BMP-2-induced osteoblast differentiation: Critical molecular switches

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Intramuscular injection of BMP-2 induces ectopic bone formation in vivo. Similarly, BMP-2 treatment blocks myogenic differentiation and induces osteoblastic transdifferentiation of premyoblastic C2C12 cells. Previous reports suggested that BMP-2-stimulated Runx2 expression could play a pivotal role in the transdifferentiation. However, increased Runx2 expression by TGF-β1 didn’t support osteoblast differentiation in vitro, and Runx2 overexpression didn’t stimulate osteocalcin expression normally associated with BMP stimulation. These results indicate that the induction of Runx2 is not sufficient to explain the BMP-induced transdifferentiation. We found that Dlx5 is specifically expressed in osteogenic cells, and is specifically induced by BMP-2 or -4 signaling but not by other osteotrophic signals. Moreover, opposing effect of TGF-β1 on BMP-2-induced osteogenic transdifferentiation was related with Dlx5 suppression. Cycloheximide treatment indicated that Dlx5 was immediately induced by BMP signaling while Runx2 and Osterix required de novo protein synthesis. Blocking or overexpressing each transcription factor indicated that Runx2 and Osterix are downstream targets of Dlx5 in BMP-2-induced osteoblast differentiation but their expression is regulated independently by Dlx5. These results indicate that Dlx5 is a key mediator of BMP-2-induced osteoblast differentiation which triggers osteogenic master genes, Runx2 and Osterix, and ultimately results in the bone marker gene expression in BMP-induced osteogenic transdifferentiation.

Next question was addressed to molecular mechanisms by which Dlx5 upregulates Runx2 expression in BMP-2 signaling. Two major isoforms of the Runx2 gene are expressed by alternative promoter usage: the Runx2-type I (Runx2-I) is derived from the proximal promoter (P2) and the Runx2-type II (Runx2-II) is produced by the distal promoter (P1). Runx2-II expression was found to be specifically stimulated by BMP-2 treatment or by Dlx5 overexpression. In addition, BMP-2, Dlx5, and Runx2-II expression clearly co-localize in osteogenic fronts and parietal bones of the developing cranial vault while Runx2-I and Msx2 are expressed in the sutural mesenchyme. Furthermore, Runx2-P1 promoter activity is strongly stimulated by Dlx5 overexpression while Runx2-P2 activity is not. Runx2-P1 promoter deletion analysis indicates that the Dlx5-specific response is due to sequences between -756 and -342 of the P1 promoter, where three Dlx5 response elements are located. Dlx5 responsiveness to these elements was confirmed by gel mobility shift assay, chromatin immunoprecipitation and site-directed mutagenesis. Moreover, Msx2 specifically suppresses the Runx2-P1 promoter, and the responsible region overlaps with that recognized by Dlx5. In sum, Dlx5 specifically transactivates the Runx2-P1 promoter, and its action on the P1 promoter is antagonized by Msx2.