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Research Progress of microRNA in the Diagnosis of Tuberculous Pleurisy

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Abstract

Tuberculosis has become a major public health and social problem threatening human health, and a large proportion of pulmonary tuberculosis patients are associated with tuberculous pleurisy (TP). Therefore, it is of great significance to find markers with high specificity and sensitivity for the rapid and accurate diagnosis and differential diagnosis of TP under the severe background of high infectivity and mortality due to the occult nature of TP. The extraction of microRNA (miRNA) from pleural effusion satisfies the characteristics of strong operability. miRNA exists not only in cells, but also in various body fluids and participates in the pathophysiological process of various diseases including infectious diseases. miRNA is a highly specific biomarker in pleural fluid in patients with TP. Therefore, this article provides a review of the research progress of mRNA in tuberculous pleurisy.

Keywords

Tuberculous, Pleurisy, miRNA, Diagnosis

1. Introduction

Tuberculosis has become a major public health and social problem threatening human health, and it is the deadliest infectious disease killer in the world. Among the 22 high-burden countries in the world announced by WHO, China ranks second in the world [1], and a large proportion of pulmonary tuberculosis patients are associated with tuberculous pleurisy (TP). Therefore, it is of great significance to find markers with high specificity and sensitivity for the rapid and accurate diagnosis and differential diagnosis of TP under the severe background of high infectivity and mortality due to the occult nature of TP.

At present, the diagnosis of TP mainly relies on medical history, clinical manifestations, imaging, routine examination of pleural effusion and observation of curative effect, and the diagnosis rate is low, especially the differential diagnosis from malignant pleural effusion is difficult [2]. Therefore, finding a method with high sensitivity, specificity, and simple and fast operation is of great significance for diagnosing TP. The extraction of microRNA (miRNA) from pleural effusion satisfies the characteristics of strong operability. miRNA exists not only in cells, but also in various body fluids and participates in the pathophysiological process of various diseases including infectious diseases [3] [4]. miRNA is a highly specific biomarker in pleural fluid in patients with TP, and Mycobacterium tuberculosis (Mtb) can regulate cytokine expression and host immune response by affecting the expression of miRNA in host cells [5]. If miRNA changes can be accurately grasped at an early stage, it can not only solve the diagnosis and treatment problems of patients by directly improving the detection rate of TP, but also help improve the development and prognosis of TP patients. Therefore, this paper attempts to make a breakthrough in the study of miRNA expression in pleural fluid, analyze on the basis that miRNA expression is involved in regulating host innate and adaptive immune response anti-Mtb, and then propose targeted solutions and methods, so as to achieve the purpose of "early detection, early diagnosis, and early treatment", and bring good news to TP patients.

In the past ten years, more and more scholars have carried out a large number of experiments and studies on miRNA, especially to explore its diagnostic direction and diagnostic value in respiratory diseases. As early as 2012, domestic research on the expression and significance of miRNAs in the peripheral blood of TP patients found that miRNAs may be closely related to the occurrence of TP and may become one of the targets of tuberculous pleural effusion [6]. Immediately afterwards, scholars proposed that miRNA, as a potential new biomarker for diagnosing tuberculosis [5], some fragments of miRNA expression are upregulated in tuberculosis patients, and some fragments show a downward trend of expression in experimental data, while other fragments express different views in different parts of tuberculosis patients. In the same year, another study of the value of miRNA-144 in the diagnosis and prediction of efficacy of tuberculous pleural effusion was conducted, and the expression of miRNA-144 in blood and pleural fluid helped distinguish tuberculous pleural effusion from malignant pleural effusion, and changes in blood expression of miRNA-144 helped predict the efficacy of TP [7]. From 2016 to 2018, some scholars screened the microRNA internal reference genes in tuberculous and malignant pleural effusions in literature [8], and found that Cel-miR-39 had good stability and moderate expression abundance, and Cel-miR-39 was suitable as an internal reference gene for the quantitative detection of miRNA expression in pleural effusions by real-time fluorescence quantitative PCR. This is the relationship between miRNA and TP that the authors have determined in the early literature, which has promoted the development of TP diagnosis to a certain extent. In 2022, literature has shown

[9] that there are significant differences in the expression of miRNAs in serum and pleural fluid exosomes in TP patients, especially members of the hsm-let-7 family are expected to become potential biomarkers for the diagnosis and treatment of TP. Exosome miRNAs may be involved in the development of diseases through signaling pathways such as MAPK and RAS. Coincidentally, diagnostic research on miRNA not only appears in TP, but also plays a role in the pathogenesis of lung cancer. The study of the specific mechanisms of different SNPs (miRNA-SNPs) in miRNAs lays the foundation for cancer treatment [10]. Two years later, other scholars also pointed out in the literature that miRNA disease characteristics can be used as a non-invasive method to objectively predict lung cancer lymph node metastasis in lung adenocarcinoma patients, and the determination of miRNA and pathway can be used as potential therapeutic targets for lung cancer treatment [11] and provide insights for future clinical applications.

In China, the number of patients with tuberculosis is large, and in actual clinical work, the diagnosis of TP is mostly clinical; however, the gold standard is to find Mtb in pleural effusion or pleura, but Mtb is usually difficult to detect in pleural effusion and pleura, and there is a lack of biochemical indicators with better specificity and sensitivity. It is often difficult to differentially diagnose atypical TP from other infectious pleural diseases and pleural effusions due to lung malignancies. Therefore, finding biomarkers with higher sensitivity and specificity is of greater value for the diagnosis of TP.

MiRNAs are a class of non-coding single-stranded RNA molecules encoded by endogenous genes with a length of approximately 22 nucleotides that are involved in the regulation of post-transcriptional gene expression in plants and animals. miRNAs can regulate target mRNA by disrupting the stability of target mRNA and inhibiting the translation of target mRNA. The breadth and diversity of miRNAs suggests that they may have a very wide variety of biological functions. Also, because the levels of miRNAs differ significantly in different tissues and developmental stages, this pattern of miRNAs expression has a differentiated loci and temporal sequence, suggesting that miRNAs have the potential to be important as molecules involved in the regulation of gene expression. MiRNA has good stability, anti acid and alkali, anti enzyme degradation, anti repeated freezing and thawing and other extreme conditions, and is a biomarker of great significance for disease diagnosis, treatment and prognosis monitoring [12].

2. Peripheral Blood and Pleural Effusion miRNA Diagnosis TP

Zhang Fan and Liu Shoujiang *et al.* found that peripheral blood miR-365a-3p expression was significantly elevated in TP patients and may be involved in the development of inflammatory response in TP. literature reported that in the two groups of peripheral blood miR-365a-3p expression, peripheral blood miR-365a-3p expression was significantly higher in patients with tuberculous pleurisy group than in healthy controls, and the difference was statistically sig-

nificant [6].

Zhang Fan and Liu Shoujiang also found that miRNA144-3p may also be involved in the occurrence and development of TP, and may have some clinical significance in the diagnosis of TP. It was reported in the literature that the expression of miRNA144-3p in peripheral blood group of combined pleurisy was significantly lower than that in peripheral blood group of patients with latent tuberculosis infection and healthy controls ($P < 0.05$), while there was no significant difference between peripheral blood groups of patients with latent tuberculosis infection and healthy controls [13].

Gao Jingli, Cui Junwei and others found that miR-29a may also play an important role in diagnosing TP. ROC curve analysis showed that the AUC of miR-29a in diagnosing TPE was 0.903 (95% CI: 0.853 - 0.954), the sensitivity and specificity were 90.50% and 79.70%, respectively. The AUC of miR-29a combined with ADA in diagnosing TPE was up to 0.943, the sensitivity and specificity were 94.90% and 92.40%, respectively, indicating that the efficacy of miR-29a combined with ADA in diagnosing TPE was significantly increased. The literature reports that miR-29a combined with ADA detection in pleural effusion has significant clinical diagnostic efficacy for tuberculous pleurisy [14].

3. miRNA Differentiates Tuberculous Pleural Effusion from Malignant Pleural Effusion

Dong Jing *et al.* found that Cel-miR-39 has the best stability and moderate expression abundance, which is suitable as an internal reference gene for quantitative detection of miRNA expression in pleural effusion by real-time fluorescent quantitative PCR. Among the 78 specimens, the Ct values detected by 7 internal reference genes miR-1268, Cel-miR-39, miR-16, miR-192, miR-20a, miR-4281 and U6 were 19.16 ± 4.40, 24.17 ± 0.73, 24.52 ± 1.65, 27.54 ± 1.36, 28.47 ± 1.72, 28.58 ± 2.09 and 30.3 ± 1, respectively 1.56; amplification efficiencies of 90%, 101%, 110%, 93%, 97%, 97%, and 90%, respectively, can be used for subsequent miRNA detection of pleural effusion; Moreover, the melting curves of the seven internal reference genes were all smooth and had only a single main peak, showing good specificity [8]. Jiao Xin and Liu Ning found that the expression level of miR-144 in blood and pleural effusion in TP patients was higher than that in patients with malignant pleural effusion, and the expression level of miR-114 in blood and pleural effusion was clinically detected to help distinguish tuberculous pleural effusion from malignant pleural effusion, miR-144 in blood may be a marker reflecting the severity of disease in TP patients, and dynamic monitoring of miR-144 changes can predict the disease outcome of TP patients. The expression of miR-144 in pleural effusions in patients with tuberculous pleural effusion and malignant pleural effusion by RT-PCR was reported. The results showed that the expression of miR-144 in plasma of patients with tuberculous pleural effusion (1.74 ± 0.43) was significantly higher than that of patients with malignant pleural effusion (1.05 ± 0.46), with a statistically significant dif-

ference ($P < 0.05$), and the expression of miR-144 in pleural effusion in patients with tuberculous pleural effusion (2.53 ± 0.83) was significantly higher than that in patients with malignant pleural effusion (1.77 ± 0.63), and the difference was statistically significant ($P < 0.05$). The expression of miR-144 in plasma before and after treatment was compared, and the results showed that the expression of miR-144 (1.01 ± 0.38) of patients whose symptoms improved significantly after one week of treatment decreased significantly, compared with that before treatment (1.74 ± 0.43), the difference was statistically significant ($P < 0.01$), the expression of miR-144 (1.70 ± 0.42) in patients whose symptoms did not improve after one week of treatment did not decrease significantly, there was no statistically significant difference compared with pretreatment) [7].

4. Challenges Faced by miRNA in Disease Detection

With the development of research technology, we have also discovered the limitations of miRNA in disease diagnosis. At present, miRNA has not been used as a common diagnostic detection method in clinical practice, which may be related to the low expression of miRNA in peripheral blood or pleural effusion, complicated PCR technology operation, long research process, many consumables, and high cost.

However, with the development of gene sequencing, more and more people have turned their attention to the research of miRNA. At present, the detection technology of miRNA is relatively mature, and the extraction amount of miRNA with low expression has been improved, and the sensitivity of PCR technology to miRNA has been improved.

Through the analysis of the current situation of miRNA research, we can find that although the detection of miRNA still faces a series of problems and challenges, with the development of medical science and technology, human beings pay more attention to diseases, and miRNA is a highly sensitive and Specific biomarkers are of great significance for the diagnosis of various diseases. In addition to the analysis of the current status of miRNA research, the status and deficiencies of clinical diagnosis of tuberculous pleurisy also play an important part in our research direction.

5. Challenges in the Clinical Diagnosis of TP

The diagnosis of TP is still a major challenge, and more and more patients are receiving CT, bronchoscopy, thoracoscopy and other examinations to determine whether they are latent tuberculosis; ADA and INF-γ are Indicators for the diagnosis of TP are accepted by most people, but their sensitivity, specificity and quality control limit their application. It should be noted that the value of ADA in diagnosing tuberculous pleurisy also needs to be combined with local epidemiology. In addition, we need to pay attention to false positives and false negatives, and there may be false positives in some nontuberculous pleurisy, and reports suggest that about 1/3 of parapneumonic effusions and 2/3 of nontuber-

culous empyema patients have ADA exceeding 40 U/L [15]. Zhou *et al.* [16] conducted a meta-analysis of seven studies on the value of γ-interferon release test in the diagnosis of tuberculous pleurisy in 2011, and the sensitivity, specificity of interferon release test in pleural fluid for the diagnosis of tuberculous pleurisy was 75% and 82%, and its misdiagnosis rate was about 20%, and the missed diagnosis rate was about 25%, and the meta-analysis published by Aggarwal *et al.* [17] suggested that the sensitivity, specificity of interferon release test in pleural fluid for the diagnosis of tuberculous pleurisy was 72% and 78%. This suggests that γ-interferon release assays have limited diagnostic value in the detection of tuberculous pleurisy. At present, in order to improve the diagnosis of TP, two or more laboratory indicators are often used. Wang Zhen *et al.* [18] found the combination of peripheral blood T-SPOT.TB and pleural effusion ADA test in patients with TBP has a high positive rate, and the combination of the two detection can improve the diagnostic sensitivity and specificity, significantly reduce the misdiagnosis rate and missed diagnosis rate, and have rapid and accurate diagnostic value for suspected TBP. There are also three or more items, such as Jin Fenhua *et al.* [19] found that the sensitivity, specificity and area under the curve of IL-33, ADA and peripheral blood T-SPOT.TB were 88.5%, 100.0% and 0.962, respectively, which could be used as effective indicators for the diagnosis of TBP. However, combined detection is not the best method for diagnosing tuberculous pleurisy, and there is a possibility of missed diagnosis and misdiagnosis. Based on this, we should continuously improve the detection technology and look for biomarkers with higher specificity and sensitivity.

In recent years, molecular diagnostic technology has developed rapidly. The use of real-time fluorescent quantitative PCR to diagnose TP has the characteristics of convenient sampling, high sensitivity and good accuracy. For slow-growing bacteria, PCR diagnosis has unique advantages, and sputum, pleural effusion, cerebrospinal fluid Various body fluids such as urine and urine can be used as specimens, which can provide a certain basis for early diagnosis of patients, dynamic monitoring during treatment and evaluation of curative effects. It has become a research hotspot to find molecular markers in pleural effusion that can indicate TP process and immune status.

Among them, miRNAs have high stability and can be rapidly and accurately quantified, and are expected to become non-invasive biomarkers to track the occurrence, development and prognosis of diseases. Therefore, we have found many auxiliary detection methods to improve the sensitivity and detection of microRNAs. These complementary approaches for exploring the function of miRNAs have been able to overcome various limitations, by altering the expression of specific noncoding RNAs to block or activate the genes they regulate, and then prevent the development of tuberculosis for therapeutic purposes.

miRNA not only plays a role in the diagnosis of TP, but also has certain guiding significance throughout its treatment. Currently, miRNAs have been found

to have the potential to be therapeutic biomarkers. MiR-144 has been found to be highly expressed in pleural fluid in patients with TP. Further transfection of T cell precursors with miR-144 has shown that miR-144 inhibits the production of TNF-α and IFN-γ [20]. Since TNF-α and IFN-γ play an important role in protective immunity, TNF and IFN can regulate the function of immune cells in the body, TNF can enhance neutrophil phagocytosis, and both have anti-infection functions. Therefore, miR-144 may have an impact on the development and prognosis of tuberculosis. By monitoring the level of miR-144 in the body, it can reflect changes in the immune function of the patient.

MiRNAs play a complex role in tuberculosis and its biology and are involved in signaling pathways expressed by enzymes and transcription factors during immune regulation. This allows miRNAs to be used as a new technology for the treatment of tuberculosis. The study found that miRNAs can enhance the activity of natural killer cells (NK cells) in the presence of Mtb. Moreover, MiR-155 can inhibit autophagy in infected macrophages by targeting brain-expressed Ras homologs (Rheb). Moreover, MiR-33/33* silencing can promote the activation of autophagy [21]. All of this suggests that miRNAs can enhance the body's immune function. Other studies have found that miR-23a specifically targets TLR2/MyD88/NF-B pathway-related genes that affect autophagy induction and Mtb survival, meaning that miR-23a may be used as a target for tuberculosis treatment. Therefore, we can treat TP by designing targeted drugs that target miR-23a. In addition, miRNAs that play a role in innate immunity or miRNAs that target cytokine-associated genes may be used for miRNA-based therapies. Certain miRNAs can regulate cytokines associated with inflammation, thereby inhibiting the production of inflammatory responses and exerting anti-inflammatory effects. For example, miR-99b blocks the release of pro-inflammatory cytokines during Mtb infection, while miR-20b inhibits the tuberculosis-induced inflammatory response in vivo via the NLRP3/caspase-1/IL-1 pathway [22] [23]. The specificity of Mirna may have further implications for future TB treatments, and recent advances in gene expression may lead to the development of therapeutic research specifically targeting miRNAs.

According to the above-mentioned many scholars' research on the basis of miRNA and the limitations of a variety of different research methods for TP diagnosis, it is not difficult to find that if an accurate and commercial miRNA combination can be used for the clinical diagnosis of tuberculosis patients in primary hospitals, it will be very helpful to change the phenomenon of TP concealment and high infectivity, improve patient survival and improve patient prognosis. The easy acquisition and large amount of pleural effusion brings certain convenience to the extraction of miRNA, if the detection technology of miRNA is mature and truly enters the clinic, it will be a small injury, low cost, easy to operate auxiliary examination.

At present, the low content and expression of miRNA in pleural effusion, the lack of quantitative detection technology has not been optimized, and the lack of

screening and validation studies of large samples make the sample coverage insufficient, which is a problem that needs to be solved in this general direction. With the discovery of more and more diagnostic markers and the diagnostic limitations of single markers are fully exposed, many scholars have gradually realized that multi-target joint diagnosis can significantly improve the diagnostic efficiency of tuberculous pleurisy, but it is limited by the optimization of various mathematical models.

However, with the development of next-generation sequencing technology and even the application of third-generation sequencing technology, the sequencing depth of miRNA for pleural effusion will become higher and higher. At present, there are mRNA pre-amplification kits developed by companies that can detect low-expression miRNAs [24]. With the rapid development of all walks of life today, it is believed that whether it is the optimization of data models or the quantitative detection of miRNAs in pleural effusion, better solutions will be obtained. In addition, the diagnosis of TP is also being studied by scholars, and different detection markers are also emerging, including IL-27, IFN-γ, miRNA, as one of them, is the most accessible and low-cost detection marker. Although miRNAs are still a long way from entering clinical diagnostics, we believe that this process is just around the corner as laboratory technology continues to evolve. Therefore, we will continue to pay attention to the research status of miRNAs and actively look for miRNA combinations with high sensitivity and specificity to contribute to the diagnosis of tuberculous pleurisy.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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