Introduction

Chronic thromboembolic pulmonary hypertension (CTEPH) is a pulmonary vascular disease caused by chronic obstruction of pulmonary arteries. Pulmonary endarterectomy (PEA) is the golden standard therapy for CTEPH patients. During PEA surgery obstructive thromboembolic material from the pulmonary arteries is removed. This material offers a unique opportunity to study CTEPH at disease side. Endothelial cells line the entire vascular system and are essential for maintaining the vascular homeostasis. Remodeling of pulmonary arteries through proliferation of endothelial cells plays a major role in the pathogenesis of pulmonary hypertension. Recent findings bring forward metabolic plasticity and mitochondrial dynamics as an interesting target for tackling CTEPH.

Aim

We aim to develop an in vitro model of CTEPH using patient derived endothelial cell (EC-CTEPH) lines after PEA and assay changes in metabolism to elucidate a phenotype that could explain vascular changes occurring in CTEPH.

Methods

Freshly obstructive thromboembolic material obtained after PEA from CTEPH patients were used for cell isolation. These PEA samples were minced into 1-2 mm pieces and cultured in 0.25% gelatin coated 6-well plates in EGM-2 endothelial medium (Lonza) at 37°C, 5% CO₂, and 95% relative humidity. Colonies appeared within 1-3 weeks of culture.

Results

Cell growth and migration

Figure 2. EC-CTEPH showed a hyperproliferative phenotype (A-B) with significantly higher and durable viability assayed by MTT assay (C). HPNE were used as controls. N=12, data are expressed as mean ± SD, *p<0.05

EC-CTEPH showed reduced capacity to migrate

Figure 3. Images of wound-healing of HPNE and EC-CTEPH after 24 hours. Representative graph showing quantification of the % of covered area over time for both EC-CTEPH and HPNE. N=12, * p<0.05

EC-CTEPH cell metabolism

Higher LDH activity in cell pellet but no significant difference in HK activity

Figure 4. Glycolytic pathway is shown with the classical glycolysis pathway intermediates. In blue highlighted the enzymes that are investigated by colorimetric assays and RT-PCR. In all graphs EC-CTEPH are compared with HPNE. Enzyme activity is expressed as mL/mg cell normalized to protein concentration. Gene expression is shown as 2^-ΔΔCT. C-CTEPH, glucose transporter-1; HK, Hexokinase; PFKFB3, 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3; LDH, lactate dehydrogenase.

Conclusion

1. Isolated EC-CTEPH cells were confirmed as being endothelial cells.
2. EC-CTEPH showed a hyperproliferative phenotype.
3. Functionally, EC-CTEPH had a reduced capacity to migrate.
4. Alterations in metabolism are being investigated. LDH activity was significantly higher in EC-CTEPH. To date, difference in gene expression was non-significant.

Future work

We believe that improved insights of the metabolism of EC-CTEPH are of great importance to better understand the disease and unravelling the metabolic profile of EC-CTEPH offers a new perspective in the search for new pharmacological interventions.

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