RESEARCH ARTICLE

The tissue expression of *MCT3, MCT8,* and *MCT9* genes in women with breast cancer

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Abstract

Background Breast cancer (BC) is a common malignancy with a high mortality rate. Malignant cell transformation is associated with metabolic changes. One group of proteins that are affected is the monocarboxylate transporters (MCTs-SLC16A). The MCTs comprise 14 members, and they play an important role in the growth, proliferation, and metabolism of cancer cells by transporting monocarboxylates such as lactate, pyruvate and thyroid hormones.

Objective We aimed to evaluate the expression of *MCT3* (SLC16A8), *MCT8* (SLC16A2) and *MCT9* (SLC16A9) genes in breast cancer samples, comparing to normal adjacent tissues.

Methods Forty paired breast cancer tumor samples, the adjacent non-tumor and five healthy tissues were collected. Three cancer cell lines (MCF-7, MDA-MB-231, and SKBR3) were also analyzed. The expression of SLC16A8, SLC16A2 and SLC16A9 were assessed using quantitative real-time PCR. The relationship between gene expression with the pathological features of the tumors, and the hormone receptors status of the patient's tumors were also analyzed.

Results There was a significantly lower expression of the *MCT3* gene in tumor samples compared to adjacent normal tissue and healthy samples (p value < 0.05). There was a significant difference in the expression of all three candidate genes between the BC tissues and normal tissues, and for the, tissues with different hormone receptor status and the molecular subtypes. Altered *MCT8* and *MCT9* gene expression was associated with a reduced survival

Conclusion MCT3 expression is significantly downregulated in breast cancer tissue. MCT3 may represent a novel therapeutic target in breast cancer patients, or in some hormone receptor subgroups.

Keywords Cancer metabolism · Monocarboxylate transporters · Lactate dehydrogenase · Breast cancer

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Abbreviations

BC	Breast cancer
ER	Estrogen receptor
HER2	Human epidermal growth factor receptor 2
MCTs	Monocarboxylate transporters
LDHs	Lactate dehydrogenases
AHD	Allan–Herndon–Dudley syndrome
HPRT1	Hypoxanthine phosphoribosyltransferase1

Introduction

Breast cancer (BC) is the second most common global cause of cancer-related mortality after lung cancer (Estiar et al. 2017; Harding et al. 2015; Moslemi et al. 2020). The mortality remains high because of the lack of effective treatments (Bogenrieder and Herlyn 2003; Harding et al. 2015; Moslemi et al. 2021; Si et al. 2015; Weigelt

et al. 2005). According to the molecular classification and microarray analysis of the patient's tumors, breast cancer may be categorized into five subgroups (Liu et al. 2014; Yersal and Barutca 2014): luminal A [estrogen receptor (ER) positive, progesterone receptor (halestrap and price) positive, and human epidermal growth factor receptor 2 (HER2) negative], luminal B [ER⁺, PR⁺, and HER2^low]. HER2 over-expression (HER2^high, ER^low, and PR^low). Basal-like usually is Triple-negative (ER⁻, PR⁻, and HER2⁻ basal marker) with the shortest survival. Normal breast tissue is ER^low, PR^low, and P53⁺ (Liu et al. 2014; Yersal and Barutca 2014).

Several factors have been reported to be risk factors for the development of some cancers, including: metabolic disorders of glucose and lactate, hormonal disorders, smoking, physical inactivity, exposure to radiation, obesity, dietary factors, alcohol, age and mutagenic substances (Stein and Colditz 2004). In cancer cells there is increased glycolysis leading to increased tissue levels of lactate, in both the absence of oxygen (anaerobic glycolysis) and in its presence (aerobic glycolysis or the Warburg effect) and this is associated with tumor progression (Draoui and Feron 2011). Increased glycolysis leads to an increased production of lactate and acidification of the tumor microenvironment (Aveseh et al. 2015), but whilst there is a reduction in extracellular pH, the intracellular pH is often normal or even in some conditions may be alkaline; this is due to expression and function of a group of membrane-proteins that called the monocarboxylate transporters (MCTs-SLC16A) and lactate dehydrogenases (LDHs) (Draoui and Feron 2011; Luz et al. 2017; Mishra and Banerjee 2019; Pinheiro et al. 2008). The MCTs family of proteins comprises 14 members, which mainly function as transporter proteins and transport monocarboxylates (lactate, pyruvate, and ketones), thyroid hormones, and carnitine (Halestrap and Meredith 2004; Halestrap and Price 1999; Van Der Deure et al. 2010).

An increased tissue expression of some MCTs has been demonstrated in breast cancer compared to normal breast tissue: for example, MCT1 is reported to be up-regulated in the basal (Pinheiro et al. 2010), luminal-like subtypes of breast cancer and the MCF7 cell line and also MCT4 is up-regulated in MDA-MB-231 cell line (Gallagher et al. 2007). MCT1 knockdown was found to reduce metastasis and angiogenesis of cancer cells (Hussien and Brooks 2010; Sonveaux et al. 2008; Végran et al. 2011). MCT1 and MCT4, two members of this family, have been extensively studied in various cancers and have been proposed as therapeutic targets for cancer, but the activity and expression of other MCTs in breast cancer have not been extensively studied (Luz et al. 2017; Pinheiro et al. 2012; Van Der Deure et al. 2010). These studies indicate the importance of this genes in cancer cell proliferation, metastasis, and since the expression of 3 members of this family MCT3, MCT8 and MCT9 have not been examined in breast cancer, we focused on these in this current study.

MCT3 protein is involved in the transport of ketones, pyruvate, and lactate and is expressed in most tissues. The gene for MCT3 (SCL16A8) is located on the long arm of chromosome 17 (Pérez-Escuredo et al. 2016). The MCT8 protein was discovered in 1994 and is encoded by a gene (SLC16A2) on the long arm of the X chromosome (Halestrap and Meredith 2004). It binds to thyroid hormone in mice and humans and whilst homologous to MCT10 (Friesema et al. 2003), does not play a role in the transport of lactate, aromatic amino acids and sulfonated iodothyronines (Van Der Deure et al. 2010). Decreased expression level of MCT8 in thyroid cancer has been suggested MCT8 as a marker of thyroid differentiation. It is also expressed in the brain, heart, kidney, liver, and testis (Badziong et al. 2017). Mutation in this gene results in the Allan–Herndon–Dudley syndrome (AHD) (Schwartz and Stevenson 2007). The MCT9 protein plays a role in the transport of substances such as carnitine and is expressed in most tissues (Halestrap and Meredith 2004; Pérez-Escuredo et al. 2016). It is encoded by a gene (SCL16A9) located on the long arm of chromosome 10 (Halestrap and Meredith 2004; Halestrap and Price 1999).

In this study, we evaluated the mRNA expression of these genes in breast cancer samples using the real-time PCR technique.

Materials and methods

Study population

Breast tumor tissues (n = 40), normal adjacent tissues (n = 40) and 5 healthy breast tissue samples were collected from the 2018 to 2020 from the Rasoul Akram Hospital, Tehran, Iran. All patients were identified to have breast cancer, based on the Laboratory and histological criteria, and have given informed written consent before surgery. The study was approved by the Ethics Committee of Iran University of Medical Sciences, Tehran, Iran with code number IR.IUMS.FMD.REC.1397.159. According to the patient's history, none of the patients had received chemotherapy or radiotherapy and had no other malignancies. Healthy breast tissues obtained from normal individuals without any history of the disease. Pathological and clinical data including breast tumor ER, PR, and HER2 status by Laboratory tests (like IHC) were obtained for each patient.

Cell lines and cell culture

The following human BC cell lines were purchased from the Pasture (Iran, Tehran) MCF-7 (ER + Luminal A-like BC

Table 1 Primer sequences were designed using Primer3.0 plus online tool used for real-time quantitative PCR

Primer	Forward sequence	Reverse sequence	Product size
HPRT	CTGGCGTCGTGATTAGTGATG	AGACGTTCAGTCCTGTCCATAA	130
MCT3	AGCCGTGAGCGTCTTCTTC	GTCAGGTAGAGCTCCAGGAG	231
MCT8	CATCTGGGCCTTCGGAATTG	CTGACACAAGACGCCCAAG	158
MCT9	TGTTCTTTGCTGGACTTGGA	CCAGCAGCAGAATAAAACCTC	135

Table 2 Patient clinical and tumor data

Variable	Clinicopathologic parameter	(%) percent of samples
Histological type	NOS	70
	NOS + comedo	13
	Others	17
Tumor stage	1	20
	2	60
	3	16.7
TNM	T1	23.3
	T2	63.3
	T3	10
Tumor type	Invasive ductal carcinoma	56.7
	Invasive ductal carcinoma + DCIS	36.7
	Other type	6.6
Family history	Yes	26.7
	No	73.3
ER(Estrogen Receptor)	NEG	40.0
	POS	60.0
PR (Progesterone Receptor)	NEG	50
	POS	50
HER2	NEG	76.7
	POS	23.3
Age	Mean = 51	Min = 30 Max = 73

cell model), MDA-MB231 (Basal like BC cell model), and SKBR3 (Her2 positive BC cell model). Cells were cultured in RPMI 1640 medium (Invitrogen, Auckland, New Zealand) supplemented with 10% heat-inactivated fetal bovine serum (Invitrogen), 2 mM L-glutamine, 100 U per ml penicillin, 100 µg per ml streptomycin (Gibco BRL, Rockville, MD, USA), and 2 g/l sodium bicarbonate (Sigma, St. Louis, MO, USA). The cells were incubated at 37 °C in a humidified atmosphere with 5% CO_2.

RNA extraction and cDNA synthesis

1.5 ml of cell culture medium and some (about 2–4 mg) of tissues stored at − 80 °C were used for RNA extraction. Total RNA was extracted from breast tissues and three cell line using RiboEX isolation reagent (GeneAll Biotech, Korea) according to the manufacturer's instructions. RNA was dissolved in DEPC-treated water and its concentration was determined with a Nano Drop one C spectrophotometer (Thermo Fisher Scientific, US). 1 µg total RNA from each sample was used for cDNA synthesis with the TAKARA kit (Primer Script™ RT reagent Kit, Japan) based on the manufacturer's protocol.

Quantitative real-time polymerase chain reaction

Quantitative real-time PCR (qtrs.-PCR) was performed on ABI Step One Sequence Detection System (Applied Biosystems, USA) using a SYBR Green-based PCR kit (Ampliqon, Denmark). The reaction was performed using a single cycle in 95 °C for 15 min followed by continual 45 cycles (95 °C for 10 s, 60 °C for 20 s and 72 °C for 30 s). The primer sequences for each gene are listed in Table 1. Each reaction was followed by melting curve analysis Involving heating
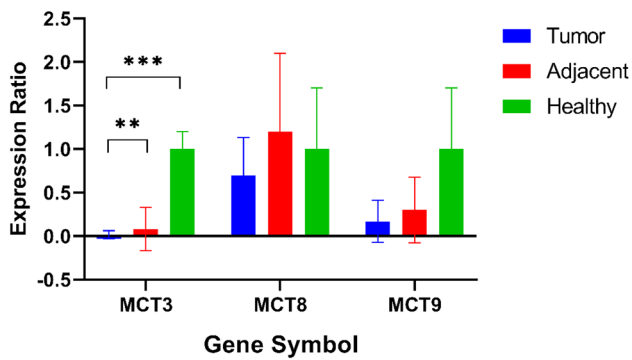
Fig. 1 The relative gene expressions of *MCT3*, *MCT8* and *MCT9* in different tissues. *MCT-3*, *MCT-8* and *MCT-9* genes are down regulated in tumor and adjacent samples compared to healthy tissues. Data shown are mean [±SD] OD values. *Value shown p value < 0.05, **value shown p value < 0.01 and ***value shown p value < 0.001
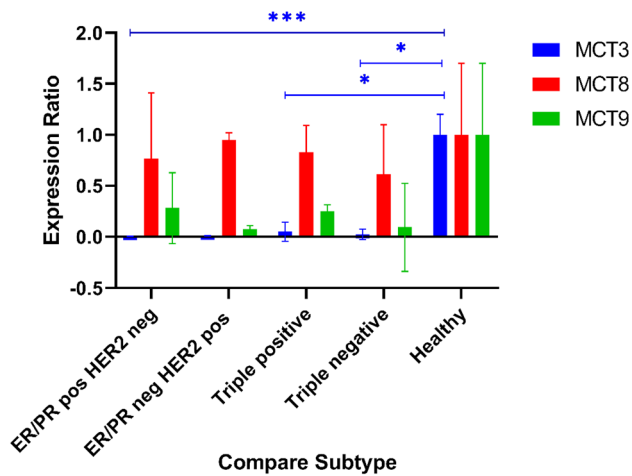

Fig. 2 Expression of genes in subtype of breast cancer. Comparing breast cancer subtypes with healthy tissue, the MCT3 gene in three subtypes of negative triple (p = 0.03), positive triple (p = 0.031) and Her2 negative (p = 0.0001) had a significant down regulation. Data shown are mean [±SD] OD values. *Value shown p value < 0.05, **value shown p value < 0.01 and ***value shown p value < 0.001

of the PCR product from 60 to 95 °C. The melt curve in the samples was compared with melt curve of positive and negative control to recognize specific amplicon from primer dimer formation.

Hypoxanthine phosphoribosyltransferase1 (HPRT1) was amplified as internal control that showed the least variation in gene expression between our normal and tumor samples.

MRNA microarray profiling data analysis

The UALCAN (http://ualcan.path.uab.edu/index.html), an interactive web resource (Chandrashekar et al. 2017) was

used to explore the mRNA expression levels of the candidate genes in tumor and normal breast tissues data.

Breast Cancer Gene-Expression Miner v4.4 (bc-Gen-ExMiner v4.4) (http://bcgenex.centregauducheau.fr), is a statistical mining tool of published annotated breast cancer transcriptomic data (DNA microarrays [n = 10001]) (Jézéquel et al. 2012, 2013). It offers the possibility to explore gene-expression of genes of interest in breast cancer. Targeted expression analysis performed with DNA microarrays data and different populations such as receptor statuses (ER, PR and HER2 by IHC), Age and molecular subtype (Table 2).

Survival analysis

To further validate, we used the Kaplan–Meier plotter database, which includes gene chip and RNA-seq data—sources for the databases include GEO, EGA and TCGA. The primary purpose of these tools are a meta-analysis based discovery and validation of survival biomarkers (Győrffy et al. 2010). Kaplan–Meier plotter database is a pan-cancer tool to analyze the relationship between gene expression of candidate genes and overall survival of 3955 BC patients. Hazard ratio (HR), 95% confidence intervals (CI) and log-rank p values were calculated.

Statistical analysis

All statistical analyses were performed using SPSS v.25 software (SPSS Inc., Chicago, IL, USA), Rest 2009 software (version 2.0.13) and Graph Pad Prism v.7.0 (Graph Pad Prism version 5.0, San Diego, USA). Rest is software for determining the relative expression results of real-time PCR that uses pair-wise fixed reallocation randomization tests to analyze data (Pfaffl et al. 2002). Descriptive statistical
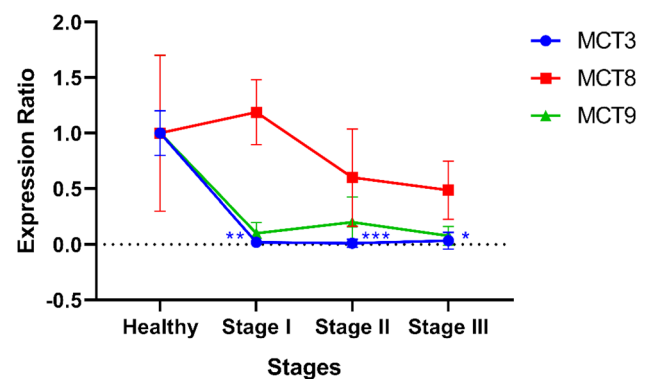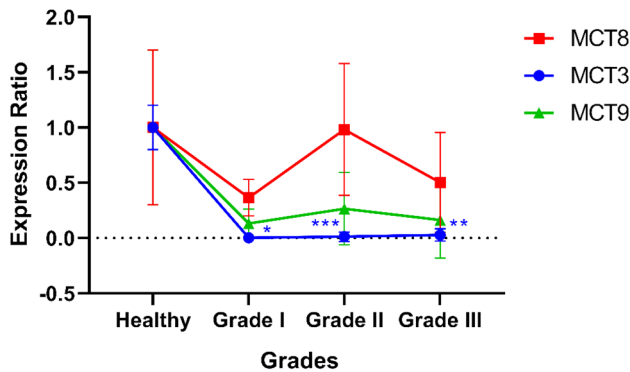

Fig. 3 Expression of genes in different stages of breast cancer comparing with healthy tissue, the MCT3 gene in three stages had a significant down regulation. Data shown are mean [±SD] OD values. *Value shown p value < 0.05, **value shown p value < 0.01 and ***value shown p value < 0.001

Table 3 Differences in the expression (expression ratio) of monocarboxylate transporters (MCTs) in tumors in relation to the clinical stage

Gene	Stage II vs Stage I	p value	Stage II vs Stage III	p value	Stage I vs Stage III	p value
MCT3	0.15 (−0.1 to 0.4)	0.029*s	0.87 (−0.4 to 2.2)	0.892	5.74 (4.6 to 6.8)	0.272
MCT8	0.17 (−0.03 to 0.4)	0.003**	0.39 (−0.01 to 0.8)	0.194	2.27 (1.8 to 2.8)	0.542
MCT9	1.77 (−0.2 to 3.8)	0.821	0.95 (−0.8 to 2.8)	0.983	0.53 (−1.3 to 2.3)	0.79

Fig. 4 Expression of genes in different grades of breast cancer comparing with healthy tissue, the MCT3 gene in three grades had a significant down regulation. Data shown are mean [±SD] OD values. *Value shown p value < 0.05, **value shown p value < 0.01 and ***value shown p value < 0.001

analysis was used to evaluate the expression of genes in the samples. p values < 0.05 were considered to be statistically significant.

Results

Clinic-pathological assessment

The mean age of patients at the time of diagnosis was 51.93 ± 2.23 (range between 30 and 73 years). According to TNM classifications, the tumors were staged by a pathologist, who had no prior knowledge of the test results. Clinicopathological factors including: age, tumor size, histological type, lymph node metastasis and TNM staging, were analyzed for evaluation of a correlation with considered genes expression (2). The subtypes were classified as follows according to ER, PR, and HER2: Triple-positive (ER positive, PR positive, HER2 positive); HER2-positive (ER negative, PR negative, and HER2 positive); HER2negative (ER positive, PR positive, and HER2 negative); and Triplenegative (ER negative, PR negative, and HER2 negative) subtypes. Most samples were of the Invasive ductal carcinoma type (93.4%). 80% of patients were involved in pathologic stage I and II (32 of 40) and 70% of samples were invasive ductal carcinoma. Immunohistochemically subtypes of the 40 samples were categorized in four groups' including: triplepositive (17%); HER2positive (14%); HER2negative (37%) and triplenegative (32%).

Expression of MCT3, MCT8 and MCT9 genes in tissue samples and their relationship to tumor subtypes

According to the obtained data, there was a significant down regulation of the *MCT3* gene in the tumor tissues compared to healthy (p value = 0.0001) and adjacent (p value = 0.004) tissues (Fig. 1). Expression level of *MCT3* gene in four
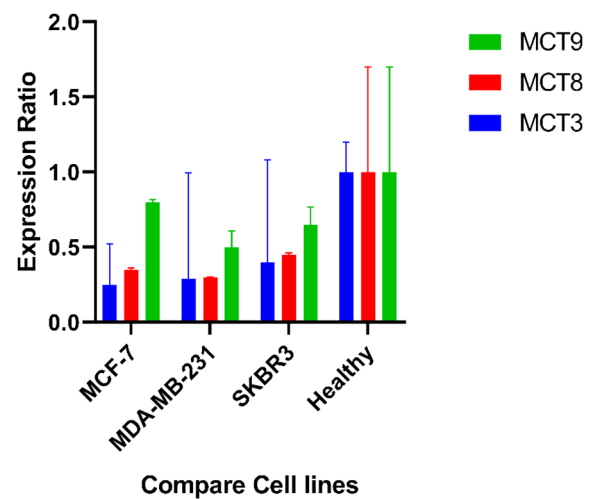

Fig. 5 The relative gene expressions of MCT3, MCT8 and MCT9 breast cancer cell lines compared to healthy tissues. Data shown are mean [±SD] OD values
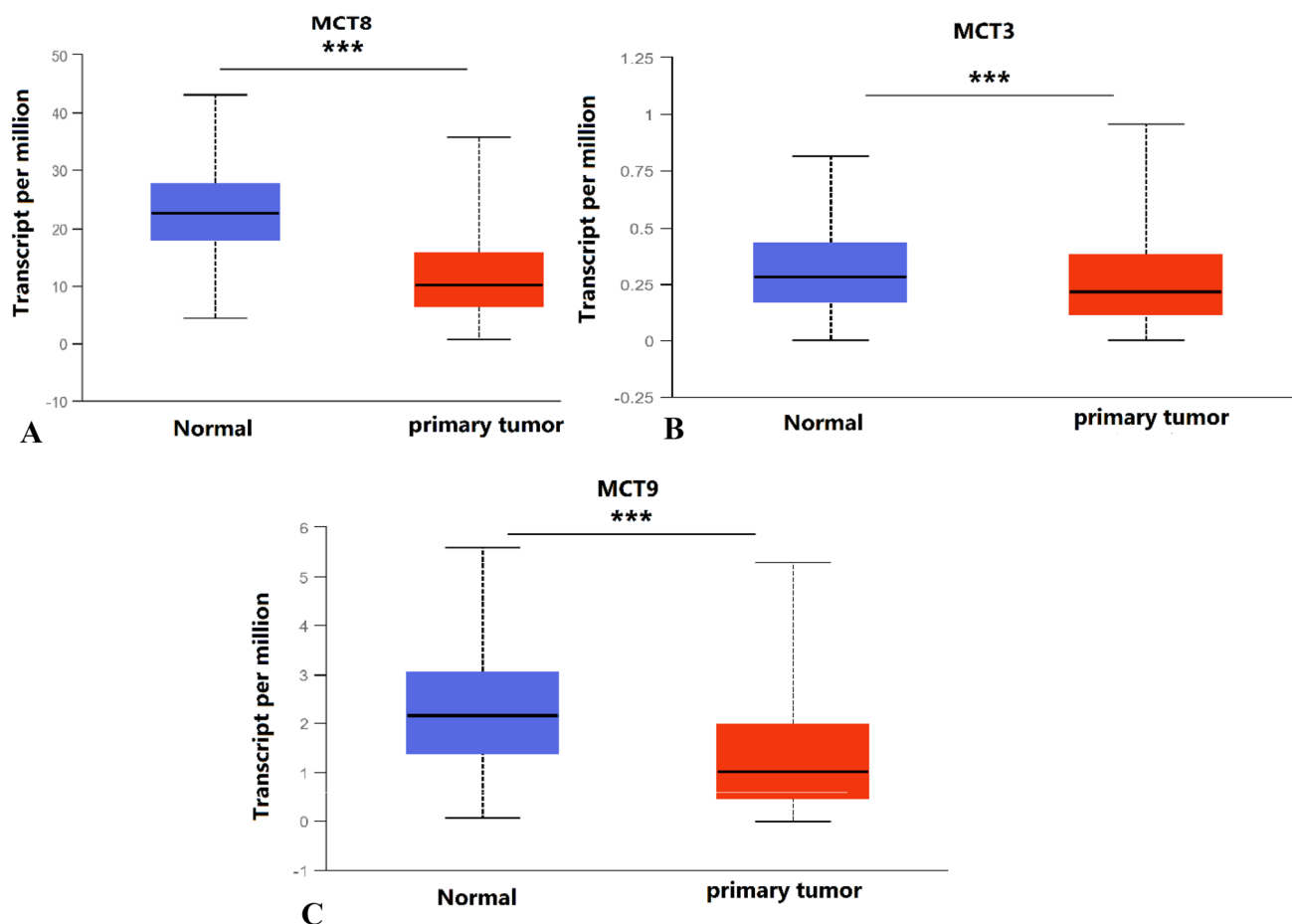
Table 4 Difference in expression (expression ratio) of monocarboxylate transporters (MCTs) in tumors in relation to the clinical grade

Gene	Grade II vs Grade I	p value	Grade II vs Grade III	p value	Grade I vs Grade III	p value
MCT3	1.445	0.73	1.057	0.946	0.732	0.875
MCT8	3.62	0.247	3.103	0.095	0.857	0.743
MCT9	2.716	0.754	9.188	0.208	3.383	0.724

Fig. 6 Expression validation in the UALCAN database for downregulated MCT8 (**A**), MCT3 (**B**) and MCT9 (**C**) genes in breast cancer samples. Blue box plot represent Normal samples (n = 114) and red box plot represent primary tumor samples (n = 1097). (Data from The Cancer Genome Atlas (TCGA) database) (***p value < 0.001)

common subgroups of breast cancer showed that triple negative compared with triple positive was not significant decreased in expression level, but in HER2 negative comparison with HER2 positive subtype and HER2 positive comparison with triple negative subtype, the expression level of *MCT3* gene over expressed non-significantly (Fig. 2).

MCT8 gene showed a non-significant down regulation in tumor tissues compared to adjacent and healthy tissues (Fig. 1). Expression level of this gene in subgroups of breast cancer showed that in HER2 negative comparison with HER2 positive subtype, the expression level of MCT8 was significantly lower (p value = 0.001). We also showed that MCT8 was significantly overexpression in comparison between HER2 positive with the triple negative subtype (p value = 0.027). The expression level of *MCT8* gene in the triple negative compared with triple positive tumor was not significantly lower in expression level (Fig. 2).

The *MCT9* gene showed a non-significant down regulation in tumor tissues compared to adjacent and healthy tissues (Fig. 1), in subgroups of breast cancer the triple positive

compared with triple negative tissue was not significant reduced in expression level, but in the HER2 negative comparison with the HER2 positive subtype and HER2 positive comparison with triple negative subtype, the expression level of *MCT9* gene was over expressed non-significantly (Fig. 2).

Expression level of MCT3, MCT8 and MCT9 genes in tumors in relation to the clinical stage and grade.

In order to evaluate the expression of *MCTs* genes in different stages and grades of breast cancer, in comparison to healthy tissue and each other, the samples were divided into six groups (stage I–III and grade I–III). The *MCTs* genes have down regulation in most stages compared to healthy tissue and down regulation of *MCT3* genes in stage I (p value = 0.009), stage II (p value = 0.0001) and stage III (p value = 0.013) has been significantly (Fig. 3). Also, the *MCT8* gene showed a non-significant over expression in stage I compared to healthy tissue. Compared in the different
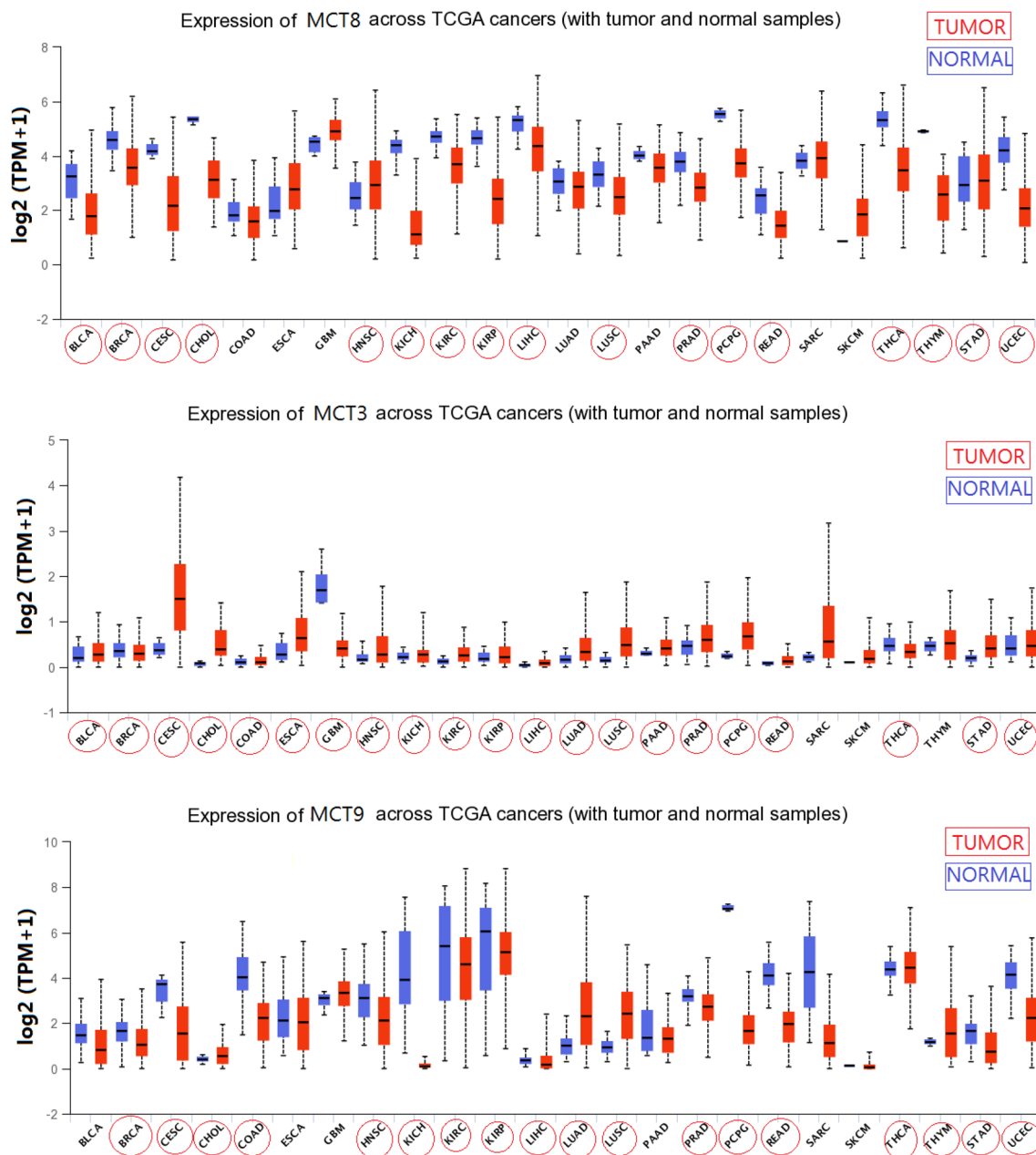
Fig. 7 TCGA data analysis of the mRNA level of MCT8, MCT3 and MCT9 genes in different types of cancer. Cancer types with significantly decreased mRNA levels are highlighted with a red circle

stages, MCT3 and MCT8 in stage II compared to stage I had a significant over expression (Table 3).

Also, The MCTs genes have down regulation in all grades compared to healthy tissue and down regulation of MCT3 genes in grade I (p = 0.027), grade II (p = 0.0001) and grade III (p = 0.005) has been significantly (Fig. 4). Compared in the different grades, none of the genes showed significant expression (Table 4).

Expression level of MCT3, MCT8 and MCT9 genes in three cell lines

MCF7 is representative of primary breast cancer cells [invasive ductal carcinoma (IDC) OR luminal A] that express E-cadherin, ER and PR receptors (Hiscox et al. 2012; Zheng et al. 2007). MDA-MB-231 is representative of metastatic, aggressive and invasive adenocarcinoma cells that are Vimentin- positive and does not express E-cadherin, ER, PR and HER2 receptors (TNBC) (Lacroix et al. 2004).

Fig. 8 bc-GenExMiner data analysis of the mRNA level of MCT8 (SLC16A2) gene in different Receptor, age and subtype statuses in DNA micro-array data. Box and whisker plot of SLC16A2 (MCT8) expression according to ER (**A**), PR (**B**) and HER2 (**C**) by IHC. Beeswarm plot of MCT8 expression in different age (**D**). Violin plot of MCT8 expression in different intrinsic molecular subtype according to PAM50 subtypes in DNA microarray data (**E**)
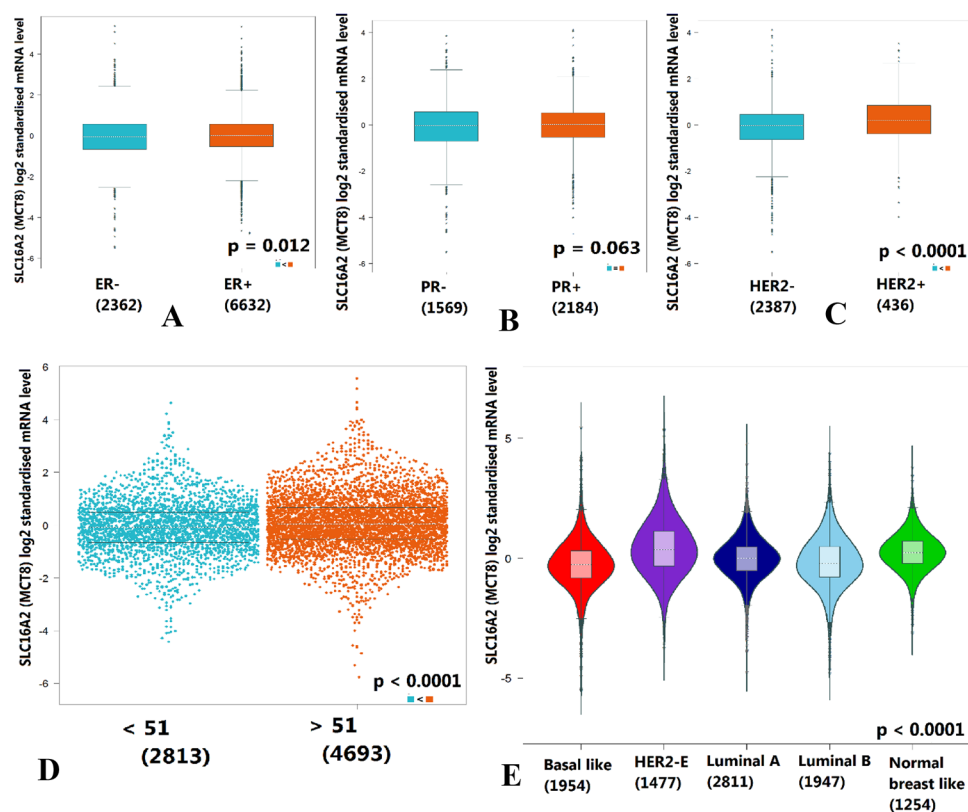
SKBR3 is representative of adenocarcinoma that over-expresses the HER2 gene product (HER2 positive) (Dai et al. 2017). All the genes in the three cell lines had non-significant decrease in expression compared to healthy tissues, Also our results showed that MCT-9 in MDA-MB-231 (p value = 0.0001) and SKBR3 (p value = 0.0001) cell lines down regulated compared to MCF-7 cell line, but MCT-3 in SKBR3 cell line (p value = 0.0001) and MCT-8 in both SKBR3 (p value = 0.0001) and MDA-MB-231 cell lines (p value = 0.0001), up regulated compared to MCF-7 cell line (Fig. 5).

Breast cancer microarray profiling data analysis

The UALCAN analysis showed that while the expression level candidate genes decreased in BC primary tumors compared with normal cases (p value < 0.001) (Fig. 6). The downregulation of candidate genes, have often been shown in a variety of cancers compared with normal cases (Fig. 7).

bc-GenExMiner analysis showed that MCT8 (SLC16A2) has significant difference in ER negative versus ER positive (p value = 0.012) and HER2 negative versus HER2 positive (p value < 0.0001) status. MCT3 (SLC16A8) has significant difference in ER negative versus ER positive (p value < 0.0001), PR negative versus PR positive (p value = 0.002) and HER2 negative versus HER2 positive (p value = 0.004) status. MCT9 (SLC16A9) has

significant difference in ER negative versus ER positive (p value < 0.0001), PR negative versus PR positive (p value < 0.0001) and HER2 negative versus HER2 positive (p value = 0.002) status.

Also All three candidate genes have significant differences between age before and after 51, and in each intrinsic molecular subtype with significantly p value (Figs. 8, 9, 10).

The expression level of *MCT8* and *MCT9* were associated with poor overall survival in Breast cancer samples

Using the Kaplan–Meier plotter database, we determined that the overall survival of 3955 BC patients (from the Kaplan–Meier plotter database) is low in cases with decreased expression of candidate genes. The follow-up time was determined in 250 months. The plots showed that low expression of SLC16A9 (MCT9) and SLC16A2 (MCT8) was associated with poor overall survival (Fig. 11).

Discussion

Nearly one in nine women develops breast cancer, and breast cancer accounts for about one-third of all cancers in women, the second most common cancer after lung cancer and the most common cause of cancer deaths among
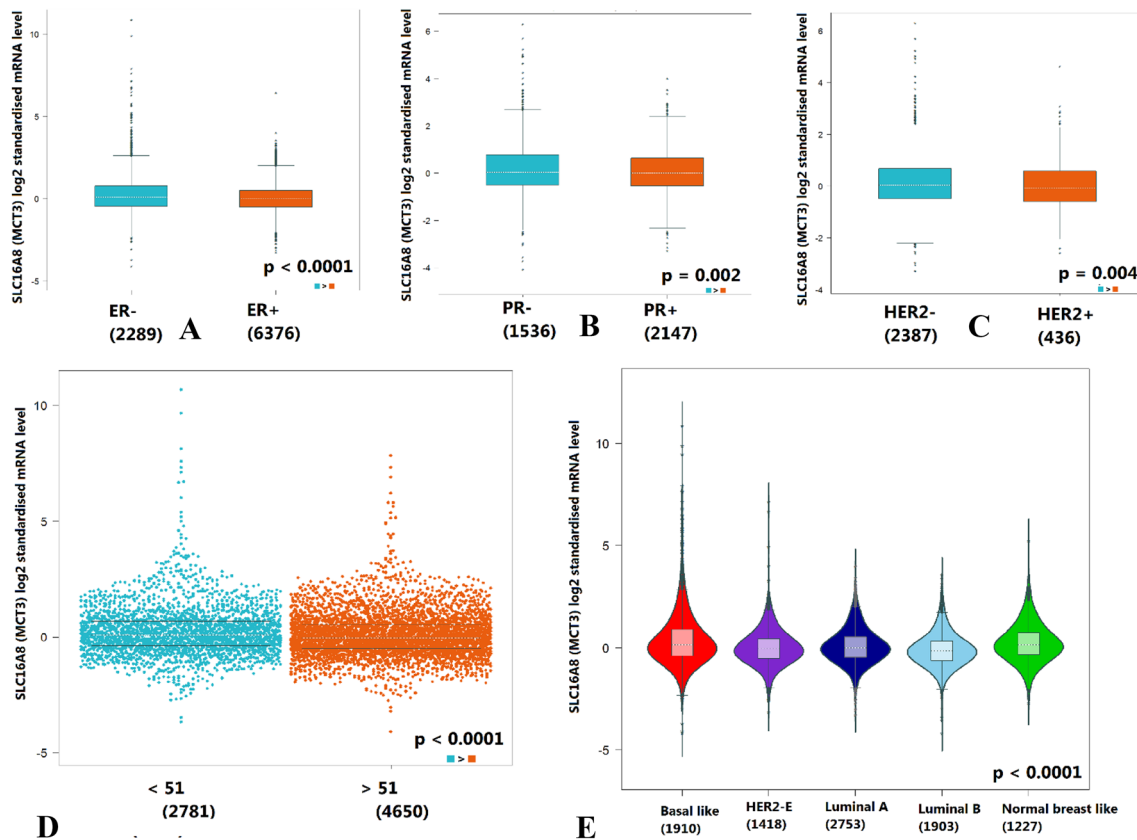
Fig. 9 bc-GenExMiner data analysis of the mRNA level of MCT3 (SLC16A8) gene in different Receptor, age and subtype statuses in DNA microarray data. Box and whisker plot of SLC16A8 (MCT3) expression according to ER (**A**), PR (**B**) and HER2 (**C**) by IHC.

Beeswarm plot of MCT3 gene in different age (**D**). Violin plot of MCT3 gene in different intrinsic molecular subtype according to PAM50 subtypes in DNA microarray data (**E**)

women (Wolman 2014). As a result, it is important to better understand and evaluate the genes and molecular markers involved in this cancer.

The important features of transporters in MCT super family are their ability to transport across membranes and their wide substrates, making them essential for many cellular processes and cancers (Halestrap 2013). *MCT3*, *MCT8* and *MCT9* genes and their substrates not yet well understood and there is very limited information on the expression and their potential roles. That's why for the first time, we examined the expression of the *MCT3*, *MCT8* and *MCT9* genes in the patients with breast cancer and MCF7, SKBR3 and MDA-MB-231 cell lines.

Some members of the MCT family can be grouped together, for example MCT1 to MCT4 are involved in the transfer of monocarboxylates (like lactate and pyruvate) and also MCT8 and MCT10 are involved in the transfer of aromatic amino acids and thyroid hormones. But other components of this family are not included in a particular group due to the lack of complete substrate recognition (Halestrap 2013). MCT1 and MCT4, are two members of this family

that have been extensively studied in various cancers such as breast cancer and have been introduced as therapeutic targets for cancer, but the activity and expression of other members of this family in breast cancer have not been studied (Halestrap 2013). MCT1 up-regulated in the basal (Pinheiro et al. 2010), luminal-like subtypes of breast cancer and MCF7 cell line and targeted MCT1 can cause decrease in metastasis and angiogenesis of the cancer cells (Hussien and Brooks 2010; Sonveaux et al. 2008; Végran et al. 2011). Also, the MCT4 and MCT2 up-regulated in MDA-MB-231 and MCF-7 cell lines compared to HMEC 184 cells (Gallagher et al. 2007). Zhu and et al. (2005) demonstrated decreased expression of MCT3, and this was associated with increased severity of atherosclerosis. The MCT3 downregulation has also been observed in the non-small cell lung cancer (NSCLC) (Eilertsen et al. 2014). Our results are also consistent with these results, showing a significant down regulation of this gene in tumor tissues compared to normal tissues, but MCT-3 in SKBR3 cell line over expressed compared to MCF-7 cell line. *MCT3* gene expression in stage II disease was significantly different from stage I and stage III disease. These
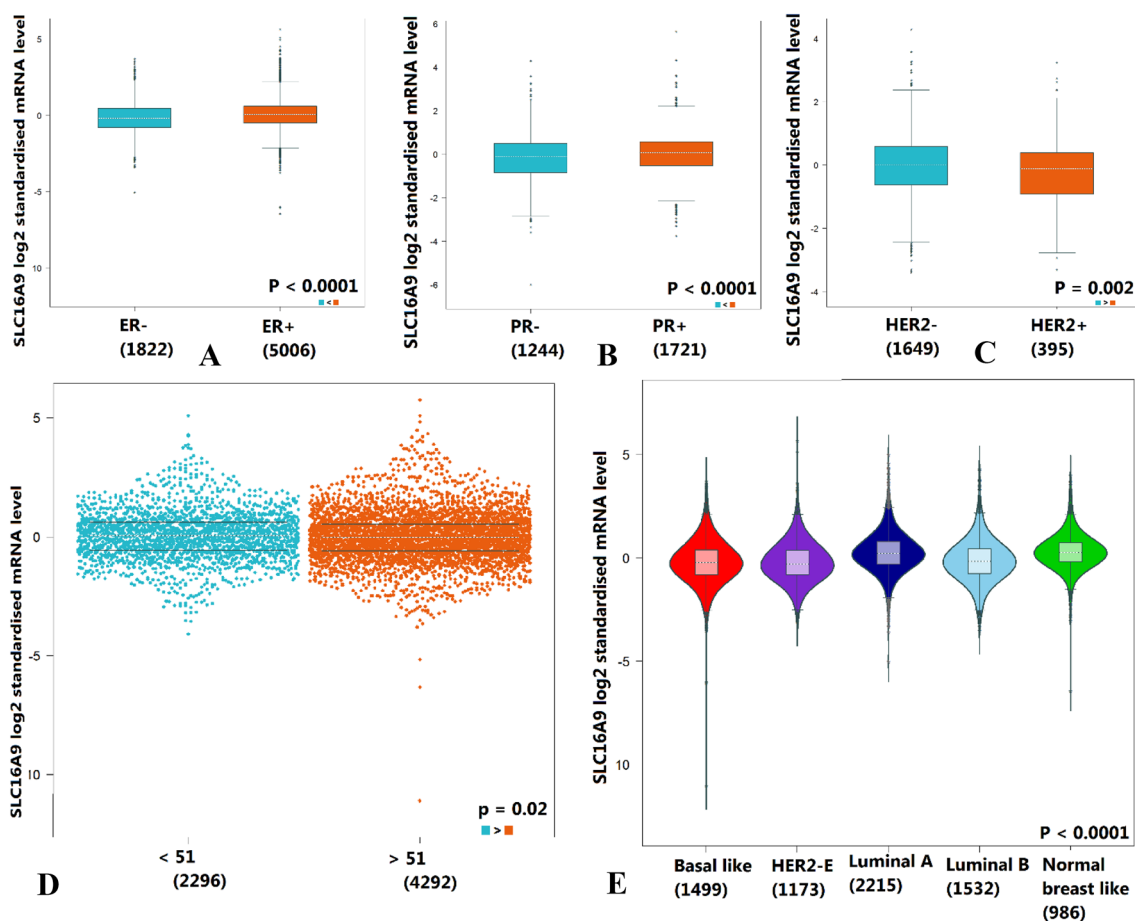
Fig. 10 bc-GenExMiner data analysis of the mRNA level of MCT9 (SLC16A9) gene in different Receptor, age and subtype statuses in DNA microarray data. Box and whisker plot of MCT9 (SLC16A9) expression according to ER (**A**), PR (**B**) and HER2 (**C**) by IHC.

Beeswarm plot of MCT9 gene in different age (**D**). Violin plot of MCT9 gene in different intrinsic molecular subtype according to PAM50 subtypes in DNA microarray data (**E**)

data suggest that in addition to being detectable in various breast cancer subtypes, these markers like HER2 (Wong and Hurvitz 2014), and EphA2 (Rezaie et al. 2020, 2019) may be used to predict the response to the treatment or disease progression.

MCT8 and MCT10 are expressed in heart, kidney, liver, skeletal muscle, placenta, testis and appears to perform a particularly useful function in the brain where it is necessary for the thyroid hormone uptake that is important for normal brain development. Badziong et al. (2017) showed that the MCT8 expression was downregulated in both benign and the malignant thyroid tumors as compared to the normal thyroid tissue, In contrast, significant upregulation of *MCT8*, *LAT2* and *LAT4* genes were found in Graves' disease, So in that research they suggested *MCT8* is a appropriate object for thyroid differentiation and MCT8 upregulation occurs in hyper functional thyroid tissue (Badziong et al. 2017). Significant down regulation of *MCT8* gene was also confirmed by Nwosu et al. (2017), with analysis of eight microarray

datasets in human hepatocellular carcinoma. In agreement with these studies, we found a significant reduction in the expression of this gene in the tumor tissues compared to the normal tissues. *MCT8* is significantly overexpression in the comparison to HER2 positive with triple negative subtype; this means that HER2 expression is positively associated with increased expression of this gene. Also, in this study we reported upregulation of the *MCT8* gene in the tumor tissue compared with the tumor adjacent tissues. This may indicate that the *MCT8* gene could help differentiate tumor cells from adjacent cells and could be the possible mechanism for these associations.

So far, the *MCT9* gene expression in various human cancers has not been studied by molecular methods such as real time PCR, but significantly down regulation in breast cancer has been identified using microarray analysis (Kim et al. 2017). The previous studies reported down regulation of the *MCT9* gene in invasive breast cancer and also CNS diffuse large B-cell lymphoma compared to non-CNS diffuse

Fig. 11 **A** Kaplan–Meier (https://kmplan.com/analysis/) survival curve showing the prognostic value of SLC16A9 (MCT9) in predicting BC using the Pan-cancer analysis. Low expression of mRNA indicated poor overall survival. **B** Low expression of SLC 16A8 (MCT3) showed no significant effect on survival (HRs; 95% confidence interval in parentheses). **C** Low expression of SLC16A2 (MCT8) showed poor overall survival
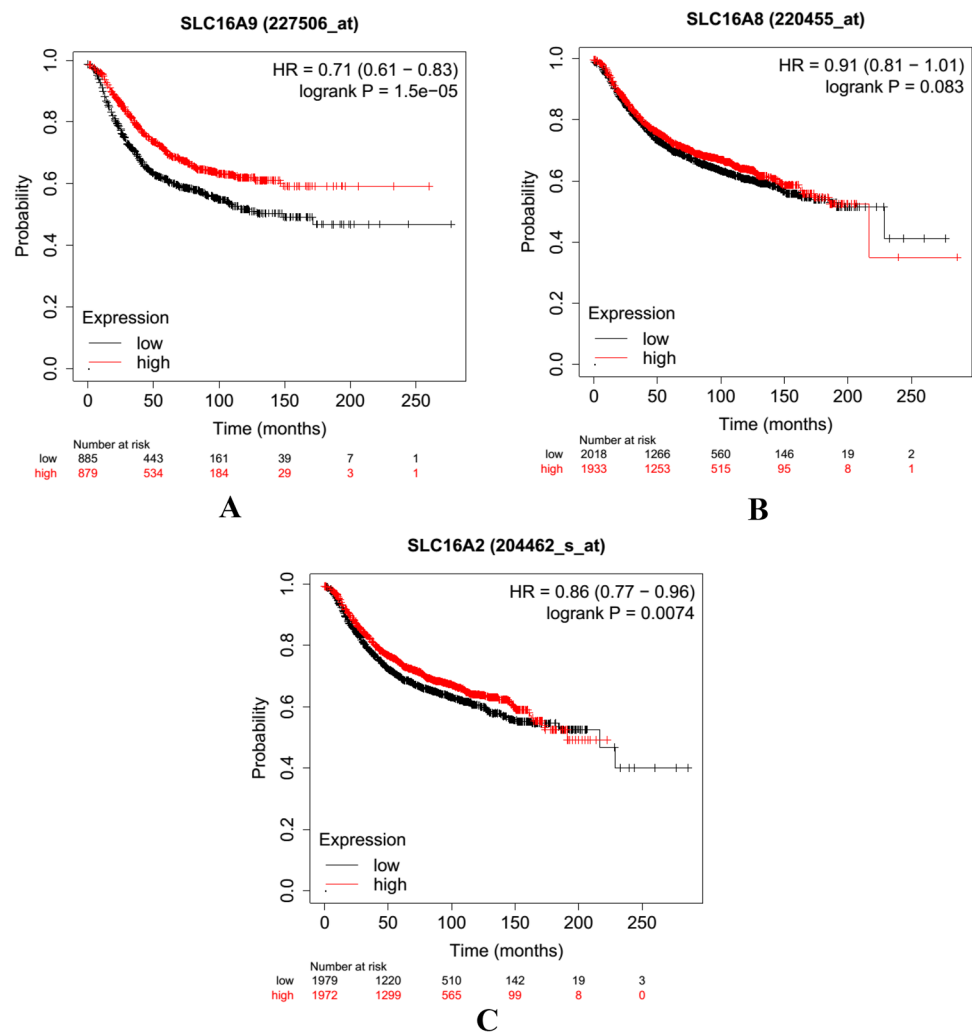
large B-cell lymphoma by analyzing microarray data (do Hyoung, 2015). MCT9 may be involved in aerobic glycolysis of malignant tumors and may be increased in certain types of lymphomas. MCT9 expression is also significantly down-regulated in MDA-MB-231 and SKBR3 cell lines compared to MCF-7 cell line.

Conclusion

Since the expression *MCT3*, *MCT8* and *MCT9* have not been examined in breast cancer previously this study may help to understand the importance of these genes in the breast cancer progression. The expression of *MCT3* may be used as a prognostic marker; however, further research is required to confirm this.

Limitations

This study had some limitations, like that sample size. In other studies with larger sample size, it might be feasible to investigate a number of issues in detail, which needs a more homogenous clinical and molecular population. Also for validating these results, the complementary techniques such as western blotting could be explored.

In conclusion, *MCT3* gene showed low expressions in tumor samples compared to adjacent normal and healthy mammary tissues. Besides, *MCT3* can represent a novel therapeutic target in breast cancer patients or some of the following subgroups such as HER2-negative or triple-negative.

Availability of data and material The data that support the findings of this study are available.

Declarations

Conflict of interest Ehsan Sohrabi, Masoumeh Moslemi, Ehsan Rezaie, Nahid Nafissi, Mansoor Khaledi, Hamed Afkhami, Javad Fathi and Ali Zekri declare that they have no conflict of interest.

Ethics approval and consent to participate The study was approved by the Ethics Committee of Iran University of Medical Sciences, Tehran, Iran with code number IR.IUMS.FMD.REC.1397.159.

Consent for publication Not applicable.

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