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Cloning and characterization of inhibitor of DNA binding 3 (ID3) promoter

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Transforming growth factor beta (TGF-β) signaling is involved in many physiological and pathological events, such as modulating immune system, regulating cell function and carcinogenesis. In macrophages TGF-β induced set of genes involved in lipid metabolism and immune response regulation. One of the strongest induced genes was Id3 which expression is known to be upregulated by bone morphogenetic proteins. The aim of current study was to investigate the mechanisms of TGF-β-dependent upregulation of Id3 mRNA expression.

First, the promoter region of Id3 gene was clone and the minimal promoter was located to as little as 155 bp upstream of transcription start site. This 155 bp comprised TATA box and Sp1 binding site. Luciferase reporter assay showed that deletion of Sp1 binding site results in a 50% reduction of the promoter activity indicating the importance of Sp1 binding site for Id3 promoter activity. Deletion of TATA-box almost completely abolished Id3 promoter activity.

To understand the signaling pathways involved in TGF-β regulated Id3 expression, human hepatocellular liver carcinoma cell line HepG2 cells were transfected with reporter plasmid containing Id3 promoter and plasmids containing, Smad-2, -3, and -4. Luciferase assay showed that the activity of Id3 promoter is regulated by TGF-β in a Smad2/3-independent manner.

Furthermore, the expression of Id3 mRNA was tested in several macrophage-like cell lines with or without TGF-β-stimulation. It was found that TGF-β-dependent Id3 expression may be observed only in primary monocyte derived macrophages but not in macrophage-like cell lines 28SC, MonoMac-6, U937 and THP-1.