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Notch signaling regulates osteogenic differentiation through different roles played by different Notch molecular components

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Osteogenic differentiation process consists of integration of several signaling pathways such as TGF-β, BMP, Wnt/β-catenin (1,2) and Notch which orchestrate to regulate differentiation of mesenchymal stem cells. To date, the role of Notch signaling pathway, which is known to regulate cell fate decision and differentiation in several cell types is still poorly understood in osteogenic differentiation, as controversial results have been described. Notch signaling is initiated by binding a Notch ligand (JAGGED and Delta-Like) to a cell surface Notch receptor (NOTCH 1–4), resulting in a multistep cleavage of receptor and releasing Notch intracellular domain (NICD) which translocates to the nucleus and activates transcription of downstream Notch target genes, such as the Hes/Hey family (3). The aim of this study was to investigate the involvement of Notch signaling in the osteogenic differentiation. To this purpose, we stimulated human immature osteoblast cell line (MG-63) with osteogenic medium for 28 days, in the absence and in the presence of DAPT, a gamma-secretase inhibitor used to block Notch signaling. At different days during differentiation, we investigated biochemical osteogenic markers such as Alkaline phosphatase (ALP) activity, osteocalcin (OC) levels, mineralization as well as gene expression of osteogenic transcription factors including Runx2, DLX5, Osterix and the gene Collagen I (CollI). Further, the gene expression of Notch receptors (Notch1-4), their ligands (Jagged1 and DLL4) and nuclear target genes (Hey1, Hey2, hes1, Hes5) was evaluated. As expected, our results showed that during osteogenic differentiation of MG63, osteogenic markers and transcription factors were upregulated. In addition, Notch2, Notch4 and Hey1 were overexpressed in different stages of differentiation. Differently, Notch1, Notch3 and Hes5 were down-regulated. No differences were observed about Notch ligands. Treatment of cells with DAPT, impaired osteogenic differentiation by reducing the main osteogenic markers such as ALP, OC, Coll I, , Runx2, DLX5 and Osterix as well as Hey1 nuclear gene. These results appear to confirm a significant role of Notch signaling during osteogenic differentiation by the activation of the canonical pathway with the main involvement of Notch 2 and Notch 4 receptors and Hey1 as nuclear target gene. In clinics these data may be relevant as they propose that Notch pathway can be used as a therapeutic target during bone defects and pathologies.

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Keywords

Osteogenic differentiation; Notch signaling; MG63.

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