Cancer Letters 588 (2024) 216700

Original Articles

STING inhibitors sensitize platinum chemotherapy in ovarian cancer by inhibiting the CGAS-STING pathway in cancer-associated fibroblasts (CAFs)

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ARTICLE INFO

Keywords:
Ovarian cancer
Chemoresistance
Cancer-associated fibroblasts
STING inhibitors
IFNB1

ABSTRACT

Chemotherapy resistance in ovarian cancer hampers cure rates, with cancer-associated fibroblasts (CAFs) playing a pivotal role. Despite their known impact on cancer progression and chemoresistance, the specific mechanism by which CAFs regulate the tumor inflammatory environment remains unclear. This study reveals that cisplatin facilitates DNA transfer from ovarian cancer cells to CAFs, activating the CGAS-STING-IFNB1 pathway in CAFs and promoting IFNB1 release. Consequently, this reinforces cancer cell resistance to platinum drugs. High STING expression in the tumor stroma was associated with a poor prognosis, while inhibiting STING expression enhanced ovarian cancer sensitivity. Understanding the relevance of the CGAS-STING pathway in CAFs for platinum resistance suggests targeting STING as a promising combination therapy for ovarian cancer, providing potential avenues for improved treatment outcomes.

1. Introduction

Ovarian cancer is one of the most lethal among gynecological malignancies globally. In the United States alone, the year 2023 is anticipated to witness 19,710 fresh cancer diagnoses and 13,270 cancer-related deaths [1]. Unfortunately, the pernicious nature of the disease leads to approximately 75% of individuals being diagnosed at an advanced stage. High-grade serous ovarian carcinoma (HGSOC) is the predominant histological subtype, constituting over 75% of epithelial ovarian cancer (EOC) cases [2]. Current standard treatment involves cytoreductive surgery followed by platinum and taxane combination chemotherapy. However, 65–80% of patients face recurrence with acquired or primary cisplatin resistance [3]. Thus, identifying new therapeutic targets and developing effective prognostic markers remain formidable challenges in overcoming cisplatin resistance.

The tumor microenvironment (TME) plays a pivotal role in cancer pathogenesis and progression. Cancer cells recruit and transform surrounding cells, creating a protective microenvironment that fosters the survival, growth, and spread of ovarian cancer [4]. This intricate interplay involves a complex network of growth factors, chemokines, cytokines, and inflammatory molecules [5]. Recently, targeting the TME strategically has gained attention as a promising tumor treatment approach, recognizing its crucial roles in tumor progression and therapeutic response modulation [6]. Central to the TME are cancer-associated fibroblasts (CAFs), a diverse group of cells with various functional and phenotypic characteristics [7]. CAFs actively contribute to tumor advancement and influence therapeutic responses through mechanisms such as inflammation control, angiogenesis, immunosuppression, drug resistance, and tumor stroma remodeling [8–10]. While the precise process of fibroblast activation into CAFs remains unclear, it is established that cancer cells can trigger the transformation of normal fibroblasts into CAFs [11].

There is a growing body of information that supports the significant involvement of the DNA-sensing CGAS-STING pathway in the innate immune response against tumors [12]. cGAS-STING pathway confirmed that it could up-regulate Type I interferon (IFN-I) through TBK1-IRF3 pathway.

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https://doi.org/10.1016/j.canlet.2024.216700
Received 14 November 2023; Received in revised form 18 January 2024; Accepted 1 February 2024
Available online 17 February 2024
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pathway and NF-κB pathway to participate in anti-tumor immunity. IFN-I is a multifaceted cytokine that exerts its influence on a wide range of immune cell populations. It promotes the multiplication of natural killer (NK) cells [13], the release of Interferon-gamma (IFN-\(\gamma\)) [14], and the enhancement of cytotoxic function [15]. It also supports the development, maturation, and migration of antigen-presenting cells derived from monocytes [16]. Furthermore, thanks to the up-regulation of PD-L1 induced by Sting agonists, a number of clinical trials have shown that the combination therapy of Sting agonists and \(\alpha\)-PD-L1/PD-L1 antibodies has achieved significant anti-tumor effects in melanoma, lung cancer, colorectal cancer and other solid tumors [17,18]. Adequate immune infiltration is necessary to initiate active T-cell therapy. However, ovarian cancer is characterized by a low gene expression profile of T-cell infiltration, and the majority of ovarian cancer patients exhibit limited infiltration of tumor tissue [19]. On the other hand, relevant studies have shown that Sting may have contrasting effects on tumor progression at different stages [18]. Ahn et al. concluded that cGAS-STING pathway activated by chronic stimulation can mediate tumor formation and metastasis through inflammation [20]. In addition, Sting signaling activation also promotes tumor growth by inducing the expansion of Breg cell subsets to form immunosuppressive TME. Therefore, it remains unclear whether patients with ovarian cancer will benefit from Sting agonists, the role of CGAS-STING pathways in ovarian cancer it is necessary to further elaboration [20–22].

Cisplatin anticancer efficacy arises from its covalent interactions with purine bases, causing DNA damage in tumor cells [23]. Existing studies propose genomic communication between cells through pinocytosis within tumors [20,24]. Understanding intercellular communication in the tumor microenvironment offers novel insights for tumor treatment. We hypothesize that platinum-based chemotherapy exacerbates genomic instability, promoting the transfer of genetic material from cancer cells to cancer-associated fibroblasts (CAFs). Activation of the CGAS-STING pathway in CAFs is triggered by genomic DNA from cancer cells, intensifying platinum resistance in cancer cells influenced by CAFs. This study comprehensively explores CAFs’ pivotal role in ovarian cancer development, progression, and platinum resistance. Correlations between Sting expression in CAFs and clinical features in ovarian cancer patients were verified. The Sting agonist MSA-2 and Sting antagonist H-151 were employed to confirm Sting’s involvement in ovarian cancer advancement and platinum resistance, both in vitro and in vivo. Mechanistic research reveals platinum chemotherapy induces genomic communication between cancer cells and CAFs, activating the CGAS-STING pathway in CAFs. Furthermore, IFN1 up-regulation promotes platinum resistance by enhancing DNA damage repair and protecting ovarian cancer cells from cisplatin-induced apoptosis and cell cycle arrest.

This study clarifies the link between the CGAS-STING pathway in cancer-associated fibroblasts (CAFs) and platinum resistance in ovarian cancer. We found that antagonizing Sting could enhance ovarian cancers responsiveness to platinum-based chemotherapy, whereas the influence of Sting agonists on anti-tumor immunity proved to be less pronounced. This underscores the potential of Sting as a promising target in combination therapy strategies, emphasizing the need for caution when considering the use of Sting agonists in ovarian cancer treatment.

2. Materials and methods

2.1. Cell lines

The human ovarian cancer cell lines HEY, SKOV3, COV362, and the mouse ovarian surface epithelial cell line ID8 were generously supplied by the Stem Cell Bank at the Chinese Academy of Sciences. The human ovarian cancer cell line OVCA8 was purchased from Shanghai Zhong Qiao Xin Zhou Biotechnology Co., Ltd. Cell lines were validated through STR profiling. The cells were subjected to conventional cell culture conditions, which included a temperature of 37 °C and a carbon dioxide concentration of 5%. The HEY, COV362, and ID8 cell lines were grown DMEM medium (Gibco, Thermo Fisher Scientific) supplemented with 10% fetal bovine serum (FBS) (LONSERA, Uruguay). The SKOV3 cell line was grown in McCoy’s 5A medium (Thermo Fisher Scientific) supplemented with 10% FBS (EXCELL, Uruguay). The OVCAR8 cell line was grown in RPMI 1640 medium (Gibco, Thermo Fisher Scientific) supplemented with 10% FBS (EXCELL, Uruguay).

2.2. Isolation and primary culture of ovarian cancer-associated fibroblasts

A total of four samples of HGSOC tissues were obtained from the Department of Obstetrics and Gynecology at Qilu Hospital, Shandong University, following the patients’ informed consent. The Ethics Committee of Shandong University provided ethical permission for this study. Patients were diagnosed with HGSOC clinicopathologically before undergoing chemotherapy and surgery. The primary cells derived from ovarian cancer were subjected to culturing techniques in accordance with established methodologies, as described in prior investigations [25]. Briefly, the tissues were cut into small pieces, and then the bacteria were eradicated using a 3% Penicillin-Streptomycin-Gentamicin Solution (Beyotime). Then, tissues were digested with 1 mg/mL collagenase type IV (Sangon Biotech) and 1 mg/mL hyaluronidase (Sangon Biotech) at 37 °C with shaking for 2–3 h. Thereafter, the dissociated tissues were collected by centrifugation at 1000 rpm for 10 min. Tissues were suspended in DMEM/F-12 with HEPES (Gibco, Thermo Fisher Scientific) and 20% FBS. The Stromal cell-enriched supernatants were then separated into the culture bottle.

2.3. Patients and tissue samples

Between 2007 and 2013, a collection of 384 formalin-fixed, paraffin-embedded (FFPE) samples of HGSOC was collected from the pathology department of Qilu Hospital, Shandong University. The utilization of all specimens was contingent upon obtaining informed consent from either the patients themselves or their legal guardians.

2.4. Immunocytofluorescence (ICF)

The cells were treated with a 4% paraformaldehyde solution for fixation and a 0.3% Triton-X 100 solution (Solarbio) for permeabilization. The primary antibodies α-SMA (1:500), FAP (1:200), EPCAM (1:200), or γH2AX (1:200) were subjected to an overnight incubation at 4 °C, followed by a secondary incubation with secondary antibodies Alexa Fluor® 488 Phalloidin (1:1000) or Alexa Fluor® 647 Conjugate (1:1000) for 1 h at room temperature in a light-restricted environment. The nucleus was labeled with DAPI (Beyotime). The images were acquired using a Sunny confocal microscope system or fluorescence microscopy.

2.5. Cell Co-cultures

Conditioned medium co-culture was performed using various combinations of human ovarian cancer cell lines and patient-derived primary cancer-associated fibroblasts. In the co-culture process, both the experimental group and the NC group maintained the same culture conditions. FBS-free supernatants of CAFs cultured for 48 h were collected. These supernatants were then mixed with normal FBS-free medium at a 1:1 ratio to create conditioned medium (CAFs-Sup). CAFs-Sup was supplemented with 10% FBS before co-culture. The HEY and OVCAR8 cell lines were treated with IC50 concentrations of cisplatin, while the control cells were treated with DMSO. Following a 48-h incubation period, the medium was subsequently substituted with FBS-free media for an additional 24 h. This step was used to mitigate any potential interference induced by cisplatin on CAFs.
Subsequently, we collected the cell supernatant from the cancer cells. The supernatant was then mixed with normal FBS-free medium at a 1:1 ratio to create conditioned medium $OC_{NC}$-Sup and $OC_{Cis}$-Sup. $OC_{NC}$-Sup and $OC_{Cis}$-Sup were then separately co-cultured with CAFs from the same patients, resulting in the labeling of the CAFs as $OC_{NC}$-CAFs and $OC_{Cis}$-CAFs. To further validate the distinction between $OC_{NC}$-CAFs and $OC_{Cis}$-CAFs in cancer progression and platinum resistance. Following a 48-h period of co-culture, the culture medium devoid of FBS was substituted. The 24-h culture supernatants were mixed with normal FBS-free medium at a 1:1 ratio to create conditioned medium $OC_{NC}$-Sup and $OC_{Cis}$-Sup. The 24-hour FBS-free supernatants were collected after 48 h of culture in H-151 (1.5 μM). These supernatants were then mixed with normal FBS-free medium at a 1:1 ratio to create conditioned medium. The conditioned medium was labeled as CAFs$_{15L}$-Sup and CAFs$_{NC}$-Sup. CAFs$_{MSA-2}$-Sup was obtained by replacing H-151 with MSA2 in the co-culture system described above.

2.6. Cell radiotherapy

$2 \times 10^5$ ovarian cancer cells were cultured overnight and treated with X-rays at a dose of 10 Gy. The FBS-free medium supernatant was collected when significant cell death was observed. The supernatant was mixed with DMEM/F-12 at a 1:1 ratio and supplemented with FBS to a final concentration of 20% for indirect co-culture with CAFs.

2.7. Cell genomic extraction

The DNA of cells was isolated utilizing the TIANamp Genomic DNA Kit (TIANGEN BIOTECH, DP304). In strict accordance with the product instructions. The extracted DNA was diluted to 2 μg/ml in DMEM/F-12 medium containing 20% FBS. It was then labeled as OCDNA-Sup and co-cultured with CAFs to verify the triggering of the CGAS-STING cascade in CAFs.

2.8. Cell proliferation and clonogenic assays

Cell proliferation was determined using the CCK-8 assay (Cell Counting Kit-8, Meilunbio). The cells were distributed in 96-well plates with a seeding density ranging from (0.8–1) $\times 10^3$ cells per well. Following incubation in different conditioned media for the designated time periods, a volume of 10 μL of CCK-8 was introduced to each well and subsequently incubated for a duration of 1–4 h at a temperature of 37 °C. The investigation involved the observation of cellular proliferation over a period of five consecutive days, with the quantification of absorbance at a wavelength of 450 nm.

In the colony formation experiments, a total of 500–800 cells were introduced onto 6-well plates and thereafter cultured at a temperature of 37 °C for a duration of 10–14 days. The colonies obtained were treated with methanol and subjected to staining using a Crystal Violet Staining Solution (Beyotime). The quantification of colony formation capabilities was the enumeration of surviving colonies with a minimum of 50 cells.

2.9. Drug resistance assay

The drug resistance assay was quantified utilizing the CCK-8 methodology, as previously indicated. The experiment involved the seeding of cells onto 96-well plates at a density of (2–3) $\times 10^3$ cells per well. These cells were then subjected to a conditioned medium that contained different concentrations of cisplatin for a duration of 48 h. The final viability was assessed utilizing the CCK-8 reagent, and subsequently, the IC50 value was determined.

2.10. Cell migration and invasion

The migratory capacity of the cells was assessed utilizing the transwell assay. Following a 48-h incubation period with different conditioned media, a total of $5 \times 10^4$ cells were resuspended in 200 μL of solution devoid of FBS. Subsequently, these cells were seeded into the upper chambers of culture inserts (Corning, 3422). Subsequently, a total volume of 600 μL of culture media, comprising 20% FBS, was introduced into the bottom chambers with the purpose of serving as chemotaxicants. The transwell was thereafter placed in an incubator set at a temperature of 37 °C for a suitable duration (12–24 h). The cells on the lower surface of the transwell were subjected to fixation in methanol for a duration of 15 min. Following fixation, these cells were stained with a solution containing 0.1% crystal violet for a period of 20 min. Subsequently, the cells were examined utilizing a light microscope. The process of invasion bears resemblance to migration, but the quantity of cells introduced during invasion was as high as $10 \times 10^7$. Additionally, a solution consisting of a 60 μL mixture of Matrigel (BD Biosciences) and the media was administered onto the membrane. The duration of the invasion was extended to 36 h.

2.11. Western blot

The cells were subjected to lysis using RIPA lysis buffer (Beyotime) supplemented with 1% PMSF and 1% NaF for a duration of 30 min at a temperature of 4 °C. The supernatant was acquired by the process of centrifugation, and the concentration of protein was assessed with the BCA Assay Kit provided by Thermo Scientific. The protein samples were separated using the PAGE Gel Fast Preparation Kit (PG112, Epizyme Biotech), with a total of 30 μg per well. The proteins were subsequently transferred onto polyvinylidene fluoride (PVDF) membranes (Merck, ISEQ00010) using the BIO-RAD Trans-blot apparatus, applying a current of 400 mA for a duration of 60 min and the membranes were subjected to blocking by immersing them in a 5% non-fat milk solution at a temperature of 25 °C for a period of 2 h. The membrane was subjected to overnight incubation at a temperature of 4 °C with the primary antibody, which was appropriately diluted. Afterwards, the membrane was rinsed with TBST and then subjected to incubation with the suitable secondary antibodies linked to horseradish peroxidase for a duration of 1.5 h at a temperature of 25 °C. The band signal was detected utilizing the Immobilon Western Chemiluminescent HRP Substrate (Merek) in conjunction with the Image Quant LAS4000 system (General Electric Company), and then quantified using the ImageJ software. The endogenous control utilized in this study was β-Actin.

2.12. siRNA transient transfection

siRNA was designed and synthesized by genomeditech. siRNA transfection was performed using Lipofectamine 3000 (Invitrogen, Thermo Fisher Scientific, USA). The sequences of siRNA used in our study were as follows: siNC sense 5'-UUCUCCGAAGGUGACGU-3', siNC antisense 5'-ACGUUACGUGAGAA-3'; siSTING1 sense 5'-CCCAUAUGGAUAGAA-3', siSTING1 antisense 5'-UUCAUAUGCACUGAGGG-3'; siSTING2 sense 5'-GGGACACUGUGUCCUGAGA-3', siSTING2 antisense 5'-GUACCUCCAGGACAGGUGCC-3'; siSTING3 sense 5'-GGU CAUAAUCAUACGUA-3', siSTING3 antisense 5'-UAUCCGAUGUAAUGACC-3'.
Figure 1: Effects of CAFs-sup on cancer cell viability, migration, and invasion.

A) HEY and OVCAR8 cells were treated with CAFs-sup or NC. Cell viability was measured at different time points.

B) Migration assay showing the movement of HEY and OVCAR8 cells in the presence of CAFs-sup and NC.

C) Bar graph showing the number of migrated cells for HEY and OVCAR8 cells.

D) HEY and OVCAR8 cells were treated with CAFs-sup and NC in a wound healing assay.

E) Invasion assay demonstrating the invasive capacity of HEY and OVCAR8 cells.

F) Bar graph illustrating the number of invaded cells in HEY and OVCAR8 cells.

G) HEY and OVCAR8 cells were treated with CAFs-sup and NC in an transwell assay.

H) Cell inhibition (%) assay showing the effect of CAFs-sup and NC on cell proliferation.

I) Relative cell counts and DNA counts from HEY and OVCAR8 cells treated with CAFs-sup and NC.

J) Flow cytometry analysis of apoptosis in HEY and OVCAR8 cells treated with CAFs-sup and NC.

K) Bar graph showing the percentage of cells in each phase of the cell cycle.

L) Western blot analysis of protein expression levels in HEY and OVCAR8 cells treated with CAFs-sup and NC.

M) Co-culture experiment with OCs and CAFs showing the effects on cell function.
procedure utilizing a surface marker to identify the presence of IFNB1 within CAFs. The samples were subsequently permeabilized and fixed utilizing the BD Cytofix/Cytoperm Fixation/Permeabilization Solution kit (BD Biosciences). Following the addition of FITC-conjugated anti-Human IFNB1 antibodies, the cells were subjected to a 30-min incubation with 4 x 10^4 cells were cultured continuously in CAFs-Sup and normal medium for 5 days, respectively. Cell viability was assessed using the CCK-8 method. After 10 h of incubation with 4 x 10^4 HEY cells and 24 h of incubation with 8 x 10^4 OVCAR8 cells, both cell types in CAFs-Sup showed increased migration towards the lower chamber. After 2 weeks of incubation, the number of colonies in CAFs-Sup and normal medium containing 5 μg/ml of cisplatin was lower.

2.16. Immunohistochemistry (IHC)

The paraffin sections underwent a baking process at a temperature of 65 °C for a duration of 2 h. Then, they were subjected to dewaxing using xylene and dehydrating using a sequence of ethanol solutions with a concentration of 2 μg/ml of cisplatin for a period of 24 h. Subsequently, the medium was substituted with a medium devoid of cisplatin. Cells were harvested when significant cell death was observed, and supernatants containing Edu-labeled DNA were obtained after removing cell debris by centrifugation at 1000 rpm. The supernatants were then co-cultured with CAFs. Following a 24-h period, the CAFs were subjected to fixation using the BD Cytofix/Cytoperm Fixation/Permeabilization Solution kit (BD Biosciences) and then incubated with the fluorescent secondary antibodies Alexa Fluor® 488 Phalloidin or Alexa Fluor® 647 Conjugate for 1 h in the dark. Ultimately, the nuclei were subjected to staining with DAPI, and further imaging was conducted with either a Sunny confocal microscope system or fluorescence microscopy.

2.17. Immunohisto-fluorescence (IHF)

After dewaxing, dehydrating, and repairing the antigen, the paraffin sections were incubated with 3% goat FBS for 30 min at 25 °C to block the antigen. The sections were incubated overnight at 4 °C with the primary antibodies STING (1:500) or α-SMA (1:500), and then incubated with the fluorescent secondary antibodies Alexa Fluor® 488 Phalloidin or Alexa Fluor® 647 Conjugate for 1 h in the dark. Ultimately, the nuclei were subjected to staining with DAPI, and further imaging was conducted with either a Sunny confocal microscope system or fluorescence microscopy.

2.18. Kaplan-meier survival analysis

The Kaplan-Meier plotter database (http://kmplot.com/analysis/) was used to examine the relationship between the survival statistics of patients with ovarian cancer and the amount of STING expression. The Qilu cohort comprised all patients with data on overall survival or progression-free survival. The immunohistochemistry staining score was utilized to identify the tissue microarray high expression group and low expression group, and GraphPad Prism 9.3.0 was employed. In the Qilu cohort, a connection between STING expression level and OS and PFS was examined.

2.19. Allograft model in C57BL/6 mice

The Laboratory Animal Ethical and Welfare Committee at Shandong University Qilu College of Medicine granted approval for the tumor allograft studies. The C57BL/6 mice used in this study were obtained from MODEL ORGANISMS. The mice were female and were 4 weeks old at the time of experimentation. Their average weight was 16.3 ± 1.57g. The mice were kept in specified pathogen-free (SPF) breeding units. A total of 1x10^5 ID8 cells were suspended in 100 μl of phosphate-buffered saline (PBS) and thereafter administered via subcutaneous injection into the right axilla of each mouse. Once the tumors attained a diameter of roughly 5 mm, the mice were allocated into six groups using a randomized block design in order to administer the appropriate medications. Drug administration was started one week after tumor formation. Cisplatin (1.5 mg/kg i.p.) was administered to the mice every three days for a total of 18 days. H-151 and MSA-2 were administered on Day 7 of cisplatin administration. H-151 (750 nmol per mouse, i.p.) was administered daily for 7 days. A single dose of MSA-2 (50 mg/kg, s.c.) was administered. The weights of the mice and the volumes of the tumors were recorded at three-day intervals. The equation used to determine the volume of a tumor is given by the formula:

\[ V = \frac{4}{3} \pi r^3 \]

where \( V \) is the volume of the tumor and \( r \) is the radius of the tumor.
Fig. 2. DNA fragmentation in ovarian cancer mediates cisplatin resistance and poor prognosis by activating the CGAS-STING pathway in CAFs. (A, B) After indirect co-culture, as shown in (Fig. 1N), Apollo staining and α-SMA staining were performed on OCGC-CAFs and OCGC-C2-CAFs. Immunofluorescence showed that OCGC-CAFs had engulfed a significant amount of DNA fragments from OCGC. In addition, the expression of α-SMA was upregulated in OCGC-CAFs. (C, D) At the protein level, OCGC-CAFs expressed higher levels of α-SMA, indicating that OCGC-CAFs were in a more activated state. (E, F) Immunofluorescence double staining of α-SMA and STING was performed on HGSOC tissues from platinum-sensitive (P-sen) (n = 12) and platinum-resistant (P-r) (n = 12) patients. α-SMA and STING showed co-localization of expression. (G, J) At the protein level, the CGAS-STING pathway was highly expressed in OCGC-CAFs compared to OCGC-CAFs and OCGC cisplatin-resistant CAFs. (H, I) The ID8 subcutaneous tumor blocks from C57BL/6 mice, treated with cisplatin for 18 days, were subjected to α-SMA and STING double immunofluorescence staining. The expression levels of α-SMA and STING in cisplatin-treated tumor tissues (n = 14) were higher than those in untreated groups (n = 14). (K) Representative images of STING immunohistochemical staining of HGSOC pathological sections from the case bank of Qilu Hospital. (L) Kaplan-Meier plotter database was used for survival analysis. The results showed that STING expression was significantly associated with a poor prognosis. (M) Immunohistochemical staining of STING in pathological sections from Qilu Hospital revealed that the proportion of high STING expression in the tumor stroma of the P-res cohort was 76.67%, which was significantly higher than that of the P-sen cohort. (N) Progression-free survival (PFS) and (O) Overall survival (OS) based on STING (stroma) expression levels. STING (stroma) expression (low vs high STING (stroma) expression: PFS, hazard ratio = 0.7821, 95% CI = 0.6185 to 0.9890 vs. hazard ratio = 1.279, 95% CI = 1.011 to 1.617, P = 0.04; OS, hazard ratio = 0.7749, 95% CI = 0.6074 to 0.9884 vs. hazard ratio = 1.291, 95% CI = 1.012 to 1.646, P < 0.05). The chi-square test yielded m, while the log-rank test yielded l, n, and o. All functional experiments were performed using three independent replicates. The paired Student’s t-test was used to determine the other plots. ns P > 0.05; *P < 0.05; **P < 0.01; ***P < 0.001.

2.20. PDX model establishment and therapy

Female NOD-scid mice, aged between four and six weeks, were acquired from GemPharmatech, a reputable supplier located in Nanjing, China. The NOD-scid mice were kept in a controlled environment known as the SPF room. The Department of Obstetrics and Gynecology at Qilu Hospital, Shandong University, provided fresh primary ovarian cancer tissues. Prior to acquiring these tissues, written informed agreement was obtained from the patients. The Animal Care and Use Committee of Shandong University granted approval for the acquisition of human tissue specimens and the implementation of the PDX experimental protocols. The tissue homogenate was combined with phosphate-buffered saline (PBS) in a 1:1 proportion and administered subcutaneously into the lower dorsal or axilla regions of NCG mice designated as P1. Once the dimensions of the tumor reached around 10 × 10 × 10 mm, it was surgically removed and subsequently transplanted into another mouse as P2, with the tumor being placed beneath the recipient’s skin.

P2 samples were obtained from the euthanized mice at tumor growth reaching dimensions of 10 × 10 × 10 mm, and subsequently processed into a homogenate. The intraperitoneal injection of the homogenate was administered to newly introduced recipient mice. Following a duration of 6 weeks, the mice were subjected to random allocation into two distinct groups, namely the cisplatin + DMSO group and the cisplatin + H-151 group. Upon completion of the treatment, the mice were euthanized, and their tumors were collected. The weights and locations of peritoneal metastases were documented and compared among several groups.

2.21. Antibodies


2.22. Statistical analysis

The studies were conducted in a manner that ensured independence, with each experiment being repeated a minimum of three times. Statistical analysis was carried out using GraphPad Prism 9.3.0. Student’s t-test and chi-square test of variance were used to determine significant differences. Survival analysis was performed utilizing the Kaplan-Meier method and the Log-rank test. Data is represented as means ± standard deviation of at least three independent experiments. Non-significant results were denoted as ns (P > 0.05), while significant results were denoted as * (P < 0.05), ** (P < 0.01), and *** (P < 0.001).

3. Results

3.1. CAFs promote platinum resistance in ovarian cancer

The human ovarian cancer-associated fibroblasts were isolated from fresh primary HGSOC specimens of four patients. The confirmation of these fibroblasts as CAFs was performed based on the expression levels of α-SMA, FAP, and EPICAM. As illustrated in Fig. S1A, the isolated CAFs exhibited high expression of α-SMA and FAP compared to EPICAM, as confirmed by immunofluorescence staining. These results revealed that CAFs of high purity were successfully isolated from HGSOC tissue specimens.

Indirect co-culture was employed to investigate the effect of CAFs on the progression of ovarian cancer. The results of the CCK-8 assay and plate clone formation assay showed that the cell proliferation ability was significantly enhanced after indirect co-culture (Fig. 1A, D, E). In addition, both HEY and OVCAR8 cells exhibited significantly increased migratory and invasive capacities when cultured in CAFs-Sup compared to their respective negative controls (NC), as shown in Fig. 1B, C, F, G. These findings suggest that CAFs can promote tumor progression through indirect non-contact mechanisms.

Ovarian cancer HEY and OVCAR8 cells were subjected to treatment with different doses of cisplatin over a duration of 48 h. The assessment of cell viability was conducted utilizing the CCK-8 method to examine the influence of CAFs on the sensitivity of cisplatin. Curves are shown in Fig. 1H. CAFs-Sup significantly inhibited the inhibition ratio of cisplatin on HEY and OVCAR8 cells at different concentrations. Comparable findings were noted in two more cell lines, SKOV3 and COV362 (Fig. S1B). The anti-tumor actions of platinum medicines are attributed to their ability to induce double-stranded DNA (dsDNA) damage in tumor cells. To investigate the specific mechanism by which CAFs contribute to chemoresistance in cancer cells, we conducted a study on the effects of CAFs-Sup on the cell cycle and apoptosis in ovarian cancer cells. Flow cytometry analysis demonstrated that CAFs significantly promoted resistance to apoptosis in HEY and OVCAR8 cells induced by cisplatin, compared to the control group (Fig. 1J, K, Figs. S1C and D). Subsequently, an evaluation was conducted on the expression levels of proteins associated with apoptosis and DNA damage repair through western blotting. The results suggested that CAFs not only resisted cisplatin-induced apoptosis but also promoted DNA repair and reduced

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A. **HEY** and **OVCAR8** bar graphs showing IFNB1 concentration (pg/mL) with statistical significance indicated.

B. **HEY** and **OVCAR8** flow cytometry analysis with histograms showing cell populations in different phases of the cell cycle.

C. **OVCAR8** bar graph showing apoptotic cell percentage with statistical significance.

D. **HEY** relative cell counts and DNA counts.

E. **HEY** percentage of cells in each phase (G0/G1, S, G2/M).

F. **HEY** and **OVCAR8** relative cell viability over time (days).

G. **HEY** and **OVCAR8** cell inhibition graph with log[Cisplatin] concentration in μg/mL.

H. Western blot analysis showing protein levels for various markers such as 53BP1, cleaved PARP, RAD51, yH2AX, cleaved caspase 8, BAX, BCL2, and β-Actin.

I. **HEY** and **OVCAR8** fluorescence images of DAPI, yH2AX, and merged views with IFNB1 concentration (ng/mL).
3.2. Cisplatin treatment increases the capacity of CAFs to promote cisplatin resistance in ovarian cancer through various mechanisms, while also promoting ovarian cancer progression.

According to our proposed hypothesis, we further investigated whether treating ovarian cancer cells with cisplatin could enhance the ability of CAFs to promote chemotherapy resistance. We still used the medium indirect co-culture system to verify the relevant conclusions. The indirect co-culture system is demonstrated in Fig. 1N. As shown in Fig. 2A–D, immunofluorescence and WB analysis revealed that the expression of α-SMA was increased in OCAc-CAFs compared to OCN-CAFs, suggesting a more activated state of OCAc-CAFs. Furthermore, we found that compared to OCN-CAFs-Sup, the clone formation, invasion, and migration abilities of ovarian cancer cells were significantly increased in OCAc-CAFs-Sup (Figs. S1F–K). The IC50 curve is shown in Fig. 1O and Fig. S1E. The OCAc-CAFs-Sup IC50 demonstrate a significant increase compared to the OCN-CAFs-Sup IC50 in HEY, OVCAR8, SKOV3 and COV362 cell lines. This indicates that OCAc-CAFs-Sup further enhances cisplatin resistance in ovarian cancer cells. The above results preliminarily support our previous hypothesis that ovarian cancer following cisplatin therapy amplifies the capacity of CAFs to facilitate resistance to cisplatin in ovarian cancer cells.

3.3. DNA fragmentation from ovarian cancer activates the CGAS-STING pathway in CAFs

Having demonstrated the functional relevance of CAFs-Sup and OCAc-CAFs-Sup, our aim was to identify the key component of the hypothesis: genomic communication. We replaced cisplatin with a whole DNA damage (Fig. 1M). CAFs-Sup abrogated cisplatin-induced G2/M cell cycle arrest in HEY cancer cells, as demonstrated in Fig. 1L. Taken together, these results indicate that CAFs can promote cisplatin resistance in ovarian cancer through various mechanisms, while also promoting ovarian cancer progression.

According to our proposed hypothesis, we further investigated whether treating ovarian cancer cells with cisplatin could enhance the ability of CAFs to promote chemotherapy resistance. We still used the medium indirect co-culture system to verify the relevant conclusions. The indirect co-culture system is demonstrated in Fig. 1N. As shown in Fig. 2A–D, immunofluorescence and WB analysis revealed that the expression of α-SMA was increased in OCAc-CAFs compared to OCN-CAFs, suggesting a more activated state of OCAc-CAFs. It was found that compared to OCN-CAFs-Sup, the clone formation, invasion, and migration abilities of ovarian cancer cells were significantly increased in OCAc-CAFs-Sup (Figs. S1F–K). The IC50 curve is shown in Fig. 1O and Fig. S1E. The OCAc-CAFs-Sup IC50 demonstrate a significant increase compared to the OCN-CAFs-Sup IC50 in HEY, OVCAR8, SKOV3 and COV362 cell lines. This indicates that OCAc-CAFs-Sup further enhances cisplatin resistance in ovarian cancer cells. The above results preliminarily support our previous hypothesis that ovarian cancer following cisplatin therapy amplifies the capacity of CAFs to facilitate resistance to cisplatin in ovarian cancer cells.

3.4. STING in stroma is linked to platinum resistance and poor prognosis in ovarian cancer

The expression of STING in the stroma was further investigated, with a focus on its correlation with patient prognosis and responsiveness to cisplatin in the Qilu cohort (n = 384). IHC analysis was employed to ascertain the levels of stromal STING expression, and Fig. 2J displays the matching typical pictures. STING was only partially expressed in ovarian cancer parenchymal or stromal cells, according to immunohistochemical pictures. STING was mostly expressed in stromal cells in 274 instances (71%), whereas parenchymal cells were the primary site of expression in 110 cases (29%). This indicates that stromal cells are predominantly responsible for STING function in ovarian cancer (Fig. S2C). Following this, we proceeded to choose the median STING expression value as the threshold to categorize the patients into two distinct groups: STING (stroma)-low (n = 172) and STING (stroma)-high (n = 212). Survival analysis from the Kaplan-Meier plotter suggested that high STING expression, without distinguishing expression regions, was associated with poor progression-free survival (PFS) (Fig. 2L). The present work assessed the expression of STING within the stroma and obtained comparable findings. We found that high STING (stroma) expression in the stroma was associated with OS and PFS outcomes among individuals diagnosed with ovarian cancer (Fig. 2N and O). The patients were categorized into two distinct groups: the P-sen group (n = 75) and the P-res group (n = 30), based on their clinical characteristics. Our findings indicate that the expression of STING (stroma) was significantly lower in the P-sen group compared to the P-res group (Fig. 2M). In addition, an examination was conducted to assess the relationship between the STING expression and the immune classification of ovarian cancer, as depicted in Fig. S2D. There was no statistically significant disparity observed in the distribution of patients with infiltrated type, excluded type, and desert type between the STING (stroma)-low and STING (stroma)-high groups. Fig. S2E displays representative...
Figure A: Western blot analysis of STING, phospho-STING (P-STING), TBK1, phospho-TBK1 (P-TBK1), IRF-3, phospho-IRF-3 (P-IRF-3), and β-Actin in CAFs. Densitometric quantification of the bands is shown below each lane.

Figure B: Immunofluorescence images showing the expression of P-IRF-3 in OVCAR8 cells treated with CAFs. The concentration of Cisplatin used is 5 μg/ml.

Figure C: Fluorescence intensity (FU) bar graph comparing the expression levels of P-IRF-3 in OVCAR8 cells treated with different CAF supernatants.

Figure D: Percentage of Merge positive cells in OVCAR8 cells treated with CAF supernatants.

Figure E: Western blot analysis showing the expression levels of STING, phospho-STING (P-STING), TBK1, phospho-TBK1 (P-TBK1), IFNβ1, and β-Actin in H-151 cells treated with varying concentrations of H-151 (μM).

Figure F: Graph showing the relative expression levels of STING, P-STING, P-TBK1, and IFNβ1 in H-151 cells treated with different concentrations of H-151 (μM). The expression levels are normalized to β-Actin.

Figure G: Western blot analysis of STING, phospho-STING (P-STING), TBK1, phospho-TBK1 (P-TBK1), IRF-3, phospho-IRF-3 (P-IRF-3), and β-Actin in CAFs. Densitometric quantification of the bands is shown below each lane.

Figure H: Immunofluorescence images showing the expression of IFNβ1 in H-151 cells treated with different CAF supernatants.

Figure I: Graph showing the cell inhibition percentage of HEY and OVCAR8 cells treated with varying concentrations of Cisplatin (μg/ml) and CAF supernatants.

Figure J: Graph showing the cell inhibition percentage of HEY and OVCAR8 cells treated with varying concentrations of Cisplatin (μg/ml) and CAF supernatants.

Figure K: Flow cytometry analysis of CAFs treated with NC, MSA-2, and H-151, showing the percentage of positive cells for CD105 (FITC-A).

Figure L: Flow cytometry analysis of OVCAR8 cells treated with CAF supernatants, showing the percentage of positive cells for CD105 (FITC-A).

Figure M: Western blot analysis showing the expression levels of MX2 and OAS2 in OVCAR8 cells treated with CAF supernatants. The concentration of MX2 and OAS2 is 82kDa and 72kDa, respectively.

Figure N: Graph showing the relative expression levels of MX2 and OAS2 in OVCAR8 cells treated with CAF supernatants.
images.

Collectively, the aforementioned findings indicate that STING may exert its biological function in ovarian cancer primarily through CAFs. Additionally, platinum-based chemotherapy can further enhance STING expression levels, which are correlated with a poor prognosis. May have exerted its biological function in ovarian cancer primarily through CAFs.

The above conclusions provide a theoretical basis for studying chemotherapy sensitization in ovarian cancer.

3.5. IFNB1 from CAFs is involved in cisplatin resistance in ovarian cancer

IFNB1 is the primary downstream product of the CGAS-STING pathway. We next investigated the contribution of IFNB1 to the progression of ovarian cancer and its role in chemoresistance to cisplatin. ELISA assay shows IFNB1 secretion in OVCAR8 cells, while CAFs exhibit a stronger α-SMA signal compared to the negative control (NC). Moreover, the expression of IFNB1 in CAFs was down-regulated by STING inhibition (Fig. 4M, N). To determine the contribution of STING in CAFs promoting platinum resistance in ovarian cancer, we used siRNA to knock down STING in CAFs and collected the supernatant and indirectly co-cultured with ovarian cancer cells (Fig. 4A). Compared with CAFs-Sup, IFNB1 could enhance the sensitivity of ovarian cancer cells to platinum and increase the nuclear damage induced by cisplatin in ovarian cancer cells. Based on this, we investigated the effects of H-151 and MSA-2 on STING expression in cells and in vivo, as well as their potential role in cisplatin resistance. As shown in Fig. 4E, F, a concentration of 1.5 μM of H-151 is optimal for inhibiting the expression of STING and α-SMA. As shown in Fig. 4G, the WB experiment demonstrated that H-151 effectively inhibited the expression of the CGAS-STING pathway in CAFs. In addition, flow cytometry confirmed that the secretion level of IFNB1 by CAFs was inhibited by H-151 (Fig. 4H). The relationship between CAFs activation and STING expression levels in CAFs was verified through immunofluorescence and WB experiments. The fluorescence signal of α-SMA in the H-151 group was weaker than the NC group, as shown in Fig. 4H, D. This indicates that a low level of STING expression in CAFs is detrimental to the maintenance of the CAFs phenotype. The same experimental conclusions were also verified in the WB experiment shown in Fig. 4D.

The study employed the indirect co-culture technique to investigate the relationship between the expression level of the CGAS-STING pathway in CAFs and the sensitivity of ovarian cancer cells to cisplatin. CAF proliferation curve, as shown in Fig. 4I and Fig. S4B, indicates that CAFs-Sup could increase the cell inhibition rate of cisplatin. The optimal concentration of H-151 is 1.5 μM, which is optimal for inhibiting STING in CAFs and collected the supernatant and indirectly co-cultured with ovarian cancer cells (Fig. 4A). Compared with CAFs-Sup, WB assay also did not observe the effect of H-151 on the expression of interferon-stimulated genes in OVCAR8 cells. All functional experiments were performed using three independent replicates. The paired Student’s t-test was performed for all statistical analyses. ns P > 0.05; *P < 0.05; **P < 0.01; ***P < 0.001.

3.6. H-151 enhances cisplatin sensitivity in ovarian cancer by inhibiting STING in CAFs

According to the aforementioned research findings, the CGAS-STING pathway is a promising candidate for modulating the malignant biological properties and response to cisplatin-based chemotherapy in ovarian cancer. To determine the contribution of STING in CAFs promoting platinum resistance in ovarian cancer, we used siRNA to knock down STING in CAFs and collected the supernatant and indirectly co-cultured with ovarian cancer cells (Fig. 4A). Compared with CAFs-Sup, IFNB1 could enhance the sensitivity of ovarian cancer cells to platinum and increase the nuclear damage induced by cisplatin in ovarian cancer cells. Based on this, we investigated the effects of H-151 and MSA-2 on STING expression in cells and in vivo, as well as their potential role in cisplatin resistance. As shown in Fig. 4E, F, a concentration of 1.5 μM of H-151 is optimal for inhibiting the expression of STING and α-SMA. As shown in Fig. 4G, the WB experiment demonstrated that H-151 effectively inhibited the expression of the CGAS-STING pathway in CAFs. In addition, flow cytometry confirmed that the secretion level of IFNB1 by CAFs was inhibited by H-151 (Fig. 4H). The relationship between CAFs activation and STING expression levels in CAFs was verified through immunofluorescence and WB experiments. The fluorescence signal of α-SMA in the H-151 group was weaker than the NC group, as shown in Fig. 4H, D. This indicates that a low level of STING expression in CAFs is detrimental to the maintenance of the CAFs phenotype. The same experimental conclusions were also verified in the WB experiment shown in Fig. 4D.

The study employed the indirect co-culture technique to investigate the relationship between the expression level of the CGAS-STING pathway in CAFs and the sensitivity of ovarian cancer cells to cisplatin. CAF proliferation curve, as shown in Fig. 4I and Fig. S4B, indicates that CAFs-Sup leads to a decline in IC50 in ovarian cancer cell lines. This finding suggests that CAFs-Sup may potentially augment the susceptibility of cancer cells to cisplatin, in contrast to CAFs-Sup-Sup. In contrast, CAFs-Sup demonstrated the ability to promote cisplatin resistance (Fig. 4C). The changes in the gene expression levels of MX2 and OAS2 inside ovarian cancer cells indicate that CAFs-Sup and CAFs-Sup may regulate the level of the interferon-activated pathway in cancer cells (Fig. 4M and N). In addition, we also explored the direct effect of H-151 on ovarian cancer cells. CCK-8 curves (Figs. S4F, H, I) and transwell assays (Figs. S4E) showed that inhibition of the CGAS-STING pathway in ovarian cancer cells did not significantly change cancer cell proliferation, migration ability, and cisplatin sensitivity. WB assay also did not observe the effect of H-151 on the response of cancer cells to cisplatin treatment (Fig. S4G). Based on the above results, H-151 can induce the quiescent phenotype of CAFs by inhibiting STING in CAFs. This inhibition also prevents CAFs from inducing platinum resistance in ovarian cancer cells in vitro.

3.7. Co-administration of H-151 increased the antitumor efficacy of cisplatin in vivo

Given the importance of the CGAS-STING pathway in antitumor
immunity, we established a syngeneic model in C57BL/6 mice using ID8 cells to investigate the potential association between the expression level of the CGAS-STING path and the prognosis of ovarian cancer. The procedure of in vivo experiments is shown in Fig. 5A. As shown in Fig. 5B and C, both the H-151 group and MSA-2 group exhibited tumor suppressive properties with monotherapy. However, H-151 demonstrated superior functionality compared to MSA-2. When H-151 was administered in combination with cisplatin, it significantly sensitized ovarian cancer cells to cisplatin. This supports the hypothesis that H-151 sensitizes cisplatin.

The CGAS-STING pathway, recognized as a classical DNA recognition pathway, assumes significant importance in probing innate immune activation[29]. Beyond its role in mediating the innate immune response against various DNA-containing pathogenic microorganisms, this pathway also senses tumor-derived DNA, contributing to the anti-tumor immune response[12,30,31]. Notably, beyond the previously studied interferons and cytokines[12], the cellular function of CGAS-STING signaling is now acknowledged as the core mediator of diverse cellular functions, including protein synthesis, autophagy, and lipid and glucose metabolism, garnering extensive attention in various biological processes[30,32]. dsDNA from various sources, such as viruses, bacteria, and their chromosomes or mitochondria, can translocate into the cytoplasm, initiating the CGAS-STING signaling cascade[33]. Prior research indicates that CGAS-STING pathway activation lacks specificity, with CGAS binding to the sugar-phosphate backbone of dsDNA in the absence of nucleotide bases. Evidence also suggests that short dsDNA, approximately 15 base pairs in length, is sufficient to bind and activate CGAS in vitro[34]. Given the intricate composition of the tumor microenvironment, the diverse functions of individual cellular components, and the varied responses to different antitumor therapies, recent studies have focused on investigating the pathway functions of specific cell populations[35,36].

While ample evidence suggests a positive impact of STING agonists on anti-tumor immunity, the research on STINGs role in tumor progression remains intricate and inconclusive. Depending on circumstances or tumor stages, STING may exhibit either inhibitory or promoting effects on tumors. Moreover, STING may fulfill diverse biological functions in distinct cell populations[21,22]. Arwert et al. proposed that genomic instability in tumor cells could activate STING in fibroblasts, influencing the therapeutic response to oncolytic viral therapy[24]. Wang et al. found that STING agonists can convert M2-like macrophages into M1-like antitumor cells, with reprogramming dependent on macrophages. Additionally, concurrent administration of STING agonists and PARPi demonstrated synergistic tumor proliferation suppression[37]. Ma et al.’s study suggests that activating STING in tumor cells or fibroblasts significantly increases IFN I production, leading to the development of SLC14A1 irCAFs and expression of stem-like genes in tumor cells[38]. As they al. proposed that STING-generated regulatory B cells hinder natural killer (NK) cell functionality in anti-tumor immune responses[13]. A recent study showed that STING activation in astrocytes contributes to brain metastasis occurrence[39]. Choosing the right time for STING agonist or inhibitor use is crucial given these multifaceted roles.

Platinum, as a first-line chemotherapy for ovarian cancer, effectively targets cancer cell DNA, inducing tumor cell death[23,40]. Previous studies have illustrated diverse intercellular communication between tumor cells and cancer-associated fibroblasts (CAFs) through both direct and indirect mechanisms. The exchange of nucleic acids, lipids, and proteins fosters mutual adaptation, creating conducive conditions for tumor development and drug resistance[20,24,41]. Building upon the recognized role of CAFs in platinum resistance in ovarian cancer, we
Fig. 6. H-151 can enhance the sensitivity of cisplatin treatment in two PDX models. (A, B) Representative images of IHC staining for α-SMA, STING, γH2AX, and Rad51 in tumor tissues. (C) Schematic diagram illustrating the establishment and application process of the PDX model. (D, E, F) Effect of H-151 combined with cisplatin on tumor growth in vivo in Patient 1 PDX model. (G, H, I) Effect of H-151 combined with cisplatin on tumor growth in vivo in patient 2 PDX model. (J, K) Representative images of IHC staining of α-SMA, STING, Rad51, and ki67 in patient 1 PDX tumor tissues. (L, M) Representative images of IHC staining of α-SMA, STING, Rad51, and ki67 in patient 2 PDX tumor tissues. All functional experiments were performed using three independent replicates. The paired Student’s t-test was performed for all statistical analyses. ns P > 0.05; *P < 0.05; **P < 0.01; ***P < 0.001.
propose that cisplatin-induced genomic instability in cancer cells may contribute to CAFs promoting platinum resistance [42–44]. Thus, we hypothesized and tested that cisplatin facilitates DNA transfer from cancer cells to CAFs. Additionally, we investigated whether CAFs can activate their CGAS-STING pathway through DNA recognition, thereby enhancing cancer cell resistance to platinum-based chemotherapy. Our study unveils a correlation between CGAS-STING pathway stimulation and platinum-based chemotherapy in CAFs, establishing that CGAS-STING pathway initiation in CAFs plays a role in developing resistance to platinum-based chemotherapy and is associated with an unfavorable prognosis in ovarian cancer patients. Concurrently, our findings open avenues for further exploring the therapeutic implications of STING inhibitors and agonists in the cancer context [45, 46].

We used primary human ovarian cancer-derived fibroblasts and employed indirect co-culture method to confirm that genomic transfer from cisplatin-treated ovarian cancer cells to CAFs amplifies CAF activation. This activation, in turn, intensifies ovarian cancer cell progression and cisplatin resistance. Interestingly, we observed the uptake of DNA by CAFs from cisplatin-treated ovarian cancer cells, accompanied by the active state of the CGAS-STING pathway within these CAFs. Examination of IFNB1 levels in the conditioned media of CAFs, through indirect co-culture experiments, indicated the involvement of IFNB1—the main output of the CGAS-STING pathway—in both ovarian cancer progression and resistance to cisplatin treatment. Moreover, we confirmed the co-expression of STING and α-SMA, and scrutinized the correlation between STING and cisplatin treatment response in ovarian cancer patients. These findings reveal a significant correlation between elevated STING expression in the ovarian cancer stroma and platinum resistance, based on data from a Qilu Hospital pathological database of 384 ovarian cancer patients. This association predicts poor progression-free survival (PFS) and overall survival (OS) outcomes. Furthermore, immunophenotyping of these 384 patients showed no significant difference in the proportion of infiltrated, excluded, and desert immunophenotypes between STING (stroma) high and low expression cohorts.

H-151, functioning as an antagonist of STING, exhibits notable efficacy against both mouse STING (mmSTING) and human STING (hsSTING), significantly suppressing STING-dependent IFNB1 synthesis [47]. Conversely, the STING agonist MSA-2 demonstrates anti-tumor efficacy in a murine tumor model [32, 48]. To explore the correlation between STING expression levels and the response to platinum treatment in ovarian cancer, both compounds were employed. H-151 alone exhibited a certain tumor inhibition effect in vivo and displayed an enhanced cisplatin-sensitizing effect when combined with cisplatin. Notably, the in vivo tests revealed that H-151 alone had a tumor inhibition effect, and its combination with cisplatin enhanced cisplatin-sensitizing effects. In the MSA-2 monotherapy group, increased expression levels of tumor growth markers were observed, along with a rise in CD8 cell population, explaining the lower tumor weight compared to the NC group. However, these results diverged from the trend observed in the cell experiments. Although no additional sensitizing or resistance effect of cisplatin was noted when combined with MSA-2, higher α-SMA expression, reduced DNA damage, and increased DNA damage repair were observed. Overall, these experiments consistently support H-151 achieving the sensitization effect of cisplatin when used in combination. However, due to the potential immune-promoting effect of MSA-2, we cannot draw a definitive conclusion, which is also a limitation of our study.

STING agonists have progressed to clinical trials as immune activators for various cancers, including non-small cell lung cancer, colon cancer, liver cancer, breast cancer [45, 49-52]. However, due to the incomplete understanding of STING function, there are still many unanswered questions that need to be addressed before STING agonists or inhibitors can be used in clinical practice. This highlights the inherent
challenges associated with using STING agonist monotherapy as a systemic therapy suggesting that STING agonists or inhibitors should be used with caution when making clinical decisions.

In summary, our study revealed that cisplatin administration induces genomic instability in ovarian cancer cells, subsequently activating the cGAS-STING cascade in cancer-associated fibroblasts (CAFs) and leading to IFNβ release. Furthermore, our findings suggest a correlation between the presence of STING in the stromal cells of ovarian cancer and a diminished response to platinum-based therapies, ultimately resulting in an unfavorable outcome. In particular, the STING inhibitor H-151 demonstrated the capacity to augment cisplatin sensitivity and synergize effectively in inducing ovarian cancer cell death.

Funding support
This study was supported by the National Key Technology Research and Development Programme of China (2022YFC2704200 and 2022YFC2704202); the "Taishan Scholars" Program for Young Expert of Shandong Province, China (tsqn20221131); the Key R&D Program of Shandong Province, China (2022QLGX03-22); and the CHINA ANTI CANCER ASSOCIATION (CORP-252).

Data and materials availability
All data are available in the main text or the supplementary materials.

CRediT authorship contribution statement
Jiale Liu: Writing – review & editing, Writing – original draft, Validation, Software, Methodology, Investigation, Formal analysis, Data curation.
Chennian Liu: Writing – review & editing, Writing – original draft, Supervision, Resources, Formal analysis. Yana Ma: Validation, Supervision, Software, Data curation. Xiyu Pan: Supervision, Software, Formal analysis.
Belhua Kong: Project administration, Investigation, Conceptualization. Qing Zhang: Writing – review & editing, Writing – original draft, Project administration, Investigation, Conceptualization. Kun Song: Validation, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest
The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

Acknowledgments
We express our profound gratitude to all individuals who participated in the study, as well as all the organizations and researchers responsible for the provision of data and program from public databases, which were utilized in this research endeavor.

Appendix A. Supplementary data
Supplementary data to this article can be found online at https://doi.org/10.1016/j.canlet.2024.216700.

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