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The expression and significance of multi-drug resistance genes in breast cancer stem cells*

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Abstract ***Objective:*** To approach the expressions of MDR1 and BCRP in breast cancer stem cells and differentiated cells. ***Methods:*** The breast cancer stem cells were separated from human breast cancer primary tissues and MCF-7 by flow cytometry. Then we measured the expressions of MDR1 and BCRP with different subset cells by Realtime-PCR. ***Results:*** Contrasted with breast cancer differentiated cells, the expressions of MDR1 and BCRP in breast cancer stem cells were higher ($P < 0.01$), and the proportion of stem cells rose after chemotherapy ($P < 0.01$). ***Conclusion:*** Contrasted with breast cancer differentiated cells, breast cancer stem cells have stronger ability of drug-resistance with higher level of multi-drug resistance genes, and it is one of key points for chemotherapy failure of breast cancer.

Key words stem cell; breast cancer; chemotherapy; multi-drug resistance

Studies in recent years have found that tumor tissue, like the normal tissue, is comprised of tumor cells of different natures, and is derived from the correspond stem cells – cancer stem cells [1–3]; as the source cells of tumor tissue, cancer stem cells are not only characterized by the infinite ability of self-renewal and reconstruction of tumor tissue, but also considered as one of the key factors to the failure and relapse of tumor chemotherapy [1, 2, 4]. Al-Hajj [2] have successfully separated and identified the human breast cancer stem cells via animal transplantation model of human breast cancer, and ascertained the specific cell phenotype – $CD44^+CD24^-$; Kondo [3] have discovered that in the tumor cell line (including MCF-7) suitable for long-term culture *in vitro*, there were similar tumor stem cells. On the basis of their studies, we aim to explore part of the mechanisms of human breast cancer stem cells' resistance to chemotherapy with tumor stem cells in the human breast cancer primary cells and MCF-7 cells as the experimental objects.

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Materials and methods

The source of cells and the flow cell sorting

Nineteen samples of breast cancer tissue were collected from patients with primary breast cancer (all of them were female) in our hospital from May 2005 to March 2006. Breast cancer cell line MCF-7 was provided by the Laboratory of Department of General Surgery in our hospital. Under the sterile condition, the collected samples of breast cancer tissue were treated according to the descriptions in the literature [2]. They were digested at 37 ℃ for 6–8 h, and subsequently filtered with 400 mesh filter screen. After cleansing for 2 times with PBS, they were made into single cell suspensions. Afterwards, the cells were marked according to the methods recorded in the literature [2]. The primary breast cancer cell were marked by CD44-APC, CD24-PE and CD2, CD3, CD10, CD16, CD18, CD31, CD64, CD140b-FITC (excluding non-breast cancer cells) and 7AAD (excluding dead cells), while MCF-7 was marked only by CD44-APC, CD24-PE and 7AAD. The cells were sorted for 2 times on each occasion to ensure the purity of every group of sorted cells > 95%.

The detection of the expressions of MDR1 and BCRP from different subgroup cells

By adoption of Real Time-PCR technique, the expressions of MDR1 and BCRP were detected. The number of the detected cells was 10^5 for both of them. β-actin was selected as internal reference. All the reagents were pur-

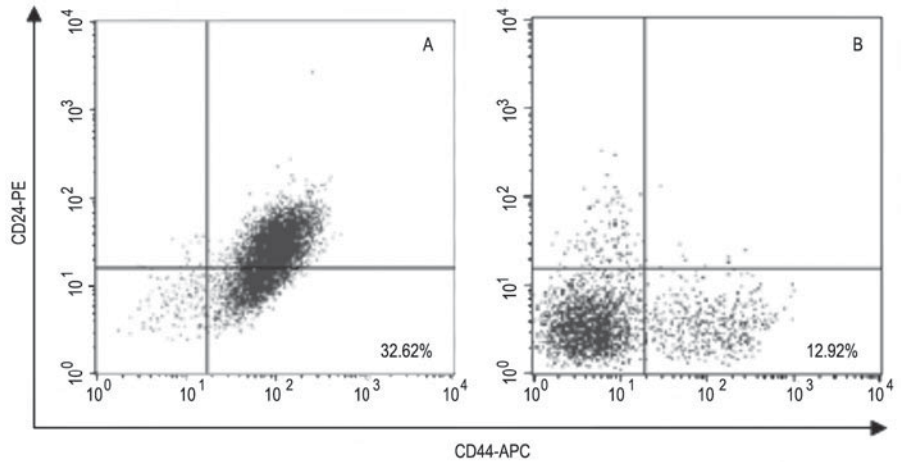

Fig. 1 CD44 and CD24 distribution of MCF-7 cells and the human breast cancer primary cells (the lower right corner for the expression of $CD44^+CD24^-$ stem cells). (A) MCF-7 cells; (B) The human breast cancer primary cells

chased from GIBCO Corporation. Reaction conditions: pre-degeneration at 94 ℃ for 2 min; afterwards, degeneration at 94 ℃ for 20 s, renaturation at 55 ℃ for 30 s, extension at 72 ℃ for 30 s (45 cycles in all).

Statistical method

Paired *t* test was performed with SPSS 11.0 software.

Results

The cellular components of human breast cancer primary cells and MCF-7 cells

Our results (Fig. 1) of the detection on the expressions of CD44 and CD24 in human breast cancer primary cells and MCF-7 cells indicated that the MCF-7 cells were mainly comprised of $CD44^+CD24^-$ stem cells (accounting for 15.05%–34.35%) and $CD44^+CD24^+$ cells (accounting for 63.97%–83.29%), while human breast cancer primary cells were mainly comprised of $CD44^+CD24^-$ stem cells (accounting for 12.26%–21.96%) and $CD44^-$ cells (accounting for 69.91%–86.41%).

The expressions of MDR1 and BCRP in different subgroups of breast cancer cells

The expression of MDR1 in human breast cancer primary cells was divided into three categories (Fig. 2A): (1) In 5 cases of samples, only the breast cancer stem cell expressed MDR1; (2) In 11 cases of samples, both groups of cells expressed MDR1, while the expression level of MDR1 in breast cancer stem cells was significantly higher than that in the breast cancer differentiated cells (14.28–23.16 times); (3) In 3 cases of samples, there was no expression of MDR1 in both groups of cells. The expression level of MDR1 in breast cancer stem cells of MCF-7 cells was 4.12 times as high as that in the breast cancer differentiated cells (Fig. 2B).

The expression of BCRP in human breast cancer primary cells fell into three categories (Fig. 3A): (1) In 10 cases of samples, both groups had high expression level of BCRP, and the expression level of BCRP in breast cancer stem cells was 9.22–15.34 times as high as that in breast cancer differentiated cells; (2) In 5 cases of samples, both groups had low expression level of BCRP, and the expression level of BCRP in breast cancer stem cells was 21.60–57.42 times as high as that in breast cancer differentiated cells; (3) In 4 cases of samples, only breast cancer stem cells expressed BCRP, and the expression level of BCRP in stem cells of MCF-7 was 3.82 times as high as that in breast cancer differentiated cells (Fig. 3B).

The changes in the expressions of MDR1 and BCRP in breast cancer stem cells after chemotherapeutic intervention

The results of further intervention by administrating chemical medicines to MCF-7 cells 60 h later indicated that the proportion of stem cells in MCF-7 prior to chemotherapy was 32.62%, while the proportion of stem cells in each group after chemotherapy increased significantly ($P < 0.01$). The proportions of stem cells in docetaxel (0.4 μg/mL), 5-FU (5.0 μg/mL), cisplatin (1.5 μg/mL), epirubicin (0.4 μg/mL) and mitomycin (1.5 μg/mL) groups were 78.28%, 78.37%, 68.08%, 89.21%, and 58.79%, respectively. Compared with pre-chemotherapy simultaneously, the expressions of stem cell MDR1 and BCRP were also increased significantly. The expression levels of MDR1 in the docetaxel, 5-FU, cisplatin, epirubicin and mitomycin groups were 20.62, 18.30, 2.35, 4.79 and 10.91 times, respectively, higher than those prior to chemotherapy, whereas the expression levels of BCRP was 32.77, 13.03, 3.08, 29.45, and 20.31 times higher than

those prior to chemotherapy, suggesting that the stem cells after chemotherapy might become more drug-resisted via gene mutation.

Discussion

The multi-drug resistance (MDR) of breast cancer is related to the persistent and excessive expressions of MDR1 and BCRP, the coding genes of the family members of ATP-binding cassette transporters [4]. The over-expressed cells of ATP-binding cassette transporter can pump out the fluorochromes (Hoechst33342 and rhodamine123) [4, 5], which is of great significance to the resistance of tumor cells to chemotherapy and the relapse of tumors. Furthermore, the previous studies suggested that the SP nature is one of the differences between tissue stem cells and tissue differentiated cells [4].

The results of this study indicated that the proportion of stem cells in the group of breast cancer cells after chemotherapy *in vitro* increased significantly compared with pre-chemotherapy. Since the generation of tumor cells after chemotherapy *in vitro* lingered in the stationary stage till the tolerance of tumor cells to the chemotherapeutic environment, the apparent increase of the proportion of breast cancer stem cells shortly after chemotherapy suggested that the breast cancer stem cells were more tolerated toward chemotherapy than the differentiated cells, and more capable of surviving in the chemotherapy. Compared with the general level of breast cancer differentiated cells, the breast cancer stem cells in both human breast cancer primary cells and MCF-7 cells had higher levels of MDR1 and BCRP expressions, indicating that breast cancer stem cells can escape chemotherapy via high expression levels of MDR1 and BCRP. Meanwhile, we believe that the three cases of expressions concerning MDR1 and BCRP in human breast cancer primary cells may suggest: (1) When both stem cells and differentiated cells in breast cancer have high levels of MDR1 and/or BCRP expression, the chemotherapy is ineffective and/or liable to relapse; (2) When only breast cancer stem cells have high levels of MDR1 and/or BCRP expression, patients can have partial remission and/or have a high risk

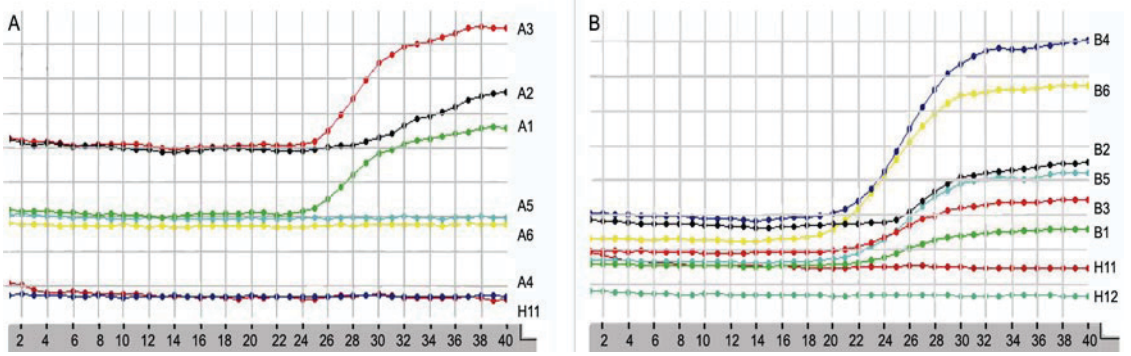

Fig. 2 (A) The MDR1 expression results in different subsets primary cells of human breast cancer cells. A1, A3 and A5 for breast cancer stem cells; A2, A4 and A6 were $CD44^-$ cells corresponding to A1, A3 and A5; H11 for negative control. (B) The MDR1 expression results in MCF-7 breast cancer stem cells after chemotherapy. B1 for control group (not received chemotherapy); B2–B6 were docetaxel, 5-FU, cisplatin, epirubicin and the MMC groups, respectively; H11 for negative control

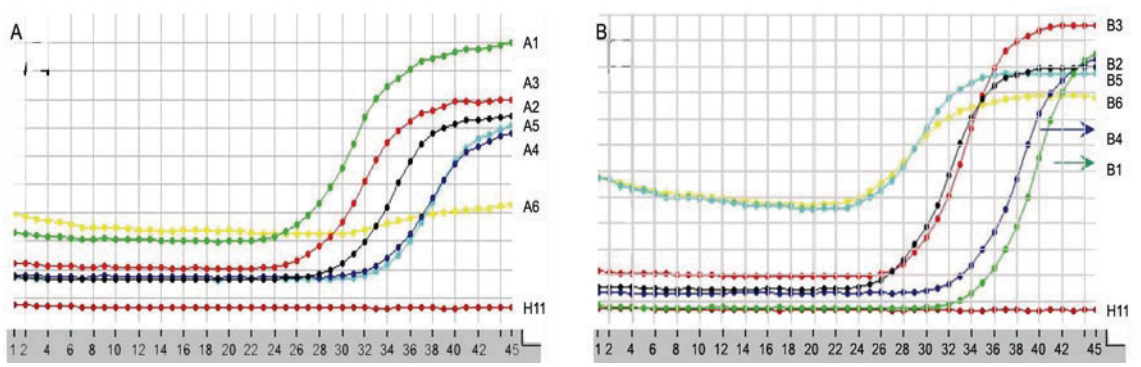

Fig. 3 (A) The BCRP expression results in different subsets primary cells of human breast cancer cells. A1, A3 and A5 for breast cancer stem cells; A2, A4 and A6 were $CD44^-$ cells corresponding to A1, A3 and A5; H11 for negative control. (B) The BCRP expression results in MCF-7 breast cancer stem cells after chemotherapy. B1 for control group (not receive chemotherapy); B2–B6 were docetaxel, 5-FU, cisplatin, epirubicin and the MMC groups, respectively; H11 for negative control

of relapse; (3) When both stem cells and differentiated cells have low or no expressions of MDR1 and BCRP, patients can have complete remission and have a low risk of relapse.

Compared with the differentiated cells, stem cells generally have more powerful abilities such as DNA reparation [4, 6], which enables the stem cells to be more adaptable to the various changes of the environment and ensures the timely reparation of tissue damage. We found that not only the breast cancer stem cells in MCF-7 were more capable of surviving after chemotherapy than differentiated cells, but also the expression levels of MDR1 and BCRP of the survived breast cancer stem cells were significantly increased compared with those before the chemotherapy, suggesting that the expression levels of MDR1 and BCRP of breast cancer stem cells, survived under the circumstances of chemotherapeutic drugs, can be induced for further increase. This is probably attributable to the mutation in some regulation mechanisms of the related drug resistance gene expression, which is ultimately conducible to the fast adaptation of the escaped breast cancer stem cells to the chemotherapeutic environment so as to wait for appropriate occasions to repair and reconstruct the damaged tumor tissues.

In sum, breast cancer stem cells can not only escape chemotherapy via high levels of MDR1 and BCRP expression, but also further promote the expressions of MDR1 and BCRP after chemotherapy to be adaptable as fast as possible to the chemotherapeutic environment and become more drug-resistant, contributing to the relapse and refractoriness of breast cancer. Hence, in the study of chemotherapy for breast cancer, breast cancer stem cells should be given further emphasis and be considered as the target cells of research and treatment.

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