Heliyon 10 (2024) e34506

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Expression pattern analysis of the *MAGE* family genes in breast cancer patients and hypomethylation activation in the MCF-7 cells

Ahmad M. Almatrafi [a,1], Salman Alamery [b], Mikhlid H. Almutairi [c,*,1]

[a] Department of Biology, College of Science, Taibah University, Medina, Saudi Arabia
[b] Department of Biochemistry, College of Science, King Saud University, P.O. Box 2455, Riyadh, 11451, Saudi Arabia
[c] Zoology Department, College of Science, King Saud University, P.O. Box 2455, Riyadh, 11451, Saudi Arabia

ARTICLE INFO

Keywords:
Cancer-testis genes
MAGE gene
Breast cancer
Expression
Biomarkers
5-Aza-2′-deoxycytidine
Hypomethylation
MCF-7

ABSTRACT

Melanoma antigen gene (MAGE) families are cancer-testis genes that normally show expression in the testes. However, their expressions have been linked with various types of human cancers, including BC. Therefore, the primary purposes of the present research were to assess the expression of *MAGE-A*, -B, and –C genes in Saudi female patients with BC and determine their regulation via the epigenetic mechanism. Ten BC samples were analyzed for the expression levels of nine *MAGE-A* genes, six *MAGE-B* genes, and three *MAGE-C* genes using the RT-PCR technique. All 18 evaluated genes except for *MAGE-A1*, *-A3*, *-A4,* and *-B5* showed weak band expressions in some BC specimens. *MAGE-A6* and *-B2* were expressed in 40 % of the BC tissue samples, and *MAGE-A9*, *-A10*, and *-B6* were expressed in 30 %. The lowest expression levels were found for *MAGE-A11*, *-B1*, *-B3*, *-B4*, *-C1*, and *-C2* in 10 % of the BC specimens and for *MAGE-A9,–B2*, and *–C3* in 20 % of the samples. The most frequently expressed gene was *MAGE-A8* (found in 70 % of the BC samples), which suggests that it may serve as - a marker for screening of BC. *In vitro* treatment, the 5-aza-2′-deoxycytidine agent led to a significant rise in mRNA expressions for all tested genes related to the *MAGE-A* family, except for *MAGE-A10*. By contrast, among the genes in the *MAGE-B* and *-C* families, only *MAGE-B1* and *-C2* exhibited detectable mRNA expression levels after treatment.

1. Introduction

Breast cancer (BC) is an inflammatory heterogeneous disease that arises from various distinct genetic and epigenetic changes that cause mammary epithelial cells to develop into aggressive malignant cells [1]. It is also the most frequently diagnosed cancer among women overall [2]. According to data from the Global Cancer in 2020, there was an estimation of over 2.3 million new patients and 685,000 deaths from BC were recorded [3]. The global incidence and mortality rates are expected to increase by 1.44 % and 0.23 % per year, respectively, with varying disease burdens between geographical regions [4]. Several risk factors interact with the development of BC, including environmental factors, race, ethnicity, and genetics [5].

In Saudi Arabia, BC has a prevalence rate of 29.7 % in female cancer, leading to the most prevalent cancer in females [6]. More than 50 % of these women are diagnosed in the late stages, which is a much higher percentage than the 20 % in developed countries [7].

* Corresponding author. Zoology Department, College of Science, King Saud University, Riyadh, Saudi Arabia.
E-mail addresses: amatrafi@taibahu.edu.sa (A.M. Almatrafi), salamery@ksu.edu.sa (S. Alamery), malmutari@ksu.edu.sa (M.H. Almutairi).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.heliyon.2024.e34506
Received 20 September 2023; Received in revised form 4 July 2024; Accepted 10 July 2024
Available online 11 July 2024

Delayed detection of BC is associated with increased mortality rates, high treatment costs, and reduced cure potential. Therefore, early detection and successful treatment strategies for BC are required to increase survival and the chance of receiving therapy [8].

In the past two decades, several studies have been conducted to identify specific tumor antigens for clinical diagnostic and immunotherapeutic targets. Among these potential antigens, cancer-testis (CT) antigens (CTAs) caught attention due to their patterns of expression being restricted to human testicular germ and embryonic stem cells. The aberrant expressions of these antigens have been found in multiple forms of cancer, including lung, colon, ovarian, and prostate cancers, melanoma, and BCs [9–11]. In fact, the human testis is a highly immunologically privileged site because of the blood testis barrier, with no expressions of the MHC class I antigens in the testicular germ cells, preventing CTAs from being recognized as non-self-structures in the immune system [12].

Melanoma antigen 1 (MAGE-A1) was the first CTA identified in 1991 in melanoma cells [13]. The expression patterns of CT genes during spermatogenesis and embryonic growth have been well documented. However, the main biological functions of several antigens in germ cells and cancers remain elusive [14].

The expressions of CT genes in cancerous cells have been linked to poor prognosis, aggressive tumorigenesis, and accelerated tumor development [12]. Until now, more than 228 CT genes have been described and categorized in 70 families, supported by their unique expressions and serological data. More than 52 % of these identified CT genes are mapped on chromosome X (CT-X), while the rest are distributed in other autosome chromosomes known as non-X CT genes [15–17]. Moreover, CT-X genes constitute nearly 10 % of X-chromosome genes and are often organized as a multigene group with well-defined clusters [18,19].

Among these CT-X genes, those in the human MAGE family, particularly the *MAGE-A*, *B*, and *C* families, have gained particular attention as novel cancer markers and Targeted immunotherapy [20,21]. Members of these families are often not active in normal human tissues but active in the testis. However, their expressions have been detected in various malignancies, including breast cancer [22–25], ovarian cancer [26,27], colon cancer [28,29], prostate cancer [30], and lung cancers [31,32] and melanoma [33,34]. New reports suggest that MAGEs are associated not only with an adverse clinico-pathological profile but additionally promote tumor development. Examples of this malignancy type which develop an addiction to MAGE expression upon phenotypic reprogramming comprise breast, lung, colon, mast cell, multiple myeloma, and melanoma cancers and its many forms [35]. In addition to these studies, in vitro analyses have shown that cancer lines expressing MAGE-A3 and -C2 exhibit high invasive potential compared to the corresponding control cells. In addition, MAGE-A3 and -A6 increase cancer cells' proliferation in soft agar and stimulate fibroblast transformation. The fact that MAGE-A6 encourages normal diploid colonic epithelial cell proliferation without anchoring is even more remarkable [36]. Also, MAGEs promote tumorigenesis when tested on living organisms. Human thyroid carcinoma cells overexpressing MAGE-A3 in orthotopic xenografts showed enhanced tumor growth and lung metastases [37], whereas *in vivo*, MAGE-C knockdown postponed tumor formation in metastatic melanoma [38]. In addition, a syngeneic mouse tumor model showed that MAGE-B suppression inhibited melanoma cell proliferation [39].

There are 12 genes in the *MAGE-A* family clustered on the Xq28 locus [40]. The expression rates of *MAGE-A* family genes in BC range from 6 % to 69 %, with higher expression patterns in patients with higher recurrence risks [41]. In 2014, Ayyoub et al. found that there was the important relationship between the gene expression status of *MAGE-A3* and *-A6* in patients with BC and tumor grade and poor prognosis [22].

In addition, the *MAGE-B* family clusters in the Xp21 region include four genes (*MAGE-B1*, *-B2*, *-B3*, *-B4*, *-B5*, and *-B6*) that share a strong homology with MAGE-A members [42]. In 2018, Vodolazhsky et al. reported overexpression of the *MAGE-B1* gene in metastatic compared with non-metastatic BC samples [43]. Administration of antitumor agents against *MAGE-B1* and *MAGE-B3* was effectively controlling metastases and residual tumor cells in a mouse BC tumor [44,45]. The MAGE-C family is comprised of *MAGE-C1*, *-C2,* and *-C3* present in chromosome Xq26–27 [46]. Overexpression of *MAGE-C1*, in addition to *MAGE-C2* genes, was noticeably associated with advanced BC stages and poor patient prognosis [23].

DNA methylation has been identified as an important regulating mechanism of several CT genes [12]. The methyl groups (CH_3) is often added to the promoter regions of certain genes by DNA methyltransferase enzymes (DNMTs). This can cause suppression of gene expression. The (DNMTs) activity controls the expressions of numerous CT genes through DNA hypermethylation [47,48]. Recent studies found that when colon cancer is treated with a DNA methyltransferase inhibitor (DNMTi) drug, it activates the expressions of *MAGE-A* and *-B* [29,49].

The mRNA expression patterns of many genes in the *MAGE-A*, *-B*, and *-C* families have not been studied in Saudi women with BC. Hence, this research aimed to assess the expression patterns of these genes in Saudi women with BC. Its secondary objective was to identify the potential regulating mechanism of these genes in the BC cell line.

2. Materials and methods

2.1. Human tissue samples for BC, with ethical approval

10 BC tissue samples were obtained from women of Saudi origin who underwent surgical treatment at the King Khalid University Hospital in Riyadh between 2020 and 2022. Not all diagnosed patients received physiotherapy, chemotherapy, or radiotherapy treatment before the tissue collection. The collected tissue samples were thenimmediately stored in Eppendorf tubes containing 800 μL of RNAlater stabilization solution (76106; Thermo Fisher Scientific). Consent forms were given and signed by each patient who participated in the study who completed a survey on their medical condition, age, history of the disease in the family, and social behavior. The institutional review board approved this research under ethical reference number (IRB No. HAPO-01-R-011, Project No. 56/2020) at the University of Al-Imam Muhammad Ibn Saud Islamic.

2.2. Gene selection and primer construction

All *MAGE-A*, *B*, and *C* gene sequences were retrieved from the NCBI. Primers were designed using the Primer-BLAST program so that each primer span had at least one intron. The anticipated fragment sizes and primer sequences for each gene are shown in Table 1.

2.3. Extraction of RNA followed by cDNA synthesis

Tissue samples were weighed, and 50 mg of each BC specimen was used for total RNA extraction. The extracted RNA quality and quantity were checked using a Drop8000 spectrophotometer (Thermo Fisher Scientific). To synthesize the cDNA, 1 μg of extracted RNA was used in conjunction with cDNA Reverse Transcription Kit (4,368,814; Applied Biosystems). The master mix for cDNA synthesis was performed in a PCR tube in a total volume of 10 μL per reaction, which was prepared as follows: 10 × RT buffer (2 μL), 25 × dNTP mix (0.8 μL), 10 × RT random primers (2 μL), MultiScribe™ Reverse Transcriptase (1 μL), and nuclease-free water (4.2 μL). The master mix was then briefly vortexed and centrifuged to mix and spin down the contents. Following that, the reaction tubes were loaded onto the thermal cycler with the following conditions: step 1 at 25 °C for 10 min, step 2 at 37 °C for 120 min, and step 3 at 85 °C for 5 min. In preparation for future use, the generated cDNA was diluted 1:10 and placed at −20 °C.

2.4. qRT-PCR and sequencing methods

The RT-PCR was carried out using 100 ng of cDNA from each specimen. PCR conditions and components were used as previously described [29,49]. The housekeeping gene *ACTB* was used as internal controls for cDNA quality and normalization. 1 % agarose gel with 0.5-g/mL ethidium bromide was used to check PCR products. For assessment of the exact PCR product size, 2.5 μL of 100-bp DNA Ladder was loaded. The RT-PCR reactions were run as follows: initial denaturation at 96 °C for 5 min (one cycle), denaturation at 96 °C for 30 s (35 cycles), primer annealing at 58 °C for 30 s (35 cycles), extension at 72 °C for 30 s (35 cycles), and final extension at 72 °C for 5 min (one cycle). A total of 15 μL of PCR products for each gene were sent to Microgen Inc. for direct sequencing. The BLAST-NCBI tool

Table 1
List of *MAGE-A*, *MAGE-B*, and *MAGE-C* gene names with their primer sequences investigated in this research.

Gene name	Primer sequence (5' → 3')	Accession number	Product size (bp)
ACTB	Sense: AGAAAATCTGGCACCACACC Antisense: AGGAAGGAAGGCTGGAAGAG	NM_001101.5	*553*
MAGE-A1	Sense: CCCACTACCATCAACTTCAC Antisense: CTCTTGCACTGACCTTGATC	NM_004988.5	*676*
MAGE-A2	Sense: CGCTTCTTCCTCTTCTACTC Antisense: GGCAATTTCTGAGGACACTC	NM_001282501.2	*317*
MAGE-A3	Sense: CTCCTCCTCTTCTACTCTAG Antisense: CCCTCAAACACCTCTAACAC	NM_005362.4	*582*
MAGE-A4	Sense: CTACCATCAGCTTCACTTGC Antisense: CTCCAGGACTTTCACATAGC	NM_001011548.1	*647*
MAGE-A6	Sense: CTCCTCCTCTTCTACTCTAG Antisense: GGCATGATCTGATTGTCACC	NM_005363.5	*474*
MAGE-A8	Sense: GTTCCAGCAGCAATGAAGAG Antisense: CATGCCCAGGACGATTATCA	NM_001166400.2	*377*
MAGE-A9	Sense: CCTCCATTTCCGTCTACTAC Antisense: CTCAACGCTTCCCAGATAAC	NM_005365.5	*475*
MAGE-A10	Sense: TGGTGCAGTTTCTGCTCTTC Antisense: CACAGAAACTCATACCGTGC	NM_001011543.3	*472*
MAGE-A11	Sense: CAGGTGAGCACTATGTTCTC Antisense: TCCCAAAGATGGCATCCATG	NM_001011544.2	*478*
MAGE-B1	Sense: CAGGAATGCTGATGCACTTC Antisense: GAGGACTTTCATCTTGGTGG	NM_002363.5	*524*
MAGE-B2	Sense: CACTGAAGCAGAGGAAGAAG Antisense: GGTCTACCTTGTCGATGAAG	NM_002364.5	*467*
MAGE-B3	Sense: GACTCCTATGTCCTTGTCAG Antisense: GCACTACTGCCATCATTGAG	NM_001386865.1	*464*
MAGE-B4	Sense: TCTTTGGCCTTGCCTTGAAG Antisense: GGAATACGCACTAGTCATGG	NM_002367.4	*524*
MAGE-B5	Sense: CAGTAGAGATGAGGAGTACC Antisense: GGGCTCTCCATAGATGTAGT	NM_001271752.1	*472*
MAGE-B6	Sense: GCGCTTAAGCAAAGATGCTG Antisense: GCCGGTAAACCACGTACTTA	NM_173523.2	*473*
MAGE-C1	Sense: GTTCTCCAGATTCCTGTGAG Antisense: CAGTAAAGTGGAGGAGAAGG	NM_005462.5	*675*
MAGE-C2	Sense: CATGGAGCTCATTCAGTGAG Antisense: CCGATACTCCAGGTAATGTC	NM_016249.4	*539*
MAGE-C3	Sense: AGTGAAGAGGAGGATACAG Antisense: GACACAGCTGCCCTTTATGA	NM_138702.1	*391*

was used for sequencing analysis.

2.5. Cell culture and analysis of DNA hypomethylation

The human BC MCF-7 cells were obtained from ATCC (Manassas, VA) and grown in RPMI-1640 media containing 10 % fetal bovine serum and 1 % penicillin-streptomycin. The cells were then exposed to an incubation temperature of 37 °C in a 5 % CO_2 environment. To investigate the effects of a demethylating agent, the MCF-7 cells were treated with two doses of 1.0- and 10.0-μM 5-aza-2′-deoxycytidine (A3656; Sigma) for 72 h. For comparison, the control cells were exposed to an equivalent concentration of the drug solvent DMSO. The culture media were refreshed every 24 h during the treatment period. Total RNA was isolated from the cells, and cDNA synthesis was performed after the procedures in Section 2.3.

2.6. Gene expression analysis via the cancer genome Atlas database

The RNA expression levels of the genes from the *MAGE-A*, *-B*, and *-C* families in breast invasive carcinoma (BRCA) tissue samples were analyzed, taking advantage of the information available in The Cancer Genome Atlas (TCGA) database and compared with those in normal breast (NB) tissue samples. The expression patterns were confirmed in both the BRCA and BC tissue samples using RNA-seq and medical data obtained from the large number of cancer cases available in OncoDB (https://oncodb.org/index.html) for each candidate gene. The RNA-seq data were divided into the BRCA and NB groups, and the difference in gene expression level between the two groups was determined using log2-fold change values and the Student *t*-test. Statistical significance was measured by setting the *p*-value at ≤ 0.05.

2.7. In silico analysis

Genes from the *MAGE-A*, *-B*, and *-C* families were analyzed for their coexpressions and gene-gene interaction networks using the GeneMANIA tool (https://genemania.org/) and their association function. In addition, the common gene networks and predicted related genes were analyzed.

2.8. Functional enrichment analysis

The functional enrichment analysis was conducted using the Gene Ontology: Cellular Component gene sets, pathway analysis in g:profiler (v.0.2.1) https://biit.cs.ut.ee/gprofiler/gost) [50] and WebGestalt (https://www.webgestalt.org/) [51]. Cytoscape (v.3.9.1) was used to visualize over-represented, significant terms (FDR <0.05) from library-unique gene sets. In this analysis, we used 20 genes, which also showed ratios of interaction and co-expression different from those of the genes. We evaluated: MAGEA-9B, MAGE-B16, MAGE-B17, MAGEB-6B, MAGE-A2B, MAGE-B10, MAGE-B18, MAGE-A12, MAGE-D4, MAGE-D4B, MAGE-E2, MAGE-L2, MAGEE1, NSMCE3, MAGEH1, MAGE-F1, TRO, MAGE-D2, NDN, and MAGE-D1. This analysis allowed us to identify enriched biological processes, molecular functions, and cellular components associated with the differentially expressed genes, including members of the MAGE gene family, in BC tissues.

3. Results

3.1. Clinical information of the patients with BC

Table 2 displays the demographic and medical data of the patients with BC who participated in this research. The mean age at the

Table 2
Clinical features of the patients with BC who participated in the research.

Characteristic	Number of patients	Percentage
Patients	10	100 %
Average of age (min-max)	51 (32–74)	
Above 51	4	40 %
Below 51	6	60 %
Estrogen Receptor (ER)		
ER+	2	20 %
ER-	8	80 %
Progesterone Receptor (PR)		
PR+	3	30 %
PR-	7	70 %
BC grades		
I	1	10 %
II	6	60 %
III	2	20 %
IIII	1	10 %

time of diagnosis for 10 patients with BC was 51 years (age range, 32–74 years). Of the patients, 60 % were younger than 51 years. Estrogen receptors were negative (ER−) in 80 % of the patients but positive (ER+) in 20 %. Progesterone receptors were negative (PR−) in nearly 70 % of the participants and positive (PR+) in 30 %. Most cases (60 %) were grade II, whereas 20 % were grade III, and 10 % were either grade I or IIII.

3.2. MAGE-A family member expressions in the testis and BC tissues

In this study, 18 genes belonging to the *MAGE-A*, *-B*, and *-C* families were selected on the basis of three main criteria. First, these selected genes were classified as testis restricted and showed no mRNA expressions in the normal human tissues, according to the NCBI database. Second, a literature search was conducted to search the RNA expression profiles of these selected genes in both BC tissues and breast cell lines [20,21,24]. Third, most selected genes were associated with malignancy [22,29].

Nine *MAGE-A* gene family members, namely *-A1*, *-A2*, *-A3*, *-A4*, *-A6*, *-A8*, *-A9*, *-A10*, and *-A11*, and their mRNA levels were verified using the RT-PCR tool in 10 human BC tissue samples. Genes that appeared in Table 1 were evaluated by RT-PCR using testis cDNA synthesized from commercial normal testis RNA (AM7972; Thermo Fisher Scientific). The *ACTB* gene was used as the quality control standard for the cDNA, and the PCR test was repeated in triplicate for each gene.

The RNA expression patterns of *MAGE- A1*, *- A3*, and *- A4* were restricted only to the normal human testis. However, these genes were not expressed in all 10 BC tissue samples. By contrast, *MAGE-A2*, *-A6*, *-A8*, *-A9*, *-A10*, and *-A11* showed weak expressions in the RT-PCR products of the BC tissue samples (Fig. 1 and Fig. S1). The most highly expressed gene in the BC tissue samples was *MAGE-A8* (70 %), followed by-*A6* (50 %), *-A9* (30 %), *-A2* (20 %), and *-A11* (10 %). The *MAGE-A10* gene showed a very weak RT-PCR expression in nearly 30 % of the specimens. The PCR products of *MAGE-A2*, *-A6*, *-A8*, *-A9*, *-A10*, and *-A11* were sequenced, followed by a comparison with the related sequences available on the NCBI Genbank (Table 3). The sequence alignment results showed a high sequence similarity to these genes.

3.3. MAGE-B family member expressions in the testis and BC tissues

Of the six genes belonging to the *MAGE-B* family, five showed various expression patterns in the BC tissue samples. The RT-PCR screening of *MAGE-B1* and *-B6* showed a medium band in 10 % and 30 % of the BC tissues, respectively (Fig. 2 and Fig. S2). Meanwhile, weak expression patterns of *MAGE-B2*, *-B3*, and *-B4* were detected in the BC tissue samples, with *MAGE-B2* having the highest expression rate (40 % of the tissue samples), followed by *MAGE-B3* (20 %) and then *MAGE-B4* (10 %). *MAGE-B5* was the only member

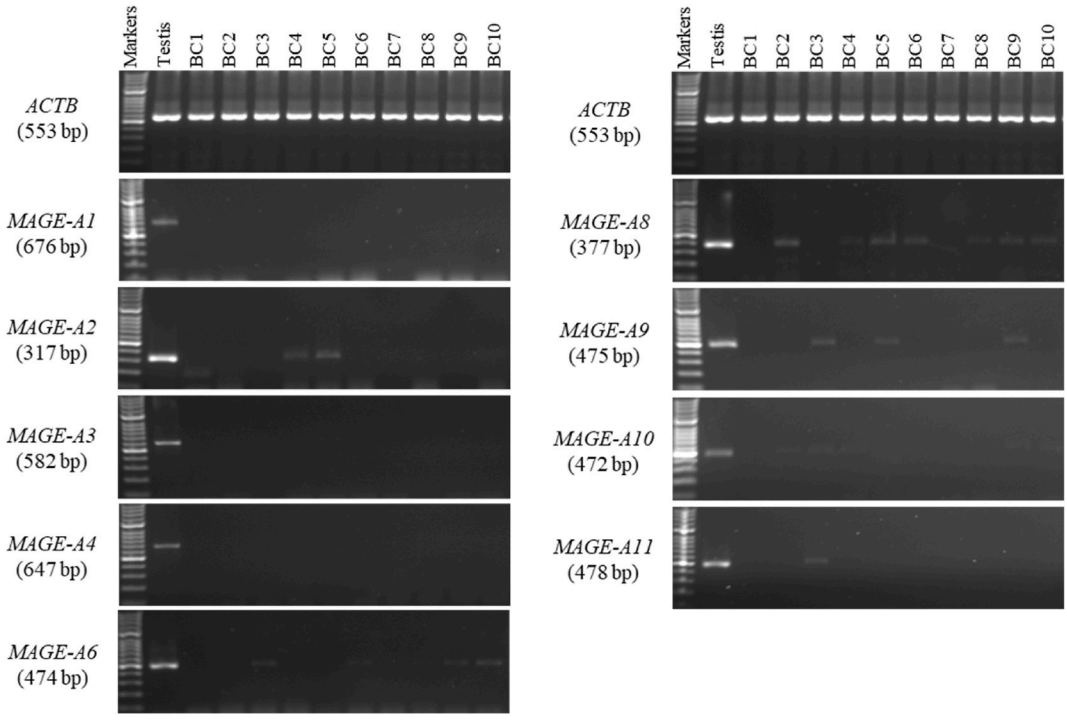


Fig. 1. RT-PCR results of nine genes belonging to the *MAGE-A* family evaluated in 10 BC specimens. *MAGE-A2*, *-A6*, *-A8*, *-A9*, *-A10*, and *-A11* showed weak PCR products, whereas *MAGE-A1*, *-A3,* and *-A4* showed no PCR product. All *MAGE-A* gene primers were analyzed on the cDNA of human testis, and each primer's performance was evaluated using *ACTB* expression. The expected size of PCR band for each candidate gene is shown between brackets on the left side.

Table 3
Summary of the sequence analysis findings for the *MAGE* family genes in the testis and BC tissue samples.

Gene name	Primer direction	Sequence in testis and BC samples		Sequence identity (%)
		Testis	BC	
MAGE-A2	Sense	Yes	–	100
	Antisense	Yes	–	100
	Sense	–	5	100
	Antisense	–	5	98
MAGE-A6	Sense	Yes	–	100
	Antisense	Yes	–	100
	Sense	–	10	99
	Antisense	–	10	98
MAGE-A8	Sense	Yes	–	100
	Antisense	Yes	–	100
	Sense	–	2	98
	Antisense	–	2	96
MAGE-A9	Sense	Yes	–	100
	Antisense	Yes	–	100
	Sense	–	3	98
	Antisense	–	3	100
MAGE-A10	Sense	Yes	–	100
	Antisense	Yes	–	100
	Sense	–	3	97
	Antisense	–	3	97
MAGE-A11	Sense	Yes	–	100
	Antisense	Yes	–	100
	Sense	–	3	97
	Antisense	–	3	99
MAGE-B1	Sense	Yes	–	100
	Antisense	Yes	–	100
	Sense	–	3	100
	Antisense	–	3	99
MAGE-B2	Sense	Yes	–	100
	Antisense	Yes	–	100
	Sense	–	5	97
	Antisense	–	5	99
MAGE-B3	Sense	Yes	–	100
	Antisense	Yes	–	100
	Sense	–	3	100
	Antisense	–	3	99
MAGE-B4	Sense	Yes	–	100
	Antisense	Yes	–	100
	Sense	–	3	96
	Antisense	–	3	99
MAGE-B6	Sense	Yes	–	100
	Antisense	Yes	–	100
	Sense	–	10	95
	Antisense	–	10	97
MAGE-C1	Sense	Yes	–	100
	Antisense	Yes	–	100
	Sense	–	3	96
	Antisense	–	3	98
MAGE-C2	Sense	Yes	–	100
	Antisense	Yes	–	100
	Sense	–	3	100
	Antisense	–	3	95
MAGE-C3	Sense	Yes	–	100
	Antisense	Yes	–	100
	Sense	–	3	96
	Antisense	–	3	96
	Sense	–	5	95
	Antisense	–	5	93

of the *MAGE-B* family that displayed no expression in the BC tissue samples and was only expressed in the testis. An unexpectedly large band (approximately 600 bp) appeared in sample 2, and the sequencing result was not related to *MAGE-B2*. Thus, the sequence data obtained for *MAGE-B1*, *-B2*, *-B3*, *-B4*, and *-B6* correct target sequences may be considered novel biomarkers of BC in Saudi women (Table 3).

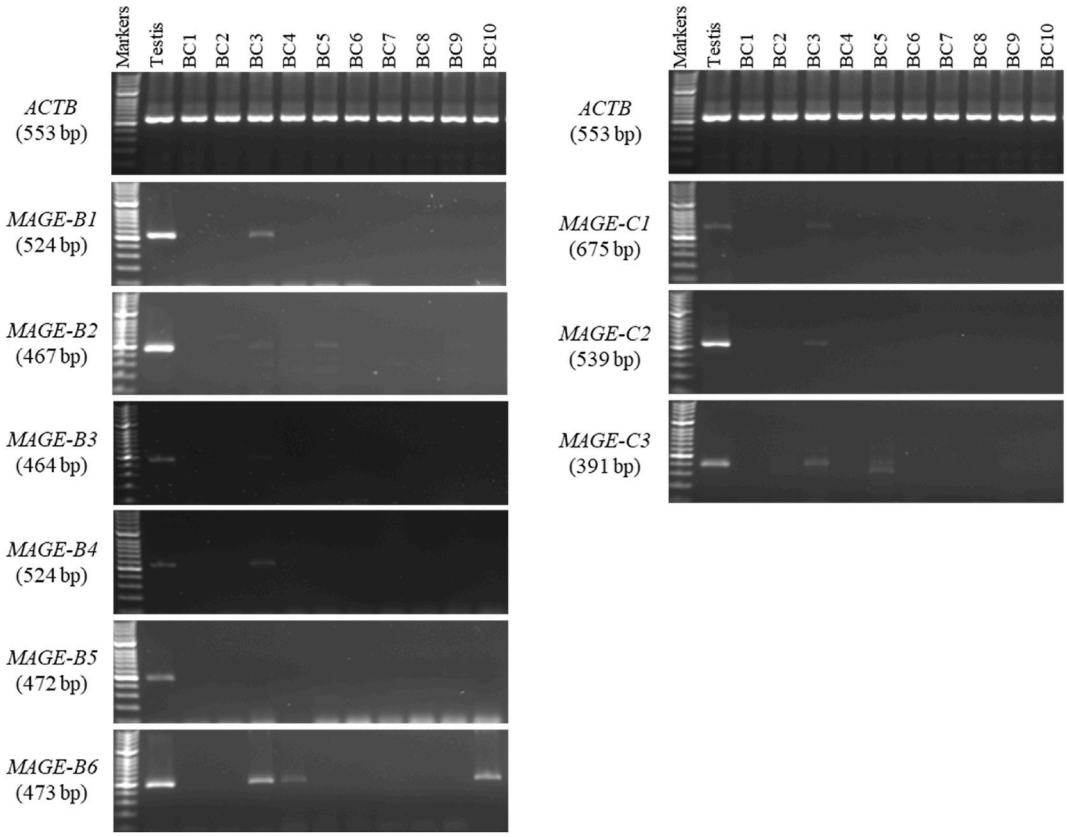


Fig. 2. RT-PCR results of six *MAGE-B* family genes (left) and three *MAGE-C* family genes (right) evaluated on 10 BC specimens. *MAGE-B1*, *-B2*, *-B3*, *-B4*, and *-B6* showed weak PCR products. No PCR product was observed on *MAGE-B5* except for normal testis cDNA. On the right side, weak PCR products can be observed in *MAGE-C1*, *-C2*, and *-C3*. All *MAGE-B* and *-C* gene primers were analyzed on the cDNA of human testis, and each primer's performance was evaluated using *ACTB* expression. The expected size of PCR band for each candidate gene is shown between brackets on the left side.

3.4. MAGE-C family members associated with RNA expression in BC tissues

The expression status of the three *MAGE-C* family genes (*MAGE-C1*, *-C2*, and *-C3*) in both the normal human testis and BC tissue samples were investigated. These genes showed weak-to-moderate expressions in the normal human testicular tissue samples and weak expressions in the BC tissue samples (Fig. 2 and Fig. S2). The mRNA level of *MAGE-C1* and *C2* were detected in nearly 10 % of the BC tissue samples, with expected sizes of 675 and 539 bp, respectively. In contrast, the mRNA level of *MAGE-C3* was detected in 20 % of the BC tissue samples. The sequencing results of *MAGE-C1*, *-C2*, and *-C3* showed high similarity to those of the target genes. However, an unexpected small fragment with a length of 300 bp) appeared in sample 5, and the sequencing result was related to *MAGE-C3*, which might suggest a novel variant for the *MAGE-C3* gene.

3.5. Analysis of MAGE-A, -B, and –C genes RNA expression levels in the NB and BC tissue samples using TCGA database

It was difficult to collect NB tissue samples in this research. Hence, the RNA seq data available in TCGA database was used to compare and contrast the expression status of the *MAGE-A*, *-B*, and-*C* genes against 1135 breast invasive carcinoma (BRCA) and 114 NB tissue samples. The results showed high RNA expression status of all *MAEG-A* family genes in the BRCA tissue samples. Still, low RNA expression levels or no RNA expression in the NB tissue samples (Fig. 3). By contrast, the RNA expression status of the *MAGE- B* family genes in the BRCA specimens ranged from high to low. *MAGE-B1* displayed high RNA expression levels in the BRCA specimens, whereas *MAGE-B5* showed weak RNA levels in the BRCA specimens. *MAGE-B4* had moderate expression in the BRCA specimens, with no RNA expression in the NB specimens. *MAGE-B6* was not found in TCGA database. Finally, the *MAGE-C* family genes also showed varied RNA expression levels in the BRCA specimens, among which *MAGE-C2* was the most promising candidate (Fig. 4).

3.6. Epigenetic regulation of MAGE-A, -B, and –C family genes in the MCF-7 cell line

DNA methylation has an important function in regulating CT gene expressions, and this is especially true for X-linked CT genes. The

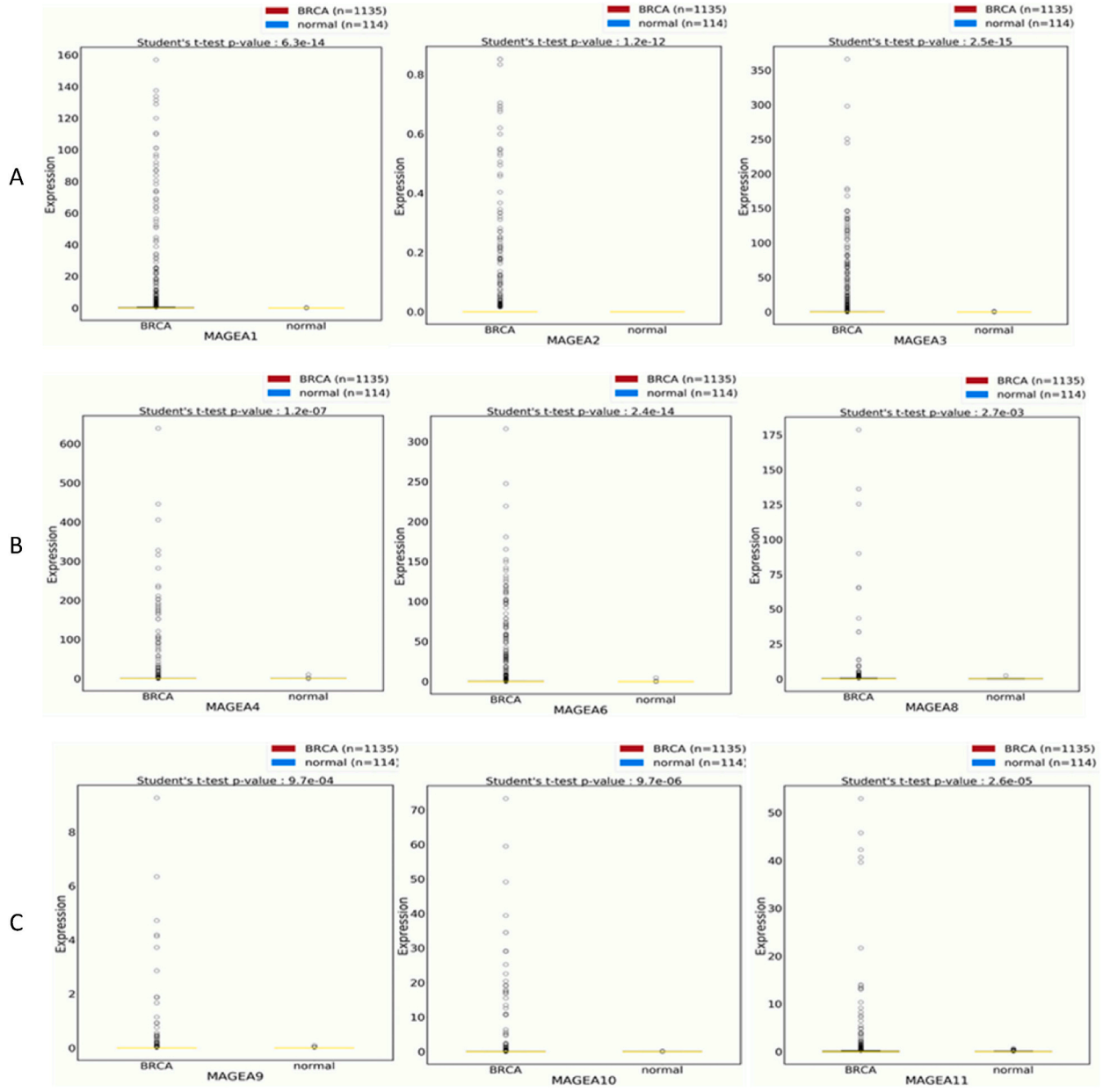


Fig. 3. The expression levels of the *MAGE- A* family genes in the BRCA and NB specimens were analyzed using the RNA seq data obtained from TCGA. *P* values ≤ 0.05 were considered significant. A: *MAGE-A1*, *MAGE-A2*, and *MAGE-A3* expression levels in BRCA and NB. B: *MAGE-A4*, *MAGE-A6*, and *MAGE-A8* expression levels in BRCA and NB. C: *MAGE-A9*, *MAGE-A10*, and *MAGE-A11* expression levels in BRCA and NB. Acronyms: BRCA: breast invasive carcinoma tissue; NB: normal breast tissue.

DNA methylation with regulatory functions in controlling the CT gene expression, particularly those located on the X chromosome, is well established [24,29,52]. These genes are typically silenced through the hypermethylation of their promoter regions [12]. Therefore, the impact of DNA demethylation using the agent 5-aza-2′-deoxycytidine on the expression statuses of the *MAGE-A*, *-B*, and *-C* family genes in MCF-7 cells was investigated. This study aimed to study whether alterations in methylation status can induce the expressions of these genes in BC.

After 72 h of treatment with 5-aza-2′-deoxycytidine at either 1.0 or 10.0 μM, no morphological differences were found between the MCF-7 cells treated with DMSO and those treated with 5-aza-2′-deoxycytidine. The cells treated with DMSO showed no expressions of the genes, as the drug was dissolved in DMSO and used as the control. The RT-PCR results revealed that *MAGE-A8* and *-A9* exhibited strong mRNA expressions in the treated MCF-7 cells at both doses (1.0 and 10.0 μM), whereas *MAGE-A2* and *-A3* showed weak-to-moderate expressions at both doses (Fig. 5 and Fig. S3). On the other hand, *MAGE–A1*, *-A4*, *-A6*, *-A11*, *-B1*, and *-C2* exhibited

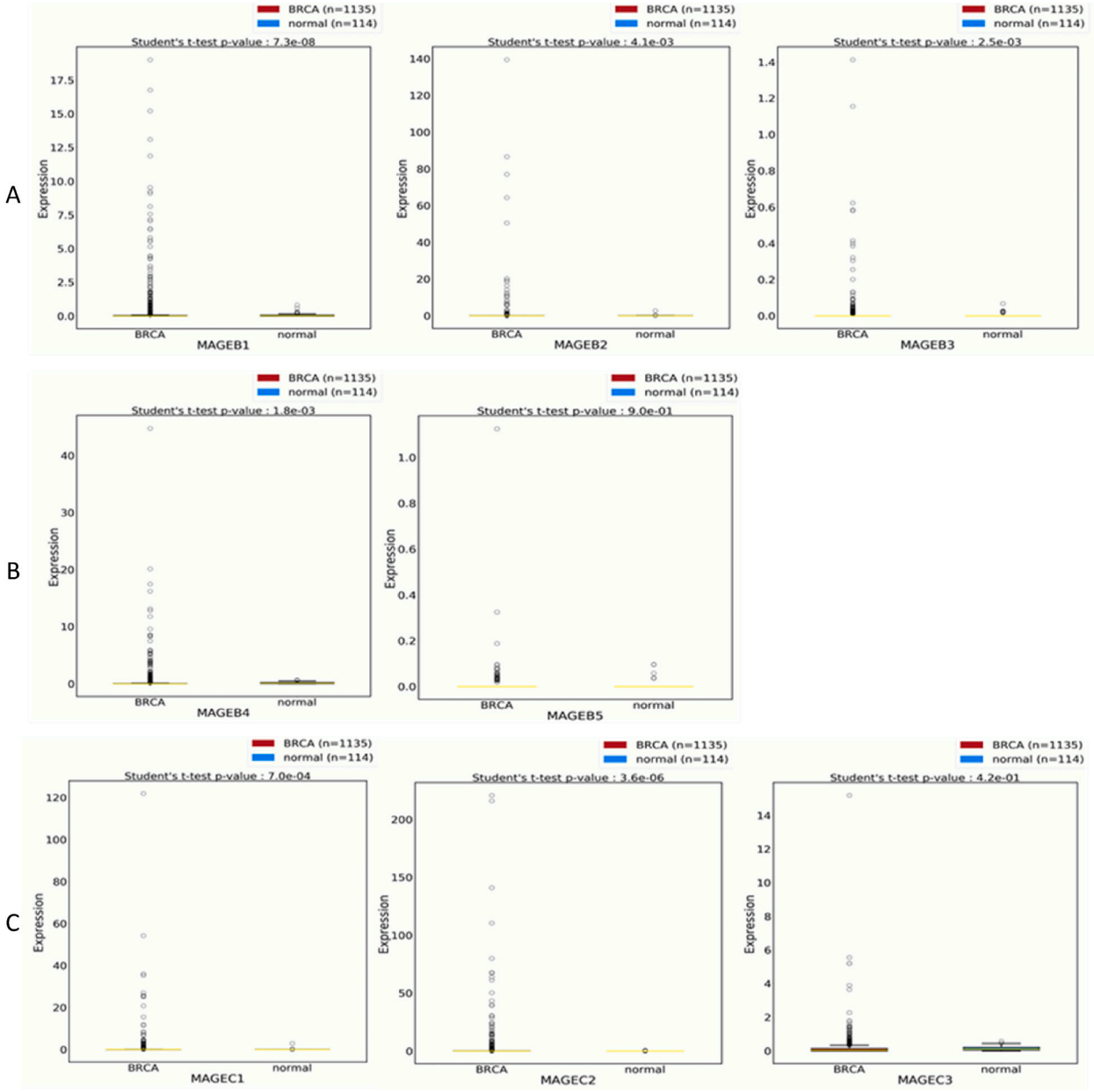


Fig. 4. The expression levels of the *MAGE-B* and *MAGE-C* family genes in the BRCA and NB specimens analyzed using RNA seq data obtained from TCGA. *P* values ≤ 0.05 were considered significant. A: *MAGE-B1*, *MAGE-B2*, and *MAGE-B3* expression levels in BRCA and NB. B: *MAGE-B4*, and *MAGE-B5* expression levels in BRCA and NB. C: *MAGE-C1*, *MAGE-C2*, and *MAGE-C3* expression levels in BRCA and NB. Acronyms: BRCA: breast invasive carcinoma tissue; NB: normal breast tissue.

mRNA expressions only in the cells treated with 10.0-μM 5-aza-2′-deoxycytidine but were not expressed in the cells treated with low-dose 5-aza-2′-deoxycytidine. No *MAGE-A10*, *-B2*, *-B3*, *-B4*, *-B5*, *-B6*, *-C1*, and *-C3* mRNA expressions were detected in the MCF-7 cells treated with either dose.

3.7. Analysis of the physical interactions and coexpressions of the MAGE-A, -B, and –C member genes

The GeneMANIA (http://www.genemania.org) was used to predict potential gene biological functions and determine the interactions of genes of interest with their functionally related genes. Our network analysis revealed strong functional associations and coexpressions between the *MAGE-A*, *-B*, and *-C* family genes investigated in this study. In addition, 20 genes also showed ratios of interaction and co-expression different from those of the genes we evaluated: *MAGEA-9B*, *MAGE-B16*, *MAGE-B17*, *MAGEB-6B*, *MAGE-*

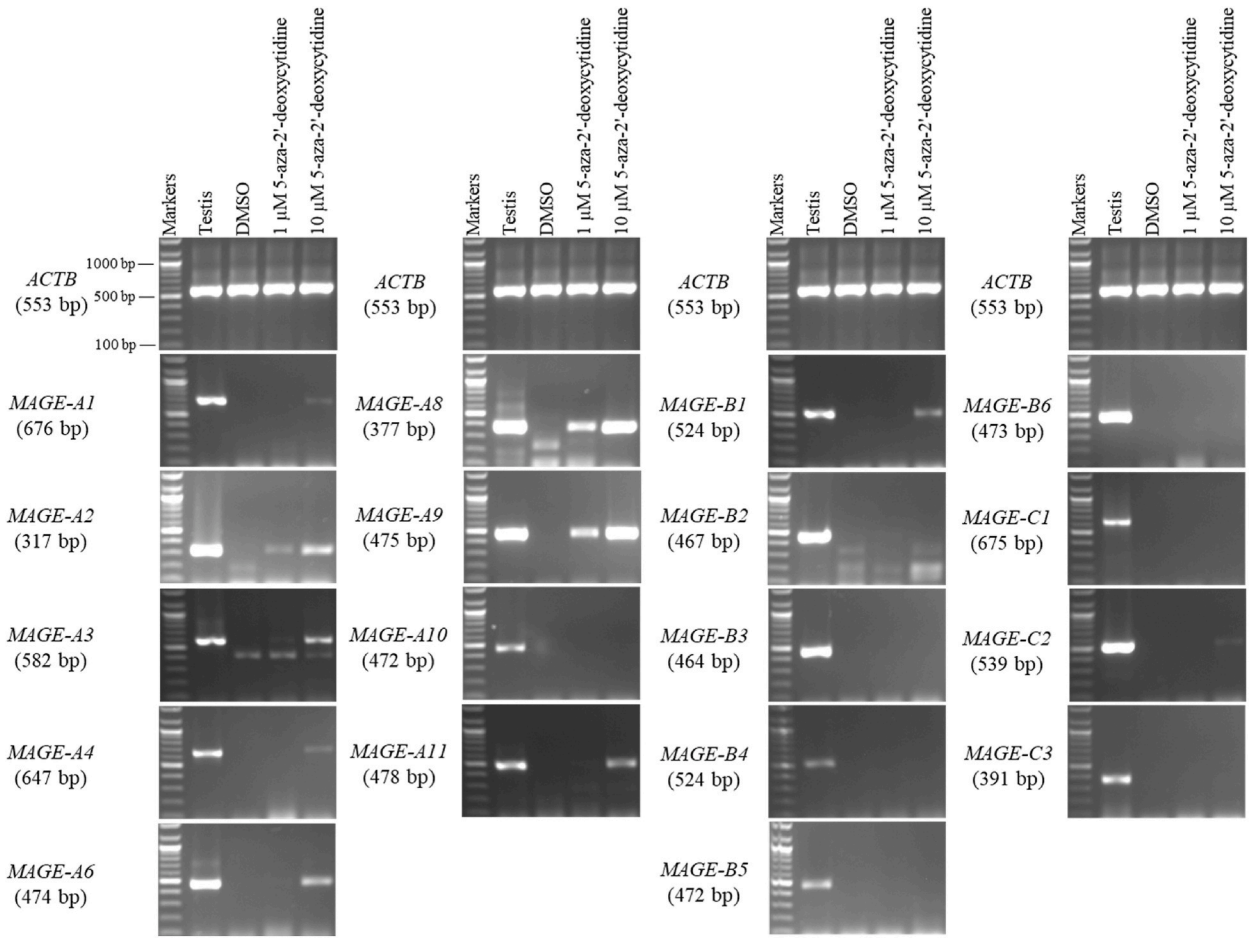


Fig. 5. The influence of DNA hypomethylation using 5-aza-2′-deoxycytidine agent on the expressions of *MAGE-A*, *-B*, and *-C* family genes on MCF-7 cells treated with two doses for 72 h. The *MAGE-A2*, *-A8*, and *-A9* expressions were highly increased in the MCF-7 cells after treatment with 5-aza-2′-deoxycytidine at both doses. *MAGE-A1*, *-A4*, *-A6*,*-A11*, *-B1*, and *-C2* showed weak-to-moderate RNA expression levels after treatment with 10 μM. By contrast, no *MAGE-A10*, *-B3*, *-B4*, *-B5*, *-B6*, *-C1*, and *-C3* RNA expressions were observed after cell treatment.

A2B, *MAGE-B10*, *MAGE-B18*, *MAGE-A12*, *MAGE-D4*, *MAGE-D4B*, *MAGE-E2*, *MAGE-L2*, *MAGEE1*, *NSMCE3*, *MAGEH1*, *MAGE-F1*, *TRO*, *MAGE-D2*, *NDN*, and *MAGE-D1* (Fig. 6). Our analysis also revealed that the relationship networks of *A*, *B*, and *C* family members were connected with physical interactions (1.92 % of members) and coexpressions (98.08 % of members).

3.8. Functional enrichment analysis

Gene lists obtained by mining high-throughput genomic data, such as enrichment analysis, can be better understood with the help of G:Profiler, a publicly available web service. Annotations for proteins were made using the Gene Ontology (GO) database of biological processes. Stratified enrichment studies were conducted using disease-specific CSF proteins. The g:SCS algorithm, designed specifically for estimating thresholds in structured functional profiling data, was used to correct enrichment findings for multiple comparisons. This algorithm is applicable to complicated and organized databases like the GO database. Significant enhancement in particular biological processes was reported when all MAGE proteins evaluated in this study were added to the background list. Without accounting for this background list, we detected an increase in biological processes associated with the binding function of histone deacetylase and the inhibition of transcription by RNA polymorphism in humans. Additionally, we found nominal associations between these processes and proteins linked to cancer risk loci (e.g., GO:0042826) histone deacetylase binding process, GO:0005634 - negative regulation of transcription by RNA polymorphism) as shown in Fig. 7. Analyzed the pathways of MAGE genes that were specifically identified in proteins linked to prostate cancer tissue datasets. The MAGE proteins exhibited a high concentration in pathways associated with extracellular vesicles and intracellular trafficking.

Additionally, certain MAGE proteins showed a high concentration in pathways connected to the plasma membrane and extracellular space (Fig. 8). The MAGE proteins underwent GO and KEGG analysis using webgestalt. The GO analysis revealed that the downstream genes of these proteins were associated with biological regulation, protein binding, and metabolic processes (Fig. 8). In

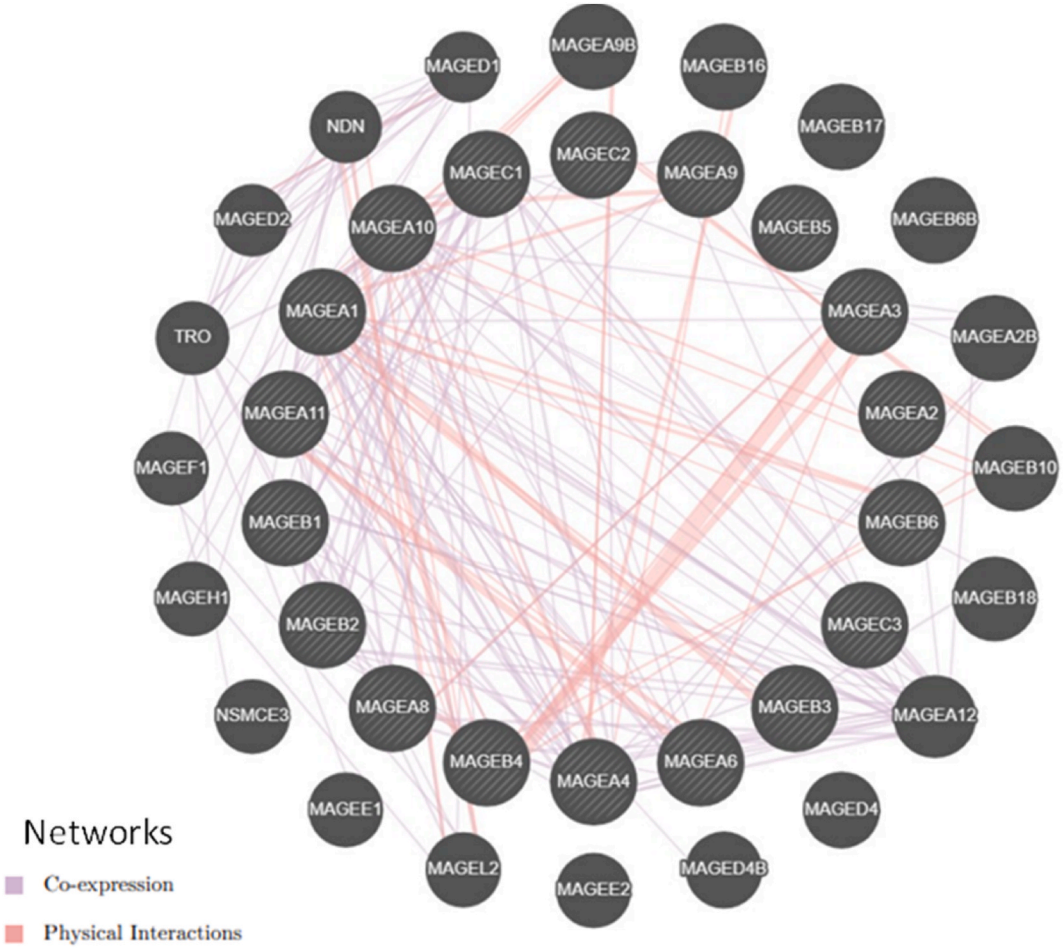


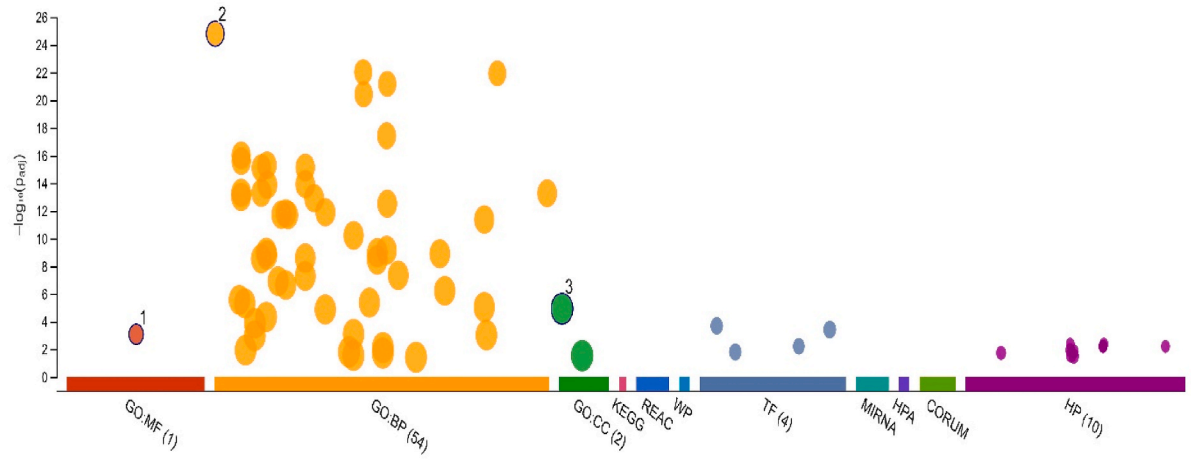
Fig. 6. Network interaction for the *MAGE-A1*, *-A2*, *-A3*, *-A4*, *-A6*, *-A8*, *-A9*, *-A10*, *-A11*, *-B1*, *-B2*, *-B3*, *-B4*, *-B5*, *-B6*, *-C1*, *-C2*, and *-C3* genes using GeneMANIA program. All *MAGE- A*, *-B*, and *-C* family members in this study are depicted as circular nodes, and the line edges indicate their physical interactions and coexpressions.

addition, the KEGG analysis revealed that MAGE proteins were associated with various biological processes, including renal sodium ion transport (GO: 0003096), negative regulation of cellular macromolecule biosynthetic process (GO: 2,000,113), female pregnancy (GO: 0007565), and multi-multicellular organism process (GO: 0044706). Furthermore, the analysis also indicated their involvement in microRNAs in cancer, transcriptional misregulation in cancer, endocytosis, and starch and sucrose metabolism, among others (Fig. 9).

4. Discussion

BC is among the most frequently reported cancers among Saudi females [6]. Saudi women with BC are often at high risk of mortality because the disease is often diagnosed at its advanced stages [7]. CT genes provide an excellent class of biomarker for detecting various forms of cancers due to their restricted expression to normal human testis and malignancies [9]. Consequently, the investigation of CT expression patterns in several BC cases might help identify new cancer biomarkers among the Saudi population, which can be used for early detection and improve the chance of receiving therapy. The MAGE gene families have been extensively studied as early detection biomarkers of cancers, which may help decrease mortality rates. Nevertheless, the RNA expression patterns of the *MAEG-A*, *- B,* and *- C* gene families in Saudi women with BC are still unclear. Therefore, this work focused on obtaining better knowledge about the RNA expression profiles of the *MAEG- A*, *- B*, and *- C* family genes in Saudi patients with BC.

For the first time, RT-PCR was used to screen 18 CT genes belonging to MAGE families in BC samples obtained from Saudi women. Among these genes, 14 showed weak PCR products in multiple BC tissue samples, while *MAGE-A8*, *MAGE-A9*, and *MAGE-B6* showed wider or stronger PCR expressions and thus might be optimal biomarkers of BC in Saudi women. *MAGE-A8* was found to be expressed in most BC specimens, and *MAGE-B6* exhibited the strongest expression in the tested samples. Previous studies have shown that patients with BC who had high *MAGE-A8* and *MAGE-B6* expression levels exhibited improved relapse-free survival; thus, these two genes might have a role as tumor suppressors in the development and occurrence of BC [25]. Conversely, the expression rate of *MAGE-A9* was



ID	Source	Term ID	Term Name	p_{adj} (query_1)
1	GO:MF	GO:0042826	histone deacetylase binding	8.386×10^{-4}
2	GO:BP	GO:0000122	negative regulation of transcription by RNA polym...	1.572×10^{-25}
3	GO:CC	GO:0005634	nucleus	1.186×10^{-5}

Fig. 7. Functional enrichment analysis of MAGE proteins. The x-axis displays the functional terms that were found to be significantly influenced, with each term color-coded according to the data source. The data sources include species and type (e.g., MF from GO is colored red), while the sources that were ignored are shown as grey. Modified enrichment p-values on a -log10 scale are displayed on the y-axis. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

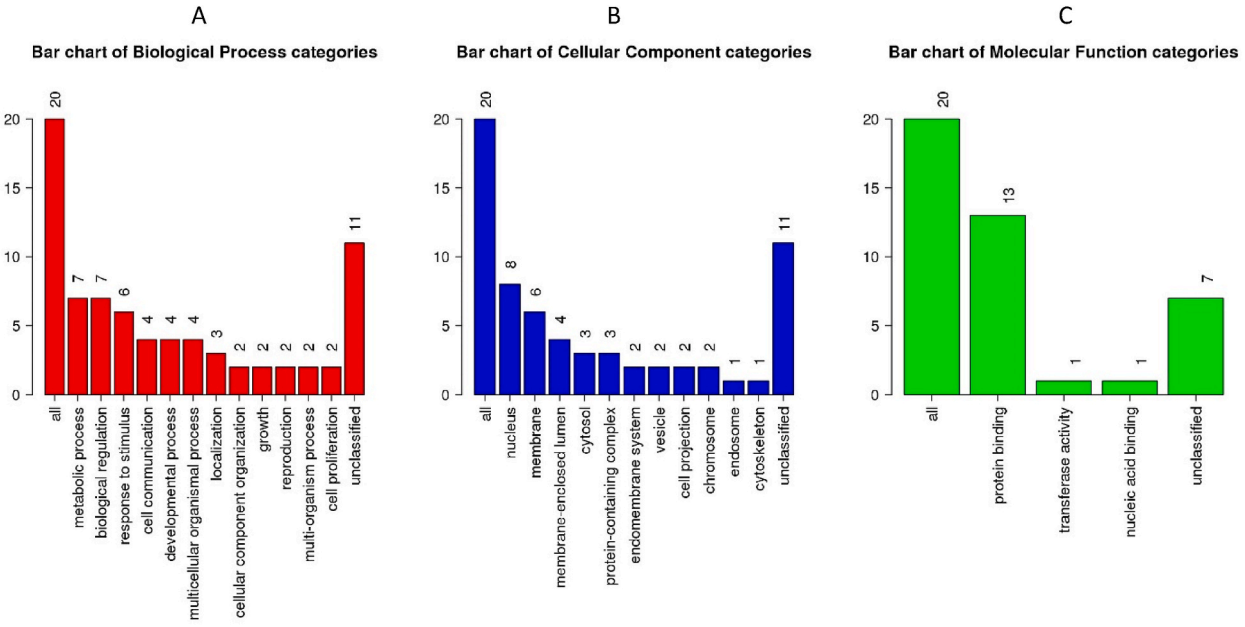


Fig. 8. Analysis of the cellular components of MAGE proteins. Each category of Biological Process, Cellular Component, and Molecular Function is represented by a red, blue, and green bar, respectively. The vertical dimension of the bar corresponds to the quantity of IDs included in both the list and the category. A: Biological process categories represented by red bars. B: Cellular component categories represented by blue bars. C: Molecular function categories represented by green bars. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

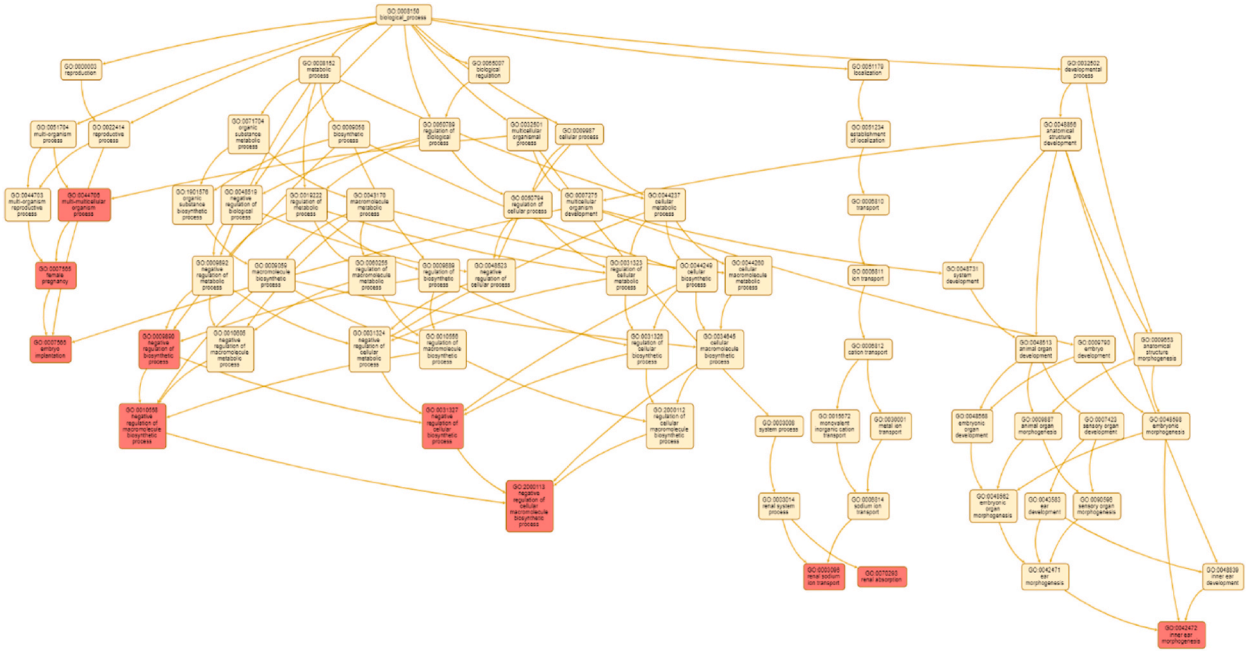


Fig. 9. The GO enrichment analysis of MAGE protein to identify which GO terms are significantly overrepresented in the MAGE protein. These terms provide insights into the biological processes, molecular functions, and cellular components associated with the MAGE proteins.

lower than previously reported [24]. A relevant study reported that a high expression level of *MAGE-A9* might be linked with poor prognosis among BC patients [53]. By contrast, the expressions of the *MAGE-A1*, *-A3*, *-A4*, and *-B5* genes were only found in the normal human testis, without evidence of RNA expression in any of the BC specimens. This might be due to the low number of collected samples in this study; thus, a large cohort of patients is required for an in-depth investigation. However, previous research found that the *MAGE-A3* and *A4* RNA expressions in BC samples were linked with mortality risk and clinicopathological parameters [54]. Doyle et al., in a recent study, found that the interaction of human Type I MAGE proteins with RING domain proteins resulted in increased ubiquitin ligase activity [55]. Given its unique expression in tumors and its remarkable ability to stimulate an immune response, Type I MAGEs are widely considered to be central to the development of tumors and the progression of cancer. Multiple clinical studies have demonstrated that the expression of CT antigen genes is associated with cancers and exhibited less differentiated or invasive capacities [56,57]. MAGE expression in BC has been demonstrated to be material in several prior study. MAGE-A valuables are often expressed in BC, as shown by Ogata et al., 2011; MAGE expression is correlated with an aggressive tumor phenotype and a poor prognosis [58]. Conley et al. conducted confirmed MAGE expression-based BC with unfavorable clinical findings. As a result, these findings indicated that MAGE expression could be potential feasible signaling/drug diagnostic markers [59]. Unlike previous findings, recent research highlighted that MAGEs display weak expression in BC samples. Thus, these results challenge the classical role of MAGEs as stimulants of tumoral development and unfavorable outcomes for patients with BC. For example, Jin et al. 2020 showed that MAGE expression in BC tissues was remarkably lower than in normal adjacent tissues. This is inconsistent with the classical understanding of MAGEs prominence in BC [60].

The findings from TCGA RNA sequencing database indicated that the RNA expression patterns of most genes in the *MAGE-A*, *-B*, and *-C* families were significantly higher in the BRCA specimens than in the NB tissue specimens. These results are in agreement with those of previous works that reported increased *MAGE-A*, *-B,* and *-C* family members' expression patterns in various cancers, including BC and colon cancer [24,28,49]. However, the qRT-PCR data in this research did not reveal the RNA expression status of the candidate genes in the matched NB specimens. Therefore, further work is needed to determine whether the results for these genes that were obtained from TCGA database are aligned with their expression in the NB specimens. In a previous study, the expression of MAGE genes were compared in smaller groups of BC patients that have been analyzed for MAGE-A1, -A2, and -A3 mRNA-level expression. Compared to their series, 15–18, n = 11–58, tumors tested positive for MAGE-A1 6 %, 4–8, n = 16–17 %, for MAGE-A2, which is lower than our series 19 %, and 10 %, 4–24, n = 15–20 %, tested positive for MAGE-3, similar to our series. Some of these variations can be explained by differences in RT-PCR techniques (e.g., primer specificity, quality of RNA used, and reverse transcription and PCR conditions), sample size, the number of MAGE-A genes tested, and statistical problems related to the use of a small number of patients [41].

The exact regulation mechanisms controlling the RNA expression levels of the *MAGE-A*, *-B*, and *-C* genes in cancerous cells are not yet fully understood [24]. However, epigenetic processes such as DNA methylation events have been observed to play a role in modulating the RNA expression levels of some members belonging to families in various cancer cells, including colon cancer [24,28,29,49,52], ovarian cancer [61], and lung cancer [62]. To examine the potential induction of *MAGE-A*, *-B*, and *-C* gene expressions

through the inhibition of DNA methylation, MCF-7 cells were exposed to two different doses of 5-aza-2′-deoxycytidine for 72 h. We hypothesized that DNA demethylation caused by 5-aza-2′-deoxycytidine may lead to changes in the chromatin structure, thereby influencing gene expression levels [47,63].

Cells treated with 5-aza-2′-deoxycytidine showed significantly increased expression of the *MAGE-A2, -A8*, and *-A9* genes. Other members such as *MAGE-A1*, *-A4*, *-A6*, *-A11*, *-B1*, and *-C2* showed weak-to-moderate RNA expression levels. These findings suggest that DNA hypomethylation appears to influence and regulate expressions of these genes. Our results align with previous reports of similar effects of DNA hypomethylation on *MAGE-A*, *-B*, and gene expressions in BC cells [23,48,64]. By contrast, no expressions of the *MAGE-A10*, *-B2*, *-B3*, *-B4*, *-B5*, *-B6*, *-C1*, and *-C3* genes were detected in the MCF-7 cells treated with either dose. This suggests that other cellular mechanisms might regulate these genes.

The present results of gene-interaction networks indicated a complex network of *MAEG-A*, *-B*, and *-C* family members. Most interacted genes involve various biological functions such as cell proliferation, cell signaling, and disease pathways. Therefore, detecting network genes associated with the *MAGE-A*, *-B*, and *-C* gene families will improve our comprehension of the pathway of these genes and facilitate the development of novel methods for detecting, preventing, and treating BC. Several studies have used gene-interaction networks to predict genes associated with colon and liver cancers [65].

This research has three limitations. First, no matching NB tissue samples were used to compare the RNA expressions of the candidate genes between the BC specimens and their matching NB tissue specimens from the same patients, as we had difficulty obtaining these tissue samples. Second, only 10 BC surgical specimens were used, and further work is required with a large number of samples. Finally, owing to sample limitations, we could not study these candidate genes at protein levels.

5. Conclusion

The current research explored the RNA expression patterns of 18 CT genes belonging to the MAGE family in BC tissues obtained from Saudi female patients. This work found that 14 genes displayed weak mRNA expression patterns in various BC specimens. The *MAGE-A8*, *-A9*, and *-B6* expression patterns suggest that they are potential markers for the early detection of BC. In addition, further research should be performed to evaluate these findings in a large cohort of patients, including a protein-level study of these genes in Saudi patients with BC compared with a matching NB group. Finally, hypomethylation has a fundamental function in the activation of the expressions of *MAGE-B1*, *-C2*, and all tested genes belonging to *MAGE-A* in BC, with the exception of *MAGE-A10*. These findings suggest that DNA methylation might play a critical role in the upregulation of the RNA expressions of these genes in BC.

Institutional review board statement

The study was conducted in accordance with the Declaration of Helsinki, and approved by the ethics committee of University of Al-Imam Muhammad Ibn Saud Islamic (IRB No. HAPO-01-R-011, Project No. 56/2020).

Informed consent statement

Informed consent was obtained from all subjects involved in the study.

Data availability statement

The data are available from the corresponding author when required.

CRediT authorship contribution statement

Ahmad M. Almatrafi: Writing – original draft, Software, Formal analysis. **Salman Alamery:** Writing – review & editing. **Mikhlid H. Almutairi:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Investigation, Funding acquisition, Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e34506.

References

[1] F. Luond, S. Tiede, G. Christofori, Breast cancer as an example of tumour heterogeneity and tumour cell plasticity during malignant progression, Br. J. Cancer 125 (2) (2021) 164–175.
[2] C. Fitzmaurice, et al., The global burden of cancer 2013, JAMA Oncol. 1 (4) (2015) 505–527.
[3] H. Sung, et al., Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, CA A Cancer J. Clin. 71 (3) (2021) 209–249.
[4] S.M. Lima, R.D. Kehm, M.B. Terry, Global breast cancer incidence and mortality trends by region, age-groups, and fertility patterns, EClinicalMedicine 38 (2021) 100985.
[5] S. Nickels, et al., Evidence of gene-environment interactions between common breast cancer susceptibility loci and established environmental risk factors, PLoS Genet. 9 (3) (2013) e1003284.
[6] W.S. Alqahtani, et al., Epidemiology of cancer in Saudi Arabia thru 2010-2019: a systematic review with constrained meta-analysis, AIMS Public Health 7 (3) (2020) 679–696.
[7] R.M. Alotaibi, et al., Breast cancer mortality in Saudi Arabia: modelling observed and unobserved factors, PLoS One 13 (10) (2018) e0206148.
[8] M.M. Rivera-Franco, E. Leon-Rodriguez, Delays in breast cancer detection and treatment in developing countries, Breast Cancer 12 (2018) 11782234177526777.
[9] J. Feichtinger, et al., Meta-analysis of clinical data using human meiotic genes identifies a novel cohort of highly restricted cancer-specific marker genes, Oncotarget 3 (8) (2012) 843–853.
[10] O. Hofmann, et al., Genome-wide analysis of cancer/testis gene expression, Proc. Natl. Acad. Sci. U. S. A. 105 (51) (2008) 20422–20427.
[11] D.K. Krishnadas, F. Bai, K.G. Lucas, Cancer testis antigen and immunotherapy, ImmunoTargets Ther. 2 (2013) 11–19.
[12] E. Fratta, et al., The biology of cancer testis antigens: putative function, regulation and therapeutic potential, Mol. Oncol. 5 (2) (2011) 164–182.
[13] P. van der Bruggen, et al., A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma, Science 254 (5038) (1991) 1643–1647.
[14] Z.A. Gibbs, A.W. Whitehurst, Emerging contributions of cancer/testis antigens to neoplastic behaviors, Trends Cancer 4 (10) (2018) 701–712.
[15] L.G. Almeida, et al., CTdatabase: a knowledge-base of high-throughput and curated data on cancer-testis antigens, Nucleic Acids Res. 37 (Database issue) (2009) D816–D819.
[16] K. Rajagopalan, et al., A majority of the cancer/testis antigens are intrinsically disordered proteins, J. Cell. Biochem. 112 (11) (2011) 3256–3267.
[17] A. Salmaninejad, et al., Cancer/testis antigens: expression, regulation, tumor invasion, and use in immunotherapy of cancers, Immunol. Invest. 45 (7) (2016) 619–640.
[18] A. Grigoriadis, et al., CT-X antigen expression in human breast cancer, Proc. Natl. Acad. Sci. U. S. A. 106 (32) (2009) 13493–13498.
[19] A.J. Simpson, et al., Cancer/testis antigens, gametogenesis and cancer, Nat. Rev. Cancer 5 (8) (2005) 615–625.
[20] M. Sang, et al., MAGE-A family: attractive targets for cancer immunotherapy, Vaccine 29 (47) (2011) 8496–8500.
[21] J.L. Weon, P.R. Potts, The MAGE protein family and cancer, Curr. Opin. Cell Biol. 37 (2015) 1–8.
[22] M. Ayyoub, et al., Expression of MAGE-A3/6 in primary breast cancer is associated with hormone receptor negative status, high histologic grade, and poor survival, J. Immunother. 37 (2) (2014) 73–76.
[23] S. Hou, et al., The expression of MAGE-C1 and MAGE-C2 in breast cancer and their clinical significance, Am. J. Surg. 211 (1) (2016) 142–151.
[24] S.Y. Hou, et al., Expressions of MAGE-A9 and MAGE-A11 in breast cancer and their expression mechanism, Arch. Med. Res. 45 (1) (2014) 44–51.
[25] B. Jia, et al., Prognostic roles of MAGE family members in breast cancer based on KM-Plotter Data, Oncol. Lett. 18 (4) (2019) 3501–3516.
[26] S. Daudi, et al., Expression and immune responses to MAGE antigens predict survival in epithelial ovarian cancer, PLoS One 9 (8) (2014) e104099.
[27] M. Sang, et al., Multiple MAGE-A genes as surveillance marker for the detection of circulating tumor cells in patients with ovarian cancer, Biomarkers 19 (1) (2014) 34–42.
[28] M.H. Almutairi, et al., Expression profile of MAGE-B1 gene and its hypomethylation activation in colon cancer, BioMed Res. Int. 2022 (2022) 6066567.
[29] M.H. Almutairi, et al., Cancer-testis gene biomarkers discovered in colon cancer patients, Genes 13 (5) (2022).
[30] L. von Boehmer, et al., MAGE-C2/CT10 protein expression is an independent predictor of recurrence in prostate cancer, PLoS One 6 (7) (2011) e21366.
[31] X. Chen, et al., Expression and prognostic relevance of MAGE-A3 and MAGE-C2 in non-small cell lung cancer, Oncol. Lett. 13 (3) (2017) 1609–1618.
[32] J.R. Tsai, et al., Differential expression profile of MAGE family in non-small-cell lung cancer, Lung Cancer 56 (2) (2007) 185–192.
[33] F. Brasseur, et al., Expression of MAGE genes in primary and metastatic cutaneous melanoma, Int. J. Cancer 63 (3) (1995) 375–380.
[34] P. Gibbs, et al., MAGE-12 and MAGE-6 are frequently expressed in malignant melanoma, Melanoma Res. 10 (3) (2000) 259–264.
[35] C.T. Pineda, et al., Degradation of AMPK by a cancer-specific ubiquitin ligase, Cell 160 (4) (2015) 715–728.
[36] S. Zhang, et al., High expression of MAGE-A9 in tumor and stromal cells of non-small cell lung cancer was correlated with patient poor survival, Int. J. Clin. Exp. Pathol. 8 (1) (2015) 541–550.
[37] W. Liu, et al., The melanoma-associated antigen A3 mediates fibronectin-controlled cancer progression and metastasis, Cancer Res. 68 (19) (2008) 8104–8112.
[38] N. Bhatia, et al., MAGE-C2 promotes growth and tumorigenicity of melanoma cells, phosphorylation of KAP1, and DNA damage repair, J. Invest. Dermatol. 133 (3) (2013) 759–767.
[39] B. Yang, et al., MAGE-A, mMage-b, and MAGE-C proteins form complexes with KAP1 and suppress p53-dependent apoptosis in MAGE-positive cell lines, Cancer Res. 67 (20) (2007) 9954–9962.
[40] E. De Plaen, et al., Structure, chromosomal localization, and expression of 12 genes of the MAGE family, Immunogenetics 40 (5) (1994) 360–369.
[41] M. Otte, et al., MAGE-A gene expression pattern in primary breast cancer, Cancer Res. 61 (18) (2001) 6682–6687.
[42] C. Lurquin, et al., Two members of the human MAGEB gene family located in Xp21.3 are expressed in tumors of various histological origins, Genomics 46 (3) (1997) 397–408.
[43] D.I. Vodolazhsky, et al., Specific features of transcription activity of cancer-testis antigens in patients with metastatic and non-metastatic breast cancer, Bull. Exp. Biol. Med. 165 (3) (2018) 382–385.
[44] R.K. Sypniewska, et al., Potential mouse tumor model for pre-clinical testing of mage-specific breast cancer vaccines, Breast Cancer Res. Treat. 74 (3) (2002) 221–233.
[45] R.K. Sypniewska, et al., Prevention of metastases with a Mage-b DNA vaccine in a mouse breast tumor model: potential for breast cancer therapy, Breast Cancer Res. Treat. 91 (1) (2005) 19–28.
[46] S. Lucas, E. De Plaen, T. Boon, MAGE-B5, MAGE-B6, MAGE-C2, and MAGE-C3: four new members of the MAGE family with tumor-specific expression, Int. J. Cancer 87 (1) (2000) 55–60.
[47] L. Sigalotti, et al., Intratumor heterogeneity of cancer/testis antigens expression in human cutaneous melanoma is methylation-regulated and functionally reverted by 5-aza-2'-deoxycytidine, Cancer Res. 64 (24) (2004) 9167–9171.
[48] F. Wischnewski, K. Pantel, H. Schwarzenbach, Promoter demethylation and histone acetylation mediate gene expression of MAGE-A1, -A2, -A3, and -A12 in human cancer cells, Mol. Cancer Res. 4 (5) (2006) 339–349.
[49] M.H. Almutairi, et al., Identification of MAGE-A Family Genes in Colon Cancer Patients and Their Expression Mechanism, vol. 34, King Saud University – Science, 2022.
[50] L. Kolberg, et al., g:Profiler-interoperable web service for functional enrichment analysis and gene identifier mapping (2023 update), Nucleic Acids Res. 51 (W1) (2023) W207–W212.
[51] Y. Liao, et al., WebGestalt 2019: gene set analysis toolkit with revamped UIs and APIs, Nucleic Acids Res. 47 (W1) (2019) W199–W205.
[52] A. Almatrafi, et al., Identification of a class of human cancer germline genes with transcriptional silencing refractory to the hypomethylating drug 5-aza-2'-deoxycytidine, Oncoscience 1 (11) (2014) 745–750.
[53] X. Xu, et al., Overexpression of MAGE-A9 predicts unfavorable outcome in breast cancer, Exp. Mol. Pathol. 97 (3) (2014) 579–584.

[54] T. Cabezon, et al., Proteomic profiling of triple-negative breast carcinomas in combination with a three-tier orthogonal technology approach identifies Mage-A4 as potential therapeutic target in estrogen receptor negative breast cancer, Mol. Cell. Proteomics 12 (2) (2013) 381–394.
[55] J.M. Doyle, et al., MAGE-RING protein complexes comprise a family of E3 ubiquitin ligases, Mol. Cell 39 (6) (2010) 963–974.
[56] A.O. Gure, et al., Cancer-testis genes are coordinately expressed and are markers of poor outcome in non-small cell lung cancer, Clin. Cancer Res. 11 (22) (2005) 8055–8062.
[57] J. Kim, et al., The clinical significance of MAGEA3 expression in pancreatic cancer, Int. J. Cancer 118 (9) (2006) 2269–2275.
[58] K. Ogata, et al., Clinical significance of melanoma antigen-encoding gene-1 (MAGE-1) expression and its correlation with poor prognosis in differentiated advanced gastric cancer, Ann. Surg Oncol. 18 (4) (2011) 1195–1203.
[59] A.P. Conley, et al., MAGE-A3 is a clinically relevant target in undifferentiated pleomorphic sarcoma/myxofibrosarcoma, Cancers 11 (5) (2019).
[60] Q. Jin, et al., High methionyl-tRNA synthetase expression predicts poor prognosis in patients with breast cancer, J. Clin. Pathol. 73 (12) (2020) 803–812.
[61] S.J. Adair, K.T. Hogan, Treatment of ovarian cancer cell lines with 5-aza-2'-deoxycytidine upregulates the expression of cancer-testis antigens and class I major histocompatibility complex-encoded molecules, Cancer Immunol. Immunother. 58 (4) (2009) 589–601.
[62] Z. Li, et al., 5-aza-2'-deoxycytidine (DAC) treatment induces the MAGE-A10 expression and improves the cytotoxicity of MAGE-A10-specific CTLs in lung cancer cells, Transl. Cancer Res. 9 (2) (2020) 1235–1245.
[63] A.R. Karpf, et al., Limited gene activation in tumor and normal epithelial cells treated with the DNA methyltransferase inhibitor 5-aza-2'-deoxycytidine, Mol. Pharmacol. 65 (1) (2004) 18–27.
[64] N. Li, et al., [Expression of melanoma-associated antigen-C2 in breast cancers and mechanism], Zhonghua Zhongliu Zazhi 43 (8) (2021) 821–826.
[65] E.K. Kim, et al., Proteomic analysis of primary colon cancer and synchronous solitary liver metastasis, Cancer Genomics Proteomics 16 (6) (2019) 583–592.