A preliminary mechanistic exploration of the effect of leptin on the docetaxel sensitivity of MDA-MB-231 triple-negative breast cancer cells

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Abstract. Breast cancer is a common tumor encountered in women, and triple-negative breast cancer (TNBC) has an extremely poor prognosis. The effect of leptin on the docetaxel sensitivity of MDA-MB-231 TNBC cells has not been investigated. The present study aimed to clarify the effect of leptin and M2 tumor-associated macrophages (TAMs) on the chemosensitivity of TNBC cell lines and its possible mechanisms. In the present study, the apoptosis of the MDA-MB-231 cell line was detected at 0, 24, 48 and 72 h using a Cell Counting Kit-8 assay to determine the appropriate concentration of docetaxel as well as the IC_{50} value. After determining the effect of leptin on TAMs, the conditioned medium with an appropriate concentration of docetaxel was collected to treat the breast cancer cells, and flow cytometry was used to detect the cell cycle distribution and apoptosis in different treatment groups. Interleukin 8 (IL-8) expression was detected using ELISA and western blot assay. The IL-8 antibody was used to neutralize IL-8, and invasion and scratch assays were used to detect changes in invasion and migration of breast cancer cells. Statistical analysis was performed using GraphPad Prism 9.0 and SPSS 22.0. It was revealed that the apoptotic rate of MDA-MB-231 cells in the leptin-treated TAMs group was lower than that in other groups. The expression of IL-8 was notably elevated in the group treated with leptin-activated TAMs compared with that in the other groups. The neutralization of IL-8 resulted in a significant reduction in the invasive migration of MDA-MB-231 cells compared with that in the non-neutralized group.

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Introduction

Breast cancer is the most common cancer in women and the second leading cause of cancer-related mortality worldwide (1). There are four primary subtypes of breast cancer: luminal A, luminal B, human epidermal growth factor receptor 2 (HER2)-positive, and triple-negative breast cancer (TNBC). These classifications are based on molecular markers, such as estrogen receptors, progesterone receptors and HER2. TNBC is the most malignant subtype of breast cancer, accounting for ~15-20% of all breast cancers (2,3). Since patients with TNBC lack relevant receptor markers, they do not benefit from established endocrine- or HER2-targeted agents. Although TNBC is the subtype that responds best to standard chemotherapy regimens, such as paclitaxel or anthracyclines, some patients with TNBC have poor outcomes. This could be due to the high heterogeneity of TNBC and the presence of primary and secondary resistance (3). Therefore, the search for effective therapeutic targets for TNBC and the mechanisms underlying its resistance to chemotherapy are of great interest.

Leptin is a hormone predominantly secreted by adipocytes, which plays a crucial role in regulating energy balance by transmitting signals from adipose tissue to the hypothalamus. The synthesis and concentration of leptin in the bloodstream are closely associated with the mass of adipose tissue (4). Leptin has been extensively studied both *in vivo* and *in vitro* for its impact on different aspects of breast cancer biology. Apart from adipose tissue, leptin is also secreted by cancer cells and its receptors are often overexpressed in these cells (4). Leptin levels have been linked to various characteristics of breast cancer, including its type, grade, stage, lymph node involvement, hormone receptor status and recurrence (5). A meta-analysis conducted by Gu *et al* (5) comprising 43 studies indicated that serum leptin might have a significant impact on the development and metastasis of breast cancer. Another meta-analysis, involving 23 studies, demonstrated that circulating leptin levels were lower in healthy individuals than in those with benign breast disease, breast cancer, or lymph node metastases, implying that leptin levels could serve as a potential diagnostic tool for tumor formation (6). Pan *et al* (7) conducted a meta-analysis involving 35 studies, suggesting that leptin could serve as a potential biomarker for breast cancer risk, particularly in overweight/obese or postmenopausal women. It may

also be a valuable biomarker for identifying individuals at high risk for breast cancer, aiding in preventive therapy (7). Furthermore, elevated serum leptin levels, coupled with increased expression of leptin receptor mRNA in breast cancer tissues, are associated with poor prognosis (8). Leptin promotes mitochondrial fusion and contributes to drug resistance in gall bladder cancer (9). Leptin also interferes with the action of tamoxifen in MCF-7 cells by inducing an increase in the nuclear expression of ERα. Thus, leptin may favor tamoxifen resistance, and inhibition of leptin expression may be a novel approach to circumvent resistance to anti-estrogen therapy (10). A previous study reported that leptin-induced microRNA-342-3p enhances gemcitabine resistance in pancreatic ductal adenocarcinoma (11). These findings underscore the possible advantage of targeting leptin signaling as a strategy to inhibit breast cancer malignancy.

Leptin has been found to influence the behavior of tumor-associated macrophages (TAMs), which are a major source of inflammatory cytokines and a key component of the tumor microenvironment (12). Stimulation of macrophages by leptin induces the release of pro-inflammatory cytokines, which can result in a pro-inflammatory reaction. Clinical evidence suggests that the presence of macrophages within the tumor microenvironment is associated with unfavorable outcomes in individuals with cancer (13). Macrophages are a highly heterogeneous group of immune cells with different functions and phenotypes that participate in both innate and adaptive immunity in the body (14). Macrophages exhibit both antitumor and pro-tumor effects. Under conditions of tumorigenesis, macrophages have an antitumor effect, whereas once a tumor has formed, they have a pro-tumor effect (15). Under the influence of the complex tumor microenvironment, macrophages can be recruited to the tumor area and polarized into either a tumor growth-inhibiting M1 state or a tumor growth-promoting M2 state (14,16). In the present study, TAMs represent M2 type-associated tumor cells. Cao *et al* (17) found that leptin promotes tumor progression and metastasis by triggering the production of the M2 macrophage-associated cytokine IL-18. These findings further establish the connection between the tumor microenvironment and breast cancer cells.

In summary, leptin plays a role in every stage of breast cancer progression and can stimulate the secretion of inflammatory factors by M2-type TAMs to influence the development of malignant tumors. However, whether it affects the docetaxel sensitivity of MDA-MB-231 TNBC cells has not been reported. Therefore, the present study was conducted to explore whether this is relevant and to initially explore the mechanism of resistance.

Materials and methods

Cell culture. MDA-MB-231 cells (cat. no. AW-CCH048; http://abiowell.com/) were cultured in DMEM/F12 medium containing 10% FBS (cat. no. D8437; Sigma-Aldrich; Merck KGaA) + 1% dual antibody. THP-1 cells (cat. no. AW-CCH098; http://abiowell.com/) were cultured in RPMI-1640 medium containing 10% FBS + 1% dual antibody + 0.05 mM β-mercaptoethanol in RPMI-1640 medium (cat. no. R8758; Sigma-Aldrich; Merck KGaA).

Western blotting (WB). Cells were washed with ice-cold PBS and 200 μl RIPA lysate was added. Afterwards, cells were scraped with a scraper, and the suspension was collected and sonicated for 1.5 min on ice. The lysate remained for 10 min on ice. The centrifuge was pre-cooled at 4˚C, and the lysate was centrifuged at 13780 x g for 15 min. The supernatant was transferred to a 1.5 ml tube. Protein concentration was determined with a BCA protein quantification kit. TEMED was mixed with 10 or 15% separating gel, and gel was sealed with isopropyl alcohol. Gel was allowed to set until stable line formed. The top layer of isopropyl alcohol was poured off, and add gelatin concentrate was added to solidify the gel. A total of 160 μl protein supernatant was mixed with 40 μl loading buffer, boiled for 5 min and left to cool on ice. The first well was spotted with 2 μl of marker, and the other wells were sampled with 10-20 μl of denatured protein. The marker and denatured protein were injected into wells, and electrophoresis was run at 75 V for 130 min. The electrophoresis was terminated when the dye reached the gel bottom. The gel was cut by molecular weight, and transferred to a nitrocellulose membrane which was subsequently washed with 1X PBST. 5% skim milk powder was prepared with 1X PBST (cat. no. AWI0130; http://abiowell.com/), and the membranes were immersed and left at room temperature for 90 min. The primary antibody (IL-8; 1:1,000; cat. no. MA5-23697; Thermo Fisher Scientific, Inc.) was diluted with 1X PBST, and the membrane was incubated at room temperature for 90 min. Subsequently, the membrane was washed three times with 1X PBST for 10 min each. HRP-labeled secondary antibodies [Goat anti-Mouse IgG (H+L) (1:5,000; cat. no. AWS0001; http://abiowell.com/) and Goat anti-Rabbit IgG (H+L) (1:5,000; cat. no. AWS0002; http://abiowell.com)] was diluted with 1X PBST, and incubated with membrane at room temperature for 90 min. The membrane was then washed three times with 1X PBST for 15 min each. The membrane was incubated with ECL solution (AWB0005; http://abiowell.com/) for 1 min. The membrane was wrapped with plastic wrap, and images were captured with gel imaging system. β-actin (1:5,000; cat. no. 66009-1-Ig; Proteintech Group, Inc.) was used as an internal control.

Cell Counting Kit-8 (CCK-8) assay. The cells were seeded into 96-well plates at a density of $5x10^3$ cells/well with 100 μl of medium per well. A total of three replicate wells were prepared for each group. After the cells were attached to the plate, they were treated as aforementioned for a specific period. Following the treatment, 10 μl of CCK-8 solution (cat. no. NU679; Dojindo Laboratories, Inc.) was added to each well. The CCK-8 solution was prepared in the complete medium by replacing the drug-containing medium, and 100 μl of medium containing CCK-8 was added to each well. The plates were incubated at 37˚C with 5% CO_2 for 4 h, and then the absorbance at 450 nm was measured using a HUISON zymography reader.

ELISA assay (IL-8; cat. no. KE00006; Proteintech Group, Inc.). Reagents were equilibrated at room temperature for 30 min. Standard and sample wells were set up; a total of 100 μl of standard/sample was added to each well, mixed well and incubated covered at 37˚C for 2 h. Then, liquid was discarded and the plate was washed 4 times with 200 μl wash solution

each, shake dry after each wash. A total of 100 μl detection antibody working solution was added to each well and incubated covered at 37˚C for 1 h. The liquid was discarded, and the plate was washed 4 times as aforementioned. HRP-labeled affinity protein working solution (100 μl) was added to each well and incubated covered at 37˚C for 40 min. The liquid was discarded, and the plate was washed 4 times as aforementioned. Substrate solution (100 μl) was added to each well and incubated at 37˚C for 15-20 min, avoiding light. The reaction was terminated by adding 100 μl termination solution to each well. OD was measured at 450 nm within 5 min. Data was analyzed using Curve Expert software (Curve Expert1.4) to create a standard curve. Sample concentration was calculated using regression equation derived from the standard curve and the sample's OD value.

Transwell assay. One day before the experiment, sterile lance tips, EP tubes, Matrigel and Transwell chambers were pre-cooled overnight at 4˚C. Matrigel was diluted with 100 μl of ice-cold, serum-free DMEM/F12 medium per well to a final concentration of 200 μg Matrigel per well. Matrigel was incubated for 30 min at 37˚C and the supernatant was removed. A total of 500 μl of 10% FBS Complete Medium was added to the lower chamber of Transwell. Treated cells were digested with trypsin to obtain single cells, resuspended in serum-free medium at a concentration of $2x10^6$ cells/ml, and 100 μl of cells was added to each well. Cells were incubated at 37˚C for 48 h. The upper chamber was removed, washed with PBS three times, and the cells were wiped off the upper chamber with a cotton ball. Subsequently, cells were fixed with 4% paraformaldehyde for 20 min at room temperature, and then the membrane was removed. The membranes were stained with 0.1% crystal violet for 5 min at room temperature, rinsed 5 times with water, placed on slides, and microscopic images were captured. Using an inverted light microscope, three randomly selected fields of view were used to observe the cells on the outer surface of the upper chamber. The chamber was removed and immersed in 500 μl of 10% acetic acid for decolorization. The absorbance (OD) value was measured at 550 nm using an enzyme standard instrument, repeating the process three times to obtain consistent results.

Wound healing assay. Horizontal lines were drawn evenly in a 6-well plate, and ~$5x10^5$ cells were added in each well after being digested with trypsin. After the cells spread all over the plate, the tip of the pipette was used to compare with the ruler and draw the horizontal line perpendicular to the horizontal line. The cells were washed 3 times with sterile PBS to remove the scratched cells and serum-free DMEM/F12 medium was added. Images of the scratch were captured at 0 h, using 3 fields of view at each time point. After incubation at 37˚C with 5% CO_2 for 24 and 48 h, images were again captured to record.

Flow cytometric assay. Cells from different treatments were collected by digestion with EDTA-free trypsin, washed twice with PBS, each time centrifuged (4˚C) at (713 x g for 5 min. Cells were collected and 500 μl of binding buffer was added to suspend the cells. A total of 5 μl of Annexin V-APC (cat. no. KGA1030; Nanjing KeyGen Biotech Co., Ltd.) and 5 μl of propidium iodide were added and mixed well at room temperature. The reaction was carried out at room temperature, protected from light for 10 min, and then observed and detected by flow cytometry [Flow cytometer (cat. no. A00-1-1102); analysis software: CytExpert_Setup-2.5.0.77; both from Beckman Coulter, Inc.)].

Induction of M2 TAMs. THP-1 cells were cultured in RPMI-1640 medium containing 10% FBS + 1% dual antibody + 0.05 mM β-mercaptoethanol and placed in a saturated humidity incubator at 37˚C with 5% CO_2. THP-1 cells in logarithmic growth were stimulated and induced to adherence using phorbol ester (PMA). Interleukin 4 (IL-4) was then added to induce monocyte differentiation into TAMs. The cells with completed induction were collected, 5 μl of CD206 (cat. no. 12-2069-42; eBioscience; Thermo Fisher Scientific, Inc.) antibody was added, mixed and incubated for 30 min at room temperature away from light. The expression of CD206 was analyzed by flow cytometry.

Neutralization of IL-8. The levels of IL-8 were detected by the ELLSA method after 0, 24 and 48 h of leptin action on TAMs. The culture medium was collected and IL-8 was neutralized with IL-8 antibody (Cell Line IL-8 antibody).

Statistical analysis. Statistical analyses were performed using GraphPad Prism 9.0 (Dotmatics) and SPSS 22.0 (IBM Corp.), and all values were the result of at least three independent measurements. Statistical analyses were performed using unpaired t-test or one-way ANOVA with Tukey's or Dunnett's as post hoc tests. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Enhancement of MDA-MB-231 docetaxel resistance in TNBC cells after leptin action on TAMs. The culture media of leptin + TAMs, leptin, TAMs and blank groups were collected following 48 h of incubation to culture the MDA-MB-231 cell line. The appropriate concentration of docetaxel was then added and culture continued for 0, 24 and 48 h, followed by the detection of apoptosis (Fig. 1A) and cell-cycle distributions of the MDA-MB-231 cells in all the groups (Fig. 1B) using flow cytometry. Compared with the other three groups, the leptin + TAMs + MDA-MB-231 group displayed a significantly lower apoptotic rate. The flow cytometric results revealed that after a combination of TAMs and docetaxel acted on MDA-MB-231 cells, there was a significant increase in G1-phase cells and a significant decrease in G2-phase cells. However, after a combination of leptin and docetaxel acted on MDA-MB-231 cells, there was a significant decrease in G1-phase cells and a significant increase in G2-phase cells (Fig. 1B).

Leptin affects IL-8 secretion by TAMs. Numerous studies have demonstrated the significant involvement of IL-8 in the development of tumor resistance to drugs (18). To investigate the possible mechanism by which leptin combined with TAMs affects the resistance of TNBC cell lines to docetaxel, the supernatants of the four groups of the aforementioned culture media were collected to detect the expression of IL-8.

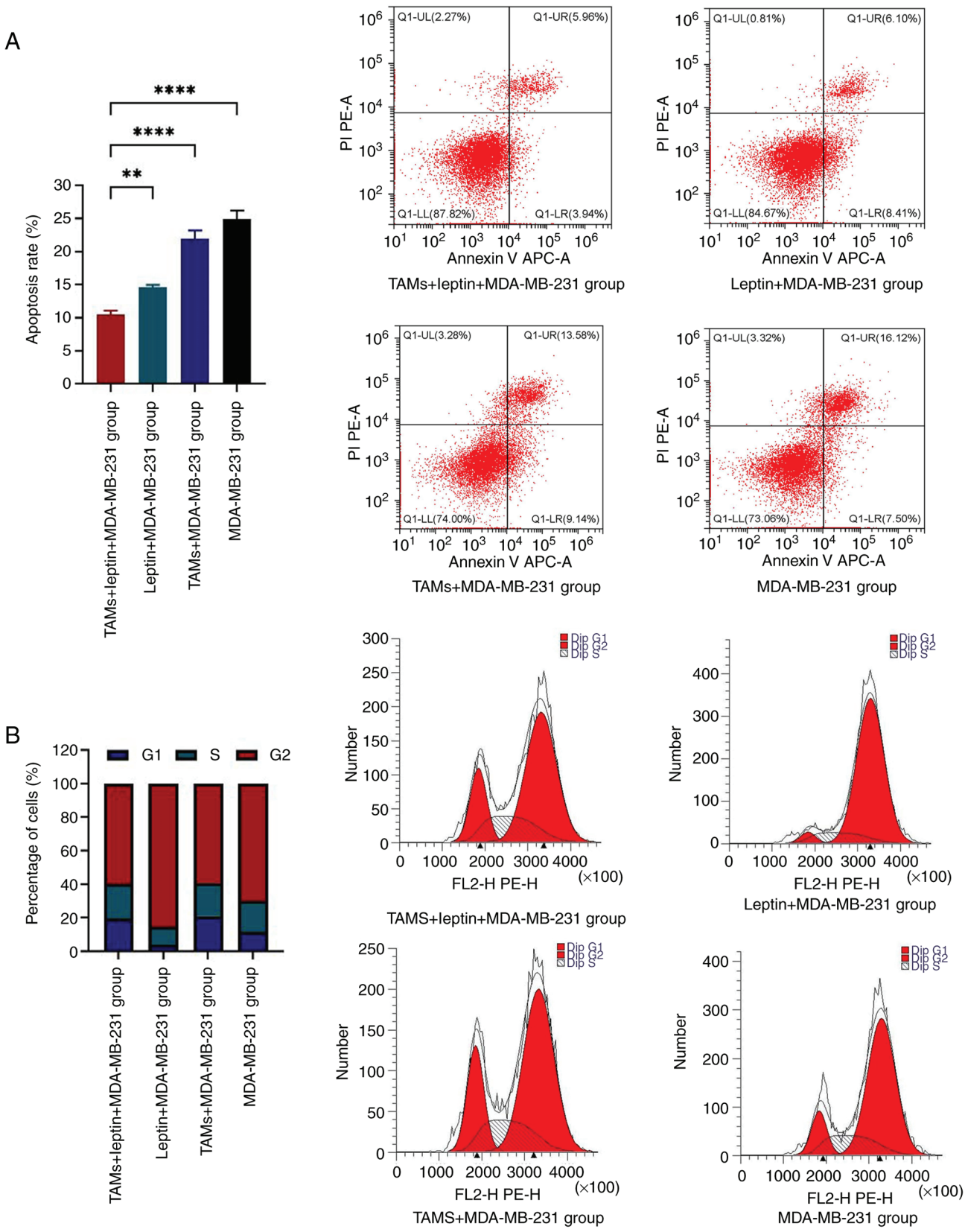

Figure 1. The combined effects of leptin with TAMs and docetaxel on cell cycle and apoptosis were evaluated. (A) Flow cytometry was performed to assess cell apoptosis. (B) Flow cytometric analysis was used to determine the cell cycle phase distribution. Data represent the mean ± standard deviation of three independent experiments. **P<0.01 and ****P<0.0001. TAMs, tumor-associated macrophages.

The ELISA results demonstrated that IL-8 expression in the leptin + TAMs group was significantly higher than that in the remaining three groups, with a statistically significant difference (Fig. 2A). WB was also used to detect the expression of IL-8, and the results supported a significant increase in IL-8 expression in the leptin + TAMs group (Fig. 2B).

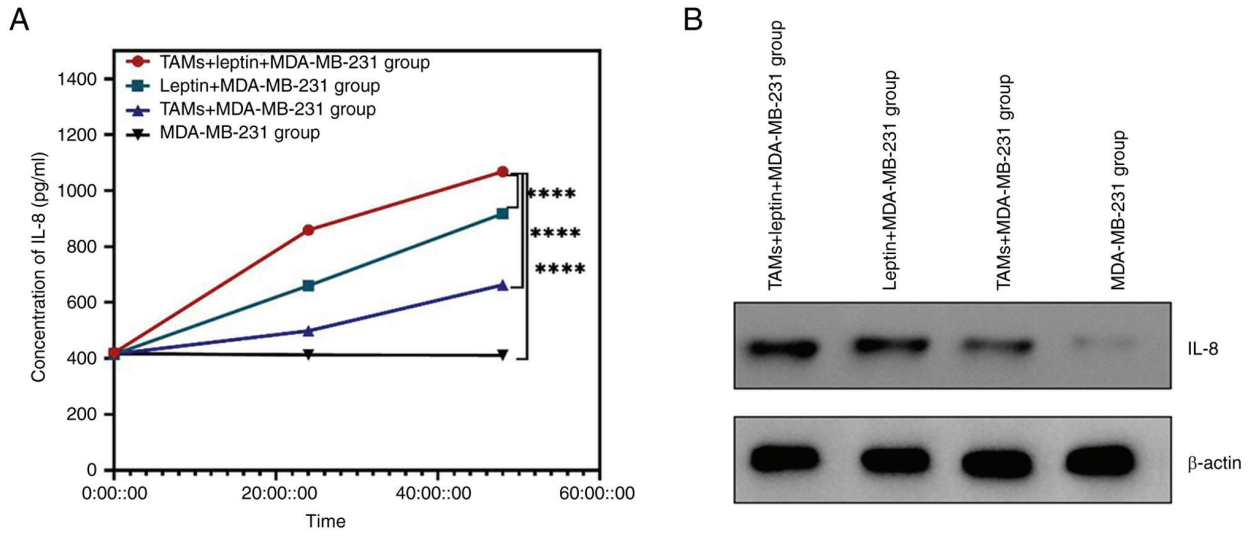

Figure 2. Leptin significantly induces an increase in IL-8 expression in TAMs. (A) ELISA analysis revealed that the leptin-treated macrophage group exhibited significantly higher levels of IL-8 expression compared with the other three groups (leptin + MDA-MB-231, TAMs + MDA-MB-231). (B) Western blot analysis of IL-8 expression in each group demonstrated consistent results with the ELISA findings. Data represent the mean ± standard deviation of three independent experiments. ****P<0.0001. TAMs, tumor-associated macrophages.

Leptin-stimulated IL-8 secretion from TAMs promotes MDA-MB-231 invasion and migration. To further investigate the effect of leptin-stimulated IL-8 secreted by TAMs on TNBC cell lines, ELISA and WB were used to detect the expression of IL-8 in the leptin + TAMs group, leptin + TAMs-neutralized and IL-8 groups, and blank group. ELISA results identified that the expression of IL-8 in the leptin + TAMs group was significantly higher than that in the other two groups. The difference in IL-8 expression between the leptin + TAMs neutralized IL-8 group and the blank group was not statistically significant, indicating that neutralization was effective (Fig. 3A). WB results were consistent with ELISA results (Fig. 3B).

The Transwell assay showed that the number of migratory cells was significantly lower in the leptin + TAMs neutralized IL-8 group than in the leptin + TAMs group (P<0.01) (Fig. 3C).

The experimental results from the cell scratch assay were consistent with those from the Transwell assay. The migration distance and number of invasive cells in the leptin + TAMs group were significantly greater than those in the leptin + TAMs neutralized IL-8 group at 24 and 48 h after scratching (P<0.01) (Fig. 3D).

Discussion

According to the latest data released by the International Agency for Research on Cancer of the World Health Organization, cancer is the leading cause of death worldwide, with nearly 10 million deaths in 2020 (1). Breast cancer accounts for the highest number of new cancer cases, at 2.26 million cases yearly, surpassing that of lung cancer. Breast cancer has now replaced lung cancer as the world's most predominant cancer, and it is one of the four leading causes of cancer-related deaths (19). Despite considerable progress and advances in the diagnosis and treatment of breast cancer, chemoresistance-induced metastatic recurrence remains a challenge for basic and clinical researchers. Leptin expression is significantly higher than normal in patients with breast cancer. Leptin and leptin receptors are expressed in normal breast epithelial cells; however, their overexpression is associated with breast cancer progression. Tumors present in the mesenchyme may include cells, such as fibroblasts, epithelial cells, macrophages, T lymphocytes, dendritic cells, neutrophils and adipocytes; structural components such as lymphatic and blood vessels; and soluble factors, including growth factors, cytokines and chemokines, in cell-tumor interactions (20). The mesenchyme normally acts as an antitumor barrier; however, it can be transformed into a tumor-promoting state, either as an intrinsic change, as in the case of an inefficient vascular system, or as an acquired change, as in the case of responsiveness to chemotherapy and radiotherapy mediated by fibroblasts and immunosuppressive cells (21). Macrophages, an important component of the tumor microenvironment, account for up to 50% of the tumor mass in some cases and are associated with poor prognosis in most cancers (22). Alterations in macrophage phenotype can occur at all stages of tumor formation, including initiation, progression and metastasis. In the present study, the effect of leptin stimulation of M2 TAMs was investigated in the tumor microenvironment on the sensitivity of TNBCs to chemotherapy and the possible mechanisms that may guide future clinical research and treatment were preliminarily explored.

A link between leptin levels, macrophage function and cancer progression is plausible. Numerous studies have highlighted the role of leptin signaling in the progression, metastasis, stemness induction, angiogenesis and therapeutic efficacy of breast, colorectal, melanoma, ovarian and other types of cancer (23). Leptin significantly promotes breast tumor growth and development of lung metastases from breast cancer; application of macrophage removers has been demonstrated to attenuate these effects of leptin (12). In the present study, leptin stimulation of M2 TAMs followed by co-culture with MDA-MB-231 triple-negative breast adenocarcinoma cells reduced sensitivity to docetaxel compared with that in

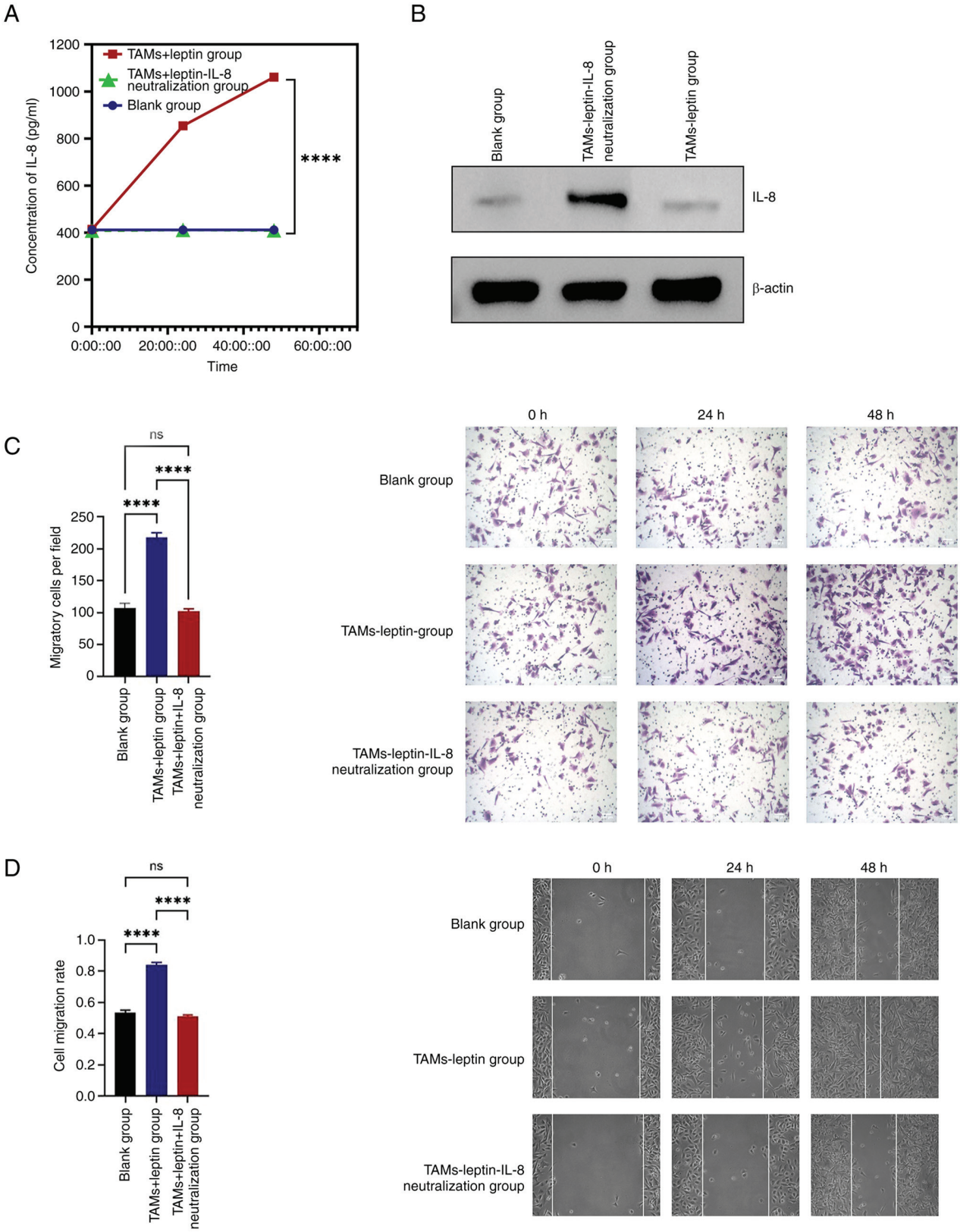

Figure 3. Effect of leptin stimulation on the secretion of IL-8 by TAMs and its impact on cell migration and invasion were investigated. (A) ELISA analysis was performed to assess the expression of IL-8 in different treatment groups. (B) Western blot analysis was conducted to validate the IL-8 expression results obtained from ELISA. (C) Cell invasion was assessed using the Transwell assay. (D) Cell migration was evaluated using the wound healing assay. Data represent the mean ± standard deviation of three independent experiments. ****P<0.0001. TAMs, tumor-associated macrophages; ns, no significance.

other groups, suggesting that the interaction between leptin and M2 TAMs enhances chemoresistance in TNBC cell lines, which is consistent with the findings of most previous studies.

Leptin induces the secretion of vascular endothelial growth factors and proinflammatory cytokines by macrophages. IL-8, acting as chemotactic factor, promotes autocrine and/or

paracrine tumors and has the potential to serve as a prognostic and/or predictive cancer biomarker. It is mainly produced by monocytes, but other cells, such as fibroblasts, epithelial cells, endothelial cells and hepatocytes, can also produce IL-8 under appropriate stimulatory conditions (24). Evidence points to a functional crossover between the leptin and estrogen signaling pathways; leptin promotes breast cancer tumor cell development and progression through activation of the JAK2/STAT3 pathway. Furthermore, leptin induces expression of the cell cycle protein D1 through STAT3 activation, which regulates the cell cycle and promotes breast cancer cell growth (25). Leptin-activated STAT3 also promotes cancer cell stemness and drug resistance through the expression of key enzymes of the acidic β-oxidation pathway. The growth-promoting effect of leptin through the ERK pathway has been demonstrated in breast cancer models (26). The PI3K/Akt signaling pathway has been implicated in regulating the leptin-induced epithelial-mesenchymal transition in breast cancer. This pathway is also considered to contribute to the upregulation of IL-8 and pyruvate kinase M2 in response to leptin (27). Collectively, these findings suggested that leptin indirectly promotes the progression of breast cancer by triggering the release of oncogenic factors from M2-type macrophages.

The present results suggested that leptin stimulates IL-8 secretion from TAMs to influence resistance to docetaxel in TNBC cell lines. Leptin-stimulated IL-8 secretion from TAMs promotes MDA-MB-231 invasion and migration. These findings provided further confirmation that the secretion of IL-8 from M2-type TAMs induced by leptin is linked to the progression of breast cancer and its resistance to drugs. It was found that IL-8 expression in the leptin-activated M2-type TAM group was significantly higher than that in the other groups.

The present findings also suggested that leptin-stimulated production of IL-8 by TAMs significantly improved the invasive ability of MDA-MB-231 TNBC cells. Upon treatment with an IL-8 neutralizing agent before culturing MDA-MB-231 TNBC cells, the invasive metastatic ability of the cell lines in the neutralized group was found to be significantly lower than that of the experimental group, with no significant difference from that of the blank control group. In summary, the interaction between leptin and M2-type TAMs may promote IL-8 production by M2-type TAMs, influencing the development of breast cancer cells. The current study suggested that there is at least one source of IL-8, which promotes the interaction of leptin with M2-type TAMs in the tumor microenvironment. Furthermore, the ability of leptin to stimulate the production of IL-8 in TAMs contributes to the progression of breast cancer. Leptin reduces the sensitivity of TNBC cell lines to docetaxel after its action on M2-type tumor macrophages. Therefore, the involvement of the leptin-TAMs-IL-8 axis in the interaction between the tumor microenvironment and breast cancer adds an additional level of intricacy that contributes to cancer progression. This interaction may also potentially correlate with chemoresistance. Therefore, the leptin-TAMs-IL-8 axis may be an important target for addressing chemoresistance in breast cancer.

Leptin is primarily produced by secretion from white adipocytes (28). It has been previously reported that obesity is not only related to metabolic diseases but is also closely associated with the development of breast cancer (29). Leptin levels are higher in more malignant breast cancers (30). Adipose tissue occupies 90% of the volume of the mammary gland and can secrete a large number of lipotropic factors, such as leptin, which can promote breast cancer invasion, metastasis, neoangiogenesis and epithelial mesenchymal transition through paracrine secretion (31). Moreover, leptin can accelerate breast cancer progression by recruiting macrophages in the tumor microenvironment (32). The effect of leptin on breast cancer also requires numerous signaling pathways to achieve. An in-depth study of the leptin signaling pathway to explore its intrinsic connection with breast cancer development will lay the foundation for the next step in finding effective targeted drugs to cut off the leptin-mediated signaling pathway.

The present study has certain strengths and weaknesses. Free doxorubicin levels may be reduced due to the higher binding of doxorubicin to proteins. The proteins are able to bind doxorubicin and form complexes that limit the availability of doxorubicin. This process may affect the action of doxorubicin on tumor cells. Therefore, to avoid the direct effect of leptin on tumor cell lines as well as docetaxel, the supernatant was collected after the action of leptin on TAMs before completing subsequent experiments. This experimental protocol minimizes the direct effects of leptin on docetaxel. It was partly proved that the action of leptin on TAM would increase the drug resistance of tumor cells, and this process might work by regulating IL-8 secretion. However, the process is not rigorous enough to directly determine the source and destination of IL-8, and further *in vivo* experiments and immunolabeling are needed for subsequent verification.

In conclusion, leptin reduces the sensitivity of TNBC cell lines to docetaxel after action on M2 tumor macrophages. The leptin-TAMs-IL-8 axis plays a role in this, potentially correlating with chemoresistance.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

SG and SD performed the experiments and collected the primary data. SG, SD and ZT wrote the main manuscript and prepared figures. All authors reviewed the manuscript. All authors read and approved the final manuscript. All authors confirm the authenticity of all the raw data.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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