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REVIEW ARTICLE

MicroRNAs: POTENTIAL BIOMARKERS IN CANCER

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ABSTRACT

microRNAs (miRNAs) are evolutionarily conserved small noncoding RNAs, also known as micromanagers of gene expression. Polymorphisms in the miRNA pathway (miR-polymorphisms) are emerging as powerful tools to study the biology of a disease and have the potential to be used in disease prognosis and diagnosis. Advancements in the miRNA field also indicate a clear involvement of deregulated miRNA gene signatures in cancers, and several polymorphisms in pre-miRNA, miRNA binding sites or targets have been found to be associated with various cancers. The miRNA polymorphisms have also been reported to influence tumor aggressiveness as well as survival of cancer patients. miRNAs have a revolutionary impact on cancer research over recent years. They emerge as important players in tumorigenesis, leading to a paradigm shift in oncology. The extensive and comprehensive use of miRNA microarrays has enabled the identification of a number of miRNAs as potential biomarkers for cancer. Many miRNAs have been identified to act as oncogenes, tumor suppressors, or even modulators of cancer stem cells and metastasis. Some studies not only reported the identified miRNA biomarkers, but also deciphered their target genes and the underlying mechanisms. The rapid discovery of many miRNA targets and their relevant pathways has contributed to the development of miRNA-based therapeutics.

KEY WORDS

Micro RNA, Cancer, Polymorphism, Epigenetic, Biomarker.

INTRODUCTION

MicroRNAs (miRNAs) are a class of small noncoding RNA molecules that regulate gene expression by Watson-Crick base pairing to target messenger RNA (mRNA). They are involved in most biological and pathological processes, including tumorigenesis. The binding of miRNAs to target mRNA is critical for regulating the mRNA level and protein expression. Since a single miRNAs can bind to 100 different target transcripts, it has been estimated that miRNAs may be able to regulate up to 30%of the protein-coding genes in the human genome (1).

A large number of miRNA genes (>1,000) have been predicted to exist in the human genome, accounting for 1% to 5% of all predicted human genes (2). Initially, they are transcribed from miRNA genes as long primary RNAs (pri-miRNAs). Since pri-miRNAs usually contain the cap structure and the poly (A) tail, it has been suggested that the transcription of miRNAs is carried out by RNA polymerase II. In the nucleus, the pri-miRNAs are processed by Drosha, a member of the RNase III enzyme family, into precursors (pre-miRNAs) of ~70 nt in length with a stem-loop structure, in conjunction with the double-stranded RNA-binding protein DGCR8/Pasha. Pre-miRNAs are exported from the nucleus to the cytoplasm by exportin-5 in a Ran-guanosine triphosphate (GTP)-dependent manner, where they are processed by another RNase III enzyme, Dicer. This causes the release of a ~22 nt double-stranded RNA duplex that is incorporated into a RNA-induced silencing complex in a manner analogous to that observed for

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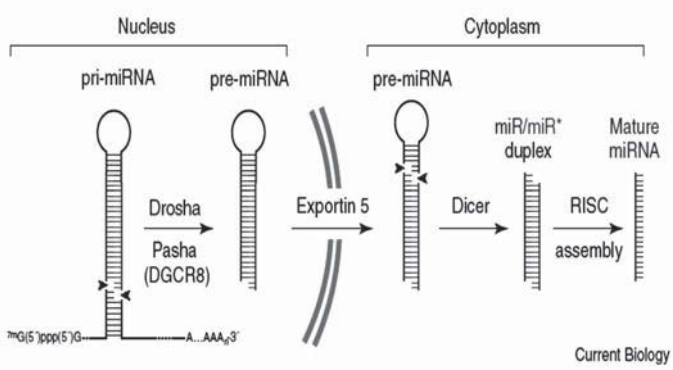
siRNA). In this complex, one strand is retained as the mature miRNA, whereas the other strand is generally degraded. This complex is now capable of regulating its target genes (3, 4).

Formation and processing of miRNA



MicroRNA-binding target

The miRNA within the RNA-induced silencing complex acts by binding to the 3' UTRs of target mRNAs (5), which inhibits their translation. The critical region for miRNA binding is nucleotides 2-8 from the 5' end of the miRNA, called the 'seed region', which binds to its target site on a given mRNA by Watson-Crick complementarity. Asymmetry is the general rule for matches between miRNA and its target, in that the 5' end of the miRNA tends to have more bases complementary to the target than the 3' end does. In a series of experiments done to determine the minimal requirements for a functional miRNA target site, it was concluded that the complementarities of seven or more bases to the 5' end miRNA is sufficient to confer regulation and that sites with weaker 5' complementarity require compensatory pairing to the 3' end, but that only extensive pairing to the 3' end of the miRNA is not sufficient to confer regulation. Additional Watson-Crick pairing to four contiguous nucleotides at nucleotides 12-17 enhances micro-RNA targeting, especially nucleotides 13-16. In truth, the existence of a seed region is not a generally reliable predictor of a real miRNA target. Because it is an energy consuming process to free the base pair within mRNA in order to make the target accessible for miRNA binding, secondary structures are also required for target recognition to occur. In this regard, Zhao et al (6) demonstrated that a common feature of most validated targets is that miRNAs preferentially target 3' UTR sites that do not have complex secondary structures and are located in accessible regions of the RNA based on favorable thermodynamics. Kertesz et al (7) systematically investigated the role of target site accessibility in miRNA target recognition and experimentally showed that mutations diminishing target accessibility substantially reduce miRNA-mediated translational repression. Current consensus is that miRNA target specificity is truly determined by both sequence matching and target accessibility. This is because even sequences with high complementarity might not be the real targets of miRNA.

Polymorphisms in miRNAs

The binding of miRNA to mRNA is critical for regulating the mRNA level and protein expression. However, this binding can be affected by single-nucleotide polymorphisms that can reside in the miRNA target site, which can either abolish existing binding sites or create illegitimate binding sites (8, 9). Therefore, polymorphisms in miRNA can have a differing effect on gene and protein expression and represent another type of genetic variability that can influence the risk of certain human diseases. Different approaches have been used to predict and identify functional polymorphisms within miRNA-binding sites. The biological relevance of these polymorphisms in predicted miRNA-binding sites is beginning to be examined in large case-control studies. Since the sequence complementarity and thermodynamics of binding play an essential role in the interaction of miRNA with its target mRNA, sequence variations such as SNPs in the miRNA-binding seed region should disrupt the miRNA-mRNA interaction and affect the expression of miRNA targets. Simply put, an SNP may either abolish or weaken a miRNA target or create a perfect sequence match to the seed of a miRNA that otherwise was not associated with the given mRNA. The increase or decrease in miRNA binding caused by the SNP variation would probably lead to a corresponding decrease or increase in protein translation (10, 11). This would make it possible to predict the effect of this type of SNP. For example, SNP-associated deregulation of the expression of an oncogene or tumor suppressor might contribute to tumorigenesis (12, 13).

Classification of miRNA polymorphism
Polymorphisms or mutations affecting miRNA biogenesis

Several proteins and protein complexes are involved in various steps of miRNA bio genesis, such as miRNA transcription, processing, export and targeting. These proteins include RNA polymerase II complex, Drosha/Pasha, Exportin-5/Ran-GTP, nuclear pore complexes, Dicer and the Argonaut protein complex/RISC complex. Polymorphisms present not only in miRNA precursors but also in the proteins involved in its biogenesis may potentially affect miRNA-mediated regulation of the cell. miR-polymorphisms/mutations affecting miRNA biogenesis can be further sub-classified in following three

categories:

1. In pri- and pre-miRNA transcripts
2. In mature miRNA sequences
3. Affecting expression of the proteins involved in various steps of miRNA biogenesis

MiR-polymorphism/mutations in miRNA target sites

In a population, miR-polymorphisms can be present either in a heterozygous or homozygous configuration, in the form of insertions, deletions, amplifications or chromosomal translocations, resulting in loss or gain of a miRNA site/ function.

Polymorphisms present in pri-, pre- and mature miRNA can potentially influence expression of hundreds of genes and pathways, broadly affecting miRNA function. Sequence variations in miRNA genes, including pri-miRNAs, pre-miRNAs and mature miRNAs, could potentially influence the processing and/or target selection of miRNAs (14). A bioinformatics approach was used to study 79 polymorphisms in the 3'-UTRs of 129 cancer associated genes, of which seven SNPs were found to be located in pre-miRNA hairpins and one in the miR-608 mature sequence (15). In a screen of 227 known human miRNAs, a total 323 SNPs were identified, of which 12 were found to be located within the miRNA precursor (14). A C>T germline alteration in the primary transcript of miR-15a/miR-16 was found in some patients with familial chronic lymphocytic leukemia (CLL) (16). The polymorphism was found to be associated with reduced expression of miR-15 and miR-16. Approximately 70% of CLL cases express low levels of these two miRNAs, suggesting an association of this genetic polymorphism to leukemogenesis (16, 17). Recently a pre-miRNA SNP (rs11614913) in miR-196a2 was found to be associated with survival in individuals with non-small-cell lung cancer (NSCLC). A significant decrease in survival was observed in individuals homozygous for the SNP (CC), suggesting that the SNP could be a prognostic marker for NSCLC. This SNP was also shown to affect the binding of miR-196a2 to its target mRNA and resulted in a significant increase in mature miR-196a2 levels with no changes in the precursor miRNA, suggesting that the mature miRNA is directly processed from the pre-miRNA (18). A more recent follow up case-control study in Chinese women with breast cancer, identified the rs11614913 (T>C) polymorphism in miR-196a2 and an A>G SNP (rs3746444) in miR-499, associated with a significant increased risk of breast cancer susceptibility (19). A common G>C polymorphism (rs2910164) in pre-miR-146a affects miRNA expression and contributes to the genetic predisposition to papillary thyroid carcinoma (PTC). Approximately 4.7% of PTC tumors showed somatic mutations of the SNP sequence, suggesting that the SNP plays a role in tumorigenesis through somatic mutation (20).

Polymorphisms in mature miRNA sequences

MiRNA binds to the target mRNA with Watson-Crick complementarity. Primarily a miRNA consists of two regions. The 5'-region of a miRNA, from positions 2-7, called as the 'seed' region, which is thought to confer much of the target recognition specificity. The other region of the miRNA, apart from the seed region, is able to tolerate mismatches to a certain extent; therefore, it was coined as 3'-mismatch tolerant region (3'-MTR) to describe this region. A miRSNP in miR-608 mature sequence has been identified *in silico*. It was demonstrated in plants, (Arabidopsis and related Brassicaceae), that mutations in the miRNA itself resulted in loss of miR-319a function, which was further compensated by other members of the miR-391 family (21).

MiRNA polymorphisms/mutations in mature miRNA sequences can be further subclassified in following categories:

i) In miRNA 5'- seed region
ii) In miRNA 3'-mismatch tolerant region (3'-MTR).
iii) miR-polymorphisms/mutations altering epigenetic regulation of miRNA genes.

i. Polymorphisms in a mature miRNA seed region

It has been suggested that the 5'-seed region is important for miRNA binding; however, this is not a reliable predictor of the actual miRNA target (22). Since the miRNA seed sequences are short and highly conserved, the probability of a 'miRNA-seed polymorphism/mutation' are expected to be lower than a 'miRNA target site polymorphism/mutation'. Indeed, one study indicated that the likelihood of a SNP occurring in a miRNA seed region is less than 1% (23). A recent study identified a polymorphism present in the seed region of miR-125a that significantly inhibited the processing of pri-miRNA to pre-miRNA, resulting in reduced miRNA-mediated translational repression. Thus, a SNP can either abolish or weaken a miRNA target, or create a perfect sequence match to the seed of a miRNA that otherwise was not associated with the given mRNA (8, 24). Although miRNA seed region polymorphisms can theoretically affect the expression of hundreds of genes, this prediction will require experimental validation. Moreover, the significance of miRNA seed region polymorphisms from the standpoint of population genetics has yet to be determined in large sets of cancer patients.

ii. Polymorphisms in mature miRNA 3'-MTR

Unlike the mRNA seed region, which is very sensitive to mismatches, it has been suggested that the 3'-MTR may tolerate mismatch SNPs to a certain extent, however, multiple SNPs, insertions, deletions or translocations in this region can potentially affect the miRNA mediated regulation of the target gene. However, this possibility needs to be further investigated. Polymorphisms affecting the expression of the proteins involved in various steps of miRNA biogenesis.

It has been proposed that polymorphisms that affect expression of proteins involved in miRNA action and biogenesis, such as Drosha, Dicer, exportin5-ranGTP and the proteins in the RISC complex, may affect miRNA-mediated regulation within the cell. Since these proteins affect global miRNA biogenesis, genetic knockout of some of these proteins are lethal in mice. Polymorphisms that affect expression of the proteins would likely deregulate miRNA biogenesis and synthesis. In Kaposi's sarcoma herpes virus (KSHV)-infected body-cavity-based lymphoma (BCBL)-1 cells, a naturally occurring polymorphism in a miRK5 viral miRNA precursor stem-loop results in reduced processing by Drosha and, therefore, lower levels of mature miRNA expression (25). Since less or more miRNA expression may have serious consequences in a cell, polymorphisms affecting the proteins involved in various steps of miRNA biogenesis can affect overall miRNA transcription, processing, export and targeting and may have deleterious effects in a cell.

iii. MiR-polymorphism in miRNA target sites

In contrast to the miR-polymorphisms in miRNA biogenesis, a miR-polymorphism located at the 3'-UTR of a target (coding) gene are more abundant in the human genome and have a more defined and limited range of effects. MiR-polymorphisms in miRNA target sites will impact only its encoded target-mRNA and its downstream effectors, hence, are more specific. A recent genome-wide association (GWA) study suggests that a gene that has more than two miRNA target sites will have increased expression variability as compared with a gene that is not regulated by a miRNA. The variability is further induced by SNPs in the miRNA target sites (26). Thus, considering the large number of less conserved 3'-UTR target sequences they will potentially harbor a higher frequency of target miR-polymorphisms, and are potentially more important from an epidemiological standpoint. MiRNA polymorphisms /mutations in miRNA-target-mRNA sites can be further subclassified in following three categories:

1. At a miRNA binding site
2. Near a miRNA binding site
3. At a miRNA binding site

The generic 3'-UTR of a gene consists of a miRNA binding site, divided into a miRNA seed region binding site and a non-seed region binding site we refer to as the 3'-MTR binding site. It is proposed that a miRNA target site polymorphism can be of two types: a polymorphism in the 5' end of the miRNA target site, where the seed region of miRNA binds, and a polymorphism in the 3'-MTR binding site.

Since the miRNA seed sequence plays an important role in target recognition and binding, it is predicted that a polymorphism in this region may have a higher probability of affecting a miRNA function, as compared with a polymorphism that is present in 3'-MTR binding region. However this concept needs to be tested experimentally. It has been proposed that polymorphisms outside the miRNA target site can be of two types: a polymorphism in the target mRNA outside the miRNA target site affecting accessibility of the miRNA. Unlike DNA-protein inter actions, mRNA-protein interactions are based on the presence or absence of secondary structure motifs in mRNAs. Most of the miRNAs binding sites in the 3'-UTRs of a target mRNA lack a complex secondary structure, thereby facilitating access for a miRNA (27).

Mutations that can create or abolish a secondary structure near a miRNA binding site may potentially influence miRNA-mediated translational repression of a target gene by affecting the accessibility of a miRNA to its binding site (7). A polymorphism near a miRNA target site could disrupt the association of miRNA with other regulatory elements present in the 3'-UTR of the target transcript more than one miRNA. Other than a miRNA binding site, a 3'-UTR harbors binding sites for cytoplasmic polyadenylation element (CPE) binding proteins and the hexanucleotide AAUAAA signal for cleavage and polyadenylation. MiRNAs are shown to promote polyadenylation by interacting with cytoplasmic polyadenylation elements and other proteins or protein complexes within the 3'-UTR (28). miR-polymorphisms may potentially affect these interactions. It has been demonstrated that under certain cellular conditions a stable secondary structure could be unfolded to provide access to a miRNA target site. This miRNA mediated regulation can be exploited by a cell during stress response or in tissue specificity (29). There is evidence that two miRNAs may bind to a target mRNA in coordination. Binding of miRNA to its target site may induce remodeling of the secondary structures in the neighboring regions, facilitating binding of miRNAs. Hence, polymorphisms near a miRNA

target site can potentially influence the accessibility of a miRNA-RISC complex by affecting the RNA structural motifs necessary for RNA-protein interaction. Further analysis of the interactions between miRNA and other regulatory elements present in 3'-UTRs will shed more light on the function of miRNA polymorphisms and will eventually establish 3'-UTR as a hotspot for pathology (10).

MiR-polymorphisms/mutations altering epigenetic regulation of miRNA genes

Various miRNA genes are affected by epigenetic silencing due to aberrant hypermethylation. Epigenetic silencing of a miRNA was found to be an early and frequent event in the development of breast cancer. Aberrant hyper methylation of miR-9-1, miR-124a3, miR-148, miR-152 and miR-663 was observed in 34-86% of cases of 71 primary human breast cancer specimens (30). MiR-polymorphism mediated epigenetic alteration of miRNA regulation is a new, unexplored area of research. It has been proposed that miR-polymorphisms or miR mutations that can alter epigenetic regulation of a miRNA (methylation or acetylation) can be a mechanism of disease progression. Gain or loss of epigenetic regulation of an oncogene or a tumor suppressor, respectively, due to a miR-polymorphism or mutation, may have devastating effects in a cell.

miRNA and Cancer

Cancer is the first leading cause of death after cardiovascular diseases. It has been shown that microRNAs are involved in carcinogenesis. The exact role of miRNAs in cancer pathogenesis was identified through expression studies of specific miRNAs which were over-expressed or knocked down and the initiation and development of different types of malignancies related to it. The first evidence that miRNAs is related to cancers came from Croce group. In their study, they found two miRNA genes (miR-15 and miR-16) are located at the chromosome 13q14 region, which is frequently deleted or down-regulated in the majority (~68%) of B cell chronic lymphocytic leukemia (B-CLL) cases (31). Subsequent investigations demonstrated that almost all cancers have alternative miRNA expression profile compared to their adjunct normal tissues. These cancer types include several important cancers, for example lung cancer, leukemia, brain cancer and breast cancer, which together cause the majority of cancer-related death in the past decades. Recognition of miRNAs that are differentially expressed between tumor tissues and normal tissues may help to identify those miRNAs that are involved in human cancers and further establish the apparent pathogenic role of miRNAs in cancers (32). As already evident from many facts, that when cells exhibit abnormal growth and loss of apoptotic ability, it usually leads to cancer formation. Cheng *et al* showed that microRNAs regulate mechanisms such as cell growth and apoptosis (33). Currently, it is well known that miRNAs can be up-regulated or down-regulated in various human cancers (34). Table 1 summarizes the alternative miRNA expression in the major cancer types.

Table 1: Cancer-related miRNAs summarizing miRNA expression in the major cancer types

Cancer	Up-regulated miRNAs	Down-regulated miRNAs
Breast cancer	miR-21, miR-155, miR-29b-2	miR-143, miR-145, miR-155, miR-200
Lung Cancer	miR-21, miR-189, miR-200b, miR-17-92 cluster	let-7 family, miR-126, miR-30a, miR-143, miR-145, miR-188, miR-331, miR-34s
Colon Cancer	miR-223, miR-21, miR-17, miR 106m, miR-34s	miR-143, miR-145, miR-195, miR-130a, miR-331
Prostate Cancer	Let-7d, miR-195, miR-203, miR-125b, miR-20a, miR-221, miR-222	miR-143, miR-145, miR-128a, miR-146a, miR-126
Brain Cancer	miR-21, miR-221	miR-181
Hepatocellular carcinoma	miR-34s, miR-224, miR-18, miR-21 miR-	miR-17-19b cluster, miR-200a, 125a, miR-199a, miR-195
Chronic lymphocytic leukemia	miR-15, miR-16	
Lung cancer	miR-17-92	let-7
Ovarian cancer	miR-200a,b,c, miR-141	miR-199a, miR-140, miR-145, miR-125b
Pancreatic cancer	miR-221, miR-181a, miR-21,	miR148a,b
Papillary thyroid carcinoma	miR-221, miR-222, miR-146, miR-181	
Stomach cancer	miR-21, miR-103, miR223	miR-218

Over-expressed miRNAs may function as oncogenes by down-regulating tumor-suppressor genes and/or genes that control cell differentiation or apoptosis, whereas the down-regulated miRNAs act as tumor-suppressor genes by negatively regulating oncogenes and/or genes that control cell differentiation or apoptosis (35-38). However, this is only the beginning to understand how miRNAs interact with classical oncogenes and tumor suppressors. Expression profiling of miRNAs has revealed that the miRNA signature is associated with tumor classification, diagnosis and progression, as well as prognosis and response to treatment (39-41). Hanahan and Weinberg (42) focused on the role of miRNAs in the hallmarks of human cancers as described below (Figure 1; Table 2).

Cancer is a multistep process of sequential alternations of several, oncogenes, tumor-suppressor genes, and miRNA genes. Genetic defects in DNA repair mechanisms and cell cycle checkpoints result in increased genomic instability and cancer predisposition (42). MicroRNAs (miRNA) can act as oncogenes or tumor suppressors and modulate the expression of approximately one third of all human genes. Several studies have reported that specific miRNA expression signatures could be used as predictors of esophageal cancer diagnosis and prognosis (43). Moreover, miRNA processing genes have also been associated with the development and survival of multiple cancers, including esophageal cancer.

Following are some examples of the miR-polymorphisms in the target mRNA that were found to be associated with cancer.

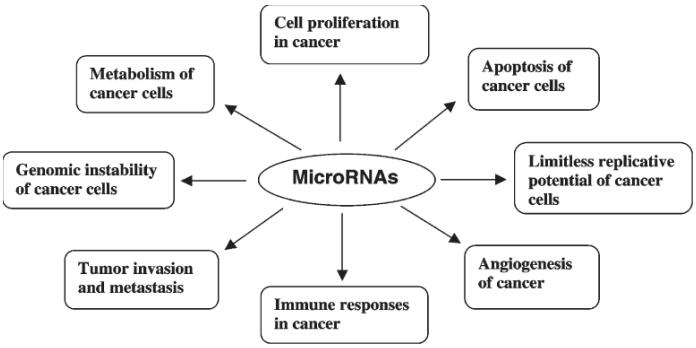


Fig 1: The role of microRNAs regulation in the hallmarks of human cancer

Colorectal cancer: A case-control study in the Czech population, which has the highest worldwide incidence of colorectal cancer, identified polymorphisms within miRNA-binding sites with a positive association with a risk of sporadic colorectal cancer (15). In the 3'-UTR of cluster of the differentiation 86 (CD86) gene, a C>G polymorphism (rs17281995) predicted to affect miR-337, miR- 582, miR-200a, miR-184 and miR-212 was significantly associated with colorectal cancer. The study also identified rs1051690 in insulin receptor (INSR) predicted to affect miR-618 and miR-612 (32).

Papillary thyroid carcinoma: A total of two polymorphisms were identified, in the miR-221/222 and miR-146a/146b miRNA binding sites in v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog (KIT) gene, associated with deregulated

Table 2: miRNAs and the hallmarks of cancer

Hallmarks of cancer	Functions of miRNAs	miRNAs
Resistance to anti-proliferation signal and independence from exogenous growth factor signals	Pro-proliferation Anti-proliferation	miR-21, miR-17 cluster, miR-221, miR-222, let-7, miR-519, miR-146a
Evasion of apoptosis	Pro-apoptosis Anti-apoptosis	miR-34 cluster, miR-29, miR-15, miR-16, miR-17-92 cluster, miR-21
Limitless replicative potential	Regulation of immortalization or senescence	miR-290, miR-24, miR-34a
Induction of angiogenesis	Pro-angiogenesis Anti-angiogenesis	miR-17-92 cluster, miR-378, miR-296, let-7f, miR-27b, miR- 130, miR-126, miR-15, miR-16, miR-20a, miR-20b
Evasion of immune system	Escape from immunosurveillance	miR-155, miR-17-92 cluster, miR-20a, miR-93, miR-106b, miR-372, miR-373, miR-520c, hcmv-miR-UL112
Tissue invasion and metastasis	Pro-metastasis Anti-metastasis	miR-10b, miR-21, miR-373, miR-520c, miR-155, let-7, miR-335, miR-206, miR-126, miR-146a, miR-101, miR-200
Genomic instability	Promote genomic Instability	Deletions or down-regulation of miRNAs, such as miR-17, miR-20a, miR-15, miR-16-1 or let-7

expression of the KIT protein contributing to papillary thyroid carcinoma (36).

Breast cancer: A C>T (rs93410170) miRSNP in the 3'-UTR of ER-alpha, resulted in stringent miR-206 mediated regulation of ER-alpha. Since ER-alpha overexpression is associated with higher risk for breast cancer, it was suggested that the SNP may be associated with breast cancer (44). An integrin-beta4 (ITGB4) SNP may influence breast tumor aggressiveness and survival, and it may have prognostic value in the clinic (45). A chromosomal translocation that was found to be associated with human tumors was shown to disrupt the let-7 miRNA mediated regulation of an oncogene, high mobility group A2 (HMGa2) (46). Recently an epidemiologic study demonstrated the association of miRNA-related genetic variants may affect bladder cancer risk (47).

Hepatocellular carcinoma (HCC): A G>C polymorphism (rs2910164) is located in the stem region opposite to the mature miR-146a sequence, which results in a change from G:U pair to C:U mismatch in the stem structure of miR-146a precursor. A case-control study was carried out to ascertain the association of this polymorphism in hepatocellular carcinoma, using 479 hepatocellular carcinoma (HCC) and 504 control subjects. The results revealed that male individuals with GG genotype were two-fold more susceptible to HCC (OR = 2.016, 95% CI = 1.056-3.848) compared to those with CC genotype. The authors next examined the influence of this polymorphism on the production of mature miR-146a and found that G-allelic miR-146a precursor displayed increased production of mature miR-146a compared with C-allelic one. Further investigations disclosed that miR-146a could obviously promote cell proliferation and colony formation in NIH/3T3, an immortalized but non-transformed cell line. These data suggested that the G>C polymorphism in miR-146a precursor may result in important phenotypic traits that have biomedical implications.

Esophageal cancer: miRNAs may affect esophageal cancer risk in general and that specific genetic variants in miRNA-related genes may affect esophageal cancer risk individually and jointly. Ye et al (43) assessed the associations between esophageal cancer risk and 41 potentially functional single nucleotide polymorphisms (SNP) in 26 miRNA-related genes in a case-control study of 346 Caucasian esophageal cancer patients (85.5% with esophageal adenocarcinoma) and 346 frequency-matched (age, gender, and ethnicity) controls and they observed significant association of higher cancer risk with seven SNPs. The most notable finding was that the SNP rs6505162, which is located in the pre-mir423 region, was associated with a per-allele odds ratio of 0.64 (95% confidence interval (95% CI= 0.51-0.80; P for trend < 0.0001). This association remained significant after correction for multiple comparisons. A common haplotype of the GEMIN4 gene was associated with a significantly reduced risk of esophageal cancer (odds ratio, 0.65; 95% CI= 0.42-0.99).

Lung cancer: Hu et al evaluated the influence 4 pre-miRNA SNPs (hsa-mir-146a rs2910164 C/G, hsa-mir-196a2 rs11614913 C/T, hsa-mir-499 rs3746444 G/A, and hsa-mir-149 rs2292832 G/T) with the overall survival of NSCLC and functional relevance (19). They found that the rs11614913 SNP in hsa-mir-196a2 was associated with survival in individuals with NSCLC. Specifically, survival was significantly decreased in individuals who were homozygous CC at SNP rs11614913. In the genotype-phenotype correlation analysis of 23 human lung cancer tissue samples, rs11614913 CC was associated with a statistically significant increase in mature hsa-mir-196a expression but not with changes in levels of the precursor, suggesting enhanced processing of the pre-miRNA to its mature form. Furthermore, binding assays revealed that the rs11614913 SNP affect binding of mature hsa-mir-196a2-3p to its target mRNA. Therefore, the rs11614913 SNP in hsa-mir-196a2 may be a prognostic biomarker for NSCLC.

Role of miRNAs in Cancer therapy

Evidences indicate that the expression of miRNAs is altered in cancer, and that certain changes may be directly implicated in the carcinogenic process. A number of miRNAs have been shown to promote cell proliferation and survival, while others diminish cell proliferation and survival (48). These two classes of miRNAs may play a central role in cancer development as novel oncogenes and tumor suppressors, respectively. In general, the majority of miRNAs are down-regulated in cancer specimens (49). Specific cancer characteristics, both genetic and epigenetic, are detectable in the plasma and serum of cancer patients and may be useful as a tool for: early detection, diagnosis and follow-up of cancer patients. miRNAs due to their small size are relatively resistant to RNase degradation (50) suggesting they would be eminently suitable candidates for detection in biological fluids. Over the last decade mRNA arrays have been investigated extensively for this use, and disease-specific and prognostic signatures have been identified. However, so far these techniques have not been implemented in clinical practice, mainly because of the requirement for fresh tumor material, problems with the reproducibility when the method is applied to different platforms, complicated bioinformatics due to massive amounts of data, and extensive costs.

To identify the tissue origin of poorly differentiated tumors with greater accuracy miRNA profiling is now being extensively used than profiles constructed using mRNAs. Furthermore, it has been suggested that the direct involvement of miRNAs in the regulation of protein expression may render miRNA expression profiles superior to mRNA expression profiles in marking cancer, since only a small fraction of mRNAs are regulatory molecules (51). miRNAs together with transcription factors generate a complex combination regulating gene expression. Thus, manipulation of miRNA-transcription factor gene networks may be provides a novel approach for developing cancer therapies (52).

miRNAs in cancer prognostication

Recent advances in human genome research have provided a wealth of knowledge and revolutionized the field of molecular epidemiology and pharmacogenomics, which in turn hold great promise for individualized medicine. Advancements in the miRNA field indicate a clear involvement of deregulated miRNA gene signatures in cancers such as papillary thyroid carcinoma (36), chronic lymphocytic leukemia (37) and breast cancer (32). Recent GWA studies suggest that variations present in regulatory sites are more likely to be associated with disease and not the variations within coding region (53) and support the notion that DNA sequence variations associated with multiple human diseases may also interfere with functions of miRNAs (54). Recently the miR-181 family of miRNAs was found to be up regulated in erythroid differentiation, and associated with the downregulation of homeobox genes, providing insights into leukemogenesis of the cytogenetically normal acute myeloid leukemia (CN-AML) molecular high-risk group (55).

The most popular therapeutic tool till date is surgical removal of the tumor with subsequent chemotherapy and/or radiation therapy. However, the cure rate is low, particularly for a majority of malignant tumors. The surprising aberrant expression of specific miRNAs in a specific cancer type suggests that those miRNAs may serve as a novel target for cancer treatment and a possible new approach for gene therapy. Although several studies demonstrated that miRNA expression profile can be used to identify and classify poor-differentiated tumors, there remains much work before it can be directly applied to clinical diagnostics. It would be more useful if majority of studies focus on the correlation between miRNA expression level with tumor subtypes besides comparing miRNA expression profiles between tumor and normal tissues. The use of tissue has magnetized the attention of scientists, and has projected some promising results (56). Although the utilization of miRNAs as cancer biomarkers has not yet been implemented in clinical practice, studies have demonstrated that the expression of several miRNAs is different in benign and malignant tissues in prostate cancer and in different stages of disease (57, 58). The dysregulation of miRNAs involved in apoptosis may provide a mechanism for cancer development and resistance to cancer therapy (59). Given mounting evidence that points to a role for miRNAs as regulators of malignancy and apoptosis, it is feasible that miRNAs play significant roles in modulating sensitivity/resistance to common cancer treatments. Recently, several studies have investigated this possibility and have demonstrated miRNAs as potential agents involved in altered sensitivity to cytotoxic therapy. A recent study (60) demonstrated a possible role for let-7 in resistance to radiation therapy in lung cancer. Let-7 has been shown to regulate the oncogenes Ras, which is commonly over-expressed in cancer and has been shown to be critical for protection from radiation induced cell death (61). The authors identified a common pattern of miRNA expression in response to radiation in both normal and tumour lung cells, with seven members of the let-7 family significantly down-regulated in response to radiation. Similarly, modulation of miRNA expression by radiation has also been demonstrated in prostate cancer. miR-521 was significantly down regulated in response to radiation in two prostate cancer cell lines suggesting a role in the radiation response. Expression of miR-521 was shown to determine response to radiation in prostate cancer cells through regulation of the DNA repair proteins (62).

Although, it is curiosity of interest that miRNA profiles can be used as substitute markers of already known molecular aberrations, it is of greater value if the miRNA expression profiles can predict the tumor's response to therapy. In almost every type of cancer, attempts have been made to identify molecular markers that could distinguish between different subtypes of tumors in order to choose the most efficient therapy, and miRNA expression signatures may turn out to be an efficient tool for identifying subgroups of patients who will benefit from alternative treatment regimens producing a desired or intended result. Perhaps one of the biggest problems facing the use of microRNAs and/or associated agents as therapeutics is their pleiotropic mode of action. As a single miRNA can target several hundred genes their perturbation may be expected to give rise to a complex phenotype that may not be readily predictable. Therefore, it is of vital importance, that such therapeutics is marked by exactness and accuracy of expression thus mitigating the effects on non-targeted cells. Significant progress has been made on the relationship between miRNAs and cancers and

the important function of miRNAs in a variety of cancers has been reviewed by several research groups (35, 39, 63, 64). More interestingly, recently studies also demonstrated tumour invasion and metastasis is also initiated by miRNAs (65). Anti-miRNAs and antisense oligonucleotides (ASO) have been employed to inhibit specific miRNA expression in vitro and in vivo for investigational and clinical purposes (66). Although miRNA-based diagnostics and gene therapy are still in their infancy, their huge potentials will meet our need for future disease diagnostics and gene therapy.

CONCLUSION

Although the miRNA field is relatively new, there are more than 3000 publications related to miRNA functions in many different organisms. However there are still many questions remaining unanswered. Are all the enzymes and proteins involved in miRNA production and processing known? What are other factors contributing to miRNA-target gene recognition, other than seed sequence complementarities? What is the regulation of miRNAs at the transcriptional level? However, it is still unclear how cancer cells manipulate miRNAs and other regulators to cooperate with other cell types in tumors to promote their own survival and growth under stressed tumor microenvironments. Current reports have determined the miRNA signature of hypoxic cancer cells, but the relationship between hypoxia and miRNA levels is not well known (67, 68). On the other hand, we are just starting to uncover the huge potential of miRNAs as novel biomarkers and therapeutic targets for medicine. Rapid progression of miRNA-related research has been revealing the huge potential of miRNAs as novel diagnostic and gene therapy tool as well as a novel class of drug targets for cancers, anti-virals and potentially many other diseases. Several human diseases, from neurological disease to heart disease to cancer, are caused or propagated by miRNA mis-expression, which has generated great interest in therapies, diagnoses and prognoses based on disease-specific miRNAs. However, many miRNA-related therapeutic fields are still in their infancy. Before miRNA therapy can become a widespread therapeutic tool for detecting and treating diseases, including cancers, new technologies and new strategies need to be developed. It is likely that much significant progress will be achieved in the therapeutic usage of miRNAs in the next few years. It was predicted that more than half of human genes might be regulated by miRNAs. Deregulation of miRNA expression is often associated with cancer. These studies may change the landscape of cancer genetics and uncover new mechanisms that contribute to cancer development. The future challenges are to learn how miRNAs are regulated in human genome and to identify more biological targets of miRNAs and the relevant signaling pathways. However, the actual in vivo function of miRNAs in humans remains to be clarified. Undoubtedly, the coming years will bring exciting new therapeutic strategies based on the targeting of miRNA in the treatment of human cancer. It is to be hoped that some of these will be efficient and will benefit future cancer patients. Taken together, further research into identifying the novel miRNAs and their target genes and into understanding the biological functions of these miRNAs, will enhance our knowledge of the roles of these novel regulators in tumorigenesis and facilitate the potential diagnosis and treatment of cancers.

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