Single-nucleotide polymorphisms among microRNA: big effects on cancer

Feng-Ju Song¹ ² and Ke-Xin Chen¹ ²

Abstract

MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression at the transcriptional or posttranscriptional level. Many miRNAs are found to play a significant role in cancer development either as tumor suppressor genes or as oncogenes. Examination of tumor-specific miRNA expression profiles in diverse cancers has revealed widespread deregulation of these molecules, whose loss and overexpression respectively have diagnostic and prognostic significance. Genetic variations, mostly single-nucleotide polymorphisms (SNPs) within miRNA sequences or their target sites, have been found to be associated with many kinds of cancers. In this review, we summarize the current knowledge of miRNAs including their biogenesis and role in cancer development, and finally, how SNPs among miRNAs affect miRNA biogenesis and contribute to cancer.

Key words Breast cancer, microRNA, target sequence, SNP, cancer risk

In 1993, Lee et al. [1] discovered a gene, lin-4, that affects development in Caenorhabditis elegans and found that its product is a small non–protein-coding RNA. After this seminal finding, the cloning and characterization of small, 20 to 22 nucleotide–long members of the non–protein-coding RNA family have led to the identification of thousands of microRNAs (miRNAs). The term “miRNA” was first introduced in 2001 in Science [2]. miRNAs are now emerging as one of the most interesting small regulatory, non-coding RNAs in molecular biology [3–4]. They perform functions as key trans-acting factors by switching off or fine tuning expression of target genes [5–6]. They are believed to function largely, although not exclusively, through base-pairing to the complementary sequences in the 3′-untranslated region (3′UTR) of target genes to suppress gene expression at the mRNA or protein levels [7–8]. There are also some exceptions. For example, microRNA-346 (miR-346) targets the 5′-untranslated region (5′UTR) of receptor-interacting protein 140 mRNA and up-regulates its protein expression [9]. miRNA regulation can be highly pleiotropic, with one miRNA able to target several hundred different transcripts, and one mRNA able to be cooperatively targeted by several miRNAs. In fact, bioinformatic prediction indicates that 30% of animal genes may be directly regulated by miRNAs [9–10]. Recent miRNA transfection experiments show strong evidence that miRNAs influence not only their targets but also non-target genes through a two-layer regulatory network in which transcription factors function as important mediators of miRNA-initiated regulatory effects [11]. miRNAs critically regulate almost all biological processes involving gene expression [12–13]. Therefore, alterations in miRNA expression have been implicated in various human diseases, including cancer [14–16]. To date, the miRNA registry (miRBase) contains more than one thousand human miRNAs. Thus far, most registered miRNAs are widely expressed and highly conserved. Deep sequencing of small RNA libraries and cell type–specific analyses are currently valuable approaches to uncovering miRNAs with less abundant expression [17]. Thus, the catalog of miRNAs is expected to grow substantially in the future, and the exploration of miRNAs in cancer development will shed more light on the causes of cancer.

MiRNA Biogenesis

miRNA genes are scattered among each of the chromosomes in humans, except for the Y chromosome. miRNAs can be encoded in independent transcription units, in polycistronic clusters, or within the introns of
protein-coding genes[18]. They are primarily derived from capped, polyadenylated RNA polymerase II transcripts, termed “primary” miRNAs (pri-miRNA) [19]. A typical metazoan pri-miRNA consists of a double-stranded RNA (dsRNA) stem of about 33 bp, with a terminal loop on one end and single-stranded RNA (ssRNA) flanking segments on the other end. The ssRNA-dsRNA junction (SD junction) is very important for pri-miRNA processing. Mature miRNAs are generated by a two-step processing mechanism, the “cropping” step and the “dicing” step [20].

In the cropping step, pri-miRNAs are first processed to stem-loop structured precursor miRNAs (pre-miRNA), typically 55 to 80 nt in length, in the nucleus. Aside from a small group of pre-miRNAs that are generated through mRNA splicing/debranching machinery termed the “miRtron” pathway [21], most pre-miRNAs are processed from pri-miRNAs by the nuclear ribonuclease (RNase) III Drosha [22], which partners with the RNA-binding protein DiGeorge syndrome critical region gene 8 (DGCIR8) [23]. Drosha is a large nuclear protein of 160 kDa in humans with two tandem RNase III domains and a dsRNA-binding domain. Drosha forms a large complex, weighing approximately 650 kDa in humans, known as the “microprocessor,” in which it interact with its co-factor, DGCIR8 [24]. Neither recombinant DGCIR8 nor Drosha alone is active in pri-miRNA processing, whereas the combination of these two proteins possesses pri-miRNA processing activity, indicating that both proteins are critical for processing.

Pri-miRNA processing consists of two sequential steps: substrate recognition and catalytic reaction. DGCIR8 recognizes the substrate by anchoring tightly at the SD junction and interacting with the approximately 33-bp stem. The sequence preceding the 5′ terminus or trailing the 3′ terminus of the pre-miRNAs forms an approximately 11-bp imperfect stem that is recognized by DGCIR8 as part of the required structure for Drosha cutting. Drosha may not be in direct contact with RNA at this stage. After the substrate recognition, Drosha may interact transiently with the stem, and catalytic reaction happens on the stem at about 11 bp from the SD junction, releasing the pre-miRNA [25,26].

A small terminal loop of pri-miRNA may impose structural constraints on the stem and affect processing, so pri-miRNA with a small loop is less active in processing. On the contrary, a large terminal loop may act as a flexible ssRNA and may contribute to tight binding by the microprocessor [27,28]. Because some large terminal loops can be seen as unstructured ssRNA segments, pri-miRNAs may be considered to have “ssRNA-dsRNA-ssRNA” structure. Because of the symmetry properties of this structure, the microprocessor appears to recognize the terminal loop as ssRNA and binds to the stem-loop in an opposite orientation. In this case, cleavage can occur at an alternative site, about 11 bp from the terminal loop. The processing at this alternative site would be “abortive” because the cleavage product does not contain miRNA sequences in full.

Pre-miRNAs are exported to the cytoplasm by exportin-5/RAN-GTP for the dicing step [29]. In this step, the pre-miRNAs are further processed in the cytoplasm by another RNase III enzyme, Dicer, that cuts the pre-miRNA at 22 bp from the terminus set by Drosha to generate a double-stranded miRNA duplex [30]. The production of miRNA/miRNA* duplexes is an essential step in miRNA biogenesis and precisely defines the termini of the mature miRNAs for preferential loading of the guide strand [31]. Recruitment of co-factors such as transactivation response element RNA-binding protein (TRBP) and protein kinase R–activating protein (PACT) increases the efficiency of pre-miRNA processing by Dicer [32]. Human Dicer is composed of an amino-terminal RNA helicase domain, followed by a PAZ (Piwi/Ago/Ago/zwille) domain, two RNase III domains, and a carboxy-terminal RNA-binding domain [33]. Despite the similarities in their basic modes of action, RNase III proteins are different in their substrate specificities. Human Dicer tends to act on any dsRNA with a simple preference toward the terminus of the molecule. The PAZ domain of Dicer interacts with the 3′ terminus and determines the processing site in a ruler-like fashion that measures approximately 22 nucleotide segments from the terminus [34,35].

For most miRNAs, only one strand (the guide strand) of the double-stranded miRNA duplex is loaded into miRNA-induced silencing complex (miRISC) [36]. The choice of the guide strand is dependent in part on the thermodynamic properties of the duplex, with the strand possessing the least thermodynamically stable 5′ terminus usually being chosen as the guide strand and eventually becoming the mature miRNA, whereas the other strand, labeled miRNA*, is usually degraded [37]. However, in cases where both the 5′ and 3′ termini of each miRNA have similar stabilities, such as in miR-142, miR-125, miR-126, and miR-342, both miRNAs have an equal chance of being selected as the guide strand [38]. Also, the fates of the miRNA guide and miRNA* strands have been shown to be tissue-dependent, with both strands being functionally active under specific conditions [39].

The mature miRNAs are used to guide miRISC to the complementary sequences in the 3′ UTR of targeted transcripts [40]. The core component of every RISC is a member of the Argonaute (Ago) protein family, containing a central PAZ domain and a carboxy-terminal PIWI domain. Structural studies have shown that the PIWI domain binds to small RNAs at their 5′ terminus, whereas the PAZ domain binds to the 3′ terminus of
ssRNAs\cite{49}. Argonaute 2 (AGO2), sometimes in cooperation with eIF6\cite{42}, is the target-cleaving endonuclease of the RISC\cite{53,54}. The result is site-specific mRNA cleavage when the pairing is nearly complete (mostly in plants) or translational inhibition when imperfect base pairing occurs (mostly in animals)\cite{42}. Several lines of evidence suggest that miRNAs may perform their function in the RNA processing bodies (P-bodies) by sequestering target transcripts to P-bodies for storage, decapping, deadenylation, and degradation\cite{46}.

For effective translational suppression, Watson-Crick base pairing between nucleotides 2 to 7 or 2 to 8 (the “seed region”) of the mRNA 5’ terminus and the 3’ UTR of the target mRNA is ubiquitously required\cite{67}. The critical role played by the seed region in most of the miRNA/mRNA interactions implies that changes in the seed region, or shift of the processing sites during biogenesis of the miRNA/miRNA duplex could result in a novel miRNA with alternative target spectra. Therefore, both the 5’ terminus of the mature miRNA that is generated from the 5’ arm of the pre-miRNA (5p) by Drosha and the 5’ terminus of the mature miRNA that is produced by Dicer from the 3’ arm of the pre-miRNA (3p) should be highly conserved. Sequences outside of the seed in the mature miRNA sequence can also impact, although less dramatically, the strength of inhibition and the spectra of targeted transcripts.

Generally, the cropping step is the most important step for miRNA biogenesis. The precise position and orientation of Drosha cleavage are critically important for the generation of mature miRNA because the second cleavage step occurs at a defined distance from the free end generated by Drosha. Therefore, Drosha cleavage determines the identity of both the 5’ and 3’ termini of the mature miRNA. The slightest error in Drosha cleavage could not only alter the seed sequence, but also invert the relative stability of the two strands, resulting in the incorporation of the improper miRNA strand into the RISC complex. Since Drosha is present in a large protein complex and the association of Drosha with co-factors besides DGCR8 present within this complex promotes the fidelity and activity of Drosha cleavage, it will be important to identify additional factors involved in the biogenesis pathway.

Most miRNAs are under the control of developmental and/or tissue-specific signaling\cite{46,48}. miRNA expression may be regulated at multiple steps of RNA biogenesis\cite{49}. Although RNA pol II-mediated transcription provides a major point of control for miRNA biogenesis, most miRNAs seem to be controlled at the posttranscriptional level. For example, the maturation of pre-miR-38 may be temporally regulated in C. elegans since miR-38 is expressed only in the embryo, whereas the pre-miR-38 is ubiquitously detected. It is also possible that the nuclear export of pre-miR-38 may be controlled by a specific developmental signal or that Dicer processing may be repressed until a certain stage; some of these deregulations have been found to contribute to cancer development\cite{55,52}. Indeed, a number of miRNAs are down-regulated in cancer though their pri-miRNAs are expressed at a relatively high level, indicating that the cropping step may be controlled dynamically during cell differentiation and tumorigenesis.

**MiRNA and Cancer**

Over the past several years, many miRNAs have been implicated in various human cancers\cite{53,54}. Both losses and gains of miRNA function have been shown to contribute to cancer development. Over half of all known human miRNA genes are located at fragile sites and genomic regions involved in cancers\cite{56}. Similarly, mouse miRNA genes are also frequently located near mouse cancer susceptibility loci. High-resolution array-based comparative genomic hybridization has revealed that the number of miRNA copies is quite abnormal in human cancers\cite{58}. miRNA profiling has revealed that most of miRNAs are significantly down-regulated in human cancers.

The first evidence of miRNA involvement in cancer was reported in 2002. During their attempts to clone a tumor suppressor gene at chromosome 13q14, a region that is frequently lost in chronic lymphocytic leukemia (CLL), Calin et al.\cite{57} found that all of the protein-coding genes present in the region of interest were not specifically altered in CLL, suggesting that they were not involved in the disease. However, they found two miRNA genes, miR-15a and miR-16-1, in the deletion. The translocation breakpoint cut the precursors of these two miRNA genes which reside in the same polycistrionic RNA. The loss of miR-15a and miR-16-1 was observed in nearly 70% of CLLs, indicating that miR-15a and miR-16-1 can function as tumor suppressor genes in CLL, as their loss is associated with the development of CLL. Calin’s group mapped the chromosomal location of all known miRNA genes and discovered that many are located in regions that are frequently involved in chromosomal alterations, such as deletions or amplifications, in many types of human cancers\cite{59}. Neither the activation of oncogenes nor the loss of tumor suppressor genes has been observed in extensive studies of these cancers, suggesting that the role of miRNAs in cancer could extend far beyond CLL. In fact, the expression of miRNAs was deregulated in many human cancers, including leukemia, lymphoma, and glioblastoma, as well as colon, lung, breast, prostate, thyroid, cervical, and ovarian cancers\cite{58,64}. The development of high-throughput miRNA quantification technologies, such as miRNA microarray,
bead-based flow cytometry, RNA-primed array-based Klenow enzyme (RAKE) assay, miRNA serial analysis of gene expression (miRAGE), and real-time RT-PCR based TaqMan miRNA assay, has facilitated the study of the global miRNA profile in the whole cancer genome. Important questions on miRNA deregulation in cancer are being addressed with the advent of these new technologies. Deregulated miRNA expression in human cancer may prove to be a powerful tool for diagnosis, classification, and prediction of clinical behavior and prognosis[62]; specific findings on miRNA expression and cancer may be categorized into the following.

First, there is a differential global expression of miRNAs between tumors and their corresponding normal tissues in most, if not all, types of cancer. Interestingly, miRNA expression is more likely to be repressed in tumors than in normal tissues. For example, colon cancer is associated with alteration in miRNA expression. Michael et al.[66] identified different miRNAs in colon adenocarcinoma and normal mucosa and found that the expression of two mature miRNAs, miR-143 and miR-145, were consistently reduced in colorectal neoplasia. Using miRNA array analyses for age-matched normal cervix and cervical cancer tissues, in combination with Northern blot verification, Wang et al.[68] identified significantly deregulated miRNAs in cervical cancer tissues, with miR-126, miR-143, and miR-145 down-regulated and miR-15b, miR-16, miR-146a, and miR-155 up-regulated.

Second, miRNA expression signatures are informative enough to identify and classify human cancers. Rosenfeld et al.[69] suggested that miRNAs can accurately identify cancer tissue origin. In their study using miRNA microarray data from 253 samples, they were able to classify 22 different types of cancer using a 48 miRNA classifier with over 90% accuracy. This classification is of great clinical importance because miRNAs may be used to identify the tissue in which cancers of unknown primary origin arose. miRNA expression profiles can also be used to distinguish two subtypes of diffuse large B cell lymphoma (DLBCL), germinal center B cell-like (GCB) and activated B cell-like (ABC) DLBCL, as miR-21, miR-155, and miR-221 were more highly expressed in ABC-type than in GCB-type DLBCL[70].

Third, miRNA expression signatures can predict biological and clinical behavior within the same cancer type. In a study regarding breast cancer, Iorio et al.[72] found that the miRNA expression patterns were significantly different between normal and neoplastic breast tissues, with miR-125b, miR-145, miR-21, and miR-155 significantly reduced in breast cancer tissues. They also observed that the expression of miRNAs was correlated with specific breast cancer clinicopathologic features, such as tumor stage, proliferation index, estrogen and progesterone receptor expression, and vascular invasion[69]. Last but not least, miRNA expression is associated with the prognosis and progression of human cancer. Takamizawa et al.[80] observed that the expression levels of let-7 were down-regulated in lung cancer, and reduced let-7 expression was significantly associated with shortened postoperative survival, independent of disease stage. Another study revealed that high expression of miR-21 was associated with poor survival and poor therapeutical outcome in colon adenocarcinomas[81]. Thus, from all the above examples we can see the important function of miRNA in cancer development, progression, and prognosis.

The underlying mechanisms of miRNA deregulation in human cancer remain a challenging topic. Recent laboratory findings have provided evidence of multiple mechanisms of miRNA deregulation in human cancers[70]. Most primary miRNAs have been well-documented to be transcribed from RNA Pol II promoter and regulated by transcription factors. Several examples showing miRNA deregulation in cancer by transcriptional deregulation have been reported[71]. Recent studies suggest that miRNA deregulation may also be attributed to the following: epigenetic alterations, such as DNA methylation and histone deacetylation[72]; single-nucleotide polymorphism (SNP) and mutation, which may affect mature miRNAs[73]; DNA copy-number abnormalities[74]; and dysfunction or deregulation of key proteins in the miRNA biogenesis pathway, which may enhance tumorigenesis[75]. In addition to these mechanisms, “dynamic” factors that modulate miRNA activity without altering mature miRNA concentrations may also contribute miRNA deregulation. Examples of dynamic factors include 3′ UTRs, which have alternative forms with different complements of miRNA target sites that can be used by miRNAs. Expression of 3′ UTR binding proteins that affect accessibility of miRNAs[76] even to those target sites that are present in a given 3′ UTR; “decoy” RNAs that sequester miRNAs away from their target or, most dramatically, a switch from target repression to target activation by miRISC itself[77,78]. Thus, taken together, these diverse mechanisms might each contribute alone or, more likely, together to miRNA deregulation in human cancer[79].

**SNPs among MiRNA**

Genetic variations range from large chromosomal anomalies to single nucleotide changes. SNPs are the most frequently identified variants in DNA sequences. miRNA-related SNPs could potentially affect the maturation of miRNAs, the silencing machinery, the structure or the expression level of mature miRNA, and
the base pairing at the target site; they may also have functional role in miRNA-mediated gene regulation, thereby affecting cancer risk. Figure 1). That this is indeed the case as has been demonstrated by a germline DNA variant (C/T) in the primary sequence of the miRNA cluster encoding miR-16-1 and miR-15a (7 bp in the 3' direction after the precursor). This DNA variant, which results in reduced mature expression of miR-16-1 and miR-15 in vitro and in vivo and is associated with deletion of the chromosome region containing this miRNA cluster, was found in 11 of 75 patients with CLL but not observed in 160 subjects without cancer. Such genetic variations are not rare, and some are proven functional. For example, a mutation in the seed region of human miR-96 is responsible for nonsyndromic progressive hearing loss; DICER1 mutations are associated with familial pleuropulmonary blastoma; and one SNP in the mature sequence of miR-30c-2 is likely to affect stem integrity. Furthermore, a G/U polymorphism (rs12975333), located at the eighth nucleotide within the mature sequence of miR-125a, has been functionally characterized to block the processing of pri-miRNA into pre-miRNA and alter the translation suppression on the lin-28 target mRNA. All these are good examples for the importance of miRNA related SNP.

Polymorphisms in pre-miRNA may influence miRNA maturation and thereby modulate miRNA expression. Several groups have tried to identify SNPs within or flanking the pre-miRNA sequences using experimental or bioinformatic approaches. In one study, 173 human pre-miRNAs in 96 Japanese individuals were sequenced, and 10 SNPs in 10 pre-miRNA hairpins were identified. In another study, a bioinformatics search identified 12 known SNPs located within 227 human pre-miRNA sequences. In a similar study, researchers screened the dbSNP database for common SNPs in 474 human pre-miRNAs. Sixty-five SNPs in 49 pre-miRNAs were found, and 3, hsa-miR-125a, hsa-miR-627, and hsa-miR-662, were located within seed regions rs12975333, rs2620381, and rs9745376, respectively, indicating that SNPs within miRNA seed region are very rare.

To assist in the identification of DNA sequence polymorphisms (DSPs) that affect miRNA-mediated regulation, Hiard et al. have searched the public domain databases for SNPs and other polymorphisms in the 3 sequence compartments involved in miRNA control: targets, miRNA precursors, and silencing machinery. The outcome of this search is browsable via the Patrocles website (http://www.patrocles.org/). DSPs are sorted depending on whether they affect the seed region, the mature miRNA outside the seed, or other parts of the pre-miRNA. In humans, for instance, 184 DSPs affecting 136 out of 676 pre-miRNAs were identified. Twelve of these mapped to the miRNA seed and 26 to the mature miRNA outside the seed. However, the 12 human miRNAs with a DSP in their seed

Figure 1. Effects of single-nucleotide polymorphisms (SNPs) among microRNA (miRNA) sequences, miRNA processing genes, and miRNA-binding sites that affect cancer risk.
sequence were either members of a seed-sharing miRNA family or more recently discovered miRNAs that are likely to be expressed at lower levels. The effect of the DSPs on pre-miRNA structure was evaluated using RNAfold and the predicted secondary structures are viewable in Patrocles[90].

The roles that SNPs among miRNA play in miRNA processing and cancer development have been studied in recent years, with the miR-146a SNP (rs2910164) within the pre-miR-146a sequence one of the most thoroughly studied examples[91]. This SNP reduced both the amount of pre-miR-146a and mature miR-146a, and affected the Drosha/DGCR8 cropping step, and was associated with papillary thyroid carcinoma (PTC), familial/sporadic breast cancer, ovarian cancer, prostate cancer, and hepatocellular carcinoma[92,93]. In an association study of 608 PTC patients and 901 controls, Jadzewsitw et al. [94] found marked differences in genotype distribution of rs2910164 (P < 0.001). The GC heterozygous state was associated with an increased risk of acquiring PTC (OR = 1.62, P < 0.001) compared with both homozygous states. Further functional analysis showed that GC heterozygotes differed from both the GG and CC homozygotes by producing three mature miRNAs, one from the leading strand (miR-146a) and the other two from the passenger strand (miR-146a*G and miR-146a*C), each with a distinct set of target genes. TaqMan analysis of paired tumor and normal samples revealed a 1.5- to 2.6-fold overexpression of polymorphic miR-146a* in 7 of 8 tumors compared with the unaffected part of the same gland. Microarray data showed that widely different transcriptomes occurred in the tumors and in unaffected parts of the thyroid from patients with GC and CC homozygotes. The affected genes were mainly involved in the regulation of apoptosis, leading to exaggerated DNA-damage response in heterozygotes and potentially explaining the predisposition to cancer[94].

We summarized in this review the SNPs within miRNA sequences, miRNA pathway genes, and miRNA binding sites that have been found to be significantly associated with cancer susceptibility[91,97-112] (Table 1). Systematic analysis of the association between miRNA-related SNPs and cancer risk has been reported predominantly in two studies. Horikawa et al. [107] evaluated SNPs in miRNA-related genes and the risk of bladder cancer and renal cell carcinoma. In their studies, in which they assessed the effects of a total of 41 SNPs in genes of the miRNA biogenesis pathway (24 SNPs), pre-miRNAs (7 SNPs), and pri-miRNAs (10 SNPs) on cancer predisposition, they found that a non-synonymous SNP in GEMIN3 and a common haplotype of GEMIN4 are associated with bladder cancer risk, whereas two SNPs in the GEMIN4 gene are associated with altered renal cell carcinoma risk. Both GEMIN3 and GEMIN4 proteins are core components of a large macromolecular complex that plays an essential role in pre-miRNA splicing and ribonucleoprotein assembly. In addition to the significant SNP/haplotype identified for the GEMIN genes, borderline significant associations with bladder cancer risk were also identified for SNPs in several other genes, including TRBP, miR-423, miR-492, miR-26a-1, and miR-124-1. In particular, the variant allele of rs784567, which is located in the 5' UTR of the TRBP gene, is associated with a 20% risk reduction (P = 0.07) [105,107]. Through a thorough searching of available bioinformatic databases, Tian et al. [98] found 4 SNPs within the pre-miRNA region (miR-146a, rs2910164; miR-149, rs2292832; miR-196a2, rs11614913; miR-499, rs3746444), with a minor allele frequency more than 0.05, from about 400 known miRNAs. They tested the association of these 4 SNPs with the risk of lung cancer[99] and breast cancer[96], as well as the prognosis of lung cancer[113]. They found that the rs11614913 SNP in miR-196a2 is associated with increased risk and shortened survival time of non–small cell lung cancer as it alters the expression of mature miR-196a and the binding activity of target mRNA. miR-196a2 rs11614913 and miR-499 rs3746444 are associated with significantly increased risk of breast cancer. Although both of these studies involved a systematic search for SNPs among miRNAs known at that time, the findings do not include some common SNPs within pre-miRNA or the miRNA biogenesis pathway, mainly because of the expanding sum of human miRNAs identified after these studies were conducted.

We previously reviewed SNPs within miRNA binding sites and cancer risk[114], followed by case control studies in this field[109]. Recently, Liang et al. [109] reported their findings that miRNA binding site SNPs may impact ovarian cancer predisposition and clinical outcome both individually and jointly with genetic variants in miRNA biosynthesis pathways. To test the hypothesis that disruption of miRNA target binding by SNPs is a widespread mechanism relevant to cancer susceptibility, Nicoloso et al. [115] analyzed SNPs known to be associated with breast cancer risk, in silico and in vitro, for their ability to modify miRNA binding sites and miRNA gene regulation. They proposed that transcribed target SNPs alter miRNA gene regulation and, consequently, protein expression, thereby contributing to the likelihood of cancer susceptibility by a novel mechanism of subtle gene regulation[113]. All of these studies have contributed to our knowledge about miRNA binding site SNPs and cancer susceptibility. However, binding site SNPs represent a large group of genetic variation that can be observed from online searching software such as Patrocles and PolymiRTS. Studies to date have been limited in the investigation of this kind of SNPs. Further systematic studies are warranted for a general view of the role this group of SNPs plays in the development of cancer.
miRNAs can also be post-transcriptionally modified, such as by RNA editing via adenosine deaminases that act on RNA (ADAR)\(^\text{[116]}\). A to I-editing of pre-miR-151 blocks its processing by Dicer/TRBP\(^\text{[117]}\). ADAR-edited pri-miR-142 is more easily degraded by Tudor-SN\(^\text{[118]}\). Edited miR-376a-5p within the middle of the seed region alters the set of targets regulated by this miRNA\(^\text{[119]}\). A survey of RNA editing of miRNAs from 10 human tissues implies RNA editing of miRNA happens quite often and is a mechanism to increase the diversity of miRNAs and their targets\(^\text{[120]}\). All the above examples show that mutant or post-transcriptional editing of miRNAs can result in alterations of processing and function and are often involved in cancer development. Hence, SNPs that occur in sequences downstream or upstream of the pre-miRNA, sequences in the terminal loop of pre-miRNA, sequences in the miRNA and miRNA* duplexes, and sequences of genes in the

| SNP category | Gene | SNP ID | Variants | Cancer | Association |
|--------------|------|--------|----------|--------|-------------|
| miRNA sequence |      |        |          |        |             |
| miR-146a     | rs2910164 | G/C | Thyroid    | 0.50(0.28, 0.89) |
| miR-219-1    | rs213210  | T/C | Esophageal | 2.39(1.36, 4.20) |
| miR-124-1    | rs531564  | C/G | Esophageal | 1.66(1.00, 2.74) |
| miR-26a-1    | rs7372209 | C/T | Esophageal | 8.79(1.06, 73.17) |
| miR-631      | rs5745925 | CT/C | Esophageal | 1.35(1.04, 1.76) |
| miR-423      | rs6505162 | C/A | Esophageal | 1.57(1.03, 2.41) |
| miR-196a2    | rs11614913| C/T | Esophageal | 0.57(0.44, 0.73) |
|              |          |      | Lung      | 1.73(1.16, 2.56) |
|              |          |      | Gloma     | 1.25(1.01, 1.54) |
|              |          |      | Breast    | 0.74(0.56, 0.98) |
|              |          |      | Gastric   | 1.48(1.06, 2.05) |
|              |          |      | Cervical  | 0.72(0.52, 0.99) |
|              |          |      | Hepatocellular | 1.37(1.06, 1.78) |
|              |          |      | Head and neck | 0.83(0.69, 0.99) |
|              |          |      | Bladder   | 2.40(1.04, 5.56) |
|              |          |      | Esophageal | 1.45(1.02, 2.06) |
|              |          |      | Renal cell | 0.68(0.47, 0.98) |
|              |          |      | Ovarian   | 0.71(0.57, 0.88) |
|              |          |      | Renal cell | 0.67(0.47, 0.96) |
|              |          |      | Ovarian   | 0.70(0.51, 0.96) |
|              |          |      | Ovarian   | 0.71(0.57, 0.87) |
|              |          |      | Esophageal | 1.84(1.16, 2.93) |
|              |          |      | Ovarian   | 0.73(0.54, 0.99) |
|              |          |      | Esophageal | 1.93(1.09, 3.40) |
|              |          |      | Ovarian   | 0.67(0.53, 0.84) |
|              |          |      | Ovarian   | 0.69(0.48, 0.99) |
|              |          |      | Ovarian   | 0.71(0.51, 0.99) |
| Binding sites |      |        |          |        |             |
| CD86         | rs17281995| G/C | Colorectal | 2.93(1.29, 6.67) |
| LAMB3        | rs25566  | C/T | Cervical   | 1.57(1.25, 1.96) |
| SET8         | rs16817496| C/T | Breast    | 1.66(1.06, 2.61) |
| ATG4A        | rs5973822 | A/G | Ovarian   | 0.42(0.24, 0.75) |
| UGT2A3       | rs17147016| T/A | Ovarian   | 1.47(1.08, 2.01) |
| COL18A1      | rs7499   | G/A | Ovarian   | 1.47(1.07, 2.02) |
| CAV1         | rs9920   | A/G | Ovarian   | 1.50(1.04, 2.17) |
| IL1R1        | rs3917328 | G/A | Ovarian   | 1.65(1.03, 2.64) |
| KRAS         | rs10771184| T/A | Ovarian   | 1.26(1.01, 1.57) |
| EZF2         | rs2075993 | A/G | Ovarian   | 1.24(1.00, 1.54) |
| IQGAP1       | rs1042538| A/T | Breast    | 0.42(0.61, 0.99) |

The data for association are presented as odds ratio (95% confidence interval).

Table 1. Single-nucleotide polymorphisms (SNPs) among microRNA (miRNA) sequences, miRNA processing genes, and miRNA-binding sites that are significantly associated with cancer risk.

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miRNA biogenesis pathway are potentially valuable predictors of cancer risk and prognosis[13,122].

In conclusion, miRNAs play a substantial role in cancer development. miRNA related SNPs are important biomarkers for their function, and the amount of this kind of SNPs is very large, most of them have not been evaluated in association studies. Therefore, we currently have little knowledge of the overall contribution of this kind of SNPs to the development of cancer. In future researches, studies specifically designed to examine the relationship between miRNA related SNPs in the whole genome and cancer risks are warranted.

Acknowledgements

This study was jointly supported by grants from the National Natural Science Foundation of China to K.C. (No. 30872172) and the Tianjin Science and Technology Committee Foundation (No. 08ZCGH202000, 09ZCZDSF04400).

Received: 2011-04-06; revised: 2011-05-10; accepted: 2011-05-10.

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