Emerging role of BAD and DAD1 as potential targets and biomarkers in cancer (Review)

YULOU LUO¹, YOU WU², HAI HUANG¹, NA YI³ and YAN CHEN³

¹First Clinical Medical College, Xinjiang Medical University, Urumqi, Xinjiang Uygur Autonomous Region 830054; ²Nursing College, Binzhou Medical University, Binzhou, Shandong 264003; ³Department of Biochemistry and Molecular Biology, School of Basic Medical Sciences, Xinjiang Medical University, Urumqi, Xinjiang Uygur Autonomous Region 830017, P.R. China

Received July 9, 2021; Accepted September 1, 2021

DOI: 10.3892/ol.2021.13072

Abstract. As key regulators of apoptosis, BAD and defender against apoptotic cell death 1 (DAD1) are associated with cancer initiation and progression. Multiple studies have demonstrated that BAD and DAD1 serve critical roles in several types of cancer and perform various functions, such as participating in cellular apoptosis, invasion and chemosensitivity, as well as their role in diagnostic/prognostic judgement, etc. Investigating the detailed mechanisms of the cancerous effects of the two proteins will contribute to enriching the options for targeted therapy, and may improve clinical treatment of cancer. The present review summarizes research advances regarding the associations of BAD and DAD1 with cancer, and a hypothesis on the feasible relationship and interaction mechanism between the two proteins is proposed. Furthermore, the present review highlights the potential of the two proteins as therapeutic targets and valuable diagnostic and prognostic biomarkers.

Contents
1. Introduction
2. Physiological characteristics and cancerous activity of BAD
3. Overview of DAD1 and its cancerous role
4. Feasible relation and interaction mechanism between BAD and DAD1
5. BAD and DAD1 as potential targets and biomarkers in cancer
6. Conclusion

1. Introduction

In recent years, the achievements of targeted therapy for cancer treatment have been self-evident (1,2). The difference between targeted therapy and conventional chemotherapy is that the cytotoxicity of normal cells is greatly reduced in targeted therapy due to its specific targeting (3,4). With the development of molecular biology and the gradual unfolding of mechanisms employed by tumor-associated factors, molecular targeted therapy will become the principal direction of antitumor treatment. However, the heterogeneity of drug resistance in tumor cells and the limited number of alternative targets are also clinical bottlenecks, which must be addressed (5). Therefore, it is urgent to identify and elucidate the abnormally activated or silenced signaling pathways in cancer cells, which are useful in exploring valuable therapeutic targets.

Tumorigenesis partly results from dysregulation of apoptosis, leading to apoptosis evasion of cells, which then become cancerous (6,7). There are three pathways to regulate apoptosis (death receptor cell death pathway, endoplasmic reticulum (ER) cell death pathway and mitochondrial cell death...
pathway), among which the mitochondrial cell death pathway is mainly regulated by the Bcl-2 family proteins (8). BAD, a member of the Bcl-2 family, acts as the main pro-apoptotic protein that regulates the cellular survival-apoptosis balance, and its phosphorylation may contribute to cancer progression (9). In addition, defender against apoptotic cell death 1 (DADI), a subunit of oligosaccharyltransferase (OST) acting on N-glycosylation residing in the ER (10), is a negative regulator of programmed cell death associated with the ER cell death pathway (11). Increasing experimental evidence has indicated deep engagement of the two proteins in tumorigenesis, particularly in cellular apoptosis, invasion, chemosensitivity and diagnostic/prognostic judgment. Therefore, their key roles in signaling transduction pathways and their close association with cellular behavior may provide insights and novel alternative molecular agents for targeted therapy of cancer.

2. Physiological characteristics and cancerous activity of BAD

Bcl-2 family and overview of BAD. The Bcl-2 family, a group of cooperative proteins, exerts a great influence on the regulation of apoptosis via the mitochondrial cell death pathway (12,13). Bcl-2 homology domains (BHI-4) have been determined to be a collective characteristic of Bcl-2 family members, and two of the member proteins can form homo- or heterodimers as an essential functional unit to promote or suppress apoptosis (14). The effects of the Bcl-2 family are antagonistic, which means that some of the members, including BAD, Bax and BH3 interacting domain death agonist, serve a pro-apoptotic role in cellular modulation, while others, including Bcl-2, myoid cell leukemin-1 (Mcl-1) and Bcl-xL, appear to suppress apoptosis (12). The mechanism underlying the regulation of the mitochondrial cell death pathway has been demonstrated to be the action of the Bcl-2 family proteins, which determine the permeability of transition pore embedded in the mitochondrial membrane (15,16). Upstream apoptosis signals make the non-selective pore an irreversible access point between the cytoplasm and mitochondrial matrix mediated by the Bcl-2 family of proteins (15,16). Mitochondrional matrix-deprived cytochrome c combines with apoptotic protease activating factor-1 and caspase-9 proenzyme to form apoposomes in the cytoplasm, which then activate caspase-9, triggering a cascade reaction of apoptotic proteases to subsequently induce apoptosis (15,16).

BAD was first cloned from a mouse cDNA library, and the homologous human gene was cloned later (17). BAD comprises 168 amino acids, of which Ser112, Ser136 and Ser155 are the three known regulatory residues, which can be sequentially phosphorylated by several kinase proteins (17). Among them, ribosomal protein S6 kinase and cAMP dependent protein kinase (PKA) mediate the phosphorylation of Ser112, Akt mediates the phosphorylation of Ser136 (18), and PKA preferentially mediates the phosphorylation of Ser155, which is located in the center of the BH3 domain (19-21). Normal phosphorylation at the three residues helps to maintain cytoplasmic sequestration of BAD, and thus, apoptosis is attenuated (22). Phosphorylation of BAD at Ser26 by the IkB kinase complex inhibits the pro-apoptotic activity of BAD (23). A novel synthetic compound, N-cyclopentyl-3-((4-(2,3-dichlorophenyl) piperazin-1-yl) (2-hydroxyphenyl) methyl) benzamide, a specific inhibitor of BAD phosphorylation at Ser99, could suppress the vitality of cancer cells in vivo and in vitro (24). Normally, phosphorylated BAD (p-BAD) combines with the amphipathic groove of chaperone 14-3-3 (25). BAD is different from most Bcl-2 family members, as it has no C-terminal transmembrane domain that anchors the outer mitochondrial membrane and nuclear envelope (17). Therefore, BAD is sequestered in the cytoplasm, and apoptosis is inhibited (22).

In the presence of survival signals, dephosphorylated BAD, which is generated by phosphatases, disassociates from 14-3-3 and begins to displace Bax in the Bcl-2/Bax or Bcl-xL/Bax heterodimer to form a Bcl-2/BAD or Bcl-xL/BAD heterodimer via the BH3 homologous domain in a concentration-dependent manner (17). Additionally, free Bax homodimerization is increased, and apoptosis is initiated by the Bax homodimer integrated into the outer mitochondrial membrane (17).

To simplify, subcellular relocation to the mitochondria of BAD that triggers apoptosis is tied to its phosphorylation status at the three amino acid residues. The switch between p-BAD and dephosphorylated BAD determines its role in the pathway, i.e., pro-apoptosis or pro-survival, wherein 14-3-3, several kinases and phosphatases are key regulators (26). In addition to apoptosis-related roles, BAD also has multiple non-apoptotic functions, such as regulation of the cell cycle (27-29), autophagy (30), immune engagement (31,32), glucose metabolism (15,33), and control of localized translation (34), all of which are closely associated with its cellular effects in cancer. Among all these pathways converging due to BAD, the phosphorylation status coordinates the multiple functions of BAD, and its BH3 domain is utilized. p-BAD manages cytoplasmic sequestration to prevent apoptosis, while other metabolic pathways, such as suppression of gluconeogenesis and activation of oxidative metabolism of glucose in the mitochondria of liver cells (33,35), are activated to promote survival according to different ligands matched with BH3. Therefore, the central status of BAD in several apoptosis-related and other metabolism-related signaling pathways may result in it being an appealing target in cancer.

Expression and function of BAD in cancer. BAD is usually expressed in the colon, stomach, prostate, kidney, brain and adipose tissues (36). The tumor-associated effects of abnormal levels of p-BAD are reflected in several aspects as described subsequently.

Cell proliferation, survival and apoptosis. Several types of cancer cells have been detected to exhibit higher levels of p-BAD than corresponding immortalized normal cells in vivo and in vitro, and cell apoptosis or survival is regulated by the BAD phosphorylation status (17,37). The effects of p-BAD on cell proliferation, survival and apoptosis are summarized in Table I.

Sastry et al (38) determined that there are two signaling pathways that phosphorylate BAD to protect prostate cancer cells from apoptosis under the stimulus of epidermal growth factor. One signaling pathway induces phosphorylation at Ser112 via Ras/MEK. Another signaling pathway induces phosphorylation at Ser136 via Ras-related C3 botulinum toxin.
substrate (Rac)/P21-activated kinase 1 (PAK1) (38). However, She et al (39) reported that phosphorylation of Ser112 and Ser136 could also be mediated by the EGFR/MEK/MAPK and phosphatidylinositol 3-kinase/Akt signaling pathways, respectively. Furthermore, survival signaling-induced kinases, such as PAK1 and Raf, promote the proliferation of cancer cells in the presence of wild-type BAD (39). Polzien et al (40) determined that Raf kinases could phosphorylate BAD at Ser134 to promote cell proliferation in B-Raf-V600E-containing tumor cells. Furthermore, replacement of Ser134 with alanine leads to phosphorylation, suggesting that BAD phosphorylation at Ser134 is essential for sufficient cell proliferation (27). Myeloproliferative neoplasms (MPNs) may result in activating mutations of the Janus kinase (JAK) gene (41-43). A previous study has reported that phosphorylation of BAD induced by JAK2 promotes cell proliferation in JAK-depleted MPN cells (44). Additionally, in cells sensitive to JAK inhibitor, treatment with JAK inhibitor results in dephosphorylation of BAD and affects its combination with Bcl-xL, initiating apoptosis (44).

Huang et al (45) revealed that overexpression of BAD inhibits the proliferation of tumor cells in vitro and reduces tumor volume in vivo by promoting cell apoptosis and suppressing cell proliferation. Smith et al (46) observed contrasting results compared with Huang et al (45) in prostate cancer cells, wherein increased BAD expression could promote cell proliferation and silencing of BAD by short hairpin RNA suppressed cell proliferation. Stickles et al (47) demonstrated that protein phosphatase 2C (PP2C) deletion leads to higher levels of p-BAD at Ser155, which is beneficial for cell proliferation in vitro. Sastry et al (9) determined that the phosphorylation of BAD is indispensable for the survival of cancer stem cells (CSCs). Deficient expression of p-BAD induces apoptosis of CSCs, which could be reversed by the BH3 mimetic ABT-737, revealing that only the BH3 homologous domain is essential in BAD (9). Furthermore, the downregulation of BAD weakens the frequency and renewal capacity of CSCs (9). Kulik (48) reported that upregulated levels of p-BAD, together with Mcl-1 mediated by the activation of the adrenocorticotropin β2 (ADRB2)/PKA signaling

| First author/s, year | Tissue/cell type | Interaction of (p-)BAD with relevant genes | Effect (Refs.) |
|----------------------|-----------------|------------------------------------------|---------------|
| Sastry et al, 2006   | Prostate cancer | Ras/MEK and Rac/PAK1 signaling pathways mediate the phosphorylation of BAD at Ser112 and Ser136, respectively. | Prevents apoptosis (38) |
| Smith et al, 2009    |                 | Silencing of BAD by shRNA. | Suppresses cell proliferation (46) |
| Kulik, 2019          |                 | Upregulation of p-BAD by the activation of the ADRB2/PKA signaling pathway. | Inhibits apoptosis (48) |
| She et al, 2005      | PTEN-deficient tumor cell lines | Phosphorylation defect of BAD at Ser112 and Ser136 via EGFR/MEK/MAPK and PI3K/Akt signaling pathways, respectively. | Promotes apoptosis (39) |
| Polzien et al, 2011  | B-Raf-mutated cancer cell lines | Raf kinase phosphorylates BAD at Ser134. | Promotes proliferation (40) |
| Winter et al, 2014   | JAK-depleted myeloproliferative neoplasms | JAK2 phosphorylates BAD. | Promotes survival (44) |
| Stickles et al, 2015 | Colorectal adenocarcinoma, breast cancer, endometrial adenocarcinoma and ovarian cancer | PP2C deletion induces higher levels of p-BAD at Ser155. | Promotes growth (47) |
| Mann et al, 2019     | Breast cancer | Phosphorylation of BAD at Ser118 increases Ser99 phosphorylation, 14-3-3 binding and Akt activation. BAD stimulates mitochondrial complex I activity. | Promotes growth and survival (49) |
| Lu et al, 2019       | Ovarian cancer | Artificial fusion p53-BAD locates to the mitochondria. | Promotes apoptosis (50) |

p-BAD, phosphorylated BAD.
pathway, are responsible for increased inhibition of apoptosis of prostate cancer cells. Furthermore, Mann et al (49) verified the two distinct mechanisms underlying the BAD-regulated increase in cell proliferation in breast cancer cells. Specifically, phosphorylation of BAD at Ser118 increases Ser99 phosphorylation, 14-3-3 binding and Akt activation, which promotes cell proliferation and survival. On the other hand, BAD stimulates mitochondrial oxygen consumption in a novel manner downstream of substrate entry into the mitochondria (49). BAD stimulates complex I activity that facilitates cell proliferation and sensitizes cells to apoptosis in response to complex I blockade, which may result in large but non-aggressive breast cancer (49). These results suggest that BAD-induced apoptosis may not only depend on mitochondrial membrane reactions between Bcl-2 family proteins, but it may also be associated with oxidative metabolism.

Lu et al (50) designed a novel chimeric gene fusion p53-BAD to overcome the dominant negative inhibition of wild-type p53 and multiple genetic aberrations in ovarian cancer (OVCA). By introducing Ser122A and Ser136A mutations to prevent phosphorylation at the two residues, p53-BAD constructs could always be located in the mitochondria. Furthermore, they observed that p53-BAD constructs exhibited higher pro-apoptotic activity, which was direct and rapid via the mitochondrial cell death pathway (50). This pro-apoptotic effect was consistent in several OVCA cell lines, regardless of the endogenous p53 status (50).

It is worth mentioning that Datta et al (32) explored the physiological significance of BAD phosphorylation for cell survival in vivo. They generated BAD(−)/mutant mice, in which the three phosphoregulatory residues were shifted to alanine; thus, endogenous BAD was not responsive to survival signaling. They demonstrated that growth factor-mediated BAD phosphorylation is indispensable to prevent cells from undergoing apoptotic stimuli. Notably, they validated that the levels of BAD phosphorylation via growth factors could raise the threshold at which mitochondria release cytochrome c in response to apoptotic stimuli. In summary, the levels of BAD phosphorylation may be a sensor that determines the extent to which cells undergo apoptosis, and it is also one of the mechanisms employed by survival factors to block apoptosis (32).

Invasion and distant metastasis. A previous study revealed that the expression levels of BAD in hepatocellular carcinoma (HCC) are associated with vascular invasion (51). Cekanova et al (52) reported that the levels of BAD and p-BAD in clinical breast cancer tissues are lower than those in normal breast tissues. The expression levels of several proteins associated with invasiveness (c-Jun, Akt and signal transducer and activator of transcription proteins), epithelial-mesenchymal-transition (EMT; transcription factor Sp1 and β-catenin) and metastasis (vascular endothelial growth factor) are decreased by BAD in BAD-overexpressing breast cancer cells (52). The novel anti-invasion and EMT inhibition functions of BAD are distinct from its traditional role in prompting the mitochondrial cell death pathway (52). Furthermore, 33 out of 60 clinical salivary gland adenoid cystic carcinoma cases exhibited high expression levels of BAD, and the expression levels of BAD were associated with distant metastasis (53).

Clinical characteristics. Hu et al (51) reported that the expression levels of BAD are decreased in clinical HCC tissues compared with non-tumorous adjacent tissues. The expression levels of BAD are negatively associated with several clinical characteristics, including α-fetoprotein levels, clinical stage and tumor size. Furthermore, subsequent multivariate analyses revealed that BAD can act as an independent indicator of overall HCC survival. This study demonstrated that BAD may act as a potential biomarker for poor prognosis in clinical HCC (51). Furthermore, Yu et al (54) reported similar results in small cell lung carcinoma (SCLC), and notably decreased expression levels of BAD were detected in clinical SCLC specimens compared with in neighboring non-tumorous tissues. The downregulated levels of BAD were significantly associated with overall survival, disease-free survival and several clinical characteristics (e.g., tumor recurrence, tumor size and clinical stage) of patients with SCLC (54). Multivariate analyses further indicated that BAD can act as an independent indicator of overall survival in SCLC (54). Another study on triple-negative breast cancer (TNBC) constructed a BAD pathway gene expression signature score system derived from principal component analysis to evaluate the overall expression and activation of the BAD pathway, and the results demonstrated that BAD pathway expression was associated with triple-negative status and overall survival (55).

Chemosensitivity. Chon et al (56) revealed that BAD phosphorylation has an important cisplatin sensitivity in endometrial cancer (EC) cells. Since BAD can be phosphorylated by PP2C, they observed that higher levels of p-BAD resulted in lower chemosensitivity to cisplatin in PKA small interfering RNA (siRNA) EC cells. However, p-BAD presented higher chemosensitivity to cisplatin in EC cells when PKA dephosphorylation was knocked down (56).

Interestingly, Hayakawa et al (57) reported that treatment of both cisplatin-sensitive and cisplatin-resistant OVCA cell lines with cisplatin could result in the phosphorylation of BAD at both Ser122 and Ser136, which was later determined to be mediated by the ERK and Akt cascades, respectively. Furthermore, they determined that inhibition of either of the two cascades could render OVCA cells more sensitive to cisplatin (57). Marchion et al (58) observed that the parallel effect of p-BAD increased with cisplatin resistance both in OVCA cells and in primary patients. Apart from the evidence presented, there are several other kinases or phosphatases derived from the BAD apoptosis pathway that are associated with the evolution of cisplatin resistance by exerting influence on p-BAD status. For example, Bansal et al (59) selected CDK1 and PP2C to validate OVCA sensitivity to cisplatin. Lower expression levels of PP2C and higher expression levels of CDK1 increased cisplatin resistance. In addition, they revealed that downregulation of CDK1 by siRNA infection increased cisplatin sensitivity (59). Taken together, these results demonstrated that inhibition of p-BAD enhanced chemosensitivity in OVCA chemotherapy (57-59).

Yu et al (60) reported that Bcl-2(-)BAD(+) breast cancer cells exhibited higher chemosensitivity to four types of anticancer
drugs (epirubicin, 5-fluorouracil, navelbine and cisplatin) than other breast cancer cells [Bcl-2(+)-BAD(-) or Bcl-2(+)-BAD(+)]. Therefore, the joint detection of Bcl-2 and BAD expression may help in chemotherapy drug selection. Boac et al (55) demonstrated that patients with TNBC express higher levels of p-BAD isoforms than patients with breast cancer that is not triple negative, and the levels of p-BAD-Ser136 are different, while the differences in p-BAD-Ser12 and p-BAD-Ser155 levels are not significant. Furthermore, the study demonstrated that targeted inhibition of kinases known to phosphorylate BAD results in increased sensitivity to nonspecific chemotherapeutic agents, such as cisplatin, in vitro (55). In a later report, BAD enhanced docetaxel sensitivity by facilitating longer mitotic arrest and activating cell death in mitosis in vivo and in vitro (61). Notably, death in mitosis has been observed to be an abnormal type of apoptosis, one that was dependent on Bcl-2 interaction and caspase activation; in fact, it was necroptosis (61). This type of BAD-enhanced docetaxel-mediated necroptotic cell death is dependent on reactive oxygen species, which indicates the chemosensitivity amplification effect of BAD in breast cancer (61).

In acute myeloid leukemia (AML), Yu et al (62) developed a system to quantify the chemosensitivity of dormant AML cells. The results revealed that two BAD mimetics, ABT-199 and ABT-737, were both able to effectively target dormant primary leukemia cells and decrease the dormant fraction of leucineaminopeptidase cells to 84 and 80%, respectively, revealing their good efficacy against cells protected by dormancy. Yiau et al (63) compared the alterations in the expression levels of CD34 and BAD in blood samples collected from patients with AML before and at day 3 after induction therapy. They observed that the average percentages of CD34 and p-BAD were higher in chemoresistant than chemosensitive samples, indicating potential CD34 signaling-associated chemotherapy resistance via p-BAD in AML (63).

Zhou et al (64) explored the relationship between low glucose levels and hypoxia-induced autophagy and chemoresistance in HCC cells. The study revealed that autophagy induced by low glucose and hypoxia in central solid tumors could reduce the protein expression levels of BAD and Bcl-2 interacting mediator of cell death (Bim), and elevated chemoresistance of HCC cells. Furthermore, they observed that chemotherapy-induced apoptosis could be reduced or promoted by RNA interference or upregulation, respectively. These results revealed that the downregulation of BAD and Bim is involved in the chemoresistance of HCC (64).

Constitutive engagement with other dominant molecules. Kim et al (65) reported that Epstein-Barr virus (EBV)-derived microRNA, miRNA-BART20-5p, inhibits BAD-mediated caspase-3-dependent apoptosis by targeting BAD in gastric carcinoma. The study demonstrated that BAD could act as a potential target of BAD in EBV-associated gastric carcinogenesis (65). Tang et al (66) revealed that downstream molecules, such as caspase-3, are influenced by BAD, together with cytokines affected by the NF-κB signaling pathway. Remodeling is performed when Akt is knocked out in primary liver cancer cells, which induces an altered inflammatory response and apoptosis. Additionally, they revealed that carnosic acid nanoparticles could activate the NF-κB signaling pathway and that the overexpression of caspase-3 can moderate inflammation, as well as promote apoptosis in Akt-knockout liver cancer cells (66). Zhao et al (67) observed that downregulation of prostate cancer associated transcript 1 in esophageal cancer cells results in upregulated BAD expression, inhibits cell proliferation, decreases migration and invasion, and enhances apoptosis. Liu et al (68) reported that inhibition of protein kinase AMP-activated catalytic subunit α1 (AMPK) with dorsomorphin (a specific AMPK inhibitor) in AMPK-driven hematological cancer types upregulates the expression levels of BAD to induce apoptosis.

Mansouri and Percival (69) observed that the anticancer effect of cranberry extract may result in a decrease in Akt induced in HL-60 cells, which leads to an increase in dephosphorylated BAD and subsequent activation of the intrinsic apoptosis pathway. Endo et al (70) demonstrated that the pro-apoptotic effect of curcumin is partly associated with BAD transfer from the cytoplasm to the mitochondrial membrane to trigger the mitochondrial cell death pathway by inhibiting the expression of 14-3-3 in the cytoplasm. An Akt-dependent manner was determined to be utilized to promote the dephosphorylation of BAD by curcumin (70). Furthermore, Gao et al (71) revealed that the JNK-p21/BAD signaling pathway may be involved in the process of cell proliferation inhibition and cisplatin resistance mediated by downregulation of cell death inducing DFFA like effector A protein in esophageal cancer.

3. Overview of DAD1 and its cancerous role

Biological characteristics and function of DAD1. DAD1 was originally cloned from a temperature-sensitive nephrogenic cell line (TSBN7) (11). Since a somatic mutation of DAD1 in a temperature-sensitive cell line was responsible for the induction of apoptosis when shifted to a non-permissive temperature, it was proposed that DAD1 may inhibit apoptosis and it was named based on this function (11). Subsequently, a number of studies have demonstrated another role of DAD1, acting as a subunit of OST, which participates in aspartic acid-mediated N-linked glycosylation (72,73). Human DAD1 gene mapping at chromosome 14q11-q12, encoding 113 amino acids (74), is widely expressed in thyroid, adrenal, kidney and lung cells (75). The molecular structure of DAD1 is conserved and stable during biological evolution, which indicates that it has an important function in cellular modulation (76).

Research on the function of DAD1 mainly focuses on two aspects: DAD1 as a crucial component of OST in catalyzing N-linked glycosylation and DAD1 as a pivotal negative regulator in programmed cell death. N-linked glycosylation is a type of co-translational or post-translational modification of proteins. The newly synthesized N-glycosylated chains are added to the asparagine residues of the peptide chains by the OST complex. However, to the best of our knowledge, the mechanism by which DAD1 regulates apoptosis remains unclear. One of the apoptotic mechanisms proposed is the DAD1 loss-induced N-linked glycosylation block (77). Researchers have demonstrated that deletion of DAD1 in hamster TSBN7 cells induces apoptosis (11). However, cycloheximide (a protein synthesis inhibitor) inhibits this process, while Bcl-2, a conventional anti-apoptotic
molecule, does not (11). Notably, apoptosis induced by DADI deletion could not be rescued by Bcl-2, suggesting that DADI may serve a pivotal role in the ER pathway rather than the mitochondrial and death receptor pathways. Brewster et al (78) and Hong et al (79) have reported that DADI is necessary for development beyond the blastocyst stage, and its deletion promotes apoptosis in mouse embryos. However, in terms of T cell development and activation, DADI enhances T cell proliferation instead of preventing apoptosis in vivo (80).

Gene cloning and/or functional exploration of heterogenetic DADI has been performed and validated in other species, including *Chlamydomonas* (81), *Hessian fly* Mayetiola destructor (82) and bay scallop Argopecten irradians (83). Furthermore, enhanced expression levels of DADI have been suggested to be accountable for unanticipated stimulus in case of cell injury or apoptosis (83). Zhang et al (76) demonstrated that DADI in *Drosophila melanogaster* (DmDADI) contributes to tissue enrichment, and upregulation of DmDADI facilitates N-linked glycosylation. Furthermore, feasible mechanisms have been proposed in terms of the deletion of DmDADI, resulting in subsequent apoptosis (76). Loss of DmDADI leads to blocked N-linked glycosylation and the accumulation of unfolded or misfolded peptide chains, and enhancement of ER stress. Defects in DmDADI, which employs the JNK pathway downstream to implement apoptosis, activate the protein kinase R-like endoplasmic reticulum kinase (Perk)/activating transcription factor 4 (Atf4) signaling pathway (76). On the other hand, compensatory proliferation of neighboring cells is driven by the Perk/Atf4 signaling pathway to sustain tissue homeostasis (76). Furthermore, Wang et al (84) cloned the homologous gene of DADI in *Chlamys farreri* (CfDADI) and observed that suppression of CfDADI with specific dsRNA injection results in increased cell apoptosis. In addition, high mRNA expression levels of CfDADI are detected in the hepatopancreas and gill, which are regarded as immune battlefields, indicating its key role in the innate immunity of scallops (84). Another study revealed the interaction between DADI and Mcl-1, an anti-apoptotic member of the Bcl-2 family, and apoptosis triggered by DADI depletion could be inhibited by Mcl-1, indicating the feasible interaction between the two apoptosis pathways (85). Notably, the anti-apoptotic effect of DADI has also been determined in humans in vivo. The expression levels of DADI are increased in neutrophils from patients with sepsis after multiple traumas (86). Furthermore, increased expression levels of the DADI gene have been observed in thymocytes of enhancer Eo/c's downstream CTCF binding sites (EACBE)⁺ mice (87). The increased DADI expression in EACBE-deleted CD4⁺CD8⁺ double-positive thymocytes can be explained by the increased interaction between enhancer Eo/c and DADI (87). EACBE is essential for the sub-topologically associating domains boundary, which separates the Tcra-Tcrld locus and the downstream region including the DADI gene (87). DADI has also been reported to be an adipokine candidate in adipose tissue (88).

### Cancerous role of DADI.

Since DADI is a negative regulator of apoptosis, the anti-apoptotic function of DADI may pose potential advantages for tumor cells to allow them to infinitely proliferate, and research on this aspect highlights the role of DADI in cancer therapy. The aberrant expressive alterations of the DADI gene in different cancer types are summarized in Table II.

| First author/s, year | Cancer type                              | Protein or mRNA alterations of DADI gene | (Refs.) |
|----------------------|------------------------------------------|-----------------------------------------|---------|
| Tanaka et al, 2001   | Hepatocellular carcinoma                 | mRNA upregulated                        | (89)    |
| Bandres et al, 2004  | Colorectal carcinoma                     | Protein upregulated                     | (90)    |
| Kulke et al, 2008    | Small bowel carcinoid tumor              | Protein upregulated                     | (91)    |
| Zhu et al, 2014      | Solid pseudopapillary tumor of pancreas  | Protein downregulated                   | (94)    |
| Schnormeier et al, 2020 | Chronic lymphocytic leukemia              | Protein upregulated                     | (95)    |
| Ayala et al, 2004;   | Prostate cancer                          | Protein upregulated                     | (96,97,100) |
| True et al, 2006;    |                                          |                                         |         |
| Bhasin, 2015         |                                          |                                         |         |
| Wang et al, 2016     | Invasive bladder cancer                  | mRNA downregulated                      | (98)    |
| Yoon et al, 2010     | Cisplatin-resistant ovarian cancer        | Protein and mRNA upregulated            | (99)    |

DADI, defender against apoptotic cell death 1.

### Table II. Aberrant expressive alterations of DADI gene in diverse cancer cells.

Cancerous expression in cancer. Tanak et al (89) identified that DADI mRNA, and antiserective factor-1, gp96 and CDC34, are highly expressed in HCC cells compared with adjacent non-tumorous liver tissues or normal liver tissues. Bandres et al (90) reported that DADI expression is upregulated in colorectal carcinoma with lymph node metastasis compared with that without lymph node metastasis, indicating the potential positive lymph node involvement. Kulke et al (91) reported that the expression levels of DADI in small bowel carcinoid tumor cells are higher than those in normal mucosa or the surrounding stroma. In addition, Wilson (92) conducted a meta-analysis to explore the genes involved in small ubiquitin-related modifier (SUMO) signaling pathways. In a meta-analysis, 10 out of 15 analyzed studies reported that DADI is co-expressed with SUMO1, which was the highest co-expression finding with SUMO1 (92). This result indicates that DADI might act as a member of the SUMO signaling pathway in cancer. Ter-Minassian et al (93)
examined genetic associations with sporadic neuroendocrine tumor (NET) risk between patients with sporadic NET and healthy controls using a custom array containing 1,536 SNPs in 355 candidate genes. Ter-Minassian et al (93) demonstrated that DAD1 contained two of the SNPs found to be associated with NET risk, including in another independent duplication set, revealing that the DAD1-associated apoptosis pathway may participate in neuroendocrine tumorigenesis. Zhu et al (94) conducted a pioneering study on the proteomics of solid pseudopapillary tumor of the pancreas (SPTP), in which isobaric
tags for relative and absolute quantitation technology integrated in liquid chromatography-tandem mass spectrometry analysis were utilized to determine differentially expressed proteins in SPTP samples compared with normal pancreatic tissues. Bioinformatics analysis resulted in 1,171 qualified proteins. Immunohistochemistry was performed to confirm the differential expression of six representative proteins and revealed the downregulation of DAD1 in SPTP specimens (94). This suggests that DADI, together with other normally expressed proteins, may be a potential biomarker of SPTP in clinical therapy. High expression levels and high variability of DAD1 have also been determined in chronic lymphocytic leukemia, one of the non-solid tumors (95).

Invasion. Ayala et al (96) observed that the expression levels of NF-xB, and its downstream agents DAD1 and pim-2 proto-oncogene, were increased in perineurial prostate cancer cells. Concurrent to the positive association between DADI expression and Gleason score in prostate adenocarcinoma, higher levels of DADI expression are associated with cancerous epithelium and perineural invasion (97). Wang et al (98) revealed that DADI is one of 21 differentially expressed genes in bladder cancer. Downregulation of the lncRNA NONHSAG045391 co-expressed with DADI has been observed in invasive bladder cancer, suggesting that NONHSAG045391 may contribute to enhanced invasiveness by targeting DADI (98). However, to the best of our knowledge, the concrete mechanism remains obscure.

Cisplatin resistance. Cisplatin treatment of a cell line derived from a clinical patient with cisplatin-resistantOVCA facilitated DADI expression at both the transcription and protein levels, indicating that cisplatin resistance might partly result from the upregulation of DADI (99).

Novel performance. The role of DADI in prostate cancer has been previously analyzed (100). First, increased DADI expression was detected in samples derived from clinical patients with prostate cancer compared with that in normal adjacent tissues. Furthermore, the study revealed that different TNM grades and Gleason grades were associated with prominent differences in DADI expression levels, which gradually increased with the progression of prostate cancer, underlying its diagnostic or prognostic role as a biomarker (100). Receiver operating characteristic curve analysis revealed that serum DADI exhibited improved specificity and sensitivity compared with prostate-specific antigen (PSA) in distinguishing low Gleason and high Gleason prostate cancer (100). Additionally, Bhasin (100) determined that ribophorin I (RPN1), another subunit of OST, is essential for DADI retention in the ER. DADI could be exocytosed with the downregulation of RPN1; thus, intervention with DADI antibody was implemented to check if DADI exocytosis was necessary. As a result, the DADI antibody exhibited markedly increased cytotoxicity compared with the control antibody in cancer cells and suppressed cancer cell survival (100). Furthermore, Bhasin (100) pointed out that this type of apoptosis was the result of extracellular DADI interacting with Fas protein. This research highlighted the potential of DADI in targeted therapy of cancer.

4. Feasible relation and interaction mechanism between BAD and DADI

Table III summarizes the genetic alterations involved in pathways with BAD and DADI participation and their effects on cellular behavior.

The apoptotic function of BAD has been greatly explored since its discovery, and the present review proposes a novel role of BAD. Al-Bazz et al (101) reported that BAD expression appeared to be nuclear in addition to its cytoplasmic location according to immunostaining in primary breast cancer. In addition, using proliferating breast cancer cell lines, Fernando et al (102) observed that endogenous BAD exists in both the cytoplasm and nucleus, whereas the levels of p-BAD in the nucleus are lower than those in the cytoplasm. Overexpression of BAD could augment the levels of p-BAD in the nucleus and inhibit the expression of cyclin D1 on the basis of phosphorylation at Ser75 and Ser99 and in combination with c-Jun (102). Using a chromatin immunoprecipitation assay, they further demonstrated that BAD could bind to the 12-O-tetradecanoylphorbol-13-acetate response element (TRE) and cAMP response element (CRE) in the promoter region of the natural cyclin D1 gene and possibly attenuate the transcriptional activity of c-Jun to suppress cyclin D1 expression (102). Activator protein 1 (API), a putative transcription factor, is a heterodimer of c-Jun and c-Fos that was also observed to be abolished by overexpression of BAD (102). These findings indicate that BAD may act as a DNA promoter binding protein and exert a transcription factor-like effect to downregulate the expression levels of targeted genes (102). Interestingly, the sequences of the DADI promoter region (~2.0 kb) were searched to predict the binding sites of transcription factors using PROMO (version 8.3; http://alggen.lsi.upc.es/cgi-bin/promo_v3/promo/promostart.cgi?dirDB=T00029). The final results are listed and shown in Table IV and Fig. 1. It was identified that there were two putative transcription factors, CRE element
binding, DNA-binding transcriptional regulator (CREB) and AP1, which are able to bind CRE and TRE, respectively, in the DAD1 promoter region. In other words, BAD, as reported by Fernando et al (102), can bind with CRE and TRE in the cyclin D1 promoter region and may also bind with the CRE and TRE elements in the DAD1 promoter region. A previous study (103) revealed that overexpression of BAD in esophageal cancer cells could inhibit DAD1 expression, which could be restored when BAD expression is downregulated. Taken together, it was hypothesized that there is a negative regulatory relationship between BAD and DAD1 [Fig. 2; (104-106)]. BAD can act as a transcription factor by binding to the promoter of DAD1 to inhibit its expression. Our hypothesis suggests that BAD can act as a transcription factor-like protein to negatively regulate the expression of targeted genes and explains the relationship between BAD and DAD1 in apoptosis regulation and the crosstalk between two apoptotic signaling pathways: the mitochondrial cell death pathway and the ER cell death pathway. Further studies should be performed to support this hypothesis.

5. BAD and DAD1 as potential targets and biomarkers in cancer

Based on the studies presented, it can be clearly inferred that BAD and DAD1 serve an indispensable role in certain types of cancer development and progression, resulting from their key regulatory functions in apoptosis pathways and a number of other abilities affecting tumorigenesis (27-33,76,83,107). Therefore, it is possible to identify the two proteins as emerging useful targets and biomarkers in carcinogenesis and tumor therapies. Aberrant expression of both proteins in cancer cells is not always the same since their expression is influenced by cancer type and several unknown factors.
However, this suggests that their ectopic expression indicates the disorder of apoptosis signaling pathways. Therefore, drugs that target BAD and DADI can be utilized to restore or suppress the activity of apoptosis signaling pathways. Furthermore, as summarized in the present review, the expression levels of BAD and DADI are associated with apoptosis (11,17,38-49,76-79), invasion enhancement and metastasis (51-53,96-98), and chemoresistance (55-64,99). Therefore, it is possible to predict the potential of apoptosis, invasion and chemoresistance by detecting the expression levels of BAD and DADI in pathological samples. Apart from their collective application value, there are several insights presented for the two proteins. As mentioned in the cell proliferation, survival and apoptosis subsection of the present review, the phosphorylation status of BAD determines the incline of the apoptosis-survival balance (17,27,37-44). Therefore, examination of the phosphorylation status of BAD may contribute to the efficacy evaluation in response to treatment. On the other hand, kinases and phosphatases associated with BAD phosphorylation status can also be exploited as potential targets due to their plausible interactions with p-BAD. Furthermore, as summarized in the clinical characteristics subsection of the present review, the expression levels of BAD are associated with a number of clinical pathological characteristics of cancer, such as overall survival, clinical stage and tumor size, and thus, BAD also acts as an independent biomarker of prognosis (51-55). In terms of DADI, the favorable proliferation inhibition by treatment with DADI antibody against prostate cancer cells in vitro has inventively demonstrated that DADI could act as a potential target in tumor therapy, together with its improved sensitivity and specificity as a diagnostic/prognostic biomarker compared with PSA (100).

6. Conclusion

The present review summarizes insights on the functional roles of BAD and DADI, particularly in cancer, and contributions to apoptosis, invasion and chemosensitivity are emphasized. However, the underlying molecular mechanisms involved require further exploration. It is gradually becoming clear that the two proteins are mainly involved in tumorigenic signaling regulation, whether as a constitutive molecule picked up by other dominant molecules or as central target of signaling pathways, affecting cellular behavior independently. Finally, a hypothesis was proposed to reveal the feasible interaction mechanism between BAD and DADI. It was highlighted that decreased DADI expression results from BAD binding to the DADI gene promoter region, exerting a novel transcription factor-like function. BAD and DADI, two emerging molecules acting as targets and biomarkers in tumorigenesis, their specific functional mechanisms and their exploitation value should be given more importance when considering the broader clinical therapeutic applications.

Acknowledgements

Not applicable.

Funding

This review was supported by Natural Science Foundation of Xinjiang Uygur Autonomous Region (grant no. 2020D01C171). The Key Discipline of the 13th Five-Year Plan (Plateau Discipline) of Xinjiang Uygur Autonomous Region also provided support to this study.

Availability of data and materials

Not applicable.

Authors' contributions

YL was the major contributor in writing of the manuscript, as well as in the preparation of the tables and figures. YW and HH searched and integrated all the supporting references, helped to refine the figures and tables and edited the manuscript. NY helped to improve the language and logic of the text in the present study. YC was responsible for the study design. Data authentication is not applicable. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. El Bali M, Bakkach J and Mechita MB: Colorectal cancer: From genetic landscape to targeted therapy. J Oncol 2021: 9918116, 2021.
2. Fan J, Shen X, Wang Y, Zhou HL, Liu G, Li YL and Xu ZX: Biomarkers for immune checkpoint therapy targeting programmed death 1 and programmed death ligand 1. Biomed Pharmacother 130: 110621, 2020.
3. Huang M, Shen A, Ding J and Geng M: Molecularly targeted cancer therapy: EGFR targeting. Front Pharmacol 12: 702445, 2021.
4. Troxell ML, Higgins JP and Kamibham N: Antineoplastic treatment and renal injury: An update on renal pathology due to cytotoxic and targeted therapies. Adv Anat Pathol 23: 310-329, 2016.
5. Sun M, Wang T, Li L, Li X, Zhai Y, Zhang J and Li W: The application of inorganic nanoparticles in molecular targeted cancer therapy: EGFR targeting. Front Pharmacol 12: 702445, 2021.
6. Eisenberg-Lerner A, Bialik S, Simon HU and Kimchi A: Life and death partners: Apoptosis, autophagy and the cross-talk between them. Cell Death Differ 16: 966-975, 2009.
7. Hanahan D and Weinberg RA: Hallmarks of cancer: The next generation. Cell 144: 646-674, 2011.
8. Blandino G and Strano S: BCL-2: The pendulum of the cell fate. J Exp Clin Cancer Res 16: 3-10, 1997.
9. Sastry KS, Al-Muftah MA, Li P, Al-Kowari MK, Wang E, Chouchane AI, Kizhakayil D, Kulik G, Marincola FM, Haoudi A and Chouchane L: Targeting proapoptotic protein BAD inhibits survival and self-renewal of cancer stem cells. Cell Death Differ 21: 1936-1949, 2014.
10. Sanjay A, Fu J and Krebich G: DADI is required for the function and the structural integrity of the oligosaccharyltransferase complex. J Biol Chem 273: 26094-26099, 1998.
Nakashima T, Sekiguchi T, Kuraoka A, Fukushima K, Shibata Y, Komiyama S and Nishimoto T: Molecular cloning of a human cDNA encoding a novel protein, BAD1, whose defect causes apoptotic cell death in hamster BHK21 cells. Mol Cell Biol 13: 3457-3467, 1993.

Czabotar PE, Lessene G, Strasser A and Adams JM: Control of apoptosis by the BCL-2 protein family: Implications for physiology and therapy. Nat Rev Mol Cell Biol 15: 49-63, 2014.

Bhola PD and Letait A: Mitochondria-judges and executioners of cell death sentences. Mol Cell 61: 695-704, 2016.

Hsu SY, Kaipa A, Zhu L and Hsueh AJ: Interference of BAD (Bcl-xL/Bcl-2-associated death promoter)-induced apoptosis in mammalian cells by 14-3-3 isoforms and P11. Mol Endocrinol 11: 1858-1867, 1997.

Danial NN: BAD: Undertaker by night, candyman by day. Oncogene 21: 533-570, 2002.

Polzenie L, Baljuls A, Rennefah UE, Fischer A, Schmitz W, Zahedi RP, Sickmann A, Metz R, Ben, et al: Identification of novel in vivo phosphorylation sites of the human proapoptotic protein BAD: Pore-forming activity of BAD is regulated by phosphorylation. J Biol Chem 284: 28004-28020, 2009.

Yang E, Zha J, Jockel J, Boise LH, Thompson CB and Korsmeyer SJ: Bad, a heterodimeric partner for Bcl-XL and Bcl-2, displaces Bax and promotes cell death. Cell 80: 285-291, 1995.

del Peso L, González-García M, Page C, Herrera R and Nuñez G: Interleukin-3-induced phosphorylation of BAD through the protein kinase Akt. Science 278: 687-689, 1997.

Tan Y, Demeter MR, Ruan H and Comb MJ: BAD Ser-155 phosphorylation regulates BAD/Bcl-2 interaction and cell survival. J Biol Chem 275: 23865-23869, 2000.

Lizcano JM, Morrice N and Cohen P: Regulation of BAD by cAMP-dependent protein kinase is mediated via phosphorylation of a novel site, Ser155. Biochem Biophys Acta 1547: 313-319, 2001.

Yang H, Masters SC, Wang H and Fu H: The proapoptotic residue BAD phosphorylation. Proc Natl Acad Sci USA 115: E10055-E10054, 2018.

Yang H, Masters SC, Wang H and Fu H: The proapoptotic protein Bad binds the amphipathic groove of 14-3-3 protein. Biochim Biophys Acta 1547: 313-319, 2001.

Hekman M, Albert S, Galmiche A, Rennefah UEE, Fueller J, Lizcano JM, Morrice N and Cohen P: Regulation of BAD by 14-3-3 protein binding domains in conjunction with 14-3-3 protein binding. Biochim Biophys Acta 1547: 313-319, 2001.

Janumyan YM, Sansam CG, Chattopadhyay A, Cheng N, Lu P, Bowman KE, Brown SM, Joklik-Mcleod A, Mann J, Githaka JM, Buckland-Twombly, ES, et al: BAD regulates mammary gland morphogenesis. Nature 424: 952-956, 2003.

Githaka JM, Tripathi N, Kirschman R, Patel N, Pandya V, Kramer DA, Montpetit R, Zhu LF, Sonenberg N, Datta SR, et al: BAD regulates mammalian glycolysis by promoting the dissociation of the glycolytic enzyme phosphofructokinase and glycogen metabolism by BAD. Cell Metab 19: 272-284, 2014.

National Center for Biotechnology Information (NCBI): BCL-2 and BCL-XL family members. NCBI, Bethesda MD, 2021. http://www.ncbi.nlm.nih.gov/gene/572. Accessed September 2, 2021.

Datta SR, Duke H, Tao X, Masters S, Fu H, Gotob O and Greenberg ME: Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery. Cell 91: 231-247, 1997.

Sastry KS, Karpova Y and Kulik G: Epidemiological growth factors protect prostate cancer cells from apoptosis by inducing BAD phosphorylation via redundant signaling pathways. J Biol Chem 281: 27367-27377, 2006.

She QB, Solit DB, Ye Q, O'Reilly KE, Lobo J and Rosen N: The BAD protein integrates survival signaling by EGFR/MAPK and PI3K/Akt kinase pathways in PTEN-deficient tumor cells. Cancer Cell 8: 287-297, 2005.

Polzenie L, Baljuls A, Albrecht M, Hekman M and Rapp UR: BAD contributes to RAF-mediated proliferation and cooperates with RAF-RAF600 in cancer signaling. J Biol Chem 286: 17934-17944, 2011.

Verstovsek S, Kantarjian H, Mesa RA, Pardanani AD, Cortes-Franco J, Thomas DA, Estrov Z, Fridman JS, Bradley EC, Erickson-Vitanen S, et al: Safety and efficacy of INCB018424, a JAK1 and JAK2 inhibitor, in myelofibrosis. N Engl J Med 363: 1117-1127, 2010.

Levine RL, Wadeleigh M, Cools J, Ebert BL, Wernig G, Hutley BJ, Boggan TJ, Woldarska IA, Clark JJ, Moore S, et al: Activating mutation in the tyrosine kinase JAK2 in polycythemia vera: Essential thrombocythemia and myelofibrosis. Cancer Cell 7: 387-397, 2005.

James C, Ugo V, Le Couédic JP, Staerk J, Delhommeau F, Lacout C, Garçon L, Raslows H, Berger R, Bennaceur-Griscelli A, et al: A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera. Nature 434: 1144-1148, 2005.

Winter PS, Saroie KS, Lin KH, Meggendorfer M, Schnittert S, Letai A and Wood KC: RAS signaling promotes resistance to JAK inhibitors by suppressing BAD-mediated apoptosis. Sci Transl Med 12: 111ra22, 2019.

Huang N, Zhu J, Liu D, Li YL, Chen BJ, He YQ, Liu K, Mo XM and Li WM: Overexpression of Bcl-2-associated death inhibitor as549 cell growth in vitro and in vivo. Cancer Biol Ther 27: 164-168, 2018.

Smith AJ, Karpova Y, D'Agostino R Jr, Willingham M and Kulik G: Expression of the Bcl-2 protein BAD promotes prostate cancer growth. PLoS One 4: e6224, 2009.

Stickle X, Marchion D, Bicaku E, Al Sawah E, Abbassi F, Xiong Y, Zghieb NB, Boac BM, Orr BC, Judson PL, et al: BAD-mediated apoptotic pathway is associated with human cancer development. Int J Mol Med 35: 1081-1087, 2015.

Kulik G: ADRB2-Targeting therapies for prostate cancer. Cancers (Basel) 11: 916, 2019.

Mann J, Githaka JM, Buckland-Twombly, ES, et al: BAD regulates mammary gland morphogenesis. Nature 424: 952-956, 2003.

Datta SR, Ranger AM, Lin MZ, Sturgill JF, Ma YC, Cowan CW, Dilkes PB, Korsmeyer SJ and Greenberg ME: Survival factor-mediated BAD phosphorylation raises the mitochondrial threshold for apoptosis. Dev Cell 3: 631-643, 2002.

Danial NN, Gramm CF, Scorrano L, Zhang CY, Krauss S, Ranger AM, Datta SR, Greenberg ME, Licklider LJ, Lowell BB, et al: BAD and glucokinesis reside in a mitochondrial complex that integrates glycolysis and apoptosis. Nature 424: 952-956, 2003.

Githaka JM, Tripathi N, Kirschman R, Patel N, Pandya V, Kramer DA, Montpetit R, Zhu LF, Sonenberg N, Fahlman RP, et al: BAD regulates mammalian glycolysis by promoting the dissociation of the glycolytic enzyme phosphofructokinase and glycogen metabolism by BAD. Cell Metab 19: 272-284, 2014.

National Center for Biotechnology Information (NCBI): BCL-2 associated aggins of cell death (Homo sapiens [human]). NCBI, Bethesda MD, 2021. http://www.ncbi.nlm.nih.gov/gene/572. Accessed September 2, 2021.
53. Zhu X, Yu Y, Hou X, Xu J, Tan Z, Nie X, Ling Z and Ge M: Expression of PIM-1 in salivary gland adenoid cystic carcinoma: Association with tumor progression and patients' prognosis. Oncol Lett 15: 2334-2338, 2018.

54. Yu Y, Zhong Z and Guan Y: The downregulation of Bcl-xL/Bcl-2-related death promoter indicates worse outcomes in patients with small cell lung carcinoma. Int J Clin Exp Pathol 8: 13075-13082, 2015.

55. Bosco RM, Collini E, Imaichi-Khan R, Xiong Y, Siddique A, Park H, Han M, Saeed-Vafa D, Soliman H, Henry B, et al: Expression of the BAD pathway is a marker of triple-negative status and poor outcome. Sci Rep 9: 17496, 2019.

56. Chen HS, Marchion DC, Xiong Y, Chen N, Bicaku E, Stickles XB, Zhgeib NB, Judson PL, Hakam A, Gonzalez-Bosquet J, et al: The BCL2 antagonist of cell death pathway influences endometrial cancer cell sensitivity to cisplatin. Gynecol Oncol 124: 119-124, 2012.

57. Hayakawa J, Ohmichi M, Kurachi H, Kanda Y, Hisamoto K, Nishio Y, Adachi K, Tatsaka K, Kanzaki T and Murata Y: Inhibition of BAD phosphorylation either at serine 112 via extracellular signal-regulated protein kinase cascade or at serine 156 via Akt cascade sensitizes human ovarian cancer cells to cisplatin. Cancer Res 60: 5968-5974, 2000.

58. Mansouri RA and Percival SS: Cranberry extract initiates caspase activation and apoptosis in human colon cancer cells by 14-3-3 protein-mediated inhibition of AKT phosphorylation. BMC Complement Altern Med 20: 71, 2020.

59. Wang MC, Zou Y, Sun K, Ma Y, Yang H, Zhang Y, Kong X and Wei L: BAD mimetics ABT-199 and ABT-737. Leuk Lymphoma 59: 185-193, 2018.

60. Bansal N, Marchion DC, Xiong Y, Chen N, Bicaku E, Fulpi WJ, Bansal N, Chon HS, Stickles XB, Khamath SG, et al: BAD phosphorylation determines ovarian cancer chemosensitivity and patient survival. Clin Cancer Res 17: 6356-6361, 2011.

61. Bansal N, Marchion DC, Bicaku E, Xiong Y, Chen N, Stickles XB, Sawah RM, Astore Y, Apont SNJ and Gonzalez-Bosquet J: BAD antagonist of cell death pathway sensitizes ovary and breast cancer cells by dorsomorphin correlates with BAD upregulation. J Basic Med Sci 19: 274-281, 2019.

62. Han M, Saeed-Vafa D, Soliman H, Henry B, et al: BAD: A subunit of the mammalian oligosaccharyltransferase (Cfdad1) from the mollusk Chlamys farreri. Invertebr Surviv Rep 4: 96‑102, 2011.

63. Zhao H, Li Z, Zhu Y, Bian S, Zhang Y, Qin L, Naik AK, He J, Zhu L, Song L, Zhang H, Zhao J, Li C and Xu W: A homologue of the defender against the apoptotic death gene (dad1) from bay scallops Argopecten irradians. Mol Biol Rep 35: 125-132, 2008.

64. Wang MQ, Wang BJ, Liu M, Jiang KY and Wang L: Molecular characterization and responsive expression in tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) induced apoptosis correlated with tumor progression and patients' prognosis. J Gynecol Oncol 23: 35-42, 2012.

65. Zhou Y, Sun X, Shen HY, Gao F, Fan YM and Sun ZJ: Expression of the apoptosis-related genes BCL-2 and BAD in human breast cancer cells and their associated relationship with chemosensitivity. J Exp Clin Cancer Res 29: 107, 2010.

66. Mann J, Yang N, Montpetit R, Kirschromn R, Lemieux H and Goping IS: BAD sensitizes breast cancer cells to docetaxel with increased mitotic arrest and necrosis. Sci Rep 10: 355-363, 2020.

67. Yu N, Song Y, Russell N, Gao F, Fan L, Zou Y, Chen N, Bicaku E, Sullivan M: Quantitative assessment of the sensitivity of dormant AML cells to BAD mimetics ABT-199 and ABT-737. Leukemia 59: 2447-2453, 2015.

68. Yousif SS, Lee C, Tohter ER, Chang KM and Abdullah M: Potential CD34 signaling through phosphorylated-BAD in chemotherapy-resistant acute myeloid leukemia. J Recept Signal Transduct Res 39: 276-282, 2019.

69. Zhou Y, Sun K, Ma Y, Yang H, Zhang Y, Kong X and Wei L: Autophagy is induced by chemotherapies-induced apoptosis through downregulating BAD and Bim in hepatic cell carcinoma. Sci Rep 4: 5382, 2014.

70. Kim H, Choi H and Lee SK: Epstein-barr virus microRNA miR-BART20-5p suppresses lytic induction by inhibiting BAD-mediated caspase-3-dependent apoptosis. Virol J 90: 1359-1369, 2016.

71. Tang B, Tang F, Wang Z, Qi G, Liang X, Li B, Yuan S, Liu J, Yu S and He S: Upregulation of Akt/NIK-xB-regulated inflammation and Akt/Bad-related apoptosis signaling pathway involved in hepatic carcinoma process: Suppression by carnosic acid nanoparticle. Int J Nanomedicine 11: 6401-6420, 2016.

72. Zhao X, Fan Y, Lu C, Li H, Zhou N, Sun G and Fan H: PCAT1 is a poor prognostic factor in endometrial carcinoma and associated with cancer cell proliferation, migration and invasion. Bosn J Basic Med Sci 19: 274-281, 2019.

73. Liu Z, Zhang G, Huang S, Cheng J, Deng T, Lu X, Aedesahakin FO, Chen Q and Wan X: Induction of apoptosis in hematological cancer cells through Caspase-3 correlates with BAD upregulation. Biochem Biophys Res Commun 522: 704-708, 2020.

74. Mansouri RA and Percival SS: Cranberry extract initiates intrinsic apoptosis in HL-60 cells by increasing BAD activity through inhibition of AKT phosphorylation. BMC Complement Altern Med 20: 71, 2020.

75. Endo H, Inoue I, Masunaka T, Tanaka M and Yano M: Curcumin induces apoptosis in lung cancer cells by 14-3-3 protein-mediated activation of Bad. Biosci Biotechnol Biochem 84: 2440-2447, 2020.

76. Gao YP, Li L, Yan J, Hou XX, Jia XY, Chang ZW, Guan XY and Qin YR: Down-regulation of SMAD3,180, p190Rho and Smad7 contributed to EMT in vascular smooth muscle cells. Gene 658: 104373, 2020.

77. Bcklue MH, Freed E, Chiang DY, Philips J, Zahrieh D, Glickman JN and Shivdasani RA: High-resolution analysis of genetic alterations in small bowel carcinoid tumors reveals areas of recurrent amplification and loss. Genes Chromosomes Cancer 47: 591-603, 2018.

78. Wilson BF: Meta-analysis of SUMO1. BMC Res Notes 1: 60, 2008.
93. Ter-Minassian M, Wang Z, Asomaning K, Wu MC, Liu CY, Paulus JK, Liu G, Bradbury PA, Zhai R, Su L, et al: Genetic associations with sporadic neuroendocrine tumor risk. Carcinogenesis 32: 1216-1222, 2011.

94. Zhu Y, Xu H, Chen H, Xie J, Shi M, Shen B, Peng C: Proteomic and genotypic analysis of solid pseudopapillary tumor of the pancreas reveals dysfunction of the endoplasmic reticulum protein processing pathway. Mol Cell Proteomics 13: 2593-2603, 2014.

95. Schnormeier AK, Pommerenke C, Kaufmann M, Drexler HG and Koeppel M: Genomic deregulation of PRMT5 supports growth and stress tolerance in chronic lymphocytic leukemia. Sci Rep 10: 9775, 2020.

96. Ayala GE, Dai H, Ittmann M, Li R, Powell M, Frolov A, Wheeler TM, Thompson TC and Rowley D: Growth and survival mechanisms associated with perineural invasion in prostate cancer. Cancer Res 64: 6082-6090, 2004.

97. True L, Coleman I, Hawley S, Huang CY, Gifford D, Coleman R, Beer TM, Gelmann E, Datta M, Mostaghel E, et al: A molecular correlate to the Gleason grading system for prostate adenocarcinoma. Proc Natl Acad Sci USA 103: 10991-10996, 2006.

98. Wang M, Xiao X, Zeng F, Xie F, Fan Y, Huang C, Jiang G and Wang L: Common and differentially expressed long noncoding RNAs for the characterization of high and low grade bladder cancer. Gene 582: 78-85, 2016.

99. Yoon J, Kim ES, Lee SJ, Park CW, Cha HJ, Hong BH and Choi KY: Apoptosis-related mRNA expression profiles of ovarian cancer cell lines following cisplatin treatment. J Gynecol Oncol 21: 255-261, 2010.

100. Bhasin N: DAD1 as potential therapeutic target and biomarker in prostate cancer (unpublished PhD thesis). Tulane University, 2015.

101. Al-Bazz YO, Underwood JC, Brown BL and Dobson PR: Prognostic significance of Akt, phospho-Akt and BAD expression in primary breast cancer. Eur J Cancer 45: 694-704, 2009.

102. Fernando R, Foster JS, Bible A, Ström A, Pestell RG, Rao M, Saxton A, Baek SJ, Yamaguchi K, Donnell R, et al: Breast cancer cell proliferation is inhibited by BAD: Regulation of cyclin D1. J Biol Chem 282: 28864-28873, 2007.

103. Meliwiweit D: The regulation mechanism study of Bad on Dad1 gene in esophageal squamous cells. MaD Thesis, Xinjiang Medical University, China 2017 (In Chinese).

104. Cell Signaling Technology (CST): Mitochondrial control of apoptosis. CST, Danvers, MA, 2021. https://www.cellsignal.com/learn-and-support/order-support?countryId=10036#collapse00. Accessed July 1, 2021.

105. Cell Signaling Technology (CST): Regulation of apoptosis. CST, Danvers, MA, 2021. http://www.cellsignal.com/pathways/regulation-of-apoptosis-pathway. Accessed July 1, 2021.

106. Cell Signaling Technology (CST): Inhibition of apoptosis. CST, Danvers, MA, 2021. http://www.cellsignal.com/pathways/inhibition-of-apoptosis-pathway Accessed July 1, 2021.

107. Bui NL, Pandey V, Zhu T, Ma L, Basappa and Lobie PE: Bad phosphorylation as a target of inhibition in oncology. Cancer Let 415: 177-186, 2018.

This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) License.