Introduction

- Human fetal membranes (amnion) are the innermost layer of the uterine cavity that protect the fetus and function as a physical barrier.
- Fetal membrane cell shedding, gap formation, and rescaling of these gaps are associated with tissue remodeling during pregnancy to maintain structural and functional integrity.
- Cellular senescence at term or in response to pathological risk factors during pregnancy can accelerate cell shedding or reduce tissue remodeling capacity, predisposing fetal membranes to be vulnerable to dysfunction or rupture.
- The fate of shed cells is unclear; however, these cells are often seen trafficking through microfractures that develop in the membranes. Microfractures are sites of tissue remodeling during normal pregnancy; however, at term and preterm, their increased number and morphometry (width and depth) likely function as channels for cell and/or amniotic fluid leak.
- The destination of shed senescent membrane cells is unclear, and it is likely that increased shedding and accumulation on the maternal side may indicate a (patho)biological condition of the fetal membranes.

Hypothesis

We hypothesize that shed amnionchorion membrane cells can reach the maternal side either through tissue layers (microfractures) or through feto-maternal circulation, and these cells can be identified in maternal blood to serve as a marker for fetal membrane physiology and function.

Objective

The objective of this study is to detect sloughed amnionchorion cells in maternal blood samples as an indicator of membrane senescence and weakening at term.

Methods

Identification of fetal membrane cell markers: Fetal membrane cells collected from normal term (not in labor) deliveries were subjected to RNAseq analysis. Markers in these cells that were differentially expressed compared to maternal blood samples were collected in Strech tubes and processed within 4 hours after collection. The gender of the fetus was determined by real-time PCR of free fetal DNA using Y-chromosome-specific genes.

Whole blood fixation and red blood cell lysis: Blood samples were fixed within 15 minutes of drawing in 2% formaldehyde in PBS. After lysing red blood cells, nucleated cells were harvested using methods reported previously.1–4

Enrichment of fetal membrane cells by Magnetic-Activated Cell Sorting (MACS): Enrichment of fetal membrane cells was performed using 9 different primary antibodies targeting 8 different markers in the fetal membrane cells, mixed together according to the manufacturer’s basic protocols with slight modifications. Markers used were proprietary of ARCEDI and UTMB and, hence, will not be revealed. The cell suspension was incubated with the antibodies for 30 minutes, washed twice with 14 mL Magnetic Activated Cell Sorting (MACS) buffer (4°C), recovered by centrifugation, and resuspended in MACS buffer.

Staining of cells inside MS columns: Enriched fetal membrane cell candidates were subsequently stained by a cocktail of cytokeratin antibodies. The cell suspension was added to a prewashed MS column. To wash the column, MACS buffer was added to the column. The MS column was then removed from the magnet and placed on a precoated 15-mL collection tube, and the cells were eluted by applying PBS to the column. The collection tube was centrifuged, and the cell pellet was resuspended in PBS.

Identification of cytokeratin-stained cells: Amniochorion cell stained with the anti-cytokeratin antibody cocktail were identified by automatic scanning using the MetaCyte scanning system developed by Metasystems. Slides were scanned at 10x magnification using a classifier developed and optimized in house for detection of cytokeratin stained cells. After scanning, cells identified by the scanner were inspected visually by automated relocation. The fetal origin of the candidate cells was confirmed by fluorescence in situ hybridization (FISH) using probes for X and Y chromosomes.

Results

- The methodologic approach of isolating fetal membrane cells from maternal blood samples is shown in Figure 1.
- Thirty-six amnion/chorion cells were enriched by MACS and characterized by staining with anti-cytokeratin antibodies from 3 blood samples from pregnant women carrying male fetuses at term (Figure 2A). Fetal sex was confirmed by FISH (Figure 2B).
- FISH was carried out with X- and Y-chromosome-specific probes. Results from the fetal membrane cell enrichment are presented in the table.
- Eight different markers chosen based on RNAseq analysis were used to enrich fetal membrane cells from maternal blood. Immunofluorescence staining of these markers was performed to localize them in fetal membrane cells. A representative staining pattern of amnion epithelial cells is shown in Figure 3 and chorion cells in Figure 4.

Identification of membrane specific markers in human primary amnion epithelial cells (AEC).

Immunostaining shows AEC co-express epithelial marker cytokeratin-18 and mesenchymal marker vimentin. While ‘membrane’ markers are seen in the cell and nucleus membrane and cytoplasm.

A) Fluorescent microscopy shows AEC express all three membrane specific markers in green and (B) an additional five membrane markers in red. Fluorescent images were captured at 20x. This figure shows one representative image from three separate experiments.

Identification of membrane specific markers in human primary chorion trophoblast (CTC).

Immunostaining shows CTC dominantly express epithelial marker cytokeratin-18 (green) but not mesenchymal marker vimentin. While ‘membrane’ markers are seen in red. Fluorescent microscopy shows CTC express all eight membrane specific markers. Fluorescent images were captured at 20x. Blue—DAPI, red—chorion membrane marker of interest, yellow—vimentin, and green—Cytokeratin-18. This figure shows one representative image from three separate experiments.

Conclusions

- Our prior reports have shown amnionchorion cell shedding during gestation and its increase at term due to cellular senescence.
- Herein we demonstrate that senescent fetal membrane shed at term can traffic from the fetal side to the maternal circulation and can be isolated using minimally invasive sampling approaches.
- Detection of these cells in early gestational periods may indicate fetal membranes’ health and serve as a biomarker for predicting conditions like preterm prelabor rupture of the membranes.

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