Therapeutic Potential of Amniotic Fluid Derived Mesenchymal Stem Cells Based on their Differentiation Capacity and Immunomodulatory Properties

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Abstract: Background: Amniotic fluid derived mesenchymal stem cells (AF-MSCs) are adult, fibroblast-like, self-renewable, multipotent stem cells. During the last decade, the therapeutic potential of AF-MSCs, based on their huge differentiation capacity and immunomodulatory characteristics, has been extensively explored in animal models of degenerative and inflammatory diseases.

Objective: In order to describe molecular mechanisms responsible for the therapeutic effects of AF-MSCs, we summarized current knowledge about phenotype, differentiation potential and immunosuppressive properties of AF-MSCs.

Method: An extensive literature review was carried out in March 2018 across several databases (MEDLINE, EMBASE, Google Scholar), from 1990 to present. Keywords used in the selection were: “amniotic fluid derived mesenchymal stem cells”, “cell-therapy”, “degenerative diseases”, “inflammatory diseases”, “regeneration”, “immunosuppression”. Studies that emphasized molecular and cellular mechanisms responsible for AF-MSC-based therapy were analyzed in this review.

Results: AF-MSCs have huge differentiation and immunosuppressive potential. AF-MSCs are capable of generating cells of mesodermal origin (chondrocytes, osteocytes and adipocytes), neural cells, hepatocytes, alveolar epithelial cells, insulin-producing cells, cardiomyocytes and germ cells. AF-MSCs, in juxtacrine or paracrine manner, regulate proliferation, activation and effector function of immune cells. Due to their huge differentiation capacity and immunosuppressive characteristic, transplantation of AF-MSCs showed beneficial effects in animal models of degenerative and inflammatory diseases of nervous, respiratory, urogenital, cardiovascular and gastrointestinal system.

Conclusion: Considering the fact that amniotic fluid is obtained through routine prenatal diagnosis, with minimal invasive procedure and without ethical concerns, AF-MSCs represents a valuable source for cell-based therapy of organ-specific or systemic degenerative and inflammatory diseases.

Keywords: Amniotic fluid, mesenchymal stem cells, cell-based therapy, regeneration, immunomodulation.

1. INTRODUCTION

The human amnion, a membrane composed of epithelial layer, basement membrane and an avascular mesenchyme, surrounds developing embryo and defends fetus against mechanical stress, pathogens and toxins [1]. The amniotic sac is filled with amniotic fluid (AF), a protective and nourishing liquid that provides normal embryo development [2]. Human AF isolated during amniocentesis is used for the routine prenatal diagnosis of broad spectrum of genetic and multifactorial fetal diseases [3]. Human AF contains water (about 98%), electrolytes, organic macromolecules (lipids, carbohydrates, proteins), suspended particles (vernix, lanugo and meconium) and stem cells [4, 5]. During the last decades, huge research efforts have been devoted to describe cellular and molecular properties of AF-derived stem cells in order to emphasize their potential use in clinical practice [4]. Accord-
ingly, herewith we summarized current knowledge and future perspective about phenotype and function of AF-derived mesenchymal stem cells (AF-MSCs) in order to describe molecular mechanisms responsible for their therapeutic effects. An extensive literature review was carried out in March 2018 across several databases (MEDLINE, EBASE, Google Scholar), from 1990 to present. Keywords used in the selection were: “amniotic fluid-derived mesenchymal stem cells”, “cell-therapy”, “degenerative diseases”, “inflammatory diseases”, “regeneration”, “immunosuppres-

2. PHENOTYPIC CHARACTERISTICS OF AF-MSCS

AF-MSCs are adult, fibroblast-like, self-renewable, multipotent stem cells with huge differentiation potential and strong immunosuppressive properties [6-8]. AF-MSCs are successfully isolated from AF samples obtained through amniocentesis under ultrasound control by well established high cell density protocols [9-14]. Accordingly, AF nowadays serves as a rich and advantageous source of MSCs in terms of number of potential donors and simplicity of harvesting procedure [8, 15-18].

Roubelakis and coworkers generated the protein map of cultured AF-MSCs by identifying 261 proteins, and compared it directly to that of cultured bone marrow-derived MSCs (BM-MSCs) [19]. AF-MSCs displayed 78 unique proteins related to increased proliferation rate and high potential for differentiation into cells of non-mesodermal origin such as hepatocytes, insulin-producing cells, keratinocytes, intestinal epithelial cells, and neuronal cells [19]. Accordingly, AF-MSCs exhibited a greater capacity for cell proliferation and self-renewal than BM-MSCs. They proliferate for more than 50 passages with an average doubling time of 1.6 days (average doubling time of BM-MSCs is 3.8 days). Despite high proliferation rate, the average lengths of the telomeres did not vary between the early and late passage cells and no obvious chromosomal rearrangements were observed, indicating that AF-MSCs maintained a normal karyotype during proliferation [13, 19-21]. AF-MSCs show senescence and longevity changes independent of telomere shortening. AF-MSCs are most likely undergoing senescence induced by oxidative stress and not normal replicative senescence in culture. Thus, longevity in cultured AF derived cells appears to be regulated more by epigenetic factors such as stress and nutrient homeostasis than by telomere length [22].

AF-MSCs express surface antigens CD29, CD44, CD73, CD90 and CD105 and do not express CD14 (marker of monocytes), CD34 (marker of hematopoietic cells), CD45 (pan-leukocyte marker), CD79a and CD19 (marker of B lymphocytes) [23]. Additionally, AF-MSCs lack expression of major histocompatibility complex (MHC) class II and co-stimulatory molecules, CD80 (B7-1), CD86 (B7-2), and CD40, suggesting low immunogenicity in vitro and in vivo [24].

Interestingly, AF-MSCs display intracellular and extracellular markers of pluripotent stem cells, such as octamer binding protein 3/4 (Oct-3/4), homeobox transcription factor Nanog, c-MYC, tumour-related antigen (TRA)-1-60, stage-specific embryonic antigen (SSEA)-3, and SSEA-4, as well as vimentin and alkaline phosphatase [8, 25-30]. AF-MSCs are considered as cells in an intermediate stage of potency, between pluripotent embryonic stem cells and lineage-restricted adult stem cells and, based on their phenotype and functional properties, have been defined as extraembryonic perinatal stem cells [31]. The presence of pluripotent markers associated with maintenance of their undifferentiated state and pluripotency indicates the risk of possible uncontrolled differentiation and malignant transformation.

Importantly, several studies confirmed that, in contrast to pluripotent embryonic stem cells, AF-MSCs are non-oncogenic in vivo [13, 32]. More precisely, it was demonstrated that AF-MSCs including late passage cells, do not form teratomas in severe combined immune-deficient (SCID) mice, indicating that they can be used in regenerative medicine [32]. Accordingly, during the last decade, the therapeutic potential of AF-MSCs, based on their huge differentiation capacity and immunomodulatory characteristics, has been extensively explored in animal models of degenerative and inflammatory diseases (Table 1).

3. THERAPEUTIC POTENTIAL OF AF-MSCS BASED ON THEIR DIFFERENTIATION CAPACITY

In accordance to the criteria formulated by the Mesenchymal and Tissue Stem Cell Committee of the International Society of Cellular Therapy (ISCT), AF-MSCs are stem cells isolated from AF that could differentiate into osteoblasts, chondroblasts and adipocytes in appropriate tissue culture-differentiating conditions [8].

Due to their chondrogenic potential, AF-MSCs were explored as cell therapy for congenital high airway obstruction syndrome [33-35]. Constructs engineered from AF-MSCs and decellularized airway scaffolds were used for prenatal tracheal reconstruction [34]. Compared to acellular implants, AF-MSCs-cellularized scaffolds exhibited full epithelialization in vivo and had a significantly greater degree of increase in elastin levels after implantation in fetal lambs with tracheal defects, indicating the potential of AF-MSCs in fetal tissue engineering and perinatal airway repair [34].

AF-MSCs were assessed for their osteogenic potential in a number of in vivo models relating to surgically created osseous defects [36, 37]. More recently, it was demonstrated that poly-L-lactic acid (PLLA) scaffolds seeded with AF-MSCs, cultured in osteogenic medium for up to 34 weeks, provided sternal and nasal bones repair [36, 37]. Chest roentgenograms showed closure of full-thickness sternal defects in leporines that received AF-MSCs seeded onto biodegradable nanofibrous scaffolds. Sternal bone repair was followed by an increase in alkaline phosphatase activity in vivo, suggesting ongoing graft remodeling [36]. Additionally, AF-MSC-scaffolds induced complete repair of full-thickness nasal bone defects and these effects were accompanied with enhanced and consistent bone mineralization, confirming the therapeutic potential of AF-MSCs-seeded scaffolds in perinatal bone reconstruction [37].

Numerous studies revealed that, in addition to differentiation into osteoblasts, chondroblasts and adipocytes, AF-MSCs were capable to generate neural cells, hepatocytes,
alveolar epithelial cells, insulin-producing cells, cardiomyocytes and germ cells in vitro indicating their huge differentiation potential and clinical application (Fig. 1) [13, 32, 38-43].

AF-MSCs have a higher capacity for neural differentiation than BM-MSCs [44]. AF-MSCs more rapidly formed and expanded neurospheres in vitro, showed higher expression of neural stemness markers following neural stem cell (NSC) differentiation (Nestin, vimentin, Mushashi, accompanied with markers of pluripotency (Oct-4, Sox2)) and produce significantly higher amount of brain-derived growth factor (BDGF) and nerve growth factor (NGF) than BM-MSCs. After terminal neural differentiation, NSCs derived from AF-MSCs generated more neurons and glial cells than NSCs derived from BM-MSCs. Importantly, AF-MSCs maintained a normal karyotype in long-term cultures after NSC differentiation and were not tumorigenic in vivo after transplantation in immunodeficient mice, indicating safeness of their therapeutic use in the treatment of neural diseases [44].

Due to their capacity for neuronal differentiation [42, 43], AF-MSCs have been proposed as one of the candidates for stem cell therapy of Alzheimer’s and Parkinson’s diseases, nerve injuries and sensorineural hearing loss [45-49].

Alzheimer's disease is characterized by the degeneration of cholinergic neurons in basal forebrain connected to the cerebral cortex [50]. Most recently, Thangnipon and colleagues defined a protocol for generation of cholinergic neurons from AF-MSCs, indicating their therapeutic potential in the treatment of Alzheimer’s disease [45]. They demonstrated that incubation of AF-MSCs with bone morphogenetic protein (BMP)-9 and N-benzylcinnamamide (PT-3) resulted in successful differentiation of AF-MSCs into functional, cholinergic neuronal-like cells in vitro [45].

Based on the results obtained in several animal studies, AF-MSCs are considered as a promising source for cell-based therapy of Parkinson’s disease, a highly complex neurodegenerative disorder developed due to the cell death of dopamine secreting neurons [46, 51, 52]. By using a rat model of Parkinson's disease, Chang and colleagues demonstrated that transplanted AF-MSCs were successfully engrafted in the brain and differentiated into functional, tyrosine hydroxylase and dopamine transporter expressing dopaminergic neurons [52]. Opposite to these results are findings obtained by Soler and co-workers who suggested that transplanted AF-MSCs were able to survive and improve the function of dopaminergic neurons only two weeks after transplantation in rat brains [46]. They showed that transplanted MSCs managed to migrate into the brain, but did not survive more than 14 days after engraftment. Within these two weeks, AF-MSCs expressed superoxide dismutase-2 and modulate the expression of pro-angiogenic IL-6 and glial cell-derived neurotrophic factor (GDNF) by host cells enabling trophic support and functional improvement of dopaminergic neurons [46]. Accordingly, Soler and colleagues suggested that injected AF-MSCs were not able to differentiate into dopaminergic neurons in vivo and propose that AF-MSC-based beneficial effects in Parkinson’s disease are mainly a consequence of AF-MSCs-dependent trophic support of dopaminergic neurons [46].

Table 1. Animal models used for studying therapeutic effects of AF-MSCs

<table>
<thead>
<tr>
<th>ANIMAL MODEL</th>
<th>PROTOCOLE</th>
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<tbody>
<tr>
<td>Parkinsonism model [46, 50]</td>
<td>Stereotactical injection of dopamine 6-hydroxydopamine (6-OHDA) into the medial forebrain bundle</td>
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<tr>
<td>Model of sciatic nerve injury [47]</td>
<td>A 5 mm resection of sciatic nerve, 10 mm from the internal obturator canal.</td>
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<tr>
<td>Sciatic nerve injury crush model [48, 53]</td>
<td>A vessel clamp (pressure 1.5 g/mm2) was applied 10 mm from the internal obturator canal for 20 minutes</td>
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<tr>
<td>Congenital neural tube defects model [54]</td>
<td>After exposure to isoflurane, chamber inhaled at 2%-4% in 100% oxygen, the dams received 60 mg/kg of all-trans retinoic acid, dissolved in olive oil at 10 mg/ml at room temperature, through gavage, on gestational day ten</td>
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<tr>
<td>Acute hepatic failure model [56]</td>
<td>Intraperitoneal administration of one dose of 100 ml/20 g body weight sun oil containing 10 ml carbon tetrachloride (CCl4) into NOD/SCID mice</td>
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<tr>
<td>Fulminant hepatic failure model [58]</td>
<td>Intraperitoneal injection of 1.5 g/kg galactosamine and 200 mg/kg lipopolysaccharide (LPS)</td>
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<td>Cigarette-smoke exposure model [59]</td>
<td>Cigarette-smoke exposure (20 cigarettes over 90 min once a day for each smoke exposure for 7 days per week for 12 weeks) and intratracheal LPS instillation</td>
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<td>Myocardial infarction model [64]</td>
<td>Ligation of the left anterior descending branch of the left coronary artery with a 7–0 polypropylene suture</td>
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<td>Colitis model [80]</td>
<td>C57Bl/6 mice received dextran sodium sulfate (DSS) in sterilized tap water (3% DSS w/v) for 5 days</td>
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<tr>
<td>Gavage-feeding with hyperosmolar formula, hypoxia and oral administration of LPS</td>
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<tr>
<td>Ovarian cancer models [83]</td>
<td>SKOV3 ovarian carcinoma cells were administered subcutaneously into the nude mouse</td>
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<tr>
<td>Bladder cancer model [84]</td>
<td>T24M bladder cancer cells were subcutaneously administered into the NOD-SCID mice</td>
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<tr>
<td>Prostate cancer model [85]</td>
<td>PC-3M human prostate carcinoma cells were injected into the dorsal prostatic lobes of nude mice</td>
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Beneficial effects of AF-MSCs were also demonstrated in nerve injuries models [47, 48, 53]. AF-MSCs transplanted intravascularly or directly in sciatic nerve crush site, improved electrophysiological function, nerve myelination and expression of neurotrophic factors [47, 48]. Post-injury regeneration was associated with the increased production of AF-MSC-derived neurotrophic factors: BDGF, GDNF, ciliary neurotrophic factor (CNTF), NGF and neurotrophin-3 (NT-3) [53].

As recently emphasized by Mohammadian and coworkers, AF-MSC-based therapy provide a new hope for the treatment of sensorineural hearing loss [49]. AF-MSCs, due to differentiation into neuronal-like cells and capacity to produce neuroprotective and trophic factors, managed to completely regenerate spiral ganglion neurons bringing back the inner ear hair-cells to functionality [49].

Several preclinical studies suggested promising role of autologous AF-MSCs applications in congenital disorders such as spina bifida. In a rat model of spina bifida, intra-amniotic injections of syngeneic AF-MSCs at embryonic day 17 resulted in partial or even complete healing of neural tube defects in vivo, suggesting AF-MSC-based therapy as a potentially new therapeutic approach in the handling of spina bifida in utero [54].

AF-MSCs, due to their capacity for differentiation into hepatocyte-like cells, have been proposed as an ideal source for cell-based therapy of acute liver failure [38]. When transplanted into CCl4-injured mice, undifferentiated AF-MSCs were integrated into the liver tissue, and they expressed hepatocyte-specific markers [55]. Although the integration of transplanted stem cells into the liver was limited (0.1-0.3% of hepatocytes), histological analysis showed liver recovery in mice that received stem cells [55]. Zheng and coworkers managed to differentiate AF-MSCs into hepatocyte-like cells in vitro using fibroblast growth factor (FGF)-4, hepatocyte growth factor (HGF), trichostatin A, dexamethasone and insulin-transferrin-selenium (ITS). Importantly, AF-MSCs had significantly higher hepatic differentiation potential then BM-MSCs making them more suit-
able for the therapy of fulminant and terminal liver diseases [38]. Accordingly, transplantation of either AF-MSCs or hepatic progenitor like cells derived from AF-MSCs (HPLs) managed to attenuate acute liver failure in mice [56]. Intravenous application of AF-MSC-derived conditioned medium also successfully alleviated liver inflammation [56], indicating that, in addition to differentiation into hepatocyte-like cells, AF-MSCs contribute to the liver regeneration through the production of hepatoprotective factors. Proteome profile analysis revealed that among AF-MSCs-derived factors, IL-1 receptor antagonist (IL-1Ra) had the most important anti-inflammatory and hepatoprotective role [56]. When AF-MSC-derived IL-1Ra binds to the IL-1 receptor (IL-1R), the interaction between IL-1 and IL-1R is prevented. Accordingly, various pro-inflammatory events, initiated by IL-1:IL-1R interaction, including the synthesis and release of chemokines and enhanced influx of neutrophils, macrophages, and lymphocytes in injured tissues are inhibited [57]. In line with these observations, Zheng and colleagues engineered AF-MSCs that overexpressed IL-1Ra [58]. These cells managed to significantly improve liver function and survival of rats suffering from fulminant hepatitis by promoting proliferation and suppressing apoptosis of hepatocytes. Additionally, overexpression of IL-1Ra enhanced incorporation of intravenously injected AF-MSCs into the injured livers and promoted their differentiation into functional, albumin-producing hepatocyte-like cells, indicating therapeutic potential of IL-1Ra overexpressing AF-MSCs in the cell-based therapy of acute liver failure [58].

Several lines of evidence suggested that AF-MSCs have the capacity to differentiate in alveolar epithelial cells and lung precursor cells contributing to the regeneration of injured lungs [39]. As described by Li and colleagues [39], appropriate induction medium which contains knockout of serum replacement, activin A and small airway basal medium could be used for successful in vitro differentiation of AF-MSCs in type II alveolar epithelial cells that play a key role in maintaining alveolar homeostasis and repair. Intratracheally administered AF-MSCs one week after emphysema induction, integrated into the lung tissue, stimulated the production of protective surfactant and decreased apoptosis of alveolar epithelial cells [59]. Additionally, Vadasz and colleagues demonstrated that mid-to late-trimester AF-MSCs could differentiate into lung precursor cells in vitro, when cultured in small airway growth medium supplemented with fibroblast growth factor-10 (FGF10) and lacking retinoic acid (RA) and triiodothyronine (T3). The same phenomenon was observed when AF-MSCs were seeded on decellularised lung scaffold, making them a promising source for an ex vivo lung tissue engineering [60].

Generation of insulin-producing β-cells is the need of the day for patients suffering from diabetic mellitus type 1 who require daily shots of insulin [61]. Most recently, Mu and coworkers defined three-stage culture strategy involving the use of nicotinamide, basic fibroblast growth factor (bFGF), epidermal growth factor (EGF) and exendin for differentiation of AF-MSCs into insulin-producing cells β-cells [62] indicating therapeutic potential of AF-MSCs in the treatment of diabetes mellitus type 1. Additionally, since IL-1Ra have potential to reduce immune cell-mediated destruction of insulin-producing β-cells [57], IL-1Ra overexpressing AF-MSCs, recently generated by Zheng and colleagues [58], should be further tested as new therapeutic agents in the treatment of this disease.

Since adult heart lacks significant intrinsic regenerative capability, replacement of injured cardiomyocytes is one of the major goals of cardiac regenerative medicine [63]. AF-MSCs are able to generate functional cardiomyocytes [64]. Zhao and colleagues showed that after stimulation with bFGF or activin A, AF-MSCs expressed Nkx2.5 (specific transcription factor for the cardiomyocytes), alpha-myosin heavy chain (cardiac-specific gene) and atrial natriuretic peptide (cardiac-specific marker) in vitro [64]. Importantly, the capacity of AF-MSCs to differentiate into cardiomyocytes was confirmed in vivo. AF-MSCs successfully engrafed in the ischemic regions of rat hearts, survived for at least 2 months and differentiated into cardiomyocyte-like cells [64]. Nearly 50% of transplanted AF-MSCs acquired cardiovascular phenotype in vivo, manifested by the expression of cardiac troponin T, Von Willebrand factor and α-smooth muscle actin, indicating therapeutic potential of AF-MSCs in regenerative cardiology [65].

AF-MSCs could be used in reproductive medicine as well. After the treatment with a germ cell maturation factor cocktail, AF-MSCs expressed germ cell markers in vitro and had the capability to, at least partially, restore ovarian function in mice with chemotherapy-induced sterility [41]. Transplanted AF-MSCs integrated into the ovaries of infertile mice and some of them managed to, through the differentiation into granulosa cells, participate in oocyte regeneration.

4. MOLECULAR MECHANISMS INVOLVED IN AF-MSC-BASED MODULATION OF IMMUNE RESPONSE

After engraftment in the inflammatory microenvironment, in the presence of high levels of pro-inflammatory cytokines (interferon gamma (IFN-γ) and tumor necrosis factor alpha (TNF-α)) or after the activation of intracellular toll-like receptor (TLR)-3 (induced by viral dsRNA), AF-MSCs adopt an immunosuppressive phenotype and regulate proliferation, activation and effector function of immune cells, through cell-to-cell contact or through the production of soluble factors (Fig. 2) [66-68]. Intercellular Adhesion Molecule 1 (ICAM-1) and Vascular cell adhesion protein 1 (VCAM-1), highly expressed on AF-MSCs, are critically important for AF-MSC-based juxtacrine suppression of immune cells since their deletion completely abrogates capacity of AF-MSCs to inhibit proliferation of immune cells in the cell to cell contact [66, 68]. Among secreted factors, AF-MSC-derived indoleamine 2, 3-dioxygenase (IDO), prostaglandin E2 (PGE2), nitric oxide (NO), transforming growth factor beta (TGF-β), HGF, IL-10, IL-1Ra and growth related oncogene (GRO) are most important for immunosuppressive effects of AF-MSCs [66, 67].

AF-MSCs had more potent immunomodulatory effects against activated T cells than BM-MSCs [69]. Presence of macrophage inflammatory protein-3α (MIP-3α), MIP-1α and activin in AF-MSCs conditioned media significantly enhanced the capacity of AF-MSCs for suppression of effector T cells [66, 67]. AF-MSCs attenuate proliferation of effector T lymphocytes in TGF-β, HGF, PGE2 and IDO-dependent
manner [69]. TGF-β suppresses activation of Janus kinase-Signal transducer and activator of transcription (Jak-Stat) signaling pathway in activated T cells causing the G1 cell cycle arrest while HGF acts synergistically with TGF-β1 further enhancing inhibition of T cell expansion [70, 71]. Suppressing effects of AF-MSC-derived PGE2 on the activation and expansion of effector T cells include the direct inhibitory effects on IL-2 production and the expression of IL-2 receptor and Jak-3 which mediate the responsiveness of T cells to IL-2 [72–74]. AF-MSC-derived PGE2 is also involved in the modulation of cytokine profile in T cells. PGE2 inhibits production of IFN-γ and IL-4 in effector CD4+ T cells attenuating Th1 and Th2 cell-driven inflammation [74].

When engrafted in Th1 and Th2 microenvironment, human MSCs most usually use IDO and its metabolites (kynurenine, quinolinic acid and 3-hydroxy-antranilic acid) to attenuate proliferation or to induce apoptosis of activated T cells, while murine MSCs usually use inducible nitric oxide synthase (iNOS)-mediated immunomodulation [75]. However, under Th17 conditions, murine MSC does not produce NO and their immunsuppressive effects are mainly mediated through the production of IDO which acts as a critical molecular switch that simultaneously blocks re-programming of Tregs into IL-17 producing effector T cells having an important role in Treg-based immunosuppression of Th17 driven inflammation [72]. We recently showed that MSCs are able to suppress generation of inflammatory, IL-17 producing T and NKT cells in IDO dependent manner and that IDO inhibitors could be used to restore MSC suppression of Th17 differentiation [76, 77]. In line with these findings, we developed immunomodulatory product (“Decellularized Multiple Allogeneic Proteins Paracrine Signaling-D-MAPPS”) which activity is based on AF-MSC-derived IDO and its interaction with several other cytokines and growth factors is capable of attenuating inflammation and promoting regeneration of injured tissues (IL-1Ra and GRO). AF-MSCs attenuate inflammation through the production of IL-1Ra while in GRO-dependent manner, attenuate maturation and antigen-presenting function of inflammatory, TNF-α, IL-12 and IL-23-producing dendritic cells (DCs) and suppress Th1 and Th17 immune response. At the same time, AF-MSC-derived GRO may promote the generation of regulatory DCs capable of producing high levels of anti-inflammatory IL-10 [66, 67, 78] creating immunosuppressive microenvironment. In line with these observations, our preliminary findings revealed that AF-MSC-based ophthalamic solution significantly attenuated dryness, grittiness, scratchiness, soreness, irritation, burning, watering and eye fatigue in patients suffering from corneal injury and dry eye syndrome indicating therapeutic potential of AF-MSCs and their products in regenerative ophthalmology.

Pathological immune response is responsible for the development of inflammatory bowel diseases (IBDs) [79]. Due

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**Fig. (2). Molecular mechanisms involved in AF-MSC-based modulation of immune response.** After engraftment in the inflammatory microenvironment, in presence of high levels of pro-inflammatory cytokines (IFN-γ and TNF-α), AF-MSCs adopt an immunosuppressive phenotype and regulate proliferation, activation and effector function of immune cells, through cell-to-cell contact or through the production of soluble factors. AF-MSCs suppress proliferation and activation of NKT cells, T lymphocytes and promote induction of regulatory phenotype in DCs. Injection of AF-MSC-CM managed to attenuate DSS-induced colitis in mice by increasing production of immunosuppressive cytokines (TGF-β and IL-10) which resulted with suppressed activity of colon-infiltrating immune cells followed by decreased production of inflammatory cytokines (TNF-α, IL-1β) in experimental animals.
to their immunosuppressive characteristics, AF-MSCs and their products have been explored as new therapeutic agents in the treatment of IBDs [80]. By using dextran sodium sulphate (DSS)-induced colitis, Legaki and colleagues showed that intraperitoneal injection of AF-MSC-derived conditioned medium (AF-MSC-CM) managed to significantly attenuate ulcerative colitis in mice [80]. Mice that received AF-MSC-CM had significantly higher levels of immunosuppressive cytokines (TGF-β and IL-10) and notably reduced levels of inflammatory cytokines (TNF-α and IL-1β) compared to DSS-only treated animals, indicating that beneficial effects of AF-MSC-CM were a consequence of the suppressed activity of colon-infiltrating immune cells (Fig. 2) [80]. Similarly, Zani and coworkers showed that AF-MSCs may improve survival of enterocytes and enhance repair of damaged intestine in necrotizing enterocolitis by suppressing gut inflammation [81].

AF-MSCs have capacity to migrate towards the site of the injury and inflammation. Homing ability of AF-MSCs decreases with the number of passages and is mainly mediated by chemokine gradient of stromal cell-derived factor 1 alpha (SDF-1α) which is produced by resident tissue cells and activated immune cells. SDF-1α specifically binds to the chemokine receptor CXCR4 expressed on the membrane of AF-MSCs inducing their migration towards the site of injury or inflammation [82]. Due to their homing specificity, AF-MSCs could be used as therapeutic vehicles for delivering cytokines or anti-cancer agents in tumor tissue. Since tumors developed at the sites of chronic inflammation and injury, AF-MSCs migrate to tumor tissues attracted by alarmins, SDF-1α and other chemokines released by tumor stromal cells or tumor-infiltrated immune cells [32, 83]. In line with these findings, Bitsika and colleagues recently used AF-MSCs to deliver IFNβ directly into bladder cancer [84]. IFNβ suppresses the growth of solid tumors by inducing apoptosis and by arresting tumor cells in the S phase of cell cycle [85, 86], but its therapeutic use is limited because of high toxicity [87]. Accordingly, AF-MSCs were used to deliver IFNβ directly to tumor tissue to avoid undesired side effects to the neighboring tissues [84]. IFNβ-delivering AF-MSCs successfully migrated and colonized the region of neoplasia in a bladder, inhibited tumor growth and prolonged survival of experimental animals [84]. Results obtained in this study strongly suggested that, due to their high proliferation rate and homing specificity, AF-MSCs represent ideal vehicles for delivering anti-cancer agents to the solid tumors. In line with these observations, recently designed clinical trial (NCT03298763) is going to test the potential of MSCs genetically modified to express TNF-related apoptosis inducing ligand (TRAIL) to enhance the efficacy of metronexed/cisplatin chemotherapy in the treatment of metastatic Non-small cell lung cancer patients [88].

Table 2. Therapeutic potential of AF-MSCs.

<table>
<thead>
<tr>
<th>REFERENCE</th>
<th>OUTCOME OF AF-MSC-BASED THERAPY</th>
<th>MECHANISM OF ACTION</th>
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<tbody>
<tr>
<td>[33-35]</td>
<td>reconstruction of prenatal tracheal defects</td>
<td>re-epithelialization; increase in elastin levels</td>
</tr>
<tr>
<td>[36, 37]</td>
<td>complete repair of full-thickness sternal and nasal bone defects</td>
<td>increase in alkaline phosphatase activity; enhanced and consistent bone mineralization</td>
</tr>
<tr>
<td>[46, 52]</td>
<td>amelioration of bladder dysfunction and behavioral improvement in Parkinson's disease model</td>
<td>differentiation in functional, tyrosine hydroxylase and dopamine transporter expressing dopaminergic neurons; AF-MSCs-mediated trophic support of dopaminergic neurons</td>
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<tr>
<td>[48, 53]</td>
<td>improved electrophysiological function and injured sciatic nerve remyelination</td>
<td>Production of neurotrophic factors: BDGF, GDNF, CNTF, NGF and neurotrophin-3</td>
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<tr>
<td>[49]</td>
<td>complete regeneration of spiral ganglion neurons</td>
<td>differentiation into neuronal-like cells; production of neuroprotective factors</td>
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<tr>
<td>[56, 58]</td>
<td>improve survival and liver function</td>
<td>differentiation into hepatocyte-like cells; promoting proliferation and suppressing apoptosis of hepatocytes through the production of anti-inflammatory and hepatoprotective factors</td>
</tr>
<tr>
<td>[39, 59, 60]</td>
<td>regeneration of injured lungs; stimulation of protective surfactant production</td>
<td>differentiation in alveolar epithelial cells and lung precursor cells</td>
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<tr>
<td>[80, 81]</td>
<td>improve survival and enhance healing of damaged intestine</td>
<td>suppression of gut inflammation by reducing levels of inflammatory cytokines TNF-α and IL-1β and increasing levels of immunosuppressive TGF-β and IL-10</td>
</tr>
<tr>
<td>[83-85]</td>
<td>inhibition of tumor growth and prolonged survival</td>
<td>apoptosis and S-phase tumor cell cycle arrest</td>
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</table>
CONCLUSION

AF-MSCs have huge differentiation and immunosuppressive potential and are capable to attenuate inflammation and promote tissue regeneration without adverse effects (Table 2). Considering the fact that AF is obtained through routine prenatal diagnosis, with minimal invasive procedure and without ethical concerns, AF-MSCs represents a valuable source for cell-based therapy of organ-specific or systemic degenerative and inflammatory diseases.

LIST OF ABBREVIATIONS

AF = amniotic fluid
AF-MSC = Amniotic Fluid Derived Mesenchymal Stem Cells
BM-MSCs = Bone Marrow-Derived MSCs
CD = Cluster of Differentiation
MHC = Major Histocompatibility Complex
Oct-3/4 = Octamer Binding Protein 3/4
TRA = Tumour-Related Antigen
SSEA = Stage-Specific Embryonic Antigen
SCID = Severe Combined Immune Immunodeficiency
PLLA = Poly-L-Lactic Acid
NSC = Neural Stem Cell
BDGF = Brain-Derived Growth Factor
NGF = Nerve Growth Factor
BMP = Bone Morphogenetic Protein
GDNF = Glial Cell-Derived Neurotrophic Factor
IL = Inter Leukin
CNTF = Ciliary Neurotrophic Factor
NT-3 = Neurotrophin-3
FGF = Fibroblast Growth Factor
HGF = Hepatocyte Growth Factor
ITS = Insulin-Transferrin-Selenium
IL-1Ra = IL-1 Receptor Antagonist
RA = Retinoic Acid
BFGF = Basic Fibroblast Growth Factor
EGF = Epidermal Growth Factor
ICAM-1 = Intercellular Adhesion Molecule 1
VCAM-1 = Vascular Cell Adhesion Protein 1
IDO = Indoleamine 2, 3-Dioxygenase
PGF = Prostaglandin E2
NO = Nitric Oxide ()
TGF-β = Transforming Growth Factor Beta
GRO = Growth Related Oncogene
MIP = Macrophage Inflammatory Protein
INOS = Inducible Nitric Oxide Synthase

DSS = Dextran Sodium Sulphate
SDF-1α = Stromal Cell-Derived Factor 1 Alpha

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

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CRH, CF, VD and NA analyzed data and wrote the paper, NJ collected and analyzed data and created figures, MG and VV designed study, analyzed data and wrote the paper.

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