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CRITICAL ROLE OF SMAD AND AP-1 COMPLEXES IN TGF-β-DEPENDENT APOPTOSIS

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INTRODUCTION. Transforming growth factor-β1 (TGF-β1) induces not only cell growth inhibition but also apoptosis in hepatocytes, myeloid cells, and epithelial cells. Smad complexes (Smad2-Smad4 and Smad3-Smad4) are identified as key signaling molecules which transmit TGF-β1 signal for growth inhibition from the TGF-β receptors to the nucleus (1, 2). However, their roles are unclear in the induction of apoptosis. Our results show here that both Smad and AP-1 complexes play a critical role in TGF-β1 signaling for apoptosis.

METHOD. Apoptosis was quantified by a photometric enzyme-immunoassay measuring the presence of cytoplasmic histone-associated DNA-fragments (mono- and oligonucleosomes) as a result of apoptosis. Nuclear extracts were prepared from TGF-β1-stimulated cells and an electrophoretic mobility shift assay was performed using a radiolabeled complementary oligonucleotides containing the consensus AP-1 binding site, TPA-responsive gene promoter element (TRE) as a probe.

RESULTS. Overexpression of a dominant-negative Smad3 mutant or inhibitory Smad7, both of which impair Smad-mediated signal transduction, inhibited TGF-β1-dependent apoptosis. Only the AP-1 complex consisting of JunD and FosB proteins (JunD-FosB) was markedly activated during TGF-β1-dependent apoptosis. FosB substantially enhanced Smad3-Smad4-dependent transcription, and dominant-negative FosB blocked TGF-β1-dependent apoptosis but not growth inhibition. Overexpression of JunD-FosB significantly enhanced induction of apoptosis by TGF-β1. Moreover, JunD-FosB bound to the AP-1 binding site, TRE and recruited Smad3-Smad4 to form a multi-component complex.
DISCUSSION. Although Smad proteins are suggested to cooperate with the AP-1 complex to regulate transcription of target genes, involvement of the AP-1 complex has not been demonstrated in cell growth inhibition or apoptosis induced by TGF-β1. We show here that not only Smad but also AP-1 complexes actually participate in TGF-β1 signaling for apoptosis. Moreover, our present results suggest synergistic cooperation between Smad and AP-1 complexes in TGF-β1-dependent apoptosis, but not growth inhibition. Smad proteins may positively or negatively modify transcription of target genes through cooperation with their DNA-binding partners to exert diverse biological activities.

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REFERENCES.