Intact mitochondria migrate in membrane tubular network connections formed between human stem cells

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Introduction

The hypothesis of mitochondrial transfer between eukaryotic animal cells is intriguing, although its route of action and physiological role is unknown. Our goal was to examine intercellular connections among several types of stem cells (human amniotic, human mesenchymal, mouse mesenchymal) and to observe whether intact functional mitochondria may travel via these connections.

Methods: Human Amniotic Stem Cell Isolation Protocol

Placenta was obtained after delivery keeping at 4°C (saline with antibiotics), processed within 4 hours. Amniotic membrane was mechanically peeled off the chorion, minced with sterile tools during, washed in PBS several times (8-10X) to remove blood. To release AE cells the minced tissue was incubated in trypsin 1X (0.05%) for 20 minutes at 37°C, digestion was stopped with FCS, cells from the first digestion were discarded to exclude debris. Remaining tissue was digested tissue in trypsin 1X (0.05%) for 20 minutes at 37°C again and stopped with FCS. After passing cell suspension through a 100 μm cell strainer, the cells were fuged for 8 minutes at 1200 RPM (150-200g). Viability of the cells was determined by exclusion of trypan blue dye and counted with a hemocytometer. Cells are cultured in DMEM supplemented with 10% FCS, penicillin (100 U/ml), streptomycin (0.1 mg/ml), epidermal growth factor (10 ng/ml)
or in EBM-2 medium (CAMBREX).

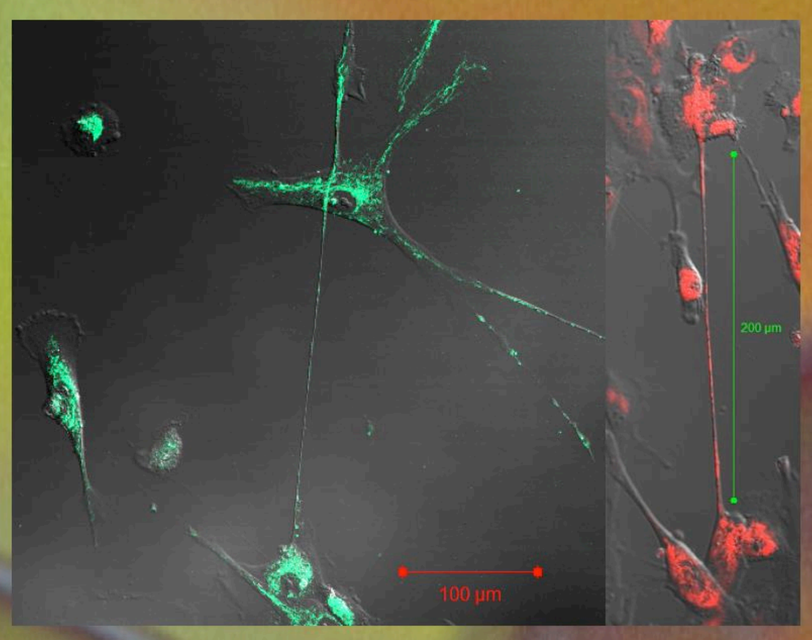

Figure 2 Mouse mesenchymal stem cell's long tubular structure using MitoTracker green and red staining (20x DIC objective).

Figure 5 Top: Human BM-derived mesenchymal stem cells can form long tubular connections with HAE cells after oxygene-glucose deprivation, OGD, 1 hour. (MitoTracker Red and Hoechst). Bottom: Human BM-derived mesenchymal stem(yellow, Vybrant DiD) cells can form long tubular connections with mouse H9C2 (green, Vybrant DiO) cardiomyocyte cells after OGD, 1 hour.

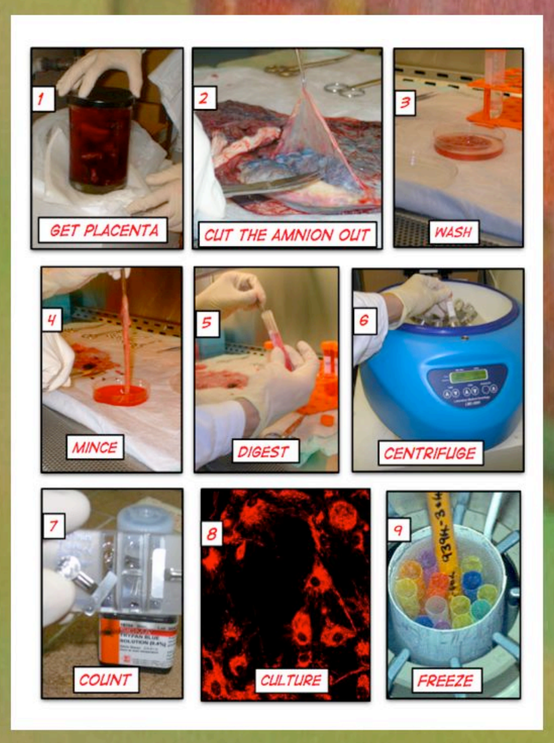

Figure 1 Isolation procedure of human epithelial amniotic cells

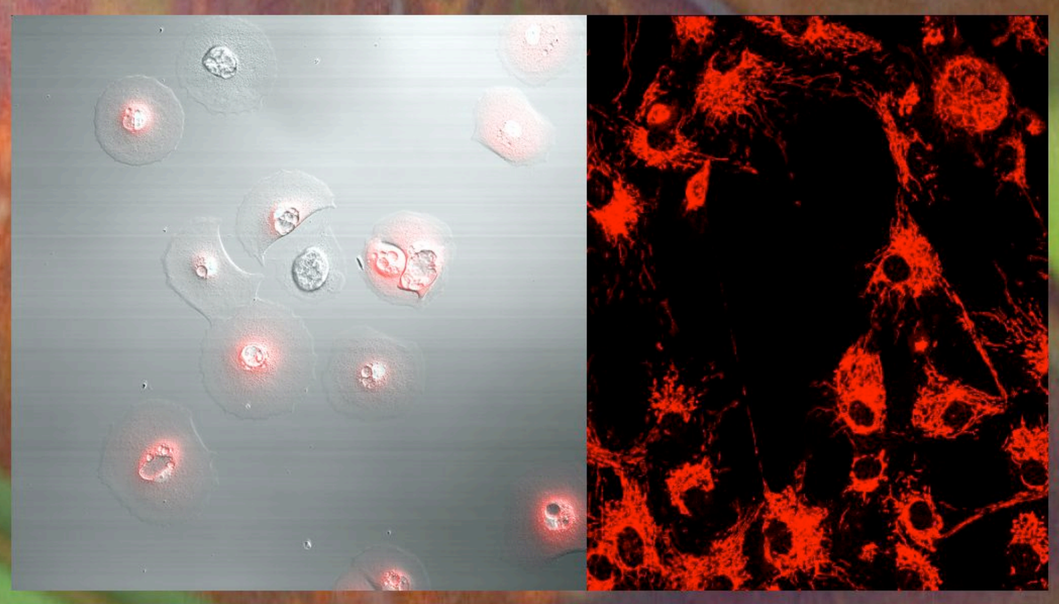

Figure 3 Freshly isolated human amniotic epithelial stem cells did not form long tubular connections (left), only after several passages (right) when the morphology of the cells had significantly altered. Left: HAE cells in passage 2 using MitoTracker red staining (20x objective). Right: Human amniotic stem cells (40x objective) after several passages of isolation form cell-to-cell connections via a tubular membrane network. Using MitoTracker red staining we observed that intact mitochondria are moving in these tubes by 20 – 60 nm/s velocity.

cell type	length (μm)
mMSC	170-280
HAE	160-220
hMSC	160-260

Figure 6 Length of membrane tubular connections between different stem cell types: mMSC and hMSC: bone marrow derived mouse and human mesenchymal stem cell, HAE: human amniotic epithelial cell.

Methods: Time-lapse Microscopy and visualization

Confocal laser scanning microscope system Zeiss LSM 510 Meta was used for time-lapse microscopy. For visualization of mitochondria and cells we labelled the mitochondria of the cells with MitoTracker red or green (Molecular Probes, 580, 490nm) while the nuclei were stained with Hoechst (340nm). Vibrant DiO (488nm) and DiD (633nm) fluorescent membrane stains were also used to visualize cells.

Methods: Oxygen glucose deprivation (OGD)

In order to find out what could be the direction of the mitochondria transfer between cells in a different redox state, an oxidative stress model is needed. Target cells were subjected to oxygen glucose deprivation (OGD). The OGD was 2 hours long in glucose free DMEM medium and in atmosphere of 0.5% O_2 and 99.5% N_2, with a 30-minute reperfusion time, when hypothetical „rescuer" stem cells were added.

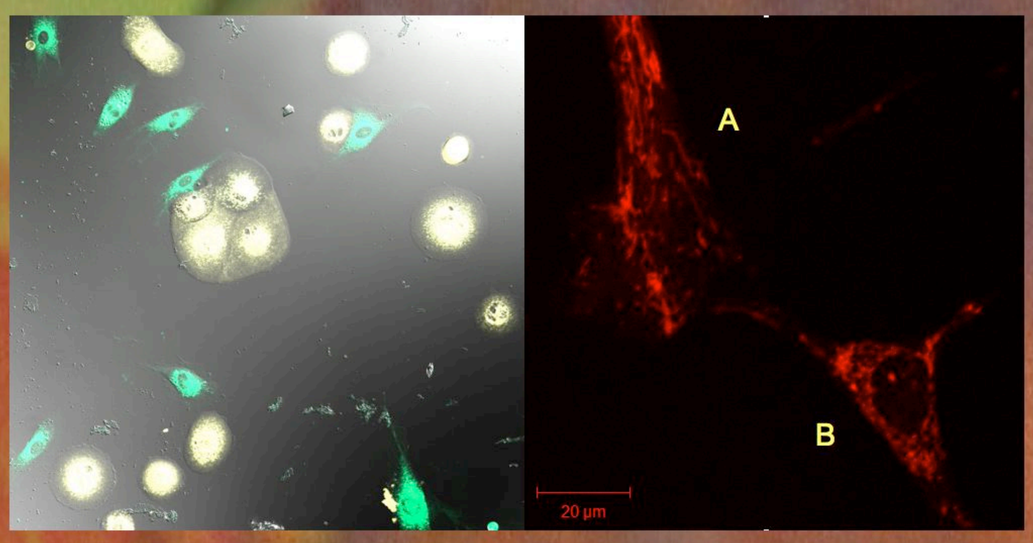

Figure 4 Left: Passage 2 early HAE (yellow, Vybrant DiD) cells did not form long tubular connections with mouse H9C2 (green, Vybrant DiO) cardiomyocyte cells after oxygene-glucose deprivation, OGD, 1 hour. Right: Tubular connection between a heart muscle cell (A) after oxidative stress, OGD, 1 hour, and a healthy human amniotic stem cell (B). Mitochondria flux in the tube towards the heart cell.

Conclusion

Human amnion-derived stem cells as well as bone marrow derived mouse and human mesenchymal stem cells form cell-to-cell connections via a tubular membrane network. These tubular connections can form between different types of cells (stem-stem, stem-differentiated), too. The maximal length of these micrometer-thick tubes is around 280 μm and the lifespan of the tubes can be several hours. Interestingly, freshly isolated amniotic epithelial stem cells did not form these connections, only after several passages when the morphology of the cells had significantly altered. Large area cell-cell contacts can be retained as long thin membrane bridges after the cells separate and *de novo* tube formation is also observed with a 60-100 nm/s growth rate. Using MitoTracker red staining we observed that intact mitochondria are moving in these tubes by 20 – 60 nm/s velocity.

Discussion

Mitochondria can leave one cell via the membrane tubes and can enter into another cell, however, when isolated mitochondria were added to the cell culture the free organelles did not enter into the cells. These results suggest that specific types of stem cells form comprehensive tubular networks among each other. One physiological role of these networks may be that mitochondria can migrate from one cell to the other, which may be a novel way of communication among stem cells.