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Research Progress on Exosomes Derived from Human Adipose Mesenchymal Stem Cells

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Abstract: Mesenchymal stem cells (MSC) have the potential of multi-directional differentiation, have stable genetic information, can self-replicate and renew, and are commonly used as seed cells in the field of regeneration[1]. Adipose mesenchymal stem cells, bone marrow mesenchymal stem cells and umbilical cord mesenchymal stem cells are the most commonly used mesenchymal stem cells. Compared with other mesenchymal stem cells, adipose mesenchymal stem cells have a wide range of sources, relatively simple acquisition methods and less trauma[2]. Therefore, adipose mesenchymal stem cells have gradually become a new favorite in stem cell research. However, due to the harsh storage conditions and inconvenient transportation of living cells, the survival rate of direct transplantation into living organisms is low, which makes it difficult for clinical application. Exosome is a kind of extracellular secretory vesicle, which exists in almost all body fluids and can be extracted from the supernatant of most cultured cells in vitro[3]. It has the characteristics of secretory cells, simple storage, convenient transportation and low immunogenicity, providing a new direction for the clinical application of adipose mesenchymal stem cells. Therefore, this paper reviews the exosomes derived from adipose mesenchymal stem cells.

Keywords: Adipose-Derived Stem Cells, ADSCs, Mesenchyma Stem Cell, Exosomes

Nomenclature and source of adipose mesenchymal stem cells

In 2001, PA zuk et al. successfully isolated a kind of human adult stem cell similar to bone marrow mesenchymal stem cell in liposuction adipose tissue and named it PLA[4].In 2002, PA zuk et al. proved that the PLA isolated had the common characteristics of mesenchymal stem cells, that is, it could conduct multidirectional differentiation under certain conditions[5]. Then scholars scrambled to name the adult stem cells from which the fat came. In 2004, the cells derived from adipose tissue that can adhere to the wall and have plastic adhesion and multidirectional differentiation were uniformly named adipose-derived stem cells（ADSCs）[6,7].

The human body is rich in adipose tissue, which can provide sufficient experimental materials for stem cell research. And with the improvement of living standards, people's obesity index is gradually rising, and the sources of adipose tissue will be more abundant. Adipose tissue can provide more stem cells than other tissues. According to research, the number of stem cells per g of bone tissue is only equal to 1/500 of stem cells per g of adipose tissue[8,9]. There are many methods to obtain adipose tissue, among which liposuction is the most important method[10]. Kevin et al. found that about 2.5 *10^5 nucleated cells could be obtained per milliliter of adipose tissue after liposuction[11], and L Aust et al. also confirmed that 4040009 ±206000 ADSCs could be isolated and cultured per milliliter of adipose tissue obtained by liposuction[12]. Other studies have shown that the amount of ADSCs in adipose tissue of different parts and layers is different, and the activity of ADSCs isolated from adipose tissue of different origins and the ability of multi-directional differentiation are also different[13].

Characteristics of adipose mesenchymal stem cells

Multidisciplinary differentiation of adipose mesenchymal stem cells

Adipose mesenchymal stem cells are derived from the mesoderm and have the ability to differentiate into other

mesoderm, that is, the ability of polydifferentiation. ADSCs can be induced and differentiated into cardiomyocytes, endothelial cells, adipocytes, osteoblasts, chondrocytes, neuro-like cells and other

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cells in vitro[14]. (1) osteoblast differentiation, ADSCs can be induced into osteoblasts under the conditions of dexamethasone + vitamin C+ -sodium glycerophosphate +10% FBS+DMEM[15], alkaline phosphatase starts to appear on the 4th day, and reaches its peak on the 7th to 14th day. Alkaline phosphatase is a marker of immature osteoblasts[16]. Type I collagen is secreted heavily in mature osteoblasts, and calcification occurs at 21-28 days after induction, forming calcium nodules[17]. (2) adipoblast differentiation. ADSCs can be induced into adipocytes under the conditions of dexamethasone + insulin +, 1-methyl-3-isobutyl-yellow piaoling + indomethacin +10% FBS+DMEM[18]. Lipid vacuoles of different sizes can be induced in the cytoplasm for 7-10 days, and the oil red O stain is red[19]. (3) chondroblast differentiation, ADSCs can be induced into chondrocytes under the condition of transforming growth factor + dexamethasone + transferrin + pyruvate + insulin +10%FBS+DMEM[20]. Type II collagen is one of the landmark substances of mature chondrocytes, which appears after 1-2 weeks of induction, and is positive for methyl blue staining[21].

The adipose derived mesenchymal stem cells are extremely capable of self-proliferation[22], and in vitro the culture of adipose mesenchymal stem cells over 14 days, the passage to the 25 generations maintains a strong proliferation ability, maintains its phenotype, and has a strong multi-differentiation potential[23].

Major markers of adipose-derived mesenchymal stem cells

At present, ADSCs have not found specific surface markers, but ADSCs are similar to bone marrow mesenchymal stem cells and can express adult stem cell phenotypes, such as CD44 and CD105, but not CD34 and CD45 from hematopoietic stem cells. CD29 and CD90 are also highly expressed in various types of mesenchymal stem cells. The difference between adipose mesenchymal stem cells and bone marrow mesenchymal stem cells is that ADSCs do not express CD106, while bone marrow mesenchymal stem cells express CD106[24].

Nomenclature and origin of exosomes

The exosome is an extracellular vesicle. The discovery of vesicles originating from cells can be traced back to 1940[25]. In 1967, the vesicles originating from cells were observed under electron microscopy[26]. The diameter of vesicles was 20-50 nm. In 1975, microvesicles with a diameter of 30-60 nm were also detected in fetal bovine serum[27].In 1987, Johnstone et al. centrifuged sheep reticulocytes in vitro for 90 minutes by 100000 g centrifugation. The vesicle was extracted and named exosome[28]. Subsequently, exosomes were gradually separated from the culture medium of other cultured cells, such as B lymphocytes[29], T lymphocytes[30], tumor cells[31], epithelial cells[32], endothelial cells[33], mesenchymal stem cells[34], oligodendrocytes[35] and so on. Therefore, most cells can release exosomes. The exosomes can also be separated from human body's natural body fluids, such as urine[36], plasma[37],breast milk[38], saliva[39], ascites[40], etc.The exosome is formed by endocytosis. First, the plasma membrane recesses inward to form an endosomes body and encapsulates the contents. Then the endosomes membrane invades partially. In the endosomal body, many vesicles are formed, called MVBs. Finally, the MVBs are combined with the cell membrane to release the intracavitary vesicles to the extracellular space, that is, the exosome[41].

Extraction and identification of exosomes

Common methods to extract exosomes include differential centrifugation, ultrafiltration, density gradient centrifugation, immunomagnetic bead method and liquid chromatography, etc. Differential centrifugation is the basis of collecting exosomes[42] and can obtain a large number of exosomes, but it will cause some damage to exosome films due to the large centrifugal force. The most commonly used density gradient method in the laboratory is the sucrose density gradient method[43], which uses the density distribution of exosomes to deposit exosomes to obtain higher purity exosomes. However, due to more preparation in the early stage and the small amount of exosomes obtained, it is not conducive to a large number of studies. With the development of technology, rapid exosome separation kits also appeared. However, due to great differences among different products, the quality and purity of the separated exosomes could not be guaranteed stably. Meanwhile, PEG may affect the exosomes obtained by using exosome separation kits when observed by transmission electron microscope.

Cell-derived vesicles are cup-shaped in shape. Cup-shaped shape is a useful feature to distinguish cell-derived vesicles from granules of similar size[25]. The diameter of microvesicles, apoptotic bodies and exosomes is mainly determined by their diameter. The diameter of microvesicles is uneven, usually concentrated in 20-10 000 nm. The diameter of apoptotic bodies is larger, concentrated in 10,000-50,000 nm, and the diameter of exosomes is between 40-100 nm[25]. Radioelectronic microscopy is the only method that can simultaneously measure vesicle size and observe vesicle morphology. It is the gold standard for identifying exosomes[7]. At the same time, there are specific markers on the surface of exosome membrane, which can also be identified by flow cytometry and Western blot. However, because of the small diameter of exosomes, the general flow cytometry can not detect them. Therefore, after enriching the exosomes by immunomagnetic beads, the exosomes can be identified by flow cytometry.

Characteristics of exosomes

The exosome is the smallest one of the extracellular vesicles. It is uniform in size and has a membrane structure between 40 and 100 nm in diameter. It has a double concave disc shape and a density between 1.13 and 1.19 g/mL. The exosome has a membrane structure,

which is different from the plasma membrane. The exosome membrane has more lipid rafts and proteins, and has its specific surface markers, such as membrane-binding proteins CD9, CD63, CD81, MHCI molecules, heat shock proteins HSP73, HPS90, etc[44,45]. The exosome contains a variety of microRNAs, proteins, cytokines, lipids, non-coding RNA[46-48], and is the basic medium of intercellular information exchange. It plays an important role in long-distance or short-distance cell signaling and material transmission[49,50]. The exosome has a relatively stable membrane structure, and its concentration is not affected by the degradation process[51]. Exosomes from different cell sources have different properties and have the characteristics of source cells. The quantity and content of exosomes are also changing according to the microenvironment of cells[51]. Stem cell-derived exosomes also have the advantages of high stability, no immune rejection, homing effect, easy control of dose and concentration[52,53]Conclusions and Future Directions

Adipose-derived mesenchymal stem cells have a wide range of sources, can be obtained in large quantities by relatively simple methods, have strong self-replication ability in vitro, can be passed on many times, and after many passages, their properties do not change, so they have obvious advantages in clinical application. Exosomes have the characteristics of source cells. They have specific proteins on their surface. They contain microRNAs, proteins, cytokines, lipids and non-coding RNA. They are important structures for intercellular material and information exchange. These three methods can be used to transfer their contents to target cells: (1) directly bind to target cells; (2) interaction between receptors and ligands; (3) endocytosis induced by endocytosis[51].The exosome membrane is relatively stable and not easy to crack. It can transport its contents to target cells more safely. Therefore, exosomes derived from mesenchymal stem cells can not only inherit the advantages of adipose-derived mesenchymal stem cells, but also have the common characteristics of exosomes, and their clinical value is immeasurable. But how to extract exosomes with high purity stably is the main problem that needs to be solved urgently at present. At the same time, how to screen exosomes containing specific content is another problem that needs to be solved in the future.

Conflict of Interest:The authors declare that they have no conflict of interest.

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