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Amniotic fluid as a source of engraftable stem cells

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Abstract:

The ability of stem cells to differentiate into various lineages has made them powerful tools of regenerative medicine and applicable to multiple human diseases. Of particular interest, amniotic fluid-derived stem cells (AFSC) have been characterized to express both adult and embryonic cell markers, indicating them as cells within an intermediate stage between embryonic and adult phenotype. AFSC can differentiate into cells of all three germ layers, including hepatic, myogenic, osteogenic, and neurogenic cell types. Furthermore, AFSC have minimal replicative senescence, retaining the ability to divide effectively for over 250 doublings. These facts indicate that amniotic fluid may exist as a promising donor source of stem cells for the treatment of multiple clinically relevant conditions. Of particular interest is the convenience of harvesting stem cells from the amniotic fluid stem for the treatment of newborns, as well as for banking or cryopreserving purposes to be used at a later date. Importantly, the promise of amniotic fluid as a source of stem cells merits ongoing research into their potential therapeutic applications. This paper is a review article. Referred literature in this paper has been listed in the references section. The datasets supporting the conclusions of this article are available online by searching various databases, including PubMed. Some original points in this article come from the laboratory practice in our research center and the authors' experiences.

Keywords:

Amniotic fluid-derived stem cells, central nervous system disorders, mesenchymal stromal cells pluripotency, regenerative medicine, stem cell therapy, traumatic brain injury

Introduction

Laboratory evidence has revealed the capacity of amniotic fluid-derived stem cells (AFSCs) to differentiate along multiple cell fate lineages,^[1-7] including the ability to produce cell types of all three germ layers.^[8-10] Human amniotic fluid, which can be safely extracted during amniocentesis, contains amniotic fluid cells (AFCs) which originate from embryonic and nonembryonic tissue sources.^[4] The phenotypic character of these cells varies depending on the time of gestations in which they are harvested. In culture, AFSC can be divided into colony-forming cells and nonadhering cells. By analyzing the morphological and growth patterns of AFSC, they can be separated into three categories – AF-type cells, epitheloid

E-type cells, and fibroblastic F-type cells.^[6] Both AF-type and E-type cells are present at the beginning of cultivation, yet E-type cells show a rapid decline as the cultivation proceeds. AF-type cells are believed to arise from mesenchymal fetal membranes and trophoblasts, E-type from fetal urine and skin tissue, and F-type cells from dermal fibroblasts and connective tissue. Evidence of their proposed trophoblastic origin, AF-type cells produce human chorionic gonadotropin, progesterone, and estrogen. As little as 1% of cells derived from human amniocentesis for genetic evaluation express the c-Kit (CD117) surface antigen, a receptor present on embryonic stem cells, primordial germ cells, and various somatic stem cells.

Despite sharing characteristics with both, AFSCs are distinct from embryonic stem cells and multipotent adult cells cell types, existing on the continuum of “stemness”

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somewhere between the two. Reflective of their intermediate properties, approximately 90% of AFSC express Oct-4, a transcription factor known to be expressed in multiple stem cells of embryonic origin including embryonic stem cells, embryonic germ cells, and embryonic carcinoma cell.^[9,11] Moreover, the expression of telomerase reverse transcriptase, which is a marker of pluripotent character, and their ability to differentiate into osteogenic and smooth muscle tissue further indicate their high stemness.^[11] Importantly, their lack of true embryonic pluripotency prohibits them from forming tumors once transplanted, a problem which has crippled embryonic stem cells harvested from the inner cell mass of blastocysts.^[9] Taken together, amniotic fluid is a promising source of safe and easily harvested stem cells and also curtails any ethical issues associated with embryonic-derived stem cells.^[1,8,9,12]

Mesenchymal Stromal Cells Derived from Amniotic Fluid

Mesenchymal stromal cells (MSCs) have been recognized as valuable tools in tissue reconstruction and cytoprotection due to their ability to differentiate into functional cells and exert various therapeutic effects. Human amniotic fluid-derived mesenchymal stem cells (AFMSCs) have been revealed to secrete important neurotrophic factors and promote peripheral nerve regeneration.^[9] Importantly, AFMSCs can be effectively harvested during second trimester amniocentesis from a quantity of 2 mL without interfering with fetal karyotyping. With phenotypic properties similar to MSCs derived from bone marrow, blood, or umbilical cord, AFMSCs are positive for mesenchymal markers including CD90, CD105, CD73, and CD166, yet do not express CD45, CD34, or CD124 hematopoietic markers.^[13] Furthermore, AFMSCs express Oct-4 mRNA and protein, an important embryonic pluripotency transcription factor.^[5,6] Separating AFSC/AFMSC subpopulations from amniocentesis cultures has been achieved by multiple protocols, including c-Kit immunoselection.^[9] Alternatively, scientists have directly cultured AFCs in media which allows the proliferation of AFMSCs, thereafter stimulating their differentiation.^[1,4,5,14] As studies have reported abnormal differentiation patterns and uncharacteristic immune elicitation in cells isolated through c-Kit immunoselection, protocols which do not require this technique of separation are favorable for their use in transplantation.^[15]

AFCs have an impressive renewal capacity, avoiding detectable chromosome length loss at over 250 doublings. AFSCs retain their ability to differentiate into cells inclusive of all three embryonic germ layers; AFCs can be stimulated into nestin-positive neural stem cells, and then fully differentiate into functional dopaminergic

neurons, or stimulated to functional osteoblasts capable of calcium mineralization. Moreover, AFSCs stimulated to hepatic lineage are capable of secreting high levels of urea and express critical liver proteins such as hepatocyte nuclear factor (HNF), hepatocyte growth factor, and alpha-fetoprotein (AFP).^[9,10]

Genetic Expression of Amniotic Fluid-derived Mesenchymal Stem Cells

As expected, AFMSCs express gene profiles and immunologic phenotypes which are characteristic of undifferentiated cells.^[16] For example, real-time polymerase chain reaction evaluation showed that AFMSCs express genes found in various tissue types such as AFP, HNF-4-alpha, nestin, and bone morphogenetic protein-4 (BMP-4), as well as human leukocyte antigen (HLA), cytokeratin 18 (CK18), avidin-biotin complex (ABC), gata binding protein-4 (GATA-4), Rex-1, stem cell factor (SCF), desmin, and fibroblast growth factor-5 (FGF-5) throughout the culture process. Nestin is a marker for neural stem cells, yet is also found in various differentiating cells such as in the myocardium and muscle. BMP-4 is suggested to play a role in chondrogenesis, bone formation, and articular cartilage regeneration, whereas desmin is known to play a role in skeletal and cardiac muscle formation. GATA-4 is a cardiac specific transcription factor, whereas FGF-5 regulates neuronal survival and differentiation. This broad expression profile implies that AFMSCs have the capacity to express pluripotent stem cell-specific genes and follow various differentiation lineages including adipocytic, osteogenic, chondrocytic, and neuronal.^[9]

Antigen Profiles of Amniotic Fluid-derived Mesenchymal Stem Cells

AFMSCs are positive for the stage-specific embryonic antigen marker expressed by embryonic stem cells, but not by adult stem cells. AFMSCs do not express the full repertoire of surface markers associated with stem cells of embryonic origin – few are weakly positive for Tra-1.^[9] AFMSCs express collagen types I-IV and XII, ICAM-1 (CD54), PECAM-1 (CD31), VCAM-1 (CD106) HCAM (CD44), von Willebrand factor, CK18, fibronectin, Fibroblast-specific proteins, alpha-smooth muscle actin, desmin, and vimentin.^[16] No variation in antigen expression has been identified with media type or gestational age.^[17] AFMSCs are positive for HLA-ABC, class I major histocompatibility antigens, and a few cells are weakly positive for HLA-DR, major histocompatibility class II antigens. Flow cytometry analysis revealed the presence of c-Kit and DAZL-expressing cells in amniotic fluid.^[18] DAZL proteins are RNA-binding proteins which are germ

cell-specific and critical for gametogenesis. The existence of key markers for embryonic germ cells within this expression profile makes it of particular interest and relevance to their stemness qualities.^[19]

Neural Lineage Cells in Human Amniotic Fluid

Among the genes expressed by AFCs are neuronal markers including nestin, neurofilament, CD133, CNPase, brain-derived neurotrophic factor, neurotrophin-3, and p75.^[4] In addition, the presence of multiple dopaminergic markers such as FGF8, sonic hedgehog, beta-catenin, and transforming growth factor beta-3 indicate the presence of cells committed to neural lineage which is highly similar to typical mesencephalic dopaminergic neurons.^[3] AFSC have the ability to migrate long distances, survive, and differentiate after transplantation – important qualities in their therapeutic applications. Indeed, transplantation of 3-week cultured AFMSCs demonstrated the ability to migrate long distances in ischemic and normal rat brains using the corpus callosum to reach various brain regions. The positive effects exerted by transplantation of AFMSCs and other MSCs may be due largely to their secretion profiles including cytokines, trophic factors, and signaling molecules which encourage the upregulation of protective factors within the host tissue. Further studies are necessary to fully characterize the histopathological and functional benefits, as well as underlying mechanisms, of AFMSC transplantation.

In addition to neurological disease, AFSC have been proposed as a possible treatment modality for other diseases. One study showed the ability of AFSCs to support mammary gland regeneration.^[20] Others have reported that transplantation promotes muscle regeneration in muscular and cardiac disorders such as ischemic heart disorder.^[1,11] AFSCs also have a broad implications in tissue engineering and other cellular therapy treatments which may have important impacts in a variety of disease.^[8,20-22]

Advantages of Amniotic Fluid-derived Stem Cells

With the ethical issues surrounding embryonic-derived stem cells, much attention has turned to alternative sources of stem cells, such as AFSC, bone marrow-derived, umbilical cord-derived, and placental stem cells.^[23] Adult bone marrow is the most widely used source of MSCs within clinical settings, yet it has significant limitations including a low yield of MSCs and invasive harvesting method. Thus, the hunt for alternative sources of MSCs is an ongoing and important scientific effort.^[2] The existence of MSCs in umbilical cord blood is debated,

and reports finding MSCs from this source describe difficult protocols with low yield and heterogeneous populations.^[24,25]

Amniotic fluid is conveniently collected during amniocentesis, an ultrasound-guided transabdominal puncture procedure used routinely in prenatal screening. The harvesting of AFSC does not require destruction of human embryos, circumventing ethical hurdles. Furthermore, AFSC can be easily grown in culture, with an expansion which supersedes that of bone marrow mesenchymal stem cells (BM-MSCs).^[2] AFSC also have high expansion capacity and have intermediate stemness qualities which indicate they can differentiate into more cell types than BM-MSCs, yet do not retain tumorigenic properties like embryonic stem cells. Finally, the gene expression profile of AFSC suggests their ability to differentiate into highly relevant cell types such as cardiomyocytes and neurons.^[9]

MSCs can also be derived from the placenta, which is an organ arising from both fetal and maternal tissue and is intimately connected to amniotic fluid. Conventionally discarded after birth, it is now recognized that the placenta may have value as a source of stem cells. As an advantage, stem cells derived from the placenta are still of fetal origin and may have fetal-derived characteristics which make them preferable to adult-derived stem cells. The placenta can also be harvested without an invasive procedure, as it is naturally expelled following childbirth. Furthermore, no ethical concerns are associated with placenta harvesting.^[2,26-28] Embryonic stem cell markers on placenta-derived (PD)-MSCs indicate the primitive characteristics of these cells, likely pointing to a renewal and differentiation capacity greater than adult MSCs.

Caveats to Amniotic Fluid-derived Stem Cells and Placenta-derived Mesenchymal Stromal Cell Use

Currently, the cost of AFSC and PD-MSCs is very high, which slows the progression of research on this cell type. Comparisons between laboratory investigations are also difficult, as the fluid and membrane are nonspecific.^[29] AFSC can also illicit immune responses, despite their origin from an immune-incompetent graft source, and have been reported to display phenotypic instability.^[15] Due to the absence of spatial signals in the development of PD-MSCs, they have been shown to have abnormal morphological development;^[30] this may pose issues with cell growth, and spontaneous differentiation. Furthermore, the existence of PD-MSC's unregulated cell divisions should be explored to ensure an absence of malignancy potential and cellular defects. In total, rigorous evaluation of the various factors which

contribute to transplantability and safety should be completed.

Conclusion

AFSC have been realized as a promising source of transplantable stem cells due to broad differentiation capacity, their ease of harvesting, and the lack of ethical concern and tumorigenicity. This has led to their rising relevance in the treatment of numerous human disease.^[9] Amniotic fluid is an accepted source of MSCs, with distinct advantages over adult-derived MSCs. The regularity of amniocentesis, the ease of harvesting, and the small quantity of fluid needed mean the use of AFSC is incredibly practical, lending to their translational value. Similarly, the placenta is now recognized as a promising source of stem cells, and specifically MSCs. While the depth of knowledge on PD-MSCs is behind that of AFSC, their fetal lineage suggests phenotypic and behavioral characteristics conducive to cell therapy.^[8,9] Regardless, more research is required to fully describe the stemness properties of these cell types and their appropriateness for various clinical applications.^[2] In all, the current evidence supports amniotic fluid present a promising source of stem cells with positive indications for their use in treating human disease.

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Conflicts of interest

There are no conflicts of interest.

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