Cloning of zebrafish BAD, a BH3-only proapoptotic protein, whose overexpression leads to apoptosis in COS-1 cells and zebrafish embryos

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摘要.

Abstract

The BH3-only proapoptotic protein, BAD, was cloned from zebrafish embryos and its properties were characterized. Zebrafish BAD (zBAD) is a protein with 147 amino acids that contains a BH3 domain and a putative 14-3-3 binding site with the sequence ofRPRSRS(84)AP, corresponding to S(136) in mouse BAD (mBAD). zBAD shares 34%, 28%, and 29% amino acid sequence identity to the human, mouse, and rat BAD, respectively. RT-PCR analysis revealed that the expression of zBAD gene is found in various partsof zebrafish tissues. The treatment with the z-VAD fmk, a broad-range caspase inhibitor, in COS-1 cells significantly increased the expression of zebrafish BAD fusion proteins (GFP-zBAD and HA-zBAD), indicating that zebrafish BAD fusion proteins maybe cleaved by caspase(s). zBAD was shown to induce apoptosis when it was overexpressed in COS-1 cells. In addition, zBAD was also expressed in muscle cells under the muscle-specific promoter from zebrafish alpha-actin gene. Abnormality in the skeletal muscles and the loss of green fluorescence signal in the same region were observed. Taken together, our results indicate that zBAD could induce apoptosis in vitro and in vivo and may have biological implications in apoptosis during zebrafish development.