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Review Article

MicroRNAs in laryngeal cancer: implications for diagnosis, prognosis and therapy

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Abstract: Laryngeal cancer is the most common head and neck cancer (skin excluded) with the increasing rates of morbidity and mortality in the world. The emerging roles of microRNAs (miRs) in laryngeal cancer have been deeply investigated in recent years. Deregulated miRs are frequently detected in tissues and cells of laryngeal cancer, which work as oncogenes or tumor supressors to regulate cancer cell proliferation, metastasis and invasion, etc. Here we reviewed the recognized roles of miRs in the diagnosis, prognosis and therapy of laryngeal cancer. Although there are lots of challenges in miRs including sensitivity, specificity, accuracy and safety, the growing improvements of miRs in laryngeal cancer remain encouraging and promising.

Keywords: MicroRNAs, laryngeal cancer, diagnosis, prognosis, therapy

Introduction

Laryngeal cancer is the most common head and neck cancer (skin excluded) and fourteenth most common cancer in male (~157,000 estimated new cases globally in 2012, less than 19,000 in women) compared to its relative rarity in female, and the death number from this cancer is about 83,000 cases [1]. Laryngeal squamous cell carcinoma (LSCC) accounts for approximately 85~90% of all laryngeal cancer. During the last decades, incidence of LSCC world wide in both gender changed differently. In the USA, the male LSCC population has declined with estimated 10,720 new cases in 2015 (10000 new cases in 2014), while in the female, it has remained constant with estimated 2,840 cases in 2015 (2630 new cases in 2014), or even tends to increase over the two decades [2]. Estimated death of LSCC patients is 2,890 and 750 in male and female respectively in 2015 [3, 4]. The data from 2010 to 2015 is illustrated in **Table 1** [3-8]. Laryngeal cancer in early-stage is often curable with modern available treatments, but most of LSCC patients with advanced disease have not retained a better outcome in the last 20 years in spite of therapeutic advancements [9]. Conventional biopsy from the primary tumor is considered to be an accurate diagnosis approach, which is often performed under local even general anesthesia. Lots of reports supported that methods, such as CT, MRI, ultrasonography, or even positron emission tomography, all of which are noninvasive imaging techniques, are not sufficient for diagnostic purposes in terms of cost-effectiveness analysis by now [10-12]. Generally, the treatment modalities used in the laryngeal cancer include surgical approaches (e.g. total laryngectomy, partial laryngectomy, supraglottic laryngectomy, supracricoid partial laryngectomy, endoscopic approach, transoral robotic surgery, transoral laser surgery) and conservative treat-

Table 1. Estimated new laryngeal cancer cases and deaths by sex, United States, 2010-2015 [3-8]

	Estimated new incidence cases			Estimated new death cases		
Year	Both Sexes	Male	Female	Both Sexes	Male	Female
2015	13,560	10,720	2,840	3,640	2,890	750
2014	12,630	10,000	2,630	3,610	2,870	740
2013	12,260	9,680	2,580	3,630	2,860	770
2012	12,360	9,840	2,520	3,650	2,880	770
2011	12,740	10,160	2,580	3,560	2,840	720
2010	12,720	10,110	2,610	3,600	2,870	730

Table 2. Summary of the upregulated miRs in laryngeal cancer

MiRs	Genomic location (Homo sapiens)	Direct targets	Ref.
miR-106b	7q22.1	RB and RUNX3	[28, 32, 34, 35]
miR-1297	13	PTEN	[30]
miR-155	21q21.3	SOCS1 and STAT3	[37]
miR-23a	19p13.13	APAF-1	[36]
miR-27a	19p13.13	PLK2	[33]
miR-423-3p	17q11.2	AdipoR2	[29]
miR-301a-3p	17q22	Smad4	[31]

Table 3. Summary of the downregulated miRs in laryngeal cancer

MiRs	Genomic location (Homo sapiens)	Direct targets	Ref.
miR-101	1p31.3; 9p24.1	CDK8	[40]
miR-129-5p	7q32.1; 11p11.2	APC	[41]
miR-139	11q13.4	CXCR4	[43]
miR-203	14q32.33	survivin	[38]
miR-205	1q32.2	Bcl-2	[48]
miR-221	Xp11.3	Apaf-1	[47]
miR-24	9q22.32; 19p13.13	XIAP	[49]
miR-34a	1p36.22	GALNT7	[42]
miR-34c	11q23.1	GALNT7 and c-Met	[39, 42]
miR-370	14q32.2	FoxM1	[50]
miR-375	2q35	IGF1R	[44]
miR-519b-3p	19q13.42	HuR and COX-2	[46]
miR-874	5q31.2	HDAC1	[45]

ment options (e.g. radiotherapy, concurrent chemo-radiotherapy and induction chemotherapy, induction therapy and target therapy). Laryngeal-preservation and survival-rates in different surgical and conservative treatment regimens were summarized in a review [13]. Usually, surgical treatment and conservative treatment modalities are often combined in advanced diseases in order to get optimal organ larynx preservation and laryngeal function, in which the quality of life is increasingly considered. Although affluent accessible curative-intent options for the patients of LSCC, the curative effect of laryngeal cancer is not steady and changes from excellent for the early stage tumor patients (≥90% for five-year survival rate for stage I-II cancer) [14-16] to a relative inferior five year survival rate (<60%) for loco-regionally advanced disease [17].

MicroRNAs (miRs) are a class of endogenous non-coding small RNAs that usually play key regulatory roles in multiple biological processes in eukaryote [18]. Mature miR is a single strand RNA with about 22 nucleotides, and the generation of miR requires two procedures: first, the long endogenous transcript pri-miR is cleavaged by Drosha enzyme to produce the 70 nucleotides pre-miR with peculiar stem-loop structure in the nucleus; second, pre-miR is then exported to the cytoplasm and processed into the single stranded mature miR by Dicer enzyme [19, 20]. MiRs mediate gene expression by regulating post-transcriptional [21]. Mature miRs integrate with argonaute proteins to form a RNA-induced silencing complex (RISC), then miRs bind with the specific target mRNA, which does not require a strict complementary base pair, thereby inhibiting target mRNA translation [20, 22, 23]. Generally, two different working modes exist in plant and animal cells respectively. In plant cells, miRs target the corresponding mRNA with precise or almost precise complementarity, resulting in the final cleavage and degradation of mRNA. While in animal cells, miRs are imperfectly complement to the 3' untranslated regions (UTRs) of mRNA, leading to translational repression of mRNA. The 2-8 highly conserved nucleotides at 5' end of miR, known as the "seed region", do not have a

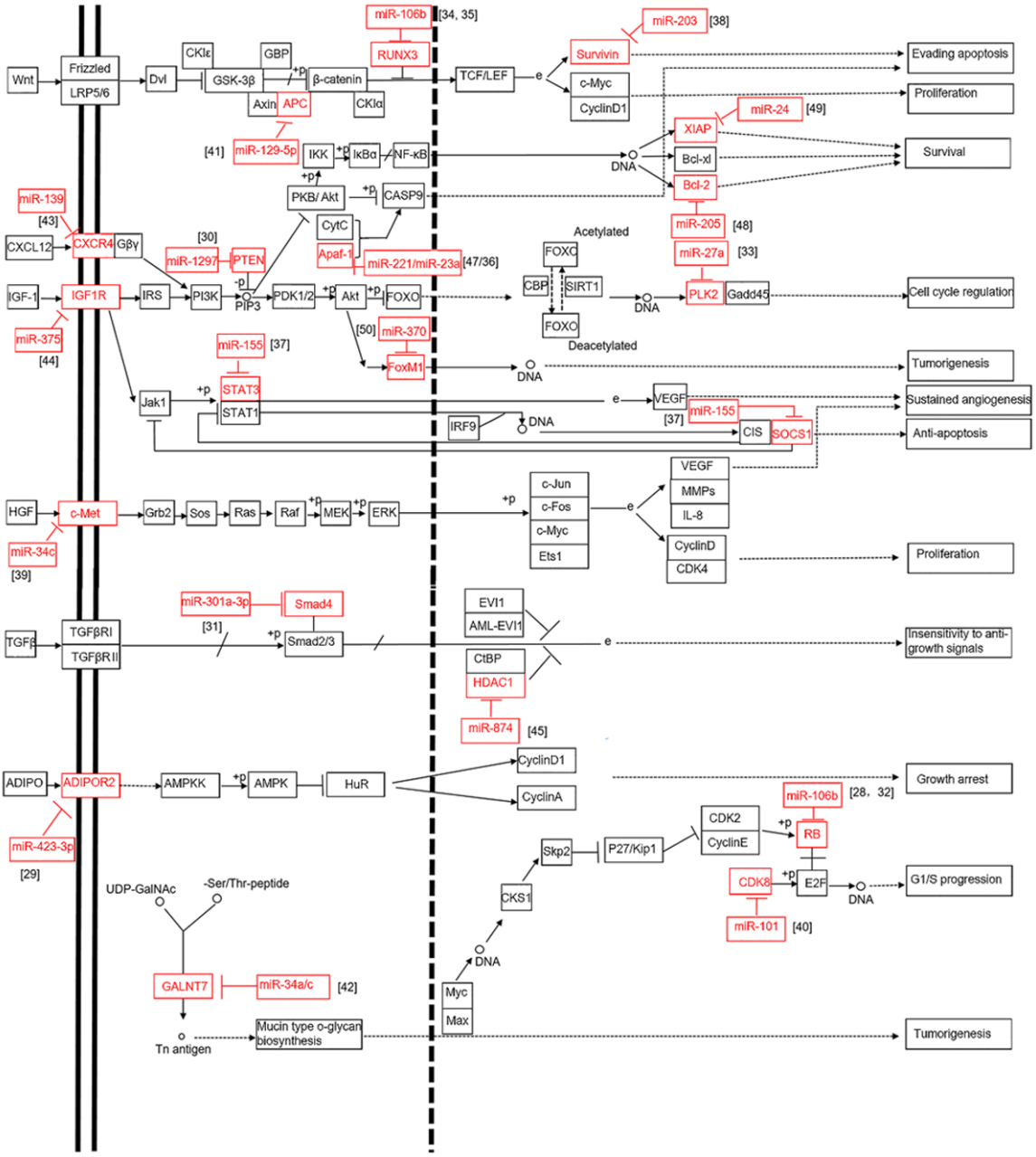

Figure 1. MiRs and their targets in signaling pathway of LSCC.

complex secondary structure. Through the seed region specifically binding to the 3' UTRs of the target mRNA, miR affects the maturation, transport or translation of mRNA [24-26]. Until now, it has discovered over 1000 known human miRs, and they control more than half of mammalian protein coding genes [27]. In this review, we comprehensively summarize the recent progress on the miRs in laryngeal cancer, including the documented upregulated miRs [28-37] and downregulated miRs [38-50] in laryngeal cancer which have validated direct targets (**Tables 2** and **3**). Eventually, we highlight the integrated miRs and their targets in signaling pathway (**Figure 1**) and contemplate their future prospects as potential biomarkers and therapeutic targets of laryngeal cancer.

It is beyond the scope of our review to conduct a discussion of all miRs that are involved in

Table 4. Summary of the potential diagnostic miRs in laryngeal cancer

MiRs	Deregulation	Molecular mechanisms	Ref.
miR-196a	Upregulation	The cancer-specific expression of miR-196a was confirmed in 89 LSCC surgical specimens.	[51]
miR-21	Upregulation	The increased exosomal expressions of miR-21 and HOTAIR were correlated with clinical stages, T classification and lymph node metastasis.	[53]
miR-27a	Upregulation	MiR-27a had a significantly increased expression in LSCC.	[33]
miR-331-3p, 603, 1303, 660-5p, 212-3p	Upregulation	MiR-331-3p, 603, 1303, 660-5p and 212-3p were only detected in LSCC and have never been reported before in plasma of any other human subject.	[25]
miR-657/miR-1287	Upregulation/downregulation	The miR-657/miR-1287 classfier displayed high sensitivity and specificity for differentiating early larynx carcinoma from normal samples.	[55]

Table 5. Summary of the potential prognosis miRs in laryngeal cancer

MiRs	Deregulation	Molecular mechanisms	Ref.
miR-101	Downregulation	Low expression of miR-101 was correlated with TNM stage, especially poorer prognosis.	[40]
miR-126	Downregulation	Elevated expression of miR-126 was closely associated with the favorable prognosis of the patients with LSCC.	[56]
miR-152	Downregulation	The expression level of miR-152 was correlated with pT stage and pN stage of supraglottic laryngeal carcinoma.	[57]
miR-19a	Upregulation	The high level of miR-19a was positively correlated with decreased overall survival.	[58]
miR-21/miR-375	Upregulation/Downregulation	High miR-21 or low miR-375 expression in tumor tissues predicted poor prognoses.	[59]
miR-23a	Upregulation	High expression of miR-23a was significantly correlated with patient five-year survival.	[60]
miR-296-5p	Upregulation	The expression of miR-296-5p was substantially related to radioresistance and tumor recurrence in early stage LSCC.	[61]
miR-34c-5p	Downregulation	Low miR-34c-5p expression was significantly associated with worse disease-free and overall survival and an increased risk of recurrence.	[62]

LSCC. Instead we shall discuss some representative examples, selected mainly depending on the depth of study, and for their utilizable features that may give clues to the future of the field.

MiRs as diagnostic biomarkers of laryngeal cancer

Koichiro Saito et al. focused on a promising cancer marker miR-196a which was validated LSCC-specific in both cancer and cancer stroma cells. Thus, it would be recognized as a potential diagnostic marker in LSCC [51]. Liu et al. investigated that miR-21 was overexpressed in laryngeal carcinoma tissues and a new target gene of miR-21, BTG2 (a pan-cell cycle regulator and tumor suppressor), was downregulated in LSCC tissues. Moreover, the loss of miR-21 inhibited the proliferation of Hep-2 cells. Therefore, the oncogenic miR-21 may act as a valuable tool for the diagnosis of LSCC [52]. Wang et al. showed that the expression of serum exosomal miR-21 and HOTAIR was significantly higher in patients with LSCC than those with vocal cord polyps. The combination of miR-21 and HOTAIR was highly sensitive (94.2) and specific (73.5%) in differentiating the malignant from benign laryngeal disease, suggesting that this combination may be a suitable screening and predicting tool for LSCC patient [53]. The results from Tian et al. shed a new sight into miR-27a, an oncogene significantly amplified in the laryngeal tumor tissues compared to the adjacent non-tumor tissues. Thus, miR-27a could provide a potential clue into the diagnosis of LSCC [33]. High-throughput real-time quantitative polymerase chain reaction revealed the expression profiles of 738 miRs in plasma from 20 LSCC patients and 44 healthy subjects and demonstrated that there were 17 miRNAs up-regulated and 9 miRNAs downregulated significantly in patients with LSCC, among which miR-331-3p, 603, 1303, 660-5p, 212-3p were LSCC specific and never detected before in plasma of any human subject, indicating that these five miRs may be potential novel non-invasive specific indicators for LSCC [54]. Using a SAM algorithm, Wang et al. found 47 miRs were significantly differentially expressed in primary larynx tumor tissues compared to normal tissues including miR-657

and miR-1287. MiR-657 and miR-1287 were overexpressed and underexpressed respectively. The miR-657-miR-1287 classifier showed highly sensitive and specific for classifying LSCC and normal esophageal mucosa tissues. It's concluded that these two miRs may act as potential biomarkers for the early diagnosis of LSCC [55]. The reported potential diagnostic miRs of LSCC are summarized in **Table 4**.

MiRs as prognostic biomarkers for laryngeal cancer

Accumulated evidences have discerned the close relationship between the expression of miRs and LSCC prognosis. For instance, miR-101 was analyzed downregulated in LSCC tissues than in adjacent normal tissues, and the low expression of miR-101 was correlated with T3-4 tumor grade, metastasis of lymph nodes, advanced clinical stage and poorer prognosis. Mechanistically, miR-101 induced cells apoptosis by directly repressing CDK8 expression. Taken together, miR-101 may be a potent tumor repressor and the detection of miR-101 may represent as a new strategy of evaluating the prognosis of LSCC patient [40]. Sun et al. measured the plasma concentrations of miR-126 in LSCC patient and reported that miR-126 expression was closely related with the prognosis of LSCC patient. In vitro assays also showed the survival rate of mice with miR-126 mimics was significantly improved. These results accounted for the potential role of miR-126 as a prognosis biomarker in LSCC [56]. Additionally, miR-152 was significantly downregulated in supraglottic laryngeal carcinoma tissues, and its expression was correlated with pT stage and pN stage, suggesting it has the potential to be a novel prognostic biomarker of supraglottic laryngeal carcinoma [57]. Inversely, Wu et al. examined the expression pattern of miR-19a in LSCC tissues by quantitative PCR and found miR-19a was overexpressed in LSCC compared with adjacent non-cancerous tissues. Furthermore, they found high miR-19a expression level was correlated with poor differentiation, lymph node metastasis or advanced clinical stages of LSCC. In addition, higher level of miR-19a was confirmed associated with decreased overall survival. These data indicated that miR-19a may contribute to the evaluation of the prognosis of LSCC patient [58]. MiR-21 and miR-375 were expressed at higher and lower levels, respectively, in the LSCC samples, compared to the normal samples. Further study showed patients with high miR-21 or low miR-375 expression in tumor tissues had poorer prognoses compared to patients with lower miR-21 or higher miR-375 expression. Favorably, the miR-21/miR-375 expression ratio was highly sensitive (0.94) and specific (0.94) for LSCC prediction. In conclusion, the expression ratio of miR-21 and miR-375 may serve as a potential biomarker with applications in the clinical setting of LSCC [59]. Zhang et al. have reported the expression level of miR-23a was significantly higher in the cancer tissues compared with normal adjacent laryngeal tissues, and patients with the elevated expression of miR-23a had a significantly greater extent of lymph node metastasis, worse clinical stage and shorter overall 5-year survival rate. Therefore, miR-23a may play a role in the prognosis prediction of LSCC [36, 60]. Danielle Maia et al. analyzed the differentially expressed-miRs in radioresistant LSCC and determined miR-296-5p was significantly related to radioresistance ($p = 0.002$) as well as an association of this marker with recurrence ($p = 0.025$) in early stage laryngeal cancer. This finding implied that miR-296-5p might serve as a prognostic marker regarding either the radioresistance or the prediction of recurrence of early stage LSCC [61]. It's surprising to find that miR-34c-5p was downregulated in LSCC and significantly correlated with worse disease-free and overall survival and increased risk of recurrence. These results suggest that low expression of miR-34c-5p in LSCC is an independent risk factor for disease-free survival and may be a potential biomarker for evaluating the risk of recurrences [62]. The reported potential prognostic miRs of LSCC are summarized in **Table 5**.

MiRs as therapeutic targets of laryngeal cancer

Recent data from pharmacological modulation of miRs in disease models supported that miRs are viable targets for therapeutics. For example, Wang et al. elucidated that miR-1 could affect the properties of growth, migration and invasion of Hep-2 cells via negatively regulating fibronectin 1 (FN1). Moreover, knocking down FN1 had the same anti-cancer effects in vitro as overexpression of miR-1. The above data implied that miR-1 may serve as a promising

Table 6. Summary of the potential therapeutic target miRs in laryngeal cancer

MiRs	Deregulation	Molecular mechanisms	Ref
miR-1	Downregulation	Suppresses the migration and invasion of LSCC cells by targeting fibronectin1	[63]
miR-129-5p	Downregulation	Inhibits growth and induces apoptosis in LSCC by directly targeting APC and possibly modulating STAT3	[41, 64]
miR-155	Upregulation	Enhances proliferation and invasion of human laryngeal squamous cell carcinoma via targeting SOCS1 and STAT3	[37]
miR-206	downregulation	Promotes proliferation and invasion of laryngeal cancer by regulating VEGF expression	[65]
miR-24	Downregulation	Functions as a tumor suppressor in laryngeal carcinoma partly through downregulation of the S100A8 protein	[66]
miR-30b	Downregulation	Improves the anti-tumor effect of p53-mediated gene therapy	[68]
miR-31-star, miR-1264, miR-3150b-5p and miR-210	Significantly expression altered	Their expressions are significantly changed after paclitaxel treatment	[67]
miR-93	Upregulation	Promotes LSCC cell proliferation, migration and invasion by directly targeting cyclin G2	[69]

therapeutic molecule deserving further research [63]. It's explained that miR-129-5p, which was downregulated in primary LSCC, had adverse effects on cell proliferation and migration, and caused cell cycle arrest in Hep-2 cell lines via targeting APC and modulating STAT3. And it alone was enough to induce apoptosis both in vivo and in vitro. More data suggested the growth of LSCC xenograft was markedly suppressed by miR-129-5p antisense oligonucleotides (ASO). These results effectively illustrated that miR-129-5p may be considered as a potent target in treatment of LSCC [41, 64]. On the contrary, miR-155 was reported significantly higher in LSCC tissues than those in the control mucosa tissues. Meanwhile, knockdown of miR-155 inhibited hep-2 cells growth, migration and invasion. Whereas the enforced expression of miR-155 enhanced hep-2 cells growth, migration and invasion through its downstream target of cytokine signaling 1 (SOCS1) and STAT3. Furthermore, the high level expression of miR-155 was closely correlated with T3 T4 stages, and poor/moderate cell differentiation. The current findings came to that miR-155 promised to be an anti-cancer target in LSCC [37]. Zhang et al. addressed that miR-206 held a lower level in LSCC tissues and aberrant expression of miR-206 was found to be inversely related with the T grade, nodal metastasis and clinical stage of LSCC. However, it's tested that the proliferation, migration, invasion and tumorigenesis in the LSCC cells were dramatically inhibited and apoptotic cells increased after miR-206 transfection. It remains more research concerning whether miR-206 could function as a therapeutic target in light of these paradoxical results [65]. Similarly, recent studies demonstrated that miR-24 was significantly lower in LSCC cells or tissues than adjacent normal tissues, and re-expression of miR-24 inhibited colony formation, enhanced apoptosis and improved LSCC sensitivity to irradiation by directly targeting X-linked inhibitor of apoptosis protein (XIAP) [49]. Guo et al. also observed that ectopic expression of miR-24 significantly induced cell morphology changes and inhibited cell proliferation and invasion by directly targeting S100A8 [66]. Based on these data, we suggested miR-24 may be a new molecular target for the treatment of LSCC. DNA microarray chips analysis showed that the expression of 49 miRs was significantly altered after the delivery of paclitaxel to LSCC patient, where the most significantly expression-changed ones were miR-31-star, miR-1264, miR-3150b-5p and miR-210, making these miRs be potential targets for paclitaxel resistance in LSCC [67]. In addition, miR-30b expression was significantly reduced in paracancerous tissues compared to surgical margins of LSCC and overexpression of microRNA-30b was well-proven to improve p53-mediated cell apoptosis obviously in LSCC via in vivo and in vitro experiments. Together, it's suggested that miR-30b could augment the anti-tumor effect of p53 gene therapy, which could provide an innovative approach for treatment in LSCC [68]. Xiao et al. have proved that miR-93, a member of the miR-106b-25 cluster, was significantly upregulated in LSCC. Moreover, in vivo and in vitro experiments demonstrated that the overexpression of miR-93

enhanced cell proliferation, migration and invasion and decreased apoptosis rates, induced cell cycle arrest by directly targeting cyclin G2 (CCNG2), which was a ubiquitous cell cycle progression inhibitor of G1 to S-phase transition. It seems likely that the key role of miR-93 is to repress several elements of the developmental program that may contribute to the treatment of LSCC if subverted [69]. The reported potential therapeutic miR targets of LSCC are summarized in **Table 6**.

Conclusion

In view of more and more miRs have been reported to unlock the mysterious door of special diagnosis and therapy of laryngeal cancer, the relatively tissue/cell specificity and the network of proteins they regulated render them promising targets for clinical medicine. It is still necessary to further explore the precise mechanisms of miRs action and whether such modest regulators can indeed be regulated in pathways of laryngeal cancer in a tissue-specific manner. Up to now, due to the limitation of measuring extracellular miRs technologies, the standardized technology of measuring these molecules intracellular and extracellular needs to be developed. While in the therapy field, several crucial issues must be addressed, such as accuracy of targets, safety and efficiency of miRs-based drug delivery. Given these challenges concerned above, the growing improvements of miRs in laryngeal cancer remain encouraging and promising.

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Disclosure of conflict of interest

None.

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