Caspase-mediated Cdk2 activation is a critical step to execute transforming growth factor-β1-induced apoptosis in human gastric cancer cells

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Background Although TGF-β1, an inhibitor of cell growth, is known to also induce apoptosis in a variety of cell types, the molecular mechanism of this apoptosis is largely undefined.

Methods To determine both the cell cycle distribution and apoptosis in SNU-16 cells, FACS analyses were performed by staining with the various reagents including PI, TUNEL, Annexin V, and BrdU. The changes of cell cycle regulatory proteins by TGF-β1 were determined using Western blot analyses in SNU-16 cells and kinase activities of cyclin-dependent kinases were also examined during TGF-β1-induced apoptosis. The associations between Cdk2 and Cdk inhibitors were determined by immunoprecipitations.

Results We identify the mechanism of TGF-β1-induced apoptosis in SNU-16 human gastric cancer cells. Cell cycle and TUNEL analysis showed that, upon TGF-β1 treatment, cells were initially arrested at the G1 phase and then driven into apoptosis. Of note, caspase-3 was activated in accordance with TGF-β1-induced G1 arrest. Activated caspase-3 is targeted to cleave p21<sup>cop1</sup>, p27<sup>cop1</sup>, and Rb, which play important roles in TGF-β1-induced G1 arrest, into inactive fragments. Subsequently, Cdk2 was aberrantly activated due to the cleavage of p21 and p27. We found that the inhibition of Cdk2 activity efficiently blocks TGF-β1-induced apoptosis, whereas it did not prevent caspase-3 activation or the subsequent cleavage of target proteins. In contrast, the suppression of caspase-3 activity inhibited the cleavage of target proteins, the activation of Cdk2, and the induction of apoptosis. Conclusion Our results suggest that activation of caspase-3 by TGF-β1 may initiate the conversion from G1 cell cycle arrest to apoptosis via the cleavage of p21, p27 and Rb, which in turn causes Cdk2 activation and, most significantly, Cdk2 activation as a downstream effector of caspase is a critical step for the execution of TGF-β1-induced apoptosis.