NKX3.1 AND PCA

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White Paper No 138
July, 2016

There has been a considerable amount of work examining the impact of the down expressing of NKX3.1, a homeobox gene. This paper reviews some of the recent work in this area.

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1 INTRODUCTION

There is a class of genes, the homeobox genes, which are transcription factors and also are involved in the developmental growth of organisms. Depending on which one is expressed a different part of an organism during developmental stage is followed.

Now in an overall context we know that DNA has promoters and repressors. These are gene products that activate and facilitate or deactivate the expression of certain genes. Activation results in the expressing of the related RNA which may eventually produce a protein which in turn may result in some action of the cell or its environment.

Let us begin with some definitions:

1. Transcription is the process whereby RNA polymerase uses the DNA template to generate the resultant RNA.

2. Transcription Factor is a protein, gene product that can directly or indirectly modify or regulate the RNA polymerase function in its process of generating RNA from the DNA.

3. Homeobox is a 180 base pair part of the DNA within a homeotic gene, part of the gene, which yields a 60 nucleic acid protein structure in a helix-turn-helix manner.

4. Homeodomain is a carboxy terminal domain of about 60 amino acid residues found in a homeobox protein that folds in a helix-turn-helix manner and binds to the DNA, and is called a helix-turn-helix motif.

5. Motif is a specific physical structural fold of the protein generated.

Now in the recent paper by Dutta et al Science makes the following summary:

The prostate and seminal vesicle have closely related developmental histories and both are regulated by the same androgenic hormones. A better understanding of the molecular mechanisms controlling the development of the two tissues could help solve why cancer arises frequently in the prostate but only rarely in seminal vesicles.

Working with cell and mouse models, Dutta et al. show that forced expression of a single gene, the homeobox gene NKX3.1, causes seminal vesicle epithelium to differentiate into prostate. NKX3.1 regulates the expression of a gene program associated with prostate differentiation by interacting with the G9a histone methyltransferase. Disruption of this regulatory network probably contributes to prostate cancer development.

The argument made in the paper is that a homeobox gene is a developmental gene. It acts during the developmental process of an organism making certain that cells develop in order and consistently. Then if one notes the closeness of seminal vesicle cells and the prostate cells one asks why the incidence of cancer is so dramatically different. Thus the examination of the presence of NKX3.1 is warranted. An observation is that the seminal vesicle cells can turn into

prostate cells with the change in NKX3.1. Thus the hypothesis is presented that this homeobox gene should be focused upon.

Now the authors state:

The NKX3.1 homeobox gene plays essential roles in prostate differentiation and prostate cancer. We show that loss of function of Nkx3.1 in mouse prostate results in down-regulation of genes that are essential for prostate differentiation, as well as up-regulation of genes that are not normally expressed in prostate.

Conversely, gain of function of Nkx3.1 in an otherwise fully differentiated nonprostatic mouse epithelium (seminal vesicle) is sufficient for respecification to prostate in renal grafts in vivo. In human prostate cells, these activities require the interaction of NKX3.1 with the G9a histone methyltransferase via the homeodomain and are mediated by activation of target genes such as UTY (KDM6c), the male-specific paralog of UTX (KDM6a). We propose that an NKX3.1-G9a-UTY transcriptional regulatory network is essential for prostate differentiation, and we speculate that disruption of such a network predisposes to prostate cancer.

The corollary to the analysis is that loss of function, namely loss of NKX3.1, which appears not only to be developmental but regulatory in nature, is essential for the establishment of a benign state. Loss of function is contributory to PCa.

Thus we examine the homeobox paradigm and in turn the specifics of NKX3.1.

2 MOTIFS

We first briefly consider the concept of motifs. Simply a motif is a specific folding or assemblage of a protein. This particular assemblage then provides across this protein or protein segment a capability to bind to DNA. The binding in turn allows the complex of which a motif is part to then act as a transcription factor which can activate or suppress a gene's expression.

The construct of motifs in genes is a portion of the gene which creates a protein segment that results in a specific and characteristic folded mechanism that allows for attachment to specific areas of DNA and in turns allows the protein so expressed to act as a transcription factor activating transcription of the targeted gene. As noted above there are a large set of such motifs. We consider herein a single class.

The following definitions are useful in understanding the resultant operations of this specific motif:

Homeobox: A region in a gene containing homeodomain motifs that code for transcription factors and are involved in patterning body axes.

Homeobox gene: This refers to any member of a group of genes containing a homeodomain. DNA binding motif these genes are very common in transcription factor genes and are often highly conserved

Homeotic gene a gene, that when mutated, causes body parts to be replaced with organs or structures from other parts of the body, i.e. Antennapedia mutations can result in legs growing from the forehead, where antennae should be.

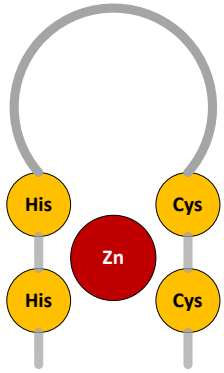
The homeobox gene NKX3.I (chromosome 8p21.2) is an androgen-regulated and mainly prostate-specific gene, which was observed primarily in secretory prostatic benign and neoplastic epithelia. It is rarely found in benign. It is found in an invasive lobular carcinomas of the breast. Some authors proposed NKX3.I as new promising prognostic marker due to its loss in the high grade PCa.

There are several significant such structural motifs:

a. Helix-turn-helix
b. Helix-loop-helix
c. Zinc finger
d. Leucine zipper

The one which we consider here is the Helix-turn-helix, of HTH motif.

The Zinc Finger is a dimer and appears as below:



The Leucine Zipper and the HLH have similar dimer and monomer conformations respectively.

3 HOMEODOMAIN

Members of this transcription factor family are characterized by the presence of a homeodomain, which is a 60 amino acid helix-turn-helix DNA-binding domain[1]. The DNA sequence that encodes the homeodomain is called the "homeobox" and homeobox-containing genes are known as "hox genes." The homeodomain is very highly conserved and consists of three helical regions folded into a tight globular structure that binds a 5'-TAAT-3' core motif. The recognition helix within the homeodomain binds the DNA major groove while the amino-terminal tail contacts the DNA minor groove. Interestingly, the homeodomain is also important for nucleocytoplasmic trafficking of hox transcription factors. Members of the hox transcription factor family can function as monomers or homodimers to directly drive transcription of target genes. The major function of hox transcription factors is to pattern the embryonic anterior-posterior body axis. More specifically, hox gene expression is crucial for normal temporospatial limb and organ development.

3.1 HOMEOBOX

The Homeobox and its related genes have played an interesting but challenging role in developmental biology and now in cancer pathways. The genes related to this 180 base pair section of DNA are the genes which control the development or organs and the time at which these development occur. Furthermore the structure of this gene collection is preserved across a dramatically large number of species, the human included. Thus it was interesting to see a paper in NEJM discussing the mutation of a specific Homeobox gene, HOX B 13, as relates to prostate cancer.

In the recent NEJM paper by Ewing et al the conclusion of the authors is stated as:

The novel HOXB13 G84E variant is associated with a significantly increased risk of hereditary prostate cancer. Although the variant accounts for a small fraction of all prostate cancers, this finding has implications for prostate-cancer risk assessment and may provide new mechanistic insights into this common cancer.

Now this appears as a significant new finding and we would like to examine this a bit. The HOX genes are quite unique in their functioning. They are built about a core Homeobox segment, which is preserved across chromosomes and species, and is hen connected with variable regions on differing chromosomes to generate some 4X13 possible genes (HOX (A,B,C,D) (1…13)). These genes are core to the morphological and embryological development of a broad range of species.

Now HOX B 13 is one of many Homeobox based genes. These genes are distributed across 4 chromosomes and have a fixed part called the homeobox part and a variable part. The gene is created as below:

[1] https://www.rndsystems.com/research-area/homeodomain--hox--transcription-factors

HOX B 13 for example uses the Homeobox segment of 180 BP and is connected to variable region creating a unique gene but the binding site is based upon the common Homeobox segment. Homeobox is preserved across species.

Homeobox genes are clustered in the chromosomes and are expressed in the body in the same order in which they occur in the chromosomal DNA. The HOX genes, the concatenation of the respective Homeobox and its variable part are named by chromosome location as A, B, C, D, and then by number 1 through 13 at present. The number reflects what makes the Homeobox genes of interest, namely the genes control the development of the embryos, namely they control what cells do as a part of the development of an entity. The process goes from head to tail, and the numbering goes from the earliest or anterior to the latest or posterior elements in the development process. Thus HOX a 1 relates to an early development and HOX B 13 would refer to a later development of the embryo. The sequencing is shown below.

	1	2	3	4	5	6	7	8	9	10	11	12	13
A	HOXA1												
B													HOXB13
C													
D													

Retinoic acid activates the Homeobox genes sequentially in development. Now the Ewing study examined patients with specific changes:

Given the consistent evidence of prostate-cancer linkage to 17q21-22 markers in our multiplex families with hereditary prostate cancer, we designed a targeted sequencing strategy to analyze 2009 exons of 202 genes contained in the most likely genomic interval defined by our fine-mapping studies. … Probands from four families were observed to have the same nonsynonymous mutation in HOXB13, a change of adenosine for guanine (transition, c.251G→A) in the second position of codon 84 (GGA→GAA), resulting in a nonconservative substitution of glutamic acid for glycine (G84E)

The question is perhaps: where does the term Homeobox come from? From Gehring and Hiromi we have the definition:

The term "homeosis" (originally spelled "homoeosis") was proposed by Bateson (8) to describe the transformation of one structure of the body into the homologous structure of another body segment. Homeotic transformation can result, for example, from abnormal regeneration of amputated structures (epigenetically) or from germ-line mutations

Thus the Homeobox genes are key to the development of embryos. They also lead to the discussions

Scott states:

Homeotic genes control cell fates during the development of all animals, as was first revealed by studies of the Drosophila homeotic gene complexes … Many of these genes contain a homeobox, a 180 bp sequence of DNA which encodes an evolutionarily conserved DNA binding domain, the homeodomain …

A plethora of mammalian homeobox genes have been reported, among which 38 are located in four clusters. A new nomenclature for the mammalian Hox genes, approved …

The new names take advantage of the elegant arrangement of the genes to provide a logical nomenclature system rather than the names given when the genes were discovered. The new system is initially designed only for vertebrate genes, although it is to be hoped that similar systems will be useful, and adopted, for other animals. In order to preserve as much clarity in the literature as possible, it has been agreed by a large number of workers in the field and by the nomenclature committees that homeobox genes not located within the Hox complexes should not be given names containing the word 'Hox'.

There are four clusters of Hox genes … now to be known as A, B, C, and D. Based on sequence similarity the genes can be sorted into 13 'paralog' groups, each group having, in most cases, a representative in each complex.

The order of paralogs along the chromosome is preserved in the four complexes. The genes within a complex are transcribed in the same direction and are numbered according to their paralog group from 1 at the 3' end to 13 at the 5' end. In several cases a representative of a paralog group is absent from a complex, in which case the corresponding gene number is omitted …

3.2 HOX GENES

HOX genes are key to the development of the embryo, it creates the head to tail and sets up the control of the development of the organs. As Lohmann and McGinnis report:

Hox genes play a major role in the morphological diversification of the anteroposterior body axis of animal embryos by switching the fates of segments between alternative developmental pathways. In their role of controlling segment diversity, Hox proteins are responsible for many different morphological structures and cell types within a given segment. But it is still largely a mystery how a single Hox gene can determine a morphological trait at a specific location within a segment, and why that trait does not appear elsewhere in the same segment or in other segments.

… morphological and transcriptional responses to Hox genes can be highly local, sometimes only in a single cell, allowing one Hox gene to control a cavalcade of different traits within one segment and between different segments, depending on the information present. Another important lesson that we can learn from the papers of Rozowski and Akam and Brodu et al. is

that, during development, Hox genes act at all levels in the developmental hierarchy. If they act very far down in the hierarchy, as in these two cases, then the output is subtle, with Hox genes acting as cell-type switches rather than as major developmental pathway switches. If they are acting (apparently) far up in the hierarchy, then the fate switch is more dramatic, which is most beautifully demonstrated in the famous four-winged fly. But even at this general level, context is still crucial: loss of Ubx in the haltere does not generate a leg, but a wing.

There are many debates still raging regarding Homeobox and Robert presents an interesting report summarizing some of them. His paper is worth the reading. It builds on the evo-devo issue, evolution and development, the ontogeny recapitulates ontogeny. Namely if the same HOX genes are present across many species, and preserved in structure, then is there really an underlying commonality across species.

We provide the details on the various HOX genes below. They all have the form as we had shown earlier and they are all numbered in a sequence consistent with what we have shown earlier.

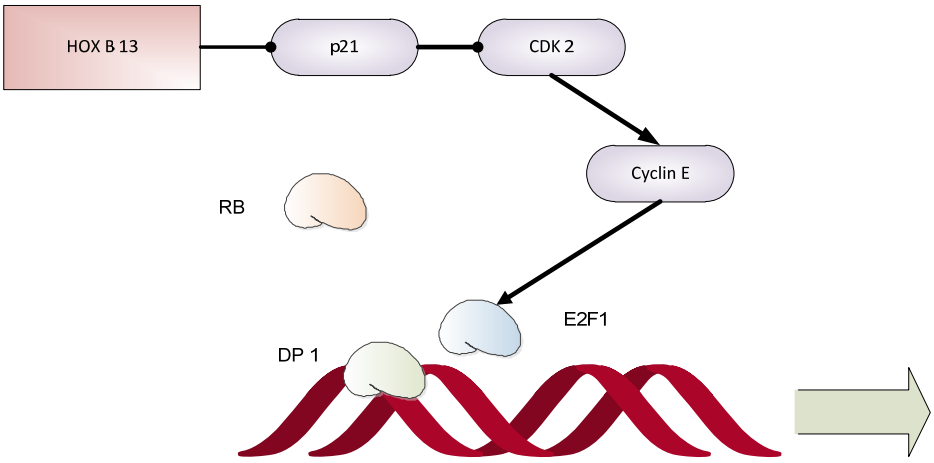
Type	Location	Genes Produced
HOX A	chromosome 7	HOXA1, HOXA2, HOXA3, HOXA4, HOXA5, HOXA6, HOXA7, HOXA9, HOXA10, HOXA11, HOXA13
HOX B	chromosome 17	HOXB1, HOXB2, HOXB3, HOXB4, HOXB5, HOXB6, HOXB7, HOXB8, HOXB9, HOXB13
HOX C	chromosome 12	HOXC4, HOXC5, HOXC6, HOXC8, HOXC9, HOXC10, HOXC11, HOXC12, HOXC13
HOX D	chromosome 2	HOXD1, HOXD3, HOXD4, HOXD8, HOXD9, HOXD10, HOXD11, HOXD12, HOXD13

Note all HOX B are from Chromosome 17. In particular HOX B 13 is 17q21-22 region [2]()

We now show from Kim et al the development of the pathway for the HOX B 13 that we have been discussing. It inhibits CDK and that in turn inhibits the activation via E2F of the cell cycle. It is the inhibition of the cell cycle that is of the most concern.

[2] see http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene&cmd=retrieve&dopt=default&rn=1&list_uids=10481

From Kim et al:
Overexpression of HOXB13 in androgen-refractory prostate tumors inhibits the expression of the p21 waf tumor suppressor and subsequently activates cyclin dependent kinase 2 (CDK2) activities. Hyperphosphorylated RB releases E2F transcription factor, which drives the genes involved in cell proliferation, and results in increased cell survivability in the absence of hormone.



As Kim et al demonstrate the HOX B 13 blocks p21 and in turn CDK2 keeping the RB pathway from entering the cell into cell cycle reproduction. They state:

Taken together, the results of this study demonstrated the presence of a novel pathway that helps understand androgen-independent survival of prostate cancer cells. These findings suggest that upregulation of HOXB13 is associated with an additive growth advantage of prostate cancer cells in the absence of or low androgen concentrations, by the regulation of p21-mediated E2F signaling.

Now Ewing at al conclude as follows:

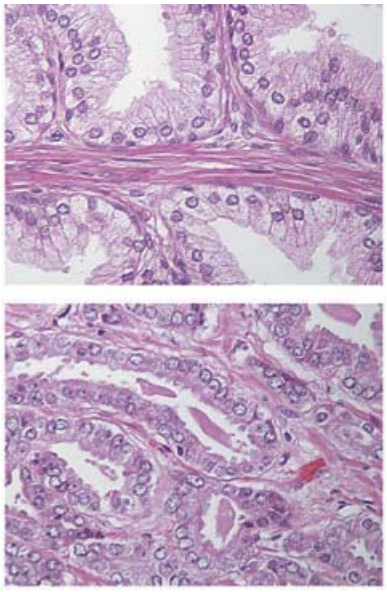
In summary, we have used linkage analysis in combination with targeted massively parallel sequencing to identify a recurrent mutation in HOXB13 that is associated with early-onset and hereditary prostate cancer.

From a clinical perspective, testing for germline mutations in BRCA1/2 is recommended in some families, since mutations in these breast-cancer–susceptibility genes are associated with elevations in the risk of prostate cancer, particularly for BRCA2.

However, neither of these genes has been shown to contribute to hereditary prostate cancer. HOXB13 G84E is associated with a significantly increased risk of hereditary prostate cancer. This work suggests that future DNA sequencing studies using next-generation technology and study populations enriched for genetic influence (as evidenced by an early age at onset and positive family history) may identify additional rare variants that will contribute to familial clustering of prostate cancer. Although HOXB13 mutations will be identified in a minority of men with prostate cancer, rare genetic lesions can identify pathways that are found to be abnormal in more common, sporadic cases.

This leaves one to somewhat guess as to have prevalent this mutation is. It also begs the question of why as a mutation which is apparently inherited the progression of the cancer is so slow. Ewing at al show that the odds ration can be as high as 32.5:1 when the mutation is present. The age at diagnosis is lower with an odds ratio of 2:1 but with the problem one sees in pathway control one wonders why the cancer does not appear much earlier as seen in BRCA.

Ewing et al have an interesting slide showing normal versus HOX B 13 prostate cells and we replicate it below from the paper.



In the top slide we see well-structured prostate cells with basal and luminal layers not showing and aberrant growth, no PIN. In the slide below from a HOX B 13 patient with a mutation of the form: GGA to GAA Glycine Glutamic acid (See Ewing et al).

From Science Daily[3]:

Expression of a single gene can convert cells lining the seminal vesicle in the pelvis into prostate cells, a new study shows. The results provide a better understanding of the molecular mechanisms controlling the development of seminal vesicle and prostate tissues, which could provide valuable insights as to why cancer arises frequently in the latter but only rarely in seminal vesicles.

Previous studies have found that loss of the gene Nkx3.1 results in impaired prostate differentiation in mice, prompting Aditya Dutta et al. to study the gene in greater detail. First, they confirmed that lack of Nkx3.1 in prostate cells results in reduced expression of a number of genes associated with prostate differentiation.

[3] https://www.sciencedaily.com/releases/2016/06/160623145934.htm

The researchers then infected seminal vesicle epithelial cells with a virus expressing Nkx3.1, finding that inducing expression of this gene caused the seminal vesicle epithelium to convert into a prostate-like state in terms of structure, histological appearance and genetic markers.

Further investigation in human prostate cells identified several components of the regulatory network involved in this "tissue respecification" process, including certain histone-modifying enzymes. The authors suggest that further exploration of this network could help researchers understand the tissue-specificity of prostate cancer.

From Science[4]:

The NKX3.1 homeobox gene plays essential roles in prostate differentiation and prostate cancer. We show that loss of function of Nkx3.1 in mouse prostate results in down-regulation of genes that are essential for prostate differentiation, as well as up-regulation of genes that are not normally expressed in prostate. Conversely, gain of function of Nkx3.1 in an otherwise fully differentiated nonprostatic mouse epithelium (seminal vesicle) is sufficient for respecification to prostate in renal grafts in vivo.

In human prostate cells, these activities require the interaction of NKX3.1 with the G9a histone methyltransferase via the homeodomain and are mediated by activation of target genes such as UTY (KDM6c), the male-specific paralog of UTX (KDM6a). We propose that an NKX3.1-G9a-UTY transcriptional regulatory network is essential for prostate differentiation, and we speculate that disruption of such a network predisposes to prostate cancer.

[4] http://science.sciencemag.org/content/352/6293/1576

4 NKX3.1

We now examine some of the details regarding how NKX3.1 functions. This will not be with regards to its developmental functions but with regards to its homeostatic functions. The observation of changing seminal to prostate cells is however a developmental function but it will not be discussed herein. Our concern is the results with a loss of function of this gene.

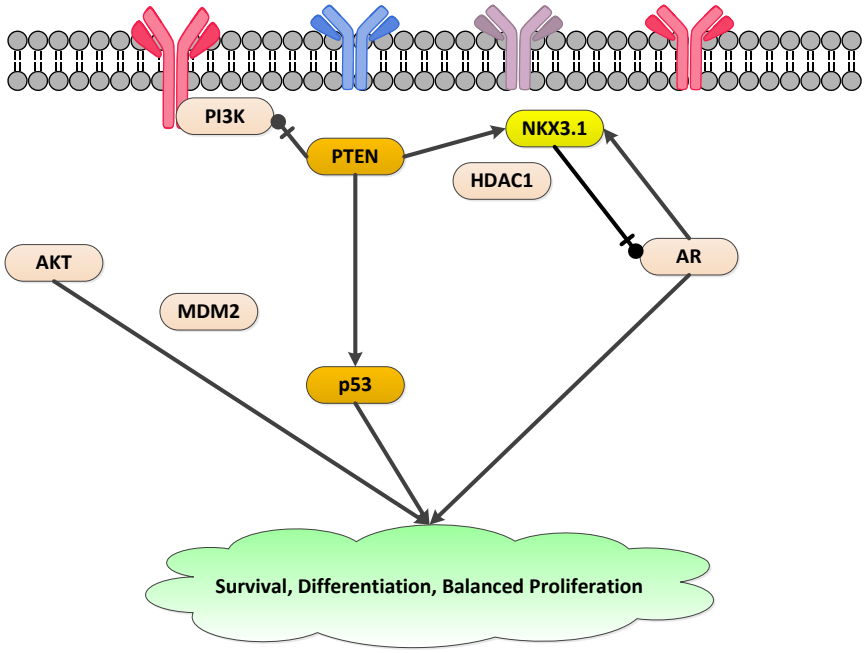
From NCBI we have[5]:

This gene encodes a homeobox-containing transcription factor. This transcription factor functions as a negative regulator of epithelial cell growth in prostate tissue. Aberrant expression of this gene is associated with prostate tumor progression.

This is a rather simplistic description. In order to fully understand this we must consider the impact on various key pathways.

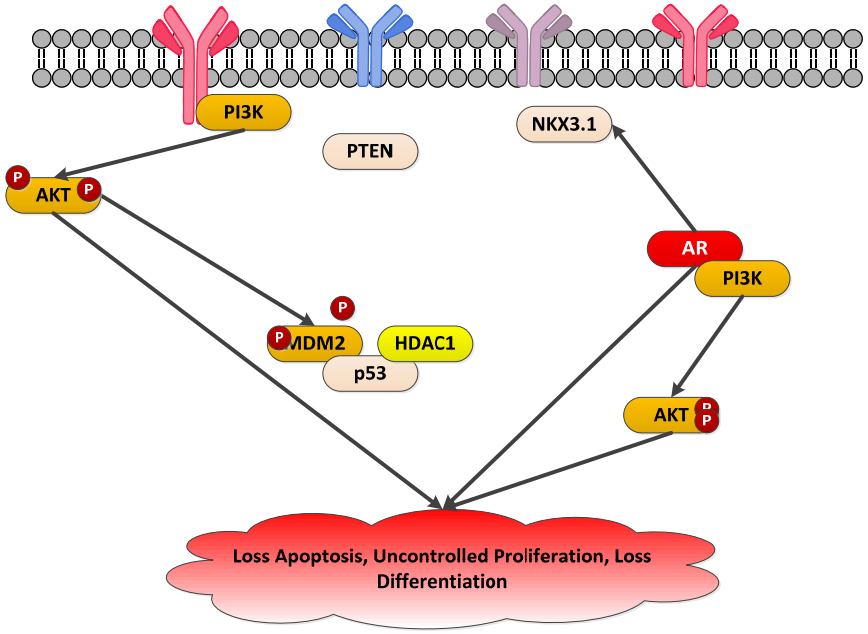
4.1 PATHWAYS

From the paper by Lei et al we have the normal case of functioning as shown below. Here NKX3.1 blocks AR and PTEN blocks the PI3K activation of AKT.

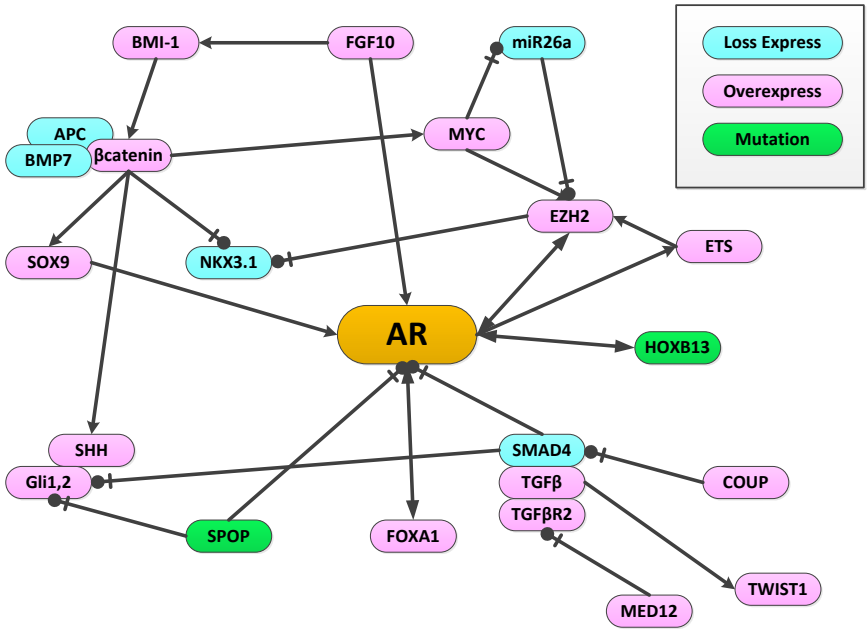


[5] http://www.ncbi.nlm.nih.gov/gene/4824

In contrast we have below the case where now AKT is activated and p53 is blocked. Here we see NKX3.1 is deactivated.



Now from the work of Shtivelman et al we have a more detailed map of the AR dynamics below and here again we see the impact of NKX3.1



Shtivelman, E., et al, Molecular pathways and targets in prostate cancer, Oncotarget, Vol. 5, No. 17, August 2014.

In the above pathway analyses we can see a significant impact that NKX3.1 can have when a loss of function, "LOF", occurs. There is no description of how this LOF occurs.

4.2 INTERACTIONS

In the paper by Iwata et al the authors examine its influence during the development of PIN. They state:

Lo-MYC and Hi-MYC mice develop prostatic intraepithelial neoplasia (PIN) and prostatic adenocarcinoma as a result of MYC overexpression in the mouse prostate. However, prior studies have not determined precisely when, and in which cell types, MYC is induced. Using immunohistochemistry (IHC) to localize MYC expression in Lo-MYC transgenic mice, we show that morphological and molecular alterations characteristic of high grade PIN arise in luminal epithelial cells as soon as MYC overexpression is detected.

These changes include increased nuclear and nucleolar size and large scale chromatin remodeling. Mouse PIN cells retained a columnar architecture and abundant cytoplasm and appeared as either a single layer of neoplastic cells or as pseudo-stratified/multilayered structures with open glandular lumina—features highly analogous to human high grade PIN.

Also using IHC, we show that the onset of MYC overexpression and PIN development coincided precisely with decreased expression of the homeodomain transcription factor and tumor suppressor, Nkx3.1. Virtually all normal appearing prostate luminal cells expressed high levels of Nkx3.1, but all cells expressing MYC in PIN lesions showed marked reductions in Nkx3.1, implicating MYC as a key factor that represses Nkx3.1 in PIN lesions.

To determine the effects of less pronounced overexpression of MYC we generated a new line of mice expressing MYC in the prostate under the transcriptional control of the mouse Nkx3.1 control region. These ''Super-Lo-MYC'' mice also developed PIN, albeit a less aggressive form. We also identified a histologically defined intermediate step in the progression of mouse PIN into invasive adenocarcinoma. These lesions are characterized by a loss of cell polarity, multi-layering, and cribriform formation, and by a ''paradoxical'' increase in Nkx3.1 protein. Similar histopathological changes occurred in Hi-MYC mice, albeit with accelerated kinetics.

Our results using IHC provide novel insights that support the contention that MYC overexpression is sufficient to transform prostate luminal epithelial cells into PIN cells in vivo. We also identified a novel histopathologically identifiable intermediate step prior to invasion that should facilitate studies of molecular pathway alterations occurring during early progression of prostatic adenocarcinomas

NKX3-1 (also NKC3.1) is a gene and Nkx3.1 its protein which is putatively a tumor suppressor gene which is primarily prostate specific. As c-Myc tends to regulate the transcription of many genes, Nkx3.1 regulates the control mechanism for the prostate cells. Even more specifically it has been argued that Nkx3.1 regulates the luminal cell growth by Iwata et al. Yet Goldstein et al would argue from similar murine models that use of up-regulated ERG translocations with Akt activation, namely putatively suppressing PTEN, allows for basal cell growth rather than luminal cell transformation.

It has been observed that diminished expression of NKX3.1 (8p21) is associated with prostate cancer progression in humans, and in mice, loss of nkx3.1 leads to epithelial cell proliferation and altered gene expression patterns. Loss of heterozygosity of 8p21 is observed in a high percentage of intraepithelial prostatic neoplasia and early carcinoma lesions, strongly implicating this region in the initial stages of prostate carcinogenesis.

The importance of NKX3.1 as a dose-dependent regulator of prostate epithelial cell growth is strongly supported by analyses of nkx3.1 knockout mice. Homozygous nkx3.1 mutant mice develop prostate epithelial hyperplasia and dysplasia that progresses with age, and lesions with histologic features strongly resembling human prostatic intraepithelial neoplasia develop in homozygous mice between 1 and 2 years of age. Importantly, both hyperplasia and prostatic intraepithelial neoplasia-like lesions also occur in a significant proportion of nkx3.1 heterozygous mutants

The question then is, is NKX3.1 a true tumor suppressor gene? Despite that loss of function of Nkx3.1 predisposes to prostate cancer, it is not sufficient for tumorigenesis as noted by Shen (2003) Moreover, while one allele of NKX3.1 is lost by means of chromosomal deletion in PIN and prostate cancer, the other allele does not undergo mutational inactivation, although protein expression is epigenetically down-regulated or lost. These features, along with the relatively subtle consequences following forced expression of Nkx3.1 in prostate cancer cells are not consistent with activities of "classic" tumor suppressor genes, such as p53, Rb, or Pten. Instead, Nkx3.1 appears to act more like a tumor modulator, serving as a regulator of differentiation, which in turn prevents cancer initiation. In this regard, further analyses of NKX3.1 can provide important insights into the relationship between regulation of differentiation and carcinogenesis.

As Iwata et al have observed:

The prevailing model of NKX3.1 expression in human prostate cancer suggests that while the protein may decrease in PIN lesions, it is much more commonly decreased in invasive adenocarcinomas, and nearly completely lost in most, if not all, metastatic prostate adenocarcinomas … there was a variable decrease in expression of Nkx3.1 in PIN lesions, and that Nkx3.1 was virtually completely lost in invasive adenocarcinomas… several observations from our group regarding NKX3.1 differ from this prevailing view. First, in a previous report, while reductions of NKX3.1 protein occurred in PIN lesions and some adenocarcinomas, the reductions were relatively minor and virtually all invasive adenocarcinomas retained significant levels of NKX3.1 protein …

More recently we have found that the majority of very high grade (Gleason score 8–10) localized prostate cancers … retain high levels of expression of NKX3.1 protein. In the present study we found that, as compared to high grade PIN, the staining for Nkx3.1 protein actually increased substantially in pre-invasive cribriform PIN/CIS lesions and in early invasive adenocarcinomas, and these levels correlated inversely with levels of MYC expression.

These results indicate the Nkx3.1 may be dynamically regulated during progression of this disease. … It is possible, therefore, that Nkx3.1 expression in invasive prostatic acini in MYC-driven mouse prostate cancers may represent a recapitulation or caricature of the process of

stromal invasion/branching morphogenesis in development, and, that Nkx3.1 may facilitate this process. …

Lei et al. found that forced restoration of Nkx3.1 expression in Pten null epithelium led to decreased cell proliferation, increased cell death, and prevention of tumor initiation... They further showed that Nkx3.1 was required to engage the p53 pathway, indicating that reduced Nkx3.1 expression can itself abrogate p53 signaling.

These findings raise the interesting possibility that the reduction in Nkx3.1 seen upon the induction of MYC in the mouse prostate prevents the induction of p53 induced apoptosis, thus facilitating MYC's ability to transform these cells. Additional studies in which Nkx3.1 expression is kept at high levels during induction of MYC in prostate epithelium will be required to address this question further. We do not know precisely how MYC is regulating Nkx 3.1 protein expression, …"

Specifically, Iwata et al state:

Since MYC may downregulate Nkx3.1 at the level of transcription …, it is possible that elevated MYC itself may be responsible for down-regulating Nkx3.1 expression.

In effect, this implies that MYC controls NKX3.1 and thus up-regulated MYC results in a down regulated NKX3.1. If NKX3.1 is controlling prostate stability then its overall regulation is via MYC. Controlling and suppressing MYC would control and up-regulate NKX3.1 and thus stabilize prostate growth. The complete pathway for this gene does not seem to be complete at this stage. Its importance is well defined however.

From Eide et al:

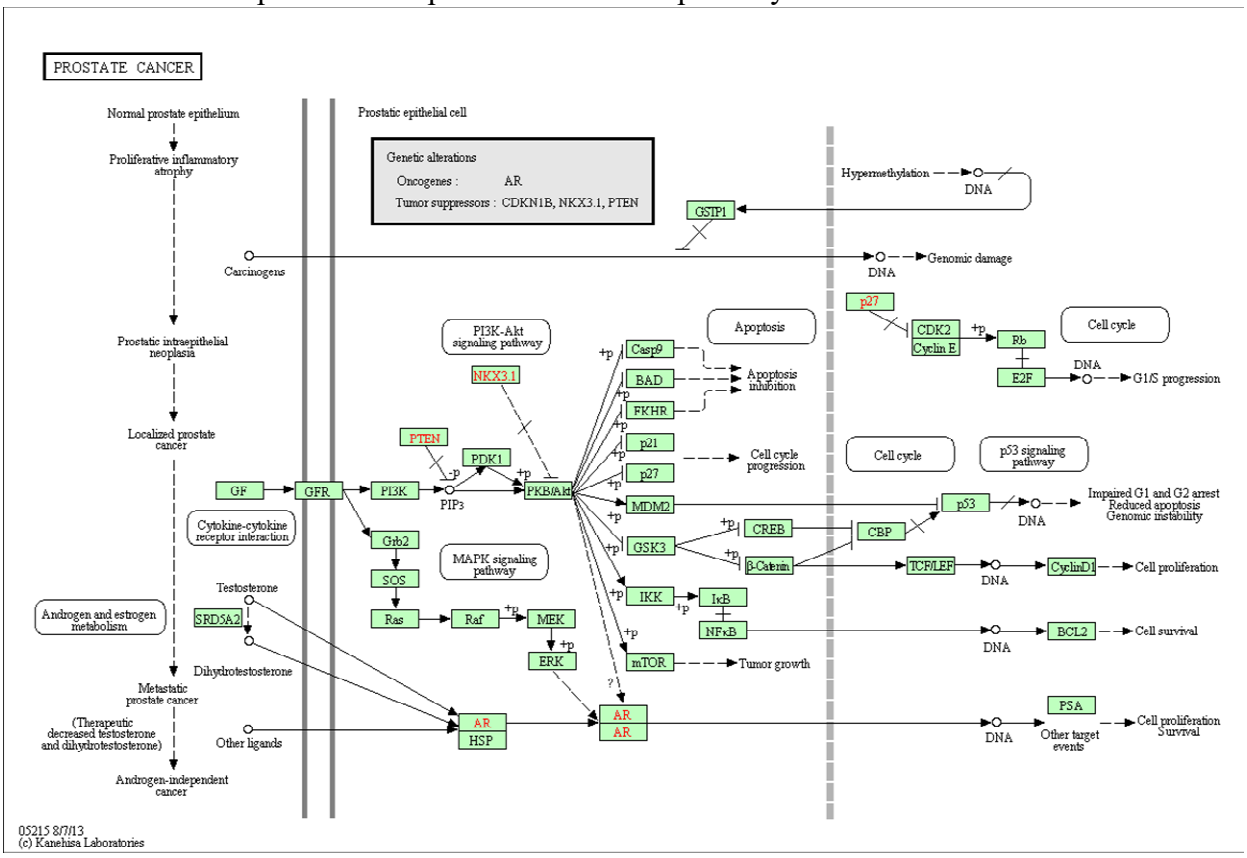
In this study we show both that TWIST1 mRNA is up-regulated by androgen via AR and that NKX3-1, a well-known androgen-regulated gene, binds the upstream regulatory region of the TWIST1 gene and represses the expression of TWIST1… The physical binding of NKX3-1 to the TWIST1 promoter might block the mesenchymal drive of TWIST1, until NKX3-1 expression is down-regulated or lost in PIN or adenocarcinoma lesions. Loss of NKX3-1 expression has been observed in ~20% of PIN lesions, ~40% of advanced prostate tumors and up to 80% of metastatic prostate cancer.

Androgen deprivation therapy as the most widely used treatment for advanced prostate cancer is likely to abolish androgen-stimulation of NKX3-1, leading eventually to down-regulation of repressor protein and de-repression of TWIST1's metastatic potential. In an attempt to identify genes whose regulation are altered by NKX3-1, Song et al. performed gene expression profiling analyses on micro dissected glands from NKX3-1- deficient prostate tissues during prostate cancer progression. They observed similarities between the expression profile of the micro dissected glands and constitutive activated AKT transgenic mice as well as PTEN-deficient mice, suggesting that the PTEN-AKT-NKX3-1 axis serve as a major molecular path of prostate tumorigenesis.

Li and Zhou showed that activation of the AKT pathway by TWIST1 is critical for the sustention of cancer stem cell like traits generated by EMT, again suggesting a link between loss of NKX3-1 expression, relive of TWIST1 expression and eventually activation of AKT pathway. Conclusions We report in this paper that TWIST1 is an androgen-regulated gene, tightly regulated by NKX3-1. We show that NKX3-1 binds to the TWIST1 promoter and that NKX3-1 overexpression reduces the activity of a TWIST1 promoter reporter construct, whereas NKX3-1 siRNA up-regulated endogenous TWIST1 mRNA in prostate cancer cells. Our finding that NKX3-1 represses TWIST1 expression emphasizes the functional importance of NKX3-1 in regulating TWIST1 expression during prostate cancer progression to metastatic disease.

4.3 PATHWAY COMPLEXITY

For the need of completeness we provide the KEGG pathway for PCa below.



5 OBSERVATIONS

There has been a multiplicity of genes related to PCa. This examination of NKX3.1 just adds to that list. What is of interest here is the use of a homeobox gene. It is not clear that this is a significant target for therapeutics but it is insightful and adds to the already substantial base of knowledge on NKX3.1

5.1 NKX3.1 SUMMARY

To summarize some key facts on NKX3.1 we refer to Lei et al. Their analysis includes the interaction between PTEN and NKX3.1. They state:

1. *PTEN loss leads to reduced NKX3.1 expression in both murine and human prostate cancers*

2. *Forced Nkx3.1 expression in Pten null epithelium using an exogenous promoter*

3. *Introducing Nkx3.1 into Pten null prostatic epithelium leads to reduced graft growth*

4. *Nkx3.1 blocks Pten null prostate cancer initiation*
5. *and progression*

6. *Forced Nkx3.1 expression leads to decreased cell proliferation and increased apoptosis of Pten null grafts*

7. *NKX3.1 negatively regulates AKT activity in an AR-dependent manner*

8. *NKX3.1 negatively regulates the AR promoter*

9. *NKX3.1 stabilizes p53 through MDM2-dependent and AKT-independent mechanisms*

10. *NKX3.1 negatively regulates AR expression in both murine and human prostatic cancer samples*

11. *NKX3.1 can physically associate with HDAC1 and promotes p53 acetylation by recruiting HDAC1 from p53-MDM2-HDAC1 complex*

It is thus clear from their analysis that NKX3.1 has a significant role to play in PCa.

5.2 ISSUES

This paper raises several questions:

1. The Homeobox mutation is a predisposing genetic risk factor. If tested and found positive for the factor what should be done next? Mastectomy is often what BRCA patients undergo, does this mean prophylactic prostatectomy?

2. The pathway seems to be somewhat understood. The E2F family control the pathway and HOX B 13 controls that pathway. It blocks it to some degree. What can happen to HOX B 13 to cause this change in non-mutated individuals?

3. Can the disease propensity be regulated by genetic pathway control, is this possible as an alternative prophylactic measure?

4. What other pathway elements should be considered.

5. Most importantly, why does it take so long for the cancer to develop, are there precursor hits somewhere and this this just eliminates other hits?

6 APPENDIX

The following is a summary of some common PCa genes[6].

Genes and alterations	***Description***	***Alterations***	***Frequency in primary versus metastatic (when known)***	***PATHWAY***
AR	Androgen receptor	Amplification and Mutations Variant splicing	Only CRPC, in majority of tumors together with cofactors	Androgen receptor signaling
AR cofactors and regulators NCOA1,2,3; NCOR1, NCOR2, TNK2 and more	Regulation of the AR activity	Amplification Mutations	About 50% in localized; >80% in CRPC	
Androgen synthesis enzymes: CYP17 etc	Steroidogenic/androgen synthesis	Overexpression, activating mutations, copy gains	Observed in CRPC	
FOXA1	AR licensing Factor	Mutations	5% of localized	
TMPRSS2:ERG, other ETS	Gene fusion involving ERG; rarely other ETS family members		40-60 % of localized	Transcription, most likely controlled by AR
NKX3.1	Homeobox, prostate specific, androgen regulated	Deletions, mutations, decreased expression	3-5% mutations, 10-20% deletions, and frequent decreased expression in localized cancers	Developmental lineage specific, transcription, AR pathway
PTEN	Phosphatase suppressor of PI3K	Deletions, rare mutations	40-50% of primary, >80% CRPC; PTEN loss is the most frequent alterations in PI3K pathway	PI3K signal transduction Co-operates with AR pathway in pathogenesis of PCa
MAGI2	PTEN interactor	Rearrangement		
PIK3CA1 catalytic subunit	PIP2 kinase	Overexpression, mutations		
PHLPP1/2	Phosphatase, inhibits AKT	Deletion, down-regulation		
Akt1	Central kinase in PI3K pathway	Point mutations (rare)		
SPOP	Speckle-type POZ domain ubiquitin ligase	Mutations	5-10% primary, same in metastatic, mutually exclusive with ERG rearrangements	Responsible for the degradation of AR cofactor NCOA3/SRC-3. AR pathway connected?
SPINK1	Serine peptidase inhibitor	Overexpression	5-10%, mutually exclusive with ERG rearrangements	Unknown

[6] http://www.cancercommons.org/researchers-clinicians/prostate-cancer/prostate-cancer-model/

MYC	Master of transcription regulation; opposes NKX3.1	Overexpressed in primary, genetic gain in metastatic	20-30% with gain in metastatic disease	Transcription
NMYC	Transcriptional regulation	Overexpression, amplification	40% of neuroendocrine PCa; 5% overall	
MED12	Regulatory component of mediator complex	Mutations	2-5%	
EZH2	Polycomb group	Elevated expression	Localized (poor prognosis) and CRPC	Transcriptional suppression
BMI	Polycomb group, transcriptional suppression	Elevated expression	Localized and metastatic	
Aurora A kinase	Mitotic kinase	Overexpression, amplification	40% of neuroendocrine PCa; 5% overall	Cell Cycle
BRAF	Serine-threonine kinase at the top of MAPK cascade	Rearrangements	1%, all	MAPK
CADM2	Cell adhesion molecule	Rearrangements	Primary and metastatic	Cell polarity, potential tumor suppressor
CHD1	Nucleosome positioning	Mutations	8%, mostly with SPOP mutations, in ETS normal	Chromatin remodeling
MLL complex (MLL2, ASH2L and more)	Epigenetic transcriptional activation	Mutations	9% CRPC	
TP53	Controls many aspects of cell cycle, apoptosis, metabolism	Loss, LOF*, GOF* mutations	30-100%, mostly in metastatic	Tumor suppressors
RB1	Cell cycle	Loss, LOF	50% metastatic	
ERCC2,4,5; ATM, XRCC4, PRKDC and more	Various genes involved in DNA repair	Losses, mutations	Mostly in metastatic	DNA damage repair
APC, BMP7, WNT family, CTNNB1 and more	WNT pathway involved on proliferation, differentiation, EMT	Losses, mutations	Metastatic	Developmental signaling pathways: WNT
EGFR, IGF1R, FGFR	Growth factor receptors	Activation	NA	Growth factor induced signaling, activation of PI3K and MAPK pathways, and AR signaling
IL6-IL6R	Cytokine receptor	Activation by IL6	NA	JAK-STAT3 pathway; activates AR
SRC	Tyrosine kinase	Activation	NA	Many signaling pathways

HSP90, HSP27Clusterin/TRPM2	Maintain stability of various signaling proteins including AR and many others		NA	Protein Chaperons

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