SIRT1, EXOSOMES, AND PROSTATE CANCER

Terrence P McGarty
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We use a recent paper regarding the suppression of the gene in Sirt1 in mice to examine a set of issues. First, we examine the issue of putative exaggeration of the extensibility of results from mice to humans. Second, we examine the relationship of miRNAs to a control mechanism in the process. Third, we consider the impact of exosomes as a putative mechanism for metastasis.

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1 INTRODUCTION

There seems to be a never ending set of claims regarding genes and gene expression as related to various cancers. In this paper we examine a recent paper relating loss of expression of SIRT1 and the resulting expansion of high grade PIN, HGPIN. This is examined in mouse models. Now this examination is of interest for a multiplicity of reasons.

First, HGPIN is often problematic. We have examined HGPIN some five years ago and demonstrated that in many cases it leads to PCa but in some anecdotally observed cases the HGPIN may actually disappear. The disappearance of HGPIN in a 24 core biopsy after a similar one none months prior is not readily explainable under the current murine models. Specifically most murine models as well as clinical studies tend to indicate that HGPIN is irreversible. However there are cases demonstrating the reverse.

Second, Sirt1 is not a generally accepted gene related to malignant or metastatic behavior. It does have multiple control points but in general it is more related to neurological controls rather than controls over adenocarcinomas. In addition it connects to certain histone acetylation mechanism and thus presents a possible epigenetic linkage via histone control of expression.

In this analysis we use the recent paper by Di Sante et al as a touchstone to examine Sirt1 and its behavior and again to use the Press around the paper as a way to examine how the researchers either directly or indirectly present their work and its implications. We all know that a great deal has been derived from murine models. However, they are not human. Humans live longer and face many more assaults on their cells than mice do. PCa for the most part is a cancer of old age. It is a cancer which putatively is driven by a multiplicity of cell assaults, resulting in genetic changes and changes in gene expression. Also we may be facing strong epigenetic alterations via methylations and acetylations. Which are these is the driving factor we really do not know.

Thus this presentation serves three purposes:

First, it is just another gene thrown on the table to be examined. The question that should and must be asked is; what is causal? Ultimately we must also understand the temporal sequences that give rise to these processes and the causal elements of those temporal sequences. How can one assemble all of these genes? The question always is; is this the right gene?

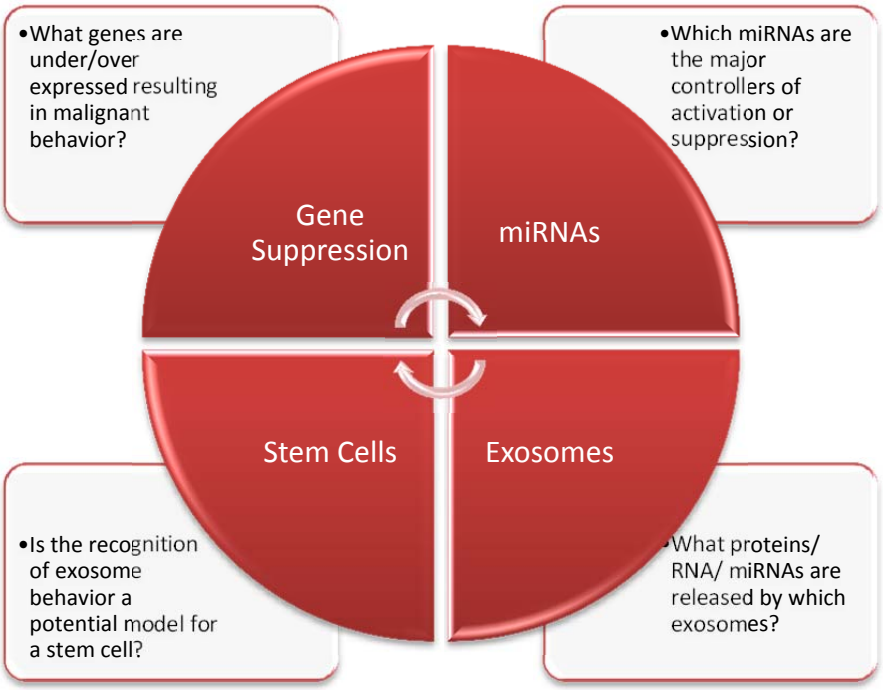
Second, it is another demonstration of how one should cautiously approach reporting results, both to the Press as well as in the literature itself. Again, as it seems with the publishing of any and all such “discoveries” we find the Press become an outlet for what may truly be exaggerated statements regarding the immediacy of use for such an observation[1]. Thus there is the key

[1] Some excellent observations have been made on this point by Lewis who states: *Those of us who read news releases daily – as science journalists do – didn’t need the recent study published in the British Medical Journal to confirm that these missives often hype research to ridiculous degrees. But it isn’t just exaggeration, headlines that suggest a study in mice is actually in humans, and use of vague terms such as “genetic engineering” that bother me about news releases….Researchers with recognizable names, who publish in journals with high impact factors, and come from prestigious institutions with media machines and relentless PR firms, will be in the news releases that*

question as to what the responsibility is to the researchers regarding balanced presentation of the results.

Third, there has been recent work examining the capability of exosomes communicating with miRNAs to other cells and creating other malignant cells. Namely it is possible that one cell has changed but that in that change it produces a set of miRNAs which cause gene suppression and this allows malignant growth. Then through the use of exosomes it can communicate to other cells the same miRNA capabilities even though the remote cells are not yet changed. Thus there may be an effective multiplier effect, namely truly genetically altered miRNA producing cells themselves multiplying and then activating unchanged cells to act in a similar manner. This may be an explanation for what we would see in a stem cell cancer environment which we have examined before.

We thus consider the issues as graphically demonstrated below:



It will be this interplay which may add insight to the development of models for cancer propagation. Most models for the systematic models of cancer propagation deal with that of cells and their mutations. This analysis affords a look at a more complex environment; an environment where one adds the communications of malignant potential from a few cells to many, via a mechanism which can be measured, monitored and perhaps managed.

dictate what the public hears and reads. http://blogs.plos.org/dnascience/2014/12/25/dislike-best-lists-eman-update-liberia/

2 RECENT CLAIMS

We now examine the results on one of the most recent genes claimed to be a PCa driver. The recent paper Di Sante et al states[2]:

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[3]:

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

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

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

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 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[4].

"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 works against inflammatory tendencies. The last statement however is critical. It is clear that the determination for human cells is still problematic. This

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

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.

3 HGPIN

HGPIN is an excessive growth of cells in the glandular regions composed of an amalgam of basal and luminal cells. It is an excess of growth within the confines of existing cells. It often appears as a hyperplasia, but since the cells do not appear exactly as they were in a normal region, they are considered as neoplasia. The luminal space surrounded by the luminal cells tends to get crowded by these new cells which tend to be confined to existing glandular locations. The growth we would see in a Gleason 1 or 2 is generally not seen, namely the cells do not start to proliferate outside of existing glands, or creating new glandular areas.

HGPIN is often observed in cases where there is a sudden increase in PSA, generally above 6.0. However HGPIN can also be present in low PSA cases, below 1.9 and it is often in these cases where it may regress.

In a paper by Lefkowitz et al the authors note:

In a high proportion of men with high grade prostatic intraepithelial neoplasia prostate cancer will develop in a 3-year interval. Our findings support the concept that high grade prostatic intraepithelial neoplasia is a precursor to prostate cancer and that repeat biopsy at a delayed interval is recommended regardless of changes in PSA….

The question is; what caused the biopsy in the first place? Generally it was due to a material increase in PSA. In the study the average PSA was about 6.5, which is low and the average age was 65. We also know that the prevalence of PCa in say the seventh decade of life if one were to biopsy the entire prostate could be well above 50%.

It is difficult to determine precisely the natural history of a single high grade prostatic intraepithelial neoplasia lesion since it is not feasible to follow-up with precision the exact areas of abnormality on repeat biopsy. Since the natural history of prostatic intraepithelial neoplasia has not been elucidated, current recommendations for serial repeat biopsy have not been validated by evidence based medicine, and several investigators have reported results of follow-up biopsies.

To our knowledge there have been no reports of follow-up interval biopsy in a cohort of men with high grade prostatic intraepithelial neoplasia independent of changes in PSA or digital rectal examination findings. We provide insight into the natural history of high grade prostatic intraepithelial neoplasia by performing an empiric follow-up interval biopsy 3 years after the initial diagnosis regardless of change in PSA or digital rectal examination.

The paucity of studies regarding HGPIN follow up appears to be the same as it was a decade ago when this paper was written.

A high proportion of men with high grade prostatic intraepithelial neoplasia will have prostate cancer, independent of changes in PSA, 3 years following initial diagnosis. Our study reaffirms the approach that men with high grade prostatic intraepithelial neoplasia and no evidence of

coexisting cancer should be followed and re-biopsied to exclude prostate cancer. Our longitudinal data in men with high grade prostatic intraepithelial neoplasia strongly support the concept that it is a risk factor for the development of prostate cancer, thereby further validating the lesion as a target for chemopreventive and therapeutic agents. We recommend a 3-year follow-up interval biopsy in men with high grade prostatic intraepithelial neoplasia, regardless of change in serum PSA.

The conclusion is critical. Namely they recommend that a 3 year biopsy be done after a positive HGPIN determination. However what if after a HGPIN determination a 9 month biopsy comes back normal, no HGPIN at all, then should one go back again, independent of PSA? That is problematic. We have argued that PSA has a normal growth pattern and like some many medical observations if things continue on the same course, slow progression, then perhaps that is a better alternative. However, it may also be prudent to perform these biopsies, albeit being quite expensive and having some modicum of morbidity. The question seems to be still unanswered.

4 SIRT1

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[5].

[5] 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*

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[6]:

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[7]:

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.

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*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.

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

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

And for PARK2 we have[8]:

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

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

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.

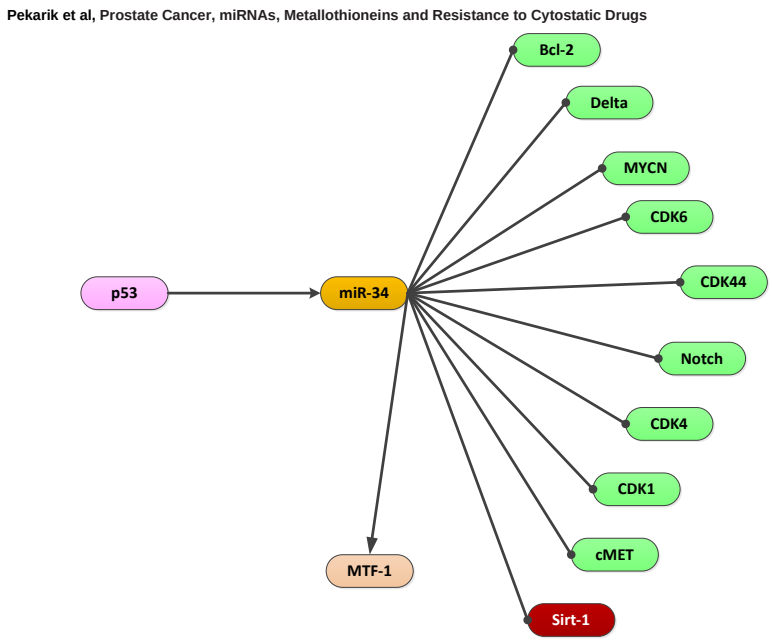
4.3 MIRNA AND SIRT1

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

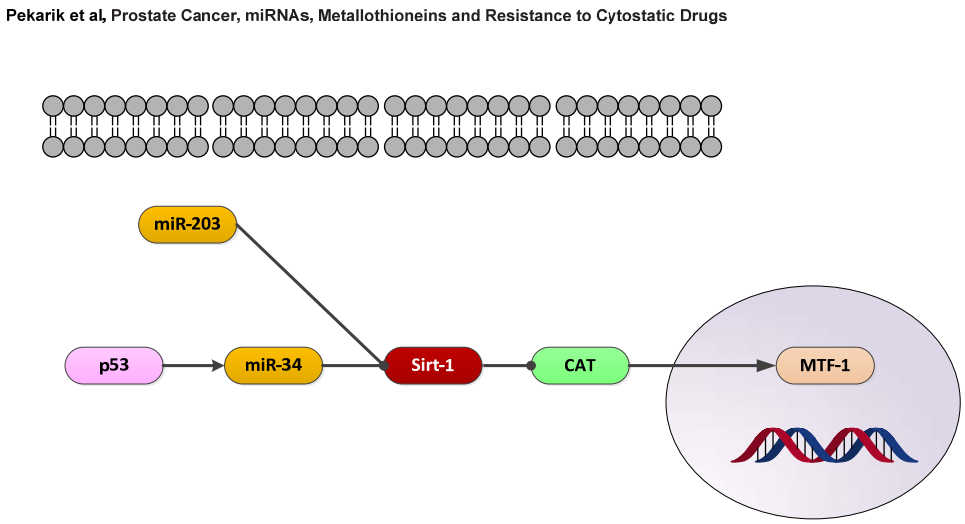
Importance of miRNAs is underscored by the fact that nearly half of the genes coding miRNAs are located at fragile sites or at regions with lost homozygozity. For example, a loss of p-arm of chromosome 1 is a common finding in sporadic colon carcinomas. Among many genes associated with DNA repair, checkpoint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

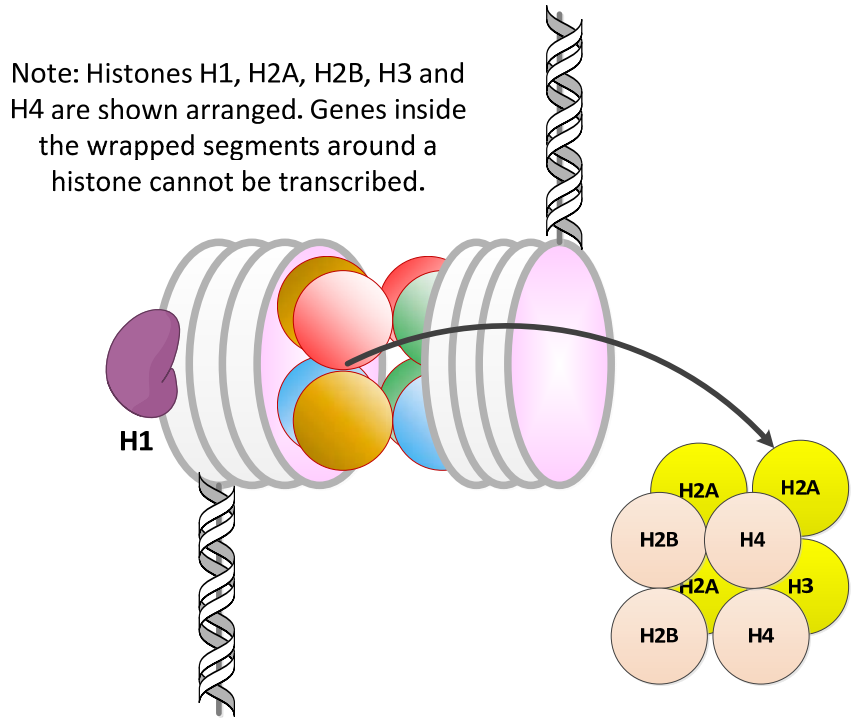
Thus, these epigenetic elements, the miRNAs, can spread throughout the body effecting changes in cells that are beyond fundamental intracellular effects. Thus the loss of Sirt1 expression may be the result of this exosomal effects.

4.4 METHYLATION FACTORS

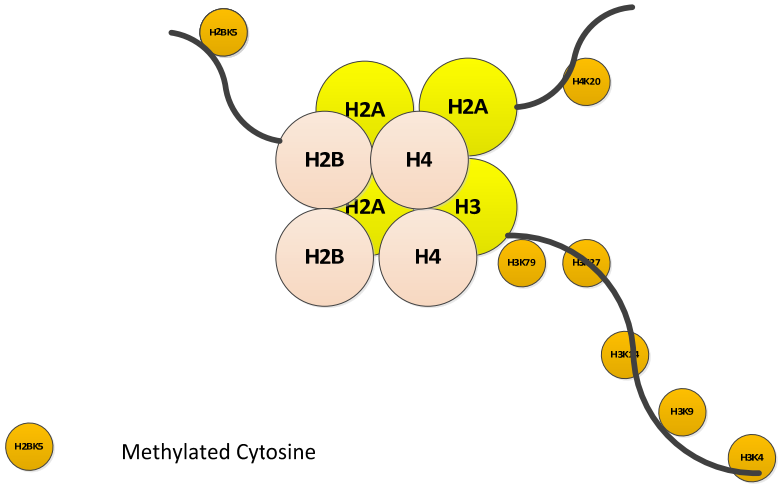
Methylation consists of the attachment of methyl groups on various elements of the genome. For our purposes we consider methylating the DNA on the CpG islands and methylation of the histones around which the DNA is wrapped. These effects have shown significant impact as well on PCa as well as many other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

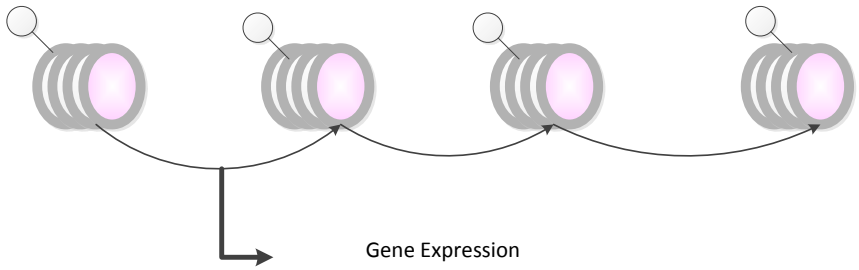


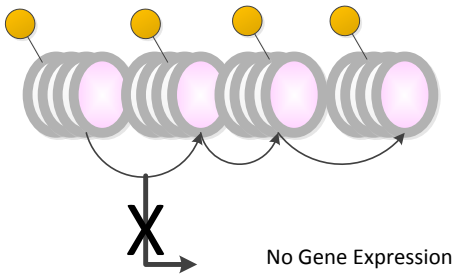
The specific arrangement of the histones is as shown below. It is not arbitrary but is a result of the specific surface charge arrangements on the histone proteins. We also depict the presence of methylated cytosines on this graphic, thus depicting the two major influences of methylation as well as acetylation,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





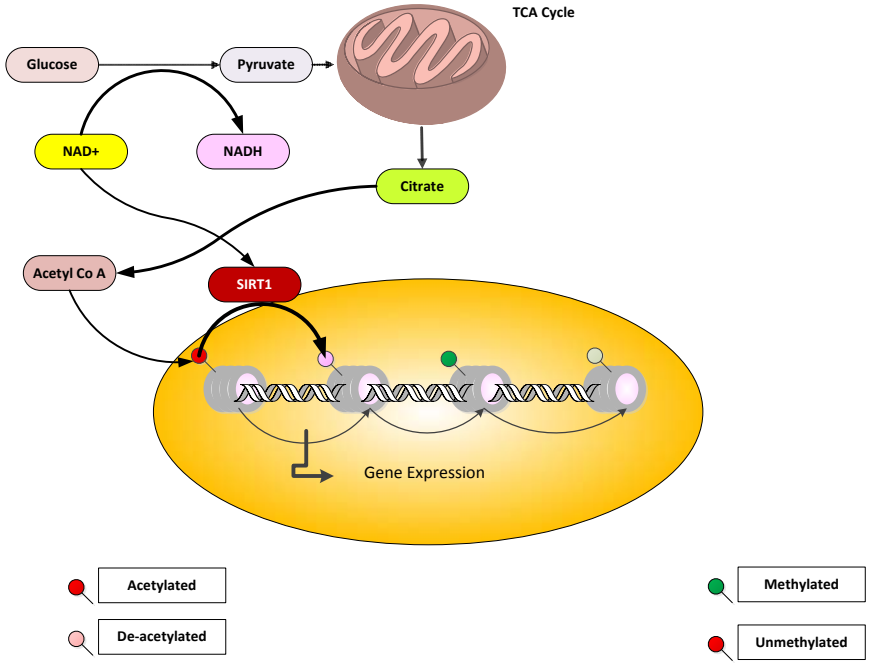
This Histone Code is shown below in the following Table.

	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[9]. 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

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

discussions of miRNA exosomes we also understand that perhaps this down regulation of Sirt1 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[10].

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.

[10] 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 FOXO3

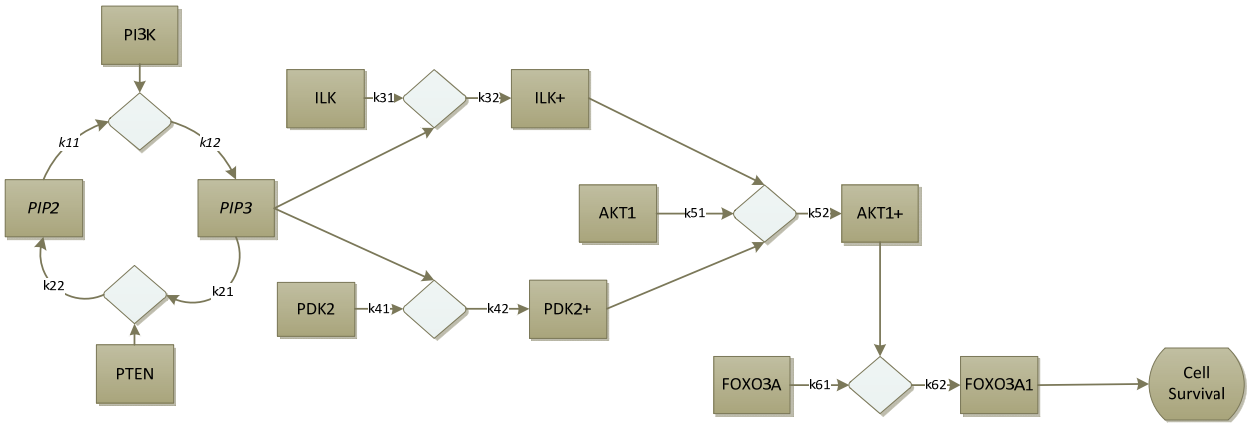
The FOXO gene, specifically FOXO3a, forkedhead box zero gene, is located at 6q21 in humans and is a key nuclear transcription regulator. It has the ability to mediate cell cycle arrest, DNA repair, and apoptosis and as such acts in many ways like a tumor suppressor gene. Loss of the FOXO gene activity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

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

5.1 FOXO PATHWAY

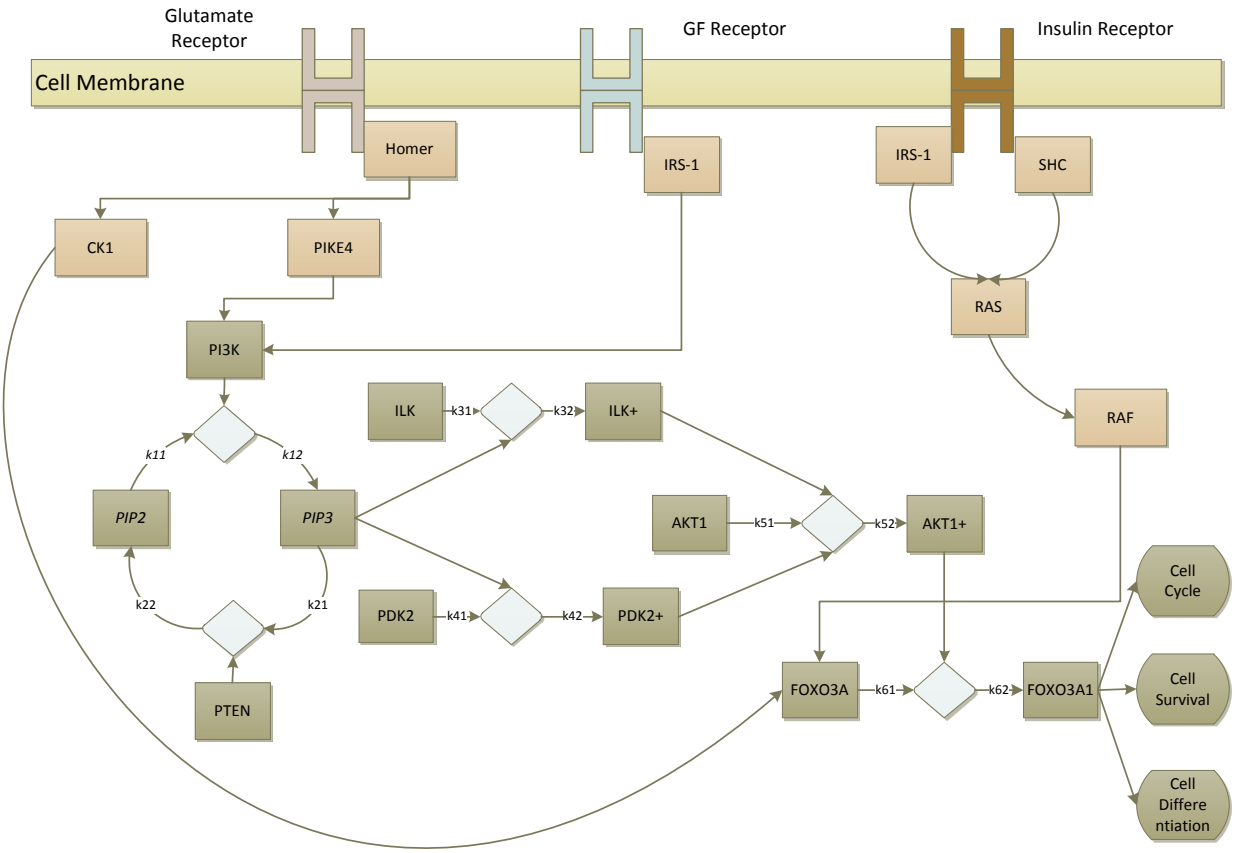
FOXO is a key element in the PI3K pathway and has its control facilitated by such elements as PTEN, growth factors, insulin and glutamate. As Essaghir et al state, in the absence of growth factors, FOXO remains in the nucleus and FOXO up-regulates genes which inhibit cell cycle such as p27 KIP1 and p21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

One view of the FOXO pathway is shown as follows[11]:



[11] Also see http://pid.nci.nih.gov/search/pathway_landing.shtml?what=graphic&jpg=on&pathway_id=200108&source=NATURE&output-format=graphic&ppage=1&genes_a=23411

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



5.2 FOXO FUNCTIONS

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

As Lam et al state:

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

Constitutive activation of the PI3K pathway, a hallmark of many cancers, is commonly a consequence of enhanced expression of genes that encode either class I PI3K subunits or PKB (protein kinase B) or is a result of genetic mutations that inhibit negative regulators of the pathway. For example, somatic deletions or mutations of PTEN (phosphatase and tensin

homologue deleted on chromosome 10), an antagonist of the PI3K pathway, have been identified in a large proportion (12–60%) of human tumours of different tissue origins.

They continue:

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

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

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

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

6 OBSERVATIONS AND RECOMMENDATIONS

In this section we first make several observations and then we conclude with some possible recommendations.

6.1 OBSERVATIONS

1. What are the roles of miRNAs?

We have examined miRNAs extensively before. Yet they seem to have an enduring role in reducing the activity of certain genes. The questions here are; what activates the miRNAs, what are the targets for suppression, and why?

2. What causes the suppression of Sirt1?

We have seen an argument for miRNA suppression of Sirt1. However there may be multiple other arguments. Is it a consequence of an already malignant process or is it a path towards such a development? Frankly we do not seem to have clear answers. This becomes a significant factor when we try to model PCa and its metastatic processes.

3. Is HGPIN the first step in loss of Sirt1 expression?

As Goldstein et al had noted regarding the cell of origin of PCa and especially HGPIN, there is a well-defined genomic alterations leading to this result but yet the details seem to be unknown.

4. Why does HGPIN at times seem reversible? Is it a temporal anomaly?

The reversion seen at times in HGPIN is a significant factor that leads one to ask; why and what is the process. Many conjecture can be made ranging from elimination of a stem cell to reduction of inflammatory states. The problem is that adequate clinical data seems to be missing from analyses.

5. What Genes are key and what Genes are there as a Consequence? And why?

We have examined many dozens of purported genes related to PCa. They continue to arise each time some researchers examine new cells. For example in a study done contemporaneously to the one in this discussion we have one by Thomsen et al regarding JUNB, a transcription factor[12]. They conclude[13]:

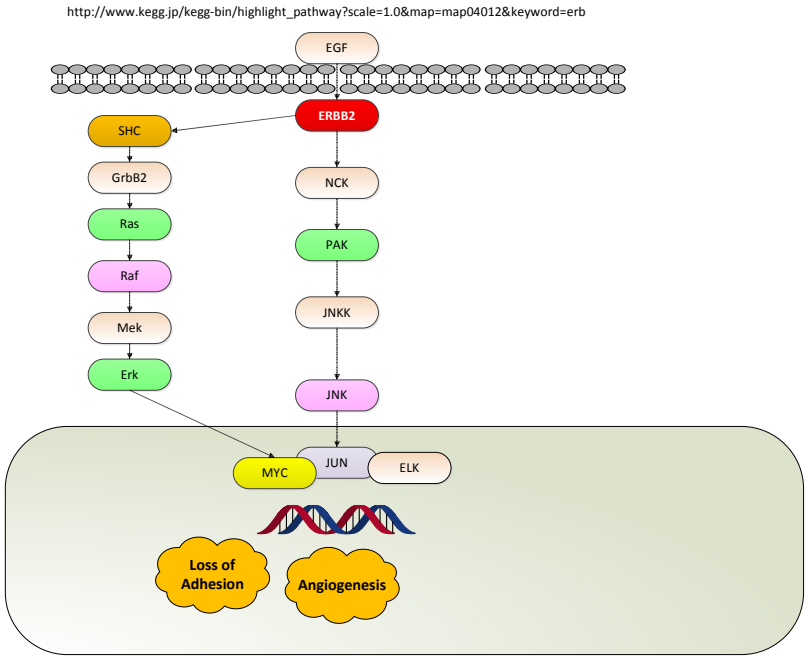
[12] http://www.nature.com/cdd/journal/vaop/ncurrent/full/cdd2014213a.html

[13] JUNB, also known as AP-1, is a proto oncogene seen in many cancers. http://www.ncbi.nlm.nih.gov/gene/3726 Also see Ott et al who state: *Activator protein-1 (AP-1) is a dimeric transcription factor composed of members of the Jun family (c-Jun, JunB and JunD), which form homodimers or heterodimers with members of the Fos family (c-Fos, Fra-1, Fra-2 and FosB) and activating transcription factor (ATF) proteins. AP-1 modulates transcription by binding to TPA-response element (TRE) or cAMP-response element (CRE) consensus elements and is involved in*

Prostate cancer is a frequent cause of male death in the Western world. Relatively few genetic alterations have been identified, likely owing to disease heterogeneity. Here, we show that the transcription factor JUNB/AP-1 limits prostate cancer progression. JUNB expression is increased in low-grade prostate cancer compared with normal human prostate, but downregulated in high-grade samples and further decreased in all metastatic samples. To model the hypothesis that this downregulation is functionally significant, we genetically inactivated Junb in the prostate epithelium of mice. When combined with Pten (phosphatase and tensin homologue) loss, double-mutant mice were prone to invasive cancer development.

Importantly, invasive tumours also developed when Junb and Pten were inactivated in a small cell population of the adult anterior prostate by topical Cre recombinase delivery. The resulting tumours displayed strong histological similarity with human prostate cancer. Loss of JunB expression led to increased proliferation and decreased senescence, likely owing to decreased p16Ink4a and p21CIP1 in epithelial cells. Furthermore, the tumour stroma was altered with increased osteopontin and S100 calcium-binding protein A8/9 expression, which correlated with poor prognoses in patients. These data demonstrate that JUNB/AP-1 cooperates with PTEN signalling as barriers to invasive prostate cancer, whose concomitant genetic or epigenetic suppression induce malignant progression.

We demonstrate the JUN action below. We have examined this behavior in previous studies as well[14].



proliferation, differentiation and apoptosis. AP-1 members may elicit divergent and even antagonistic effects via a cell-type-specific regulation of target genes…

[14] See the White Papers we have included at the end of this section as examples of details on other genes and especially transcription factors.

But is this gene, a transcription factor causal or consequential? The same can be said about the gene Sirt1 as discussed herein. The list of putative PCa related genes seems to grow by the day.

6. Why do researchers all too often make claims which are at best a stretch?

To best understand this point, which we have made several times, let us examine another Press release. As noted in Eureka[15]:

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 (Dec 18th) 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.

Let us examine the clarity of this statement in light of what we have presented.
They are:

i) The individual genes driving prostate cancer. Do we understand them? We do some, but we also have a plethora of dozens of others whose increase or decrease is somewhat correlated with PCa.

ii) Developing prevention. One develops prevention if and only if one understand the cause or causes and one can then mitigate the processes which lead to those aberrant actions. Frankly at best we can say that inflammation may be a problem but then what part of the complex inflammatory process do we address?

iii) Yes we know Sirt1 and its system genes (proteins) act in certain ways in a wide variety of ailments. But recognizing is presence to cause to prevention is still a long and uncertain process.

Thus the opening statement is rant with exaggerations at best.

"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 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."

It was not clear that they had shown a therapeutic that allowed for the re-expression of Sirt1 in mice not less than in humans. The process of Sirt1 suppression was not identified and thus

[15] http://www.eurekalert.org/pub_releases/2014-12/tju-hdp121114.php

suppressing the suppression is uncertain. At least that is what one understands reading the available literature.

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).

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.

SOD2 is supported by Sirt1 and thus we see the HGPIN build up. One suggestion is to examine those patients who have HGPIN regression, to see if it has been sustained and moreover what expressions were reactivated or suppressed.

More importantly there is now clear data regarding the hype associated with Press Releases and Academic findings. In the BMJ they state[16]:

40% (95% confidence interval 33% to 46%) of the press releases contained exaggerated advice, 33% (26% to 40%) contained exaggerated causal claims, and 36% (28% to 46%) contained exaggerated inference to humans from animal research. When press releases contained such exaggeration, 58% (95% confidence interval 48% to 68%), 81% (70% to 93%), and 86% (77% to 95%) of news stories, respectively, contained similar exaggeration, compared with exaggeration rates of 17% (10% to 24%), 18% (9% to 27%), and 10% (0% to 19%) in news when the press releases were not exaggerated. Odds ratios for each category of analysis were 6.5 (95% confidence interval 3.5 to 12), 20 (7.6 to 51), and 56 (15 to 211). At the same time, there was little evidence that exaggeration in press releases increased the uptake of news … Exaggeration in news is strongly associated with exaggeration in press releases. Improving the accuracy of academic press releases could represent a key opportunity for reducing misleading health related news.

Thus there is a propagation of many of these exaggerated results to a much wider audience than one may suspect. That propagation has substantial negative effects. The authors and their institutions have duty to present the facts, cautiously and correctly. All too often the authors themselves may participate in the hype and that behavior is quite questionable.

7. Diet and PCa: The focus on Sirt1 has also allowed a focus on glucose metabolism. We know that diets have an impact on PCa and we also know that excess glucose causes mini-inflammatory states. Thus we can see clearly the nexus between this excess and suppression of Sirt1 via acetylation and the Histone code. Perhaps we can build upon this linkage in a more complete and systematic manner.

[16] http://www.bmj.com/content/349/bmj.g7015

8. Micro RNAs, Exosomes and PCa: The use of exosomes to move miRNAs about the body adds another dimension to understanding the rapidity of metastatic behavior as well as a means to develop targeted therapeutics. miR34 is a known powerful blocker or various genes. That is, we can assess the amount or even the presence of this in exosomes perhaps we can then develop powerful prognostic tests as well as targeted therapeutics.

9. PCa Stem Cells: Perhaps the mechanism of exosomes releasing miRNAs into cells then suppressing genes like Sirt1 could be a viable mechanism for metastatic spread. If so then they could also become therapeutic targets. An ancillary question is; does a quasi-malignant cell responding to an exosome revert to normal when the exosome is removed?

6.2 RECOMMENDATIONS

Based upon the above we would make the following recommendations.

1. HGPIN Regression study: We would recommend a detailed HGPIN Regression Study. Simply records of multiple biopsies of HGPIN patients should be considered and examination of those with regression within the next biopsy period should be examined. Then retrospective examination of the patient and their behavior should also be examined. This is a simple first step. It can be accomplished by simple data gathering and no Trial structure is required.

2. HGPIN Regressed Genes Presence

One of the challenges in prostate biopsies is the ability to return to the same location from which the original cells were obtained. Thus even with a high density biopsy of say 24 cores we cannot be certain that we have resampled the original dysplasia. his is even the case with ultrasound guidance, yet it would seem possible to have recorded the location and to have a sophisticated ultrasound system replace the new sample to close proximity to the old. That way there could be a resampling of the same cells.

7 REFERENCES

1. Anastasiadou, E., F. Slack, Malicious exosomes, Science, 19 DECEMBER 2014 SCIENCE sciencemag.org VOL 346 ISSUE 6216, pp 1459-1460.

2. Berghe W., Epigenetic impact of dietary polyphenols in cancer chemoprevention: Lifelong remodeling of our epigenomes, Pharmacological Research 65 (2012) 565– 576

3. Braicu, C., et al, Exosomes as divine messengers: are they the Hermes of modern molecular oncology?, Cell Death and Differentiation (2015) 22, 34–45

4. Brooks, C., W. Gu, How does SIRT1 affect metabolism, senescence and cancer?, Nature Reviews Cancer 9, 123-128 (February 2009)

5. da Silva, H., et al, Dissecting Major Signaling Pathways throughout the Development of Prostate Cancer, Hindawi Publishing Corporation Prostate Cancer Volume 2013, Article ID 920612, 23 pages

6. Di Sante, G., et al, Loss of *Sirt1* Promotes Prostatic Intraepithelial Neoplasia, Reduces Mitophagy, and Delays Park2 Translocation to Mitochondria

7. Dominy, J., et al, The Deacetylase Sirt6 Activates the Acetyltransferase GCN5 and Suppresses Hepatic Gluconeogenesis, Molecular Cell 48, 900–913, December 28, 2012

8. Goldstein, A., et al, Identification of a Cell of Origin for Human Prostate Cancer, Science, Vol 329, July 2010, p 568.

9. Guatente, L., Sirtuins, Aging, and Medicine, NEJM, Vol 364 June 2011

10. Hines, Z., et al, The cAMP/PKA Pathway Rapidly Activates SIRT1 to Promote Fatty Acid Oxidation Independently of Changes in NAD+, Molecular Cell 44, 851–863, December 23, 2011

11. Labbe, D., et al, Role of Diet in Prostate Cancer, the Epigenetic Link, Oncogene, 2014.

12. Lam, E., et al, FOXO Transcription Factors, Biochem Soc Trans 2006, pp 722-726.

13. Lefkowitz, G., et al, Follow Up Interval Prostate Biopsy of High Grade Prostatic Intraepithelial Neoplasia is Associated with High Likelihood of Prostate Cancer Independent of Change in Prostate Specific Antigen Levels, Journal of Urology, Vol 168, Oct 2002.

14. Melo et al, Cancer Exosomes Perform Cell-Independent MicroRNA Biogenesis and Promote Tumorigenesis, Cancer Cell, Volume 26, Issue 5, p707–721, 10 November 2014.

15. McGarty, T., Prostate Cancer Genomics, DRAFT 2013. www.telmarc.com

16. Ott, R., et al, JunB is a gatekeeper for B-lymphoid leukemia, Oncogene (2007) 26, 4863–4871

17. Pastell, R., M. Nevalainen, Prostate Cancer, Humana (Totowa) 2008.

18. Pekarik, V., et al, Prostate Cancer, miRNAs, Metallothioneins and Resistance to Cytostatic Drugs, Current Medicinal Chemistry, 2013, 20, 534-544

19. Powell, M., et al, Disruption of a Sirt1 -Dependent Autophagy Checkpoint in the Prostate Results in Prostatic Intraepithelial Neoplasia Lesion Formation, Cancer Res Published OnlineFirst December 28, 2010.

20. Pulla, V., et al, IRT1 Pathway in T2DM, https://www.researchgate.net/publication/259223485_SIRT1_Pathway_in_T2DM

21. Roy, S., et al, Inhibition of PI3K/AKT and MAPK/ERK pathways causes Activation of FOXO Transcription Factor, Jrl Mol Sig 2010, pp 1-13.

22. Shackelford, D., R. Shaw, The LKB1–AMPK pathway: metabolism and growth control in tumour suppression, Nature Reviews, Cancer Volume 9, August 2009, 563

23. Slaby, O., et al, MicroRNAs in colorectal cancer: translation of molecular biology into clinical application. Mol Cancer. 2009 Nov 14;8:102. doi: 10.1186/1476-4598-8-102.

24. Thomsen, M., et al, Loss of JUNB/AP-1 promotes invasive prostate cancer, Cell Death & Differentiation , (19 December 2014) doi:10.1038/cdd.2014.213.

25. van der Heide, L., et al, The Ins and Outs of FOXO Shuttling, Biochem Jrl 2004, pp 297-309.

8 RELATED WHITE PAPERS

The following are related White Papers we have written in the recent past which may facilitate the material contained herein. http://www.telmarc.com/White%20Papers/default.html these papers are working papers and reflect observations at the time they were prepared. The intent of the papers below is to make observations of recent research and to attempt to place that in some context in terms of the ability to construct a model for PCa and other cancers.

No. 120 CNVs and Prostate Cancer

No. 119 SNPs and Prostate Cancer

No. 118 Vitamin D and Prostate Cancer

No. 117 SPDEF, ETS Transcription Factors and PCa

No. 116 Methylation, Prostate Cancer, Prognostics

No. 112 Prostate Cancer: miR-34, p53, MET and Methylation

No. 111 CRISPR and Cancer

No. 110 ERG and Prostate Cancer

No. 108 Cancer Cell Dynamics

No. 107 Prostate Cancer Genetic Metrics

No. 106 Divergent Transcription

No. 104 Prostate Cancer and Blood Borne Markers

No. 103 Prostate Cancer Indolence

No. 101 Exosomes and Cancer

No. 100 lncRNA and Prostate Cancer

No. 99 SNPs and Cancer Prognostics

No. 98 CCP and Prostate Cancer

No. 95 MER Tyrosine Kinase Receptors and Inhibition

No. 93 Cancer Cell Dynamics Methylation and Cancer

No. 91 Methylation and Cancer

No. 88 Extracellular Matrix vs. Intracellular Pathways

No. 87 Prostate Cancer Prognostic Markers

No. 86 Cancer Models for Understanding, Prediction, and Control

No. 85 Prostate Cancer Stem Cells

No. 84 Epistemology of Cancer Genomics

No. 83 Prostatic Intraepithelial Neoplasia

No 82 Prostate Cancer: Metastatic Pathway Identification

No 80 PSA Evaluation Methodologies

No 79 The PSA Controversy