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Targeted therapy against Bcl-2-related proteins in breast cancer cells

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Received: 2 Feb 2005 Revisions requested: 3 Mar 2005 Revisions received: 25 Aug 2005 Accepted: 31 Aug 2005 Published: 28 Sep 2005

Breast Cancer Research 2005, **7**:R940-R952 (DOI 10.1186/bcr1323)
This article is online at: http://breast-cancer-research.com/content/7/6/R940

Abstract

Introduction Bcl-2 and Bcl-xL confer resistance to apoptosis, thereby reducing the effectiveness of chemotherapy. We examined the relationship between the expression of Bcl-2 and Bcl-xL and chemosensitivity of breast cancer cells, with the aim of developing specific targeted therapy.

Methods Four human breast cancer cell lines were examined, and the effects of antisense (AS) *Bcl-2* and AS *Bcl-xL* phosphorothioate oligodeoxynucleotides (ODNs) on chemosensitivity were tested *in vitro* and *in vivo*. Chemosensitivity was evaluated by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) assay, and the antitumor effect was assessed *in vivo* by the success of xenograft transplantation into athymic mice.

Results Treatment with AS *Bcl-2* and *Bcl-xL* ODNs resulted in a sequence-specific decrease in protein expression, compared with controls. Treatment of BT-474, ZR-75-1, and MDA-MB-231 cells with AS *Bcl-2* increased chemosensitivity to doxorubicin (DOX), mitomycin C (MMC), paclitaxel (TXL), and docetaxel (TXT). Transfection of the *Bcl-2* gene into MDA-MB-453 cells decreased sensitivity to DOX and MMC. Treatment of MDA-MB-231, BT-474, and ZR-75-1 cells with AS *Bcl-xL* increased chemosensitivity to DOX, MMC and taxanes to a smaller extent than AS *Bcl-2*. This occurred in the setting of increased Bax and cleaved poly(ADP-ribose) polymerase, as well as decreased Bcl-2 and pAkt. AS *Bcl-2* ODNs induced splenomegaly in association with increased serum IL-12, which was attenuated by methylation of the CpG motifs of AS *Bcl-2*; however, methylated CpG failed to negate the increased antitumor effect of AS *Bcl-2*. Bcl-2 and Bcl-xL, to a smaller extent, are major determinants of chemosensitivity in breast cancer cells.

Conclusion Targeted therapy against Bcl-2 protein with the use of AS ODNs might enhance the effects of chemotherapy in patients with breast cancer.

Introduction

Bcl-2 and Bcl-xL proteins are inhibitors of the mitochondrial apoptosis pathway; they exert their action by blocking their proapoptotic counterparts, including Bid and Bax, thereby preventing the release of cytochrome *c* and the activation of caspase [1,2]. Bcl-xL shows remarkable homology to Bcl-2 and inhibits apoptosis as effectively as Bcl-2 in some cells. Furthermore, Bcl-xL is capable of preventing cell death when Bcl-2 fails to do so, suggesting that these proteins exert independent effects on the mitochondrial apoptotic pathway [3]. Given that Bcl-2 and Bcl-xL are capable of inhibiting anticancer drug-induced apoptosis, which is mediated by the voltage-dependent anion channel (VDAC) in the outer mitochondrial membrane, overexpression of Bcl-2 and Bcl-xL might confer resistance to chemotherapy [4]. In fact, overexpression of Bcl-2 and Bcl-xL is observed in several cancers, including hematologic malignancies, as well as a range of solid tumors, including nasopharyngeal, colorectal, prostate, and breast cancer [5-7].

Antisense oligodeoxynucleotides (AS ODNs) are short, synthetic stretches of DNA that hybridize with specific mRNA

ANOVA = analysis of variance; AS = antisense; CREB = cyclic-AMP-responsive element-binding protein; DMSO = dimethyl sulfoxide; DOX = doxorubicin; ER = estrogen receptor; IL = interleukin; LSD = least significant difference; MM = mismatch control; MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide; ODN = oligodeoxynucleotide; PARP = poly(ADP-ribose) polymerase; RC = random control; TXL = paclitaxel; TXT = docetaxel; VDAC = voltage-dependent anion channel.