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ORIGINAL

HSA-MIR-124-3P: A POTENTIAL PROGNOSTIC MARKER IN ATHLETES FOR EARLY DETECTION AND MANAGEMENT OF MULTIPLE MYELOMA

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ABSTRACT

Purpose: The role of Exosomal RNAs in the bone marrow microenvironment and their prognostic significance in multiple myeloma is not fully understood, especially in physically active populations such as athletes. This study aims to evaluate specific exosomal RNAs, including hsa-miR-124-3p, as potential prognostic markers for multiple myeloma in athletes. **Experimental Design:** Bone marrow fluid was collected from athletes diagnosed with multiple myeloma, showing varying treatment responses. Exosomes were isolated and characterized through dynamic light scattering, transmission electron microscopy, and Western blot analysis. The effect of these exosomes on PRMI8226 cell migration and proliferation was observed. MiRNA sequencing of the exosomes was performed, followed by bioinformatics analysis to identify differentially expressed miRNAs related to treatment outcomes. Clinical sample validation was conducted to ascertain the relevance of these findings in a broader athletic context. **Results:** The study revealed that bone marrow-derived exosomes from athletes with multiple myeloma exhibit varied impacts on PRMI8226 cells based on the therapeutic response. Exosomes associated with poor prognosis were found to enhance PRMI8226 cell proliferation and migration. MiRNA sequencing identified distinct miRNA expressions in exosomes, with certain miRNAs enriched in cancer-related pathways. Notably, exosomal hsa-miR-124-3p was highly expressed in athletes exhibiting poor therapeutic responses. **Conclusions:** This study highlights the crucial role of exosomal miRNA in the progression and treatment of multiple myeloma in

athletes. Specific miRNAs, such as hsa-miR-124-3p, hsa-miR-451b, and hsa-miR-509-3p, were predominantly expressed in athletes with less favorable treatment outcomes. Among these, hsa-miR-124-3p shows significant potential as a prognostic marker for therapeutic efficacy in athletes, providing a new avenue for early detection and management of multiple myeloma in this specific population.

Keywords: Athlete Health; Multiple Myeloma in Athletes; dynamic light scattering, transmission electron microscopy, and Western blot analysis; miRNA

1. INTRODUCTION

Athletes, renowned for their dedication to physical excellence and strenuous training regimens, represent a unique population with distinct health considerations. The pursuit of peak athletic performance often entails rigorous physical exertion, leading to physiological adaptations in response to training stressors. While these adaptations can enhance athletic abilities, they also introduce a complex interplay between exercise and health, including the potential risks associated with intense physical activity (Kalluri & LeBleu, 2020). (Bernardi & Farina, 2021).

Among the health concerns that have recently come to the forefront is the connection between intense physical training and the development of multiple myeloma, a hematological cancer characterized by the abnormal proliferation of plasma cells within the bone marrow. Multiple myeloma is notorious for its insidious onset, with symptoms often remaining latent until advanced stages of the disease. Early detection is paramount for effective management and improved prognosis. (Bento et al., 2019; Shi, Ding, Qu, Tang, & Hao, 2020).

In this context, microRNAs (miRNAs) have emerged as promising candidates for biomarkers associated with various cancers. MicroRNAs are small non-coding RNA molecules that play crucial roles in the regulation of gene expression(Hideshima & Anderson, 2021).. Dysregulation of specific miRNAs has been linked to cancer initiation, progression, and prognosis, making them valuable tools for early detection and prognostication. (Srivastava, Rathore, Munshi, & Ramesh, 2021).

One such miRNA, HSA-MIR-124-3p, has garnered attention for its potential as a prognostic marker in athletes for the early detection and management of multiple myeloma. Its role in modulating cancer-related pathways and gene expression suggests its relevance as a biomarker in individuals engaged in intense physical activity. Investigating the association between HSA-MIR-124-3p expression levels and the risk of multiple myeloma in athletes can offer valuable insights into its utility as a diagnostic tool for early

detection(Bernardi & Farina, 2021) (Rocchi, Chiti, Maiese, Turillazzi, & Spinetti, 2020).

This study delves into the intricate relationship between HSA-MIR-124-3p, multiple myeloma, and the unique context of athletes. By exploring the expression patterns and diagnostic significance of this miRNA in athletes who subject their bodies to demanding physical training(Chen, Moscvin, & Bianchi, 2020) (Lopes et al., 2021), we aim to contribute to the emerging field of personalized healthcare for athletes. .(Jiménez-Avalos, Fernández-Macías, & González-Palomo, 2021; Wu et al., 2021). The potential identification of HSA-MIR-124-3p as a prognostic marker may not only enhance early detection but also facilitate targeted interventions, ultimately improving the health and well-being of athletes facing the risk of multiple myeloma (Sirkeci & Přívara, 2017). As the worlds of sports science and medicine converge, the investigation of HSA-MIR-124-3p's role in multiple myeloma holds promise for advancing our understanding of athlete health and underscores the importance of tailored healthcare strategies in the context of intense physical activity.(Moser-Katz, Joseph, Dhodapkar, Lee, & Boise, 2021).

2. MATERIALS AND METHODS

2.1 Patients and Samples

During April 2019, bone marrow fluid was collected from 1 patient with multiple myeloma with good prognosis and 1 patient with poor prognosis of multiple myeloma, exosomes were isolated, and cell function experiments and sequencing were performed. During March 2020-2021, collect the bone marrow fluid of patients with good prognosis of multiple myeloma (n=10) and patients with good prognosis of multiple myeloma (n=10) to verify the selected potential biomarkers, from Each subject obtained written informed consent, and all aspects of the study were approved by the Ethics Committee of Shenzhen Luohu District People's Hospital. The research was conducted in accordance with relevant guidelines.

2.2 Isolation and Identification of Exosomes

The bone marrow's exosome is isolated by ultracentrifugation. The isolated process needs to be kept at four °C throughout the process. Western Blot (WB) analysis identified the isolated exosomes' surface proteins, such as CD63、TGS101. The size of exosomes is analyzed by nanoparticle tracking analysis. The exosomes' morphology is identified through transmission electron microscopy.

2.3 Migration Assays

Migration rate was measured by wound healing assay. The wound was

made using a pipette tip, and pictures were taken immediately and 24h after wounding. The distance migrated by the cell was measured during the period.

2.4 Cell Proliferation

CCK8 is used to evaluate the effects of different sources of bone marrow exosomes on the proliferation of PRMI8226 cells. In short, PRMI8226 cells were seeded into a 96-well plate, the cell density was 5000 cells/well, and bone marrow exosomes containing different sources were added for stimulation. After 12, 24, 36, and 48 hours, each Add 10μLCCK8 solution to the wells and incubate the plate in an incubator for 1-4 hours. Then, use a microplate reader to measure the wells at 450 nm.

2.5 miRNA library sequencing and analysis

Total RNAs from exosomes was used for miRNA library preparation and sequencing. RNA-seq was conducted on an Illumina HiSeq 2500 platform. The quality of the sequencing data was analyzed by the bioinformatics pipeline. Briefly, read sequence quality checks were performed by FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Adapters from the 3′ ends of reads were trimmed using Cutadapt with a maximum allowed error rate of 0.1. Reads shorter than 17 nucleotides in length were excluded from further analysis. Reads were mapped to human hg19 using Bowtie version 1.1.1. Then reads were annotated to human miRNAs from miRBase Release 21 using miRDeep2 (version 2.007). The number of reads in each miRNA was enumerated by miRDeep2, and differences in expression between groups were statistically assessed by DeSeq (version 1.16.0). Differential expression miRNAs were determined based on log2 fold change (log2 fold change) and false discovery rate (FDR) with |log2 fold change| ≥ 1 and FDR ≤ 0.05. Pathway analysis were constructed in GO and KEGG.

2.6 qRT-PCR Analysis for Evolution and Validation of Candidate Serum Exosomal miRNA

The candidate miRNAs were further confirmed with quantitative real-time polymerase chain reaction (qRT-PCR). qRT-PCR was performed using the QuantiFast® SYBR® Green PCR Master Mix in an LightCycler® 96 qPCR system (Roche Life Science, USA). The relative gene expression values of the target miRNAs were normalized to that of cel-miR-39-3p (U6) and the differences were calculated using the $2-\Delta\Delta Ct$ method.25.

3. RESULTS

3.1 The Characterization of Exosomes from the bone marrow

Exosomes are separated from the patient's bone marrow culture fluid

using ultracentrifugation. The TEM analysis showed that exosomes were cup-like vesicles (Figure1.A). WB shows the surface protein of exosomes contain the CD63 and TSG101 protein (Figure1.B). The NTA analysis shows that the particle size of the exosome is between nm-nm (Figure1.C).

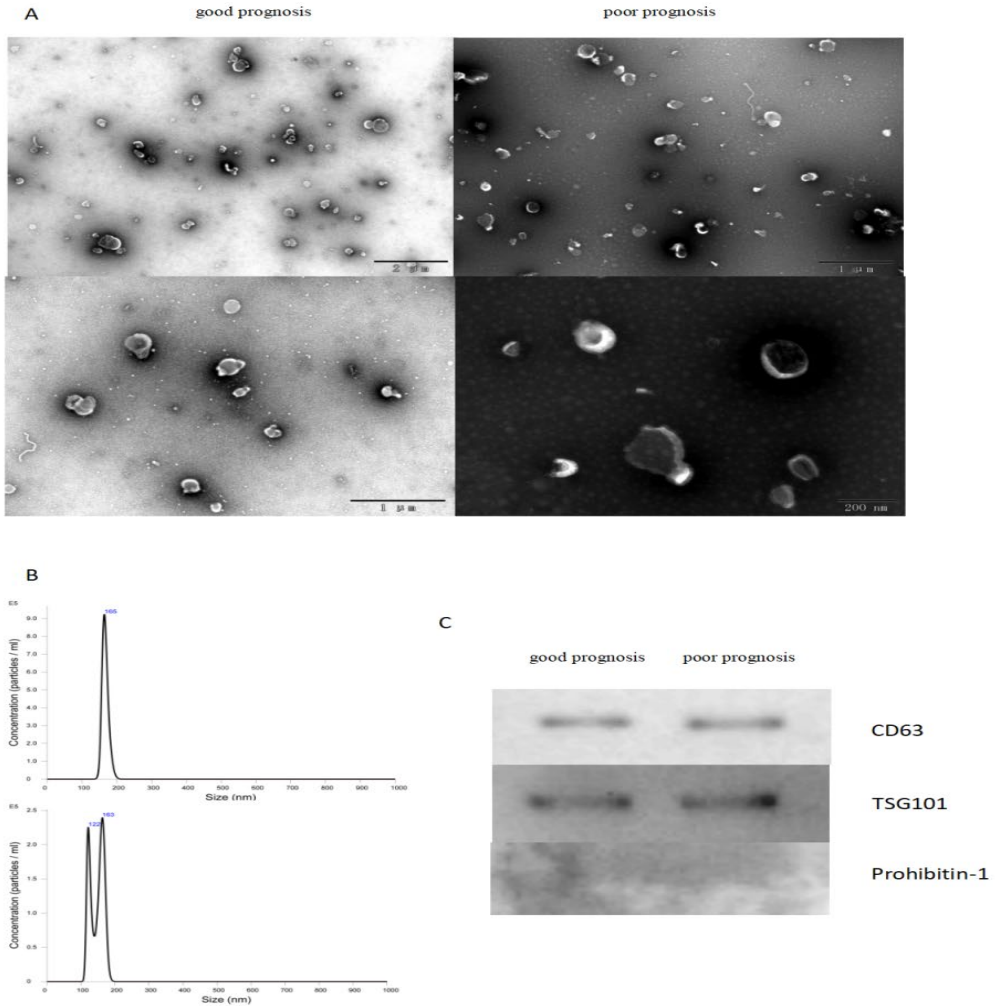

Figure1： Identification of exosomes. Exosomes were isolated from the bone marrow of the patient and morphological characterization was observed by Transmission Electron Microscope (A). The surface protein markers (CD63、TSG101) were identified by Western blot analysis (B). The isolated exosomes' size is between nm and nm. The poor prognosis' exosome is mainly concentrated in 1 nm, and the excellent prognosis' exosome is concentrated primarily in 1 nm.(C).

3.2 Different exosomes affect the biological function of PRMI8226 cells

We observed the effects of exosomes from different patients on PRMI8226 cells and found that the exosomes of patients with poor prognosis can significantly promote the proliferation and migration of PRMI8226 cells. The exosomes of patients with a good prognosis have no such effect. (Figure2.A-C).

Effects of different types of exosomes on the proliferation and migration of PRMI8226 cells. (A) Cell proliferation for PRMI8226 cells after the exosome stimulation. (B and C) Migration of PRMI8226 cells after the exosome

stimulation，evaluated by wound healing assay.

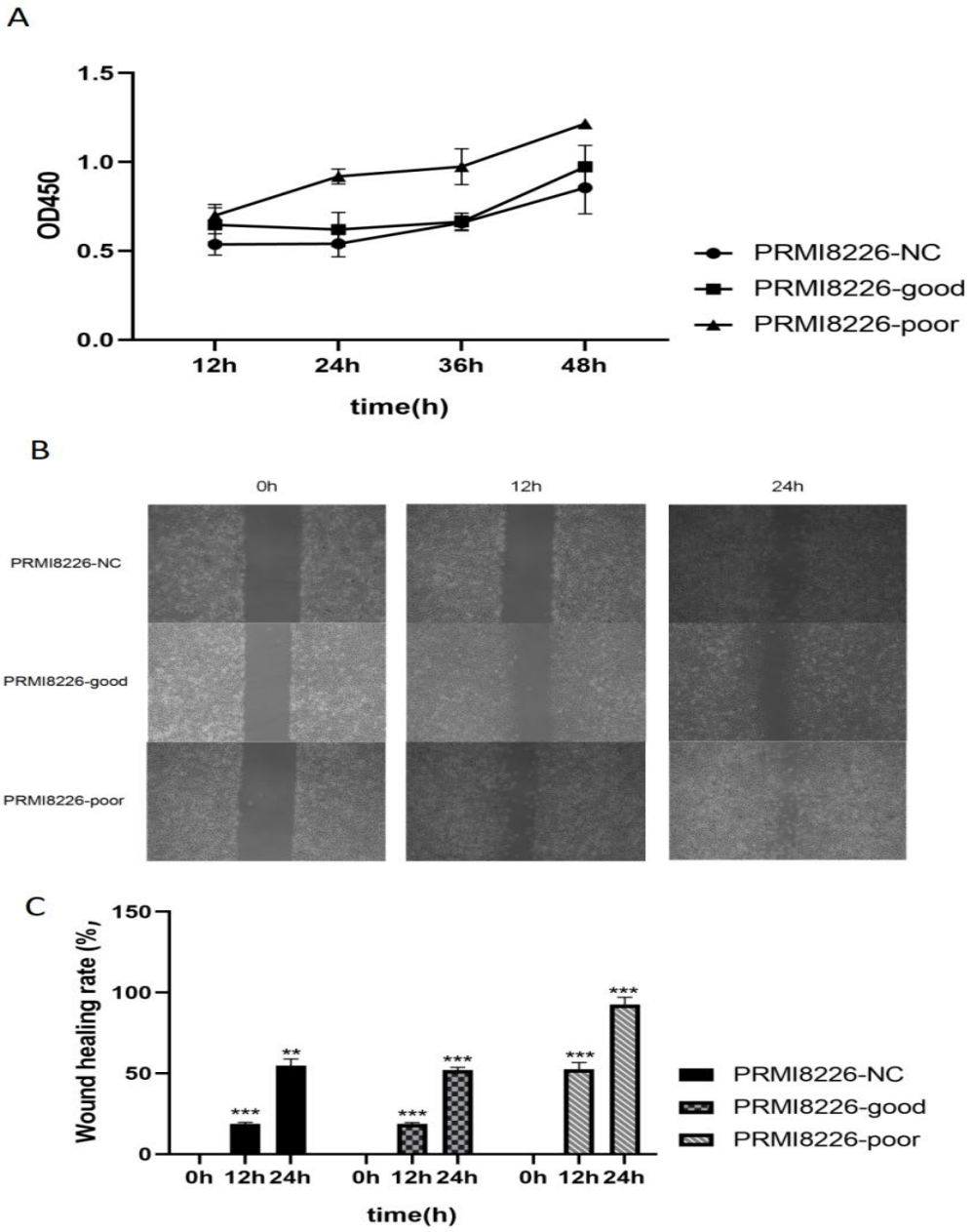

Figure 2

3.4 miRNA expression profile

Exosomes have been demonstrated to contain both mRNAs and miRNAs, which can be delivered to other cells and affect cellular function. we compared the expression level of miRNAs in HXR-TDE and YRJ-TDF. Using a 2-fold change and qvalue < 0.05 as the threshold cutoff, we found 12 miRNAs were significantly up-regulated and 24 miRNAs were significantly down-regulated (Figure3.A). The differentiated expressed microRNAs were clustered (Figure3.B). To further investigate the internal mechanisms on exosomes miRNA, pathway analysis of target genes was performed. For the results, some

up-regulated genes were most enriched in PI3K−Akt signaling pathway, Ras signaling pathway and Metabolic pathways (Figure3.C), while down-regulated genes enriched in MAPK signaling pathway, Transcriptional misregulation in cancer and so on (Figure3.D).

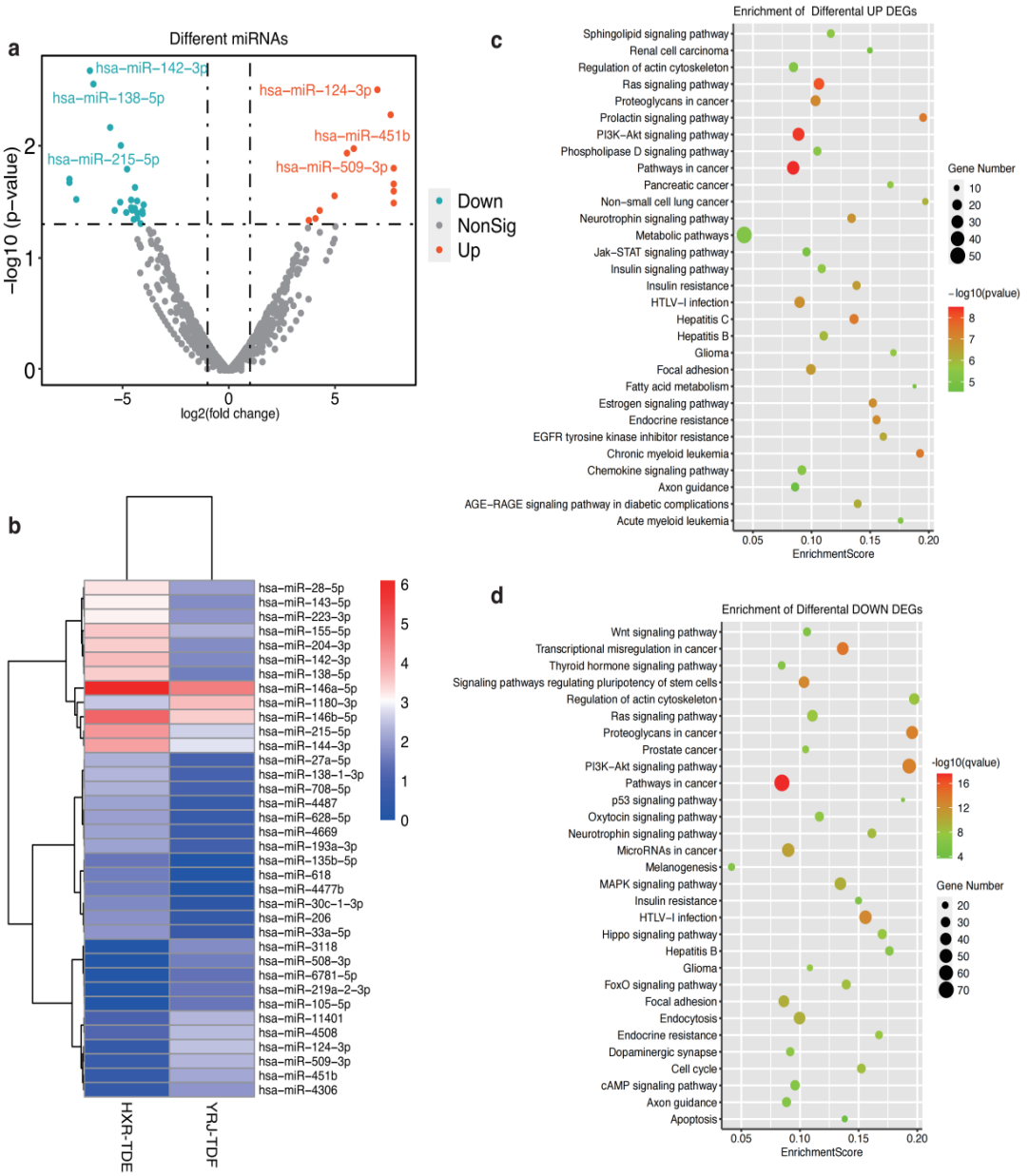

Figure 3

3.5 Selection and Verification Serum Exosomal miRNA as the Diagnostic Biomarker

Three miRNAs (miR-124-3p, miR-451b, miR-503-3p) with the most significant increase in expression were selected as Multiple myeloma biomarkers for miRNA verification. Among patients with different prognosis types, no significant differences were observed between miR-451b and miR-503-3p, while significant differences were observed for miR-124-3p. (P = 0.025).

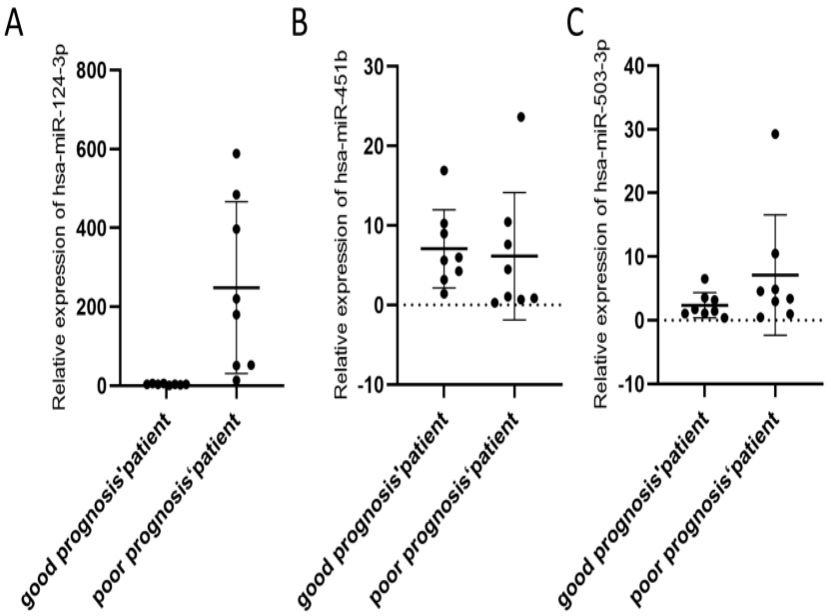

Figure 4： Three miRNAs (miR-124-3p, miR-451b, miR-503-3p) with the most significant increase in expression were selected as Multiple myeloma biomarkers for miRNA verification. Among patients with different prognosis types, no significant differences were observed between miR-451b and miR-503-3p（B and C）, while significant differences were observed for miR-124-3p. (A).

4. DISCUSSION

In clinical trials, the drug resistance of patients with multiple myeloma is still a problem (Moloudizargari, Hekmatirad, Mofarahe, & Asghari, 2021). Some patients use the most conventional drugs to achieve sound therapeutic effects, and some patients use the most advanced drugs to be still unable to control their condition effectively. Suppose a diagnostic biomarker that can effectively predict the effect of the patient's treatment can be found. In that case, it will be helpful for the personalized treatment of patients and greatly help develop patients' drug sensitivity, which can help researchers develop new drugs to avoid the drug resistance and improve the therapeutic effect.

Exosomes are one of the essential tools for mutual "communication" between cells. Many studies have shown that exosomes can be related to the occurrence, development and drug resistance of tumors. Luan W et al. found that melanoma transfers mir-106b-5p to melanocytes by secreting exosomes, promoting the EMT, migration, invasion, and adhesion of melanocytes (Cohen et al., 2021). Senlin Zhao et al. found that exosomes secreted by colorectal cancer cells can induce M2 macrophage polarization to promote liver metastasis of colorectal cancer (Luan et al., 2021). The bone marrow microenvironment can promote the survival and drug resistance of myeloma

cells (Zhao et al., 2020). We studied the BM exosomes of MM and found that different patients' BM exosomes have different effects on the proliferation and migration of PRMI8226 cells. The exosomes of patients with poor prognosis can bring stronger proliferation and migration ability to PRMI8226 cells. Therefore, our research results are consistent with previous studies and mutually verify each other.

So how do exosomes affect the occurrence, development and drug resistance of tumors, thereby affecting the treatment effect of patients? Mirna is closely related to cancer. Farah Hady El Kilany and others proposed that miR-744 promotes breast cancer progression through miR-744/eNOS/NO axis. Xiaohui Tan confirmed that whether it is esophageal squamous cell carcinoma (EAC), the exosomes secreted in vivo and in vitro are guaranteed to contain a large amount of miR-196b, which directly targets the tumor suppressor ephrin type-A receptor 7 (EPHA7), Activate tumor cells to generate EMT (Andrews, Kabrah, May, Donaldson, & Morse, 2013).We performed miRNA sequencing on the bone marrow fluid-derived exosomes of patients with different multiple myeloma, and we found the expression of miRNA in BM exosomes in patients with different therapeutic effects has a difference. Through the enrichment of KEGG pathways, we found that these differential miRNAs are concentrated in tumor development pathways, such as transcription disorders and tumor microenvironment-related proteoglycans. This result proves that the bone marrow-derived exosomes of patients with multiple myeloma are likely to affect the drug resistance of multiple myeloma cell through miRNA (Fan et al., 2021).

After verification, we found that mir-138-5p and mir-142-3p are significantly low-expressed in patients with poor prognoses. Mir-138-5p has been proven to be a tumor suppressor in lung cancer, glioblastoma, bladder cancer, and breast cancer, which can inhibit cell proliferation and invasion and slow down tumor progression (Song et al., 2020) .Mir-124-3p has been proven to be a prognostic biomarker for prostate cancer and endometrial cancer (Li, Qian, Zhang, & Shi, 2019) (Rasoolnezhad, Safaralizadeh, Hosseinpourfeizi, Banan-Khojasteh, & Baradaran, 2021). It has been proven to target the ZFX gene to inhibit tumor progression in non-small cell lung cancer. Therefore, we believe that the expression of mir-138-5p and mir-142-3p in BM exosomes is closely related to patients' treatment effects.

5. Conclusion

In conclusion, the study of HSA-MIR-124-3p as a potential prognostic marker in athletes for the early detection and management of multiple myeloma holds significant promise. Our investigation into the dysregulation of this microRNA in athletes engaged in intense physical activity has revealed its potential diagnostic significance. By focusing on athletes, who are susceptible to unique health challenges due to their demanding training regimens, we have

taken a step toward personalized healthcare in the realm of sports and physical fitness. Early detection of multiple myeloma through the assessment of HSA-MIR-124-3p levels could lead to timely interventions, potentially improving the prognosis and quality of life for athletes facing this hematological cancer. As we continue to unravel the intricate relationship between intense physical activity, biomarkers, and cancer risk, we pave the way for more targeted and effective strategies for athlete health management. The potential implications of our findings extend beyond sports and into the broader landscape of cancer research and early detection, highlighting the valuable intersection of sports science and medical innovation.

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