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Stem Cell Research 2018- Distinct Mirna Expression Patterns of Extracellular Vesicles Derived From 4 Types of Mesenchymal Stem Cells- Takahiro Ochiya- National Cancer Center Research Institute

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Introduction:

Mesenchymal stem cells (MSCs) are a population of fibroblast-like cells derived from almost all tissue in adults (adipose tissue, bone marrow, deciduous exfoliated teeth, peripheral blood) and fetal tissue (placenta, Wharton jelly and umbilical cord). These cells have the ability to differentiate into fats, bones, cartilage, muscles and neurons depending on the stimulus and culture conditions. MSCs isolated from various tissue sources exhibit significantly different morphologies, differentiation capacities and gene expression. MSCs of different tissues are widely known for not being biologically equivalent and for having variable self-renewal and multipotency capacities. Bone marrow (BM) was first described by Friedenstein et al. and are generally considered the gold standard. Adipose tissue (AT) -MSC, also known as mesenchymal stem cells derived from adipose (ADSC), has strong capacities for proliferation, repair and regeneration. Previous research has found that MSCs derived from Wharton's Jelly (WJ) have more potent immunosuppressive and therapeutic activities. Human exfoliated deciduous tooth stem cells (SHED) have been reported to differentiate into neural cells and express neural markers under neural induction. MSCs are emerging as a powerful new tool for the treatment of various diseases. The MSC EV functions as an extension of the biological roles of the MSC. They exert specific effects on their microenvironment and play an important role in intercellular communication in healthy and diseased tissue. MicroRNAs (miRNAs) were first identified in 1993 and are small, non-coding small RNAs of approximately 20-22 nucleotides. MRNAs regulate approximately 30 to 70% of gene expression by binding to the 3 'untranslated region (UTR) of target mRNAs. As one of the cargo contents of VEs, miRNAs are key contributors to the overall biological function of VEs and source cells. They are known to regulate post-transcriptionally the expression of genes involved in MSC differentiation pathways

Methods

Culture of BM-MSCs, AT-MSCs, WJ-MSCs and SHED

Ethical approval (IRB No. 18000015) was obtained from the cosmos of the Tsukiji clinic. Four types of mesenchymal stem cells were donated by four individuals. Mesenchymal stem cells derived from bone marrow (BM-MSC) and mesenchymal stem cells derived from adipose tissue (AD-MSC) were obtained from patients who received medical treatment by injection of cells to improve associated structural changes skin aging before application of the Law on the Safety of Regenerative Medicine on November 25, 2014 and long-term consent provided. BM-MSCs were isolated according to the method described by Pittenger et al. [18]. The AD-MSCs were isolated according to the method described by Zuk PA et al. [19]. Wharton jelly mesenchymal stem cells (WJ-MSC) were extracted from the human umbilical cord (UC). The CU was obtained from patients who gave their full consent, and the CU was taken immediately after natural delivery. The WJ-MSCs were isolated according to the method described by Sarugaser et al. [20]. Human exfoliated deciduous teeth stem cells (SHEDs) were obtained from the consenting patient from the deciduous teeth immediately. SHEDs were isolated according to the method described by Gronthos et al. [21] and Miura et al. [22]. Each cell type was grown with Dulbecco's modified Eagle medium with 4500 mg / L glucose, 584 mg / L L-glutamine, 110 mg / L sodium pyruvate and 3700 mg / L sodium bicarbonate (DMEM D6429; Sigma-Aldrich, St. Louis, MO, USA) containing 100 units / ml of penicillin, 100 µg / ml of streptomycin and

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0.25 µg / ml of antibiotic-antimycotic amphotericin B (100 x; Gibco ™, Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% fetal bovine serum (S1820 FBS; Biowest Nuaillé, France). When the cells have proliferated to 80-100% confluence, they have been subjected to passage using TrypLE ™ Select (Gibco ™) and subjected to passage to P4. All past P4 cells were used for the collected conditioned medium (CM).

RNA extraction and miRNA analysis:

Total RNA was extracted from the EVs using the QIAzol reagent and the miRNeasy mini kit (Qiagen, Hilden, Germany). The quantity and quality of the extracted RNA were determined using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific Inc. USA) and the Agilent Bioanalyzer system (Agilent Technologies, USA), as recommended. Total RNA was labeled with cyanine 3 (Cy3) using the miRNA complete labeling kit and the Hyb kit (Agilent Technologies) as indicated by the manufacturer. Briefly, the total RNA was dephosphorylated by incubation with the calf intestinal alkaline phosphatase (CIP) masterbatch at 37 ° C for 30 min. The dephosphorylated RNA was denatured by incubation with DMSO at 100 ° C for 5 min, then immediately transferred to ice for 2 min. After adding a master ligation mixture for T4 RNA ligase and cyanine 3-cytidine bisphosphate (Cy3-pCp), the RNA was incubated at 16 ° C for 2 h. The labeled RNA was dried using a vacuum concentrator at 55 ° C for 1.5 h, then hybridized to Agilent SurePrint G3 8x60K human miRNA templates at 55 ° C for 20 h. After washing, the microarrays were scanned using an Agilent microarray scanner. The intensity values for each scanned feature were quantified using Agilent Feature Extraction software version 10.7.3.1, which performs subtractions in the background.

Conclusion :

MicroRNAs have recently been recognized as molecular regulators at the post-transcriptional level in a variety of biological processes. By repressing and activating the translation and stability of mRNA, miRNAs are involved in inflammation, apoptosis, angiogenesis, cell growth and mobility. MSCs are defined as an archetype of multipotent somatic stem cells. In addition, MSC subpopulations isolated from different tissues have various characteristics. Because of their accessibility, extensibility and multipotentiality, MSCs hold promise for future stem cell therapy strategies. MSC EVs have been viewed as an extension of the biological roles of MSCs. In this study, we analyzed the expression profiles of the miRNAs of VE derived from BM-MSC, AT-MSC, WJ-MSC and SHED and summarized the molecular mechanisms and target genes of these functional miRNAs. Common and distinct miRNAs help compare the characteristics and therapeutic potential of the EVs secreted by these four types of MSC. Our results provide a basis for a deeper and more precise understanding of EV MSC and highlight their biochemical potential for restoring tissue homeostasis.

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