Upregulating MMP-1 in carcinoma-associated fibroblasts reduces the efficacy of Taxotere on breast cancer synergized by Collagen IV

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Abstract. Chemotherapy is an important comprehensive treatment for breast cancer, which targets micro-environment of tumors as well as their characterisitcs. A previous microarray analysis revealed that matrix metalloproteinase (MMP)-1 was highly upregulated in carcinoma-associated fibroblasts (CAFs) prior to and following treatment with Taxotere under co-culture conditions. However, whether the chemotherapeutic effects of Taxotere were influenced by the changes in MMP-1 remained unclear. The purpose of the present study was to investigate the impact and mechanism of CAFs in regulating the efficacy of Taxotere on breast cancer cells. CAFs isolated from primary invasive ductal human breast tumors following surgical resection, were used in co-culture with MDA-MB-231 cells to simulate the tumor micro-environment. Following the addition of Taxotere, changes in MMP-1 gene and protein expression were assessed by reverse transcription-quantitative polymerase chain reaction and western blot analysis, respectively. Proliferation, invasion and apoptosis assays revealed that when MMP-1 was upregulated in CAFs, the therapeutic efficacy of Taxotere was reduced in breast cancer cells. Chemosensitivity was significantly increased when MMP-1 expression was inhibited by GM6001. In addition, Collagen IV was upregulated in CAFs following chemotherapy and protected breast cancer cells against chemotherapeutic side effects. Collagen IV expression significantly decreased, as well as MMP-1 expression when GM6001 was added. Proliferation and invasion assays demonstrated that the exogenous addition of Collagen IV weakend the chemotherapeutic effect of Taxotere on breast tumor cells. Overall, the results revealed that in CAFs, MMP-1 synergized with Collagen IV as a key gene in regulating the chemotherapeutic effect of Taxotere on breast tumor cells and served an important role in reducing the efficacy of Taxotere on breast cancer, potentially via the transforming growth factor-β signaling pathway. These fidings provide a theoretical basis for the mechanism of CAFs in reducing the chemotherapeutic effect of Taxotere on breast cancer cells and a novel approach for enhancing the chemosensitivity of tumors.

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Introduction

Breast cancer is one of the most common carcinoma in females worldwide, accounting for approximately 1,384,155 new cases, and 459,000 deaths annually (1). Chemotherapy is considered one of the most effective treatment for patients with breast cancer and can improve overall survival in patients (2). Nevertheless, due to chemotherapy resistance and the lack of effective predictors, the clinical efficacy of chemotherapy is limited. Disease relapse may occur in months or years, due to chemotherapeutic resistance acquired during treatment (3,4). There are two types of chemotherapy resistance, the first is intrinsic resistance which is predominantly related to the heterogeneity of tumor cells due to tumor stem cells (5,6), and the other is acquired resistance, which occurs over the course of treatment (7).

Previous studies have focused on the tumor itself, while ignoring the effects of tumor surroundings on tumor growth (4). Recently, emerging evidence has indicated that as the predominant components of stroma cells in the tumor environment, carcinoma associated fibroblasts (CAFs) have been reported to be crucial in the progression and chemotherapy effect of breast cancer (8-11). Dangi-Garimella *et al* (12), reported that CAFs promote gemcitabine resistance in pancreatic cancer through MT1 and matrix metalloproteinase (MMP) in CAFs mediated expression of HMGA2 by secreted Collagen I. Additionally, some researchers have demonstrated that inhibition of the p53 response in CAFs can improve the efficacy of anticancer treatment by increasing the anti-angiogenic effects of chemotherapy and radiotherapy in mice (13). Given the relationship between CAFs and chemotherapy resistance, we hypothesized that CAFs may be impaired, and the expression

of secreted factors involved in chemotherapy resistance may be altered, following chemotherapeutic treatment. However, which factors have changed and whether these changes will influence the chemotherapeutic effect of Taxotere on breast cancer cells remain unclear.

Our previous microarray analysis showed that MMP-1 in CAFs under co-culture conditions is upregulated before and after Taxotere treatment (14). However, whether the overexpressed MMP-1 and its target protein Collagen IV could affect the chemotherapeutic effect of Taxotere on mammary tumor cells and its specific mechanism were not in-depth investigate at that time. MMP-1 in tumor cells can promote growth, invasion and metastasis of tumors, and is closely related to the prognosis (15-17). However, MMP-1 in CAFs has not been reported that involved in the chemotherapy of tumor with Taxotere yet, which secreted from CAFs served as ECM proteins.

Based on our previous work, we hypothesized that highly expressed MMP-1 in CAFs is a key gene that regulates the chemotherapeutic effect of Taxotere on tumor cells. The aim of the present study was to further investigate the function and molecular mechanism of CAFs in protecting breast cancer cells against chemotherapeutic treatment and possibly providing novel predictors for chemotherapeutic efficiency and feasible for targeted therapy.

Materials and methods

Ethics statement. The present study was performed with the approval of the Institutional Review Board and Human Ethics Committee of Xuanwu Hospital of Capital Medicine University, China, and was carried out in accordance with The Declaration of Helsinki of the World Medical Association. Written informed consent was obtained from all patients prior to surgery to collect the samples for research purposes. And these patients did not receive any form of chemotherapy, endocrine therapy or radiotherapy treatments prior to their surgery.

Breast cancer cell line and cell culture of CAFs. The tumor removal was performed by Professor Kang at Xuanwu Hospital, Beijing, China. Three breast cancer tissue samples were obtained between January and February 2016, then were immediately placed in DMEM (SH30022.01; HyClone; GE Healthcare Life Sciences, Logan, UT, USA) supplemented with 10% fetal bovine serum (FBS, 10099-141; Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) and antibiotics and incubated in a vacuum cup filled with ice (only tissues more than those needed for clinical diagnoses were harvested for the present study). And all samples' histopathological diagnoses were determined as triple negative breast cancer by pathologist. Tissues were minced into pieces, washed with PBS three times and digested for 8-12 h at 37˚C in prepared reagent containing 0.1% collagenase type I and 0.1% hyaluronidase (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany). The digested pellet was resuspended in fresh DMEM containing 10% FBS (and all steps were performed under sterilized condition) (14,18).

The human breast cancer cell line MDA-MB-231 was obtained from the Laboratory of Xuanwu Hospital, Capital Medical University and was cultured in DMEM supplemented with 10% FBS at 37˚C in a humidified atmosphere of 5% CO_2 according to a standard procedure. Cell counting was performed with a VWR hemocytometer (Hausser Scientific Company, Horsham, PA, USA).

Reagents. GM6001, a specialized MMP inhibitor (Abcam, Cambridge, MA, USA) was prepared from lyophilized powder and the final solvent concentration in the medium was 2 μM. The lyophilized powder of type IV collagen (Sigma-Aldrich; Merck KGaA) was reconstituted in sterile PBS, and the final solvent concentration in the medium was 20 μg/ml at 4˚C. MMP-1 (anti-rabbit monoclonal antibody; Abcam), Collagen IV (Col IV;, anti-rabbit polyclonal antibody; Abcam) and GAPDH antibodies were purchased from Abcam, and secondary antibodies (anti-rabbit IgG-HRP; Abcam) were purchased from Santa Cruz Biotechnology, Inc., Dallas, TX, USA. The final solvent concentration of Taxotere (Sanofi, Shanghai, China) in the medium was 20 ng/ml based on the IC50 value from our previous experiment (14).

Immunohistochemistry (IHC). IHC staining for α-smooth muscle actin (α-SMA), multi-cytokeratin (CK) and Vimentin (all purchased from ZSGB-Bio, Beijing, China) was performed. CAFs were seeded in chamber slides for 24 h and fixed in cold acetone for 10 min. After antigen retrieval and blocking of endogenous peroxidase in 3% hydrogen peroxide, the CAFs were incubated with primary antibodies at 4˚C in a moist chamber overnight (PBS was used as a control). Specific signals were visualized by incubation with a peroxidase-coupled secondary antibody for 10 min, followed by incubation with 3, 3'-diaminobenzidine (DAB). Counterstaining was performed with hematoxylin and 0.1% hydrochloric acid (HCL) for 5 min, and the slides were cover slipped.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). RT-PCR was performed to confirm differential gene expression in cultured CAFs before and after Taxotere treatment (20 ng/ml for 24 h), using a Bio-Rad IQ5 Real-Time PCR System (Bio-Rad Laboratories, Inc., Hercules, CA, USA). RNA was isolated from fibroblasts using TRIzol reagent (Thermo Fisher Scientific, Inc.). cDNA was synthesized using 1 μg total RNA, oligo (dT), and SuperscriptTM III reverse transcriptase (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. The primers for the candidate genes (COL4A1, COL4A2, COL4A3, COL4A4, COL4A5, COL4A6, MMP-1) were designed with Primer Express Software (Applied Biosystems; Thermo Fisher Scientific, Inc.). Predicted PCR product sequences were verified using BLAST for recognition of target and non-target sequences: Human COL4A1, Forward 5'-CTCCACGAGGAGCACAGC-3' and Revese 5'-CCTTTT GTCCCTTCACTCCA-3'; Human COL4A2, Forward 5'-GCC AGTGCTACCCTGAGAAA-3' and Revese 5'-CGGGGA ATCCTTGTAATCCT-3'; Human COL4A3, Forward 5'-CAG GTGCTCCTGCTGCC-3' and Revese 5'-GCACTGGCCTTT GTCTTTACA-3'; Human COL4A4, Forward 5'-TGTGTT CCTGAAAAGGGGTC-3' and Revese 5'-CCTTTCTCTCCT GAAAGCCC-3'; Human COL4A5, Forward 5'-TACTGG CCCTGAGTCTTTGG-3' and Revese 5'-TTTCCCCTTTTA TGCCACTG'; Human COL4A6, Forward 5'-CTGCTCCTG GTTACGTTGTG-3'andRevese 5'-GGAAAACACTGACAG

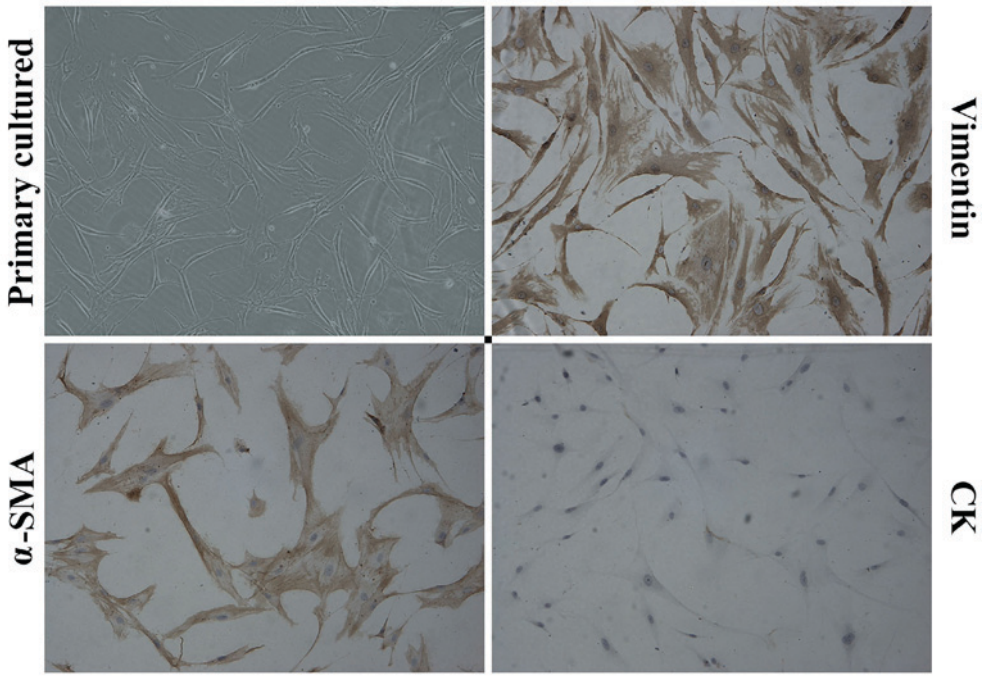

Figure 1. Characterization of primary cultured CAFs. The cultured cells were flat spindle shaped, rich in cytoplasm and flat ovoid nuclear in morphology. Immunostaining revealed that the primary cultured CAFs demonstrated positive expression of α-SMA and Vimentin, with negative expression of CK (magnification, x100). CAF, carcinoma-associated fibroblast; α-SMA, α-smooth muscle actin; CK, multi-cytokeratin.

CTCCC'; Human MMP-1, Forward 5'-TTCGGGGAGAAG TGATGTTC-3' and Revese 5'-TTGTGGCCAGAAAACAGA AA-3'; Conditions for the Real-time PCR reactions were as follows: 10 min at 95˚C followed by 40 cycles of 15 sec at 95˚C; 15 sec, 58˚C and 35 sec, 72˚C. The mRNA expression level was determined using the $2^{-\Delta\Delta Cq}$ method, in which relative quantification of mRNA expression level was calculated using β-actin as the internal reference.

Western blot analysis. The cell culture, drug treatment and group classification protocols were the same as those for the RT-PCR analyses. Cells were lysed using RIPA buffer with protease and phosphatase inhibitors. Following SDS-PAGE analyses, proteins were transferred to nitrocellulose membranes, blocked and incubated with primary antibodies. Secondary antibodies were detected with streptavidin-horseradish peroxidase (HRP). Chemi-luminescent detection was achieved using Western Lightning ECL reagent (Thermo Fisher Scientific, Inc.). The target bands of the gel were semi-quantified by densitometric analysis using an image software program.

Proliferation assay (CCK-8). Cell proliferation was detected by the Cell Counting Kit-8 (CCK-8) assay. The harvested MDA-MB-231 cells were diluted with DMEM at a concentration of $5x10^4$ cells/insert, while CAFs were diluted to a concentration of $2x10^4$ cells/insert. For the proliferation assay, cells were divided into four groups: Control group presented mono-culture MDA-MB-231, CO group presented MDA-MB-231 co-cultured with CAFs, CO+GM6001 group presented CO group with GM6001 treatment, CO+Col IV group presented CO group with Collagen IV treatment. Then, MDA-MB-231 cells were added to each lower chamber, and CAFs were added to the upper chamber. The 0.4 μm pore transwell inserts (Costar, USA) were used for this assay. After incubation for 24 h, all four groups were treated with Taxotere. Then, the cells were cultured for 24, 48 or 72 h, the old medium was discarded, and 10 μl CCK-8 (Dojindo Molecular Technologies, Inc., Kumamoto, Japan) in 100 μl culture medium was added to each well and incubated for another 3 h. The absorbance was measured at wavelength of 450 nm.

Flow cytometry (FCM). For analysis of apoptosis, cell culture, drug treatment and the group classification methods were performed as described above. After being resuspended in 500 μl binding buffer, the MDA-MB-231 cells were stained with 5 μl Annexin-V-FITC and 1 μl PI (Alexa Fluor 488 Annexin V/Dead Cell Apoptosis kit; Invitrogen; Thermo Fisher Scientific, Inc.), in the dark at room temperature for 15 min. Finally, cell apoptosis was measured by a FACSAria flow cytometer (Cytoflex; Beckman Coulter, Inc., Shanghai, China).

Invasion assay. Invasion assays were performed using 8 μm pore transwell inserts (Costar). The harvested MDA-MB-231 cells were used at a concentration of $2x10^4$ cells/insert, while CAFs were used at a concentration of $1x10^4$ cells/insert. Matrigel (356237; BD Biosciences, Franklin Lakes, NJ, USA) was equilibrated with serum-free DMEM at a 1:3 ratios on ice, and 50 μl/cm^2 matrigel was added to each filter. The group classification, cell culture and drug treatment protocols were the same as those for the proliferation assay. The MDA-MB-231 cells were added to the upper chamber, and CAFs were added to the lower chamber. At the end of the incubation period, the cells on the upper filters were removed with a cotton swab, and the filters were fixed in 4% formaldehyde and stained with 1% crystal violet. The number of cells that invaded into the lower

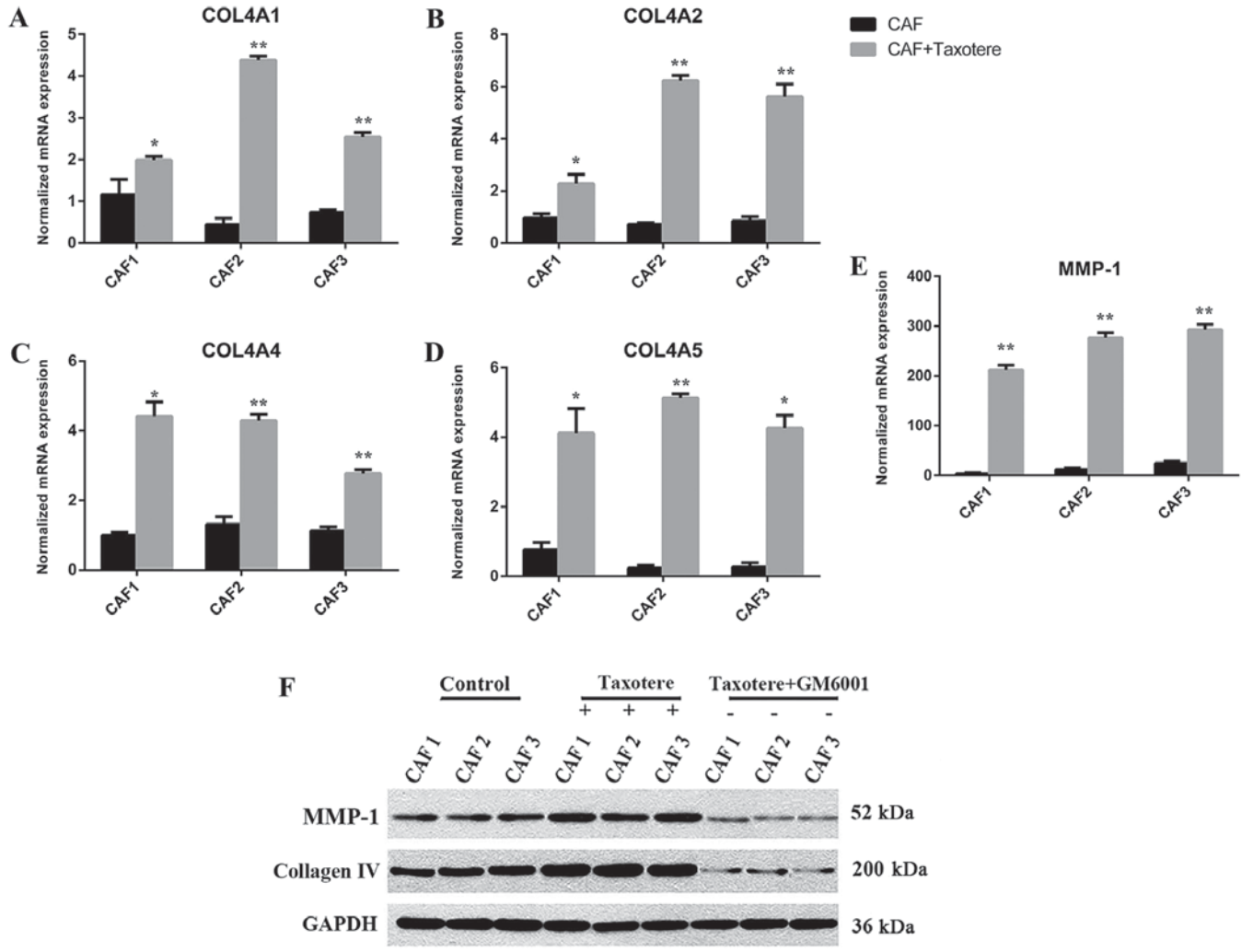

Figure 2. Chemotherapy induces high MMP-1 and collagen IV expression in CAFs. Following treatment with Taxotere (20 ng/ml), RT-qPCR revealed that the mRNA level of (A) COL4A1, (B) COL4A2, (C) COL4A4, (D) COL4A5 and (E) MMP-1 were significantly upregulated in CAFs. (F) Western blot analysis revealed the protein expression of MMP-1 and Collagen IV following treatement with Taxotere and GM6001. *P<0.05 and **P<0.01 vs. the CAF group. Data are presented as the mean ± standard deviation. MMP, matrix metalloproteinase; CAF, carcinoma associated-fibroblast; RT-qPCR, reverse transcription-quantitative polymerase chain reaction.

surface of the membrane were counted from 5 randomized fields at 100 times magnifications using an inverted microscope (Olympus IX70; Olympus Corporation, Tokyo, Japan). The assay was performed twice, each time in triplicate.

Statistical analysis. All statistical analyses were performed using SPSS v.22.0 (SPSS, Inc., Chicago, IL, USA) and GraphPad Prism software (GraphPad Software, Inc., La Jolla, CA, USA). Data are presented as the mean ± standard deviation for three independent experiments. One-way ANOVA with Bonferroni post hoc analysis was used for multiple group parametric comparisons of proliferation, invasion and apoptosis assays; Student's t-test for two groups parametric comparisons of RT-qPCR. P<0.05 was considered to indicate a statistically significant difference and is indicated by an asterisk.

Results

Characterization of primary cultured CAFs. Primary cells were cultured from the surgically resected breast cancer tumors, which were confirmed as triple negative breast cancer by pathologist. The obtained primary cells showed stable characteristic and the fourth or fifth passage cells were used for our experiment. The cultured cells had a flat spindle shape, abundant cytoplasm and an ovoid nuclear morphology. Immunostaining showed that the primary cultured CAFs expressed α-SMA and Vimentin, which are CAF-specific biomarkers, but not CK, an epithelial cell biomarker (Fig. 1).

Chemotherapy induced MMP-1 and collagen IV expression in CAFs. After Taxotere treatment, the gene expression levels of MMP-1 and synthesis of Collagen IV (COL4A1, COL4A2, COL4A4, COL4A5, P<0.05) were highly upregulated in CAFs as shown by RT-PCR, MMP-1 in particular showed significantly increased expression (P<0.01; Fig. 2A-E), while there were no statistical significant in COL4A3 (CAF1, P=0.026; CAF2, P=0.114; CAF3, P=0.083), COL4A6 (CAF1, P=0.02; CAF2, P=0.18, CAF3, P=0.846). Western blotting was performed to assess the protein levels of MMP-1 and

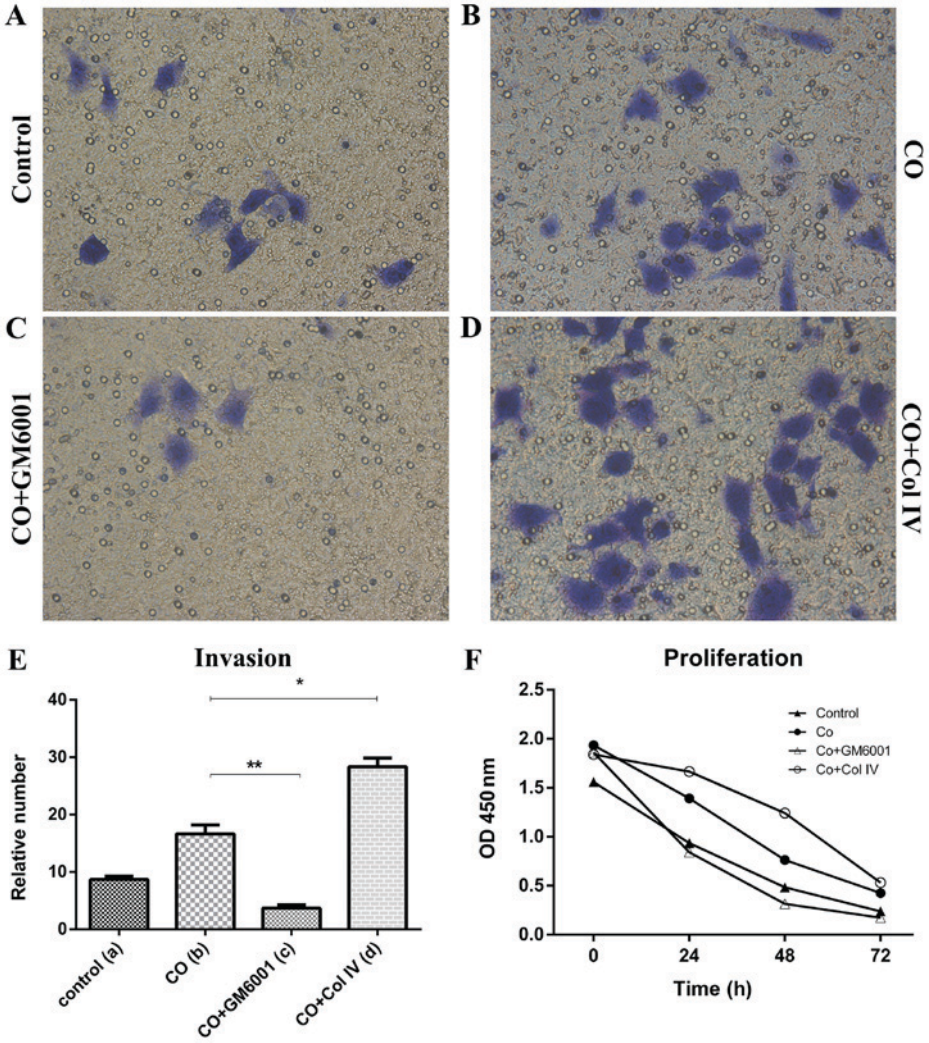

Figure 3. Proliferation and invasion of MDA-MB-231 cells. An invasion assay was performed on cells in the (A) control, (B) CO, (C) CO+GM6001 and (D) CO+ColIV groups and (E) the results were quantified. (F) A proliferation assay was performed on cells from each group. Magnification, x100. *P<0.05 and **P<0.01. Data are presented as the mean ± standard deviation. CO, MDA-MB-231 cells co-cultured with CAFs; ColIV, Collagen IV.

Collagen IV. The results showed that protein levels in CAFs were significantly different before and after chemotherapy (P=0.00; Fig. 2F). The variations in gene and protein expression of MMP-1 and Collagen IV were consistent.

CAFs promoted breast cancer cells resistance to chemotherapeutic effects. After Taxotere treatment, CO group displayed increased proliferation (24 h, P=0.01, 48 h, P=0.036) and invasion (P=0.00; Fig. 3E and F), but decreased apoptosis was decreased dramatically (P=0.002), compared with those of Control group (Fig. 4E). Thus, CAFs could protect breast cancer cells against the effects of chemotherapy.

GM6001 increased chemosensitivity of breast cancer cells. To further investigate the role of MMP-1 in CAFs on breast cancer chemotherapy, we used GM6001, an inhibitor of MMP-1, to decrease the expression of MMP-1 in CAFs. After GM6001 was added to the Taxotere-treated CAFs (CO+GM6001), MMP-1 in CAFs protein expression was substantially decreased, compared to that of CO group as shown by western blot analysis. Additionally, MDA-MB-231 cell proliferation (24 h, P=0.00, 48 h, P=0.02) and invasion (P=0.00) showed significant decreased (Fig. 3E and F), but apoptosis was significantly increased (P=0.013) between CO+GM6001 and CO group (Fig. 4E). Thus, we concluded MMP-1 plays an important role in CAF induced protecion of breast cancer cells against chemotherapy (Taxotere). Upregulated expression of MMP-1 in CAFs increased the chemotherapy resistance, while decreased MMP-1 expression in CAFs promoted chemosensitivity of breast cancer cells.

Collagen IV promoted breast cancer cells resistance to chemotherapy. To evaluate the effect of Collagen IV secreted from CAFs on MDA-MB-231 cells chemotherapeutic effect, we treated co-cultured cells with Collagen IV after chemotherapy (CO+Col IV). The proliferation (24 h, P=0.035; 48 h, P=0.01) and invasion assays (P=0.00) showed that there were significant differences, compared to that of the CO group (Fig. 3E and F). The proliferation and invasion of MDA-MB-231 cells were strongly enhanced by addition of Collagen IV after chemotherapy. These results indicated that Collagen IV could promote resistance of breast cancer cells to Taxotere. However, the apoptosis assay indicated that there

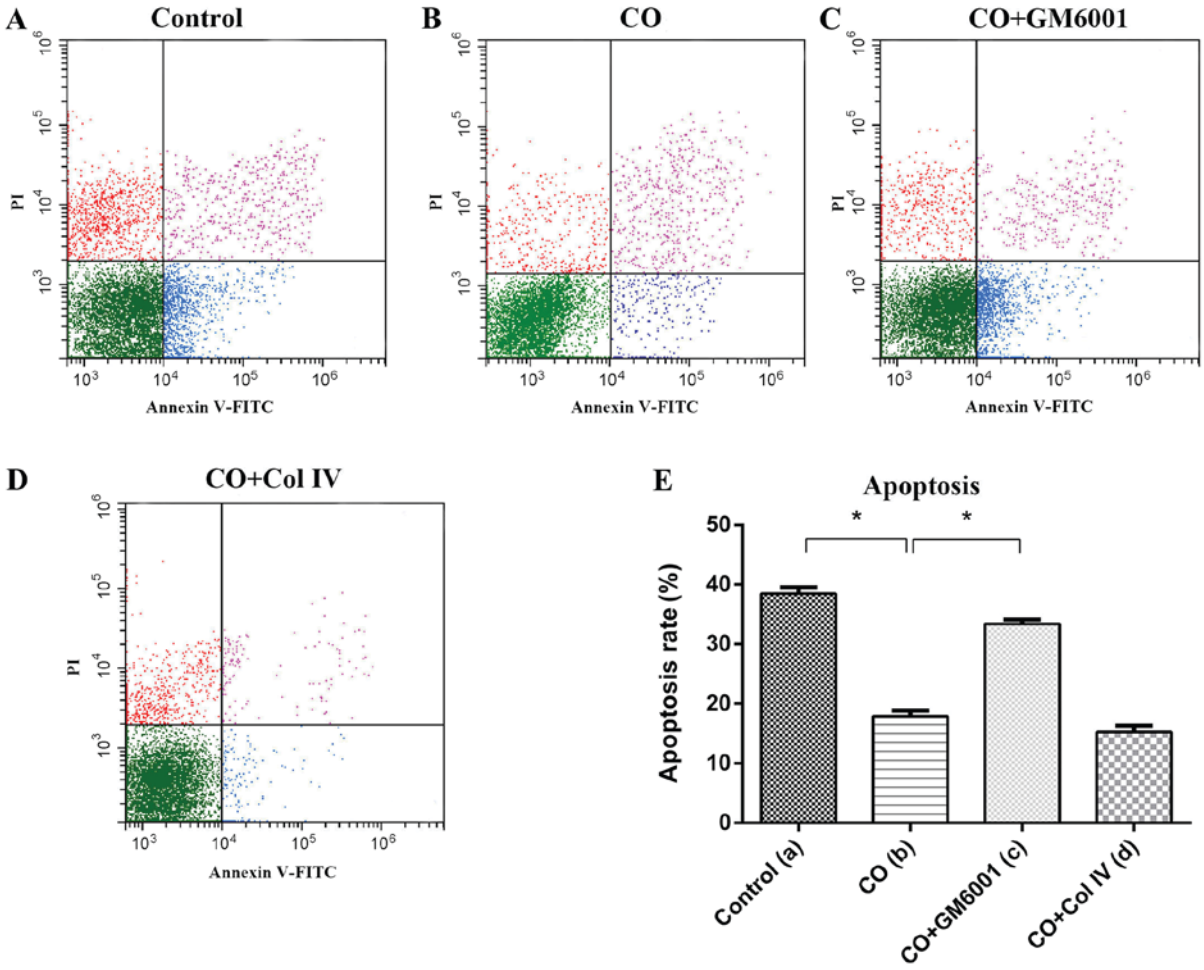

Figure 4. Apoptosis of MDA-MB-231 cells. Flow cytometry was performed to analyse the apoptosis rate in the (A) control, (B) CO, (C) CO+GM6001 and (D) CO+ColIV groups and (E) the results were quantified. Data are presented as the mean ± standard deviation. *P<0.05. CO, MDA-MB-231 cells co-cultured with CAFs; ColIV, Collagen IV.

was no significant difference between CO and CO+Col IV group after chemotherapy (P=0.487; Fig. 4E).

Discussion

Breast cancer is a complex disease, involving tumor cells themselves and the tumor microenvironment, which includes multiple components of the extracellular matrix (ECM), such as collagen, as well as cellular components, such as fibroblasts (19,20). Many investigations have shown that CAFs play an important role in tumor initiation, progression, apoptosis and chemotherapy resistance (19,21,22). Overall, CAFs are induced to adapt to the drugs used for treatment, which is consistent with the hypothesis that CAFs co-evolve along with the tumor cells (23). Moreover, the resistance of breast cancer to a comprehensive range of chemotherapeutic drugs and the lack of useful predictive markers of drug response are ongoing problems (24,25). As the majority of researchers have focused on endo-crinotherapy and CAFs after treatment, conversely, few studies have examined CAF induced chemotherapy resistance (26-28). And the molecular mechanism underlying CAF-mediated chemotherapy resistance is still ambiguous.

The aim of the present study was to further investigate the function and molecular mechanism of CAFs in protecting breast cancer cells against chemotherapeutic treatment. Moreover, based on our previous work, we hypothesized that highly expressed MMP-1 is a key gene that regulates the chemotherapeutic effect of Taxotere on breast tumor cells.

To verify this hypothesis, CAFs were first isolated from primary invasive ductal human breast tumors following surgical resection and were used with MDA-MB-231 cells for co-culture to simulate the tumor growth microenvironment. After addition of Taxotere, high expression in MMP-1 gene and protein levels were observed by RT-PCR and Western blot analyses, respectively. After Taxotere treatment, CO group displayed increased proliferation and invasion, but the apoptosis was decreased dramatically, compared with those of Control group. Hence, we concluded that up-regulated MMP-1 in CAFs under co-cultured conditions decreased the therapeutic efficacy of Taxotere on breast cancer cells. These assays also indicated that chemosensitivity was significantly increased when MMP-1 expression was inhibited by GM6001. These results are consistent with the hypothesis. MMP-1 is predominantly produced by CAFs, and it could be increased following stimulation (15). MMPs play a crucial role in proliferation, invasion, metastasis and apoptosis of tumor cells (15-17). Faller WJ founded that MMPs have a significant effect on tumor resistance to

gemcitabine (29). These research results were consistent with our data. Thus, based on our data, as the main component of ECM, MMP-1 was hypothesized to directly affect tumor cells and increase the ECM abundance by regulating the synthesis of collagen and reducing blood flow to limit the transport of drugs.

Thus, we continue to study the change of MMP-1 targeted protein: Collagen. And the results showed that Collagen IV was upregulated in CAFs after chemotherapy and enhanced breast cancer cell resistance to chemotherapeutic effects, Collagen IV expression significantly decreased, along with MMP-1 expression, after GM6001 was added. Proliferation and invasion assays showed that addition of exogenous Collagen IV weakened the chemotherapeutic effect of Taxotere on breast tumor cells. Collagen IV is a member of super-collagen family of proteins, predominantly secreted by stromal cells and plays an important role in tumor progression and drug effects and is consist of 3 peptide chains, which encoding genes including COL4A1-COL4A6 (30-33). So, we verified the 6 encoding genes; while, only COL4A1, COL4A2, COL4A4, COL4A5 had statistically significance. It was reported Collagen IV was highly expressed in ovarian cancer after gemcitabine treatment and promoted the acquisition of ovarian cells resistance to gemcitabine (33). The results indicated that Collagen IV, which is secreted by CAFs and is a component of the ECM, may induce cell adhesion-mediated drug resistance by interacting with integrin receptors of cancer cells and then reducing the chemotherapy effects. Interestingly, our results showed that Collagen IV protein expression significantly decreased after treatment with GM6001, which is consistent with the alteration in MMP-1. This phenomenon contrasts with the finding that MMPs degraded Collagen. We inferred that the degradation and synthesis of the Collagen protein are in a dynamic equilibrium, and under certain conditions or stimuli, the synthesis of Collagen protein is greater than the degradation by MMPs. It was also demonstrated that MMP-1 could directly affect tumor cells and be treated as not only a kind of ECM proteolytic enzymes, but also should be taken as an enzyme that involved in the signaling between cells and cells, cells and stoma, even as a protein equipped signal potential amplification ability. TGF-β is involved in the classical pathway of collagen secretion and could be activated with latent TGF-β (LTBP-1) by MMPs (34,35). Bates *et al* (36) founded that the collagen levels significantly decreased after TGF-β neutralizing antibody was added to block the TGF-β pathway. Hence, combining these findings with our study results, it was suggested TGF-β pathway maybe the reactive regulator between MMP-1 and Collagen IV: After Taxotere treatment, CAF secreted MMP-1 synergized with Collagen VI to decrease the chemotherapeutic effect of Taxotere on breast cancer cells by the TGF-β pathway.

There are some limitations in our study. Collagen IV was added exogenously, and no negative intervetions were taken to decrease Collagen IV expression. Thus, Collagen IV can not be identified as the key gene to regulate the effet of Taxotere on tumor cells. In addition, the relationship between MMP-1 and Collagen IV should be further verified by TGF-β pathway study. What'more, the present study was focused on triple-negative breast cancer. What's the influence of Taxotere on hormone receptors of breast cancer needed to be discussed in the future. However, MMP-1 synergized with Collagen IV in CAFs should be confirmed as the key regulator that regulates the chemotherapeutic effect of Taxotere on tumor cells by *in vitro* experiments in the present study.

In summary, we cultured CAFs of primary breast cancer samples, and provided new evidence showing that CAFs induced breast cancer cell resistance against the chemotherapeutic effect of Taxotere and elucidated the underlying molecular mechanism. The observation that high expression of MMP-1 synergy with Collagen IV in CAFs plays an important role in reducing the efficacy of Taxotere in breast cancer cells and maybe react via the TGF-β pathway. This provides a theoretical basis for the chemotherapeutic effect of CAFs on breast tumor cells and a novel approach to enhance the chemosensitivity of tumors.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

HK designed the experiments, provided critical reagents and experimental expertise and supervised the study. QC designed and performed the experiments, generated the figures and wrote the manuscript. BW and KL collected the tumor tissues. HS, YZ and TH assisted with some of the experiments.

Ethics approval and consent to participate

The study was approved by the Institutional Review Board and Human Ethics Committee of Xuanwu Hospital of Capital Medicine University. Written informed consent was obtained from all patients prior to their inclusion within the study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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