DOI:10.1158/0008-5472.CAN-04-3502

Research Article

Predominant Bcl-XL Knockdown Disables Antiapoptotic Mechanisms: Tumor Necrosis Factor–Related Apoptosis-Inducing Ligand–Based Triple Chemotherapy Overcomes Chemoresistance in Pancreatic Cancer Cells *In vitro*

Jirong Bai,[1] Jianhua Sui,[2] Aram Demirjian,[1] Charles M. Vollmer Jr.,[1] Wayne Marasco,[2] and Mark P. Callery[1]

[1]Department of Surgery, Beth Israel Deaconess Medical Center and [2]Department of Cancer Immunology and AIDS, Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts

Abstract

Pancreatic cancer is lethal because of its invasiveness, rapid progression, and profound resistance to chemotherapy and radiation therapy. To identify the molecular mechanisms underlying this, we have examined the expression and potency of three major death receptors: tumor necrosis factor receptor (TNF-R), TNF-related apoptosis-inducing ligand receptor (TRAIL-R), and Fas in mediating cytotoxicity in four invasive pancreatic cancer cell lines. We have analyzed the expression of major antiapoptotic factors, cell cycle regulators and death receptor decoys (DcR) in comparison with normal pancreas tissues and five other human malignant tumor cell lines. We have found that different pancreatic cancer cell lines coexpress high-level TRAIL-R, Fas, and TNF-R1 but are strongly resistant to apoptosis triggered by the death receptors. DcR2 and DcR3 overexpression may partly contribute to the resistance of pancreatic cancer cells to TRAIL-R– and Fas-mediated cytotoxicity. Bcl-XL and Bcl-2 are predominantly overexpressed in pancreatic cancer cell lines, respectively. Bcl-XL is also predominantly overexpressed in prostate, colorectal, and intestinal cancer cells. The knockdown of the predominant Bcl-XL overexpression significantly reduces the viability of pancreatic cancer cells to TNFα- and TRAIL-mediated apoptosis by sublethal-dose single and combined antitumor drugs, including geldanamycin, PS-341, Trichostatin A, and doxorubicine. Geldanamyin and PS-341 synergistic

suicide program that is highly conserved among all species. This regulated cell death process is primarily mediated by three different types of death receptors: tumor necrosis factor receptor (TNF-R), TNF-related apoptosis-inducing ligand receptor (TRAIL-R), and Fas and plays a critical role in removing unwanted cells during embryogenesis, immune responses, and tissue homeostasis (4). These natural surveillance mechanisms against tumorigenesis are severely compromised in pancreatic cancer.

RelA/p50, a heterodimeric component of NFκB transcription factors (5, 6), is constitutively activated in the majority of pancreatic cancer cell lines and tumors but not in normal pancreatic tissues (7). The activation of NFκB deregulates the biological functions of its downstream antiapoptotic factors (8), including Bcl-2 and Bcl-XL (9–12), inhibitors of apoptotic proteins (IAP; refs. 13–15), Fas-associated phosphatase-1 (Fap-1; ref. 16), and cFLIP (8, 13), as well as death receptor decoys (DcR; refs. 17, 18). These factors cooperatively and negatively regulate apoptotic pathways in tumor cells by blocking proapoptotic functions and suppressing activation of caspase cascades. Bcl-XL overexpression is detected in pancreatic cancer tissues (19) and is linked to abnormal NFκB signaling (11). However, its role in pancreatic cancer chemoresistance has not been established.

In this study, we hypothesize that down-regulation of a predominantly overexpressed antiapoptotic factor could disable antiapoptotic mechanisms in pancreatic cancer cells. We have asked whether death receptor–based apoptotic pathways remain potent in pancreatic cancer cells. Which antiapoptotic factors are