Histol Histopathol (2010) 25: 91-98

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

Review

The amniotic membrane as a source of stem cells

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Summary. Cellular therapy has emerged as a new potential tool for curing a wide range of degenerative diseases and tissue necrosis. Embryonic stem cells possess potential for differentiation into a wide range of cell lineages, but the ethical issues associated with establishment of this human cell line have to be resolved prior to any use. The bone marrow (BM) is the usual source of adult stem cells for hematopoietic stem cell transplants and cellular therapy, but the BM harvest is a surgical procedure that requires general anesthesia or sedation, and there seems to be a reduction of the proliferative potential and differentiation capacity of the marrow mesenchymal stem cells in older donors. For these reasons there is an increasing interest in other sources of stem cells from adult and fetal tissues. The amniotic membrane (AM) or amnion is a tissue of particular interest because its cells possess characteristics of stem cells with multipotent differentiation ability, and because of low immunogenicity and easy procurement from the placenta, which is a discarded tissue after parturition, thus avoiding the current controversies associated with the use of human embryonic stem cells. Therefore, amniotic membrane has been proposed as a good candidate to be used in cellular therapy and regenerative medicine.

Key words: Amnion, Stem cell, Amniotic epithelial stem cell, Amniotic mesenchymal stem cell, Differentiation

Introduction

Bone marrow (BM) has been the most studied tissue as a source of progenitor cells. It contains at least two cell populations: the hematopoietic stem cells capable of regenerating the peripheral blood cell lines and the immune system, and the mesenchymal stem cells, capable of giving rise to the mesodermal cell lines: osteocyte, chondrocyte and adipocyte (Herzog et al., 2003), and as supported by several studies give rise to ectodermal (neurons) (Moraleda et al., 2006), endodermal (hepatocytes) (Schwartz et al., 2002) and endothelial cell lines (Reyes et al., 2001). For these reasons BM has been safely used in clinical medicine since 1970 for bone marrow transplantation (Copelan, 2006), and more recently in clinical and preclinical regeneration assays (Horwitz et al., 2002; Koc et al., 2002; Moraleda et al., 2006; Smith et al., 2006). However, the BM harvest is a surgical procedure that requires general anesthesia or sedation, and as reported by Stenderup (2003) there seems to be a reduction of the proliferative potential and differentiation of the marrow mesenchymal stem cells in older donors (Stenderup et al., 2003). Consequently, there is increasing interest in other sources of stem cells from adult and fetal tissues, such as granulocyte-colony stimulating factor, peripheral blood, connective and adipose tissue, skeletal muscle, dental pulp, umbilical cord blood, amniotic fluid and placenta, among others. The placenta and its membranes have recently received particular attention as a source of adult stem cells with multi and pluripotential ability for differentiation, not only due to its reduced immunogenic capacity and easy procurement from a discarded tissue, but also because its use does not imply the ethical issues associated with the establishment of human Embryonic Stem Cell lines (ESC).

In this review we will summarize the new information about the amniotic membrane (AM) progenitor cells emphasizing the characteristics that make this tissue a good candidate to be used in cellular therapy and regenerative medicine.

The placenta and the amniotic membrane structure

The placenta is a structure of fetal-maternal origin, with a round shape, 15 to 20 cm in diameter and 2 to 3 cm in thickness. It has two sides: one is the chorionic

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plate that represents the fetal compartment, faces the amniotic cavity and is formed by the amnion and the chorion. The other side, called the basal plate, is of maternal origin and is formed by the deciduas (basal and parietal). In the placental disc the chorionic villi keep the two plates together, connecting its cytotrophoblastic cells with the basal deciduas. Fetal membranes (amnion and chorion) go further from the placental disc to cover the fetus in the amniotic cavity, protect it during its development and offer a safe surrounding, where it can grow without being distorted by the pressure of neighboring structures (Frank and Kaufman, 2006; Parolini and Soncini, 2006).

The AM is the most internal layer of fetal membranes. It consists of a thin epithelium, a basement membrane and a stroma of avascular connective tissue called amniotic mesoderm (Fig. 1). The epithelium makes a simple, continuous, uninterrupted line of columnar, cuboid or flat cells, in contact with the amniotic fluid. It sits over a resistant well defined basement lamina connected with the amniotic mesoderm where three structures can be identified: an acellular compact layer made up of collagen I, III and fibronectin; a netting of mesenchymal cells called fibroblastic layer; and an intermediate layer or sponge zone, rich in proteoglycans, glycoproteins and a non fibrilar collagen type III, which is loosely connected to the chorion.

In humans the development of the placenta and its membranes begins early with the implantation of the blastocyst 5 to 6 days after fertilization. At this point the blastocyst is formed by a group of internal cells (which give rise to the embryo), a blastocyst cavity that later will develop into extra-embryonic structures, and an external wall of trophoblastic cells, which attaches to the endometrium, proliferates rapidly and differentiates into cytotrophoblast (internal layer) and syncytiotrophoblast (external layer). In the second week after fertilization, as the implantation progresses, the group of internal cells or embryonic stem cells (ESC) differentiates into epiblast and hypoblast, while the amniotic cavity appears. The epiblast gives origin to the amniotic epithelium that surrounds the amniotic cavity, and the hypoblast originates the extra-embryonic mesoderm that gives rise to the connective tissue that surrounds the amniotic epithelium; this means that the amniotic membrane has an epithelium of epiblastic origin and a mesoderm of hypoblastic origin, and that the chorion has a mesoderm of hypoblastic origin and a trophoblast of trophoblastic

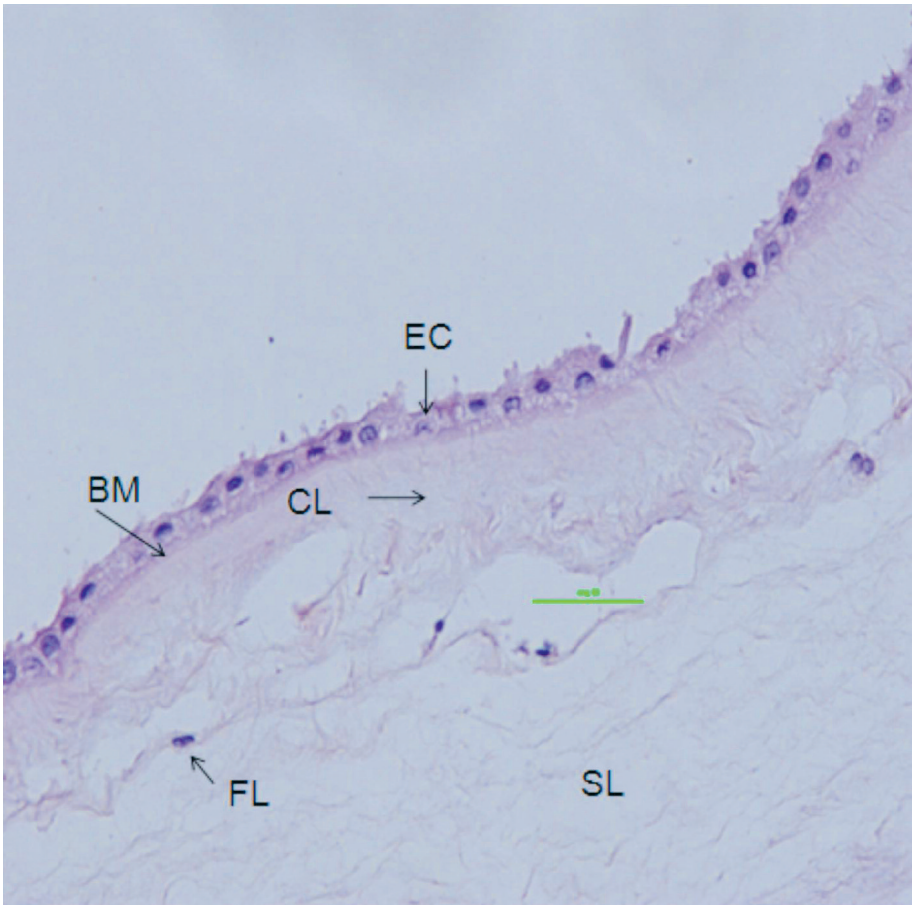

Fig. 1. Haematoxylin and eosin stained section of human amniotic membrane. EC: Epithelial cells; BM: Basement membrane; CL: Compact layer; FL: Fibroblastic layer; SL: Sponge layer

origin. The different degrees of plasticity observed in stem cells isolated from different parts of the placenta are probably related to embryologic origin. During gastrulation (day 15 to 17 of fertilization) the epiblast differentiates into the three germinal layers (ectoderm, mesoderm and endoderm) and the destiny of the cells is specified (Frank and Kaufman, 2006; Parolini et al., 2008).

Stem cells from the amniotic membrane (AM)

The amniotic epithelial cells

They are also known as hAEC (human Amniotic Epithelial Cells). Tamagawa et al. (2004) were the first to propose the pluripotenciality of these cells. They isolated epithelial and mesenchymal cells form a human AM and were able to create "in vitro" a xenogenic chimera with mice embryo cells, showing the contribution of human cells in the up-bringing of the three germinal layers (Tamagawa et al., 2004). Later, other researchers studied the stem cell characteristics of the amniotic epithelium and demonstrated their ability to differentiate to the three germinal layers: ectoderm, mesoderm and endoderm (Miki et al., 2005; Miki and Strom, 2006).

Different methods to isolate hAEC have been published (Miki and Strom, 2006; Miki et al., 2007). All of them are based on the separation of the AM from the underlying chorion through the spongy layer and the subsequent exposure to trypsin or other digestive enzymes, in different concentrations and for different periods of time, which detach the cells from the basal membrane. An average of $100x10^6$ hAEC (range 80 - $300x10^6$) may be obtained from one AM with any of these methods. Freshly isolated hAEC stained with May Grünwald / Giemsa are medium size cells with a central or eccentric nucleus, one or two nucleoli and abundant cytoplasm, usually vacuolated (Miki et al., 2007).

Although the hAEC have a homogenous morphologic appearance, the isolated cells show a heterogeneous phenotype, suggesting that there might be different subpopulations of cells (Table 1). The hAEC cells show surface markers associated with embryonic stem cells (ESC) such as SSEA 3 (stage specific embryonic antigen 3) and SSEA 4; TRA 1-60 (tumor rejection antigen) and TRA 1-81; other antigens such as the ABCG 2/BCRP, a member of the ATP-binding cassette superfamily that works as an efflux pump, CD9, CD24, E-Cadherin, Integrin α6 and ß, c-met (receptor growth factor of the hepatocyte). They do not express SSEA-1, CD34 (markers of hematopoietic and endothelial stem cells), nor CD133 (expressed in hematopoietic stem cells, endothelial cells, glioblastoma cells, etc), and are negative or weakly positive for CD117 (c-kit) and CCR4 (chemokine CC receptor) (Miki et al., 2005; Miki and Strom, 2006).

The recently isolated and cultured hAEC also express transcription factors specific for pluripotential stem cells, such as OCT-4 (octamer binding protein 4), SOX-2, NANOG, and REX-1; which are also expressed in the ESC, epiblast, embryo carcinoma cells and embryonic germinal cells (Miki et al., 2005; Miki and Strom, 2006).

The hAEC are routinely cultured in Dulbecco modified Eagle's media, supplemented with 5 to 10% of fetal bovine serum and Epidermal Growth Factor (EGF). The cells proliferate showing numerous mitotic events and form a confluent single layer with typical cuboid epithelial morphology (Fig. 2). It is possible to make 2 to 6 passes, before the proliferation stops. Without EGF, the proliferation stops early and giant multinuclear cells are formed. If cells are cultured in low densities, the senescence occurs before, apparently due to less cellular interaction and complex molecular steps related to EGF receptor and the integrins. Immunohystochemistry stains show that virtually all cultured epithelial cells are positive to low molecular weight cytokeratin and negative to vimentin, which confirms its epithelial nature (Miki and Strom, 2006; Miki et al., 2007).

Under appropriate conditions of high density and long term culture, the hAEC makes spheroid structures or "clusters" of cells over the single layer of adjacent cells, similar to the embryoid bodies described in the cultures of ESC. These spheroid structures keep their stem cell characteristics, they do not require nutrients derived from other cells to maintain the expression of OCT-4 and NANOG, do not express telomerase, show a normal karyotype, and are not oncogenic after transplant. All this is in contrast to the embryoid bodies of the ESC, which have telomerase activity, become aneuploid in cultures, and can produce teratomas when

Table 1. Phenotype and *in vitro* differentiation of human Amniotic Epithelial Cells.

Cell type	Phenotype	Ectodermal lineages	Mesodermal lineages	Endodermal lineages
hAEC (human Amniotic Epithelial Cells).	Positive: SSEA-3, SSEA-4,TRA 1-60,TRA-1-81, ABCG 2, CD9, CD24, E-Cadherin, Integrin α6 and ß, c-met (13) Negative: SSEA-1, CD34, CD133, CD117, CCR4 (13)	Expression of neuronal and glial markers: Nestine, GAD,GFAP,CNP (13,14) Synthesis and release of acetylcholine, catecholamine, dopamine (16,17)	Expression of cardiac-specific genes and transcription factors: MLC-2A, MLC-2V, GATA and NKx2.5 (13)	Expression of pancreatic α and ß cell markers: PDX-1, PAX-6, NKX2.2, insulin and glucagon (13, 19). Expression of hepatocytes related genes: Albumin,α-1 AT, α fetoprotein (13, 20)

injected into mice with severe combined immunodeficiency (Miki and Strom, 2006).

The hAEC can be induced under appropriate conditions "in vitro" to differentiate in cells of the three germinal layers (Table 1). In the assays performed by Miki et al. (2005) (leaders in this field), the differentiation to neural tissue (ectodermic line) was evident by RT-PCR expression of specific neural genes, some of them are present before the culture, similar to that observed with human embryonic stem cells (hESC). However, the expression of nestin (an intermediate filament protein expressed in nerve cells) and glutamic acid descarboxylase (GAD), a very important enzyme in GABA biosynthesis, increased in 7 days. The cells induced to neural differentiation acquired an elongated shape, neuronal morphology, 90% reacted to GFAP (glial fibrilar acid protein) antibody, a marker for glial cells lineage, and 5% to CNP (cyclic nucleotide phosphodiesterase), a marker of oligodendrocyte lineage (Miki et al., 2005).

Other investigators also demonstrated the differentiation of the hAEC to neural cells with capacity to synthesize and release acetylcholine, catecholamines and dopamine (Elwan and Sakuragawa, 1997; Kakishita et al., 2000). These findings support the hypothesis that the human amnion could be part of the neural formation during the early development phase, and outlines the possibility that the cells derived from this tissue could offer ways for important advances in the treatment of neural degenerative diseases. In fact, some studies have already been published showing promising results in animal models with Parkinson's Disease and Muco-polysaccaridosis type VII (Kosuga et al., 2000).

The pancreatic differentiation (endodermal lineage) was demonstrated by Miki et al. (2005) through RT-PCR by early expression of the transcription factor PDX-1 and later by the factors PAX-6, NKX2.2 and the expression of genes of mature cells such as insulin and glucagon (Miki et al., 2005). This differentiation was also pointed out by Wei et al, who demonstrated that the transplant of cultured hAEC in the spleen of diabetic mice with combined severe immunodeficiency, normalized the levels of serum glucose for several months after the transplant, suggesting that these cells have the potential to become insulin secreting ß cells "in vivo" and that they could represent a therapeutic weapon for diabetes mellitus type I (Wei et al., 2003).

The hepatic differentiation (endodermal lineage) was investigated by quantitative RT-PCR of mRNA of albumin and α-1 antitrypsine (α-1 AT) genes, the production of proteins and tests of functional activity (Miki et al., 2005). Sakuragawa et al. (2000) reported that cultivated hAEC produced albumin and α fetoprotein, and that cells similar to hepatocytes, immunocytochemically positive for albumin and α fetoprotein, could be identified as integrated in the liver parenchyma of mice with severe combined immunodeficiency, after the hAEC transplant in the liver (Sakuragawa et al., 2000). All these findings suggest a potential role for hAEC in regenerating hepatic tissue.

The differentiation to cardiac cells (mesodermal line) was also evaluated for the first time by Miki et al (2005), through the determination by RT-PCR of genes related with cardiomyocytes. They demonstrated the presence of genes of the light chain of myosin, specific of the atrium and ventricle (MLC-2A and MLC-2V) and of the transcription factors GATA-4 and NKx2.5 when the cells were cultured in specific media during 14 days. The immunocytochemistry analysis using the specific anti α actinin antibody showed the expression of α actinin with an identical pattern to the one reported for cardiomyocytes derived from ESC (Miki et al., 2005).

Human amniotic mesenchymal stromal cells (hAMSC)

By agreement of the First International Workshop on Placenta Derived Stem Cells held in Italy in March 2007 (Parolini et al., 2008), these cells are known as hAMSC, in concordance with the denomination given to the Multipotent Mesenchymal Stromal Cells, derived from Bone Marrow (BM-MSC) by the International Society for Cellular therapy (ISCT) (Horwitz et al., 2005; Dominici et al., 2006).

The MSC have generated great interest in the scientific community because of their high potential of expansion "in vitro", their self-renewal capacity and

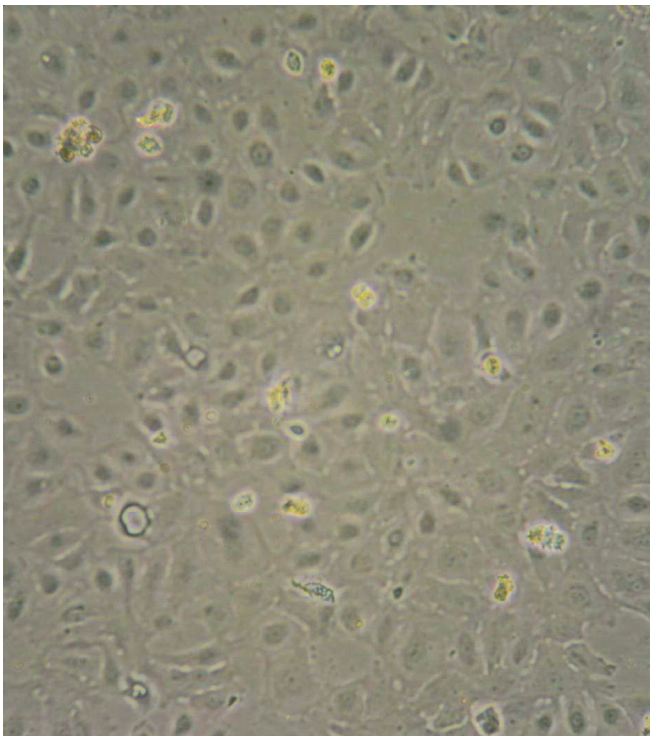

Fig. 2. Morphology of cultured human Amniotic Epithelial Cells (hAEC).

their immunomodulatory properties. The MSC can be isolated from different tissues such as the bone marrow, peripheral blood, umbilical cord blood, adult connective tissue, placenta, dental pulp, and others. More recently it has been demonstrated that the MSC can differentiate "in vitro" to cells of all the germinal layers: ectoderm (neural), mesoderm (skeletal muscle, cardiomyocyte, endothelial, etc.,) and endoderm (pancreatic, hepatic cells) (Alviano et al., 2007; Dazzi and Marelli-Berg, 2008; Parolini et al., 2008).

The Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy established in 2006 the minimal criteria to define the human MSC that included: adhesion to plastic, expression of specific surface antigens and capacity of multipotential differentiation "in vitro" (chondrogenic, adipogenic and osteogenic), demonstrated by staining of cellular cultures. The immunophenotypic definition includes the requirement that more than 95% of the population of MSC must express the CD105, CD 73 and CD90 surface antigens by flow cytometry and be negative or positive less or equal to 2% for CD45 (pan-leukocyte marker), CD34 (primitive hematopoietic progenitor and endothelial cells), CD14 or CD11 (expressed mainly in monocytes and macrophage), CD79α or CD19 (marker for B cells) and HLA class II (Dominici et al., 2006). In 2007, in the First International Workshop on Placenta Derived Stem Cells (Parolini et al., 2008), these criteria were extended to include the necessity to verify the fetal origin of the cells isolated from the placenta, using sensitive methods able to find maternal contamination of 1% or less (Table 2).

hAMSC were isolated for the first time from AM of the second and third trimester of gestation by In't Anker et al. (2004), who demonstrated their potential of differentiation to osteogenic and adipogenic cells (In't Anker et al., 2004). Later, Portmann-Lanz et al. (2006) pointed out their capacity for differentiation to chondrogenic, myogenic and neurogenic lines (Portmann-Lanz et al., 2006). In 2007, Alviano et al. confirmed this and gave the first evidence of their angiogenic potential (Alviano et al., 2007).

As has been mentioned hAMSC are located in the amniotic mesoderm. They can be obtained by exposure of AM to collagenase, alone or combined with DNAase (Steigman and Fauza, 2007). After 3 to 4 weeks of culture it is possible to obtain a population of adherent mesenchymal cells, morphologically identical to the mesenchymal cells obtained from BM. These cells are easy to expand "in vitro" for at least 15 passes without morphologic alteration (Fig. 3).

As well as in other stem cells, the presence of the transcript OCT-4, which codifies a protein involved in the maintenance of the renovation capacity, is detected in hAMSC by RT-PCR analysis. According to Alviano et al (2007), the level of expression of OCT-4 and the proliferation capacity are higher in hAMSC than in BM-hMSC (Alviano et al., 2007).

hAMSC are capable of differentiating "in vitro",

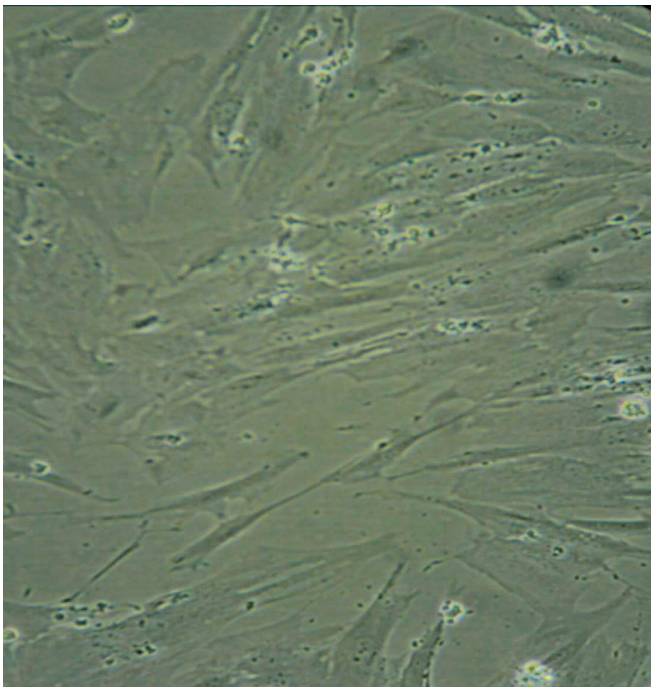

Fig. 3. Morphology of cultured human Amniotic Mesenchymal Stromal Cells (hAMSC).

Table 2. Phenotype and *in vitro* differentiation of human Amniotic Mesenchymal Stromal Cells.

Cell type	Phenotype	Osteoblastic Differentiation	Adipogenic differentiation	Chondrogenic differentiation	Myogenic differentiation	Angiogenic differentiation
hAMSC (human Amniotic Mesenchymal Stromal Cells).	Positive: CD105, CD90, CD73 Negative: CD34, CD45, CD14, CD11, CD79_, CD19, HLA class II (22) Fetal origin (11)	Morphologic changes and calcium deposits showed with the von Kossa´s coloration (23,24,27)	Simple multi vacuolated adipocytic cells, small and large colonies stained with Red oil O dye (23)	Abundant extra-cellular matrix (Collagen) proved by the Alcinos´ blue coloration (23,26)	Myogenic transcription factor: Myo D and Myogenin and the protein desmin evidenced by immuno-cytochemical staining (23,26)	Structures similar to capillaries, receptor to VEGF-1, CEGF2, ICAM, cytoplasmic von Willebrand factor (23)

under standard culture conditions, towards the three basic lines demanded by the Committee of Stem Cells of Tissue and Mesenchymal of the International Society for the Cellular Therapy (Dominici et al., 2006): osteoblasts, adipocytes and chondroblasts (Table 2).

Osteoblastic differentiation has been evidenced in the first weeks of induction assays by morphologic changes and later by the formation of a mineralized matrix. The cells flattened and showed calcium deposits with the von Kossa's coloration (Steigman and Fauza, 2007).

Adipogenic differentiation was evidenced by the presence of simple multi-vacuolated adipocytic cells joined together in small and large colonies, whose size grew with the induction time. The aggregates of bigger size secreted drops of neutral lipids that stained intensively with the Red oil O dye (In't Anker et al., 2004).

Chondrogenic differentiation was demonstrated after 3 weeks of induction by the emergence of an abundant extra-cellular matrix, whose chondrogenic nature was proved by the Alcinos' blue coloration or by immunocytochemistry analysis to human collagen type II (Portmann-Lanz et al., 2006).

Myogenic differentiation has been evaluated by RT-PCR for the specific myogenic transcription factors: Myo D and Myogenin. Myo D appeared after seven days of culture, and Myogenin a week later. The skeletal protein desmin was detected on the third week of induction. This is in agreement with the fact that the specific cytoskeleton filaments were synthesized during late biogenesis. Positive desmin cells were evidenced by immunocytochemical staining (Portmann-Lanz et al., 2006).

The matrigel assays (semisolid medium) provided evidence of the hAMSC angiogenic differentiation potential when specific media were used. In this assay, morphologic changes were observed in the initial 4 hours, and structures similar to capillaries in 20 hours. In angiogenic cultures, when the media was supplemented with vascular endothelial growth factor (VEGF), the formation of structures similar to capillaries increased at 2, 4 and 20 hours of incubation, and the flow cytometry analysis revealed a boost in the expression of the receptors for vascular endothelial growth factor 1 and 2 (VEGF-1 and VEGF-2), as well as ICAM-1, and the appearance of CD34 positive hAMSC, which confirmed the endothelial differentiation of these cells. This was also observed by immunofluorescence which detected a clear granular cytoplasmic positivity for the von Willenbrand factor in comparison with the non treated cells (Alviano et al., 2007).

Other characteristics of the amniotic membrane

Other characteristics of the AM include low immunogenicity, anti-inflammatory, anti-fibrotic and anti-microbial properties. These pleiotropic functions are related in part to its capacity to synthesize and release biologically active substances, including cytokines and signalling molecules such as Tumor Necrosis Factor, Interferon, Transforming Growth Factor α (TGF α), Transforming Growth Factor ß (TGF ß), Basic Fibroblastic Growth Factor (bFGF), Epidermal Growth Factor (EGF), IL-4, IL-6, IL-8, natural inhibitors of Metallo-proteases, ß defensins, Prostaglandins, etc. (Li et al., 2005; Parolini et al., 2008).

Low immunogenicity

It has been shown that the cells of the fetal membranes have low immunogenicity and immuno-regulatory properties (Li et al., 2005). Thus AM has been used to treat skin ulcers, burns injuries, eye chronic ulcers, without producing rejection reactions, even though immune suppression therapy is not given. In vitro studies have demonstrated that cells isolated from amnion and chorion do not trigger immuno allogeneic or xenogenic responses, actively suppress the proliferation of T lymphocytes and inhibit the differentiation of monocytes. The human AM survive for long periods in immunocompetent animals and the cells are grafted persistently in various organs and tissues, observing human mycro chimerism, especially in BM, lung and thymus, after intraperitoneal o intravenous transplant in rat neonates (Akle et al., 1981; Changdong et al., 2007). These results are consistent with the AM stem cell immunonophenotype which expresses the non-classic and little polymorphic HLA-G molecules (class I b) and lacks the highly polymorphic antigens HLA A-B-C- (class I a), HLA DR (class II) and the T cell co-stimulatory molecule B-7 (Hunt et al., 2005).

The HLA-G molecule displays at least four inhibitory functions relevant to immune responses: 1) it can bind directly to inhibitory receptors found in NK cells and other leucocytes; 2) it possesses the appropriate leader peptide for binding to HLA-E, which will in turn inhibit the NK cells via their CD94/NKg2 receptor; 3) it can induce apoptosis of activated CD8+ T cell; and 4) it can inhibit CD4+ T cell proliferation (Hunt et al., 2005).

MSC may suppress immune reactions in vitro and in vivo in a major histocompatibility complex (MHC) independent manner (Rasmusson, 2006; Bifari el al., 2008). Interestingly, the immune regulatory properties of MSC do not appear to be related to the stemness, and are expressed not only by bone marrow MSC, but also by MSC derived from other tissues (Jones et al., 2007).

Recently, it has been shown that ex-vivo expanded MSC, isolated from placenta and from other tissues, have a direct immunosuppressive effect on T-cells from human peripheral blood (PB) and umbilical cord blood in vitro (UCB). MSC do not induce allogeneic PB lymphocyte proliferation and may inhibit mixed lymphocyte reaction (MLR). Expanded placental MSC have an inhibitory effect on UCB CD4+ and CD8+ proliferation triggered by allogenic PB lymphocytes or PHA. However, the way they modulate the immune system is unclear (Changdong et al., 2007).

This immunoregulatory feature strongly implies that hAMSC have a potential application in allograft transplantation. Since the placenta and UCB can be obtained from the same donor, AM is an attractive source of MSC for co-transplantation in conjunction with UCB-derived hematopoietic stem cells to reduce the potential of graft versus host disease in recipients (Changdong et al., 2007).

MSC have been proposed in the treatment of graft versus host disease (GVHD) after allogeneic hematopoietic stem cell transplantation, although the path to clinical efficacy is hindered by the limited understanding of how these cells work and how best to use their potential. It has been shown, using animal models, that MSC can treat GVHD only if administered in the presence of active acute disease and that this requirement is strictly related to the presence of IFN γ (interferon gamma) (Dazzi and Marelli-Berg, 2008).

Non tumorigenicity

The studies of tumorigenicity performed by injection of hAEC into the rear leg muscles and/or the testis of severe combined immunodeficiency (SCID) mice did not show any evidence of tumor formation after seven months of follow-up, whereas injections of the transformed cell line HepG2 developed tumors in approximately 2-3 weeks (Tamagawa et al., 2004). Akle et al. (1981) reported that there was no evidence of tumorigenicity when isolated amniotic cells were transplanted either into human volunteers to examine their immunogenicity, or into patients treated with amniotic cells in an attempt to correct lysosomal storage disease. Still, open questions are the tumorigenic potential of MSC infusion and the MSC-mediated support of developing cancers by preventing specific anti-cancer immune responses (Bifari el al., 2008).

Few ethical problems

Because the AM is discarded after parturition and it is easy to obtain without harming mothers or babies, the ethical issues associated with the use of embryonic stem cell would be overcome. However, the use of human amniotic membrane has to be approved by the Ethical Committee of each Institution and a written informed consent has to be obtained from the mothers.

Conclusion

The opportunity of having a fetal tissue rich in cells with stem cell characteristics and the capacity to differentiate towards the three germinal cells lines, with low immunogenicity, non tumorigenicity and non ethical barriers, make the AM an alternative source of stem cells particularly interesting in the context of cell based therapy applications such as transplantation and regenerative medicine.

Acknowledgements. This work was supported by grants FIS EC07/90762, RETICS RD/0010/2012 from the ISCIII, the Junction Program of Biomedical Research in Advanced Therapies and Regenerative Medicine among the ISCIII and FFIS 2007, and by the Fundación Séneca (grant 08859/PI/08).

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Acceepted June 8, 2009