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PRINCIPAL INVESTIGATOR: Xiao-kun Zhang, Ph.D.

CONTRACTING ORGANIZATION: The Burnham Institute
La Jolla, CA 92037

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**5. AUTHOR(S)**
Xiao-kun Zhang, Ph.D.

**6. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)**
The Burnham Institute
La Jolla, CA 92037

**7. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)**
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**14. ABSTRACT**
Most anticancer agents ultimately kill cancer cells primarily by inducing apoptosis, a programmed cell death. We propose that Bcl-2 undergoes a conformational change in response to chemotherapeutic agents in breast cancer cells in vitro and in vivo and that a Bcl-2-conformation-sensitive antibody can be used to better predict and evaluate the responses of breast cancer cells to therapies. We have characterized an epitope-specific anti-Bcl-2 antibody that specifically recognizes pro-apoptotic Bcl-2 conformation. By using this antibody, we show that a number of chemotherapeutic agents, including paclitaxel, retinoid-related molecules, and nonsteroidal anti-inflammatory drugs, induce breast cancer cell apoptosis by modulating Bcl-2 conformation. Induction of Bcl-2 conformational change involves Bcl-2 phosphorylation. Moreover, apoptosis induction of breast tumor grown in animal by a Nur77-derived peptide correlates with Bcl-2 conformational change. Together, we have developed a novel epitope-specific anti-Bcl-2 antibody that can be used to predict and evaluate the response of breast cancer cells to certain chemotherapeutic agents in vitro and in animal.

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# Table of Contents

- Cover .................................................................................................................1
- SF 298 ...............................................................................................................2
- Table of Contents .............................................................................................3
- Introduction .......................................................................................................4
- Body .....................................................................................................................4
- Key Research Accomplishments ......................................................................4
- Reportable Outcomes ........................................................................................12
- Conclusions .......................................................................................................13
- References ..........................................................................................................13
- Appendices .........................................................................................................14
Introduction

Recent studies have indicated that most anticancer agents ultimately kill cancer cells by inducing apoptosis, a programmed cell death (Reed, 1996). Overexpression of the anti-apoptotic protein Bcl-2 is implicated in breast cancer development, tumor progression and drug resistance. Paradoxically, the levels of Bcl-2 correlate with favorable outcomes following endocrine treatment in almost all the published reports or chemotherapy in some cases (Daidone et al., 1999). Recently, we reported that Bcl-2 manifests opposing biological activities, survival and death, depending on its protein conformation (Lin, 2004). Upon binding to Nur77 (also known as TR3 or NGFI-B), an orphan member of the nuclear receptor superfamily, Bcl-2 undergoes a conformational change that exposes its otherwise hidden BH3 domain, resulting in its conversion from a protector to a killer (Lin, 2004). We proposed a novel concept that Bcl-2 mediates the clinical responses (apoptosis) by changing its conformations. The purpose of this Concept application was to determine whether Bcl-2 underwent a conformational change in response to chemotherapeutic agents in breast cancer cells \textit{in vitro} and \textit{in vivo} and whether a Bcl-2-conformation-sensitive antibody could be used to better predict and evaluate the responses of breast cancer cells to therapies.

There are two objectives of this application: 1) To determine whether Bcl-2 undergoes a conformational change in breast cancer cell lines \textit{in vitro}; and 2) To determine whether Bcl-2 undergoes a conformational change in breast tumor formed in nude mice. With the funding from BCRP, we have successfully conducted the proposed experiments. A significant amount of exciting results that support our concept have been obtained. They are summarized below and are in the preparation for publications.

Key Research Accomplishments

1. An epitope-specific anti-Bcl-2 antibody can be used to detect pro-apoptotic Bcl-2 protein conformation

Our first objective was to determine whether Bcl-2 underwent a conformational change in breast cancer cell lines \textit{in vitro}. To this end, we have evaluated a number of chemotherapeutic agents in breast cancer cells for their apoptotic effect and induction of Bcl-2 conformational change. Based on our previous finding that Bcl-2 BH3 domain is exposed in the pro-apoptotic Bcl-2 conformation, we have conducted several experiments, including immunoprecipitation, immunostaining, and flow cytometry, to characterize an anti-Bcl-2 antibody raised against the Bcl-2 BH3 domain (\(\alpha\)Bcl-2 BH3 domain). Using Nur77/ADBD, a Nur77 mutant protein known to bind Bcl-2 and induce its conformational change (Lin, 2004), we found that the anti-Bcl-2 antibody was very sensitive in detecting pro-apoptotic Bcl-2 conformation by using several technologies, including flow cytometry, immunostaining, and immunoprecipitation (Figure 1). This is the first conformation-sensitive anti-Bcl-2 antibody reported, which detects the pro-apoptotic Bcl-2 conformation.
Figure 1. Epitope-specific anti-Bcl-2 antibody for detecting Bcl-2 conformational change.
a. Bcl-2 conformational change detected by flow cytometry. Bcl-2 was co-transfected into HEK293T cells with GFP or GFP-Nur77/ADBD expression vector for 14 hr. Cells were immunostained with a polyclonal antibody against the Bcl-2 BH3 domain (αBcl-2 BH3-domain), followed by SRPD-conjugated secondary antibody. Transfected (GFP-positive) cells were identified by flow cytometry. Bcl-2 fluorescence from the transfected cells (Green histogram) was compared to that from the non-transfected cells (purple histogram). Similarly fluorescence-positive cells were gated to compare Bcl-2 fluorescence on GFP or GFP-Nur77/ADBD co-expression. Numbers represent % of transfected cells showing Bcl-2 immunofluorescence compared to the auto-fluorescence of the non-transfected cells from the same transfection.

b. Detection of Bcl-2 conformational change by epitope-specific anti-Bcl-2 antibodies using immunostaining. HEK293T cells transfected with Nur77/ADBD were immunostained with αBcl-2-BH3 domain and visualized by fluorescence microscopy.

c. Detection of Bcl-2 conformational change by immunoprecipitation. HEK293T cells transfected with Nur77/ADBD were incubated with αBcl-2 BH3 domain antibody. Immunoprecipitates were subjected to immunoblotting using anti-Bcl-2 antibody (Santa Cruz). For comparison, cells extracts were also immunoprecipitated by two other anti-Bcl-2 antibodies: rabbit polyclonal antibody against the whole protein (αBcl-2), mouse monoclonal antibody against the Bcl-2 hydrophobic groove (αBcl-2 (BH3-pocket)) (BD Transduction Labs).

2. Bcl-2 conformation change as an indicator of apoptosis of breast cancer cells in vitro in response to paclitaxel and other chemotherapeutic agents

After characterizing epitope-specific anti-Bcl-2 antibody, we then used the antibody to evaluate a number of chemotherapeutic agents for their induction of Bcl-2 conformational change in breast cancer cells. Several compounds, including paclitaxel, 3-Cl-AHPC (a compound that potently induces apoptosis of hormone-dependent and independent breast cancer cells (Dawson et al., 2001)), and SDX-308 (an analog of nonsteroidal anti-inflammatory drug Etodolac, which selectively induces tumor cell apoptosis (Kolluri and Cottam, 2005), were found to induce Bcl-2 conformational change in breast cancer cells (Figure 2). These results demonstrate that immunostaining using the epitope-specific anti-Bcl-2 antibody to determine Bcl-2 conformational change is very simple and sensitive and may have clinical application.

![Chemotherapeutic agents induce a Bcl-2 conformational change](image)

Figure 2. Chemotherapeutic agents induce a Bcl-2 conformational change. MDA-MB231 breast cancer cells were treated with or without paclitaxel, 3-Cl-AHPC, SDX-308 overnight. Cells were immunostained with anti-Bcl-2 antibody (αBcl-2 BH3 domain) and visualized by fluorescence microscopy.

Our observation that paclitaxel induced a Bcl-2 conformational change is very interesting, since paclitaxel (Taxol), a naturally occurring antineoplastic agent, has shown great promise in the treatment of a variety of tumor types, including breast tumor (Esteva, 2002). Recent studies demonstrate that paclitaxel exerts its anti-cancer effects through induction of apoptosis and that phosphorylation of Bcl-2 by paclitaxel through its activation of Jun N-terminal kinase (JNK) is a potential cause of taxane-induced apoptosis (Ferlini et al., 2003; Haldar et al., 1995; Srivastava et al., 2005).
In vitro, paclitaxel treatment of tumor cell lines can result in the increased phosphorylation of Bcl-2, occurring mainly at Ser70, Ser87, and Thr69 in the Bcl-2 loop region (Srivastava et al., 1999; Yamamoto et al., 1999). In MDA-MB231 breast cancer cells, deletion of the loop region from Bcl-2 prevented paclitaxel-induced Bcl-2 phosphorylation and apoptosis (Srivastava et al., 1999). In addition, substitution of Ser70, Ser87, and Thr69 with alanine conferred more anti-apoptotic activity to Bcl-2 following treatment with paclitaxel (Srivastava et al., 1999). Intriguingly, several studies have also suggested that paclitaxel binds directly to Bcl-2. Screening of a random library of phage displayed peptide revealed that paclitaxel is able to bind a panel of peptides showing a high degree of homology with the Bcl-2 loop region. In addition, paclitaxel was able to dock to the loop region using a homology model for Bcl-2. These results suggest that both the level of Bcl-2 expression and its phosphorylation state may modulate the apoptotic response to paclitaxel and serve as prognostic indicators for responsiveness to paclitaxel. However, their value in predicting the survival of breast cancer patients remains controversial.

Because paclitaxel and its analogs are currently evaluated for treating breast cancer patients, we therefore focused on characterization of its effect on Bcl-2 conformational change in order to provide a proof-of-concept that Bcl-2 conformational change can be used as a prognostic indicator for breast cancer response to paclitaxel. Induction of Bcl-2 conformational change by paclitaxel could be observed in other breast cancer cells, as treatment of MCF-7 breast cancer cells also resulted in immunostaining of pro-apoptotic Bcl-2 conformation (Figure 3).

![Figure 3. Paclitaxel induces a Bcl-2 conformational change in MCF-7 breast cancer cells. Immunostaining of apoptotic Bcl-2. H460 cells were treated with or without paclitaxel (50 nM) overnight. Cells were immunostained with anti-Bcl-2 antibody against Bcl-2 BH3 domain and visualized by fluorescence microscopy.](image)

We have also conducted several experiments to determine how paclitaxel induced a Bcl-2 conformational change, in order to further validate our concept. Paclitaxel induces apoptosis of breast cancer cells through phosphorylation of Bcl-2 (Ferlini et al., 2003; Haldar et al., 1995; Srivastava et al., 1999). To study whether paclitaxel induced Bcl-2 conformational change by modulating Bcl-2 phosphorylation, we treated MDA-MB231 and MCF-7 breast cancer cells with paclitaxel and Bcl-2 phosphorylation was examined. Figure 4 shows that treatment of breast cancer cells with paclitaxel strongly induced Bcl-2 phosphorylation in a dose- and time-dependent manner. To study the effect of Bcl-2 phosphorylation on its conformation, we used Jurkat cells stably expressing Bcl-2 (Jurkat/Bcl-2). Treatment of Jurkat/Bcl-2 cells with paclitaxel also enhanced Bcl-2 phosphorylation (Figure 5a). By using anti-Bcl-2 BH3 domain antibody, we observed that the antibody immunoprecipitated phosphorylated Bcl-2 while it had no reaction with unphosphorylated Bcl-2 (Figure 5b). Moreover, paclitaxel strongly enhanced Bcl-2 immunostaining detected by the antibody (Figure 5c). These results suggest that the Bcl-2 BH3 domain epitope is available in phosphorylated Bcl-2 but not in unphosphorylated Bcl-2 protein. Thus, phosphorylation of Bcl-2 by paclitaxel induces a Bcl-2 conformational change, resulting in exposure of its BH3 domain and
apoptosis. These results further support that Bcl-2 conformational change is an indicator of therapeutic responses of breast cancer cells to certain chemotherapeutic agents, such as paclitaxel.

Figure 4. Paclitaxel induces Bcl-2 phosphorylation in breast cancer cells. a. Time-course analysis of paclitaxel effect on Bcl-2 phosphorylation in breast cancer cells. MCF-7 or MDA-MB231 breast cancer cells were treated with paclitaxel (50 nM) the indicated time. Cell lysates were prepared and analyzed by immunoblotting using anti-Bcl-2 antibody. b. Dose-dependent effect of paclitaxel on Bcl-2 phosphorylation in breast cancer cells. MCF-7 or MDA-MB231 breast cancer cells were treated with the indicated concentration of paclitaxel for 12 hr. Cell lysates were prepared and analyzed by immunoblotting using anti-Bcl-2 antibody.

Figure 5. Bcl-2 protein phosphorylation induced by paclitaxel undergoes a conformational change. a. Jurkat cells stably transfected with Bcl-2 were treated with 50 nM paclitaxel for the indicated times, and immunoblotting was conducted using anti-Bcl-2 antibody (Santa Cruz) recognizing the whole length Bcl-2 protein. b. Hyperphosphorylated Bcl-2 undergoes a conformational change. Jurkat/Bcl-2 cells were treated with paclitaxel. Lysates were prepared and subjected to immunoprecipitation using epitope-specific anti-Bcl-2 antibody. Bcl-2 was detected by immunoblotting using anti-Bcl-2 antibody. c. Detection of paclitaxel-induced Bcl-2 conformational change by immunostaining. Jurkat/Bcl-2 cells treated with or without paclitaxel were immunostained with the epitope-specific anti-Bcl-2 antibody and visualized by fluorescence microscopy.

To study whether paclitaxel-induced Bcl-2 phosphorylation and conformational change were responsible for its apoptotic effect in breast cancer cells, we first determined whether JNK activation by paclitaxel accounted for Bcl-2 phosphorylation. Treatment of breast cancer cells with JNK inhibitor or serine protease inhibitor TPCK largely abrogated the inducing effect of paclitaxel on Bcl-2 phosphorylation and apoptosis of breast cancer cells (Figure 6). Thus, Bcl-2 phosphorylation and conformational change contribute to the apoptotic effect of paclitaxel in breast cancer cells.
Figure 6. Inhibition of paclitaxel-induced Bcl-2 phosphorylation and apoptosis by JNK inhibitor II and serine kinase inhibitor TPCK in breast cancer cells.  

a. Inhibition of paclitaxel-induced Bcl-2 phosphorylation by JNK inhibitor II and serine kinase inhibitor TPCK. MDA-MB231 cells were treated with or without 10 μM TPCK or JNK inhibitor II (4 μg/ml) for 2 hr, followed by paclitaxel (50 nM) for 24 hr. Lysates were prepared and subjected to immunoblotting using anti-Bcl-2 antibody.  
b. Inhibition of paclitaxel-induced Bcl-2 apoptosis by JNK inhibitor II and serine kinase inhibitor TPCK. MDA-MB231 cells were treated with or without 10 μM TPCK or JNK inhibitor II (4 μg/ml) for 2 hr, followed by paclitaxel (50 nM) for 24 hr. Apoptosis was determined by DAPI staining and scored by examining 300 cells for nuclear fragmentation and/or chromatin condensation.

Our finding that Bcl-2 phosphorylation and conformational change contributed to the apoptotic effect of paclitaxel offered an opportunity to enhance its therapeutic effect. Since retinoic acid (RA) is known to sensitize breast cancer cells to certain chemotherapeutic agents and there is a significant interest in using combinational approach for treating breast cancer involving RA, we studied whether RA could synergize with paclitaxel for inducing apoptosis through the Bcl-2 pathway. Combination treatment of breast cancer cells with 9-cis-RA and paclitaxel synergistically induced apoptosis of breast cancer cells, which was accompanied by a strong induction of Bcl-2 phosphorylation (Figure 7). These results provide another example that Bcl-2 conformational change can be a sensitive indicator of therapeutic response, again supporting our concept.
Figure 7. Retinoic acid sensitizes breast cancer cells to apoptosis induction and Bcl-2 phosphorylation by paclitaxel. (a-b). ZR-75-1 (A) or MDA-MB231 (B) cells pretreated with 9-cis-RA (0.1 μM) for 24 hr were treated with paclitaxel (1 μM) for 24 hr. Apoptosis was determined by DAPI staining for nuclear morphology change. (c-d). RA enhances paclitaxel-induced Bcl-2 phosphorylation. MDA-MB231 (c) and Jurkat/Bcl-2 (d) cells were treated with RA (10⁴ M) and paclitaxel (50 nM) as indicated for 6 hr. Lysates were prepared and subjected to immunoblotting using anti-Bcl-2 antibody.

Together, these studies demonstrate that Bcl-2 conformational change is an important event in the apoptosis of breast cancer cells, and the epitope-specific anti-Bcl-2 antibody can be used to determine the responsiveness of breast cancer cells to certain chemotherapeutic agents, such as paclitaxel and retinoids.

3. Bcl-2 conformational change as an indicator of apoptosis of breast tumor formed in animal

We have recently identified a peptide, Nur77-derived Bcl-2 converting peptide (NuBCP) (Figure 8a), based on a fragment in Nur77 protein, which binds Bcl-2 and induces Bcl-2 conformational change and apoptosis (Lin, 2004). NuBCP potently inhibited the growth (Figure 8b) and apoptosis (Figure 8c) of ZR-75-1 breast cancer cells.

Figure 8. Apoptosis induction by NuBCP in breast cancer cells. a. Location of NuBCP in Nur77. b. Growth inhibitory effect of NuBCP in ZR-75-1 breast cancer cells. ZR-75-1 cells were exposed to the indicated concentration of NuBCP (fused with polyarginine for cell penetration) for 16 hr, and cell morphology is shown. c. Apoptosis induction by NuBCP in ZR-75-1 cancer. ZR-75-1 cells were exposed to the indicated concentration of NuBCP. Apoptosis was determined by Annexin V staining, followed by flow cytometry analysis.

NuBCP induced apoptosis by binding to Bcl-2, as illustrated by our co-immunoprecipitation assays (Figure 9). DNA sequences encompassing NuBCP (Nur77/489-497) were cloned into a vector containing the green fluorescence protein (GFP). When transfected into HEK293T cells, GFP-NuBCP was precipitated by anti-Bcl-2 antibody only when Bcl-2 was co-expressed (Figure 9a). Interestingly, the D-enantiomer of NuBCP (D-NuBCP) also potently induced apoptosis of breast cancer cells (data not shown) and interacted with Bcl-2 (Figure 9b).
The interaction of D-NuBCP with Bcl-2 was illustrated by its ability to inhibit the binding of Bcl-2 with Nur77/ΔDBD, a Nur77 mutant lacking its DNA-binding-domain (DBD) (Lin, 2004) (Figure 9b). Like Nur77, both L-NuBCP and D-NuBCP targeted mitochondria (Figure 9c). GFP-NuBCP fusion colocalized extensively with RFP-Mito, a red fluorescence protein (RFP) fused with a classical mitochondria-targeting sequence. FITC-D-NuBCP accumulated in cells also displayed extensive colocalization with RFP-Mito, showing the ability of the D-NuBCP-9 to associate with mitochondria.

Figure 9. NuBCP interacts with Bcl-2 and targets mitochondria. a. Binding of NuBCP with Bcl-2. DNA sequences corresponding with NuBCP (Nur77/489-497) were cloned as a GFP fusion, which was transfected into HEK293T cells together with or without Bcl-2 expression vector. Cell lysates were prepared and analyzed for interaction of NuBCP with Bcl-2 by co-immunoprecipitation assay. b. Binding of D-NuBCP with Bcl-2. To study the binding of D-Nur77 peptide to Bcl-2, its ability to compete with Nur77/ADBD for interaction with Bcl-2 was examined by co-immunoprecipitation. c. NuBCPs target mitochondria. GFP-NuBCP and mitochondria-targeted Red Fluorescent Protein (RFP-mito) were transfected into H460 cells for 16 h. Confocal microscopy analysis showed that in H460 cells GFP-NuBCP colocalized extensively with that of RFP-Mito, a red fluorescence protein (RFP) fused with a classical mitochondrial targeting sequence. To determine whether D-NuBCP targeted mitochondria, the RFP-mito transfected H460 cells were treated with polyarginine-conjugated FITC-D-NuBCP-9 peptide. Polyarginine-conjugated FITC-D-NuBCP-9 accumulated in H460 cells also displayed extensive colocalization with RFP-Mito.

Our identification of NuBCP peptide that binds Bcl-2 and targets mitochondria offered another excellent opportunity to evaluate whether Bcl-2 conformational change can be used as an indicator of breast cancer cell apoptosis in response to NuBCP in vitro and in animal, as proposed in our application. We first evaluated whether Bcl-2 conformational change was an indicator of apoptosis in vitro when cells were exposed to NuBCP. For this purpose, HEK293T cells transfected with Bcl-2 were exposed to peptides and subsequently stained with anti-Bcl-2 BH3 domain antibody. Fluorescence microscopy showed that the antibody failed to stain Bcl-2 in control cells (Figure 10a). By contrast, cells exposed to NuBCP or D-NuBCP were strongly stained. Thus, both peptides were able to trigger BH3 domain exposure, a pro-apoptotic Bcl-2 conformation. Consistent with its inability to induce apoptosis, NuBCP/AA, a NuBCP mutant, failed to induce Bcl-2 immunostaining. Flow cytometry analysis also demonstrated that exposure of cells to NuBCP-9, or D-NuBCP resulted in strong Bcl-2 immunofluorescence, whereas cells exposed to NuBCP/AA did not (Figure 10b). The effect of NuBCPs on Bcl-2 conformation was further examined by immunoprecipitation assays (Figure 10c), showing that the anti-Bcl-2 BH3 domain antibody precipitated endogenous Bcl-2 in cells treated with NuBCP-9 or D-NuBCP, but not NuBCP/AA and other control peptides, t-Bid, or Bad peptides. These results provide further evidence that Bcl-2 conformational change can evaluate the response of breast cancer cells to chemotherapeutic drugs.
Figure 10. Bcl-2 conformational change as an indicator of apoptotic effect of NuBCP in vitro. a. Fluorescence microscopy analysis. HEK293T cells were transfected with Bcl-2 expression vector for 16 h. Cells were then exposed to the indicated peptide (20 μM) for 16 h, immunostained by anti-Bcl-2/BH3 domain antibody or DAPI, and examined by fluorescence microscopy. b. Flow cytometry analysis. H460 cells were exposed to the indicated peptide (20 μM) for 16 h and were immunostained with anti-Bcl-2/BH3 domain antibody, followed by SRPD-conjugated secondary antibody (Southern Biotech). Bcl-2 fluorescence from peptide-treated cells (Green histogram) was compared to that from the non-treated cells (purple histogram). Numbers represent % of treated cells showing Bcl-2 immunofluorescence compared to the auto-fluorescence of the non-treated cells from the same experiment. c. Immunoprecipitation assay. H460 cells were exposed to the indicated peptide (10 μM) for 16 h. Bcl-2 was then precipitated by anti-Bcl-2/BH3 domain antibody and Western blotting was done using a polyclonal anti-Bcl-2 antibody raised against the whole protein.

Figure 11. Bcl-2 conformational change as an indicator of breast tumor apoptosis in animal. a. Induction of apoptosis of MDA-MB435 breast cancer cells by NuBCPs. MDA-MB435 cells were exposed to the indicated peptide (20 μM) for 16 h and apoptosis was determined by Annexin V staining. b. Inhibition of tumor growth by NuBCPs. MDA-MB435 cells were inoculated subcutaneously in the flank region of SCID mice. In days 10 and 13, NuBCP-9/AA, NuBCP or D-NuBCP peptide in 50 μL PBS was injected into tumor, and tumor volumes were measured every week for two months. c. Induction of apoptosis of tumor by NuBCPs. Tumor tissues from animal treated
with the indicated peptide were stained by TUNEL. d. Correlation of apoptosis induction and Bcl-2 conformational change in animal. Apoptosis in the tumor tissues from animals treated for two days with the indicated peptide was determined by TUNEL staining. In addition, Conformational change in Bcl-2 induced by NuBCPs was detected in the same tissues by immuno-staining with aBcl-2/BH3 domain antibody. Nuclei were visualized by DAPI staining.

Our second objective of the Concept Award was to determine whether Bcl-2 conformational change could be used as an indicator of apoptosis of breast tumor grown in animal. For this purpose, we examined whether BuCBPs induced Bcl-2 conformational change in breast tumor grown in animal and whether the conformational change is associated with apoptosis in breast tumor. MDA-MB435 breast cancer cells are very sensitive to NuBCPs (Figure 11a) and rapidly form tumor in SCID mice. We inoculated MDA-MB435 breast cancer cells subcutaneously into the fat pads of SCID mice. MDA-MB435 tumors appeared 7 days after inoculating into SCID mice. On days 10 and 13, peptides were injected into tumors. Comparison of NuBCP with control (NuBCP/AA) peptides demonstrated that the injection of NuBCPs dramatically suppressed the growth of tumor (Figure 11b), suggesting that NuBCPs were active in animal. D-NuBCP was more effective than NuBCP-9, likely reflecting its enhanced stability in vivo. A TUNEL assay showed that tumors injected with either NuBCP or D-NuBCP, but not the control peptide, exhibited extensive apoptosis (Figure 11c). Immunostaining of tumor tissues by anti-Bcl-2 BH3 domain antibody showed that Bcl-2 immunofluorescence stained by the antibody overlapped extensively with TUNEL staining (Figure 11d). These results demonstrate that Bcl-2 conformational change can be used as an indicator of apoptosis of breast tumor in animal.

Reportable Outcomes

Abstracts

Era of Hope Meeting
June 8-11, 2005
Philadelphia, PA
Bcl-2 Conformational Change as an Indicator for Chemotherapy Response.
Bingzhen Lin, Xihua Cao, and Xiao-kun Zhang

Era of Hope Meeting
June 8-11, 2005
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Nur77-derived Bcl-2-converting Peptides (NuBCPs) Induce Breast Cancer Cell Apoptosis.
Xiao-kun Zhang, Arnold C. Satterthwait, Siva Kumar Kolluri, and Xiuwen Zhu

Keystone Symposia on Tissue-Selective Nuclear Receptors
September 18-22, 2005
Beaver Run Resort, CO
Retinoic Acid and Paclitaxel Synergistically Induce Apoptosis by Modulating Bcl-2
Phosphorylation and Conformation
Nathalie Bruey-Sedano, Bingzhen Lin, and Xiao-kun Zhang

Manuscripts in Preparation
Conclusion

In conclusion, we have conducted all studies proposed in our Concept application. With the support from BCRP, we have obtained a significant amount of results that support our concept that Bcl-2 conformational change can be used as an indicator of breast cancer cell apoptosis induced by certain chemotherapeutic agents. We have characterized an epitope-specific anti-Bcl-2 antibody that specifically recognizes pro-apoptotic Bcl-2 conformation. We have used this antibody to show that Bcl-2 conformational change is an indicator of breast cancer cell apoptosis induced by a number of chemotherapeutic agents in vitro. Our studies show for the first time that paclitaxel induces breast cancer cell apoptosis by modulating Bcl-2 conformation. We also demonstrate that Bcl-2 conformational change is responsible for apoptosis induced by combinational treatment of paclitaxel and retinoic acid. Moreover, our results show that Bcl-2 conformational change is indicative of apoptosis of breast tumor grown in animal. Thus, our studies strongly suggest that further studies are conducted to determine the feasibility of using Bcl-2 conformational change as a diagnostic factor for breast cancer patients, which is expected to be more sensitive and reliable.

References


antiinflammatory drug etodolac binds retinoid X receptor and induces tumor-selective apoptosis. PNAS.


Personnel

Xiao-kun Zhang, Ph.D. - Principal Investigator
Nathalie Bruey-Sedano, Ph.D. - Postdoc

Appendices

Manuscripts in Preparation
Era of Hope Meeting - Philadelphia, PA
June 8-11, 2005
Xiao-kun Zhang, Arnold C. Satterthwait, Siva Kumar Kolluri, and Xiwen Zhu
Nur77-derived Bcl-2-converting Peptides (NuBCPs) Induce Breast Cancer Cell Apoptosis
Abstract

Orphan nuclear receptor Nur77 (also known as TR3 or NGFI-B) plays a critical role in regulating breast tumor metastasis and responses of cancer patients to chemotherapy. Our recent discovery (Cell 116, 527, 2004) that Nur77 converts Bcl-2, a potent anti-apoptotic molecule, from a protector to a killer through their physical interaction offers an opportunity to design drugs that mimic Nur77 action, which are likely to be effective against cancer cells with high Bcl-2 levels. We report here the identification of a Nur77-derived Bcl-2-converting peptide (NuBCP) with only 9 amino acids, which fully mimics the Nur77 function. NuBCP binds to Bcl-2, leading to a Bcl-2 conformational change and extensive apoptosis of breast cancer cells. The apoptotic effect of NuBCP-9 is not inhibited but rather potentiated by Bcl-2 overexpression. In addition, the functional activities of NuBCP-9 are completely retained in its all D-amino acid analog, which is resistant to proteolytic degradation. Moreover, both NuBCP and D-NuBCP peptides effectively inhibit the growth of breast tumor in animal by inducing Bcl-2 conformational change and apoptosis. Our results suggest that NuBCP-9 and its protease-resistant D-enantiomer may have therapeutic value against Bcl-2-overexpressing cancer cells.

Era of Hope Meeting – Philadelphia, PA
June 8-11, 2005
Bingzhen Lin, Xihua Cao, and Xiao-Kun Zhang
Bcl-2 Conformational Change as an Indicator for Chemotherapy Response.

Abstract

Interest in translational studies on breast cancer is presently devoted to identifying biological predictors of disease prognosis and response to treatment. Unfortunately, no tumor biological factor is available for predicting and evaluating chemotherapy response in breast cancer, except the estrogen receptor status, which predicts the response to endocrine therapy. Recent studies have indicated that most anticancer agents ultimately kill cancer cells primarily by inducing apoptosis, a programmed cell death. Overexpression of the anti-apoptotic protein Bcl-2 is implicated in breast cancer development, tumor progression and drug resistance. Paradoxically, the expression of Bcl-2 correlates with favorable outcomes following endocrine treatment in almost all the published reports or chemotherapy in some cases. Recently, we reported that Bcl-2 manifests opposing biological activities, survival and death, depending on its protein conformation. Upon binding to Nur77 (also known as TR3 or NGFI-B), an orphan member of the nuclear receptor superfamily, Bcl-2 undergoes a conformational change that exposes its otherwise hidden BH3 domain, resulting in its conversion from a protector to a killer. Interestingly, the N-terminal loop region, located between the BH4 and BH3 domains of Bcl-2 is responsible for Bcl-2 binding to Nur77. Paclitaxel (Taxol), a naturally occurring antineoplastic agent, has shown great promise in the treatment of a variety of tumor types, including breast tumor. Paclitaxel potently induces apoptosis of breast cancer cells in part through its phosphorylation of the loop region of Bcl-2. Intriguingly, deletion of the loop region of Bcl-2 blocks paclitaxel-induced apoptosis in breast cancer cells, thereby suggesting the requirement of the loop region for its apoptotic effect. We studied whether Bcl-2 undergoes a conformational change in response to paclitaxel in breast cancer cells. Treatment of breast cancer cells with paclitaxel strongly induced Bcl-2 phosphorylation, which was suppressed by Jun N-terminal kinase inhibitor and serine protease inhibitor TPCK. Using anti-Bcl-2 antibody recognizing the Bcl-2 BH3 domain, we observed that it immunoprecipitated phosphorylated Bcl-2 while it had no reaction with unphosphorylated Bcl-2. These results suggest that the Bcl-2 BH3 domain epitope is available in phosphorylated Bcl-2 but not in unphosphorylated Bcl-2 protein. Thus, phosphorylation of Bcl-2 by paclitaxel may induce a Bcl-2 conformational change, resulting in exposure of its BH3 domain and apoptosis. Together, our results demonstrate that Bcl-2 conformational change may be indicative of therapeutic responses to certain chemotherapeutic agents.