

SMAD, FAK AND ERK1/2 ACTIVATION DURING BMP-6-INDUCED OSTEOGENESIS AND CHONDROGENESIS OF AMCS

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Introduction Adipose-derived mesenchymal cells (AMCs) are a promising cell source for regeneration of bone and cartilage. However, their clinical application requires successful strategies for osteo- and chondrogenesis as well as a detailed understanding of the pathways responsible for commitment to a particular lineage. BMP-6 elicits a strong chondrogenic effect in AMCs, but the osteogenic effect has not been studied. Using a single medium, we examined the role of BMP-6 and culture conditions on the osteo- and chondrogenic differentiation of AMCs and evaluated subsequent activation of the SMAD, FAK and ERK1/2 pathways.

Methods AMCs were isolated from 25-30d mice in accordance with the Notre Dame Animal Care and Use Committee approved protocol and expanded in growth medium (DMEM, 10% FBS and 1% pen-strep). Cells were seeded in monolayer at 1300 cells/cm² or pelleted by centrifugation at 200,000 cells/well in polypropylene round-bottom 96-well plates. AMCs in both culture conditions were differentiated in growth medium supplemented with 100 µg/mL ascorbic acid, 10 mM β-glycerophosphate and 0 or 100 ng/mL BMP-6. To assess osteogenesis, alkaline phosphatase and mineralization were quantified at 7d and 14d, respectively. To evaluate chondrogenesis, proteoglycan accumulation was measured at 12d via sGAG assay and frozen sections were stained with Alcian blue. Gene expression of *Runx2*, *Ocn*, *Sox-9* and *Agc* was assessed at 7d via quantitative real-time PCR from both conditions and compared. Protein expression of SMAD, FAK and MAPK pathways was assessed via western blot.

Results BMP-6 induced bone-like mineralization in AMCs in monolayer while enhancing cartilage matrix deposition in AMCs in pellet (Fig 1). BMP-6 upregulated *Runx2* and *Ocn* in monolayer and *Agc* in pellet, however, *Sox-9* was equally upregulated in both conditions. Canonical BMP-6 signaling via SMAD1/5 was observed in both culture conditions (Fig 2). FAK and ERK1/2 pathways were activated only in monolayer and expression was not mediated by BMP-6 in either condition (Fig 2).

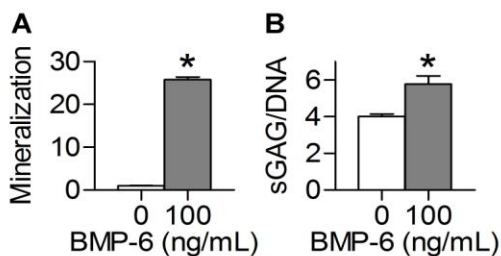


Figure 1. BMP-6 enhances osteogenesis and chondrogenesis in AMCs. AMCs were seeded in monolayer (M) or pellet (P) and cultured with 0 or 100 ng/mL BMP-6. A) Mineralization in monolayer. B) Proteoglycan accumulation in pellet.* indicates difference from control, $p < 0.05$.

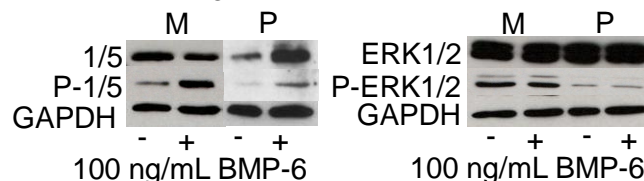


Figure 2. Activation of SMAD1/5 and ERK1/2. AMCs were seeded in monolayer (M) or pellet (P) and differentiated with 0 or 100 ng/mL BMP-6. Activation was detected by phosphorylation (P). Equal loading confirmed by GAPDH.

Conclusion Our data indicate that BMP-6 is both osteogenic and chondrogenic in AMCs and that this action is specific to culture conditions. Because BMP-6 signals via the canonical pathway in both monolayer and pellet culture, cellular interpretation of the SMAD signal may be modified by cues from the culture conditions to drive commitment to a particular lineage. FAK and ERK1/2 pathways are both modulated by culture conditions; future studies will examine the role of these and other signaling pathways in BMP-6-induced osteo- and chondrogenic differentiation.