The human amniotic membrane's metabolic landscape

Banerjee A[1,2#], Weidinger A[1,2#], Lindenmair A[1,2], Hennerbichler S[2,4], Eibl J[3], Kozlov AV[1,2], Redl H[1,2], Wolbank S[1,2] # equal contribution

1 Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, AUVA Research Center, Donaueschingenstraße 13, 1200 Vienna, Austria

2 Austrian Cluster for Tissue Regeneration, Austria; 3 Bio-Products & Bio-Engineering AG, Schottenring 10, 1010 Vienna, Austria

4 Red Cross Blood Transfusion Service of Upper Austria, Krankenhausstraße 7, 4017 Linz, Austria

asmita.banerjee@trauma.lbg.ac.at, adelheid.weidinger@trauma.lbg.ac.at

Aims

The human amniotic membrane (hAM) is a rich source of stem cells. To exploit the regenerative capacity to a full extent, the hAM is considered to be applied in its vital form, containing vital cells with functional cell organelles. We investigated ATP concentrations and levels of reactive oxygen species (ROS) in two sub-regions of vital hAM, placental and reflected amnion.

Conclusion

Investigations of viable hAM demonstrated distinct metabolic differences of placental and reflected amnion. Taking advantage of this knowledge about sub-regional differences of the human amniotic membrane could allow a more customized clinical application tailored to specific pathological situations.

1 Topology of human amniotic membrane

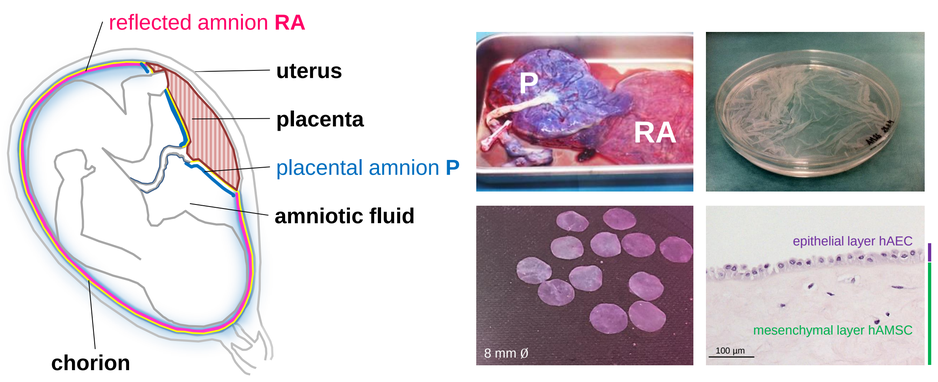

Figure 1. Placental amnion covers placenta, and reflected amnion covers the uterine wall. Amnion of the sub-regions were prepared separately.

2 Metabolic activity

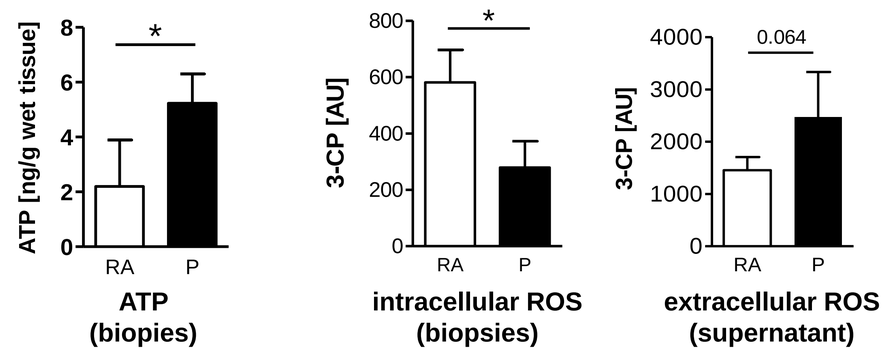

Figure 2. Despite higher adenosine triphosphate (ATP) concentrations in placental amnion, intracellular reactive oxygen species (ROS) levels were lower. (n=4, mean±SD)

3 Mitochondrial staining of epithelial and mesenchymal cells

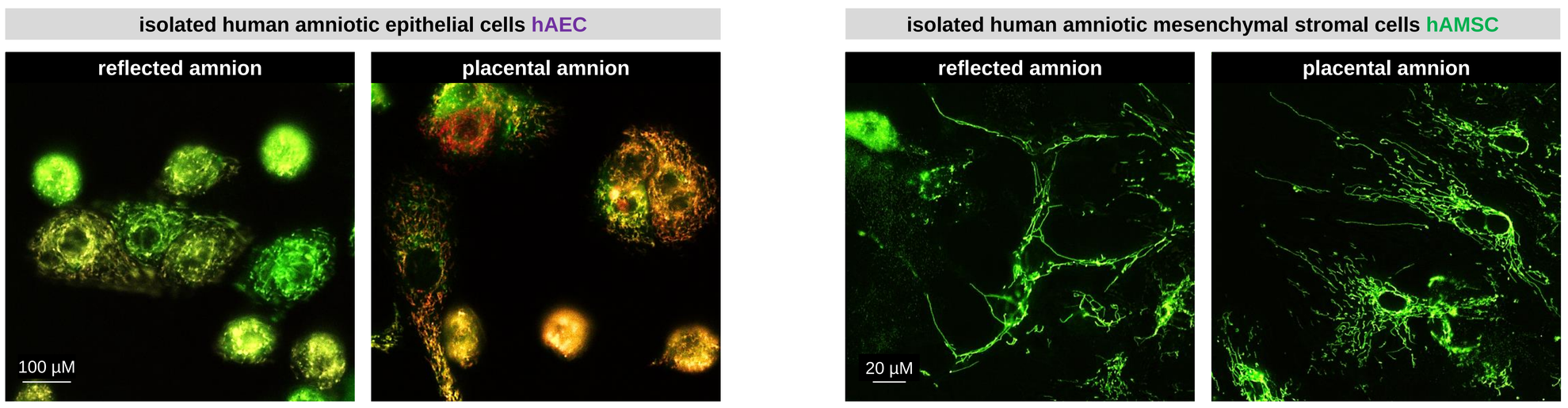

Figure 3. Different mitochondrial morphology and membrane potential in human amniotic epithelial cells (hAEC) and mesenchymal stromal cells (hAMSC), isolated from placental and reflected amnion. Mitochondria were stained with MitoTracker Green FM and TMRM. Merged confocal images.

Methods

ATP concentrations were assessed with Bioluminescence Assay Kit CLS II (Roche, Switzerland). Levels of ROS were measured with electron paramagnetic resonance spectrometry (Miniscope MS200, Magnettech, Germany). Mitochondria were stained with 250 nM MitoTracker Green FM (MTG; Molecular Probes, USA) and 500 nM tetramethylrhodamin-methylester (TMRM; VWR, USA) and observed with LSM510 (Carl Zeiss, Germany).

1 Banerjee *et al.* 2015

Results

In line with with our previous findings of higher mitochondrial respiration[1], we also found higher ATP concentrations in fresh placental amnion biopsies. Despite higher respiratory activity, we found lower levels of intracellular reactive oxygen species (ROS) in placental amnion, however, higher levels of extracellular ROS, suggesting different regulatory mechanisms in the placental and reflected amnion. Isolation of epithelial and mesenchymal cells show different mitochondrial morphologies.