Mini Review

Roles of BCL-2 and BAD in Breast Cancer

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Abbreviations

AIF: Apoptosis Inducible Factor; AP-1: Activator Protein-1; AKT: Protein Kinase B; Apaf-1: Apoptosis Protease Activating Factor-1; BAD: Bcl-2-Associated Death Promoter; BCL-2: B-Cell Lymphoma 2; BCLxL: B-Cell Lymphoma-Extra Large; BH3: Bcl-2 Homology Domain 3; BRCA1: Breast Cancer Type 1 Susceptibility Protein; CDK4: Cyclin-Dependent Kinase-4; CXCL12/SDF1: Stromal Cell-Derived Factor-1; CXCR4: Chemokine Receptor Type 4; EMT: Epithelial-Mesenchymal Transition; ERα: Estrogen Receptor α; ERβ: Estrogen Receptor β; ERK: Extracellular Signal-Regulated Kinases; FADD: Fas-Associated Protein with Death Domain; P: Phospho; Ras/MEK/ERK: MAPK Signaling Pathway; JNK: c-Jun kinase; MCL1: Myeloid Leukemia Cell Differentiation Protein-1; MMP10: Metalloproteinase-10; MTA3: Metastasis-Associated Protein-3; Sp1: Specificity Protein-1; STAT: Signal Transducer and Activator of Transcription; TMA: Tissue Microarrays; TIMP2: Metallopeptidase Inhibitor 2; TRE: Transcription Response Elements; VEGF: Vascular Endothelial Growth Factor.

Apoptotic Regulators Have Multiple Roles

Apoptosis has a major role in cancer as it is an essential regulator of cell mass in tumor and normal tissue and is controlled by a variety of proteins, as depicted in Figures 1A and 1B [1] and its role in cancer is described in excellent reviews [1-3].

Among the multitude of proteins that have critical roles in apoptosis many have non-apoptotic functions, e.g. cytochrome C, a key player in the intrinsic apoptosis pathway, is required for oxidative phosphorylation-linked electron transport. Functions for caspases in cell-cycle entry, cell maturation, immune system function [4,5] differentiation [6], and other apoptosis-unrelated functions [7,8] have been described in addition to their well-established roles in apoptosis. Other pro-apoptotic molecules have pro-survival effects, e.g. apoptosis inducible factor (AIF), Endo G and Omi [9,10], and developmental functions, such as of Fas-associated protein with Death Domain (FADD) *in vivo* [11,12]. Additionally, Apoptosis protease activating factor-1 (Apaf-1) functions in the DNA checkpoint [7] and the permeability transition pore complex proteins also regulate cell metabolism and survival [7]. BCL-2 has a variety of other non-apoptotic functions *in vitro* [2,13-19]. Bcl-2 is known *in vitro* to inhibit cell cycling independent of apoptosis and retards transit through G1 phase [13,18,19] and activates a programme of premature senescence in human carcinoma cells [15]. MCL1 may inhibit cell cycle transit as well [14]. In accordance with cell cycle regulation, BH3 proteins, BIM and BCL-2 have been localized to the nucleus [3,20,21] as well, this data suggests that BCL-2 family proteins may have nuclear functions. However the traditional view is that apoptotic regulators including BCL-2 family members, are typically localized to the intracellular membranes, cytoplasm, or mitochondria [2,3]. Recently, BID was demonstrated to have a role in inflammation and immunity independent of apoptosis [22].
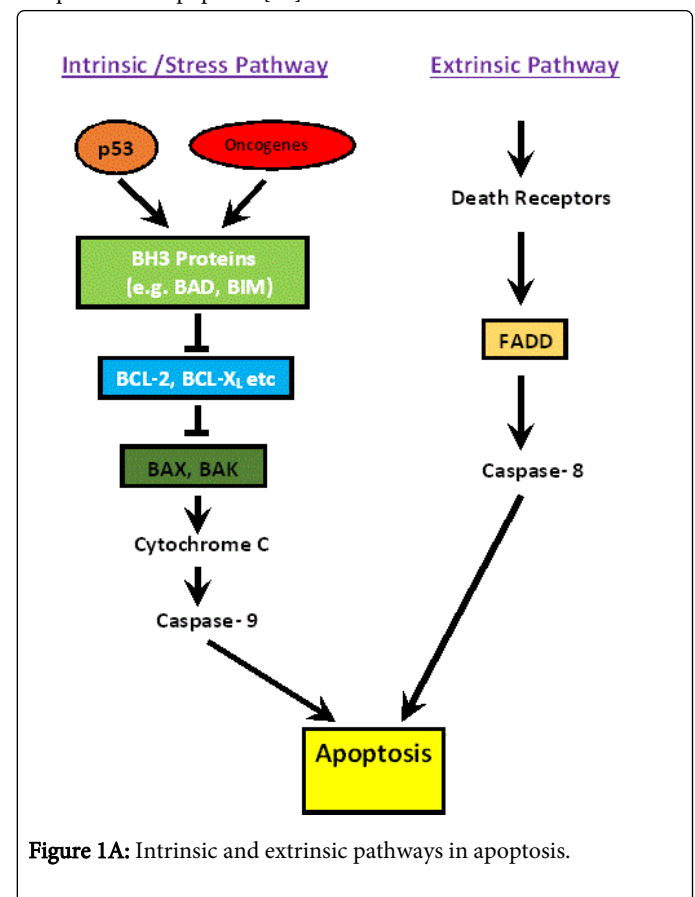

Figure 1A: Intrinsic and extrinsic pathways in apoptosis.

The Role of BCL-2 in Clinical Breast Cancer

The role of BCL-2 in clinical breast cancer is in marked contrast to its well-known anti apoptotic effects *in vitro*. Many clinical studies suggest that BCL-2 expression, measured both by immunohistochemistry and PCR, is a strong predictor of overall and disease-free survival in breast cancer patients. BCL-2 is a favorable and superior prognostic marker [23-26], independent of lymph node status, tumor size, grade, and other biomarkers including estrogen

receptor α (ERα) Recently [26] data was presented which showed that the Ki67/BCL-2 ratio is a superior prognostic marker than either alone in ERα positive breast cancer.
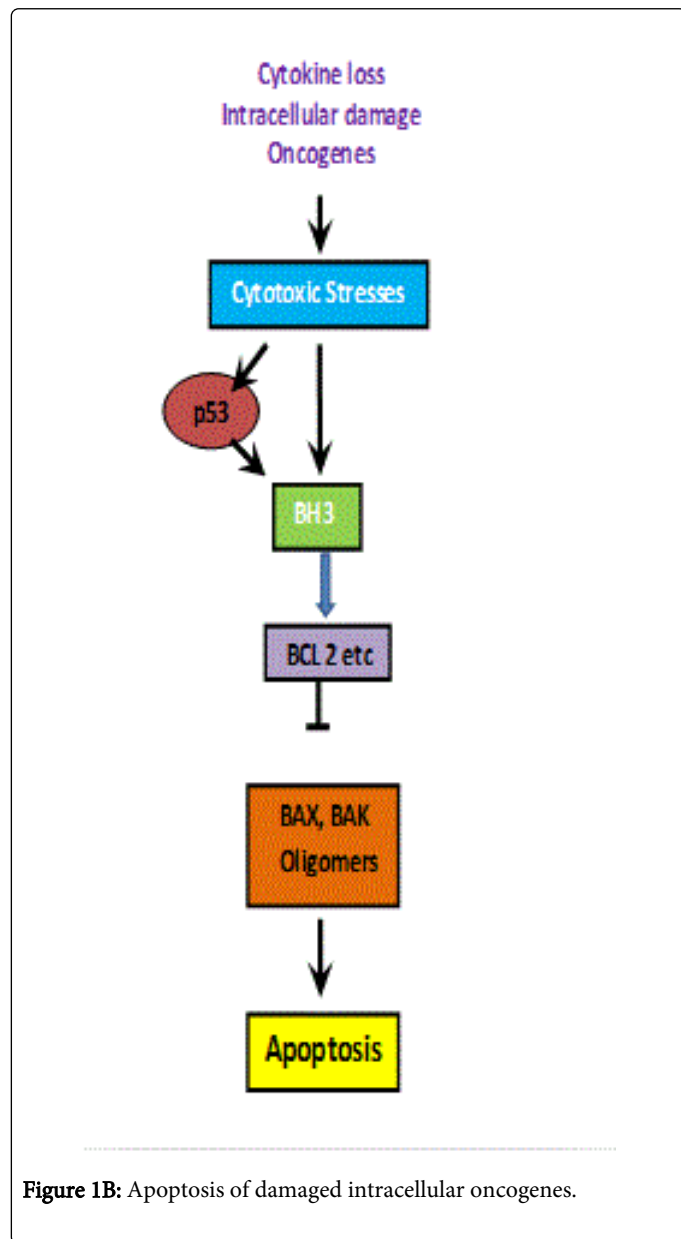

Figure 1B: Apoptosis of damaged intracellular oncogenes.

The Role of BAD in Breast Cancer

In this brief review we will consider the role of BCL-2 antagonist BAD in breast cancer and cite evidence that both of these proteins have similar roles *in vivo*. Recently we found that BAD was able to regulate several proteins that may have roles in the epithelial-mesenchymal transition (EMT) and BAD inhibited extra-cellular matrix invasion by breast cancer cells *in vitro* [27]. To our knowledge, this was the first demonstration of both anti-invasive and EMT inhibiting effects of BAD, or any other BCL-2 family proteins in breast cancer cells. Recent studies also demonstrate non-apoptotic roles of BAD: blood glucose regulation, cooperation with p53 at mitochondria, cell cycle regulation, and pro-survival functions [28-31].

BAD regulates the cell cycle through Cyclin D1

We had previously demonstrated that BCL-2 antagonist, BAD is localized to the nucleus, in addition to the cytoplasm in normal human breast tissue and that BAD prevents cyclin D1 transcription [32]. Decreased synthesis of cyclin D1 resulted in decreased CDK4 activity as evidenced by decreased Rb phosphorylation and blockade of G1 to S phase transition and cell cycle progression. Furthur, BAD is able to inhibit both CRE- and TRE-luciferase activities through the inhibition of c-Jun binding to these elements [27,32], resulting in inhibition of cyclin D1 expression. Unpublished data showed that phosphorylation of S112&S136 in BAD were required for the suppressive effects of BAD on the cyclin D1 promoter. Since BAD interacts selectively with un-phosphorylated c-Jun [32], we investigated whether BAD could also regulate signal pathways that phosphorylate c-Jun. BAD inhibited the Ras-MEKK-MEK-ERK and JNK pathways selectively, resulting in decreased c-Jun-mediated activation of TRE and CRE in the cyclin D1 promoter [32]. c-Jun is phosphorylated and activated by JNK, ERK, and a variety of other kinases [33,34]. The inhibitory effects of BAD on the activation of JNK/c-Jun by E2 are broadly similar to that of serum. Thus the mitogenic effects of estradiol exerted via induction of cyclin D1 in addition to regulation of p27kip1 [35] were antagonized by BAD. Other inducers of cyclin D1 were also blocked: β-catenin mRNA and protein expression, a significant down-regulation of both phosphorylated and total STAT1 and STAT3 in BAD over-expressing cells resulting in a reduced activation, similarly a reduced p/T ratio STAT5 suggested reduced activation of STAT5. STATs are well known regulators of cyclin D1 [36,37].

The mechanisms by which BAD regulates expression of several proteins remains to be elucidated and may relate at least in part to its ability to bind with c-Jun and inhibit the latter's transcriptional effects. Furthermore, BAD may indirectly effect gene regulation through inhibition of cyclin D1, which is known to control transcription [38-41] and also through β-catenin. The transcription factor Sp1that regulates SNAIL expression [42] is another activator of the cyclin D1 promoter [43]. Its inhibition by BAD could potentially be yet another mechanism by which BAD regulates the expression of cyclin D1 in cancer cells. These results collectively demonstrate the ability of the cell to utilize complex mechanisms to counteract the expression of pro-tumorigenic cyclin D1 through regulation of molecules like BAD. In breast tumor tissues, the expression of cyclin D1 in the cytoplasm was significantly lower compared to its normal counterpart [27], the significance of cytoplasmic cyclin D1 is unknown even though others have shown similar data [44,45] cytoplasmic localization may correlate with functions of cyclin D1 in the cytoplasm, such as in cell migration [46-48]and mitochondrial metabolism [38,49], in addition to its cell cycle-related function. Unpublished data also suggested a positive correlation between nuclear ERβ and BAD and the former may decrease the tumor promoting role of nuclear ERα. [50-52].

BAD inhibits breast cancer invasion *in vivo*

Al-Bazz et al. also reported that BAD is localized to both the nucleus and cytoplasm in breast cancer tissue, suggesting a nuclear role of BAD. Significantly less staining intensities for BAD and p-BAD were found in cancer than in normal breast tissue and in both cases the expression of BAD in the cytoplasm exceeded that of nuclei. High BAD expression is associated with longer disease free survival, overall survival [53], and longer time to relapse in tamoxifen-treated breast cancer patients [54]. Premenopausal breast carcinoma in younger women is more aggressive with a higher potential for invasion and

metastasis and poor prognosis compared to postmenopausal breast carcinoma [55]. Interestingly, expression of both BCL-2 and BAD in premenopausal breast carcinoma was significantly lower than in post-menopausal breast carcinoma and this decrease correlated with the progression from Grade I to III [56]. It is noteworthy that both BCL-2 and its antagonist BAD decreased with increasing severity of the disease. Since patient survival is directly related to metastasis, it is likely that expression of BAD protein as well as BCL-2 could be associated with a lowered metastatic potential. In our study we found that in Grade II cancers, decreased expression of p-BAD was found in 47% of cytoplasm and 80% of nuclei compared to normal tissue [27].
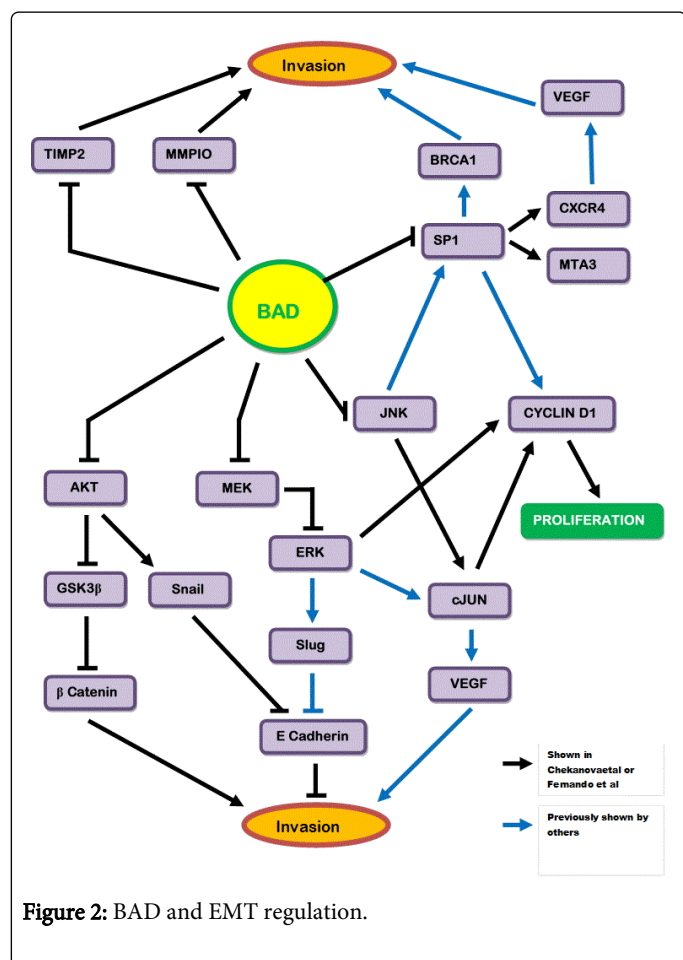

Figure 2: BAD and EMT regulation.

BAD and EMT regulation

Since cyclin D1 and c-Jun have been shown to promote breast cancer cell invasion [38,46,47,49,57] and BAD inhibits both these proteins [32] we investigated whether BAD may regulate invasion *in vitro*; Over-expression of BAD significantly reduced MCF7 cell (a cell line established from a pleural effusion) invasion through Matrigel. BAD decreased MMP10 secretion coupled with increased secretion of the MMP inhibitor TIMP-2 that correlates with the survival in breast cancer patients [58] and the secretion of pro-angiogenic VEGF, which has been shown to correlate with cancer metastasis was significantly reduced by BAD [59]. Overexpression of BAD significantly decreased activities of several EMT related proteins including CXCL2/SDF and its receptor CXCR4 as well,this system is important in tumor cell migration [60] (Figure 2).

A well characterized EMT inducer SNAIL [61], expression was decreased as demonstrated by mRNA and protein expression. Further, the expression of an upstream regulator of SNAIL, MTA3 [62], was also blocked by BAD.A reversal or inhibition of EMT by BAD was further evident by its stimulatory effect on E-cadherin expression. Increased E-cadherin expression correlates with better prognosis in many cancer types [61], and E-cadherin is inhibited by SNAIL at the transcriptional level [61]. BAD also decreased AKT activation which promotes migration of breast cancer cells [63], this effect was nullified by BAD Si RNA. Further phosphorylation of β-catenin,a prognostic marker in breast cancer [64] by GSK-3β induces its degradation [65], and GSK-3β is inactivated by AKT/PKB mediated phosphorylation. Therefore, we measured the effects of BAD on GSK-3β and BAD overexpression significantly activated GSK-3β presumably due to AKT inactivation.

The role of BAD in other cancers

A role for BAD as a good prognostic indicator has also been reported for gastric, hepatocellular and colon carcinomas as well [54,66-70]indicating an anti-invasive role *in vivo*. However, contrasting results have been described as well, where BAD over-expression accelerated tumor growth in prostate cancer C4-2 xenografts [71].

The mechanisms by which BAD regulates expression of several proteins remains to be elucidated and may relate at least in part to its ability to bind with c-Jun and inhibit the latter's transcriptional effects. Furthermore, BAD may indirectly effect gene regulation through inhibition of cyclin D1, which is known to control transcription [38-41,48]and also through β-catenin. The transcription factor Sp1that regulates SNAIL expression is another activator of the cyclin D1 promoter [43]. Its inhibition by BAD could potentially be yet another mechanism by which BAD regulates the expression of cyclin D1 in cancer cells. These results collectively demonstrate the ability of the cell to utilize complex mechanisms to counteract the expression of pro-tumorigenic cyclin D1and c-jun through regulation of molecules like BAD.

Conclusion

In vitro data support a pro-invasive role for BCL-2 and its pro-survival partner BCLxL [33,72,73]. Data which suggests an anti-invasive role for BCL-2, *in vitro* was also reported [74]. Most *in vitro* results suggest an anti-apoptotic role for BCL-2, which would predict an adverse prognosis, in contrast to the actual situation in patients where BCL-2 clearly has a beneficial role (vide infra). It is also important to note that BCL-2 and its antagonist partner BAD have similar protective roles in patients with breast cancer. Taken together, the above findings and our data clearly indicate that detailed studies on the role of BCL-2 family proteins in EMT and metastasis are urgently required. The mechanisms, by which BAD or BCL-2 decrease the metastatic potential of breast cancer cells *in vivo*, are currently unknown; perhaps their inhibitory effects on the cell cycle regulation of EMT proteins may impede the emergence of invasive clones or their precursor cancer stem cells. Given the data discussed above, treatment of breast cancer with synthetic BCL-2 antagonists may have effects contradictory to that anticipated, and actually could result in an adverse clinical outcome.

Acknowledgement

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