Commentary

BAD: a good therapeutic target?

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Abstract

The major goal in cancer treatment is the eradication of tumor cells. Under stress conditions, normal cells undergo apoptosis; this property is fortunately conserved in some tumor cells, leading to their death as a result of chemotherapeutic and/or radiation-induced stress. Many malignant cells, however, have developed ways to subvert apoptosis, a characteristic that constitutes a major clinical problem. Gilmore *et al.* recently described the ability of ZD1839, a small-molecule inhibitor of the epidermal growth factor receptor (EGFR), to induce apoptosis of mammary cells that are dependent upon growth factors for survival. Furthermore, they showed that the major effector of the EGFR-targeted therapy is BAD, a widely expressed BCL-2 family member. These results are promising in light of the role of the EGFR in breast cancer development.

Keywords: apoptosis, ErbB1 receptor, insulin growth factor-1 receptor, mitogen-activated protein kinase, ZD1839

Introduction

Cells are naturally endowed with mechanisms that induce apoptosis, a characteristic that underlies the successful use of standard cancer drugs and radiation. The growth of a tumor, however, is exquisitely dependent upon overcoming the normal tendency of a cell to die under stress conditions. A major clinical problem is thus the ability of malignant cells to evade cancer drugs, either by altering expression of surveillance molecules or by upregulating signaling pathways capable of promoting cell survival and proliferation, even in the presence of an apoptosis-inducing agent.

A recent paper from the laboratory of Charles Streuli described the ability of ZD1839, a small-molecule inhibitor of the epidermal growth factor receptor (EGFR), to induce apoptosis of normal mammary cells [1]. In this system, insulin-like growth factor-1 (IGF-1) and epidermal growth factor (EGF) are two important survival factors. Interestingly, ZD1839 was effective in inducing cell death not only in the presence of EGF, but also in the presence of IGF-1. Furthermore, the authors shed light on the mechanisms underlying ZD1839-mediated apoptosis, showing that BAD, a BCL-2 family member, is an important survival target of the EGFR in mammary cells. The results are promising, considering the importance of this receptor in breast cancer development. In the future, it will be essential to understand the mechanisms by which apoptosis can be triggered in tumors in order to achieve the desired efficacy in cancer therapeutics.

Receptor tyrosine kinases and cancer

Members of the ErbB family of receptor tyrosine kinases play important roles in normal biology of the breast and in cancer. Upon ligand binding to the extracellular domain, these receptors form homodimers and heterodimers leading to activation of the intracellular kinase domain and phosphorylation on specific tyrosine residues. These tyrosines then serve as docking sites for molecules that further transduce the signal, leading to activation of the mitogen-activated protein kinase (MAPK) pathway and the phosphoinositide-3 kinase (PI3K)/protein kinase B

BH = BCL-2 homology; EGF = epidermal growth factor; EGFR = epidermal growth factor receptor; IGF-1 = insulin-like growth factor-1; IGF-1R = insulin growth factor-1 receptor; mAb = monoclonal antibody; MAPK = mitogen-activated protein kinase; PI3K = phosphoinositide-3 kinase.

pathway, among others [2]. Alterations in expression and activation of the EGFR and ErbB2 are relatively common in breast cancer. Furthermore, these receptors have also been implicated in the development of many other types of solid tumors [3,4].

The IGF-1 receptor (IGF-1R), like the insulin receptor, is composed of α and β chains, and ligand binding induces the formation of a heterotetrameric complex. The activated IGF-1R phosphorylates a major substrate, IRS1, on multiple sites, forming a signaling platform that stimulates mitogenic and survival pathways, including MAPK and PI3K [5]. IGF-1 expression is a prognostic factor for increased risk of developing breast cancer [6]. Moreover, overexpression of the IGF-1R and elevated serum IGF-1 levels have been observed in breast cancer patients [6].

Because of their evident role in cancer, the receptor tyrosine kinases (in particular, ErbB receptors) have been vigorously pursued as promising targets. Specific mAbs were among the earliest described therapeutics; these include C225 (EGFR specific) and Herceptin (ErbB2 specific) [4]. Small molecular weight ErbB kinase inhibitors are also in various stages of clinical development [3,4]. ZD1839 (Iressa), an anilinoquinazoline that blocks EGFR activity, was used in the studies of Gilmore *et al.* [1].

ZD1839 causes apoptosis of normal mammary cells

Apoptosis, a highly regulated process, is triggered by different mechanisms. They are broadly classified as 'extrinsic' when occurring via activation of a death receptor, or 'intrinsic' when the mitochondria and members of the BCL-2 family take part [7]. This large protein family is comprised of anti-apoptotic and pro-apoptotic molecules whose members share up to four BCL-2 homology (BH) domains. Very generally, the members can be divided into three subclasses: those like the multidomain BCL-2 and BCL-X_L, which are anti-apoptotic; those like the multidomain BAX and BAK, which are pro-apoptotic; and, finally, the BH3 domain-only pro-apoptotic proteins, such as BID, BIK and BAD. The latter group uses the BH3 domain to interact with the multidomain BCL-2 family members, and can be further divided based upon preferred binding partners. Recent results [8] suggest that the BH3 domain-only proteins function to induce apoptosis by two mechanisms: the BID-like BH3 domains activate the pro-apoptotic BAX and BAK; and the BAD-like BH3 domains bind anti-apoptotic BCL-2 or BCL-X_L, thus interfering with their activity. The activity of the BH3 domain-only proteins is controlled by various mechanisms ranging from transcriptional to protein modification.

BAD, the protagonist of Gilmore *et al*.'s publication, is regulated by phosphorylation. Growth factors and cytokines stimulate BAD phosphorylation on specific serine residues: S112, S136, S155 and S170. Depending upon the cell type and the inducing agent, various kinases phosphorylate BAD, including p90Rsk-1, protein kinase A, protein kinase B and p70S6 kinase [9–14]. Phosphorylation of BAD on these residues promotes its binding to, and subsequent cytosolic sequestration by, 14-3-3 proteins [15]. Importantly, these modifications prevent BAD from associating with BCL-2 or BCL-x_L on the mitochondrial outer membrane, leaving these proteins free to exert their anti-apoptotic function [15].

The EGFR and the IGF-1R have survival and proliferative roles in normal mammary cells; in serum-free conditions, primary mammary cells must be grown in EGF or IGF-1 to prevent apoptosis. The results of Gilmore *et al*. show that BAD is the target of signaling pathways emanating from both receptors [1]. EGF induces phosphorylation of S112 via MAPK activation; and IGF-1 induces both S112 and S136 phosphorylation, the latter via the PI3K pathway. ZD1839 blocks the EGFR and the downstream MAPK activity, leading to a decrease in S112 phosphorylation and induction of apoptosis. Intriguingly, IGF-1-mediated survival of primary mammary cells is also blocked by ZD1839. These results suggest that IGF-1R transactivation of the EGFR, which occurs in the mammary cell line, is the main mechanism by which IGF-1 maintains survival of primary mammary cells.

What influences BAD activity in breast cells?

Considering the importance of BAD inactivation in promoting survival of normal mammary cells, as shown by Gilmore *et al*. [1], it is interesting to consider factors that might influence BAD's activity, particularly in cancer cells. BAD is widely expressed [16] and its level might play an important role in treatment outcome. For example, loss of or decrease in BAD expression during the course of tumor development might allow the prosurvival proteins BCL-2 or BCL-x_L to function unopposed to this potent inhibitor. In fact, in normal human breast cells, BAD levels are relatively high in comparison with those in other organs [16], suggesting that BAD might have a special role in the breast. Indeed, Gilmore *et al*. [1] showed that transient BAD overexpression enhances the sensitivity of mammary cells to apoptosis, perhaps because the S112 and S136 kinases become limiting. On the other hand, primary cultures of $BAD^{-/-}$ mammary cells are no longer sensitive to ZD1839-induced apoptosis, suggesting that BAD might be the important pro-apoptotic effector downstream of the EGFR.

Tumor cells might also develop mechanisms to circumvent BAD-induced apoptosis. While primary mammary cells appear to use mainly MAPK for survival, both in response to EGF as well as to IGF-1 [1], tumor cells might use multiple survival pathways that could impinge on BAD. In fact, in the presence of IGF-1, the FSK mammary cells are not as sensitive to ZD1839-induced apoptosis as the primary mammary cells. Their results show that these cells

rely on both the MAPK and the PI3K pathway for survival, a mechanism that is likely to be quite common in tumor cells.

Can receptor tyrosine kinase inhibitors trigger apoptosis in malignant breast cells?

The results of Gilmore *et al.* [1] show that normal mammary cells are exquisitely dependent upon survival signals emanating from the EGFR. Can these findings be put to use for breast cancer therapy? In other words, are breast tumor cells equally dependent upon these pathways for their survival?

It has been well documented that agents targeting members of the EGFR family generally yield satisfactory growth inhibitory results [3,4,17,18]. Apoptosis, however, appears to be triggered mainly when a combined therapy is employed. Turning to *in vivo* tumor models, a humanized EGFR antibody (ABX-EGF) appears to cause tumor cell death [19]. Furthermore, EGFR inhibitors have been shown to have growth inhibitory effects on ErbB receptor-driven orthotopic tumor models [20,21] and, in the latter, to induce apoptosis, although the effectors have not yet been identified.

Returning to the results of Gilmore *et al.* [1], they showed that metastatic breast cancer cells, isolated from one patient who had failed multiple therapies, underwent apoptosis in the presence of ZD1839. This suggests that EGFR kinase inhibitors can potentially cause apoptosis of malignant breast cells, and highlights the potential efficacy of such therapy in this tissue.

Future goals

The success of future therapies depends on their ability to interfere with distinct pathways leading to apoptosis. Considering BAD's facility to function as a switch between life and death, and its regulation via both phosphorylation and localization, the development of inhibitors of multiple signaling proteins, as well as of molecules that interfere with protein–protein interactions, would be valuable. In this regard, it has been reported that synthetic peptides that mimic the BH3 domain of the pro-apoptotic family members BAX and BAK were able to induce apoptosis [22,23]. In addition, small molecules that inhibit the binding of BAK to BCL-x_L, by specifically blocking the BH3-mediated interaction with the survival molecule, resulted in cell death both *in vitro* and *in vivo*.

Finally, the interaction between BAD and 14-3-3 proteins might itself be a target for intervention. Intriguingly, a recent report shows that, in postmitotic neurons, Cdc2-mediated phosphorylation of BAD on S128 inhibits the interaction of phospho-S136 with 14-3-3, leading to apoptosis [24]. It might thus be possible to develop small molecules that directly block the ability of phospho-BAD to interact with 14-3-3, thereby freeing its pro-apoptotic function.

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