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REVIEW

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Translating amniotic fluid-derived stem cells for transplantation in stroke

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Abstract

This article discusses possible applications of cells derived from human amniotic fluid in regenerative medicine, specifically in stroke therapy. Recent studies have evaluated amniotic fluid as a viable source for mesenchymal stem cells in the expansion of cell-based transplantation. Laboratory data have demonstrated the ability of amniotic fluid stem cells (AFSC) to act as biobridges or subdural patch-like networks when treating traumatic brain injury (TBI). Also AFSCs have been shown to differentiate along the neuronal lineage following transplantation in animal models of brain disorders. In addition to the cells' many clinical applications, AFSCs can be harvested without raising any ethical concern. This paper evaluates the characteristics of AFSCs, along with the functional benefits of using the cells in animal stroke models, reinforcing the potential advantages of deriving stem cells from amniotic fluid, for stroke treatment.

Keywords: Placenta, Stroke, Adult stem cells, Transplantation

Background

Recent studies have identified human amnion and amniotic fluid as potential stem cell sources with clinical significance in the field of regenerative medicine. Several investigations have evaluated the differentiation potential of cells from both the amnion and amniotic fluid, demonstrating that these cells exhibit high plasticity [1]. The majority of studies target amnion stem cells, revealing their tendency to promote re-epithelization, modulate differentiation and angiogenesis, and reduce inflammation, apoptosis, and fibrosis following transplantation [1–4]. Accumulating evidence have now similarly shown stemness of the lesser studied amniotic fluid-derived stem cells (AFSCs). Unlike AMSCs, AFSCs have a different mode of collection, allowing for different treatment options for diseases with different time-frames of stem cell administration. Here, we discuss AFSCs' significance for stroke therapy in the clinical setting. Moreover, we compare the benefits and drawbacks of AFSCs and amnion membrane stem cells.

Stemness of cells derived from the amniotic fluid

The presence of particular pluripotency markers and genes in cells obtained from amniotic fluid classifies these cells as stem cells. In a recent study by Antonucci et al. [5], molecular analysis revealed that human second trimester AFSCs express Fragilis, Stella, Vasa, c-Kit, and Rnf17, genes coordinating the early stages of germ cell development, along with OCT4 and SOX2, indicators of pluripotency. Aggregated AFSCs form embryoid bodies (EBs) which regain pluripotency potential and they modestly retain features of early stage embryogenesis. In addition, alternate spliced exons explicit of pluripotent stem cells have been observed in cells from AFSC-derived EBs, such as the b isoform of Sall4 and the exon 10 of DNMT3B. These exons display markers of the three embryonic germ layers, such as GATA4, GATA6, AFP and Nestin, and X chromosome inactivation appears to be lacking [5]. The reactivation of the dormant X chromosome may be associated with genomic reprogramming events, further supporting the significant role of AFSCs in embryogenesis [5]. CD117-negative populations of human amniotic fluid mesenchymal stromal cells (AFMSCs) are not only readily abundant for

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therapeutic use, but can also easily differentiate into induced pluripotent stem cells (iPSCs) with the use of nonintegrating Sendai viral vectors encoding for OCT4, SOX2, KLF4 and cMYC [6]. Moreover, Jiange et al. [6] have revealed the potential of iPSCs to imitate human embryonic stem cells, noting their ability to form relatively homogenous populations of neural progenitors, and to show engraftment potential in vivo. In vitro, these neural progenitor cells display the ability to differentiate into astrocytes and mature neurons [6].

In a similar study conducted by Antonucci et al. AFMSCs were shown to have gene expression profiles similar to those in undifferentiated cells [7]. RT-PCR analysis revealed that AFMSCs express genes for SCF, GATA-4, Rex-1, CK18, vimentin, HLA ABC, and FG-5 in culture, along with BMP-4, nestin, HNF-4α, and AFP [7]. This is a notable discovery, because the aforementioned genes regulate a diverse array of cell types, indicating the ability of AFMSCs to differentiate into a multitude of cell types including adipocytes, chondrocytes, neuronal cells and osteocytes [7]. These observations suggest that AFMSCs can exhibit many pluripotent stem cell specific genes and proliferate well through *ex vivo* expansion [7].

Although laboratory findings provide evidence of therapeutic potential of AFMSCs based on the cells' ability to differentiate into all three germ layers in vitro, AFMSCs are known for having low immunogenicity and therefore are still being tested for their immunologic characteristics. Studies have shown AFMSCs to express immunosuppressive factors such as CD59 (protectin) and HLA-G, resulting in AFMSCs being resistant to rejection [8]. CD59 hinders the complement membrane attack complex by binding C5b678 and obstructing C9 from binding and polymerizing, therefore averting the complement from damaging cells [8]. HLA-G, is present within the placenta which is distinct from HLA-A and HLA-B genes, and stands as an important factor in immune tolerance in pregnancy, thereby allowing AFMSC graft resistance [8]. Additionally, other studies have shown the ability of AFMSCs to modulate the immune system, leading to restriction of T lymphocyte proliferation [8]. Moreover, increased CD105+ levels were found in the late-passage of the cell culture in contrast to the early-passage ASFC culture [9]. This provides evidence of AFSCs role as a mesenchymal precursor, in that the prevalence of CD105 and the long-term culture conditions permit mesenchymal cell growth [9]. A recent in vitro investigation determined the gestational age (i.e., those derived from first-, second-to third-trimester) that influence AFSCs' function in regulating the proliferation of lymphocytes [10]. Interestingly, first-trimester AFSCs considerably hindered natural killer cell and T cell proliferation, while second- and third-trimester AFSCs were far less effective and only the inflammatory-primed second-trimester AFSCs could restrict B-cell proliferation [10].

As noted above, AFSCs exhibit features of both embryonic and adult stem cells, but such phenotypic characteristics may vary dependent on the donor [11]. Notwithstanding, these stemness characteristics of AFSCs do not inhibit the differentiation capacity of AFMSC preparation, although the ectopic expression of Oct-4 in hAFMSCs could be a secondary factor in achieving pluripotency [12], while the selective expression of SOX9 and introduction of Wnt signaling can be used to explicitly differentiate cells into neurons and promote neurogenesis [13, 14]. However, before these procedures can be initiated, an appropriate cyropreservative protocol must be identified, such as a slow freezing solution [15]. In their recent study, Zong et al. not only found the ability of AFSCs to differentiate into functional neurons using inner stem cells as a feeder layer, but also identified the Wnt signaling pathway as an integral aspect of initiating neurogenesis [16]. These diverse properties and applications of AFSCs continue to suggest their essential role in stroke therapy.

AFSCs and stroke

Stroke accounts for an outstanding number of deaths each year, one of every nineteen deaths [17], but treatment options are often limited. Intravenous recombinant tissue plasminogen activator (tPA) is the only nationally approved treatment for acute ischemic stroke, and while this thrombolytic therapy affords positive aspects, for instance, the reduction of deaths and decrease of dependence for daily activities, it also has several detrimental side effects [18]. Thrombolytic therapy can only be administered in a 4.5-h window post stroke and also results in increased mortality at 7–10 days following treatment, along with elevated risks of intracranial hemorrhage resulting in death at 3 to 6 months following the initial ischemic event [18].

An emerging treatment option for stroke is intravenous delivery of bone marrow and perinatal-derived cells, which have been shown in the laboratory as successfully arresting the secondary cell death, even when initiated within the first week post-stroke onset, thus markedly extending the therapeutic window [19]. Nonetheless, optimizing the timing of cell transplantation must be taken into account since the stroke brain, unlike peripheral organs that succumb to ischemic insults, presents with immediate cell death processes from the onset that rapidly progress to debilitating brain dysfunctions [20].

Transplantation of AFSCs has been explored in brain disorders, demonstrating their ability to differentiate along a neural lineage [7, 21–25], along with other regenerative features that promote restorative mechanisms, such as

angiogenesis, immunomodulation, neurogenesis and provide functional improvement [20]. Although AFSC transplantation stands as a promising clinical treatment for stroke, few studies have explored these cells as a source for transplantation in stroke. In one experimental stroke study, the transplantation of AFSCs led to a significant reduction of brain damage that accompanied behavioral recovery [26]. AFSCs may be an alternative graft source to embryonic neuronal stem cells, which are often difficult to study due to ethical concerns [26]. Because of the inherent anti-inflammatory effects of AFSCs, they appear as appropriate donor cells for stroke and other neurological disorders characterized by a substantial neuroinflammation [27]. Preclinical data also reveal that AFSC transplantation improves cardiac function, suggesting that these cells can be used on patients with stroke presenting with cardiovascular etiology [28]. In translating AFSCs for clinical application, consideration on the phase of stroke (acute or chronic) may dictate the cell delivery route of AFSCs. In addition, the timing of transplantation of AFSCs, regardless of the stroke phase, may depend on the ready availability of cryopreserved cells [27]. Our laboratory has investigated AFSC therapy in chronic stroke rats, i.e., 1 month after induction of experimental ischemic insult, and showed that AFSC transplantation reduces deficits in memory and learning, decreases infarct volume and neuron damage, and increases cell proliferation [29].

Advantages & disadvantages of amniotic fluid versus amnion membrane stem cells

AFSCs and amnion membrane-derived stem cells (AMSCs) have various advantages and disadvantages. One notable advantage of AFSCs over AMSCs is that amniotic fluid can be collected via amniocentesis, allowing the cells to be isolated, cultured and amplified before childbirth, which may permit the child's own stem cells to be immediately used for therapeutic purposes in any emergency treatment following delivery. Such ready availability of AFSCs enables for a more efficient therapeutic window. In contrast, it takes weeks to amplify AMSCs from the amnion membrane, limiting the feasibility of autologous AMSCs when contemplating of treatment intervention at childbirth. In this regard, the child can benefit from his/her own AFSCs, as autologous transplantation due to the cells' earlier harvesting period. On the other hand, AMSCs pose as feasible graft source for allogeneic transplantation because of the delayed harvest (i.e., after childbirth), as well as the lengthy time required for cell proliferation. Additionally, distinct safety issues arise for both the mother and child for each distinct harvesting method. Amniocentesis for harvesting AFSCs may be associated with risks of harming the mother and the child, while AMSCs can easily be collected following childbirth without such risk of injury. Alternatively, AFSCs can be collected following childbirth, but this delayed harvest negates the aforementioned benefits of early harvesting. In addition, it is easier to culture and amplify stem cells derived from the amniotic membrane, because the amniotic membrane contains more initial stem cells. However, despite the initial low yield of stem cells derived from amniocentesis, the time interval prior to childbirth allows more time for cell amplification. Lastly, because AFSCs are harvested from the fluid without specific landmark features of the originating tissue source, phenotypically defining these cells as a homogeneous cell population may present as a technical challenge [5, 30–32]. Compared with AMSCs, discrete regions of the amnion membrane are largely well defined. For example, most AMSCs are of epithelial and mesenchymal origins [33]. This understanding of the amnion membrane permits for simpler methods to isolate and further differentiate these cells into specific phenotypes. However, recent studies have shown that secreted trophic factors, rather than the differentiated stem cells themselves, mediate most therapeutic effects [25, 34]. Thus, therapeutic outcomes may be achieved regardless of generating a homogenous stem cell population.

Tissue engineering & regenerative potential of amniotic fluid stem cells

AFSCs and AFMSCs may be applied to tissue engineering and regenerative medicine for stroke due to their therapeutic properties. Both AFSCs/AFMSCs may act as biobridges or subdural patch-like networks when treating traumatic brain injury (TBI). AFMSCs may support biobridge formation, which is demonstrated by the notch-induced human bone marrow derived MSCs during regeneration in rat brain following TBI incidence [35]. Biobridges allow both endogenous and exogenous stem cells to transverse non-neurogenic tissue to the site of injury, which can aid the suppression of damaging inflammation. Additionally, no immune response is triggered following the graft-host integration of the biobridge formation, providing further evidence that amniotic fluid subdural patches are a possible therapeutic option for regenerative medicine. Such biobridge formation induced by bone marrow MSCs in TBI may be extended to stroke using AFSCs/AFMSCs.

Despite potential graft-versus-host disease issues arising from cell transplantation between species, xenografts remain as a treatment option for a vast array of health disorders, notably neurological diseases. Foreign cell and tissue transplants are often rejected by the host's immune system. After transplantation of xenografts, the graft is immediately rejected as xenoreactive antibodies lead to complement activation and systemic inflammation [36, 37]. Many commonplace immune tolerance

techniques, like neonatal desensitization, have failed to increase the viability of the foreign transplant [38]. To circumvent such poor xenograft survival, treatment with circulating inflammatory alpha-1-antitrypsin (ATT) in combination with anti-CD4/CD8 therapy may promote graft acceptance [39]. In the same vein, AFMSCs' anti-inflammatory features may be honed to similarly suppress xenograft rejection by co-transplantation of these immunosuppressive amniotic fluid stem cells with xenografts. The ability of AFMSCs to abrogate the immune and inflammatory reactions linked to xenografts may also improve the therapeutic outcome of allograft transplantation. The acceptance of an allograft is defined as a lack of damaging reaction by the host's immune system against the foreign alloantigens of the graft. The immunomodulatory properties of AFMSCs may support allograft long-term acceptance by hindering both the innate immune response and the donor-specific adaptive immune response, initiated by T-cell recognition of foreign alloantigen [40]. Such blockade of deleterious effects of graft rejection by AFMSCs may also circumvent the need for chronic immunosuppression of the allograft [8, 41].

Conclusion

AFSCs have great clinical potential as graft source for cell therapy in stroke. The simple isolation, amplification, multiple opportunities to harvest, either through amniocentesis or after birth, the increased amplification time, the ability to differentiate into many distinct cell lines, their immunomodulatory effects and lack of ethical concerns associated with their therapeutic use make AFSCs an effective source of stem cells. Transplantation of AFSCs may be used in regenerative medicine, notably in the therapeutic treatment of ischemic stroke due to its neurogenesis, angiogenesis and immunomodulation characteristics. However, further research must be conducted to fully understand the vast therapeutic range AFSCs may have in the clinical setting for stroke patients. Investigating the potential of AFSCs could guide further progress in the study of regenerative medicine, eventually leading to their application in stroke.

Abbreviations

AFMSC: Amniotic fluid mesenchymal stromal cells; AFSC: Amniotic fluid-derived stem cells; AMSC: Amnion membrane-derived stem cells; ATT: Alpha-1-antitrypsin; EB: Embryoid bodies; iPSC: Induced pluripotent stem cells; TBI: Traumatic brain injury; tPA: Tissue plasminogen activator

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Availability of data and materials

This paper is a review article. Referred literature in this paper has been listed in the references part. The datasets supporting the conclusions of this article are available online by searching the PubMed. Some original points in this article come from the laboratory practice in our research centers and the authors' experiences.

Authors' contributions

The authors contributed equally to the conceptualization and write-up of this manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

All authors approved the publication of this manuscript.

Ethics approval and consent to participate

Not applicable.

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