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ORIGINAL ARTICLE

Study of differential gene expression between invasive multifocal/multicentric and unifocal breast cancer

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Summary

Purpose: *To investigate the differential gene expression pattern between invasive multifocal/multicentric (MMBC) and unifocal breast cancer (UFBC) with cDNA array and to discover the potential outlier genes associated with the incidence of MMBC and also to provide a guidance for clinical treatment and prognosis prediction.*

Methods: *This retrospective study analyzed the gene expression pattern alteration in breast cancer. We collected 156 MMBC (136 cases with 2 foci, 20 cases with 3 foci) and 130 UFBC samples from patients hospitalized in Yuhuangding Hospital, Yantai, from January 2005 to December 2015. The outlier genes were screened by cDNA expression microarray and validated by RT-PCR.*

Results: *18 overexpressed and 22 underexpressed genes were identified in the differential analysis, including family genes ABCC11, ABCB5 and PRODH, PROL1. Noteworthily, ABCC11 was significantly upregulated, while ABCB3 was downregulated, which were confirmed by RT-PCR results.*

Conclusion: *The differential expression pattern of ABCC11 and ABCB5 genes may serve as outliers, potentially associated with incidence of MMBS.*

Key words: *ABCB5, ABCC11, differential expression, multifocal/multicentric, unifocal breast cancer*

Introduction

Breast cancer is the most common malignant tumor in females [1]. Many lines of research were dedicated to tumorigenesis, pathology, therapy, and prognosis of UFBC [2-4]. Meanwhile, an ever increasing number of reports are focused on MMBC. Some researchers defined MMBC as cancer with more than one focus of invasive carcinoma separated by benign tissue whether in the same or a different quadrant and regardless of the distance between the foci [5]. A study also reported that the majority of MMBC had aggregate dimensions over 2 cm [6]. In comparison with UFBC, MMBC features bigger tumor cumulative diameter, higher histological grade, higher incidence of lymph node metastasis and higher level of Ki67 proliferation index, which all indicate more aggressive biological behavior that was noticed by pathologists and clinicians [6,7]. Up until now, most of the research over MMBC has focused on the imaging, pathology and surgery with conclusions usually controversial and confusing, and with lack of molecular studies [8-10]. Thus we set out to analyze the differential gene expression between MMBC and UFBC in fresh patient samples and identify the potential outlier genes tightly associated with MMBC, which could provide guidance for clinical therapy and serve as prognostic marker.

The human ATP-binding cassette (ABC) transporters is a large family of transmembrane

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proteins and encompasses 7 subgroups and 48 members in total, based on the sequence similarities [11]. All the ABC transporters share common structural domains including two transmembrane regions (TMRs) and two nucleotide-binding regions (NBRs) [12]. The hydrophobic TMRs consist of six helices and feature diverse configuration and substrates specificity, while NBRs are highly conserved and responsible for ATP binding and hydrolyzation, which provides energy for influx or efflux of endogenous or exogenous substrates [13]. The ABC proteins function as either monomer or dimer, which significantly increase the substrate spectrum and specificity.

ABCB5 was first identified as a regulator of cell differentiation in skin progenitor cells and melanoma stem cells [14-16]. Four major forms of ABCB5 have been identified with putative and distinct physiological function and organ distribution [17]. It was reported that ABCB5 was actively expressed in a variety of human cancers including malignant melanoma, breast cancer, colorectal cancer, hepatocellular carcinoma and leukemia [15,18-20]. It was known that ABCB5 could promote melanoma metastasis and enhance metastasis and epithelial-mesenchymal transition (EMT) in breast cancer cells [18,20].

The ABCC11 gene encodes the multidrug resistance-associated protein 8 (MRP8) [21], the allelic genotype of which was associated with earwax phenotype [22]. The transmembrane protein participates in the physiological transportation of bile acids, conjugated steroids and cyclic nucleotide and maintain the appropriate intracellular homeostasis of its substrate molecules [23]. Diseases associated with ABCC11 include Acrofacial Dysostosis 1, Nager Type and Breast Cancer [24,25]. Consistent with its efflux pump function, several studies addressed the association of high expression of ABCC11 with therapeutic resistance in different cancers [26-29].

In this study, with utilization of microarray technology on MMBC and UFBC samples, we unexpectedly discovered a dramatic change of ABC family genes associated with breast cancer subtypes, which may indicate potential clinical exploitation as early diagnosis biomarker, molecular subtyping, therapy guide and prognosis and warrants further investigation.

Methods

Patients

The pathological documents of 3597 patients diagnosed with invasive ductal carcinoma in Yuhuangding Hospital, Yantai, from January 2005 to December 2015 were carefully reviewed by two experienced pathologists to screen for MMBC cases and the following standards were employed: (1) two or more separate carcinoma foci in the same breast regardless of distance and sector; (2) only invasive ductal carcinomas were selected while all the other forms of invasive carcinoma were excluded from further investigation. One hundred and thirty UFBCs were selected for comparison. All the clinical data and immunohistochemical staining sections were analyzed in accordance with the WHO Breast Cancer Classification Guide (2012). Written informed consent was obtained from the patients and this study was approved by the ethics committee of Yuhuangding Hospital (No.20150028).

Microarray

RNA was extracted from 0.4g of each sample by Trizol (Invitrogen, USA) method in accordance with the manufacturer's instruction. The first strand cDNA was synthesized through reverse PCR (First Strand cDNA Synthesis Kit, Clontech, USA) and labeled with Cy3 fluorescence marker (CDNA Microarray Labeling and Hybridization Kit, Biotech, China). After purification and quantification, the labeled cDNA was subjected to hybridization and scanning by the ScanArray 4000 (Packard Biochip Technologies, USA). The microarray used in this study was purchased from Beijing Capital-Biotech and the expression profiling and analysis was provided.

Real-time PCR

The primers used in this study were synthesized by Shanghai Sangon and listed as follows:
ABCC11 Forward primer: 5'- GTCTGGGTTCTCATCCACACATCC-3'; ABCC11 Reverse primer: 5'- CCAGAGCTTTGCTGGGGTCTTGTA-3'; ABCB5 Forward primer: 5'-TAATACGACTCACTATAGGGATGTCTGGCTTTTCCCTTCTTGAC-3'; ABCB5 Reverse primer: 5'-GATTTAGGTGACACTATAGAAATTCAAGCTGGACGAATGACCCCA-3'.

The total RNA was extracted with Trizol reagent and the first strand cDNA were synthesized following the manufacturer's instruction. The reaction with set up according to the direction (RT-PCR Kit, Promega, USA). The PCR conditions were as follows: 95°C denature for 30s, 52°C annealing for 30s, 55°C extension for 45s by 35 cycles, 72°C extra extension for 45s. The melting curve was plotted post-amplification and the relative expression was calculated by the $2^{-\Delta\Delta Ct}$ method and normalized to β-actin. Each measurement was independently triplicated.

Statistics

The Feature Extraction Software was employed for preprocessing analysis. The differential expression and statistical significance of p value was calculated with Gene Spring GX Software. The p values were determined using T-test and $p<0.05$ was considered as statistically significant.

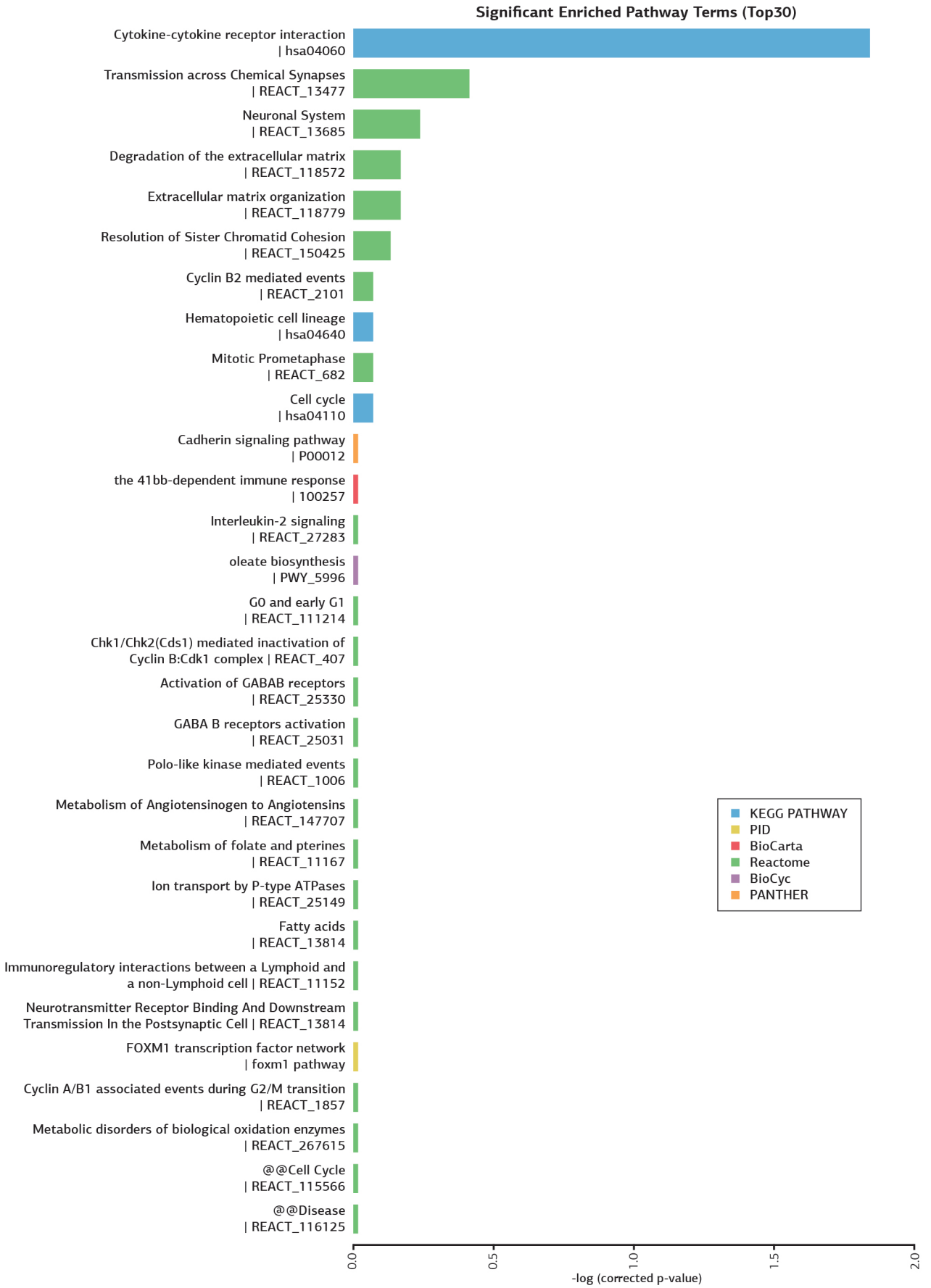

Figure 1. Pathway function enrichment analysis searched between MMBC and UFBC patients. The results show that differentially expressed genes were mostly manifested in the cytokine-cytokine receptor interaction, transmission across chemical synapses and neuronal system.

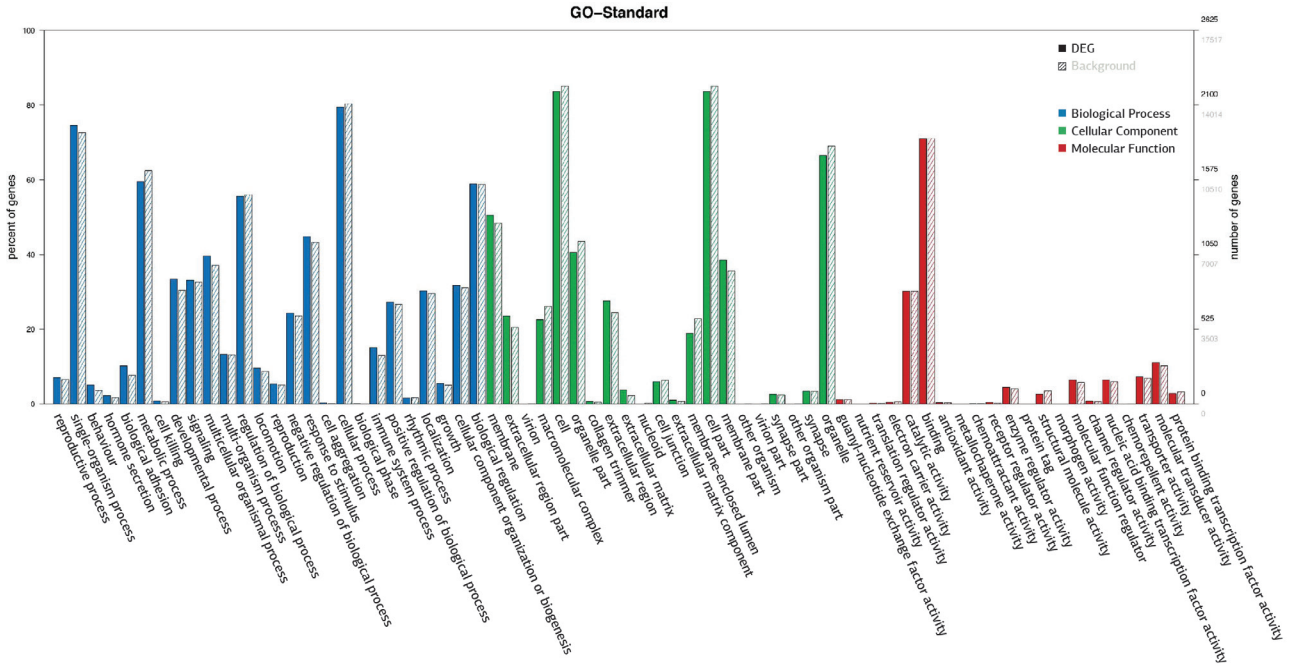

Figure 2. Differential expression genes were analyzed by GO analysis between MMBC and UFBC. The outcome demonstrated there were 40 differential expression genes in MMBC vs UFBC. In MMBC, 18 genes were up- and 22 were down-regulated (fold change>2). The differential genes were focused on the biological process, cellular component and molecular function.

Results

The differential expression genes in MMBC vs UFBC

Comparing the gene expression between MMBC and UFBC, the pathway analysis (Figure 1) demonstrated that the differential expression genes were mostly manifested in the cytokine-cytokine receptor interaction pathway, followed by transmission across chemical synapses, and neuronal system.

Gene ontology (GO) analysis (Figure 2) showed that in comparison to UFBC, the biological process in MMBC was mainly manifested in single-organism process, metabolic process, and regulation of biological processes. In the cellular component, the expression genes were focused on the membrane and organelles. Different binding functions were noticed in the molecular function between MMBC vs UFBC.

Among the screened differential expression of genes in MMBC vs UFBC, 18 were up- and 22 were down-regulated (Tables 1 and 2). It is noteworthy that ABCC11 (ATP-binding cassette, sub-family C (CFTR/MRP), member 11), ABCB5 (ATP-binding cassette, sub-family C (CFTR/MRP), member 5), PRODH (proline dehydrogenase (oxidase) 1), PROL1 (proline rich, lacrimal 1) belonged to the same family. ABCC11 and PRODH were overexpressed while ABCB5 and PROL1 were underexpressed in the MMBC patients. To our knowledge, this is the first report that suggested a potential association of PRODH/PROL1 with MMBC.

Validation of ABCC11 and ABCB5 expression by RT-PCR

The expression of ABCC11 and ABCB5 in 12 MMBC and 12 UFBC samples was determined by RT-PCR and quantified by densitometry, with

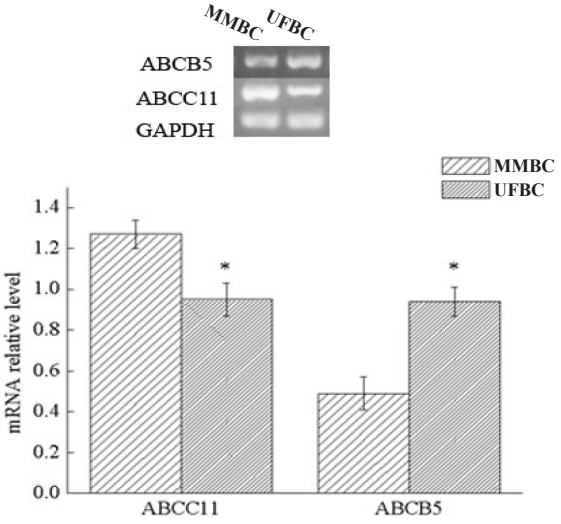

Figure 3. Expression of ABCC11 and ABCB5 mRNA was detected by RT-PCR in MMBC and UFBC patients. Compared with MMBC patients, the levels of ABCC11 were significantly decreased in the UFBC group ($p<0.05$). However, the expression of ABCB5 was remarkably increased in the UFBC patients ($p<0.05$). *Compared with the MMBC, $p<0.01$.

Table 1. Overexpression of genes in MMBC (fold change >2)

Gene symbol	*Gene name*	*Sequence*
POF1B	premature ovarian failure, 1B	ATGCACACATTACAAATAATATAAGGGGAAGTTTTATTAGCTTAGTAGGAAATTGTTATT
SDR16C5	short chain dehydrogenase/ reduc tase family 16C, member 5	TGCCCTCACTTTGGATCTATTGTATTCTTACAGTTTAATCAACTGGCAAAGTGCTTTCAT
LOC101929056	uncharacterized LOC101929056	GACAGTTTAGCGTCGGTAGCCGCGAGAAATATATAAAGAACACGAGTCAAATGACATTAA
CEACAM6	carcinoembryonic antigen-related cell adhesion molecule 6 (non-specific cross reacting antigen)	ACACTCATCTGACTCATTCTTTATTCTATTTTAGTTGGTTTGTATCTTGCCTAAGGTGCG
LRRC31	leucine rich repeat containing 31	TGAGCTGAGGAAATTAGATCTTTCCTGCAATAAGGATCTAGGTGGAGGTTTTGAAGACTC
TMEM63C	transmembrane protein 63C	ATGCTGAACCTTCTTGTTAATGCTATGACCGTGCCTTGAATAAACAAGTCCTCCCAACCT
FBXL16	F-box and leucine-rich repeat protein 16	ATGCGGAAGATATAACCTTATATTTGGTAAGTGTTTCTTGTGCTATTTTATCACGTGACC
DNAH5	dynein, axonemal, heavy chain 5	CTGTCATAAGGATTTATGCAGAAAACAATACTTTACGAGATCCTCGGTTTTACTCCTGTC
CYP4B1	cytochrome P450, family 4, subfamily B, polypeptide 1	CATAGAAATAGGTGTAAGATATTGTTAAGACGCACTATCTTAGCATGAGACGGGACTTCT
CAPN9	calpain 9	TGGATTAACCTTTTCCTTCGGTTTGATGCTGACAAGTCCGGCACCATGTCTACCTATGAA
ABCC11	ATP-binding cassette, sub-family C (CFTR/ MRP), member 11	AGTCTGCGACCTTCTTGTTTGGAGATGAGAACTTCTCCTGGAAGCAGGGGTAAATGTAGG
LOC339260	uncharacterized LOC339260	TGAATATTTCTGTCCATAAGACTATTAGAATCCCCCAAAGTGGGATACCAGTGAGGACCT
SLC9C1	solute carrier family 9, subfamily C (Na+-transporting carboxylic acid decarboxylase), member 1	GGAGAAATAATAGGAGAGATAAACTGCTTAACTAATGAACCTATGAAATATTCTGCCACC
CLEC3A	C-type lectin domain family 3, member A	CCACTTTGCAAACTTTAACTACACATGCTTGGAATTAAGTTTTAGCTGTTTTCATTGCTC
CYP21A2	cytochrome P450, family 21, subfamily A, polypeptide 2	TATCTGCTTGGCCTGACTCAGAAATTCGGGCCCATCTACAGGCTCCACCTTGGGCTGCAA
XLOC_ l2_013730		TTGTCTCAACACCTCTGATGAACAGTAGCGGAAGAAAGGACTCTTTCCACATGAGTTCCT
CEACAM7	carcinoembryonic antigen-related cell adhesion molecule 7	AATGCACGGTGGTCCCTGTTAGAGTTAGACCTCTAGACTCACCTGTTCTCACGCCCTGTT
TFF3	trefoil factor 3 (intestinal)	GCTGCTTTGACTCCAGGATCCCTGGAGTGCCTTGGTGTTTCAAGCCCCTGCAGGAAGCAG
PRODH	proline dehydrogenase (oxidase) 1	GCAGCGGAAGTTCAATGTGGAGAAGCCGCTCATCTTCAACACATACCAGTGCTACCTCAA

Table 2. Overexpression of genes in MMBC (fold change >2)

Gene symbol	*Gene name*	*Sequence*
CECR2	cat eye syndrome chromosome region, candidate 2	AACAAATTGGCACTAGAAGTGGAATAAGAGGACCTTTCCAGGAAATGTACAGACCATCAG
KCNC2	potassium channel, voltage gated Shaw related subfamily C, member 2	AGTACACAGAGTGACACATGTCTGGGCAAAGACAATCGACTTCTGGAACATAACAGATCA
GHRH	growth hormone releasing hormone	CTGACCCACTTTTTCCTTTGAAAATACAATAAAATTCCCCCATACCGGTGTGCATTTAAA
CRISP2	cysteine-rich secretory protein 2	TACTACTATGTTTGCCAATATTGTCCTGCTGGTAATAATATGAATAGAAAGAATACCCCG
SCNN1G	sodium channel, non voltage gated 1 gamma subunit	AGAGCTAGGATTTGACCCCAGACAATCTGACTTCATGATTGTGGCATCCAATTCGTGTCT
PROL1	proline rich, lacrimal 1	TCTCAACTCTTTCCATTGGAATCTATTAGACAACCTCGACTCTTTCCGGGTTATCCAAAC
BEX1	brain expressed, X-linked 1	TGAACCAGTCTGTAAGATTTTTGTTAGCAGAAGAATTTTACCTATTGCATGGAAAGATGC
FAM25A	family with sequence similarity 25, member A	GCCATCACCCATGCAGCAGAGAGTCTGGACAAACTTGGACAGTGAGTGCACCTGCTACCA
SEMG2	semenogelin II	AAGGCAGTATTTCGATCCAAACTGAAGAGCAAATACATGGCAAGTCTCAAAACCAGGTAA
ZNF695	zinc finger protein 695	CCCTTGGTGAGGATAGCTTCAATATGCAATTCCTATTTCACAGTCTTGCTATGTCTAAGC
FGG	fibrinogen gamma chain	CACTATGAAGATAATCCCATTCAACAGACTCACAATTGGAGAAGGACAGCAACACCACCT
CRISPLD1	cysteine-rich secretory protein LCCL domain containing 1	CCAGGAATTTTCCTGTGATTCTCCTTGTAAGAATTAAATGCTCTCTAATATGATATCCCT
HS6ST3	heparan sulfate 6-O-sulfotransferase 3	TCTGTACAATAATATGTATGAACTCAGTTACTAGGGGACTGTATTGTGACATTATCAACC
lnc-CBWD3-1	lnc-CBWD3-1:1	GGCAAATCAACTGTTAACTTTGCAGTTTTTATTTAATCTCCCAGGAATGTAACCCTTAAC
CECR7	cat eye syndrome chromosome region, candidate 7 (non-protein coding)	TGCACGATGCAGAAAATTAGACCTGCATGGCACGTTTAGCCCTAAATTTAGGAATCTACC
BAAT	bile acid CoA:amino acid N-acyltransferase	GCATTTGGCTGCATAGCTACAATGATGGCATTTTAACTTATTCATGATTAGAGATGATGA
TMEM108	transmembrane protein 108	CGACTATAGAGACACTGGGATGGTCCTTGTTAACCCCTTCTGTCAAGAAACACTGTTTGT
ANKRD62	ankyrin repeat domain 62	GTACTATCTGAAACAGACAAAACCAAATCACAGTTAGAGCATCAGAATCTTGAAAGGAAA
GHRH	growth hormone releasing hormone	GGGATGAAGATTCCTCCTGTGACCCGGGCTACCTGTAGCCAAAATGCAACTGGATCCAGT
PI15	peptidase inhibitor 15	CCTGTGTAAACCTCTGTCCTTATGAACCATAACAAATGTAGCTTTTTAAAGTCCATTGTA
HYDIN	HYDIN, axonemal central pair apparatus protein	CGGGTACAGCATAATCATCCCCTTCAAGAATGTCTTCTATCACATGGTGACCTTCTCCAT
MDGA2	MAM domain containing glycosylphosphatidylinositol anchor 2	GGACATTGAACAAACAAACTACCAAAGATTCCTCCACTGACTACTGACTCAAAAATAAAA
FUT9	fucosyltransferase 9 (alpha (1,3) fucosyltransferase)	AACATAACACTTTAAGGCAGCTAAGCAAATATTTTAATAAGCCATGAAAGGCAAGATGCC
ABCB5	ATP-binding cassette, sub-family B (MDR/TAP), member 5	CCGCAGTATTTTCATCCACTGGTAATCACTTTAGGTAATCAAAACTAGTTCAGACTTTTA

GAPDH serving as loading control. As shown in Figure 3, the levels of ABCC11 in MMBC were significantly increased compared with the UFBC group ($p<0.05$). However, compared with the UFBC group, the levels of ABCC5 were remarkably decreased in the MMBC group ($p<0.05$), which was consistent with the microarray screening.

Discussion

Breast cancer is a complex and heterogeneous disease in regard to its clinical, pathological and biological aspects [2,4,30]. MMBC is common nowadays with a documented incidence of 9-75%, partly owing to the advancement of medical imaging technology with higher sensitivity and resolution [31]. MMBC refers to two or more separate tumors in different sections. However, the practical definition and classification of MMBC is relatively ambiguous and sometimes controversial, which reflects our current limited knowledge of this disease [5]. Herein, we sought to analyze the differential expression in MMBC compared to UFBC patients and attempt to identify the potential outlier genes for precise molecular subtyping and better target therapy.

Based on our preliminary microarray results and followed by the RT-PCR validation, we identified 38 genes with significant change in expression. Pathway function and gene ontology analysis demonstrated that the outlier genes enriched in several biological concepts such as cytokine-receptor interaction, chemical synapses mediated transmission, neuronal system, etc. Remarkably, two gene families ABC and PRO encompassed more than one outlier gene, which may corroborate its significance in MMBC.

Although literature retrieval showed PRODH and PROL1 are not related to tumor, several studies have investigated the likely function of ABCC11 and ABCB5 in breast cancer [25,32]. The ABCC11 is a member of the superfamily of ATP-binding cassette (ABC) transporters [12,33], and participates in physiological processes involving bile acids, conjugated steroids and cyclic nucleotides, and also enhances the cellular exportation of cAMP and cGMP [23,34]. A study has reported a single nucleotide polymorphism (SNP), 538G>A (Gly180Arg), in ABCC11, which was previously characterized as tightly associated with the phenotype of human earwax, linked to high risk of breast cancer occurrence [33]. Although transcript level varied, Sosonkina et al. demonstrated remarkable decrease of ABCC11 protein in breast cancer in comparison with healthy controls, which strongly suggested a tumor suppressor function of ABCC11 in this setting [32]. Clinically, the ABCC11 is intimately linked to multi-drug resistance [12]. The association between ABCC11 expression and breast cancer risk is still under debate, and the heterogeneity of breast cancer may account for this discrepancy. Besides therapeutic resistance and prognostic value, our results suggested that ABCC11 could play a unique role in tumorigenesis and progression of MMBC.

The ABCB5 is a transmembrane protein, especially in stem cells, and transports a diverse variety of molecules including ions, sugars, peptides and more complex organic molecules [35]. Several studies proposed that ABCB5 promoted melanoma metastasis though enhancing NF-kB p65 protein stability [36]. For example, the c-MYC-ABCB5 axis plays a pivotal role in 5-fluorouracil resistance in human colon cancer [37]. In melanoma ABCB5 favors stem-like cells through modulation of a pro-inflammatory cytokine signaling circuit [38]. Targeting ABCB5 with microRNA-522 restored the drug sensitivity to doxorubicin in resistant HT29 colon cancer cell clones [39]. Until now, the possible role of ABCB5 in MMBC has not been addressed. The markedly under-presentation in MMBC indicates a potential tumor suppressor function of ABCB5 during oncogenesis regardless of drug resistance.

In summary, we have identified several outlier genes in our carefully characterized MMBC vs UFBC breast cancer patients. Our results demonstrated that the expression of ABCC11 and ABCB5 were significantly changed in MMBC in comparison to UFBC subtype. In addition to the well-known biological functions related to drug resistance and cell stemness, the divergent roles of ABCC11 and ABCB5 in tumorigenesis of MMBC were suggested. Further investigation of the unique relevance of ABC family genes with MMBC is warranted. In addition, our gene expression data, which were critically confirmed by RT-PCR, would serve the research community in this area in regard to distinct tumorigenesis and progression of MMBCs.

Conflict of interests

The authors declare no conflict of interests.

References

1. Harbeck N, Gnant M.Breast cancer. Lancet 2016;21: 51-65.
2. Cao SS, Lu CT. Recent perspectives of breast cancer prognosis and predictive factors. Oncol Lett 2016;12:3674-8.
3. Kelly D. A new direction for breast cancer therapy. Science 2016;354:1017-8.
4. Killock D. Breast cancer: Genetic signature might spare 100,000 women annually from chemotherapy. Nat Rev Clin Oncol 2016;13:589.
5. Cserni G, Bori R, Sejben I et al. Unifocal, multifocal and diffuse carcinomas: a reproducibility study of breast cancer distribution. Breast 2013;22:34-8.
6. Lang ZQ, Wu YQ, Li CY et al. Multifocal and multicentric breast carcinoma: a significantly more aggressive tumor than unifocal breast cancer. Anticancer Res 2017;37:4593-8.
7. Buggi F, Folli S, Curcio A et al. Multicentric/multifocal breast cancer with a single histotype: is the biological characterization of all individual foci justified? Ann Oncol 2012;23:2042-6.
8. Nijenhuis MV, Rutgers EJ. Conservative surgery for multifocal/multicentric breast cancer. Breast 2015;24:S96-9.
9. Wolters R, Wöckel A, Janni W et al. BRENDA Study Group. Comparing the outcome between multicentric and multifocal breast cancer: what is the impact on survival, and is there a role for guideline-adherent adjuvant therapy? A retrospective multicenter cohort study of 8,935 patients. Breast Cancer Res Treat 2013;142:579-90.
10. Weissenbacher T, Hirte E, Kuhn C et al. Multicentric and multifocal versus unifocal breast cancer: differences in the expression of E-cadherin suggest differences in tumor biology. BMC Cancer 2013;13:361.
11. Stavrovskaya AA, Stromskaya TP. Transport proteins of the ABC family and multidrug resistance of tumor cells. Biochemistry (Mosc) 2008;73:592-604.
12. van Veen HW, Konings WN. The ABC family of multidrug transporters in microorganisms. Biochim Biophys Acta 1998;1365:31-6.
13. Chandra BR, Yogavel M, Sharma A. Structural analysis of ABC-family periplasmic zinc binding protein provides new insights into mechanism of ligand uptake and release. J Mol Biol 2007;367:970-82.
14. Frank NY, Pendse SS, Lapchak PH et al. Regulation of progenitor cell fusion by ABCB5 P-glycoprotein, a novel human ATP-binding cassette transporter. J Biol Chem 2003;278:47156-65.
15. Schatton T, Murphy GF, Frank NY et al. Identification of cells initiating human melanomas. Nature 2008;451:345-9.
16. Luo Y, Ellis LZ, Dallaglio K et al. Side population cells from human melanoma tumors reveal diverse mechanisms for chemoresistance. J Invest Dermatol 2012;132:2440-50.
17. Frank NY, Frank MH. ABCB5 gene amplification in human leukemia cells. Leuk Res 2009;33:1303-5.
18. Wang S, Tang L, Lin T et al. ABCB5 promotes melanoma metastasis through enhancing NF-Kβ p65 protein stability. Biochem Biophys Res Commun 2017;492:18-26.
19. Cheung ST, Granulin-epithelin precursor and ATP-dependent binding cassette (ABC)B5 regulate liver cancer cell chemoresistance. Gastroenterology 2011;140:344-55.
20. Yao JT, Yao X, Tian T et al. ABCB5-ZEB1 axis promotes invasion and metastasis in breast cancer cells. Oncol Res 2017;25:305-16.
21. Bera TK, Lee S, Salvatore G, Lee B, Pastan I. MRP8, a new member of ABC transporter superfamily, identified by EST database mining and gene prediction program, is highly expressed in breast cancer. Mol Med 2001;7:509-16.
22. Sato T, Amano T, Ono H et al. Allele frequencies of the ABCC11 gene for earwax phenotypes among ancient populations of Hokkaido, Japan. J Hum Genet 2009;54:409-13.
23. Chen ZS, Guo Y, Belinsky MG, Kotova E, Kruh GD. Transport of bile acids, sulfated steroids, estradiol 17-beta-D-glucuronide, and leukotriene C4 by human multidrug resistance protein 8 (ABCC11). Mol Pharmacol 2005;67:545-57.
24. Prokop-Prigge KA, Mansfield CJ, Parker MR et al. Ethnic/racial and genetic influences on cerumen odorant profiles. J Chem Ecol 2015;41:67-74.
25. Ota I, Sakurai A, Toyoda Y et al. Association between breast cancer risk and the wild-type allele of human ABC transporter ABCC11. Anticancer Res 2010;30:5189-94.
26. Oba T, Izumi H, Ito KI. ABCB1 and ABCC11 confer resistance to eribulin in breast cancer cell lines. Oncotarget 2016;7:70011-27.
27. Uemura T, Oguri T, Ozasa H et al. ABCC11/MRP8 confers pemetrexed resistance in lung cancer. Cancer Sci 2010;101:2404-10.
28. Oguri T, Bessho Y, Achiwa H et al. MRP8/ABCC11 directly confers resistance to 5-fluorouracil. Mol Cancer Ther 2007;6:122-7.
29. Toyoda Y, Ishikawa T. Pharmacogenomics of human ABC transporter ABCC11 (MRP8): potential risk of breast cancer and chemotherapy failure. Anticancer Agents Med Chem 2010;10:617-24.
30. Loibl S, Gianni L. HER2-positive breast cancer. Lancet 2016;pii:S0140-6736(16)32417-5.
31. Desmedt C, Fumagalli D, Pietri E et al. Uncovering the genomic heterogeneity of multifocal breast cancer. J Pathol 2015;236:457-66.
32. Sosonkina N, Nakashima M, Ohta T, Niikawa N, Starenki D. Down-regulation of ABCC11 protein (MRP8) in human breast cancer. Exper Oncol 2011;33:42-6.
33. Huang Y, Anderle P, Bussey KJ et al. Membrane transporters and channels: role of the transportome in cancer chemosensitivity and chemoresistance. Cancer Res 2004;64:4294-301.
34. Guo Y, Kotova E, Chen ZS et al. MRP8, ATP-binding

cassette C11 (ABCC11), is a cyclic nucleotide efflux pump and a resistance factor for fluoropyrimidines 2',3'-dideoxycytidine and 9'-(2'-phosphonylmethoxyethyl)adenine. J Biol Chem 2003;278:29509-14.

35. Moitra K, Scally M, McGee K, Lancaster G, Gold B, Dean M. Molecular evolutionary analysis of ABCB5: the ancestral gene is a full transporter with potentially deleterious single nucleotide polymorphisms. PLoS One 2011;6:e16318.
36. Wilson BJ, Schatton T, Zhan Q et al. ABCB5 identifies a therapy-refractory tumor cell population in colorectal cancer patients. Cancer Res 2011;71:5307-16.
37. Kugimiya N, Nishimoto A, Hosoyama T et al. The c-MYC-ABCB5 axis plays a pivotal role in 5-fluorouracil resistance in human colon cancer cells. J Cell Mol Med 2015;19:1569-81.
38. Wilson BJ, Saab KR, Ma J et al. ABCB5 maintains melanoma-initiating cells through a proinflammatory cytokine signaling circuit. Cancer Res 2014;74:4196-4207.
39. Yang G, Jiang O, Ling D et al. MicroRNA-522 reverses drug resistance of doxorubicin-induced HT29 colon cancer cell by targeting ABCB5. Mol Med Rep 2015;12:3930-6.