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REVIEW

MicroRNA binding site polymorphisms as biomarkers in cancer management and research

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Abstract: MicroRNAs (miRNAs) are important regulators of eukaryotic gene expression. They have been implicated in a broad range of biological processes, and miRNA-related genetic alterations probably underlie several human diseases. Single nucleotide polymorphisms of transcripts may modulate the posttranscriptional regulation of gene expression by miRNAs and explain interindividual variability in cancer risk and in chemotherapy response. On the basis of recent association studies published in the literature, the present review mainly summarizes the potential role of miRNAs as molecular biomarkers for disease susceptibility, diagnosis, prognosis, and drug-response prediction in tumors. Many clues suggest a role for polymorphisms within the 3′ untranslated regions of *KRAS* rs61764370, *SET8* rs16917496, and *MDM4* rs4245739 as SNPs in miRNA binding sites highly promising in the biology of human cancer. However, more studies are needed to better characterize the composite spectrum of genetic determinants for future use of markers in risk prediction and clinical management of diseases, heading toward personalized medicine.

Keywords: miRSNP, 3′-UTR target binding site, cancer risk, biomarkers

Introduction

MicroRNAs (miRNAs) are short, noncoding RNAs of 22–27 nucleotides that regulate gene expression through binding to cognate sequences, preferentially 3′ untranslated regions (UTR) regions, of mRNAs. The degree of complementarity around nucleotides 2–7 of a miRNA, the "seed" region, is the most important known determinant of recognition of an mRNA by a targeting miRNA.[1] Based on the importance of seed pairing, multiple bioinformatics algorithms have been developed to predict miRNA-binding sites in mRNA sequences.[2–6] Alterations of miRNA::mRNA interactions, although restricted to a few nucleotides, can have profound effects on the control of gene expression, as showed by Clop et al.[7] These authors report, for the first time in mammals, that the G to A transition within the 3′-UTR of *GDF8* creates a target site for mir-1 and mir-206, causing a translational inhibition of the myostatin gene and, hence, contributing to the muscular hypertrophy of Texel sheep.[7] Following this and other evidence, it was hypothesized that a miRNA::mRNA pairing could be affected by a number of factors, including miRNA expression levels, the presence of SNPs within miRNA genes, and the presence of SNPs located within miRNA-binding target sites, now defined as miRSNPs and typically located at the 3′-UTR of mRNAs. In the same year Clop et al published their report, Chen et al found that negative selection in humans is stronger on computationally predicted conserved miRNA binding sites than on other conserved sequence motifs in 3′-UTRs, providing independent support

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for the target prediction model and explicitly demonstrating the contribution of miRNAs to Darwinian fitness.[8] In 2007, Mishra et al demonstrated that variant *DHFR* 829C>T (rs34764978), falling near a miR-24 binding site, causes the overexpression of human dihydrofolate reductase, contributing to methotrexate resistance.[9] A year later, for the first time, a putative miRSNP was also associated with the risk for colorectal cancer among Caucasians.[10] Thus, in recent years, there has been increasing interest in the role of post-transcriptional regulation of gene expression by miRNAs[11] and in the influence of miRSNPs on cancer risk[12,13] and clinical outcomes.[14–16] Actually, a growing number of studies have suggested that miRSNPs constitute a promising novel class of polymorphic variations worth investigation, with the potential of opening new areas of research in cancer biology and clinical oncology.[17] Moreover, it has been suggested that miRSNPs could be employed as useful biomarkers in the study of disease progression, patient prognosis,[18] and treatment efficacy of cancer.[19,20]

This article focuses on the studies of miRSNPs related to cancer. The review includes case–case studies in which the associations between miRSNPs and clinical outcome were evaluated. Moreover, case-control association studies were also included, with the aim of suggesting a possible role for miRSNPs in increased susceptibility to cancer. A concise resume of the results is reported in Table 1.

Lung cancer

miRSNPs as biomarkers of prognosis in lung cancer

Lung cancer (LC) continues to be the leading cause of cancer-related deaths worldwide because of its high incidence, malignant behavior, and lack of major advancements in treatment strategy. Non-small-cell lung cancer (NSCLC) accounts for about 80% of all cases, with less than 15% of patients surviving beyond 5 years.[21] Thus, specific prognostic biomarkers to be added to the standard tumor, node, and metastasis staging system may improve the medical care of patients with NSCLC.[22–25] Because *SET8* is found overexpressed in various types of tumor, including LC,[26] Takawa et al evaluated whether *SET8* rs16917496 T>C, a miRSNP falling within miR-502 binding site, could be associated with the overall survival (OS).[26] Indeed, in a Chinese population, the CC genotype was associated with longer OS and reduced risk for death for NSCLC.[27] The results are consistent with the in vitro observations that the variant C allele may decrease the expression of *SET8* through enhancing the binding capacity of miR-502.[27]

The same SNP was further analyzed in 44 cases and 44 controls, and the C allele was confirmed to be independently associated with longer OS in NSCLC patients.[28] Another study provided the evidence that the rs1564483 A allele, located within the 3′-UTR of *BCL2*, was associated with a significantly lower risk for LC in male Chinese patients and with a favorable OS in advanced NSCLC patients.[29] This effect was more obvious in smoking patients, in stage IIIA patients, and in patients without surgery undergone to chemotherapy or radiotherapy. The authors speculated that the rs1564483 G-to-A substitution might change the stem-loop structure of 3′-UTR or introduce a miRNA binding site, which may affect *BCL2* mRNA stability or its expression levels.[29] Several miRNAs, including miR-181b,[30] miR-200bc/429,[31] and miR-204,[32] have been reported to bind to the *BCL2* 3′-UTR in correspondence with the miRSNP, thereby modulating *BCL2* mRNA levels. When the expression of BCL2 is decreased, the balance between pro- and antiapoptotic pathways could be shifted in favor of a proapoptotic activity, thus contributing to protecting lung cells from genotoxicants and carcinogenesis.[33,34]

Another miRSNP, the rs2240688, within the 3′-UTR of *CD133* was significantly associated with a decreased risk for LC in Asian patients: compared with individuals with the AA genotype, those patients with CA or CC genotypes showed a 20% decreased risk in an exploratory sample set, confirmed in an independent sample set of validation.[35] In addition, the C allele conferred a significantly favorable prognosis, with the median OS of CC + CA patients significantly longer than that found in patients carrying the AA genotype. Functional assays revealed that the A>C transversion creates a new binding site for miR-135a/b and causes a decreased expression of the *CD133* mRNA.[35] *CD133* has been identified as a pleiotropic marker of cancer cells stemness in various human tumors. Both clinical analyses and laboratory studies have shown that *CD133* plays a critical role in tumorigenesis and tumor progression.[36,37] Increased *CD133* expression was observed in several human cancer tissues[38,39] and was reported to be associated with poor prognosis. In another study on NSCLC, significant differences in time to recurrence were found when Caucasian patients were analyzed for *KRT81* rs3660 (20.3 months for the CC genotype versus 86.8 months for the CG + GG genotypes), especially among patients at stage I.[17] The SNP is located within predicted binding sites of several miRNAs, including miR-17, miR-93, miR-20b, miR-519d, miR-520g, miR-520h, miR-519c-3p, miR-519b-3p, miR-519a, and miR-765, some of which were shown to be deregulated in NSCLC.[40] *KRT81* encodes

Table 1 List of micro-RNA binding sites evaluated in epidemiologic studies

Single nucleotide polymorphism ID	Gene	Race	Information	Predicted binding site for	Disease	Cases	Controls	Endpoint	Best result	*P*-value	Assay	Ref
rs16917496	*SET8*	AS	T>C (0.32)	miR-502	NSCLC	576		OS (months)	41.0 vs 58.0	0.031	Gene reporter in vitro assay and IHC on tissues	27
								OS (RR; 95%CI)	0.44 (0.26–0.74)	0.006		
rs16917496	*SET8*	AS	T>C (0.32)	miR-502	SCLC	44		OS (RR)	0.45 (0.22–0.94)	0.035		28
rs16917496	*SET8*	AS	T>C (0.32)	miR-502	BC	1,100		AO (years)	54.6 vs 47.7			76
rs16917496	*SET8*	AS	T>C (0.32)	miR-502	HCC	142	142	Risk (OR; 95%CI)	NS	NS	IHC in HCC tissues	107
								OS (RR)	0.17 (0.05–0.58)	0.004		
rs1564483	*BCL2*	AS	G>A (0.29)	miR-181b	NSCLC	1,017	1,017	Risk (OR)	0.73 (0.54–0.98)	0.007		29
				miR-204		1,017		MST (months)	11.7 vs 15.2	0.036		
				miR-200bc/429								
rs2240688	*CD133*	AS	A>C (0.27)	miR-135a/b	LC	773	778	Risk (OR)	0.80 (0.65–0.98)	0.027	Gene reporter in vitro assay + mRNA and IHC on tissue	35
						503	623	Risk (OR)	0.80 (0.63–1.00)	0.05		
						559		MST (months)	10 vs 12	0.02		
						331		MST (months)	14 vs 18	0.081		
rs3660	*KRT81*	CAU	C>G (0.41)	miR-20a/b	NSCLC	175		TTR (months)	20.3 vs 86.8	0.003	In silico prediction + IHC on tissues	17
				miR-106a/b	SCC	89		TTR (months)	23.9 vs 100.2	0.002		
				miR-17								
				miR-93								
				miR-519d								
rs2239680	*BIRC5*	AS	T>C (0.23)	miR-335	LC	600	600	Risk (OR)	3.43 (2.04–5.77)	<0.01	Gene reporter in vitro assay + mRNA and IHC on tissues	44
						1,000	1,000	Risk (OR)	1.63 (1.17–2.26)	<0.01		
rs61764370	*KRAS*	HISP	T>G (0.03)	let-7	NSCLC	325	325	Risk (OR)	2.3 (1.1–4.6)	0.02	Gene reporter in vitro assay	45
		CAU	T>G (0.08)			2,205	1,497	Risk (OR)	1.36 (1.07–1.73)	0.01		
rs61764370	*KRAS*	CAU	T>G (0.08)	let-7	NSCLC	218		OS		NS		46
rs61764370	*KRAS*	CAU	T>G (0.08)	let-7	CRC	409 (stage I, II)		Risk (RR)	0.68 (0.49–0.94)			51
						182 (stage III)		OS (HR)	0.46 (0.18–1.04)	0.038		
						69 (stage IV)		NS				
								NS				
rs61764370	*KRAS*	CAU	T>G (0.08)	let-7	CRC	100		Responder (%)	31.9 vs 0.0	0.004		55
rs61764370	*KRAS*	CAU	T>G (0.08)	let-7	BC	268 (*BRCA1* carriers)	797	Risk (OR)	1.47 (1.05–2.06)	0.025		94
						89 (*BRCA2* carriers)		Risk (OR)	NS			
						685 noncarriers		Risk (OR)	NS			
rs61764370	*KRAS*	CAU	T>G (0.08)	let-7	OC	100	101	Risk (OR)	2.38 (1.16–5.09)	0.02		104
						308	322	Risk (OR)	2.01 (1.36–2.99)	0.0005		
rs61764370	*KRAS*	CAU	T>G (0.08)	let-7	HNC	513	597	Risk (OR)	NS			126
								OS (HR)	2.5 (1.3–4.9)	<0.01		

(*Continued*)

Table 1 (*Continued*)

Single nucleotide polymorphism ID	Gene	Race	Information	Predicted binding site for	Disease	Cases	Controls	Endpoint	Best result	*P*-value	Assay	Ref
rs2735383	*NBS1*	AS	G>C (0.39)	miR-629	LC	1,056 503	1,056 623	Risk (OR) Risk (OR)	1.47 (1.15–1.86) 1.55 (1.12–2.15)	0.008 0.026	Gene reporter in vitro assay + WB + chromosome aberration challenge assay	47
rs465646	*REV3L*	AS	T>C (0.17)	miR-25 miR-32	LC	500 572	517 547	Risk Risk	0.69 (0.53–0.90) 0.72 (0.55–0.94)	0.007 0.016	Cell focus formation assay and gene reporter in vitro assay	48
rs3134615	*MYCL1*	AS	G>T (0.02)	miR-1827	SCLC	666	758	Risk (OR)	2.08 (1.39–3.21)	0.0004	Gene reporter in vitro assay	50
rs1534862	*NEIL2*	CAU	T>C (0.05)		CRC	718 229		OS (HR)	1.66 (1.18–2.34)	0.003	In silico prediction	57
rs2233921	*SMUG1*	CAU	G>T (0.46)	miR-770-5p miR-665 miR-455-3p	CRC	718 229		OS (HR)	0.54 (0.36–0.81)	0.003	Gene reporter in vitro assay	57
rs10082466	*MBL2*	AA	A>G (0.39)	miR-27a	CRC	103	127	Risk (OR)	3.17 (1.57–6.40)	0.001	Gene reporter in vitro assay	1
rs696	*NFkBIA*	AS	A>G (0.37)	miR-449 miR-34	CRC	1,001	1,005	Risk (OR)	1.38 (1.14–1.66)	0.0008	Gene reporter in vitro assay	63
rs709805	*KIAA0182*	CAU	G>A (0.26)	miR-324-3p	CRC	717	739	Risk (OR)	1.57 (1.06–2.7)	0.027	Gene reporter in vitro assay	64
rs354476	*NUP210*	CAU	T>C (0.47)	miR-125a miR-125b	CRC	717	739	Risk (OR)	1.36 (1.02–1.82)	0.0045	Gene reporter in vitro assay	64
rs17281995	*CD86*	CAU	G>C (0.12)	miR-337 miR-582 miR-200a miR-184 miR-212	CRC	697	624	Risk (OR)	2.74 (1.24–6.04)	0.013	In silico prediction	10
rs1051690	*INSR*	CAU	G>A (0.14)	miR-618 miR-612	CRC	697	624	Risk (OR)	1.94 (1.03–3.66)	0.03	In silico prediction	10
rs7356	*RPA2*	CAU	A>G (0.38)	miR-3149 miR-1183	CRC	1,098	1,469	Risk (OR)	1.33 (1.01–1.75)	0.04	In silico prediction	71
rs4596	*GTF2H1*	CAU	G>C (0.37)	miR-518a-5p miR-527 miR-1205	CRC	1,098	1,469	Risk (OR)	0.79 (0.64–0.99)	0.03	In silico prediction	71
rs115160714	*TOPBP1*	CAU	C>T (0.005)	miR-3138 miR-4302 miR-1207-5p	BC	534	556	Risk (OR) Grading (G3)	3.54 (1.56–8.39) 6.83 (2.75–16.86)	0.002 0.0001	In silico prediction and mRNA expression + protein on tissue	78
rs1044129	*RYR3*	AS	A>G (0.44)	miR-367	BC	1,532 1,125	1,600	Risk (OR) PFS (HR)	1.26 (1.03–1.54) 2.20 (1.03–4.70)	0.028 0.042	Gene reporter in vitro assay and IHC on tissues	84

rs743554	*ITGB4*	CAU	G>A (0.14)	miR-34a	BC	749	1,493	Risk (OR)	7.65 (1.44–40.7)	0.017	In silico prediction	85
						244		Grading (G3)	2.26 (1.17–4.37)	0.015		
								HR	2.11 (1.21–3.68)	0.0085		
								OS (HR)				
rs4245739	*MDM4*	AS	A>C (0.05)	miR-191	BC	1,100	1,400	Risk (OR)	0.55 (0.40–0.76)	0.00023	Gene reporter in vitro assay	87
						1,100	1,400	Risk (OR)	0.41 (0.25–0.67)	0.00031		
rs4245739	*MDM4*	AS	A>C (0.05)	miR-191	OC	154	154	Risk (OR)		NS	Gene reporter in vitro assay + mRNA expression and IHC on tissues	102
						113		PFS (months)	52 vs 82	0.042		
rs4245739	*MDM4*	AS	A>C (0.05)	miR-191	ESCC	540	550	Risk (OR)	0.54 (0.35–0.82)	0.004	mRNA expression on tissue	137
						588	600	Risk (OR)	0.68 (0.44–0.99)	0.049		
rs7963551	*RAD52*	AS	T>G (0.19)	let-7 b	BC	878	900	Risk (OR)	0.85 (0.71–1.07)	0.12	Gene reporter in vitro assay	88
						914	967	Risk (OR)	0.81 (0.68–0.97)	0.02		
rs799917	*BRCA1*	CAU	C>T (0.34)	miR-638	BC	166 (sporadic)	186	Risk (OR)	2.81 (1.40–5.61)	0.003	Gene reporter in vitro assay	90
						169 (familial)	186	Risk (OR)	1.26 (0.59–2.71)	NS		
rs334348	*TGFBR1*	CAU	A>G (0.23)	miR-628-5p	BC	166 (sporadic)	186	Risk (OR)	2.67 (1.19–6.03)	0.002	Gene reporter in vitro assay	90
						169 (familial)	186	Risk (OR)	1.26 (0.59–2.71)	0.55		
rs1042538	*IQGAP1*	AS	A>T (0.43)	miR-124	BC	1,541	1,598	Risk (OR)	0.78 (0.61–0.99)	0.049	mRNA and protein expression in frozen tissues	91
rs2747648	*ESR1*	CAU	T>C (0.042)	miR-453	BC	1,223	1,495	Risk (OR)	0.73 (0.54–0.97)	0.029	In silico prediction	95
						721 (<50 years old)	713	Risk (OR)	0.60 (0.41–0.89)	0.010		
						438 (high risk fam)	659	Risk (OR)	0.53 (0.35–0.79)	0.002		
rs10889677	*IL23R*	AS	A>C (0.33)	Let-7e	BC	491	502	Risk (OR)	1.40 (1.09–1.79)	0.0084	In silico prediction	99
				Let-7f		491		AO (years)	46.0 vs 50.6	0.0114		
rs17147016	*UGT2A3*	CAU	T>A (0.13)	miR-224	OC	417	417	Risk (OR)	1.47 (1.08–2.01)	0.015	In silico prediction	103
rs7499	*COL18A1*	CAU	G>A (0.47)	miR-594	OC	417	417	Risk (OR)	1.47 (1.07–2.02)	0.017	In silico prediction	103
rs3917328	*IL1R1*	CAU	C>T (0.04)	miR-335	OC	417	417	Risk (OR)	1.65 (1.03–2.64)	0.037	In silico prediction	103
rs10771184	*KRAS*	CAU	T>A (0.45)	miR-544	OC	417	417	Risk (OR)	1.26 (1.01–1.57)		In silico prediction	103
						417		OS (HR)	0.56 (0.38–0.84)	0.005		
rs1425486	*PDGFC*	CAU	G>A (0.34)	miR-425	OC	417		OS (HR)	2.69 (1.67–4.33)	4.2×10^{-5}	In silico prediction and gene reporter in vitro assay	103
rs1047920	*SNAI1*	CAU	C>T (0.08)	miR-24	OC	417		OS (HR)	1.96 (1.30–2.97)	0.0038	In silico prediction	103
rs7869402	*TLR4*	CAU		miR-539	OC	417		OS (HR)	2.16 (1.31–3.57)	0.002	In silico prediction	103
rs17875871	*IFNAR1*	AS	Del (0.12)	miR-1231	HCC	420	420	Risk (OR)	1.84 (1.18–2.84)	0.006	In silico prediction	108
rs3783553	*IL1A*	AS	In (0.29)	miR-122	HCC	403	434	Risk (OR)	0.30 (0.17–0.54)	0.0001	Gene reporter in vitro assay	112
				miR-378		1,074	1,239	Risk (OR)	0.75 (0.57–0.98)	0.0328		
rs56228771	*SGSM3*	AS	In ND	miR-151-5p	HCC	502	513	Risk (OR)	0.55 (0.42–0.73)	1.03×10^{-5}	In silico prediction and mRNA expression on tissue	113
rs6147150	*EERB4*	AS	Del ND	let-7c	HCC	270	270	Risk (OR)	1.59 (1.22–2.07)	0.003	In silico prediction	114

(*Continued*)

Table 1 (*Continued*)

Single nucleotide polymorphism ID	Gene	Race	Information	Predicted binding site for	Disease	Cases	Controls	Endpoint	Best result	*P*-value	Assay	Ref
rs8679	*PARP1*	CAU	T>C (0.21)	miR-145 miR-105 miR-630 miR-302a	BIC	752	704	Risk (OR)	1.29 (1.02–1.62)	0.05	In silico prediction	116
rs7180135	*RAD51*	CAU	A>G (0.44)	miR-197	BIC	202		OS (HR)	0.52 (0.31–0.87)	0.01	In silico prediction	116
rs1417608	*HSD3B2*	CAU	A>G (0.07)	miR-423-5p	BIC	563	863	Risk (OR)	1.94 (1.36–2.75)	0.001	In silico prediction	117
				miR-1914 miR-3658		497	957	Risk (OR)	3.66 (1.06–12.63)	0.04		
rs9299	*HOXB5*	CAU	A>G (0.37)	miR-7	BIC	391	391	Risk (OR) Grading (T2–T4)	2.05 (1.06–3.94) 2.25 (1.33–3.79)	0.031 0.003	Gene reporter in vitro assay	121
rs884225	*EGFR*	AS	A>G (0.39)	miR-214	BIC	908	1,239	Risk (OR)	1.40 (1.09–1.80)	0.008	Gene reporter in vitro assay	122
rs3747238 G allele	*SMC1B*	CAU	T>C (0.46)	miR-609 miR-124a	HNC	150 with recurrence 300 w/t recurrence		ROR (HR) TTR (months)	1.74 (1.19–2.54) >93 vs 75.8	0.004 0.019	In silico prediction	125
rs8126	*TNFAIP2*	CAU	T>C (0.37)	miR-184	HNC	1,077	1,073	Risk (OR)	1.48 (1.06–2.05)	0.02	Gene reporter in vitro assay + mRNA expression on tissue	127
rs8126	*TNFAIP2*	CAU	T>C (0.37)	miR-184	GC	301	313	Risk (OR)	2.00 (1.09–3.64)	0.024		130
rs712	*KRAS*	AS	T>G (0.18)	let-7	GC	118	674	Risk (OR)	3.05 (1.53–6.08)	0.00153		131
rs12537	*MTMR3*	AS	C>T (0.23)	miR-181a	GC	500	502	Risk (OR) OS (HR)	1.72 (1.36–2.16) 1.38 (1.03–1.83)	3.99×10^{-5} 0.029	Gene reporter in vitro assay	132
rs6573	*RAP1A*	AS	C>A (0.11)	miR196a	ESCC	536	608	Risk (OR)	0.43 (0.21–0.91)	0.02	Gene reporter in vitro assay	135
rs1131445	*IL16*	AA	T>C (0.25)	miR-135a/b	PC	256		OS (HR)	3.0 (1.23–7.12)	0.014		137
rs11902171	*ITGAv*	AS	G>C (0.08)		PC	347	367	Risk (OR)	0.57 (0.35–0.93)	0.024		141
rs1434536	*BMPR1B*	AS	C>T (0.34)	miR-125b	PC	247	278	Risk (OR)	1.90 (1.15–3.15)	0.015	Gene reporter in vitro assay	143

Notes: The results are reported for the less common (underlined) allele, using the most common allele as reference. The table resumes the best associations reported in the cited study.

Abbreviations: ID, identification number; Ref, reference; AS, Asian; T, timeline; miR, microRNA; NSCLC, non-small-cell lung cancer; OS, overall survival; RR, relative risk; IHC, immunohistochemistry; SCLC, small cell lung cancer; BC, breast cancer; AO, age of onset; HCC, hepatocellular carcinoma; OR: odds ratio; NS, statistically nonsignificant; LC, lung cancer; MST, median survival time; mRNA, messenger RNA; CAU, Caucasian; SCC, squamous cell carcinoma; TTR, time to recurrence; HIS, Hispanic; HR, hazard ratio; CRC, colorectal cancer; OC, ovarian cancer; HNC, head and neck cancer; WB, western blot; AA, African American; PFS, progression-free survival; ND, not detected; BIC, bladder cancer; ROR, risk of recurrence; GC, gastric cancer; ESCC, esophageal cancer; PC, prostate cancer ; vs, versus; fam, familiar; In, insertion; Del, deletion; w/t, without.

for a protein known as Hb-1, a type of hair keratin that is physiologically expressed in hair shafts. Keratins are proteins expressed in all types of epithelial cells,[41] with different expression patterns among different carcinomas,[42] and they are extensively used as diagnostic markers.

In addition, survivin was studied for its miRSNPs. Survivin is overexpressed in many types of human cancer, including LC, and is considered a promising therapeutic target.[43] Interestingly, the expression of its encoding gene, *BIRC5*, was found to be correlated with a SNP (rs2239680) within the 3′-UTR in normal lung tissues.[44] This putative miRSNP was evaluated in two independent sets of samples from a Chinese Han population, and the C allele was associated with a significantly increased risk for LC and advanced staging. Furthermore, a reporter gene assay showed that rs2239680 T>C change caused an altered regulation of *BIRC5* mRNA expression through the effect on miR-335::mRNA pairing.

miRSNPs as biomarkers of susceptibility to LC

A study showing the importance of miRSNPs within the 3′-UTR of the protooncogene *KRAS* was presented in 2008.[45] In this region, there are at least 10 different target sites for one of the first discovered miRNAs (let-7), and one of them, rs61764370, within the let-7 complementary site 6 (LCS6) was extensively studied in relation to several types of cancer, including LC. This miRSNP was evaluated for its association with the risk for NSCLC in two independent sample sets: one consisting of 325 cases and 325 controls from New Mexico[45] and other replicating the findings on 2,205 cases and 1,497 controls of Caucasian origin.[45] The LCS6 variant allele was significantly associated with increased risk for NSCLC among moderate smokers. However, rs61764370 was not found to be associated with OS in LC patients, suggesting a poor clinical utility in NSCLC.[46]

The CC genotype of rs2735383, a functional miRSNP within the 3′-UTR of the *NBS1* gene, was associated with a significantly increased risk for LC when compared with GG or GC genotypes in a study consisting of 1,559 cases and 1,679 controls, all Han Chinese.[47] The CC genotype caused a decrease of the mRNA expression through the alteration of the miR-629 binding site. In turn, a deficient expression of *NBS1* may induce deficiencies in the DNA repair and increased mutagen sensitivity, providing a possible explanation for its relationship with the risk for LC. In another Chinese study (1,072 patients and 1,064 cancer-free controls), the variant C of rs465646 within the 3′-UTR of *REV3L* was associated with decreased risk for LC.[48] REV3Lp constitutes the catalytic subunit of DNA polymerase zeta, the major participant in trans-lesion DNA synthesis, one error-prone DNA repair system.[49] Consistent with this role, T allele showed a stronger binding affinity for miR-25 and miR-32, resulting in significantly weaker reporter expression levels, as confirmed by additional experiments.[48] Another case-control association study showed that rs3134615 T allele within the 3′-UTR of *MYCL1* was associated with a significantly increased risk for small cell lung cancer (SCLC).[50] This miRSNP is located within the binding site for miR-1827. The G>T change may inhibit the interaction of miR-1827 with *MYCL1* mRNA, resulting in higher expression of *MYCL1*. Because *MYCL1* is a member of the *MYC* oncogene family, which plays a critical role in carcinogenesis, individuals carrying the rs3134615 T allele are expected to have elevated risk for the development of SCLC. miR-1827 may play a role in lung carcinogenesis by functioning as a tumor suppressor, and further studies of this miRNA in cancer are warranted.

Colorectal cancer

miRSNPs as biomarkers of prognosis in colorectal cancer

Colorectal cancer (CRC) is the second most common cancer and the fourth-leading cause of cancer death worldwide.[51] The tumor node, and metastasis staging system is currently the main tool to provide prognostic information, being highly predictive for prognosis at the extremes, although less predictive for intermediate stages.[52,53] According to current guidelines, adjuvant chemotherapy is not administered to early-stage patients (T1-3-N0-M0), as 5-year OS is more than 70%. Nevertheless, 20%–30% of them will die of CRC within 5 years. Unfortunately, molecular markers enabling us to detect these aggressive forms are lacking. Several authors undertook studies to evaluate whether miRSNPs could represent prognosis markers to be used to this end.

The *KRAS* rs61764370 (within LCS6) was evaluated in the early CRC stages in the prospective Netherlands Cohort Study.[51] The T allele was associated with a higher CRC risk and a shorter OS compared with the G allele. In patients with advanced disease, no clear associations were observed.

Response to therapy

rs61764370 also was associated with the response to cetuximab, a monoclonal antibody directed toward the *epidermal growth factor receptor* (*EGFR*).[54] In a recent study on Caucasians, it was shown that among patients with wild-type KRAS, 31.9% of those with the rs61764370 TT genotype presented a complete or a partial response, whereas

none of those with the TG + GG genotypes responded.[55] This finding adds knowledge of the role of *KRAS* in the cure of the disease. However, these findings were not in agreement with the results obtained in a previous work.[56] In summary, *KRAS* genotypes deserve further validation as prognostic biomarkers and consideration in therapy decision-making, especially for early-stage patients.

Concerning the most commonly used therapies based on 5-fluorouracil (5-FU), miRSNPs in BER genes were evaluated, and interesting results were found for miRSNPs rs1534862 within *NEIL2* and rs223392 within *SMUG1*.[57] Both miRSNPs were found to be associated with OS, with the stronger association for TT homozygotes of rs223392 after stratification for 5-FU-based chemotherapy. This is in agreement with the fact that SMUG1 and NEIL2 are among the main DNA glycosylases involved in the response to damages induced by 5-FU.[58–61] A functional in vitro assay showed that the *SMUG1* T allele, compared with the G allele, caused a reduced expression of a reporter gene. Thus, it was suggested that *SMUG1* excision activity, modulated by miRSNP rs223392, could affect the toxicity caused by 5-FU.[58]

miRSNPs as biomarkers of susceptibility in colorectal cancer

Chronic intestinal inflammation has been identified as a risk factor for CRC.[62] Therefore, it is possible that functionally important genetic variants of inflammatory mediators, such as mannose-binding lectin 2 (*MBL2*), are also associated with susceptibility to CRC. Four *MBL2*-specific allele variants in linkage disequilibrium located in the 3′-UTR region of the gene were associated with a higher risk for CRC in African-Americans.[1] In particular, C allele of rs10082466 was associated with increased risk, and it was predicted to create a novel binding site for miR-27a and miR-27b. The increased binding affinity predicted for the C allele of rs10082466 was reflected by a significant decrease in normalized luciferase activity compared with the negative control. More important, the C allele of rs10082466 was associated with lower plasma MBL levels and activity in cases and controls, as would be expected for a regulatory interaction involving a germ-line polymorphism. miR-27a binds more efficiently to the C allele, which is consistent with the observed lower plasma MBL levels and activity.

Nuclear factor kB (*NFkB*) plays a key role in the regulation of apoptosis. The function of NFkB is inhibited by binding to the NFkB inhibitor, and the disruption of the balance of NFkB and the NFkB inhibitor is related to the development of many diseases, including tumors. Therefore, it was hypothesized that SNPs within the 3′-UTR of *NFkBIA* were associated with CRC susceptibility.[63] Both A>G polymorphisms (rs696) were associated with an increased risk for CRC among Chinese patients. For rs696, the GG genotype was associated with a statistically significantly increased risk compared with AA + GA. Moreover, the authors found that the change from A to G in the 3′-UTR of *NFkBIA* decreased luciferase activities, as assessed by an in vitro reporter assay. These experiments suggested that NFkBIA 2758 A>G variants may affect mRNA stability, likely generating a novel seed site for miR-449a. More experiments showed that miR-449a reduced the relative luciferase activities via the *NFkBIA* 3′-UTR target site created by the A allele. The results indicate that A allele strengthens the binding of miR-449a with 3′-UTR of *NFkBIA*, which in turn inhibits the expression of *NFkBIA*. This polymorphism could be a genetic marker for susceptibility to CRC.

In another case-control association study on Caucasians, the AA homozygotes for rs709805 (within the predicted gene *KIAA0182*) showed an increased CRC risk compared with in the GG + GA group, and the CC homozygotes for rs354476 (*NUP210*) had an increased risk compared with the TT + TC group.[64] In vitro assays carried out to test the differences between the common and variant 3′-UTRs of *NUP210* and *KIAA0182* showed that only the T allele of rs354476 was associated with a reduced expression of the reporter gene. *NUP210* encodes the nuclear pore glycoprotein 210 involved in the structural organization of the nuclear pore complex.[65] During mitosis, Ser1880 of glycoprotein 210 is phosphorylated by the cyclin B-p34cdc235.[66] An increased expression of *NUP210* was found also in other types of cancer, such as cervical cancer.[67]

Positive associations between risk for CRC and two miRSNPs (*CD86* rs17281995 and *INSR* rs1051690) were also found in another study on Caucasians.[10] Five different miRNAs (miR-337, miR-582, miR-200a, miR-184, and miR-212) bind to a target site that contains the same polymorphism within *CD86*. CD86, with CD80, is a costimulatory ligand expressed on the surface of the antigen-presenting cells (dendritic cells, macrophages, and B cells) in the immune system.[68] Two different miRNAs bind to the same polymorphism within *INSR*: miR-612 and miR-618. *INSR* encodes for insulin receptor, and after the binding of insulin to the extracellular portion, a second messenger system diverges into two separate pathways that regulate distinct biological effects: the phosphoinositide-3-kinase pathway and the mitogen-activated protein kinase pathway.[69]

In addition, SNPs residing within the 3′-UTRs of genes involved in pathways such as DNA repair, DNA signaling,

or apoptosis may indirectly contribute to affecting the individual risk of developing CRC.[45,70] The role of miRSNPs in genes specifically involved in the nucleotide excision repair (NER) pathway was investigated. rs7356 within *RPA2* and rs4596 within *GTF2H1* were associated with rectal cancer risk in Caucasian patients.[71] rs4596 is located within a target region of the predicted miR-518a-5p and miR-527 that showed the highest energy-binding level to 3′-UTR with the G allele. Interestingly, findings from association studies showed that G-allele carriers were at decreased cancer risk. The GTF2H1 (general transcription factor IIH, polypeptide 1, 62 kDa) encodes for a component of the core-TFIIH basal transcription factor involved in NER and, when in complex with Cdk-activating kinase (CAK), also is involved in RNA transcription. Variant A allele of rs7356 in *RPA2* (replication protein A 32 kDa subunit) was associated with increased risks. The G allele of rs7356 is more prone to bind miR-3149 and miR-1183, eventually resulting in a stronger negative regulation on target gene expression.

Breast cancer

miRSNPs as biomarkers of prognosis in breast cancer

Breast cancer (BC) is the most frequently diagnosed cancer and one of the leading causes of cancer death among women worldwide.[72,73] Germ-line mutations in *BRCA1* and *BRCA2* account for only 5% of all BC cases in the general population.[74] Other low-penetrance genetic variants, especially in as-yet-unknown combinations, are expected to explain most BC incidence.[75] Investigators have hypothesized that the 3′-UTRs of miRNA target genes may harbor part of these variants. The genotype CC of *SET8* rs16917496, a BC candidate gene, was associated with earlier age of onset when compared with TT in Asian patients.[76] Another candidate miRSNP, rs115160714, within *TOPBP1*, encoding for topoisomerase IIb binding protein 1 (TopBP1), was evaluated in relation to BC by Forma et al in Caucasian patients.[77,78] Heterozygotes (CT) and homozygotes (TT) had significantly increased risk for BC compared with common homozygotes (CC). Moreover, patients with a tumor classified as high grade (G3) or T2-T4N1M0 were carriers of the variant allele (T) more often than expected. In agreement with these findings, *TOPBP1* mRNA and protein expression were found to be increased in individuals with the CT or TT genotype. Three candidate miRNAs, miR-3138, miR-4302, and miR-1207-5p, were predicted to bind to the 3′-UTR of TopBP1. Thus, the study raised the hypothesis that a genetic variation of TopBP1 may be involved in the etiology of BC. The biological bases for explaining its role in BC could rely on the fact that TopBP1 shares structural functional similarities with BRCA1 and is involved in cell survival, DNA replication, DNA damage repair, and cell cycle checkpoints.[79,80]

Because calcium and vitamin D intake were associated with mechanisms of carcinogenesis of the mammary gland,[81,82] and in addition, breast calcifications are an important risk factor for BC,[83] Zhang et al[84] evaluated the role of miRSNPs within the 3′-UTR of *RYR3,* a CICR (calcium-induced calcium release) protein playing a crucial role in cellular Ca^{2+} homeostasis. After the analysis of 1,532 breast cancer cases and 1,600 healthy Chinese women, rs1044129 was found to be associated with BC risk, calcification, and progression-free survival. These findings were also supported by in vitro assays showing that miR-367 binds more tightly to the A allele of rs1044129 than to the G allele and represses *RYR3* expression more strongly.

Concerning the role of integrins for BC, a Swedish study evaluated whether miRSNPs within the 3′-UTR of *ITGA3*, *ITGA6*, *ITGAv*, *ITGB3*, *ITGB4*, and *ITGB5* genes could be associated with BC clinical outcome and risk.[85] Detailed clinical data of 749 Swedish incident patients with follow-up within 15 years were evaluated and compared with data from 1,493 matched controls. The strongest association was observed between the rare A allele of the SNP rs743554 within *ITGB4* and the risk for estrogen receptor-negative carcinomas. The same allele also was associated with worse OS compared with the common allele. None of the remaining putative miRSNPs were significantly associated with BC risk. In silico analysis predicted that A allele may cause a loss of the binding site for the miR-34a. The association between the *ITGB4* and hormone-receptor status may be explained by the fact that integrin-mediated signal transduction pathways regulate estrogen receptor α (ER-α) in mouse mammary epithelial cells.[86]

miRSNPs as biomarkers of susceptibility in breast cancer

Several studies investigated the role of miRSNPs as possible risk factors for BC. In 1,100 BC cases and 1,400 controls from two regions of China, the AC and CC genotypes of *MDM4* rs4245739 were significantly associated with decreased BC risk compared with the AA genotype.[87] In another Chinese study, the variant C allele rs7963551 within the 3′-UTR of *RAD52* was associated with a reduced BC risk.[88] Luciferase activity assay showed a higher expression level for C allele compared with A allele, which might be a result of a reduced inhibition from a weakened binding capacity of miRNA to

the 3′-UTR of *RAD52* harboring C allele. These findings suggested that rs7963551, one of the miRSNPs located with a let-7 binding site, may alter expression of *RAD52,* contributing to the development of BC. *RAD52* could play a crucial role for BC, considering its role in the homologous recombination repair in cooperation with *BRCA1* and *BRCA2*.[89] For these latter genes, Nicoloso et al[90] reported that T allele rs799917 within the 3′-UTR of *BRCA1* is associated with susceptibility to BC in Caucasians and that this risk is particularly increased for the sporadic form. The authors showed that miR-638 interacts more strongly with C allele of SNP rs799917 than with the T allele; this difference was also confirmed at the protein level.[90]

Concerning the pathway of the mitogen-activated protein kinase, several pieces of evidence suggested that deficiencies of IQGAP1 (IQ motif-containing GTPase-activating protein 1) could be related to cancer development. Thus, the genotypes of *IQGAP1* were evaluated in a Chinese association study on 1,541 BC patients and 1,598 controls.[91] The TT genotype of the putative miRSNP rs1042538 was associated with a significantly lower risk for BC compared with the AA genotype. The results were consistent with the finding that the expression levels of IQGAP1 protein were significantly higher in the TT genotype. Lim et al[92] verified experimentally that the A-to-T variant disrupts a miRNA target site for miR-124, and thus the A allele causes miR-124 to bind more tightly with *IQGAP1* mRNA, leading to the down-regulation of the encoded protein. Functional studies established that IQGAP1 interacts with and regulates the actin-Cdc42/Rac1-mitogen-activated protein kinase pathway, contributing to its role in cell migration and invasion.[93] In the same pathway, the association between miRSNPs *KRAS* rs61764370 and BC risk also was evaluated. A German study was carried out on 268 *BRCA1*-positive families, 89 *BRCA2*-positive families, 685 *BRCA1*/*BRCA2*-negative families, and 797 geographically matched controls.[94] The allele frequency of the *KRAS* variant was found to be increased only among patients with BC positive for *BRCA1* mutations (compared with controls). However, when a larger sample set was analyzed by including other family members in addition to the index cases, the association could not be replicated.

In another German study of 1,223 BC families and 1,495 unrelated controls, a significant association was revealed for the T allele within the 3′-UTR of *ESR* rs2747648, and in particular, among premenopausal women.[95] According to in silico analyses, T allele attenuates the binding of miR-453, leading to higher ESR1 protein levels. *ESR1* is a member of the nuclear receptor family, a group of hormone-inducible transcription factors that activates gene expression by recruiting multiple coactivators. Clinical studies have shown that depletion of *ESR1* significantly reduces BC risk, providing further support for the observed protective effect of the C allele in premenopausal women.[96,97]

Other studies on BC susceptibility focused on the role of immunity and inflammation. Interleukin 23 (*IL23*) and its receptor (*IL23R*) guide T cells toward a T-helper type 17 (Th17) phenotype characterized by IL-17A production.[98] A microenvironment constituted by tumor-infiltrating cells releasing high levels of IL-17 is a poor prognostic factor for BC. Thus, the effect of the miRSNP *IL23R* rs10889677 was evaluated in Chinese Han women, and the A allele was associated with an increased risk for BC.[99] Zwiers et al reported that A allele could determine the loss of binding capacity for the miRNAs let-7e and let-7f.[100]

Ovarian cancer

miRSNPs as biomarkers of prognosis in ovarian cancer

Epithelial ovarian cancer (EOC) is the fifth most common cancer in women.[101] At the time of diagnosis, more than 80% of patients present late-stage malignancies with a survival rate less than 30% at 5 years.[102] A recent study on Chinese patients hypothesized that *MDM4* could affect chemosensitivity and progression of EOC.[102] The authors found that the common AA genotype rs4245739 is more frequent in patients with high-grade carcinomas and that when the analysis was stratified considering cases not expressing the estrogen receptor, patients with the AA genotype have an increased risk for recurrence and tumor-related death. Further analyses showed that the miRSNP creates a putative target site for miR-191 (a miRNA highly expressed in normal and tumor tissues) and that this acquisition causes downregulation of *MDM4* expression, thereby significantly delaying ovarian carcinoma progression and tumor-related death.

In another study on 417 Caucasian cases and controls, the authors investigated 238 SNPs from eight miRNA processing genes and 134 genes for EOC predisposition and association with clinical outcome and treatment response.[103] Four miRSNPs (*UGT2A3* rs17147016, *COL18A1* rs7499, *IL1R1* rs3917328, and *KRAS* rs10771184, alias rs12245) were associated with increased risks. The variant allele of *KRAS* was also associated with longer OS and favorable treatment outcome. Moreover, the variant alleles within *PDGFC* rs1425486 and *TLR4* rs7869402 were associated with poor response to therapies. *PDGFC* is a member of the platelet-derived growth factor family, which encodes a mitogenic factor for cells of mesenchymal origin, and with an in vitro reporter assay, the authors demonstrate

that rs1425486 alleles could differentially affect miR-425 targeting in ovarian cancer cells, suggesting that *PDGFC* is a putative target for miR-425.

miRSNPs as biomarkers of susceptibility to ovarian cancer

The Connecticut Ovarian Cancer Case-Control study, consisting of 320 patients and 328 controls, mostly from Northern Italy, showed a significant increased risk of developing EOC for carriers of *KRAS* G allele rs61764370.[104] In EOC, *KRAS* overexpression was shown to disrupt the EGFR-signaling pathway, a pathway found frequently deregulated in the disease. This could provide a rationale for explaining the association. However, no increased risks were observed in a larger study of 8,669 cases and 10,012 controls, mostly from Northern Europe and Northern America.[101] Although false-positive results of the Connecticut study could not be ruled out because of the small sample size, positive associations could be also ascribed to regional differences. It is quite impressive that when the analysis was stratified considering patients affected by a familial form of EOC and negative for mutations within *BRCA1* or *BRCA2* (ie, 31 diagnosed for hereditary breast and ovarian cancer syndrome), the frequency of the carriers of the variant allele was much higher (61%) compared with what was expected (14.5%).[104] Although the study was somewhat limited by the small number of uninformative patients, these findings support the hypothesis that the G allele could be a genetic marker of increased risk for hereditary breast and ovarian cancer.

Hepatocellular carcinoma

mirSNPs as biomarkers of prognosis to hepatocellular carcinoma

Hepatocellular carcinoma (HCC) is the fifth most common malignancy worldwide and the third leading cause of cancer death.[105] Because of its high fatality, its incidence and mortality rates are almost equal. The incidence of HCC is rising steeply in Asia and Africa, where hepatitis B and C viruses (HBV and HBC) are more prevalent.[106] The SNP in the miR-502 binding site of the *SET8* 3′-UTR was examined for its predictive power relative to HCC outcomes.[107] The *SET8* CC genotype was associated with longer postoperative OS in Asian patients with HCC and with reduced SET8 protein levels, according to the immunostaining of 51 HCC tissue samples. C allele located within the seed region was perfectly matched with G allele in miR-502. These data suggest that an altered expression of *SET8*, at least in part ascribed to miR-502, affects HCC outcome. Thus, an analysis of genetic polymorphisms within miRNA binding sites may help identify patient subgroups with poor prognosis.

miRSNPs as biomarkers of susceptibility to hepatocellular carcinoma

HBV and HCV are the main etiological risk factors for HCC worldwide.[106] Thus, several studies were undertaken to ascertain the role of miRSNPs involved in modulating the inflammation and/or in the immune response to these infections. A study of 420 Chinese patients and unrelated controls showed that the 4-bp In/Del miRSNP rs17875871 within the 3′-UTR of *IFNAR1* was associated with the risk for HCC and that the association was more pronounced in a subgroup of patients positive for B-hepatitis.[108] In silico predictions suggested that rs17875871 is located in the seed region of a miR-1231 predicted target sequence. *IFNAR1* encodes for a membrane protein needed to compose the receptors for interferon alpha and beta. The binding with the ligand activates STAT1 and STAT2 cascades.[109] Several pieces of evidence showed the involvement of IFNAR1 in HBV and HCV replication and chronic infection.[110] Genetic polymorphisms within *IFNAR1* were also found to be associated with clinical presentation and outcomes after HBV infection.[111] Thus, *IFNAR1* may affect the inflammatory process related to HBV and HCV infections, thereby contributing to HCC susceptibility.

In another study, the miRSNP rs3783553 within *IL1A* 3′-UTR was evaluated in association with HCC in two independent Asian populations. The variant allele consisting of a 4-bp (TTCA) insertion was associated with decreased risks.[112] In addition, it was shown in vitro and in vivo that this allele disrupts the binding sites for miR-122 and miR-378, thereby increasing the expression of IL-1a. These findings suggest that functional polymorphism rs3783553 could contribute to HCC susceptibility. Considering that IL-1a affects not only various phases of the malignant process, such as carcinogenesis, tumor growth, and invasiveness, but also patterns of interactions between malignant cells and the host's immune system, these results indicate that IL-1a may be a promising target for immunotherapy, early diagnosis, and intervention of HCC.

Concerning pathways of intracellular signaling, a Chinese study carried out on rs56228771 found an association between a 4-bp In/Del miRSNP within the 3′-UTR of *SGSM3* and a significantly decreased risk for HCC.[113] Tissue samples with In/In genotype had the highest levels of *SGSM3*, about 1.52-fold and 2.93-fold higher than that with In/Del and Del/Del genotype, respectively. Bioinformatics predictions showed that the insertion allele disrupts a binding site for

miRNA-151-5p and, thus, causes *SGSM3* to upregulate. Moreover, an association between HCC susceptibility and a 12-bp In/Del polymorphism rs6147150 (within the 3′-UTR of *ERBB4*) was found: carriers of the Del allele had a 1.59-fold increased risk for HCC. Bioinformatics analysis suggests that rs6147150 lies within a predicted binding site for let-7c, and it could be hypothesized that let-7c tightly binds *ERBB4* transcripts containing the 12-bp deletion allele, negatively regulating *ERBB4* expression.[114] These findings suggested that common genetic polymorphisms in *ERBB4* may affect HCC risk, at least in part via let7c-mediated regulation, which may be involved in the pathogenesis of HCC.

Bladder cancer

miRSNPs as biomarkers of prognosis or response to therapy in bladder cancer

Bladder cancer (BlC) is the fourth most common cancer among men in the United States and accounts for 3% of the cancer deaths.[72] The fact that BlC incidence is three to four times higher in men suggests that risk may be modified by hormone levels.[115] Among BlC patients of Caucasian origins undergoing radiotherapy, carriers of the *RAD51* rs7180135 minor allele showed longer OS.[116] miR-197 was predicted to bind more tightly to the G allele, likely resulting in a reduction of *RAD51* expression. RAD51 is involved in DSB homologous recombination repair; thus, in carcinoma, alteration of RAD51 expression could potentiate radiosensitization. Low *RAD51* mRNA expression has previously been associated with lower local recurrence and improved survival after adjuvant chemotherapy and radiotherapy treatments in BC.[116] The results suggest that the *RAD51* rs7180135 genotype, through the alteration of miR-197 binding site, may affect radiosensitivity and radiotherapy outcome in BlC. If successfully validated, this might be used clinically as a predictive marker of radiotherapy outcome. In the same study, rs8679 in 3′-UTRs *PARP1* was associated with risk for BlC. The variant was predicted to decrease the strength of binding with miR-145, possibly increasing *PARP1* expression.

miRSNPs as biomarkers of susceptibility to bladder cancer

In agreement with the hypothesis that a key role in modulating this risk could be played by hormone level, the A allele of miRSNP rs1417608 within the 3′-UTR of the hormone regulation gene 3-beta-hydroxysteroid dehydrogenase type 2 (*HSD3B2*) was associated with nearly a 2-fold increased risk for BlC in patients of Caucasian origins.[117] *HSD3B2* encodes a NAD+, dependent microsomal enzyme that catalyzes biosynthesis of dihydrotestosterone and dihydroprogesterone. Interestingly, this association was confirmed in another study (the Texas BlC Study), in which the SNP rs1341015, in linkage disequilibrium with rs1417608, was evaluated. Moreover, two other SNPs in strong linkage disequilibrium with rs1417608 (rs1819698 and rs1538989) were associated with significantly increased risks for prostate cancer in people of European descent.[118] The variant allele of rs1819698 was also computationally predicted to disrupt a miRNA binding site for miR-3658. In summary, all these observations support the notion that hormone synthesis deregulation, through *HSD3B2*, may be important in the etiology of BlC.

In recent years, the *HOX* gene family has also been associated with human diseases, especially cancers. For instance, *HOXB5* has been reported to be related to human diseases, including acute myeloid leukemia,[119] EOC,[120] and urological carcinomas. In BlC, the frequency of the G allele of *HOXB5* rs9299, a miRSNP falling within miRNA-7 binding site, was higher among Caucasian patients with BlC compared with healthy controls, and it was found to be correlated with the risk for high grade and high stage.[121] The expression of the *HOXB5* mRNA with the G allele was significantly higher than the mRNA with the A allele in both cancer tissues and cell lines. In summary, the results suggest this miRSNP may affect *HOXB5* expression, which in turn may affect bladder tumorigenesis.

In another study, the association between *EGFR* miRSNP rs884225 T>C and BlC risk was examined among Chinese patients.[122] Results showed that the CC genotype was associated with a significantly increased risk compared with TT + TC genotypes. In addition, luciferase reporter gene assay confirmed that T-to-C substitution could increase the *EGFR* expression. On the basis of the bioinformatics analysis, rs884225 polymorphism lies within a predicted binding site for miR-214; however, in vitro experiments could not confirm such a prediction, and thus it is unclear whether rs884225 is a functional polymorphism or is a proxy for other variations nearby.[122] In any case, much evidence shows that overexpression of EGFR plays an important role in regulating carcinogenesis by mediating cell mortality, apoptosis, tumor invasion, and metastasis. Thus, *EGFR* differential regulation at the 3′-UTR level might constitute a susceptibility factor that warrants being explored further.

Head and neck cancer

miRSNPs as biomarkers of prognosis in head and neck cancer

Squamous cell carcinoma of the head and neck (HNSCC), which includes cancers of the oral cavity, pharynx, and

larynx, is one of the six most common cancers worldwide, accounting for 35% of all cancers in the United States.[123] Most early-stage patients can be cured with surgery, radiotherapy, and chemotherapy. However, second primary tumors (SPTs) and local-regional recurrence negatively affect their long-term prognosis. It has been reported that 15%–25% of HNSCC patients will develop SPT/recurrence during the first 5 years after initial diagnosis.[124] Thus, the development of clinical biomarkers predicting SPT/recurrence could be very important for the surveillance and targeted chemoprevention of high-risk patients. Genetic variations in miRNA-binding sites are reported to be associated with the risk for HNSCC and with SPT/recurrence in Caucasian patients with early stages.[125] In particular, the rare homozygous genotype of the miRSNP rs3747238 within *SMC1B* was associated with an increased SPT/recurrence risk and reduced OS. The variant allele is predicted to create de novo binding sites for miR-609 and miR-124a, resulting in lower *SMC1B* expression. *SMC1B* is involved in chromosome structure maintenance during meiosis and mitosis, and its reduced expression could be related to potentially increased genome instability and greater cancer progression risk. For the same type of cancer, the genotype *KRAS* rs61764370, the miRSNP affecting a let-7 miRNA-binding site, was evaluated. Although no significant associations with the risk were described, the variant G allele was associated with a significantly reduced OS compared with a common allele.[126] This observation suggested that the miRSNP could be associated with tumor progression, rather than initiation. Moreover, in the presence of the variant allele, *KRAS* expression was increased. Amplified *KRAS* promotes the growth in HNSCC, and its immune-histochemical positivity for K-ras protein was associated with late stages and increased tumor size.

miRSNPs as biomarkers of susceptibility in head and neck cancer

In HNSCC, the C allele of rs8126 within *TNFAIP2* was associated with increased risks among Caucasians.[127] This allele was also associated with higher mRNA expression levels of *TNFAIP2* compared with the T allele in blood lymphocytes of 64 cases. rs8126 C allele was predicted to disrupt a miR-184 binding site, providing a possible explanation for the altered *TNFAIP2* expression level.[127] *TNFAIP2* mRNA is detectable in many human tissues and most hematopoietic cell lines.[128] Although the function of the encoded protein is unknown, it was involved in apoptosis, and the gene was found induced by the tumor necrosis factor α in human endothelial cells.[129] Moreover, *TNFAIP2* was found to be highly expressed in nasopharyngeal carcinoma tumor cells when compared with adjacent normal tissues, and the increased expression of *TNFAIP2* was significantly associated with shorter OS in nasopharyngeal carcinoma patients without distant metastasis.[127]

Other tumors

Gastric and esophageal cancer

Gastric cancer (GC) is one of the most common malignancies worldwide, accounting for 8% of total cancer cases and 10% of total cancer deaths, although both its incidence and mortality have been declining in the latest decade.[72] Not many studies have investigated the role of miRSNPs in GC. However, interestingly, the genotype CC of rs8126 within *TNFAIP2*, already associated with increased risk of HNSCC, was also associated with increased risks for GC among Caucasians.[130] Studies carried out on other miRSNPs revealed that the TT genotype of *KRAS* rs712, another miRSNP falling within a let-7 binding site within the 3′-UTR, was associated with an increased risk among Asian patients.[131] Moreover, increased risks for GC were also found, among Asian patients, for the variant allele of rs12537, located within the 3′-UTR of *MTMR3*.[132] This allele was also associated with poor OS, and GC tissues from carriers of the T allele showed lower *MTMR3* mRNA expression levels than CC homozygotes. Luciferase assay revealed that miR-181a directly targeted *MTMR3*, and its suppressive effect was enhanced when the C allele was substituted by its T-variant. MTMR3, myotubularin-related protein 3, is ubiquitously expressed and has been demonstrated to regulate autophagy.[133] However, little is known about its role on cancer.

Esophageal cancer (ESCC) is one of the most aggressive cancers, and its incidence worldwide has significantly increased in recent years.[72] After complete surgical removal of the primary tumor, the 5-year survival rate is 50%–80% for stage I, 10%–40% for stage II, and 10%–15% for stage III disease. Patients with distant metastases (stage IV) who are treated with palliative chemotherapy have a median survival of less than 1 year.[134] In an association study of Chinese patients, authors found that the CC genotype rs6573 within the 3′-UTR of *RAP1A* was associated with an increased risk compared with CA or AA genotypes.[135] In addition, the C allele was more frequently represented among patients with stage III or IV disease. The change A>C in the binding site for miR-196a, was associated with a high constitutive expression of *RAP1A*. *RAP1A* is involved in a wide range of biological processes, including cell proliferation, differentiation, and cell motility and an abnormal *RAP1A* activation contributes

to the tumorigenic processes. Expression of *RAP1* at high levels can morphologically transform Swiss 3T3 fibroblasts and form tumors when injected into nude mice.[136]

rs4245739, a SNP within *MDM4*, was another miRSNP associated with the risk of developing ESCC.[137] C allele had a significantly decreased risk compared with A allele in a Chinese population. In the genotype-phenotype correlation analysis of 29 human ESCC and paired esophagus tissue samples, AC + CC genotypes were associated with a statistically significant decrease of *MDM4* mRNA expression. The change C to A was predicted to disrupt the interaction between miR-191 and mRNA, thereby increasing *MDM4* expression in cancer cells.

Prostate cancer

Prostate cancer (PC) remains the second leading cause of cancer-related death in men in the United States.[138] Androgen deprivation therapy is the most commonly used first-line treatment for advanced PC. Despite a generalized positive response, within 2–3 years, the disease progresses to a castration-resistant status in 20% of patients, and the life expectancy becomes approximately 16–18 months. A variety of prediction parameters, such as tumor stage, Gleason score, and prostate-specific antigen kinetics, have been used in clinical practice to define the presentation of PC and adapt the treatment strategy.[139] However, their prognostic capabilities are still limited and might be improved by the incorporation of other markers. In the following work, the authors explored the role of miRSNPs in *ALOX15*, *RAF1*, *IL-16*, and *IL-18* (genes of biologic relevance to PC) and were evaluated in a longitudinal screening of high-risk people.[140] Analysis showed a statistically borderline association between *IL-16* rs1131445 TT genotype and earlier age at diagnosis only among African-Americans, not Caucasians. Another study, carried out evaluating miRSNPs in five integrin genes, failed to show any association with PC prognosis; however, GC carriers of rs11902171 within 3′-UTR of *ITGAv* were associated with decreased risk among Chinese patients.[141] Integrins have been implicated in the genesis and development of many tumors and act as indispensable partners of oncogenes in the transformation of normal cells.[142] The transversion G to C in rs11902171 may alter targets for the mir-382, mir-30a-3p, and mir-30e-3p. Finally, the T allele of rs1434536 within the 3′-UTR of *BMPR1B* was found associated with the risk for localized PC in Asian.[143] The association was stronger among patients older than 70 years, suggesting that rs1434536 was a more appropriate predictor for PC in older people. The C allele gave a reduced luciferase activity relative to the T allele, likely because the C-to-T substitution causes a reduced binding of miR-125b to *BMPR1B* mRNA. This gene encodes a member of the bone morphogenetic protein receptor family of transmembrane serine/threonine kinases, and its regulation could affect PC cells homing and growth at distant metastatic sites.[144]

Leukemia

Chronic lymphocytic leukemia is a B-cell malignancy and one of the most common non-Hodgkin lymphomas. About 69,740 cases of non-Hodgkin lymphoma are expected to be diagnosed in the United States in 2013.[145] In the present study on 745 chronic lymphocytic leukemia Caucasian cases and 1,521 controls, the strongest association with chronic lymphocytic leukemia risk was observed with a common SNP located within the 3′-UTR of *IRF8* (rs1044873, log additive odds ratio =0.7; $P=1.81\times10^{-6}$).[146] rs1044873 is located in the 3′-UTR of the *IRF8* gene and therefore is potentially located within a target region for miRNA. However, bioinformatics analysis did not support this. According to PolymiRTS, rs1044873 is not within any validated miRNA target. In addition, in acute myeloid leukemia, a polymorphic nucleotide T deletion is present in the 3′-UTR of *NPM1*, was associated with adverse outcomes, and could independently predict shortened survival in patients with de novo acute myeloid leukemia.[147] In particular, patients carrying a homozygous delT genotype had higher relapse rates (59% versus 31%; $P=0.051$) and significantly shortened OS (median, 9 months versus 12 months; $P=0.016$) and relapse-free survival (median, 5 months versus 12 months; $P=0.007$) than patients carrying a non homozygous genotype. The nucleotide T deletion created an illegitimate binding NPM1 for miR-337-5p, which was widely expressed in different acute myeloid leukemia subtypes and inhibited NPM1 expression.

Discussion

MiRSNPs represent a promising class of genetic variations worth being deeply investigated as markers of individual susceptibility to complex diseases, to prognosis, and in clinically relevant decision-making. Although it should be acknowledged that most of the illustrated studies have not been replicated in independent laboratories yet, it also should be noticed that some of the findings reported here were consistent among different tumor types and had some experimental evidence based on in vitro assays. In particular, several variants within the 3′-UTR of the protooncogene *KRAS* were of interest. It has been shown that there are ten different LCSs within the 3′-UTR of *KRAS* mRNA to induce KRAS downregulation. A miRSNP within the sixth site (also termed KRAS-LCS6) has been identified and demonstrated to affect

KRAS expression. The rs61764370 determines the change of the ancestral T allele to a G allele, disrupts the let-7 miRNA binding site, and causes an increased KRAS expression.[45] This natural change might represent a mechanism of KRAS activation. This variant is relatively uncommon; in fact, is almost absent in Native Americans and East Asians, is very rare in Africans, and has a minor allele frequency of about 7% in the European populations.[148]

This variant allele is associated with increased risk for NSCLC,[46] BC, and EOC. Other groups had also reported that the *KRAS* variants were associated with decreased OS in HNSCC and increased OS in NSCLC and, in addition, seem to modulate therapeutic responses in CRC patients.[51,54] It is also worth noting that rs712 and rs10771184, miRSNPs falling within other LCSs of KRAS mRNA, were associated with increased risks for GC and EOC and OS. Thus, overall, there are many clues to the role of polymorphisms within KRAS 3′-UTR in the biology of human cancer. A miRSNP highly promising for its role in human tumors is rs16917496. This miRSNP, within the 3′-UTR of *SET8*, is associated with longer OS in patients with NSCLC[27] and HCC[107] and with the age at diagnosis of BC.[76] The rational linking of the regulation of *SET8* to cancer could reside in the multiple functions of the encoded protein that is involved in the advancement of cell cycle through the S-phase[149] and in the transcriptional regulation,[150] genome stability,[151] apoptosis, and cell-cycle arrest.[152] Most of these actions can be explained by SET8 having a well-defined function in the p53 pathway by mono-methylating p53 at lysine 382 and suppressing the p53-mediated transcription activation of target genes.[153]

Finally, it is worth mentioning here miRSNP rs4245739 within *MDM4*. The A allele was associated with the risk for BC[87] and ESCC,[132] as well as an unfavorable prognosis in EOC.[103] Mdm4 was originally discovered as a p53-interacting protein through screening of a mouse embryo cDNA expression library.[154] The p53-binding domain of Mdm4 interacts with the transactivation domain of p53 to represses its transcriptional activity. High levels of Mdm4 are also found in a variety of human cancers: HNSCC, retinoblastoma, melanoma, and BC. Mdm4 upregulation in malignancies is mostly ascribed to *MDM4* gene amplification.[155] However, the C-to-A substitution also could constitute an alternative mechanism of activation. In fact, it could disrupt the interaction between miR-191 and mRNA, thereby increasing *MDM4* expression. In summary, a new emerging field of study supports the notion that gene regulation through miRNAs could explain part of the phenotypic variability observed in humans. More studies are needed to better characterize the composite spectrum of genetic determinants for a futuristic use of markers in risk prediction and clinical management of diseases, moving toward personalized medicine.

Disclosure

The authors report no conflicts of interest in this work.

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