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Research Article

Diagnostic Implication of Bone Marrow Mesenchymal Stem Cells (BMSC) Exosomal MicroRNA-632 on Chronic Atrophic Gastritis

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Abstract

Evidence has indicated the significance of microRNAs (miRNAs) derived from Bone marrow mesenchymal stem cells (BMSC)exosomes (BMSC-exo). We aimed to investigate the potential of miR-632 as a therapeutic target for diagnosis and treatment for chronic atrophic gastritis. We collected 96 serum samples from patients with chronic atrophic gastritis and the expression level of miR-632 was investigated in these samples by RT-qPCR. The relationship between the miRNA level and prognosis and patients' characteristics was evaluated. The expression of miR-632 was significantly not associated with the sex, age, pathogenic site, and the size of serum BMSC-exo ($p > 0.05$), but miR-632 expression was related to the development of chronic atrophic gastritis and neoplastic progression: gastric cancer of intestinal type group> chronic atrophic gastritis complicated with intraepithelial neoplasia group> chronic atrophic gastritis complicated with intestinal metaplasia group> chronic non-atrophic gastritis group ($p < 0.05$). Downregulated expression of miR-632 predicted greater survival (124.23 ± 12.43 months) compared to the higher miR-632 expression with survival time of 68.34 ± 10.90 months ($p < 0.05$). Expression level of miR-632 relates to the inflammation-mediated carcinogenesis and prognosis of chronic atrophic gastritis, which still further requires systematic investigation.

Keywords: BMSC; Chronic Atrophic Gastritis; MicroRNA-632; Inflammation-mediated Carcinogenesis; Exosome

Introduction

Both China Cancer in 2017 and China Cancer in 2015 reported that chronic atrophic gastritis increase cancer risk or progression and it is considered as precursor lesion for gastric cancer [1]. The chronic atrophic gastritis has received extensive attention in recent years, but the casuality of chronic atrophic gastritis in lung cancer has not been fully elucidated. Early diagnosis is key to treatment and survival, decelerating or preventing the progression of the disease [2]. Considering the significance of inflammation for cancer, it is meaningful to clarify the mechanism whereby early

chronic atrophic gastritis evolves into lung cancer to underlie treatments against tumors, which is a key scientific issue and deserves much attention [3]. The guidelines propose that patients with chronic atrophic gastritis are supposed to accept follow-up endoscopy with biopsy [4]. However, patients usually cannot bear the cost and invasiveness of lifetime endoscopy. At present, there are few non-invasive biomarker tools for management of precancerous conditions and lesions in the stomach (MAPS) [5], among which clinical application of PG I/II is retrained by regional differences, limited sensitivity and expense [6]. Identifying novel non-invasive biomarkers for MAPS thereby is necessary. Of note, the diagnostic value of bone marrow-derived mesenchymal stem (BMSCs)-derived exosomal microRNA (miRNAs), exosomes (exo), and exo-miR has been frequently implicated in gastric cancer [7]. As for the correlation between BMSC-exo miRNAs and chronic atrophic gastritis, a study detected the aberrant expression of BMSC-exo miRNAs in chronic atrophic gastritis using database, and identified 57 up-regulated and 42 down-regulated miRNAs, whilst miR-632 was significantly down-regulated by 8.25 times [8]. Oncogenic function of miR-632 has been noted in many tumors, such as gastric, ovarian, and pancreatic cancer [9]. Therefore, it is speculated that BMSC-exo miR-632 could work as a molecular marker for the pathogenesis of chronic atrophic gastritis. In this study, we focused on the role of miR-632 in the disorder and its impact on the inflammation-mediated carcinogenesis.

Materials and Methods

Sample collection

A total of 150 patients with gastritis or gastric cancer were enrolled in this study who were admitted in the Endoscopy Center and Pathology Department of our hospital, according to the Guidelines for the Diagnosis and Treatment of Primary Gastric Cancer 2017.v1, including cases of gastric cancer of intestinal type, chronic atrophic gastritis, chronic non-atrophic gastritis, chronic atrophic gastritis complicated with intraepithelial neoplasia, and chronic atrophic gastritis complicated with intestinal metaplasia (n = 30, each group). All patients were pathologically confirmed and gastric mucosa samples were collected under gastroscopy before treatment. The participants comprised 80 males and 70 females with a median age of 61.34 ± 5.87 years (range, 42-78 years old). The specimens were collected during the operation and then directly transferred to a refrigerator. The peripheral blood of patients was collected, and the follow-up was carried out once a month.

Reagents and instruments

Reagents

qPCR SuperMix-UDG (Gibco, USA); TaqMix (Abeam, USA); TaqMan miRNA (Guangzhou Ruibo Biotech); PCR reagents (BD biosciences); RNA extraction kit (Milipore, USA); DEPC (BD bioscience); ethanol, Isopropanol and chloroform (Guangzhou Ruibo Biological Co. Ltd.) were used for analysis.

Instruments

CO_2 constant temperature incubator, protein extraction kit, a PCR machine (Bio-TEK, Japan); UV spectrophotometer (Bio-TEK Instruments, USA); a high-speed refrigerated centrifuge (Zhengzhou Purification Equipment Factory).

Flow cytometry

BMSCs were tested for cell purity by flow cytometry. After washing with PBS, the cells were separated and incubated with antibodies against CD34 and CD90 for 1 hour. The levels of CD34 and CD90 were measured by flow cytometry.

BMSC-exo extraction and identification [10]

The BMSCs at passage 3 were seeded at a density of 5×10^6 cells/75 cm^2, and the culture medium was centrifuged at 10 000 r/min for 30 min and the precipitation obtained was the exos. The specific surface antigens (CD9 and CD63) were detected and the particles were observed using the Tunable resistive pulse sensing (TRPS) method.

RT-qPCR

Total RNA was extracted from tissue and detected in ABI ViiA™7qRT-PCR using the miRNeasy Kit kit. The relative expression of miR-632 was determined through RT-qPCR.

Statistical analysis

Data were analyzed by SPSS 23.0 and analyzed by the Wilcoxon signed-rank test, with t test for normal distribution. p <0.05 indicated the significant difference between groups.

Results and Discussion

Correlation between the miR-632 level in serum BMSC-exo and clinicopathological characteristics

Among 150 included cases, miR-632 expression level of 80 ones was higher than the average level and the other 70 had lower level of miR-632. The expression of miR-632 was significantly not as-

sociated with the sex, age, pathogenic site, and the size of BMSC-exo (P > 0.05). But its expression level increased as the condition developed, including metaplasia, and intraepithelial neoplasia. The miR-632 level was even highest in the group of gastric cancer patients, indicating that the miR-632 expression is related to the risk of inflammation-mediated carcinogenesis, see table 1.

Clinicopathological characteristics	n	(%)	miR-632 expression (χ ± s)	P value
Gender				
Male	80	53.33	1.47 ± 0.16	0.334
Female	70	46.67	1.38 ± 0.20	
Age				
> 60	76	50.67	1.48 ± 0.21	0.753
≤ 60	74	49.33	1.46 ± 0.15	
Type				
Chronic non-atrophic gastritis group	30	20.00	1.38 ± 0.16	0.026
Chronic atrophic gastritis group	30	20.00	1.49 ± 0.23	
Chronic atrophic gastritis with intestinal metaplasia group	30	20.00	1.56 ± 0.26	
Chronic atrophic gastritis with intraepithelial neoplasia group	30	20.00	1.62 ± 0.25	
Intestinal gastric cancer group	30	20.00	1.75 ± 0.19	
Pathogenic site				
Gastric antrum	40	26.67	1.49 ± 0.17	0.423
Stomach	40	26.67	1.28 ± 0.13	
Cardia	40	26.67	1.46 ± 0.23	
Whole stomach	30	20.00	1.41 ± 0.15	
Lesion size				
≤ 5 cm	70	46.67	1.41 ± 0.18	0.124
> 5 cm	80	53.33	1.48 ± 0.21	

Table 1: The correlation between miR-632 and clinicopathological characteristics.

The relationship between miR-632 in serum BMSC-exos and clinical features

As demonstrated by Logistic regression analysis, clinical features were related to the expression of miR-632 and consistently, the increase in expression was accompanied with the risk of triggering cancer (P = 0.004, Table 2).

Clinicopathological characteristics	OR(95%CI)	P value	Revised OR (95%CI)	P value [a]
Gender				
Male	0.92 (0.25~3.17)	0.90	0.45 (0.09~2.01)	0.93
Female	1.00		1.00	
Age				
60	1.08 (0.45~3.03)	0.97	0.91 (0.25~3.19)	0.32
≤60	1.00		1.00	
Malignant transformation				
No	1.00	0.04	1.00	0.03
Yes	3.51 (2.13~9.27)		5.16 (1.27~20.83)	
Lesion				
Gastric antrum	1.00	0.41	1.00	0.91
Others	1.79(0.81~6.59)		1.16 (0.21~6.82)	

Table 2: The miR-632 expression and clinical case factors.

The correlation between the expression of miR-632 and prognosis

Higher expression of miR-632 indicated poor prognosis. It was evident that the survival of high expression group (68.34 ± 10.90 months) was longer than that of low expression survival time (124.23 ± 12.43 months), suggesting that miR-632 expression is closely related to prognosis, as shown in figure 1.

Discussion

MiRNAs are the most studied non-coding RNAs. miRNAs participate in various pathways of mammals and cell organisms as well as cancer-related processes such as proliferation, metabolism, cell cycle, apoptosis and differentiation [9]. They also trigger the imbalance of gene post-transcription during tumor formation [10]. Of note, miRNAs relative to mRNAs are more stable and less likely to degrade [11]. Establishment of miRNAs expression data bring great value and contribute to further applications of biomarkers to diagnosis and treatment for cancers [12].

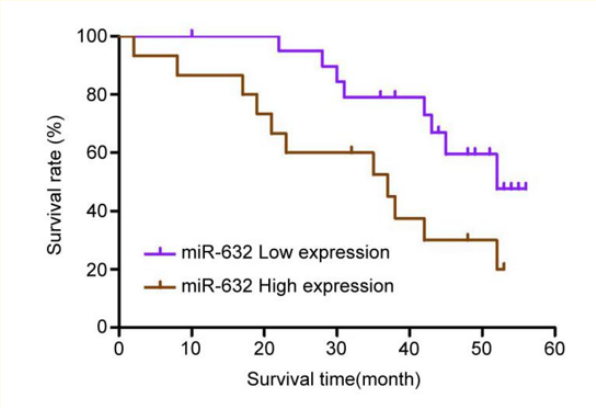

Figure 1: The previous correlation between the relative expression of microRNA-632 and the survival rate.

Liye Ma., *et al.* figure 1.

Exo are phospholipid bilayer particles present in various body fluids and tissues of the human body [13], usually carrying multiple functional substances such as protein, DNA, mRNA, miRNA, lncRNAs [14]. They play a large role in intercellular communication by transferring lncRNA, DNA, or protein, functioning as a resource library of compiled proteins and RNAs [15]. The substances of exo are protected by lipid membranes, leaving the stability of exosomal miRNAs relative to other miRNAs and resistance to RNase enzyme activity [16]. Therefore, miR-exo are expected as promising tumor detection tool for identifying tumor phenotypes.

Chronic atrophic gastritis and intestinal metaplasia increase the risk of gastric cancer [17], and thus are considered as precursor lesions for gastric cancer. Follow-up endoscopic examinations with strict biopsy are required for these patients [18]. However, the cost and invasiveness of lifetime endoscopic review make it difficult for patients to accept. At present, there are few non-invasive biomarker tools for MAPS [19]. Importantly, we have screened out the differentially expressed gene miR-632 in atrophic gastritis. Previously, dysregulated expression of miR-632 has been noticed in serum BMSC-exo of different tumors [20] and thus miR-632 has potential predictive effects for prognosis. In bladder cancer, the up-regulation of BMSC-exo miR-632 can act as a marker molecule [14]. Its implication on prognosis is also indicated in endometrial cancer where cisplatin treatment effectively mediate the expression of miR-632 [21].

Besides, through accumulating evidence has depicted the role of miRNAs, exo, and Exo-miR in gastric cancer, but only a few studies involve the precancerous state. The current study unveiled that the BMSC-exo miR-632 expression was not correlated with age, sex, location of onset, and exo size ($p > 0.05$), but associated with condition of the chronic atrophic gastritis and process of malignant transformation. The expression of miR-632 was significantly overexpressed in chronic atrophic gastritis cells and was positively correlated with the prognosis of the disease. Overexpression of miR-632 brings the risk of malignant transformation; the patients with high expression of miR-632 have a shorter survival time of 68.34 ± 10.90 months when compared to the patients with low expression (124.23 ± 12.43 months). MiR-632 expression is closely related to the prognosis, and plays a role in the diagnosis of early chronic atrophic gastritis.

Conclusion

In conclusion, our work demonstrates that the decreased expression of BMSC-exo miR-632 in chronic atrophic gastritis is related to the prognosis and malignant transformation, so miR-632 may become potential markers and therapeutic targets. However, this study is still in the observation stage without clinical verification of large samples. Further comprehensive study and in-depth exploration of the mechanism are still required.

Ethical Compliance

Research experiments conducted in this article with animals or humans were approved by the Changhai Hospital, Naval Military Medical University following all guidelines, regulations, legal, and ethical standards as required for humans or animals.

Conflicts of Interest

There are no conflicts to declare.

Bibliography

1. Zhuang XM and B Zhou. "Exosome secreted by human gingival fibroblasts in radiation therapy inhibits osteogenic differentiation of bone mesenchymal stem cells by transferring miR-23a". *Biomed Pharmacotherapy* 131 (2020): 110672.

2. Zhu L., *et al*. "Mesenchymal stem cells-derived exosomes ameliorate nucleus pulposus cells apoptosis via delivering miR-

142-3p: therapeutic potential for intervertebral disc degenerative diseases". *Cell Cycle* 19.14 (2020): 1727-1739.

3. Li Y., *et al*. "Chronic Atrophic Gastritis: A Review". *Journal of Environmental Pathology, Toxicology, and Oncology* 37.3 (2018): 241-259.

4. Zhou X., *et al*. "Downregulated miR-150 in bone marrow mesenchymal stem cells attenuates the apoptosis of LPS-stimulated RAW264.7 via MTCH2-dependent mitochondria transfer". *Biochemical and Biophysical Research Communications* 526.3 (2020): 560-567.

5. Zhang Z., *et al*. "Non-invasive detection of gastric cancer relevant d-amino acids with luminescent DNA/silver nanoclusters". *Nanoscale* 9.48 (2017): 19367-19373.

6. Zheng J., *et al*. "lncRNA-TINCR Functions as a Competitive Endogenous RNA to Regulate the Migration of Mesenchymal Stem Cells by Sponging miR-761". *BioMed Research International* 2020 (2020): 9578730.

7. Link A and J Kupcinskas. "MicroRNAs as non-invasive diagnostic biomarkers for gastric cancer: Current insights and future perspectives". *World Journal of Gastroenterology* 24.30 (2018): 3313-3329.

8. Zheng C., *et al*. "Long noncoding RNA XIST regulates osteogenic differentiation of human bone marrow mesenchymal stem cells by targeting miR-9-5p". *Mechanisms of Development* 162 (2020): 103612.

9. Zhang T., *et al*. "Suppression of miR-10a-5p in bone marrow mesenchymal stem cells enhances the therapeutic effect on spinal cord injury via BDNF". *Neuroscience Letter* 714 (2020): 134562.

10. Zhang L., *et al*. "MiR-20a-containing exosomes from umbilical cord mesenchymal stem cells alleviates liver ischemia/reperfusion injury". *Journal of Cellular Physiology* 235.4 (2020): 3698-3710.

11. Zhai Z., *et al*. "High glucose inhibits osteogenic differentiation of bone marrow mesenchymal stem cells via regulating miR-493-5p/ZEB2 signalling". *Journal of Biochemistry* 167.6 (2020): 613-621.

12. Zha JP., *et al*. "MiR-920 promotes osteogenic differentiation of human bone mesenchymal stem cells by targeting HOXA7". *Journal of Orthopaedic Surgery and Research* 15.1 (2020): 254.

13. Yuan B., *et al*. "Effect of exosomes derived from mir-126-modified mesenchymal stem cells on the repair process of spinal cord injury in rats". *European Review for Medical and Pharmacological Sciences* 24.2 (2020): 483-490.

14. Ying H., *et al*. "Extracellular vesicles carrying miR-193a derived from mesenchymal stem cells impede cell proliferation, migration and invasion of colon cancer by downregulating FAK". *Experimental Cell Research* 394.2 (2020): 112144.

15. Yang R., *et al*. "IFN-gamma promoted exosomes from mesenchymal stem cells to attenuate colitis via miR-125a and miR-125b". *Cell Death and Disease* 11.7 (2020): 603.

16. Yan J., *et al*. "Effects of miR-26a on Osteogenic Differentiation of Bone Marrow Mesenchymal Stem Cells by a Mesoporous Silica Nanoparticle - PEI - Peptide System". *International Journal of Nanomedicine* 15 (2020): 497-511.

17. Yan G., *et al*. "m (6)A Methylation of Precursor-miR-320/RUNX2 Controls Osteogenic Potential of Bone Marrow-Derived Mesenchymal Stem Cells". *Molecular Therapy - Nucleic Acids* 19 (2020): 421-436.

18. Baek DH., *et al*. "Gastric epithelial dysplasia: characteristics and long-term follow-up results after endoscopic resection according to morphological categorization". *BMC Gastroenterology* 15 (2015): 17.

19. Liu T., *et al*. "Visualization of exosomes from mesenchymal stem cells in vivo by magnetic resonance imaging". *Magnetic Resonance Imaging* 68 (2020): 75-82.

20. Xiong L., *et al*. "Exosomes from Bone Marrow Mesenchymal Stem Cells Can Alleviate Early Brain Injury After Subarachnoid Hemorrhage Through miRNA129-5p-HMGB1 Pathway". *Stem Cells and Development* 29.4 (2020): 212-221.

21. Xiao J., *et al*. "Osteogenic differentiation of rat bone mesenchymal stem cells modulated by MiR-186 via SIRT6". *Life Science* 253 (2020): 117660.

Volume 5 Issue 9 September 2021