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The tissue transglutaminase: a potential target regulating MDR in breast cancer

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Abstract. – OBJECTIVE: Multi-drug resistance (MDR) is the main obstacle influencing the anti-tumor effect in breast cancer. To date, no proper potential targets are found to overcome MDR. Here, tTG was explored to show whether it is a potential target to regulate MDR in breast cancer.

MATERIALS AND METHODS: tTG was silenced by small interfere siRNA. After that, the mRNA level of CD44, CD24, LRP, MRP and MDR1 were detected by RT-PCR. The Western blot analysis was used to detect the expression of LRP, P-gp and MRP. In addition, the impact of tTG on cell apoptosis, as well as cell proliferation were observed. Finally, to evaluate the role of tTG in BALB/c nude mice, the growth of tumor was performed, and the immunohistochemistry analysis was used to observe the expression of LRP, P-gp and MRP *in vivo*.

RESULTS: In MCF-7/ADR, Compared to MCF-7, tTG expression was highly increased. After silencing tTG, the mRNA level and the protein level of P-gp, MRP, LRP were both differently decreased. The mRNA level of CD44 and CD24 was also down-regulated after silencing tTG. In addition, the cell proliferation was significantly inhibited in the ADR + tTG siRNA+Adriamycin group ($p<0.05$), and the tumor growth was prevented in a time-dependent situation. Cell apoptosis was significantly strengthened in the ADR+tTG siRNA+Adriamycin group ($p<0.05$). *In vivo*, the growth of tumors was reduced after silencing tTG, and the LRP, P-gp and MRP expression were significantly down-regulated in ADR + tTG SiRNA +adriamycin group ($p<0.05$).

CONCLUSIONS: It is concluded that the tTG may be a potential target regulating the MDR by regulating LRP, P-gp and MRP expression as well as the expression of CD44CD24 to improve the MDR in breast cancer.

Key Words:
Breast cancer, tTG, Multi-drug resistance, CD44, CD24.

Abbreviations

tTG: Tissue transglutaminase; MDR: multi-drug resistance; ECM: extracellular matrix; ABC: ATP-binding cassette transporters; P-gp: P-glycoprotein; MRP: MDR-related protein; LRP: lung resistance-related protein; CSCs: cancer stem cells; BCSCs: breast cancer stem-like cells; NABC: non-ATP-binding cassette transporters; siRNA: small interfering RNA; MVP: major vault protein.

Introduction

Female breast cancer is very common in women worldwide. Early diagnosis and systematic treatment lead to a general downward trend in the death rate of the disease, of which chemotherapy is greatly important. However, recurrence of cancer still accounts for more than 90% as a result of multi-drug resistance (MDR) to chemotherapy and finally results in the failure of treating the disease[1]. Until now, there are still no effective targets that can overcome MDR.

Tissue transglutaminase (tTG, TGM2), as one member of the transglutaminase family, is a kind of Ca^{2+}-dependent ubiquitous multifunctional enzyme. It is reported that tTG can catalyze the GTPase and cross-linking activity through different active sites[2]. tTG is involved and plays an important role in a series of important medical course, such as cell apoptosis, growth, migration, by promoting interaction with the extracellular matrix (ECM)[3]. It is reported that tTG had a relationship with drug resistance in cancer cells[4]. Han et al[5] showed that tTG expression was associated with increased invasion and resistance to chemotherapy in prostate cancer cells. Mehta et al[6] demonstrated that both multidrug-resistant breast cancer cells and the primary tumors from patients

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exhibited high levels of tTG. tTG was important for drug metabolism, which has the ability to catalyze irreversible conjugation of several amines to glutamine residues of certain proteins[7]. A few anti-cancer drugs, such as adriamycin (ADR), actinomycin D and bleomycin have also been demonstrated to serve as amine substrates for tTG. It is probable that the efficacy of the drugs is closely correlated with the presence of tTG in targeted cells[8].

At the same time, the membrane ATP-binding cassette transporters (ABC), as a widely studied mechanism of MDR, can increase the efflux of chemotherapeutic agents from cells to influence drug metabolism, leading to MDR. P-gp and MRP are the most widely researched in the family of ABC. P-gp is the best characterized protein of the ABC family because of its important role in conferring a MDR phenotype. In many types of cancer cells, the highly expression of P-gp could cause the up-regulation of drug efflux pumps, leading to the adaptation to the presence of xenobiotics, as well as a prolonged survival. In addition to the function of drug efflux pumps, P-gp may be correlated with several signal way, such as Erk1/2 pathway, to induce drug resistance[9]. MRP was also involved in ATPdependent efflux of xenobiotics across the cell membrane. Moreover, MRP is mainly lipophilic anionic transporter and is reported to transport free or conjugates of glutathione (GSH). In the presence of free GSH, MRP can transport neutral organic drugs in free form[10]. Lung resistance-related protein (LRP), not belonging to ABC, is considered to be human of the major vault protein (MVP)[11]. It is reported that LRP expression has a direct relationship with MDR through mainly mediating the anti-tumor drug transported from the nucleus to cytoplasm[12].

Therefore, we speculate tTG might be associated with ABC (such as P-glycoprotein, MDR-related protein) and NABC (such as LRP). In this study, we observed a relation between tTG and P-gp, MRP, LRP. Of note, the over-expression of tTG is demonstrated to confer cancer stem cells (CSCs) traits in breast cancer cells. He et al[13] indicated that tTG may be a potential target for drug sensitivity of chemotherapy. As a result, we assume that tTG might have an effect on the marker of BCSCs. The relation between tTG and the marker of BCSCs was also explored. The mechanism of tTG regulating the MDR in breast cancer was preliminarily researched in the content.

Materials and Methods

Cell Culture

The MCF-7 cells and the MCF-7/ADR cells were obtained from KGI Biological Technology Development Company (Nanjing, China). They were cultured in Roswell Park Memorial Institute-1640 (RPMI-1640) (KGM31800S-500) (HyClone, South Logan, UT, USA) including 10% fetal bovine serum (FBS) (Gibco, Rockville, MD, USA) at 37°C and 5% CO_2. Apart from the condition above, the phenotype of multidrug resistance was kept with 1 μg/mL. Before use, they were grown in drug-free medium for two days or more.

tTG silencing by siRNA

A small interfering RNA (siRNA) duplex targeting TGM2, (forward: 5'-GGCUGAAGAUCAGCACUAATT-3', reverse: 5'-UUAGUGCUGAUCUUCAGCCTT-3') (Invitrogen, Carlsbad, CA, USA) was embedded into the cells by X-tremeGENE siRNA transfection reagent according to the manufacturer's instruction. 6 h, 36 h 48 h after that, the cells were collected, and immunofluorescence detection was performed. Cells were incubated with X-tremeGENE siRNA transfection reagent and the Stealth Negative control was regarded as the negative control. To verify the efficiency of transfection, we used negative control FAM (forward: 5'-UUCUCCGAACGUGUCACGUTT-3', reverse: 5'-ACGUGACACGUUCGGAGAATT-3'). According to the manufacturer's instruction, immunofluorescence was used to detect transfection efficiency after 12 h.

The Cell Proliferation Detection by MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyl-Tetrazolium Bromide) Assay

The MCF-7 and the MCF-7/ADR cells were inoculated at $1x10^4$ cells/well in 96 well culture plates. The cells were divided into 5 groups: MCF-7 group, ADR group, ADR+adriamycin group, ADR + tTG siRNA group, and ADR + tTG SiRNA +adriamycin group. The condition was kept at 37°C for 24, 48, 72 h. Each well added 20 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT, 5 mg/mL, Sigma-Aldrich, St. Louis, MO, USA), and then hatched at 37°C for 4 h. A microplate reader (Bio-Rad, Hercules, CA, USA) was used to measure absorbance values at 490 nm. The courses were repeated four times in the same way.

Apoptosis Assay

The Cells were inoculated at $6x10^4$ cells/well in 6-well plates. The cells were divided into 5 groups: MCF-7 group, ADR group, ADR+adriamycin group, ADR + tTG siRNA group, and ADR + tTG siRNA +adriamycin group. After that, they were resuspended using 1x binding buffer at a concentration of 1×10^6 cells per 1 mL, then transferred 100 μL of the solution into a 5 mL culture tube, and added 5 μL of annexin V-FITC and 5 μL of propidium iodide (PI). After the cells vortexed gently, they were incubated for 15 min at room temperature in the dark. At last, 400 μL of 1x binding buffer was added to each tube, and FACScan flow cytometry was used to analyze the samples. According to the manufacturer's instructions of the Annexin V-FITC Apoptosis Detection Kit (BD Biosciences, Mountain View, CA, USA), the analysis of annexin V binding was carried out. BD FACS Calibur (BD Biosciences, Mountain View, CA, USA) was employed to analyze samples.

RNA Extraction As Well As Reverse Transcription Quantitative Polymerase Chain Reaction (RT-PCR)

The mRNA levels of CD44, CD24, as well as LRP, MDR1, MRP were measured by RT-PCR. The MCF-7, ADR and ADR + tTG siRNA groups were washed twice with cold phosphate-buffered saline (PBS) and gathered by scraping. Total RNA was separated from 6-well plates by the TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocols, and then subjected to a reverted fist strand complementary deoxyribose nucleic acid (cDNA) synthesis kit and realmaster mix (TaKaRa, Otsu, Shiga, Japan). Next, resultant cDNA was dissolved in 20 μL diethylpyrocarbonate (DEPC)-H_2O used in the following PCR reactions. The primers used in the study are listed in Table I. The RT-PCR reaction was detected through the SYBR green detection system (Thermo Scientific, Waltham, MA, USA). β-Actin served as a negative control. All cycle threshold values were ensured in real time using CFX96™ Real-Time PCR Detection (Bio-Rad, Hercules, CA, USA). The data were analyzed by $2^{-\Delta\Delta Ct}$.

Western Blot Analysis

The cell lysates were prepared in radioimmunoprecipitation assay (RIPA) buffer (Beyotime, Shanghai, China). Protein concentrations were confirmed through bicinchoninic acid (BCA) assay (Beyotime, Shanghai, China) using spectrophotometer at 562 nm. The proteins were isolated on 8% sodium dodecyl sulphate (SDS)-polyacrylamide gel and shifted onto the polyvinylidene difluoride (PVDF) membranes (Bio-Rad, Hercules, CA, USA). After enclosing with 5% nonfat dry milk, we washed the membranes by Tris-Buffered Saline and Tween (TBST) including 0.1% Tween 20 and incubated with 1:500 diluted mouse anti P-gp antibody and 1:5000 diluted mouse anti β-actin antibody all night at 4°C. The membranes were washed and then incubated for 2h with respective peroxidase conjugated anti mouse secondary antibodies (1:2,000), washed through Tween 20 with 0.1% in TBS. The blots were observed using strengthened chemiluminescence reagents (Bio-Rad, Hercules, CA, USA). The relative photographic density was quantitated by a gel documentation and analysis system (Glyko, Hayward, CA, USA). The membranes of the LRP were incubated with 1:1000 diluted rabbit anti LRP antibody, and the anti-rabbit antibodies (1:200) were as secondary antibodies. The membranes of the MRP were incubated with 1:1000 diluted rabbit anti LRP antibody, and the secondary antibodies were anti rabbit antibodies (1:2000). The tTG membranes were incubated with 1:1000 diluted rabbit anti LRP antibody, and anti-rabbit antibodies (1:2000) were as the secondary antibodies. Densitometric analysis of the bands was performed through image J software (NIH, Bethesda, MD, USA) and all values were normalized to β-actin.

Xenograft Tumor Model Study

To investigate the role of tTG in regulating MDR *in vivo*, animal experiments were carried out. Male BALB/c nude mice with weight 18-22 g and 6-8 weeks were obtained from the SPF Laboratory Animal Center of Beijing Vital River Laboratory. The tumor models were made by injecting 1×10^6 MCF-7 /ADR cells from medium containing 50% Matrigel into the right axilla of the nude mice. The volume of tumors reached 100 mm^3, and the nude mice were divided into 4 groups (n=5) randomly. Methods of the treatment were divided into four group as follows: ADR group, ADR + tTG SiRNA group, ADR+adriamycin group, and ADR + tTG SiRNA (1 nmol/20 g) +Adriamycin (once weekly 4 mg/kg by intraperitoneal injection) group. All mice were injected 12 times over a 24-hr interval intravenously. To observe anti-tumor efficacy, all mice were killed, and then tumors were excised. The weight of the tumors was measured, and the tumors were pho-

tographed at the end of experiment (28 days). All experiments above were performed in compliance with NIH guidelines for caring and using research animals and approved by the Animal Ethics Committee of Binzhou Medical University. Moreover, the expression of tTG and LRP P-gp, MRP was evaluated with the method of SP immunohistochemical through rabbit-anti-human monoclonal antibody, as well as an Histostain™- SP Kit (SPN 9001 ZSGB-BIO, Beijing, China). The expression of tTG was detected 3 times through serial sections from the same paraffin-embedded tissues, and 3 different places under the microscope (×200) for every slide were randomly selected for semi-quantitative scoring by 2 pathologists in a blinded manner. Then, the scores were recorded as means±SD (standard deviation) and analyzed by one-way analysis of variance (ANOVA).

Statistical Analysis

The whole data were expressed as means ± SD. The Student's *t*-test was analyzed to compare two groups. Comparison between multiple groups was done using One-way ANOVA test followed by Post-Hoc Test (Least Significant Difference). Values of $p<0.05$ were deemed to be significant.

Results

Immunofluorescence Was Used to Detect Transfection

The cellular uptake of FAM-siRNA complexes was analyzed to determine the transfection efficiency in MCF-7/ADR. We observed FAM-siRNA within MCF-7/ADR after 6 hours of incubation. The immunofluorescence microscopy technology showed that siRNA was successfully transfected into cells and the transfection rate reached 90% (Figure 1A). The expression of tTG and its encoded gene TGM2 was down-regulated in MCF-7/ADR through RT-PCR (Figure 1B), Western blot respectively after transfection (Figure 1C and Figure 1D).

Cell Growth of MCF-7/ADR and MCF-7 Cells

The MCF-7/ADR, as well as MCF-7 cells, were cultured in the four groups for 24, 48 or 72 h. The data showed that the MCF-7+ adriamycin group cells growth was inhibited more significantly. The cell growth was significantly inhibited in the ADR + tTG SiRNA +adriamycin group, with the difference between ADR + tTG siRNA +Adriamycin and the other two groups (ADR+adriamy-

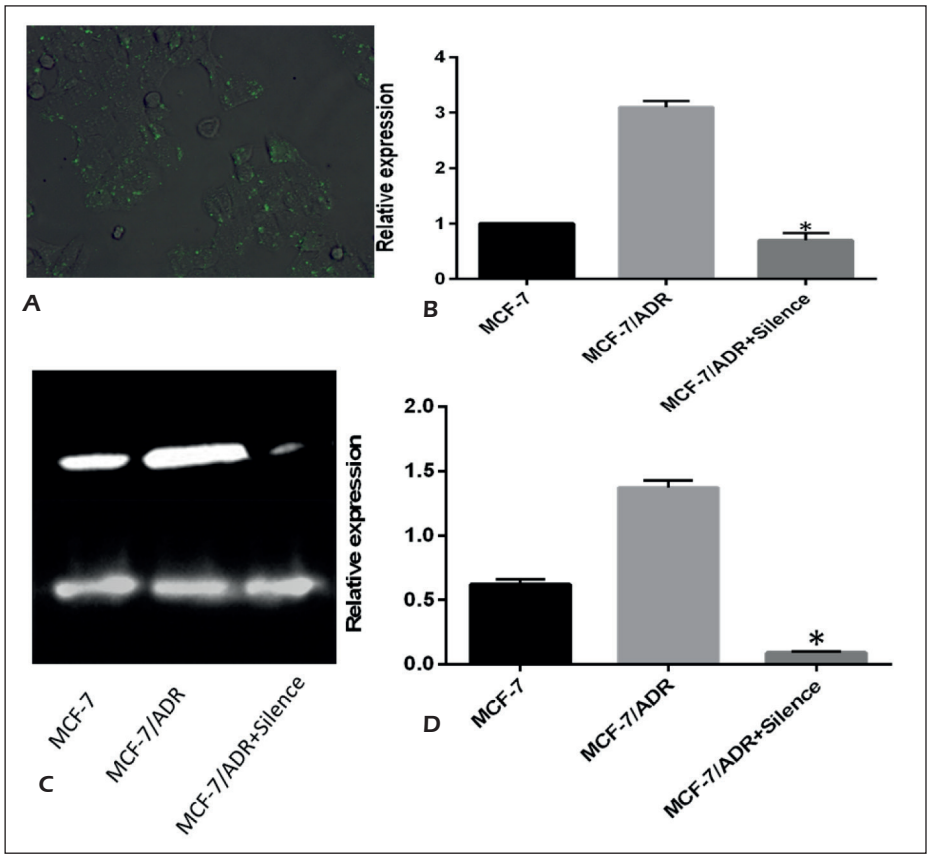

Figure 1. The successful transfection was performed. **A**, A small interfering RNA is used to silencing TGM2 gene. The immunofluorescence microscopy technology showed that siRNA was successfully transfected into cells. **B**, TGM2 was detected by real time-PCR in MCF-7, MCF-7/ADR, and MCF-7/ADR + silence. The expression of TGM2 was significantly down-regulated after tTG silencing. **C-D**, The expression of tTG in MCF-7, MCF-7/ADR and MCF-7/ADR+silence was detected by the Western blot. The figure showed that the expression of tTG was significantly down-regulated by silencing.

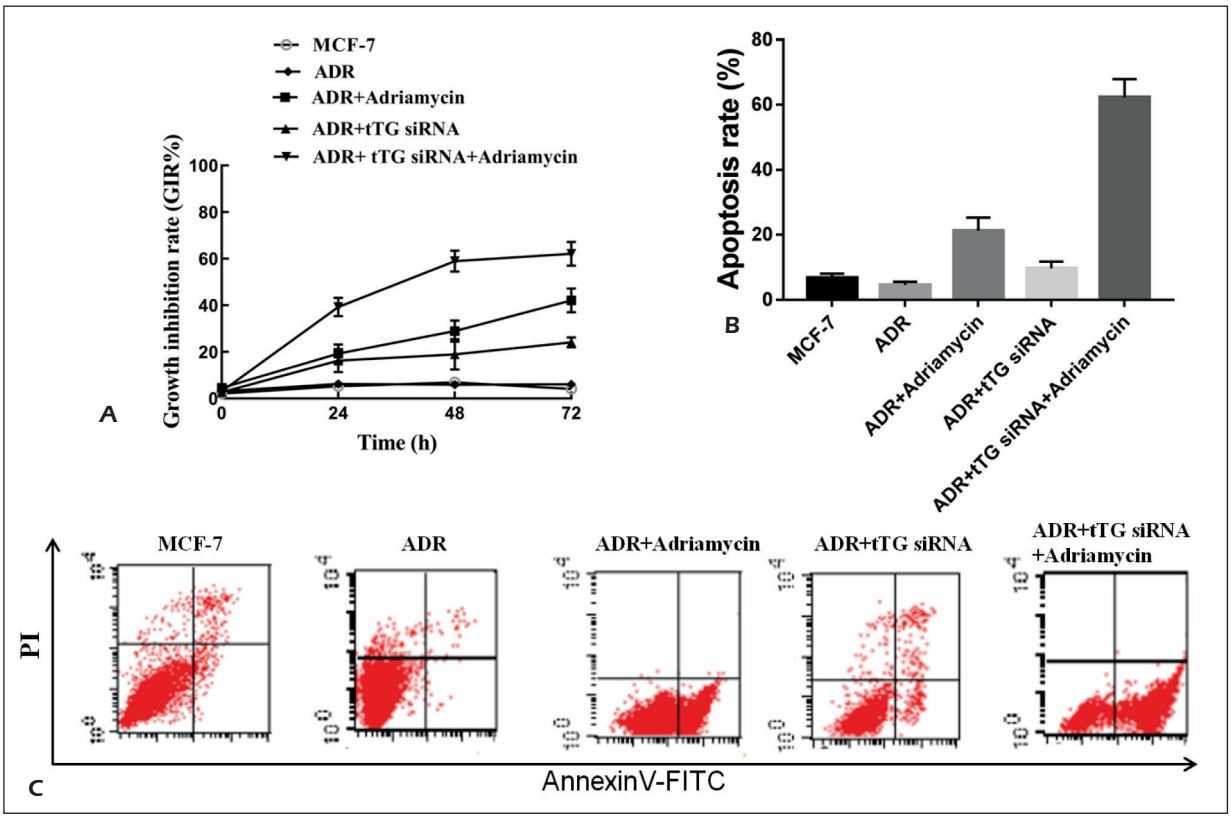

Figure 2. A, The growth inhibition rate was detected by MTT in the five groups (MCF-7, ADR, ADR+adriamycin group, ADR + tTG SiRNA, and ADR + tTG SiRNA +adriamycin). The growth of the cells was significantly inhibited in the ADR + tTG SiRNA +adriamycin group ($p<0.05$). The growth inhibiting rate increased in a time-dependent manner. **B-C**, The apoptosis of MCF-7/ADR cells was detected by flow cytometry. The apoptosis of MCF-7/ADR cells was significantly enhanced in the ADR + tTG SiRNA +adriamycin (64.38±5.27%) compared with the ADR+Adriamycin (24.93±1.1%), ADR + tTG SiRNA group (12.39±0.67%) ($p<0.05$).

cin group and ADR + tTG SiRNA group) was significant at 72 h ($p<0.05$). Furthermore, the growth inhibiting rate was increased in a time-dependent manner (Figure 2A).

Cell Apoptosis of MCF-7/ADR and MCF-7

The cell apoptosis of MCF-7/ADR was significantly enhanced in the ADR + tTG siRNA+Adriamycin (64.38±5.27%) compared with the ADR+Adriamycin (24.93±1.1%), ADR + tTG siRNA group (12.39±0.67%) ($p<0.05$). ADR (2.56±0.13%) also enhance the apoptosis, but the effect of the ADR + tTG siRNA +adriamycin group was the strongest. And the apoptosis rate of MCF-7 was 3.15±0.18% (Figure 2B and Figure 2C).

tTG Silencing Down-Regulated LRP, P-gp, MRP Expression and Their Corresponding Encoded Gene

To detect whether tTG silencing affects the mRNA levels of LRP, MDR1, MRP, as well as LRP, P-gp, MRP expression, RT-PCR and the Western-blot were performed, respectively. Western-blot showed that LRP, P-gp, MRP expression was significantly down-regulated after successful tTG silencing (Figure 3A and Figure 3B). The RT-PCR results showed that the mRNA levels of LRP, MDR1, and MRP were significantly decreased after successful tTG silencing (Figure 3C).

tTG Silencing Down-Regulated the Levels of CD44, CD24

To detect whether tTG silencing affects CD44 and CD24 in MCF-7/ADR. We used RT-PCR to test the levels of CD44 and CD24. The results show that the levels of CD44 and CD24 were down-regulated significantly after successful tTG silencing (Figure 3D).

Silencing tTG Enhanced the Sensitivity to Adriamycin on MCF-7/ADR Xenografts

tTG expression within tumors was detected. Immunohistochemistry was used to detect tTG expression in tissue samples of the xenograft. tTG

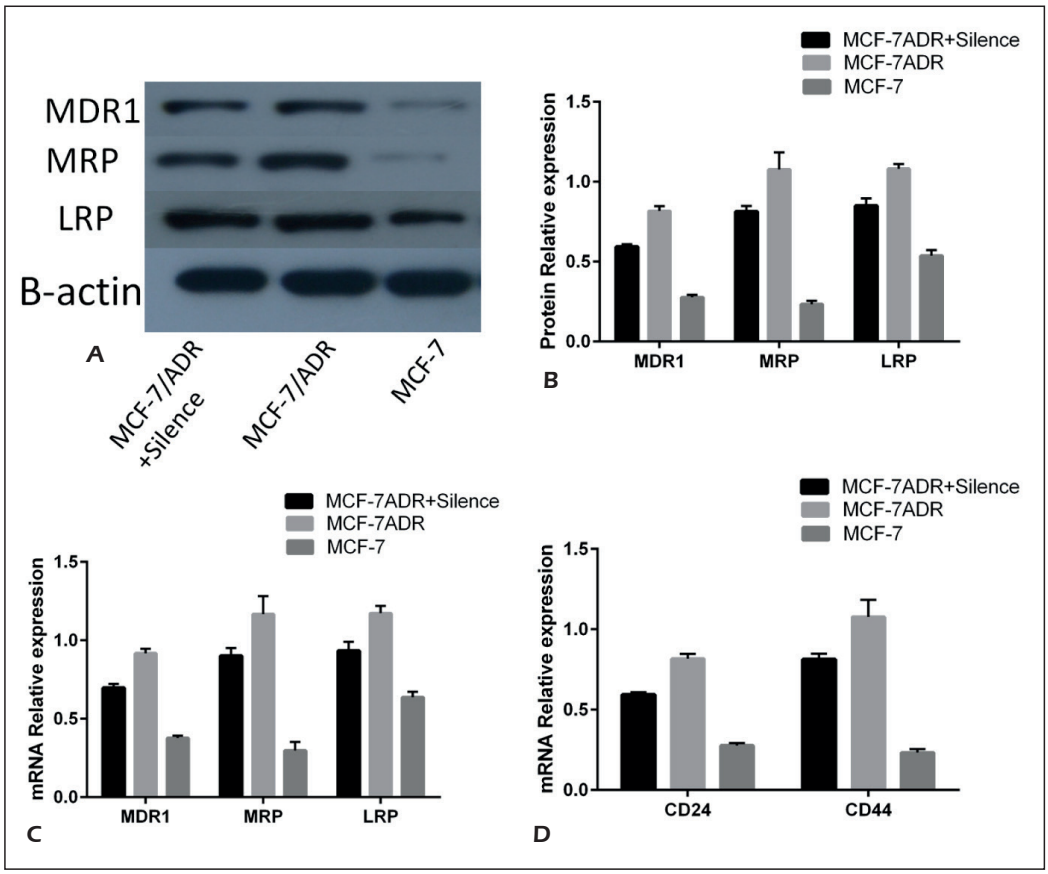

Figure 3. **A-B**, The expression of P-gp, MRP, LRP was detected by the Western blot before and after tTG silencing, respectively. The figure showed that the expression of P-gp, MRP, LRP was significantly down-regulated after tTG silencing ($p<0.05$). **C**, The encoded gene of P-gp (ABCB1), MRP (ABCC1), LRP were detected by the RT-PCR before and after tTG silencing respectively. The figure showed that the encoded gene of P-gp (ABCB1), MRP (ABCC1), LRP were significantly decreased after tTG silencing ($p<0.05$). **D**, The RT-PCR was performed to test the levels of CD44 and CD24. The levels of CD44 and CD24 were down-regulated significantly after tTG silencing ($p<0.05$).

was significantly expressed in the xenograft tumors, and tTG was significantly down-regulated in ADR+tTG siRNA group and ADR+tTG siRNA+adriamycin group. The weights of tumors were 1.31 ± 0.12 g (ADR group), 0.86 ± 0.08 g (ADR+tTG-siRNA group*), 0.61±0.04 (ADR+adriamycin group), 0.43±0.06 (ADR+tTG-siRNA+adriamycin group*), respectively (Figure 4A). LRP, P-gp and MRP expression was down-regulated after tTG silencing in ADR + tTG SiRNA +adriamycin ($p<0.05$) (Figure 4B and Figure 4C).

Discussion

There is a downward trend in death rate of breast cancer with the development of chemotherapy, endocrine-therapy and radiotherapy. To most of the patients with breast cancer, chemotherapy is necessary to improve prognosis of breast cancer. However, MDR is still an important obstacle, as well as a main cause of recurrence in breast cancer patients. tTG is a ubiquitous multifunctional enzyme, which plays a role in many fields. Some studies showed that tTG expression probably strengthen MDR in many types of cancer cells, and it was 150 times greater in MCF-7/ADR than the wild-type MCF-7[14]. In this study, tTG expression and its encoding gene TGM2 was significantly elevated in MCF-7/ADR, compared to MCF-7. This preliminarily indicated that tTG may play a part in the drug-resistance phenotype. Herman et al[15] shows similar results that tTG might play a part in the drug-resistance phenotype of breast cancer. Furthermore, tTG was silenced by siRNA in order to explore its role in drug resistance. The results of the immunofluorescence showed that siRNA was successfully transfected into cells.

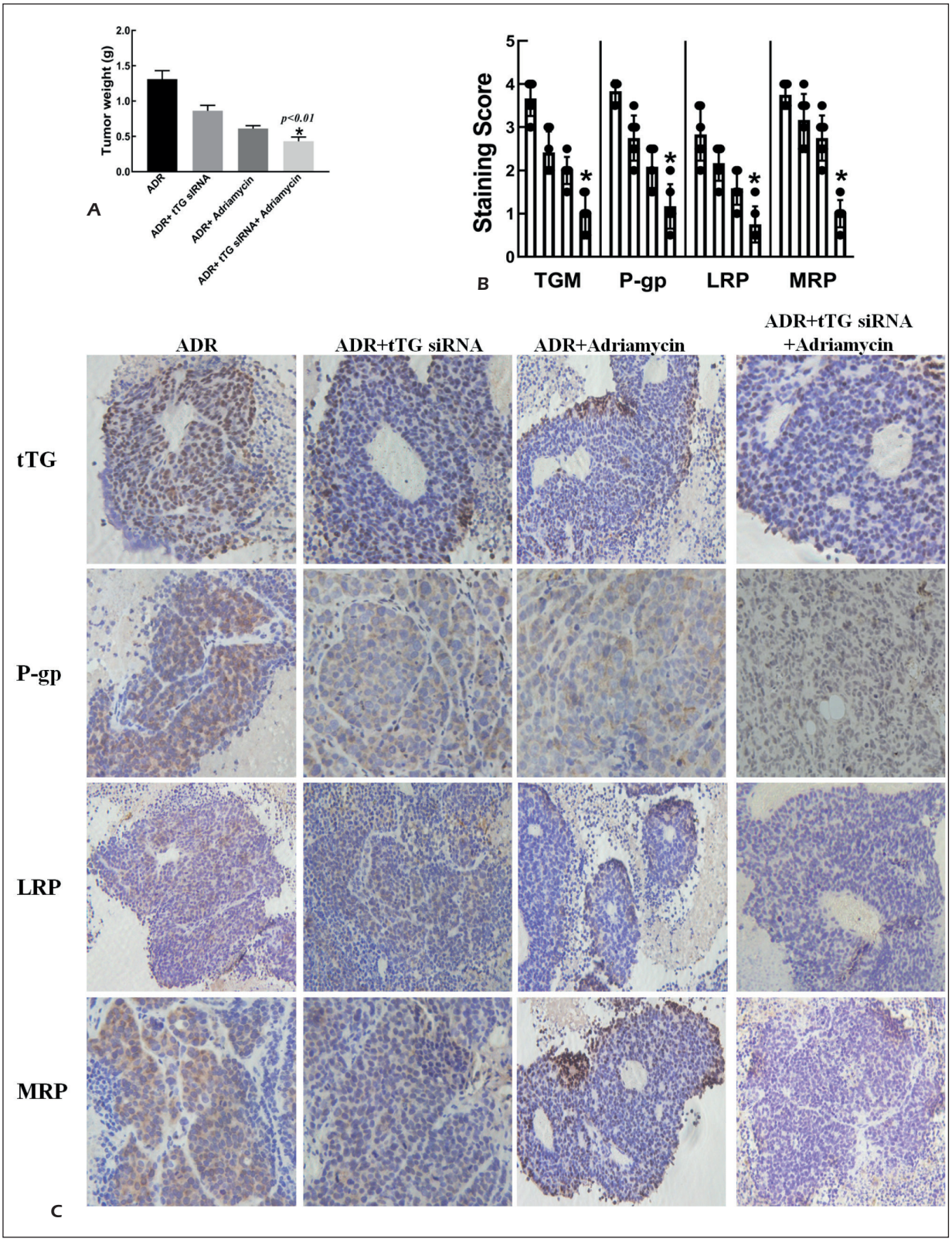

Figure 4. A, The weight of tumors in the mice of all groups. The tumor was significantly inhibited in ADR+tTG siRNA+adriamycin group ($p<0.05$). **B**, The statistic figure of immunohistochemical analysis. There is significant difference between ADR+tTG siRNA+adriamycin group and the other three groups. **C**, Immunohistochemical analysis for the expression of tTG, P-gp, MRP and LRP (magnification, 400x) for mice of all groups. After tTG silencing, the expression of tTG, P-gp, MRP and LRP were significantly down-regulated within tumors.

After silencing, the expression of tTG was greatly down-regulated with the down-regulation of its encoded gene TGM2. These suggested that transfection is a successful course. *In vivo*, tTG was significantly down-regulated after successful silence. To explore the cell growth inhibition, we used MTT assay to measure the growth inhibition rate in the five groups. The results of the MTT assay revealed that silencing tTG only may inhibit cell proliferation in MCF-7/ADR. After silencing tTG, treatment with a combination of adriamycin reduced the viability of MCF-7/ADR effectively *in vitro*. Simultaneously, the cell growth was inhibited in a time-dependent way. The results showed that silencing tTG might have effects on reversing the MDR in MCF-7/ADR.

Yuan et al[16] identified that tTG may be a potential target to promote cell death and chemosensitivity in glioblastomas. Hwang et al[17] reported that tTG siRNA combined with docetaxel had a greater effect than that control with docetaxel in ovarian cancer. In this study, the cell apoptosis of MCF-7/ADR was detected to assess the effect of tTG. The results suggested that down-regulation of tTG expression might promote cell apoptosis compared to ADR group. It is found that tTG siRNA combined with adriamycin had a more significant efficacy than adriamycin alone in MCF-7/ADR. Data demonstrated that silencing tTG is correlated with an enhanced anti-tumor role in chemotherapy. Fraij et al[18] revealed the similar results that transfection of tTG in tumor cells could induce apoptosis and sensitize the MCF-7, as well as T47D cells to chemotherapeutic treatment.

One of the major factors causing MDR is ABC, such as P-gp, MRP, which promote chemotherapeutic drug efflux contributing to failure in treatment finally[19]. Except for ABC above, LRP, belonging to non- ATP-binding cassette transporters (NABC), is another factor responsible for MDR. It is found first in a MDR cell line, regarded as a major vault protein. It is showed that doxorubicin is redistributed from the nucleus to the cytoplasm as a result of LRP over-expression in colon carcinoma[20]. Krisnamurti et al[21] showed that LRP, MRP P-gp were expressed both in MDR cell lines of breast cancer and in breast cancer patients clinically. As a result, searching for the reversal agents to overcome transporter-mediated MDR and improve the sensitivity of diverse chemical drugs could be a valuable strategy. tTG, encoded by TGM2, is located on human chromosome 20q11-12. It is a complex protein with multiple functions in intracellular and extracellular environment. Just as the complexity of tTG, the function, structure, and /or stability of many intracellular and extracellular proteins may be catalyzed by interaction with tTG. So, we speculated that tTG might have an effect on the ABC and NABC. In this study, the mRNA levels of LRP, MDR1, MRP and LRP, P-gp, MRP expression were down-regulated when tTG was silenced by siRNA. As a result, it is suggested that tTG may play a part in reversing MDR by down-regulating LRP, P-gp, MRP expression, as well as the mRNA levels of MDR1, MRP, LRP, leading to reduce the ability of efflux. MRP and P-gp belong to ABC, but LRP was NABC. tTG may probably have exerted a wide influence over reversing MDR by regulating both ABC and NABC. *In vivo* study, the same results were showed as that *in vitro* study. The weights of tumors were significantly decreased, and the immunohistochemical detection suggested that LRP, P-gp, MRP expression were also down-regulated in xenografts after silencing tTG. Compared to ADR group, LRP, P-gp, MRP expression were down-regulated in ADR + tTG siRNA group. This showed that silencing tTG may reverse MDR by regulating LRP, P-gp and MRP. Simultaneously, there is difference between ADR+tTG siRNA+adriamycin and ADR+ adriamycin group. This shows that silencing tTG may enhance the sensitivity of adriamycin. As a result, it is demonstrated that tTG may be a potential target on reversing MDR to improve the sensitivity of adriamycin in breast cancer.

Cancer stem cells are another important reason for MDR[22]. The CD44+/CD24−/low phenotype is considered to be characterized as BCSCs. Though the CD44+/CD24−/low cells account for a small group in total cancer cells, they are probably the source of cancer relapse and metastasis, and resistance to therapy. Li et al[23] showed that the MCF-7 cells with CD44+/CD24−/low caused drug resistance by undergoing adaptive changes after therapy. Our team demonstrated that the MCF-7 cells with CD44+/CD24−/low may be resistant to chemotherapy as a result of the cells with CD44+/CD24−/low being in the G0/G1 cell cycle[24]. In addition, ABC including P-gp and MRP are over-expressed on BCSCs. LRP expression was found to be significantly enhanced in lung cancer stem cells. Hence, the elimination of BCSCs may reverse MDR and improve the sensitivity to chemotherapy. Identifying some genes over-expressed in cancer stem cell can be a good strategy that changes the characteristic of CSCs to improve the curative effect of cancer. Kumar et al[22] demonstrated that tTG conferred stem

cell like properties, and sustained expression of tTG was associated with increase in CD44+/CD24low/- subpopulation in mammary epithelial cells. The down-regulation of tTG was associated with a decrease in the number of CD44+/CD24low/- subgroup. tTG plays an important part in keeping cancer stem cell survival, invasive, and metastatic behavior. In this study, the expression of CD44 was markedly decreased after tTG silenced. The results suggested that silencing tTG caused a decreased expression of CD44. And the number of CD44+/CD24low/- cells may be probably decreased. In addition, LRP, P-gp, MRP expression and their corresponding encoded gene were down-regulated after tTG was silenced, and the cell apoptosis of MCF-7/ADR was significantly increased in the ADR + tTG siRNA + Adriamycin group compared to the ADR + Adriamycin group. It is suggested that the decreased expression of CD44+/CD24low/- may contribute to reversing MDR and improving the sensitivity to chemical drug to some degree. The characteristic of breast cancer stem cell may also be changed as a result of the down-regulated CD44 expression, and more cancer stem cells may be killed to decrease recurrence and failure of treatment. Of note, the expression of CD24 was also down-regulated. Breast cancer is a complex and heterogeneous disease that has kinds of subpopulation cells. CD24+ cells in breast cancer were studied to be associated with aggressive phenotype[25]. Here, silencing tTG may improve MDR by down-regulating CD24 expression in breast cancer. Onishi et al[26] showed that inhibiting CD24 expression may also modulate chemo-sensitivity according to drug type. In this study, it was the first time that tTG may regulate MDR by influencing the expression of LRP, P-gp, MRP, as well as the marker of BCSCs. This may provide a new strategy to overcome MDR.

Conclusions

On the whole, tTG is a ubiquitous multifunctional enzyme. tTG may regulate MDR by influencing ABC (P-gp and MRP), NABC (LRP) and the markers of BCSCs. In addition, after tTG silencing, cell proliferation was inhibited and apoptosis was enhanced. As a result, it is concluded that tTG may be an important potential target to regulate MDR in breast cancer. Further studies are needed to explore more effects of tTG in the future. From the content of our work, we provided new insights into tTG in MDR. Silencing tTG combined with chemotherapeutic agent adriamycin could reduce the required dose of chemotherapy and enhance the anti-tumor effect.

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Conflict of Interests

The authors declare that they have no conflict of interests.

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