

Towards an ideal source of mesenchymal stem cell isolation for possible therapeutic application in regenerative medicine

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Background. The possibility of obtaining mesenchymal stem cells (MSCs) from fetal tissue such as amniotic fluid, chorionic villi and placenta is well-known and a comparison between MSCs originating in different sources such as fetal tissue and those from bone marrow in terms of yield and function is a topical issue. The mesenchymal stem cells isolated from bone marrow are well-characterized. Unfortunately the low quantitative yield during isolation is a major problem. For this reason, other tissue sources for MSCs are of paramount importance.

Conclusion. In this review, starting from a description of the molecular and cellular biology of MSCs, we describe alternative sources of isolation other than bone marrow. Finally, we describe the potential therapeutic application of these cells.

Key words: mesenchymal stem cells, stem cell markers, regenerative medicine

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THE SPECIFIC CELLULAR AND MOLECULAR BIOLOGY OF MSCs

Mesenchymal stem cells (MSCs) are stromal cells with a fibroblastoid morphology, capable of growing adherent to plastic in culture¹.

The expression of specific surface antigens can distinguish MSCs from other cell types and hematopoietic stem cell markers CD105, CD90, CD73, CD44, CD29 and CD13 are the most common antigens expressed by MSCs which are instead negative for all hematopoietic markers including CD34, CD45, CD11b, CD33, CD117 and HLA-DR (ref.^{2,3}).

Regarding gene expression, all MSCs express embryonic markers such as Oct-4, Nanog and Sox-2; the expression of these factors is crucial for maintaining transcriptional self-renewal and pluripotency⁴ (Fig. 1).

In embryonic stem cells, Oct-4 plays, for example, a central role in maintaining self-renewal and differentiation into specific cell lines⁵. A decreased expression of Oct-4 induces differentiation into cell types of the extraembryonic trophoblast. In contrast, an increased expression leads to differentiation into mesoderm and endoderm. Finally no expression of Oct-4 causes developmental arrest of the embryo itself⁶.

Another feature used to distinguish MSCs is their ability to differentiate *in vitro* into several mesenchymal cell

lines such as osteoblasts, chondrocytes and adipocytes² after administration of growth factors or when cultured in media supplemented with dexamethasone and ascorbate⁷⁻¹¹.

LOOKING FOR A SUITABLE SOURCE OF SUPPLY OF MSCs

Adipose tissue

In clinical practice, the main source of MSCs is adult bone marrow. However, harvesting of bone marrow mesenchymal cells is beset by several problems: it is difficult to access and it involves the use of invasive techniques. The percentage of MSCs in the bone is very low (0.001-0.01%) and it decreases with ageing¹². For these reasons it is important to find alternative sources of MSCs.

A possible alternative source of mesenchymal stem cells is adipose tissue which offers a greater number of cells and is easily accessible². Adipose tissue-derived cells are applicable for soft tissue repair and used in the clinic for the coating of scaffolds, in breast augmentation surgery and in fistula repair. Many other applications are under clinical trials¹³.

Fetal tissues

Fetal tissues represent a new alternative source of mesenchymal stem cells. They can be isolated from the fetus itself or from extraembryonic structures like umbilical cord, amniotic fluid, the Wharton's jelly, the chorionic villi and placenta¹⁴.

Many studies indicate that stem cells derived from these new sources have features both of pluripotent embryonic stem cells and multipotent adult stem cells¹⁵. Hematopoietic stem cells isolated from umbilical cord, for example, are well characterized, and their application in transplantation, especially in children, is well documented¹⁶. The non-hematopoietic cells from umbilical cord blood are instead a recent discovery. These cells grow in adhesion in vitro in the absence of cytokines or growth factors for more than ten cell passages, showing a CD34 negative, CD45 negative immunophenotype. These cells form fibroblast-like colonies and have features comparable to adult mesenchymal stem cells¹⁵. The amount of MSCs isolated from umbilical cord blood is about 20% of the total, probably because few of these cells go into the bloodstream¹⁷.

Wharton's jelly

The Wharton's jelly is the outer part of the umbilical cord and is composed of umbilical cord matrix stem cells, umbilical cord perivascular cells and stromal umbilical cord blood cells¹⁴. The cells with greater proliferative potential are located at the interface with the amniotic fluid, while the fibroblast-like cell types are close to blood vessels¹⁸. Stem cells isolated from Wharton's jelly have a typical expression profile of adult mesenchymal cells and they are able to differentiate into adipocytes, chondro-

cytes, osteocytes, cardiomyocytes and neurons¹⁹. These cells also express embryonic stem cells markers such as Oct-4, Sox-2, Nanog and Rex-1 (ref.^{20,21}).

Amniotic fluid

The amniotic fluid is usually used for prenatal diagnosis through amniocentesis but recently its potential as a source of stem cells has been highlighted. The population of cells obtained from amniotic fluid is heterogeneous, in fact it is derived from all three germ layers and it contains several partially differentiated cell types; the main cell type is the epithelial one. Tsai et al.²² developed a protocol for isolation and expansion of stem cells from amniotic fluid samples at second trimester of pregnancy, in particular between the sixteenth and twentieth week. These cells are positive for CD105 and CD90 antigens, but negative for hematopoietic antigens. In addition, they express Oct-4 (ref.²³) and they are able to differentiate into osteocytes²⁴, adipocytes and neuronal cells²⁵. All together these features strongly suggest that amniotic fluid-derived stem cells are MSCs.

Other studies have confirmed and extended these data, revealing the positivity of amniotic fluid cells for other embryonic markers including SSEA-4 and Nanog. In addition the expression of Oct-4 maintains high levels up to twenty culture passages demonstrating the ability of these cells to maintain the undifferentiated state for a long time²⁶.

The amniotic stem cells can be cultured for over eight months, maintaining a high rate of proliferation, with a doubling time of about 36 h without degradation of karyotype²⁷.

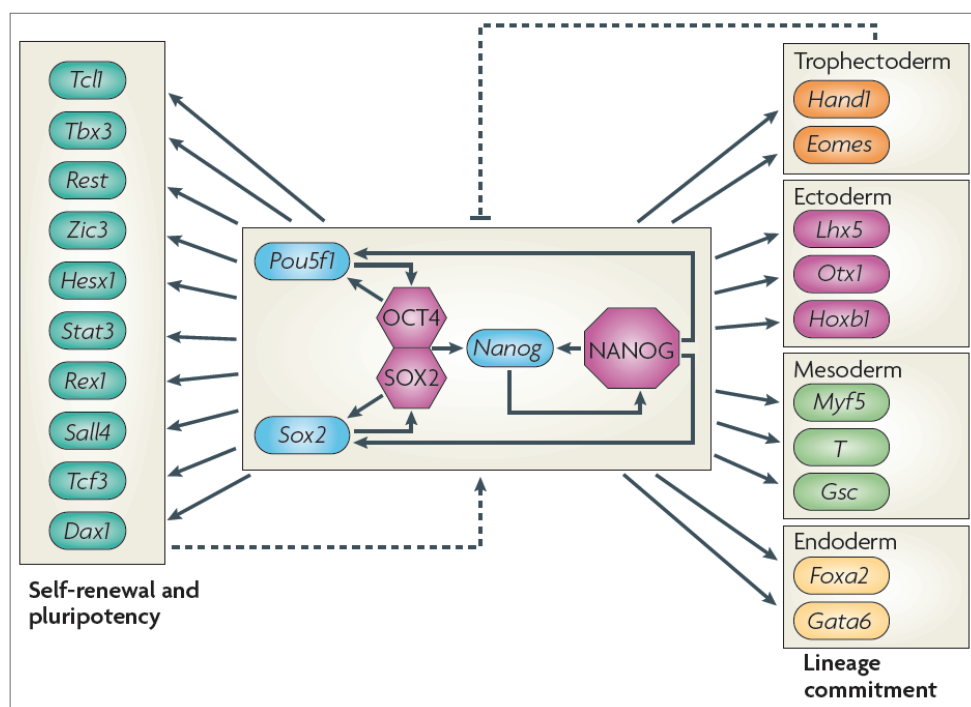


Fig. 1. Schematic representation of the transcriptional circuit of mesenchymal stem cells. (Modified from Ben D. MacArthur et al. Systems biology of stem cell fate and cellular reprogramming. Nature Review Molecular Cell Biology 2009;10,672-81. doi:10.1038/nrm2766).

Unfortunately, the great therapeutic potential of the amniotic fluid-derived cells is greatly undermined by *in vivo* experiments which showed a lack of capacity of differentiation, implying the request of reprogramming *ex vivo*¹⁴.

Amniotic membrane

The amniotic membrane, or amnion, contains a stem cell population. This membrane, without blood vessels, results from the epiblast eight days after fertilization and consists of three parts: an insert of epithelial cells called amniotic epithelial cells, a cell-free intermediate and an exterior part, juxtaposed to the chorion, which contains amniotic mesenchymal cells²⁸.

Since these cells originate from epiblast, it is reasonable that they have features of embryonic stem cells. Recent studies have shown that these features are restricted to the population of amniotic epithelial cells²⁹.

Chorionic villi

The disintegration of the chorionic villi, obtained by chorionic villus sampling for prenatal analysis, allows to isolate a cell population with fusiform morphology. Poloni et al.²⁹ characterized a population of cells positive for mesenchymal surface antigens as CD105, CD90, CD73, CD44, CD29 and CD13, but negative for hematopoietic ones. The undifferentiated state of this cell population is shown by the expression of several stem cell markers as Oct-4, Rex-1, GATA-4 and nestin. The chorionic villi cells are also able to differentiate into osteocytes, adipocytes, chondrocytes and neuronal cells, they have a high proliferative potential and undergo alterations in karyotype³⁰.

Placenta

In addition to supporting fetal development, the placenta is an important reserve of stem and progenitor cells.

The placenta is a maternal-fetal organ that originates from the trophoblast and includes parts of cytotrophoblast and syncytiotrophoblast³¹. The populations of stem cells derived from placenta can be divided in chorionic trophoblast cells and chorionic mesenchymal cells; both populations show remarkable plasticity³². Recent studies have demonstrated the presence of stem cells also in the mother's side of the placenta, the decidua, isolated at the time of delivery. These cells express stem cell markers as SSEA-4, SSEA-1, Nanog, Rex-1 and Oct-4 (ref.³³).

MSCs isolated from placenta showed features comparable to those isolated from bone marrow¹⁴. These placenta-derived cells seem to be able to differentiate into endothelial cells as well as mesenchymal cells, suggesting the existence of resident endothelial progenitors during embryonic development³⁴.

Furthermore, it appears that the tissues of the placenta located between the vitelline yolk and aorta are an independent site for the regeneration of CD34+ hematopoietic stem cells³⁵, which together with the CD31+ and CD133+ endothelial progenitors, support angiogenesis in the fetus³⁴.

POSSIBLE THERAPEUTIC APPLICATIONS OF MSCs

Thanks to their properties, the use of MSCs could represent a breakthrough in regenerative medicine. Mesenchymal stem cells are easily isolated from the donor, can be expanded in culture maintaining genetic stability, are little immunogenic and thus suitable for allogeneic transplants^{36,37}.

The potential of mesenchymal stem cells goes beyond the conventional differentiation in different cell lines and it includes the mesodermal cells differentiation into liver, kidney, muscle, epithelial, neuronal and cardiac cells^{38,39}. More than 120 clinical trials are in progress, using stem cells for different therapeutic applications. So far they find application in the repair of bone and cartilage or in immunomodulation which takes advantage of the immunosuppressive capacity of MSCs³⁷ which are able to inhibit the T-lymphocytes, probably through the production of nitric oxide⁴⁰, counteracting Graft versus Host Disease^{41,42}. It is not yet enough clear the molecular mechanism by which MSCs exert their immunomodulatory properties, but it is interesting to note that these cells have both immunosuppressive capacity and the ability to trigger the immune response.

In vivo, MSCs seem to be able to migrate into damaged tissues (homing) (ref.⁴³) and to repair them differentiating into tissue-specific phenotypes, or favoring the repair triggered by endogenous cells⁴⁴; it has also been proposed that bone marrow-derived mesenchymal stem cells could exert a crucial role in kidney transplant outcome⁴⁵. The migration ability of MSCs allows to administer them intravascularly, in a non-invasive way⁴⁶. Mesenchymal stem cells are able to migrate into ischemic tissues in a chemotaxis-driven way, proliferating and differentiating into specific cellular types⁴⁷. These abilities make them suitable for the treatment of damage caused by ischemia such as myocardial infarction or cerebral ischemia⁴⁸.

In vitro studies have shown that MSCs can be induced to differentiate into cardiomyocytes⁴⁹ even if, *in vivo*, the beneficial effect of MSCs seems to result from the secretion of cytokines in the environment surrounding the lesion rather than from their differentiation. MSCs can also differentiate into neural cellular types in the presence of EGF (Epidermal Growth Factor) and BDNF (Bone Derived Neurotrophic Factor) and they have the ability to increase the proliferation of indigenous neural stem cells⁵⁰.

In vivo studies have shown that therapy based on the use of MSCs restores blood flow and the integrity of the blood-brain barrier following cerebral ischemic injury⁵¹.

CONCLUSION

MSCs could be suitable for several applications in regenerative medicine. For this reason it is important to find novel sources that can offer an abundance of cells in a non invasive manner. MSCs derived from fetal append-

ages could represent a good alternative compared to bone marrow ones. According to our current research, it was not a surprise that the evidence that MSCs isolated from fetal appendages when cultured show a higher proliferation rate than those isolated from bone marrow. What is surprising is that MSCs isolated from bone marrow, despite being adult cells, show phenotypic features and gene expression profile perfectly comparable to fetal stem cells. MSCs isolated from different sources are comparable to each other and therefore can be considered an alternative for research and clinical applications. However, these cells tend to reflect their origin, particularly those isolated from amniotic fluid; another important issue to be addressed is the high heterogeneity of the samples which usually contain different cell types.

More studies have to be conducted for well-characterized stem cells from fetal appendages and, in our opinion, the future goal could be isolation and characterization mesenchymal stem cells from placenta, the only source of mesenchymal stem cells that can be exploited in a completely non-invasive way, being a discarded tissue at the moment of birth.

CONFLICT OF INTEREST STATEMENT

Author's conflict of interest disclosure: *None declared.*

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