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Clinicopathological and immunohistochemically thesis of MAGE C2 antigen expression in set of breast cancer

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Abstract
Background: Breast cancer is the most commonly diagnosed malignant disease and the leading cause of cancer death among females, with approximately 2.3 million new cases and 685,000 deaths worldwide. Recent studies indicate that Melanoma-associated gene C2 (MAGEC2) expression is linked to poor prognosis, aggressive clinical course, chemotherapy resistance, and poor outcomes in breast cancer patients. MAGEC2, part of the MAGE family on the X chromosome, interacts with STAT3 in tumor cells, preventing its degradation and enhancing transcriptional activity. Objective Evaluation of the prognostic role of MAGEC2 expression in women with breast cancers and its association with other prognostic factors.
Method: This study examined 46 formalin-fixed paraffin-embedded breast cancer tissue blocks of varying grades and histopathological features. Hematoxylin and eosin staining assessed histopathological diagnosis, while polyclonal antibody for MAGEC2 was used for immunohistochemical staining. MAGEC2 expression was scored by staining intensity (0-3) and percentage (0-100%), with semiquantitative scores indicating no expression (-ve), mild (+1), moderate (+2), and high expression (+3).
Results: In this study of 46 invasive breast cancer cases, 23.9% had (3+) MAGEC2 expression, 47.8% had (2+), 23.9% had (1+), and 4.4% had no expression. MAGEC2 over-expression was significantly associated with higher tumor grade (p=0.019), but not with other clinicopathological parameters like patient age, histology type, or hormonal receptor status (PR, ER, HER-2).
Conclusion: MAGEC2 according to these results proves to be a powerful marker in predicting the prognosis of breast cancer. With a further work up and with more studies, it may be of routine use in the near future.

Keywords: Clinicopathological, immunohistochemically, MAGE, C2 antigen, breast cancer

Introduction

Breast cancer remains the most frequently diagnosed malignancy globally, accounting for one in eight cancer diagnoses. In 2020, there were approximately 2.3 million new cases and 685,000 deaths worldwide. Notably, the prevalence of breast cancer is higher in nations undergoing economic transitions, but these transitioning countries disproportionately suffer from higher mortality rates. Projections suggest that by 2040, the incidence of breast cancer will rise to over 3 million new cases with 1 million fatalities [1]. Globally, breast cancer represents one in every six cancer deaths among women [2]. The response from public health systems, especially in low- and middle-income countries, has been inadequate in managing this burden, jeopardizing both health outcomes and economic growth [3]. In Iraq, breast cancer has been the leading type of cancer since 1986, surpassing lung cancer. In 2021, the Iraqi cancer registry reported 35,815 cancer cases, with breast cancer constituting 7,246 cases, making up 30.63% of the top ten cancers in both genders and 47.96% among Iraqi women [4]. The highest incidence rates occur in women aged 45-49, with peak age-specific rates in the 50-54 age group [4, 5]. The urgency for enhanced national efforts to curb breast cancer's progression is evident, particularly in developing countries where the lack of comprehensive national cancer programs leads to delayed diagnoses and high mortality rates [6]. Melanoma-associated gene C2 (MAGEC2) is part of the MAGE family, consisting of nineteen members divided into four subfamilies (A to D) based on chromosomal location and protein similarity [7]. MAGEC2 is a cancer/testis antigen highly expressed in several cancers.

Its functions include promoting cell cycle progression, enhancing the migration and invasion of malignant cells, inducing tumor cell morphological changes, and fostering metastasis. The activation of Signal transducer and activator of transcription 3 (STAT3) by MAGEC2 leads to increased transcriptional activity due to the inhibition of STAT3 degradation [8]. Cancer-testis antigens, expressed only in the testis and malignant cells, have become pivotal in cancer therapy, especially in the era of immune-based treatments. These antigens are valuable for their specificity to cancer cells, making them prime targets for therapeutic interventions [9]. The MAGE family, a prominent group of cancer-testis antigens, has seen 24 of its 60 newly identified proteins expressed across various cancers [10]. MAGE proteins, associated with aggressive clinical behaviors, chemotherapy resistance, and poor outcomes, are overexpressed in various cancers, including melanoma, colon, brain, breast, and prostate cancers [11, 12]. Specifically, MAGEC2 is preferentially expressed in high-grade invasive ductal breast cancer and hormone receptor-negative cancers, linking it to enhanced oncogenic activities and increased metastatic potential [13]. This expression profile underscores the potential of MAGEC2 as a target for advanced therapeutic strategies aimed at mitigating tumor progression and improving clinical outcomes in breast cancer and other malignancies.

Objectives: to analyze the expression pattern of MAGEC2 and evaluate its prognostic significance and its association with various clinicopathological prognostic parameters in women with breast cancer**.**

Method

This retrospective cross-sectional study was conducted at the Babylon Training Center for Pathology from December 2022 to December 2023. The study involved 46 formalin-fixed paraffin-embedded tissue blocks from randomly selected breast cancer cases, which included different types of biopsies such as tru-cut, excisional biopsy, and mastectomy specimens. These cases were re-evaluated by an expert pathologist to confirm diagnoses, and clinical data along with ER, PR, and HER-2 statuses were collected from Teba Hospital for Specialized Surgeries and Al-Hilla Teaching Hospital. The control group consisted of positive control sections from the liver processed alongside each set of MAGEC2 immunostaining, and negative controls which were sections untreated with the primary MAGEC2 antibody. The inclusion criteria targeted adult females with varying histological grades and hormonal receptor statuses of tumor cells, while the exclusion criteria ruled out benign breast tumors, male breast cancer, and inadequate biopsy samples. Key materials used in this study included the primary antibody MAGEC2, a polyclonal rabbit antibody with a concentration of 0.9 mg/mL, stored at -20 °C in PBS with 0.05% sodium azide and 50% glycerol. The study also employed a range of equipment such as water baths, humidity chambers, microwaves, hot plates, and an Olympus light microscope. The hematoxylin and eosin staining procedure involved deparaffinizing and rehydrating 5-micron sections, staining with Harris hematoxylin, differentiating in 1% acid alcohol, washing, and then staining with eosin followed by a series of dehydration steps, clearing with xylene, and mounting with DPX. Immunohistochemical staining began with deparaffinization and rehydration of 5 µm thick slices, followed by epitope retrieval using a citrate buffer solution. The sections were treated with a Poly Detector Peroxidase Blocker, primary antibody (dilution 1:50), PolyDetector Plus Link, PolyDetector HRP Label, and a DAB substrate chromogen solution. Hematoxylin was used for counterstaining. A positive immunohistochemical reaction was indicated by dark brown precipitation in the nucleus and/or cytoplasm of the cells. The MAGE C2 protein staining in tumor cells was scored from 0 (no staining) to 3 (strong staining), with the percentage of staining designated from 0-100%. Scores were calculated by multiplying intensity and percentage values, classifying the expression levels from no expression to high expression. Data analysis was performed using SPSS version 27, presenting continuous variables as means ± SD and categorical variables as frequencies and percentages. Fisher's exact test was utilized to determine the significance of associations between categorical variables, with a p-value ≤ 0.05 considered statistically significant.

Results

In a study of 46 breast cancer cases, the expression of MAGEC2 in human breast cancer cells was examined, alongside correlations with age, tumor grade, histopathological types, and hormonal status. The age of the patients ranged from 31 to 80 years, with a mean age of 52.83 ± 12.31 years. Using the Nottingham modification of the Bloom–Richardson system, the tumor grades were classified as grade 1 in 6 cases (13.0%), grade 2 in 28 cases (60.9%), and grade 3 in 12 cases (26.1%). The majority of the cases, 39 (84.8%), were diagnosed with invasive ductal breast cancer, while 7 (15.2%) had invasive lobular breast cancer. The hormonal profile showed that 28 cases (60.9%) were estrogen receptor (ER) positive, 23 cases (50.0%) were progesterone receptor (PR) positive, and 20 cases (43.3%) were HER-2 positive. The age distribution of the patients was as follows: <40 years and 40-50 years comprised 8 patients (17.4%), 51-60 years included 15 patients (32.6%), 61-70 years included 10 patients (21.7%), and >70 years included 5 patients (10.9%). Various specimen types used in the study included excisional biopsy, tru-cut biopsy, and mastectomy. As show in Fig 1.

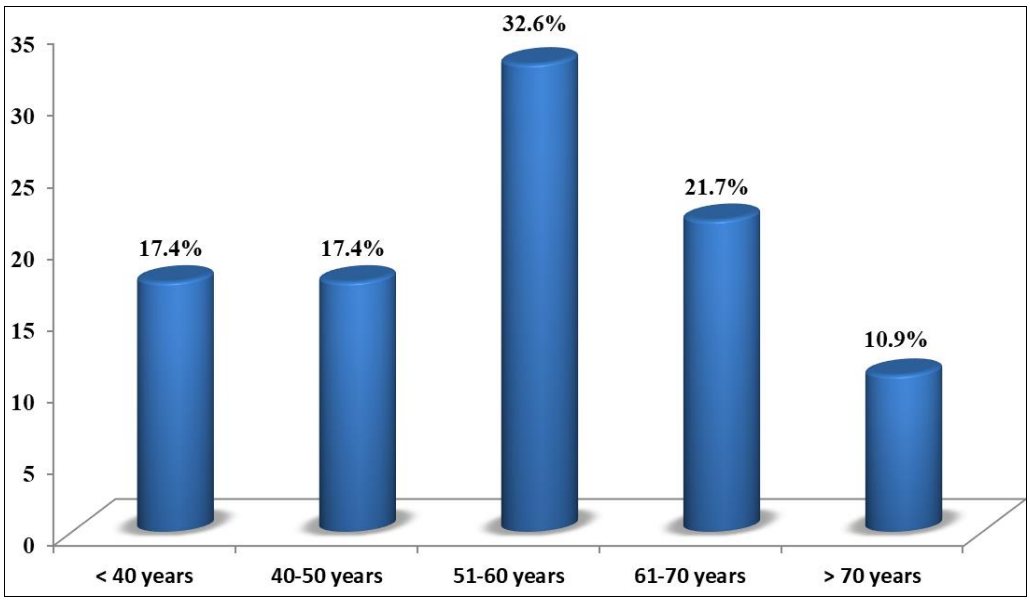

Fig 1: Distribution of breast cancer patients according to age (N=46)

Distribution of breast cancer patients according to Types including (Invasive ductal carcinoma and Invasive lobular carcinoma). Invasive ductal carcinoma represents majority of patients (N= 39, 84.8%) and Invasive lobular carcinoma represent only 7 patients (15.2%), as shown in Figure (2)

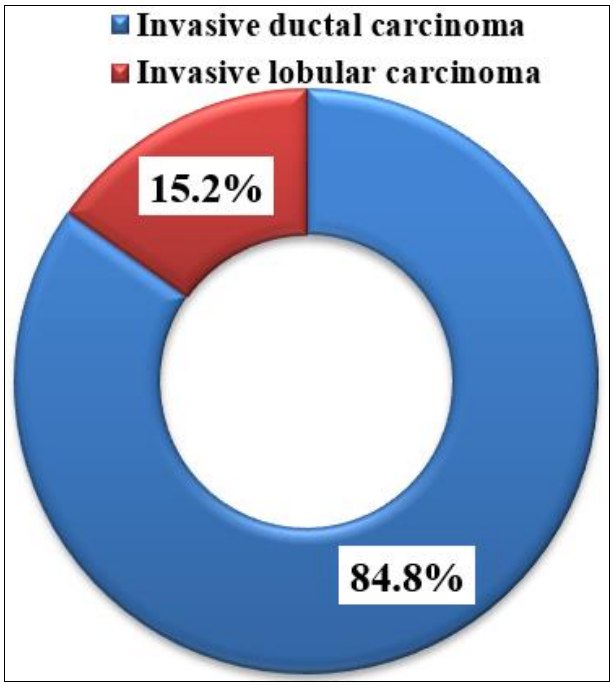

Fig 2: Distribution of breast cancer patients according to Types (N=46)

Distribution of breast cancer patients according to grade including (grade I, grade II and grade III). Grade I represent only 6 patients (13.0%). Grade II represent majority of patients (N=28, 60.9%) and grade III represent only 12 patients (26.1%), as shown in Table 1.

Table 1: Distribution of breast cancer patients according to grade (N=46)

Grade	Number	%
Grade I	6	13.0%
Grade II	28	60.9%
Grade III	12	26.1%
Total	46	100.0%

Distribution of breast cancer patients according to Hormone receptor and HER-2-neu status Distribution of breast cancer patients according to hormone receptor status including (ER, PR and HER-2). Positive ER represent 28 patients (60.9%). Positive PR represent 23 patients (50.0%) and positive HER-2 represent 20 patients (43.5%), as shown in Figure 3.

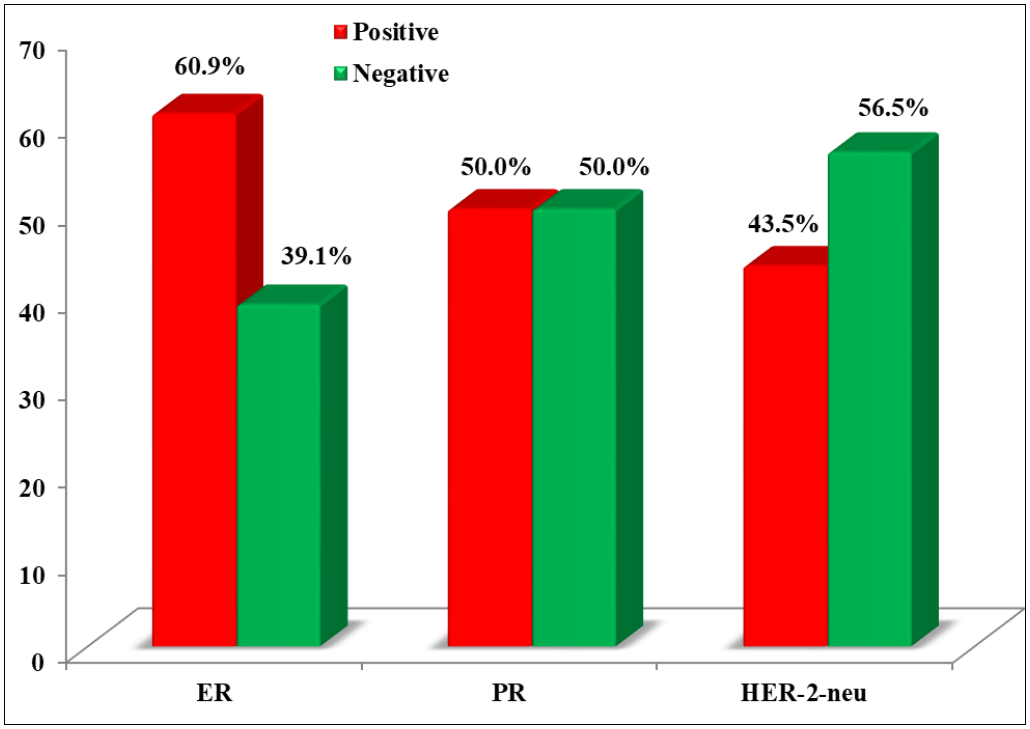

Fig 3: Distribution of breast cancer patients according to Hormone receptor and HER-2-neu status (N=46).

Distribution of breast cancer patients according to Melanoma associated gene C2 expression including (negative, (+1), (+2) and (+3)). Negative MAGE C2 represent only 2 patients (4.4%). MAGE C2 (+1) represent 11 patients (23.9%). MAGE C2 (+2) represent less than half of patients 22 patients (47.8%) and MAGE C2 (+3) represent 11 patients (23.9%), as shown in Figure 4. associated gene C2 expression (N=46)

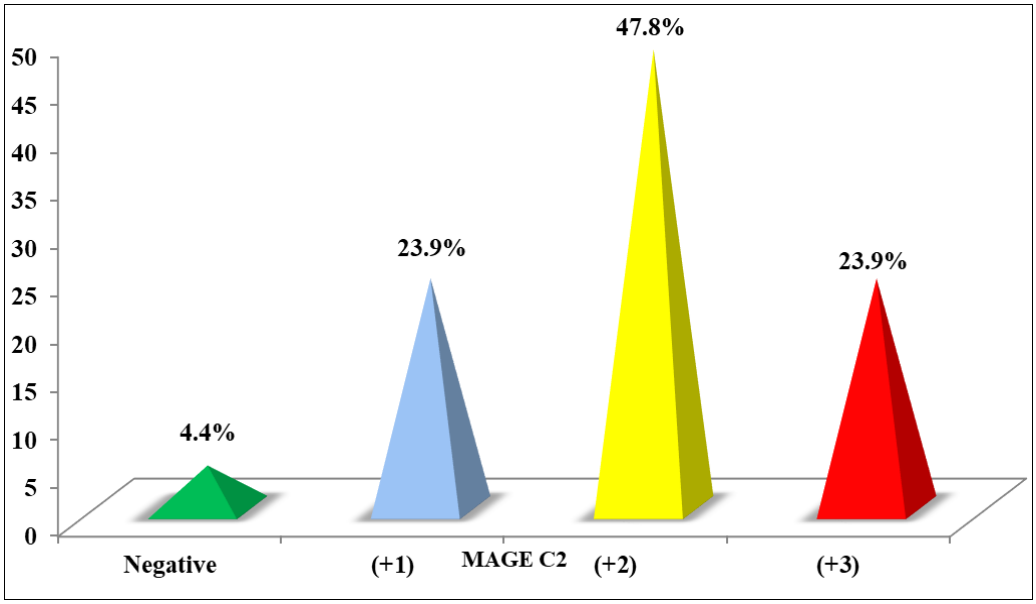

Fig 4: Distribution of breast cancer patients according to Melanoma associated gene C2 expression

The association between Melanoma associated gene C2 expression including (Negative, (+1), (+2) and (+3)) and study variables including (age, diagnosis and type of specimen) among study patients with breast cancer. There was no significant association between Melanoma associated gene C2 expression age and diagnosis, as shown in Table 2.

Table 2: The association between MAGE C2, age and diagnosis (N=46)

Study variables	MAGE C2				Total (N=46)	P value
	Negative (N=2)	+1 (N=11)	+2 (N=22)	+3 (N=11)		
Age (Years)	0 (0.0)	2 (18.2)	4 (18.2)	2 (18.2)	8 (17.4)	0.836
< 40 years						
40-50 years	0 (0.0)	2 (18.2)	5 (22.7)	1 (9.1)	8 (17.4)	

51-60 years	1 (50.0)	3 (27.3)	5 (22.7)	6 (54.5)	15 (32.6)	
61-70 years	0 (0.0)	3 (27.3)	6 (27.3)	1 (9.1)	10 (21.7)	
>70 years	1 (50.0)	1 (9.1)	2 (9.1)	1 (9.1)	5 (10.9)	
Total	2 (100.0)	11 (100.0)	22 (100.0)	11 (100.0)	46 (100.0)	
Diagnosis						
Invasive ductal carcinoma	2 (100.0)	10 (90.9)	19 (86.4)	8 (72.7)	39 (84.8)	0.688
Invasive lobular carcinoma	0 (0.0)	1 (9.1)	3 (13.6)	3 (27.3)	7 (15.2)	
Total	2 (100.0)	11 (100.0)	22 (100.0)	11 (100.0)	46 (100.0)	

The association between Melanoma associated gene C2 expression including (negative, (+1), (+2) and (+3)) and grade of breast cancer including (grade I, grade II and grade III). There was significant association between Melanoma associated gene C2 expression and grade of breast cancer. Majority of patients with Melanoma associated gene C2 expression (+3) (N=10, 91.0%) presented with grade II and Grade III, as shown in Table 3

Table 3: The association between MAGE C2 and grade of breast cancer (N=46)

Grade of breast cancer	MAGE C2				Total (N=46)	P value
	Negative (N=2)	+1 (N=11)	+2 (N=22)	+3 (N=11)		
Grade I	1 (50.0)	3 (27.3)	1 (4.5)	1 (9.0)	6 (13.0)	0.019
Grade II	0 (0.0)	8 (72.7)	15 (68.2)	5 (45.5)	28 (60.9)	
Grade III	1 (50.0)	0 (0.0)	6 (27.3)	5 (45.5)	12 (26.1)	
Total	2 (100.0)	11 (100.0)	22 (100.0)	11 (100.0)	46 (100.0)	

The association between Melanoma associated gene C2 expression including (negative, (+1), (+2) and (+3)) and hormone receptor status including (ER, PR) and HER-2/neu status. There was no significant association between Melanoma associated gene C2 expression hormone receptors and HER-2/neu status, as shown in Table (4).

Table 4: The association between MAGE C2, hormone receptors and HER-2/neu status (N=46)

Hormone receptor and HER-2/neu status	MAGE C2				Total (N=46)	P value
	Negative (N=2)	+1 (N=11)	+2 (N=22)	+3 (N=11)		
ER						
Positive	1 (50.0)	9 (81.8)	12 (54.5)	6 (54.5)	28 (60.9)	0.413
Negative	1 (50.0)	2 (18.2)	10 (45.5)	5 (45.5)	18 (39.1)	
Total	2 (100.0)	11 (100.0)	22 (100.0)	11 (100.0)	46(100.0)	
PR						
Positive	0 (0.0)	7 (63.6)	10 (45.5)	6 (54.5)	23 (50.0)	0.471
Negative	2 (100.0)	4 (36.4)	12 (54.5)	5 (45.5)	23 (50.0)	
Total	2 (100.0)	11 (100.0)	22 (100.0)	11 (100.0)	46(100.0)	
HER-2						
Positive	1 (50.0)	4 (36.4)	11 (50.0)	4 (36.4)	20 (43.5)	0.892
Negative	1 (50.0)	7 (63.6)	11 (50.0)	7 (63.6)	26 (56.5)	
Total	2 (100.0)	11 (100.0)	22(100.0)	11 (100.0)	46(100.0)	

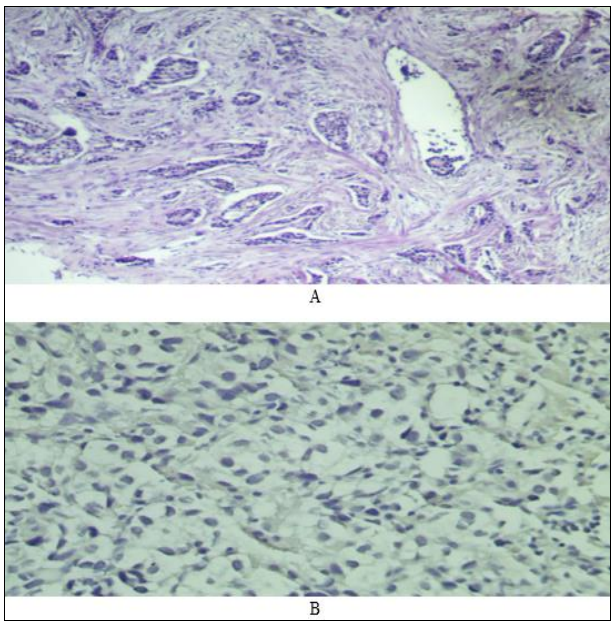

Fig 5: Invasive ductal carcinoma, grade 1 A: H&E stained slide section (X10). B: MAGEC2 stained slide section showing no expression (X40).

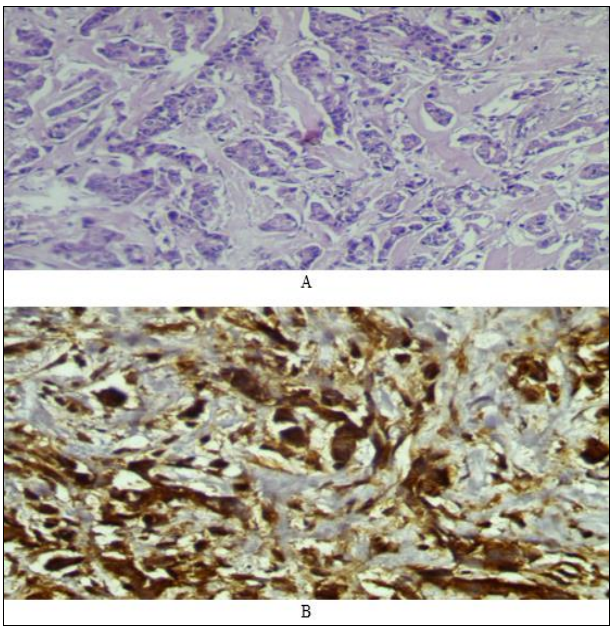

Fig 6: Invasive ductal carcinoma, grade 3, A: H&E stained slide section (X10). B: MAGEC2 stained slide section with diffuse dark brown nuclear and cytoplasmic staining, score +3 (X40)

Discussion

Breast cancer is the most frequently diagnosed malignant tumor among women and a leading cause of death. Early detection of breast carcinoma highlights the need for effective diagnostic biomarkers to improve prognosis and therapeutic outcomes. Tumor-specific antigens, such as cancer-testis antigens (CTAs), are a promising method in immune-based cancer therapy, as they redirect immune cells to target these specific antigens. Some CTAs are used as molecular targets in pharmacological and immune-targeted therapies for various cancers [1, 9]. MAGEC2, a CTA, is highly expressed in many cancers and is shown to accelerate cell cycle progression, invasion, and migration of cancer cells, thereby promoting metastasis [8]. Recent studies have found that MAGEC2 is preferentially expressed in high-grade invasive ductal breast cancer and hormone receptor-negative cancers [13]. This study aimed to evaluate MAGEC2 expression in breast cancer cells and its correlation with clinicopathological parameters, including patient age, histological type, tumor grade, and ER, PR, and HER2/neu status. Several previous studies were reviewed, including those by Yang F, *et al*. (China, 2014; 540 breast cancer and 23 noncancerous breast tissues) [14], Hou S, *et al*. (China, 2015; 60 primary breast cancer specimens and 60 tumor-free breast specimens) [15], and Zhao Q, *et al*. (China, 2016; 110 tumor samples) [16]. In this study, MAGEC2 expression was positive in 95.7% of invasive breast cancer cases and negative in 4.4%. The patients' ages ranged from 31 to 80 years, with the highest percentage in the 50-60 age group (32.6%) and the lowest in the >70 age group (10.9%). There were no significant differences in MAGEC2 expression relative to patient age (p=0.836), consistent with Yang F, *et al*. [14], Hou S, *et al*. [15], and Zhao Q, *et al*. [16]. MAGEC2 expression was strongly associated with higher tumor grades. In grade 3 tumors, 91.7% showed moderate to strong MAGEC2 expression, compared to 71.1% in grade 2 and 33.3% in grade 1, suggesting more aggressive and less differentiated carcinomas. This finding aligns with studies by Yang F, *et al*. [14] and Hou S, *et al*. [15]. Notably, lower-grade tumors exhibited cytoplasmic staining, whereas high-grade tumors showed both nuclear and cytoplasmic staining. Regarding hormonal profiles, the study found that MAGEC2 expression was highest in ER-negative patients (83.3% moderate to strong expression), compared to 64.3% in ER-positive patients. This is consistent with Yang F, *et al*. [14] and Hou S, *et al*. [15]. For PR status, MAGEC2 expression was higher in PR-negative cases (73.9%) compared to PR-positive cases (69.6%), aligning with Hou S, *et al*. [15] but not with Yang F, *et al*. [14]. HER-2 positive cases also showed higher MAGEC2 expression (75% moderate to strong) compared to HER-2 negative cases (69.2%), consistent with Hou S, *et al*. [15] but not Yang F, *et al*. [17]. Statistically, there was no significant association between MAGEC2 expression and ER, PR, and HER-2/neu status (p=0.413, 0.471, and 0.892 respectively), consistent with Hou S, *et al*. [15]. Interestingly, the highest MAGEC2 expression was found in invasive lobular carcinoma (85.7% moderate to strong) compared to invasive ductal carcinoma (69.2%). This contradicts Hou S, *et al*. [15], which may be due to differences in tumor histological features or methodology. There was no significant association between MAGEC2 expression and histopathological types (p=0.688), inconsistent with Hou S, *et al*. [15]. Variations among studies may result from different antibody clones, measurement methods, or procedures used to assess protein expression in tumor cells. This study underscores the potential role of MAGEC2 as a biomarker and therapeutic target in breast cancer, given its strong association with higher tumor grades and specific hormonal profiles. Further research and standardization are needed to clarify its clinical implications.

Conclusion

The aggressive behavior of high-grade malignancies is significantly influenced by the overexpression of the melanoma-associated gene (MAGEC2). The expression of MAGEC2 is not significantly correlated with the status of hormonal receptors, histologic type, or age. The melanoma associated gene (MAGEC2) can be employed as a prognostic marker.

Conflict of Interest

Not available

Financial Support

Not available

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