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Dissociation of Bax from a Bcl-2/Bax Heterodimer Triggered by Phosphorylation of Serine 70 of Bcl-2[1]

Miki Shitashige,[*,†] Masakazu Toi,[‡] Takeo Yano,[§] Masao Shibata,[§] Yoshinobu Matsuo,[‖] and Futoshi Shibasaki[*,2]

[*]Department of Molecular Cell Physiology, The Tokyo Metropolitan Institute of Medical Science, 3-18-22 Honkomagome, Bunkyo-ku, Tokyo 113-8613; [†]Japan Science and Technology Corporation, 4-1-8 Honcho, Kawaguchi, Saitama 332-0012; [‡]Department of Surgery, Tokyo Metropolitan Komagome Hospital, 3-18-22 Honkomagome, Bunkyo-ku, Tokyo 113-8677; [§]Medical Biological Lab., 1063-103 Terasawaoka-Azaohara, Nagano 396-0002; and [‖]Fujisaki Cell Center, Hayashibara Biochemical Labs Inc., 675-1 Fujisaki, Okayama 702

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Serine 70 in the loop region of Bcl-2 is specifically phosphorylated by paclitaxel-treatment in tumor cells and BHK cells expressing Bcl-2. The phosphorylation of serine 70 of Bcl-2 (pS70-Bcl-2) peaks 24 to 48 h after paclitaxel treatment and accelerates apoptosis. Phosphorylation is effectively inhibited in the presence of actinomycin D or cycloheximide, which restore cell viability to the same level as control cells not expressing Bcl-2. These results indicate that paclitaxel-induced kinase(s) and/or its activator(s) are synthesized *de novo* and play an important role in paclitaxel-induced apoptosis by phosphorylating Bcl-2. In binding assays using the phosphorylation-specific antibody against pS70-Bcl-2, the induction of serine 70 phosphorylation 70 results in a loss of the binding ability of Bcl-2 to Bax, a pro-apoptotic partner, and induces subsequent cell death. When the pS70-Bcl-2 antibody was added to human breast cancer tissue, serine 70 phosphorylation was also detected, even prior to treatment with anticancer agents. Further study of breast cancers revealed 83% of tumors with high pS70-Bcl-2 expression responded to paclitaxel or docetaxel treatment, whereas 57% of those with low expression not respond. These findings suggest that pS70-Bcl-2 might be a predictive factor for prognosis and sensitivity to paclitaxel treatment for breast cancer.

Key words: apoptosis, Bcl-2, breast cancer, paclitaxel (Taxol), phosphorylation.

The expression of Bcl-2 averts cell death induced by a variety of apoptotic inducers such as anti-tumor agents and glucocorticoids (*1*). Recent studies have demonstrated that paclitaxel (Taxol) phosphorylates Bcl-2 at several sites in a 60-aa loop region between the α1 and α2 helices. This drug stabilizes microtubules and arrests cells in the G_2/M phase of the cell cycle (*2, 3*). Mutagenic studies clearly identified serine 70 in the loop region as a target phosphorylation site of paclitaxel-induced kinases (*4*). A single amino acid mutant of S70 to alanine (S70A) is resistant to apoptosis induced by paclitaxel (*5*). Human lymphoma, breast cancer and prostate cancer cells (*6, 7*) exposed to paclitaxel undergo apoptosis in correlation with the appearance of a phosphorylated form of Bcl-2, suggesting that the phosphorylation of Bcl-2 may inhibit Bcl-2 function. Several kinases have been proposed as Bcl-2 kinases so far. Raf-1 kinase, protein kinase C, protein kinase A, c-Jun N-terminal kinase, and CDC2 kinase, all phosphorylate Bcl-2 protein at different sites and with distinct effects on its kinetics (*8*). These kinases might be activated partly by drug stimuli or partly in a cell cycle–dependent manner (*9*). In contrast, calcineurin, a serine/threonine phosphatase that is normally involved in T-cell regulation (*10, 11*), has been reported to interact with Bcl-2 (*12*). The interaction between calcineurin and Bcl-2 causes mobility shifts of Bcl-2 in immunoblotting, suggesting that calcineurin might affect the anti-apoptotic function of Bcl-2 through dephosphorylation. These results support the hypothesis that the anti-apoptotic function of Bcl-2 might be regulated in the balance between kinases and phosphatases.

It is generally accepted that Bcl-2 exerts its anti-apoptotic effects mainly by heterodimerization with pro-apoptotic members of the Bcl-2 family such as Bax and Bad (*13*). This means that the phosphorylation of Bcl-2 might reduce heterodimer formation with Bax (*7, 14*) to decrease or inactivate the Bcl-2 anti-apoptotic function, although the physiological relevance of phosphorylated Bcl-2 remains unclear. In the present study, we raised a specific antibody against the phosphorylated serine 70 of Bcl-2 in order to

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[2] To whom correspondence should be addressed. Phone: +81-3-3823-0090, Fax: +81-3-3823-0085, E-mail: fshibasa@rinshoken.or.jp

Abbreviations: pS70-Bcl-2, phosphorylated serine 70 of Bcl-2; BHK, baby hamster kidney; Tet, tetracycline; Tet-Bcl-2 BHK, Tet-inducible BHK cells expressing Bcl-2; Cytc, cytochrome *c*; Ab, antibody; Abs, antibodies; Tax, paclitaxel (Taxol®); Taxt, docetaxel (Taxotere®); PR, progesterone receptor; ER, estrogen receptor; wt, wild-type; AcD, actinomycin D; CHX, cycloheximide; CyPA, cyclophilin A.