The functional role of microRNAs in laryngeal carcinoma

Laryngeal carcinoma is one of the most common malignancies in the head and neck. Laryngeal cancer is the eleventh most common cancer worldwide and accounts for 2.4% of new malignancy every year [1]. Laryngeal squamous cell carcinoma represents about 85% to 90% of all larynx tumors [2]. Perhaps because intractable factors such as lymph node metastasis affect the prognosis of laryngeal cancer, the survival rate of advanced laryngeal cancer has not appreciably improved in the past 30 years, remaining around 30% to 40% [3]. Traditional surgical treatments, radiotherapy and chemotherapy have not been able to improve the overall survival rate of patients with laryngeal cancer, which has even declined in some groups of patients [4]. The occurrence of laryngeal carcinoma is associated with activation of oncogenes (such as BCL2, c-MYC) and inactivation of tumor suppressor genes (such as p53, RB) [5-8]. However, the molecular mechanism of laryngeal cancer is still unclear. MicroRNA (miRNA) is a small molecule non-coding RNA with a length of about 19-25 nucleotides that regulates gene expression by binding to messenger RNA, leading to transcript degradation or translational inhibition [9]. Bioinformatics analysis predicts that miRNA regulates more than 30% of protein-coding genes. The continued accumulation of evidence suggests that abnormal expression of miRNAs is associated with a variety of malignancies, including laryngeal squamous cell carcinoma [10]. In this review, we will survey some of the research progress on miRNAs involved in the development of laryngeal cancer.

2 Abnormal expression of miRNAs in laryngeal carcinoma

Laryngeal cancer is one of the most common malignancies in the head and neck, and a variety of aberrant miRNAs involved in its development. Cao et al. [11] screened the expression of miRNAs in 6 tissue pairs (laryngeal squamous cell carcinoma tissue and adjacent normal tissue) using miRNA array. They found differences between the tissues involving 29 miRNAs, of which 6 were confirmed, including upregulation of miR-21, miR-93, miR-205, and miR-708 and downregulation of miR-125b and miR-145. In 48 pairs of laryngeal carcinoma and adjacent normal tissue, six miRNAs were confirmed, including upregulation of miR-21, miR-93, miR-205 and miR-708, and downregulation of miR-125b and miR-145 [11]. Sun et al. found 38 miRNAs had abnormal expression in laryngeal squamous cell carcinoma tissue, 22 of which were upregulated and 16 of them were downregulated [12]. Lu et al. found that miR-21-3p and miR-106b-3p were upregulated and let-7f-5p, miR-10a-5p, miR-125a-5p, miR-144-3p, miR-195-5p, and miR-203 were downregulated in freshly frozen laryngeal carcinoma tissue compared with adjacent normal tissue.
with surrounding normal tissue specimens by using microarray technology combined with RT-PCR [13]. These results show that there are many abnormal expressions of miRNA in laryngeal carcinoma and miRNAs may play an important role in the development and progression of laryngeal carcinoma.

3 MiRNAs and the initiation and development of laryngeal carcinoma

The molecular mechanism of the initiation and development of laryngeal carcinoma has not been entirely illuminated. A large number of related genes appear to be involved in the progression of laryngeal carcinoma. MiRNAs can regulate the expression of a variety of related genes, so the miRNAs may play the role of oncogene or tumor suppressor gene. Some miRNAs are upregulated in laryngeal carcinoma and may function as oncogenes. For example, some upregulated miRNAs are able to promote cancer cell proliferation. In contrast, some miRNAs in laryngeal squamous cell carcinoma may be downregulated and these have the functions of tumor suppressor genes. The abnormal expression of microRNAs is closely associated with the development, metastasis and prognosis of laryngeal carcinoma.

4 MiRNAs promote the progression of laryngeal cancer

There have been many studies of the function of miR-21 in laryngeal cancer. The miR-21 is located in the chromosome fragile region 17q23.2 and can regulate the expression of multiple target genes that accelerate the progression of laryngeal cancer. Ren et al. found that the migration ability of Hep-2 cells significantly decreased after inhibiting the expression of miR-21 by transfecting antisense oligonucleotides (ASO), while ASO-miR-21 could inhibit the expression of MMP-2 and Ras protein, leading to cell cycle arrest [14]. B cell translocation gene 2 (BTG2) is a kind of cell cycle regulator and tumor suppressor that can inhibit the proliferation of tumor cells [15]. The abnormal expression of miR-21 was able to promote the cancer process by downregulating the expression of BTG2 in laryngeal squamous cell carcinoma. In vitro experiments have confirmed that increased expression of miR-21 in Hep-2 cells could enhance its proliferation ability. However, miR-21 inhibition caused cell number reduction, which was due to disordered G1-S phase transition [16]. Additionally Liu et al. [16] found that miR-21 could also inhibit the proliferation and invasion ability of laryngeal cancer cells by negatively regulating the expression of tumor suppressor gene PTEN. The results showed that miR-21 was negatively correlated with PTEN expression [17]. PTEN is able to inhibit cell migration and invasion, and induce apoptosis as well. The main target of PTEN is PI3K. MiR-21 can inhibit expression of PTEN by targeting 3'UTR of PTEN mRNA. However, PTEN can block PI3K / AKT bypass and inhibit cancer cell growth, migration, and invasion. It has been found that miR-1297 is overexpressed in laryngeal carcinoma tissue and Hep-2 cell lines. The miR-1297 also plays a role in carcinogenesis by regulating the expression of tumor suppressor gene PTEN. Reducing expression of miR-1297 can inhibit cancer cell proliferation, migration and tumorigenesis [18]. These results suggest that a complex miRNA gene regulatory network is involved in the development of laryngeal cancer. MiRNAs suppress gene expression by binding to mature mRNA transcripts and promoting mRNA degradation, by inhibiting translation, or both. A miRNA can regulate multiple target genes and one gene can be regulated by multiple miRNAs.

5 MiRNAs inhibit laryngeal cancer cell proliferation

In contrast to upregulated miRNAs, downregulated miRNAs play a tumor suppressor role, and are more involved in complex regulatory networks. MiR-203 is an antitumor miRNA that is downregulated in laryngeal squamous cell carcinoma. Its expression is inversely related to the expression of the survivin gene [19]. Survivin is a typical inhibitor of cell apoptosis, which plays a role by inhibiting activity of caspase-3 and caspase-7. Survivin has little or no expression in normal cells but has high expression in a variety of tumor cells. Survivin is a specific target molecule of miR-203 [20]. After transfecting miR-203, the expression of survivin will be downregulated, which will induce cell apoptosis, inhibit cell proliferation, and reduce tumor invasion [21]. Additionally members of the miR-34 family can regulate survivin protein too, functioning as tumor suppressors, including miR-34a, miR-34b and miR-34c [22]. They are a class of highly conserved miRNAs in which miR-34a and miR-34c negatively regulate survivin proteins and their expression levels are associated with tumor differentiation, lymph node metastasis, clinical stage, and survival. MiR-34a mimics significantly suppressed cell proliferation by arresting cells at G0/G1 phase in Hep-2 cells [23]. GALNT7 is another target gene
for miR-34a and miR-34c, regulated by miR-34a and miR-34c. GALNT7 plays a key role in the migration and pre-invasion behavior of cancer cells. MiR-34a and miR-34c also play a role in suppressing cancer by downregulating the expression of GALNT7 [24]. MiR-519b-3p and miR-519a have been found to be downregulated in laryngeal squamous cell carcinoma. It has been reported that elevated expression of miR-519b-3p significantly inhibited Hep-2 cell proliferation and decreased the percentage of cells in the S phase [25]. After increase in the level of microRNA-519a in laryngeal squamous cell carcinoma human epithelial type 2 cells, cell growth was inhibited and the cell cycle was arrested in the G2/M phase, which was attributed to downregulated expression of HuR gene [26]. In head and neck squamous cell carcinoma (HNSS), downregulation of miR-874 is a frequent event. Expression level of miR-874 was significantly downregulated in HNSCC tissues (including oral, pharyngeal and laryngeal SCCs) compared with normal epithelia. Luciferase reporter assays showed that miR-874 directly regulated HDAC1, and silencing of the HDAC1 gene leads to inhibition of cell proliferation and induced G2/M arrest and cell apoptosis in SAS cells [27]. MiR-205 was reported to be involved in the proliferation and apoptosis of laryngeal squamous cell carcinoma (LSCC). MiR-205 was able to downregulate the proliferative markers of dihydrofolate reductase and proliferating cell nuclear antigen and apoptotic regulator of Bcl-2 [28]. MiR-1 and miR-206 have been thought to be muscle-specific miRNAs, but some studies have shown that the two miRNAs are downregulated in laryngeal squamous cell carcinoma [29,30]. Fibronectin 1 (FN1) is an extracellular matrix glycoprotein that plays an important role in the development of tumor [31]. FN1 is a direct target gene of miR-1, which inhibits the growth, migration and invasion of laryngeal cancer cells by negative regulation of FN1. The expression level of miR-206 is negatively correlated with TNM staging of laryngeal carcinoma. The tumor-suppressing effect is mainly caused by the regulation of vascular endothelial growth factor (VEGF).

6 MiRNAs are associated with differentiation and metastasis of laryngeal carcinoma

Abnormal expression of miRNA is also associated with differentiation, metastasis and prognosis of laryngeal carcinoma, such as the miR-21 and miRNA-519a and so on. MiRNA-155 was upregulated in laryngeal squamous cell carcinoma and was significantly higher in well-differentiated carcinoma [32]. MiRNA-155 is located on chromosome 21 and interacts with the downstream target gene SOCS1-STAT3. The elevated expression of miR-155 is associated with downregulation of SOCS1 and increased STAT3 expression. The activation of STAT3 can promote the development and differentiation of laryngeal squamous cell carcinoma [33]. This suggests that miR-155 exerts a carcinogenic effect by regulating the expression of the target gene SOCS1-STAT3. Expression level of miR-106b is also associated with the proliferation and metastasis of laryngeal cancer cells [34]. MiR-106b downregulates the expression of RUNX3 by binding to the 3'-UTR region of RUNX3 gene. RUNX3 is a tumor suppressor. The abnormal methylation of RUNX3 CpG island and translation silencing can lead to the proliferation and metastasis of laryngeal cancer cells [35]. The downregulation of miR-126 is also associated with prognosis in laryngeal squamous cell carcinoma. Deficiency in miR-126 induces aberrant expression of Camsap1. Abnormal upregulation of Camsap1 can promote the migration and invasion of tumor cells by promoting the formation and aggregation of microvessels [36]. In addition, reduced expression of miR-203 is associated with overall 5-year survival rate, clinical stage, and lymph node metastasis [21]. The above results show that the abnormal expression of miRNAs are closely related to metastasis and differentiation, as well as clinical stage of laryngeal cancer. Detecting the expression level of these miRNAs will provide a reference value for the prognosis of laryngeal cancer patients.

7 MiRNAs and diagnosis of laryngeal cancer

At present, the invasive endoscopy combined with pathological biopsy diagnosis technology gives patients a high degree of discomfort, which hinders the large scale screening of laryngeal cancer. With such shortcomings of diagnostic techniques, most laryngeal cancer patients are middle or advanced stage at time of diagnosis. Early detection and early treatment can significantly improve the survival rate and cure rate of patients. Therefore, it is imperative to find effective tumor markers for early diagnosis. Existing studies indicate miRNA is a very effective new tumor marker. It has been found that miR-657 and miR-1287 are highly sensitive and specific for the identification of early laryngeal, which indicates that miR-657 and miR-1287 expression levels have potential as a method of non-invasive early screening and early diagnosis of laryngeal cancer [37]. In addition, Ayaz and his colleagues found that 17 miRNAs were expressed only
in laryngeal squamous cells, and five miRNAs (miR-331-3p, miR-603, miR-1303, miR-660-5p and miR-212-3p) were first found in plasma. These data indicates that these miRNAs may be peculiar to laryngeal cancer [38]. Detection of these miRNAs associated with laryngeal squamous cells in plasma will provide convenience for diagnosis of laryngeal cancer. Detection of miRNA expression level also helps to identify the metastasis potential of laryngeal cancer and therefore predict prognosis. For example, the expression level of miR-16 is related to the migration ability of laryngeal carcinoma cells [39]. Zyxin is a type of tumor suppressor gene associated with the movement of cells [40]. In laryngeal carcinoma, miR-16 reduces cell adhesion by negative regulation of zyxin, and further promotes the metastasis of laryngeal carcinoma. Detection of specific or abnormal expression of miRNA will provide an important reference for the early diagnosis of laryngeal cancer.

8 Prospects

The incidence of laryngeal cancer increases year by year, with little new progress in treatment. Some studies have found that miRNAs, as gene regulatory regulators, play an important role in development, diagnosis and treatment of cancer. Due to the complex regulatory network of miRNAs, functional research of miRNAs is premature in laryngeal cancer. Therefore, in-depth study of miRNAs involved in laryngeal cancer will not only help to explore molecular mechanism of laryngeal cancer, but also provide new molecular markers for the early diagnosis of laryngeal cancer.

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