OBSTETRICS

Human amniotic fluid: a source of stem cells for possible therapeutic use

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Stem cells are undifferentiated cells with the capacity for differentiation. Amniotic fluid cells have emerged only recently as a possible source of stem cells for clinical purposes. There are no ethical or sampling constraints for the use of amniocentesis as a standard clinical procedure for obtaining an abundant supply of amniotic fluid cells. Amniotic fluid cells of human origin proliferate rapidly and are multipotent with the potential for expansion in vitro to multiple cell lines. Tissue engineering technologies that use amniotic fluid cells are being explored. Amniotic fluid cells may be of clinical benefit for fetal therapies, degenerative disease, and regenerative medicine applications. We present a comprehensive review of the evolution of human amniotic fluid cells as a possible modality for therapeutic use.

Key words: amniotic fluid, stem cell, therapy, transplantation

Stem cells have become the focus of great interest because of their potential for therapy in a wide variety of conditions. Conditions possibly treatable by stem-cell transplants include genetic defects, tissue and organ replacement, autoimmune disease, and malignancies. A stem cell is an undifferentiated cell that is capable of prolonged self-replication without differentiation. There is a spectrum of stem cells that ranges from multipotent cells that have the potential to differentiate into 1 or 2 lineages, pluripotent cells that may differentiate into many lineages, and totipotent cells that have unlimited differentiation potential. A totipotent stem cell should have the potential to differentiate into lineages for mesodermal, ectodermal and endodermal tissues (such as osteogenic, neurogenic, and hepatic lineages).[1]

Stem cells are characterized by the presence of surface markers and transcription factors that are associated with self-renewal without differentiation as seen in early embryonic states. Some examples of these surface markers are SSEA3, SSEA4, Tra-1-60, Tra 1-81, CD117, and CD90. Some important transcription factors that are associated with embryos are Oct3, Oct4, Sox2, Nanog, and Rex1. Flow cytometry has been the primary technique for the identification of stem cells by detection of these markers.

Stem-cell therapy introduces stem cells into selected tissue environments to prevent and treat injury or repair abnormal tissue. Stem cells from various sources have been considered as potential therapeutic alternatives to organ replacement and other therapies that require transplantation. They can be studied in vitro by modeling pathophysiologic processes of inflammation, disease, and treatments that would occur in vivo. They are introduced intravenously or directly into tissue to study the prevention of injury or the repair of a damaged system or congenital defect. Stem cells are well-known to exist in embryonic tissue and bone marrow. Additional sources of stem cells in humans have been identified: umbilical cord blood, umbilical cord cells, placenta, amniotic membranes, amniotic fluid, peripheral blood, and somatic cells induced to pluripotency. For induced pluripotent cells that are derived from human somatic cells, Oct4, SOX2, KlF4, and c-myc also have been used to transduce somatic cells to develop a pluripotent cell population. There has been significant controversy regarding the various sources about safety in transplantation, accessibility, and ethical issues when human embryos are the source, tumorigenesis with embryonic and transduced cells, and expansion and reliability of all these sources for clinical use.[2] Therefore, amniotic fluid—derived cells might be a practical alternative source because they are readily available, grow well in culture, and avoid these issues.

Alternative sources of stem cells

Embryonic stem (ES) cells are totipotent but have been shown to form tumors in immunodeficient mice. ES cells grow as teratocarcinomas in vivo and frequently acquire chromosomal aberrations.[3,4] They have restricted differentiation capacity and have been shown to yield both genetic and epigenetic abnormalities in culture. Significant ethical issues arise regarding the use of embryos as well.

Cord blood cells are expanded easily but are primarily hematopoietic lineage cells with <1% multipotent cells. There is substantial clinical experience with cord blood transplantation for

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hematologic indications and growing interest in other applications.[5,6] Umbilical cord and placental stem cells exist in the middle ground between ES cells and adult stem cells and might yield desirable expansion with lack of tumorigenesis. However, expansion and culture studies are limited at present.

Bone marrow is the most common source of adult stem cells for clinical transplantation. Restricted lineage, low proliferation rates because of limited telomerase expression, and restricted differentiation potential limit their clinical application so far. They are, however, less tumorigenic than ES cells and do not have ethical concerns. There is extensive clinical experience with their use, establishing standard protocols for transplantation.

Amniotic fluid is readily available, is noncontroversial, and is obtained routinely by amniocentesis. It is analyzed for prenatal genetic diagnosis in the second trimester, for evidence of fetal pulmonary maturity in the third trimester, and for infection at any gestational age. It is made up of a heterogeneous population of cells that routinely is retrieved clinically and cultured for genetic studies. It has been shown that a significant percentage of cells that are obtained from amniocentesis exhibit stem-cell markers. These cells are cultured easily, expanded, and remain viable over many passages, tolerating cryopreservation very well.[2,7-10]

A great deal of research has been performed on second-trimester amniotic fluid cells (AFC), but there is little known about whether third-trimester AFC closely resemble those of the midtrimester.[11] You et al[11] retrieved amniotic fluid at the time of elective cesarean delivery at term. AFC were isolated in culture and found to be positive for surface markers CD29, CD73, CD90, and CD105 as well as Oct-4. Proliferation potential was verified, and differentiation into osteogenic lineage was reported as successful. A more recent study aimed to characterize human AFC from the early third trimester, where 3 samples were analyzed from gestations from 28-34 weeks. Third-trimester AFC expressed comparable levels of Oct-4 and Nanog, but lower levels of SOX2 and Rex-1. Samples were also successfully differentiated to adipocytes, osteoblasts, chondroblasts, myocytes, and neural-like cells, although third-trimester samples showed poor differentiation potential to myocytes and stronger potentiation to neural lineage.[12] Further studies on not only third-trimester amniotic fluid but also AFC at term are warranted, because these findings are promising but inconclusive.

AFC

In early studies, amniotic fluid from pregnant ewes was isolated and expanded to mesenchymal, fibroblast/myofibroblast cell lineages. These cells were noted to proliferate significantly faster than surrounding cells, showed little cell death, and could be isolated consistently from amniotic fluid.[13] AFC showed stem-cell potential when they were found to contain Oct-4, which is a known marker specific for human embryonic stem cells that are associated with maintenance of the undifferentiated state and pluripotency.[14,15] Besides their rapid proliferation, human AFC have been differentiated successfully into all embryonic germ layers, thereby demonstrating pluripotency. After multiple passages in culture, human AFC remained chromosomally stable and did not form teratomas or undergo malignant change.[2,7] These qualities of human AFC suggested significant advantages as a potential source of cells for clinical transplantation.

ES cell molecular markers are molecules that specifically are expressed by stem cells and are critical to the characterization and identification of their pluripotency. There are a wide range of cell-surface proteins, transcription factors, and molecular markers indicative of stemness (Table). These surface markers are usually glycosphingolipids or membrane proteins. Research on stem cells has used the technique of flow cytometry. Flow cytometry separates cells using their cell surface markers, thereby identifying viable cells. This process then permits the formation of clonal cultures from specifically identified cells. Flow cytometry can also be used to identify transcription factors in cell nuclei that are related to stem-cell behavior. However, in contrast to the process of identification of cell surface markers, the cells are no longer viable and cannot be cultured after transcription factor identification.

Human amniotic fluid—derived stem cells were identified as expressing Oct-4, SOX2, Nanog, Rex1, cyclin A, and mesenchymal markers that include CD90, CD105, CD73, CD166, CD133, and CD44.[15-19] There is a higher percentage of stem-cell transcription factors Oct-4, Nanog, and SOX2 in AFC from fluid that is obtained from 15-17 weeks gestation vs later gestational ages. Cell surface markers do not appear to vary with gestational age among the second-trimester samples, but individual samples' expression varies greatly and may overshadow this effect.[10] The ability for adipocyte, osteocyte, and neuronal cell generation were also demonstrated.[20,21] De Coppi et al[21] postulated that CD117 was a marker for selection of AFC from human amniocentesis cultures. However, subsequently, it was shown that CD117 is actually a clonal marker that was present in only approximately 0.5-2% of AFC in cultures that used the same media.[10,22] Another variety of human AFC, mesenchymal stem cells have been identified with distinct populations of varying differentiation potential.[23]

Our studies on human AFC have shown the presence of CD117, 133, 90, 15, 44, 29, 9, 73, as well as SSEA1, SSEA3, SSEA4, Tra-1-60, Tra-1-81, Oct4, Rex1, Nanog, and SOX2.[24-28] Our laboratory selected SSEA4, Tra-1-60, and CD90 for subsequent investigations because they were the most highly expressed ES cell markers in our patient samples. These studies have also shown clonal populations bearing all 3 markers, combinations of 2 different stem cell markers (eg, SSEA4/CD90, SSEA4/TRA-1-60, and Tra-1-60/CD90), and populations with just 1 marker.[10] Clones with different combinations of markers may vary in their properties of stemness. This is an area for further investigation that has not yet been explored. Thus, there is a mixture of cells with varying potential