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Role of high mobility group A1 and body mass index in the prognosis of patients with breast cancer

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Abstract. The high mobility group A1 (HMGA1) protein is associated with poor prognosis in patients with a wide range of cancers. However, the affect of HMGA1 on the risk of mortality from breast cancer (BC) has not been fully characterized. In the present retrospective multiple center study, the HMGA1 expression level was determined by performing immunohistochemistry on surgical tissue samples of 273 BC specimens from the Second Affiliated Hospital of Zhejiang University (Zhejiang, China) and 310 BCs from the National Engineering Center for Biochip (Shanghai, China). Kaplan-Meier analysis and Cox proportional hazard model were employed to analyze the survivability. HMGA1 expression was significantly associated with tumor histological degree and body mass index (BMI). However, HMGA1 expression showed no prognostic value in patients with BC. Combined evaluation of HMGA1 expression and high BMI (≥24 kg/m^2) predicted worse overall survival of BC. Therefore, HMGA1 and BMI were considered to serve synergistic roles in the development and progression of BC, and combined evaluation of HMGA1 expression and high BMI may be an effective marker in predicting poor prognosis of BC patients.

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Introduction

Breast cancer (BC) represents the leading cause of cancer-associated mortality in females worldwide (1). As a heterogeneous disease, a series of genetic markers have been evaluated and associated with clinical prognostic parameters in patients with BC (2-4). However, these markers are not yet effective enough to be used in clinical practice, and additional studies are required to produce effective targets that can be used to predict prognosis and drug resistance (5).

The high mobility group A1 (HMGA1) proteins are architectural non-histone chromatin factors, which form stereospecific multiprotein complexes termed enhanceosomes on the promoter/enhancer regions of genes that regulate gene transcription. Each HMGA1 protein has three AT-hook domains that bind to the minor groove of AT-rich DNA sequences and interact with various transcription factors to enhance or inhibit gene transcription (6,7). HMGA1 is involved in a variety of cellular processes, including embryogenesis, cell cycle regulation, senescence, differentiation and DNA repair (8-11). HMGA1 protein overexpression is a feature of malignant tumors, including pancreas, breast and colorectal cancers (12-18). A previous *in vitro* study provided evidence that HMGA1 exerts an important role in the pathogenesis of breast cancer; exogenous expression of HMGA1 in normal human breast cells may lead to malignant phenotype transformation (19). HMGA1 promoted metastatic processes in breast cancer cells through enhancing cell proliferation, the Hippo signaling pathway and epithelial-to-mesenchymal transition (20-25). In addition, HMGA1 expression in breast cancer cells diminished cellular DNA repair activity by inducing enhanced apoptosis and sensitizing cells to cisplatin-induced death (26). Knockdown of HMGA1 expression altered breast cancer cells to a more differentiated phenotype and reduced breast tumorigenesis (21,27).

A high body mass index (BMI) is an independent risk factor for cardiovascular disease (28,29) and cancer (30,31). Several studies have demonstrated that BMI influences the outcomes of patients with BC and is considered a prognosis factor (32-35). Furthermore, HMGA protein expression in tumors may also be associated with BMI (36).

To elucidate the role of HMGA1 and BMI in the prognosis of BC, HMGA1 protein expression was evaluated by immunohistochemical staining in two large cohorts of BC samples. It was identified that HMGA1 expression indicated an advanced BC malignancy, while its expression did not show significant prognostic value. However, the combined evaluation of HMGA1 expression and high BMI may serve as a biomarker of poor prognosis in patients with BC.

Materials and methods

Patients. The eligible BCs were collected based on inclusion and exclusion criteria. Inclusion criteria: BCs with pathological diagnosis; informed consent obtained or waiver of consent; and follow-up information available. Exclusion criteria: Failed to get informed consent; multiple cancers; lack of histological diagnosis; and no follow-up information. A total of 273 BCs who received surgical operation in the Second Affiliated Hospital of Zhejiang University (Zhejiang, China) were entered as the training set. The validation set, which consisted of 310 patients with BC who received surgical operation were collected from the National Engineering Center for Biochip (Shanghai, China). In the training set, all patients who received surgical operation between January 2004 and September 2010 were followed up until August 2015. The 310 BCs in the validation set received operations between January 2001 and December 2008, and the last follow-up time was July 2014.

Construction of tissue microarray (TMA). Formalin-fixed and paraffin-embedded tumor specimens were prepared for TMA using the Beecher Manual Tissue Arrayer (Beecher Instruments, Inc., Sun Prairie, WI, USA). Briefly, one core tissue biopsy with a diameter of 1 mm was taken from a representative region of an individual paraffin-embedded BC sample and placed into a new recipient paraffin block. Every sample included 2-3 tissue cores for biomarker analysis. Consecutive sections of 4-5 mm were cut from TMA blocks and placed on glass slides for subsequent immunohistochemical analysis. The tumor blocks also contained tumor and normal breast tissue samples as positive and negative controls for each IHC staining.

HMGA1 immunohistochemistry. Paraffin sections of 5-6 μm were deparaffinized and antigen was retrieved by boiling for 15 min in 0.1 M citrate buffer. The endogenous peroxidase activity was blocked with 3% hydrogen peroxide for 15 min. Array slides were then incubated with normal goat serum (catalog no. ZLI-9021; ZSGB-Bio, Beijing China). for 15 min. The primary antibody HMGA1 (catalog no. ab129153; dilution, 1:250; Abcam, Cambridge, UK) was incubated overnight at 4˚C in a humidified chamber. The rabbit antibody against HMGA1 (catalog. no. A380388; dilution, 1:5,000) used in the present study was purchased from ALEXIS Biochemicals (San Diego, CA, USA). PBS was used as a negative control. The array slides were incubated with horseradish peroxidase-labeled polymer conjugated with corresponding antibodies for 30 min. Diaminobenzidine (catalog no. D8230; Solarbio, Beijing, China) was then applied for 5 and 10 min, respectively. Each slide was counterstained with hematoxylin (Dako; Agilent Technologies, Inc., Santa Clara, CA, USA).

Scoring of HMGA1 expression. HMGA1 staining was assessed for the percentage of nuclear immunoreactivity in tumor cells by two independent observations. Results were grouped into the following categories: No nuclear staining (-); with nuclear staining <20% (+); 20-50% of nuclear positive cells (++); and >50% of nuclear positive cells (+++). All clinicopathological data (pathological diagnosis, grade and tumor node metastasis stage) and immunohistochemical data [estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor 2 (HER2)] were reevaluated by pathologists from the Department of Pathology (Second Affiliated Hospital, Zhejiang University School of Medicine, Zhejiang, China). BMI scores were divided by 24 according to the Chinese standard which determines that >24 kg/m^2 is categorized as overweight or obese (37).

Statistical analysis. SPSS 21.0 software (IBM SPSS, Armonk, NY, USA) was used for statistical analysis. The association between HMGA1 expression/BMI and clinicopathological factors was estimated using the Pearson's χ^2 test. Overall survival (OS) curves were constructed using the Kaplan-Meier method by the log-rank test. Univariate analysis was performed with the log-rank test, and Cox's regression test was applied for multivariable analysis. The factors of ER status, PR status, HER2 status, tumor size and lymph node involvement were excluded when performed multivariable analysis, as these factors have the collinear relation with TNBC and TNM stage. Hazard ratios (HRs) were reported with 95% confidence intervals (CIs). P<0.05 was considered to indicate a statistically significant difference.

Results

HMGA1 expression and clinicopathological characteristics in BC. Associations between HMGA1 expression and clinicopathological characteristics of BC patients are shown in Table I. HMGA1 expression was observed in 105/273 (38.5%) patients with BC in the training set and 191/310 (61.6%) in the validation set. HMGA1 staining was negative in all the normal samples (Fig. 1A). The association between positive HMGA1 expression and clinicopathological parameters was then analyzed. It was identified that HMGA1 overexpression was significantly associated with histological grade in the training set (P=0.031) and the validation set (P<0.001). However, no significant difference in HMGA1 expression, according to age, tumor location, stage of disease, triple-negative BC (TNBC) or other parameters, was observed (Table I).

BMI and clinicopathological parameters. A total of 158/273 patients with BC in the training set were recorded with BMI, the association between BMI and associated clinicopathological parameters was analyzed. BMI did not show any association with age, location, tumor stage or other parameters (Table II), however, high BMI (>24 kg/m^2) was significantly associated with HMGA1 expression (P=0.033).

Survival analysis. In order to clarify whether HMGA1 affects the prognosis of patients with BC, Kaplan-Meier analysis was performed, and it was revealed that HMGA1 level did not predict survival significance in patients with BC. As shown

Table I. HMGA1 protein expression and clinicopathological characteristics in breast cancer.

	Training set (ZJU, n=273)			Validation set (SBC, n=310)		
Characteristics	Patients, n	HMGA1$^+$, n (%)	P-value	Patients, n	HMGA$^+$, n (%)	P-value
Age			0.863			0.983
≤50 years	136	53 (39.0)		117	72 (61.5)	
>50 years	137	52 (38.0)		193	119 (61.7)	
Tumor location[a]			0.377			0.604
Left	147	53 (36.1)		136	86 (63.2)	
Right bilateral	126	52 (41.3)		174	105 (60.3)	
Histological grade[b]			0.031			<0.001
I	45	13 (28.9)		51	18 (35.3)	
II	119	45 (37.8)		195	121 (62.1)	
III	24	14 (58.3)		64	52 (81.3)	
Tumor size			0.188			0.614
T1	125	45 (36.0)		78	41 (52.6)	
T2	129	50 (38.8)		199	131 (65.8)	
T3 and T4	19	10 (52.6)		33	19 (57.6)	
Lymph node involvement			0.631			0.073
N (-)	148	55 (37.2)		145	97 (66.9)	
N (+)	125	50 (40.0)		165	94 (57.0)	
AJCC stage			0.133			0.664
I	82	26 (31.7)		41	24 (58.5)	
II	128	52 (40.6)		181	117 (64.6)	
III	63	27 (42.9)		88	50 (56.8)	
ER status			0.798			0.192
Negative	104	39 (37.5)		113	75 (66.4)	
Positive	169	66 (39.1)		197	116 (58.9)	
PR status			0.510			0.501
Negative	116	42 (36.2)		156	99 (63.5)	
Positive	157	63 (40.1)		154	92 (59.7)	
HER2 status			0.609			0.075
Negative	214	84 (39.3)		208	121 (58.2)	
Positive	59	21 (35.6)		102	70 (67.6)	
Triple-negative			0.740			0.442
TNBC	68	25 (36.8)		46	26 (56.5)	
Others	205	80 (39.0)		264	165 (62.5)	

[a]In the validation set there are three bilateral breast cancers. [b]In the training set there were 85 cases without data. Student's t test was used for comparisons between 2 groups of experiments, and one-way ANOVA analysis was used for comparisons among 3 or more groups. P<0.05 was considered significant. ZJU, Zhejiang university; SBC, Shanghai Biochip Center; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor 2; TNBC, triple-negative breast cancer; AJCC, American joint committee on cancer.

in Fig. 2A and B, HMGA1 did not affect the OS of patients with BC in the training set (P=0.382) or the validation set (P=0.570). The results of Cox's regression test are presented in Table III. As expected, the univariate analysis revealed that tumor stages 3 and 4, lymph node involvement, American Joint Committee on Cancer (AJCC) stages II and III, histological grade III and TNBC subtype were associated with unfavorable prognosis; while ER (+) and PR (+) were associated with favorable prognosis in the training cohort. These results were confirmed in the validation cohort. Multivariate analysis indicated that the AJCC stage in the training set (HR, 5.01; CI, 2.19-11.47) and the validation set (HR, 2.02; CI, 1.20-2.89), and TNBC status in the training set (HR, 4.35; CI, 1.61-11.75) and the validation set (HR, 2.77; CI, 1.69-4.56) were the independent prognostic risk of patients with BC. HMGA1 expression was not associated with OS in the training set (HR, 0.79; CI, 0.29-2.14; Fig. 2C) or in the validation set (HR, 0.78; CI, 0.49-1.23; Fig. 2D).

The association between BMI and prognosis in the group of 158 patients with BC was then analyzed, and it was identified

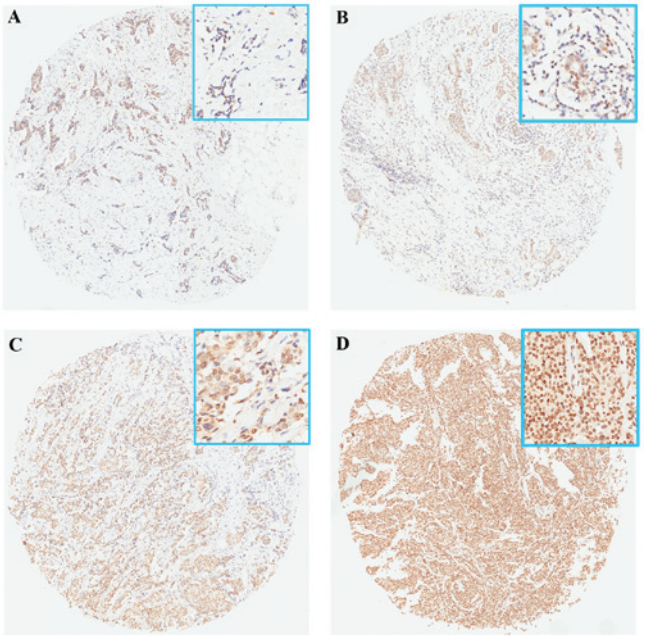

Figure 1. Immunohistochemical analysis of HMGA1 in breast cancer tissues with (A) negative, (B) weakly positive, (C) moderately positive and (D) strongly positive staining. Each slide was investigated in with low magnification (40x) and then with high magnification (200x) under a microscope (Olympus, Tokyo, Japan).

that high BMI was associated with poor OS (P=0.024; Fig. 3A), but not associated with disease-free survival (DFS; P=0.733; Fig. 3B). In addition, BMI and HMGA1 combined (HMGA1 positive and BMI >24 kg/m^2) evaluation had a stronger association with OS (P=0.004; Fig. 3C) and DFS (P=0.074; Fig. 3D). Cox's regression test was then performed (Table IV). BMI (HR, 2.23; CI, 1.09-4.56) and BMI/HMGA2 combined score (HR, 2.83; CI, 1.25-5.95) had a significant adverse prognosis value for OS (HR, 1.32; CI, 0.70-2.50), but not with DFS (HR, 1.86; CI, 0.93-3.73) in univariate analysis. However, the prognostic value of the combined BMI and HMGA1 score was dampened in multivariate analysis; the HRs of the high BMI-HMGA1 combined score with DFS and OS were 1.82 (CI, 0.58-5.62) and 4.21 (CI, 0.61-29.00), respectively.

Discussion

In the present study, HMGA1 expression in 583 patients with BC was retrospectively analyzed from two medical centers, to clarify the expression patterns of HMGA1 in BC samples. In total, 38.5% of patients with BC in the training set and 61.6% of patients in the validation set showed HMGA1 expression. The discrepancy of HMGA1 expression ratio may be due to the difference of baseline characteristics of patients from these two sets. In the training set, 93.0% of patients were stage I and II, and 24.9% of patients had TNBC, while in the validation set, 83.2% of patients were identified as stage I and II, and only 14.8% of patients were classified as TNBC. The oncogenic protein HMGA1 has been established as the prognostic and predictive marker of survival in various types of cancers (16,38,39). Its expression preceded the appearance of the malignant phenotype, as only 40% of hyperplastic lesions with cellular atypia were stained for HMGA1, while 62% of breast carcinomas were HMGA1 expression (12). In the present study, HMGA1 expression determined by

Table II. Association between body mass index and clinicopathological characteristics of patients with breast cancer.

	BMI, kg/m^2		
Characteristic	<24	≥24	P-value
Age (years)			0.249
≤50	51	22	
>50	57	28	
Tumor location[a]			0.249
Left	52	29	
Right bilateral	56	21	
Histological grade[b]			0.080
I	25	8	
II	53	18	
III	8	7	
Tumor size			0.437
T1	53	25	
T2	48	20	
T3 and T4	7	5	
Lymph node involvement			0.455
N (-)	63	26	
N (+)	45	24	
AJCC stage			0.851
I	34	15	
II	55	21	
III	19	14	
ER status			0.410
Negative	38	21	
Positive	70	29	
PR status			0.384
Negative	46	25	
Positive	62	25	
HER2 status			0.169
Negative	97	41	
Positive	11	9	
Triple-negative			0.893
TNBC	81	37	
Others	27	13	
HMGA1 status			0.033
Negative	65	21	
Positive	43	29	

[a]In the validation set there are 3 bilateral breast cancers. [b]In the training set there were 85 cases without data. Student's t-test was used for comparisons between 2 groups of experiments, and one-way ANOVA analysis was used for comparisons among 3 or more groups. P<0.05 was considered significant. BMI, body mass index; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor 2; TNBC, triple-negative breast cancer; AJCC, American joint committee on cancer; HMGA1, high mobility group A1.

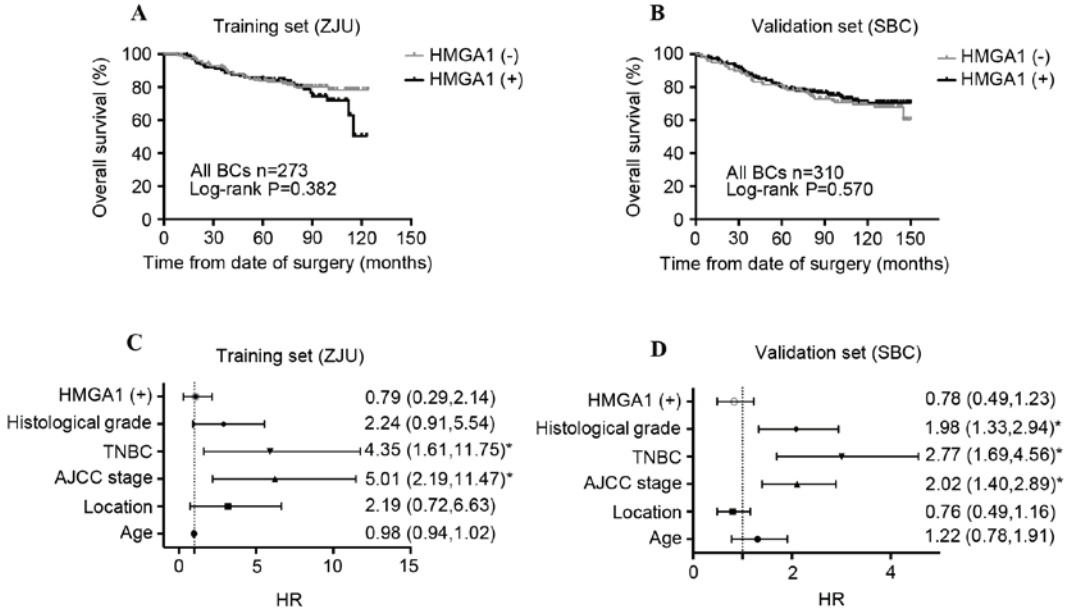

Figure 2. Prognostic significance of HMGA1 expression in breast cancer. (A) Kaplan-Meier OS analysis of HMGA1 expression for all patients in the training and (B) validation cohort. (C) Forest plots of the results of multivariate Cox analysis for OS in the training and (D) validation cohort. OS, overall survival; HMGA1, high mobility grade A1; ZJU, Zhejiang university; SBC, Shanghai biochip center; TNBC, triple-negative breast cancer; HR, hazard ratio. *P<0.05.

Table III. Univariate and multivariate Cox analysis for high mobility group A1 and survival of breast cancer.

	Training set (ZJU, n=273)		Validation set (SBC, n=310)	
Characteristic	Univariate, HR (95% CI)	Multivariate, HR (95% CI)	Univariate, HR (95% CI)	Multivariate, HR (95% CI)
Age (>50 vs. ≤50 years)	1.02 (0.99-1.04)	0.98 (0.94-1.02)	1.10 (0.71-1.70)	1.22 (0.78-1.91)
Location (right vs. left)	1.19 (0.69-2.03)	2.19 (0.72-6.63)	0.86 (0.57-1.29)	0.76 (0.49-1.16)
ER status (+ vs. -)	0.33 (0.19-0.58)[a]		0.60 (0.39-0.91)[a]	
PR status (+ vs. -)	0.28 (0.16-0.50)[a]		0.54 (0.35-0.83)[a]	
HER2 status (+ vs. -)	1.53 (0.84-2.79)		1.50 (0.98-2.31)	
Tumor size (T3/4 vs. T1/2)	2.01 (1.35-2.99)[a]		1.73 (1.23-2.44)[a]	
Lymph node involvement (+ vs. -)	1.98 (1.58-2.48)[a]		1.46 (1.19-1.80)[a]	
AJCC stage (II/III vs. I)	3.62 (2.35-5.58)[a]	5.01 (2.19-11.47)[a]	2.10 (1.47-2.99)[a]	2.02 (1.40-2.89)[a]
TNBC (TNBC vs. non-TNBC)	3.32 (1.94-5.70)[a]	4.35 (1.61-11.75)[a]	2.45 (1.52-3.94)[a]	2.77 (1.69-4.56)[a]
Histological grade (III vs. I/II)	3.65 (1.61-8.31)[a]	2.24 (0.91-5.54)	1.70 (1.19-2.42)[a]	1.98 (1.33-2.94)[a]
HMGA1 (+ vs. -)	1.05 (0.61-1.82)	0.79 (0.29-2.14)	0.88 (0.58-1.35)	0.78 (0.49-1.23)

[a]P<0.05. HR, hazard ratio; CI, confidence interval; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor 2; TNBC, triple-negative breast cancer; AJCC, American joint committee on cancer; HMAG1, high mobility grade A1.

immunohistochemistry did not show any association with OS in patients with BC, while HMGA1 expression was significantly associated with histological grade of patients with BC, which is consistent with previous studies (12,40,41).

BMI is a simple measurement based on individual weight and height, it is widely used to define overweight and obesity. High BMI has been identified as a major risk of type 2 diabetes mellitus (T2DM) (42). The presence of a functional variant of the HMGA1 gene was also associated with T2DM (43), and this HMGA1 variant positively associated with BMI (44). From these results, it was inferred that an association may exist between HMGA1 and BMI. Notably, HMGA1 expression was found to be significantly associated with BMI in patients with BC; 62.5% of HMGA1 positive patients were overweight or obese (BMI ≥24 kg/m^2), while only 17.8% of HMGA1 negative patients were overweight. Survival analysis resulted in poor OS for BC patients with high BMI (≥24 kg/m^2). Although HMGA1 expression did not indicate any prognostic value in patients with BC, the HMGA1 expression and BMI combined score had a stronger prognostic value for OS (Fig. 3B, P=0.004). Similarly, BMI did not have prognostic value for DFS (P=0.733), but the HMGA1 expression and BMI combined score showed a trend in association with DFS (P=0.074). It was hypothesized that HMGA1 and BMI may

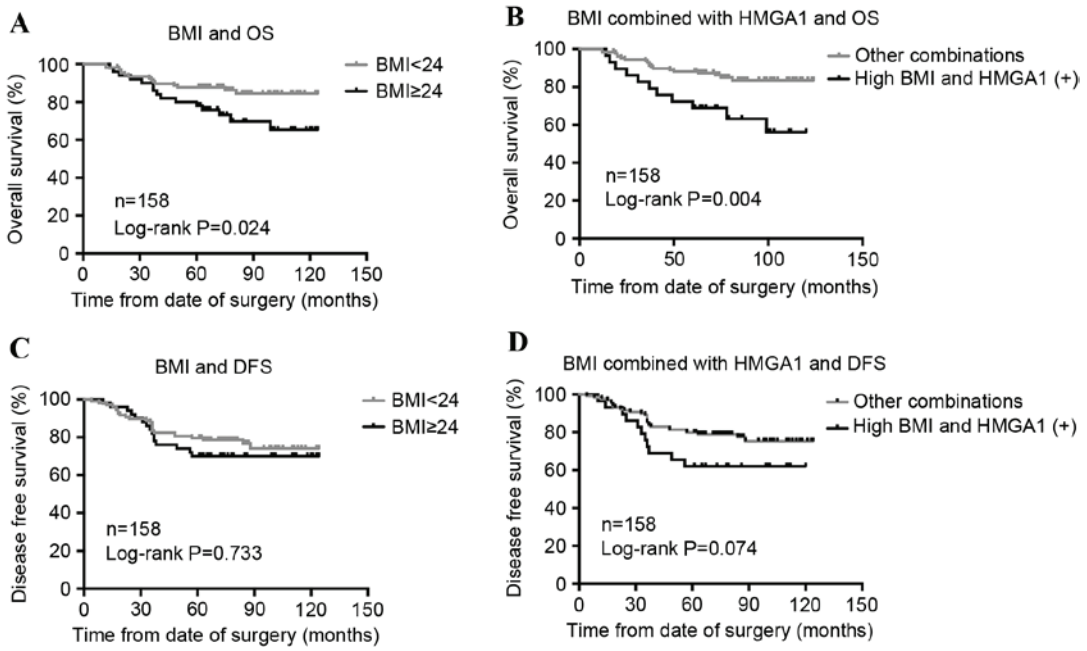

Figure 3. (A) is showing BMI and OS by Kaplan-Meier analysis. (B) is showing BMI and DFS by Kaplan-Meier analysis. (C) is showing HMGA1 and BMI combine score and OS by Kaplan-Meier analysis. (D) is showing HMGA1 and BMI combine score and DFS by Kaplan-Meier analysis; HMGA1, high mobility grade A1; DFS, disease-free survival; BMI, body mass index.

Table IV. Univariate and multivariate Cox analysis of prognostic factors for disease-free survival and overall survival in 158 patients with breast cancer.

	Disease-free survival		Overall survival	
Factors	Univariate, HR (95% CI)	Multivariate, HR (95% CI)	Univariate, HR (95% CI)	Multivariate, HR (95% CI)
Location (right vs. left)	1.15 (0.62-2.14)		1.31 (0.64-2.70)	
HMGA1 (+ vs. -)	1.55 (0.83-2.90)		1.90 (0.92-3.95)	
BMI (≥24 vs. <24)	1.32 (0.70-2.50)		2.23 (1.09-4.56)[a]	
Age (>50 vs. ≤50 years)	1.03 (1.01-1.05)[a]	1.01 (0.98-1.05)	1.03 (1.00-1.06)[a]	0.96 (0.88-1.04)
AJCC stage (II/III vs. I)	2.94 (1.83-4.71)[a]	1.66 (0.82-3.37)	4.95 (2.69-9.11)[a]	2.76 (0.68-11.25)
TNBC (TNBC vs. non-TNBC)	3.58 (1.93-6.67)[a]	3.12 (1.25-7.83)[a]	4.63 (2.24-9.55)[a]	5.07 (0.95-27.12)
Histological grade (III vs. I/II)	1.69 (0.79-3.61)	1.06 (0.47-2.41)	14.63 (3.43-62.52)[a]	8.70 (1.21-62.28)[a]
BMI-HMGA1 combined score (high vs. low)	1.86 (0.93-3.73)	1.82 (0.58-5.64)	2.83 (1.35-5.95)[a]	4.21 (0.61-29.00)

[a]P<0.05. BMI, body mass index; TNBC, triple-negative breast cancer; HMGA1, high mobility grade A1; AJCC, American joint committee on cancer.

perform a synergistic role in the process of tumorigenesis. HMGA1 protein directly binds to an adipose-specific promoter CCAAT-enhancer-binding protein-β to exert a critical role in adipocyte hemostasis, and suppression of HMGA1 expression impaired adipocytic differentiation and decreased fat tissue development (45). Adipocytes promoted the secretion of peptide hormone cholecystokinin of cancer cells and enhanced the proliferation of prostate cancer stem cells (46). In addition, the leptin, which was produced by adipocytes, was suggested to contribute to tumor development and progression through activating the Janus kinase/signal transducers and activators of transcription, phosphatidylinositol 3-kinase/AKT and extracellular signal-related kinase signaling pathways (47,48). It was hypothesized that HMGA1 may enhance the proliferation of adipocytes, particularly adipocytes around the cancer cells, which may interact with cancer cells by secreting specific cytokines to promote the malignant biological properties of cancer cells. However, additional studies are required to elucidate the particular molecular mechanisms underlying this connection among HMGA1, obesity and BC.

The BC tissues included in the present study were collected from two medical centers; however, complete pathological

BMI and DFS data could not be obtained for all samples. Therefore, the potential selection bias and confounding bias was inevitable. BMI and HMGA2 combined score had a significant adverse prognosis value for OS in univariate analysis. However, it did not indicate an independent risk in multivariate analysis. A lack of enough samples of patients with BMI, the presence of collinearity between BMI-HMGA1 combined score and other clinicopathological parameters, or other confounding factors, may affect the reliability of the results.

In conclusion, the present study demonstrated that HMGA1 expression in BC is positively associated with pathological differentiation. However, HMGA1 expression is not prognostic of survival in patients with BC. The combined evaluation of HMGA1 expression and high BMI can be a more effective marker in predicting poor prognosis of patients with BC. HMGA1 and BMI may play a synergistic role in the development and progression of BC.

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