PROSTATE CANCER: MIR-34, P53, MET AND METHYLATION

Terrence P McGarty
White Paper No 112
May, 2014

We examine several recent works which discuss an amalgam of genetic and epigenetic factors and prostate cancer. Unlike the approaches taken by many at looking at solely one factor, such as pathway elements, this approach looks at a multiplicity of them. It does not however look at the cell in the complexity of the ECM.

Notice

This document represents the personal opinion of the author and is not meant to be in any way the offering of medical advice or otherwise. It represents solely an analysis by the author of certain data which is generally available. The author furthermore makes no representations that the data available in the referenced papers is free from error. The Author also does not represent in any manner or fashion that the documents and information contained herein can be used other than for expressing the opinions of the Author. Any use made and actions resulting directly or otherwise from any of the documents, information, analyses, or data or otherwise is the sole responsibility of the user and The Author expressly takes no liability for any direct or indirect losses, harm, damage or otherwise resulting from the use or reliance upon any of the Author's opinions as herein expressed. There is no representation by The Author, express or otherwise, that the materials contained herein are investment advice, business advice, legal advice, medical advice or in any way should be relied upon by anyone for any purpose. The Author does not provide any financial, investment, medical, legal or similar advice in this document or in its publications on any related Internet sites.

Contents

1 INTRODUCTION

There has been an explosion in genetic "causes" for many cancers and prostate cancer, PCa, is not the exception. One of the most significant factors has been the ability by some to take metrics of multiple gene expressions and allege that with the proper weightings these single dimensional metrics are prognostic. The problem with the metrics is often that they do not relate to actual genetic control mechanisms. We consider here an example in PCa of several genes and miRNAs which taken together create a putative malignant state.

Specifically we examine three elements:

1. p53, the classic oncogene which is a control element for keeping cells in a homeostatic state and avoiding malignant changes.

2. miRNA 34, or miR-34 which is a micro RNA and is also found to have a controlling effect upon a cell.

3. MET, a tyrosine kinase receptor which can be activated by HGF, the hepatocellular growth factor ligand, and which can activate multiple pathways and if activated and done so in an uncontrolled manner can result in malignancies.

This examination is predicated on a recent paper by Cheng et al (2014) which discusses the joint regulation effects of p53 and miR-34.

This section discusses the micro RNA process and its impact on PCa. Micro RNAs, miRNA, are small single stranded RNAs which when in the cytoplasm may often bind to other RNA on complement binding sites and thus change or incapacitate the mRNA to which it binds from being translated into a protein. Craig Mello was awarded the Nobel Prize in 2006 for the discovery and his Nobel Lecture provides an excellent overview of the early stages of miRNA investigation.

In a recent paper by Cheng et al (2014) they state:

The miR-34 family was originally found to be a direct target of p53 and is a group of putative tumor suppressors. Surprisingly, mice lacking all mir-34 genes show no increase in cancer formation by 18 months of age, hence placing the physiological relevance of previous studies in doubt.

Here, we report that mice with prostate epithelium-specific inactivation of mir-34 and p53 show expansion of the prostate stem cell compartment and develop early invasive adenocarcinomas and high-grade prostatic intraepithelial neoplasia, whereas no such lesions are observed after inactivation of either the mir-34 or p53 genes alone by 15 months of age.

Consistently, combined deficiency of p53 and miR-34 leads to acceleration of MET-dependent growth, self-renewal, and motility of prostate stem/ progenitor cells.

Our study provides direct genetic evidence that mir-34 genes are bona fide tumor suppressors and identifies joint control of MET expression by p53 and miR-34 as a key component of prostate stem cell compartment regulation, aberrations in which may lead to cancer

This is a murine model which putatively demonstrates that a blocking of both miR-34 and p53 leads to PCa. Specifically, this is MET pathway dependent growth.

As noted in Bioscience Technology[1]:

Previous research at Cornell and elsewhere has shown that another gene, called p53, acts to positively regulate miR-34. Mutations of p53 have been implicated in half of all cancers. Interestingly, miR-34 is also frequently silenced by mechanisms other than p53 in many cancers, including those with p53 mutations.

The researchers showed in mice how interplay between genes p53 and miR-34 jointly inhibits another cancer-causing gene called MET. In absence of p53 and miR-34, MET overexpresses a receptor protein and promotes unregulated cell growth and metastasis.

This is the first time this mechanism has been proven in a mouse model, said Alexander Nikitin, a professor of pathology in Cornell's Department of Biomedical Sciences and the paper's senior author. Chieh-Yang Cheng, a graduate student in Nikitin's lab, is the paper's first author.

In a 2011 Proceedings of the National Academy of Sciences paper, Nikitin and colleagues showed that p53 and miR-34 jointly regulate MET in cell culture but it remained unknown if the same mechanism works in a mouse model of cancer (a special strain of mice used to study human disease).

The findings suggest that drug therapies that target and suppress MET could be especially successful in cancers where both p53 and miR-34 are deficient.

Also, the number of stem cells in mice with both p53 and miR-34 silenced increased substantially compared with control mice or mice with only miR-34 or p53 independently silenced.

"These results indicated that together [miR-34 and p53] regulate the prostate stem cell compartments," said Nikitin.

This is significant, as cancer frequently develops when stem cells become unregulated and grow uncontrollably, he said.

[1] http://www.biosciencetechnology.com/news/2014/03/gene-family-proven-suppress-prostate-cancer also http://www.news.cornell.edu/stories/2014/03/gene-family-proven-suppress-prostate-cancer

Researchers further found that p53 and miR-34 affect stem cell growth by regulating MET expression. In absence of p53 and miR-34, MET is overexpressed, which leads to uncontrolled growth of prostate stem cells and high levels of cancer in these mice.

From Tang's Lab at MD Anderson we have[2] (see Liu et al):

Cancer stem cells (CSCs), or tumor-initiating cells, are involved in tumor progression and metastasis1. MicroRNAs (miRNAs) regulate both normal stem cells and CSCs and dysregulation of miRNAs has been implicated in tumorigenesis6. CSCs in many tumors—including cancers of the breast, pancreas, head and neck, colon, small intestine, liver, stomach, bladder and ovary—have been identified using the adhesion molecule CD44, either individually or in combination with other marker(s).

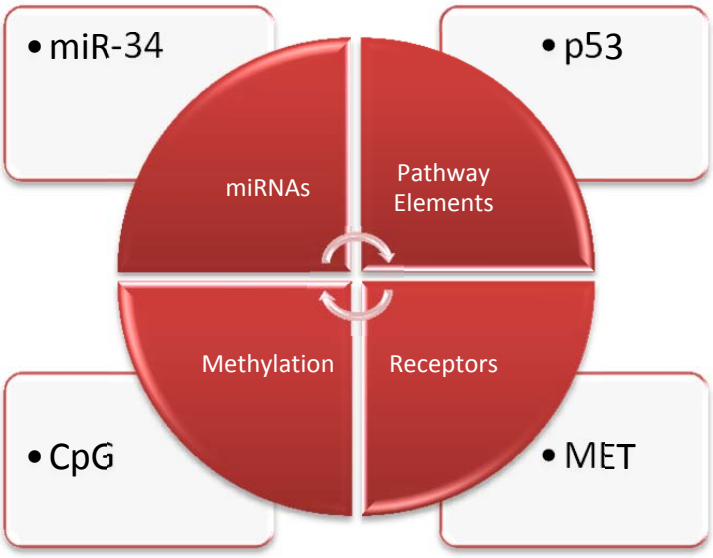
Prostate CSCs with enhanced clonogenic17 and tumor-initiating and metastatic capacities are enriched in the CD44+ cell population, but whether miRNAs regulate CD44+ prostate cancer cells and prostate cancer metastasis remains unclear. Here we show, through expression analysis, that miR-34a, a p53 target was underexpressed in CD44+ prostate cancer cells purified from xenograft and primary tumors.

Enforced expression of miR-34a in bulk or purified CD44+ prostate cancer cells inhibited clonogenic expansion, tumor regeneration, and metastasis. In contrast, expression of miR-34a antagomirs in CD44− prostate cancer cells promoted tumor development and metastasis. Systemically delivered miR-34a inhibited prostate cancer metastasis and extended survival of tumor-bearing mice.

We identified and validated CD44 as a direct and functional target of miR-34a and found that CD44 knockdown phenocopied miR-34a overexpression in inhibiting prostate cancer regeneration and metastasis. Our study shows that miR-34a is a key negative regulator of CD44+ prostate cancer cells and establishes a strong rationale for developing miR-34a as a novel therapeutic agent against prostate CSCs.

Overall we examine here a four part set of elements related to PCa; receptors, pathway elements, mi RNAs and methylation. We outline this graphically below:

[2] http://staging-www.nature.com/nm/journal/v17/n2/full/nm.2284.html



Note that in the above each plays a role in the development of PCa.

This has been known for a while. We see in Yamamura et al (2012) that they observed:

MicroRNA-34a (miR-34a), a potent mediator of tumor suppressor p53, has been reported to function as a tumor suppressor and miR-34a was found to be downregulated in prostate cancer tissues. We studied the functional effects of miR-34a on c- Myc transcriptional complexes in PC-3 prostate cancer cells. Transfection of miR-34a into PC-3 cells strongly inhibited in vitro cell proliferation, cell invasion and promoted apoptosis. Transfection of miR-34a into PC-3 cells also significantly inhibited in vivo xenograft tumor growth in nude mice. miR-34a downregulated expression of c-Myc oncogene by targeting its 39 UTR as shown by luciferase reporter assays. miR-34a was found to repress RhoA, a regulator of cell migration and invasion, by suppressing c-Myc–Skp2–Miz1 transcriptional complex that activates RhoA.

Overexpression of c-Myc reversed miR-34a suppression of RhoA expression, suggesting that miR-34a inhibits invasion by suppressing RhoA through c-Myc. miR-34a was also found to repress c-Myc-pTEFB transcription elongation complex, indicating one of the mechanisms by which miR- 34a has profound effects on cellular function. This is the first report to document that miR-34a suppresses assembly and function of the c-Myc–Skp2–Miz1 complex that activates RhoA and the c-Myc-pTEFB complex that elongates transcription of various genes, suggesting a novel role of miR-34a in the regulation of transcription by c-Myc complex.

It is interesting to see that we have a miRNA as a tumor suppressor. It is a key change in the way we can understand the overall pathway control paradigm. Thus the miRNA acts in a powerful manner to modulate cell growth and proliferation.

2 MICRO RNA

The development of our understanding of micro RNAs has evolved from that of elements just left over to key control factors in major pathway expression. From Pekarik et al:

Among all previously described factors involved in the initiation and development of prostate cancer another element interconnecting several cellular processes may be traced. This element is represented by microRNAs (miRNAs), short non-coding regulatory molecules involved in multitude of processes in eukaryotic cells. They play a role in virtually each step of tumour formation and progression. miRNAs networks affect apoptotic pathways, cellular growth, responsiveness to growth factors and anticancer drugs, inhibit expression of tumour suppressor genes or permit expression of oncogenes.

Classical textbooks refer to carcinogenesis as a harmonic process caused by a loss of function of tumour suppressor genes and simultaneous activation of oncogenic genes. Recent progress in miRNAs function studying did not change this definition substantially; it only extended our understanding of regulation of this intrinsic network by miRNAs which can be likewise characterised as oncogenic miRNAs and antitumour miRNAs.

Indeed, we now see that tumor growth is a highly complex amalgam of genetic elements and supra-genetic elements as well. We have also argued that in many cases we see extracellular matrix interactions as well as free radical excitation of cells as well.

Oncogenic miRNAs are those that directly or indirectly suppress gene expression of tumour suppressors or proapoptotic genes and vice versa anti-tumorigenic miRNAs are those that reduce expression of oncogenic proteins. miRNAs are involved in nearly all types of cancer studied so far and they target classical oncological pathways. However, certain miRNAs were specifically associated with defined tumour types suggesting that they are involved in specific processes related to a cancer type or a tissue of origin. With regard to the number of genes regulated by miRNAs it is not surprising that these small regulatory molecules play a role also in the resistance of cancer cells to various anti-cancer drugs. In that respect, miRNAs become very attractive target for potential therapeutic interventions.

Recent research has revealed existence of miRNAs circulating in human blood serum. More surprisingly, it was found that levels of various miRNAs are altered in response to various physiological changes and some of these changes are well correlated with tumour existence. This makes circulating miRNAs a very attractive non-invasive cancer biomarker.

miRNAs have come to the fore as one of the several epigenetic factors which can precipitate various malignancies. The added factor of methylation as a silencing mechanism also adds to but further complicates the understanding of cancer progression. Thus, when we see loss of a miRNA, we may actually be indirectly observing the effects of methylation of the CpG region about that miRNA encoding region.

The relationship between miRNAs and pathway control elements is now being better understood. From Yamamura et al:

MicroRNAs (miRNAs) are highly conserved, single stranded, non-coding RNAs of approximately 22 nucleotides that regulate gene expression by posttranscriptional silencing of specific target mRNAs, by repressing translation or cleaving RNA transcripts. miRNAs regulate diverse cellular processes such as cell-cycle progression, proliferation, apoptosis and development. miRNAs have been shown to function as oncogenes or tumor suppressor genes.

The p53 tumor suppressor is deleted or mutated in more than 50% of human tumors and is a key molecule which suppresses malignancies. p53 has been found to target the miR-34 family and the ectopic expression of miR-34 genes has drastic effects on cell proliferation and survival. Ectopic miR-34a causes cell-cycle arrest in the G1 phase and apoptosis. As p53 has been found to target miR-34a and since, cell-cycle arrest and apoptosis are also end points of p53 activation, the miR-34a gene may be a mediator of p53 function. The proto-oncogene c-Myc regulates cell proliferation and transformation both transcriptionally and non-transcriptionally and is frequently deregulated in human cancers

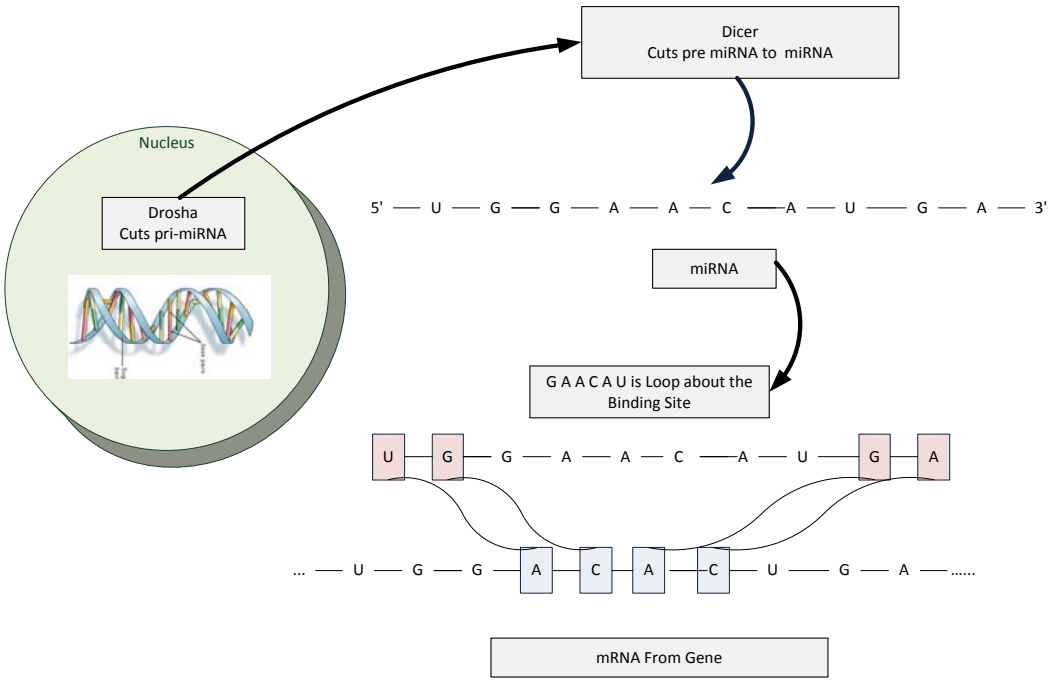
We examine these factors in some detail herein.

2.1 MIRNA PRODUCTION AND ACTION

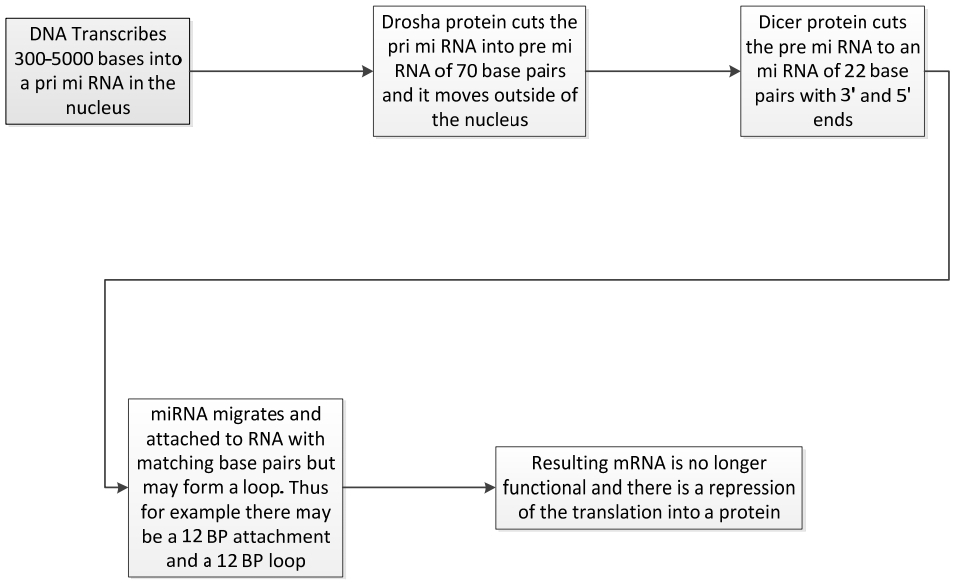
We now briefly examine the miRNA production and action. This is graphically demonstrated below. From segments of the DNA, segments not containing genes, a long segment called a pri-miRNA is generated and it is then cut to a shorter segment called a pre-miRNA and transported to the cytoplasm outside of the nucleus. Then another protein called Dicer cuts up the pre miRNA into about 22 base single-stranded pair segments which are the miRNA,

Then as we show below the small miRNA can bind to mRNA at complement sites, and in fact the binding may allow for a loop which extends out from the binding sites composed of non-complement base pairs. This binding then inactivates the mRNA and prevents its translation to a protein.

The process continues as follows (See Garcia and Miska in Appasani) and we show below the Drosha, Dicer, Pasha slicing that is a key element to this process.



The process is described in some detail below. Here we describe the steps one at a time as is currently understood (an alternative view of this is in the paper by He and Hannon, 2004).



It is also possible for the miRNA to target more than one mRNA since the miRNA may bind in its complement binding with many other such sites on other mRNAs. It is currently not clear what the affinity of binding is for a miRNA and any possible mRNA.

Also miRNA may be obtained from introns as well as exons. The former is called intronic and the latter called exonic. Now the exonic miRNA goes through the pri and preprocess whereas the intronic miRNA is cut directly to a pre miRNA segment (see Ying et al in Appasani).

miRNAs have been identified and currently there are well over 1,000. They are named in a simple numerical order such as miRNA 34.

2.2 SIRNA AND MIRNA

miRNA is a single stranded product of the process above. An alternative double stranded product is called small-interfering RNA or siRNA. siRNA usually trigger mRNA degradation whereas miRNA may cause degradation or suppression of translation to proteins. For this section we shall not focus a great deal on the siRNA functions.

2.3 DYNAMICS OF MIRNA

Now there may be some dynamics associated with this miRNA process as well. The model above assumes a simple one to one matching of miRNA and mRNA. However the generation of the two RNAs can be continuous and we should be looking at the concentrations. Thus we define:

[miRNA] to be the concentration of the miRNA

and

[mRNA] the concentration of the targeted mRNA

then we have a dynamic process. Namely we can see a process such as follows:

If [miRNA] < [mRNA] then there will be excess mRNA and its product protein P will have a [P] >0. Otherwise the miRNA will bind to all mRNA and there will be no resultant protein.

One may view miRNA as a buffer agent which controls the [P] of its associated [mRNA]. One can see in dynamic form the following model:

$$\frac{d[miRNA_i]}{dt} = K_{mi,i}[Pro_{mi,i}]$$

and

$$\frac{d[mRNA_i]}{dt} = K_{m,i}[Pro_{m,i}] - \kappa_i[miRNA_i]$$

where

$$[Pro] = Concentration\ of\ related\ promoter$$

Now since the binding is not necessarily 1:1, namely the miRNA may bind to several mRNA, then we may want to expand the above as follows:

$$\frac{d[miRNA_i]}{dt} = K_{mi,i}[Pro_{mi,i}]$$

and

$$\frac{d[mRNA_i]}{dt} = K_{m,i}[Pro_{m,i}] - \sum_{n=1}^{N} \kappa_{i,n}[miRNA_{i,n}]$$

where

$[Pro] = Concentration\ of\ related\ promoter$

3 MIR-34

miR-34 is one of now hundreds of micro RNAs, which are short, generally 22 base pairs, and non-coding RNA segments. They are now well known as control elements in the expression of genes and have significant control mechanisms.

3.1 MIR-34 STRUCTURE

From NCBI[3] (1p36.22; 1p36.22):

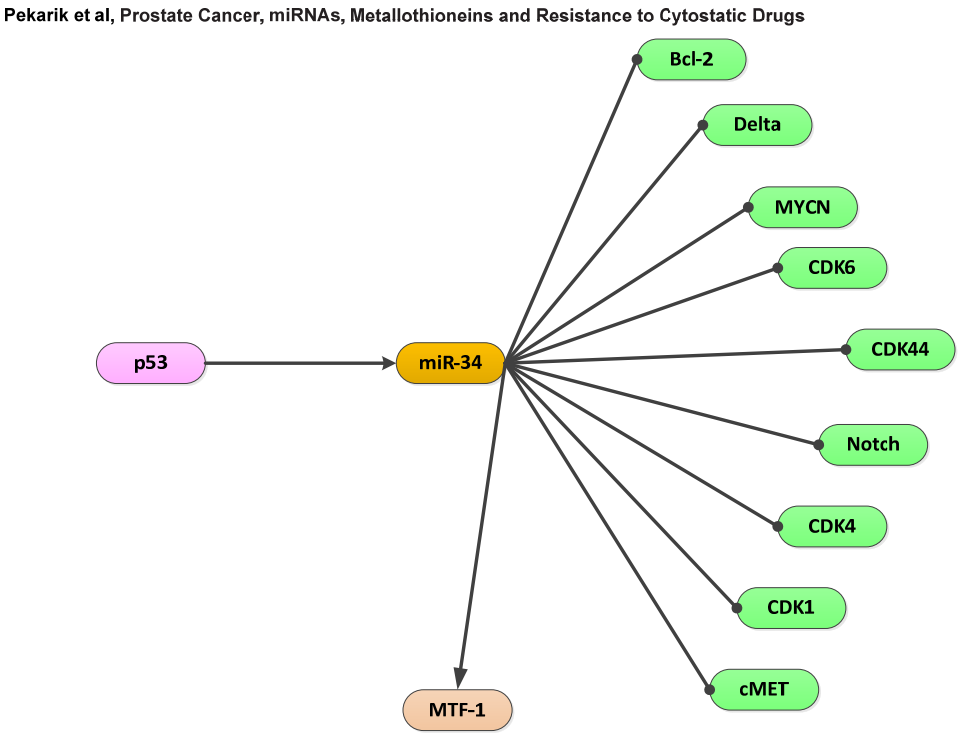
microRNAs (miRNAs) are short (20-24 nt) non-coding RNAs that are involved in post-transcriptional regulation of gene expression in multicellular organisms by affecting both the stability and translation of mRNAs. miRNAs are transcribed by RNA polymerase II as part of capped and polyadenylated primary transcripts (pri-miRNAs) that can be either protein-coding or non-coding.

The primary transcript is cleaved by the Drosha ribonuclease III enzyme to produce an approximately 70-nt stem-loop precursor miRNA (pre-miRNA), which is further cleaved by the cytoplasmic Dicer ribonuclease to generate the mature miRNA and antisense miRNA star (miRNA) products. The mature miRNA is incorporated into a RNA-induced silencing complex (RISC), which recognizes target mRNAs through imperfect base pairing with the miRNA and most commonly results in translational inhibition or destabilization of the target mRNA.*

3.2 MIR-34 PATHWAY CONTROL

miR-34 is an integral control element in cell homeostasis. The driver if p53 and then through miR-34 there are significant controls and activations. We demonstrate this graphically below based upon the work of Pekarik et al.

[3] http://www.ncbi.nlm.nih.gov/gene/407040

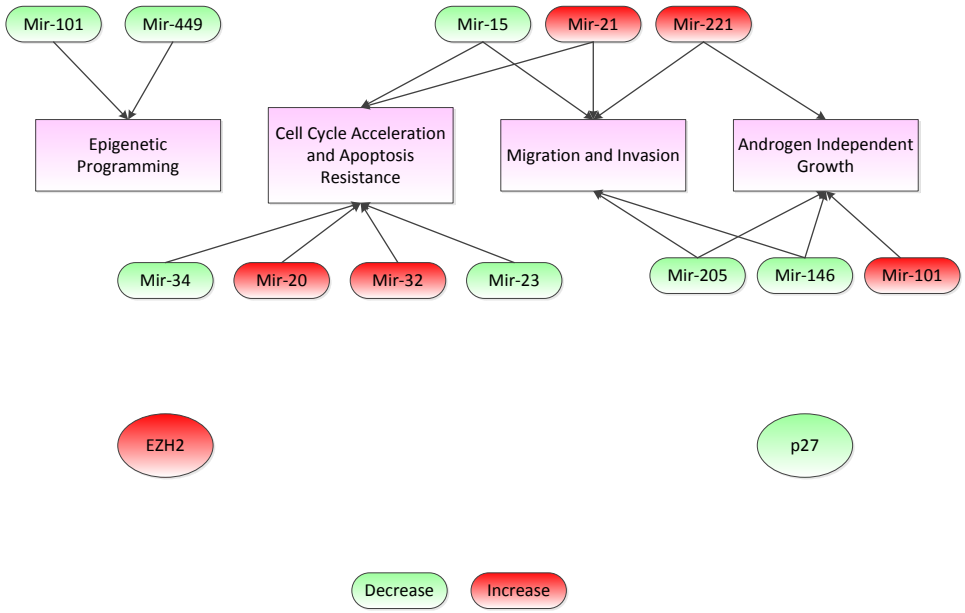

Pekarik et al, Prostate Cancer, miRNAs, Metallothioneins and Resistance to Cytostatic Drugs
Bcl-2
Delta
MYCN
CDK6
CDK44
Notch
CDK4
CDK1
cMET
p53
miR-34
MTF-1

4 MIRNA AND CANCER

There has been a great amount of research regarding the impact of miRNA on cancer and especially on PCa. miRNAs may downregulate tumor suppressor genes such as PTEN. This has been seen in miRNA 21. Colin and Croce have provided several review article regarding miRNA and their influence on cancers. They argue that miRNA alterations are heavily involved in the initiation of many cancers. Their focus had been on CLL, chronic lymphocytic leukemia, and its initiating miRNAs, miR 15 and miR 16. Coppola et al (2010) provide a detailed summary of miRNAs and PCa.

4.1 FUNCTIONS

The graphic from Coppola et al is shown below where it depicts a collection of miRNAs which impact various parts of the PCa process.



For example miR34 can cause the activation and recapitulate p53 which in turn induces cell cycle arrest and apoptosis. Loss of the miR34 can result in the impairment of the p53 control of apoptosis and permit the cells to proliferate. Coppola et al perform a detailed analysis of all of the above related miRNAs and their resultant impact on PCa. miR-21 up-regulation leads to PTEN loss and thus is an oncogene.

Recent work by Poliseno et al has shown that PTEN can be down regulated via miR-106b. It had already been known that PTEN could be down-regulated by miR-22, miR-25 and miR-302. Their work demonstrated that miR-22 and miR-106b are overexpressed in PCa miR-106b is an intronic miRNA. The work of Poliseno thus has demonstrated a proto-oncogenic miRNA dependent network that regulates PTEN and thus can have a significant role in initiating PCa.

Micro RNAs are regulators of mRNA, the post transcriptional result which is then used to generate via translation the operative protein. Currently there are nearly 1,000 identified miRNAs. They are generally 22 nucleotides long, short segments, and they usually target specific mRNA and silence it. Each one of the miRNA may act upon many mRNAs.

As He and Hannon state:

Non-coding RNAs participate in a surprisingly diverse collection of regulatory events, ranging from copynumber control in bacteria[1] to X-chromosome inactivation in mammals[2].MicroRNAs (miRNAs) are a family of 21–25-nucleotide small RNAs that, at least for those few that have characterized targets, negatively regulate gene expression at the post-transcriptional leve.

Members of the miRNA family were initially discovered as small temporal RNAs (stRNAs) that regulate developmental transitions in Caenorhabditis elegans[6]. Over the past few years, it has become clear that stRNAs were the prototypes of a large family of small RNAs, miRNAs, that now claim hundreds of members in worms, flies, plants and mammals.

The functions of miRNAs are not limited to the regulation of developmentally timed events. Instead, they have diverse expression patterns and probably regulate many aspects of development and physiology. Although the mechanisms through which miRNAs regulate their target genes are largely unknown, the finding that at least some miRNAs feed into the RNA INTERFERENCE (RNAi) pathway has provided a starting point in our journey to understand the biological roles of miRNAs.

miRNAs are simple yet complex entities and key players in the epigenetics which control gene expression.

It is clear from the above that miRNAs can positively and negatively impact many elements in the pathways we have considered in HGPIN and PCa. Coppola et al review several of the key ones. For example:

- miR-146: Down regulates the AR.
- miR-34: Can recapitulate p53 resulting in apoptosis and arrest.
- miR-23: can result in c-myc overexpression and cell proliferation.

In a recent paper by Poliseno et al they have identified several others:

- miR-106b: Down-regulates PTEN and triggers PIN in murine models.
- miR-22, miR-25, miR-302: Down-regulating of PTEN.

Similarly the papers by Petrocca et al and that by Calin and Croce detail many of the miRNAs and their impacts on many cancers. As seen in the above graphic these are but a few in the overall targeting of PCa control genes. As Coppola et al state:

The hypothesis that miRs can be regarded as new broad-spectrum oncogenes or tumor suppressor genes has opened a revolutionary field of research with exciting diagnostic and therapeutic perspectives.

The compelling hint of a widespread miR deregulation in cancer pathogenesis came from the analysis of the genomic distribution of 186 miR. In this study, it was demonstrated that more than half of them mapped in cancer-associated genomic regions, namely in chromosomal sites prone to deletions, amplifications or recombinations. These aberrations can result in miR down- or up-regulation, conferring selective advantages to mutated cells.

Additional mechanisms of miR deregulation include altered expression of miRs as a consequence of excessive or deficient processing; aberrant transcription of the precursors by epigenetic silencing of miR promoters or as a result of the activity of oncogenic transcription factors; and more rarely, point mutations in mature miRs or in target sequences that can interfere with normal target recruitment

The problem that we will have in any modeling of HGPIN and PCa is not only do we have issues regarding the somewhat well-known genes but the impact of the epigenetic factors is unknown, complex, and possibly random.

Furthermore miRNAs can act in a positive or negative manner depending upon the cell and the activated networks in the cell. From Croce (2009) we have:

Importantly, miRNAs should not be described as oncogenes or tumor suppressor genes, unless the tissue or cell type involved in their action is specified. For example, miR-221 and miR-222 target an oncogene, KIT, and inhibit the growth of erythroblastic leukaemia[30], and therefore function as tumor suppressors in erythroblastic cells. but they also target at least four important tumor suppressors −phosphatase and tensin homologue (PTEN), p27, p57 and tissue inhibitor of metalloproteinases 3 (TIMP3) −and function as oncogenic miRNAs by suppressing these tumor suppressors in various human solid tumours[31] (TABLE 1). Therefore, before describing an miRNA as a tumor suppressor or an oncogene, it is necessary to specify in which cell or tissue, as cellular context is crucial for the function of miRNAs….

Recent work on miR-34 has demonstrated its impact on p53 (Rokhlin et al) and the fact that miR-34 significantly mediates the role of p53 in apoptosis in AR dependent PCa.

As Sevli et al state:

The miRNAs have critical functions in gene expression and their dysregulation may cause tumor formation and progression. Today, it is known that tumors possess widespread deregulated miRNA levels. Over-expression or down-regulation of specific miRNAs in different tumor types make them potential therapeutic targets and diagnostic markers. Up-regulated miRNAs inhibiting tumor suppressor genes in tumor cells are commonly termed as oncogenic miRNAs or oncomirs. The miRNAs whose down-regulation promotes tumor progression are tumor suppressor miRNAs. One type of mRNA may possibly be targeted by multiple different miRNAs

with variable efficiencies. Conversely, a single miRNA may target more than one mRNA. Thus, to be able to observe a tumorigenic phenotype, some significant changes should occur in microRNome content of the cells.

4.2 MIRNA AND STEM CELLS

As we have indicated elsewhere, the concept of the cancer stem cell has received significant attention. There has also been a great deal of work on the area of linking miRNAs and the stem cell model for PCa. In a recent work by Liu et al (2011) the authors demonstrate the nexus between miR-34a and its ability to inhibit PCa stem cells by directly repressing CD44. They observe that cancer stem cells have been observed in many solid cancers by using the fact that CD44 adheres to the cell surface. PCa stem cells with enhance clonogenic and tumor initiating and metastatic capacities are often enriched with CD44+ cell population. The work of Liu et al demonstrated that the administration of miR-34a to PCa cells inhibited PCa metastasis and inhibited PCa regeneration. This is one of the first uses of miRNA as a tumor suppressor.

In a recent paper by Xia (2008) the author states:

The key characteristics of stem cells are that they are capable of self-renewal and differentiation. The mechanisms by which stem cells maintain self-renewal and differentiation are complicated. In the past years, protein-coding genes had been broadly investigated in stem cell self-renewal and differentiation.

Recent studies indicate miRNAs as one of the most abundant classes of post-transcriptional regulators proved to be crucial in a wide range of biological processes, which suggest that miRNAs may also play essential roles in stem cell self-renewal and differentiation. Disruption of Dicer function in murine ESs influences miRNA processing and greatly impairs their ability to differentiate …

Cancer stem cells (CSCs) are the cells within a tumor that possess the capacity to self-renew and to produce the heterogeneous lineages of cancer cells that comprise the tumor. CSCs can thus only be defined experimentally by their ability of self-renewal and tumor propagation.

The implementation of this approach explains the use of alternative terms in the literature, such as "tumor-initiating cells" to describe putative CSCs. …

The identification of growth and differentiation pathways responsible for CSC proliferation and survival will help in the discovery of novel therapeutic targets. Previous studies have shown that many signal pathways may participate in regulating CSC functions, including Wnt/β-catenin, Notch, and Sonic hedgehog homolog (SHH). The canonical Wnt cascade has emerged as a critical regulator of stem cells and activation of Wnt signalling has also been associated with various cancers …

CSC maintenance is dependent on β catenin signaling. Moreover, because Wnt/β-catenin signalling is not essential for normal epidermal homeostasis, such a mechanistic difference may

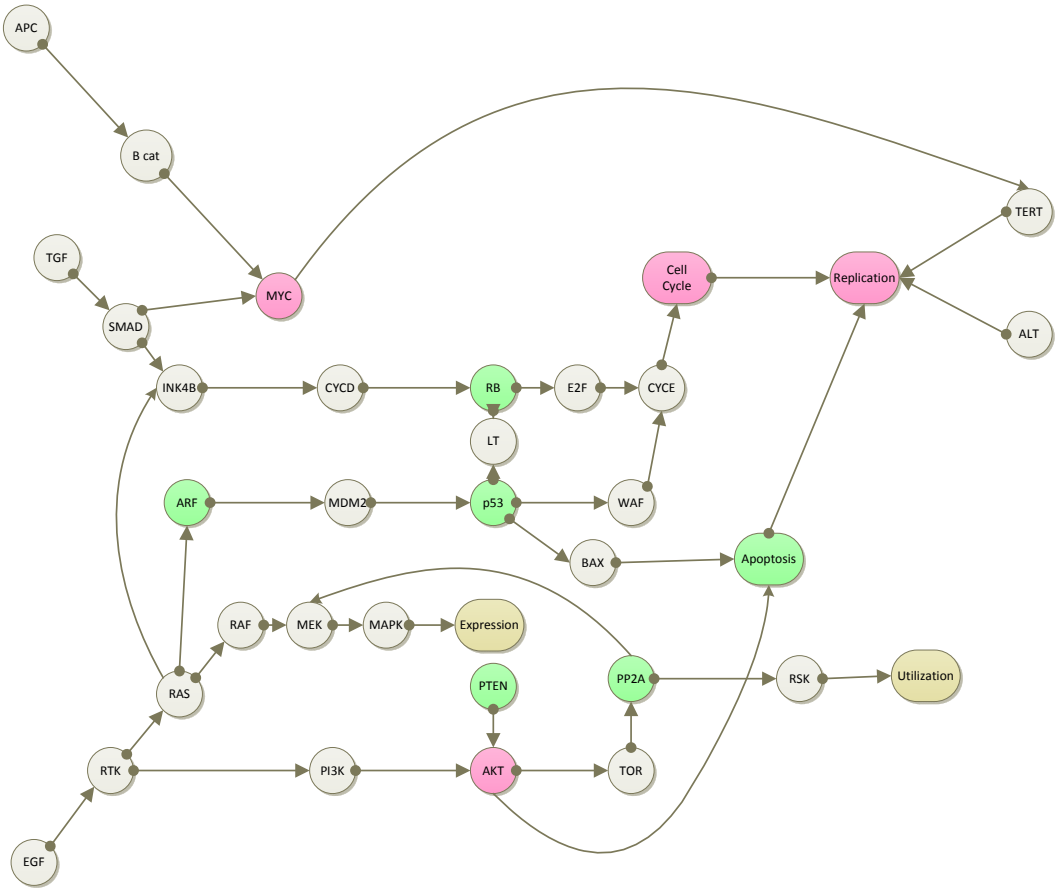
thus be targeted to eliminate CSCs and consequently eradicate squamous cell carcinomas. It is therefore hypothesized that inhibition of Wnt signaling may provide an effective way to reduce the unwanted stem cell renewal which results in cancers.

Inhibition of Wnt signalling may prove to be an effective road to inhibit the uncontrolled cell renewal that drives cancer. Acting as novel and pivotal regulators of protein-encoding genes, miRNAs will have great potential in regulating CSCs' biological functions by targeting CSCs-related signal pathway molecules.

We have performed various analyses of CSCs especially for PCa. This is a critical area for ongoing research and most likely will prove quite useful.

5 P53 PATHWAYS

The pathway issues here revolve around p53, a classic oncogene. The figure below shows some of the major linkages of p53 with other genes.



Focus on other pathway defects is continuing and there has been recent focus on MDM4, which is a control element of p53, the product of TP53 which is a key control element of proliferation and apoptosis. In a recent paper by Gembarska et al the authors state the following[4]:

The inactivation of the p53 tumor suppressor pathway, which often occurs through mutations in TP53 (encoding tumor protein 53) is a common step in human cancer. However, in melanoma—a highly chemotherapy-resistant disease—TP53 mutations are rare, raising the possibility that this cancer uses alternative ways to overcome p53-mediated tumor suppression. Here we show that Mdm4 p53 binding protein homolog (MDM4), a negative regulator of p53, is upregulated in a substantial proportion (~65%) of stage I–IV human melanomas and that melanocyte-specific

[4] http://www.nature.com/nm/journal/vaop/ncurrent/pdf/nm.2863.pdf

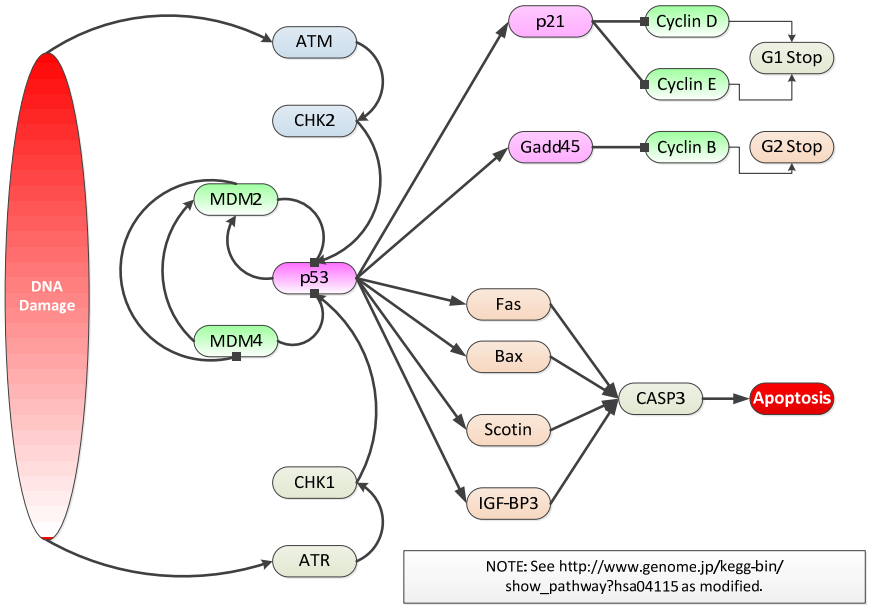
Mdm4 overexpression enhanced tumorigenesis in a mouse model of melanoma induced by the oncogene Nras.

MDM4 promotes the survival of human metastatic melanoma by antagonizing p53 proapoptotic function. Notably, inhibition of the MDM4-p53 interaction restored p53 function in melanoma cells, resulting in increased sensitivity to cytotoxic chemotherapy and to inhibitors of the BRAF (V600E) oncogene. Our results identify MDM4 as a key determinant of impaired p53 function in human melanoma and designate MDM4 as a promising target for antimelanoma combination therapy.

Now MDM4, also called Mdm4 p53 binding protein homolog, is located at 1q32. It acts in a somewhat complex manner to control p53 functions. From NCI we have the following description of the gene and its product :

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

The sources for information on p53 pathway and its relation to MDM4 are extensive . Specific details of the p53 pathway are shown in the NCI data bases for pathways. However, we shall present a simplified description based upon KEEG pathway data. This we do below (We combine from the KEGG genome database) We can further examine some of the p53 functions in the Figure below:



Note in the above that p53, when functioning properly, can detect DNA damage and correct it or lead the cell to apoptosis.

6 MET

MET is a tyrosine kinase receptor. It is activated by HGF the hepatic growth factor and it in turn activates a multiplicity of pathways. It is considered a proto-oncogene and thus is of general concern.

From NCBI[5]:

The proto-oncogene MET product is the hepatocyte growth factor receptor and encodes tyrosine-kinase activity. The primary single chain precursor protein is post-translationally cleaved to produce the alpha and beta subunits, which are disulfide linked to form the mature receptor. Various mutations in the MET gene are associated with papillary renal carcinoma.

MET is located on 7q31. We now examine the MET structure and then examine its control over several pathways.

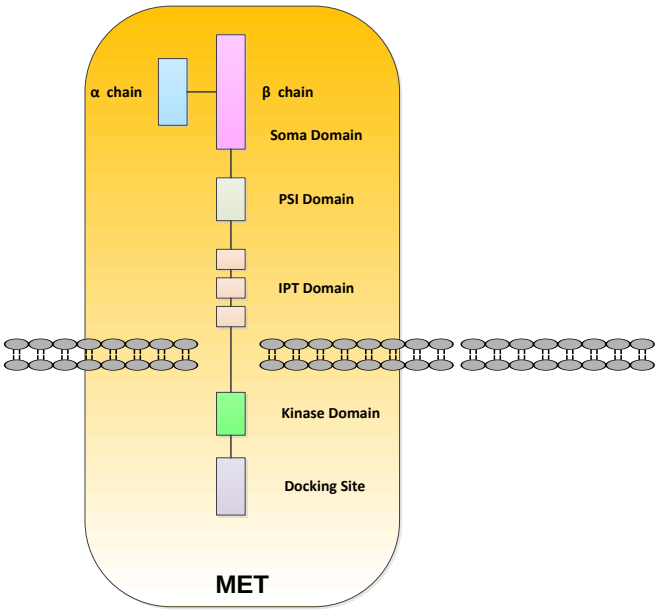
From Benvenuti and Comoglio we have:

Both MET and RON are tyrosine kinases crucially involved in the control of the ''invasive growth'' (Giordano et al., 2002). Under physiological conditions such as embryonic development and organ regeneration, they contribute to establishing the normal tissue patterning by orchestrating cellular proliferation, disruption of intercellular junctions, migration through the EMC and protection from apoptosis. In transformed tissues, receptor deregulation is responsible for cancer progression and metastasis formation and dissemination. Either upon ligand stimulation or receptor constitutive activation, cancerous cells are induced to leave the primary tumor, degrade the basal membrane, move towards different organs and there give rise to metastasis

6.1 MET STRUCTURE

We show the structure of MET below which shows the internal and external elements of the receptor.

[5] http://www.ncbi.nlm.nih.gov/gene/4233



See Organ and Tsao, Adv Med Onc

Organ and Tsao indicate the details above as follows:

The extracellular portion of c-MET is composed of three domain types.

*(i) The N-terminal 500 residues fold to form a large semaphorin (**Sema**) domain, which encompasses the whole a-subunit and part of the b-subunit.*

*The plexin semaphorin integrin (**PSI**) domain follows the Sema domain, spans approximately 50 residues and includes four disulphide bonds.*

*(iii) This domain is connected to the transmembrane helix via four immunoglobulin plexin transcription (**IPT**) domains, which are related to immunoglobulin-like domains.*

Intracellularly, the c-MET receptor contains;

(i) a tyrosine kinase catalytic domain flanked by distinctive …sequences. This portion of c-MET contains the catalytic tyrosines …which positively modulate enzyme activity, …

(ii) The multifunctional docking site in the C-terminal tail contains tyrosines …which recruit several transducers and adaptors when c-MET is active.

From Benvenuti and Comoglio we have:

MET tyrosine kinase is a disulphide-linked heterodimer originated from the proteolytic cleavage of a single chain precursor. The heterodimer is formed by a single-pass transmembrane beta chain (145 kDa) and a completely extracellular alpha chain (50 kDa). The extracellular segment

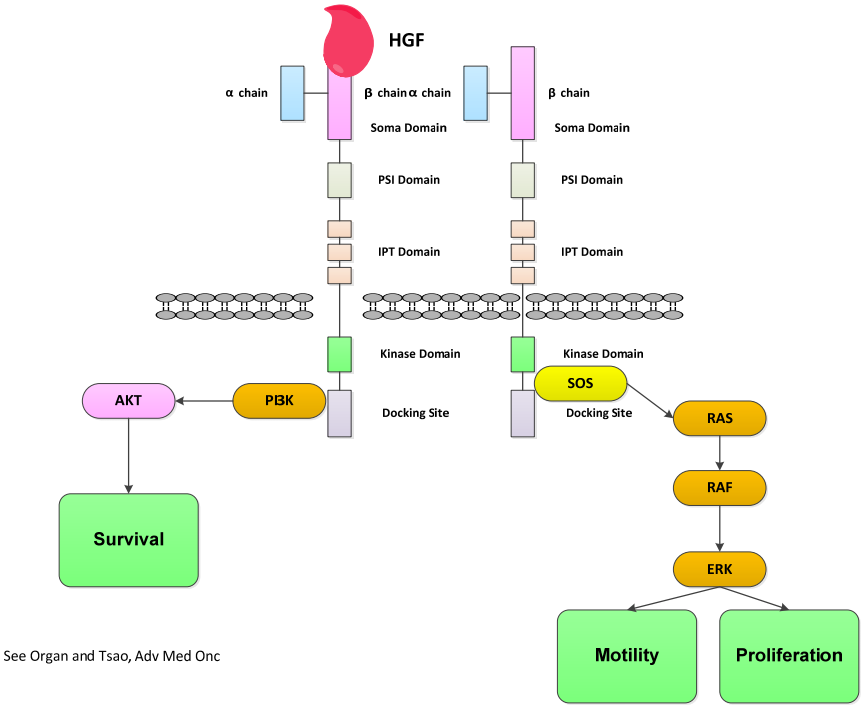
contains a Sema domain, an atypical motif made by over 500 amino acids, which has a low affinity binding activity for the ligand.

The extracellular portion comprises also a cysteine-rich domain (Cys domain) known as Met-related sequence (MRS), and four immunoglobulin-like structures (IPT domain), a typical protein–protein interaction region. The intracellular portion of the receptor is made of a juxtamembrane section followed by a catalytic site and a C-terminal regulatory tail.

The MET structure is thus a typical receptor and especially a typical kinase receptor.

6.2 MET CONTROLS

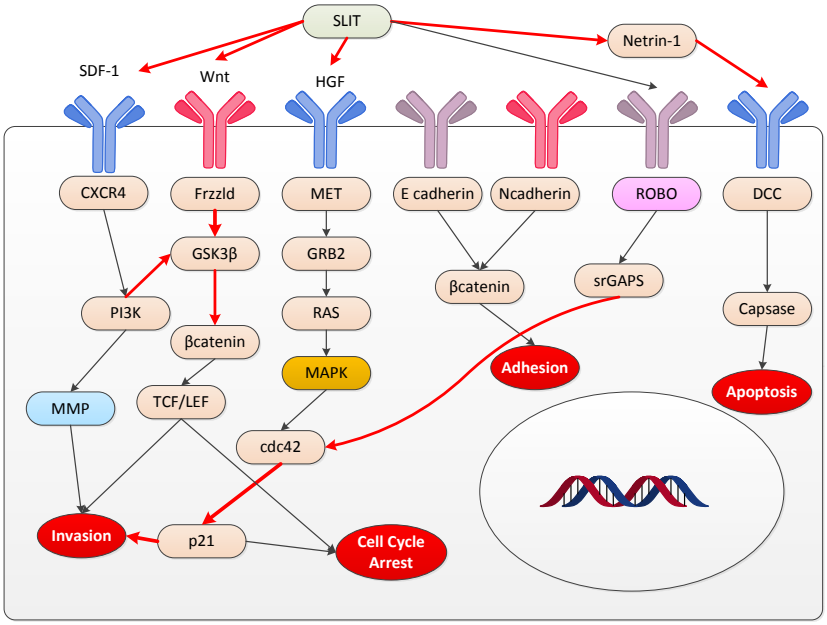
We now briefly examine MET pathway controls.



The above demonstrates the three control factors; survival, motility, and proliferation. All three are factors in cancer metastasis.

6.3 MET AND PATHWAYS

MET controls many pathway elements in a cell. We show some of them in the Figure below from Dickinson and Duncan. Note the HGF binds to MET and thus it activates a set of pathways facilitating invasion and stopping cell cycle arrest.



From: Dickinson and Duncan

The above demonstrates the MET pathway and its relationship to the many other key pathways.

6.4 MET AND CANCER

MET can be over expressed and over activated and the result is a malignant growth. Thus MET has the potential for becoming a significant factor in cancer development. From Benvenuti and Comoglio:

It has been extensively demonstrated that when used in a deviant cellular environment and without spatial and temporal regulation, MET exerts a major role in tumor formation and progression. Cells which over-express either MET or HGF are tumorigenic when implanted into nude mice and become extremely metastatic, moreover transgenic mice for either MET or HGF develop metastatic tumors while, on the contrary, endogenously expressing cancer cells become less aggressive when MET is switched off.

In the above the issue is over expression. The question is; what is driving that over expression? Is it truly an excess production or a loss of control or modification? Is this on a cell by cell basis or is it pandemic? They continue:

Accordingly, it was demonstrated that short hairpin RNA (shRNA) mediated MET knockdown in rabdomyosarcomas (RMS)-derived cell lines greatly affects cell proliferation, survival and invasion. Furthermore in xenograft models of RMS MET silencing produced a dramatic reduction of tumor mass. Similar results were obtained silencing MET in lung cancer cell lines harboring MET amplification. In those cell lines receptor silencing (once more achieved by shRNA technology) induced a significant growth inhibition; notably the silencing sorted no effects on cell lines that did not display receptor gene amplification.

This seems to answer the question regarding complete cell line activation.
It has been extensively described, both in animal models and in normally occurring human cancers, that constitutive activation of MET can be achieved in three different ways:

(i) with establishment of ligand-receptor autocrine loops;

(ii) via receptor over-expression, and

(iii) in presence of activating point mutations in the receptor coding sequence.

Ligand-receptor autocrine circuits make cells independent from the need of growth factors; receptor over-expression triggers receptor oligomerization and reciprocal activation even in absence of ligands; point mutations generate constitutively active receptors.

This last event is extremely uncommon; however, some missense point mutations have been described in MET coding sequence in certain human cancers.

The above discussion describes the ways in some detail. The causes of over expression could then be addressed as a therapeutic methodology. They continue:

Particularly missense mutations located in the tyrosine kinase domain of MET were described in patients who suffer from hereditary and sporadic papillary renal-cell carcinomas and head and neck squamous-cell carcinomas, whereas alterations in the juxta-membrane region were mainly found in human gastric and lung cancers

The above does also present the issue of mis-sense mutations, changes that may not change anything but may cause a cessation of genetic progression.

7 OBSERVATIONS

The paper which we have used to initialize the focus on this report is one which combines: mir-34, MET, p53, and methylation. It is an amalgam of receptors of the kinase inhibitor variety, key pathway oncogenes, miRNAs and methylation. It is an interplay between all of the complex elements which are now known in cancer genetics.

The Cheng et al results are simply as follows:

1. miR-34 Cooperates with p53 in Suppression of Prostate Carcinogenesis
2. p53 and miR-34 Cooperate in the Control of Prostate Stem/Progenitor Cell Activity
3. p53 and miR-34 Regulation of Stem/Progenitor Cells Depends on MET

However in their conclusions we have also introduced the methylation effects as well. They conclude:

Our study provides direct genetic proof that miRNAs of the miR-34 family may act as tumor suppressors in concert with other genes, such as p53. These findings offer a solid physiological basis for the rational design of diagnostic and therapeutic approaches. Because the lack of mir-34 genes alone is insufficient for cancer initiation, their downregulation is likely to occur at some point during tumor progression.

However, the preexistence of mir-34 methylation in some normal cells cannot be excluded. Further genomic studies in conjunction with animal modeling should be able to address this question. Although our current studies have been focused on prostate cancer, tissue-specific inactivation of mir-34 and p53 in other tissues will address likely interactions of these genes in other cell lineages.

Thus we have exhibited here a complex interplay between types of cell control mechanisms. The challenge will be how best to model this complex interplay. In our prior analyses we have let epigenetic factors be secondary and considered almost as noise. Here, however, they are pari passu with all other elements and must be considered expressly.

Also Liu et al from Tang's Lab state:

We have shown that miR-34a is underexpressed in tumorigenic CD44+ prostate cancer cells and that it has potent antitumor and antimetastasis effects. Our results establish miR-34a as a key negative regulator of CD44+ prostate cancer cells and CD44 as an important target of miR-34a. Our findings suggest that reduced expression of miR-34a in prostate CSCs contributes to prostate cancer development and metastasis by regulating expression of CD44 and the migratory, invasive and metastatic properties of CSCs

Tang's Lab has done extensive work on PCa CSC and the implications of reduced miR-34 are significant. The issue here is several fold. First, the measure of miR-34 activity can be prognostic. Second, the reasons for reduced miR-34 is of prime concern. As we shall note later, the cause may be methylation of CpG clusters. Thus if one were to try anti-methylation drugs, would that assist? There is always a concern here since anti-methylation therapeutics are non-selective.

8 REFERENCES:

1. Abba, M., et al, MicroRNAs in the Regulation of MMPs and Metastasis, Cancers 2014, 6, 625-645; doi:10.3390/cancers6020625.
2. Araud, T., Overview of the miRNA Pathways, Thesis, U Geneva, 2008.
3. Armstrong, L., Epigenetics, Garland (New York) 2014.
4. Bader, A. et al, Developing therapeutic microRNAs for cancer, Gene Therapy, 2011, V 18.
5. Bartel, D., MicroRNAs: Genomics, Biogenesis, Mechanism, and Function, Cell 2004 pp 281-297.
6. Benvenuti, S., P., Comoglio, The MET Receptor Tyrosine Kinase in Invasion and Metastasis, J. Cell. Physiol. 213: 316–325, 2007
7. Calin, G., C. Croce, MicroRNA Signatures in Human Cancers, Nat Rev 2006 pp 857-866.Calin, G., C. Croce, Chromosomal Rearrangements and microRNAs, Jrl Clin Inves 2007 pp 2059-2066.
8. Cheng, C. Y., et al, miR-34 Cooperates with p53 in Suppression of Prostate Cancer by Joint Regulation of Stem Cell Compartment, Cell Reports (2014), http://dx.doi.org/10.1016/j.celrep.2014.02.023
9. Coppola, V., et al, MicroRNAs and Prostate Cancer, Endo Rel Canc 2010 pp F1-F17.
10. Croce, C., Causes and Consequences of microRNA Dysregulation in Cancer, Nature 2009 pp 704-714.
11. Gottwein, E., B. Cullen, Viral and Cellular MicroRNAs as Determinants of Viral Pathogenesis and Immunity, Cell Host 2008, pp 375-387.
12. He, L., G. Hannon, MicroRNAs: Small RNAs with a Big Role in Gene Regulation, Nature, 2004 pp 522-532.
13. Huang, C., et al, A Study of Cancer Related MicroRNAs through Expression Data and Literature Search, World Acad Sci & Engr, 2009 254-256.
14. Jones, P., S. Baylin, The Epigenomics of Cancer, Cell, 2007 pp 683-692.
15. Kasinski, A., F., Slack, miRNA-34 Prevents Cancer Initiation and Progression in a Therapeutically Resistant K-ras and p53-Induced Mouse model of Lung Adenocarcinoma, Cancer Res Published OnlineFirst September 10, 2012.
16. Kim, N., et al, A p53/miRNA-34 axis regulates Snail1-dependent cancer cell epithelial–mesenchymal transition, J. Cell Biol. Vol. 195 No. 3 417–433, 2011.
17. Lawrie, C., MicroRNAs in Medicine, Wiley (Hoboken, NJ) 2014.
18. Liu et al, The microRNA miR-34a inhibits prostate cancer stem cells and metastasis by directly repressing CD44, Nature Medicine, 17, 211–215 (2011)

19. Liu, C., et al, The microRNA miR-34a inhibits prostate cancer stem cells and metastasis by directly repressing CD44, nature medicine VOLUME 17, NUMBER 2, FEBRUARY 2011
20. Mello, C., Return of the RNAi World: Rethinking Gene Expression and Evolution, Nobel Laureate Lecture 2006.
21. Organ, S., M. Tsao, An overview of the c-MET signaling pathway, Therapeutic Advances in Medical Oncology 3 (1), 2011, http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3225017/pdf/10.1177_1758834011422556.pdf
22. Pekarik, V., et al, Prostate Cancer, miRNAs, Metallothioneins and Resistance to Cytostatic Drugs, Current Medicinal Chemistry, 2013, Vol. 20, No. 4
23. Peruzzi, B., D. Battero, Targeting the c-Met Signaling Pathway in Cancer, Clin Cancer Res 2006;12:3657-3660.
24. Petrocca, F., et al, Emerging Role of miR-106b-25/miR-17-92 Clusters in the Control of Transforming Growth Factor β Signalling, Can Res 2008, pp 8191-8194.
25. Poliseno, L., et al, Identification of the miR-106b-25 MicroRNA Cluster as a Proto-oncogenic PTEN-targeting Intron That Cooperates with Host Gene MCM7 in Transformation, Sci Sig 2010 .
26. Powell, E., et al, Contribution of p53 to Metastasis, Cancer Discovery, April 2014.
27. Rakhlin, O. et al, MicroRNA-34 Mediates AR-dependent p53-indiced Apoptosis in Prostate Cancer, Can Bio & Therapy 2008 pp 1288-1296.
28. Sevigani, C., et al, Mammalian microRNAs: A Small World for Fine-tuning Gene Expression, Mam Gene V 17 2006, pp 189-201.
29. Sevli, S., et al, The function of microRNAs, small but potent molecules, in human prostate cancer, Prostate Cancer and Prostatic Diseases (2010) 13, 208–217
30. Steffan, J., et al, HGF-induced invasion by prostate tumor cells requires anterograde lysosome trafficking and activity of Na+-H+ exchangers, Journal of Cell Science 123, 1151-1159, 2010.
31. Stephen, A., et al, Dragging Ras Back in the Ring, Cancer Cell 25, March 17, 2014.
32. Xia, H., Great Potential of MicroRNA in Cancer Stem Cell, Jrl Can Mol 2008 pp 79-89.
33. Yamamura, S., et al, MicroRNA-34a Modulates c-Myc Transcriptional Complexes to Suppress Malignancy in Human Prostate Cancer Cells, PLOS One, January 2012, Volume 7, Issue 1, e29722