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Review Article

Amniotic fluid derived stem cells as an organ specific regenerative medicine: Review

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Abstract

Stem cells are now becoming part of regenerative medicines due to pluripotency, but as some of the major risk factors and ethical considerations; use of amniotic fluid for extracting stem cells have now gained importance in recent years. Much of research is focused on derivatisation of stem cells obtained from amniotic fluid into various organs. Amniotic fluid derived stem cells (AFDSC) are heterogeneous population, devoid of carcinoma development and have no ethical debates. Moreover amniotic fluid (AF) stem cells have high in vitro proliferation potential. AFDSC may guarantee source of stem cell with matching immune profile. AF stem cells may become invaluable source for direct treatment of genetic disorders. The isolation techniques used for AF stem cells extraction got importance for its ease and no need of feeder cells. The work showed use of AF derived stem cells for various organ regeneration like lungs, kidneys, heart muscles, even nowadays scientist have proved its use in Alzheimer's disease as well and other brain disorders. Use of AF stem cells in hematopoietic system got attention. Recent studies now involve use of AF stem cells for neurotransmitter system also. It is now believed that AF stem cells are most efficient among others stem cells for therapeutic purposes. Risk factors are devoid in case of AF stem cells, moreover, due to plasticity, lack of immunogenicity, lack of tumorogenicity showed AFDSC as safe for therapy and regenerative treatment.

Key words

Amniotic fluid, Stem cell, Plasticity, Carcinoma, Immunogenicity.

Introduction

Most of cells of amniotic fluid are derived from skin, digestive, urinary and pulmonary tracts of fetus and from surrounding amnion. As it's known that AF contain carbohydrates, proteins, peptides, lipids, lactate, pyruvate, electrolyte, enzymes and hormones for development.

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Erythropoietin, epidermal growth factor (EGF) and lactoferrin (LF) are also found in AF. In 2004 [1], first technique was reported to derive hAFS cells with two stage culture technique. With protocol, non adherent cells from routine amniocentesis were used for hAFS cell derivation, but they showed heterogeneity in yield within hAFS cells population. Then in 2006 [2], established an optional protocol following two stage culture method for generating high population purity by constructing a clonal hAFS cell line from a single AF cell.

The studies and isolation of amniotic fluid was initiated in 20th century. Moreover interest was gained for characterization and culture of cells in amniotic fluid in 1960's and 1970's. Many of studies were conducted, focused on using of amniotic fluid for cells they contained to determine health of fetus, or for providing of general characterization of amniotic fluid. This has now turned attention of researchers and clinicians. AFDSC have more preference as it is antiquated for any ethical debates and facile storage at minimum cost. AFDSC have storage capability for longer time periods with no adverse effects and can be easily expanded, they are heterogeneous population. It can be noted that amniotic fluid has been considered as important source for undifferentiated or partially differentiated cells, due to its contact with fetus. Nevertheless we still lack in knowledge of amniotic fluid cell composition, new insights are given on amniotic fluid cell composition and cell differentiation can give better understanding of mechanism lying therein.

Amniotic cavity

The amniotic fluid is contained in the amniotic cavity that, in humans, starts forming as early as seven days post fertilization, and is delimited by a membrane called amnion. The formation of the amniotic cavity is a result of the cavitation of the epiblast. The amnion is formed by the cells of the epiblast, by the side facing the cytotrophoblast. This is the first appearance of the amniotic ectoderm, and at this stage it is still a continuum of the portion of the epiblast that will form the embryo. The amnion formation is completed at fourteen days post fertilization and is constituted of two layers: the amniotic ectoderm (inner layer facing the amniotic fluid) and the amniotic mesoderm (outer layer). The amnion has the important function of protecting the embryo and controlling the composition and the volume of the amniotic fluid. In humans, after seventeen weeks of gestation the amnion becomes surrounded and fused with another membrane, the chorion, and is therefore incorporated into the placenta. At the beginning of the formation of the amniotic cavity, active transport of solutes from the amnion, followed by passive movement of water, comprise the amniotic fluid.

Amniotic fluid

The amniotic fluid is the liquid present in the amniotic cavity and is constituted of about 98% water. This volume and composition change continuously during the different gestational stages. The volume of the amniotic fluid at the beginning of the pregnancy is multiple times the volume of the fetus, but at the end of gestation, at forty weeks, it will represent only a quarter of the volume of the fetus. Early during development, when the fetus has not yet started urination and deglutition, the plasma from the mother is surmised to play an important role in the composition of the amniotic fluid, and even though the mechanism is not completely understood, active transport of solutes is probably present between the amnion into the amniotic cavity, therefore creating a gradient for water recruitment [42]. The exchange of fluid through the skin that

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occurs until keratinization is also an important contributor to the osmolarity of the amniotic fluid. After keratinization, urination, swallowing and secretion due to breathing events also contributes to the composition of the amniotic fluid. Urine start to be part of the composition of the amniotic fluid at about eight weeks and its amount will increase during gestation, reaching a flow rate of up to 900 ml/day at the end of gestation [5]. Similarly, at approximately eight weeks, the fetus begins swallowing and secreting material including lung fluid and urine. Secretion of lung fluid is due to an active transport of chloride through the epithelium of the lung [7]. Sampling of amniotic fluid at later stages of the pregnancy is used to monitor lung development via the presence or absence of surfactant lipids and proteins secreted into the amniotic fluid.

The cells present in the amniotic fluid have both embryonic and extra embryonic origins. Approximately forty years ago, researchers attempted to characterize these cells by cytological and biochemical parameters. Early characterization distinguished [6] four epithelial cell types in the amniotic fluid: large eosinophilic cells, large cyanophilic cells, small round cyanophilic cells, and polygonal eosinophilic cells [8]. Some cells may also be derived from the mother, passing through the placenta into the fluid itself. Embryonic stem cells – Differentiation and pluripotent alternatives 496 range from 6 um to 50 um and the shape can vary notably from round to squamous in morphology [9].

Composition and functions of AF

AF contains carbohydrates, proteins and peptides, lipids, lactate, pyruvate, electrolytes, enzymes, and hormones. Prior to keratin production in fetal skin, amino acids diffuse from the placenta through the placental membranes into AF and from the fetal circulation through the fetal skin into AF. Later in pregnancy diffusion through the placental membranes persists and is augmented by fetal urinary excretion of amino acids [10]. Like breast milk, AF is rich in taurine which is found in greater quantity in AF than in maternal serum, while most other amino acids have lower concentrations in AF than in maternal and fetal blood. Glutamine is an essential precursor for nucleic acid biosynthesis in all cells and is particularly important in rapidly dividing cells such intestinal mucosa cells. In fetal sheep, the uptake of glutamine from the AF by the fetal intestine is an active process [11]. Arginine also plays an essential role in fetal and placental development. Arginine is hydrolyzed to ornithine, which is then converted into the polyamines, putrescine, spermine, and spermidine, which are key regulators of placental angiogenesis, trophoblast growth, and embryogenesis. In sheep, the concentrations of arginine, ornithine, and polyamines increase rapidly in both allantoic and amniotic fluids early in gestation and remain elevated in AF throughout pregnancy. As gestational age increases, the swallowed polyamines in AF support proliferation and differentiation of intestinal epithelial cells [12].

The role of swallowed carbohydrates and lipids in AF is less well defined. Growth restricted rabbit fetuses were given infusions of dextrose or dextrose with amino acids directly into AF, and there was no improvement in growth, while an infusion of bovine AF did improve organ and somatic growth [13]. In a fetal rabbit model with esophageal ligation, the infusion of graded amounts of glucose or glucose with amino acids into AF enhanced organ weights and fetal growth [14]. No studies have yet demonstrated reversal of fetal growth restriction (FGR) by intra-amniotic infusion of nutrient solutions. Insulin-like growth factor I (IGF-I) is found in

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human milk and AF. High levels of epidermal growth factor (EGF) are found in human milk and AF. Erythropoietin (EPO) is found in human AF.

The role of swallowed EPO in the human fetus and neonate is not clear. It is puzzling that concentrations of EPO are significant in AF and actually increase in human milk with the length of breast feeding. Granulocyte colony-stimulating factor (G-CSF) is found in human AF. High levels of epidermal growth factor (EGF) are found in and AF The concentration of EGF in amniotic fluid is four-fold higher than that found in fetal urine suggesting that the site of production is the amnionic membranes.

Transforming growth factor alpha (TGF-α) has a structure similar to EGF and binds to the same receptor. TGF-α is present in AF. Transforming growth factor beta-1 (TGF-β1) is found in rat AF and human breast milk, but is found in human AF only during the late stages of gestation. TGF-β1 is believed to induce terminal differentiation of intestinal epithelial cells and to accelerate the rate of healing of intestinal wounds by stimulating cell migration. TGF-β1 may also stimulate IgA production. Lactoferrin (LF) is a glycoprotein with two binding sites for ferric ion. LF is appears in human AF at 20 weeks gestation increasing in concentration with gestation.

Isolation, expansion and characterization of amniotic fluid cells

The possibility of using amniotic fluid derived pluripotent and multi potent stem cells has been found appealing due to the relative easiness and safe procedure required to retrieve the cells from its source. Furthermore, the use of multi potent progenitors has been considered an attractive alternative to the use of pluripotent cells due to their already committed phenotype. Cells can be isolated from the liquid collected by amniocentesis. Briefly, prior to amniocentesis, an ultrasound is performed to confirm fetal viability, gestational age, number of fetuses, placental location, volume, fetal anatomical survey, uterine cavity abnormalities and to evaluate the best needle insertion site.

A 20 cc syringe is used to aspirate the liquid. The first 2 cc collected should be discharged and then using another syringe, additional 15 to 20 cc are aspirated. Removal of the fluid generally takes less than 1 minute. After collection, the cells are seeded with specific culture media and the adherent fraction is expanded.

Contact between amniotic fluid and compartments of the developing fetus, such as lung and gastrointestinal tract can explain the presence of different types of cells. Moreover, cells detaching from the forming kidney or exfoliating from the fetal skin may contribute significantly to cellular composition. In particular, the presence of mature cell lines derived from all three germ layers has been identified [17]. Mesenchymal and hematopoietic progenitor cells have also been shown to exist before the 12th week of gestation in humans [44] together with cells expressing proteins and various genetic markers from specific tissue types including brain, heart, and pancreas have all been discovered [3, 15, 16] .

Fauza, et al. reported the successful isolation and expansion of un fractioned mesenchymal stem cells (AFMC) from human samples between 20 and 37 weeks of gestation, confirming the presence of a multi potent mesenchymal cell types over the progression of gestation [18]. A fully characterization of amniotic fluid pluripotent cell population has first been reported by Atala in 2007 [4]. This newly isolated stem cell population (AFSC) is characterized by expression of c-kit, a surface

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marker expressed by stem cells of mesenchymal origin. AFSC express some surface markers and transcription factors distinctive of ESC such as OCT-4 and SSEA-4 indicating they can actually posses some important characteristics that also ESC have, showing their pluripotential capability. They stained positively for a number of surface markers characteristic of mesenchymal and/ or neural stem cells, including CD29, CD44 (hyaluronan receptor), CD73, CD90 and CD105 [4].

The first technique to derive hAFS cells was developed in 2004 by Tsai, et al. [1], who reported a two stage culture technique. With the protocol, non-adherent cells from routine amniocentesis were used for hAFS cell derivation, but the yield showed heterogeneity within the hAFS cell population. In 2006, Tsai, et al. [2] established an optional protocol following the two stage culture method for generating high population purity by constructing a clonal hAFS cell line from a single hAFS cell. Subsequently, Kim, et al. presented a protocol for deriving hAFS cells. The technique is performed by prolonging an in vitro hAFS cell culture with subsequent subculturing until a stem cell population with a homogeneous morphology can be obtained.

Why use AFSC in regenerative medicine?

When selecting a stem cell population for use in a regenerative or therapeutic capacity, there are a myriad of factors that need to be considered. The pluripotentiality, the ability of the cells to differentiate into different germ layers and tissue types, is of fundamental importance if one is isolating cells to treat diseases or developmental deficiencies in which progenitor cells within the patient are compromised or overwhelmed. Additionally, the plasticity of the cells and their ability to differentiate to repopulate different populations within an organ, and repopulate them correctly is crucial. Furthermore, the behavior of the cells after injection must be carefully studied and characterized. Tumorogenicity, immunogenicity and the propensity to form teratomas and further exacerbate a disease state can rule out various cellular therapies simply due to risk. To date, amniotic fluid stem cells have demonstrated the ability to meet all of these criteria and behave remarkably well in a regenerative and therapeutic capacity. Amazingly pluripotent, less immunogenic, and not prone to teratoma formation, AFSCs have quickly risen near the top of the list of stem cell therapies to continue developing.

Amniotic fluid cells and organ specific regenerative medicine

Due to an easy and safe collection procedure, amniotic fluid has quickly gained interest as a potential source of pluripotent/ multipotent cells for regenerative medicine purposes. Amniotic fluid stem cells have been shown to be easily cultured and expanded upon collection and isolation [4, 19, 3, 18] and Arnhold, et al. [20] proved that after c-kit selection these cells are still exhibiting optimal growth rate. Their potential for differentiation has been proved in many published works and cells can be retrieved from different species like humans [4, 19, 3], goat [21], mouse [4] and buffalo [45].

A recent report following a comparative analysis of AFSC and BM-MSC cells on proliferative potential and immunogenicity analysis showed the AFSC are less immunogenic and harbor a higher proliferation rate than BM-MSC [46].

Amniotic fluid cells and kidney regeneration

The complexity of the kidney and the multiple functions of the renal compartment are a great

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challenge to a successful therapeutic approach for its recovery and the regeneration. Beside the use of endogenous stem cells and other traditional and advanced therapies, the administration of exogenous stem cells, including AFSC has been proposed [12]. In the recent past, Perin, et al. showed the capability of AFSC to participate in vitro to the development of embryonic kidneys. In particular, cells labeled with the surface marker CMDil were shown able to integrate within the structures of the developing kidney. Integration into the metanephric structures was additionally confirmed by the migration of the injected cells to the periphery of the embryonic kidney. This data strongly correlates to the centrifugal pattern of induction, morphogenesis and differentiation of the metanephros, proceeding from the centre to the periphery of the embryonic organ [19]. Moving into an in vivo model, the same group for the first time proved the potential of human AFSC to participate to the regeneration of kidneys undergoing acute tubular necrosis [23]. After intra renal injection, cells were showed to survive, integrate into renal structures, and differentiate into tubular cells expressing proximal as well as distal epithelial tubular markers and persist over the long term. However, as the Authors highlight in their study, the main mechanism of action seems to lie into the ability of AFSC to modulate the immune response by lowering pro-inflammatory cytokines while stimulating the expression of anti inflammatory molecules, and by lowering apoptosis and increasing endogenous proliferation. On a different model of acute renal injury, Camussi's research group confirmed the positive results and the comparable efficacy between BM-MSC and AFSC [47]. Beside the use of pluripotent cells, in 2010, we reported the isolation and characterization of more committed Amniotic Fluid derived Kidney Progenitor Cells (AFKPC) [3]. Cells expressing both CD24 and OB-Cadherin were sorted and characterized for a wide range of kidney markers such as PAX-2, LIM-1, GDNF, ZO-1. Additional selections were performed on the CD24+OB-cadherin+ cells to isolate cells committed to mesangial differentiation, podocyte differentiation, mesenchymal to epithelial transition cells and vascular progenitors. Characterization of marker expression for these subpopulations showed significant differences in gene expression, confirming their different commitment to renal fate.

Amniotic fluid cells and lung regeneration

In uterus, the developing lungs of the fetus are filled with fetal lung liquid which is actively secreted into the amniotic fluid. In the late gestational period, surfactant produced by the fetal lungs contributes to the composition of amniotic fluid and can be measured to determine the developmental stage of the surfactant system within the organ. Contact between the developing lung and the fluid make it a possible important reservoir for cells to be used in lung regenerative medicine. In fact, AFSC were shown able to integrate and proliferate into mouse embryonic lung and express human lung epithelial cell markers [24] .Following hyperoxia injury, a tail vein injection of cells into nude mice showed localization in the distal lung with expression of both TTF1 and type II pneumocyte marker surfactant protein C. In the same work, specific Clara cells damage through naphthalene injury was followed by integration and differentiation of AFSC at the bronchio alveolar and bronchial positions with expression of specific Clara cell 10-kDa protein [14] . The positive results obtained by Warburton's research group were the first to prove the use of AFSC for in vivo organ regeneration. However, as underlined by the author, the number of cells homing and integrating within the lung was considerably low and the effects on tissue

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regeneration may be due on mechanisms different from integration and proliferation. However, our knowledge on this field is still lacking and more studies should be performed to clarify molecular pathways and suggest a plausible mechanism of action.

Amniotic fluid cells and heart refunction

Heart failure remains one of the major causes of mortality in the United States [25]. Stem cells have been proposed as an alternative, innovative approach for the treatment of heart disease and cardiac differentiation. AFSC have been tested in the past years for their potential of becoming functional cardiomyocytes. Hoerstrup's research group used amniotic fluid derived cells to successfully repopulate heart valves. After isolation, CD133- and CD133+ cells were isolated, characterized and subsequently seeded onto tissue engineered scaffolds. Feasible heart valve leaflets were obtained in vitro with the use of both fibroblast-like and endothelial like cells [26]. However, Chiavegato, et al., in 2007 showed that injections of human AFSC into a rat normal or ischemic myocardium was ineffective and cells were targeted by the immune response with consequent rejection of the xenotransplanted cells. On the other hand, the use of a xenotransplantation model, even when cells were injected in immune deficient animals, may not be ideal for immunogenicity studies.

New insights on the cardiomyogenic potential of amniotic fluid cells have been published in 2010 by Soker, et al. showing the in vitro capability of AFSC to be differentiated into cardiac cells when co-cultured with rat cardiomyocytes [48]. Along with this work, Sung's research group reported the differentiation of AFMC into cardiomyocytes and endothelial cells [38]. Bollini, et al. in two different works demonstrated the potential of AFSC to differentiate into cardiomyocytes both in vitro and in vivo showing their cardioprotective effect following acute myocardial infarction [27].

Amniotic fluid cells and hematopoietic system

C-kit positive/ Lin – cells derived from both human and mouse, have been shown to have hematopoietic potential [29]. These cells were capable of differentiating into erythroid, myeloid, and lymphoid lineages in vitro as well as in vivo, in the peripheral blood of irradiated mice. Furthermore, single cells analysis was able to assess the expression of several genes important during different stages of hematopoietic differentiation.

Amniotic fluid cells and pancreas refunctioning

The occurrence of pancreatic damage and diabetes has dramatically increased in the last years. The rise of this emergency has strongly encouraged physicians and scientist to search for alternative therapeutic approaches. In 2009, it was suggested that stem cells derived from amniotic fluid could be of use for pancreatic regeneration [30]. However, the first attempts to differentiate amniotic fluid cells into functional pancreatic cells were unsuccessful. In fact, the use of obestatin, a molecule proven to efficiently increase expression of pancreatic beta cell genes, was unable to stimulate pdx-1 expression these cells [31].

A better knowledge of developmental pathways and gene cascades involved in pancreatic specification brought, a year later, to a growing number of successes. In fact, differentiation into pancreatic cells was proven using a variety of different procedures. In particular, transfection

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with the PDX-1 gene was able to induce pancreatic features on cells from AFMC [32].

With an interesting approach, Li, et al. were able to prove differentiation into insulin producing cells by silencing several neuronal genes by use of small interference NRSF RNA. This was shown as crucial for pancreatic differentiation and for the expression of pancreatic markers including Pdx1, Hnf4, Isl-1, Nkx6 [1]. A different approach was taken by Zou, et al. Knowing that the expression of particular surface markers can identify cell populations with specific traits and defined commitment, a CD44+/ CD105+ population was isolated and successfully differentiated into pancreatic cells expressing PDX-1 [33]. The increasing number of studies reported in the last two years suggests that the interest for amniotic fluid cells for beta cell differentiation is a growing research subject. Moreover, differentiation into pancreatic beta cells is been proven as possible in vitro settings. However, no in vivo studies have been published reporting their potential in acute and chronic pancreatic diseases.

Amniotic fluid cells in brain function

The differentiation of pluripotent and multi-potent cells into neural cells has been considered fundamental for understanding brain differentiation and for the establishment of innovative approaches for the healing of brain injuries. Many different studies have been performed on amniotic fluid cells and their expression of neuronal markers. However, their ability to differentiate into functional brain cells has being highly debated.

First reports on amniotic fluid progenitor cells commitment to neuronal cell lineage were published in 2006. In fact, McLaughlin's research group reported the ability to isolate and expand them in culture. Their studies showed that these novel progenitors are committed to mesencephalic dopaminergic neurons [15]. AFMC were shown to be able to differentiate into brain cells both in vitro [1, 2] and in vivo [34]. Selection of specific cell population based on specific surface marker expression didn't show to really improve the neuronal potential of amniotic fluid cells. Cells isolated by use of different surface markers like c-kit [4], sox-2 [35] were shown able to differentiate into neuronal like cells. However, in 2009 was reported the inability of AFSC to differentiate into dopamine neurons both in vitro and in vivo assays [49]. An interesting recent study, investigated the impact of extracellular signals on neural differentiation, where it was confirmed that extracellular matrix has an essential role on neurogenic differentiation and therefore regulates its efficiency [36]. While many studies seems to prove that differentiation of amniotic fluid stem cells, either AFSC or AFMC, into neural cell types, there are still too many open questions about functionality of the differentiation, ideal cell population and best differentiation cocktail. While the current status of the research gives great hope for the future, to confirm of deny the possible use of amniotic fluid cells for brain regeneration more in vitro and in vivo data are certainly required.

Amniotic fluid cells and liver functioning

Only a few studies have been reported that investigate the potential of amniotic fluid derived cells for hepatocyte differentiation. Zheng, et al. in their work, claimed that AFSC had a better response to the differentiation when compared with BM-MSC under the same conditions [21]. Later on, differentiation into the hepatic lineage was successfully obtained by Gasbarrini research group [37] that showed the equal potential of adult and fetal derived cells, including AFSC, for liver regeneration. However, beside these encouraging results, more studies

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are required prior to confirm the suitability of amniotic fluid stem cells for liver therapy.

Amniotic fluid cells and bone

In 2010, it was reported a positive effect of transient ethanol exposure during early differentiation of AFSC into osteoblasts [50]. Papaccio's research group showed the ability of AFMC to differentiate into bone cells when co-cultured with dental pulp cells proving potential for bone engineering [51]. Osteogenic progenitors have been found within amniotic fluid [52]. In this work, they were able to obtain calcium mineralization and osteogenic differentiation of AFMC. Expression of various osteogenic markers after 30 days in culture was demonstrated. Similar results were obtained by two other research groups [53, 54].

Peister in 2011 showed that AFSC were capable of a greater differentiation potential compared to mesenchymal stem cells although the latter response to the differentiative cocktail was occurring at earlier times [55]. However, in vivo data are still lacking and the osteogenic potential of amniotic fluid cells in a complex environment should be undisclosed.

Amniotic fluid cells and tissue regeneration

A. Chondrocytes

The regenerative capacity of the cartilage is limited. The ability to differentiate stem cells into cartilage may provide a better alternative to primary culture of chondrocytes that in vitro dedifferentiate losing their characteristics [38].

Fauza's research group demonstrated the ability of ovine AFMC to successfully differentiate into chondrocytes on 3D scaffolds expressing several markers of cartilage. Atala's group showed [39] the ability of AFSC to differentiate into chondrocytes [4]. However, no functional studies were performed to confirm the possible use of these amniotic fluid derived cartilage cells.

B. Adipocytes

Adipocyte differentiation was proven in 2007 for AFSC when these cells were first characterized and tested for their pluripotentiality [4]. In addition, adipogenic differentiation for goat derived AFMC was shown in 2011 proving their differentiative potential.

C. Myocytes

Muscular tissue is well known to harbor endogenous stem cells that help recovering the tissue after an injury. However, the differentiation potential of these pluripotent stem cells and when the extent of the injury, due to an acute or chronic insult, is too heavy, muscular degeneration occurs with loss of motility and impaired function. The study of cells feasible for muscular differentiation and regeneration has been considered essential for a successful therapeutic approach.

Amniotic fluid cells have been studied for their capability to differentiate into functional muscular cells. In particular, Streubel reported using non-hematopoetic AFMC for the conversion of amniocytes into myocytes [41]. De Coppi showed the ability of AFSC to differentiate into myocytes in vitro by expression of markers expressed by the differentiating and mature muscle fibers [4] and the results were later confirmed by studies both in vitro and in vivo on scid mice [40].

D. Amniotic fluid derived cells and their role as cytokine modulators

In the last years, new evidences have been found that correlates the administration of stem cells with the modulation of inflammatory and fibrotic processes through cytokine mediated

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cross-talk between the pluripotent cells and the surrounding environment. New studies have highlighted the possibility that the same mechanism of action can be used to explain the effect of amniotic fluid stem cells in many diseases. In particular, Perin showed that in a murine model of acute tubular necrosis, the expression of inflammatory cytokines is strongly regulated after injection of AFSC [23]. Down regulation of pro-inflammatory molecules and up-regulation of pro-regenerative and anti-flogistic cytokines resulted in a faster regeneration of the damaged tissue with higher proliferation rate, lower apoptosis and an overall better physiological profile of different renal parameters. A broad study performed by Yoon [56] investigated the in vitro production of cytokines by AFMC in the cultured media. Presence of several inflammatory molecules was reported such as IL-8, IL-6, TGF, TNFRI, VEGF, and EGF and other molecules involved in the TGFB/ SMAD2 pathway. The conditioned culture media proved to be useful for enhancing wound healing in an in vivo murine model. While studying the angiogenic potential of AFSC, Teodolinda, et al. [42] reported the ability of the cells to produce and release several cytokines and chemo-attractant molecules that are able to modulate not only the vessel growth but also the activity of macrophages/ monocytes and other cells involved in inflammation and immune response.

Conclusion

Thus the studies revealed that method discussed above could prove efficient to derive stem cells from amniotic fluid of even good grade and also facilitate for effective growth to be used for regeneration medicine. Of various sources, this is one of best source to derive stem cells. Considering such scope of stem cells, it is expected to increase in practical aspect of humans to treat various diseases. Furthermore, its functioning discussed above also assure about its safety after transplantation into humans. In this very same direction, the establishment of protocols and differentiative media will better allow us to compare the different populations and understand their mechanism of action. In addition, knowledge about how the different compartments of the developing fetus are contributing to the cellular composition may disclose important information about the development and the amniotic fluid composition. These cells were used to regenerate liver, brain, kidneys, bones, blood, lungs, and in other disorders. Better results are obtained using amniotic derived stem cells in specific organ regeneration and its function was found to be compatible with that of original organ. These stem cells are thus can be further studied to avoid risk of human use. From this, it is obvious that amniotic fluid stem cells are most current topic of research from last few years for their potential use in future medicine to develop disease resistant species through mutations in cells.

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