CITED2 regulates trophoblast glycoprotein expression in the adventitial pericyte and migration

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**Introduction**
Hyoxia induces migration in adventitial pericytes (APCs), conferring them with therapeutic potential towards the acquisition of a proangiogenic pericyte-like phenotype. Hypoxic activation of HIF1alpha can regulate epithelial-to-mesenchymal transitions (EMT), including the mesenchymal-to-pericyte transition. TPBG is an EMT marker which is associated with migration. CITED2 regulates the HIF1alpha hypoxia-induced genes, mainly under hypoxic conditions. We aim to demonstrate the role of TPBG on hypoxic-dependent migration of APCs and to identify the mechanism underpinning TPBG transcription.

**Methods & Results:** CD34/NG2 positive APCs were isolated from saphenous vein leftovers (from CABG). APCs were conditioned for 24, 48 and 72h under normoxia or hypoxia (2% O2). Hyoxia induces the mRNA expression of classic EMT markers (SNAIL1/2, ZEB2, TIMP1/2) and TPBG (1.97 ± 0.27 mRNA fold change after vs. normoxia; 2.63 ± 0.62 protein fold change vs. normoxia, both p<0.05). TPBG loss-of-function (LOF) by siRNA or gain-of-function (GOF) by TPBG overexpression were used to validate the role of TPBG in migration. LOF resulted in reduced scratch assay migration (0.67±0.03, p<0.01), whilst GOF resulted in increased migration (5.40±0.60, p<0.01). CITED2 was found progressively down-regulated after 48h of hypoxic conditioning at the transcript level but not at the protein level (1.41 ± 0.07-fold change after 48h of hypoxia vs. normoxia, p <0.05). CITED2/TPBG interaction was further studied by over-expressing CITED2. ChIP analysis evidenced the binding of CITED2 to TPBG at the TSS -1985/-1836 and +482/+555 under normoxia, and that was considerably enhanced by hypoxia (both p <0.05). LOF experiments were performed in hypoxic APCs by silencing CITED2 (70nM). A pool of 4 scrambled siRNAs was used as control in parallel. Knocking-down effect was validated by qPCR in 4 APC lines showing 0.30 ± 0.13-fold change expression of the CITED2 transcript, p<0.001 (qPCR, 2-DDCT method). That was confirmed by ICC showing a nuclear location with a modest but significant reduction of immunoreactive CITED2. CITED2 LOF was associated with a significant down-regulation of TPBG at protein levels after 48 or 72h depending on the cell line and by an overall 0.78 ± 0.10, p= 0.02 compared to siRNA control. Wound healing experiments additionally showed that silencing of CITED2 was associated with a lower migration profile in the APC (0.70 ±0.03, p<0.01).

**Conclusion**
TPBG plays a crucial role in APC migration. CITED2 significantly affects TPBG expression in hypoxic APCs and thus hypoxia-induced migration in APCs. Discordant protein and transcript levels suggest a tight regulation of the CITED2 stability, pinpointing an important role of CITED2 in the APC. These findings might be relevant for regenerative medicine applications due to the association among APC migration and TPBG expression.

**Conflict of interest**
N/A