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SNPing cancer in the bud: microRNA and microRNA-target site polymorphisms as diagnostic and prognostic biomarkers in cancer

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Abstract

MicroRNAs are master regulators of gene expression and control many biological pathways such as cell growth, differentiation and apoptosis. Deregulation of microRNA expression and activity results in a myriad of diseases including cancer. Recently, several reports have indicated that single nucleotide polymorphisms (SNPs) in microRNAs and microRNA-target sites impact microRNA biology and associate with cancer risk, treatment response and outcome. In this review we will describe these findings and discuss the possible future of utilizing these SNPs as diagnostic and prognostic markers in the clinic.

Keywords

MicroRNA; 3′ untranslated region; microRNA-target site; single nucleotide polymorphism; human disease; cancer; cancer risk; treatment response; personalized medicine

1. Introduction

Sequence analysis of the human diploid genome estimates that human populations are 99.5% identical at the DNA level (Levy et al., 2007; J. Wang et al., 2008). Therefore, factors leading to human diversity must arise from the remaining 0.5% of variable genetic information, comprised primarily of single nucleotide polymorphisms (SNPs). Approximately 10 million SNPs have been identified in the human genome, occurring at a frequency of approximately 1–3% (or 1 out every 100–300 nucleotides) in the normal population (Levy, et al., 2007; Sachidanandam et al., 2001; J. Wang, et al., 2008). SNPs can occur in coding and non-coding regions of the genome. While the vast majority of SNPs located in non-coding regions of the genome were believed to be silent, new evidence suggests that SNPs coincident with cis-regulatory elements play a critical role in defining human diversity and disease by regulating the nature and timing of gene expression (Buonocore et al., 2010; Dimas et al., 2009; Pastinen, Ge, & Hudson, 2006).

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5. Conflict of Interest Statement

Dr. Weidhaas has patented IP surrounding SNP rs61764370 through Yale University, and has co-founded a company that has licensed this IP.

Cis-regulatory elements are sequence motifs in DNA and RNA that control gene expression (Pastinen, et al., 2006; Pastinen & Hudson, 2004). Cis-regulatory elements are often controlled by the concomitant expression of a requisite trans-acting factor. Transacting-factors function in response to stimuli and allow cells to fine tune gene expression and adapt to environmental or extracellular cues. Uncovering the relationship between cis-regulatory elements and the trans-acting factors that govern their expression is important to further our understanding of normal biological processes as well as disease.

2.1 MicroRNAs

MicroRNAs are a class of trans-acting RNAs found in eukaryotic organisms that bind to a cis-regulatory element in a target mRNA and regulate gene expression by inhibiting protein translation (Hobert, 2004). The first microRNAs discovered, lin-4 (R. C. Lee, Feinbaum, & Ambros, 1993; Wightman, Ha, & Ruvkun, 1993) and let-7 (Reinhart et al., 2000), were identified in *C. elegans* for their ability to control developmental timing and cell fate specification. The discovery that let-7 homologs displayed temporal expression in flies and mice indicated that microRNAs may have similar functions in higher order species (Pasquinelli et al., 2000). This prompted cloning efforts, which elucidated hundreds of new genes that encode for these trans-acting, small RNAs in worms, flies, mice and humans (Lagos-Quintana, Rauhut, Lendeckel, & Tuschl, 2001; Lagos-Quintana, Rauhut, Meyer, Borkhardt, & Tuschl, 2003; Lagos-Quintana et al., 2002; Lau, Lim, Weinstein, & Bartel, 2001).

MicroRNA genes are catalogued in the miRbase database (Griffiths-Jones, Grocock, van Dongen, Bateman, & Enright, 2006). According to the most recent release of miRbase, 21,264 precursor microRNAs and 25,141 mature microRNAs have been identified in 193 eukaryotic species as well as viruses (Kozomara & Griffiths-Jones, 2011). Of these, 1,600 precursor microRNAs and 2,042 mature microRNAs were cloned from human sources (Kozomara & Griffiths-Jones, 2011). MicroRNAs represent approximately 2% of the amount of protein-coding genes (Griffiths-Jones, 2004). MicroRNAs are believed to regulate up to 30% of all protein-coding genes (John et al., 2004; Krek et al., 2005; Lewis, Shih, Jones-Rhoades, Bartel, & Burge, 2003; Lim et al., 2005). As microRNA discovery extends to various cell, tissue and tumor types with the aide of deep-sequencing, the amount of annotated microRNAs will likely increase.

While many microRNAs display cell and tissue-specific expression patterns (Blower et al., 2007; Landgraf et al., 2007; Wienholds et al., 2005), elucidating the factors that govern microRNA expression in response to particular environmental cues and the specific mRNAs that are regulated in response to these cues remains a critical challenge to understanding how microRNAs function in human biology. Several recent studies have begun to uncover how extracellular stimuli such as growth factors (Seike et al., 2009; Suarez, Fernandez-Hernando, Pober, & Sessa, 2007), hormones (Klinge, 2009; Porkka et al., 2007), hypoxia (Kulshreshtha et al., 2007), DNA damage (Wagner-Ecker, Schwager, Wirkner, Abdollahi, & Huber, 2010; Weidhaas et al., 2007; Zhou et al., 2010) effect microRNA expression. Identifying the particular microRNAs and requisite mRNA targets that are sufficient to elicit a context-dependent, microRNA-mediated cellular response is critical, as they will likely provide useful diagnostic and prognostic biomarkers. Furthermore, uncovering how microRNA associated SNPs play a role in altering the normal biological processes in response to these cues is critical to understanding the molecular basis of how these variants play a role in disease onset and progression and will allow for the development of targeted therapeutics in the future.

MicroRNAs play a role in regulating many biological pathways including cell growth, differentiation and apoptosis (reviewed by (Esquela-Kerscher & Slack, 2006; He & Hannon,

2004) all of which are deregulated in cancer. MicroRNAs can function as both oncogenes and tumor suppressors (Croce, 2009; Hammond, 2006; B. Zhang, Pan, Cobb, & Anderson, 2007). Conditional deletion (He et al., 2007) or over-expression (Hayashita et al., 2005; Medina, Nolde, & Slack, 2010) of single microRNA genes is sufficient to drive tumorigenesis in mice. Consistent with these findings, it was found that 50% of all microRNA genes are in fragile regions of the genome that are frequently deleted, amplified and mis-expressed in human cancers (Calin & Croce, 2006; Calin et al., 2002; Calin et al., 2004). The role of SNPs in microRNAs and their binding sites are not surprisingly critical in cancer as well, as will be discussed in this review.

2.2 MicroRNA biogenesis

MicroRNA genes are located in the introns of protein-coding genes as well as in intergenic regions of the genome previously thought to be transcriptionally inactive (Saini, Griffiths-Jones, & Enright, 2007). About 45% of human microRNA genes are clustered together in groups of 2 or more and are individually generated from the polycistronic transcript (Saini, et al., 2007).

In mammalian systems, microRNAs are transcribed from the genome by RNA polymerase-II as a long primary transcript (or pri-microRNA) that is capped and polyadenylated (Cai, Hagedorn, & Cullen, 2004; Y. Lee et al., 2004). The pri-microRNA folds into a stem-loop structure and is bound by the double-strand RNA binding protein DGCR8 at the base of the stem (Han et al., 2004; Han et al., 2006). DGCR8 associates with the RNaseIII enzyme Drosha, which cleaves both strands of the pri-microRNA stem generating a shorter ~70 nucleotide stem-loop called the pre-microRNA (Gregory et al., 2004; Y. Lee et al., 2003) (Figure 1).

Exportin5 cooperatively binds the pre-microRNA hairpin and Ran-GTP and facilitates export of the RNA from the nucleus to the cytoplasm (Yi, Qin, Macara, & Cullen, 2003; Zeng & Cullen, 2004). In the cytoplasm the pre-microRNA is bound by the RNaseIII enzyme Dicer (Bernstein, Caudy, Hammond, & Hannon, 2001; Bernstein et al., 2003), which measures approximately 2-helical turns (22 nucleotides) up from the base and cleaves both strands of the stem generating a 22 nucleotide microRNA duplex (Macrae et al., 2006; H. Zhang, Kolb, Jaskiewicz, Westhof, & Filipowicz, 2004) (Figure 1).

The duplex is unwound by an RNA helicase (Chu & Rana, 2006; Salzman, Shubert-Coleman, & Furneaux, 2007) and the mature microRNA is loaded into 1 of 4 Argonuate proteins (Ago1–4) (Carmell, Xuan, Zhang, & Hannon, 2002; Farazi, Juranek, & Tuschl, 2008; Peters & Meister, 2007). The other strand of the duplex (or microRNA*) is often degraded. However in some cases, like miR-199 and miR-199* both strands of the microRNA duplex are loaded into an Ago protein (Czech et al., 2009; Okamura, Liu, & Lai, 2009). Ago is the heart of the microRNA-induced silencing complex (miRISC), which is guided by the microRNA to complementary elements in the 3′ UTR of a target mRNA (Carmell, et al., 2002; Peters & Meister, 2007). The miRISC negatively regulates gene expression by either mRNA cleavage or inhibiting translation (Valencia-Sanchez, Liu, Hannon, & Parker, 2006).

2.3 Determinants for microRNA target selection

To better understand how SNPs may be important in disrupting microRNA regulation of targets, it is important to understand the complexity of microRNA target selection. Target selection is based predominantly on the extent of Watson-Crick base pairing between the microRNA and mRNA and this is linked directly to the mechanism by which the mRNA is silenced. Nucleotides 2–7 (from the 5′ end of the microRNA), also called the microRNA

'seed', are a major determinant of mRNA target selection (Lewis, et al., 2003). Mutation(s) in either the seed or seed-complementary site inhibited microRNA activity and could be rescued with a compensatory mutation(s) highlighting the importance of seed sequence complementarity (Vaucheret, Vazquez, Crete, & Bartel, 2004; Vella, Choi, Lin, Reinert, & Slack, 2004). Complementarity in the seed region leads to translation repression (Pillai et al., 2005).

While Watson-Crick base pairing in the seed is absolutely critical for target recognition, there are other enhancing features that can strengthen microRNA target selection. Complementarity at position 8 of the microRNA, and the presence of an adenosine residue in the target mRNA opposite of nucleotide 1 enhance target recognition (Lewis, Burge, & Bartel, 2005). Additionally, more than 4 contiguous Watson-Crick base pairs between nucleotides 12–17 at the 3′ end of the microRNA also enhance target recognition (Grimson et al., 2007).

MicroRNA-directed cleavage occurs by hydrolysis of the phosphodiester backbone in the target mRNA opposite nucleotides 10 and 11, when there is complementarity between (at least) nucleotides 2–15 (Meister et al., 2004). While 1 or 2 single nucleotide mis-matches or G:U wobbles are tolerated, canonical Watson-Crick base pairing is absolutely critical between nucleotides 9–12 (Felice, Salzman, Shubert-Coleman, Jensen, & Furneaux, 2009; Martinez & Tuschl, 2004). However, it was recently demonstrated that centered pairing requiring 11–12 contiguous Watson-Crick base pairs, between nucleotides 4–15 of the microRNA, is also sufficient to direct target RNA cleavage (Shin et al.). While examples of microRNA-directed mRNA cleavage can occur in humans, computational analysis indicated that the amount of target sites predicted to fit this criteria are extremely rare (John, et al., 2004; Yekta, Shih, & Bartel, 2004).

3.1 SNPs in microRNAs and microRNA target sites

Because microRNA biogenesis and target selection is highly sequence dependent, germline sequence variants (such as SNPs) and posttranscriptional base modifications (such as ADAR editing) in either the microRNA or microRNA-target site can have profound effects on microRNA function. Interestingly, the first evidence that a microRNA-associated SNP could elicit gross morphologic defects was inherent to the initial discovery of the let-7 microRNA. The temperature sensitive let-7(n2853) mutation that results in *C. elegans* lethality is in fact a single nucleotide G>A point mutation at position 5 of the microRNA (Reinhart, et al., 2000). This mutation inhibits let-7 from targeting the lin-41 mRNA and results in reiteration of larval cell divisions in the adult worm (Reinhart, et al., 2000). While the let-7(n2853) mutation is chemically induced, it provides proof of principle evidence for this concept.

Sequencing analysis showed that microRNAs and microRNA target sites are highly conserved through evolution (Chen & Rajewsky, 2006). Furthermore, SNPs in microRNA genes are relatively rare (Saunders, Liang, & Li, 2007). These findings indicate that trans-acting microRNAs and the requisite cis-regulatory elements they regulate were under selective pressure during evolution. This suggests that the repertoire of SNPs that have been identified in microRNAs and microRNA target sites may represent a class of functional variants.

In theory, microRNA-associated SNPs can elicit cancer phenotypes by either creating a loss-of-function scenario whereby the expression, activity or targeting of a tumor suppressor microRNA is inhibited; or a gain-of-function scenario where by the expression, activity or targeting of an oncogenic microRNA is enhanced. SNPs can have director or indirect effects on microRNAs. Direct effects include SNPs in the pri-microRNA, pre-microRNA or mature microRNA that impair or enhance microRNA processing or function (Figure 3). Indirect

effects include SNPs in microRNA promoters that affect transcription (Figure 2) and SNPs in an mRNA that create or destroy a target site (Figure 4). In the later half of this review we will discuss cancer-associated microRNA SNPs that affect microRNAs via three different mechanisms; microRNA transcription; microRNA precursor processing, and microRNA-mRNA binding.

3.2 MicroRNA promoter SNPs

MicroRNA transcription is regulated by the same mechanisms that control protein-coding genes (Hobert, 2004; Marson et al., 2008). A number of well-characterized transcription factors control both mRNA and microRNA expression (He, He, Lim, et al., 2007; O'Donnell, Wentzel, Zeller, Dang, & Mendell, 2005). Transcription factors bind to conserved sequence motifs in the genome (typically) upstream of the gene for which they are controlling the transcription of (Orphanides, Lagrange, & Reinberg, 1996; Roeder, 1996). Because the recognition of a transcription factor to a particular DNA locus is sequence dependent (el-Deiry, Kern, Pietenpol, Kinzler, & Vogelstein, 1992; Maniatis, Goodbourn, & Fischer, 1987), variants in transcription factor binding sites could potentially alter microRNA expression (Figure 2). Computational analysis predicts that there are over 20,000 SNPs coincident with microRNA promoters in humans (Schmeier, Schaefer, MacPherson, & Bajic, 2011). However, the functional and phenotypic relevance of virtually all of these SNPs remains unclear.

The miR-34 family is transcriptionally upregulated following exposure to cytotoxic stress in a p53-dependent manner (He, He, Lowe, & Hannon, 2007; Hermeking, 2007). Mapping of the miR-34a and miR-34b/c promoters indicated that there are conserved p53 binding sites upstream of the miR-34 family that are required for transcription (Chang et al., 2007; He, He, Lim, et al., 2007). Loss of miR-34 function attenuates p53-mediated cell cycle arrest and apoptosis (He, He, Lim, et al., 2007; Raver-Shapira et al., 2007), which is congruent with increased cellular transformation and sensitivity to cytotoxic therapy (Kato et al., 2009). The rs4938723 T>C SNP located 423 nucleotides upstream of miR-34b/c is located in a transcription factor binding site and is predicted to attenuate GATA binding (Y. Xu et al., 2011). The rs4938723 SNP is associated with an increased risk of hepatocellular carcinoma in a case-control study of 501 Chinese individuals (Y. Xu, et al., 2011). While the precise mechanism in which the rs4938723 SNP associated with increased HCC risk is unknown, it phenocopies a *TP53* loss-of-function mutation, and therefore likely inhibits expression of the miR-34 family resulting in enhanced cellular transformation.

3.3 SNPs in microRNA precursors

MicroRNA biogenesis proceeds through sequential processing steps mediated by the RNaseIII enzymes Drosha and Dicer (Kim, 2005) (Figure 1). These processing events rely heavily on proper folding of the precursor RNAs into a stem-loop structure (Han, et al., 2006). Variants in the pri-microRNA and/or pre-microRNA could alter secondary structure and inhibit or enhance pri-microRNA processing (Figure 3).

Patients with chronic lymphocytic leukemia (CLL) frequently have homozygous deletions at chromosome 13q13.4 (Calin, et al., 2002). This genomic locus encodes a polycistronic transcript from which, miR-15a and miR-16-1 are processed (Calin, et al., 2002; Lagos-Quintana, et al., 2001). These microRNAs are dynamically expressed during the cell cycle (Rissland, Hong, & Bartel, 2011) and target genes involved in regulating cell cycle progression (Linsley et al., 2007; Liu et al., 2008). They have also been shown to target genes involved in apoptosis and function as tumor suppressors (Bonci et al., 2008; Cimmino et al., 2005). In 2005, Carlo Croce's group identified a homozygous C>T SNP coincident with the pri-miR-16-1 locus in two CLL patients with intact 13q13.4 (Calin et al., 2005).

This SNP associated with decreased miR-16 expression in CLL cell lines derived from patients and inhibited pri-miR-16 processing *in vitro* (Calin, et al., 2005). Moreover, this SNP is associated with CLL-like disease in mice (Raveche et al., 2007), highlighting the importance of these microRNAs in tumorigenesis.

The miR-146 family, comprised of *miR-146a* and *miR-146b*, is transcriptionally activated in THP1 cells in response to LPS stimulation in an *NF-κB*-dependent manner (Taganov, Boldin, Chang, & Baltimore, 2006). *NF-κB*-mediated upregulation and ectopic expression of *miR-146a/b* inhibits migration and invasion of breast (Bhaumik et al., 2008; Hurst et al., 2009) and pancreatic (Li et al., 2010) cancer cell lines. Inhibition of migration and invasion attributed to targeting of *IRAK-1* leading to subsequent inhibition of *NF-κB* by miR-146a in a negative feedback loop (Bhaumik, et al., 2008; Hurst, et al., 2009). A G>C SNP (rs2910164) in the pre-miR-146a was identified by associating with an increased risk of papillary thyroid carcinoma (Jazdzewski et al., 2008). Subsequent analysis showed association of the rs2910164 SNP with hepatocellular carcinoma (T. Xu et al., 2008), prostate cancer (B. Xu et al., 2010), and esophageal squamous cell carcinoma (Guo et al., 2010) in Han Chinese individuals. In a recent study the rs2910164 SNP was shown to associate with a decreased risk of bladder cancer and reduced risk of recurrence (M. Wang et al., 2012). Functional analysis established that the G>C alteration attenuates Drosha-mediated processing resulting in reduced miR-146a expression (Jazdzewski, et al., 2008). Interestingly, several reports have linked decreased *miR-146a* expression to androgen-independent prostate cancer (Lin, Chiang, Chang, & Ying, 2008; B. Xu et al.). The rs2910164 SNP is associated with hormone-refractory prostate cancer (B. Xu, et al., 2010). Therefore, it is plausible that *miR-146a* governs prostate cancer biology. However, in breast (Hurst, et al., 2009) and bladder (M. Wang, et al., 2012) cancer cell lines the rs2910164 SNP leads to upregulation of miR-146a expression. The discrepancy in data regarding the affect of the rs2910164 SNP on miR-146a expression highlights the ability of SNPs to dynamically alter microRNA expression in a tissue specific manner.

A homozygous T>C SNP (rs11614914) in *pre-miR-196a-2* is associated with an increased risk of lung (Tian et al., 2009), breast (Hoffman et al., 2009) and gastric cancer (Peng et al., 2010) in Chinese populations, whereas, in Caucasian populations where the allele frequency is reversed, a C>T homozygous variant of the rs11614914 SNP associated with an increased risk of oesophageal cancer in non-smokers (Ye et al., 2008). While the T>C variant reduces the expression of *miR-196a-2* (Hoffman, et al., 2009), the target genes sufficient to drive tumorigenesis are unknown and it is possible that this microRNA can function as both a tumor suppressor and oncogene. Homozygous rs11614914 T>C variants were associated with poor survival in patients diagnosed with non-small cell lung cancer indicating the importance of these SNPs as possible diagnostic markers for cancer prognosis (Hu et al., 2008). Taken together these observations indicate that SNPs in pre-microRNA regions can play a dynamic role in microRNA processing and cancer biology.

3.4 SNPs in microRNA target sites

Sequence complementarity is a major determinant for microRNA-target recognition (Bartel, 2009). Therefore, SNPs in mRNAs can alter microRNA binding by either creating a new site or destroying an existing target site (Figure 4). Computational analysis indicates that there are approximately 20,000 SNPs coincident with conserved human microRNA target sites with putative functionality (Chen & Rajewsky, 2006). Michel George's group was the first to report that a 3′ UTR SNP could create an aberrant microRNA target site. They showed that a homozygous SNP in the 3′ UTR of myostatin caused muscular hypertrophy in Texel sheep (Clop et al., 2006). The G>A SNP creates an aberrant target site for miR-1 and miR-206, which are highly expressed in skeletal muscle and specifically target the variant allele (Clop, et al., 2006). Whereas, Matthew State's group showed that a Tourette's

syndrome associated SNP in the 3′ UTR of SLITRK1 destroyed a target site for miR-189, and was the first evidence that a 3′ UTR SNP could inhibit microRNA binding (Abelson et al., 2005). Since these seminal observations, microRNA binding site SNPs have been identified in cancer and appear to function as biomarkers for disease risk, treatment response and outcome.

Let-7 is a tumor suppressor microRNA that regulates the expression of the KRAS (Johnson et al., 2005), MYC (Kumar, Lu, Mercer, Golub, & Jacks, 2007) and HMGA2 (Y. S. Lee & Dutta, 2007; Mayr, Hemann, & Bartel, 2007) oncogenes. Let-7 expression is frequently down regulated in many types of cancer and this is associated with poor prognosis in lung cancer (Karube et al., 2005; Takamizawa et al., 2004). A heterozygous T>G SNP (rs61764370) in the 3′ UTR of KRAS associates with an increased risk for non-small cell lung carcinoma in 2 case-controlled studies (Chin et al., 2008), as well as, increased risk for ovarian cancer (E. Ratner et al.), triple negative breast cancer (Paranjape et al.), melanoma (Chan et al.), and hereditary breast and ovarian cancer (Pilarski et al.). The T>G variant is coincident with a let-7 target site in the 3′ UTR of KRAS and attenuates let-7-mediated suppression (Chin, et al., 2008), resulting in KRAS overexpression.

Furthermore, the rs61764370 SNP was recently shown to associate with resistance to platinum-based therapy in ovarian cancer and increased cancer specific death in these patients (E. S. Ratner et al., 2011). Additionally, evidence from Graziano et al., indicated that metastatic colorectal cancer patients with the rs61764370 SNP undergoing salvage cetuximab-irinotecan therapy displayed chemotherapy resistance, with poor overall survival and progression-free survival (Graziano et al., 2010). These findings suggest that this germline, non-coding sequence variant in the KRAS 3′ UTR phenocopies somatic, activating (gain-of-function) KRAS mutations found in the open-reading-frame in treatment response. This data highlights the potential utility for the rs61764370 SNP as a companion diagnostic in the clinic.

While the rs61764370 SNP was identified by direct sequencing of the KRAS 3′ UTR, other groups have utilized in silico analysis to identify candidate SNPs in microRNA target sites for genotype-phenotype correlations (Nicoloso et al., 2010; Sethupathy, Giang, Plotkin, & Hannenhalli, 2008). Analysis of microRNA target site SNPs in genes associated with the DNA damage repair pathway demonstrated that a heterozygous T>C SNP (rs8679) in the PARP1 3′ UTR associated with increased risk for developing bladder cancer, but not breast cancer (Teo et al., 2012). This SNP is coincident with several predicted microRNA target sites in the PARP1 3′ UTR, in particular miR-145, which is frequently down-regulated in bladder cancer (Ichimi et al., 2009). It is possible that the rs8679 SNP in combination with reduced miR-145 expression contribute to increased bladder cancer risk.

In this same report a heterozygous A>G SNP (rs7180135) in the RAD51 3′ UTR associated with a favorable response (improved 5-year cancer specific survival) to radiation therapy in muscle-invasive bladder cancer (Teo, et al., 2012). This SNP is predicted to be coincident with a miR-197 target site and disrupt microRNA targeting. Interestingly, miR-197 is downregulated in cells following exposure to ionizing radiation (Weidhaas, et al., 2007). It is possible that the rs7180135 SNP and downregulation of miR-197 following IR therapy work synergistically to enhance the cellular DNA damage response, resulting in increased survival. These reports indicate that microRNA target site SNPs can function similarly to protein-coding mutations that associate with not only disease risk, but treatment response and outcome as well.

4. Looking into the future: utilizing SNPs as companion diagnostics

There is sufficient proof-of-principle evidence that microRNA SNPs can play a critical role in predicting cancer risk, treatment response and outcome. Understanding the factors that contribute to cancer risk can be a powerful future tool for clinicians and genetic counselors, as well as in advancing our understanding of cancer biology. If a risk allele is identified clinicians could advise patients to begin earlier, more frequent and intensive screening or even stronger preventative measures, in hopes of preventing disease or catching it at an earlier and more treatable stage. More interestingly, as microRNAs are stimulated by external stimuli, it may also be possible to manage patients with such SNPs by modifying lifestyle factors to maintain homeostasis of their inherited differences. This is an avenue of active research that may prove most promising.

While assessing an individual's risk can be a useful tool to catch cancer at an earlier time, the question regarding what is the best treatment for individual cancer patients still remains. There is mounting evidence that microRNA SNPs can predict treatment response and outcome. For example, the miR-34 family protects cells against cytotoxic therapy (Kato, et al., 2009). Therefore, the miR-34b/c promoter SNP (rs4938723) that inhibits miR-34 expression (Y. Xu, et al., 2011), could be hypothetically utilized as a companion diagnostic with treatment. The KRAS 3′ UTR SNP (rs61764370) associates with poor response to platinum-based therapy in ovarian cancer (E. S. Ratner, et al., 2011) and cetuximab-irinotecan treatment in colorectal cancer patients (Graziano, et al., 2010). Consistent with these findings patients harboring the SNP displayed poor outcome and poor overall survival. These results indicate the potential utilization of microRNA-associated SNPs as companion diagnostics. Application of these SNPs into treatment decisions will require further confirmation in prospective randomized trials, yet the evidence for their potential as a new class of inherited markers that could bring clinicians one-step closer to providing tailored/personalized care for the treatment of cancer is already very promising.

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Abbreviations

DNA	deoxyribonucleic acid
RNA	ribonucleic acid
miRNA	microRNA
SNP	single nucleotide polymorphism
5′ UTR	5′ untranslated region
3′ UTR	3′ untranslated region
C. elegans	Caenorhabditis elegans
DGCR8	DiGeorge syndrome critical region 8 (gene)
Ago	Argonuate
SLITRK1	SLIT and NTRK-Like family member 1 (gene)
KRAS	V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (gene)
HMGA2	high-mobility group AT-hook 2 (gene)

PARP1	Poly [ADP-ribose] polymerase 1 (gene)
HCC	hepatocellular carcinoma
CLL	chronic lymphocytic leukemia
BCL2	B-cell CLL/lymphoma 2 (gene)
BRCA1	breast cancer 1, early onset (gene)
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells (gene)
THP1	Human acute monocytic leukemia cell line

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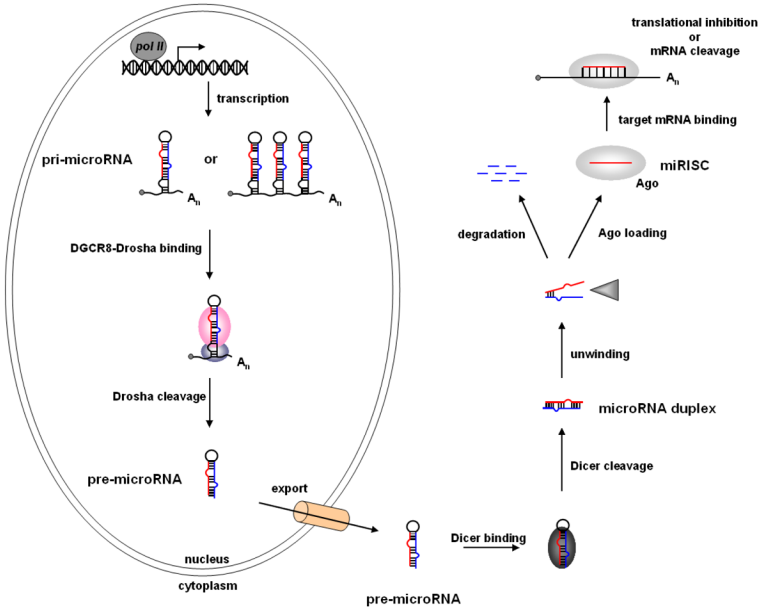

Figure 1.
The microRNA biogenesis pathway.

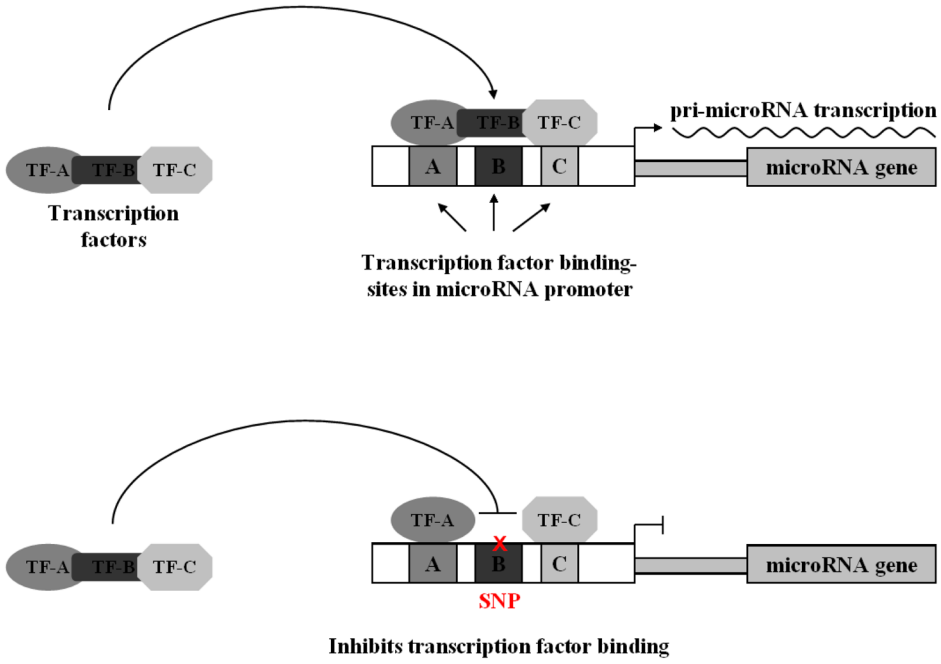

Figure 2.
SNPs in microRNA promoters can prevent transcription factor binding and inhibit pri-microRNA transcription.

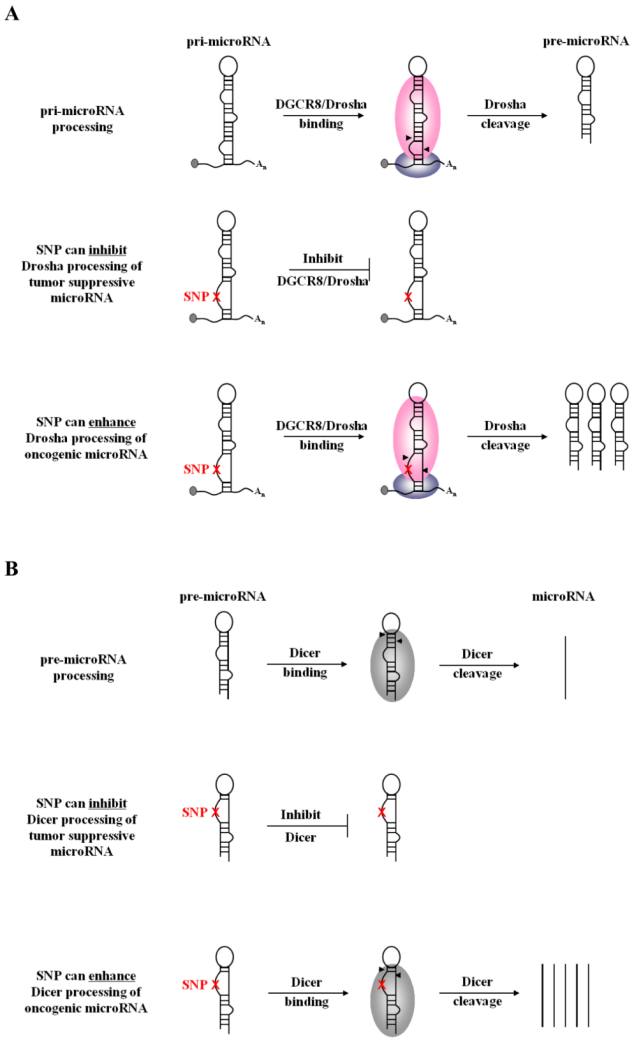

Figure 3.
SNPs in pri-microRNA (A) or pre-microRNA (B) precursors can inhibit the processing of a tumor suppressor microRNA or can enhance the processing of an oncogenic microRNA.

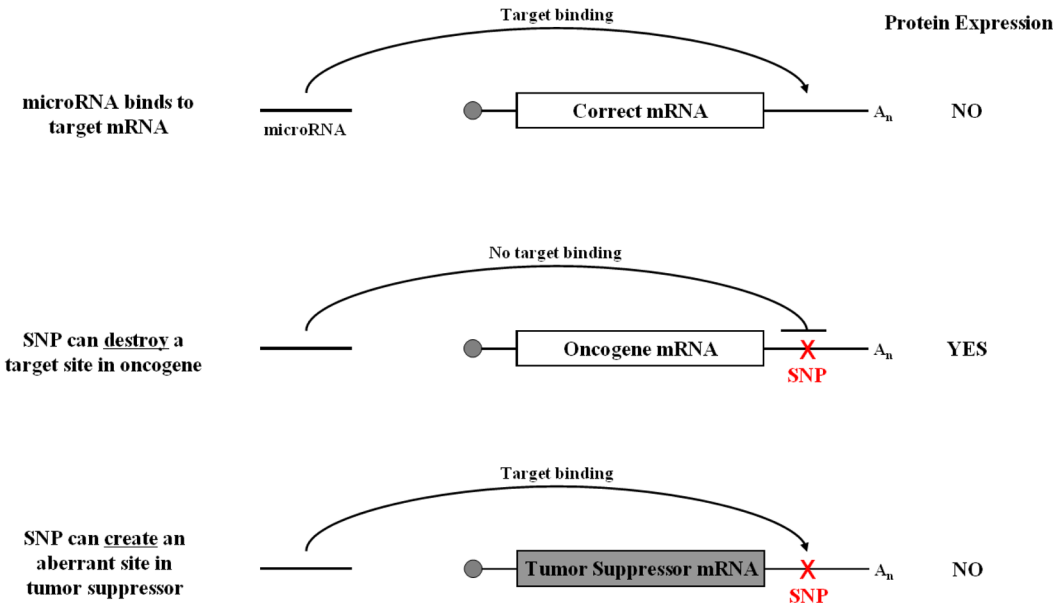

Figure 4.
SNPs in microRNA-target sites can either destroy an existing target site or create a target site in the wrong mRNA.