REVIEW

The functional significance of *microRNA-375* in human squamous cell carcinoma: aberrant expression and effects on cancer pathways

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MicroRNAs (miRNAs) are a class of small, non-coding RNA molecules consisting of 19–22 nucleotides that are involved in a variety of biological processes, including development, differentiation, apoptosis and cell proliferation. In cancer research, a growing body of evidence has indicated that miRNAs are aberrantly expressed in many types of human cancers and can function either as tumor suppressors or oncogenes. Bioinformatic predictions suggest that miRNAs regulate more than 30% of protein-coding genes. Aberrant expression of miRNAs in cancer cells causes destruction of miRNA-regulated messenger RNA networks. Therefore, the identification of miRNA-regulated cancer pathways is important for understanding the molecular mechanisms of human cancer. Searching for the aberrant expression of miRNAs in cancer cells is the first step in the functional analysis of miRNAs in cancer cells. Genome-wide miRNA expression signatures can rapidly and precisely reveal aberrant expression of miRNA in cancers. The miRNA expression signatures of human cancers have revealed that *miR-375* is significantly downregulated in cancer cells. Our recent data on maxillary sinus, hypopharyngeal and esophageal squamous cell carcinomas have suggested that *miR-375* is frequently downregulated and functions as a tumor suppressor that targets several oncogenic genes in cancer cells. In this review, we focus on several types of human squamous cell carcinoma and describe the aberrant expression of miRNAs and the cancer pathways they regulate in these diseases.

INTRODUCTION

Head and neck squamous cell carcinoma (HNSCC), the sixth most common malignancy worldwide, arises in the oral cavity, oropharynx, larynx and hypopharynx.[1] In spite of considerable advances in multimodal therapy, including surgery, radiotherapy and chemotherapy, the overall 5-year survival rate for patients with HNSCC is only 40–50%.[2] Furthermore, the survival rate has not markedly improved in recent decades because of locoregional recurrences, distant metastases and second primary tumors.[2] Esophageal cancer is similarly common, being the eighth most common cancer worldwide and ranks sixth among the leading causes of cancer mortality.[3] Esophageal cancer can be divided into two main forms with distinct pathological characteristics: squamous cell carcinoma and adenocarcinoma. Esophageal squamous cell carcinoma (ESCC) is the most common type in eastern Asia, including Japan.[4] Patients with early stage disease who undergo curative surgery have a 5-year overall survival rate of 30–35%.[5] Patients in the advanced stages seldom survive more than 5 years, despite aggressive chemotherapy or chemoradiotherapy.[5]

To develop evidence-based novel therapies for HNSCC and ESCC, understanding at the molecular level is indispensable. However, most studies on human cancers have focused mainly on protein-coding genes, and our understanding of alterations in non-protein-coding sequences in cancer is largely unclear. In the post-genome sequencing era, it is crucial to find novel molecular mechanisms based on recent genome-wide studies, including non-coding RNA in human oncogenesis, including HNSCC and ESCC.

RNA can be divided into two categories: protein-coding RNA and non-coding RNA.[6] MicroRNAs (miRNAs) are a class of small, non-coding RNA molecules, consisting of 19–22 nucleotides, that are involved in a variety of biological processes, including development, differentiation, apoptosis and cell proliferation.[7] They regulate gene expression through translational repression and messenger RNA cleavage.[8] Bioinformatic predictions suggest that miRNAs regulate more than 30% of protein-coding genes.[7] So far, 1527 human miRNAs have been registered in miRBase release 18.0 (http://microrna.sanger.ac.uk/).

In the cancer research field, a growing body of evidence suggests that miRNAs are aberrantly expressed in many types of human

Table 1 Differentially expressed miRNAs in squamous cell carcinoma

Sites of SCC	Tumor and normal control	Platform	Differentially expressed miRNAs	Reference
Uvula, larynx, floor of mouth and tongue	16 tumor tissues and 5 non-diseased epithelial tissues	mirVANA microRNA Bioarrays platform v2 for 662 miRNAs	Upregulated in tumors: miR-21, miR-181d, miR-181b, miR-491, miR-455, miR-18a, miR-130b, miR-221, miR-193b, miR-181a, miR-18b	18
			Downregulated in tumors: **miR-375**	
Larynx, oropharynx and hypopharynx	51 tumor tissues and 4 normal laryngeal epithelial tissues	TaqMan MicroRNA Assays Human Panel for 322 miRNAs	Upregulated in tumors: 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	19
			Downregulated in tumors: miR-125b, **miR-375**, let-7a, miR-10a, miR-140, miR-100, miR-143, miR-99a, miR-30c, miR-365, miR-127, let-7c, miR-199b, let-7e, miR-26a	
Hypopharynx	10 tumor tissues and adjacent normal epithelial tissues	TaqMan Low Density Array Human MicroRNA Panel v1.0 for 365 miRNAs	Upregulated in tumors: miR-517c, miR-196a, miR-7, miR-196b, miR-650, miR-18a, miR-452, miR-183, miR-432, miR-301a, miR-21	13
			Downregulated in tumors: 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	
Oral cavity, oropharynx and hypopharynx	49 tumor tissues and 39 normal pharyngeal and oral cavity tissues	Affymetrix miRNA array chips for 847 miRNAs	Upregulated in tumors: 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*	20
			Downregulated in tumors: **miR-375**, miR-1224-5p, miR-617, miR-99a, miR-125b, miR-378, miR-27b, miR-125b-2*	
Maxillary sinus	Five tumor tissues and adjacent normal epithelial tissues	TaqMan Low Density Array Human MicroRNA Panel v2.0 for 667 miRNAs	Downregulated in tumors: 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	15
Oral cavity	Four tumor tissues and adjacent normal epithelial tissues	TaqMan low density array v1.0 Early Access assays for 368 miRNAs	Upregulated in tumors: miR-200abc, miR-141, miR-205	21
			Downregulated in tumors: **miR-375**, miR-127	
Esophagus	10 tumor tissues and adjacent normal epithelial tissues	TaqMan Low Density Array Human MicroRNA Panel v1.0 for 365 miRNAs	Downregulated in tumors: **miR-375**, let-7c, miR-145, miR-143, miR-100, miR-133a, miR-99a, miR-133b, miR-1, miR-30a-3p, miR-504, miR-139-5p, miR-204, miR-203, miR-326	12
Esophagus	44 tumor tissues and adjacent normal epithelial tissues	miRNA microarray chips version 3 for 329 miRNAs	Upregulated in tumors: miR-21, miR-223, miR-146b, miR-224, miR-155, miR-7-2, miR-181b-1, miR-146a, miR-181-1, miR-181-2, miR-181d, miR-181c, miR-7, miR-16-1, miR-122a, miR-125a, miR-16-2	22
			Downregulated in tumors: miR-203, **miR-375**, miR-133a-1, miR-133a-2, miR-1-2, miR-200b, miR-378, miR-143, miR-145, miR-1-1, miR-125b-2, miR-30c-1, miR-499, miR-320, miR-220, miR-99a, miR-126, miR-30c-2, miR-30b, miR-29c, miR-202	
Esophagus	Five tumor tissues and adjacent normal epithelial tissues	miRCURY LNA array for 850 miRNAs	Upregulated in tumors: miR-424, miR-422a, miR-199a-5p, miR-199a-3p, miR-199b-3p, miR-181a, miR-21, miR-455-3p, miR-665, miR-483-5p, miR-127-3p	23
			Downregulated in tumors: **miR-375**, miR-194, miR-215, miR-192, miR-203, miR-1249, miR-29b, miR-1281, miR-9, miR-126, miR-142-5p, miR-1	

Abbreviation: miRNA, microRNA. Based on the miRNA expression profiling studies, differentially expressed miRNAs in human squamous cell carcinoma were listed. miR-375 was commonly downregulated in all of the studies (bold).

cancers, including HNSCC and ESCC, and that they have significant roles in initiation, development and metastasis of these cancers.[9,10] Some highly expressed miRNAs could function as oncogenes by repressing tumor suppressors, whereas low-level miRNAs could function as tumor suppressors by negatively regulating oncogenes.[10] Aberrant expression of miRNAs in human cancer cells causes destruction of miRNA-regulated messenger RNA networks. Therefore, identification of the miRNA-regulated pathways is important for the next generation of human cancer research.

Genome-wide miRNA expression signatures can rapidly and precisely reveal aberrant expression of miRNA in cancers. Thus, we have conducted miRNA expression signature analyses and searched

for tumor-suppressive miRNAs in various types of cancers.[11–15] Our previous studies of HNSCC and ESCC signatures found that *miR-375* was significantly reduced in cancer tissues[13,15] and functioned as a tumor suppressor.[16,17] Other research groups also showed downregulation of *miR-375* in HNSCC and ESCC and other types of human cancers.[18–31]

In this review, we focus on HNSCC and ESCC and describe the aberrant expression of miRNAs in these cancers and the cancer pathways they may regulate in these diseases.

CLONING OF miR-375 AND ITS FUNCTION IN NORMAL CELLS

miR-375 was originally cloned and identified as an evolutionarily conserved pancreatic islet-specific miRNA.[32] Northern blot analysis showed that *miR-375* was highly expressed in mouse pancreatic islet cells, and not found in other tissues, including the liver, lung, fat, intestine, brain, kidney, spleen, heart and testis.[32] In zebrafish embryo, *miR-375* is expressed in pancreatic islet and pituitary gland.[33] So, the functions of *miR-375* in normal cells have been investigated mainly in pancreatic islet cells. In murine pancreatic beta cells, overexpression of *miR-375* suppressed glucose-induced insulin secretion and, conversely, that inhibition of endogenous *miR-375* function enhanced insulin secretion. *miR-375* negatively regulated glucose-stimulated insulin secretion by directly regulating the expression of *myotrophin*, a gene involved in actin depolymerization and, potentially, vesicular fusion.[32] *miR-375* also negatively regulates the expression of *3′-phosphoinositide-dependent protein kinase 1* (*PDK1*) in pancreatic beta cells and, subsequently, decreases insulin transcription.[34] *miR-375*$^{-/-}$ mice were found to develop hyperglycemia and hyperglucagonemia, with increased pancreatic alpha cells and decreased pancreatic beta cells.[35] These studies show that *miR-375* has an important role in insulin secretion and glucose metabolism, and may thereby become a novel therapeutic target in diabetes. *miR-375* is also reported to contribute to adipocyte differentiation. Overexpression of *miR-375* enhanced 3T3-L1 adipocyte differentiation through modulation of the ERK-PPARγ2-aP2 pathway.[36] Functional analysis of *miR-375* in normal cells is important, and elucidation of the molecular networks regulated by *miR-375* in several types of human cells is necessary.

ABERRANT EXPRESSION AND FUNCTIONAL SIGNIFICANCE OF miR-375 IN HNSCC AND ESCC

Screening for differentially expressed miRNAs in cancer cells is important as a first step in understanding the role of miRNAs in cancer. Genome-wide miRNA expression signatures can rapidly and precisely reveal aberrant expression of miRNA in cancer cells. As cases in point, several groups have conducted searches for miRNA expression signatures in HNSCC using PCR-based, microarray-based and sequencing-based methods. In this review, we have listed nine miRNA expression signatures in HNSCC and ESCC.[12,13,15,18–23] Differentially expressed miRNAs are summarized based on the nine signatures in Table 1. Recently, we conducted miRNA expression signature analyses in hypopharyngeal-SCC, maxillary sinus SCC and ESCC using clinical specimens.[12,13,15] Our expression signatures revealed that *miR-1*, *miR-30a-3p*, *miR-139-5p*, *miR-143*, *miR-204* and *miR-375* were commonly downregulated in cancer tissues when compared with adjacent, non-cancerous tissues. These commonly downregulated miRNAs have been selected by other research groups, as well, suggesting that it is important to investigate their functional significance and to identify the molecular targets of these miRNAs to provide novel information on human SCC oncogenesis.

Among the miRNAs identified in our expression signature analysis, *miR-375* was found to be the most commonly downregulated, being downregulated in all signatures in HNSCC and ESCC. In spite of the difference in methods of expression analysis, *miR-375* was commonly downregulated in those signatures that suggests downregulation of *miR-375* is important for SCC oncogenesis. Based on this conclusion, the functional significance of *miR-375* in HNSCC was recently investigated by our group. The restoration of *miR-375* caused significant inhibition of cell proliferation and induction of cell apoptosis in SAS and FaDu cell lines derived from HNSCC.[16] In maxillary sinus SCC, restoration of *miR-375* also inhibited cell proliferation and invasion in IMC-3 cells.[17] Similarly, another group demonstrated that transfection of *miR-375* into FaDu, UTSCC-8 and UTSCC-42a cell lines reduced proliferation and clonogenicity.[19]

miRNAs are unique in their ability to regulate many protein-coding genes. Bioinformatic predictions suggest that miRNAs regulate more than 30% of protein-coding genes.[7] The elucidation of new molecular pathways regulated by tumor-suppressive *miR-375* is

Table 2 *miR-375* function and its target genes in cancer

Type of cancer	miR-375 function	Validated target gene	Reference
HNSCC	TS	Metadherin (*MTDH*)	16,24
HNSCC	TS	Lactate dehydrogenase B (*LDHB*)	17
Esophageal SCC	TS	Insulin-like growth factor 1 receptor (*IGFR1*)	23
Esophageal cancer	TS	3′-Phosphoinositide-dependent protein kinase-1 (*PDK1*)	25
Gastric cancer	TS	PDK1, tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, ζ (*YWHAZ*)	26
Gastric cancer	TS	Janus kinase 2 (*JAK2*)	27
Pancreatic cancer	TS	Insulin-like growth factor-binding protein 5 (*IGFBP5*), caveolin 1 (*CAV1*)	28
HCC	TS	Yes-associated protein 1 (*YAP1*)	29
HCC	TS	MTDH	30
Melanoma	TS	ND	31
Lung cancer	Inducing NE phenotype	YAP1	39
ILC of breast	Oncogenic	ND	40
Breast cancer	Upregulation of ERα	Ras, dexamethasone-induced 1 (*RASD1*)	41
Prostate cancer	ND	Sec23 homolog A (*S. cerevisiae*) (*SEC23A*)	42

Abbreviations: ERα, estrogen receptor alpha; HCC, hepatocellular carcinoma; HNSCC, head and neck squamous cell carcinoma; ILC, invasive lobular carcinoma; ND, not determined; NE, neuroendocrine; SCC, squamous cell carcinoma; TS, tumor suppressive.
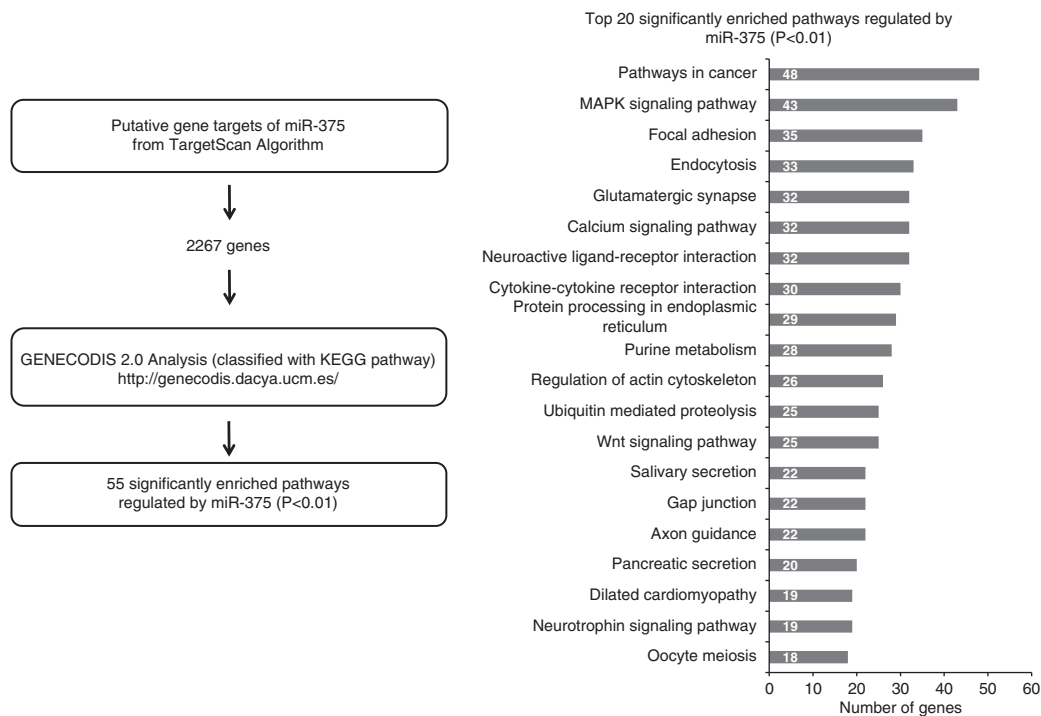
Figure 1 Workflow for the *in silico* analysis of gene targets of *miR-375*. A total of 2267 genes were identified by the TargetScan program as predicted targets of miR-375. The genes were then analyzed and categorized with KEGG and pathways by the GENECODIS program (left). Twenty significantly enriched signaling pathways are shown in descending order of the number of genes contained in each pathway (right).

important for our understanding of human SCC oncogenesis. Based on this view, we also performed molecular target searches for *miR-375* in cancer cells by using genome-wide gene expression analysis and luciferase reporter assays in *miR-375* transfectants. Our data revealed that the *metadherin* (*MTDH*) and *lactate dehydrogenase B* were direct targets of *miR-375* in HNSCC and maxillary sinus SCC.[16,17] Silencing of the *MTDH* gene caused significant inhibition of cancer cell proliferation, suggesting *MTDH* functions as an oncogene in HNSCC.[16] Very interestingly, another group also reported that *MTDH* was regulated by *miR-375* and that both *miR-375* overexpression and *MTDH* knockdown in HNSCC cell lines resulted in significant decreases in tumor formation in SCID mice.[24]

In ESCC, expression signatures revealed that *miR-375* was significantly reduced in cancer cells.[12,22,23] The downregulation of *miR-375* was caused by hypermethylation of the promoter region, and ectopic expression of *miR-375* inhibited tumor cell growth and metastasis *in vitro* and *in vivo* by targeting *insulin-like growth factor 1 receptor*.[23] Another study also showed a tumor-suppressive function for *miR-375* in esophageal cancer cell lines and identified *PDK1* as a target of *miR-375*.[25]

The prognosis of both HNSCC and ESCC patients at advanced stages is very poor, despite advances in medical treatments. For this reason, the search for new therapeutic targets is important. Tumor-suppressive *miR-375*-mediated pathways provide new potential therapeutic targets in HNSCC and ESCC.

Next, we describe the clinical applications of *miR-375* in HNSCC and ESCC. Several groups have reported that reduced expression of *miR-375* could be a biomarker for HNSCC.[18,21,37] The expression levels of *miR-375* were found to be lower in oral SCC patient oral rinse and saliva compared with those in healthy controls.[21] The expression ratio miR-221:miR-375 could be useful for distinguishing

HNSCC from normal epithelium.[18] Another report showed that low *miR-375* expression levels significantly correlated with cancer survival and distant metastasis.[37] In ESCC, plasma concentrations of *miR-375* were shown to be significantly lower in ESCC patients than in controls.[38] Furthermore, low expression levels of *miR-375* were significantly correlated with advanced stage, distant metastasis, poor overall survival and disease-free survival in ESCC patients.[23]

Recent topics of miRNA study are the existence of circulating miRNAs in the blood and body fluids of humans, especially cancer patients. Thus, a number of reports in the past several years have shown that circulating miRNAs in plasma or urine might be used as disease biomarkers and prognostic predictors. These studies will continue to provide evidence for which of the circulating miRNAs may be useful biomarkers in cancer.

ABERRANT EXPRESSION OF *miR-375* AND ITS TARGET GENES IN OTHER TYPES OF HUMAN CANCERS

Aberrant expression of *miR-375* and indications of its involvement in other types of cancer have also been reported (Table 2).[16,17,23–31,39–42] Downregulation of *miR-375* has been reported for gastric cancer, cervical cancer, pancreatic ductal adenocarcinoma and hepatocellular carcinoma.[26,27,29,30,43–45] In gastric cancer, ectopic expression of *miR-375* in cancer cells reduced cell viability and induced apoptosis by targeting *PDK1* and *tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein ζ* (*YWHAZ*).[26] Overexpression of *miR-375* suppressed gastric cancer cell proliferation *in vitro* and *in vivo* by targeting *Janus kinase 2*.[27] In hepatocellular carcinoma, it was reported that restoration of *miR-375* in cancer cells decreased cell proliferation, clonogenicity, migration and invasion, and induced G1 arrest and apoptosis.[30] Interestingly, this report demonstrated that *MTDH* was directly regulated by *miR-375*, as it is in HNSCC.[16,24,30]

Table 3 *miR-375* target genes assigned to pathways in cancer

Entrez gene ID	Gene symbol	Gene name
1956	EGFR	Epidermal growth factor receptor (erythroblastic leukemia viral (v-erb-b) oncogene homolog, avian)
7423	VEGFB	Vascular endothelial growth factor B
2064	ERBB2	v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian)
2475	MTOR	Mechanistic target of rapamycin (serine/threonine kinase)
3815	KIT	v-kit Hardy–Zuckerman 4 feline sarcoma viral oncogene homolog
2247	FGF2	Fibroblast growth factor 2 (basic)
4193	MDM2	Mdm2 p53-binding protein homolog (mouse)
3480	IGF1R	Insulin-like growth factor 1 receptor
7428	VHL	Von Hippel–Lindau tumor suppressor
1030	CDKN2B	Cyclin-dependent kinase inhibitor 2B (p15, inhibits CDK4)
331	XIAP	X-linked inhibitor of apoptosis
5979	RET	Ret proto-oncogene
1021	CDK6	cyclin-dependent kinase 6
2246	FGF1	Fibroblast growth factor 1 (acidic)
867	CBL	Cas-Br-M (murine) ecotropic retroviral transforming sequence
2335	FN1	Fibronectin 1
3479	IGF1	Insulin-like growth factor 1 (somatomedin C)
4437	MSH3	mutS homolog 3 (Escherichia coli)
7048	TGFBR2	Transforming growth factor, beta receptor II (70/80 kDa)
5156	PDGFRA	Platelet-derived growth factor receptor, alpha polypeptide
7189	TRAF6	TNF receptor-associated factor 6
330	BIRC3	Baculoviral IAP repeat-containing 3
7474	WNT5A	Wingless-type MMTV integration site family, member 5A
2252	FGF7	Fibroblast growth factor 7 (keratinocyte growth factor)
1488	CTBP2	C-terminal-binding protein 2
7976	FZD3	Frizzled homolog 3 (Drosophila)
5154	PDGFA	Platelet-derived growth factor alpha polypeptide
7855	FZD5	Frizzled homolog 5 (Drosophila)
5578	PRKCA	Protein kinase C, alpha
3066	HDAC2	Histone deacetylase 2
3675	ITGA3	Integrin, alpha 3 (antigen CD49C, alpha 3 subunit of VLA-3 receptor)
8074	FGF23	Fibroblast growth factor 23
8030	CCDC6	Coiled–coil domain containing 6
7175	TPR	Translocated promoter region (to activated MET oncogene)
1286	COL4A4	Collagen, type IV, alpha 4
1871	E2F3	E2F transcription factor 3
5579	PRKCB	Protein kinase C, beta
1288	COL4A6	Collagen, type IV, alpha 6
861	RUNX1	Runt-related transcription factor 1
4286	MITF	Microphthalmia-associated transcription factor
6654	SOS1	Son of sevenless homolog 1 (Drosophila)
8322	FZD4	Frizzled homolog 4 (Drosophila)
8325	FZD8	Frizzled homolog 8 (Drosophila)
3915	LAMC1	Laminin, gamma 1 (formerly LAMB2)
2257	FGF12	Fibroblast growth factor 12
9978	RBX1	Ring-box 1
8554	PIAS1	Protein inhibitor of activated STAT, 1
6921	TCEB1	Transcription elongation factor B (SIII), polypeptide 1 (15 kDa, elongin C)

Another report indicated that *miR-375* inhibits proliferation and invasion of hepatocellular carcinoma cells by targeting *yes-associated protein* (*YAP*).[29]

We have described *miR-375* function as a tumor suppressor in human cancers. However, in some types of cancer, *miR-375* functions as an oncogene. In a breast cancer study, *miR-375* was upregulated in estrogen receptor alpha-positive cell lines, and *miR-375* overexpression, as well as facilitating cell proliferation, induced estrogen receptor alpha upregulation through regulation of *RASD1*, a negative regulator of estrogen receptor alpha.[41] miRNA expression profiling in invasive lobular carcinoma of the breast showed that *miR-375* was upregulated when compared with normal breast epithelium.[40] Ectopic expression of *miR-375* in non-tumorigenic breast epithelial MCF-10 A cells induced loss of cellular organization and a hyperplastic phenotype.[40] In prostate cancer (PC), both deep sequencing and microarray analysis of miRNA expression revealed that *miR-375* was significantly upregulated in tumor tissues.[46] Serum levels of *miR-375* in PC patients are upregulated compared with those of healthy
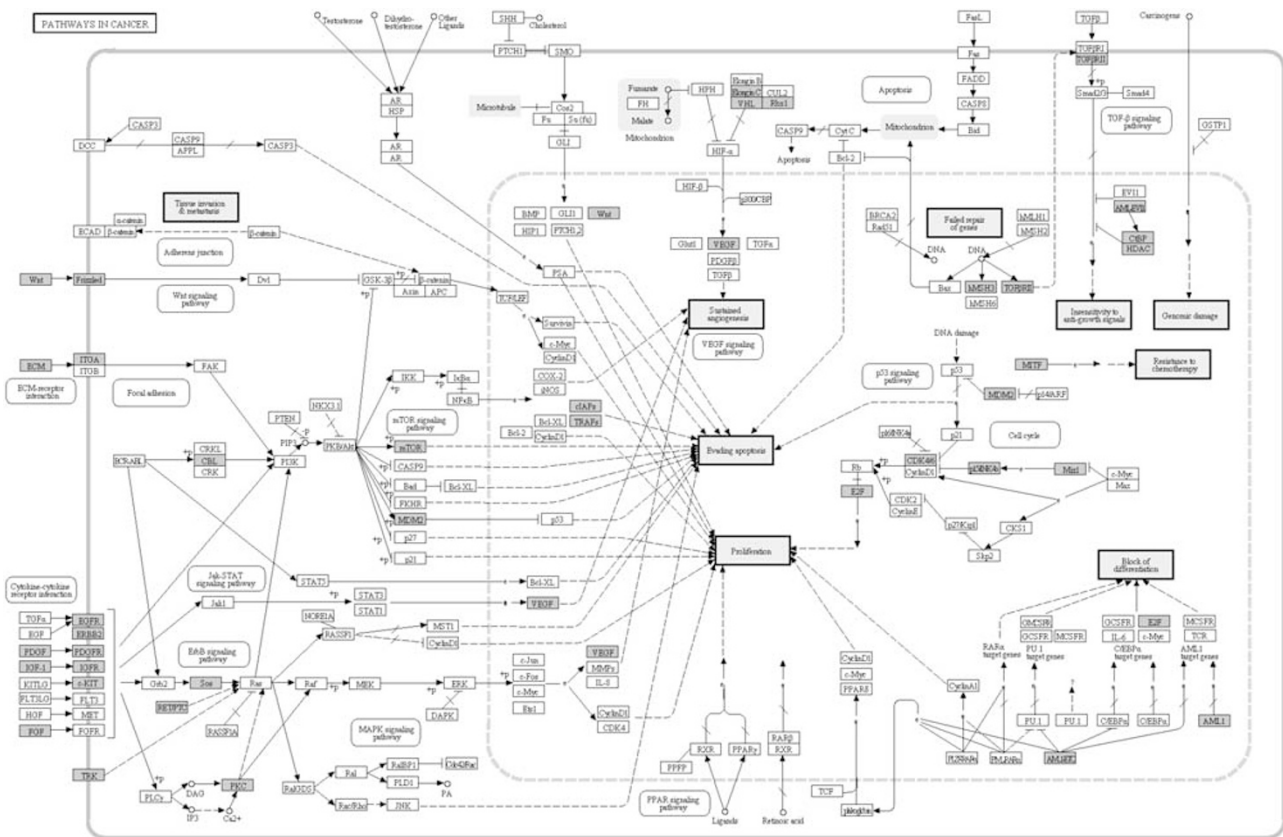
Figure 2 Putative *miR-375* target genes in 'Pathways in cancer' from KEGG. The genes highlighted in gray are putative *miR-375* target genes, as defined by the KEGG pathway and determined through GENECODIS analysis.

controls.[47] In addition, *miR-375* was significantly upregulated in serum exosome fractions of metastatic PC patients relative to those of patients with non-recurrent PC.[48] In lung cancer with neuroendocrine features, the transcription factor *ASH1/ASCL1* is a key regulator in this disease.[39] This study demonstrated that *miR-375* was induced by *ASH1/ASCL1* in lung cancer cells. Furthermore, it was indicated that *miR-375* directly regulated *YAP1*, which had growth inhibitory activities in neuroendocrine-lung cancer cells.[39] These findings indicate *miR-375* can function as an oncogene in some types of cancer.

Is the function of *miR-375* an oncogene or a tumor suppressor? This phenomenon of seemingly opposing activities of *miR-375* has been described in several articles, yet it is very difficult to explain this phenomenon at present. Individual miRNAs may have different roles and regulate different target genes in different types of cancer, so it is necessary to investigate the expression patterns of both miRNAs and messenger RNAs in specific cancers to understand how miRNAs regulate cancer networks in the future.

IN SILICO ANALYSIS OF miR-375-REGULATED PATHWAYS
miRNAs are unique in their ability to regulate many protein-coding genes. One miRNA can have hundreds or even thousands of potential gene targets, and bioinformatic predictions suggest that miRNAs regulate more than 30% of protein-coding genes.[7] Our interest is in the elucidation of the miRNA-regulated molecular network in several types of cells.

In this review, we have applied *in silico* analysis to identify the biological processes or pathways potentially regulated by *miR-375.*

Using the TargetScan database, a total of 2267 putative gene targets of *miR-375* were identified and are listed (Supplementary Table 1). Among 2267 genes, we investigated differentially expressed genes that were regulated by *miR-375* in HNSCC cells. *miR-375*-regulated genes were identified based on our expression signatures of *miR-375* transfectants in HNSCC cancer cells (Supplementary Table 1). These data were registered on the GEO database (accession number: GSE26032). Next, these candidate target genes were assigned to pathways using GENECODIS software analysis,[49,50] and statistically enriched pathways were identified. The GENECODIS software assigned a great many of the putative *miR-375* targets to known pathways in KEGG (Kyoto Encyclopedia of Genes and Genomes),[51] and these data facilitate the understanding of miRNA-regulated molecular networks in human cells. According to the GENECODIS software analysis, several pathways were identified, such as 'pathways in cancer', 'MAPK signaling pathway', 'focal adhesion' and 'cytokine-cytokine receptor interaction' (Figure 1).

We focused on the 'pathways in cancer', as it was identified as the most enriched pathway among the *miR-375* target genes. Among 2267 genes (these genes have putative target site(s) for *miR-375* in their 3′ untranslated regions), 48 were assigned to this pathway (Table 3) and are highlighted in the KEGG MAP (Figure 2). When we analyzed the 'pathways in cancer', several genes were found to have been implicated in HNSCC. For example, overexpression of *epidermal growth factor receptor* has been shown in HNSCC,[52,53] and overexpression of *epidermal growth factor receptor* is a negative prognostic factor in HNSCC patients.[53,54] The *mammalian target of rapamycin (mTOR)* pathway was shown to be overactivated in HNSCC by investigating

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the phosphorylation status of the ribosomal protein S6, a well-known downstream target of *mTOR*.[55] These genes have been used to develop molecularly targeted therapeutic agents for human cancer, such as Gefinitib (Iressa, also known as ZD1839), Erlotinib (marketed as Tarceva) and Cetuximab (marketed as Erbitux), which target *epidermal growth factor receptor*[56], and Everolimus (marketed as Afinitor) and Temsirolimus (marketed as Torisel), which target *mTOR*.[57] Cetuximab received Food and Drug Administration approval for treatment of locally advanced HNSCC in combination with radiotherapy. Overexpression of the genes targeted by these antagonists may result in the reduction of *miR-375*. In this way, the analysis of molecular pathways that started with identification of cancer-related miRNAs has provided new insights into human oncogenesis.

CONCLUSIONS

In this review, we have described aberrant expression of *miR-375* in human cancers. In HNSCC and ESCC, *miR-375* is frequently down-regulated in cancer cells and functions as a tumor suppressor. Identification of molecular targets regulated by miRNAs is a big challenge for current cancer research. The oncogenic and/or tumor-suppressive miRNA-regulated novel cancer pathways could provide new information into the molecular mechanisms of human oncogenesis, and cancer progression and metastasis.

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