Characterization of the human *E2F4* promoter region and its response to 12-*O*-tetradecanoylphorbol-13-acetate

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The E2F transcription factors (TFs), which control the progression of the cell cycle in response to DNA damage and various stresses, are known to interact with a tumour suppressor, Retinoblastoma 1 (RB1). The response of the human *RB1* promoter to a 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in HL-60 cells is mediated by a duplicated GGAA motif, which is also present in the 50-upstream of the *E2F* family genes. The motifs are especially rich in the 50-upstream of the *E2F4* gene. In the present study, we constructed luciferase expression vectors containing a 466 bp of the 50-upstream of the human *E2F4* gene. The transfection of this plasmid and deletion/mutation-introduced derivatives into HL-60 cells and a Luc reporter assay showed that duplicated and triplicated GGAA (TTCC) motifs in the *E2F4* promoter respond to TPA. As expected, electrophoretic mobility shift assay indicated that SPI1 (PU.1) binds to the GGAA motif-containing element. A quantitative RT-PCR and western blotting showed that the *E2F4* transcripts and its encoding proteins accumulate during the differentiation of HL-60 into macrophage-like cells. In contrast, the expression of the *E2F1* gene and the protein, which possibly acts as a cell cycle accelerator, was greatly diminished.