Characterization of amnion-derived mesenchymal cells from human placenta

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Keywords: endothelial progenitor cells, mesenchymal cells, placenta, stem cells

Background:
Recent research in stem cell biology identified the fetal membranes of the human placenta as a new source of cells which show phenotypical similarities to bone marrow-derived mesenchymal progenitor cells. These findings raise hope for new applications in cell therapy due to the multipotent differentiation potential and immunomodulatory properties of these cells [1]. Even though amnionic membrane - human mesenchymal stromal cells (AM-hMSC) currently play an important role in stem cell research, their characterization is mostly limited to immunodetection by flow cytometry. Under *in vitro* conditions, these cells present a highly variable profile of cell surface antigens and the distinction to amnionic epithelial cells (AEC) remains unclear. Therefore, we performed morphological and immunohistochemical studies to compare the *in situ* with the *in vitro* situation.

Methods:
For the isolation of AM-hMSC, the amnion of human term placentas was separated from the chorion manually and treated with collagenase/DNase. Subsequently, the remaining tissue was incubated with trypsin/EDTA to obtain the AEC. Cells were cultured in DMEM under standard conditions and further characterized by phase-contrast microscopy and scanning electron microscopy. Both cultured cells and placental cryosections were examined immunohistochemically.

Results and discussion:
In situ, AM-hMSC are separated from AEC by an acellular compact layer (Fig. 1A). AM-hMSC cultures consist of larger cells with a fibroblastic morphology and smaller, more polygonal cells with an epithelial-like morphology (Fig. 1B, C). Ultrastructural examinations show cells with a varying number of microvilli at their surface and with many pseudopodial-like and filopodial contact zones to neighboring cells (Fig. 1D). AM-hMSC are not only positive for the mesenchymal marker vimentin, but some cells also express the epithelial marker cytokeratin *in situ* and *in vitro*. In addition, the expression of the embryonic stem cell markers Oct-4 and SSEA-4 indicates a multipotent differentiation potential.
AM-hMSC as well as AEC express CD54 (ICAM-1), CD90 (Thy-1), CD73 (ecto-5'-nucleotidase), and CD105 (endoglin) *in situ* and *in vitro,* common markers used to identify mesenchymal progenitor cells [1]. Apart from CD90, all of these markers are also expressed on endothelial cells. However, both cell types are negative for the endothelial marker von Willebrand factor (vWF). Interestingly, AM-hMSC but not AEC express vascular endothelial growth factor receptor-2 (VEGFR-2). The absence of vWF confirms the amnion to be an avasular tissue with no mature endothelial cells present. The expression of VEGFR-2 on AM-hMSC does not only distinguish them from epithelial cells, but also points to an endothelial

DOI: 10.3217/978-3-85125-062-6-306,

progenitor cell potential, as this receptor can also be found on vasculogenic and angiogenic precursor cells (hemangiogenic stem cells) [2].

Conclusion:
We conclude that AM-hMSC display a hybrid epithelial-mesenchymal phenotype with a multipotent differentiation potential. The expression of VEGFR-2 on AM-hMSC is a distinctive feature with regard to AEC and may indicate a superior appliance of AM-hMSC for the development of vascular grafts.

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3. The excellent technical assistance of Elisabeth Bock and Rudolf Schmied is gratefully acknowledged.

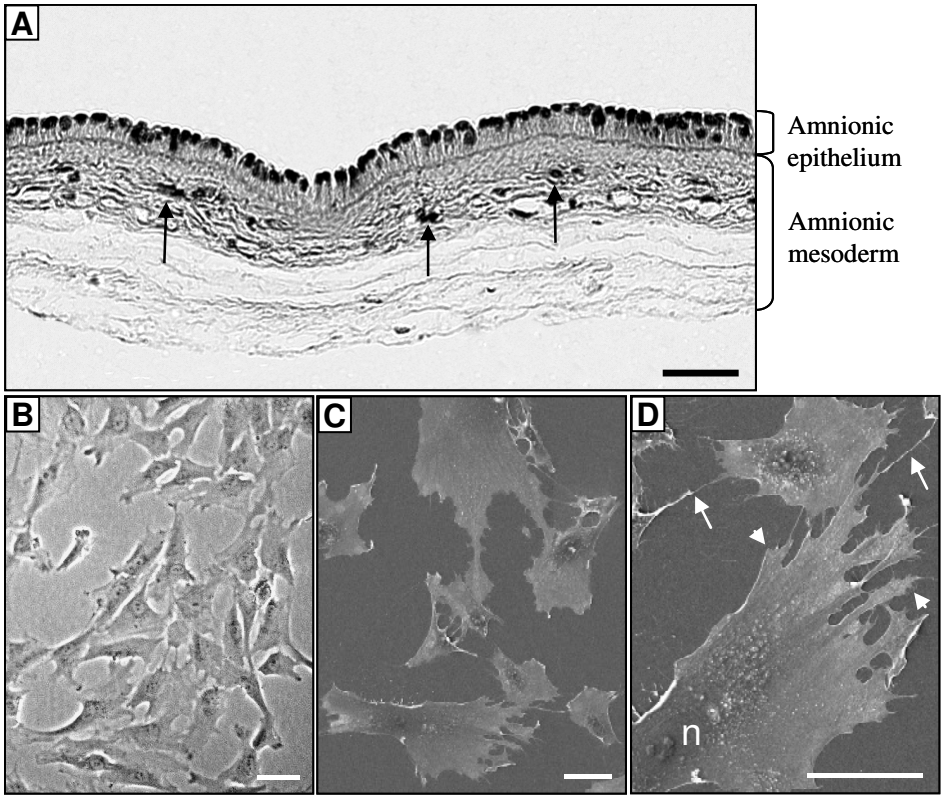

Figure 1.
(A) Typical features of placental amnion as seen in paraffin sections after H&E staining. The amnion epithelium covers the underlying amnionic mesenchyme, consisting of an acellular compact layer followed by mesenchymal cells (arrows) and connective tissue fibers embedded in stromal matrix.
(B) Phase-contrast micrograph and (C, D) scanning electron micrographs show the mesenchymal phenotype of isolated AM-hMSC: The cells have a smooth surface with a varying density of microvilli and grow in loose arrangements. They are connected by thin cytoplasmic projections (arrows) and filament-rich pseudopodial-like extensions (arrowheads). The central zone contains the nucleus (n) and most of the organelles. The peripheral margins are characterized by low cytoplasmic density. Scale bar: 50µm.

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