BIOCELL
2023 47(1): 133-141

miR-103-3p regulates the differentiation of bone marrow mesenchymal stem cells in myelodysplastic syndrome

Ningyu LI[1,2,#]; Xiaofang CHEN[2,#,§]; Suxia GENG[2]; Peilong LAI[2]; Lisi HUANG[2]; Minming LI[2]; Xin HUANG[2]; Chengxin DENG[2]; Yulian WANG[2]; Jianyu WENG[2,*]; Xin DU[1,2,*]

[1] School of Medicine, South China University of Technology, Guangzhou, 510006, China
[2] Department of Hematology, Guangdong Provincial People's Hospital, Guangdong Academy of Medical Sciences, Guangzhou, 510080, China

Key words: Myelodysplastic syndrome, Mesenchymal stem cells, *miR-103-3p*, Osteogenic differentiation, Adipogenic differentiation

Abstract: The pathogenesis of myelodysplastic syndrome (MDS) may be related to the abnormal expression of microRNAs (miRNAs), which could influence the differentiation capacity of mesenchymal stem cells (MSCs) towards adipogenic and osteogenic lineages. In this study, exosomes from bone marrow plasma were successfully extracted and identified. Assessment of *miR-103-3p* expression in exosomes isolated from BM in 34 MDS patients and 10 controls revealed its 0.52-fold downregulation in patients with MDS compared with controls (NOR) and was downregulated 0.55-fold in MDS-MSCs compared with NOR-MSCs. Transfection of MDS-MSCs with the *miR-103-3p* mimic improved osteogenic differentiation and decreased adipogenic differentiation *in vitro*, while inhibition of *miR-103-3p* showed the opposite results in NOR-MSCs. Thus, the expression of *miR-103-3p* decreases in MDS BM plasma and MDS-MSCs, significantly impacting MDS-MSCs differentiation. The *miR-103-3p* mimics may boost MDS-MSCs osteogenic differentiation while weakening lipid differentiation, thereby providing possible target for the treatment of MDS pathogenesis.

Introduction

Bone marrow loss and a high proclivity to leukemic progression are the characteristics of myelodysplastic syndrome (MDS). Recent research has revealed that the mesenchymal niche plays a significant role in the onset and progression of the MDS (Chen *et al.*, 2021; Pronk and Raaijmakers, 2019).

The bone marrow microenvironment (BMME) is a complex network system primarily comprising stromal cells such as mesenchymal stem cells (MSCs), osteoprogenitor cells, vascular endothelial cells, monocytes, macrophages, and cytokines. It maintains normal hematopoietic function by interacting with hematopoietic stem cells (HSCs) to control their proliferation and differentiation (Morrison and Scadden, 2014). Considering that an abnormal BMME is essential for myeloid tumor cell invasion and anti-apoptosis, cloning and amplifying MDS cells, poor hematopoiesis of bone marrow, and disease progression, BMME may be a possible therapeutic target (Matsuda *et al.*, 2004; Mei *et al.*, 2018).

The bone marrow stromal cell (BMSC) population, which makes up a large part of the hematopoietic microenvironment, encompasses a variety of adherent cell types. The three most important types of BMSCs in the BMME are mesenchymal stem cells, endothelial cells, and osteoblasts, and all three play important roles in hematopoietic regulation (Chen *et al.*, 2017; Psaila *et al.*, 2012). Adipocytes, osteoblasts, astrocytes, cardiomyocytes, chondrocytes, hepatocytes, muscles, and neurons can all develop from MSCs (Doan and Chute, 2012). MSCs have a lower ability to differentiate into osteoblasts but a higher ability to differentiate into adipocytes, which could be linked to myeloid malignancies (Woods and Guezguez, 2021). However, more research into this mechanism is needed.

Recent research has suggested that the faulty activities of MDS-MSCs may be related to abnormalities in the expression of microRNA (Meunier *et al.*, 2020). For example, *DICER1* expression is downregulated in MDS-MSCs and MSCs from acute myeloid leukemia (AML) patients, and microRNA (miRNA) expression is dysregulated compared with those in controls (NOR-MSCs), and MSCs from both MDS and AML patients are changed, thus limiting stromal assistance to HSCs (Ozdogan *et al.*, 2017; Santamaría *et al.*, 2012). *MiR-7977* causes an abnormal decrease in hematopoietic

*Address correspondence to: Xin Du, Xind_gdph2020@163.com; Jianyu Weng, wsswjy@sina.com
#These authors contributed equally to this work
§Present address: Department of Hematology, Guangzhou First People's Hospital, School of Medicine, South China University of Technology, Guangzhou, 510180, China
Received: 17 February 2022; Accepted: 24 May 2022

growth factors in MSCs, leading to a reduction in the hematopoietic supportive capacity of CD34+ cells in the bone marrow (Horiguchi *et al*., 2016). Previous studies have shown that aberrant microRNA expression can affect MDS-MSCs, which can then affect MDS progression.

MicroRNAs (miRNAs) are non-coding RNAs with a length of 18–25 nucleotides and always function as a passive precursor hairpin over target gene expression by degrading or suppressing mRNA translation (Bartel, 2004). Several recent studies have shown the relevance of miRNAs in controlling the hematopoietic microenvironment (Machova *et al*., 2011). However, the involvement of miRNAs in the shift in the differentiation of MSCs from osteoblasts to adipocytes in MDS is yet to be investigated (Rhyasen and Starczynowski, 2012). We studied the database GSE139471 by GEO-2R (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE139471) and found reduced expression of *miR-103-3p* in MDS-MSCs.

In the bone marrow supernatant, miRNAs are mostly secreted by MSC-derived exosomes and are involved in cell-to-cell communication, cell signaling, and altering cell or tissue metabolism. The contents of MSC-derived exosomes are not static but depend on MSCs tissue origin, their activities, and their immediate intercellular neighbors. Hence, abnormal miRNA levels in exosomes are of great significance when MSCs are cultured with tumor cells or the *in vivo* tumor microenvironment (Muntión *et al*., 2016).

Therefore, in addition to the evidence provided by the database, we detected the expression level of *miR-103-3p* in exosomes from BM supernatant of MDS patients.

MiR-103 is associated with endometrial cancer, breast cancer, rectal cancer, and other tumors (Chung *et al*., 2009; Yu *et al*., 2012; Nonaka *et al*., 2015) and is also relevant for the proliferation of leukemia cells (Kfir-Erenfeld *et al*., 2017). *MiR-103* plays a key role in facilitating the differentiation of MSCs into adipocytes and promotes preadipocyte ER stress and apoptosis by inhibiting the canonical Wnt/β-catenin pathway (Zhang *et al*., 2018).

Osteogenic differentiation is regulated by: runt-related transcription factor 2 (*RUNX2*). *RUNX2* is an essential regulator of bone formation and osteogenic differentiation of MSCs (Almalki and Agrawal, 2016). It has a significant impact on the transformation of bone marrow mesenchymal stem cells to osteoblasts (Zhao *et al*., 2021) and has been identified as a major player in the maintenance of bone homeostasis by promoting osteogenic differentiation of MSCs (Djouad *et al*., 2017). A number of miRNAs have been recently identified in the regulation of *RUNX2* expression/activity, thus affecting the process of osteogenesis. MiRNAs targeting *RUNX2* corepressors favor osteogenesis, while those targeting *RUNX2* coactivators inhibit osteogenesis (Narayanan *et al*., 2019).

Adipogenesis relies on the regulation of the expression of several transcription factors, including peroxisome proliferation-activated receptor γ (*PPARG*). *PPARG* is one of the master regulators of adipogenic differentiation (Zhuang *et al*., 2016) and is expressed in both osteoblasts and adipocytes, as well as in MSCs, suggesting its crucial role in regulating adipocyte formation and osteoblast development (Giaginis *et al*., 2007), and it is considered a validation marker of the adipogenic differentiation of MSCs.

In this study, following our hypothesis that *miR-103-3p* is related to the function of MDS-MSCs, we detected the expression and level of *miR-103-3p* and investigated the effect of *miR-103-3p* on the differentiation of MSCs obtained from the bone marrow of MDS patients.

Materials and Methods

Patients

The MDS patients were recruited from the Department of Hematology, Guangdong Provincial People's Hospital, Guangdong Academy of Medical Sciences, Guangzhou, Guangdong, China. All the patients diagnosed with MDS were over 18 years of age. Baseline characteristics of the study population included sex, age, peripheral blood cell count, WHO classification, and IPSS-R risk group (Table 1).

Exosome isolation and identification from bone marrow plasma

The Total Exosome Isolation kit (Thermo Scientific, Waltham, MA, USA) was used to extract exosomes from bone marrow plasma. We mixed 1 mL plasma with 0.5 mL PBS and mixed well using a vortex. Then, we added 0.05 mL Proteinase K and 0.2 mL exosome precipitation reagent to the sample and mixed them thoroughly. After incubation of the sample for 10 min at room temperature, we centrifuged the samples for 5 min at room temperature and 10,000 g and discarded the supernatant. The exosomes were trapped in a pellet at the tube's bottom.

Exosomes are formed when the plasma membrane is doubly invaginated, forming intracellular multivesicular bodies (MVBs) containing intraluminal vesicles. These vesicles are finally released as exosomes with diameters varying from 40 to 160 nm because of MVB fusion to the plasma membrane and exocytosis. Their average diameter is 100 nm. In the realm of exosome research, nanoparticle tracking analysis (NTA) has been recognized as one of the methods for exosome characterization (Kalluri and LeBleu, 2020).

Isolation, culture, and identification of bone marrow mesenchymal stem cells

Fresh bone marrow was used to isolate the mononuclear cells. The cells were then placed in a T75 flask, and the culture medium used was the human MesenCult proliferation kit (STEMCELL Technologies Canada). When the confluence reached 90%, cell passage was accomplished with 0.25% trypsin, usually on the 14th day.

The surface markers of the cultured MSCs were detected by flow cytometry (BD Biosciences, USA). The MSCs were positive for CD73, CD90, and CD105 and negative for CD34, CD45, and CD14 (eBiosciences, USA) (Mushahary *et al*., 2018), and suitable isotypic controls were used to characterize the MSCs.

Differentiation analysis

MSCs differentiate into adipogenic and osteogenic lineages (Malvicini *et al*., 2019). A total of 2 × 105 cells were seeded per well in six-well plates containing an osteogenic induction medium, the OriCell® Adult Bone Marrow Mesenchymal Stem Cell Osteogenic Induction and Differentiation Kit (Cyagen), for 21 days; the medium was changed every three days for osteogenic differentiation. On the 21st day, the cells were fixed for 1 h with paraformaldehyde and then stained with Alizarin Red S solution at room temperature for 30 min. An adipogenic

TABLE 1

Baseline demographics and disease characteristics

	Healthy individuals (n = 10)	Patients with MDS (n = 34)
Sex, *n* (%)		
Male	6 (60%)	21 (61.8%)
Female	4 (40%)	13 (38.2%)
Median age (years)	31 (25~42)	56 (22~71)
WHO classification, *n* (%)		
MDS-SLD		3 (8.8%)
MDS-MLD		5 (14.7%)
MDS-EB-1		7 (20.1%)
MDS-EB-2		9 (21.4%)
MDS 5q-		2 (5.9%)
MDS-U		5 (14.7%)
MDS-RS		3 (8.8%)
IPSS-R risk group, *n* (%)		
Very low		6 (17.6%)
Low		7 (20.6%)
Intermediate		9 (26.5%)
High		7 (20.6%)
Very high		5 (14.7%)
Hematology, median (min, max)		
Hemoglobin (g/L)	149 (134~154)	72 (44~152)
Leukocyte count (109/L)	5.69 (3.43~7.57)	2.54 (0.62–6.96)
Platelet count (109/L)	248 (192~286)	78 (5~362)
Neutrophil count (109/L)	3.25 (1.45~4.93)	1.51 (0.32–4.22)

Notes: MDS-SLD (MDS with single lineage dysplasia); MDS-MLD (MDS with multilineage dysplasia); MDS-EB-1 (MDS with excess blasts-1); MDS-EB-2 (MDS with excess blasts-2); MDS 5q-(MDS with isolated del(5q)); MDS-U (MDS, unclassifiable); MDS-RS (MDS with ring sideroblasts); WHO, the world health organization; RAEB, refractory anemia with an excess blast; IPSS-R, revised International Prognostic Scoring System.

induction medium, the OriCell® Human Bone Marrow Mesenchymal Stem Cell Adipogenic Differentiation Basal Medium (Cyagen), was used for adipogenic differentiation. A total of 2 × 105 cells were plated per well in six-well plates containing the full lipid-induced differentiation medium. For each cycle, MSCs were induced to differentiate in medium A for 72 h and then cultured in medium B for 24 h. After 3–5 induction cycles (21 days), the cells were dyed with an oil red solution at room temperature for half an hour.

miRNA transfection

miR-103-3p mimic, inhibitor, and negative control (mir-NC) were synthesized by Ribo Biotechnology Company, Guangzhou, China. When the MSCs passed to the third generation and reached 60%–70% confluence, Lipofectamine 3000 Transfection Reagent (Thermo Scientific, Waltham, MA, USA) was used to transfect human bone marrow MSCs with *miR-103-3p* mimic, *miR-103-3p* inhibitor, and mir-NC all labeled by fluorescent. The transfection working solution was discarded after 48 h, and the MSCs were washed three times with phosphate buffer saline (PBS).

Gene expression analysis

After routine culture for one week, total RNA was extracted from the cells using the miRNeasy Mini Kit (Qiagen, Germany). The extracted RNA was reverse transcribed into cDNA using miRNA 1st strand cDNA synthesis kit AG11717 (Accurate Biology, Hunan, China) following the manufacturer's instructions. The RNA expression levels of *PPARG*, *RUNX2*, and *miR-103-3p* were determined using quantitative polymerse chain reaction (qPCR). The reference gene for *miR-103-3p* was *U6*. The qPCR cycling conditions were as follows: 95°C for 30 s followed by 40 cycles of 95°C for 5 s and 60°C for 30 s.

Statistical analysis

GraphPad Prism 7.0 (GraphPad Software, San Diego, CA) was used to perform statistical analysis and prepare graphs. The means of the two samples were compared by independent-sample or paired-sample t test, depending on the specific situation.

Results

miR-103-3p expression in exosomes generated from the bone marrow of patients with myelodysplastic syndrome

Exosomes were extracted from the BM supernatant of MDS patients, and the exosomal RNA was used for qPCR to detect the expression level of our target microRNA. The expression of *miR-103-3p* was noticeably reduced (0.52-fold, $\Delta\Delta C_T$ = 1.994458422) in exosomes from bone marrow plasma of 34 patients with MDS compared with healthy controls ($P \leq 0.01$) (Fig. 1A).

Several methods, including electron microscopy, flow cytometry, and western blot analysis, have been used to characterize exosomes (Mateescu *et al.*, 2017). Herein, electron microscopy revealed exosome structures in all the samples (Fig. 1B). As shown by NTA, exosomes were basically

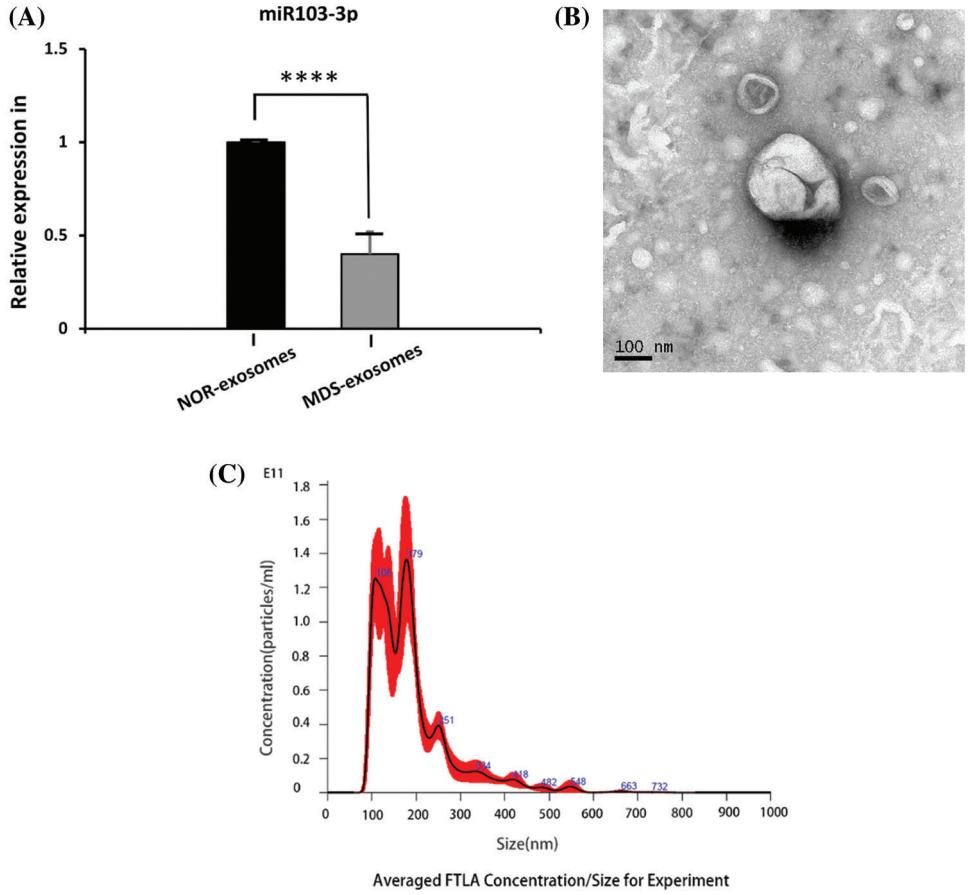

FIGURE 1. (A) Expression of *miR-103-3p* in exosomes derived from the bone marrow plasma samples from 10 healthy controls and 34 patients with MDS ****$P \leq 0.01$. (B) Under the electron microscope, exosome structures were observed in all samples. (C) The measured average particle size of the samples was 189.4 ± 2.6 nm, and exosomes were basically in the distribution range of 30–200 nm.

in the distribution range of 30–200 nm (Fig. 1C). The measured average particle size of the exosome was 189.4 ± 2.6 nm.

The expression of miR-103-3p in mesenchymal stem cells derived from bone marrow

MSCs express CD90, CD73, and CD105, but not CD34, CD14, or CD45, and are plastic-adherent in culture. MDS-MSCs collected from five samples (P3) expressed >90% CD105, CD90, and CD73 surface markers, and did not express CD45, CD34, and CD14 surface markers (Fig. 2A). In early passage (P3), both MDS-MSCs (n = 5) and NOR-MSCs (n = 5) had typical fibroblast-like morphology (Fig. 2B).

Analysis of the database GSE139471 by GEO-2R (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE139471) revealed that the reduction of *miR-103-3p* was the highest and was statistically significant in MDS-MSCs among the 384 microRNAs tested, and quantitative real-time-polymerase chain reaction (qRT-PCR) demonstrated a noticeable decrease in expression of *miR-103-3p* (0.55-fold, $\Delta\Delta C_T$ = 3.201984678) in BM MSCs of 19 MDS patients, compared with healthy donors ($P \leq 0.01$) (Fig. 2C).

Inhibition of adipogenic differentiation due to high expression of miR-103-3p and enhanced osteogenic differentiation of myelodysplastic syndrome-mesenchymal stem cells

To determine miRNA transfection concentration and overexpression in MDS and downregulated in NOR, we tried transfecting with gradient concentrations (50, 100, 160 nM) miRNA to find the concentration with the highest transfection success rate by measuring fluorescence signal and a transfection concentration of 160 nM of the mimic achieved the highest transfection success rate, that is, more than 90% (Fig. 3A).

To evaluate whether the aberrant expression of *miR-103-3p* could affect the function of MSCs in MDS, the *miR-103-3p* mimic was transfected along with Lipo3000 into 16 MDS-MSCs. After transfection, there was a noticeable improvement in the expression of *miR-103-3p* in MDS-MSCs (average fold change 7722.52, $P \leq 0.05$) (Fig. 3B). Meanwhile, we transfected the inhibitor of *miR-103-3p* into five NOR-MSCs.

Additional testing was needed to confirm the impact of *miR-103-3p* on osteogenic differentiation of MDS-MSCs. Therefore, we overexpressed *miR-103-3p* in MDS-MSCs and downregulated *miR-103-3p* in NOR-MSCs. Adipogenic differentiation was significantly inhibited in the MDS-MSCs +mimic group and enhanced in the NOR-MSCs+inhibitor group (Fig. 3D). The opposite was observed for adipogenic differentiation. Meanwhile, the Oil O Red staining revealed decreased lipid content in the MDS-MSCs+mimic group, while alizarin red staining revealed a significant reduction in the formation of mineralized nodules in the NOR-MSC +inhibitor group (Fig. 3C). For lipid formation, staining in MDS-MSCs was stronger than NOR-MSCs, and weaker in

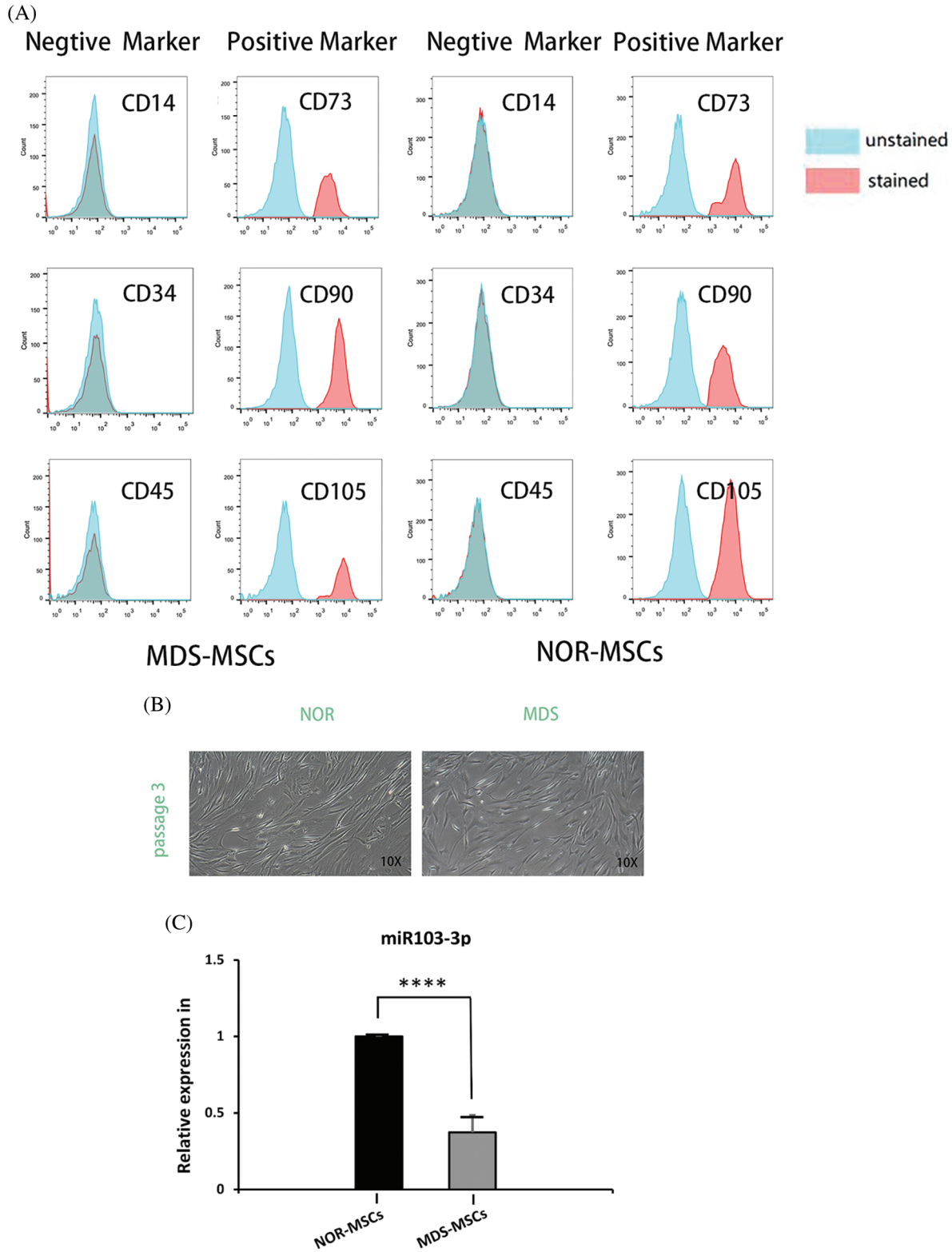

FIGURE 2. (A) Phenotypic characterization of bone marrow-mesenchymal stem cells (BM-MSCs) from control (NOR-MSCs) and those of myelodysplastic syndrome (MDS) ($n = 5$, P3). (B) Both MDS-MSCs ($n = 5$) and NOR-MSCs ($n = 5$) showed typical fibroblast-like morphology in the early passage (P3). (C) The expression of *miR-103-3p* was obviously decreased in the bone marrow of MSCs of patients with MDS, $n = 19$, $^{****}P \leq 0.01$.

bone formation than NOR-MSCs (Figs. 3C and 3D). These data indicated that the high expression of *miR-103-3p* significantly impeded the advancement of adipogenic differentiation of MDS-MSCs. Overexpression of *miR-103-3p* inhibited osteogenic differentiation of hMSCs. Following the overexpression of *miR-103-3p* in MDS-MSCs, there was a significant increase in the expression of *RUNX2*, whose activity is associated with osteoblast differentiation (Narayanan *et al.*, 2019). However, the expression of *PPARG*, an indispensable factor of adipogenesis whose pathway promotes the formation of

adipocytes (Meyer *et al.*, 2016), decreased slightly (Fig. 3E). Under decreased *miR-103-3p* expression in NOR-MSCs, the opposite phenomenon was observed (Fig. 3F).

Discussion

One of the many types of endogenous non-coding, short, small RNAs, known as miRNAs, control the expression of certain genes posttranscriptionally by limiting protein translation or speeding up the degradation of messenger RNA. The main RNA-induced silencing complex is formed by complementary binding with 3′UTR of the target gene mRNA (Bartel, 2004). Over 60% of the human genome contains potential miRNA-binding sites, so the powerful potential of miRNA cannot be ignored (Friedman *et al.*, 2009). We demonstrated that *miR-103-3p* was poorly expressed in exosomes and MSCs derived from the patients with MDS. However, the knowledge of the pathogenetic role of *miR-103-3p* in MDS is still lacking. MDS-MSCs may secrete miRNA into microvesicles, a newly discovered method for communication and control between cells (Chen *et al.*, 2010). Therefore, we first detected the expression level of *miR-103-3p* in exosomes of MDS BM supernatant and observed its reduced expression. Then, the same results were observed when we detected the level of *miR-103-3p* expression in MDS-MSCs. These results prove that the decrease in *miR-103-3p* levels in bone marrow was due to the release of MSC-derived exosomes into the BMME. Next, we discussed the role of *miR-103-3p* in MDS-MSCs in the BMME.

MDS is characterized by ineffective hematopoiesis, which results in considerable morbidity and mortality. MDS-MSCs have been shown to support hematopoiesis through physiological processes, although their role in the pathophysiology of MDS is still unknown. MDS-derived MSCs have much less osteogenic differentiation ability, as evidenced by cytochemical staining and lower expression levels of Osterix and osteocalcin (Geyh *et al.*, 2013). In contrast, MDS-MSCs have a higher proclivity to adipogenic differentiation (Wu *et al.*, 2017). Therefore,

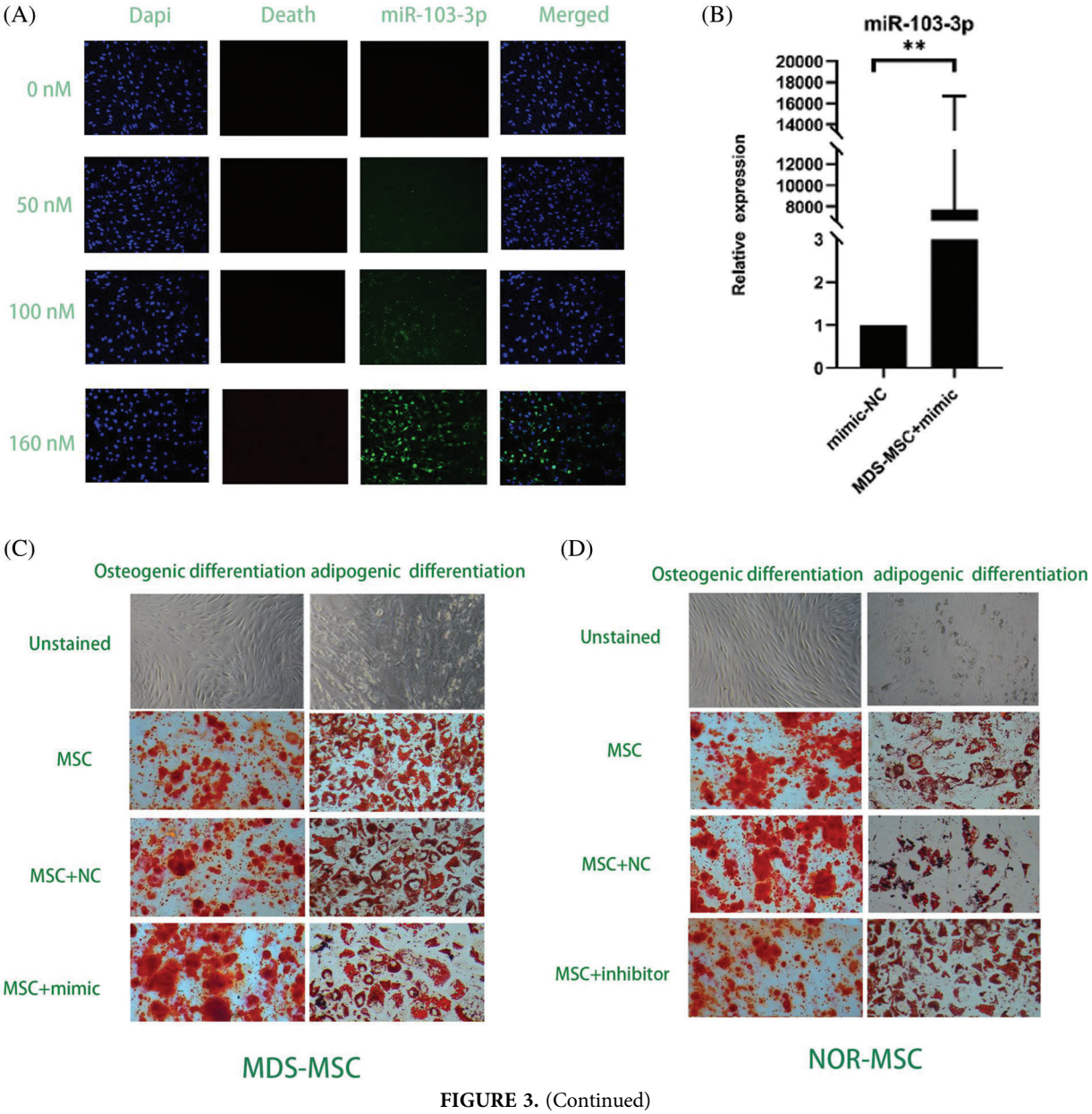

FIGURE 3. (Continued)

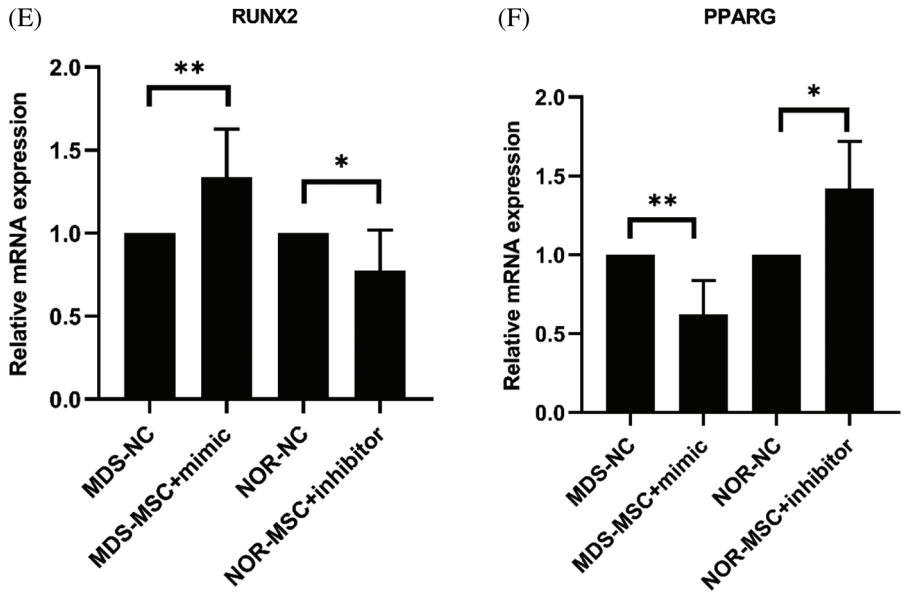

FIGURE 3. (A) At160 nM concentrations of the *miR-103-3p* mimic and the inhibitor, the transfection rate of myelodysplastic syndrome-mesenchymal stem cells (MDS-MSCs) was more than 90%. (B) After the transfection, the expression of *miR-103-3p* obviously increased in MDS-MSCs. (C) To compare the degree of differentiation of the cells in each of the groups (as in the figure above), oil red and alizarin red staining were conducted. Compared with the NC group, the alizarin red color in the MDS-MSC+mimic group as greatly enhanced, whereas oil red staining was significantly reduced. (D) Compared with the NC group (control), the NOR-MSC+inhibitor group showed much less calcification. The density of lipid droplets increased considerably. (E) Quantitative real-time polymerase chain (qRT-PCR) analysis of mRNA expression levels of *RUNX2* in MDS-MSCs and NOR-MSCs transfected with the *miR-103-3p* mimic or inhibitor. $n = 6$, $^*P \leq 0.01$. (F) Q-PCR analysis of mRNA expression levels of *PPARG* (coding for peroxisome proliferation-activated receptor γ) in MDS-MSCs and NOR-MSCs transfected with the *miR-103-3p* mimic and the *miR-103-3p* inhibitor. $n = 6$, $^* P \leq 0.01$.

through the differentiation experiment to explore the effect of *miR-103-3p* on the hematopoietic microenvironment of MDS, we could verify the effect of *miR-103-3p* on the differentiation ability of MDS-MSCs.

Our findings show that downregulation of *miR-103-3p* markedly decreased calcium deposition and increased lipid content. However, there was a significant increase in calcium deposition and a decrease in lipids in MDS-MSCs transfected with the *miR-103-3p* mimic. Silencing of *miR-103-3p* by an inhibitor increased the amount of lipids and decreased calcium deposition. Overexpression of *miR-103-3p* promoted the expression of the osteoblastic marker *RUNX2* and decreased the expression of the adipogenic marker *PPARG* compared with the cells treated with mimic NC on day 21 after being differentiated. These data suggest that the low expression of *miR-103-3p* suppressed the osteogenic differentiation of MDS-MSCs and promoted adipogenic differentiation. Histological stainings showed significantly reduced calcium deposition in MDS-MSCs compared with that in NOR-MSCs, while there were significantly more lipid droplets in MDS-MSCs than in NOR-MSCs, which is in agreement with various earlier studies (Geyh *et al.*, 2013; Wu *et al.*, 2017). Osteoblasts support hematopoietic progenitors and comprise a crucial component of the HSC niche ('endosteal niche') (Arai *et al.*, 2004; Calvi *et al.*, 2003; Shiozawa *et al.*, 2008; Taichman and Emerson, 1994), and adipogenic differentia which leads to ineffective hematopoiesis (Shiozawa *et al.*, 2008). However, after the after the level of *miR-103-3p* in MDS was further reduced, the lipid-forming ability of MSCs was weakened, and the osteogenic ability was enhanced. Therefore, we believe that the function of MSCs is improved toward hematopoietic, which is beneficial to the hematopoietic microenvironment of MDS and enhancing the expression of *miR-103-3p* can enhance the hematopoietic support capacity of MSCs.

In summary, *miR-103-3p* is poorly expressed in plasma exosomes and MSCs derived from bone marrow of patients with MDS. Promoting the expression of *miR-103-3p* could stimulate osteogenic differentiation and inhibit adipogenic differentiation of MDS-MSCs *in vitro*, which can explain the invalid hematopoiesis of MDS. *miR-103-3p* has not been studied earlier for its role in MDS, and no evidence of hematopoietic association has been reported. Although the specific mechanism is not yet clear, the relationship between *miR-103-3p*, MDS-MSCs, and exosomes is worth further studies, and abnormal miRNA expression from MDS-MSC-derived exosomes can be considered a new therapeutic approach for the treatment of MDS.

Conclusion

The results showed that the expression level of *mir-103-3p* in MDS exosomes and mesenchymal stem cells was significantly reduced, and *mir-103-3p* could significantly affect the differentiation function of MDS-MSCs *in vitro*, but the effect of *mir-103-3p* on hematopoietic needs further study.

Acknowledgement: The authors thank the Central Laboratory of Guangdong Provincial People's Hospital (Guangdong 308 Academy of Medical Sciences).

Availability of Data and Materials: The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Author Contribution: The authors confirm contribution to the paper as follows: study conception and design: Xiaofang Chen, Ningyu Li, Xin Du; data collection: Ningyu Li; analysis and interpretation of results: Ningyu Li; draft manuscript preparation: Ningyu Li, Xiaofang Chen. All authors reviewed the results and approved the final version of the manuscript.

Ethical Approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The Research Ethics Committee: Guangdong Provincial People's Hospital, Guangdong Academy of Medical Sciences; Ethical approval code: 2019646H; Date of approval: November 15, 2019.

Informed Consent: Informed consent was obtained from all individual participants included in the study.

Funding Statement: This work was supported by The Nature Science Foundation of China (Nos. 82070176, 82070128, 81900132) and the Medical Science and Technology Research Fund of Guangdong Province (No. A2020585).

Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

References

Almalki SG, Agrawal DK (2016). Key transcription factors in the differentiation of mesenchymal stem cells. *Differentiation* **92**: 41–51. DOI 10.1016/j.diff.2016.02.005.

Arai F, Hirao A, Ohmura M, Sato H, Matsuoka S, Takubo K, Ito K, Koh GY, Suda T (2004). Tie2/angiopoietin-1 signaling regulates hematopoietic stem cell quiescence in the bone marrow niche. *Cell* **118**: 149–161. DOI 10.1016/j.cell.2004.07.004.

Bartel DP (2004). MicroRNAs: Genomics, biogenesis, mechanism, and function. *Cell* **116**: 281–297. DOI 10.1016/S0092-8674(04)00045-5.

Calvi LM, Adams GB, Weibrecht KW, Weber JM, Olson DP et al. (2003). Osteoblastic cells regulate the haematopoietic stem cell niche. *Nature* **425**: 841–846. DOI 10.1038/nature02040.

Chen TS, Lai RC, Lee MM, Choo AB, Lee CN, Lim SK (2010). Mesenchymal stem cell secretes microparticles enriched in pre-microRNAs. *Nucleic Acids Research* **38**: 215–224. DOI 10.1093/nar/gkp857.

Chen X, Li N, Weng J, Du X (2021). Senescent mesenchymal stem cells in myelodysplastic syndrome: Functional alterations, molecular mechanisms, and therapeutic strategies. *Frontiers in Cell and Developmental Biology* **8**: 617466. DOI 10.3389/fcell.2020.617466.

Chen X, Yi H, Zhang B, Liu Y, Wang S, Liao T, Deng Z, Zeng F, Jing Z, He L (2017). Caffeic acid phenethyl ester promotes haematopoietic stem/progenitor cell homing and engraftment. *Stem Cell Research & Therapy* **8**: 255. DOI 10.1186/s13287-017-0708-x.

Chung TK, Cheung TH, Huen NY, Wong KW, Lo KW et al. (2009). Dysregulated microRNAs and their predicted targets associated with endometrioid endometrial adenocarcinoma in Hong Kong women. *International Journal of Cancer* **124**: 1358–1365. DOI 10.1002/ijc.24071.

Djouad F, Ipseiz N, Luz-Crawford P, Scholtysek C, Krönke G, Jorgensen C (2017). PPARβ/δ: A master regulator of mesenchymal stem cell functions. *Biochimie* **136**: 55–58. DOI 10.1016/j.biochi.2016.11.011.

Doan PL, Chute JP (2012). The vascular niche: Home for normal and malignant hematopoietic stem cells. *Leukemia* **26**: 54–62. DOI 10.1038/leu.2011.236.

Friedman RC, Farh KK, Burge CB, Bartel DP (2009). Most mammalian mRNAs are conserved targets of microRNAs. *Genome Research* **19**: 92–105. DOI 10.1101/gr.082701.108.

Geyh S, Oz S, Cadeddu RP, Fröbel J, Brückner B et al. (2013). Insufficient stromal support in MDS results from molecular and functional deficits of mesenchymal stromal cells. *Leukemia* **27**: 1841–1851. DOI 10.1038/leu.2013.193.

Giaginis C, Tsantili-Kakoulidou A, Theocharis S (2007). Peroxisome proliferator-activated receptors (PPARs) in the control of bone metabolism. *Fundamental & Clinical Pharmacology* **21**: 231–244. DOI 10.1111/j.1472-8206.2007.00486.x.

Horiguchi H, Kobune M, Kikuchi S, Yoshida M, Murata M et al. (2016). Extracellular vesicle miR-7977 is involved in hematopoietic dysfunction of mesenchymal stromal cells via poly(rC) binding protein 1 reduction in myeloid neoplasms. *Haematologica* **101**: 437–447. DOI 10.3324/haematol.2015.134932.

Kalluri R, LeBleu VS (2020). The biology, function, and biomedical applications of exosomes. *Science* **367**: 6977. DOI 10.1126/science.aau6977.

Kfir-Erenfeld S, Haggiag N, Biton M, Stepensky P, Assayag-Asherie N, Yefenof E (2017). miR-103 inhibits proliferation and sensitizes hemopoietic tumor cells for glucocorticoid-induced apoptosis. *Oncotarget* **8**: 472–489. DOI 10.18632/oncotarget.13447.

Machová PK, Lopotová T, Klamová H, Burda P, Trněný M, Stopka T, Moravcová J (2011). Expression patterns of microRNAs associated with CML phases and their disease related targets. *Molecular Cancer* **10**: 41. DOI 10.1186/1476-4598-10-41.

Malvicini R, Santa-Cruz D, Pacienza N, Yannarelli G (2019). OCT4 silencing triggers its epigenetic repression and impairs the osteogenic and adipogenic differentiation of mesenchymal stromal cells. *International Journal of Molecular Sciences* **20**: 3268. DOI 10.3390/ijms20133268.

Mateescu B, Kowal EJ, van Balkom BW, Bartel S, Bhattacharyya SN et al. (2017). Obstacles and opportunities in the functional analysis of extracellular vesicle RNA-an ISEV position paper. *Journal of Extracellular Vesicles* **6**: 1286095. DOI 10.1080/20013078.2017.1286095.

Matsuda M, Morita Y, Hanamoto H, Tatsumi Y, Maeda Y, Kanamaru A (2004). CD34+ progenitors from MDS patients are unresponsive to SDF-1, despite high levels of SDF-1 in bone marrow plasma. *Leukemia* **18**: 1038–1040. DOI 10.1038/sj.leu.2403301.

Mei Y, Zhao B, Basiorka A A, Yang J, Cao L, Zhang J, List A, Ji P (2018). Age-related inflammatory bone marrow microenvironment induces ineffective erythropoiesis mimicking del(5q) MDS. *Leukemia* **32**: 1023–1033. DOI 10.1038/leu.2017.326.

Meunier M, Guttin A, Ancelet S, Laurin D, Zannoni J et al. (2020). Extracellular vesicles from myelodysplastic mesenchymal stromal cells induce DNA damage and mutagenesis of hematopoietic stem cells through miRNA transfer. *Leukemia* **34**: 2249–2253. DOI 10.1038/s41375-020-0738-8.

Meyer MB, Benkusky NA, Sen B, Rubin J, Pike JW (2016). Epigenetic plasticity drives adipogenic and osteogenic differentiation of marrow-derived mesenchymal stem cells. *Journal of Biological Chemistry* **291**: 17829–17847. DOI 10.1074/jbc.M116.736538.

Morrison SJ, Scadden DT (2014). The bone marrow niche for haematopoietic stem cells. *Nature* **505**: 327–334. DOI 10.1038/nature12984.

Muntión S, Ramos TL, Diez-Campelo M, Rosón B, Sánchez-Abarca LI et al. (2016). Microvesicles from mesenchymal stromal cells are involved in HPC-microenvironment crosstalk in myelodysplastic patients. *PLoS One* **11**: e0146722. DOI 10.1371/journal.pone.0146722.

Mushahary D, Spittler A, Kasper C, Weber V, Charwat V (2018). Isolation, cultivation, and characterization of human mesenchymal stem cells. *Cytometry Part A* **93**: 19–31. DOI 10.1002/cyto.a.23242.

Narayanan A, Srinaath N, Rohini M, Selvamurugan N (2019). Regulation of *RUNX2* by MicroRNAs in osteoblast differentiation. *Life Sciences* **232**: 116676. DOI 10.1016/j.lfs.2019.116676.

Nonaka R, Miyake Y, Hata T, Kagawa Y, Kato T et al. (2015). Circulating miR-103 and miR-720 as novel serum biomarkers for patients with colorectal cancer. *International Journal of Oncology* **47**: 1097–1102. DOI 10.3892/ijo.2015.3064.

Ozdogan H, Gur Dedeoglu B, Oztemur Islakoglu Y, Aydos A, Kose S et al. (2017). DICER1 gene and miRNA dysregulation in mesenchymal stem cells of patients with myelodysplastic syndrome and acute myeloblastic leukemia. *Leukemia Research* **63**: 62–71. DOI 10.1016/j.leukres.2017.10.006.

Pronk E, Raaijmakers M (2019). The mesenchymal niche in MDS. *Blood* **133**: 1031–1038. DOI 10.1182/blood-2018-10-844639.

Psaila B, Lyden D, Roberts I (2012). Megakaryocytes, malignancy and bone marrow vascular niches. *Journal of Thrombosis and Haemostasis* **10**: 177–188. DOI 10.1111/j.1538-7836.2011.04571.x.

Rhyasen GW, Starczynowski DT (2012). Deregulation of microRNAs in myelodysplastic syndrome. *Leukemia* **26**: 13–22. DOI 10.1038/leu.2011.221.

Santamaría C, Muntión S, Rosón B, Blanco B, López-Villar O et al. (2012). Impaired expression of DICER, DROSHA, SBDS and some microRNAs in mesenchymal stromal cells from myelodysplastic syndrome patients. *Haematologica* **97**: 1218–1224. DOI 10.3324/haematol.2011.054437.

Shiozawa Y, Havens AM, Pienta KJ, Taichman RS (2008). The bone marrow niche: habitat to hematopoietic and mesenchymal stem cells, and unwitting host to molecular parasites. *Leukemia* **22**: 941–950. DOI 10.1038/leu.2008.48.

Taichman RS, Emerson SG (1994). Human osteoblasts support hematopoiesis through the production of granulocyte colony-stimulating factor. *The Journal of Experimental Medicine* **179**: 1677–1682. DOI 10.1084/jem.179.5.1677.

Woods K, Guezguez B (2021). Dynamic changes of the bone marrow niche: Mesenchymal stromal cells and their progeny during aging and leukemia. *Frontiers in Cell and Developmental Biology* **9**: 714716. DOI 10.3389/fcell.2021.714716.

Wu Y, Aanei CM, Kesr S, Picot T, Guyotat D, Campos Catafal L (2017). Impaired expression of focal adhesion kinase in mesenchymal stromal cells from low-risk myelodysplastic syndrome patients. *Frontiers in Oncology* **7**: 164. DOI 10.3389/fonc.2017.00164.

Yu D, Zhou H, Xun Q, Xu X, Ling J, Hu Y (2012). microRNA-103 regulates the growth and invasion of endometrial cancer cells through the downregulation of tissue inhibitor of metalloproteinase 3. *Oncology Letters* **3**: 1221–1226. DOI 10.3892/ol.2012.638.

Zhang Z, Wu S, Muhammad S, Ren Q, Sun C (2018). miR-103/107 promote ER stress-mediated apoptosis via targeting the Wnt3a/β-catenin/ATF6 pathway in preadipocytes. *Journal of Lipid Research* **59**: 843–853. DOI 10.1194/jlr.M082602.

Zhao W, Yang H, Chai J, Xing L (2021). *RUNX2* as a promising therapeutic target for malignant tumors. *Cancer Management and Research* **13**: 2539–2548. DOI 10.2147/cmar.s302173.

Zhuang H, Zhang X, Zhu C, Tang X, Yu F, Shang GW, Cai X (2016). Molecular mechanisms of PPAR-γ governing MSC osteogenic and adipogenic differentiation. *Current Stem Cell Research & Therapy* **11**: 255–264.