mi RNA and Melanoma

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by

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Micro RNAs and Melanoma

Contents

1 INTRODUCTION

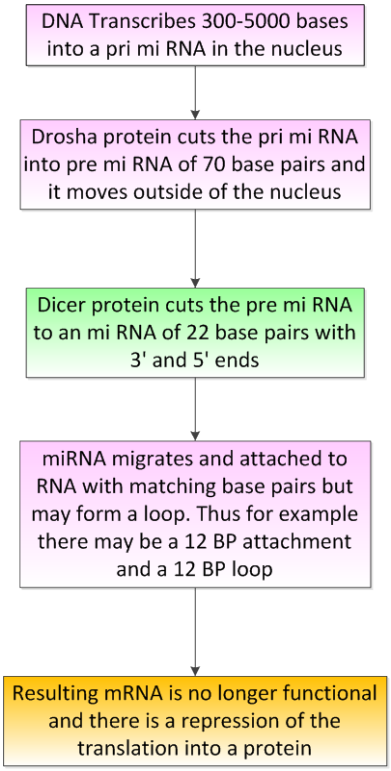
We have previously examined the impact of miRNAs in the development of cancers from several perspectives. In this brief analysis we take a recent finding regarding melanoma and a specific miRNA and then use it as a baseline to examine miRNAs in a broader context, focusing specifically on melanoma. The interest here is twofold; first, as a potential therapeutic target and second as a potential prognostic marker.

miRNAs have been examined for the past twenty years but just the last decade have they been understood specifically as elements in cancer control. Even more so, only in the past five years has their full impact been understood and the ability to manipulate certain miRNA paths controlled.

This section details many of the elements of miRNA as regards to cancer and metastatic control as well as the therapeutic control via miRNAs. What is of most significant interest is that miRNAs have such a pervasive set of control paths via activating oncogenes and suppressing genes which control metastatic growth. The miRNAs are not just control elements in select paths but appear to be control elements in the day to day paths of cellular homeostasis. This makes modeling of pathways significantly more complex.

It is critical to understand that as we have seen genomic models built around proteins, genes and pathways, we have also not seen the clear presence of miRNAs as integral parts of this process. One need just look at the many papers on pathway dynamics and almost to each one there is a total absence of miRNAs. We had proposed about five years ago that we look at miRNAs as noise, as at best epigenetic accidents which result in loss of expression. Now however it may be argued that they play as significant a role as the well-known pathways, albeit not yet fully understood.

Let us recall that the miRNA functions in a manner shown below:



We shall detail this process later in the document. However it is good to understand the nature of the miRNA. One key factor is that reproducing and introducing miRNAs appears to be rather straightforward. This perhaps they represent a powerful tool in the therapeutic arsenal.

The specific focus here is on miRNA-26a[1]. There are many databases now with a great deal of information regarding the miRNAs and we refer to them as in course.

2 RECENT OBSERVATIONS

We begin by examining a recent paper regarding miR-26a. As we shall discuss later this miRNA is found to be aberrant in multiple cancers and in the case of melanoma the disruption associated with several pathways is somewhat clearly understood. In a recent paper by Reuland et al the authors make the following observations[2]:

Melanoma is an aggressive cancer that metastasizes rapidly and is refractory to conventional chemotherapies. Identifying microRNAs (miRNAs) that are responsible for this pathogenesis is therefore a promising means of developing new therapies. We identified miR-26a through microarray and quantitative reverse-transcription–PCR (qRT-PCR) experiments as a miRNA that is strongly downregulated in melanoma cell lines as compared with primary melanocytes.

[1] http://www.mirbase.org/cgi-bin/mirna_entry.pl?acc=MI0000083

[2] http://www.nature.com/jid/journal/vaop/ncurrent/full/jid2012400a.html

Treatment of cell lines with miR-26a mimic caused significant and rapid cell death compared with a negative control in most melanoma cell lines tested.

In surveying targets of miR-26a, we found that protein levels of SMAD1 (mothers against decapentaplegic homolog 1) and BAG-4/SODD were strongly decreased in sensitive cells treated with miR-26a mimic as compared with the control.

The luciferase reporter assays further demonstrated that miR-26a can repress gene expression through the binding site in the 3′ untranslated region (3′UTR) of SODD (silencer of death domains). Knockdown of these proteins with small interfering RNA (siRNA) showed that SODD has an important role in protecting melanoma cells from apoptosis in most cell lines sensitive to miR-26a, whereas SMAD1 may have a minor role. Furthermore, transfecting cells with a miR-26a inhibitor increased SODD expression. Our findings indicate that miR-26a replacement is a potential therapeutic strategy for metastatic melanoma, and that SODD, in particular, is a potentially useful therapeutic target.

The observations focus on several key areas:

1. The impact of miRNAs on melanoma metastasis. As we will discuss there have been many previous studies implicating many miRNAs in this area. Thus seems to expand the results.

2. There appears to be a therapeutic approach to the issue by increasing the miRNA26a to further reduce by binding to the SODD facilitator product. There again have been several studies along this line recently. SODD is an interesting controlling gene/protein complex and the control via miR-26a is of significance.

3. There may be a prognostic indicator here as well. Again there has been a great deal of work in this field.

First we examine both the miRNA26a and SODD respectively and then we examine the issues discussed above in some detail. This represents just another of many studies regarding the use of miRNAs for the potential control of melanoma.

Before continuing it is useful to examine some of the additional comments the authors of the referred to article have made to the trade press relating to the release of the paper. Now one trade press article states[3]:

A University of Colorado Cancer Center study in this month's edition of the Journal of Investigative Dermatology describes a new target and potential treatment for melanoma, the most dangerous form of skin cancer. MicroRNA can decide which genes in a cell's DNA are expressed and which stay silent. Melanoma tends to lack microRNA-26a, which makes the gene SODD go silent.

3 http://medicalxpress.com/news/2012-12-serendipity-potential-therapy-melanoma.html also http://medicalxpress.com/news/2012-12-serendipity-potential-therapy-melanoma.html#jCp

"It's a double negative," says Yiqun Shellman, PhD, investigator at the CU Cancer Center, associate professor at the CU School of Medicine, and the study's co-senior author. "miR-26a works to stop the growth of cancer. You turn off this thing that should stop growth, and you have growth." When Shellman, David Norris and colleagues reintroduced microRNA-26a to melanoma cell lines that lacked it, they saw a marked decrease in cancer cell survival. MicroRNA-26a killed melanoma cells while leaving healthy cells unharmed. In fact, the discovery started back a couple steps.

First the group compared microRNA expression in healthy cells to that of microRNA expression in melanoma cells. "We hoped the difference between microRNA expression in healthy and melanoma cells would show which ones were contributing to tumorgenesis," Shellman says.

The microRNA most consistently different between healthy and cancerous cells was 26a. The discovery of how it works and what exactly it does was serendipitous. "We started by testing the effect of microRNA-26a on known gene targets to see if it was effecting the expression of logical, cancer-causing pathways, but none of them seemed affected in melanoma," Shellman says.

"We were working with the SODD gene in an unrelated project, and SODD has a putative but not high-scored binding site for miR-26a, and thought, why not test it? Sure enough, it turned out to be the target – microRNA-26a downregulates this gene." Shellman hopes this robust finding in cell cultures will help pave the way for future work with microRNA-26a as a therapeutic target in animal models and eventually a human trial.

"The first step is to further pinpoint the genetic signatures of the patients likely to benefit from microRNA-26a replacement therapy," Shellman says, noting that only some and not all melanoma cells were killed by miRNA replacement. "Maybe it's simply the downregulation of microRNA-26a itself, or maybe we can use SODD expression as the biomarker," Shellman says.

Once Shellman and colleagues discover the characteristics of a melanoma susceptible to microRNA-26a treatment, they hope funding will allow the lab to follow the promising therapy up the evolution from cells to humans.

As can be seen from the conversation above, there still may exist some questions of the details of the process. What is critical, however, is the fact that the miRNA plays such a prominent role, that one may target the miRNA, and that a pathway is a fundamental part of the development of a putative therapeutic. But fundamentally the last sentence above does diminish the ultimate enthusiasm.

The critical observations made here is the relationship between the controlling proteins, their related mRNA and the interference coming from miRNA. This has not been explored in significant detail until of late.

Another trade press review states as follows[4]:

[4] http://www.redorbit.com/news/health/1112752907/genetic-culprit-for-melanoma-found-122112/

Researchers from the University of Colorado Cancer Center say that they have discovered a new, more targeted way of treating melanoma, the most deadly form of skin cancer. The findings, described in a recent edition of the Journal of Investigative Dermatology, describe how small pieces of genetic material known as MicroRNA can choose the genes in a DNA cell that are either expressed or kept silent. With melanoma in particular, the researchers discovered a deficiency of microRNA-26a that usually silences the gene SODD.

"It's a double negative," explained the study's co-senior author Yiqun Shellman, an investigator at the University of Colorado Cancer Center and associate professor at the University of Colorado School of Medicine, in a prepared statement. "MiR-26a works to stop the growth of cancer. You turn off this thing that should stop growth, and you have growth."

In the study, melanoma cell lines that lacked microRNA-26a were reintroduced to the cell in a lab. As a result, there was a reduction in cancer cell survival and the microRNA-26a eliminated melanoma cells while leaving healthy cells alive. The team of investigators was able to compare the expression of microRNA in healthy cells to the expression of microRNA in melanoma cells.

"We hoped the difference between microRNA expression in healthy and melanoma cells would show which ones were contributing to tumorgenesis," continued Shellman in the statement. The researchers saw that the expression of micro-RNA-26 was consistently different between healthy and cancerous cells. Some, but not all, of the melanoma cells were eliminated by the replacement introduction of mRNA.

"The first step is to further pinpoint the genetic signatures of the patients likely to benefit from microRNA-26a replacement therapy," noted Shellman in the statement. "Maybe it's simply the downregulation of microRNA-26a itself, or maybe we can use SODD expression as the biomarker."

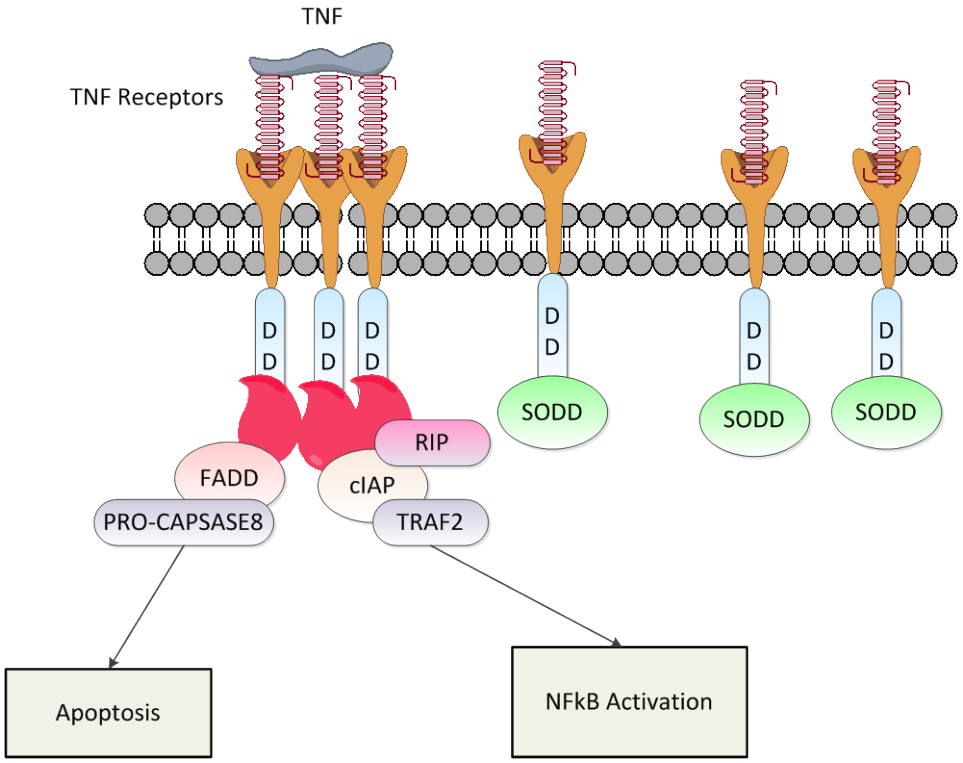
Moving forward, Shellman believes that her team's discovery of the role of MicroRNA in the development of carcinoma in cell cultures may eventually help develop new therapeutic techniques that could be used in real cancer patients.

This above statement is a simple reiteration of some of the prior work. Again it is clear that although experimentally observed, one is still quite a way from clinical reality.

3 SODD PATHWAY

Let us first consider the SODD pathway. From the Biocarta database we have the following graphic with the text noted below[5].

[5] http://www.biocarta.com/pathfiles/h_soddpathway.asp



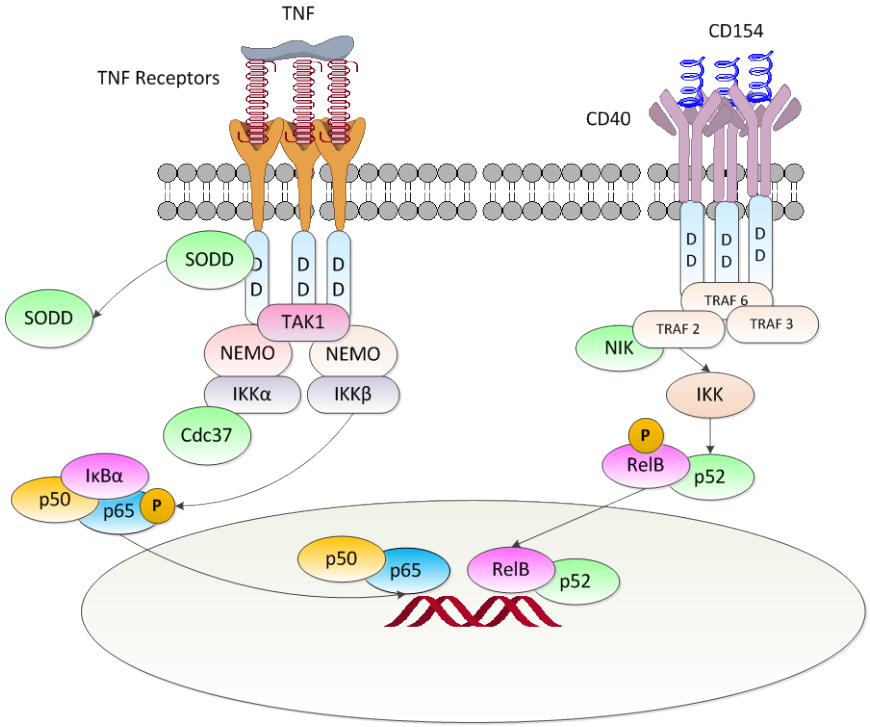
The tumor necrosis factor (TNF) receptor superfamily contains several members with homologous cytoplasmic domains known as death domains (DD). The intracellular DD are important in initiating apoptosis and other signaling pathways following ligand binding by the receptors. In the absence of ligand, DD-containing receptors are maintained in an inactive state.

TNF RI contains a cytoplasmic DD required for signaling pathways associated with apoptosis and NF-kB activation. Jiang et al. identified a widely expressed 60 kDa protein, named SODD (silencer of death domains), associated with the DD of TNF RI and DR3. Overexpression of SODD suppresses TNF-induced cell death and NF-kB activation demonstrating its role as a negative regulatory protein for these signaling pathways.

TNF-induced receptor trimerization aggregates the DD of TNF RI and recruits the adapter protein TRADD. This in turn promotes the recruitment of the DD-containing cytoplasmic proteins FADD, TRAF2 and RIP to form an active TNF RI signaling complex (Figure 1A). In contrast, SODD acts as a silencer of TNF RI signaling and does not interact with TRADD, FADD, or RIP (Figure 1B).4 It is associated with the DD of TNF RI and maintains TNF RI in an inactive, monomeric state. TNF-induced aggregation of TNF RI promotes the disruption of the SODD-TNF RI complex.

SODD does not interact with the DD of other TNF receptor superfamily members such as Fas, DR4, DR5, or TNF RII. SODD association with TNF RI may represent a general model for the prevention of spontaneous TNF signaling by other DD-containing receptors.

In a recent paper by Viatour et al, the authors examine the specifics of the NF-kB pathway elements regarding inflammation and cancer. We take the figure below as modified from their work. It demonstrates the SODD impact as an integrated whole.



As Viatour et al state regarding the above Figure[6] which is detailed in the footnote[7]:

The classical (blue arrows), alternative (green arrows) and atypical (purple arrows) NF-κB-activating pathways as illustrated by the TNF-α-mediated, CD40-mediated and DNA-damage-mediated NF-κB activation pathways, respectively. In the classical NF-κB-activating pathway, upon binding of TNFα to TNFR1, SODD is released from the receptor and triggers the sequential recruitment of the adaptors TRADD, RIP and TRAF2 to the membrane.

Then, TRAF2 mediates the recruitment of the IKK complex – composed of IKKα, IKKβ and NEMO – to the TNFR1 signalling complex. Hsp90 and Cdc37 are also part of the IKK complex and are required for the TNFα-induced IKK activation and shuttling of the IKK complex from the cytoplasm to the membrane, and ELKS connects IκBα to the IKK complex [83].

6 http://www.sciencedirect.com/science/article/pii/S0968000404002993

7 Abbreviations: CK2, casein kinase 2; ELKS, Glu-Leu-Lys-Ser; Hsp90, heat shock protein 90; IκB, inhibitor of NF-κB; IKK, IκB kinase; NEMO, NF-κB essential modulator; NF-κB, nuclear factor-κB; NIK, NF-κB-inducing kinase; RIP, receptor-interacting protein; RSK1, ribosomal S6 kinase 1; SODD, silencer of death domains; TNF-α, tumour necrosis factor α; TNFR1, TNF receptor 1; TRADD, TNF-receptor-associated death domain protein; TRAF, TNF-receptor-associated factor; Ub, ubiquitin.

Activation of the IKK complex leads to the phosphorylation of IκBα at specific residues, ubiquitination through binding of ubiquitin proteins and degradation of this inhibitory molecule via the proteasome pathway. Then, the heterodimer p50–p65 is released and migrates to the nucleus where it binds to specific κB sites and activates a variety of NF-κB target genes, including IL-8, IL-6, TNFα and many more.

The alternative pathway is triggered by binding of the CD40 ligand to its receptor, leading to recruitment of TRAF proteins and the sequential activation of NIK and IKKα, which then induces the processing of the inhibitory protein p100. p100 proteolysis releases p52 which forms heterodimers with RelB. This pathway is NEMO-independent and relies on IKKα homodimers. The atypical pathway, which is triggered by DNA damage such as UV, relies on sequential p38 and CK2 activations, and involves phosphorylation and subsequent IκBα degradation via an IKK-independent pathway.

Subsequently, free NF-κB moves into the nucleus to activate its target genes. Note that the DNA-damaging agent doxorubicin also triggers p65 phosphorylation via a p53- and RSK1-dependent pathway (not shown). Phosphorylation of the signalling molecules in addition to NF-κB and IκB proteins are illustrated.

Tschopp et al discuss apoptosis as in which follows[8]:

Spontaneous signaling from death-domain-containing receptors can result in inappropriate cell death. An inhibitory protein has recently been identified, called silencer of death domains (SODD), that binds to the death domain of tumor necrosis factor receptor 1, thereby negatively regulating downstream signaling.

Members of the tumor necrosis factor (TNF) ligand family are critically involved in the regulation of infections, inflammation, autoimmune diseases and tissue homeostasis. Binding of these ligands to their respective receptors leads to the triggering of diverse signaling pathways, including the activation of caspases, the activation and nuclear translocation of nuclear factor κB (NF-κB), and the activation of mitogen-activated protein kinases such as Jun N-terminal kinase (JNK). As each of these pathways leads to different outcomes, TNF-related ligands can promote apoptosis, differentiation, or proliferation.

The TNF-related factors are synthesized as trimeric type II transmembrane molecules that are capable of oligomerizing (trimerizing) the corresponding receptor. Consequently, ligand-bound receptors transmit the signal across the membrane by bringing their cytoplasmic portions into close proximity, leading to the recruitment and activation of downstream effector proteins. Those members of the TNF receptor family that induce apoptosis have in their cytoplasmic region a sequence of approximately 70 amino acids called the death domain, which is essential for signal transmission.

These domains represent protein–protein interaction motifs that have an intrinsic propensity to oligomerize and to interact with other death domains in a homophilic fashion. At present, six

[8] http://www.sciencedirect.com/science/article/pii/S0960982299802334

death-domain-containing receptors have been identified — Fas, TNF receptor 1 (TNF-R1), death receptor 3 (DR3; also known as TRAMP, Wsl, APO-3 and LARD), the two receptors for TNF-related apoptosis-inducing ligand (TRAIL) TRAIL-R1/DR4 and TRAIL-R2/DR5, and DR6

They previous figure has demonstrated the processes referred to above. Now from NCBI we have the definition and description of SODD (SODD (silencer of death domains) ; or BAG-4 8p11.23, BAG4 BCL2-associated athanogene) as follows[9]:

The protein encoded by this gene is a member of the BAG1-related protein family. BAG1 is an anti-apoptotic protein that functions through interactions with a variety of cell apoptosis and growth related proteins including BCL-2, Raf-protein kinase, steroid hormone receptors, growth factor receptors and members of the heat shock protein 70 kDa family.

This protein contains a BAG domain near the C-terminus, which could bind and inhibit the chaperone activity of Hsc70/Hsp70. This protein was found to be associated with the death domain of tumor necrosis factor receptor type 1 (TNF-R1) and death receptor-3 (DR3), and thereby negatively regulates downstream cell death signaling.

The regulatory role of this protein in cell death was demonstrated in epithelial cells which undergo apoptosis while integrin mediated matrix contacts are lost. Alternatively spliced transcript variants encoding distinct isoforms have been identified

The critical observation is the role of SODD in apoptosis. If it is inhibited then cell death is prevented.

Similarly for SMAD, NCBI states[10] (SMAD1 SMAD family member 1 or BSP1; JV41; BSP-1; JV4-1; MADH1; MADR1 at 4q31):

The protein encoded by this gene belongs to the SMAD, a family of proteins similar to the gene products of the Drosophila gene 'mothers against decapentaplegic' (Mad) and the C. elegans gene Sma. SMAD proteins are signal transducers and transcriptional modulators that mediate multiple signaling pathways. This protein mediates the signals of the bone morphogenetic proteins (BMPs), which are involved in a range of biological activities including cell growth, apoptosis, morphogenesis, development and immune responses.

In response to BMP ligands, this protein can be phosphorylated and activated by the BMP receptor kinase. The phosphorylated form of this protein forms a complex with SMAD4, which is important for its function in the transcription regulation. This protein is a target for SMAD-specific E3 ubiquitin ligases, such as SMURF1 and SMURF2, and undergoes ubiquitination and proteasome-mediated degradation. Alternatively spliced transcript variants encoding the same protein have been observed.

9 http://www.ncbi.nlm.nih.gov/gene/9530

10 http://www.ncbi.nlm.nih.gov/gene/4086

4 MIRNA-26A IN OTHER CANCERS

miRNA miR-26a has been found to play a role in other cancers. We examine a few of these malignancies here based upon work in the literature.

4.1 LUNG CANCER

Dang et al state regarding miR-26a the following[11]:

MicroRNAs (miRNAs) are a class of 21-23 nucleotide RNA molecules that play critical roles in the regulation of various cancers, including human lung cancer.

Among them, miR-26a has been identified as a tumor-related regulator in several cancers, but its pathophysiologic properties and correlation with the development of human lung cancer remain unclear. In this study, it was determined that miR-26a expression is clearly down-regulated in human lung cancer tissues relative to normal tissues.

Meanwhile, the overexpression of miR-26a in the A549 human lung cancer cell line dramatically inhibited cell proliferation, blocked G1/S phase transition, induced apoptosis, and inhibited cell metastasis and invasion in vitro. In contrast, a miR-26a inhibitor was used to transfect A549 cells, and the inhibition of endogenous miR-26a promoted cell metastasis and invasion. In addition, miR-26a expression inhibited the expression of enhancer of zeste homolog 2 (EZH2) and transactivated downstream target genes, including disabled homolog 2 (Drosophila) interacting protein gene (DAB2IP) and human Runt-related transcription factor 3 (RUNX3), which suggests that EZH2 is a potential target of miR-26a as previously reported. In conclusion, miR-26a plays an important role as an anti-oncogene in the molecular mechanism of human lung cancer and could potentially be used for the treatment of lung cancer.

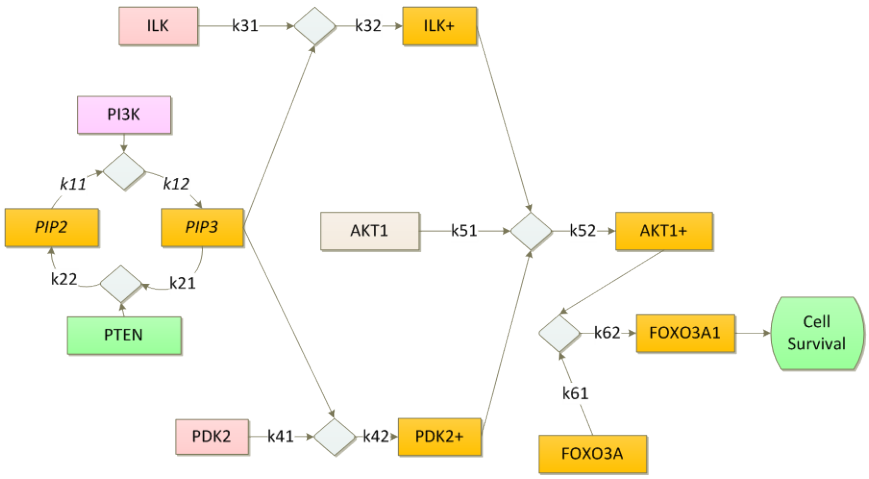
4.2 GLIOMA

Huse et al have examined miRNA26a in glimoas. Specifically they state:

Activated oncogenic signaling is central to the development of nearly all forms of cancer, including the most common class of primary brain tumor, glioma. Research over the last two decades has revealed the particular importance of the Akt pathway, and its molecular antagonist PTEN (phosphatase and tensin homolog), in the process of gliomagenesis.

Before continuing we present part of the AKT pathway as related to cell survival as below:

[11] http://www.ncbi.nlm.nih.gov/pubmed/22469510



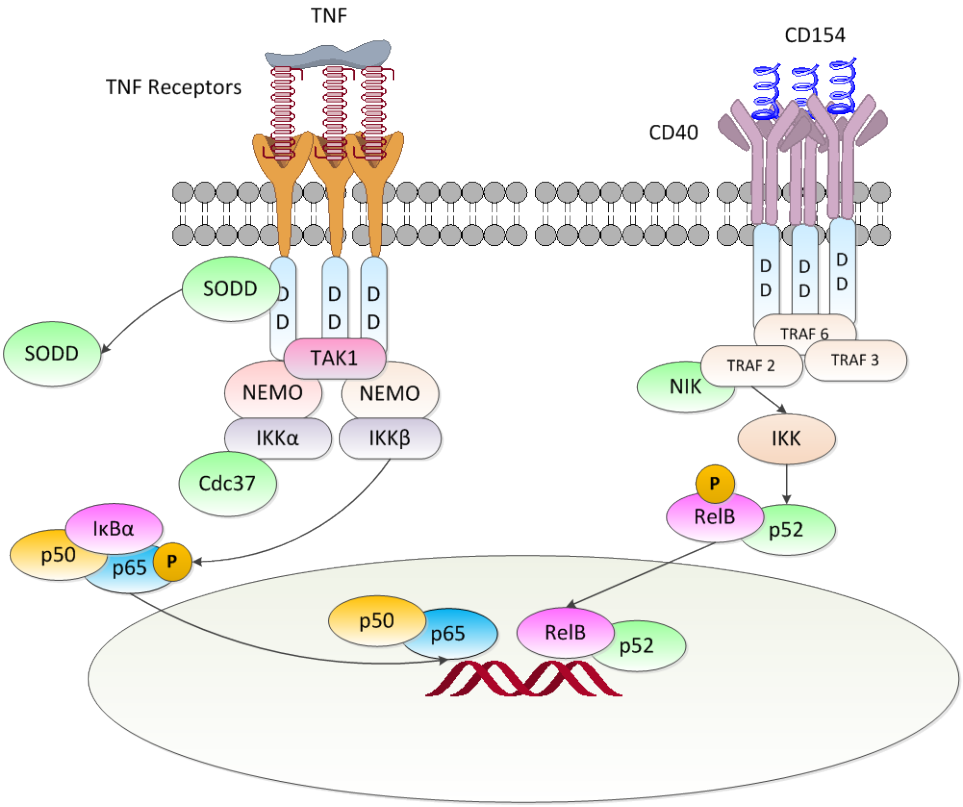
Note in this above graphic we show putative reaction rates associated with AKT and its up and down stream control elements. We have not depicted any miRNA control elements. The reason for this is that when this was developed there was no full recognition for how miRNA did interfere. We continue with the article on gliomas as follows:

Recent studies have also demonstrated that microRNAs (miRNAs) may be responsible for the modulation of cancer-implicated genes in tumors. Here we report the identification miR-26a as a direct regulator of PTEN expression. We also show that miR-26a is frequently amplified at the DNA level in human glioma, most often in association with monoallelic PTEN loss.

Finally, we demonstrate that miR-26a-mediated PTEN repression in a murine glioma model both enhances de novo tumor formation and precludes loss of heterozygosity and the PTEN locus. Our results document a new epigenetic mechanism for PTEN regulation in glioma and further highlight dysregulation of Akt signaling as crucial to the development of these tumors.

Chario presents the following graphics, as we have modified and simplified it, to describe the process[12]:

[12] http://www.sciencedirect.com/science/article/pii/S0962892409001342

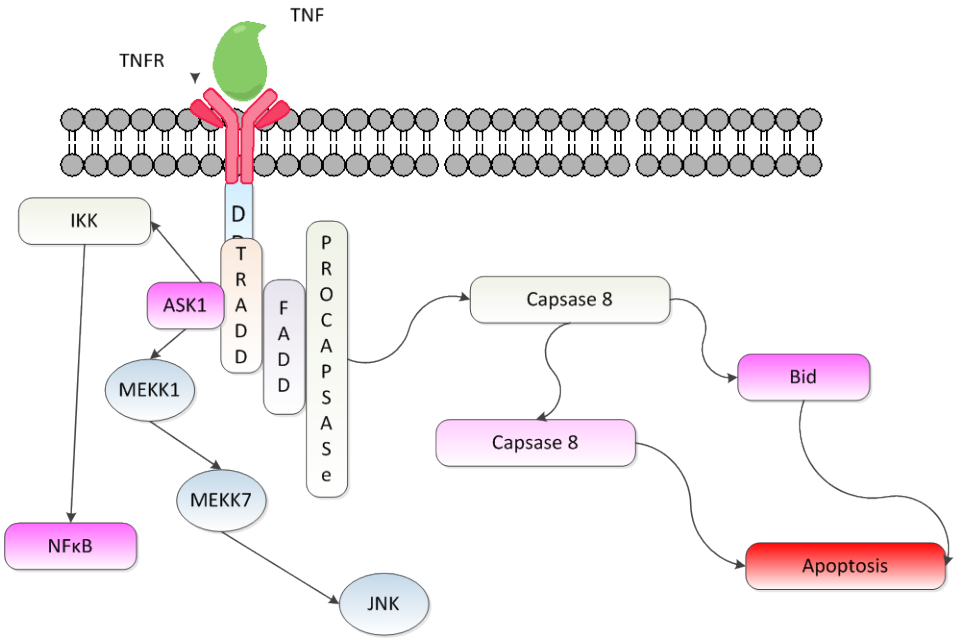


Chario then discusses some details of these pathways. We have examined them in detail before. He starts by stating

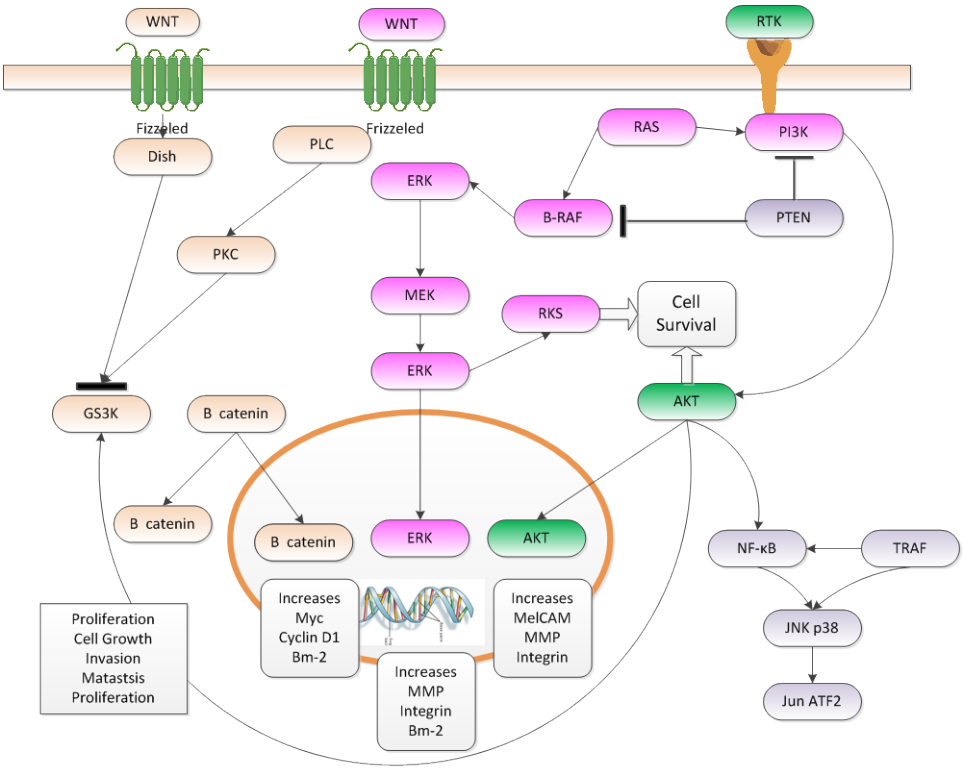
The IκB kinase (IKK) complex is involved in transcriptional activation by phosphorylating the inhibitory molecule IκBα, a modification that triggers its subsequent degradation, enabling activation of nuclear factor kappa B (NF-κB). Importantly, recent reports indicate that multiple cytoplasmic and nuclear proteins distinct from the NF-κB and IκB proteins are phosphorylated by the catalytic subunits of the IKK complex, IKKα or IKKβ.

Here, I describe how IKK subunits can have crucial roles in allergy, inflammation and immunity by targeting proteins such as SNAP23 and IRF7, but also in cancer by phosphorylating key molecules such as p53, TSC1 and FOXO3a through NF-κB-independent pathways. Thus, these recent findings considerably widen the biological roles of these kinases and suggest that a full understanding of the biological roles of IKKα and IKKβ requires an exhaustive characterization of their substrates.

Now Chario considers several cases in detail. The graphic above sets the general stage. He considers both immune and cancerous effects on the NF-κB functions. First recall the NF-κB production as shown below (see Barrett):



A more detailed view is shown below:



We can see the many well accepted pathways being processed.

Now Chario relates three IKK pathway details. We use the above diagrams as points of reference.

4.2.1 The IKKα- and IKKβ-dependent NF-κB-activating pathways.

On the left is the TNFα-dependent signalling pathway. Upon binding of TNFα to the TNFR1, SODD is released from the receptor and triggers the sequential recruitment of the adaptors TRADD, RIP and TRAF2 to the membrane. Then, TRAF2 mediates the recruitment of the IKK complex, composed of IKKα, IKKβ and NEMO, to the TNFR1 signalling complex. The scaffold proteins TAB2 and TAB3 subsequently bind to Lys63-polyubiquitylated substrates, such as RIP1, resulting in TAK1 and then IKKβ activations.

Whereas the receptor-proximal events that include the recruitment and activation of TAK1 rely on Lys63-linked polyubiquitylations, linear ubiquitin (Ub) chains are preferentially sensed by the UBAN motif of NEMO (not illustrated, see text for details). Hsp90 and Cdc37 are also part of the IKK complex and are required for the TNFα-induced IKK activation and shuttling of the IKK complex from the cytoplasm to the membrane. Activation of IKKβ leads to IκBα phosphorylation on specific residues, polyubiquitylation through binding of ubiquitin proteins and its degradation through the proteasome pathway.

Then, the heretodimer p50–p65 binds to specific κB sites and activates a variety of NF-κB target genes coding for pro-inflammatory cytokines (IL-6) and chemokines. A variety of proteins including TAK1, IKKβ, NEMO and p65 are also phosphorylated upon TNFα stimulation.

On the right is the alternative NF-κB-activating pathway. Binding of CD154 triggers the classical NEMO-dependent pathway (not illustrated) and the NEMO-independent cascade. This pathway relies on the recruitment of TRAF6 and the heterodimer TRAF2–TRAF3 to the CD40 receptor. NIK is subsequently activated by phosphorylation and polyubiquitylated in a degradative manner by the E3 ligase TRAF3. IKKα homodimers are activated by NIK and phosphorylate the inhibitory molecule p100, the partial processing of which generates the NF-κB protein p52. This latter transcription factor moves into the nucleus as heterodimer with RelB to regulate the expression of genes involved in lymphoid orgagenesis or coding for chemokines (BLC) or cytokines (BAFF).

4.2.2 The IKKα- and NF-κB-independent pathways.

Activation of the TLR9-dependent pathway triggers IKKα-mediated IRF7 phosphorylation in the cytoplasm and ultimately leads to IFN-α production. Nuclear IKKα phosphorylates the co-repressor SMRT, which triggers its nuclear export with HDAC3 and its degradation.

*As a result, co-activators such as CBP are recruited and the expression of NF-κB-dependent genes is induced. CBP is also phosphorylated by IKKα in the nucleus and this modification enhances its binding to NF-κB proteins and limits the expression of the p53-dependent target genes. Upon estrogen treatment (not represented), the co-activator SRC-3 is also phosphorylated by IKKα, a modification that is required for the expression of hormone-responsive genes (*cyclin D1, c-myc*) and, consequently, for breast cancer cell proliferation.*

4.2.3 The IKKβ- and NF-κB-independent pathways and their relevance in cancer.

TRAF2 is targeted by the deubiquitine ligase CYLD upon stimulation by TNFα and this post-translational modification facilitates the non degradative (Lys63-linked) polyubiquitylation of TRAF2. IKKβ also phosphorylates 14-3-3β upon TNFα stimulation and releases the 14-3-3β–TPP complex from ARE sequences found in multiple mRNAs.

As a result, those mRNAs coding for cytokines and chemokines are stabilized. TNFα also triggers the IKKβ-mediated phosphorylation of TSC1, which results in mTOR activation, S6K1 and 4EBP1 phosphorylations, and ultimately to the stimulation of angiogenesis through the transcriptional induction of the VEGF-encoding gene. FOXO3a is also a target of IKKβ and this phosphorylation triggers its nuclear export and its degradative polyubiquitylation in breast cancer cells harbouring constitutive IKKβ activity.

Dok1 is also phosphorylated by IKKβ upon TNFα stimulation and this modification positively regulates cell motility. IKKβ-mediated Aurora A phosphorylation causes its subsequent degradative polyubiquitylation, a pathway that is required for genome integrity.

The tumour-suppressor protein p53 is inactivated through an IKKβ-mediated phosphorylation and subsequent degradation through the proteasome pathway.

4.3 HEPATOCELLULAR CARCINOMAS

Zhu has examined miRNAs in the context of hepatocellular carcinomas. His work states that miR-26a is also present and it details other pathway control elements as well. Specifically Zhu states:

MicroRNAs (miRNAs) belong to a class of endogenously expressed, small non-coding RNAs that cause translational repression and/or mRNA destabilization by binding to the 30-untranslated regions (30-UTRs) of the target mRNAs (1). Approximately 40% of all miRNAs are located within intronic regions of protein-coding transcriptional units (TUs) (2).

Analysis of 175 human miRNAs across 24 different human organs reveals that the expression of intronic miRNAs largely coincides with the transcription of their host TUs (3), indicating that the intronic miRNAs and their host genes may be co-regulated and are generated from a common precursor transcript. Emerging evidence suggests that intronic miRNAs may be functionally associated with their host genes. Few reports suggest that there is an antagonizing effect of the intronic miRNA on the function of its host gene, as with miR-218 (4) and miR-10 (5,6).

The secreted Slit ligands and their Robo receptors constitute a Slit–Robo signaling pathway that controls the directed migration of neurons and vascular endothelial cells during embryonic development. miR-218 is localized in the intron of the Slit gene, and similar expression patterns are observed between miR-218 and Slit in different tissues.

We conducted serum starvation stimulation assays in primary fibroblasts and two-thirds partial-hepatectomies in mice, which revealed that miR-26a/b and CTDSP1/2/L were expressed

concomitantly during the cell cycle process. Specifically, they were increased in quiescent cells and decreased during cell proliferation. Furthermore, both miR-26 and CTDSP family members were frequently downregulated in hepatocellular carcinoma (HCC) tissues. Gain- and loss-of-function studies showed that miR-26a/b and CTDSP1/2/L synergistically decreased the phosphorylated form of pRb (ppRb), and blocked G1/S-phase progression.

Further investigation disclosed that miR-26a/b directly suppressed the expression of CDK6 and cyclin E1, which resulted in reduced phosphorylation of pRb. Moreover, c-Myc, which is often upregulated in cancer cells, diminished the expression of both miR-26 and CTDSP family members, enhanced the ppRb level and promoted the G1/S-phase transition. Our findings highlight the functional association of miR-26a/b and their host genes and provide new insight into the regulatory network of the G1/S-phase transition.

The cyclin presence is anticipated as regards to proliferation via lost cell cycle control. The G1/S phase transition is often a major transition in malignancies.

4.4 MELANOMA

Other researchers have examined miRNAs and melanoma as well. For example the work of Segura et al (2012) state:

Melanoma incidence and associated mortality continue to increase worldwide. The lack of treatments with durable responses for stage IV melanoma may be due, at least in part, to an incomplete understanding of the molecular mechanisms that regulate tumor initiation and/or progression to metastasis. Recent evidence supports miRNA dysregulation in melanoma impacting several well-known pathways such as the PI3K/AKT or RAS/MAPK pathways, but also underexplored cellular processes like protein glycosylation and immune modulation.

There is also increasing evidence that miRNA can improve patient prognostic classification over the classical staging system and provide new therapeutic opportunities. The integration of this recently acquired knowledge with known molecular alterations in protein coding genes characteristic of these tumors (i.e., BRAF and NRAS mutations, CDKN2A inactivation) is critical for a complete understanding of melanoma pathogenesis.

Here, we compile the evidence of the functional roles of miRNAs in melanomagenesis and progression, and of their clinical utility as biomarkers, prognostic tools and potential therapeutic targets. Characterization of miRNA alterations in melanoma may provide new angles for therapeutic intervention, help to decipher mechanisms of drug resistance, and improve patient classification for disease surveillance and clinical benefit.

The above work readily complements the work upon which we have focused this analysis.

Additional melanoma analyses has been done by Zehavi et al. Zehavi et al state[13]:

[13] http://www.molecular-cancer.com/content/11/1/44

We show that the expression of miRNAs from a large cluster on human chromosome 14q32 is significantly down-regulated in melanoma cell lines, benign nevi and melanoma samples relative to normal melanocytes. This miRNA cluster resides within a parentally imprinted chromosomal region known to be important in development and differentiation. In some melanoma cell lines, a chromosomal deletion or loss-of-heterozygosity was observed in the cis-acting regulatory region of this cluster.

In several cell lines we were able to re-express two maternally induced genes and several miRNAs from the cluster with a combination of de-methylating agents and histone deacetylase inhibitors, suggesting that epigenetic modifications take part in their silencing.

Stable over-expression of mir-376a and mir-376c, two miRNAs from this cluster that could be re-expressed following epigenetic manipulation, led to modest growth retardation and to a significant decrease in migration in-vitro. Bioinformatic analysis predicted that both miRNAs could potentially target the 3'UTR of IGF1R.

Indeed, stable expression of mir-376a and mir-376c in melanoma cells led to a decrease in IGF1R mRNA and protein, and a luciferase reporter assay indicated that the 3'UTR of IGF1R is a target of both mir-376a and mir-376c. Our work is the first to show that the large miRNA cluster

Note in the above the selection and determination of other miRNAs as well. It is not expected that any single miRNA will be considered the sole controlling element. In fact one may anticipate a progression as the tumor develops. The setting off of miRNAs as the tumor stage changes would be an interesting by-product of this analysis.

Another quite useful analysis of miRNAs and melanoma has been done by Taveira da Cruz and Jasiulionis. In their work the two authors state:

miRNAs are non-coding RNAs that bind to mRNA targets and disturb their stability and/or translation, thus acting in gene posttranscriptional regulation. It is predicted that over 30% of mRNAs are regulated by miRNAs. Therefore these molecules are considered essential in the processing of many biological responses, such as cell proliferation, apoptosis, and stress responsiveness.

As miRNAs participate of virtually all cellular pathways, their deregulation is critical to cancer development. Consequently, loss or gain of miRNAs function may contribute to tumor progression.

Little is known about the regulation of miRNAs and understanding the events that lead to changes in their expression may provide new perspectives for cancer treatment. Among distinct types of cancer, melanoma has special implications. It is characterized as a complex disease, originated from a malignant transformation of melanocytes.

Despite being rare, its metastatic form is usually incurable, which makes melanoma the major death cause of all skin cancers. Some molecular pathways are frequently disrupted in melanoma, and miRNAs probably have a decisive role on these alterations.

Therefore, this review aims to discuss new findings about miRNAs in melanoma fields, underlying epigenetic processes, and also to argue possibilities of using miRNAs in melanoma diagnosis and therapy.

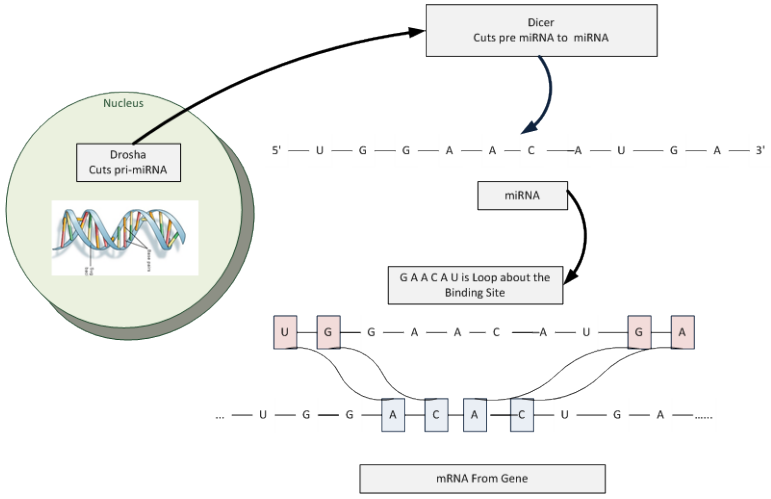
The conclusions drawn from the above paper are considerable. After just a few years there is now a well-accepted understanding of how miRNAs function and that they play critical roles in pathways. However, and this is a very significant however, we do not understand what precipitates them nor do we fully understand their relationship in pathway analysis. What is clear is that they are found in a multiple set of cancers, that that are pathway control elements, but the complex interactions we would anticipate are still unknown.

5 MICRO RNAS

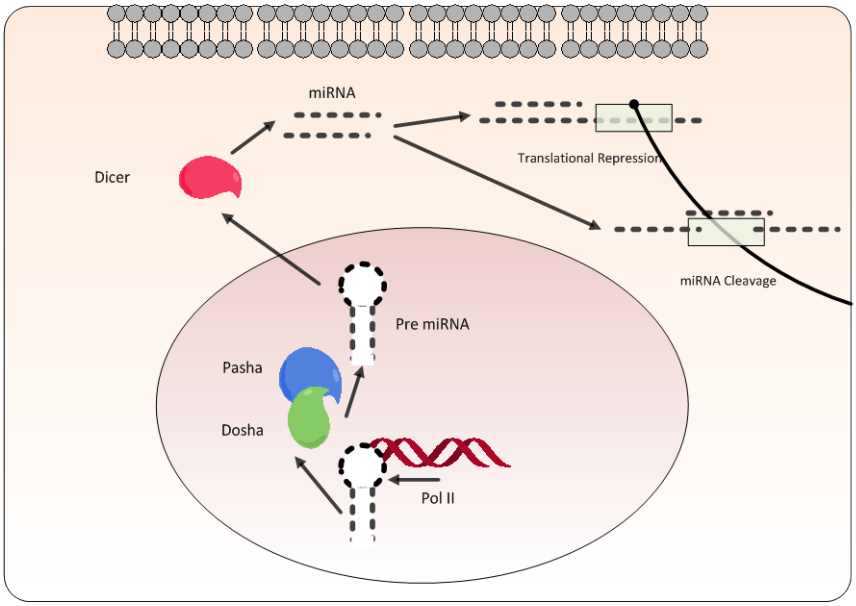
miRNAs are small (19-25 nucleotide single strand RNA) which have been created off intron sections of the DNA of a cell through pol II or pol III. They then operate on mRNA from exons which have escaped from the nucleus and are putatively maturing to proteins in the cytoplasm. Some of the proteins may be beneficial and some may not. The miRNAs seem to be secondary, and in some cases primary, pathway control elements. miRNAs contain RNA nucleotides, U, A, C, G. Thus simply stated if any possible combination is available there could be 4^{22} such miRNAs or about one trillion, equal to the national debt each year! This is a simplistic statement but it does provide a metric. We have discovered just more than a 1,000 miRNAs to data, with variants on some. Therefore a great deal more can be determined.

To demonstrate the recent occurrence of miRNA, it was not until the 6^{th} edition of Watson's Biology of the Gene in 2008 that we see a Chapter on controlling RNAs with miRNA (See Chapter 18). In addition even some of the recent literature lends miRNAs a place as a curiosity. In fact the more they are understood the more powerful they become.

In the classic review paper by Esquela-Kerscher, A. and F, Slack, they present an excellent discussion on miRNAs. First we present the overall construct. miRNAs are produced like all RNA and then pass through the Drosha/Pasha complex and emerge from the nucleus as a double RNA with a loop. Dicer cuts the loop creating single strand short RNAs which are the miRNA.



Now from the paper we have the more detailed description where we show how miRNA can interfere with RNA translation by either inhibiting it or by slicing the RNA and in turn also inhibiting it. We depict that below.



We rely upon that here, They state:

The biogenesis of microRNAs. *MicroRNA (miRNA) genes are generally transcribed by RNA Polymerase II (Pol II) in the nucleus to form large pri-miRNA transcripts, which are capped (7MGpppG) and polyadenylated (AAAAA). These pri-miRNA transcripts are processed by the RNase III enzyme Drosha and its co-factor, Pasha, to release the ~70-nucleotide pre-miRNA*

precursor product. (Note that the human let-7a-1 miRNA is shown here as an example of a pre-miRNA hairpin sequence. The mature miRNA sequence is shown in red.)

RAN–GTP and exportin 5 transport the pre-miRNA into the cytoplasm. Subsequently, another RNase III enzyme, Dicer, processes the pre-miRNA to generate a transient ~22- nucleotide miRNA:miRNA duplex.*

This duplex is then loaded into the miRNA-associated multiprotein RNA-induced silencing complex (miRISC) (light blue), which includes the Argonaute proteins, and the mature single-stranded miRNA (red) is preferentially retained in this complex. The mature miRNA then binds to complementary sites in the mRNA target to negatively regulate gene expression in one of two ways that depend on the degree of complementarity between the miRNA and its target. miRNAs that bind to mRNA targets with imperfect complementarity block target gene expression at the level of protein translation (lower left).

However, recent evidence indicates that miRNAs might also affect mRNA stability (not shown). Complementary sites for miRNAs using this mechanism are generally found in the 32 untranslated regions (3' UTRs) of the target mRNA genes. miRNAs that bind to their mRNA targets with perfect (or nearly perfect) complementarity induce target-mRNA cleavage (lower right). miRNAs using this mechanism bind to miRNA complementary sites that are generally found in the coding sequence or open reading frame (ORF) of the mRNA target.

They further detail it as follows:

MicroRNAs can function as tumour suppressors and oncogenes.

a. In normal tissues, proper microRNA (miRNA) transcription, processing and binding to complementary sequences on the target mRNA results in the repression of target-gene expression through a block in protein translation or altered mRNA stability. The overall result is normal rates of cellular growth, proliferation, differentiation and cell death.

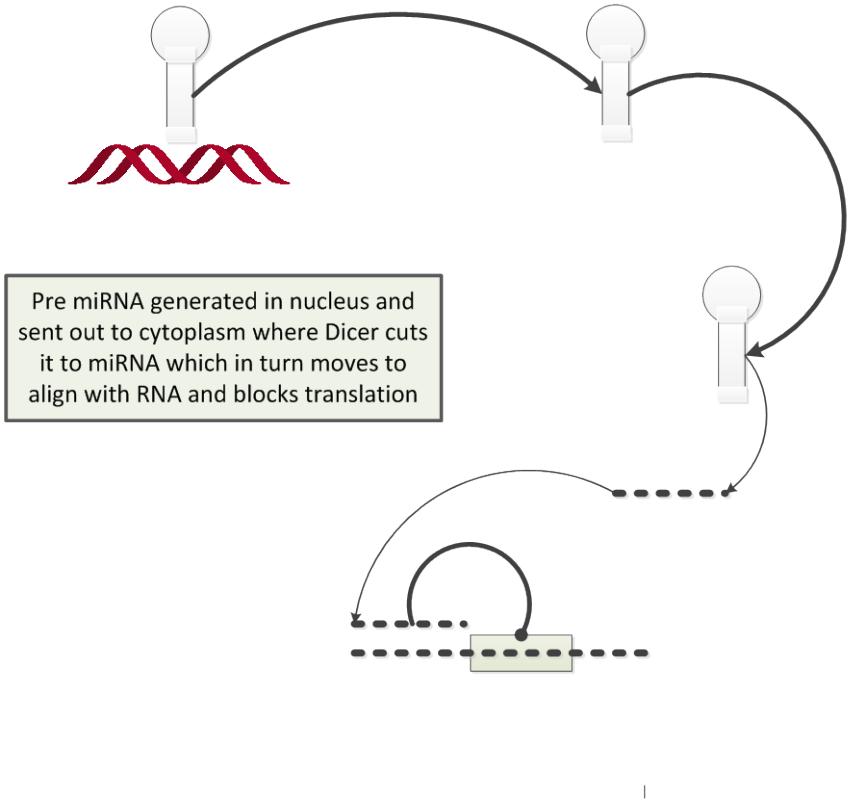
b. The reduction or deletion of a miRNA that functions as a tumour suppressor leads to tumour formation.

A reduction in or elimination of mature miRNA levels can occur because of defects at any stage of miRNA biogenesis (indicated by question marks) and ultimately leads to the inappropriate expression of the miRNA-target oncoprotein (purple squares). The overall outcome might involve increased proliferation, invasiveness or angiogenesis, decreased levels of apoptosis, or undifferentiated or de-differentiated tissue, ultimately leading to tumour formation.

The amplification or overexpression of a miRNA that has an oncogenic role would also result in tumour formation. In this situation, increased amounts of a miRNA, which might be produced at inappropriate times or in the wrong tissues, would eliminate the expression of a miRNA-target tumour-suppressor gene (pink) and lead to cancer progression.

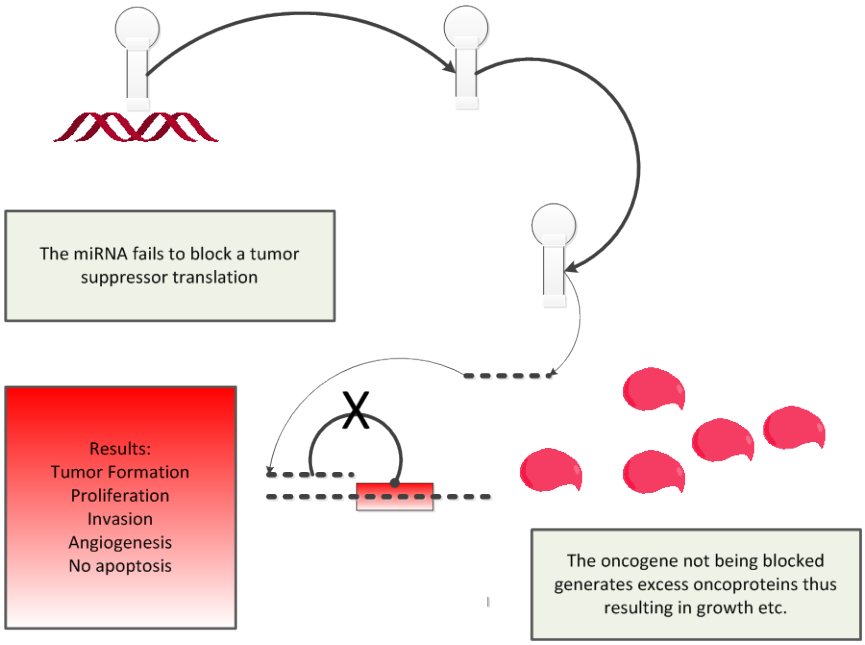
Increased levels of mature miRNA might occur because of amplification of the miRNA gene, a constitutively active promoter, increased efficiency in miRNA processing or increased stability of the miRNA (indicated by question marks). ORF, open reading frame.

We depict these three cases shown as follows. First, miRNA acting in a normal manner. This is below:

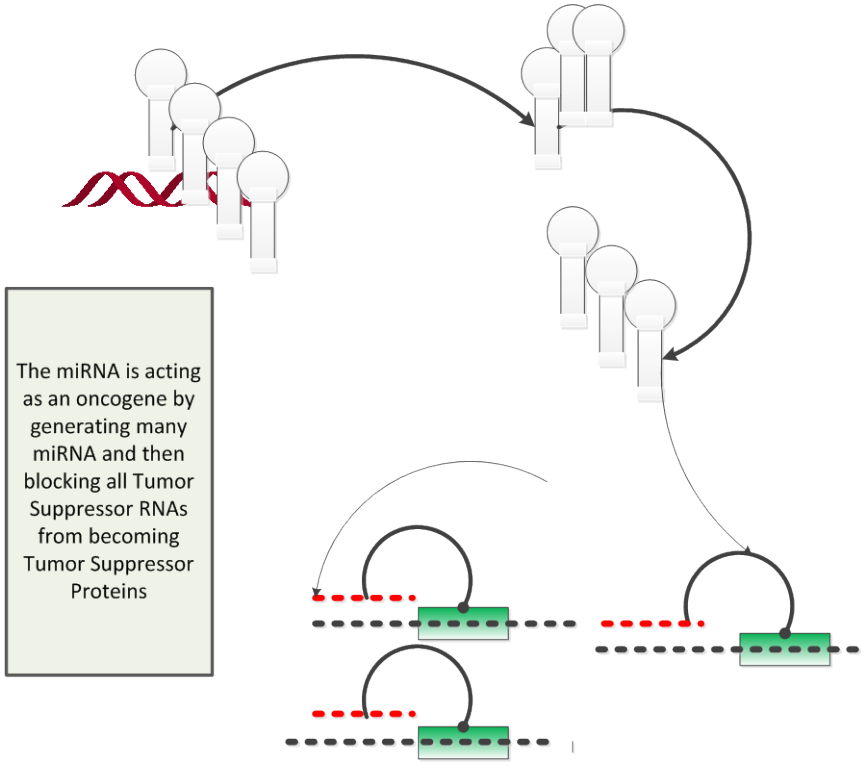


Notice above the miRNA is assumed to be a normal part of the control mechanism of the control of the conversion of the mRNA into a protein. It block the conversion.

Second, we now consider the second case. Here we have an oncogene which is not blocked by the miRNA and it results in many oncoproteins as shown below.



Third and finally in case 2 we have a massive explosion of miRNAs acting as onco activators as shown below.



These methods demonstrate in a somewhat simple manner how the miRNA functions in the case of certain cancers. It also demonstrates how the miRNA can become a target for therapeutics.

6 MEASUREMENTS OF MIRNA

There are many ways in which we can measure miRNAs. The technology has developed greatly in the past decade. An excellent paper by Pritchard et al presents an overview.

Pritchard et al comment on recovery of miRNA. This is an important observation in that it may generally be readily obtained:

It is possible to extract high-quality miRNA from a wide range of cell and tissue sources, including cell lines, fresh tissues, formalin-fixed paraffin-embedded (FFPE) tissues, plasma, serum, urine and other body fluids[27,28] (TABLE 1). The principles for isolating miRNA are, in general, the same as for isolation of total RNA, except that miRNA isolation protocols are often slightly modified to retain (and sometimes to enrich) the small RNA fraction[28]. Widely used commercially available products are generally based on chemical extraction using concentrated chaotropic salts, such as guanidinium thiocyanate (for example, Trizol and QIAzol reagents), followed by a solid-phase extraction procedure on silica columns. miRNAs may also be analysed using total RNA isolated by traditional chemical extraction with Trizol

Pritchard et al summarize the many profiling techniques. Specifically on Figure 3 of their paper (it is worth looking at the paper rather than trying to replicate what they have accomplished) they depict the following:

Approaches to microRNA profiling.

a. Quantitative reverse transcription PCR (qRT-PCR). In TaqMan qRT-PCR, the reverse transcription reactions use stem–loop primers that are specific to the 3! end of the microRNA (miRNA) for specificity (top left). Amplicons are generated using an miRNA-specific forward primer and a reverse primer. As the DNA polymerase proceeds along the template, the TaqMan probe is hydrolysed and fluorescent dye is freed from the quencher, resulting in light emission (top middle). In SYBR-green-based qRT-PCR, miRNA is typically polyadenylated at the 3! end, and oligo-d(T) is used as a reverse transcription primer (bottom left). An miRNA-specific forward primer and a reverse primer that anneals to the 3! portion of the miRNA sequence as well as to the poly(A) tail enable PCR amplification with dsDNA-intercalating SYBR green dye as the detector (bottom middle). Both TaqMan and SYBR-green-based qRT-PCR are available in 'array' format (right).

b. miRNA microarray. DNA-based capture probes (which may or may not incorporate LNA-modified bases) are used to capture fluorescently tagged miRNAs; this is followed by scanning of slides and quantification of fluorescence.

c. Nanostring nCounter. A bridge oligonucleotide templates ligation of an miRNA to a specific tag. Capture and detection is done by two target-specific probes: a capture probe containing biotin to allow adsorbance to the solid phase via streptavidin and a second reporter probe with an individual colour-coded sequence. No amplification or labelling of miRNA is required with this method.

d. RNA sequencing (RNA-seq). Currently established RNA-seq platforms begin with reverse transcription of miRNA to a cDNA library. Adaptor ligation then allows the library either to be affixed to a solid phase, as in the Illumina platform, or to beads for emulsion PCR, as in the Roche and ABI platforms (for details of sequencing chemistry

7 MIRNA MELANOMA TARGETS

Profiling of common nevi CN and atypical nevi AN by miRNA has been reported by Holst et al. They conclude:

MicroRNAs (miRNAs) are small non-coding RNAs, which regulate gene expression through base pairing with mRNA and which are crucially involved in carcinogenesis (the so-called oncomiRs). We compared the miRNA signature between acquired melanocytic nevi showing clinical atypia (atypic nevi, AN) and common acquired nevi (common nevi, CN).

We obtained miRNA profiles from 41 biopsies (22 AN and 19 CN) and showed that AN could be differentiated from CN on the basis of the expression of 36 miRNAs (false discovery rate <0.05). OncomiRs were present in this group, and we further confirmed the differential expression of miR-125b and let-7c by qRT-PCR. Our data suggest that miRNAs are functionally involved in the pathogenesis of nevi and possibly malignant melanoma. …

Our study showed that AN can be differentiated from CN on the basis of the expression of 36 miRNAs. Furthermore, AN showed a tendency to further clustering into two groups, which could define different subtypes of AN. The fact that AN are molecularly heterogeneous has also been suggested by Scantolini et al. (24) on the basis of mRNA expression profiles in melanocytic lesions.

Among the differentially expressed miRNA, we found the known oncomiRs let-7a, let-7c and miR-125b. The expression pattern showing a reduced miR-125b expression indicates a more 'malignant' miRNA signature of AN in comparison with CN. Because N-RAS and P53, known to be involved in MM, can be targeted by these miRNAs (the list of other predicted targets is shown in the supplementary material), further studies should identify their functional significance in the pathogenesis of melanocytic lesions.

In another paper by Segura et al the authors find:

MiRNA signatures have potential as clinically relevant biomarkers of prognosis in metastatic melanoma. Our data suggest that molecularly based models of risk assessment can improve the standard staging criteria and support the incorporation of miRNAs into such models.

They state:

MiRNAs significantly associated with post-recurrence survival. Eighteen miRNAs found overexpressed in metastatic tissues of patients with longer survival (≥1.5 y) compared with

patients with shorter survival (<1.5 y). Fold change, FDR, Cox regression coefficient (d), chromosome location, and host genes (when pertinent) are indicated.

They then present a set of miRNA and calculate their influence.miRNA	Score (d) Cox Regression Coefficient	Location	Host Gene
has-miR-150	−3.98	19q 13.33	Intergenic
has-miR-455-3p	−3.52	9q32	COL27A1
has-miR-145	−3.06	5q 33.1	Intergenic
has-miR-342-3p	−2.9	14q 32.2	EVL
has-miR-497	−2.87	17p 13.1	AC027763.1
has-miR-155	−2.77	21q 21.3	Intergenic
has-miR-342-5p	−2.66	14q 32.2	EVL
has-miR-143	−2.61	5q 33.1	Intergenic
has-miR-193a-3p	−2.43	17q 11.2	Intergenic
has-miR-146b-5p	−2.38	10q 24.32	Intergenic
has-miR-28-3p	−2.32	3q 28	LPP-201
has-miR-10b	−2.14	2q 31.1	HOXD3
has-miR-193b	−2.08	16 p3.12	Intergenic
has-miR-28-5p	−1.87	3q 28	LPP-201
has-miR-142-5p	−1.86	17q 22	Intergenic
has-miR-143	−1.73	5q 33.1	Intergenic
has-miR-126	−1.73	9q34.3	EGFL7
has-miR-214	−1.72	1q 24.3	DNM3

They conclude with:

Many of the miRNAs from our signature are located in genomic regions previously reported as altered in melanoma, such as loss of 9q32 (miR-455-3p) in melanoma cell lines, gain of the 5q locus (miR-145) in acral melanoma, and gain of 21q (miR-155) in uveal melanoma (Supplementary Table S8).

In conclusion, our results show the potential of miRNAs as clinically useful markers of prognosis in metastatic melanoma patients. A six-miRNA signature was able to improve risk stratification for stage III patients, suggesting that miRNAs may serve as a useful molecular adjunct to the current morphologic staging system in identifying high-risk patients who might benefit from adjuvant therapy.

Differential expression of most miRNAs from the predictor signature was also observed in the matched-pair primary tissue, suggesting that the miRNA signature may also play a role in prognosis of early lesions. Further studies with a larger cohort of primary melanoma patients are needed to better define the role of the signature in predicting the development of aggressive disease.

Recent work by Luo also details similar markers (p 66).Luo also details a more complex causative analysis of miR-101. Luo also does a detailed analysis of MITF as well as EZH2 which we have discussed in previous literature analyses. Luo concludes:

To test whether miR-101 is able to down-regulate MITF protein expression, we transfected Ma-Mel-79b and -86b cells with miR-101 mimic and performed Western blot analysis using antibody against MITF. As shown … MITF protein was decreased to 62 % and 56 % in Ma-Mel-79b and -

86b cells at 72 hr after transfection of miR-101. We also tested the expression of EZH2, which has been reported to be an oncogene and a target of miR-101 in various types of. Indeed, overexpression of miR-101 down-regulated EZH2 in the two tested melanoma cell lines. Interestingly, overexpression of miR-101 in both cell lines also caused a cleavage of poly-(ADP-ribose) polymerase (PARP) which is one of the targets of caspase-3 and serves as a indicator for apoptosis

Targets for ascertaining prognosis are critical. Not only for melanoma, but they are critical for all types of cancer. miRNAs seems to be such a set of targets.

8 THERAPEUTIC APPLICATION

There is always the desire to find therapeutic methodologies to control cancers and particularly melanomas. With miRNA we have a simple RNA structure, some 22 nucleotides, which can be reproduced or modified to control aberrant pathways. Rather than trying to match to either complex RNAs or proteins we could possibly find more readily targeted miRNAs.

Kota et al present an analysis of the application of miRNA in the area of therapeutics. The have discussed how this may apply to hepatocellular carcinomas. They state:

Therapeutic strategies based on modulation of microRNA (miRNA) activity hold great promise due to the ability of these small RNAs to potently influence cellular behavior. In this study, we investigated the efficacy of a miRNA replacement therapy for liver cancer. We demonstrate that hepatocellular carcinoma (HCC) cells exhibit reduced expression of miR-26a, a miRNA that is normally expressed at high levels in diverse tissues.

Expression of this miRNA in liver cancer cells in vitro induces cell-cycle arrest associated with direct targeting of cyclins D2 and E2. Systemic administration of this miRNA in a mouse model of HCC using adeno-associated virus (AAV) results in inhibition of cancer cell proliferation, induction of tumor-specific apoptosis, and dramatic protection from disease progression without toxicity. These findings suggest that delivery of miRNAs that are highly expressed and therefore tolerated in normal tissues but lost in disease cells may provide a general strategy for miRNA replacement therapies.

There seems to be quite a bit more work being done in this area and it represents an exciting breakthrough segment for therapeutics.

9 OBSERVATIONS

Much of what we know about miRNAs and their functions has evolved in the past five years to a decade at most. In fact in the past decade one has seen a great opening to RNAs in general. Before that it could be said that RNAs were the poor cousin in the process, the glory given the DNA and then the pathway dynamics dominated by proteins. We now appear to have opened a door on control mechanisms at the RNA level, dominated by miRNA and their control of mRNA before it becomes a protein. Thus RNA is somewhat exciting, and the miRNA have presented an added level of complexity to our modeling of complex cellular dynamics.

Based upon the analysis herein:

9.1 miRNA present new Paradigms for Cell Signalling

The most significant result from the explosion of miRNA effects is that what we have seen as now classic pathways may have significant undercurrent resulting from the miRNAs. Are miRNAs dominant control elements, is so where do they impact the most. We have seen many of the miRNA discoveries as just incidental to studying pathways. In our prior analysis we assumed them to be just noise. Now we can no longer accept such a proposition. In fact they seem to play significant if not dominant roles.

9.2 miRNA provide opportunistic targets for Therapeutics

The use of miRNAs as therapeutic targets is of significant interest. We have discussed some of the results and we have tried to place miRNAs in context of a broad therapeutic approach. The true reason is the simplicity of the miRNA structure. It is not a complex protein of hundreds of nucleic acids folded in a complex manner. The miRNA is just some 22 nucleotides on a sugar backbone.

9.3 miRNA expand the Interest in Introns as Control Elements

We have been trained to ignore the introns. It was the trash heap of evolution, perhaps of some use in the past. However since miRNAs are intro sourced, we now have a new window on the importance of introns.

9.4 miRNA may have Broader Roles in Metastatic Growth

We have looked at such proteins as PTEN, p53, and others as the control element. We looked at kinases and receptors and instigating ligands as part of that process. When we examine miRNA we see control coming from within. What instigates the processing and release of miRNAs. What are the feedback loops, if any, between the surface changes on receptors and the activation of miRNAs.

9.5 miRNA can be a Significant Target for Diagnostic and Prognostic Analysis

One of the problems we have in many cancers is both diagnosis and prognosis. In melanoma unfortunately prognosis may often be dire, but not always. In addition diagnosis of pigmented lesions is often problematic. Take a simple melanoma in situ, where it is diagnosed based on upward movement of the melanocyte. Are there differences in the MIS? Namely is each MIS identical, just losing its stability, say through loss of E-cadherin, and if not are there simple miRNAs which can be targeted and profiled.

There are many more observations which will evolve as we better understand miRNAs. Since we are at the beginning of understanding them we must keep in mind the ever changing field of play, and thus any analysis must include miRNAs as significant participants.

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