High BIM mRNA levels are associated with longer survival in advanced gastric cancer

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Abstract. Chemotherapy drugs, including 5-fluorouracil (5-FU), oxaliplatin and docetaxel, are commonly used in the treatment of gastric cancer (GC). Apoptosis-relevant genes may be associated with drug resistance. In the present study, the messenger RNA (mRNA) expression levels of B-cell lymphoma 2 interacting mediator of cell death (BIM), astrocyte elevated gene-1 (AEG-1) and AXL receptor tyrosine kinase (AXL) were investigated in 131 advanced GC samples, and the expression levels of these genes were correlated with patients' overall survival (OS). All 131 patients received first-line FOLFOX combination chemotherapy with folinic acid and 5-FU, in which 56 patients were further treated with second-line docetaxel-based chemotherapy. A correlation between the mRNA expression levels of BIM and AEG-1 was observed (r_s=0.30; P=0.002). There was no association between the mRNA expression levels of any of the individual genes analyzed and OS in patients only receiving first-line FOLFOX chemotherapy. In a subgroup of patients receiving docetaxel-based second-line chemotherapy, those with high or intermediate levels of BIM exhibited a median OS of 18.2 months [95% confidence interval (CI), 12.8-23.6], compared with 9.6 months (95% CI, 8.9-10.3) in patients with low BIM levels (P=0.008). However, there was no correlation between the mRNA expression levels of AEG-1 or AXL and OS. The risk of mortality was higher in patients with low BIM mRNA levels than in those with high or intermediate BIM mRNA levels (hazard ratio, 2.61; 95% CI, 1.21-5.62; P=0.010). Therefore, BIM may be considered as a biomarker to identify whether patients could benefit from docetaxel-based second-line chemotherapy in GC.

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Introduction

The incidence of gastric cancer (GC) ranks as the fifth most frequent among all types of cancer worldwide (1). Nearly 40% of all GC cases occur in China, and are often diagnosed in advanced stages (2). The median overall survival (OS) for GC patients remains <12 months with first-line oxaliplatin, 5-fluorouracil (5-FU) and folinic acid treatment (3). Of all GC patients, ~1/2 could be candidates for second-line treatment at the time of failure of first-line chemotherapy (4). Docetaxel is among the most frequently used agents for GC second-line treatment (5). In a previous study by the present authors, the median OS was 25.8 months for patients with high messenger RNA (mRNA) expression levels of breast cancer susceptibility gene 1 (BRCA1) treated with second-line docetaxel-based chemotherapy (6). Recent evidence also suggests that an underlying cause of drug resistance may be the failure of drug-induced apoptosis (7-9). Platinum treatment initiates apoptosis through the formation of DNA adducts, which primarily form intrastrand crosslinks that activate the apoptotic pathway, eventually resulting in cell death (10,11). The most recognized mechanism of docetaxel-based regimen is the binding to microtubules, which arrests the cell cycle in G2/M and eventually leads to cell death (12).

B-cell lymphoma 2 (BCL-2) interacting mediator of cell death (BIM) belongs to the BCL-2 protein family, and is also a member of the BCL-2-homology 3-only (BH3-only) family (13). BIM is expressed in a wide variety of tissues, including GC, and acts as a pivotal regulator of the mitochondrial apoptosis pathway (14). Abnormal levels of BIM have been recognized to affect the chemotherapy response (15). Platinum-resistant cancer cells conserved sensitivity to BH3-induced mitochondrial apoptosis (16). In line with that, BH3-mimetic compounds such as ABT-737 were able to sensitize cancer cells to platinum (17). In addition, overexpression of BIM enhanced the *in vitro* sensitivity to docetaxel of non-small cell lung cancer (NSCLC) (18). Consistent with this finding, downregulation of BIM by small interfering RNA (siRNA) delayed paclitaxel-mediated apoptosis, indicating that low BIM expression levels were responsible for resistance to paclitaxel (19). Notably, pre-treatment mRNA levels of BIM strongly predicted the capacity of epidermal growth factor receptor (EGFR), human EGFR 2 (HER2) and phosphoinositide 3-kinase (PI3K) inhibitors to induce apoptosis in EGFR-mutant, HER2-amplified and phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha-mutant tumors, respectively (20). In a previous study, the present authors observed that patients with high BIM expression achieved longer survival in EGFR-mutant NSCLC treated with erlotinib or chemotherapy (21).

Astrocyte elevated gene-1 (AEG-1) was originally identified as a novel gene induced by human fetal astrocytes following infection with human immunodeficiency virus 1 (22). AEG-1 does not impact the uptake or retention of chemotherapy drugs; instead, AEG-1 increases chemoresistance by enhancing cell survival (23). Overexpression of AEG-1 suppresses apoptosis through phosphorylation of substrates of the anti-apoptotic protein kinase B (also known as AKT) (24), and is important in promoting cancer malignant behavior (25). In previous studies, AEG-1 overexpression correlated with poor prognosis in GC (25) and NSCLC (26). It has been confirmed that AEG-1 contributed to resistance to chemotherapeutic drugs such as 5-FU in hepatocellular carcinoma cell lines (27). Furthermore, knockdown of AEG-1 sensitized breast cancer cell lines to paclitaxel *in vitro* and *in vivo* (23). Low AEG-1 expression was associated with longer progression-free survival in platinum-based chemotherapy in NSCLC (28). In addition, AEG-1 mRNA expression correlated with BRCA1 expression (28), which induced sensitivity to docetaxel (6).

AXL receptor tyrosine kinase (AXL) belongs to the Tyro3, AXL and Mer family (29). Growth arrest-specific gene 6 (Gas6) is the ligand of AXL (30). In conjunction with each other, Gas6/AXL signaling may enhance cell survival (31). Activation of Gas6/AXL signaling induced the activation of the PI3K signaling pathway, which increased the expression of anti-apoptotic proteins such as BCL-2 and BCL-extra large (BCL-XL) (32). Overexpression of AXL was responsible for tumor growth in mesothelioma (33), lung cancer (34) and breast cancer (35). Furthermore, increased AXL activation has been linked with cisplatin resistance in ovarian cancer (36).

In the present study, the mRNA expression levels of BIM, AEG-1 and AXL were examined in 131 advanced GC samples. In addition, the expression levels of the above genes were correlated with patients' clinicopathological features and OS to first-line FOLFOX combination chemotherapy with folinic acid and 5-FU, with or without second-line docetaxel-based chemotherapy.

Patients and methods

Study population. A total of 131 advanced GC samples in which BRCA1 mRNA expression levels had been previously determined (6) were included in the present study. Patients' clinical characteristics are indicated in Table I. All patients received a combination of oxaliplatin, 5-fluorouracil (FU) and folinic acid (FOLFOX) as first-line therapy (85 mg/m^2 oxaliplatin plus 200 mg/m^2 folinic acid and 600 mg/m^2 5-FU every for 2 weeks until disease progression) for a median of 3 cycles (range, 1-8 cycles). A total of 34 patients received single-agent docetaxel (35 mg/m^2), and the remaining 22 patients were treated with docetaxel-based doublets (6 patients received 35 mg/m^2 docetaxel plus 100 mg/m^2 irinotecan weekly for 3 weeks, every 4 weeks until disease progression; 11 patients received 35 mg/m^2 docetaxel weekly for 3 weeks plus 1,000 mg/m^2 capecitabine daily for 2 weeks, every 4 weeks until disease progression; and 5 patients received 35 mg/m^2 docetaxel weekly for 3 weeks plus 6 mg/m^2 hydroxycamptothecin on days 1 and 5, every 4 weeks until disease progression) for a median of 3 cycles (range, 1-7 cycles). Following progression, 56 patients further received docetaxel-based second-line chemotherapy. A total of 34 patients received single-agent docetaxel, and the remaining 22 patients were treated with docetaxel-based doublets, based on their response to first-line chemotherapy, Eastern Cooperative Oncology Group (ECOG) performance status (PS) and patient consent. Survival was calculated from the starting date of first-line treatment to the date of last follow-up or mortality from any cause. Approval was obtained from the patients and from the ethics committee of Drum Tower Hospital (Nanjing, China).

Gene expression analysis. Gene expression profiling was performed on RNA isolated from macrodissected tumor tissues containing ≥80% of tumor cells, in accordance with a proprietary procedure (European patent publication no. EP1945764-B1). Primers and probes for gene expression analysis of BIM, AEG-1 and AXL are indicated in Table II. The mRNA levels of BIM, AEG-1 and AXL were measured by reverse transcription-quantitative polymerase chain reaction (RT-qPCR) using Taqman® Universal PCR Master Mix (Applied Biosystems; Thermo Fisher Scientific, Inc., Waltham, MA, USA), according to the comparative Cq method (37). β-actin was used as an endogenous control, and commercial RNA controls (Stratagene; Agilent Technologies, Inc., Santa Clara, CA, USA) were used as calibrators. RT-qPCR was conducted in a 7900HT Fast Real-Time PCR System (Applied Biosystems; Thermo Fisher Scientific, Inc.). The reactions were initiated by heating to 50˚C for 2 min and then to 95˚C for 2 min, followed by 40 cycles of 95˚C for 15 sec and 60˚C for 60 sec.

Statistical analysis. Gene expression levels were analyzed as categorical variables by terciles. Correlations between gene

Table I. Patient characteristics.

Characteristics	All patients N (%)	Patients receiving only first-line chemotherapy N (%)	Patients receiving second-line chemotherapy N (%)	P-value
Total, N (%)	131 (100.0)	75 (100.0)	56 (100.0)	
Age, years				0.330
<60	63 (48.1)	32 (42.7)	31 (55.4)	
≥60	68 (51.9)	43 (57.3)	25 (44.6)	
Gender				0.410
Female	31 (23.7)	20 (26.7)	11 (19.6)	
Male	100 (76.3)	55 (73.3)	45 (80.4)	
Tumor site				0.270
Distal stomach	50 (38.2)	25 (33.3)	25 (44.6)	
Proximal stomach	38 (29.0)	24 (32.0)	14 (25.0)	
Whole stomach	42 (32.1)	25 (33.3)	17 (30.4)	
Unknown	1 (0.8)	1 (1.4)	0 (0.0)	
Stage				0.550
III	79 (60.3)	44 (58.7)	35 (62.5)	
IV	52 (39.7)	31 (41.3)	21 (37.5)	
ECOG PS				0.390
0-1	119 (90.8)	66 (88.0)	53 (94.6)	
2	12 (9.2)	9 (12.0)	3 (5.4)	
Histological grade				0.070
G2	35 (26.7)	20 (26.7)	15 (26.8)	
G2-3	35 (26.7)	17 (22.7)	18 (32.1)	
G3	59 (45.0)	37 (49.3)	22 (39.3)	
Uknown	2 (1.6)	1 (1.3)	1 (1.3)	

ECOG, Eastern Cooperative Oncology Group; PS, performance status.

Table II. Sequences of primers and probes.

Gene	Primers	Probes
β-actin	F 5'-TGAGCGCGGCTACAGCTT-3' R 5'-TCCTTAATGTCACGCACGATTT-3'	6-FAM 5'-ACCACCACGGCCGAGCGG-3' TAMRA
AEG-1	F 5'-GGGGAAGGAGTTGGAGTGAC-3' R 5'-GTAGACTGAGAAACTGGCTCAGCAG-3'	6-FAM 5'-AATATTTTCTGGCATTGGGTCTA-3' MGB
AXL[b]	F 5'-CAGCGCAGCCTGCATGT-3' R 5'-GCGTTATGGGCTTCGCAG-3'	6-FAM 5'-CAGGGCTGAACAAGAC-3' MGB
BIM	Assay-on-Demand BIM (ID#Hs00708019_s1)[a]	

[a]Assay-on-Demand BIM (ID#Hs00708019_s1; catalogue no. 4331182; Life Technologies; Thermo Fisher Scientific, Inc.). [b]AXL is a receptor tyrosine kinase that belongs to the Tyro3, AXL and Mer family. AEG-1, astrocyte elevated gene-1; BIM, B-cell lymphoma 2 interacting mediator of cell death; 6-FAM, 6-carboxyfluorescein; TAMRA, tetramethylrhodamine; MGB, minor groove binder.

expression and clinicopathological parameters were analyzed with the χ^2 test. Correlations among different genes were conducted using Spearman's correlation coefficient analysis. Stratified log-rank tests were used to assess the median OS. Estimation of survival curves was performed with the Kaplan-Meier method. A multivariate analysis was performed using the Cox proportional hazards regression model. All analyses were performed with SPSS version 17.0 software (SPSS, Inc.,Chicago, IL, USA). Two-sided $P<0.05$ was considered to indicate a statistically significant difference.

Table III. Association between gene expression and clinicopathological characteristics in all patients.

Characteristics	Patients, N (%)	Median overall survival, months (95% confidence interval)	P-value
Age, years			0.300
<60	63 (48.1)	12.5 (7.8-17.1)	
≥60	68 (51.9)	10.9 (8.7-13.0)	
Gender			0.630
Female	31 (23.7)	11.7 (7.2-16.2)	
Male	100 (76.3)	11.3 (8.9-13.6)	
Tumor site			0.110
Distal stomach	50 (38.2)	11.3 (8.9-13.6)	
Proximal stomach	38 (29.1)	15.9 (11.0-20.9)	
Whole stomach	42 (32.1)	9.9 (9.0-10.7)	
Unknown	1 (0.8)	-	
Stage			0.001
III	79 (60.3)	12.9 (9.1-16.7)	
IV	52 (39.7)	9.6 (7.5-11.6)	
ECOG PS			<0.001
0-1	119 (90.8)	12.5 (9.9-15.1)	
2	12 (9.2)	6.3 (0.7-11.9)	
Histological grade			0.070
G2	35 (26.7)	12.6 (2.2-23.0)	
G2-3	35 (26.7)	12.4 (7.2-17.6)	
G3	59 (45.0)	10.6 (9.1-11.1)	
Unknown	2 (1.6)	-	
BIM mRNA levels			0.170
Low	34 (25.9)	11.6 (8.4-14.7)	
Intermediate	35 (26.7)	18.2 (3.9-32.5)	
High	35 (26.7)	10.6 (8.1-13.0)	
Not detected	27 (20.7)	-	
AEG-1 mRNA levels			0.360
Low	42 (32.1)	10.9 (8.9-12.8)	
Intermediate	40 (30.5)	10.0 (6.9-13.1)	
High	42 (32.1)	12.5 (9.4 -15.7)	
Not detected	7 (5.3)	-	
AXL mRNA levels[a]			0.250
Low	41 (31.3)	12.8 (10.9 -14.7)	
Intermediate	43 (32.8)	12.4 (7.9-17.0)	
High	43 (32.8)	10.7 (8.9-12.5)	
Not detected	4 (3.1)	-	
Second-line chemotherapy			0.060
Yes	56 (42.7)	15.0 (8.3-11.9)	
No	75 (57.3)	10.1 (13.0-17.0)	

[a]AXL is a receptor tyrosine kinase that belongs to the Tyro3, AXL and Mer family. ECOG, Eastern Cooperative Oncology Group; PS, performance status; BIM, B-cell lymphoma 2 interacting mediator of cell death; AEG-1, astrocyte elevated gene-1; mRNA, messenger RNA.

Results

Distribution and clinicopathological features of all patients. A total of 131 advanced GC samples were included in the study, of which, 100 were males and 31 females. The median age of all patients enrolled was 59.6 years (range, 22-84 years). All patients were pathologically confirmed as adenocarcinoma, of which, 79 patients (60.3%) were confirmed with stage III and 52 patients (39.7%) with stage IV disease (Table I).

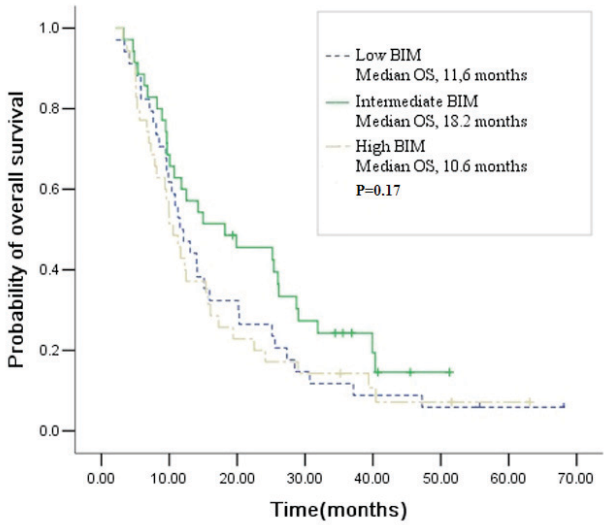

Figure 1. Kaplan-Meier estimates of overall survival in all patients according to the messenger RNA levels of B-cell lymphoma 2 interacting mediator of cell death. BIM, B-cell lymphoma 2 interacting mediator of cell death; OS, overall survival.

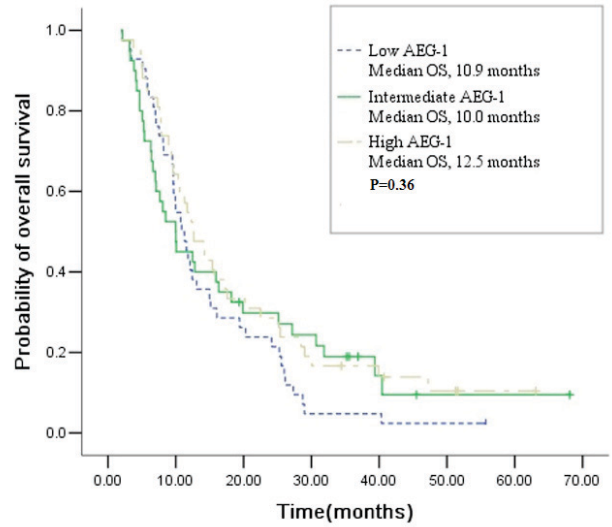

Figure 2. Kaplan-Meier estimates of overall survival in all patients according to the messenger RNA levels of astrocyte elevated gene-1. AEG-1, astrocyte elevated gene-1; OS, overall survival.

Correlations among different genes. A correlation was observed between BIM and AEG-1 mRNA expression (r_s=0.30; P=0.002). However, no associations were observed between BIM, AXL and AEG-1 mRNA expression (P=0.100 and 0.140 respectively).

Association between OS and clinicopathological characteristics. The median OS was 11.6 months (95% CI, 9.8-13.6) in all patients. Among the patients with stage III disease, the median OS was 12.9 months, compared with 9.6 months among the patients with stage IV disease (P=0.001). Notably, the median OS was ~12.5 months for patients with ECOG PS=0-1 vs. 6.3 months for patients with ECOG PS=2 (P<0.001). There was no significant association between OS and age (P=0.300), gender (P=0.630), tumor site (P=0.110) or histological grade (P=0.070) (Table III).

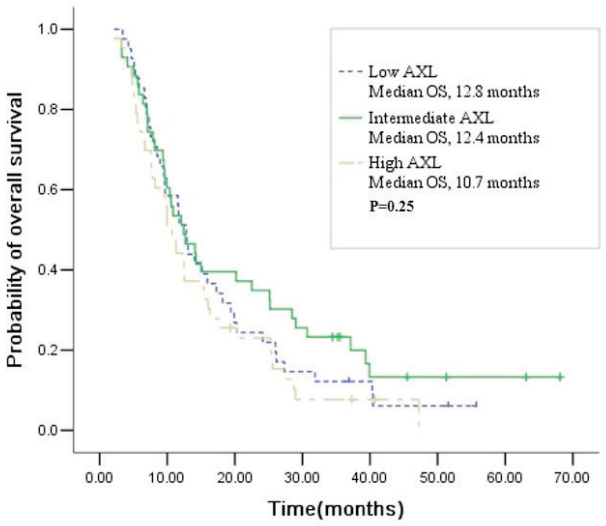

Figure 3. Kaplan-Meier estimates of overall survival in all patients according to the messenger RNA levels of AXL. OS, overall survival.

Survival for GC patients according to mRNA expression levels. No association was observed between OS and the mRNA expression levels of BIM (P=0.170), AEG-1 (P=0.360) and AXL (P=0.250) in all 131 patients, respectively (Figs. 1-3).

Among the 75 patients receiving only first-line FOLFOX chemotherapy, a trend towards longer survival was observed in those with low BIM levels (P=0.080). However, there was no difference in survival according to their AEG-1 (P=0.810) or AXL mRNA expression levels (P=0.350).

Among the 56 patients receiving additional docetaxel-based second-line chemotherapy, the median OS was 9.6 months (95% CI, 8.9-10.3) for patients with low levels of BIM, 25.2 months (95% CI, 12.5-37.9) for those with intermediate BIM levels and 15.7 months (95% CI, 9.4-22.0) for those with high BIM levels (P=0.021). Considering the obvious trend of a longer OS in patients with higher BIM expression, high and intermediate expression groups were merged into a whole group for further analysis (Fig. 4). Patients with high or intermediate levels of BIM exhibited a median OS of 18.2 months (95% CI, 12.8-23.6), while patients with low BIM exhibited a median OS of just 9.6 months (95% CI, 8.9-10.3; P=0.008). Longer survival was also observed in patients with high levels of AEG-1 (P=0.080), although the difference was not significant. There was no difference in OS according to the expression levels of AXL (P=0.600).

To further understand the role of BIM as a predictive biomarker, multivariate analysis of OS was performed. Patients with low BIM mRNA levels had higher mortality than those with high or intermediate BIM mRNA levels (HR of mortality, 2.61; 95% CI, 1.21-5.62; P=0.010). Lower risk of mortality was observed in patients with ECOG PS=0-1 compared with those with ECOG PS=2 (HR, 0.17; 95% CI, 0.04-0.65; P=0.010) in patients with stage III tumors, compared with patients with

Table IV. Median OS and HRs for risk of mortality in all patients.

	Only first-line FOLFOX chemotherapy				First-line FOLFOX plus second-line docetaxel-based chemotherapy			
Variable	Median OS (95% CI)	P-value	Risk of mortality, HR (95% CI)	P-value	Median OS (95% CI)	P-value	Risk of mortality, HR (95% CI)	P-value
Stage		0.180				<0.001		
III	10.9 (8.6-13.1)		0.66 (0.34-1.26)	0.210	20.3 (12.5-28.1)		0.37 (0.17-0.82)	0.010
IV	8.9 (6.3-11.5)		1.00 (reference)		9.7 (7.8-11.6)		1.00 (reference)	
ECOG PS		0.001				0.110		
0-1	10.6 (8.4-12.7)		0.34 (0.14-0.82)	0.020	15.1 (11.8-18.4)		0.17 (0.04-0.65)	0.010
2	6.3 (0.0-15.3)		1.00 (reference)		8.1 (0.6-15.7)		1.00 (reference)	
Histological grade		0.240				0.140		
G2	12.1 (10.3-13.9)		0.65 (0.30-1.41)	0.270	20.2 (1.9-38.5)		0.90 (0.33-2.45)	0.840
G2-3	7.5 (6.4-8.7)		1.57 (0.73-3.40)	0.250	17.3 (12.1-22.4)		0.60 (0.26-1.33)	0.200
G3	10.0 (9.0-11.0)		1.00 (reference)		11.3 (6.3-16.3)		1.00 (reference)	
BIM levels		0.180				0.008		
Low	12.1 (9.0-15.2)		0.79 (0.42-1.47)	0.450	9.6 (8.9-10.3)		2.61 (1.21-5.62)	0.010
Intermediate and high	9.6 (7.3-11.9)		1.00 (reference)		18.2 (12.8-23.6)		1.00 (reference)	

ECOG, Eastern Cooperative Oncology Group; PS, performance status; BIM, B-cell lymphoma 2 interacting mediator of cell death; OS, overall survival; HR, hazard ratio; CI, confidence interval; FOLFOX, folinic acid and 5-fluorouracil.

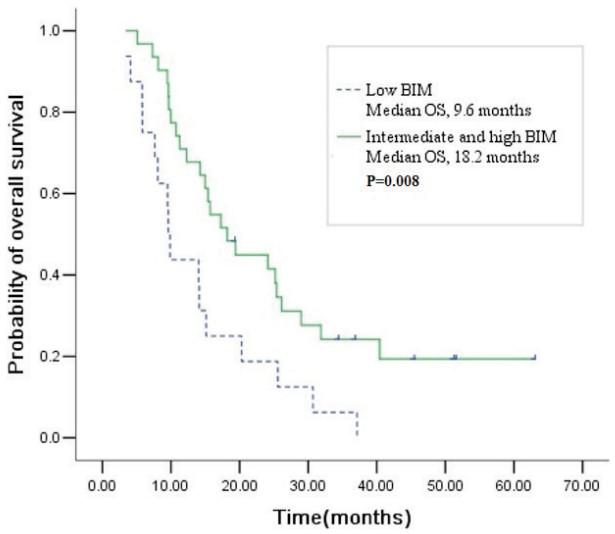

Figure 4. Kaplan-Meier estimates of overall survival in patients receiving docetaxel-based second-line chemotherapy according to the messenger RNA levels of B-cell lymphoma 2 interacting mediator of cell death. BIM, B-cell lymphoma 2 interacting mediator of cell death; OS, overall survival.

stage IV tumors (HR, 0.37; 95% CI, 0.17-0.82; P=0.010) (Table IV).

Discussion

Following the failure of first-line chemotherapy, several drugs are recommended for second-line regimens, including paclitaxel, docetaxel and irinotecan (38). The median OS of patients receiving second-line docetaxel-based regimens ranges from 3.5 to 10.9 months, which is still dismal (5). The present authors previously observed that GC patients with high BRCA1 levels could benefit from receiving second-line docetaxel-based chemotherapy (6). In addition, the median OS was further prolonged for patients with high levels of BRCA1 and multiple myeloma SET domain (39).

The DNA damage caused by chemotherapy leads to cell cycle arrest, DNA repair or commitment to apoptosis (40). Failure of drug-induced apoptosis is a vital reason for chemoresistance (41). A previous study identified that overexpression of genes involved in apoptosis appeared to contribute to docetaxel sensitivity in breast cancer through high-throughput screening of thousands of genes (42). It is commonly known that taxanes interfere with the dynamics of the microtubules and induce apoptosis through the mitochondrial apoptotic pathway (43).

In the present study, a marked difference in OS (18.2 vs. 9.6 months) was observed in patients receiving second-line docetaxel-based chemotherapy, according to their BIM mRNA expression levels in univariate analysis (P=0.008). In addition, this association was also significant in multivariate analysis, which further confirmed the role of BIM as a predictive biomarker. Patients with low BIM mRNA levels exhibited higher mortality than those with high or intermediate BIM mRNA levels (HR, 2.61; 95% CI, 1.21-5.62; P=0.010). These results were consistent with previously published data suggesting that overexpression of BIM was accompanied by a collateral increase in sensitivity to taxanes (19), which may translate into prolonged OS.

BIM is an important mediator of tumor cell death (15). Other studies have previously demonstrated that several kinase-driven tumors, including chronic myelogenous leukemia and NSCLC, maintain a survival advantage by suppressing BIM transcription and by targeting BIM protein for proteasomal degradation (44-46). Numerous studies have clearly demonstrated that activation of the PI3K/AKT signaling pathway could regulate BIM expression (44,47). The PI3K/AKT signaling pathway triggers a cascade of cell responses, including cell cycle progression, programmed cell death and DNA damage repair in cancer (48). Furthermore, the PI3K/AKT signaling pathway is closely associated with the development and recurrence of cancer (49). The class O of forkhead box (FOXO) transcription factors are downstream effectors of the PI3K/AKT signaling pathway (50). When active, FOXOs induce cell cycle arrest and apoptosis, acting as anti-proliferative factors (51). BIM is mainly regulated by FOXO3a, a member of the FOXO family (52). Following an apoptotic-stress event, BIM translocates to the mitochondria, and is essential to mediate the release of cytochrome *c* from the mitochondria, which in turn activates the effector caspase-9 and the formation of the apoptosome (53). Previous studies reported that the PI3K inhibitor LY294002 could increase BIM expression and cell death, which partly demonstrated a modulating role of PI3K/AKT signaling on BIM expression (47). The aforementioned results are in agreement with the pattern of FOXO3a dephosphorylation and nuclear translocation. The dephosphorylation status of AKT inhibits the nuclear export of its substrate FOXO3a to the cytoplasm, which transactivates the main target gene, BIM, to cause cell cycle arrest and cell death (52). In a previous study, BIM mRNA levels could be increased by upregulation of FOXO3a following paclitaxel treatment, leading to apoptosis in breast cancer cells and contributing to tumor sensitivity to paclitaxel (54). Thus, the impact of BIM and other BH3-only proteins in GC patients through apoptosis pathways should be further investigated.

AEG-1 was identified as an oncogene that caused detrimental effects to patients' OS through preventing cancer cells from undergoing apoptosis (55). Overexpression of AEG-1 leads to the activation of the PI3K/AKT pro-survival signaling pathway and the downregulation of BCL-2 associated agonist of cell death (BAD), p21, p27 and FOXO3a (24). In addition, increasing expression and activation of FOXO3a by AEG-1 knockdown further confirms this mechanism (56). AXL, a receptor tyrosine kinase, was originally cloned from cancer cells (57). A crucial step in AXL-dependent signal transduction is the activation of PI3K/AKT (58). The activation of AXL protects cells from apoptosis and increases the expression of the anti-apoptotic proteins BCL-2 and BCL-XL (59), as well as the phosphorylation of BAD (60). AXL is also implicated in angiogenesis (61) and immune response (62). Preclinical findings and retrospective studies have illustrated that overexpression of AEG-1 and AXL confers broad drug resistance to chemotherapeutic agents, including paclitaxel (63), cisplatin (64,65) and 5-FU (27). However, no correlations were observed in the present study between AEG-1 and AXL mRNA expression levels and patients' outcome to chemotherapy, either in first-line or second-line chemotherapy. This reflects

the complexity of tumor drug response and the fact that single genes may not be sufficient to predict the therapeutic effect.

The present study has certain limitations. First, the number of patients included in the study is relatively small, which may cause bias in data analysis. In addition, the study is retrospective in nature. Furthermore, multiple gene models or signatures may be more effective than single biomarkers, as gene expression patterns associated with drug resistance and sensitivity are complex.

In conclusion, based on the significantly prolonged OS among patients with high or intermediate BIM mRNA expression in the present study, BIM may act as a potential biomarker in second-line docetaxel-based chemotherapy for GC. The findings in the current study pave the way for personalized chemotherapy in GC.

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