The functional role of exosome microRNAs in lung cancer

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Abstract: Lung cancer causes the highest incidence and mortality rates of cancer disease worldwide. Despite obvious advances in lung cancer research, a better understanding of the disease is urgently needed to improve early detection and correct diagnoses. Exosomes are released from cancer cells and modulate cell-cell communication. Exosomes transfer a wide variety of molecules including microRNAs. MicroRNAs (miRNAs) are single-stranded, small non-coding RNAs that regulate gene expression. Accumulating evidence indicates that miRNA expression patterns represent the status of physiology and disease. The focus of this review is to provide an update on the progress of miRNAs of cancer-derived exosome as potential biomarkers for lung cancer.

Keywords: exosome; microRNA; lung cancer; biomarker

Introduction

Lung cancer is the leading cause of cancer death worldwide [1]. Lung cancer is divided into two categories of non-small cell lung cancer (NSCLC) and small cell lung cancer. The clinical incidence of NSCLC is up to 75% [2]. Due to the lack of early detection of NSCLC, more than 80% of patients were diagnosed during the middle and late term [3]. Despite the continuous improvement of medical technologies such as surgical resection, adjuvant radiotherapy and chemotherapy, molecular targeted therapy and immunotherapy, the 5-year survival rate of NSCLC patients is only 17% [4]. Therefore, there is a pressing need to find more promising treatment methods to improve the survival rate and quality of life of patients with NSCLC. In addition, we need to find new biomarkers to surveil the development of lung cancer.

MicroRNAs (miRNAs) are a class of small, noncoding RNAs, which regulate post-transcriptional gene expression by binding to the 3′-UTR or ORF region of target mRNAs [5]. Many biological functions of miRNAs has been well elucidated, including involved in the processing of cancer, such as apoptosis, metabolism, differentiation [6]. Much evidence has indicated that miRNAs are able to be packed into exosomes, which will contribute to guaranteeing their stability and protect miRNAs from degradation [7].

Exosomes are 30-100 nm membrane vesicles of endocytic origin that are secreted by most cells [8]. Exosomes are present in body fluids including plasma, urine, saliva, and malignant effusions [9]. After the receptor cells uptake exosomes, the signal substances of exosomes, including proteins, lipids, miRNAs and mRNAs, are also transferred into the recipient cells, thereby altering the molecular composition of receptor cells. Recently, the origin, structure and function of exosomes have been investigated more thoroughly by using proteomics and genomics. The study of exosomes in cancer detection, diagnosis and treatment has become a hot topic. Accumulating evidence indicates that miRNA expression patterns of cancer-derived exosomes represent physiological and pathological conditions in the progression of cancer. What’s more, miRNAs are stable in the cancer-derived exosome, so they are being developed as biomarkers for cancer. The focus of this review is to provide an update on the progress of miRNAs as potential biomarkers for lung cancer.

2 Exosomes and lung cancer

The occurrence of lung cancer is a multi-step process. A variety of epigenetic and genetic changes are involved in the progression of lung cancer. Cancer-derived exosomes have multiple functions in the occurrence and developmental processes of lung cancer.
2.1 Exosomes mediate lung cancer immune escape

Exosomes are involved in the immune regulation of tumors and play an important role in the development and progression of NSCLC. The epidermal growth factor receptor (EGFR) carried by exosomes of lung cancer can induce immune-resistance of dendritic cells and CD8+ T cells inhibited by tumor-specific regulatory T cells, which will promote immune escape of tumor cells [10].

2.2 Exosomes mediate lung cancer invasion – metastasis

Exosomes mediate tumor microenvironment interaction with cancer. Exosomes can transmit mutant EGFR into tumor-associated vascular endothelial cells and activate MAPK signaling, which will promote endothelial cell proliferation [11]. Studies by Wang et al found that TGF-β and IL-10 from cancer-derived exosomes are also involved in the regulation of cell proliferation and metastasis and their high expression can promote lung cancer cell growth and metastasis [12]. Rahman et al showed that exosomes that are from high invasive potential lung cancer cells can induce the expression of vimentin in recipient cells, which will drive the recipient cells to undergo epithelial transformation, resulting in upregulation of invasion and migration ability [13].

2.3 Exosomes mediate lung cancer resistance

Cancer-derived exosomes may also mediate cisplatin chemotherapy resistance of lung cancer. In vitro experiments showed that a large number of exosomes released from A549 cells of cisplatin resistance were uptaken by other peripheral A549 cells to induce intercellular transmission of drug-resistant substances [14]. Exosomes derived from gefitinib-treated PC9 cells decreased the antitumor effects of cisplatin and inhibition of exosome secretion resulted in a modest synergistic effect when cisplatin and gefitinib were co-administered [15].

3 MiRNAs and lung cancer

3.1 Application of miRNA in the diagnosis of lung cancer

The miRNA expression profiles are different between lung cancer tissues and normal tissues, which makes it possible to use miRNA detection for early diagnosis of lung cancer. Yanaihara et al used miRNA gene chips to analyze 104 cases of non-small cell lung cancer tissue and normal lung tissue, and found that 15 miRNAs were up-regulated and 28 miRNAs were down-regulated [16]. Studies have shown that five miRNA combinations (miR-34c-5p, miR-34a, miR-25, miR-191 and let-7a) can accurately differentiate lung adenocarcinoma and lung squamous cell carcinoma [17]. Lebanean et al found miR-205 is a highly specific biomarker of lung squamous cell carcinoma by comparing expression profiles of 122 lung cancer cases (62 squamous cell carcinoma cases, 60 adenocarcinoma cases) [18].

3.2 Application of miRNA in the prognosis of lung cancer

The current study has found that miRNA can also be used as a prognostic indicator of lung cancer. Takalmzawa et al found that lung cancer patients with reduced let-7 expression showed shorter survival than patients with overexpression let-7 [19]. Yanaihara et al found that high expression of hsa-miR-31 was associated with poor survival in 60 squamous cell carcinoma patients using Kaplan–Meier analysis [16]. Chen Y et al found that low expression of miRNA-148a was strongly associated with high tumor grade, lymph node (LN) metastasis of lung cancer [20].

4 Exosomal miRNAs and lung cancer

4.1 Exosomal miRNAs mediate lung cancer invasion – metastasis

Exosomes contain a large number of bioactive substances, such as protein, messenger RNA (mRNA), microRNA (miRNA) and lipids [21]. Exosomal miRNAs are widely involved in the regulation of lung cancer invasion and metastasis [22]. As such, miR-126 has been found to reduce adherence, migration and invasion of lung cancer cells by regulation of Crk linker proteins. In addition, miR-126 is able to regulate stromal cell-derived factor 1α to reduce mesenchymal stem cells and inflammatory monocytes recruited by cancer cells [23]. Gibbons et al found that the forced expression of miR-200 downregulated the capacity of lung cancer cells to undergo epithelial-to-mesenchymal transition (EMT), invade, and metastasize [24].
4.2 Exosomal miRNAs as diagnostic and predictive biomarkers in lung cancer

The function of exosomal miRNAs to distinguish lung cancer patients from healthy individuals has been shown in some studies. Cazzoli et al used 30 plasma samples, including 10 from lung adenocarcinoma patients, 10 from lung granuloma patients and 10 from healthy smokers matched for age and sex as negative controls to perform microRNAs analysis. They found that miR-378a, miR-379, miR-139-5p and miR-200b-5p can distinguish the lung adenocarcinoma group from that of healthy former smokers [25]. Munagala et al found that miR-21 and miR-155 were significantly upregulated in exosome of recurrent lung cancers compared to primary cancers [26]. Liu and colleagues used a quantitative polymerase chain reaction (qPCR) array panel to analyze 84 plasma exosomal miRNAs in 10 lung adenocarcinoma patients and 10 matched healthy controls. The results showed that increased levels of exosomal miR-23b-3p, miR-10b-5p and miR-21-5p were independently related to poor overall survival [27]. Rabinowits et al evaluated the expression levels of specific exosomal miRNAs in patients with and without lung adenocarcinoma and they though circulating exosomal miRNA might be useful as an acceptable marker for diagnosis and prognosis in patients with lung cancer [28]. Dejima and colleagues investigated expression profiling of miRNAs derived from plasma exosome based microarrays. In their miRNA microarray analyses, the exosomal miR-21 and miR-4257 levels of the lung cancer patients showed obvious upregulation compared with those without recurrence and healthy individuals. In addition, they found that exosomal miR-21 was significantly associated with tumor size and tumor-node-metastasis (TNM) stage and exosomal miR-4257 showed an obvious association with lymphatic invasion, histological type and TNM stage of lung cancer. Additionally, the disease-free survival (DFS) rates of high expression exosomal miR-21 patients were significantly worse than those of low exosomal miR-21 patients, and the DFS rates of patients with high exosomal miR-4257 levels were significantly worse than those with low exosomal miR-4257 levels [29].

5 Conclusions

Lung cancer is one of the most common malignancies all the world. To improve the survival rate of lung cancer patients, we need to find more effective diagnosis and treatment pathways. The characteristics of lung cancer are slow cell diffusion and metastasis. Because of a lack of early screening and atypical symptoms, more than 80% of patients were diagnosed in the middle and late terms [30]. This also means that lung cancer patients missed the best treatment time, which led to unsatisfactory treatment and prognosis [31]. To date, the serum markers of lung cancer include cytokeratin 19 fragments, squamous cell carcinoma antigens, carcinoembryonic antigens, neuron-specific enolases, sugar chain antigens and so on [32]. But these markers have limited significance for the diagnosis of lung cancer. Clinicians usually rely on the pathological results of invasive tissue biopsy to diagnosis lung cancer. Therefore, we urgently need to find more effective serum tumor markers.

The tumor-derived exosome has multiple functions including immune suppression, promoting tumor growth, metastasis and drug resistance. Some researchers suggest that tumor-derived exosome is one of cancer pathogenesis. Tumor-derived exosome activates the tumor metastasis signal through enhancing the expression of proinflammatory factors, such as TAK1, which is a central target of exosome-mediated miRNA and is associated with the spontaneous development of hepatocellular cancer related to aberrant responses to inflammatory [33]. The exosome protects the MiRNA from degradation of RNAase, which contributes to maintaining the biological activity of MiRNA as a disease diagnostic marker [34]. The MiRNA is a kind of endogenous non-coding small molecule, which involves many physiological processes including cell differentiation, proliferation, apoptosis and so on. Many studies have shown that miRNAs play an important role in immune response, neurodevelopment and DNA repair [35]. According to the located cellular environment of miRNAs and their target genes, more and more studies have suggested that miRNAs play an important role in oncogenes or tumor suppressor genes [36]. Their aberrant expression is closely related to a variety of human tumors, such as lung cancer, gastric cancer, colorectal cancer, thyroid cancer, breast cancer [37]. The occurrence and disease process of lung cancer are accompanied by dynamic changes of miRNA expression levels [38]. However, abnormal expression of miRNAs in a variety of tumors does not imply poor specificity of miRNA. In contrast, miRNAs are expressed in specific tissues and at specific stages. From a large-scale miRnome analysis on 540 samples including lung, breast, stomach, prostate, colon, and pancreatic tumors, miRNA signatures are composed of a large portion of overexpressed miRNAs [39]. In terms of lung cancer, there are currently specific miRNA expression profile databases. The TCGA program is a joint project initiated by the National Cancer and
Oncology Institute (NCI) and the National Human miRNA Group (NHGRI) in 2006, which includes mRNA and miRNA expression profiles, copy number variants, mutations and other large-scale data. TCGA showed miR-182, miR-451, let-7 was an obvious abnormal expression in lung cancer.

Over the past decade, rapid progress has been made in miRNA and lung cancer research. Accumulated studies have identified some miRNAs with differential expression levels in lung cancer tissues compared to normal tissues. Meanwhile, abnormal expression profiles of miRNAs in lung cancer patients are not only found in tumor tissues but also in serum exosomes. Several studies suggest that miRNAs are promising biomarkers for the diagnosis and prediction of lung cancer. However, before they can be applied by clinicians, there are still some problems that need to be solved: we find numerous miRNA candidates whose expression varies in lung cancer tissue compared to the normal tissue or exosomes compared to the healthy persons by using miRNA array analysis, but we do not have a gold standard to evaluate their effectiveness. Although exosomal miRNAs may be promising diagnostic markers for lung cancer, we do not have a standard method for isolating exosomal miRNAs. In summary, the study of exosome miRNAs offers a new strategy, which will contribute to identify the molecular mechanisms of lung cancer biology. Further studies are still needed to find more accurate biomarkers of lung cancer.

Conflict of interest: Authors state no conflict of interest

References

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[34] Lesley C., Robyn A.S., Benjamin J.S., Andrew F.H., Exosomes provide a protective and enriched source of miRNA for biomarker profiling compared to intracellular and cell-free blood, Journal of extracellular vesicles, 2014, 3.


