Derivation and characterisation of endothelial cells from patients with chronic thromboembolic pulmonary hypertension

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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, including intima and superficial media. 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.

Aim

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

Methods

Freshly obstructive thromboembolic material obtained after PEA from CTEPH patients is used for cell isolation. These PEA samples are minced into 1-2 mm pieces and cultured in 0.2% gelatine coated 6-well plates in EGM-2 endothelial medium (Lonza) at 37°C, 5% CO_2 and 95% relative humidity. Colonies appeared within 1-3 weeks of culture.

Figure 1. Material obtained after PEA from CTEPH patients

- Phenotypic characterisation: immunohistochemistry and flow cytometry quantified expression of endothelial markers.
- Cell growth kinetics: cells were plated at a concentration of $3x10^4$ in 24-well gelatine coated plates in 1mL of EGM-2 media. At each subsequent passage, cells were counted and plated again at the same cell concentration. Cell migration was measured by wound-healing assay and expressed as the % of covered area.
- Metabolic changes were examined using RT-PCR.

Results

Phenotypic characterisation by immunohistochemistry

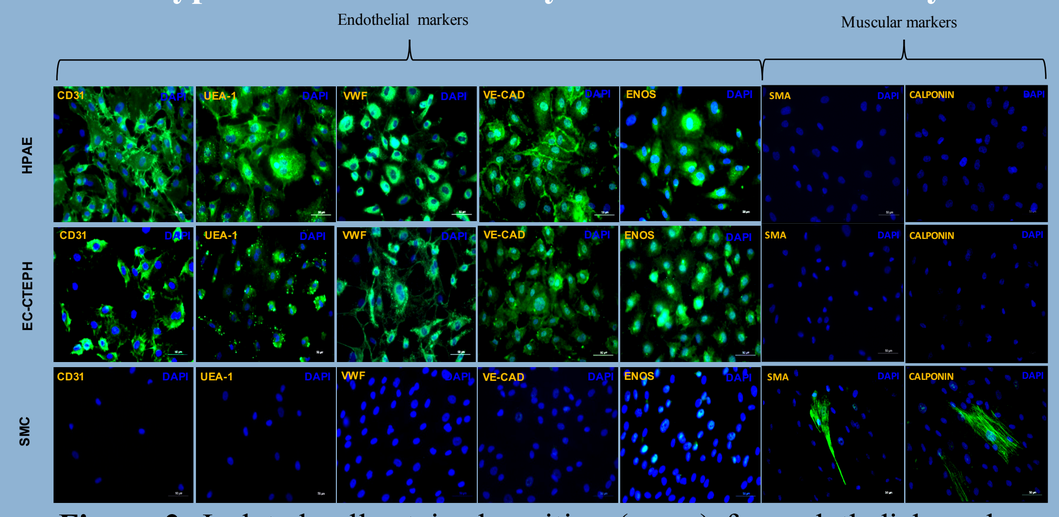

Figure 2. Isolated cells stained positive (green) for endothelial markers and negative for muscular markers. Nuclei were stained by DAPI (blue). AF488 Goat Anti-mouse fluorophore-conjugated antibody was used. HPAE (human pulmonary arterial endothelial cells); SMC (smooth muscle cells).

Phenotypic characterisation by flow cytometry

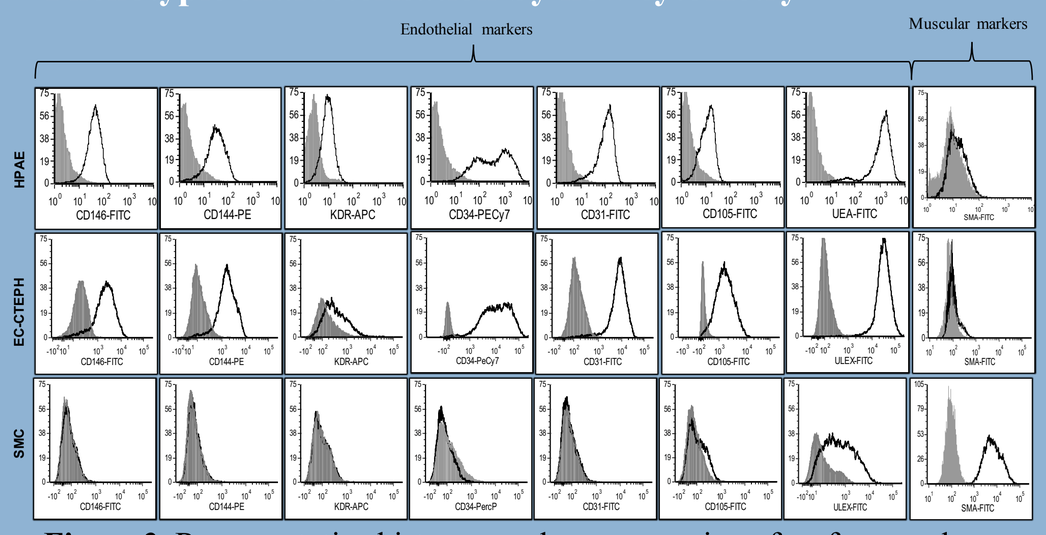

Figure 3. Representative histograms show expression of surface markers associated with endothelial cells and muscular cells on HPAE, SMC and CTEPH. EC-CTEPH were positive for all endothelial markers and negative for muscular markers.

Cell growth and migration

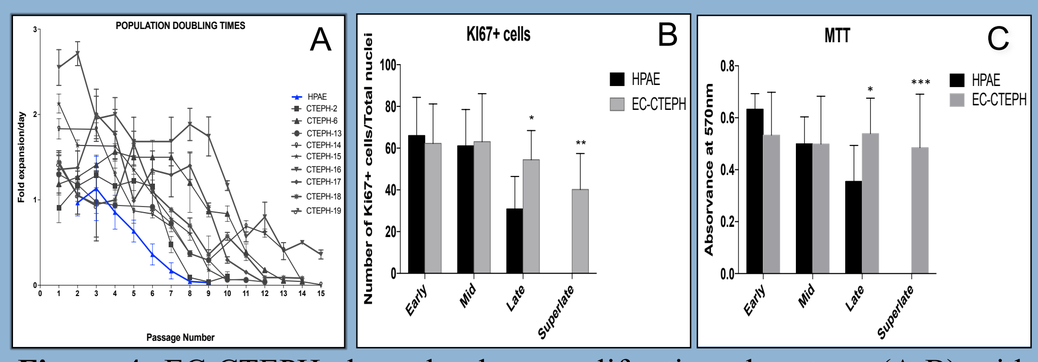

Figure 4. EC-CTEPH showed a hyperproliferative phenotype (A-B) with significantly higher and durable viability assayed by MTT assay (C). HPAE were used as controls. N=10, data are expressed as mean ± SD. * $p<0.05$

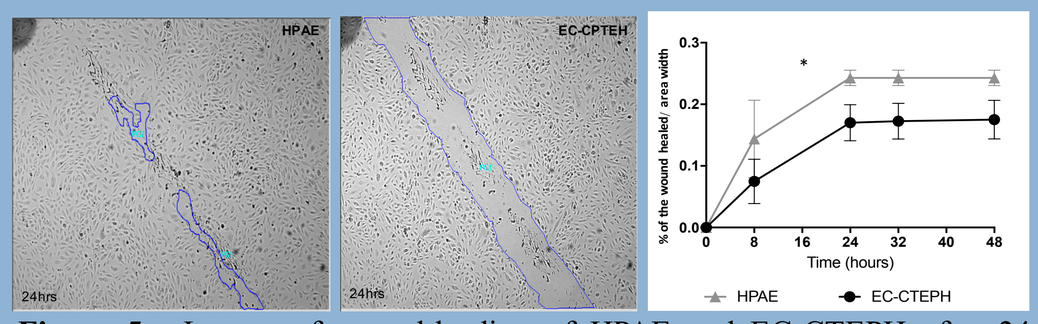

Figure 5. Images of wound-healing of HPAE and EC-CTEPH after 24 hours. Representative graph showing quantification of the % of covered area over time for both EC-CTEPH and HPAE. N=10, * $p<0.05$.

Metabolism

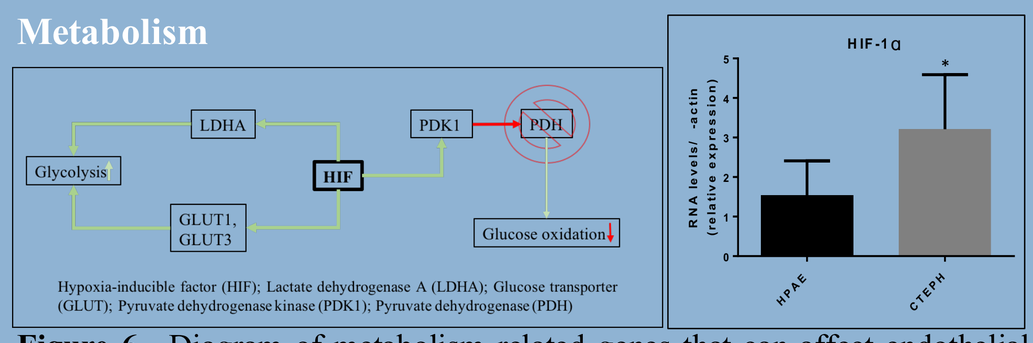

Figure 6. Diagram of metabolism related genes that can affect endothelial cell metabolism towards glycolysis. HIF-1α is higher expressed in CTEPH patient cells assayed by RT-PCR. * $p<0.05$

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Conclusion

1. Isolated CTEPH cells are confirmed as being endothelial cells.
2. EC-CTEPH show a hyperproliferative phenotype.
3. Functionally, EC-CTEPH have a reduced capacity to migrate.
4. EC-CTEPH show alterations in metabolism towards anaerobic glycolysis.