Gain-of-Function Alk2 Mutation Enhances Chondrocyte Differentiation and Promotes Heterotopic Endochondral Ossification

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ALK2 (activin receptor-like kinase 2) is an evolutionarily conserved type I bone morphogenetic protein (BMP) receptor that responds to exogenous ligand to mediate downstream BMP signaling. Gain-of-function mutations in ALK2 cause the rare genetic disorder fibrodysplasia ossificans progressiva (FOP), characterized by progressive heterotopic (extra-skeletal) endochondral ossification. We hypothesized that the mildly activating FOP patient mutation in Alk2 (R206H) predisposes or accelerates chondrogenic differentiation of progenitor cells and induces heterotopic endochondral ossification within soft connective tissues. To test this hypothesis, mouse embryonic fibroblasts (MEFs) were harvested from wild-type and knock-in $Alk2^{R206H/+}$ embryos as a progenitor cell model. $Alk2^{R206H/+}$ MEFs showed increased canonical BMP signaling as detected by phosphorylation of Smads1/5/8 and increased expression of BMP responsive genes in both the absence and presence of BMP ligand. Under chondrogenic culture conditions, the addition of BMP ligand increased the sensitivity of $Alk2^{R206H/+}$ MEFs toward chondrogenesis. Consistent with these results, $Alk2^{R206H/+}$ MEFs showed earlier appearance of chondrocyte morphology and accelerated onset with increased abundance of early chondrogenic transcripts during BMP-induced differentiation. We determined that Alk2 mRNA is most abundant in undifferentiated MEFs and decreased upon differentiation, suggesting important roles during early differentiation. Loss of Alk2 prior to chondrogenic culture severely inhibited differentiation of MEFs. MEFs implants in hind-limb muscle of wild-type mice demonstrated that $Alk2^{R206H/+}$ MEFs promote heterotopic endochondral ossification *in vivo*. Our data show that heterozygous expression of Alk2 (R206H) in progenitor cells enhances chondrogenic differentiation *in vitro*, promotes heterotopic ossification *in vivo*, and supports early chondrogenic differentiation as an important therapeutic target for preventing heterotopic bone formation in FOP patients.

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