Notch 3 receptor is localized and activated from an intracellular compartment in embryonic lung vascular smooth muscle cells

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Background: Notch receptor signaling plays a fundamental role in development by regulating cell fates. In canonical Notch signaling, the receptor is activated on the cell surface after engagement with ligands expressed on homotypic or heterotypic cells. Recently in *Drosophila*, however, intracellular activation of Notch receptor has been demonstrated to occur in ligand dependent and independent mechanisms. Notch 3 signaling is implicated in arterial vascular smooth muscle cell (VSMCs) proliferation, differentiation, and cell identity, and may play an important role in the pathogenesis of pulmonary hypertension. Here we investigated the expression and activation dynamics of the Notch 3 receptor during pulmonary artery development.

Methods and Results: Immunohistochemical studies showed that the Notch 3 receptor is expressed exclusively in lung vascular, but not bronchial smooth muscle cells. Flow cytometry analysis indicated that Notch 3 is localized solely in the cytoplasm of VSMCs, and not on the cell surface. Confocal microscopy confirmed this finding and revealed that Notch3 is localized within endosomes. To determine the dynamics of Notch 3 signaling in lung VSMCs, the activated cleaved form of the Notch 3 receptor (NICD) and the induction of Notch 3 down-stream target genes HRT-1 and HRT-2 were determined by immunohistochemistry (IHC) and real time PCR (qPRC), respectively. These data show that Notch 3 signaling is active at E18.5 and continues to around post-natal day 5. These findings raise the possibility that Notch 3 receptor activation occurs in the interior of VSMCs. To determine the possible Notch 3 receptor ligand in lung VSMCs, qPCR and IHC was performed. We found that Jagged 1 is the predominant Notch ligand expressed in embryonic VSMCs. Confocal microscopy demonstrated that Jagged 1 colocalizes with the Notch 3 receptor inside the cell, and immunoprecipitation followed by western blot analysis confirmed a physical association between Jagged 1 and Notch 3 receptor. To validate the ligand-dependent activation of Notch 3 in lung VSMCs, Jagged 1 was selectively knocked-out (KO) in smooth muscle cells. In Jagged 1 KO mice, a down regulation of the Notch 3 down-stream target genes HRT-1 and HRT-2 was observed, consistent with suppression in Notch 3 signaling in VSMCs.

Conclusion: This report demonstrates for the first time an intracellular ligand dependent Notch receptor activation in a mammalian cell. We speculate that this signaling system plays an important role in the maturation of the pulmonary artery.