Journal of Clinical & Cellular Immunology

Wimalasena et al., J Clin Cell Immunol 2015, 6:4
http://dx.doi.org/10.4172/2155-9899.1000352

Mini Review Open Access

Roles of BCL-2 and BAD in Breast Cancer

Wimalasena J[1*], Cekanova M[2], Pestell RG[3] and Fernando RI[4]

[1]*Department of Physiology, Faculty of Medicine, The University of Peradeniya, Peradeniya, Sri Lanka*

[2]*The University of Tennessee, College of Veterinary Medicine, 2407 River Drive A122, Knoxville, Tennessee, 37996, USA*

[3]*Department of Cancer Biology, Sidney Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, PA, USA*

[4]*Laboratory of Tumor Immunology and Biology, Center for Cancer Research, NCI, NIH, Bethesda, MD, USA*

[*]**Corresponding author:** Wimalasena J, Department of Physiology, Faculty of Medicine, The University of Peradeniya, Peradeniya, Sri Lanka, Tel: +94812392501; E-mail: jwimalas@gmail.com

Received date: May 25, 2015; **Accepted date:** August 28, 2015; **Published date:** August 29, 2015

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].

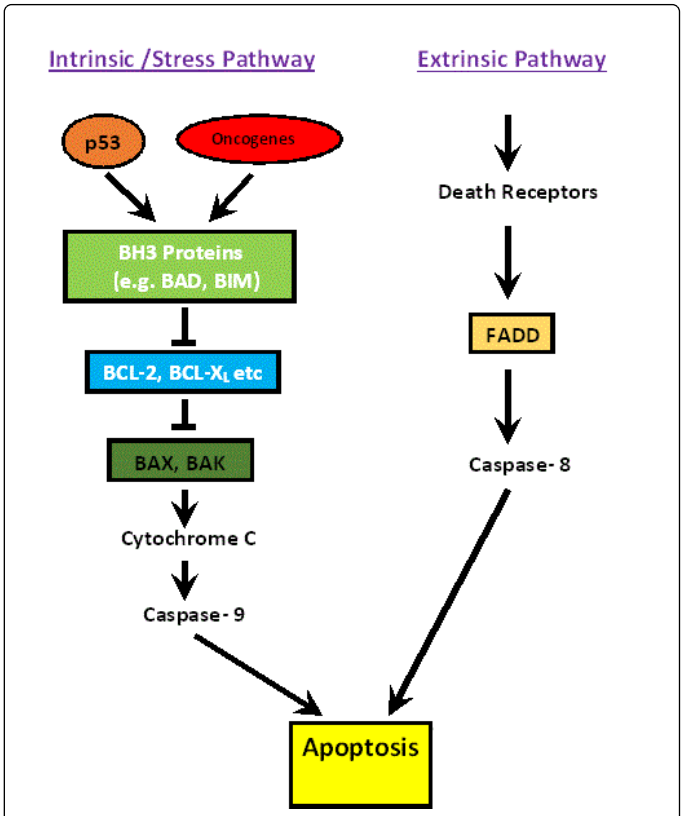

Figure 1A: Intrinsic and extrinsic pathways in apoptosis.

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.

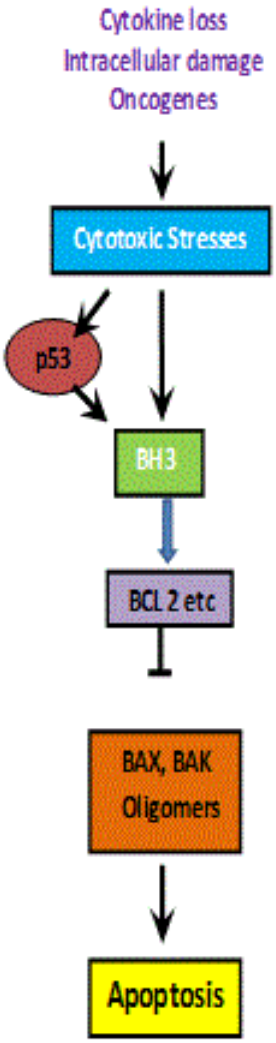

Figure 1B: Apoptosis of damaged intracellular oncogenes.

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].

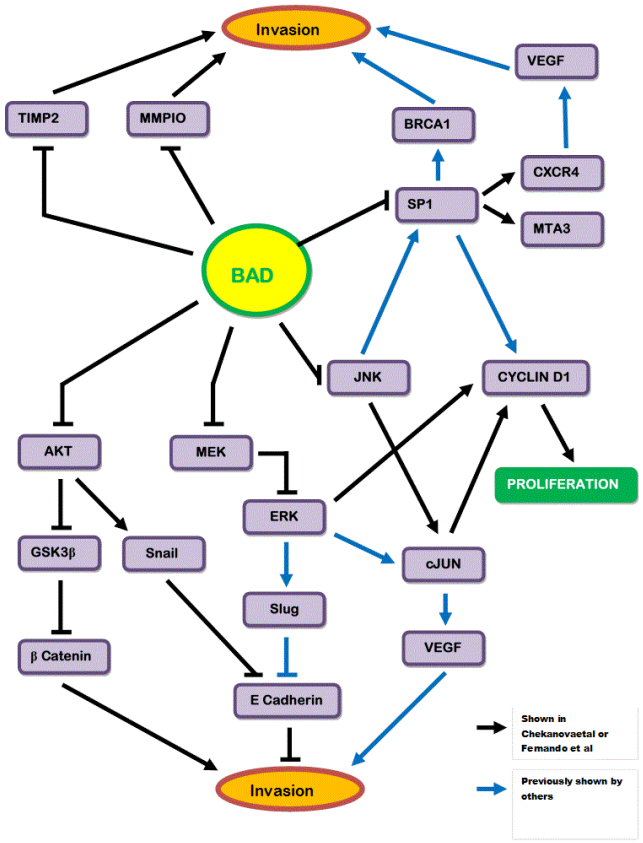

Figure 2: BAD and EMT regulation.

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

JW would like to thank Dr Shalitha Samarakoon, Department of Forensic Medicine, University of Peradeniya for preparing the figures.

References

1. Adams JM, Cory S (2007) The Bcl-2 apoptotic switch in cancer development and therapy. Oncogene 26: 1324-1337.
2. Cory S, Huang DC, Adams JM (2003) The Bcl-2 family: roles in cell survival and oncogenesis. Oncogene 22: 8590-8607.
3. Strasser A, O'Connor L, Dixit VM (2000) Apoptosis signaling. Annu Rev Biochem 69: 217-245.
4. Nhan TQ, Liles WC, Schwartz SM (2006) Physiological functions of caspases beyond cell death. Am J Pathol 169: 729-737.
5. Chun HJ, Zheng L, Ahmad M, Wang J, Speirs CK, et al. (2002) Pleiotropic defects in lymphocyte activation caused by caspase-8 mutations lead to human immunodeficiency. Nature 419: 395-399.
6. Miura M, Chen XD, Allen MR, Bi Y, Gronthos S, et al. (2004) A crucial role of caspase-3 in osteogenic differentiation of bone marrow stromal stem cells. J Clin Invest 114: 1704-1713.
7. Galluzzi L, Joza N, Tasdemir E, Maiuri MC, Hengartner M, et al. (2008) No death without life: vital functions of apoptotic effectors. Cell Death Differ 15: 1113-1123.
8. Saleh M, Vaillancourt JP, Graham RK, Huyck M, Srinivasula SM, et al. (2004) Differential modulation of endotoxin responsiveness by human caspase-12 polymorphisms. Nature 429: 75-79.
9. Vahsen N, Candé C, Brière JJ, Bénit P, Joza N, et al. (2004) AIF deficiency compromises oxidative phosphorylation. EMBO J 23: 4679-4689.
10. Huang KJ, Ku CC, Lehman IR (2006) Endonuclease G: a role for the enzyme in recombination and cellular proliferation. Proc Natl Acad Sci U S A 103: 8995-9000.
11. Zhang Y, Rosenberg S, Wang H, Imtiyaz HZ, Hou YJ, et al. (2005) Conditional Fas-associated death domain protein (FADD): GFP knockout mice reveal FADD is dispensable in thymic development but essential in peripheral T cell homeostasis. J Immunol 175: 3033-3044.
12. Yeh WC, de la Pompa JL, McCurrach ME, Shu HB, Elia AJ, et al. (1998) FADD: essential for embryo development and signaling from some, but not all, inducers of apoptosis. Science 279: 1954-1958.
13. Huang DC, O'Reilly LA, Strasser A, Cory S (1997) The anti-apoptosis function of Bcl-2 can be genetically separated from its inhibitory effect on cell cycle entry. EMBO J 16: 4628-4638.
14. Chattopadhyay A, Chiang CW, Yang E (2001) BAD/BCL-[X(L)] heterodimerization leads to bypass of G0/G1 arrest. Oncogene 20: 4507-4518.
15. Crescenzi E, Palumbo G, Brady HJ (2003) Bcl-2 activates a programme of premature senescence in human carcinoma cells. Biochem J 375: 263-274.
16. Linette GP, Li Y, Roth K, Korsmeyer SJ (1996) Cross talk between cell death and cell cycle progression: BCL-2 regulates NFAT-mediated activation. Proc Natl Acad Sci U S A 93: 9545-9552.
17. Trisciuoglio D, Gabellini C, Desideri M, Ziparo E, Zupi G, et al. (2010) Bcl-2 regulates HIF-1alpha protein stabilization in hypoxic melanoma cells via the molecular chaperone HSP90. PLoS One 5: e11772.
18. Vairo G, Innes KM, Adams JM (1996) Bcl-2 has a cell cycle inhibitory function separable from its enhancement of cell survival. Oncogene 13: 1511-1519.
19. Vairo G, Soos TJ, Upton TM, Zalvide J, DeCaprio JA, et al. (2000) Bcl-2 retards cell cycle entry through p27(Kip1), pRB relative p130, and altered E2F regulation. Mol Cell Biol 20: 4745-4753.
20. Krajewski S, Tanaka S, Takayama S, Schibler MJ, Fenton W, et al. (1993) Investigation of the subcellular distribution of the bcl-2 oncoprotein: residence in the nuclear envelope, endoplasmic reticulum, and outer mitochondrial membranes. Cancer Res 53: 4701-4714.
21. Akao Y, Otsuki Y, Kataoka S, Ito Y, Tsujimoto Y (1994) Multiple subcellular localization of bcl-2: detection in nuclear outer membrane, endoplasmic reticulum membrane, and mitochondrial membranes. Cancer Res 54: 2468-2471.
22. Yeretssian G, Correa RG, Doiron K, Fitzgerald P, Dillon CP, et al. (2011) Non-apoptotic role of BID in inflammation and innate immunity. Nature 474: 96-99.
23. Park SH, Kim H, Song BJ (2002) Down regulation of bcl-2 expression in invasive ductal carcinomas is both estrogen- and progesterone-receptor dependent and associated with poor prognostic factors. Pathol Oncol Res 8: 26-30.
24. Castiglione F, Sarotto I, Fontana V, Destefanis M, Venturino A, et al. (1999) Bcl-2, p53 and clinical outcome in a series of 138 operable breast cancer patients. Anticancer Res 19: 4555-4563.
25. Callagy GM, Webber MJ, Pharoah PD, Caldas C (2008) Meta-analysis confirms BCL-2 is an independent prognostic marker in breast cancer. BMC Cancer 8: 153.
26. Ali HR, Dawson SJ, Blows FM, Provenzano E, Leung S, et al. (2012) A Ki67/BCL-2 index based on immunohistochemistry is highly prognostic in ER-positive breast cancer. J Pathol 226: 97-107.
27. Cekanova M, Fernando RI, Siriwardhana N, Sukhthankar M, De la Parra C, et al. (2015) BCL-2 family protein, BAD is down-regulated in breast cancer and inhibits cell invasion. Exp Cell Res 331: 1-10.
28. Jiang P, Du W, Heese K, Wu M (2006) The Bad guy cooperates with good cop p53: Bad is transcriptionally up-regulated by p53 and forms a Bad/p53 complex at the mitochondria to induce apoptosis. Mol Cell Biol 26: 9071-9082.
29. Danial NN (2008) BAD: undertaker by night, candyman by day. Oncogene 27 Suppl 1: S53-70.
30. Danial NN, Gramm CF, Scorrano L, Zhang CY, Krauss S, et al. (2003) BAD and glucokinase reside in a mitochondrial complex that integrates glycolysis and apoptosis. Nature 424: 952-956.
31. Seo SY, Chen YB, Ivanovska I, Ranger AM, Hong SJ, et al. (2004) BAD is a pro-survival factor prior to activation of its pro-apoptotic function. J Biol Chem 279: 42240-42249.
32. Fernando R, Foster JS, Bible A, Ström A, Pestell RG, et al. (2007) Breast cancer cell proliferation is inhibited by BAD: regulation of cyclin D1. J Biol Chem 282: 28864-28873.
33. Vogt PK (2002) Fortuitous convergences: the beginnings of JUN. Nat Rev Cancer 2: 465-469.
34. Eferl R, Wagner EF (2003) AP-1: a double-edged sword in tumorigenesis. Nat Rev Cancer 3: 859-868.
35. Fernando RI, Wimalasena J (2004) Estradiol abrogates apoptosis in breast cancer cells through inactivation of BAD: Ras-dependent nongenomic pathways requiring signaling through ERK and Akt. Mol Biol Cell 15: 3266-3284.
36. Matsumura I, Kitamura T, Wakao H, Tanaka H, Hashimoto K, et al. (1999) Transcriptional regulation of the cyclin D1 promoter by STAT5: its involvement in cytokine-dependent growth of hematopoietic cells. EMBO J 18: 1367-1377.
37. Bromberg JF, Wrzeszczynska MH, Devgan G, Zhao Y, Pestell RG, et al. (1999) Stat3 as an Oncogene. cell 98: 295-303.
38. Pestell RG (2013) New roles of cyclin D1. Am J Pathol 183: 3-9.
39. Fu M, Rao M, Bouras T, Wang C, Wu K, et al. (2005) Cyclin D1 inhibits peroxisome proliferator-activated receptor gamma-mediated adipogenesis through histone deacetylase recruitment. J Biol Chem 280: 16934-16941.
40. Aggarwal P, Vaites LP, Kim JK, Mellert H, Gurung B, et al. (2010) Nuclear cyclin D1/CDK4 kinase regulates CUL4 expression and triggers neoplastic growth via activation of the PRMT5 methyltransferase. Cancer Cell 18: 329-340.
41. Bienvenu F, Jirawatnotai S, Elias JE, Meyer CA, Mizeracka K, et al. (2010) Transcriptional role of cyclin D1 in development revealed by a genetic-proteomic screen. Nature 463: 374-378.

42. Fukase K, Ohtsuka H, Onogawa T, Oshio H, Ii T, et al. (2008) Bile acids repress E-cadherin through the induction of Snail and increase cancer invasiveness in human hepatobiliary carcinoma. Cancer Sci 99: 1785-1792.
43. Marampon F, Casimiro MC, Fu M, Powell MJ, Popov VM, et al. (2008) Nerve Growth factor regulation of cyclin D1 in PC12 cells through a p21RAS extracellular signal-regulated kinase pathway requires cooperative interactions between Sp1 and nuclear factor-kappaB. Mol Biol Cell 19: 2566-2578.
44. Hibberts NA, Simpson DJ, Bicknell JE, Broome JC, Hoban PR, et al. (1999) Analysis of cyclin D1 (CCND1) allelic imbalance and overexpression in sporadic human pituitary tumors. Clin Cancer Res 5: 2133-2139.
45. Pelosio P, Barbareschi M, Bonoldi E, Marchetti A, Verderio P, et al. (1996) Clinical significance of cyclin D1 expression in patients with node-positive breast carcinoma treated with adjuvant therapy. Ann Oncol 7: 695-703.
46. Li Z, Jiao X, Wang C, Ju X, Lu Y, et al. (2006) Cyclin D1 induction of cellular migration requires p27(KIP1). Cancer Res 66: 9986-9994.
47. Jiao X, Katiyar S, Willmarth NE, Liu M, Ma X, et al. (2010) c-Jun induces mammary epithelial cellular invasion and breast cancer stem cell expansion. J Biol Chem 285: 8218-8226.
48. Fujita N, Kajita M, Taysavang P, Wade PA (2004) Hormonal regulation of metastasis-associated protein 3 transcription in breast cancer cells. Mol Endocrinol 18: 2937-2949.
49. Sakamaki T, Casimiro MC, Ju X, Quong AA, Katiyar S, et al. (2006) Cyclin D1 determines mitochondrial function in vivo. Mol Cell Biol 26: 5449-5469.
50. Yan M1, Rayoo M, Takano EA; kConFab Investigators, Fox SB (2011) Nuclear and cytoplasmic expressions of ERβ1 and ERβ2 are predictive of response to therapy and alters prognosis in familial breast cancers. Breast Cancer Res Treat 126: 395-405.
51. Shaaban AM, Green AR, Karthik S, Alizadeh Y, Hughes TA, et al. (2008) Nuclear and cytoplasmic expression of ERbeta1, ERbeta2, and ERbeta5 identifies distinct prognostic outcome for breast cancer patients. Clin Cancer Res 14: 5228-5235.
52. Yan M, Rayoo M, Takano EA; kConFab Investigators, Fox SB (2011) Nuclear and cytoplasmic expressions of ERβ1 and ERβ2 are predictive of response to therapy and alters prognosis in familial breast cancers. Breast Cancer Res Treat 126: 395-405.
53. Al-Bazz YO, Underwood JC, Brown BL, Dobson PR (2009) Prognostic significance of Akt, phospho-Akt and BAD expression in primary breast cancer. Eur J Cancer 45: 694-704.
54. Cannings E, Kirkegaard T, Tovey SM, Dunne B, Cooke TG, et al. (2007) Bad expression predicts outcome in patients treated with tamoxifen. Breast Cancer Res Treat 102: 173-179.
55. Agrup M, Stal O, Olsen K, Wingren S (2000) C-erbB-2 overexpression and survival in early onset breast cancer. Breast Cancer Res Treat 63: 23-29.
56. Yu B, Sun X, Shen HY, Gao F, Fan YM, et al. (2010) Expression of the apoptosis-related genes BCL-2 and BAD in human breast carcinoma and their associated relationship with chemosensitivity. J Exp Clin Cancer Res 29: 107.
57. Katiyar S, Jiao X, Wagner E, Lisanti MP, Pestell RG (2007) Somatic excision demonstrates that c-Jun induces cellular migration and invasion through induction of stem cell factor. Mol Cell Biol 27: 1356-1369.
58. Nakopoulou L, Katsarou S, Giannopoulou I, Alexandrou P, Tsirmpa I, et al. (2002) Correlation of tissue inhibitor of metalloproteinase-2 with proliferative activity and patients' survival in breast cancer. Mod Pathol 15: 26-34.
59. Bergers G, Hanahan D (2008) Modes of resistance to anti-angiogenic therapy. Nat Rev Cancer 8: 592-603.
60. Arya M, Ahmed H, Silhi N, Williamson M, Patel HR (2007) Clinical importance and therapeutic implications of the pivotal CXCL12-CXCR4 (chemokine ligand-receptor) interaction in cancer cell migration. Tumour Biol 28: 123-131.
61. Zeisberg M, Neilson EG (2009) Biomarkers for epithelial-mesenchymal transitions. J Clin Invest 119: 1429-1437.
62. Fujita N, Kajita M, Taysavang P, Wade PA (2004) Hormonal regulation of metastasis-associated protein 3 transcription in breast cancer cells. Mol Endocrinol 18: 2937-2949.
63. Ju X, Katiyar S, Wang C, Liu M, Jiao X, et al. (2007) Akt1 governs breast cancer progression in vivo. Proc Natl Acad Sci U S A 104: 7438-7443.
64. Geyer FC, Lacroix-Triki M, Savage K, Arnedos M, Lambros MB, et al. (2011) β-Catenin pathway activation in breast cancer is associated with triple-negative phenotype but not with CTNNB1 mutation. Mod Pathol 24: 209-231.
65. Wu D, Pan W (2010) GSK3: a multifaceted kinase in Wnt signaling. Trends Biochem Sci 35: 161-168.
66. Galmiche A, Ezzoukhry Z, François C, Louandre C, Sabbagh C, et al. (2010) BAD, a proapoptotic member of the BCL-2 family, is a potential therapeutic target in hepatocellular carcinoma. Mol Cancer Res 8: 1116-1125.
67. Jeong EG, Lee SH, Kim SS, Ahn CH, Yoo NJ, et al. (2007) Immunohistochemical analysis of phospho-BAD protein and mutational analysis of BAD gene in gastric carcinomas. APMIS 115: 976-981.
68. Yoo NJ, Lee JW, Jeong EG, Soung YH, Nam SW, et al. (2006) Expressional analysis of anti-apoptotic phospho-BAD protein and mutational analysis of pro-apoptotic BAD gene in hepatocellular carcinomas. Dig Liver Dis 38: 683-687.
69. Lee JW, Soung YH, Kim SY, Nam SW, Kim CJ, et al. (2004) Inactivating mutations of proapoptotic Bad gene in human colon cancers. Carcinogenesis 25: 1371-1376.
70. Sinicrope FA, Rego RL, Foster NR, Thibodeau SN, Alberts SR, et al. (2008) Proapoptotic Bad and Bid protein expression predict survival in stages II and III colon cancers. Clin Cancer Res 14: 4128-4133.
71. Smith AJ, Karpova Y, D'Agostino R Jr, Willingham M, Kulik G (2009) Expression of the Bcl-2 protein BAD promotes prostate cancer growth. PLoS One 4: e6224.
72. Pinkas J, Martin SS, Leder P (2004) Bcl-2-mediated cell survival promotes metastasis of EpH4 betaMEKDD mammary epithelial cells. Mol Cancer Res 2: 551-556.
73. Rahman KM, Sarkar FH, Banerjee S, Wang Z, Liao DJ, et al. (2006) Therapeutic intervention of experimental breast cancer bone metastasis by indole-3-carbinol in SCID-human mouse model. Mol Cancer Ther 5: 2747-2756.
74. Ke H, Parron VI, Reece J, Zhang JY, Akiyama SK, et al. (2010) BCL-2 inhibits cell adhesion, spreading, and motility by enhancing actin polymerization. Cell Res 20: 458-469.