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ORIGINAL ARTICLE

Correlations of MRP1 gene with serum TGF-β1 and IL-8 in breast cancer patients during chemotherapy

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Summary

Purpose: *To investigate the expressions of multidrug resistance-associated protein 1 (MRP1) gene, serum transforming growth factor beta-1 (TGF-β1) and interleukin-8 (IL-8) in patients with breast cancer during chemotherapy, and to analyze their correlations in chemotherapy.*

Methods: *346 breast cancer patients admitted to the Department of Surgery (Breast) of Nanjing Drum Tower Hospital from March 2015 to December 2017 were included as study subjects. All selected patients received chemotherapy in our hospital. Quantitative reverse transcription- polymerase chain reaction (qRT-PCR) and enzyme-linked immunosorbent assay (ELISA) were adopted to detect the expression levels of MRP1 mRNA, as well as MRP1, TGF-β1 and IL-8 proteins in patients before chemotherapy and at 1, 2, 4 and 8 weeks after chemotherapy. Correlations of MRP1 protein/mRNA with clinical features of patients were analyzed, and Pearson's correlation analysis was performed to examine correlations of MRP1 protein/mRNA with TGF-β1 and IL-8 proteins.*

Results: *The expressions of MRP1 mRNA as well as MRP1, TGF-β1 and IL-8 proteins were increased with the prolongation of chemotherapy time, and there were statistically significant differences between the two time points ($p<0.05$). No correlations of MRP1 with the clinical patient features with breast cancer were found. Pearson's correlation analysis showed that the expression level of MRP1 was positively correlated with the expression levels of TGF-β1 ($r=0.732$, $p=0.012$) and IL-8 ($r=0.709$, $p=0.018$), and the expression level of TGF-β1 was positively related to that of IL-8 ($r=0.714$, $p=0.015$).*

Conclusion: *With the prolongation of chemotherapy time in breast cancer patients, the expression level of MRP1 also increased which may affect the therapeutic effect of chemotherapy in breast cancer patients and lead to drug resistance. TGF-β1 and IL-8 may be closely associated with the mechanism of drug resistance in MRP1-guided breast cancer chemotherapy.*

Key words: *breast cancer, drug resistance, IL-8, MRP1, TGF-β1*

Introduction

Breast cancer is a very common malignant tumor in females in China, which seriously jeopardizes the health and life of patients. The incidence rate of breast cancer has risen rapidly in recent years, with an incidence rate of 378,6/100 million and a mortality rate of 114/100,000. Breast cancer accounts for about 17% of all malignant tumors in females [1,2]. At present, surgical excision as well as postoperative radiotherapy, chemotherapy and hormonotherapy are the main treatment methods for breast cancer. However, in recent years, many new chemotherapy drugs and regimens were proved unsatisfactory in improving the prognosis of breast cancer patients, with 10-year overall survival rate not exceeding 60% [3,4]. Therefore, research on biomolecules that affect the therapeutic effect of breast cancer has important scientific significance and clinical application value.

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Multidrug resistance (MDR) of tumor cells is a major barrier affecting treatment, and multidrug resistance associated protein 1 (MRP1) is one of the major members of MDR [5,6]. In recent years, many studies have reported that MRP1 is highly expressed in many malignant tumors such as gastric cancer, lung cancer and leukemia, and the therapeutic effect of chemotherapy in patients with high expression of MRP1 is obviously poorer than those with low expression of MRP1 [7-9]. There are few reports on the expression level of MRP1 in breast cancer and its effect on the chemotherapy outcome of breast cancer patients, and the mechanism of action of MRP1 in this cancer is not elucidated in studies. Transforming growth factor beta-1 (TGF-β1) is a protein regulating cell growth and differentiation [10]. Interleukin-8 (IL-8) regulates human reproductive physiological and pathological processes by binding to specific receptors [11]. In recent years, studies have reported that TGF-β1 and IL-8 are correlated with the drug resistance mechanisms of tumors [12,13].

In this study, changes in the expression level of MRP1 in breast cancer patients during chemotherapy were examined, and its correlations with the expression levels of TGF-β1 and IL-8 were analyzed so as to provide a basis for guiding the clinical breast cancer chemotherapy.

Methods

Study subjects

A total of 346 patients with breast cancer who were admitted to our hospital from March 2015 to December 2017 were selected. *Inclusion criteria*: 1) patients with an average age of 52.64±14.05 years; 2) female patients pathologically diagnosed with breast cancer; 3) patients with no previous tumor history; 4) patients undergoing primary surgery and chemotherapy in our hospital for the first time, without receiving endocrine therapy; 5) patients with no liver, kidney and other organ dysfunction, and no abnormal hemorrhage or abnormal coagulation function; and 6) patients with complete medical records and follow-up data. *Exclusion criteria:* 1) patients with distant metastasis; 2) patients with hypertension, heart disease or diabetes; 3) patients with lung, chest or other diseases; or 4) patients dying of other diseases. This study was approved by the Medical Ethics Committee of our hospital, and patients or their family members signed the informed consent.

Extraction of the total RNA from cells

Fasting peripheral venous blood was collected from all patients in the early morning, and TRIzol reagent (Shanghai Mingjin Biology Co., Ltd., Shanghai, China) was used to extract the total RNA from the patient serum according to the kit instructions. A micro-ultraviolet ND-1000 spectrophotometer (NanoDrop Technologies, Inc., USA) was applied to analyze the concentration and purity of the extracted RNA. The ratio of 1.8-2.0 indicated that the purity of the extracted RNA met the experimental requirements. Three percent agarose gel electrophoresis (gel electrophoresis kit was purchased from Shanghai Jingke Science and Technology Co., Ltd.) was used to analyze RNA integrity.

Detection of MRP1 mRNA via qRT-PCR

The above-mentioned total mRNA extracted was reversely transcribed and synthesized into complementary deoxyribonucleic acid (cDNA) according to the instructions of the fluorescence qPCR kit (Thermo Fisher Scientific, China Co., Ltd.). Reaction: at 37°C for 45 min and 95°C for 5 min. The amplification reaction system of cDNA was 25 μL. PCR conditions: at 95°C for 5 min, followed by circular reaction at the same condition using the two-step method, 95°C for 10s and 60°C for 34s for a total of 40 cycles. U6 was taken as the internal reference. All samples were repeared in 3 wells, and the results were analyzed via the $2^{-\Delta Ct}$ method. The qRT-PCR primers were synthesized by Suzhou Syobio Technologies Co., Ltd. (Suzhou, China). Primer sequences are shown in Table 1.

Enzyme-linked immunosorbent assay (ELISA)

MRP1, TGF-β1 and IL-8 proteins were detected by ELISA according to the kit instructions. Besides, all the detection kits were purchased from Shanghai Jingkang Biological Engineering Co., Ltd. (Shanghai, China).

Observational indexes

Changes in the expression levels of MRP1 mRNA as well as MRP1, TGF-β1 and IL-8 proteins before chemotherapy and at 1, 2, 4 and 8 weeks after chemotherapy were evaluated and analyzed. Correlations of MRP1 with clinicopathological features of patients and correlations among MRP1, TGF-β1 and IL-8 were assessed and analyzed.

Statistics

SPSS 19.0 [AsiaAnalytics (formerly SPSS China)] was applied. Count data were expressed as percentage, and compared using the x^2 test. Quantitative data were expressed as mean±standard deviation, and analyses

Table 1. Primer sequences

	Forward primer	*Reverse primer*
MRP1	5'-ATACCTGCTGTTCGGATTT-3'	5'-CGCATAGTGGATGGCTTT-3'
U6	5'-CGCTTCGGCAGCACATATAC-3'	5'-TTCACGAATTTGCGTGTCAT-3'

among multiple groups were performed using repeated analyses of variance (ANOVA). Correlations of MRP1 with patient clinicopathological features were analyzed via cross tabulation analysis. Pearson's correlation analysis was conducted to analyze correlations among MRP1, TGF-β1 and IL-8. P<0.05 showed that the difference was statistically significant.

Results

General data

There were 346 patients with breast cancer, including 161 (46.53%) cases aged less than 50 years and 185 (53.47%) aged more than 50 years. The tumor diameter was less than 5 cm in most cases (n=328; 94.80%), while 18 cases (5.20%) had a tumor diameter greater than 5 cm. Patients were histologically classified into grade I (85; 24.57%), grade II (111; 32.08%) and grade III (150; 43.35%). According to TNM staging system, patients were divided into stage I (132; 38.15%), stage II (128; 36.99%) and stage III (86; 24.86%). Among 346 patients, there were 146 (42.20%) cases with lymph node metastasis, 101 (29.19%) with blood vessel invasion and 48 (13.87%) with nerve invasion (Table 2).

QRT-PCR detection results of MRP1 mRNA

According to qRT-PCR detection results, the expression level of MRP1 mRNA was increased with the prolongation of chemotherapy time. The mean expression levels of MRP1 mRNA before chemotherapy and at 1, 2, 4 and 8 weeks after chemotherapy were 0.717±0.124 ng/mL, 0.759±0.126 ng/mL, 0.779±0.125 ng/mL, 0.834±0.131 ng/mL and 0.901±0.262 ng/mL, respectively, and there were statistically significant differences in the expres-

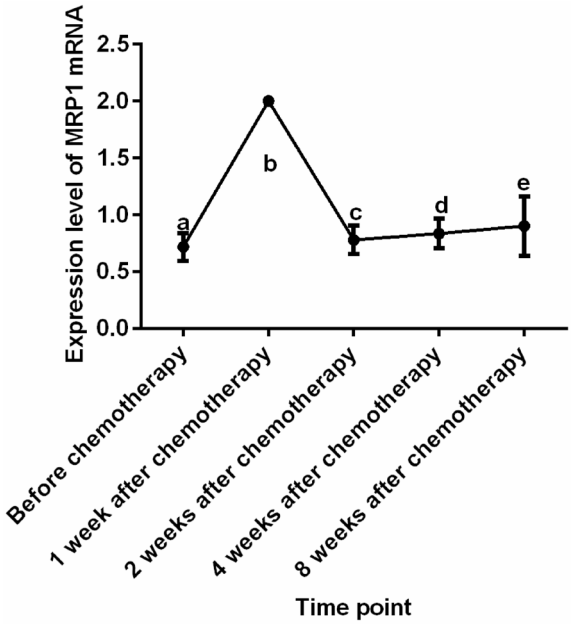

Figure 1. QRT-PCR detection results of MRP1 mRNA. QRT-PCR detection results show that the expression level of MRP1 mRNA is increased with the extension of chemotherapy time, and p<0.05 in pairwise comparisons among a, b, c, d and e.

Table 2. General information

Information	*n (%)*
Age, years	
≤50	161 (46.53)
>50	185 (53.47)
Tumor diameter (cm)	
≤2	209 (60.40)
>2, ≤5	119 (34.39)
>5	18 (5.20)
TNM staging	
I	132 (38.15)
II	128 (36.99)
III	86 (24.86)
Histological grading	
I	85 (24.57)
II	111 (32.08)
III	150 (43.35)
Lymph node metastasis	
Yes	146 (42.20)
No	200 (57.80)

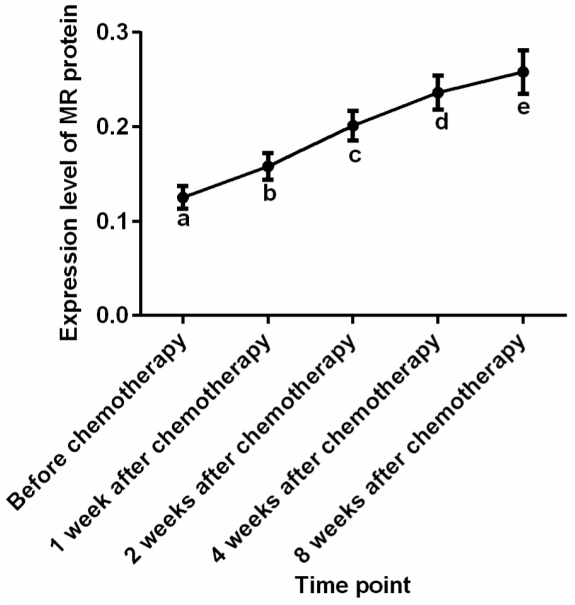

Figure 2. ELISA detection results of MRP1 protein. ELISA detection results reveal that the expression level of MRP1 protein goes up with the prolongation of chemotherapy time, and p<0.05 in pairwise comparisons among a, b, c, d and e.

sion level of MRP1 mRNA between each time points (p<0.05) (Figure 1).

ELISA detection results of MRP1 protein

Results of the ELISA detection revealed that the expression level of MRP1 protein rose up in parallel with the prolongation of chemotherapy time. The mean expression levels of MRP1 protein before chemotherapy and at 1, 2, 4 and 8 weeks after chemotherapy were 0.125±0.012 ng/mL, 0.158±0.014 ng/mL, 0.201±0.016 ng/mL, 0.236±0.018 ng/mL and 0.258±0.023 ng/mL, respectively, displaying statistically significant differences in the expression level of MRP1 protein at two time points (p<0.05) (Figure 2).

Correlations of MRP1 with clinical features of patients with breast cancer

The median of MRP1 mRNA expression level before chemotherapy was 0.134 ng/mL, and patients with expression level lower than the median were included into the low expression group 1, while those with the expression level higher than the median were included into the high expression group. Cross tabulation analysis results revealed that the high expression levels of MRP1 mRNA before and after chemotherapy were not related to age, tumor diameter, TNM stage, histological grade and lymph node metastasis (p>0.05) (Table 3).

ELISA detection results of TGF-β1

ELISA detection results revealed that the expression level of TGF-β1 protein went up with the prolongation of chemotherapy time. The mean expression levels of TGF-β1 protein before chemotherapy and at 1, 2, 4 and 8 weeks after chemotherapy were 5.26±1.38 ng/mL, 5.93±1.56 ng/mL, 6.47±1.76 ng/mL, 6.83±1.78 ng/mL and 7.65±2.01 ng/mL, respectively, with statistically significant differences in the expression level of TGF-β1 protein between each two time points (p<0.05) (Figure 3).

ELISA detection results of IL-8

ELISA detection results indicated that the expression level of IL-8 protein went up as the chemotherapy time prolonged. The mean expression levels of IL-8 protein before chemotherapy and at 1, 2, 4 and 8 weeks after chemotherapy were 76.56±13.42 pg/mL, 82.37±14.36 pg/mL, 88.12±15.25 pg/mL, 92.18±16.17 pg/mL and 101.98±18.44 pg/mL, respectively. The differences were statistically significant in the expression level of IL-8 protein between two time points (p<0.05) (Figure 4).

Table 3. Correlations of MRP1 protein expression with clinicopathological features of patients with breast cancer

Features	*n*	*Before chemotherapy*			*After chemotherapy*		
		Low expression group 1	*High expression group 1*	*p value*	*Low expression group 1*	*High expression group 1*	*p value*
Age, years				0.493			0.523
≤50	161	121	40		35	126	
>50	185	133	52		42	143	
Tumor diameter (cm)				0.467			0.619
≤2	209	146	63		58	151	
>2	137	101	36		34	103	
TNM staging				0.934			0.775
I	132	99	33		36	96	
II	128	91	37		41	87	
III	86	60	26		25	61	
Histological grading				0.862			0.114
I	85	60	25		30	55	
II	111	82	29		26	85	
III	150	110	40		51	99	
Lymph node metastasis				0.298			0.262
Yes	146	111	35		46	100	
No	200	142	58		52	148	

Correlations among MRP1, TGF-β1 and IL-8

According to Pearson's correlation analysis, the expression level of MRP1 mRNA was positively correlated with the expression levels of TGF-β1 (r=0.732, p=0.012) and IL-8 (r=0.709, p=0.018), showing a positive correlation between the expression level of TGF-β1 and that of IL-8 (r=0.714, p=0.015).

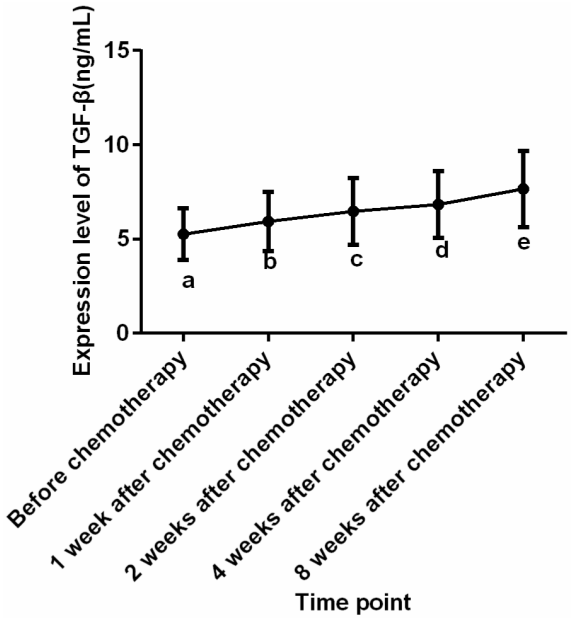

Figure 3. ELISA detection results of TGF-β1. ELISA detection results indicate that the expression of TGF-β1 protein rises up with the increase in chemotherapy time, and p<0.05 in pairwise comparisons among a, b, c, d and e.

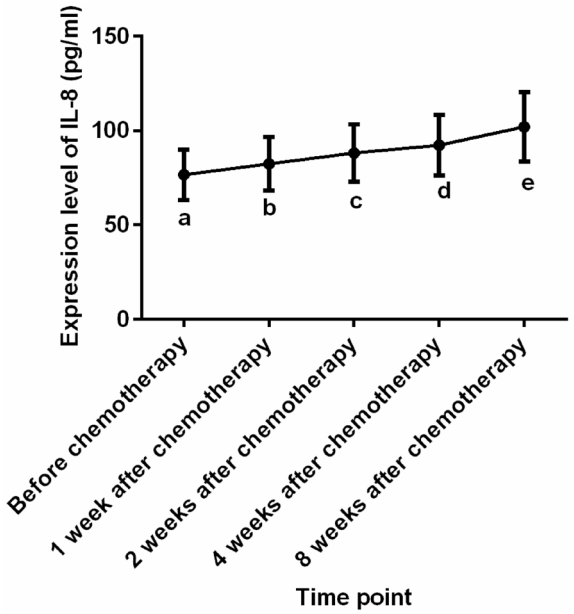

Figure 4. ELISA detection results of IL-8. ELISA detection results illustrate that the expression level of IL-8 protein is increased as the chemotherapy time is prolonged, and p<0.05 in pairwise comparisons among a, b, c, d and e.

Discussion

With the gradual increase in the incidence rate of breast cancer and the frequent application of chemotherapy, the therapeutic effect of chemotherapy for breast cancer has gradually become unsatisfactory [14,15]. MRP1 is a molecule that has become popular in recent years, which can protect tumor cells from being recognized and killed by immune cells and reduce the sensitivity to drugs [16,17]. However, there are few reports on the influence of MRP1 on the therapeutic effect of breast cancer chemotherapy, and its mechanism is still not clear. TGF-β1 has important regulatory effects on cell growth, differentiation and immune function [10]. Lin et al. [18] reported that TGF-β1 can participate in drug resistance in osteosarcoma cells by inducing miR-202 to inhibit apoptosis in human osteosarcoma cells. The main biological activity of IL-8 is to attract and activate neutrophils [11], but in recent years it has been found to be associated with tumor drug resistance. Fernando et al. [13] reported in a study that blocking IL-8 signal transduction can improve the acquired resistance to epidermal growth factor receptor (EGFR) inhibition in lung cancer patients, and enhance the chemotherapy outcome. There are few reports on the roles of TGF-β1 and IL-8 in the mechanism of drug resistance in breast cancer chemotherapy. This study aimed to investigate the mechanism of drug resistance in breast cancer through analyzing the changes in MRP1, TGF-β1 and IL-8 expression levels in breast cancer patients during chemotherapy, thus providing theoretical guidance for clinical breast cancer chemotherapy.

In this study, the expression levels of MRP1, TGF-β1 and IL-8 before chemotherapy and at 1, 2, 4 and 8 weeks after chemotherapy in 346 breast cancer patients undergoing chemotherapy were evaluated. The study results illustrated that the expression levels of MRP1, TGF-β1 and IL-8 gradually increased with the prolongation of chemotherapy time. Statistical analyses revealed that there were statistically significant differences in the expression levels of MRP1, TGF-β1 and IL-8 between two adjacent time points among 5 time points (before chemotherapy and at 1, 2, 4 and 8 weeks after chemotherapy). The degree of resistance to chemotherapy in breast cancer patients was not constant but increased with the prolongation of chemotherapy time [19,20]. Kim et al. [21] ascertained in a study that MRP1 can induce sensitivity of breast cancer cells to chemotherapy, and added that the results revealed that MRP1 expression level in breast cancer tissues increased after chemotherapy. Oshimori et al. [12] reported

in a study that TGF-β1 promotes drug resistance in squamous cell carcinoma. Wang et al. [22] also reported that inhibiting TGF-β1 can increase the sensitivity of lung cancer cells to chemotherapy. In their study Limpakan et al. [23] detected that as chemotherapy continues, the drug resistance in gastric cancer cells is gradually increased, but the IL-8 level is relatively low in gastric cancer cells remaining sensitive to chemotherapy. Their results are similar to those of the present study. Correlations among MRP1, TGF-β1 and IL-8 were further analyzed in this study, which showed that the expression level of MRP1 was positively correlated with the expression levels of TGF-β1 and IL-8. Therefore, it was believed that MRP1 might affect the therapeutic effect of chemotherapy for breast cancer patients and lead to drug resistance. TGF-β1 and IL-8 might be closely related to the mechanism of drug resistance in MRP1-mediated breast cancer chemotherapy. In order to detect the dynamic changes in the expression levels of MRP1, TGF-β1 and IL-8 in breast cancer patients during chemotherapy, the changes in the expression levels of MRP1, TGF-β1 and IL-8 in serum of patients with breast cancer were examined, which were different from the above studies but obtained similar results. This indicates that the detected roles of MRP1, TGF-β1 and IL-8 expression levels in the serum of breast cancer patients are similar to those in tumor tissues, which lays a theoretical foundation for clinically detecting and assessing the therapeutic effect of chemotherapy in breast cancer patients using serological tests in the future. However, due to the short research time, it was impossible to collect data on the therapeutic effect of chemotherapy in all patients, and the prognosis of all patients could not be evaluated more accurately. In addition, correlations of the expression level of MRP1 with clinical features of breast cancer patients were not noticed in this study, which might be due to the lack of serum detection and the insufficient number of patients included. These issues are expected to be explored in future studies to supplement the conclusions of this research.

In summary, with the prolongation of chemotherapy time for breast cancer patients, the expression level of MRP1 is also increased, which may affect the therapeutic effect of chemotherapy in breast cancer patient and lead to drug resistance. Besides, TGF-β1 and IL-8 may be closely related to the mechanism of drug resistance in MRP1-mediated breast cancer chemotherapy.

Conflict of interests

The authors declare no conflict of interests.

References

1. Lambertini M, Boni L, Michelotti A et al. Ovarian suppression with triptorelin during adjuvant breast cancer chemotherapy and long-term ovarian function, pregnancies, and disease-free survival: a randomized clinical trial. JAMA 2015;314:2632-40.
2. Moore HC, Unger JM, Phillips KA et al. Goserelin for ovarian protection during breast-cancer adjuvant chemotherapy. N Engl J Med 2015;372:923-32.
3. Courneya KS, Segal RJ, Vallerand JR et al. Motivation for different types and doses of exercise during breast cancer chemotherapy: A randomized controlled trial. Ann Behav Med 2016; 50:554-63.
4. Coates AS, Winer EP, Goldhirsch A et al. Tailoring therapies-improving the management of early breast cancer: St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2015. Ann Oncol 2015;26:1533-46.
5. Hiramatsu K, Katayama Y, Matsuo M et al. Multi-drug-resistant Staphylococcus aureus and future chemotherapy. J Infect Chemother 2014;20:593-601.
6. Wilson APR, Livermore DM, Otter JA et al. Prevention and control of multi-drug-resistant Gram-negative bacteria: recommendations from a Joint Working Party. J Hosp Infect 2016;92:S1-S44.
7. Yan J, Dang Y, Liu S et al. LncRNA HOTAIR promotes cisplatin resistance in gastric cancer by targeting miR-126 to activate the PI3K/AKT/MRP1 genes. Tumor Biol 2016;37:16345-55.
8. Liu H, Wu X, Huang J et al. miR-7 modulates chemoresistance of small cell lung cancer by repressing MRP1/ABCC1. Int J Exp Pathol 2015;96:240-7.
9. Wang H, Jia XH, Chen JR et al. HOXB4 knockdown reverses multidrug resistance of human myelogenous leukemia K562/ADM cells by downregulating P-gp, MRP1 and BCRP expression via PI3K/Akt signaling pathway. Int J Oncol 2016;49:2529-37.
10. Wei Y, Kim TJ, Peng DH et al. Fibroblast-specific inhibition of TGF-β1 signaling attenuates lung and tumor fibrosis. J Clin Invest 2017;127:3675-88.
11. Shang HG, Yu HL, Ma XN, Xu X. Multidrug resistance and tumor initiating capacity of oral cancer stem cells. JBUON 2016;21:461-5.
12. Oshimori N, Oristian D, Fuchs E. TGF-β promotes heterogeneity and drug resistance in squamous cell carcinoma. Cell 2015;160:963-76.
13. Fernando RI, Hamilton DH, Dominguez C et al. IL-8 signaling is involved in resistance of lung carcinoma cells to erlotinib. Oncotarget 2016;7:42031.

14. Untch M, Jackisch C, Schneeweiss A et al. Nab-paclitaxel versus solvent-based paclitaxel in neoadjuvant chemotherapy for early breast cancer (GeparSepto-GBG 69): a randomised, phase 3 trial. Lancet Oncol 2016;17:345-56.
15. Kirshner JJ, McDonald MC, Kruter F et al. NOLAN: a randomized, phase 2 study to estimate the effect of prophylactic naproxen or loratadine vs no prophylactic treatment on bone pain in patients with early-stage breast cancer receiving chemotherapy and pegfilgrastim. Supportive Care Cancer 2018;26:1323-34.
16. Jakacki R. Treatment strategies for high-risk medulloblastoma and supratentorial primitive neuroectodermal tumors. Review of the literature. J Neurosurg 2005;102:44-52.
17. Kovacevic Z, Sahni S, Lok H et al. Regulation and control of nitric oxide (NO) in macrophages: Protecting the "professional killer cell" from its own cytotoxic arsenal via MRP1 and GSTP1. J BBA-GEN Subjects 2017;1861:995-9.
18. Lin Z, Song D, Wei H et al. TGF-β1-induced miR-202 mediates drug resistance by inhibiting apoptosis in human osteosarcoma. J Cancer Res Clin 2016;142:239-46.
19. Perez EA, Romond EH, Suman VJ et al. Trastuzumab plus adjuvant chemotherapy for human epidermal growth factor receptor 2–positive breast cancer: planned joint analysis of overall survival from NSABP B-31 and NCCTG N9831. J Clin Oncol 2014;32:3744-52.
20. Swain SM, Baselga J, Kim SB et al. Pertuzumab, trastuzumab, and docetaxel in HER2-positive metastatic breast cancer. N Engl J Med 2015;372:724-34.
21. Kim B, Stephen SL, Hanby AM et al. Chemotherapy induces Notch1-dependent MRP1 up-regulation, inhibition of which sensitizes breast cancer cells to chemotherapy. BMC Cancer 2015;15:634.
22. Wang J, Chen Y, Xiang F et al. Suppression of TGF-β1 enhances chemosensitivity of cisplatin-resistant lung cancer cells through the inhibition of drug-resistant proteins. Artif Cells Nanomed Biotechnol 2017;18:1-8.
23. Limpakan S. Chemotherapy sensitivity, cytokine IL-8 level, and genetic expression mRNA IL-8 in gastric cancer treatment response prediction. Cancer Res 2017;77:2052.