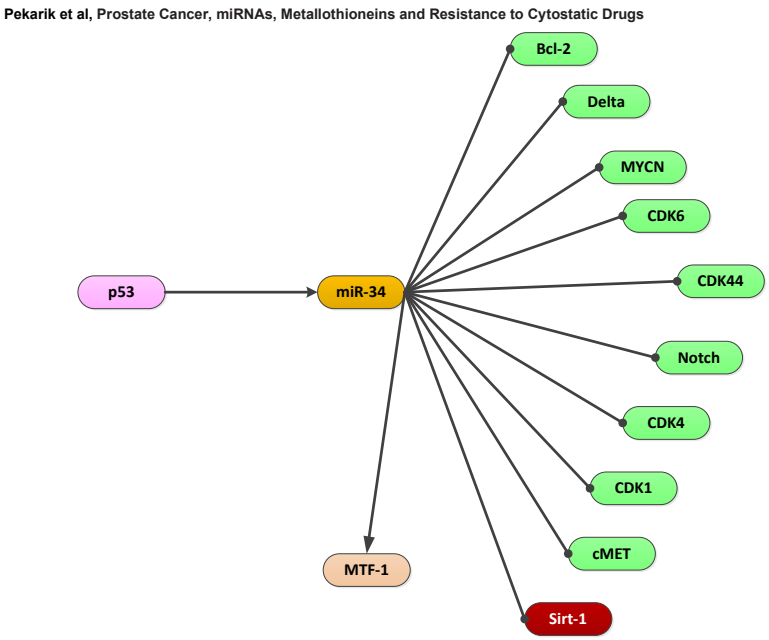
4.3 MIRNA AND SIRT1

The control of Sirt1 may be done via miRNAs. As Pekarik et al note:

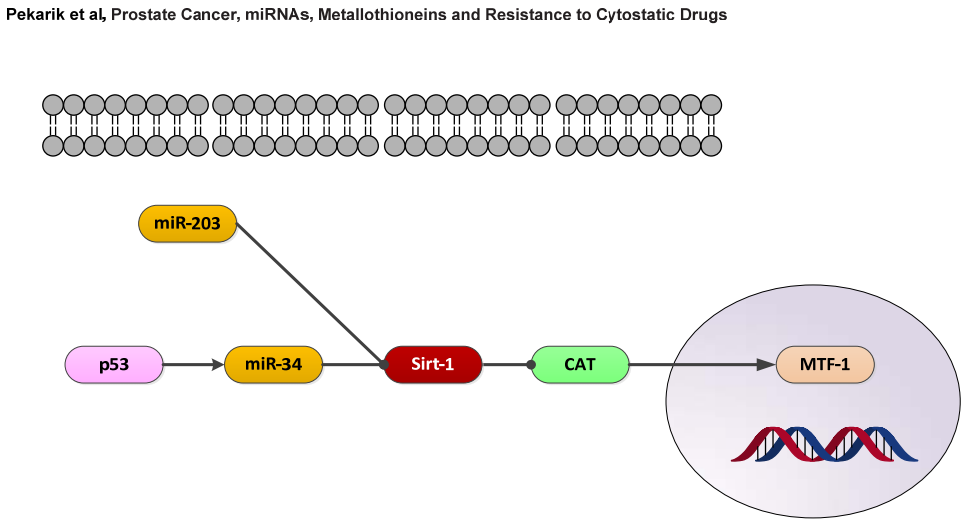
Importance of miRNAs is underscored by the fact that nearly half of the genes coding miRNAs are located at fragile sites or at regions with lost homozygozity. For example, a loss of p-arm of chromosome 1 is a common finding in sporadic colon carcinomas. Among many genes associated with DNA repair, checkpoint functions, tumour suppressors, etc. are also multiple miRNAs.

The most critical is miR-34a, directly regulated by tumour suppressor gene p53 and classified now as tumour suppressor itself. Ectopic miR-34a expression induces apoptosis and a cell cycle arrest in G1 phase. Downstream targets of miR-34 are Bcl2, MYCN, NOTCH1, Delta1, CDK4 and 6, Cyclin D1, Cyclin E2, c-Met, SIRT1, and E2F3, all the genes involved in apoptosis or proliferation and cell growth control…

We have discussed miRNAs and especially mrR-34 as part of PCa process. The control Sirt1 by miR-34 is a key observation It links back to a cause. Thus one may surmise that this is a potential initiator and the miR-34 expression generated in some feedback manner with the inflammation which would have been controlled by Sirt1. We demonstrate that below.



And then we demonstrate the controlling process:



In addition miRNAs have also recently been shown to be facilitators of metastasis. There is a short review by Anastasiadou and Slack in Science which states:

Interestingly, exosomes contain messenger RNA (mRNA) and miRNA that can be transferred to other cells and regulate gene expression of the target cell. Likewise, miRNAs are present in apoptotic bodies (small membrane vesicles that are produced by cells undergoing programmed cell death), or they are in the plasma, associated with Argonaute2 (AG02), the key effector protein of a miRNA-mediated gene silencing mechanism. However, miRNAs detected in human

serum and saliva are mostly concentrated inside exosomes. Virally encoded miRNAs are also found in exosomes, indicating how oncogenic viruses could manipulate the tumor microenvironment. …

Melo et al. reveal a role of exosomes in cell-independent miRNA biogenesis that affects cancer progression. The authors show that only exosomes derived from cancer cells, but not those derived from normal cells, contain key enzymes involved in miRNA biogenesis such as Dicer, TAR (trans-activation response) RNA-binding protein (TRBP), and AGO2.

The exosomes also contain the membrane protein CD43, which plays a role in accumulating Dicer in cancer exosomes. The study also shows that Dicer-containing cancer exosomes process precursor miRNAs into mature miRNAs (including oncomiRs) over time, and upon encounter with normal human mammary epithelial, cells induces them to become cancerous.

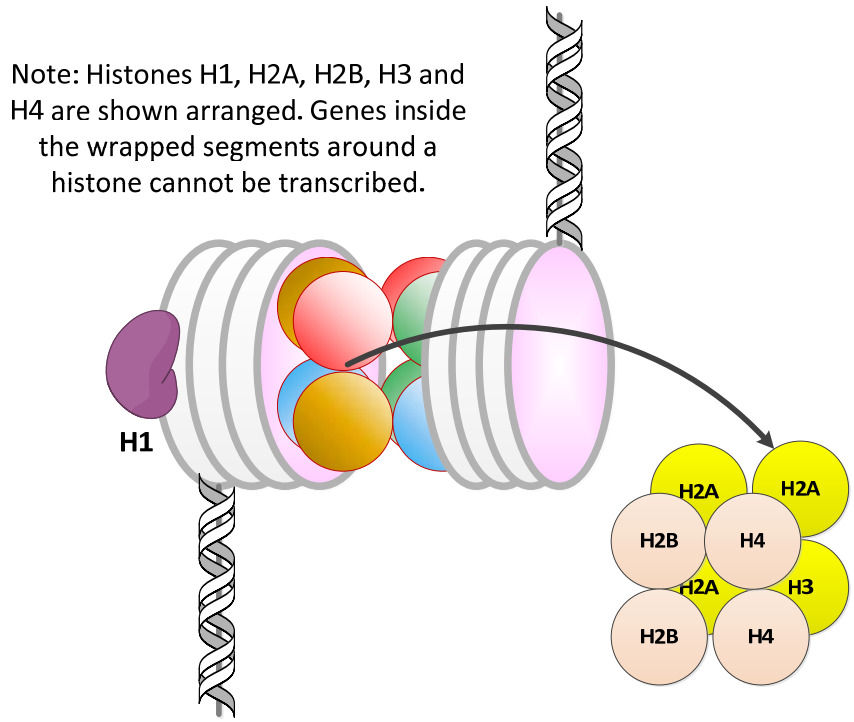
Thus, these epigenetic elements, the miRNAs, can spread throughout the body effecting changes in cells that are beyond fundamental intracellular effects. Thus the loss of Sirt1 expression may be the result of this exosomal effects.

4.4 METHYLATION FACTORS

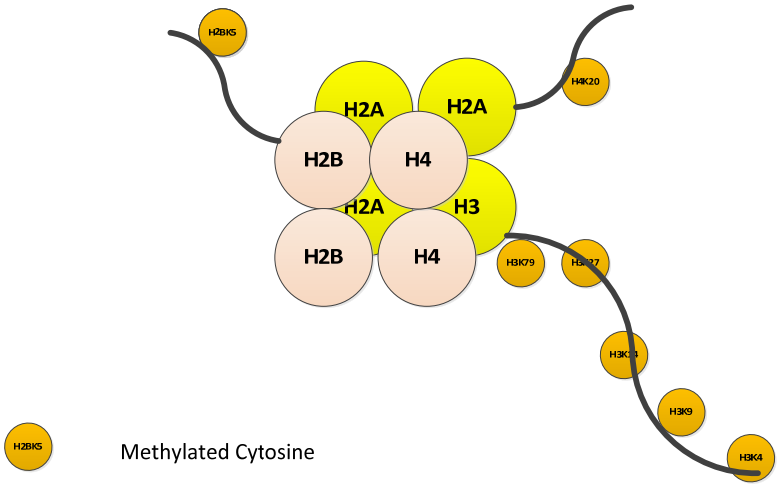
Methylation consists of the attachment of methyl groups on various elements of the genome. For our purposes we consider methylating the DNA on the CpG islands and methylation of the histones around which the DNA is wrapped. These effects have shown significant impact as well on PCa as well as many other cancers.

We have now described methylation, a rather simple process, and now we seek to discuss its influence on DNA. We start first at the top level of DNA, namely the chromosome. The DNA is often wrapped around histones, which are large protein masses that arrange themselves in a specific group. There are five main histones, H1, H2A, H2B, H3, and H4. They arrange themselves as shown below.

It appears as if one has eight large globes, each a histone, and they then allow the DNA to coil about them and in effect make certain that that specific segment of DNA is not read. Histones are another mechanism for DNA expression. They must be released so the DNA can be opened and then read in order for it to be expressed.

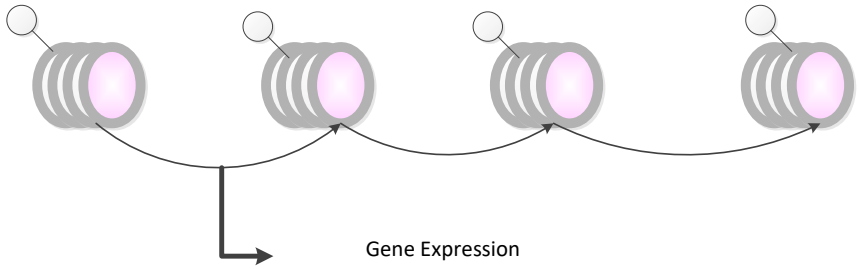


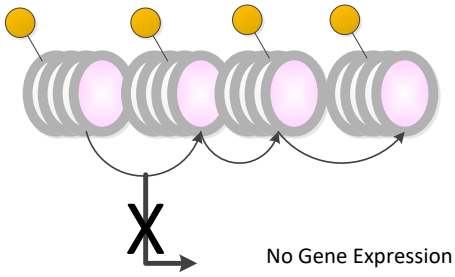
The specific arrangement of the histones is as shown below. It is not arbitrary but is a result of the specific surface charge arrangements on the histone proteins. We also depict the presence of methylated cytosines on this graphic, thus depicting the two major influences of methylation as well as acetylation, which we shall discuss.



Now what can happen is that the histone tails may become methylated, or acetylated, and when this occurs the histones may bind together or open up, depending on which lysine on the tail is affected. The open and close as a result of a methylation or acetylation is also called the histone

code. Methylate or acetylate the right ones and the DNA is curled and not expressible and do another set and the DNA can be expressed.





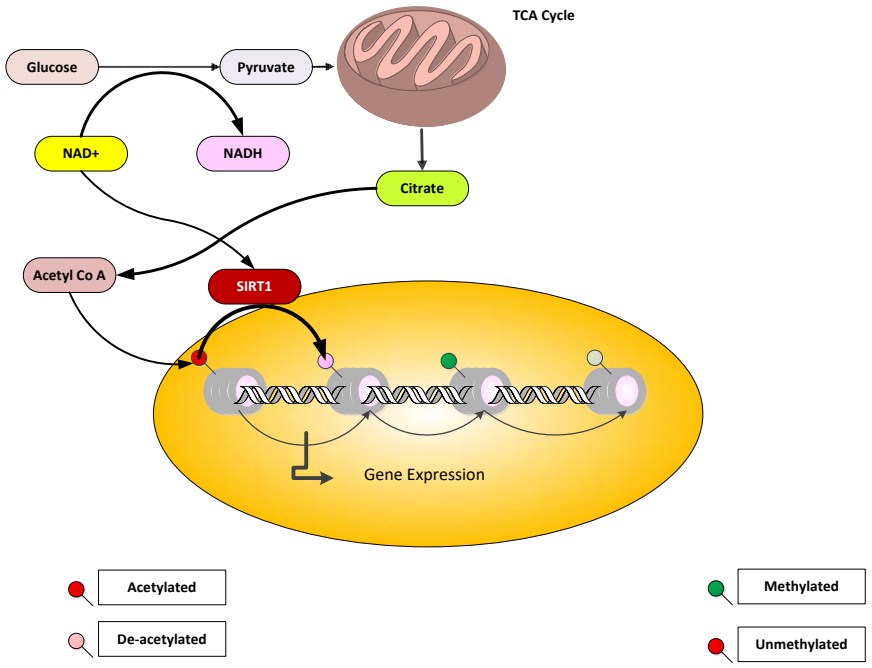
This Histone Code is shown below in the following Table.

	H3K4	H3K9	H3K14	H3K27	H3K79	H4K20	H2BK5
Mono-meth	Active	Active		Active	Active	Active	Active
Di-meth		Repress		Repress	Active		
Tri-meth	Active	Repress		Repress	Active		Repress
Acetyl		Active	Active		Repress		

Now we can use the above to understand the impact of these epigenetic factors via the interactions between Sirt1 and diet. In a recent paper by Labbe et al the authors examine dies and PCa. In particular they discuss the effect of Sirt1[13]. We show a modification of the Figure in the paper below. Glucose is converted to pyruvate via the action of NAD+ to NAH. Likewise this activates citrate to Acetyl-Co A and acetylates the histone changing its code but Sirt1 then deacytylates it to the ground state again. Thus loss of Sirt1 can potentially allow excess acetylated states which in turn does not allow the related genes to be expressed. Now from our discussions of miRNA exosomes we also understand that perhaps this down regulation of Sirt1

[13] http://www.nature.com/onc/journal/vaop/ncurrent/pdf/onc2014422a.pdf

could be a result of metastatic spread of deregulating miRNAs. Although conjecture, the spread of miR34 via exosomes would result in suppression of Sirt1 as well as many other critical genes.



The authors state as flows in their paper:

SIRT1 activity depends on the NAD+/NADH ratio modulated by glycolysis, while O-linked N-acetylglucosamine transferase uses GlcNAc produced by the hexosamine pathway. Pyruvate entering the tricarboxylic acid (TCA) cycle produces alpha-ketoglutarate, a critical cofactor for Jumonji domain-containing histone demethylase and TET. Acetyl-CoA is converted from the citrate generated by the TCA cycle and used as a donor by histone acetyltransferases.

Finally, the increase in ATP/ADP ratio from the TCA cycle also inactivates AMPK…. Under low-nutrient conditions, the NAD+/NADH ratio increases, activates SIRT1, which in turn de-acetylates and triggers ACECSs activity. Therefore, the pool of acetyl-CoA, which is governed by nutrient availability, controls the acetylation of metabolic enzymes as well as of histones at any given time.

As Melo et al state:

Exosomes are secreted by all cell types and contain proteins and nucleic acids. Here, we report that breast cancer associated exosomes contain microRNAs (miRNAs) associated with the RISC-Loading Complex (RLC) and display cell-independent capacity to process precursor microRNAs (pre-miRNAs) into mature miRNAs. Pre-miRNAs, along with Dicer, AGO2, and TRBP, are present in exosomes of cancer cells. CD43 mediates the accumulation of Dicer specifically in cancer exosomes.

Cancer exosomes mediate an efficient and rapid silencing of mRNAs to reprogram the target cell transcriptome. Exosomes derived from cells and sera of patients with breast cancer instigate nontumorigenic epithelial cells to form tumors in a Dicer-dependent manner. These findings offer opportunities for the development of exosomes based biomarkers and therapies.

It would be expected that this may be found elsewhere, especially in PCa, since both PCa and Breast Cancer have great similarity[14].

Moreover, Braicu et al have presented a more comprehensive understanding of exosomes. Their observations are as follows:

Exosomes are key elements that facilitate intercellular communication; depending on their vesicular content ('cargo'), they can modulate tumor cells by influencing major cellular pathways such as apoptosis, cell differentiation, angiogenesis and metastasis. This communication can involve the exchange of molecules such as small noncoding RNAs (e.g. miRNAs) between malignant, non-transformed and stromal cells (in all directions). Exosomal miRNAs represent ideal candidates for biomarkers, with multiple applications in the management of an array of pathologies such as cancer. Manipulating exosomal miRNAs suggests new alternatives for patient-tailored individualized therapies.

They continue:

MiRNAs are short single-stranded (19–25 nucleotides in length) nonprotein-coding RNA transcripts (ncRNA) that are initially produced in the nucleus and then transported into the cytoplasm, where they undergo a series of steps to acquire maturation. Mature miRNAs regulate gene expression by binding (through watsonian complementarity) to the sequence of a target mRNA. This interaction results in translational repression and/or mRNA cleavage, which consequently decreases the levels of the mRNA coding protein. MiRNAs have been found to be aberrantly expressed in many diseases. For example, in cancer, the tumor microenvironment contains deregulated miRNA levels, and a reason for their altered levels is because they are being actively secreted as membrane-bound vesicular content.

Finally they state:

Immediately after their synthesis, exosomes are released and can remain in the extracellular space near the cell they originated from. Alternatively, they can also travel through body fluids such as blood, urine, amniotic fluid, saliva, lung surfactant, malignant effusions or breast milk. The end result of this dynamic process is a variety of regulative molecules being transported to different tissues in different places, and influencing cellular processes. Exosomes have been shown to carry proteins, many of which have the potential to influence multiple regulatory mechanisms. For example, exosomes can transport annexins that have the ability of altering the dynamics of the cytoskeleton.

[14] See Telmarc White Paper 112 Prostate Cancer: miR-34, p53, MET and Methylation for detailed analysis.

Thus it is well understood that exosomes have not only the potential to allow one to see inside the cell, not only to transport to other cells but more importantly to act and a distributed means of control.

5 OBSERVATIONS

The issue of neuroendocrine cells in PCa has received a considerable amount of attention. De novo NE PCa is very aggressive and has a very high mortality rate in less than just one year. However NE PCa is fortunately rare. Yet NED in metastatic PCa leads to CRPC, namely androgen blocking no longer works. In this paper we have reviewed some of the key issues and have tried to do so by assembling the empirically provided data and then logically creating a rational system structure amenable for a therapeutic attack.

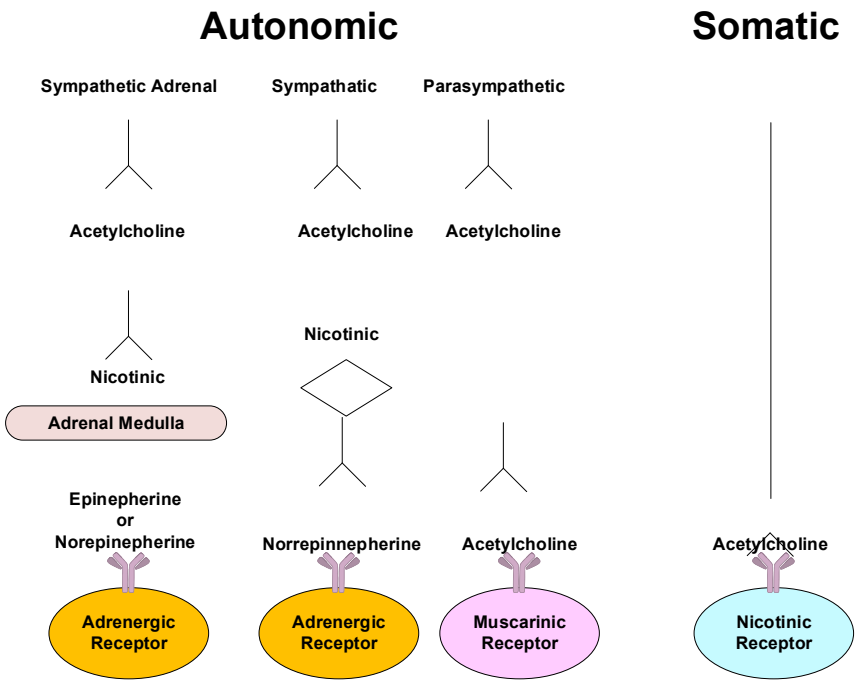
5.1 BETA BLOCKERS APPEARS TO HAVE SOME EFFICACY

Beta blockers have been used for decades. Typical ones are propranolol and timolol. As Lu et al have noted in a meta study regarding the use of the blockers:

In summary, though there are some limitations in this study, we observed reduced cancer-specific mortality among prostate cancer patients taking beta-blockers. However, we did not observe any effect of beta-blocker use on all-cause mortality in this meta-analysis. Taken together with studies in other cancer types and in preclinical models, our findings indicate a beneficial effect of beta-blockers on survival in patients with prostate cancer. Therefore, beta-blockers may be considered a promising therapeutic approach for adjuvant therapy in prostate cancer. Further clinical trials must be explored in larger patient cohorts.

The question is: is the receptor we have focused on herein the most effective one? Recall that the neurotransmitters we have discussed work as follows[15]:

[15] See Clark et al, Pharmacology, 5th Ed, Lippincott, 2012, p 43



Thus the flow of control can be readily intercepted via a beta blocker. There are several Beta receptors (labeled 1, 2, 3) but we should ask if the pathways are fully defined.

5.2 THERE IS A FUNDAMENTAL LOGICAL BASIS FOR THE EFFECT

As we noted above, accepting the targeting of the Beta adrenergic receptors, we are doing so because we are led logically to understand their role in controlling promotor proteins which in turn generate proteins that effect growth outside of the endocrine cell. That is we have demonstrated the pathway logic leading to the neuroendocrine paradigm initially introduced. As Braadland et al note:

The reports on effects of β-blockers on mortality in other cancer types brings forth an important question: are the in vivo effects of β-blockers mediated by common tissue specific/non-specific attributes, or are the effects indirect (i.e., systemic or neural effects facilitated by other local or distant tissue expressing ADRBs)? β- blockers probably have an effect on immune responses, hormone levels, angiogenesis, neurogenesis, and at the metastatic niche. In the prostate, stromal cells proximal to tumor tissue express ADRBs, and may exert the effect, which may also explain the discrepancy between cell line results and in vivo data.

It is also worth noting that the majority of β-blockers are targeting β1-adrenergic receptors or both β1- and β2-adrenergic receptors, whereas ADRB2 has been the receptor mediating the effects on cancer cells. Another plausible explanation lies in the antagonistic mechanism of action.

Propranolol, for example, a commonly used antagonist in vitro, has been shown to function as an inverse agonist, and can thus lower the β-adrenergic receptor's activity below its' basal level. In clinical practice, however, numerous β-blockers are used, and their mechanisms of action vary. Furthermore, the differences observed could be dose-dependent, as it is difficult to measure the dose in patient tissue, whereas this parameter can be controlled in cell lines and animal models.

We anticipate that ADRB antagonists will reduce the development of neuroendocrine prostate cancers, but this has not yet been addressed in any publications. More studies are needed to unravel whether β-blockers can play a role in future tailored prostate cancer therapy.

Thus as we asked at first, the logical basis, there seems to be a putative reason for the efficacy of a beta blocker.

5.3 OTHER DRIVERS MAY ALSO HAVE MERIT

Is this the best target or are there many others which may be used separately or in parallel? As Qi et al have previously noted:

Neuroendocrine (NE) phenotype, seen in >30% of prostate adenocarcinomas (PCa), and NE prostate tumors are implicated in aggressive prostate cancer. Formation of NE prostate tumors in the TRAMP mouse model was suppressed in mice lacking the ubiquitin ligase Siah2, which regulates HIF-1a availability. Cooperation between HIF-1a and FoxA2, a transcription factor expressed in NE tissue, promotes recruitment of p300 to transactivate select HIF-regulated genes, Hes6, Sox9, and Jmjd1a. These HIF-regulated genes are highly expressed in metastatic PCa and required for hypoxia-mediated NE phenotype, metastasis in PCa, and the formation of NE tumors.

Tissue-specific expression of FoxA2 combined with Siah2-dependent HIF-1a availability enables a transcriptional program required for NE prostate tumor development and NE phenotype in PCa. Our results provide insight into regulation and function of the FoxA2/HIF-1a complex in determining NE prostate tumor formation and NE phenotype, an important component of metastatic prostate adenocarcinomas. These results also point to a role for Siah2 in determining tumor differentiation.

Siah2 loss has little effect on development and growth of the prostate luminal epithelium but decreases initiation of NE carcinomas and, consequently, the metastatic burden in the TRAMP model. We show that partial deletion of Siah1a on a Siah2 null background fully ablated NE tumor formation, suggesting that both Siah2 and Siah1 are required to enable the development of prostate NE tumors. As HIF-1a is stabilized under hypoxia and FoxA2 is expressed in NE tissues, our findings suggest conditional and spatial cooperation between these two factors under specific tissue and oxygen requirements.

Siah2-dependent regulation of HIF coupled with NE-specific expression of FoxA2 provides a framework for a specific tumor differentiation program associated with a highly metastatic phenotype.

Thus there is a certainty regarding the NE Type being an aggressive indicator but the question remains is the ADRB2 receptor the primary driver and is VEGF the primary subsequent driver. The above brief discussion opens the door for a substantial expansion of activity. Notwithstanding this, however, this observation does present an interesting path.

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