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REVIEW

The microRNA signatures: aberrantly expressed microRNAs in head and neck squamous cell carcinoma

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microRNAs (miRNAs) are responsible for fine tuning the normal expression of RNA networks in human cells. Accumulating studies have demonstrated that abnormally expressed miRNAs have pivotal roles in the development of head and neck squamous cell carcinoma (HNSCC). Specifically, expression signatures of miRNAs in HNSCC have revealed dysregulated production of miRNAs and the resultant abnormal production of mRNAs and proteins. In this review, we discuss current findings regarding aberrantly expressed miRNAs and their contribution to HNSCC molecular pathogenesis.
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HEAD AND NECK SQUAMOUS CELL CARCINOMA

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer worldwide. It arises in the oral cavity, nasopharynx, oropharynx, hypopharynx and larynx.[1,2] HNSCC is associated with smoking tobacco, consumption of strong alcoholic beverages and infection by human papillomavirus.[2,3] Even with current advances in multimodality therapy, the overall survival rate for HNSCC patients is poor and the mortality rates for this disease have not improved in the past 40 years.[4,5] Local recurrence and distant metastasis after advanced treatment appear to be major contributing factors in the poor survival rate of HNSCC patients.[6]

HNSCC is a heterogeneous disease with accumulated genetic alterations, such as chromosomal abnormalities, inactivation of tumor suppressors and activation of oncogenes.[7] For example, there is frequent silencing of tumor suppressor genes (for example, *p16ink4a, p14ARF* and *TP53*) and activation of oncogenic genes (for example, *CCND1*, *RB1*, *PI3K* and *EGFR*).[8] Dysregulated gene expression networks might contribute to malignant transformation and the invasive malignancy of HNSCC.

Recent advances in whole-exome sequencing have provided new insights into the molecular pathogenesis of HNSCC. These data have shown that multiple antitumor pathways (*TP53, RB1/INK4/ARF* and *NOTCH*) participate in tumor initiation and aggressiveness.[9] The Cancer Genome Atlas (TCGA) study showed the presence of chromosomal amplifications in 3q26-3q28, a region involving HNSCC-promoting genes *TP53* and *SOX2* and the oncogene *PIK3CA*.[10,11] Moreover, in smoking-related HNSCCs, studies have demonstrated loss-of-function of both *TP53* and *CDKN2A* as well as frequent copy-number amplification of 3q26-3q28 and 11q13-11q22.[12] Whole-exome sequencing has demonstrated that mutations in the PI3K pathway were frequently involved in HNSCC.[13]

IDENTIFICATION OF ABERRANTLY EXPRESSED MICRORNAS BASED ON EXPRESSION SIGNATURES OF HNSCC

In normal cells, miRNAs tightly regulate both protein-coding and protein-non-coding genes.[14] A single miRNA can control thousands of targeted RNAs, and >60% of protein-coding genes may be influenced by miRNAs.[15] Dysregulated miRNA expression disrupts the normal RNA networks present in healthy cells, leading to oncogenic development.[15,16] Aberrantly expressed miRNAs can be divided into two classes depending on their expression status.[17] Overexpressed miRNAs can act as oncogenes if they repress tumor suppressor genes. In contrast, miRNAs with antitumor properties can enhance the development of cancer cells when they are downregulated (Figure 1). Strategies to identify abnormal expression of miRNAs and miRNA-mediated cancer pathways offer new directions in cancer research.

Cytogenetic alterations constitute early events in the progression of cancer development. For example, changes in chromosomal structure can alter the expression of miRNAs. Chromosomal regions that are subject to amplification or loss may result in miRNAs with oncogenic behavior or loss of tumor-suppressive properties.[18,19] Recent evidence suggests that epigenetic alterations (heritable changes in gene expression without DNA sequence alteration) may lead to aberrant expression of miRNAs in HNSCC cells.[20–22] It is well known that DNA hypermethylation of CpG islands leads to the inactivation of tumor-suppressive miRNA in cancer cells.[19,23] In oral cancer cells, *miR-34b*, *miR-137*, *miR-193a* and *miR-203* function as tumor

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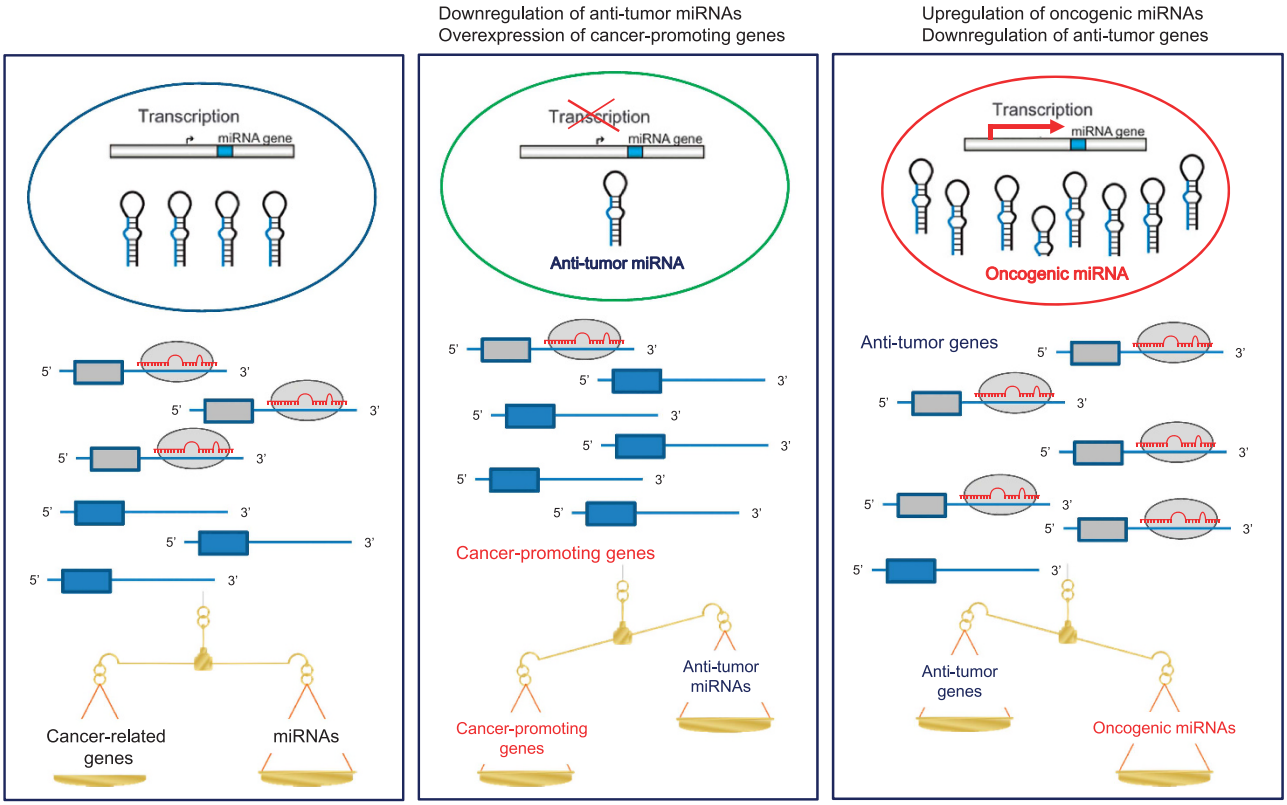

Figure 1 Oncogenic microRNA (miRNA) and tumor-suppressive miRNA in cancer cells. miRNAs can be separated into two main classes: those that are oncogenic and those that are tumor suppressive. Overexpressed miRNAs can act as oncogenes by repressing tumor suppressor genes, whereas underexpressed miRNA may normally function as antitumor miRNA by negatively regulating cancer-promoting genes.

suppressors and these miRNAs are located on CpG islands and silenced through aberrant DNA methylation.[24]

Activation of DNA methyltransferases modulates the expression of both protein-coding and non-coding genes. Three DNA methyltransferases are particularly important: DNMT3A, DNMT3B and DNMT1.[25] These methyltransferases are regulated by specific miRNAs, leading to demethylation of specific genomic sequences.[26] Thus, downregulation of DNA methyltransferases permits expression of protein-coding and non-coding genes. Other important epigenetic gene controls are exerted by histone modification, such as histone acetylation (associated with active gene transcription) and methylation of histone H3 lysine 9 (inactivation of gene expression).[21,22] Evidence indicates that DNA methylation and histone modification cooperatively regulate transcription of the human genome[21,22,26] and that epigenetic modifications affect cancer pathogenesis. Thus, it is important to elucidate the miRNA networks that control the expression of protein-coding and non-coding genes in cancer cells.

Advanced molecular technologies can identify abnormally expressed miRNAs in various types of cancer cells. To seek out differentially expressed miRNAs in HNSCC cells, we used HNSCC clinical specimens to establish microarray-based, PCR-based and deep sequencing-based miRNA expression signatures.[27–30] Moreover, we have demonstrated the roles of miRNAs in human SCC pathogenesis.[31,32] In this review, we highlight aberrantly expressed miRNAs in HNSCC based on 11 miRNA expression signatures from previously published studies.[27–30,33–39] Differentially expressed miRNAs identified from signatures are summarized in Table 1. The signatures exhibit considerable variability in the differential expression of miRNAs. The variety of aberrantly expressed miRNAs may depend on technical aspects, patient populations and analysis platforms for miRNA signatures. However, there are certain miRNAs that frequently observed to be up- or downregulated among the 11 signatures. These data suggest that these miRNAs may contribute substantially to HNSCC pathogenesis (Tables 2 and 3).

ABERRANTLY EXPRESSED MIRNAS IN HUMAN CHROMOSOMES

High-resolution arrays for comparative genomic hybridization have used to document HNSCC features. Combined genome-wide gene expression studies have revealed candidate tumor suppressors or oncogenes that contribute to HNSCC initiation, progression and metastasis.[40] It has hypothesized that novel cancer-related genes or miRNAs might be present in chromosomal regions that have deleted or amplified. To investigate the correlation between chromosomal alterations and miRNA expression in HNSCC cells, we have mapped dysregulated miRNAs in human chromosomes, merging the data from current array-based comparative genomic hybridization analysis (Figure 2).[41–45]

Six miRNAs (*miR-127*, *miR-411*, *miR-376c*, *miR-376a*, *miR-410* and *miR-487b*) are located within chromosomal region 14q32 (Figure 2). In that region, large miRNA clusters are present and 42 intergenic miRNAs are located within 10 kb of one another.[46] Past studies have indicated that this chromosomal region has pivotal roles in embryonic development.[46,47] Several reports showed that tumor-suppressive miRNAs were clustered there in several types of cancers. Among them, *miR-410* inhibited cancer cell proliferation and invasion by

Table 1 microRNA expression profiles in HNSCC

Author	*Year*	*Journal*	*Location*	*Sample*	*Methods*	*No. of tissues (tumor/normal)*	*Downregulated microRNA*	*Upregulated microRNA*
Kikkawa	2010	*Br. J. Cancer*	Hypopharyngeal	Clinical tissue	TaqMan Low Density Array Human MicroRNA Panel v1.0 for 365 miRNAs	20 (10/10)	*miR-1, miR-375, miR-139-5p, miR-504, miR-125b, miR-199b, miR-100, miR-497, let-7c, miR-30a*, miR-218, miR-10b, miR-126*, miR-378, miR-328, miR-204, miR-143, miR-126, miR-99a, miR-195, miR-489, miR-203, miR-140-5p, miR-29a, miR-26a, miR-214,miR-30a, miR-26b, miR-30e*, miR-30b, let-7b*	*miR-517c, miR-196a, miR-7, miR-196b, miR-650, miR-18a, miR-452, miR-183,miR-432, miR-301a, miR-21*
Hui	2010	*Clin. Cancer Res.*	Larynx, oro-pharynx and hypopharynx	Clinical tissue	TaqMan MicroRNA Assays human Panel for 322 microRNAs	55 (51/4)	*let-7f,miR-142-3p, miR-324-5p, miR-368, miR-370, miR-373*, miR-422b, miR-424, miR-9, miR-16–2, miR-140-5p, miR-miR-1, miR-133a*	*miR-423, miR-93, miR-106b, miR-16, miR-20a, miR-155, miR-193a, miR-25, miR-92,let-7i, miR-17-5p, miR-19b, miR-223, miR-27a,miR-142-3p, miR-210, miR-106a, miR-15a, miR-21, miR-29b, miR-130b, miR-205, miR-422b*
Liu	2010	*Cancer Res.*	Head and neck	Clinical tissue	TaqMan Low Density Array Human MicroRNA Panel for 154 miRNAs	20 (10/10)	*miR-100, miR-328, miR-99a, miR-124, miR-149, miR-139, miR-124a, miR-204, miR-211*	*miR-31, miR-34c, miR-187, miR-135b, miR-372, miR-34b, miR-21, miR-371, miR-216, miR-301, miR-10a, miR-155, miR-130b, miR-223, miR-373, miR-96, miR-224, miR-147, miR-128b, miR-104, miR-183, miR-182*
Nohata	2011	*Br. J. Cancer*	Maxillary sinus	Clinical tissue	TaqMan Low Density Array Human MicroRNA Panel v2.0 for 667 miRNAs	10 (5/5)	*miR-874, miR-133a, miR-375, miR-204, miR-1, miR-139-5p, miR-145, miR-143,miR-486-3p, miR-146a, miR-410, miR-126, miR-539,miR-134, miR-218, miR-146b-5p, miR-140-3p, miR-30a-3p,miR-191, miR-186, miR-148a, miR-30e-3p, miR-29c*	data not shown
Lajer	2011	*Br. J. Cancer*	Oral cavity	Clinical tissue	Affymetrix miRNA array chips for 847 miRNAs	47 (30/17)	*miR-375, miR-1224-5p,miR-617, miR-99a, miR-125b, miR-378, miR-27b,miR-125b-2**	*miR-31, miR-21, miR-223, miR-503, miR-187, miR-1246, miR-146b-5p, miR-146a, miR--155, miR-424*, miR-181a, miR--181b, miR-27a*,miR-132, miR-106b*, miR-345, miR-21**
Severino	2013	*BMC Cancer*	Oral cavity	Clinical tissue	IIlmina miRNA arrays version 1.0	30 (15/15)	*miR-1,miR-30a-3p,miR-139, miR-133a,miR-486,miR-135a, miR-204,miR-206,miR-411, miR-499,miR-10b,miR-99a, miR-299-5p,miR-379,miR-100, miR-30a-5p,miR-95,miR-378, miR-218,miR-368,miR-363, miR-128a,miR-655,miR-376a, miR-628,miR-487b,miR-410, miR-140,miR-801,miR-376a*, miR-154,miR-432*	*miR-196a,miR-33,miR-19a, miR-33b,miR-142-5p,miR-503, miR-31,miR-7,miR-19b, miR-135b,miR-632,miR-504, miR-187, miR-339,miR-302d, miR-34b, miR-34c,miR-455,miR--9,miR-296,miR-301,miR-130b, miR-196b,miR-200a,miR-210, miR-17-3p,miR-302b*,miR-224, miR-183,miR-138,miR-188, miR-92b, miR-182,miR-144, miR-146b, miR-182*,miR-149, miR-141,miR-610*
Fukumoto	2014	*Br. J. Cancer*	Hypopharyngeal	Clinical tissue	MiRCURY LNA microRNA Array	22 (11/11)	*miR-1,miR-133a,miR-133b, miR-29c-3p,miR-451a,miR-206, miR-378a-3p,miR-29a-3p, miR-378d,miR-125b-5p, miR-101-3p,miR-1184,miR-4328,*	*miR-21-5p,miR-4732-5p, miR-4776-3p*

Table 1 (Continued)

Author	Year	Journal	Location	Sample	Methods	No. of tissues (tumor/normal)	Downregulated microRNA	Upregulated microRNA
							miR-126-3p,miR-145-5p,let-7c, miR-4324,miR-203a,miR-4462, miR-29b-3p,miR-659-5p, miR-5000-3p,miR-4638-5p,	
Fukumoto	2014	*Br. J. Cancer*	Oral cavity	Clinical tissue	TaqMan Low Density Array Human MicroRNA Panel v2.0 for 667 miRNAs	10 (5/5)	*miR-126-5p,miR-145-5p, miR-145-3p,miR-26b-5p, miR-26a-5p,miR-204,miR-29c, miR-195,miR-30c,miR-10b, miR-656,miR-30e-3p,miR-140-5p,miR-23b,miR-10b,miR-126-3p, miR-143,miR-30d,miR-139-5p, miR-19b-1-5p,miR-598, miR-885-5p,miR-376c,miR-487b, miR-101,miR-886-5p, miR-140-3p,miR-30e, miR-125b,miR-378a-5p, miR-320,miR-136-3p, miR-26a-1-3p,miR-127-3p, miR-411,miR-30a-3p,miR-29c-5p, miR-376a,miR-26b-3p, miR-770-5p,miR-433,miR-375*	data not shown
Zhang	2014	*Genomics*	Larynx	Clinical tissue	Illumina platform for analyzing transcriptomes employing a 100-bp paired end library	12 (10/2)	*miR-34c*	*miR-1301, miR-15b, miR-182, miR-183, miR-184, miR-224, miR-450a-1, miR-450a-2, miR-9-3, miR-96*
Victoria	2015	*Oncotarget*	Head and neck	Clinical tissue	Illumina HiSeq 2000 instrument to generate 50-base reads	14 (7/7)	*miR-191-5p, miR-26a-5p, miR-181-5p, miR-150-5p, let-7f-5p, miR-93-5p, let-7a-5p, miR-30c-5p, miR-28-5p, miR-26b-5p, miR-30b-5p, miR--122-5p, miR-98-5p, miR-183-5p, miR-224-5p*	*miR-205-5p, miR-145-5p, miR-27b-5p, miR-103a-3p, miR-107, miR-320a, miR-320b, miR-486-5p, miR-100-5p, miR-32-5p, miR-215-5p, miR-148-5p, miR-99a-5p*
Wang	2016	*J. Exp. Clin. Cancer Res.*	Nasopharyngeal	Clinical tissue	Illumina high-throughput next-generation sequencing	20 (12/8)	*miR-92b-3p, miR-375, miR-34c-5p, miR-449c-5p*	*miR-27a-5p, miR-193b-3p, miR-92a-3p, miR-205-5p*

targeting Wnt-7β, an activator of the Wnt-β-catenin pathway.[48]

Some miRNAs are grouped closely together within the human genome, that is, at distances <5 Kb pairs. These so-called 'clustered miRNAs' have studied to determine their functional role in human cancers.[49] Several such clusters are downregulated in several signatures of HNSCC, including the *miR-143/miR-145* cluster (5q32), the *miR--30*/miR-30c-2* cluster (6q13), the *miR-1-2/miR-133a-1* cluster (18q11.2), the *miR-1-1/miR-133a-2* cluster (20q13.3) and the *miR--99a/let-7c* cluster (21q21) (Figure 2). The expression of clustered miRNAs has regulated by the same transcriptional mechanisms. In some miRNA clusters, all of the members of the clustered miRNAs control identical target genes.[50]

There is a consensus that certain clustered miRNAs (*miR-145* and *miR-143)* are frequently reduced in a broad range of human cancers, and that these miRNAs possess tumor-suppressive activities.[51–54] Several reports showed that *miR-145* and *miR-143* targeted the same genes (*GOLM1*, *HK2* and *FSCN1*).[51–53] Tumor suppressor *TP53* transcriptionally regulates the antitumor *miR-145* by direct interacting with the *miR-145* promoter region.[55–58] Interestingly, the *MYC* oncogene is directly repressed by *miR-145*.[55,59,60] Research indicates that antitumor *miR-145* participates in *TP53* regulatory pathways, and contributes to the direct suppressor of *MYC* oncogenes.

DOWNREGULATED MIRNAS ACT AS TUMOR SUPPRESSORS IN HNSCC

We and other researchers have used gain-of-function studies to investigate the functional roles of miRNAs as tumor suppressors.[29–32] Tumor-suppressive miRNAs and their target genes are summarized in Table 4.

We have identified tumor-suppressive miRNAs in HNSCC based on expression signatures.[27–30] From those data, *miR-375* was the most frequently downregulated miRNA in HNSCC cells. Restoration of *miR-375* markedly suppressed cancer cell aggressiveness, suggesting

Table 2 Downregulated microRNAs in HNSCC

				Overlapping transcripts		
Number	*Hsa-mature sequence*	*Stem-loop sequence*	*Locus*	*Sense*	*Antisense*	*Clustered microRNA (within 10Kbp)*
6	hsa-miR-375	hsa-miR-375	2q35			
5	hsa-miR-99a	hsa-miR-99a	21q21.1	*MIR99A, LINC00478*		*hsa-miR-let-7c*
5	hsa-miR-125b	hsa-miR-125b-1	11q24.1		*MIR100HG*	
		hsa-miR-125b-2	24q21.1	*LINC00478*		
5	hsa-miR-139-5p	hsa-miR-139	11q13.4		*PDE2A*	
5	hsa-miR-204	hsa-miR-204	9q21.12		*TRPM3*	
4	hsa-miR-1	hsa-miR-1-1	20q13.33	*C20orf166*		
		hsa-miR-1-2	18q11.2	*MIR133A1HG*	*MIB1*	*hsa-miR-133a-1*
4	hsa-miR-26a-5p	hsa-miR-26a-1	3p22.2	*CTDSPL*		
4	hsa-miR-30a-3p	hsa-miR-30a	6q13			
4	hsa-miR-100	hsa-miR-100	11q24.1		*MIR100HG*	*hsa-let-7a-2*
4	hsa-miR-126-3p	hsa-miR-126	9q34.3	*EGFL7*		
4	hsa-miR-140-3p	hsa-miR-140	16q22.1	*WWP2*		
4	hsa-miR-143	hsa-miR-143	5q32	*MIR143HG*		*hsa-miR-145*
3	hsa-let-7c	hsa-let-7c	21q21.1	*MIR99A, LINC00478*		*hsa-miR-99a*
3	hsa-miR-10b	hsa-miR-10b	2q31.1			
3	hsa-miR-26b-5p	hsa-miR-26b	2q35	*CTDSP1*		
3	hsa-miR-30c-5p	hsa-miR-30c-1	1p34.2	*NFYC*		*hsa-miR-30e*
		hsa-miR-30c-2	6q13			
3	hsa-miR-133a	hsa-miR-133a-1	18q11.2	*MIB1*	*MIR133A1HG*	*hsa-miR-1-2*
		hsa-miR-133a-2	20q13.33	*C20orf166*		
3	hsa-miR-145-5p	hsa-miR-145	5q32	*MIR143HG*		*hsa-miR-143*
3	hsa-miR-218	hsa-miR-218-1	4p15.31	*SLIT2*		
		hsa-miR-218-2	5q34		*SLIT3*	
		hsa-miR-26a-2	12q14.1		*CTDSP2*	
3	hsa-miR-378a-3p	hsa-miR-378a	5q32	*PPARGC1B*		
2	hsa-miR-101-3p	hsa-miR–101-1	1p31.3			*hsa-miR-3671*
		hsa-miR–101-2	9p24.1	*RCL1*		
2	hsa-miR-124	hsa-miR-124-1	8p23.1			
		hsa-miR-124-2	8q12.3			
		hsa-miR-124-3	20q13.33			
2	hsa-miR-126-5p	hsa-miR-126	9q34.3	*EGFL7*		
2	hsa-miR-127-3p	hsa-miR-127	14q32.2		*RTL1*	*hsa-miR-337, hsa-miR-665, hsa-miR-431, hsa-miR-433,hsa-miR-432, hsa-miR-136*
2	hsa-miR-140-5p	hsa-miR-140	16q22.1	*WWP2*		
2	hsa-miR-195	hsa-miR-195	17p13.1		*MIR497HG*	*hsa-miR-497*
2	hsa-miR-199b	hsa-miR-199b	9q34.11	*DNM1*		*hsa-miR-3154*
2	hsa-miR-206	hsa-miR-206	6p12.2			*hsa-miR-133b*
2	hsa-miR-29a-3p	hsa-miR-29a	7q32.3		*LOC646329*	*hsa-miR-29b-1*
2	hsa-miR-29c-3p	hsa-miR-29c	1q32.2			*hsa-miR-29b-2*
2	hsa-miR-30a-5p	hsa-miR-30a	6q13			
2	hsa-miR-30e-3p	hsa-miR-30e	1p34.2	*NFYC*		*hsa-miR-30c-1*
2	hsa-miR-328	hsa-miR-328	16q22.1	*ELMO3*		
2	hsa-miR-376a	hsa-miR-376a-1	14q32.31			*hsa-miR-543, hsa-miR-495, hsa-miR-376c, hsa-miR-376a-2, hsa-miR-654, hsa-miR-376b, hsa-miR-300, hsa-miR-1185-1, hsa-miR-1185-2. hsa-miR-381, hsa-miR-487b, hsa-miR-539, hsa-miR-889, hsa-miR-544a, hsa-miR-655*
		hsa-miR-376a-2	14q32.31			*hsa-miR-543, hsa-miR-495, hsa-miR-376c, hsa-miR-376a-1, hsa-miR-654, hsa-miR-376b, hsa-miR-300, hsa-miR-1185-1, hsa-miR-1185-2. hsa-miR-381, hsa-miR-487b, hsa-miR-539, hsa-miR-889, hsa-miR-544a, hsa-miR-655*
2	hsa-miR-410	hsa-miR-410	14q32.31			

Table 2 (Continued)

				Overlapping transcripts		
Number	*Hsa-mature sequence*	*Stem-loop sequence*	*Locus*	*Sense*	*Antisense*	*Clustered microRNA (within 10Kbp)*
2	hsa-miR-411	hsa-miR-411	14q32.31			*hsa-miR-323b, hsa-miR-154, hsa-miR-496, hsa-miR-377, hsa-miR-541, hsa-miR-409, hsa-miR-412, hsa-miR-369, hsa-miR-656 hsa-miR-379, hsa-miR-299, hsa-miR-380, hsa-miR-1197, hsa-miR-323a, hsa-miR-758, hsa-miR-329-1, hsa-miR-329-2, hsa-miR-494, hsa-miR-1193, hsa-miR-543*
2	hsa-miR-487b	hsa-miR-487b	14q32.31	*MIR381HG*		*hsa-miR-543, hsa-miR-495, hsa-miR-376c, hsa-miR-376a-1, hsa-miR-376a-2, hsa-miR-654, hsa-miR-376b, hsa-miR-300, hsa-miR-1185-1, hsa-miR-1185-2. hsa-miR-381, hsa-miR-539, hsa-miR-889, hsa-miR-544a, hsa-miR-655*
2	hsa-miR-30b-5p	hsa-miR-30b	8q24.22			*hsa-miR-30d*
2	hsa-miR-34c-5p	hsa-miR-34c	11q.23.1			*hsa-miR-34b*
2	hsa-miR-191-5p	hsa-miR-191	3p21.31	*NDUFAF3*	*DALRD3*	*hsa-miR-425*

Table 3 Upregulated microRNAs in HNSCC

				Overlapping transcripts		
Number	*Hsa-mature sequence*	*Stem-loop sequence*	*Locus*	*Sense*	*Antisense*	*Clustered microRNA (within 10Kbp)*
4	hsa-miR-21	hsa-miR-21	17q23.1			
4	hsa-miR-183	hsa-miR-183	7q32.2			*hsa-miR-96, hsa-miR-182*
3	hsa-miR-31	hsa-miR-31	9p21.3		*MIR31HG*	
3	hsa-miR-182	hsa-miR-182	7q32.2			*hsa-miR-96, hsa-miR-183*
3	hsa-miR-223	hsa-miR-223	Xq12			
2	hsa-miR-27a-5p	hsa-miR-27a	19p13.12			*hsa-miR-23a, hsa-miR-24-2*
2	hsa-miR-96	hsa-miR-96	7q32.2			*hsa-miR-182, hsa-miR-183*
2	hsa-miR-130b	hsa-miR-130b	22q11.21			*hsa-miR-301b*
2	hsa-miR-135b	hsa-miR-135b	1q32.1		*BLACAT1*	
2	hsa-miR-155	hsa-miR-155	21q21.3	*MIR155HG*		
2	hsa-miR-187	hsa-miR-187	18q12.2			
2	hsa-miR-196b	hsa-miR-196b	7p15.2	*HOXA10-AS, HOXA-AS4*	*HOX10-HOXA9*	
2	hsa-miR-301	hsa-miR-301a	17q22		*SKA2*	
		hsa-miR-301b	22q11.21			*hsa-miR-130b*
2	hsa-miR-503	hsa-miR-503	Xq26.3		*MIR503HG*	*hsa-miR-424, hsa-miR-542, hsa-miR-450a-1, hsa-miR-450a-2, hsa-miR-450b*

this miRNA acts as a tumor suppressor.[61–63] Our study showed that the metadherin (*MTDH*) and lactate dehydrogenase B genes were directly regulated by *miR-375* in HNSCC cells.[61] Similarly, another study showed the regulation of *MTDH* by *miR-375*. Moreover, silencing of *MTDH* in HNSCC cell lines resulted in significantly reduced tumor formation.[64] Hypermethylation of the promoter regions of *miR-375* silenced *miR-375* expression in cancer cells.[65,66]

The *miR-99* family (*miR-99a*, *miR-99b* and *miR-100*) is evolutionarily ancient. The origin of the family precedes bilaterian ancestors.[67] miRNAs in the same family have nearly identical sequences and target the same sets of genes.[67] Among *miR-99* family members, *miR-99a* and *miR-100* are frequently downregulated in several HNSCC signatures (Table 2). The deregulation of *miR-99* family members has been reported frequently in several types of cancers, and they have an important role in regulating cancer cell development and progression.[68,69] In a recent study, *miR-99* family members were identified using a mouse dermal wound healing model. These miRNAs regulate cell proliferation and migration of skin and oral mucosa epithelial cells by regulating AKT/mTOR signaling.[70] The same group showed that a *miR-99* family member directly controlled *HOXA1* in embryonic development.[67]

The *miR-100* gene is mapped on human chromosome 11q24.1, which is frequently deleted in several types of cancer.[71] Several reports showed that downregulation of *miR-100* was involved in human cancers.[72] *miR-100* regulates the PI3K/AKT pathway, which is a key signaling system that promotes cancer cell proliferation and suppresses apoptosis in bladder cancer.[73] On the other hand, *miR-100* inhibits invasion through regulating *HOXA1* in breast cancer.[74] Because of the remarkable stability of *miR-100* in blood, several reports revealed that

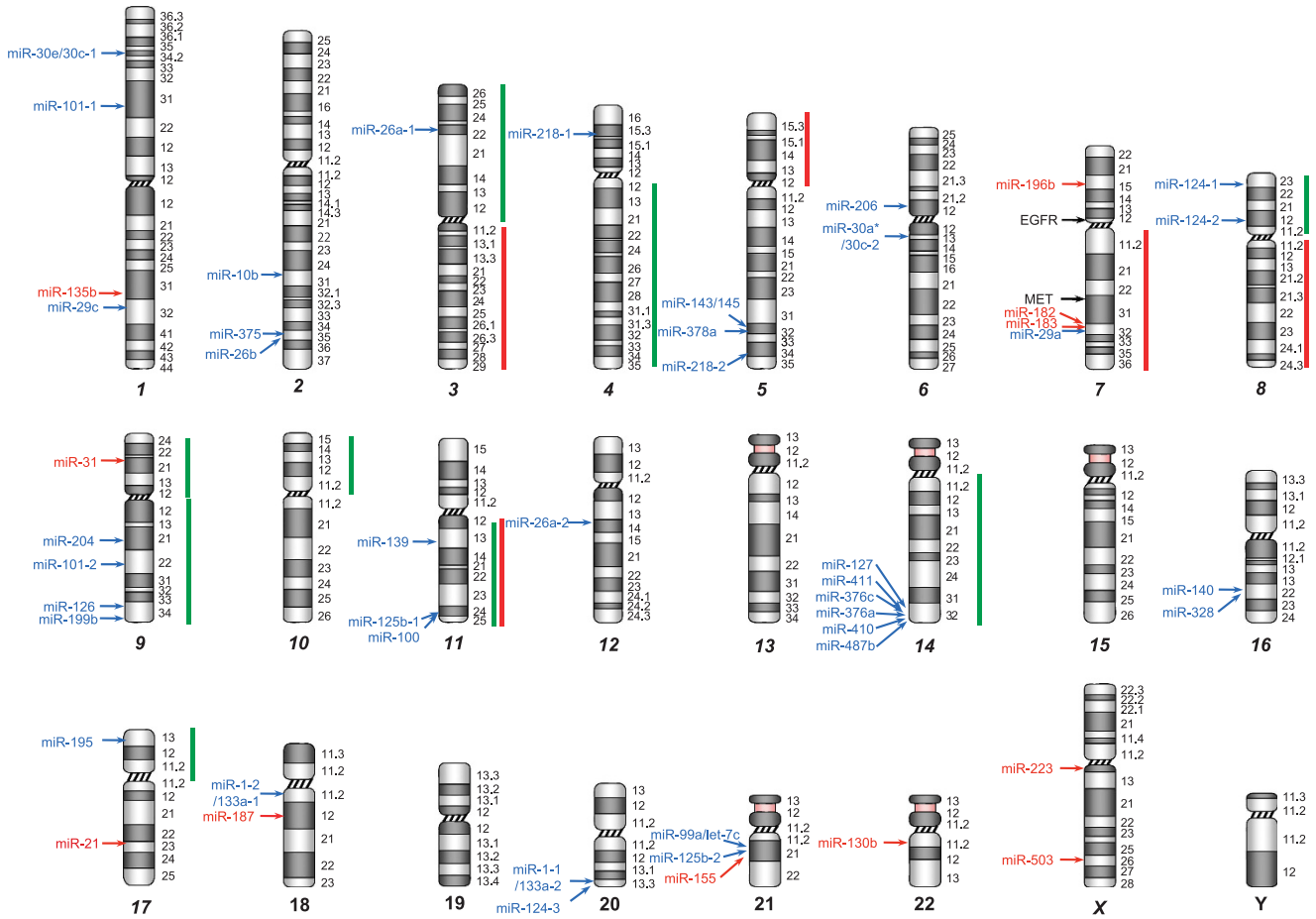

Figure 2 Chromosome mapping of aberrantly expressed microRNA (miRNA) in head and neck squamous cell carcinoma. Downregulated and upregulated miRNAs in chromosomes, merging the data from array for comparative genomic hybridization analysis (green bars are amplified regions whereas red bars show regions of loss). The blue arrows indicate downregulated miRNAs found in multiple profiling studies, and the brown arrows indicate upregulated miRNAs found in multiple signatures.

the expression levels can be used as a biomarker for diagnosis and prognosis.[72]

The *miR-125* family consists of several members, *miR-125a* (chromosome 11q24) and *miR-125b* (chromosome 24q21.1), with distinct seed sequences.[75] A large number of studies have found that *miR-125b* is dysregulated in multiple types of cancers and has pivotal roles in cancer pathogenesis.[76–79] Overexpression of *miR-125b-1* inhibited HNSCC cell aggressiveness via targeting of tumor-associated calcium signal transducer 2 (*TACSTD2*) as a glycoprotein.[80]

miR-125b regulates the *ErbB* genes, which are tyrosine kinase receptors.[81] Surprisingly, *miR-125b* can also promote cell proliferation through its targeting of *p53* expression.[82] In fact, upregulated *miR-125b* promotes cancer cell aggressiveness in many cancers.[75,83–86]

miR-139 is located on chromosome 11q13.4. It acts as a tumor suppressor in colorectal cancer, hepatocellular cancer, breast cancer and non-small cell lung cancer,[87–90] and it may be a promising biomarker.[91] Moreover, one report showed that *miR-139* inhibited proliferation and metastasis via targeting of *CXCR4* in laryngeal squamous cell carcinoma.[92]

The *miR-204* gene is located in the cancer-associated genomic region (CAGR) 9q21.12. It exhibits a high frequency of loss of heterozygosity in various cancers including HNSCC.[93] Furthermore, the expression levels of *miR-204* in HNSCC were downregulated and it suppressed HNSCC cell migration, adhesion and invasion in HNSCC.[93]

We recently showed that six miRNAs (*miR-26a*, miR-26*b*, *miR-29a*, *miR-29b*, *miR-29c* and *miR-218*) markedly inhibited metastasis-related genes or pathways in HNSCC.[30–32] For example, *miR-26a/b*, *miR-29a/b/c* and *miR-218* commonly targeted lysyl oxidase-like 2 (*LOXL2*), which promotes metastasis in several types of cancers.[94] Furthermore, the 11 signatures revealed that *miR-26a-5p*/*miR-26b-5p*, *miR-29a-3p*/*miR-29c-3p* and *miR-218* were downregulated in HNSCC. Therefore, we will focus on these families below.

The three members of the *miR-26*-family are distributed as follows: *miR-26a-1* (chromosome 3p22.2), *miR-26a-2* (chromosome 12q14.1) and m*iR-26*b (chromosome 2q35). The seed sequences of these miRNAs are identical, suggesting that all *miR-26* family members regulate the same human genes. Interestingly, MYC protein directly binds to promoter regions of these miRNAs and MYC negatively suppresses the expression of these miRNAs.[95] Expression of *miR-26a* and *miR-26b* was significantly downregulated in oral cancer tissues and restoration of both *miR-26a* and *miR-26b* significantly inhibited cancer cell migration and invasion.[30,87] *miR-26a* and *miR-26b* were reported to possess antitumor functions in several types of cancers.[96–100]

Table 4 Validated target genes of tumor-suppressive microRNA in HNSCC

MicroRNA	*Author*	*Year*	*Journal*	*Location*	*Target genes*	*Method*
miR-375	Luo	2014	*Biomed. Res. Int.*	Laryngeal	*IGF1R*	PCR, western blot, luciferase assay
miR-375	Kinoshita	2012	*Int. J. Oncol.*	Maxillary sinus	*LDHB*	PCR, western blot, luciferase assay
miR-375	Nohata	2011	*J. Hum. Genet.*	Head and neck	*AEG-1, MTDH*	PCR, western blot, luciferase assay
miR-375	Hui	2011	*Clin. Cancer Res.*	Head and neck	*MTDH*	PCR, western blot
miR-99a	Kuo, Y	2014	*Oral Dis.*	Oral cancer	*MTMR3*	qRT-PCR, western blot
miR-99a	Chen, Z	2012	*Oral Oncol.*	Head and neck	*IGF1R, mTOR*	qRT-PCR, western blot
miR-99a	Yan, B	2012	*Mol. Med. Rep.*	Oral cancer	*mTOR*	Western blot, Luciferase assay
miR-125b	Nakanishi	2014	*Oncogene*	Head and neck	*TACSTD2*	Western blot, Luciferase assay
miR-125b	Shiiba	2013	*Br. J. Cancer*	Oral cancer	*ICAM2*	qRT-PCR, Luciferase assay
miR-139	Luo	2014	*Med. Oncol.*	Laryngeal	*CXCR4*	qRT-PCR, western blot, Luciferase assay
miR-204	Lee, Y		*PloS Comput. Biol.*	Head and neck	*SPARC*	qRT-PCR
miR-204	Ma, L	2014	*FEBS Lett.*	Nasopharyngeal	*CDC42*	Western blot, Luciferase assay
miR-26a/b, miR-29a/b/c, miR-218	Fukumoto	2015	*J. Hum. Genet.*	Head and Neck	*LOXL2*	PCR, western blot, luciferase assay
miR-26a	Yu	2013	*Oncol. Lett.*	Nasopharyngeal	*EZH2*	PCR, western blot, IHC
miR-26a	Jia	2014	*Int. J. Cancer*	Tongue	*DNMT3B*	PCR, western blot, luciferase assay
miR-26a/b	Fukumoto	2014	*Br. J. Cancer*	Oral cavity	*TMEM184B*	PCR, western blot, luciferase assay
miR-29b	Yang	2014	*Oral Oncol.*	Oral cavity	*CX3CL1*	PCR, western blot, luciferase assay
miR-29b	Jia	2014	*Oral Oncol.*	Oral cavity	*Sp1*	PCR, western blot, luciferase assay
miR-29c	Liu	2013	*Cancer Lett.*	Nasopharyngeal	*TIAM1*	PCR, western blot, luciferase assay
miR-29a	LU	2013	*Biomed. Pharmacother.*	Oral cavity	*MMP2*	PCR, western blot, luciferase assay
miR-29a/b/c	Kinoshita	2013	*Br. J. Cancer*	Head and Neck	*LAMC2, ITGA6*	PCR, western blot, luciferase assay
miR-218	Kinoshita	2012	*Oncotarget*	Head and Neck	*LAMA3, LAMB3, LAMC2*	PCR, western blot, luciferase assay
miR-218	Uesugi	2011	*Cancer Res.*	Oral cavity	*RICTOR*	PCR, western blot, luciferase assay
miR-218	Wu	2014	*Carcinogenesis*	Oral cavity	*PXN*	PCR, western blot
miR-218	Alajez	2011	*Cancer Res.*	Nasopharyngeal	*ROBO1, BIRC5*	PCR, western blot, luciferase assay

Abbreviations: LDHB, lactate dehydrogenase B; MTDH, metadherin.

The four members of the *miR-29* family consist of two miRNA clusters, one located at 7q32 (*miR-29b-1* and *miR-29a*) and the other at 1q32 (*miR-29b-2* and *miR-29c*).[101] Downregulation of *miR-29s* was reported in esophageal cancer, hepatocellular cancer, gastric cancer and colon cancer.[101] Furthermore, our past report revealed that expression of *miR-29s* significantly downregulated and inhibited cancer cell migration and invasion in HNSCC, prostate cancer, renal cell carcinoma and lung cancer.[32,102–104] On the other hand, *miR-29* was upregulated in diffuse large B lymphoma.[105]

The *miR-218 family* is divided between two chromosomal regions: *miR-218-1* at 4p15.31 and *miR-218-2* at 5q34.[106] Considerable evidence suggests that downregulation of *miR-218* occurs in various cancers and that it normally acts as an antitumor miRNA, such as in colorectal cancer.[107] Ectopic expression of *miR-218* significantly suppressed HNSCC cell aggressiveness through targeting of genes involved in focal adhesion pathways, such as laminins and integrins.[31]

UPREGULATED MIRNAS ACT AS ONCOGENIC GENES IN HNSCC

Upregulated microRNAs may possess oncogenic activities if they target tumor-suppressive genes. In the 11 microRNA profiles, *miR-21* and *miR-183* were the most frequently upregulated in HNSCC clinical specimens.

The *miR-21* gene is located at 17q23.1. Several reports revealed that *miR-21* was upregulated in various cancers including HNSCC, and this miRNA acts as a key promoter of oncogenic processes.[108] Moreover, *miR-21* was a prognostic marker and was associated with clinicopathological characteristics in HNSCC.[109]

miR-183, *miR-96* and *miR-182* are clustered microRNAs at 7q32.2.[110] Although past reports showed that *miR-183* acted as an oncogene in gastric cancer and colon cancer,[111,112] no report has shown that *miR-183* is an oncogene in HNSCC.

The *miR-223* gene is generated from a site located at Xq12. In the profiles, *miR-223* was one of the most highly upregulated in HNSCC. Upregulated expression levels of *miR-223* are reported in various types of cancer.[113,114] However, *miR-223* suppresses proliferation and migration through targeting *MAFB* in nasopharyngeal carcinoma cells.[115] Moreover, our past study revealed that *miR-223* inhibited migration and invasion via its targeting of *ITGB4* in prostate cancer.[116]

miR-31 is located at 9p21.3, and its expression status varies according to the cancer type. Upregulation of *miR-31* was reported in EBV-associated nasopharyngeal carcinoma, lung cancer and ovarian cancer.[117] Furthermore, expression of *miR-31* was significantly upregulated in patients with early stage OSCC, suggesting that salivary *miR-31* was a biomarker for this disease.[118] On the other hand, our past report revealed that *miR-31* was downregulated in prostate cancer tissues.[119]

miR-182 is transcribed from a locus at 7q32.2, and it is clustered with *miR-183* and *miR-96*.[110] *miR-182* was overexpressed in papillary thyroid cancer, prostate cancer, breast cancer and lung cancer.[110] Furthermore, the serum expression level of *miR-182* is diagnostic with prognostic potential in ovarian cancer patients.[120] In HNSCC, the expression level of *miR-182* was upregulated in human papillomavirus-associated oropharyngeal carcinoma and related to cancer invasion and drug resistance.[121]

CONCLUSIONS

The discovery of miRNAs has opened new approaches in cancer research, providing insights into novel pathological processes underlying oncogenic transformation. Aberrantly expressed miRNAs

disrupt tightly controlled RNA networks in normal cells and thereby promote pathologic events. The present review highlighted recent findings in HNSCC miRNA expression signatures. The identification of aberrant miRNA-regulated cancer networks is an exciting new development in cancer research and suggests new therapeutic approaches.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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