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HJURP Derived from Cancer-Associated Fibroblasts Promotes Glutamine Metabolism to Induce Resistance to Doxorubicin in Ovarian Cancer

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Cancer-associated fibroblasts (CAFs) are closely associated with tumor drug resistance. This study intended to delineate how CAFs induced DOX resistance in ovarian cancer. Differential gene expression analysis of ovarian cancer CAFs was completed using Gene Expression Omnibus database. CAFs and normal fibroblasts (NFs) were isolated from ovarian cancer tissues and adjacent normal tissues. The expressions of Holliday Junction Recognition Protein (HJURP), *α*-smooth muscle actin (*α*-SMA), and fibroblast activation protein alpha (FAP) were assessed by quantitative reverse transcription polymerase chain reaction and Western blot (WB), *α*-SMA and FAP were detected by immunofluorescence. A2780 cells were treated with CAF or NF conditioned medium (CM), and protein expression of HJURP was assessed by WB. A2780-DOX cells were constructed and cultured with CAF or NF CM, and cell viability and IC_{50} value of DOX were assayed by Cell Counting Kit-8. Kits were used to test glutamine metabolism and mitochondrial tricarboxylic acid (TCA) cycle products, while WB was utilized to assess expressions of amino acid transporters. mRNA and protein levels of HJURP in CAFs derived from ovarian cancer were significantly higher than those in NFs. Culturing ovarian cancer cells with CAF CM could increase protein expressions of HJURP. HJURP derived from CAFs significantly enhanced viability of A2780-DOX cells and DOX resistance. CAF-derived HJURP fostered glutamine metabolism and mitochondrial TCA cycle in ovarian cancer resistant cells ultimately leading to ovarian cancer DOX resistance. CAF-derived HJURP drove ovarian cancer glutamine metabolism and DOX resistance.

Keywords: cancer-associated fibroblasts; doxorubicin resistance; glutamine metabolism; Holliday Junction Recognition Protein (HJURP); ovarian cancer
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Introduction

Ovarian cancer is a prevalent gynecological disease. According to the Global Cancer Data, approximately 314,000 new cases of ovarian cancer were diagnosed in females in 2020, resulting in approximately 207,000 female deaths (Sung et al. 2021). Surgical intervention, chemotherapy, radiation therapy, targeted therapy, and immunotherapy are treatment avenues for ovarian cancer (Veneziani et al. 2023). Doxorubicin (DOX) is initially a synthesized antibiotic from *Streptomyces peucetius caesius* (Mattioli et al. 2023). It is now commonly functioned as an anthracycline chemotherapy drug for ovarian cancer (Zhang et al. 2023). The anticancer action of DOX is mediated by two primary mechanisms: first, excessive generation of free radicals during DOX metabolism results in cell damage; second, DOX can insert into host DNA and impede topoisomerase IIA activity, thereby initiating a cascade of apoptosis (Al-Malky et al. 2020). However, ovarian cancer patients often develop resistance to DOX. Understanding the processes underlying DOX resistance is crucial for improving treatment effectiveness in ovarian cancer patients.

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Cancer-associated fibroblasts (CAFs) are among the most abundant stromal cells in the tumor microenvironment, and they can increase cancer cell resistance through varying mechanisms (Biffi and Tuveson 2021). CAF could activate different signaling cascades in tumor cells by releasing cytokines or secreting extracellular vesicles, ultimately leading to drug resistance in cancer cells (Guo et al. 2019). For example, CAFs can deliver miR-98-5p through exosome and target Cyclin Dependent Kinase Inhibitor 1A (CDKN1A) expression in cancer cells, thereby hindering apoptosis and cell cycle in cisplatin-treated ovarian cancer cells, ultimately leading to development of cisplatin resistance (Guo et al. 2019). Therefore, a more thorough knowledge of the processes by which CAFs drive tumor cell resistance is necessary in order to combat chemoresistance and malignant development in ovarian cancer.

Cellular metabolic reprogramming contributes to development of drug resistance in tumors (Cocetta et al. 2020). The human body contains large amounts of glutamine, an amino acid that may be used as a carbon source to assist the tricarboxylic acid (TCA) cycle and glutathione synthesis (Yoo et al. 2020). Tumor cells need to consume a large amount of glutamine and obtain the required energy through glutamine metabolism (Reinfeld et al. 2021). In addition, metabolic reprogramming of glutamine is particularly pivotal for drug-resistant cells. Zhang et al. (2021) conducted a metabolomic sequencing analysis on DOX-resistant or sensitive colorectal cancer cells and found that DOX-resistant cell group had higher levels of D-glutamine and glutamate. Therefore, targeting glutamine metabolism may help drug-resistant cells become more sensitive to medications. According to Kim et al. (2022), blocking glutamine uptake inhibits mTORC1/S6K signaling, and makes the ovarian cancer resistant cells sensitive to paclitaxel therapy again. To sum up, abnormal glutamine metabolism in cancer is closely related to development of drug resistance.

However, the relationship between CAF as an active participant in tumor metabolism and the glutamine metabolism and resistance of ovarian cancer still needs elucidation. In this study, HJURP was overexpressed in ovarian cancer CAFs and HJURP derived from CAF could promote glutamine metabolism in ovarian cancer cells, thereby increasing their resistance to DOX.

Methods

Cell culture

Ovarian cancer cells A2780 (BNCC351906) were accessed from BNCC (China) and cultured in RPMI-1640 medium (Gibco, Grand Island, New York, USA) containing 10% fetal bovine serum (FBS) (Gibco). By gradually increasing concentration of DOX in A2780 culture medium, DOX-resistant ovarian cancer cells (A2780-DOX) were constructed. Cells were grown in a cell incubator with 5% CO_2 at 37°C.

Isolation and culture of primary fibroblasts

Written informed consent was obtained from patients with ovarian cancer samples and adjacent normal tissues in this study, and the research protocol was approved by the Affiliated Jinhua Hospital, Zhejiang University School of Medicine Institutional Review Board. Tumor tissue samples and adjacent normal tissue samples from 10 ovarian cancer patients were collected and washed several times with sterile PBS (1.5 mM KH2PO4, 135 mM NaCl, 2.7mM KCl and 8mM K2HPO4, pH7.4). Samples were sliced into small cubes and incubated with an appropriate amount of 10 ml PBS and 10ml 0.25% trypsin/25 mM EDTA at a 1:1 ratio, shaking at 37°C for 30 minutes. The solution containing suspended cells was taken out and filtered through a 40 μm cell filter, followed by centrifugation at 1,500 rpm for 10 minutes. The collected normal fibroblast (NF) and CAF suspensions were cultured with DMEM/F12 (Gibco) supplemented with 10% FBS (Gibco) and 1% penicillin/streptomycin (Gibco). Experiments were conducted using primary fibroblasts with no more than four passages. Culture medium of NFs or CAFs, which had been cultured for 3 days, was collected and mixed with fresh DMED/F12 medium at a 1:1 ratio to culture A2780 cells as conditioned mediums (CM-NF, CM-CAF). To investigate the effect of CAFs on A2780-DOX cell resistance, CAF culture media (CM-sh-HJURP, CM-sh-NC) transfected with sh-HJURP or sh-NC were collected, and CMs were configured as described above to culture A2780-DOX cells for 72 hours. To delineate impact of CAFs on glutamine metabolism in A2780-DOX cells, A2780-DOX cells treated with DMSO or the amino acid transporter inhibitor GPNA (MCE, USA) were cultured in different CMs. The above cells were cultured in a highly humidified (80%) incubator containing 5% CO_2 (v/v) at 37°C (Deying et al. 2017).

Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

Cells were plated in a six-well plate (1×10^6 cells/well) and treated variously following experimental protocol. Total RNA was extracted from cells using TRIzol (Invitrogen, Carlsbad, California, USA). Using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA), the concentration and purity of RNA were ascertained. qRT-PCR was done on Applied Biosystems™ 7500 Real-Time PCR System (Thermo Fisher Scientific) using the CellsDirect™ One-Step qRT-PCR Kit (Invitrogen). mRNA levels of HJURP, α-smooth muscle actin (α-SMA), and fibroblast activation protein alpha (FAP) were analyzed using $2^{-\Delta\Delta Ct}$ method with Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) as reference gene. Primers are listed in Table 1.

Immunofluorescence

After cells were treated with different methods, they were fixed with 4% paraformaldehyde for 10 minutes, then permeabilized with 0.1% TritonX-100 for 10 minutes. Cells

Table 1. qRT-PCR primer sequence.

Gene	Primer sequence (5′→3′)
HJURP	F: CATGCAGCGGCTGATAGAGA
	R: AGGCTGGATCTCTCCTTCGT
α-SMA	F: GTGACTACTGCCGAGCGTG
	R: ATAGGTGGTTTCGTGGATGC
FAP	F: AGAACCATGCTTTGGAGATACT
	R: TTTACTCCCAACAGGCGACC
GAPDH	F: AATCCCATCACCATCTTC
	R: AGGCTGTTGTCATACTTC

were blocked with 1% BSA at room temperature for 1 hour. Subsequently, the fixed and permeabilized cells were incubated with Alexa Fluor 488-Anti-α-SMA (ab202295, Abcam, Cambridge, UK) or FITC-FAP antibody (AA311-410, antibodies-online GmbH, Aachen, Germany) separately overnight at 4°C, followed by incubation with secondary antibodies at 37°C in the dark for 1 hour. The cell nucleus was examined using DAPI staining, sealed with fluorescence quencher (Beyotime, Shanghai, China), and examined using a confocal microscope (Nikon, Tokyo, Japan).

Western blot (WB)

Cells were plated in six-well plates (1×10^6 cells/well) and treated following protocol. Total protein was extracted with RIPA lysis buffer (Thermo Fisher Scientific), and the protein concentration was determined by BCA kit (Thermo Fisher Scientific). Protein was separated in equal amounts using SDS-PAGE electrophoresis and then put onto a PVDF membrane. After rapid blocking, the membrane wasleft overnight at 4°C to be treated with the primary antibody. After washing away the excess primary antibody with TBST, the secondary antibody was incubated at room temperature for 2 hours. After TBST rinses, protein bands were assessed on fluorescence and chemiluminescence imaging system (Clinx, Shanghai, China) according to ECL chemiluminescence detection kit (epizyme, Shanghai, China). Antibody information was as follows: Rabbit anti-FAP antibody (ab314456, Abcam, 1:1,000); Rabbit anti-α-SMA antibody (ab124964, Abcam, 1:10,000); Rabbit anti-SLC38A2 antibody (PA5-113241, Invitrogen, 1:1,000); Rabbit anti-SLC7A5/LAT1 antibody (ab305251, Abcam, 1:1,000); Rabbit anti-SLC1A5/ASCT2 antibody (ab237704, Abcam, 1:1,000); Rabbit anti-HJURP antibody (ab233541, Abcam, 1:1,000); Goat anti-rabbit IgG H&L (HRP) antibody (ab6721, Abcam 1:5,000).

Cell transfection

Fragments containing HJURP were inserted into pcDNA3.1 expression vector (V79020, Invitrogen) or pGPU6/Neo plasmid (GenePharma, Shanghai, China) to construct oe-HJURP and sh-HJURP plasmids, with oe-NC and sh-NC as empty plasmids. Cells were transfected using Lipofectamine 3000 reagent (Invitrogen).

Cell Counting Kit-8 (CCK-8) assay

Cell proliferation ability and IC_{50} were assessed by CCK-8 kit (Beyotime). A2780-DOX cells were seeded in a 96-well plate (5,000 cells/well) and cultured with NF or CAF CMs under different treatments for 0, 12, 24, 48, and 72 hours. Then, 10 μL of CCK-8 detection reagent was added to each well, and the absorbance at 450 nm was measured after incubation for 4 hours. For the determination of IC_{50} for DOX, A2780-DOX cells were seeded in a 96-well plate (20,000 cells/well) and left overnight with NF or CAF CMs treated differently. Cells were treated with 0, 1, 10, 25, 50, 100, and 150 μM DOX for 24 hours. Absorbance was measured and IC_{50} value of A2780-DOX was calculated using the above method.

Bioinformatics analysis

Gene Expression Omnibus (GEO) database was searched for expression chip GSE40595 related to ovarian cancer CAFs to screen for differentially expressed genes, and the expression of HJURP in CAF was examined using the Wilcoxon test. In addition, we analyzed signaling pathways enriched by HJURP through GSEA.

Detection of glutamine metabolism

Glutamine Assay Kit (Abcam) was utilized to assess glutamine uptake, Glutamate Assay Kit (Abcam) was employed to test glutamate levels, and α-KG Assay Kit (Abcam) was used to measure α-KG levels. A2780-DOX cells were seeded in a 6-well plate (2×10^5 cells/well) and treated with DMSO or GPNA, and NF-CM or CAF-CM was used. Cells were harvested and added to buffer, and the supernatant was collected after thorough homogenization. Protein was removed using a 10kDa spin column (Abcam), and the reaction solution was added sequentially for detection and analysis by comparing with the standard curve.

Detection of metabolite content

NADPH/NADP+ assay kit (Abcam) was utilized to detect ratio of NADPH/NADP+, glutathione assay kit (Beyotime) was used to test ratio of GSH/GSSG, aspartate assay kit (Sigma-Aldrich, St. Louis, Missouri, USA) was employed to assess content of aspartate (Asp), and oxaloacetate assay kit (Sigma-Aldrich) was taken to analyze level of oxaloacetate (OAA). Cell treatment was performed as described in section 2.9. Cells were harvested and supernatant was extracted. Reaction solution was sequentially added for comparative analysis and calculation.

Statistical analysis

Every experiment was conducted three times. GraphPad Prism was used to analyze and plot the data, which were then shown as mean ± standard deviation. Student t-text was used for single comparison, and one-way ANOVA was used for multiple comparisons. Statistics are deemed significant when $P < 0.05$.

The research protocol was approved by the Affiliated Jinhua Hospital, Zhejiang University School of Medicine Institutional Review Board (No.2023-201).

Results

Specific upregulation of HJURP in fibroblasts derived from ovarian cancer

First, we performed mRNA differential expression analysis of fibroblasts in ovarian cancer and normal tissues in GEO-GSE40595 dataset by bioinformatics methods. 2,441 differentially expressed genes were obtained, including 1,075 significantly upregulated genes and 1,366 significantly downregulated genes. Wilcoxon test revealed significant overexpression of HJURP in ovarian cancer fibroblasts. We selected HJURP as the target gene for our investigation since the role of HJURP in ovarian cancer is not well characterized. To determine mRNA levels of HJURP in ovarian cancer fibroblasts, we isolated CAFs and NFs from tumor tissues or adjacent normal tissues of 10 ovarian cancer patients. CAF, as activated fibroblasts, differed from NF in that it could secrete various mesenchymal-specific proteins such as α-SMA, FAP, etc. We determined the separation of CAFs and NFs by detecting the mRNA and protein expression of α-SMA and FAP using qRT-PCR, WB, and immunofluorescence staining. As illustrated in Fig. 1A, B, mRNA and protein expressions of α-SMA and FAP in CAFs were significantly higher than those in NFs. As presented in immunofluorescence staining images, fluorescence intensity of α-SMA and FAP was weak in NFs, while it was obvious in CAFs (Fig. 1C).

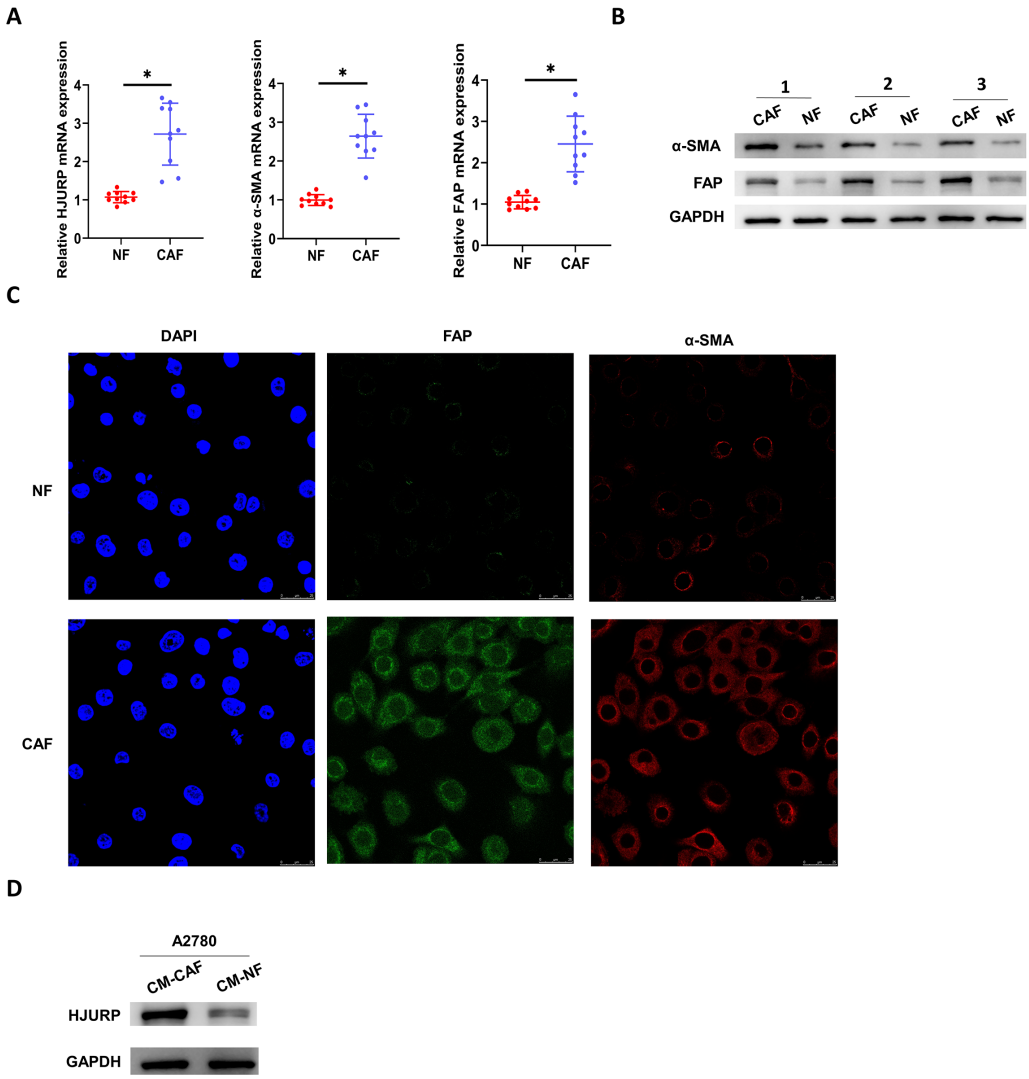

Fig. 1. Expression of HJURP in CAFs.
CAFs and NFs were isolated from tumor tissues and adjacent normal tissues of 10 ovarian cancer patients, respectively. A: qRT-PCR was used to detect mRNA expression levels of HJURP, α-SMA, and FAP in NFs and CAFs. B: WB was used to detect the protein expression levels of α-SMA and FAP in NFs and CAFs. C: Immunofluorescence staining was used to detect the protein expression levels of α-SMA and FAP in NFs and CAFs (Representative images). Scale bar was 25 μm. D: CM-CAF and CM-NF were used to culture ovarian cancer cell line A2780, and WB was used to detect the protein expression levels of HJURP. *represents $P < 0.05$.

These results indicated that we have successfully isolated primary NFs and CAFs. qRT-PCR revealed that mRNA level of HJURP in CAFs was significantly higher than that in NFs (Fig. 1A). Since CAFs can affect neighboring tumor cells in multiple ways (Dasari et al. 2018), we hypothesized that CAFs could secrete HJURP and thereby affect HJURP expression in ovarian cancer cells. Ovarian cancer cells were cultured with CM-CAF or CM-NF, and WB detection revealed that the protein expression level of HJURP in ovarian cancer cells treated with CM-CAF was much higher than that in cells treated with NF matrix (Fig. 1D). In summary, significant overexpression of HJURP was observed in CAFs, and HJURP derived from CAF could promote HJURP protein expression in ovarian cancer cells.

HJURP derived from ovarian cancer fibroblasts enhances resistance to DOX in ovarian cancer.

A previous study has reported that approximately 276 genes are differentially expressed in CAFs and are associated with chemoresistance in ovarian cancer (Han et al. 2022). In addition, HJURP can facilitate DOX resistance in triple-negative breast cancer (Mao et al. 2022). Therefore, we intended to investigate whether HJURP derived from ovarian cancer fibroblasts promoted ovarian cancer cell DOX resistance. A drug-resistant cell line A2780-DOX was built by gradually increasing concentration of DOX in culture medium. qRT-PCR presented that mRNA expression of HJURP in A2780-DOX was significantly higher than that in A2780 (Fig. 2A). Then, CM-NF and CM-CAF, the CM for NF or CAF, were collected and incubated with A2780-DOX cells separately. WB revealed that CAF CM significantly increased protein level of HJURP in A2780-DOX cells (Fig. 2B). Cell viability and IC_{50} were assayed by CCK-8. As depicted in Fig. 2C, viability of A2780-DOX cells in CM-CAF group was significantly higher than that in CM-NF group. In addition, A2780-DOX cells cultured in CM-CAF presented increased tolerance to DOX (Fig. 2D). Therefore, CAF could enhance resistance of ovarian cancer cells to DOX. To elucidate that impact of CAFs on ovarian cancer DOX resistance was mediated by HJURP, we knocked down HJURP in CAFs and cultured A2780-DOX cells in CM. WB unveiled that knocking down HJURP in CAFs significantly reduced protein level of HJURP in A2780-DOX cells (Fig. 2E). CCK-8 testing revealed that sh-HJURP significantly repressed cell viability of A2780-DOX in CM-CAF (Fig. 2F), and sh-HJURP could eliminate CAF-induced DOX resistance in ovarian cancer cells (Fig. 2G). These data indicated that HJURP in the CAF CM could facilitate DOX resistance in ovarian cancer cells.

Fibroblast-derived HJURP mediates glutamine metabolism to promote resistance to DOX in ovarian cancer

To clarify the mechanism of HJURP derived from fibroblasts promoting DOX resistance in ovarian cancer, we did GSEA on HJURP. As shown in Fig. 3A, HJURP was significantly enriched in the GOBP_GLUTAMINE_METABOLIC_PROCESS signaling pathway. HJURP was significantly positively correlated with glutamine metabolism marker genes SLC10A3, SLC1A5, SLC11A2, etc.

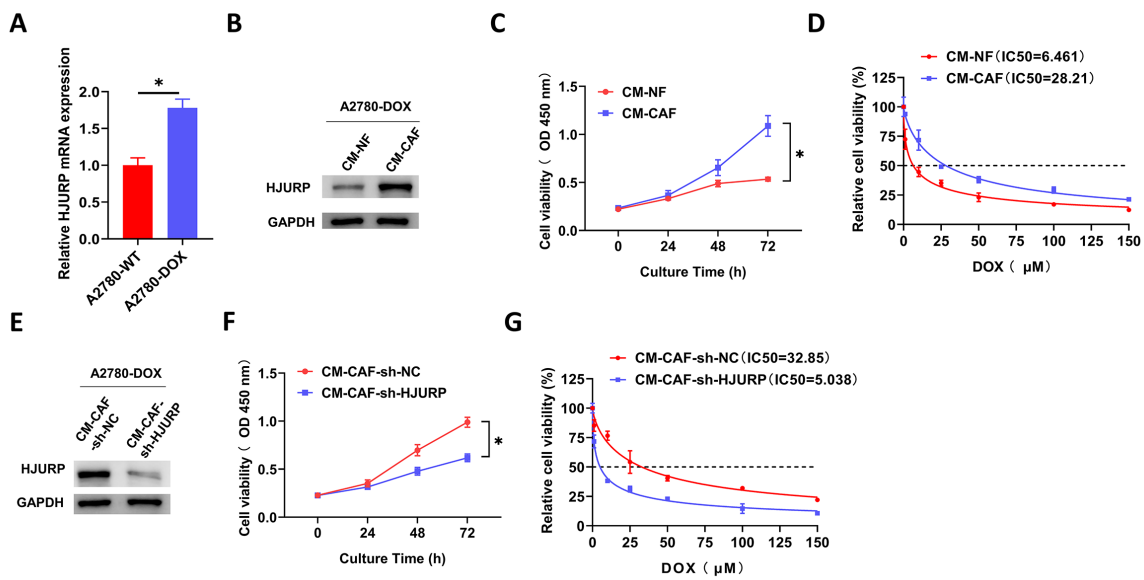

Fig. 2. Effect of CAF-derived HJURP on DOX resistance in ovarian cancer.
A: Construction of A2780 drug-resistant cells (A2780-DOX), qRT-PCR detection of HJURP mRNA levels in DOX-sensitive cells (A2780) and A2780-DOX cells; B: WB detection of the effect of CM-NF or CM-CAF cultured A2780-DOX cells on HJURP protein expression levels; C-D: CCK-8 detection of cell viability and DOX IC_{50} after CM-NF or CM-CAF cultured A2780-DOX cells; CAF cells were transfected with sh-NC or sh-HJURP, and A2780-DOX cells were cultured with CM-sh-NC or CM-sh-HJURP, E: WB detection of HJURP protein expression levels in each group; F-G: CCK-8 detection of cell viability and DOX IC_{50} in each group. *represents $P < 0.05$.

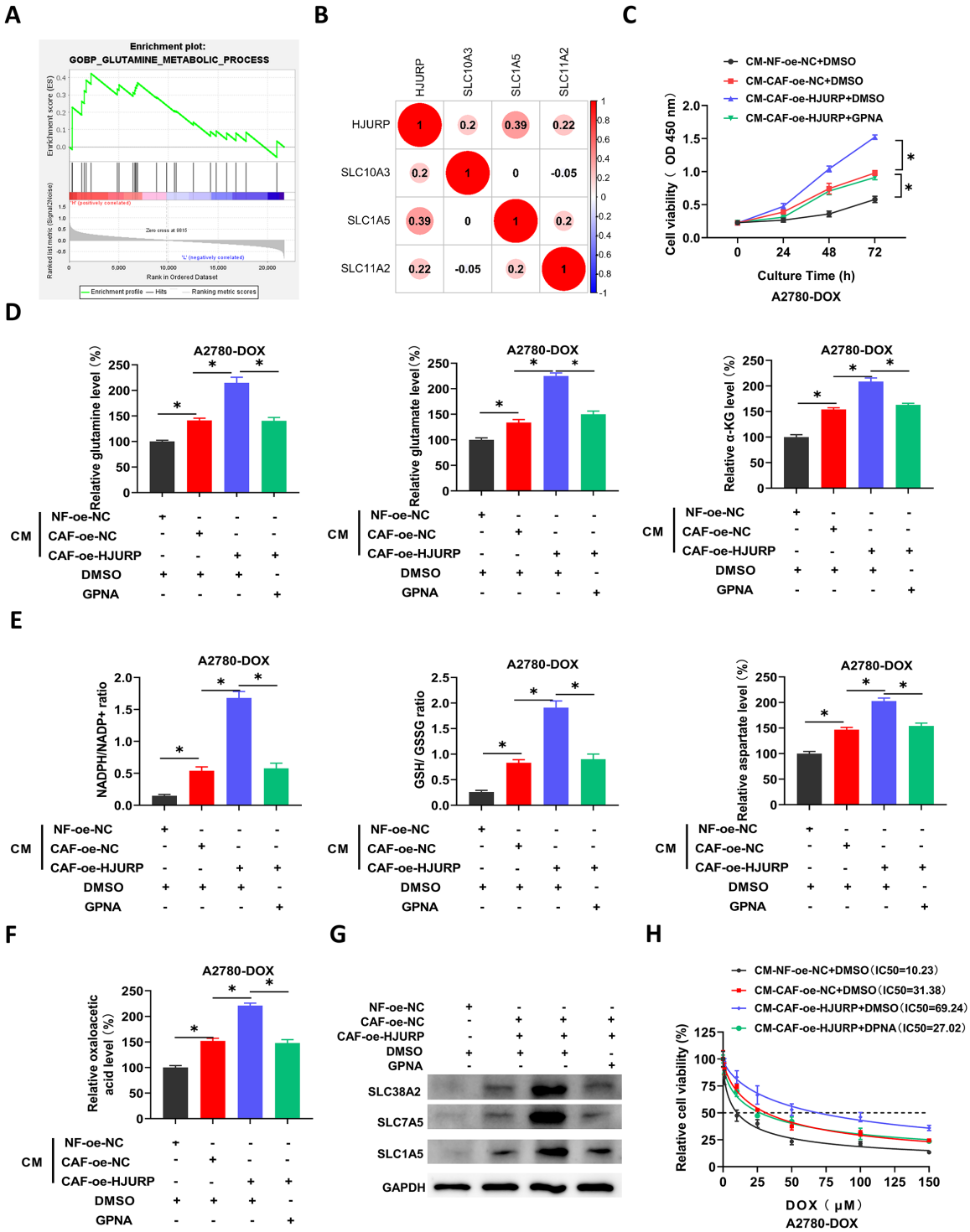

Fig. 3. Impact of CAF-derived HJURP on glutamine metabolism in ovarian cancer cells.
A: GSEA results of HJURP; B: Correlation plot of HJURP with glutamine metabolism marker genes; A2780-DOX cells cultured with CM-NF-oe-NC, CM-CAF-oe-NC, CM-CAF-oe-HJURP and treated with DMSO or GPNA, C: Cell viability detected by CCK-8 assay in each group; D: Contents of glutamine, glutamate, and α-KG in cells of each group detected by assay kits; E-F: Ratios of NADPH/NADP+ and GSH/GSSG, as well as contents of aspartate and oxaloacetate in cells of each group detected by assay kits; G: Protein expression levels of SLC38A2, SLC7A5, and SLC1A5 in cells of each group detected by WB; H: DOX IC_{50} of cells in each group detected by CCK-8 assay. *represents $P < 0.05$.

(Fig. 3B). Relationship between glutamine metabolism and tumor resistance has drawn a lot of attention from researchers (Hu et al. 2021). We hypothesized that HJURP derived from ovarian cancer fibroblasts led to ovarian cancer DOX resistance by affecting glutamine metabolism in ovarian cancer cells. GPNA was a glutamine analog metabolism inhibitor that suppressed activity of ASCT2, SNAT family, and LAT1/2. A2780-DOX cells were treated with DMSO or GPNA and cultured with fibroblast cell CM from different sources. Cell viability of A2780-DOX cells in different treatment groups was measured by CCK-8. As depicted in Fig. 3C, upregulation of HJURP in CAF increased viability of A2780-DOX cells after CM-CAF treatment, while culturing A2780-DOX cells treated with GPNA in the same way significantly reduced cell viability. The glutamine metabolism in ovarian cancer cells was examined using different assay kits. Compared to NF CM treatment, cell uptake of glutamine, synthesis of glutamate and α-KG significantly increased after CAF CM treatment (Fig. 3D), and levels of intracellular NADPH/NADP+, GSH/GSSG, aspartate, and oxaloacetate also significantly increased. These results indicated that CAF activated glutamine metabolism in ovarian cancer resistant cells. Compared with CAF treated with oe-NC, the production of related metabolites of glutamine metabolism and mitochondrial TCA cycle-related substances in A2780-DOX cells incubated with CAF overexpressing HJURP significantly increased, and this trend was restored after treatment with GPNA (Fig. 3E, F). In addition, protein expression of glutamine transporters SLC38A2, SLC7A5, and SLC1A5 was assessed by WB. As plotted in Fig. 3G, expressions of transporters were upregulated in group with strong glutamine metabolism, ultimately enhancing the influx of glutamine in ovarian cancer resistant cells. We also examined the changes in A2780-DOX resistance under different treatments. Upregulation of HJURP in CAF enhanced resistance of ovarian cancer cells to DOX, while inhibition of glutamine uptake in ovarian cancer cells by GPNA reversed this trend (Fig. 3H). Overall, these data suggested that HJURP derived from ovarian cancer-associated fibroblasts affected DOX resistance by increasing glutamine metabolism in ovarian cancer cells.

Discussion

This work revealed significant overexpression of HJURP in ovarian cancer cells and ovarian CAFs through bioinformatics analysis. HJURP derived from ovarian CAFs fostered glutamine metabolism in ovarian cancer by upregulating amino acid transporters, thereby increasing resistance of ovarian cancer to DOX. For the aim of overcoming DOX resistance in ovarian cancer, targeting HJURP generated from CAFs may therefore constitute a potential therapeutic target.

We discovered abnormal expression of HJURP in ovarian cancer and CAFs through bioinformatics analysis. HJURP is an adverse prognostic factor and is significantly upregulated in prostate cancer (Lai et al. 2021), non-small cell lung cancer (Wei et al. 2019), breast cancer (Hu et al. 2010), hepatocellular carcinoma (Chen et al. 2019), pancreatic cancer (Wang et al. 2020), and other cancers. Drug resistance is the greatest challenge encountered in cancer treatment. In triple-negative breast cancer, HJURP can bind to the YAP1 protein and prevent its ubiquitination, which lowers the cancer cell sensitivity to DOX (Mao et al. 2022). This study also found a correlation between HJURP and DOX resistance in ovarian cancer cells, and experimentally verified that the expression level of HJURP in DOX-resistant ovarian cancer cells was significantly higher than that in DOX-sensitive ovarian cancer cells. This is consistent with previous findings suggesting that HJURP may act as a tumor-promoting factor. We also unraveled that HJURP derived from ovarian cancer CAFs could further enhance resistance of ovarian cancer DOX-resistant cells. Similarly, Dou et al. (2022) reported that HJURP overexpression is associated with poor prognosis in serous ovarian cancer, and HJURP may indirectly regulate WEE1 to promote cisplatin chemoresistance in ovarian cancer through the transcription factor MYC. Therefore, HJURP is a key factor for drug resistance of ovarian cancer cells, and difference between our experiment and previous studies lies in our exploration of the impact of CAF-derived HJURP on the DOX resistance of ovarian cancer. CAFs are the most abundant stromal cells in the tumor microenvironment and are inseparable from tumor drug resistance and complex metabolism.

CAF plays a key role in initiation and development of tumors (Fiori et al. 2019; Ma et al. 2022; Kennel et al. 2023). Thus, deep understanding of interaction mechanism between CAFs and cancer cells has significant clinical implications. CAFs can communicate with cancer cells through cell-cell contact, secretion of cytokines, or exosome (Li et al. 2021). In this study, CAF affected protein level of HJURP in ovarian cancer cells by secreting HJURP. HJURP in the CAF CM also increased uptake of glutamine by ovarian cancer cells via upregulation of amino acid transporters, thereby facilitating production of metabolic products glutamate and α-KG and providing substrates for TCA cycle. Glutamine has significant effects on cell cycle, apoptosis, proliferation, glycolysis, oxidative stress, and chemoresistance in ovarian cancer (Fasoulakis et al. 2023). We manifested that CAF-derived HJURP fostered TCA cycle in ovarian cancer by affecting glutamine metabolism, further lowering sensitivity of ovarian cancer cells to DOX. However, Guo et al. (2021) pointed out that methylation of glutaminase in cisplatin-resistant ovarian cells leads to a decrease in levels of TCA cycle metabolites, and this reprogramming of glutamine metabolism results in cisplatin resistance in ovarian cancer. This may be due to different mechanisms of glutamine metabolism reprogramming in ovarian cancer DOX-resistant cells caused by CAF-derived HJURP, which also fully reflects the diversity of tumor drug resistance.

Our research results suggested the importance of CAF-derived HJURP in modulating cancer glutamine metabolism and ovarian cancer chemotherapy resistance, and targeting CAF-derived HJURP could provide new ideas for enhancing sensitivity of ovarian cancer chemotherapy. Although we have preliminarily revealed the molecular mechanism of HJURP-induced DOX resistance in ovarian cancer cells derived from CAFs, the specific mechanism of HJURP overexpression in CAFs remains unclear. In addition, how CAF-derived HJURP enters ovarian cancer cells and through which mechanism it affects ovarian cancer cell glutamine metabolism are still elusive. Notwithstanding limitations, the results demonstrated that the secretion of HJURP by CAF may serve as an attractive target in cancer therapy.

Author Contributions

(I) Conception and design: Yanfang Lan. (II) Provision of study materials or patients: Hao Xu and Yanfang Lan. (III) Collection and assembly of data: Hao Xu. (IV) Data analysis and interpretation: Lanying Jin. (V) Manuscript writing: Lanying Jin and Hao Xu. (VI) Final approval of manuscript: All authors.

Conflict of Interest

The authors declare no conflict of interest.

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