Enrichment of CD44+/CD24+ cells predicts chemoresistance in luminal breast cancer

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Abstract

Purpose: Chemoresistant cells are pre-existing and adaptively selected by chemotherapy. The biomarkers of chemoresistant cells in luminal breast cancer remain unknown. The expression of CD44 and CD24 are associated with breast cancer progression. This study aimed to explore the proportion change of differently expressed CD44/CD24 tumor cells during the induction of chemoresistance.

Methods: Tumor specimens of patients with luminal breast cancer resistant and sensitive to chemotherapy drugs regimens were collected. The proportion of CD44+/−CD24+/− tumor cells in the two groups of samples was compared by immunofluorescence staining. MCF-7 and T47D variants resistant to chemotherapy drugs were induced by pulse selection of the parental cells with the IC90 value of the two drugs for 7 weeks. Changes in the proportion of CD44+/−CD24+/− cells during the induction of chemoresistance were observed and compared using flow cytometry.

Results: The proportion of CD44+CD24+ cells in the tumor tissues of chemoresistant patients was significantly higher than chemosensitive patients. At the endpoint of chemoresistance induction, the proportions of CD44+CD24+ cells reached 86.27%, 84.41%, 83.03%, and 85.12% in MCF-7/EPI, MCF-7/DOC, T47D/EPI, and T47D/DOC, respectively. The proportion of CD44+CD24− cells increased briefly and then decreased to nearly the pre-induction level (3.01%). The proportion of CD44−CD24− and CD44−CD24+ cells in the four chemoresistant variants was less than 5% at the endpoint of chemoresistance induction.

Conclusions: The enrichment of CD44+CD24+ cells predicts chemoresistance in luminal breast cancer. CD44+CD24+ is a biomarker for chemoresistant luminal breast cancer cells.

Introduction

Breast cancer is the most common and lethal cancer among women worldwide [1]. Luminal breast cancer (estrogen receptor [ER] and/or progesterone receptor [PR] positive, ER+PR+/−) accounts for more than 70% of all breast cancers [2]. Although most patients with luminal breast cancer are sensitive to endocrine therapy, chemotherapy should also be considered for high-risk patients [3]. However, in clinical practice, patients with luminal breast cancer show more drug resistance and multidrug resistance to chemotherapy than patients with other molecular subtypes, which leads to chemotherapy failure and increases the risk of tumor recurrence and metastasis [4-8]. Some studies have reported that resistant genotypes are pre-existing and adaptively selected by chemotherapy [9]. However, biomarkers of chemoresistant cells in luminal breast cancer remain unknown.

According to the cancer stem cell (CSC) theory, CSCs are the springhead of tumorigenesis, metastasis, recurrence, and even chemoresistance, and CD44+CD24− is a biomarker for luminal breast CSCs [10-13]. However, breast CSCs (BCSCs) alone cannot explain the clinical chemoresistance, as the large number of residual tumor cells after chemotherapy cannot all be BCSCs in chemoresistant breast cancer. Therefore, there should be many other phenotypic cells, except CD44+CD24− tumor cells, resistant to chemotherapy

in luminal breast cancer. Considering that a significant association between CD24 expression and shortened patient overall survival and disease-free survival has been demonstrated in many types of cancer, including breast cancer [14-18], and that several studies have confirmed that CD44+CD24+ cells are related to chemoresistance in gastric cancer, pancreatic adenocarcinoma, and cervical cancer [19~21], we hypothesized that CD44 and/or CD24 expression biomarkers are associated with breast cancer chemoresistance. In this study, we first analyzed CD44 and CD24 expressions in tumor specimens from patients with chemoresistant and chemosensitive luminal breast cancer. Furthermore, we induced chemoresistance in luminal breast cancer cell lines and explored the CD44+/−CD24+/− tumor cell proportion change during the induction of chemoresistance to verify the association between CD44+/−CD24+/− tumor cell enrichment and breast cancer chemoresistance.

Methods

Tumor samples

A total of 64 specimens were obtained from patients with invasive breast cancer who received neoadjuvant therapy at the Department of Breast and Thyroid Surgery, Southwest Hospital, Third Military Medical University (Army Medical University) between 2012 and 2016. All patients met the following criteria: (1) patients pathologically diagnosed with invasive ductal carcinoma with ER positivity, PR positivity, and human epidermal growth factor receptor 2 (HER2) negativity; (2) patients with IIB to IIIC tumor-node-metastasis staging on admission; and (3) patients receiving four to six cycles of neoadjuvant chemotherapy based on epirubicin (EPI) and/or docetaxel (DOC). The patients enrolled in this study were divided into the chemosensitive (29 patients) and chemoresistant (35 patients) groups based on their response to EPI and/or DOC regimens. The response to these drugs was evaluated according to the standard guidelines of the Response Evaluation Criteria in Solid Tumors [22] as follows: complete response (CR) was defined as disappearance of all lesions in both primary tumor and lymph nodes, partial response (PR) was defined as at least a 30% reduction in the sum of the longest diameter of target lesions, progressive disease (PD) was defined as at least a 20% increase in the sum of the longest diameter of target lesions, and stable disease (SD) was defined as neither sufficient shrinkage to qualify as PR nor sufficient increase to qualify as PD. CR and PR were classified as chemosensitive, whereas SD and PD were classified as chemoresistant [22]. This study was approved by the Southwest Hospital Research Ethics Committee. Written informed consent was obtained from all the participants. All methods were performed in accordance with the approved guidelines.

Cells and culture

The luminal breast cancer cell lines MCF-7 and T47D were purchased from the American Type Culture Collection. MCF-7 cells were cultured in Dulbecco's Modified Eagle's Medium (Gibco, USA) supplemented with 10% fetal bovine serum (FBS, Gibco, USA). T47D cells were cultured in Roswell Park Memorial

Institute-1640 medium supplemented with 10% FBS. All the cells were cultured in a humidified 5% CO2 incubator at 37°C.

Immunofluorescence and hematoxylin and eosin staining

Immunofluorescence was performed as previously described [22-24]. Briefly, paraffin-embedded samples were sectioned at a thickness of 4 μm. After being warmed for 30 min at 60°C, the slides were subjected to dewaxing in xylene for 3–4 min and rehydrated by passing them through graded alcohol in decreasing concentration order as 100%, 90%, 80%, 70%, 50%, and 30% for 1–2 min in each and rinsed in distilled water. Antigen retrieval was performed using a pressure cooker for 15–20 min in 0.01 M citrate buffer (pH, 6.0) to remove aldehyde links formed during initial fixation of tissues. The slides were then blocked with 2% bovine serum albumin for 1 h at room temperature. Subsequently, the sections were incubated with mouse-anti-human CD24 (1:1000, Abcam, ab31622) and rabbit anti-human CD44 (1:1000, Abcam, ab157107) primary antibodies overnight at 4°C. Following incubation with secondary antibody goat anti-mouse IgG H&L conjugated with Alexa Fluor 488 (1:1000, Abcam, ab150113) or goat anti-rabbit IgG H&L conjugated with Alexa Fluor 568 (1:1000, Abcam, ab175471) for 1 h at room temperature and counterstaining with 4',6-diamidino-2-phenylindole, at least three randomly selected microscopic fields were obtained using a laser scanning confocal microscope under a high-power lens (LSM780, Zeiss) and processed using a software (ZEN 3.1, Zeiss). Hematoxylin and eosin (H&E) staining was performed to identify tumor cell positions in histology to perform image registration. After immunofluorescence staining and confocal laser scanning microscopic analysis, the sections were immersed in 0.5% (v/v) hydrochloric acid for 1–2 s followed by the diluted ammonia water for several times and rinsed in tap water. After dehydration with graded alcohol, the sections were dipped in eosin solution for 30 s to 1 min, rinsed with absolute alcohol, and immersed in xylene solution for 30 s. After the excess xylene was drained and mounted with DPX, the sections were observed under a whole-slide histological scanner (Axio Scan.Z1, Zeiss) and processed using a software (Image-Pro Plus software 6.0) [25]. Quantification of the proportion of CD44+CD24− and CD44+CD24+ tumor cells in clinical samples was determined by expression analysis of CD44 and/or CD24 in tumor cells identified by H&E staining.

Induction and characterization of chemoresistant cells

EPI- and DOC-resistant variants of MCF-7 and T47D cells were generated by pulse selection [26, 27]. To generate resistant variants, the IC90 values of EPI and DOC in MCF-7 and T47D cells were determined. MCF-7 and T47D cells were seeded in 24-well plates at 2 × 105/well overnight. Different concentrations of EPI (Selleck, s1223) and DOC (Selleck, s1148) (0, 5, 10, 20, 100, 200, 400 ng/mL) were then added. After 72 h, the cells were counted using an automatic cell counter (Bio-Rad TC10, Bio-Rad, USA). Based on the growth inhibition curves, IC90s were calculated. MCF-7 and T47D cells were exposed to IC90 concentrations of EPI or DOC for 4 h and once a week for 7 weeks, respectively, to obtain resistant variants MCF-7/EPI, MCF-7/DOC, T47D/EPI, and T47D/DOC. At the endpoint of induction, the resistance

index (RI), which refers to the ratio of the IC50 of resistant variants to that of their parental cells, was determined for each resistant variant by drawing growth inhibition curves, as described above.

Quantitative reverse transcription polymerase chain reaction

The expression of multidrug resistance-associated protein I (MRPI) in MCF-7/EPI, MCF-7/DOC, T47D/EPI, and T47D/DOC was detected using quantitative reverse transcription polymerase chain reaction. The assay was performed using the SYBR Premix Ex Taq kit (TaKaRa, Japan) according to the manufacturer's instructions. The forward primer for MRPI was (5'-3'): AACCTGGACCCATTCAGCC. The reverse primer for MRPI was (3'-5'): GACTGGATGAGGTCGTCCGT. Data were collected and analyzed using a Bio-Rad RT-PCR CFX96.

Flow cytometry analysis

During induction, a small number of cells from each of the four resistant variants were marked with CD44 and CD24 antibodies and investigated by flow cytometry. Moreover, 1 × 106 cells were resuspended in phosphate-buffered saline (PBS) containing 1% FBS and stained with APC anti-human CD44 (1:400, BioLegend, 338806) and PE anti-human CD24 (1:400, BioLegend, 311106) antibodies for 30 min at 4°C. The cells were washed with assay buffer for flow cytometry analysis. Specimens were subsequently analyzed using a NovoCyte flow cytometer (ACEA Biosciences, Inc.) [28, 29].

For multidrug resistance protein 1 (MDR1) expression, 1 × 106 cells of four resistant variants (MCF-7/EPI, MCF-7/DOC, T47D/EPI, T47D/DOC) and their parental cells (MCF-7 and T47D) were resuspended in PBS containing 1% FBS and stained with APC anti-human MDR1 (BioLegend, 348607) for 30 min at 4℃. The cells were washed with an assay buffer for flow cytometry analysis. Specimens were subsequently analyzed using a NovoCyte flow cytometer (ACEA Biosciences, Inc.).

Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences version 17.0 and GraphPad Prism version 8. Results are expressed as mean±standard error of the mean. Moreover, $P<0.05$ was considered statistically significant. Student's t-test (two-tailed) was used to determine the significance of the differences between the two groups of data.

Results

CD44+CD24+ cells are increased in tumor tissues of chemoresistant patients.

We examined CD44 and CD24 in tumor tissues of chemoresistant (n=29) and chemosensitive (n=35) patients with luminal breast cancer using immunofluorescence staining (Fig. 1A). H&E staining of the same sample with immunofluorescence staining was performed to distinguish tumor cells from stromal cells on the slides. There were 85.07±4.11% of CD44+CD24+ cells in the tumors of chemoresistant patients, which was four times higher than 20.46±6.09% of CD44+CD24+ cells in tumors±chemosensitive patients (Fig. 1B, P<0.001). There were no differences in the proportion of CD44+CD24− cells in tumor tissues between chemoresistant (3.90±1.39%) and chemosensitive (4.14±1.29%) patients (Fig. 1C, P>0.05). Notably, there was no difference between the chemosensitive and chemoresistant groups in terms of age, tumor size, and lymph node metastasis grade on admission (Fig. 1D, E, F and Table 1, P>0.05). These results indicate that CD44+CD24+ cells may be related to chemoresistance in patients with luminal breast cancer.

Table 1

Characteristics of chemoresistant and chemosensitive patients

Patients	Chemoresistance(n=29)	Chemosensitivity(n=35)
Age	49.97±9.18	47.11±7.64
Pre-chemotherapy		
Tumor size in mm	27.07±6.34	27.49±6.74
Lymph node metastasis grade	1.59±0.50	1.57±0.56
Distant metastasis	0	0
Post-chemotherapy		
Tumor size in mm	28.28±6.02	11.29±3.32***
Lymph node metastasis grade	1.62±0.49	1.49±0.61
Distant metastasis	0.10±0.31	0
Miller-Payne grade	1.1±0.31	3±0.27***

Mean ± SEM, chemoresistance vs chemosensitivity ***P<0.001

CD44+CD24+ subset cells are enriched in chemoresistant luminal breast cancer cells in vitro.

To verify these results, we generated chemoresistant luminal breast cancer cell lines. Anthracycline EPI and paclitaxel DOC are two types of drugs with different mechanisms of action and are commonly used in high-risk luminal breast cancer chemotherapy [30, 31]. Therefore, we induced EPI- and DOC-resistant variants (MCF-7/EPI, MCF-7/DOC, T47D/EPI, and T47D/DOC). The growth inhibition of EPI and DOC in the four chemoresistant cells was significantly lower than that in their parental cells (Fig. 2A, $P<0.001$). The RIs of MCF-7/EPI, MCF-7/DOC, T47D/EPI, and T47D/DOC to their parental cells were 13.5±0.039, 14.2±0.058, 15.23±0.053, and 12.75±0.063, respectively (Table 2). This suggested that we successfully induced resistant cells. The relative expressions of MRPI in MCF-7/EPI, MCF-7/DOC, T47D/EPI, and T47D/DOC were 28.73±1.11, 32.22±0.53, 30.78±0.73, and 36.26±0.57 times to their parental cells, respectively (Fig. 2B, $P<0.001$). The relative expressions of MDR1+ cells in MCF-7/EPI, MCF-7/DOC, T47D/EPI, and T47D/DOC were 67.33±2.57%, 66.46±1.67%, 67.48±1.58%, and 66.67±1.86%, respectively (Fig. 2C). The MDR1+ cells MCF-7/EPI, MCF-7/DOC, T47D/EPI, and T47D/DOC were significantly higher than their parent cells (Fig. 2D, $P<0.001$). These results further proved that chemoresistant cells were successfully obtained.

Table 2

The resistance index of epirubicin (EPI)- and docetaxel (DOC)- resistant variants of luminal breast cancer cells

Drug	Cell	IC50 (μg/mL)	RI
EPI	MCF-7	0.152±0.018	13.5±0.039
	MCF-7/EPI	2.053±0.045***	
	T47D	0.186±0.062	14.2±0.058
	T47D/EPI	2.641±0.105***	
DOC	MCF-7	0.197±0.012	15.23±0.053
	MCF-7/DOC	3.001±0.049***	
	T47D	0.218±0.076	12.75±0.063
	T47D/DOC	2.779±0.094***	

Based on the induced EPI- or DOC-resistant variants, we detected the proportion changes of cell subsets of CD44+/−CD24+/− cells in the induction of chemoresistance in MCF-7 and T47D cells (Fig. 3A and B). First, we observed the changes in the proportion of CD44+CD24− cells during induction. In general, CD44+CD24− cells experienced a brief increase and then decreased to nearly the pre-induction level (Fig. 3C and D). Taking MCF7/EPI as an example, the proportion of CD44+CD24− cells increased to a peak value of 26.57±2.09% in the third week after induction and then decreased to 3.01±1.28% at the endpoint. We found that the proportions of CD44−CD24− and CD44−CD24+ cells in the four chemoresistant

variants were all less than 5% at the endpoint. The only cell subset that continuously increased and eventually accounted for more than 80% of the induction was CD44+CD24+ cells. For MCF-7/EPI and MCF-7/DOC, in the first 2 weeks of induction, the proportion of CD44+CD24+ cells increased slowly and then increased significantly from 3 to 5 weeks. At 6 weeks, the proportion of CD44+CD24+ cells reached approximately 80% and tended to reach a dynamic equilibrium. At the endpoint, the proportion of CD44+CD24+ cells in MCF-7/EPI and MCF-7/DOC reached 86.27±2.04% and 84.41±1.59%, respectively, which were two to three times higher than 24.86±1.71% in the parental cells (Fig. 3E, $P<0.001$). This was also true for T47D/EPI and T47D/DOC. During induction, the proportion of CD44+CD24+ cells increased continuously and reached equilibrium after 6 weeks of induction. At the endpoint, the proportions of CD44+CD24+ cells in T47D/EPI and T47D/DOC reached 83.03±1.01% and 85.12±1.30%, respectively, which were significantly higher than 15.37±1.62% in the parental cells (Fig. 3F, $P<0.001$). These results suggested that chemoresistance is accompanied by CD44+CD24+ cell enrichment.

Discussion

Breast CSCs, marked with CD44+CD24− in luminal breast cancer, are recognized as the key source of drug resistance. CD44+CD24− luminal breast cancer cells have multiple chemoresistant mechanisms, such as drug efflux, antiapoptotic pathway, DNA damage repair, cycle arrest, survival promoting signaling pathway, and aldehyde dehydrogenase expression [4, 32~34]. A previous study reported that large numbers of CD44+CD24− cells could be generated by in vivo passage of HER2-positive breast cancer cells SKBR3 in NOD/SCID mice treated with chemotherapy [27]. In our study, we found that nearly 80% of CD44+CD24+ cells were found in tumors of patients with chemoresistant luminal breast cancer. In addition, the proportion of CD44+CD24+ cells increased to 83% at the endpoint of chemoresistant MCF-7 and T47D cell induction. Through histological and cytological experiments, we were the first to find that CD44+CD24+ cells rather than CD44+CD24− cells were enriched in luminal breast cancer cells during chemotherapy. CD44+CD24+ may be a biomarker for chemoresistant cells in luminal breast cancer. We hypothesized that the differences in biomarkers of chemoresistant cells in different breast cancers might be related to the molecular types. ER, PR, and/or HER2 causes the differential expression of resistance-related genes during chemotherapy. For example, P-glycoprotein (P-gp) and topoisomerase 2 have a high expression in luminal B breast cancer cells, whereas thymidylate synthase and glutathione-S-transferase-π (GST-π) are highly expressed in HER2-positive breast cancer [35~38]. In future studies, we will induce chemoresistant cells in other types of breast cancer cell lines to prove this hypothesis.

What is the mechanism of enrichment of CD44+CD24+ cells during chemotherapy? Some studies have reported that resistant cells are adaptively enriched by chemotherapy [9]. Some studies have reported a dynamic balance between different cell subtypes. In chemotherapy, this balance is disrupted, and a new balance is formed through mutual transformation between different subtypes [39]. In future studies, we will describe the fate of each subtype of CD44+/−CD24+/− cells in the formation of chemoresistance using a mathematical model to explore whether the enrichment mechanism of CD44+CD24+ cells is caused by the proliferation of the rare CD44+CD24+ cells with primary chemoresistance, or the transformation of cells of other phenotypes or both.

Based on our results, we believe that there are several possible mechanisms for the resistance of CD44+CD24+ cells to chemotherapy. First, the high expressions of MRPI and MDR1 suggest that CD44+CD24+ cells have the ability of drug efflux and drug resistance. MRPI is distributed in the membrane of tumor cells and can induce drug resistance by mediating intracellular drug excretion and altering intracellular drug redistribution [40, 41]. MDR1 plays a major role in drug resistance in breast cancer. MDR1-encoded proteins, such as P-gp, GST-π, and P53, are all involved in drug resistance [42, 43]. Second, during the induction of chemoresistance, we found that the cell cycle was prolonged to 7 days. Prolonged cell cycle and slowed cell growth rate might be helpful for CD44+CD24+ cells to resist chemotherapy. Finally, chemotherapy-induced senescent cancer cells engulf other cells to enhance their survival [44]. We observed that cells often gathered into clusters during induction. However, the phagocytic function of CD44+CD24+ cells must be verified. Given the important role of chemoresistant cells in tumor recurrence and metastasis, it is necessary to study the ability of CD44+CD24+ cells to migrate and invade. In addition, analyzing the difference in transcription between enriched CD44+CD24+ cells and their parents and CD44+CD24+ cell pre-induction chemoresistance is helpful for understanding the molecular mechanism of chemoresistance and metastasis of enriched CD44+CD24+ cells.

In this study, we found that the proportion of CD44+CD24+ cells significantly increased by more than 80% in the tumors of chemoresistant patients and cell lines. The progression of chemoresistance in luminal breast cancer is accompanied by the enrichment of CD44+ CD24+ cells. Therefore, there are some potential applications based on our study. First, the enrichment of CD44+CD24+ cells can predict the chemoresistance of luminal breast cancer, which may be used as a supplement to existing clinical methods, such as mammography, ultrasonography, and magnetic resonance imaging, to monitor and predict the therapeutic effect of chemotherapy. Second, because CD44 and CD24 are surface markers of chemoresistant cells, CD44 and CD24 targeted drugs can be developed for complementary treatment of chemoresistant luminal breast cancer. Finally, detecting the molecules promoting CD44+CD24+ cell enrichment in tumors of patients with breast cancer is helpful to predict whether the patient can benefit from chemotherapy.

Conclusion

The enrichment of CD44+CD24+ cells predicts chemoresistance in luminal breast cancer. CD44+CD24+ is a biomarker for chemoresistant luminal breast cancer cells.

Abbreviations

BCSCs: breast cancer stem cells

CR: complete response

CSC: cancer stem cell

DOC: docetaxel

EPI: epirubicin

ER: estrogen receptor

FBS: fetal bovine serum

GST-π: glutathione-S-transferase-π

H&E: hematoxylin and eosin

HER2: human epidermal growth factor receptor 2

MDR1: multidrug resistance protein 1

MRPI: multidrug resistance-associated protein I

PBS: phosphate-buffered saline

PD: progressive disease

PR: partial response

PR: progesterone receptor

SD: stable disease

Declarations

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Statements and Declarations

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

PT contributed substantially to the conception and design, and gave final approval of the version to be published. JW, QHM, LZ, and QQC collected and analyzed the data and performed the expression verification of clinical samples. JW, MHW, and WL wrote the manuscript. YZY and YH critically revised the manuscript. All authors contributed to the article and approved the submitted version.

Data Availability

Ethics approval

All procedures performed in this study were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Consent to Participate

Informed consent was obtained from all individual participants included in the study.

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Figures

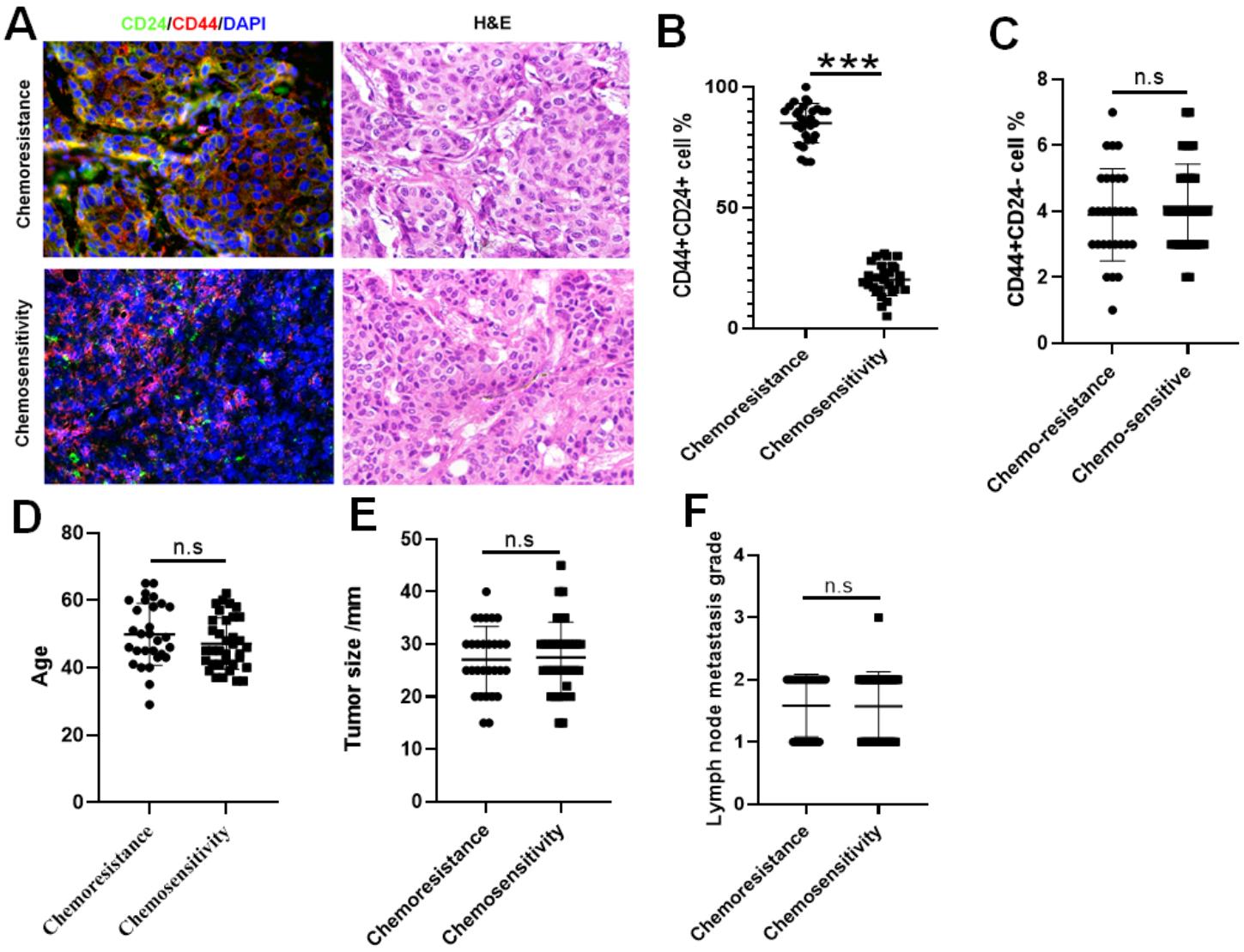

Figure 1

Increased CD44+CD24+ cells in tumor tissues of chemoresistant patients. (A) Representative images of hematoxylin and eosin staining and immunostaining of CD44 and CD24 in chemoresistant (n=29) and chemosensitive (n=35) breast cancer biopsies (×200). (B) The percentage of CD44+CD24+ cells in chemoresistant and chemosensitive breast cancer biopsies. Mean±standard error of the mean (SEM), $^{ppp}P<0.001$. (C) The percentage of CD44+CD24− cells in chemoresistant and chemosensitive breast cancer biopsies. Mean±SEM, n.s, no significance. There are no differences between the chemoresistant and chemosensitive patient groups in age (D), tumor size (E), and lymph node metastasis grade (F) on admission. Mean±SEM, n.s, no significance

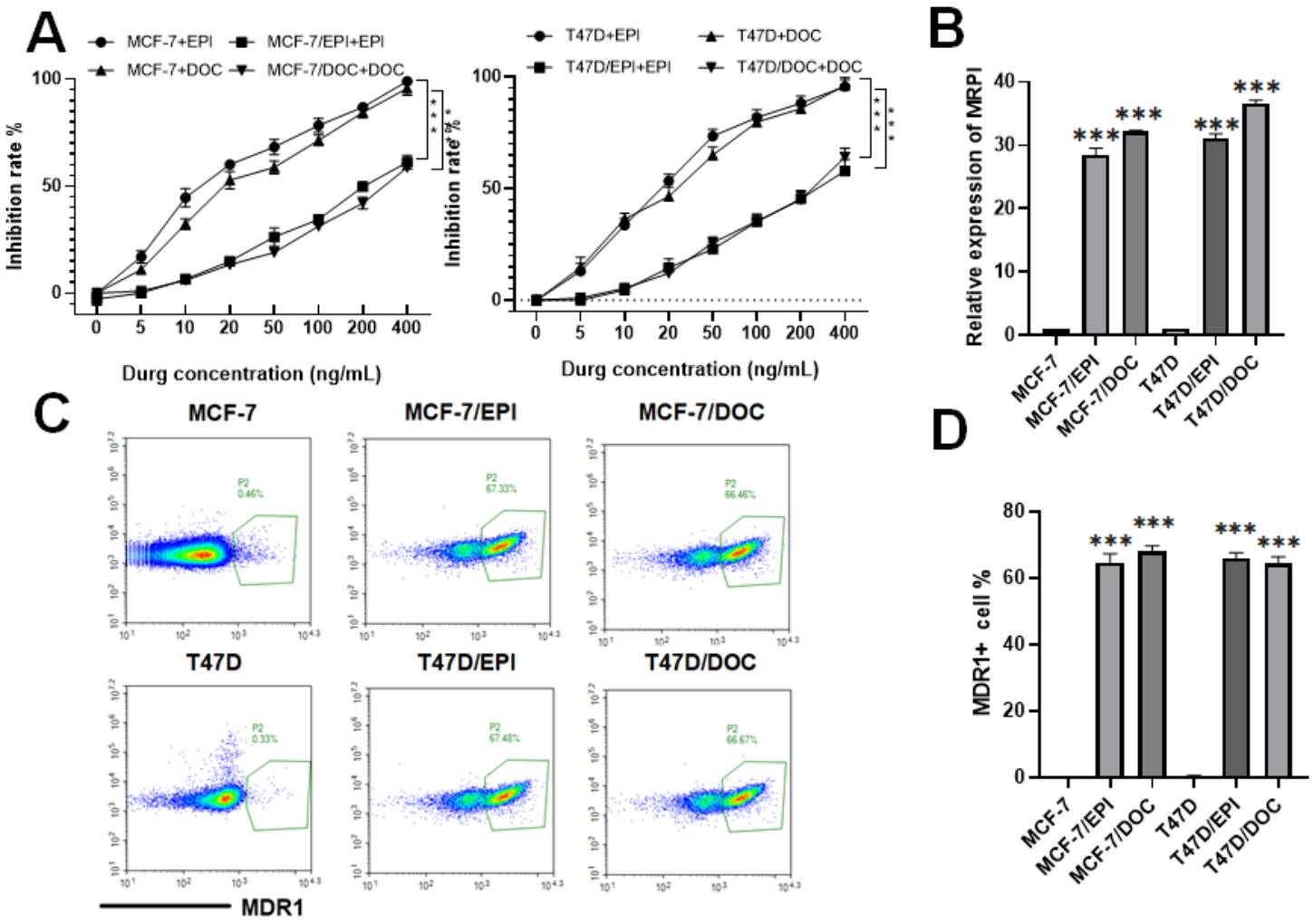

Figure 2

The characteristics of chemoresistant cells. (A) The growth inhibition of chemoresistant cells MCF-7/epirubicin (EPI), MCF-7/docetaxel (DOC), T47D/EPI, and T47D/DOC and their parents. Mean±standard error of the mean (SEM), $^{bbb}P<0.001$. (B) The relative expression of multidrug resistance-associated protein I of chemoresistant cells. Mean±SEM, $^{bbb}P<0.001$. (C, D) The proportion of multidrug resistance protein 1+ cells in chemoresistant cells. Mean±SEM, $^{bbb}P<0.001$

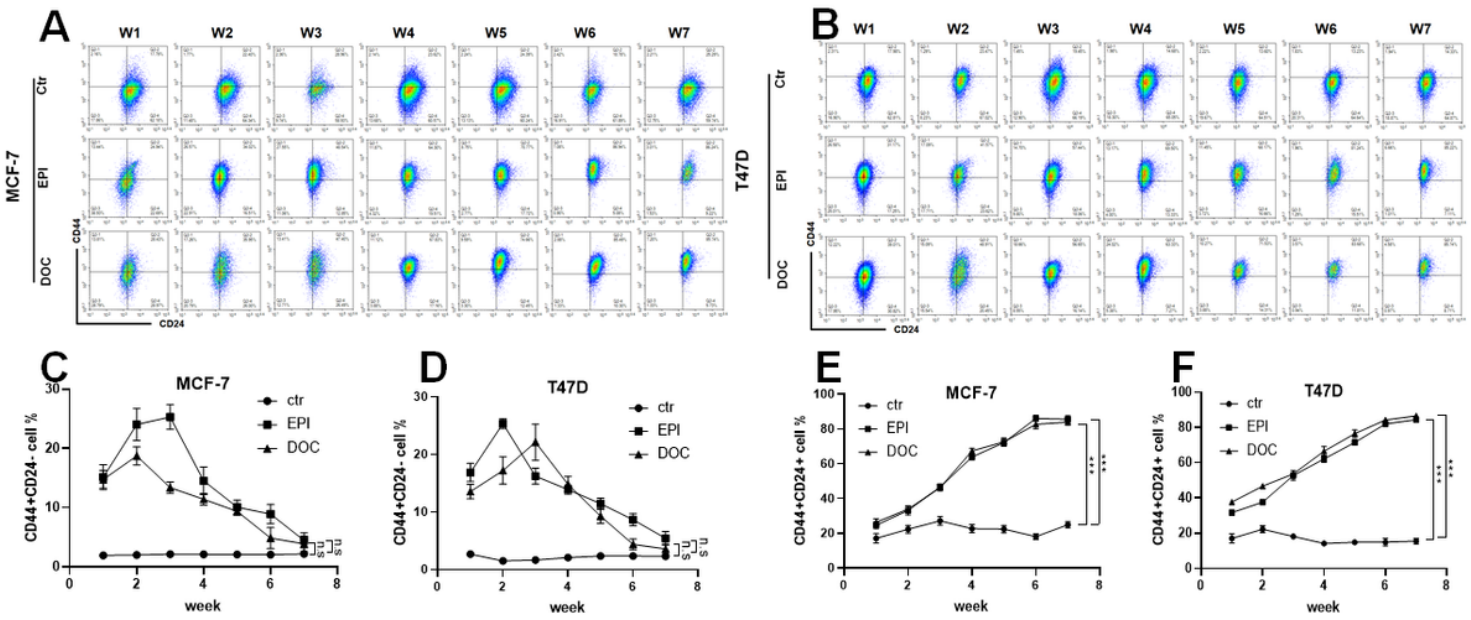

Figure 3

Chemoresistance of luminal breast cancer cells was accompanied by enrichment of CD44+CD24+ cells. (A) Fluorescence-activated cell sorting (FACS) detection of CD44+/−CD24+/− during induction of MCF-7/ epirubicin (EPI) and MCF-7/ docetaxel (DOC). (B) FACS detection of CD44+/−CD24+/− during induction of T47D/EPI and T47D/DOC. (C) The change in the proportion of CD44+CD24− cells during induction of MCF-7/EPI and MCF-7/DOC. Mean±standard error of the mean (SEM), n.s, no significance. (D) The change in the proportion of CD44+CD24− cells during induction of T47D/EPI and T47D/DOC. Mean±SEM, n.s, no significance. (E) The change in the proportion of CD44+CD24+ cells during induction of MCF-7/EPI and MCF-7/DOC. Mean±SEM, ^{ØØØ}P<0.001. (F) The change in the proportion of CD44+CD24+ cells during induction of T47D/EPI and T47D/DOC. Mean±SEM, ^{ØØØ}P<0.001