Exploration and application of exosomes as diagnostic markers for IBD

Wanying Wu*

Major in Clinical Medicine, School of Medicine, Yan'an University, Yan'an City, 716000, China

Abstract: In Asia, the prevalence of inflammatory bowel disease (IBD) is high, resulting in significant physical and mental burdens for patients, including chronic pain, persistent diarrhea, fatigue, and psychological stress. Early detection and treatment of IBD can lead to better remission outcomes. However, currently, there is no non-invasive, simple, and cost-effective method for the early diagnosis of IBD. Recently, both domestic and international scholars have suggested that intraluminal incision can serve as a valuable index for non-invasive diagnosis. This paper reviews various proteins from different tissues and tissue sections, highlighting the importance of liquid tissue sections in the diagnosis of IBD.

1. Introduction

Inflammatory bowel disease (IBD) is a chronic intestinal disorder that includes Crohn's disease (CD) and ulcerative colitis (UC). Symptoms of CD and UC include chronic diarrhea, abdominal pain, weight loss, and are often accompanied by fatigue and psychological stress, which contribute to the significant physical and mental burdens experienced by patients. UC primarily affects the colon, while CD can involve multiple areas of the intestine [1]. Additionally, IBD can manifest with extra-intestinal symptoms affecting muscles, eyes, skin, and other areas, leading to serious complications.

The global incidence of IBD is increasing in both developed and developing countries [2]. In mainland China, the prevalence rate of CD was 0.28 per 100,000 individuals, and for IBD, it was 0.85 per 100,000 individuals [3]. Middle-aged and adolescent patients are particularly prone to severe complications, causing significant burdens for families and society. Early diagnosis and treatment are crucial for reducing the occurrence of IBD.

Current diagnosis of IBD predominantly involves invasive medical procedures such as enteroscopy, which can be uncomfortable and expensive, along with limited laboratory tests that may not always provide definitive answers. These methods, while essential, present challenges in terms of patient compliance and the need for a more accessible and less intrusive diagnostic approach. This underscores the need for non-invasive, simple, and cost-effective methods that can improve early detection and treatment outcomes for IBD patients. Enteroscopy, an invasive and expensive procedure, is crucial for diagnosing and treating IBD. However, patient compliance is often low, and some IBD patients cannot receive a definitive diagnosis [4]. There is a need for a more sensitive, specific, and cost-effective method for detecting IBD.

Exosomes, small vesicles released by cells, have gained interest as potential biomarkers and delivery vehicles for various diseases. They carry functional RNA, proteins, drugs, and molecules and have shown clinical potential in tissue repair, disease diagnosis, and prognosis [5].

Ongoing research is exploring the use of exosomes for diagnosing and treating IBD, and this review emphasizes their diagnostic significance in IBD. Among the emerging diagnostic approaches, intraluminal incision has garnered attention. This technique involves a minimally invasive procedure where a small portion of the intestinal lining is incised to obtain a tissue sample. It is considered promising due to its potential to offer a less invasive alternative to traditional biopsy methods, reducing patient discomfort and the associated risks while still providing valuable diagnostic information.

2. Introduction, production process and application of exosomes

Extracellular vesicles (EVs) are secreted by diverse organisms, including animals [6], plants [7], and microorganisms [8], and are present in various bodily fluids. They can be classified based on their size as exosomes (30-200nm), microvesicles (200-1000nm), and apoptotic bodies (1000-5000nm) [9], with exosomes being the most extensively studied subtype. Exosomes are lipid bilayer structures that contain lipids, proteins, and nucleic acids, including disease-specific nucleic acids like DNA, mRNA, and lncRNA, rendering them potential diagnostic markers [10].

Due to their low immunogenicity and high stability in circulation, exosomes have recently been investigated for their role in diagnosing inflammatory bowel disease. In

*Corresponding author's e-mail: 2190027118@qq.com

the following discussion, we will explore the significance of exosomes in diagnosing IBD. As show in figure 1.

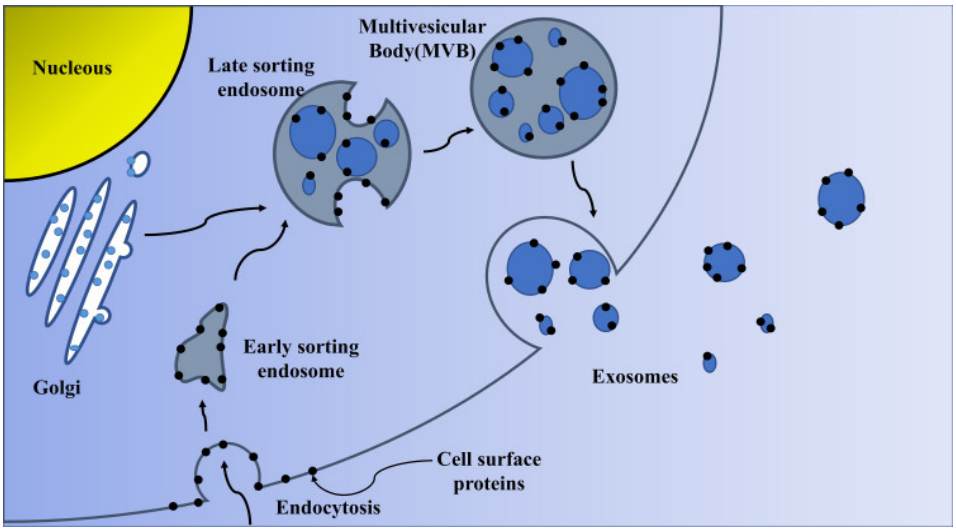

Fig. 1. Biogenesis of exosomes

3. Exosomes as Diagnostic markers of IBD

Currently, the clinical diagnosis of IBD mainly relies on serological testing, imaging techniques, and biopsies. However, these methods have their own drawbacks. Serological examinations, like monitoring C-reactive protein levels, lack specificity and are not accurate in predicting postoperative recurrence of IBD. Imaging techniques and biopsies are invasive, costly, and often result in low patient compliance.

In contrast, exosomes exhibit characteristics such as non-toxicity and biocompatibility. They can serve as diagnostic markers, addressing the limitations of other diagnostic methods. Hence, exosomes have the potential to be utilized as diagnostic biomarkers for IBD.

3.1. Exosomal micrornas (miRNA) as a diagnostic marker for IBD

MicroRNAs (miRNAs) are small non-coding RNA molecules, typically 19-25 nucleotides long, that play a regulatory role in the development of various diseases. Several miRNAs have been found to be upregulated in IBD, making exosomal miRNAs promising candidates for IBD diagnosis.

In IBD patients, specific miRNAs have shown altered expression levels in various tissues compared to healthy individuals. Examples of upregulated miRNAs in IBD include miR-29b , miR-7-5p miR-301a, miR-155 miR-214 and miR-223. Conversely, downregulation of miRNAs such as miR-181b-5p, miR-192, miR-375 and miR-422b has also been observed in IBD. Additionally, the expression patterns of exosomal miRNAs differ between ulcerative colitis (UC) and Crohn's disease (CD). For instance, miR-16 and miR-548 are found to be highly expressed in UC patients compared to CD patients. Another study by Schaefer et al. using microarray analysis identified miR-101 as overexpressed in CD patients, while miR-21, miR-31, and miR-142-3p were overexpressed in UC patients. These specific miRNAs offer potential for differentiating between UC and CD.

Assessing the expression levels of miRNAs in IBD holds great importance for exploring novel diagnostic and therapeutic approaches for the disease. As diagnostic markers, miRNAs have the capacity to overcome the limitations of conventional examinations like endoscopy and enable early detection of IBD. Therefore, further investigations are warranted to broaden the repertoire of miRNAs used for diagnosing IBD and to enhance the clinical application of exosomal miRNAs.

3.2. Exosomal circular RNA (circRNA) as a diagnostic marker for IBD

Circular RNA (circRNA) is a distinctive RNA molecule that forms a closed loop structure and exhibits increased stability due to its resistance to degradation by nucleases. The altered expression of circRNAs in exosomes has been observed in individuals with inflammatory bowel disease (IBD), indicating their potential as diagnostic markers for the condition. Several circRNAs, including circRNA 103765, circQTL SNPs , circ 103516, and circHIPK, have been identified with dysregulated expression levels in IBD patients. Moreover, distinct circRNA expression profiles can differentiate between ulcerative colitis (UC) and Crohn's disease (CD). For instance, reduced expression of circ 0007919 in UC patients promotes inflammation, whereas circRNA 092520, circRNA 102610, circRNA 103124, circRNA 102610, and circRNA 004662 are upregulated in CD patients. Notably, circRNA 004662 exhibits higher expression in CD compared to UC, indicating its potential as a discriminatory marker for distinguishing between these two conditions. Further research in this field holds the potential to deepen our understanding of the role circRNA plays in IBD and its clinical applicability as a diagnostic tool.

3.3. Exosomal long non-coding RNA (lncRNA) as a diagnostic marker for IBD

Exosomal long non-coding RNA (lncRNA) refers to a specific class of RNA molecules that reside in exosomes and have a transcript length exceeding 200 nucleotides. Several lncRNAs, including PCA3, PCGEM1, and PCAT-1, have been identified as highly specific diagnostic markers for prostate cancer , while HOTAIR has shown diagnostic potential for breast cancer. Similarly, in the context of IBD, lncRNAs are closely associated and often exhibit overexpression. One such lncRNA is nuclear-enriched abundant transcript 1 (NEAT1) , primarily localized in the nucleus and involved in the innate immune response. NEAT1 has been found to be highly expressed in various tissues of IBD patients and contributes to the inflammatory reaction by regulating the intestinal epithelial barrier and exosome-mediated macrophage polarization. Other overexpressed lncRNAs in IBD include CRNDE, H19, SPRY4-IT1, BC012900, KIF9-AS1, LINC01272 , and colon cancer-associated transcription-1 (CCAT1). Conversely, lncRNA DIO3OS is down-regulated in IBD. Monitoring the expression levels of these lncRNAs has the potential to serve as diagnostic and prognostic markers for IBD. Thus, exosomal lncRNAs may hold promise as diagnostic markers for IBD in clinical settings.

3.4. Exosomal protein as a diagnostic marker for IBD

Exosomal proteins have demonstrated efficacy as diagnostic biomarkers for monitoring the occurrence and progression of IBD. For instance, studies have revealed increased levels of salivary exosomal protein PSMA7 in IBD patients, with higher levels observed in UC compared to CD patients. Therefore, PSMA7 can serve as a diagnostic marker for IBD. Additionally, other salivary exosomal proteins like CD9, CD63, and CD81, along with biomarkers such as pregnancy zone protein (PZP) and annexin A1 (ANXA1), have been found to be elevated in IBD patients. These substances may also function as diagnostic markers for IBD. Furthermore, cytokines like IL-1β, IL-6, and TNF-α, which are elevated in the saliva of CD patients. Similarly, elevated concentrations of IL-6, IL-8, and MCP-1 have been observed in the saliva of UC patients. These cytokines can be utilized to differentiate between UC and CD. Overall, exosomal proteins and other biomarkers present in saliva offer promising diagnostic markers for IBD.

4. Cases Study

4.1. Case 1: diagnosis of ulcerative colitis

This case study focuses on the use of exosomes in the diagnosis of ulcerative colitis (UC). Ulcerative colitis is a chronic inflammatory bowel disease that primarily affects the colon and rectum, and its symptoms include abdominal pain, diarrhea, and bloody stools. Early and accurate diagnosis is essential for disease management and prevention of complications.

In this case, we selected a group of UC patients and healthy controls, and collected blood and stool samples for exosomes isolation and analysis. Using a nanotechnology platform and a polymer-based precipitation method, we successfully isolated exosomes from the samples and analyzed the miRNA expression profiles in the exosomes using high-throughput sequencing. Exosomes from UC patients showed significantly higher levels of expression of specific miRNAs, such as miR-16 and miR-548, compared to healthy controls. These miRNAs are considered to play a key role in the pathogenesis of UC, and may participate in the occurrence and development of the disease by regulating inflammatory response and immune cell function. Furthermore, the expression differences of these miRNAs were verified by quantitative real-time PCR (qRT-PCR), further confirming their potential as biomarkers for UC diagnosis. By analyzing miRNA expression patterns in patient exosomes, we were able to distinguish UC patients from healthy individuals with high accuracy. This exosome-based diagnostic method is not only non-invasive, but also provides a simple and reliable detection method due to the stability of miRNA in plasma and feces. We also observed significant changes in miRNA expression levels in exosomes of UC patients after treatment, suggesting that exosomal miRNAs may be used to monitor disease activity and therapeutic efficacy. This finding provides a new perspective for the individualized treatment of UC.

In conclusion, this case study demonstrates the potential of exosomal miRNAs for clinical use in the diagnosis of UC. With further clinical validation and methodological optimization, the exosome-based diagnostic platform is expected to become a powerful tool for the early diagnosis and management of UC.

4.2. Case 2: diagnosis of Crohn's disease

Crohn's disease (CD) is a chronic inflammatory bowel disease that can affect the entire digestive tract, and its diagnosis is challenging because it is similar to ulcerative colitis in clinical presentation and pathological features, but its location and characteristics are different. This case study aims to explore the potential use of exosomes in the diagnosis of CD. A group of patients with endoscopically and histologically confirmed CD and a group of age-and sex-matched healthy controls were included in this study. Blood and stool samples were collected from all participants. Exosomes were isolated by size-exclusion centrifugation and immunomagnetic beads to ensure high purity of exosomes. Subsequently, the expression profiles of miRNAs and lncRNAs were performed on the isolated exosomes using next-generation sequencing (NGS). The results showed that a series of specific miRNAs (e.g., miR-101) and lncRNAs (e.g., NEAT1) were significantly up-regulated in plasma exosomes in CD patients. The abnormal expression of these molecules is closely related to the inflammatory activity and lesion extent of CD. MiR-101, in particular, was found to be expressed at more

than 3-fold higher levels in CD patients than in healthy controls, showing higher sensitivity and specificity. Analysis of exosomes in stool samples revealed specific protein markers associated with CD, such as the saliva-derived exosomal protein PSMA7, which was expressed at significantly higher levels in CD patients than in UC patients and healthy controls. This finding suggests that PSMA7 may serve as a potential biomarker to distinguish CD from other IBD subtypes. The expression patterns of miRNAs and lncRNAs in exosomes are associated with the clinical course of CD and response to treatment. Significant changes in the expression levels of specific miRNAs and lncRNAs in the exosomes of CD patients after treatment with biologics suggest that exosomal molecules may be used to monitor disease activity and assess therapeutic efficacy.

The conclusions of the case study highlight the potential of exosomal molecules in the diagnosis of CD. Comprehensive analysis of exosomal miRNA, lncRNA and protein markers can not only improve the diagnostic accuracy of CD, but also provide a new perspective for disease monitoring and treatment. Future studies will expand the sample size and further validate the clinical utility of these biomarkers in multicenter clinical trials. This case study provides empirical support for the use of exosomes in the diagnosis of CD and sets the stage for future research directions and clinical applications. With the development of exosomal research, we expect that these non-invasive biomarkers will become an important tool for early diagnosis, disease monitoring and personalized treatment of CD.

4.3. Case 3: Disease surveillance and treatment response assessment for IBD

The complexity of inflammatory bowel disease (IBD) is not only reflected in its diagnosis, but also in its continuous disease monitoring and evaluation of treatment response. A cohort of patients with IBD, including patients with Crohn's disease (CD) and ulcerative colitis (UC) in active and remission, was included in this case. All patients received standard treatment regimens, and blood and stool samples were collected before and after treatment. Exosomes were isolated by ultracentrifugation and characterized by electron microscopy and nanoparticle tracking analysis (NTA). The RNA and protein composition of exosomes was assessed by high-throughput sequencing and proteomic analysis.

It has been found that exosomes are significantly increased in patients with active IBD and carry specific miRNAs and lncRNAs associated with disease activity. In particular, miR-21 and miR-146a were up-regulated in exosomes of patients with active IBD, and the expression levels of these miRNAs significantly decreased after treatment, suggesting that exosomal miRNAs may serve as biomarkers of disease activity. In addition, the changes of specific proteins in exosomes in fecal samples also provide a new perspective for the monitoring of IBD.

The results of this case study indicate that exosomes have important application potential in disease surveillance and therapeutic response assessment in IBD. The miRNA and protein composition of exosomes can be used as a reflection of disease activity, providing clinicians with a new non-invasive monitoring tool. Exosomal analysis provides more real-time and dynamic disease information than traditional serology and endoscopy. There are still some challenges in the application of secretion analysis in IBD monitoring. First, the techniques for the isolation and analysis of exosomes need to be further standardized to ensure the reproducibility and reliability of results. Second, the clinical relevance and predictive value of exosomal biomarkers need to be validated in a larger patient population. Finally, the cost-effectiveness ratio of exosomal analysis is also an important factor in determining whether it can be widely used in clinical practice.

Despite the challenges, the use of exosomes in disease surveillance and therapeutic response assessment in IBD is promising. Future research should focus on the development of more efficient and cost-effective exosomal analysis techniques and the validation of exosomal biomarkers in multicenter, large-scale clinical trials.

5. Conclusions and perspective

In recent years, there has been an increasing interest in the development of non-invasive diagnostic techniques for inflammatory bowel disease (IBD) to reduce the need for invasive procedures like endoscopy, which can be uncomfortable for patients. Exosomes have emerged as promising diagnostic markers for IBD, providing a simpler, safer, and more cost-effective approach to diagnosis.

Although there are already diagnostic markers used in clinical settings, such as serum C-reactive protein and inflammatory cytokines, their low specificity limits their standalone use in IBD diagnosis. Future studies could explore the combined use of exosomes with these diagnostic markers to enhance diagnostic accuracy. Researchers can investigate the specific exosomes that can be incorporated with existing markers to improve the overall diagnostic rate for IBD. Additionally, integrating exosomes with other established diagnostic methods for IBD should be considered. Another crucial aspect to consider is the association between inflammation and cancer. Refractory IBD may lead to cancer development, and elevated levels of exosomes could serve as indicators of IBD progression. Therefore, future research should focus on exploring the diagnostic role of exosomes in monitoring IBD development and its potential relationship with cancer. Lastly, advancements in exosome enrichment and separation methods are necessary. Consistent and large-scale isolation of highly purified exosomes is essential for their clinical application as diagnostic markers for IBD. Ongoing research efforts should aim to enhance these methods to ensure reliable and efficient isolation of exosomes. In conclusion, exosomes show significant potential as non-invasive diagnostic markers for IBD, offering a simpler and more

patient-friendly approach to diagnosis. Further research is warranted to optimize and expand their utility in clinical settings.

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