Extraembryonic Mesenchymal Stromal

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Extra-embryonic mesenchymal stromal cells (MSC) are characterized by robust and constitutive anti-inflammatory and anti-fibrotic properties, indicating as therapeutic agents for inflammatory conditions such as liver fibrosis or advanced cirrhosis, as well as chronic inflammatory settings or deranged immune responses. MSC are certainly the most diffused and largely variable cell products generated and described during the past decades in cell-based therapy approaches. These cells have been defined and collected under a large umbrella of acronyms and nomenclature.

extraembryonic membranes umbilical cord

1. Amniotic Fluid

Amniotic fluid (AF) is an essential ingredient of amnion sac, crucial for the fetus' growth, development, and protection [1]. Such fluid is commonly harvested by amniocentesis at 15–20th week of the gestation for early diagnosis of the fetal genetic abnormalities [2] or can be collected during caesarian surgical procedure at the end of pregnancy [3]. Over the past several years, purification procedures and cytological descriptions have described the presence of cells floating in AF, primarily of fetal origin (due to skin or intestine exfoliation) or excreted within the fetal urine [4]. Amniotic fluid cells are classified into three main categories based on their morphological and biomedical activity, including the epithelioid type, AF type, and fibroblastic type cells [5]. Epithelioid types are derived from the skin and urine of the fetus and indicate a round shape and slow-growing properties. AF-type cells originated from the placenta and are associated with estrogen, human chorionic gonadotropin (hCG), and progesterone production, and fibroblast-like cells descended from mesenchymal tissue with no hormonal activity and a spindle-shaped morphology [6].

Amniotic liquid is still considered an important source of MSC for cell-based therapy [7]. AF-MSC have been largely described and fulfilling all the criteria needed (surface markers and gene expressions) to be defined as multipotent MSC [8][9][10]. AF-MSCs are accessible and easily isolated/purified in a less invasive manner than other extraembryonic and somatic MSCs. However, the accessibility of these cells is associated with some concerns: first, AF-MSCs can be obtained both at mid-term through amniocentesis and at full-term delivery. However, amniocentesis procedure is considered not free from risk for the fetus and the mother; thus, such a diagnostic procedure is going to be rapidly substituted by less invasive biomolecular and serological analysis. The harvest of AF-MSC at the end of pregnancy has been shown to be attainable, but limited to caesarian section [11]. Such a practice ideally does not introduce any risk either for the newborn or the mother, but it is still poorly offered since

the priority is commonly given to the baby and mother, limiting quality and quantity of AF devoted to cell purification.

Functional analysis of AF-MSC, in comparison with other sources of mesenchymal cells, proved expressions of ECM remodeling genes and adhesive factors; secretion of growth and anti-inflammatory factors have also been measured at the same level as somatic MSCs, while the expression of prostaglandins and oxytocin receptors are much higher in AF-MSC [12]. AF-MSCs proliferative capacity has also been shown at higher levels in AF-MSC, as well as their engraftment and adhesion efficiency. The same study also highlighted the reduced immunogenicity of AF-MSCs in comparison to other fetal or adult MSC [13]. Furthermore, AF-MSCs possess important ability to adapt against genotoxic stress, replicative senescence. These fetal-derived MSC have proved superior potential for DNA repair in comparison with adult bone-marrow MSCs, encouraging their application in innovative clinical setting [14].

The clinical efficacy of AF-MSC has been mainly ascribed to their paracrine effects, such as secretion of soluble mediators (i.e., TGFβ1 and IL-10), trophic mediators, or angiogenic factors. AF-MSCs are capable to secrete soluble proteases (matrix metalloproteinases (MMP)-2, -9, and -14) responsible for ECM remodeling and fibrosis reversal [15][16][17][18]. AF-MSC effects have been proved in preclinical and clinical settings, where such allogeneic cells have been offered regenerative effects for cardiovascular, renal, musculo-skeletal, gastrointestinal, hematopoietic, respiratory, neurological, and urinary diseases [19]. Furthermore, immunomodulatory and anti-oxidative effects of AF-MSCs have also been reported and described in different regenerative medicine applications.

2. Amniotic Membrane

The human amnion or amniotic membrane (AM) is an avascular tissue, characterized in histological analysis by a thick stroma with embedded scarce MSC, while on the surface in direct contact with the fetus, epithelial cells line the surface [20]. Amnion epithelial cells (AECs) originate from epiblasts, during the second week of gestation, before gastrulation and attachment to the uterus; amnion MSC (AMSC) rise from the primitive streak of the trophectoderm, after the three germ layers have been originated [21].

Mechano-enzymatic procedures have a proven effectiveness in isolating human AMSCs from full-term amniotic membrane [22]. Freshly isolated or cryopreserved human AMSCs have been reported to express somehow stemness genes such as octamer binding transcription factor (Oct)-3/4, SRY (sex-determining region Y)-box (SOX)-2, Myc, Rex-1, and Nanog in addition to the angiogenic genes PECAM-1, bFGF, and VEGF. Such an expression pattern has been described decreasing during serial passages, and limited to cells at the early passage in vitro [23][24].

Flow cytometric analysis on human AMSC confirmed constitutive expression of surface antigens widely accepted as identity markers for MSCs (CD73, CD90, CD105) [25]. The absence of surface markers such as CD31, CD34, CD45, CD106 supports hAMSC identity and homogeneity [26]. Besides, AMSCs express human leukocyte antigen (HLA) class Ia, but lack class II (HLA-DR) [27]. These surface molecules and ectoenzymes are critical mediators to

grant AMSC tolerogeneity in allogeneic settings. Human AMSC modulate activation and proliferation of host immune cells, such as T and B cells, or natural killer (NK) cells. Furthermore, extraembryonic AMSCs modulate the production of pro-inflammatory cytokines such as interferon-gamma (IFN-γ), tumor necrosis factor-α (TNF-α), and interleukin (IL)-1β, IL-5, IL-6, IL-9, IL-13, IL-17A, and IL-22 by the innate and adaptive immune cells [28].

Amnion-derived MSCs have also been confirmed as multipotent cells, capable of differentiating into adipocytes, osteoblasts, and chondrocytes [29]. The administration of AMSCs has been described as supportive and beneficial in treating neurological, cardiovascular, and gastrointestinal disorders, but also helpful in a few cancers [30].

3. Chorionic Plate

Chorionic plate MSC (CP-MSC) can be isolated from the chorionic layer of the human placenta by exposing tissue to enzymatic activity. CP-MSC possess similar properties to other extraembryonic or adult MSCs, including the ability for self-renewal and mesoderm differentiation, in addition to “classical” identity proven by selective surface markers [31][32]. The CP-MSC proliferative rate has been described superior to the afore described AMSC [33]. Furthermore, chorionic MSC present enhanced adipogenic potential [34], described as superior to other extraembryonic MSC such as AMSC (whose osteogenic potential is instead reported preferable) [35] or umbilical-cord-derived MSC (prevalently chondrogenic) [36]. Several preclinical studies have described CP-MSC differentiation in to neuronal, pancreatic, angiogenic, and cardiomyocyte-like cells [37].

Recent reports highlighted notable immunomodulatory properties possessed by CP-MSC and peculiar gene expressions and differentiation capacity [38]. CP-MSC have been shown particularly active in reducing T-cell proliferation and IFN-γ secretion [39]. Additionally, it has been reported that CP-MSC secrete high levels of IL-10 and TGFβ1 [40].

4. Umbilical Cord

The umbilical cord (UC) is a multi-layer tissue, characterized by a thick stroma with embedded blood vessels. Human UC consist of two arteries, and one vein enclosed by a gelatinous material called Wharton’s Jelly [41]. Several cells can be isolated from full-term UC, including the most described and commonly used hematopoietic stem cells floating in the umbilical cord blood [42]. Once cord blood is drained out, selective and consecutive enzymatic digestions may facilitate isolation of MSC from the Wharton’s Jelly (WJ-MSC) [43], endothelial cells from the umbilical vein (UVEC) [44], and umbilical cord perivascular cells (UCPVC) [45], and very small embryonic-like stem cells [46]. UC-MSC can be enzymatically isolated from Wharton’s Jelly, perivascular tissue, and umbilical membrane [47][48]. Both natural delivery and caesarean section birth have been offering quality tissues for UC-MSC manufacturing. UC-MSCs are multipotent MSCs, and as all the other somatic or extraembryonic MSCs, present characteristic morphology, plastic adherence, and certain surface markers [49][50]. Notably, gene expression analysis in human UC-MSC resulted in highly angiogenetic and neurogenic pattern profiles compared to other adult MSCs [51].

Human UC-MSCs have been applied in regenerative models where they enhanced innate repair capacity, induced secretion of anti-inflammatory cytokines, modulated recipient's immune recognition and rejection, and inhibited tissue apoptosis as indicated by increased Bcl-xl/Bax protein ratio and decreased cleaved caspase 3 levels [52][53].