Basic Science Category

BMPR2 Silencing Activates ERK1/2-AP1 Signaling Through Upstream Effects of RAF

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**Background:** Pulmonary arterial hypertension (PAH) is a rare disease characterized by a pro-proliferative endothelial cell phenotype, vascular inflammation and pulmonary vessel remodeling culminating in right ventricular failure and ultimately death. Mutations in bone morphogenetic protein type II receptor (BMPR2), a member of the TGF-β superfamily, have been identified in the majority of patients with heritable PAH. Loss of normal BMPR2 function has been implicated in the abnormal endothelial cell growth and the formation of plexiform lesions characteristic of advanced PAH. We hypothesized that BMPR2 deficiency could alter the interactions of cytoskeletal scaffolding proteins and the RAF-ERK1/2-AP1 signaling pathway, leading to its dysregulated activation.

**Results:** BMPR2 siRNA gene silencing in pulmonary artery endothelial cells (PAEC) significantly activated ERK1/2 signaling. Microarray analysis found that BMPR2 knockdown (KD) suppressed Sprouty1, an inhibitor of ERK1/2 signaling and induced RAF-1, an upstream activator of ERK1/2. Western blotting confirmed that BMPR2 KD increased the expression of all three RAF family members, RAF-1, A-RAF and B-RAF. In addition to total protein, phosphorylated RAF, an activating event, was increased by BMPR2 silencing across all three family members. Inhibitors of MEK1/2 and RAF proteins reduced the activation of ERK1/2 and the expression of FRA1, an AP1 family member and target of ERK1/2 signaling. Notably, scaffolding proteins are known to regulate the intensity, amplitude, and spatial specificity of ERK1/2 signaling. Receptor for activated C-kinase (RACK1) has been shown to interact with BMPR2 and co-immunoprecipitation confirmed the interaction between BMPR2 and RACK1 and among RACK1, A-RAF and B-RAF proteins in PAECs.

**Conclusions:** Activation of the RAF-ERK1/2-AP1 signaling pathway by BMPR2 silencing is reinforced by downstream induction and suppression of regulatory molecules. In addition, BMPR2 directly interacts with upstream partners of RAF-ERK1/2 and its loss may alter their function. Disordered RAF-ERK1/2-AP1 signaling may contribute to the vascular remodeling characteristic of PAH.