Transforming growth factor β1 inhibits bone morphogenic protein (BMP)-2 and BMP-7 signaling via upregulation of Ski-related novel protein N (SnoN): possible mechanism for the failure of BMP therapy?

Bone morphogenic proteins (BMPs) play a key role in bone formation. Consequently, it was expected that topical application of recombinant human (rh)BMP-2 and rhBMP-7 would improve the healing of complex fractures. However, up to 36% of fracture patients do not respond to this therapy. There are hints that a systemic increase in transforming growth factor β1 (TGFβ1) interferes with beneficial BMP effects. Therefore, in the present work we investigated the influence of rhTGFβ1 on rhBMP signaling in primary human osteoblasts, with the aim of more specifically delineating the underlying regulatory mechanisms. BMP signaling was detected by adenoviral Smad-binding-element-reporter assays. Gene expression was determined by reverse transcription polymerase chain reaction (RT-PCR) and confirmed at the protein level by western blot. Histone deacetylase (HDAC) activity was determined using a test kit. Data sets were compared by one-way analysis of variance. Our findings showed that Smad1/5/8-mediated rhBMP-2 and rhBMP-7 signaling is completely blocked by rhTGFβ1. We then investigated expression levels of genes involved in BMP signaling and regulation (for example, Smad1/5/8, TGFβ receptors type I and II, noggin, sclerostin, BMP and activin receptor...
membrane bound inhibitor (BAMBI), v-ski sarcoma viral oncogene homolog (Ski), Ski-related novel protein N (SnoN) and Smad ubiquitination regulatory factors (Smurfs)) and confirmed the expression of regulated genes at the protein level. Smad7 and SnoN were significantly induced by rhTGFβ1 treatment while expression of Smad1, Smad6, TGFβRII and activin receptor-like kinase 1 (Alk1) was reduced. Elevated SnoN expression was accompanied by increased HDAC activity. Addition of an HDAC inhibitor, namely valproic acid, fully abolished the inhibitory effect of rhTGFβ1 on rhBMP-2 and rhBMP-7 signaling. rhTGFβ1 effectively blocks rhBMP signaling in osteoblasts. As possible mechanism, we postulate an induction of SnoN that increases HDAC activity and thereby reduces the expression of factors required for efficient BMP signaling. Thus, inhibition of HDAC activity may support bone healing during rhBMP therapy in patients with elevated TGFβ serum levels.