Title: The hypoxia-induced miR-130 increases proliferation of pulmonary arterial smooth muscle cells by targeting the tumour suppressor CDKN1A (p21)

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Body: Background. Vascular remodelling is one of the hallmark features in the pathogenesis of pulmonary hypertension (PH) and is mainly driven by overshooting proliferation of pulmonary arterial smooth muscle cells (PASMCs). We have shown previously that the microRNA (miRNA) family miR-130 (i.e. miR-130a and miR-301a) is upregulated in the murine model of hypoxia-induced PH. Here we addressed the effects of miR-130 on the proliferation of PASMCs in vitro and identified novel targets of miR-130. Methods. For inhibition of the miR-130 family LNA-modified seed blockers were designed. BrdU proliferation assay was employed in PASMCs transfected with miRNA precursor molecules or LNA seed blockers. Expression of cyclin-dependent kinase inhibitor (CDKN)1A was measured on RNA and on protein level. Reporter gene assay was used to proof direct miRNA-target interaction. Results. Proliferation of PASMCs was significantly increased by overexpression of miR-130a and miR-301a (by 37.9% and 62.4%, respectively) and decreased by transfection of seed blockers (reduction of 65.4%). Inhibition of the miR-130 family increased the expression of the tumour suppressor CDKN1a (by 55.7% on mRNA level, and by 72.2% on protein level). Reporter gene assays showed that miR-130 family directly targets CDKN1A. Conclusion. Our results show that the miR-130 family triggers proliferation of PASMCs probably mediated through repression of CDKN1A. Since levels of the miR-130 family are induced by hypoxia in vivo, these data emphasize the potential role of miR-130 in the process of vascular remodelling in PH.