ABCB1 regulates myeloid-derived suppressor cells-related immune factors in breast cancer

Han-Kun Chen
Chi Mei Medical Center

Yi-Ling Chen
Chia Nan University of Pharmacy and Science

Chih-Yang Wang
Taipei Medical University

Wei-Pang Chung
National Cheng Kung University Hospital

Jung-Hua Fang
National Cheng Kung University

Ming-Derg Lai
National Cheng Kung University

Hui-Ping Hsu (✉ hphsu@mail.ncku.edu.tw)
National Cheng Kung University Hospital  https://orcid.org/0000-0002-3285-5543

Research Article

Keywords: Breast cancer, ABC transporters, ABCB1, myeloid-derived suppressor cells, patient-derived xenograft

Posted Date: September 7th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-2008518/v1

License: ☕️ This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

**Purpose** Resistance to standard chemotherapy is a critical problem for breast cancer patients. The ATP-binding cassette (ABC) superfamily transporters actively pump out drugs and play an important role in chemoresistance. ABCB1 (ABC subfamily B, member 1, also named as multidrug resistance protein 1, MDR1) and suppressive myeloid-derived suppressor cells (MDSCs) potentially involve in chemoresistance of breast cancer. The relationship between ABCB1 and MDSC is less studied.

**Methods** Microarray or RNA sequencing data was obtained from The Cancer Genome Atlas Breast Invasive Carcinoma in Genomic Data Commons Data Portal (GDC TCGA-BRCA) and GEO database. Expression of ABCB1 and MDSC-related genes was compared. Patient-derived xenograft (PDX) from HER2-enriched breast cancer was established to investigate the association of ABCB1 and MDSC-related genes in breast cancer.

**Results** Expression of ABCB1 was increased in doxorubicin-selected MCF-7/ADR cells. High expression of ABCB1 mRNA was correlated with lymph node metastasis and worse overall survival of breast cancer patients. ABCB1 was positively correlated with IL6, CSF1, CSF3, or PTGS2 and negatively correlated with VEGF. PDX model from HER2-enriched stage IIA breast cancer was established. Treatment with doxorubicin or paclitaxel suppressed growth of P2 tumors and expression of ABCB1. Expression of IL6, CSF1, CSF3, PTGS2 was suppressed by paclitaxel, but not by doxorubicin. Intrasplenic MDSCs, including CD11b^+Ly6G^+ and CD11b^+Ly6C^+ cells, were higher than intratumor MDSCs in PDX-carrying nude mice. Clinically, the patient developed cancer recurrence after adjuvant chemotherapy with doxorubicin-based regimen and was well-controlled after paclitaxel-trastuzumab combined therapy.

**Conclusions** ABCB1 is a poor predictor of breast cancer patients. Regulation of MDSC-related immune factors by ABCB1 and immune response to chemotherapeutic agents also contributes to cancer recurrence and treatment effect. PDX model is suitable to test expression of targeting genes and potential interaction with immune cells.

Background

Chemoresistance is one of the major challenges for breast cancer treatment [1]. Mechanism of chemoresistance is complex. Crosstalk between receptor tyrosine kinases and downstream pathways, deregulation of cell cycle and apoptosis regulators, modulation of tumor-infiltrating immune cells are major mechanisms of chemoresistance [2]. Besides, the ATP-binding cassette (ABC) superfamily is one of the largest family proteins of membranous transporters in human. ABC transporters actively pump out substrates outside the cell membrane by the energy from ATP hydrolysis. ABC transporters also function in cell apoptosis, energy metabolism, and material transport. In cancer cells, ABC transporters participate detoxification and drug resistance [1]. Multidrug resistance protein 1 (MDR1; gene: ABC subfamily B, member 1, ABCB1), multidrug resistance associated protein (MRP; gene: ABC subfamily C, member 1, ABCC1), breast cancer resistance protein (BCRP; gene: ABC subfamily G, membrane 2, ABCG2) are
studied in chemoresistance breast cancer. Increased expression of ABCB1 protein is detected in recurrent breast cancer and the patients with ABCB1-positive cancer fails to response to chemotherapy [3]. ABCB1 is also one of metastatic markers for triple negative breast cancer [4]. ABCB1 is overexpressed in doxorubicin-resistant MCF7 breast cancer cells [5]. Theoretically, inhibition of ABC transporters during cancer therapy improves sensitivity of cancer cells to cytotoxic agents. Several clinical trials combining with ABCB1-reversing agents and chemotherapeutic drugs are applied in cancer patients currently. However, the results of these agents are not satisfactory. No effective ABCB1-reversing drug without significant toxicity has been approved [6, 7]. In addition, treatment with doxorubicin induced ABCB1 expression and enhances drug efflux potential through FOXO3a signaling in K562 leukemia cells [8]. Downstream of ABCB1 is finely regulated. Further study about ABCB1 in cancer should be investigated for potential effective therapeutic agents.

The ability of escaping from immune surveillance is important for cancer cells. Cancer cells secreted specific cytokines to recruit and activate suppressive immune cells in tumor microenvironment, including regulatory T cells, myeloid-derived suppressor cells (MDSCs) and M2 tumor-associated macrophages [9]. In literature, 16 genes of MDSC-related immune factors are expressed by human solid tumor cell lines, including transforming growth factor beta (TGF-β, gene TGFB1), interleulin-1 beta (IL-1β, gene IL1B), IL-4, IL-6, IL-10, granulocyte-macrophage colony-stimulating factor (GM-CSF, gene CSF2), macrophage colony-stimulating factor (M-CSF, gene CSF1), indoleamine 2,3-dioxygenase (IDO), fms-related tyrosine kinase 3 ligand (FLT3L), c-kit ligand (KITLG), inducible NO synthase (iNOS, gene NOS2), arginase-1 (ARG1), TNF-alpha, cyclooxygenase 2 (COX2, gene PTGS2), vascular endothelial growth factor (VEGF), and G-CSF [10, 11]. Cancer cells may regulate suppressive MDSCs through these 16 cytokines.

Patient-derived xenograft (PDX) is created by engrafting tumor tissues in immunodeficient mice. The tissue structure, cell morphology, genetic features, and molecular biology are similar to surgical specimens. Gradual infiltration of host immune cells is detected after 3–5 passages [12]. Currently, PDX model is used to identify therapeutic agents, study carcinogenesis, investigate cancer heterogenicity, or develop precision medicine [13]. Engraftment rate of PDX depended on tumor burden and cancer characteristics, ranges from 20–90%. The advanced cancer with malignant potential has the highest establishment rate; for example, colorectal cancer, pancreatic cancer, head and neck cancer and ovarian cancer. Breast cancer has the relatively lowest success rate of PDX engraftment (from 21–37%) [14]. The triple negative breast cancer or HER2+ subtype has higher successful rate than estrogen receptor positive cancers [15]. PDX tumors display comparable therapeutic responses to corresponding clinical observation [16]. PDX models exhibit superior predictive values to cell line xenografts or genetically engineered mouse models [17]. In the present study, we hypothesized that ABCB1 predicts poor prognosis of breast cancer patients and regulates secretion of MDSC-related cytokines. To test this hypothesis, we studied the RNA sequencing data from The Cancer Genome Atlas Breast Invasive Carcinoma in Genomic Data Commons Data Portal (GDC TCGA-BRCA) and examined gene expression of ABC transporters and MDSC-related cytokines. In addition, PDX model was used to test ABCB1, MDSC-related genes, and immune cells in breast cancer.
Methods

Patients and PDX

One PDX was obtained from a 67-year-old female patient with invasive ductal carcinoma after curative resection in 2018. The characteristics of the patient was listed in Table 1. Written informed consent was obtained and the present study was approved by the Institutional Review board of the National Cheng University Hospital (NCKUH IRB no. A-ER-106-157). Clinical outcome was also recorded.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Patient characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age at surgery</strong></td>
<td>67 years old</td>
</tr>
<tr>
<td>Gender</td>
<td>Female</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>Invasive ductal carcinoma</td>
</tr>
<tr>
<td>Operation methods</td>
<td>Total mastectomy and sentinel lymph node biopsy</td>
</tr>
<tr>
<td>Tumor size</td>
<td>2.6 cm</td>
</tr>
<tr>
<td>Histology grade</td>
<td>Grade III</td>
</tr>
<tr>
<td>Extensive intraductal component</td>
<td>Present</td>
</tr>
<tr>
<td>Lymphatic tumor emboli</td>
<td>Absent</td>
</tr>
<tr>
<td>Tumor stage</td>
<td>pT2</td>
</tr>
<tr>
<td>Nodal stage</td>
<td>pN0</td>
</tr>
<tr>
<td>AJCC TNM stage</td>
<td>pT2N0, stage IIA</td>
</tr>
<tr>
<td>Subtype</td>
<td>HER2-positive</td>
</tr>
<tr>
<td>Estrogen receptor</td>
<td>Negative (&lt; 1%)</td>
</tr>
<tr>
<td>Progesterone receptor</td>
<td>Positive (5%)</td>
</tr>
<tr>
<td>Ki-67</td>
<td>20%</td>
</tr>
<tr>
<td>Recurrence at postoperative 23 months</td>
<td>Left upper lung nodule, metastatic carcinoma of breast origin, negative ER, weak positive PR (~ 2%), HER2 3 + by IHC staining</td>
</tr>
<tr>
<td></td>
<td>Right lower lung nodule in chest computed tomography</td>
</tr>
<tr>
<td>Survival</td>
<td>Partial response 43 months after first operation</td>
</tr>
</tbody>
</table>

Abbreviations: AJCC TNM stage, American Joint Committee on Cancer tumor-node-metastases staging system; ER, estrogen receptor; IHC, immunohistochemistry; PR, progesterone receptor.
The experimental protocol and animal housing was followed the institutional guidelines of National Laboratory Animal Center, Tainan, Taiwan, and Laboratory Animal Center, College of Medicine, National Cheng Kung University. Subcutaneous transplant of cancer specimen over flank in NOD.Cg-Prkdc<sup>scid</sup>Il2rg<sup>tm1Wjl</sup>/YckNarl mice was performed. Tumor growth was measured of two perpendicular diameters once a week. Tumor volumes were calculated as length · width<sup>2</sup>/2. Relative tumor volume of each tumor was calculated as the ratio of current volume in relation to the initial volume. After tumor volumes close to 1000 mm<sup>3</sup>, the PDX cancer tissues were removed and the mice were sacrificed. The cancer tissues were minced into small pieces and implanted into subcutaneous fat of non-obese diabetic/severe combined immunodeficiency disorder (NOD/SCID) mice to establish next generation of PDX mice. The PDX mice was maintained until passage 13.

Seventeen P2 PDX mice was treated intraperitoneally with normal saline (n = 3), doxorubicin (18 mg/kg, titrate to 12 mg/kg, n = 5), paclitaxel (10 mg/kg, n = 3), trastuzumab (30 mg/kg, n = 3), or AZD8055 (mTOR inhibitor, 20 mg/kg, n = 3), twice a week for 21 days. Tumor size was recorded and the mice was sacrificed at Day 28 after first dose of treatment. The cancer specimens were obtained for other experiments.

Two P7 and two P9 PDX was established in athymic nude mice. The cancer tissues and spleen were obtained for studying immune cells by flow cytometry.

**Quantitative real-time polymerase chain reaction (qPCR)**

The fresh cancer tissues from P2 PDX mice after drug treatment were obtained. Total RNA was extracted using RNeasy<sup>®</sup> Mini kit according to the manufacturer’s instruction. Single-stranded complementary DNA (cDNA) was synthesized from 2 µg of total RNA using M-MuLV reverse transcriptase (Roche) and oligo-dT random primers. The cDNA was amplified with specific genes and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as the endogenous control. Primers were designed to be specific for the ABCB1 (NM_001348945.2), IL6 (interleukin-6, NM_00600.5), CSF1 (colony stimulating factor 1, NM_000757.6), CSF3 (colony stimulating factor 3, NM_000759.4), PTGS2 (prostaglandin-endoperoxide synthase 2, NM_000963.4), VEGFA (vascular endothelial growth factor A, NM_001025366.3), and GAPDH (NM_002046.3) genes and listed in supplementary Table 1. SYBR green fluorescence was added. PCR parameters were as follows: 95°C for 10 min; 95°C for 15s, 60°C for 1 min and total 40 cycles. Fluorescence increase of fluorescein was automatically measured during PCR. All samples were amplified in duplicate and the C<sub>T</sub> value was recorded. The 2-delta-delta C<sub>T</sub> value was calculated following GAPDH normalization.

**Bioinformatics**

RNA sequencing of 1217 breast cancer samples was performed by Illumina platform and raw data was downloaded from The Cancer Genome Atlas Breast Invasive Carcinoma in Genomic Data Commons Data Portal (GDC TCGA-BRCA) [18]. The results were re-analyzed using the latest Human Genome Assembly hg38 and re-organized by University of California Santa Cruz Xena team [19]. The upper quartile of the
fragments per kilobase of transcript per million mapped reads (HTSeq-FPKM-UQ) was selected. Expression of \textit{ABCB1}, ABC transporters, and MDSC-associated genes was extracted for further analysis. Survival status was also obtained.

The cBioPortal platform collects multidimensional cancer genomics and datasets [20, 21]. A total of 15 breast cancer datasets with 10,928 samples were selected from cBioPortal, including those of primary and metastatic cancer. Breast fibroepithelial tumors, xenografts of breast cancer, metaplastic breast cancer, juvenile papillomatosis, or adenoid cystic breast cancer were excluded due to different tumor pathophysiology and tumor growth conditions. Amplification, mutation, or deletion of \textit{ABCB1} genes were explored.

Gene expression data of breast cancer were collected from the GEO database (http://www.ncbi.nlm.nih.gov/geo/). Doxorubicin-selected MCF-7/ADR cells was compared with parental MCF-7 cells in GSE24460 dataset to study ABC transporters in chemoresistant cells [22]. Gene expression was examined by Affymetrix Human Genome U133A 2.0 Array. Raw data were obtained and normalized according to the robust multichip average (RMA). The RMA signal was computed for gene-level probe set summaries using the Affymetrix Expression Console (version 1.3) (Affymetrix, Santa Clara, CA, USA) and R (version 3.2.0) (www.r-project.org). Heatmap of ABC transporters was drawn. ABCB1-overexpressing human mammary epithelial cells was compared with parental cells [23]. High-throughput RNA sequencing was performed (Illumina, San Diego, CA, USA) and 162 genes were differentially expressed. We uploaded the raw data into Gene Set Enrichment Analysis (GSEA) platform and analyzed by BIACARTA pathways [24].

\section*{Flow cytometry}

Fresh cancer tissues and spleen from nude mice was finely minced into small pieces, then grinded, filtered, and centrifuged. Detached cells were washed by PBS and incubated with commercially available anti-CD4, anti-CD25, anti-CD8, anti-CD11b, anti-Ly6G, or anti-Ly6C antibodies (BD Biosciences) under 4°C overnight. Then 1 ml of staining buffer was added and cells are analyzed by flow cytometry (BD Biosciences). Data analysis was performed using WinMDI 2.9.

\section*{Statistical analysis}

All statistical analyses were carried out using STATA version 16.0 (StataCorp LLC, Texas, USA). Continuous variables with normal distribution were compared between two groups using the Student’s t-test. Mean ± standard deviation (SD) was expressed. Nonparametric Wilcoxon rank-sum or Kruskal-Wallis test for two or more groups of continuous variables. Correlation between two continuous variables was calculated by Spearman’s method and expressed as rho (\(\rho\)) with 95% confidence interval (95% CI). Survival curves were estimated using the Kaplan-Meier method, and compared between groups using the log-rank test. Median split was used for turning a continuous variable into binary variable to visualize the difference between groups in Kaplan-Meier method. A \(P\) value < 0.05 was considered statistically significant.
Results

**ABC transporters in breast cancer**

There are total 48 ABC transporters in 7 suprafamily [1]. RNA sequencing data of 48 ABC transporters was obtained from TCGA datasets and compared between survivors (censored) and non-survivors (expired). Lower expression of \( \text{ABCA3}, \text{ABCA7}, \text{ABCB2}, \text{ABCB8}, \text{ABCF3}, \) and \( \text{ABCG1} \) was detected in non-survivors. In contrary, expression of \( \text{ABCA1}, \text{ABCA5}, \text{ABCA6}, \text{ABCA8}, \text{ABCA9}, \text{ABCA10}, \text{ABCB1}, \text{ABCB5}, \text{ABCB7}, \text{ABCC2}, \text{ABCC9}, \text{ABCD2}, \text{ABCE1}, \text{ABCG2}, \) and \( \text{ABCG4} \) was higher in non-survivors than in survivors (Supplementary Fig. 1). In addition, we also got consist data and found that compare to the MCF-7 parental cells, ABCB1 ranked top 1 highest expression genes of ABC transporters in breast doxorubicin-selected MCF-7/ADR cells, from GSE24460 dataset (Supplementary Fig. 2).

In these ABC transporters, ABCB1 is the most famous one related to drug resistance. Genetic mutation rate of \( \text{ABCB1} \) was 1.7% in cBioPortal database. Amplification of \( \text{ABCB1} \) gene is the most common form of gene alteration in breast cancer (Supplementary Fig. 3).

RNA sequencing data from 1217 breast cancer samples and corresponding patients' outcomes were obtained from TCGA. The patients with lymph node metastasis had a higher level of \( \text{ABCB1} \) expression (mean ± SD = 13.93 ± 0.06) than those without lymph node metastasis (mean ± SD = 13.67 ± 0.07) (Fig. 1A). The patients with higher \( \text{ABCB1} \) expression had a trend of worse overall survival than those with lower \( \text{ABCB1} \) expression (Fig. 1B).

**Expression of MDSC-related genes in ABCB1\textsuperscript{high} breast cancer**

RNA sequencing data of \( \text{ABCB1} \) and MDSC-related genes was obtained from TCGA dataset. Spearman's rank-order correlation was calculated and correlation coefficient (\( \rho \)) was expressed. Positive correlation of \( \text{ABCB1} \) with \( \text{IL6}, \text{CSF1}, \text{CSF3}, \) or \( \text{PTGS2} \) was detected with Spearman's \( \rho \) larger than 0.3 (Fig. 2D, F, H, O). Negative correlation between \( \text{ABCB1} \) and \( \text{VEGF} \) was found with Spearman's \( \rho \) less than −0.3 (Fig. 2P).

Since we found ABCB1 had high expression in doxorubicin-selected breast MCF-7/ADR cells. We further used BI OCARTA pathway analysis for the upregulated genes of \( \text{ABCB1} \)-overexpressing human mammary epithelial cell in GSE173411 dataset. The data indicated that Cytokines and Inflammatory Response related genes, including \( \text{IL6}, \text{CSF1}, \) and \( \text{CSF3}, \) were significantly associated with \( \text{ABCB1} \)-overexpressing cells (Supplementary Fig. 4).

**Clinical courses of patient and establishment of PDX**

We used PDX model to verify the significance of \( \text{ABCB1} \) and MDSCs-related genes in breast cancer. The 67-year-old female patient with invasive ductal carcinoma undergoing total mastectomy and sentinel lymph node biopsy in 2018. Postoperative pathological report showed HER2-enriched stage IIA cancer. Adjuvant therapy with cyclophosphamide-Epirubicin-5-flourouracil (500-75-500 mg/m\(^2\)) every 3 weeks for 6 courses then adjuvant endocrine therapy with letrozole. Left upper lung nodule was detected by chest
computer tomography 23 months after mastectomy. Right lower lung nodule was found 28 months after mastectomy. Video-assisted thoracoscopic surgery confirmed HER-2 enriched metastatic carcinoma of breast origin. Salvage therapy with docetaxel-pertuzumab-trastuzumab (60 mg/m²-840 mg-6 mg/kg) every 3 weeks for 6 courses and pertuzumab-trastuzumab for total one year. The patient still kept trastuzumab therapy currently 43 months after mastectomy with partial response (Table 1).

Passage 1 (P1) xenograft was grown in NOD.Cg-Prkdc<sup>scid</sup> Il2rg<sup>tm1Wjl</sup>/YckNarl mice at Day 50 after transplantation (Supplementary Table 2). The mice were maintained at National Laboratory Animal Center, Tainan, Taiwan. Twenty P2 PDX mice were transferred to Laboratory Animal Center, College of Medicine, National Cheng Kung University. Three P2 mice were maintained until Day 49 after implantation for further passage. Then, the mice were sacrificed and cancer tissues were re-implanted into subcutaneous fat of NOD/SCID mice to establish next passage of PDX. The growth rate of P2 ~ P6 was better than rate of P7 ~ P12 PDX (Fig. 3).

Phosphoinositide 3-kinases/protein kinase B/mammalian target of rapamycin (PI3K/AKT/mTOR) signaling is downstream of HER2 signaling [25]. We treated standard chemotherapy drugs (paclitaxel and doxorubicin) and anti-HER2 agents (trastuzumab and AZD8055) in other seventeen P2 mice (Fig. 4). AZD-8055 is a selective ATP-competitive mTOR kinase inhibitor. There was no effect on tumor growth by treatment with AZD8055 (blue line in Fig. 4) and only minimal tumor suppressive effect by treatment with trastuzumab (green line in Fig. 4). Treatment with doxorubicin suppressed tumor growth after post-transplantation day 43 (red line in Fig. 4). However, significant loss of body weight (more than 10%) was recorded in the doxorubicin group and lifespan of these mice was shorter. Treatment with paclitaxel successfully suppressed growth of P2 xenograft at post-implantation day 43 and after day 48 (orange line in Fig. 4). The mice were sacrificed and the xenografts were removed at post-implantation day 62 (12 days after first dose of treatment). The cancer tissues were collected for qPCR study.

In the P2 xenografts of present study, expression of ABCB1 and immune-related genes were detected by qPCR and compared with GAPDH. ΔC<sub>T</sub> was recorded and subtracted by the result of normal saline group. Expression of ABCB1 was decreased in the xenograft after treatment with paclitaxel, doxorubicin or AZD8055. Expression of ABCB1 in xenograft of trastuzumab group was similar to normal saline group (Fig. 5A). Expression of IL6, CSF1, CSF3, PTGS2 was increased in the xenograft after treatment with doxorubicin, but decreased in paclitaxel, trastuzumab group. Expression of VEGF was suppressed in all groups. Treatment with AZD8055 inhibited expression of IL6, CSF1, CSF3, VEGF, but not PTGS2.

We also established PDX model in nude mice to examine innate immunity in cancer-bearing mice. Intrasplicenic and intratumor immune cells were analyzed by flow cytometry. Level of CD4<sup>+</sup>CD25<sup>+</sup> and CD8<sup>+</sup> cells was similar in spleen and xenograft (Fig. 6). Mouse MDSCs include CD11b<sup>+</sup>Ly6G<sup>+</sup> and CD11b<sup>+</sup>Ly6C<sup>+</sup> cells. Intrasplicenic CD11b<sup>+</sup>Ly6G<sup>+</sup> and CD11b<sup>+</sup>Ly6C<sup>+</sup> cells were higher than intratumor MDSCs (Fig. 6).

**Discussion**
Early recurrence is one of the critical issues in treatment of breast cancer patients. PDX model is established for testing drugs and studying mechanism of carcinogenesis or cancer heterogeneity. In our data, expression of ABCB1 was correlated with lymph node metastasis and poor survival of 1217 breast cancer patients in GDC TCGA-BRCA database. Increased expression of ABCB1 gene was detected in doxorubicin-resistant MCF-7/ADR cells. High expression of ABCB1 was correlated with increased level of IL6, CSF1, CSF3, PTGS2 and decreased level of VEGF. Cytokines and Inflammatory Response related genes, including IL6, CSF1, and CSF3, were significantly associated with ABCB1-overexpressing cells. We also used PDX model to prove the significance of ABCB1 and MDSC-related cytokines. Treatment with doxorubicin and paclitaxel suppressed growth of PDX tumor; however, anti-HER2 monoclonal antibodies (trastuzumab) and mTOR inhibitor (AZD8055) failed to suppress tumor growth. Expression of ABCB1 and VEGF was repressed after treatment of doxorubicin, paclitaxel, trastuzumab and AZD8055. Expression of IL6, CSF1, CSF3, PTGS2 was increased after treatment with doxorubicin, but reduced in paclitaxel and trastuzumab group. Expansion of splenic MDSCs (CD11b+Ly6G+ and CD11b+Ly6C+ cells) was detected in PDX-implanted nude mice. The donor patient of PDX developed cancer recurrence and lung metastasis 23 months after curative resection and adjuvant chemotherapy with anthracycline-based regimen. The metastatic cancer was well-controlled after taxanes-based regimen and anti-HER2 therapy, which was compatible with suppression of ABCB1 and MDSC-related genes in PDX model.

PDX model is established to study cancer heterogeneity and preclinical drug-testing [26]. The successful rate of PDX engraftment in breast cancer ranges from 21–37%, which is relatively lower than other kinds of cancer. The take rate is highest in TNBC (51.3%), followed by HER2+ cancer (26.5%) [14]. Our patient has pT2N0, stage IIA, HER2+ invasive ductal carcinoma. Cancer recurrence with lung metastasis developed after curative surgery and adjuvant anthracycline-based chemotherapy. Drug resistance to anthracycline involves complicated mechanism and activation of MDSCs to inhibit cytotoxic T cells is reported [27]. Increased circulating MDSCs is correlated with cancer stage and treatment with doxorubicin-cyclophosphamide. By contrast, level of MDSCs is decreased to pretreatment status after paclitaxel [28]. Our finding was compatible with the change of MDSC level in previous report. MDSC-related genes are increased in PDX tumor after treatment with Doxorubicin and reduced in paclitaxel group. Implantation of PDX in nude mice was also success to study immune cells in circulation or tumor microenvironment; the former was considered as intrasplenic cells and the latter as intratumor cells. Increased level of intrasplenic MDSCs (CD11b+Ly6G+ and CD11b+Ly6C+ cells) was detected. These results confirmed the application of PDX model to study MDSCs and cytokines.

Chemoresistance is one of the major challenges for treatment of breast cancer patients. Mechanism of chemoresistance is complex. Membranous transporters, ABC superfamily, actively pump out substrates outside the cell membrane by the energy from ATP hydrolysis. ABC transporters also function in cell apoptosis, energy metabolism, and material transport. In cancer cells, ABC transporters participate detoxification and drug resistance [1]. MDR1 (gene: ABCB1, also named as P-glycoprotein, P-gp) is important in chemoresistance of breast cancer. Increased expression of ABCB1 protein is detected in recurrent breast cancer and the patients with ABCB1-positive cancer fails to response to chemotherapy
ABCB1 genes are overexpressed in doxorubicin-resistant MCF7 breast cancer cells [4]. Other studies report that loss of ABCB1 protein in immunohistochemistry staining is correlated with TNBC, lymph node metastasis, larger tumor size, and poor prognosis of patients [29]. The reason of these contradictory results is possible due to different experimental methods. In our data, we collected RNA sequencing data from 1217 breast cancer samples and expression of ABCB1 gene was higher in non-survivors and the patients with lymph node metastasis. The correlation between ABCB1 expression and overall survival of patients was not strong because of overlapping survival curves in Kaplan-Meier survival analysis. Treatment of doxorubicin, paclitaxel, or AZD8055 in PDX mice suppress ABCB1 expression in tumor. From our results, ABCB1 performed as a tumor promoter in breast cancer.

There is no direct evidence linking expression of ABCB1 and secretion of cytokines except several related reports. Increased expression of PTGS2 genes was detected by ABCB1-expressing normal human mammary epithelial cells and PTGS2 is encoding the cyclooxygenase-2 protein [30]. Treatment with paclitaxel suppress secretion of VEGF from cancer cell lines [31]. In our data, expression of IL6, CSF1, CSF3, PTGS2 was increased in the PDX tumor after treatment with doxorubicin, but decreased in paclitaxel, trastuzumab group. Expression of VEGF was suppressed in all groups. Increased number of intrasplenic MDSCs also detected in PDX-carried nude mice.

The pitfall of present study was the limited sample size. The successful rate of PDX engraftment was quite low and we only had one PDX model. To solve this problem, we used RNA sequencing data from 1217 breast cancer samples to study correlation between ABCB1 and MDSC-related genes. High expression of ABCB1 was correlated with increased level of IL6, CSF1, CSF3, PTGS2 and decreased level of VEGF. Big data of RNA sequencing could provide evidence for a correlation study and PDX is an operable model for a causal relation. The two experimental methods are complementary.

Conclusion

ABCB1 mRNA expression was correlated with lymph node metastasis and poor survival of breast cancer patients. High expression of ABCB1 was correlated with increased level of IL6, CSF1, CSF3, PTGS2 and decreased level of VEGF. Treatment with doxorubicin and paclitaxel suppressed growth of PDX tumor and expression of ABCB1 and VEGF was repressed. Expression of IL6, CSF1, CSF3, PTGS2 was increased after treatment with doxorubicin, but reduced in paclitaxel and trastuzumab group. The result of PDX model was compatible with clinical course of the donor patient. Combination of big data from RNA sequencing and experimental result from the PDX model is the foundation of precision medicine.

Declarations

Authors’ contributions

H.K.C., C.Y.W., and H.P.H. designed the studies; W.P.C. and H.P.H. collected the clinical data; Y.L.C. and J.H.F. assisted the animal experiments; H.K.C. and H.P.H. wrote the initial manuscript draft; M.D.L. edited
the manuscript; and all authors read and approved the final manuscript.

Acknowledgments

The authors are thankful to the patient who participated in the study. We thank the Laboratory Animal Center, College of Medicine, National Cheng Kung University and Taiwan Animal Consortium for the technical support. We were blessed with support from the late superintendent, Professor Pin-wen Lin. We also wish to thank Dr Po-Hsien Huang and Miss Ya-Li Hsiao for their support.

Funding

The study was supported by the Ministry of Science and Technology (MOST) of Taiwan (grant No. 109-2314-B-006-018-MY3 to H.P.H.), the National Cheng Kung University Hospital (grant No. NCKUH-10902031 & NCKUH-11002013 & NCKUH-11102007 to H.P.H.), and the Chi Mei Medical Center (grant No. CMNKCKU11004).

Conflict of interest

None.

Ethics approval and consent to participate

This study was approved by the Institutional Review Board of National Cheng Kung University Hospital (A-ER-106-157). Written informed consent was obtained from all participants.

References


Figures
Figure 1

Expression of $ABCB1$ mRNA in breast cancer samples. (A) Correlation of $ABCB1$ expression with lymph node metastasis of breast cancer patients. (C) Overall survival of patients with high and low expression of $ABCB1$ mRNA is shown. The patients with lower expression of $ABCB1$ tended to have better prognosis. FPKM, fragments per kilobase of transcript per million mapped reads.
Figure 2

Correlation between $ABCB1$ gene and $MDSC$-related cytokines in 1217 breast cancer samples from TCGA. (A) $TGF\beta1$, (B) $IL1B$, (C) $IL4$, (D) $IL6$, (E) $IL10$, (F) $CSF1$ (M-CSF), (G) $CSF2$ (GM-CSF), (H) $CSF3$, (I) $FLT3$, (J) $KITLG$, (K) $NOS2$, (L) $ARG1$, (M) $IDO1$, (N) $TNF$, (O) $PTGS2$, and (P) $VEGF$. 
Figure 3

Relative tumor volume of serial passages of PDX, compared between P2 ~ P6 and P7 ~ P12. The standard deviation is presented as upward error bars. Nonparametric Wilcoxon rank-sum test is used to analyze the difference between two groups. Statistically significant difference is detected at post-implantation day 17, 28, 33, and 36. *$P < 0.05$. 

$\text{P2 } \sim \text{P6 (n = 17)}$

$\text{P7 } \sim \text{P12 (n = 16)}$
Figure 4

Relative tumor volume of P2 PDX mice after treatment with normal saline (black), doxorubicin (red), paclitaxel (orange), trastuzumab (green), or AZD8055 (blue).
Figure 5

Expression of ABCB1 in cancer samples from PDX mice after drug treatment.

Figure 6

MDSCs in spleen and tumor samples from PDX-implanted nude mice. Intrasplenic immune cells were represented as circulatory immune cells and intratumor immune cells were corresponded to tumor microenvironment. *$P < 0.05$.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.
• SupplementaryData.docx