Identification of diagnostic and prognostic biomarkers for cancer: Focusing on genetic variations in microRNA regulatory pathways (Review)

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Abstract. MicroRNAs (miRNAs) are a highly conserved class of small, noncoding RNAs, which regulate gene expression by post-transcriptional degradation or translational repression. miRNAs are involved in the regulation of cell apoptosis, proliferation, differentiation and other physiological processes. They have been increasingly recognized to be involved in the initiation and progression of human carcinogenesis. More recently, it has been proposed that the genetic variations in miRNA genes, those encoding their biogenesis pathway and target binding sites, may affect the miRNA processing machinery and/or targeting. Polymorphisms in the miRNA regulatory pathway may result in the loss or gain of a miRNA function, which can function as an oncogene or tumor suppressor. Increasing evidence has suggested a marked association between miRNA polymorphisms and cancer diagnosis, treatment efficacy and prognosis. Progress in current understanding of genetic polymorphisms of miRNA regulatory pathways have important implications, not only understanding the pathogenesis of various types of cancer, but also in identifying biomarkers for their diagnosis and prognosis. In the present review, a comprehensive list of potentially functional miRNA-associated single nucleotide polymorphisms are presented, and their importance as candidate cancer biomarkers is discussed.

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1. Introduction

MicroRNAs (miRNAs) are small, single-stranded, 21-23 nucleotide-long, independent functional units of noncoding RNA (1-3). Often referred to as the 'micromanagers of gene expression', miRNAs are evolutionarily well-conserved. Mature miRNAs regulate the expression of ~30% of all human genes involved in fundamental biological processes at a post-transcriptional level by sequence-specific binding to 3'-untranslated regions (3'-UTRs) of multiple target messenger RNAs (mRNAs), leading to their degradation or translational suppression (4,5). Increasing evidence has suggested that miRNAs are important in a broad range of biological processes, including embryonic development, cellular proliferation, differentiation, apoptosis and other physiological processes (6,7).

miRNAs are synthesized in a precisely coordinated manner. Briefly, the miRNA gene is transcribed by RNA polymerase II, resulting in a hairpin-shaped primary miRNA (pri-miRNA), which is ~500-3,000 base pairs in length. This pri-miRNA is further processed by Drosha/Pasha (DGCR8) to form a 60-70 nucleotide-long precursor miRNA (pre-miRNA), which is transported from the nucleus to the cytoplasm through nuclear pore complexes, with the assistance of Exportin-5 (XPO5) (6,8). The pre-miRNA is further cleaved in the cytoplasm by the RNase III endonuclease, Dicer, to release two complementary short RNA molecules (9). The argonaut protein complex selectively binds to the guide strand and facilitates the formation of a miRNA-mRNA-induced silencing complex (RISC) assembly, which consists of HIWI, GEMIN3 and GEMIN4. Upon miRNA

binding, the RISC complex is activated and, by a mechanism that remains to be fully elucidated, locates its binding site in the 3'-UTR of the target mRNA and contributes to regulation of the expression of the gene (8-10).

Advancements in investigations of miRNA have indicated the involvement of miRNAs in the genesis, progression (proliferation, migration and invasion) and prognosis of multiple types of human malignancy (11). Of note, ~50% of all annotated human miRNA genes are located in fragile sites or areas of the genome, which are frequently deleted, amplified and mis-expressed in human cancer (12). The conditional deletion or overexpression of a single miRNA is sufficient to drive tumorigenesis in mice (13). It has been suggested that the single nucleotide polymorphisms (SNPs) in miRNAs, which encode their biogenesis pathway and target binding sites, may affect the regulatory capacity of miRNAs by affecting miRNA processing and/or miRNA-mRNA interactions (14). Polymorphisms in miRNA regulatory pathways may result in the loss or gain of an miRNA function, which can act as an oncogene or tumor suppressor. Previously, several studies have demonstrated a marked association between miRNA-polymorphisms and the risk, treatment response and outcome in patients with cancer (15,16). Polymorphisms in miRNA regulatory networks are a novel class of functional polymorphisms in the human genome (11). These enable investigation of the biology of cancer and have the potential for use as biomarkers in cancer diagnosis and prognosis. The present review provides a brief outlook on the biogenesis and biology of miRNAs, and the functional effects of miRNA-associated SNPs.

2. Genetic polymorphisms in the miRNA biogenesis pathway

Several proteins and protein complexes are involved in various stages of miRNA biogenesis, including miRNA transcription, processing, export and targeting (7). These proteins include the RNA polymerase II complex, Drosha/Pasha, Exportin-5, nuclear pore complexes, Dicer and the Argonaut protein/RISC complex, as shown in Fig. 1. As the underexpression or overexpression of miRNA may have serious consequences in a cell, polymorphisms in core components of miRNA biogenesis may impair or enhance miRNA processing efficiency or function, resulting in altered levels of mature miRNAs and deleterious effects (4). Several lines of evidence have supported that SNPs in the biogenesis pathway of miRNAs are associated with development and progression in a several types of tumor (Table I).

DROSHA. Drosha is an RNAse III enzyme, which mediates the processing of pri-miRNAs into pre-miRNAs with DGCR8 (8). In a previous *in vitro* functional investigation, a reduction in miRNA processing efficacy, which was induced by the knockdown of *DROSHA*, was found to reduce the levels of mature forms of tumor-suppressive miRNAs and facilitate the invasion of breast cancer cells (17). Several studies have indicated the role of Drosha in breast cancer. A case-control study demonstrated that two SNPs in *DROSHA, rs644236* and *rs7737174*, may contribute to the risk of breast cancer in postmenopausal women (18). Jiang *et al* also suggested *rs2291109* as a predictor for breast cancer risk, however, the association was not confirmed (19). In addition, patients with breast cancer carrying the *DROSHA rs874332* C allele are at increased risk of mortality (20). As *rs874332* is located in the 3'-UTR of *DROSHA* mRNA and a predicted miRNA binding site, it is possible that *rs874332* may be correlated with the translational repression and mRNA destabilization of *DROSHA* through an miRNA-mRNA interaction. However, data from Sung *et al* (21) involving east Asian women, including 5,066 cases and 4,337 controls, failed to identify an association between the SNPs in *DROSHA* and breast cancer risk. In addition, *DROSHA rs6877842* has been reported to reduce the risk of recurrence in patients with renal cell carcinoma by 36%, and haplotypes of *DROSHA* (*rs6877842/rs10719*) have been associated with survival rates (22). *rs10719* may also affect the risk of malignant peripheral nerve sheath tumors through increasing the expression level of *DROSHA* (23).

DGCR8 (Pasha). DGCR8, as a component of the multiprotein complex with the RNAse III enzyme, Drosha, is a double stranded RNA-binding protein, which is involved in the processing of pri-miRNAs into pre-miRNAs (9). Impaired miRNA processing through the knockdown of *DGCR8* also facilitates the invasion of breast cancer cells (17). The *rs9605062* in *DGCR8* may upregulate the level or timing of gene expression (20), and it has been reported that *rs9606250* is significantly associated with poor disease-free survival (DFS) rates in breast cancer (20). In addition, the interruption of miRNA binding of *rs417309,* located at the binding sites of miR-106b and miR-579 in the 3'-UTR of *DGCR8,* has been found to increased the risk of breast in the Chinese population (19). Another two linked SNPs, *rs2073778* and *rs720012*, in *DGCR8* have also been shown to be significantly associated with tumor progression in bladder cancer (24).

XPO5. XPO5 is located in the nuclear membrane, and mediates the transport of pre-miRNAs to regulate miRNA expression (10). The XPO5-mediated nuclear export of pre-miRNAs may be a rate-limiting step in miRNA biogenesis. The overexpression of *XPO5* has been shown to result in enhanced miRNA activity (25), whereas the loss of *XPO5* leads to reduced expression and function of pre-miRNAs (26). Among the SNPs in *XPO5*, *rs11077* has received the most attention. Located in the 3'-UTR of *XPO5*, *rs11077* may affect mRNA stability, alter the expression of XPO5 and, consequently, affect the expression of miRNAs, including those specific for drug metabolism, altering the response to chemotherapy and affecting survival rates of patients with advanced non-small-cell lung cancer (NSCLC) and small-cell lung cancer (27,28). In addition, *rs11077* has been associated with poor progression in hepatocellular carcinoma and renal cell carcinoma (22,29). A mutation in *rs11544382* in a functionally conserved region of *XPO5* may also alter the protein structure of XPO5, resulting in altered nucleocytoplasmic transport activity (30), and this SNP has been associated with an increased risk of breast cancer (30).

DICER. Dicer is an enzyme responsible for the cleavage of miRNA precursors, and has been implicated in the oncogenic process of several types of cancer. Increasing evidence has supported the role of *DICER rs1057035* in cancer susceptibility. This SNP has been associated with a decreased risk of oral cancer (31), cervical carcinoma (32) and hepatocellular

Table I. Polymorphisms in microRNA biogenesis pathways and functional variations.

Biogenesis gene	SNP site	Tumor type (population, n)	Description	Risk (95% CI)	Ref
DROSHA	rs874332 (C>T)	Breast cancer (Korean, 488)	OS	HR=2.24 (1.21-4.17)	20
	rs644236 (C>T)	Breast cancer (Korean, 559/567)	Susceptibility	OR=1.27 (0.94-1.73)	18
	rs7737174 (A>G)	Breast cancer (Korean, 559/567)	Susceptibility	OR=1.63 (1.01-2.64)	18
	rs2291109 (A>T)	Breast cancer (Chinese, 878/900)	Susceptibility	OR=0.81 (0.66-0.99)	19
	rs6877842 (C>G)	Renal cell carcinoma (Caucasian, 316)	Recurrence	HR=0.36 (0.13-0.98)	22
	rs10719 (C>T)	Malignant peripheral nerve sheath tumor (Chinese, 156/200)	Susceptibility	OR=1.64 (1.23-2.20)	23
DGCR8	rs9606250 (A>T)	Breast cancer (Korean, 488)	DFS	HR=0.21 (0.05-0.84)	20
	rs417309 (A>G)	Breast cancer (Chinese, 878/900)	Susceptibility	OR=1.50 (1.16-1.93)	19
	rs2073778 (C>T)	Bladder cancer (non-Hispanic, 421)	Progression	HR=4.00 (1.53-10.46)	24
XPO5	rs11077 (A>C)	NSCLC (Chinese, 112)	OS	RR=0.457 (0.251-0.831)	27
		SCLC (Chinese, 42)	OS	RR=2.469 (1.088-5.603)	28
		Hepatocellular carcinoma (Chinese, 108)	OS	RR=0.395 (0.167-0.933)	29
		Renal cell carcinoma (Caucasian, 316)	Recurrence	HR=0.36 (0.16-0.85)	22
	rs11544382 (A>G)	Breast cancer (Caucasian, 441/479)	Susceptibility	OR=1.59 (1.06-2.39)	30
DICER	rs1057035 (C>T)	Head and neck cancer (Chinese, 397/900)	Susceptibility	OR=0.65 (0.46-0.92)	31
		Cervical carcinoma (Chinese, 1,486/1,549)	Susceptibility	OR=0.962 (0.805-1.149)	32
		Hepatocellular carcinoma (Chinese, 1300/1344)	Susceptibility	OR=0.79 (0.64-0.96)	33
		Breast cancer (Korean, 488)	DFS	HR=1.72 (0.99-2.99)	20
			OS	HR=2.08 (1.01-4.28)	
GEMIN4	rs7813 (C>T)	Renal cell carcinoma (Caucasian, 316)	OS	HR=1.74 (1.15-2.62)	22
		Prostate cancer (Chinese, 300/244)	Susceptibility	OR=2.53 (1.07-6.28)	34
		Malignant peripheral nerve sheath tumor (Chinese, 156/200)	Susceptibility	OR=0.50 (0.34-0.72)	23
		Ovarian cancer (Caucasian, 339/349)	Susceptibility	OR=0.71 (0.57-0.87)	35
	rs2740348 (C>G)	Renal cell carcinoma (Caucasian, 279/278)	Susceptibility	OR=0.67 (0.47-0.96)	36
		Prostate cancer (Chinese, 300/244)	Susceptibility	OR=0.68 (0.47-0.98)	34
AGO2	rs2292779 (C>G)	Breast cancer (Korean, 488)	DFS	HR=1.42 (1.06-1.92)	20
			OS	HR=2.94 (1.52-5.69)	
	rs11786030 (A>G)	Breast cancer (Korean, 488)	DFS	HR=2.62 (1.41-4.88)	20
			OS	HR=2.41 (1.05-5.50)	
	rs3864659 (A>C)	Breast cancer (Korean, 559/567)	Susceptibility	OR=0.67 (0.46-0.96)	18

NSCLC, non-small-cell lung cancer; SCLC, small-cell lung cancer; OS, overall survival; DFS, disease-free survival; HR, hazard ratio; OR, odds ratio; RR, relative risk.

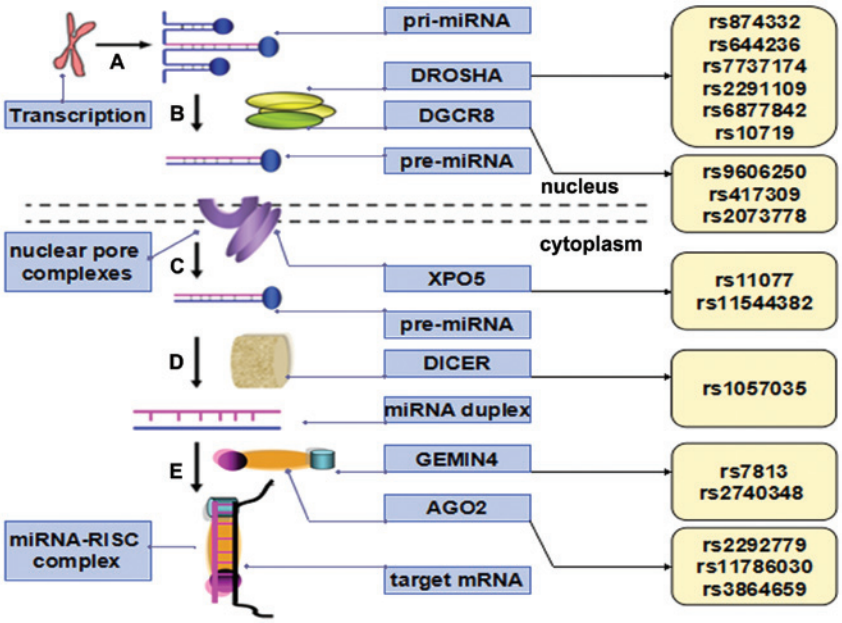

Figure 1. Illustrative overview of polymorphisms in miRNA biogenesis pathways. (A) miRNA gene is transcribed by RNA polymerase II; (B) pri-miRNA is further processed by Drosha/Pasha; (C) pre-miRNA is transported to the cytoplasm by Exportin-5; (D) pre-miRNA is cleaved by Dicer, releasing two complementary short RNA molecules; (E) miRNA-RISC complex binds to the 3'-untranslated reguin of the target mRNA and contributes to translational inhibition or mRNA degradation. Polymorphisms, which affect the expression of proteins involved in miRNA action and biogenesis, including Drosha, Pasha, Dicer, Exportin 5 and proteins in the RISC complex, may affect miRNA-mediated regulation of mRNA expression and protein translation in the cell, consequently, contributing to the susceptibility and prognosis of various types of cancer. miRNA, microRNA; pri-miRNA, primary miRNA; pre-miRNA, precursor miRNA; RISC. RNA-induced silencing complex.

carcinoma (33). This polymorphism is located in the 3'-UTR of *DICER* and a predicted binding site of miR-574-3p, which may affect the binding of miR-574-3p, and result in decreased mRNA expression levels of *DICER* (31). Of note, this SNP has been shown to be associated with a 1.72- and 2.08-fold increased risk of progression and cancer-associated mortality, respectively, among patients with breast cancer (20).

GEMIN4. The GEMIN4 protein is referred to as an important molecule in the RISC complex, which is involved in the maturation process of miRNAs, and the recognition and repression of target mRNAs (7). The protein expression level of GEMIN4 is closely associated with the biogenesis of associated miRNAs (7). *Rs7813* in the exons of *GEMIN4* has been frequently identified as a predictive biomarker in several types of cancer, including renal cell carcinoma (22), prostate cancer (34), malignant peripheral nerve sheath tumor (23) and ovarian cancer (35). Another non-synonymous SNP, *rs2740348*, which is located in the functional region of the *GEMIN4* gene has been demonstrated to decrease the risks of prostate cancer and renal cell carcinoma by 36 and 33%, respectively (34,36). Notably, Wan *et al* found that *rs2740348* and *rs7813* were significantly associated with cell growth and DNA repair in a heptacellular carcinoma cell line (37), suggesting that the amino acid changes caused by these SNPs may have a physiological significance on the development of cancer.

AGO2. AGO2 is important in miRNA-mediated gene silencing, as a component of the RISC complex that directly binds miRNAs and mediates the cleavage of target mRNAs (7). Emerging evidence from *in vitro* analysis and clinical samples has indicated that the abnormal expression or enzymatic function of AGO2 is associated with cancer development and progression. In breast cancer cell lines, the overexpression of AGO2 induces the transformed phenotype (38). Sung *et al* indicated that *AGO2 rs3864659* may have a protective effect on breast cancer risk (18). In addition, two further SNPs in *AGO2 rs11786030* and *rs2292779* have been significantly associated with poor DFS and poor overall survival (OS) rates in breast cancer (20). Variations in the genomic structure of *AGO2*, including changes in copy number or frameshift mutations, have also been reported to be associated with several types of cancer, including multiple myeloma, gastric cancer and colorectal cancer (39,40).

3. Genetic polymorphisms in miRNA genes

SNPs in miRNA genes are considered to exert their effects by one of three mechanisms: Through transcription of the primary transcript; through pri-miRNA and pre-miRNA processing; and through effects on miRNA-mRNA interactions (11). In general, sequence variations in miRNA genes, including pri-miRNAs, pre-miRNAs and mature miRNAs, have the potential of affect the processing efficiency and/or target selection of miRNAs, leading to aberrant expression of hundreds of genes in different biological pathways (11). As miRNAs are highly conserved, SNPs in miRNA genes are relatively rare. The majority of studies have followed a biologically-based candidate gene approach to identify SNPs in miRNAs, which may affect cancer susceptibility, relying on a knowledge of the functional link between a particular miRNA and gene target (Table II).

Pre-miR-27a. The pre-miR-27a, *rs895819*, has been frequently investigated in the development of cancer, however, the results remain contradictory rather than conclusive (41-46). To inte-

grate all individual studies and comprehensively analyze the role of *rs895819* in tumorigenesis, several meta-analysis have been performed. Previous overall meta-analysis suggested no association between the pre-miR-27a *rs895819* polymorphism and cancer susceptibility (47-49). In a stratified analysis, according to the type of cancer, individuals with the variant G allele were consistently found to be at a reduced risk of breast, renal cell and nasopharyngeal cancer, but at an increased risk of digestive tract cancer (47). In addition, subgroup analysis according to ethnicity revealed that the *rs895819* AG genotype was associated with a decreased risk of cancer in Caucasian individuals (48). As this SNP is located at the terminal loop of pre-miR-27a, it may have an effect on the secondary structure of pre-miR-27a (42). The substitution of G for A in *rs895819* may reduce the size of the loop and alter the minimum free energy, consequently inhibiting cleavage and resulting in low expression levels of mature miR-27a (42).

miR-196a2. *rs11614913* in the mature sequence of miR-196a2 has been increasingly identified a predictor for various types of cancer (50-52). The results from several meta-analyses, each containing thousands of subjects, have demonstrated that *rs11614913* may contribute to the risk of developing breast cancer (53), lung cancer (54,55), hepatocellular carcinoma (56) and cancer of the digestive system (57,58). In addition, the SNP was positively correlated with improved recurrence-free survival (RFS) in patients with stage II and stage III NSCLC (59). The polymorphism may negatively affect endogenous processing of either miR-196a2 precursor to its mature form, and the levels of mature miR-196a2 are lower in CC carriers, compared with TT carriers (60). Furthermore, binding assays have revealed that this SNP can affect the binding of mature miR-196a2 to its target mRNA (60).

miR-146a. miR-146a, first identified in the mouse, has been shown to be important in tumorigenesis, by promoting cell proliferation and colony formation in NIH/3T3 cells (61). However, it also exhibits an antitumor property, by suppressing metastatic ability, in breast cancer and prostate cancer (62,63). The G-C substitution (*rs2910164*), located in the middle of the stem hairpin on the passenger strand of the precursor miR-146a, has a lower transcriptional activity due to decreased nuclear processing efficiency, leading to low level of mature miR-146a in cells (64). Although three meta-analyses consistently found that *rs2910164* was not involved in overall cancer risk, stratified analysis by ethnicity has shown a close association between *rs2910164* and overall cancer risk in the Caucasian population (50-52). Jiang *et al* reported that the *rs2910164* GG homozygote was a protective genotype, in terms of susceptibility to acute-on-chronic hepatitis B liver failure (65). However, results from another meta-analysis showed the C variant to be associated with decreased hepatocellular carcinoma risk (56). As for patients with NSCLC, variants of *rs2910164* were found to be positively correlated with RFS (59). However, in the development of cervical squamous cell carcinoma, the G allele of *rs2910164* was associated with a significantly increased risk, as well as reduced tumor differentiation and a decline in lymph node status (66).

miR-499. It is known that the secondary structure of miRNA is critical to mRNA-miRNA interactions and gene regulation (67). *The rs3746444* polymorphism may affect miR-499 maturation and regulate the expression of its target genes through directly altering its secondary structure. Zhou *et al* provided evidence that *rs3746444* may contribute to the susceptibility to cervical squamous cell carcinoma (66). Of note, Liu *et al* demonstrated that *rs3746444* has a protective effect in the development of head and neck cancer (68). In addition, the T allele of *rs3746444* was associated with a decreased risk of breast cancer among Asian individuals, however, a follow-up meta-analysis suggested risk was increased in Caucasians individuals, suggesting ethnic differences in the consequences of SNPs (69). A further meta-analysis failed to identify any significant correlation between the miR-499 polymorphism and risk of hepatocellular carcinoma (56).

miR-218. The expression level of miR-218 is associated with infection with high-risk human papilloma virus (HPV), and is involved in the pathogenesis of cervical cancer (70). *The rs11134527* in miR-218 has been shown to upregulate the expression of miR-218, and inhibit the expression of its target gene, *LAMB3,* by interfering with the mRNA-miRNA interaction. The overexpression of *LAMB3* induces carcinogenesis by increasing carcinoma cell migration and disturbing tumor microenvironment, therefore, this polymorphism has been implicated in the infective process of high-risk HPV, thus contributing to cervical carcinogenesis (66,71). Another study evaluated the role of *rs11134527* in hepatocellular carcinoma, which noted that the AG genotype of *rs11134527* was associated with family history and elevated levels of serum α-fetoprotein, suggesting that the AG genotype may be associated with genetic predisposition in patients with hepatocellular carcinoma (72).

4. Genetic polymorphisms in miRNA target sites

The disruption of miRNA-dependent regulation by SNPs in the miRNA binding site of target mRNAs has been confirmed as a mechanism for altered gene expression in cancer. In contrast to the miRNA-polymorphisms in the miRNA biogenesis pathway, the polymorphisms located at the 3'-UTR of an miRNA target gene are more abundant in the human genome, and affect only the expression of the target gene and its downstream effectors, resulting in a more defined and limited range of effects (73). The majority of the miRNA binding sites in the 3'-UTRs of a target mRNA lack a complex secondary structure, thereby facilitating access for an miRNA. Polymorphisms at or close to these binding sites, through creating or eradicating secondary structure, may affect the accessibility of an miRNA-RISC complex, and the coordination of miRNAs with other regulatory elements in the 3'-UTR of the target transcript (11). Among the 120,000 known SNPs that occur in 3'-UTRs, ~17% destroy putative conserved or non-conserved miRNA-binding sites, and 8.6% create novel predicted target sites, according to the Patrocles database (74). Several examples of SNPs located in the 3'-UTR of target mRNAs, and their clinical significance, are presented in Table III.

FAS is a cell surface receptor of the tumor necrosis family, which is important in the regulation of apoptosis (75). The *rs2234978* SNP in the 3'-UTR of *FAS* has been reported to

Table II. Polymorphisms in miRNA genes and their clinical significance.

miRNA	SNP site	Tumor type (population, n)	Description	Risk (95% CI)	Ref
pre-miR-27a	rs895819 (A>G)	Breast cancer (Chinese, 264/255)	Susceptibility	OR=0.535 (0.321-0.891)	42
		Renal cell carcinoma (Chinese, 594/600)	Susceptibility	OR=0.71 (0.56-0.90)	44
		NSCLC (Chinese, 576)	OS	HR=1.71 (1.12-2.26)	45
			Chemotherapy response	OR=0.54 (0.32-0.91)	
miR-196a2	rs11614913 (C>T)	Breast cancer (meta, 2588/3260)	Susceptibility	OR=0.906 (0.825-0.995)	53
		Lung cancer (meta, 2219/2232)	Susceptibility	OR=1.13 (0.98-1.29)	55
		Hepatocellular carcinoma (meta, 3437/3437)	Susceptibility	OR=0.90 (0.83-0.98)	56
		Digestive system cancers (meta, 4999/7606)	Susceptibility	OR=1.29 (1.10-1.50)	58
		NSCLC (Korean, 388)	RFS	HR=0.60 (0.38-0.94)	59
miR-146a	rs2910164 (C>G)	Acute-on-chronic hepatitis B liver failure (Chinese, 717/251)	Susceptibility	OR=0.496 (0.309-0.797)	65
		NSCLC (Korean, 388)	RFS	HR=0.48 (0.28-0.80)	59
		Cervical squamous cell carcinoma (Chinese, 226/309)	Susceptibility	OR = 2.10 (1.22-3.59)	66
miR-499	rs3746444 (C>T)	Cervical squamous cellcarcinoma (Chinese, 226/309)	Susceptibility	OR= 1.78 (1.24-2.56)	66
		Head and neck cancer (non-Hispanic white, 1109/1130)	Susceptibility	OR=0.83 (0.69-0.99)	68
pri-miR-218	rs11134527 (A>G)	Cervical carcinoma (Chinese, 1584/1394)	Susceptibility	OR=0.77 (0.63-0.95)	71
		Hepatocellular carcinoma (Chinese, 302/513)	Susceptibility	OR=2.96 (1.16-7.56)	72

miRNA, microRNA; SNP, single nucleotide polymorphism; NSCLC, non-small-cell lung cancer; meta, meta-analysis; OS, overall survival; HR, hazard ratio; OR, odds ratio; RFS, recurrence-free survival; Ref, reference.

Table III. Polymorphisms in miRNA target sites and the effects of the variability.

Target gene	SNP site	miRNA	Tumor type (population, n)	Description	Risk (95% CI)	Ref
FAS	rs2234978 (C>T)	miR-561	NSCLC (Caucasian, 535)	OS	HR=0.59 (0.44-0.77)	74
FZD4	rs713065 (A>G)	miR-494	NSCLC (Caucasian, 535)	OS	HR=0.46 (0.32-0.65)	74
SP1	rs17695156 (C>T)	miR-545	NSCLC (Caucasian, 535)	Recurrence	HR=3.36 (1.62-6.69)	74
MDM4	rs4245739 (A>C)	miR-191	Esophageal squamous cell carcinoma (Chinese, 1128/1150)	Susceptibility	OR=0.54 (0.35-0.82)	76
		miR-191	Ovarian carcinoma (Caucasian, 113)	Mortality	HR=5.5 (1.5-20.5)	77
				Recurrence	HR=4.1 (1.2-13.5)	
SGSM3	rs56228771 (insertion/deletion)	miR-151-5p	Hepatocellular carcinoma (Chinese, 502/513)	Susceptibility	OR=0.55 (0.42-0.73)	78
COL1A2	rs3917 (insertion/deletion)	miR-382 let-7g	Hepatocellular carcinoma (Chinese, 207/245)	Susceptibility	OR=1.76 (1.03-3.01)	79

miRNA, microRNA; SNP, single nucleotide polymorphism; NSCLC, non-small-cell lung cancer;OS, overall survival; HR, hazard ratio; OR, odds ratio; Ref, reference.

create a novel miRNA-binding site for miR-561, and ultimately result in decreased expression of FAS. Patients with NSCLC, who carry the variant allele, appear to have a better overall survival (OS), independent of treatment regimen (75). This may be explained by higher expression levels of *FAS* due to the SNP, which may increase tumor cell death. Similarly, *rs713065* in the 3'-UTR of *FZD4* may downregulate the expression of FZD4 by creating an miR-494 binding site, leading to enhanced survival through decreased WNT signaling (76). By contrast, *rs17695156* in the 3'-UTR of *SP1* is predicted to disrupt a conserved miR-545 binding site and alter the expression of SP1 by affecting mRNA stability or post-transcriptional regulation, and patients with NSCLC patients carrying at least one variant allele of *rs17695156* have a shorter median RFS, compared with patients with a common homozygous genotype (75). The *rs4245739* SNP in the 3'-UTR of *MDM4* has been noted to create an miR-191 target site and results in decreased expression of MDM4 (76). As MDM4 is key in the P53 tumor suppressor pathway, by negatively regulating P53 function, this polymorphism may contribute to reduced susceptibility to esophageal squamous cell carcinoma (77). In addition, AA genotype carriers, who do not express the estrogen receptor, have a 4.2-fold increased risk of recurrence and a 5.5-fold increased risk of tumor-associated mortality in ovarian cancer (78).

In addition to SNPs, the insertion/deletion polymorphisms in a target gene can also create or destroy a binding site. SGSM3 is involved in the small G protein-coupled receptor signal transduction pathway. It has been reported that a 4-bp insertion/deletion polymorphism (*rs56228771*) in the 3'-UTR of *SGSM3* can affect the susceptibility of hepatocellular carcinoma, reducing decreased risk of ins/del+ins/ins genotypes by ~45% (79). In addition, a 7-base pair deletion polymorphism (*rs3917*) in the 3'-UTR of *COL1A2* has been associated with a 1.73-fold increased risk of hepatocellular carcinoma (80). The *rs3917* lies within a predicted binding site for miR-382 and let-7g, and the deletion allele may alter the affinity of miRNA-mRNA binding, by disrupting the local structure of *COL1A2* mRNA, possibly upregulating the expression of COL1A2 (80,81).

5. Scope and challenges

Further investigations. To date, the majority of the studies in this field are case-control studies, based on a candidate gene approach (15). Although several positive results have been reported, inconsistent findings and non-replication of previous results have frequently occurred (15). This may be attributed to several reasons, including the heterogeneity of patient groups, different experimental designs, insufficient sample size or unclear disease biology (15).

Heterogeneity in clinical confounding factors and endpoint phenotypes between initial and replication studies can undermine the opportunity to compare among them. It is essential to account for all confounding factors, which may predispose to a given phenotype, in order to estimate the residual phenotype that is likely due to genetics. As a small sample size can provide imprecise or incorrect estimates of the magnitude of an observed effect, sufficient sample size is necessary to accurately distinguish a suggested effect from a lack of effect (82). As several initial studies have been reported in populations of European descent, the challenge remains to extend investigations to include other ethnic populations (82).

Well-planned investigations are required, providing sufficient statistical power and stringency to detect and quantify a modest impact of the investigated SNPs. Follow-up epidemiological association investigations are important to validate previous findings in multiple independent large and homogenous samples. The National Cancer Institute-National Human Genome Research Institute working group on replication in association studies has published a comprehensive set of guidelines, providing a number of essential criteria for establishing positive replication studies (83).

Investigation of biological mechanisms. In addition, further functional investigations are required to clarify the underlying mechanism. Several miRNAs are found in CpG islands, and miRNA expression can also be affected by DNA methylation and histone deacetylase inhibitors, providing another example of the bivalent roles of how miRNAs in malignancy (84). For example, two well-defined tumor suppressors, miR-124 and miR-34, are subject to epigenetic silencing by aberrant DNA hypermethylation, affecting cell cycle pathways in tumors, whereas the downregulation of miR-34 affects the Notch pathway, which is involved in cell invasion and apoptosis (85-87). Furthermore, DNA methylation profiles in miRNA promoter regions can be useful as a diagnostic and prognostic marker. For example, miR-23b, an miRNA with tumor suppressor activity in prostate cancer, is downregulated through DNA hypermethylation of its promoter region, and its expression level is correlated with OS and RFS (88). In addition to SNPs, structural variations, including insertions, deletions, inversions and copy number variants, with important implications on tumor biology (79,80). Evaluating the link among genetic variants, epigenetic modifications and disease predispositions is currently an active area of investigation (79,80).

Of note, there are several ways in which the processes of miRNA production, stability and maturation can be orchestrated (89-91). A semi-miRNA of12 nucleotides in length, which correspond to the 5' region of the miRNA, let-7, is generated along the miRNA pathway, and may be involved in the control of gene expression by regulating the activity of mature miRNAs *in vivo* (92). Novel mechanisms for miRNA biogenesis have been described, and may be important as cancer drivers (93). Winter *et al* provided the first evidence that a small number of miRNAs are generated from single-stranded loop regions of human pre-miRNA hairpins, termed loop-miRs (94). In addition, an alternative miRNA processing pathway has been found in *Drosophila melanogaster* and *Caenorhabditis elegans*, which bypasses *DROSHA* and uses a splicing technique to generate miRNA precursors from short intronic sequences (95,96). The genetic polymorphisms and the functional implications of these novel pathways require further investigation.

6. Conclusion

The present review focused on the predictive role of genetic variations in miRNA regulatory networks on inherited cancer

risk and progression. Although the biological mechanisms underlying their effects on miRNA maturation and cancer development remain to be fully elucidated, our knowledge of the myriad of pathways in malignancy has improved, and further investigations of miRNA polymorphisms hold promise in advancing knowledge in the field of pharmacogenomics, molecular epidemiology and personalized medicine.

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