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Cancer-associated fibroblasts-derived IL-8 mediates resistance to cisplatin in human gastric cancer

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ABSTRACT

Chemoresistance remains the major obstacle to achieve optimal prognosis in gastric cancer patients, and the underlying molecular mechanisms of cancer-associated fibroblasts (CAFs) in gastric cancer chemoresistance remain poorly understood. We identified the high pretherapeutical serum IL-8 level in gastric cancer patients was associated with poor response to platinum-based therapy, and it increased gradually during neoadjuvant chemotherapy and it decreased after radical surgery. Immunohistochemistry assays showed that IL-8 was highly expressed in gastric cancer tissues in chemoresistant patients, and located in CAFs. Primary CAFs produced more IL-8 than the corresponding normal fibroblasts, and human stomach fibroblast line Hs738 secreted more IL-8 after co-cultured with conditioned media from AGS or MGC-803 cells. IL-8 increased the IC50 of cisplatin (CDDP) in AGS or MGC-803 *in vitro*. Simultaneously, IL-8 treatment enhanced the expression of PI3K, phosphorylated-AKT (p-AKT), phosphorylated-IKb (p-IKb), phosphorylated-p65 (p-p65) and ABCB1, and ABCB1 and p-p65 were overexpressed in tumor tissues of chemoresistant patients. Collectively, CAFs derived IL-8 promotes chemoresistance in human gastric cancer *via* NF-κB activation and ABCB1 up-regulation. Our study provides a novel strategy to improve the chemotherapeutical efficacy and the prognosis of gastric cancer.

1. Introduction

Although the incidence has been declining in most parts of the world in the last decades, stomach carcinoma remains a prominent cancer worldwide and is responsible for over 1,000,000 new cases in 2018 and an estimated 783,000 deaths, making it the fifth most frequently diagnosed cancer and the third leading cause of cancer death [1]. In 2014, 410,400 new stomach cancer cases and 293,800 cancer-associated deaths were estimated to have occurred in China, accounting for 10.79% and ranking third in incidence of all cancers combined, which indicates that stomach cancer remains a major contributor to the cancer burden among the Chinese population [2]. Gastrectomy with D2 lymph node dissection has become the global standard procedure for locally advanced gastric cancer to maximally reduce locoregional recurrence, particularly in East Asia [3]. However, even after curative surgery and adequate lymphadenectomy the survival of advanced gastric cancer remains poor. Multimodality therapy including neoadjuvant chemotherapy (NAC) and postoperative adjuvant chemotherapy (POAC) has been studied and applied to improve survival [4]. Based on the evidence of the ACTS-GC [5] and the CLASSIC trials [6], POAC after curative D2 gastrectomy is the current standard strategy. However, approximately 20%–30% of patients still develop locoregional or

Abbreviations: NAC, neoadjuvant chemotherapy; POAC, postoperative adjuvant chemotherapy; POC, perioperative chemotherapy; ECF, epirubicin/cisplatin/infusional 5-FU; FLOT, docetaxel/oxaliplatin/5-fluorouracil; GEJ, gastroesophageal junction; TME, tumor microenvironment; CAFs, Cancer-associated fibroblasts; CT, computed tomography; EUS, endoscopic ultrasonography; RECIST, response evaluation criteria in solid tumors; NFs, normal fibroblasts; ELISA, enzyme-linked immunosorbent assay; CCK-8, cell counting kit-8; α-SMA, α-smooth muscle actin; IHC, immunohistochemistry; CDDP, cisplatin; ABCB1, ATP-binding cassette subfamily B member 1; NF-κB, nuclear factor kappa B; ECM, extracellular matrix; MSCs, mesenchymal stem cells; FSP-1, fibroblast-specific protein-1; FAP, fibroblast-activated protein; TEM1, tumor endothelial marker 1

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Table 1
Correlation between the pretherapeutic serum levels of IL-6 and IL-8 and clinicopathological features of gastric cancer patients.

variable	IL-6				IL-8			
	N	normal (%)	abnormal (%)	P	N	normal (%)	abnormal (%)	P
Age (years)				0.274				0.426
< 60	54	36 (66.7)	18 (33.3)		54	18 (33.3)	36 (66.7)	
≥60	57	42 (73.7)	15 (26.3)		57	21 (36.8)	36 (63.2)	
Gender				0.456				0.104
male	78	66 (84.6)	12 (15.4)		78	24 (30.8)	54 (69.2)	
female	33	27 (29.0)	6 (33.3)		33	15 (45.5)	18 (54.5)	
Lauren classification				0.115				0.000
intestinal	33	21 (23.6)	12 (36.4)		33	3 (9.1)	30 (90.9)	
diffuse and mixed	78	60 (76.9)	18 (23.1)		78	36 (46.2)	42 (53.8)	
Tumor size (cm)				0.593				0.121
≥3	93	36 (38.7)	57 (61.3)		93	30 (32.3)	63 (67.7)	
< 3	18	7 (38.9)	11 (61.1)		18	9 (50.0)	9 (50.0)	
Tumor location				0.328				0.401
upper third	27	18 (66.7)	9(33.3)		27	12 (44.4)	15 (55.6)	
middle third	42	34 (81.0)	8(19.0)		42	15 (35.7)	27 (64.3)	
lower third	42	29 (69.0)	13 (31.0)		42	12 (28.6)	30 (71.4)	
Depth of tumor invasion				0.420				0.017
localized in subserosa	9	4 (44.4)	5 (55.6)		9	0 (0.0)	9 (100.0)	
beyond subserosa	102	55 (53.9)	47 (46.1)		102	39 (38.2)	63 (61.8)	
Lymph node metastasis				0.419				0.202
N0	24	21 (87.5)	3 (12.5)		24	12 (50.0)	12 (50.0)	
N1− N3	87	72 (82.8)	15 (17.2)		87	27 (31.0)	60 (69.0)	
pTNM stage				0.459				0.104
I/II	33	27 (87.9)	6 (18.1)		33	15 (45.5)	18 (54.5)	
III/IV	78	66 (84.6)	12 (15.4)		78	24 (30.8)	54 (69.2)	
Lymphovascular invasion				0.060				0.310
absence	39	36 (92.3)	3 (7.7)		39	12 (30.8)	27 (69.2)	
presence	72	57 (79.2)	15 (20.8)		72	27 (37.5)	45 (62.5)	
Chemotherapy				0.440				0.000
sensitive	42	36 (85.7)	6 (14.3)		42	24 (57.1)	18 (42.9)	
resistant	69	57 (82.6)	12 (17.4)		69	15 (21.7)	54 (78.2)	

metastatic recurrences even after these postoperative adjuvant chemotherapies, especially in those with pathological stage III disease [7]. NAC, or perioperative chemotherapy (POC), consisting of three courses of ECF (epirubicin/cisplatin/infusional 5-FU) combined with surgery and three postoperative courses of ECF, has been the standard treatment for resectable gastric cancer in Europe [8]. Recently, the German FLOT4 trial established the perioperative FLOT regimen (docetaxel/oxaliplatin/5-fluorouracil) as the new treatment standard for resectable adenocarcinoma of the gastroesophageal junction (GEJ) and the stomach [9]. In a large series of patients with stage II/III resectable gastric/GEJ adenocarcinomas, patients receiving POC were shown to survive longer than those receiving postoperative chemoradiotherapy [10]. An updated meta-analysis also indicated that NAC is associated with significant improvement in the outcomes of survival and disease progression for gastric cancer patients [11]. However, resistance to chemotherapy remains the major obstacle to achieve optimal results in gastric cancer patients.

Chemotherapeutic resistance, whether intrinsic or acquired, is a complex and multifactorial phenomenon that is associated with tumor cells as well as with the tumor microenvironment (TME) [12,13]. A variety of factors have been demonstrated to be involved in chemoresistance, including a reduction in drug uptake, enhanced drug efflux [14], a reduced prodrug activation, alterations in drug targets [15], the dysregulation of cell survival and death signaling pathways [16], and interactions between cancer cells and TME.

Increasing evidence has demonstrated that TME plays a major role in the pathogenesis of multiple cancer types. Cancer-associated fibroblasts (CAFs) are the predominant cell type in the tumor-associated stroma and contribute to tumorigenesis by secreting growth factors, modifying the extracellular matrix, supporting angiogenesis, and suppressing antitumor immune responses, and fostering resistance to therapy [17]. CAFs have also been reported to predict poor outcome of many cancer types, including gastric cancer [18]. Currently, CAFs has gained attention as a promising target for cancer therapy.

Of note, CAFs contribute to chemoresistance via multiple mechanisms. The dense extracellular matrix synthesized by CAFs can act as a physical barrier to drug delivery [19], and CAFs create a microenvironmental niche that accelerates cancer cell proliferation [20,21], resists apoptosis [22], and promotes cancer stem cell maintenance [20]. However, the underlying molecular mechanisms of CAFs in gastric cancer chemoresistance remain poorly understood [19].

In this present study, we investigated the clinicopathological significance of the pretherapeutic serum proinflammatiory cytokine levels, including IL-6 and IL-8, in patients with advanced primary gastric cancer, and found that the high IL-8 level was associated with the poor response to neoadjuvant chemotherapy. The high serum IL-8 was determined to be highly expressed in CAFs in the tumor microenvironment gastric cancer. Functional studies revealed that IL-8 mediated gastric cancer resistance to cisplatin via NF-κB activation and ABCB1 up-regulation. Our study highlights the importance and its underlying

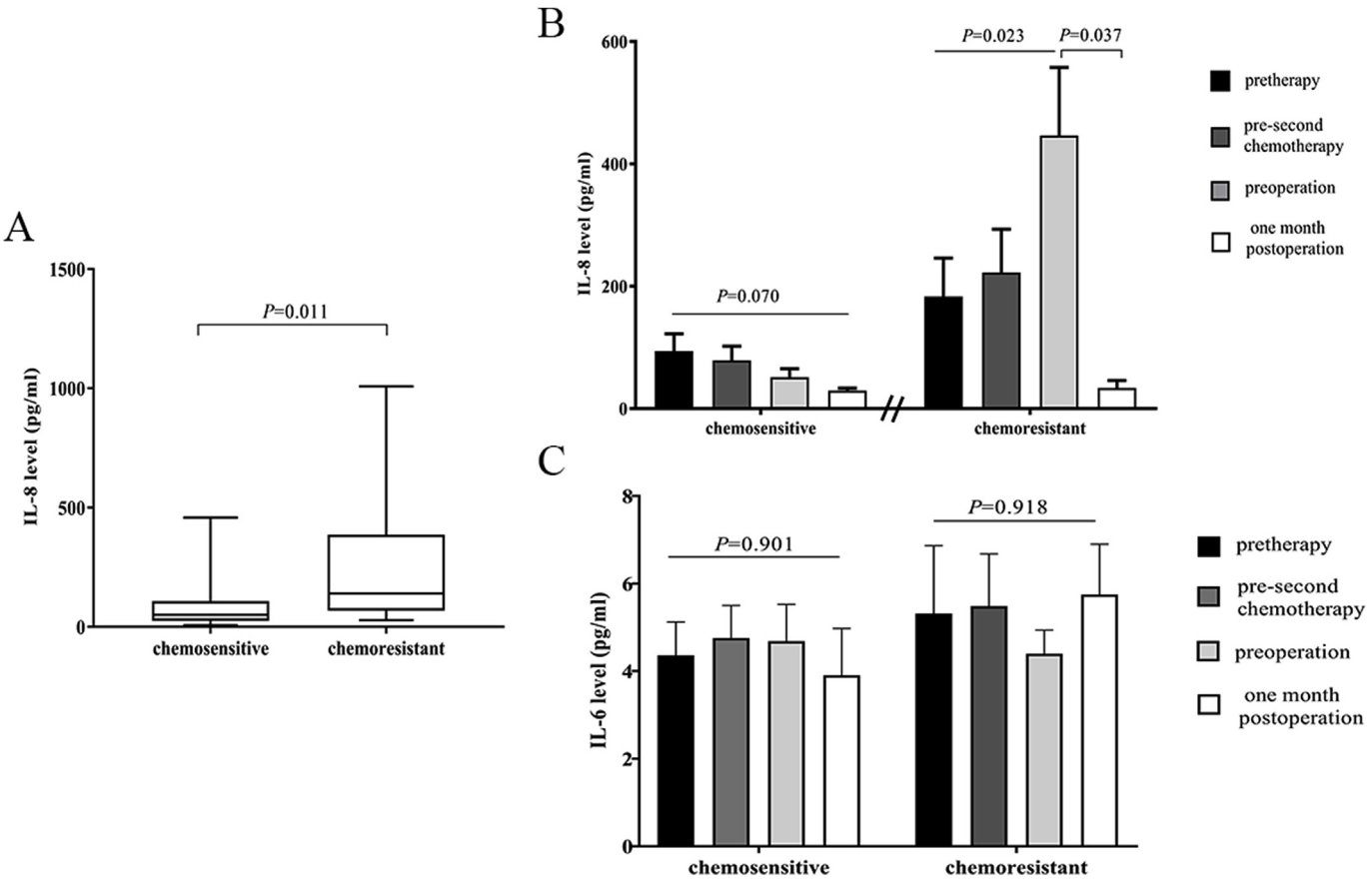

Fig. 1. The serum IL-8 level was associated with chemoresistance of gastric cancer patients. **A.** The pretherapeutic serum IL-8 level in chemoresistant gastric cancer patients (282.00 ± 65.18 pg/ml) was higher than that in chemosensitive patients (85.77 ± 30.67 pg/ml, $P = 0.011$). **B.** During NAC, the serum IL-8 levels in the chemoresistant patients increased gradually ($P = 0.023$), and it decreased dramatically post radical surgery ($P = 0.037$); however, it decreased slightly in chemosistantive patients ($P = 0.070$). **C.** The serum IL-6 level remained nearly unchanged in the chemosensitive patients ($P = 0.901$) and the chemoresistant patients ($P = 0.918$).

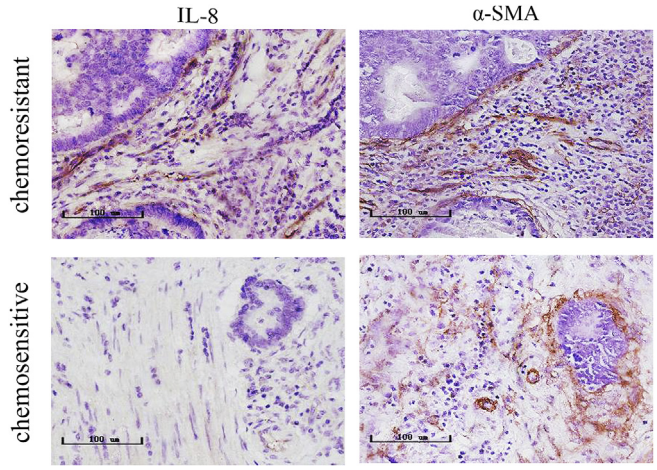

Fig. 2. IL-8 was highly expressed in CAFs in gastric cancer tissues. In the tissue of chemoresistant patient, IL-8 was highly expressed in CAFs, which expressed α-SMA, in the stroma of gastric tumor tissues. In the chemosensitive patient who obtained CR after NAC, α-SMA was expressed in the stroma, but there was no IL-8 immunoactivity found.

mechanisms of CAFs in gastric cancer chemoresistance, and provides a new indicator for evaluating the response to chemotherapy in gastric cancer, as well as a novel strategy to improve the chemotherapeutical efficacy and the prognosis in gastric cancer patients.

2. Materials and methods

2.1. Human serum and tissue specimens

A total of 111 patients, diagnosed with primary advanced gastric adenocarcinoma by endoscopy and pathological examination, were enrolled in this retrospective study. All these patients were evaluated as cT2 ~ 3N1 ~ 2M0 stage by abdominal computed tomography scan (CT) or endoscopic ultrasonography (EUS). After systemic evaluation, these patients underwent two cycles NAC containing platinum-based drugs, and they were performed radical surgery after the chemotherapy. Tumor response rate was determined by tumor size as described by Response Evaluation Criteria in Solid Tumors (RECIST) [23]. There were no severe complications associated with chemotherapy and surgery in these patients. The serum specimens were collected at pretherapy, pre-second chemotherapy, preoperation and one month postoperation respectively. The gastric cancer tissues and the corresponding non-cancerous mucosal tissues were collected from all patients immediately after resection, and were snap frozen in liquid nitrogen, and were transferred to laboratories anonymously. All patients provided written informed consent. The study protocol was approved by the Institutional Review Board of Nanjing University of Chinese Medicine, and complied with the Helsinki Declaration.

2.2. Cell culture and reagents

Human gastric adenocarcinoma cell lines, AGS (ATCC, VA, USA) and MGC-803 (CBTCCCAS, Shanghai, China), and human stomach

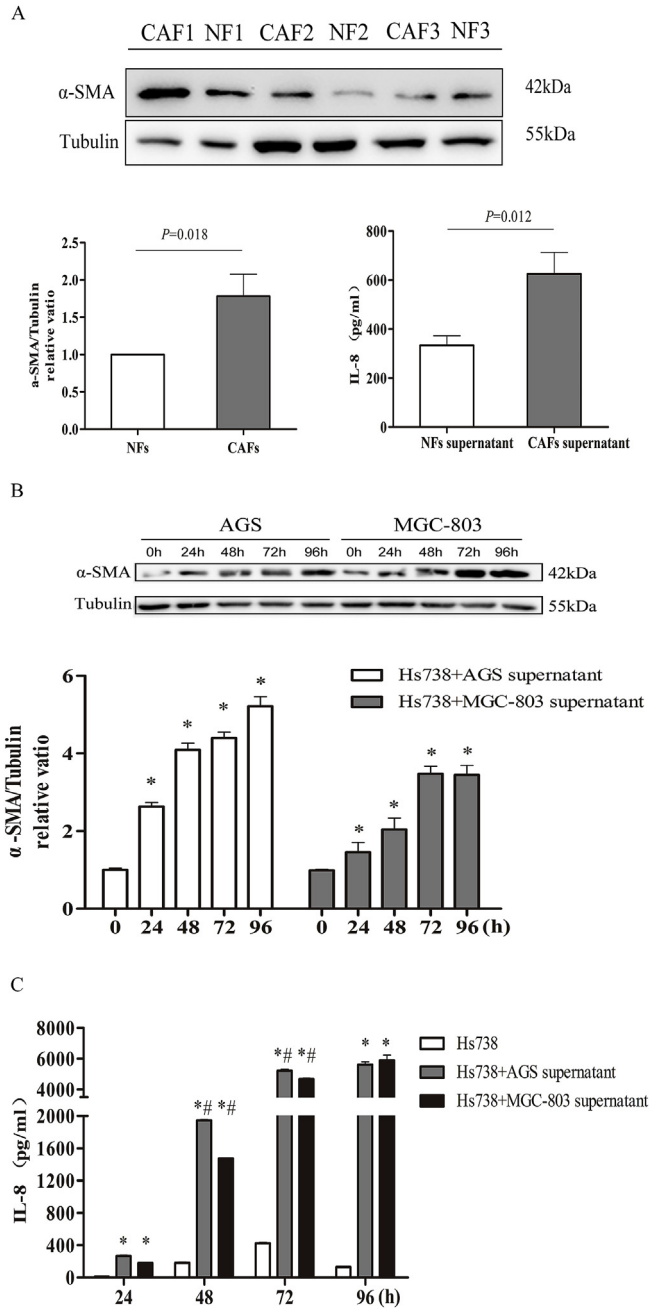

Fig. 3. CAFs produced more IL-8. A. We obtained three pair of primary cultured CAFs and corresponding NFs from three patients, and α-SMA was overexpressed in CAFs compared with NFs ($P = 0.018$), and higher level of IL-8 was detected in the cell supernatant of CAFs rather than NFs ($P = 0.012$). **B.** Both AGS supernatant and MGC-803 supernatant upregulated the α-SMA expression in Hs738 (*: $P < 0.05$ *vs* 0 h). **C.** The IL-8 levels in AGS supernatant and MGC-803 supernatant were very low (17.91 ± 1.12 pg/ml and 15.54 ± 0.27 pg/ml respectively). Activated Hs738 by these supernatant produced more IL-8 (*: $P < 0.05$ *vs* Hs738 at each time point; #: $P < 0.05$ *vs* corresponding group at 24 h).

fibroblat line Hs738 (ATCC, VA, USA) were cultured in the complete DMEM (ATCC, VA, USA) with 10% fetal calf serum. Cisplatin and human IL-8 were obtained from Sigma-Aldrich (USA).

2.3. Isolation and culture of primary fibroblasts

Primary CAFs were isolated from gastric carcinoma tissue samples, and primary normal fibroblasts (NFs) were isolated from the noncancerous mucosa tissues at least 5 cm from the outer tumor margin in the same patient according to reported protocol [24]. Briefly, fresh samples were washed with serum-free DMEM, cut into small pieces, and were transferred to a 0.15% collagenase IV solution, followed by incubation at 37 °C for 40 min. Digested cells were filtered through a 40-mm cell strainer (Milex-GP) and centrifuged at 1500 rpm for 10 min. The single-cell suspension was incubated in a Fibroblast Medium Kit (Cat. No. P60108, Innoprot) for 24 h, allowing fibroblasts to attach on culture plates. Unattached cells were removed after 24 h incubation, and the adherent cells were further cultivated for experiments. Cultured CAFs and NFs less than five passages were used for our experiments.

2.4. ELISA

The enzyme-linked immunosorbent assay (ELISA) (Invitrogen, CA, USA) was used to detect IL-6 and IL-8 in the patient serums and in the cell culture supernatants. Each experiment was repeated at least three times.

2.5. Cell proliferation assay

Cell proliferation was analyzed using a Cell Counting Kit-8 (CCK-8) assay (Dojindo, Japan) according to the manufacturer's protocol. The results were plotted as mean ± standard error (SE) of three separate experiments for each experimental condition.

2.6. Immunohistochemistry

The expression of α-smooth muscle actin (α-SMA) and IL-8 in gastric tissue specimens were detected by immunohistochemistry (IHC). A mouse monoclonal anti-IL-8 antibody (Abcam, Cambridge, UK), and a mouse monoclonal anti-α-SMA antibody (Cell Signaling Technology, MA, USA) were used. IHC was performed on paraffin-embedded formalin-fixed tissues according to standard protocols.

2.7. Western blotting

Protein expression levels of the indicated molecules were detected using the western blotting [25]. The antibodies used for the analyses were as following: rabbit monoclonal anti-α-SMA antibody, rabbit monoclonal anti-PI3 Kinase antibody, rabbit monoclonal anti-AKT antibody, rabbit monoclonal anti-p-AKT antibody, mouse monoclonal anti-IKKα antibody, rabbit monoclonal anti-p-IKKα/β antibody, mouse monoclonal anti-IkBα antibody, rabbit monoclonal anti-p-IkBα antibody, rabbit monoclonal anti-p65 antibody, rabbit monoclonal anti-p-p65 antibody, rabbit monoclonal anti-ABCB1 antibody, rabbit monoclonal anti-β-actin antibody, rabbit monoclonal anti-Tubulin antibody (Cell Signaling Technology, MA, USA). The relative levels were quantified and normalized against β-actin or Tubulin in the same sample with a densitometric analysis.

2.8. Statistical analysis

Data are expressed as mean ± standard error. In experiments involving protein expression, the data were representative of three independent experiments. The associations between the protein levels and various clinicopathological parameters were analyzed with the Pearson's $\chi 2$ test. Quantitative data were compared between the control and treatment groups by analysis of variance. All analyses were performed with SPSS software (version 19.0; SPSS Inc., Chicago, IL). Values of $P < 0.05$ were considered to indicate statistical significance.

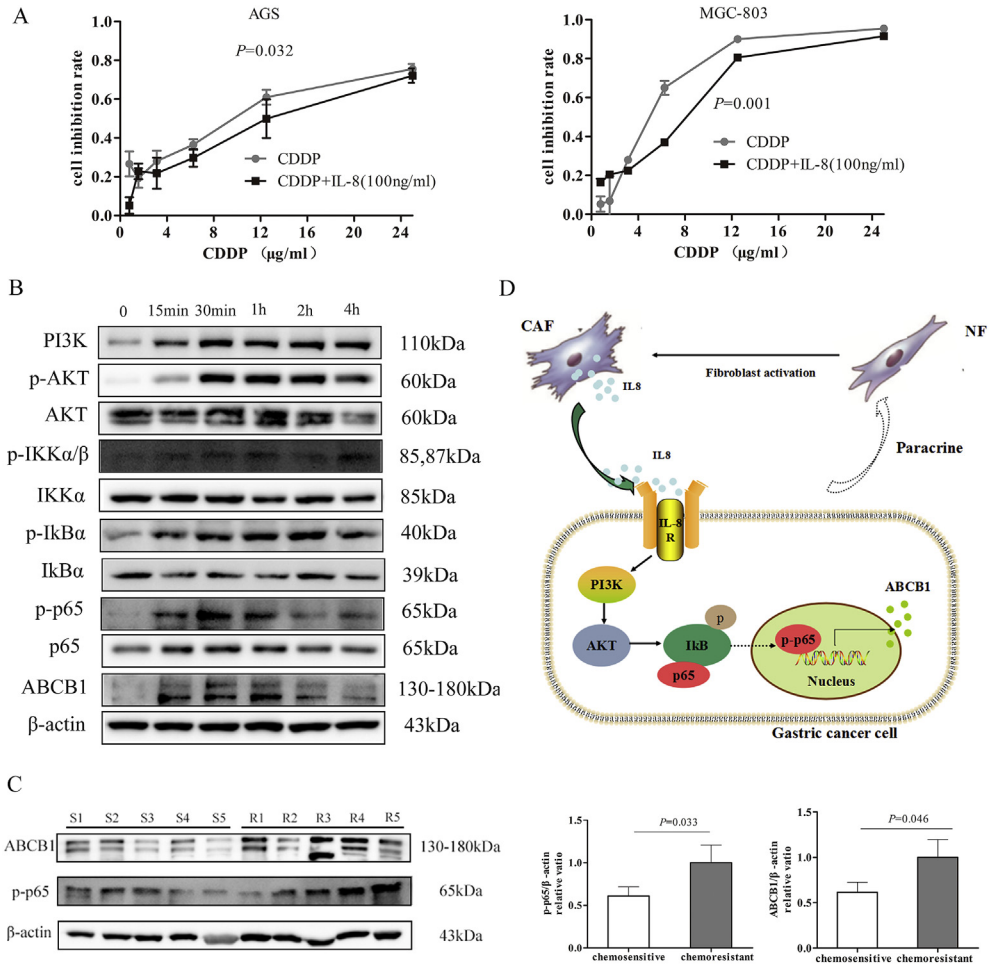

Fig. 4. IL-8 mediates chemoresistance to cisplatin in human gastric cancer cells via NF-κB activation and ABCB1 up-regulation. **A.** IL-8 (100 ng/ml) increased the IC50 of CDDP in both AGS ($P = 0.032$) and MGC-803 cells ($P = 0.001$), meaning that IL-8 promotes chemoresistance in human gastric cancer cells. **B.** IL-8 treatment enhanced the expression of PI3K, p-AKT, p-IKb, p-p65 and ABCB1. **C.** ABCB1 ($P = 0.046$) and p-p65 ($P = 0.033$) were overexpressed in tumor tissues of five chemoresistant patients (R1 ~ R5) rather than tissues of five chemosensitive patients (S1 ~ S5). **D.** Schematic representation of the mechanism of IL-8 in chemoresistance of gastric cancer.

3. Results

3.1. High serum IL-8 level in gastric cancer patients is associated with poor chemotherapy response

In order to investigate the clinical significance of the pretherapeutic serum IL-6 and IL-8 levels in patients diagnosed with primary advanced gastric cancer, 111 cases, undergoing two cycles neoadjuvant chemotherapy containing platinum-based drugs, were enrolled in this study, and all these patients were performed radical surgery after neoadjuvant chemotherapy. Overall, 48 patients obtained complete response or partial response (chemosensitive group), and 63 patients underwent stable or progressive disease (chemoresistant group) based on RECIST. As shown in Table 1 and Fig. 1A, the serum IL-8 level was associated with Lauren diffuse and mixed type ($P = 0.000$), advanced tumor invasion ($P = 0.017$) and poor response to platinum-based chemotherapy ($P = 0.000$); however, the serum IL-6 level had no effect on the clinicopathological features ($P > 0.05$).

Of great interest and importance, continuous monitoring indicated that the serum IL-8 levels in the chemoresistant patients increased gradually during NAC ($P = 0.023$), and it decreased dramatically post radical surgery (446.71 ± 111.07 pg/ml *vs* 33.74 ± 12.27 pg/ml, $P = 0.037$); however, it decreased slightly in chemosensitive patients ($P = 0.070$) (Fig. 1B). The serum IL-6 level remained nearly unchanged in these two groups ($P > 0.05$) (Fig. 1C). Thus, it may be considered that the serum IL-8, rather than IL-6, in gastric cancer patients is involved in chemoresistance, and high IL-8 level may be produced by tumor tissues.

3.2. IL-8 is highly expressed in CAFs in the tumor microenvironment of gastric cancer

To determine whether IL-8 was expressed in tumor tissues in these patients and which cells IL-8 located in, we performed immunohistochemistry assays. As shown in Fig. 2, IL-8 was highly expressed in gastric tumor tissues in the chemoresistant patients rather than in the chemosensitive patients. Cytologically, IL-8 exclusively located in the fibroblasts in the tumors, which expressed α-SMA, namely IL-8 was highly expressed in CAFs.

Furthermore, we obtained the primary cultured CAFs from gastric cancer tissues and normal fibroblasts (NFs) from the corresponding noncancerous mucosa tissues in these patients, and we evaluated the secretion of IL-8 in these CAFs and NFs. Fig. 3A showed that α-SMA was overexpressed in CAFs by comparison with NFs ($P = 0.018$), indicating these primary cultured fibroblasts were activated. Higher level of IL-8 was detected in the cell supernatant of CAFs rather than NFs ($P = 0.012$).

It has been well established that cancer cell supernatant may activate fibroblasts. Thus, we used the conditioned media from human gastric cancer cell lines, AGS and MGC-803, to co-culture human stomach fibroblast line Hs738. As shown in Fig. 3B, both AGS supernatant and MGC-803 supernatant upregulated the α-SMA expression in Hs738 ($P < 0.05$), namely gastric cancer cell supernatant activates fibroblasts. Importantly, the activated Hs738 produced more IL-8 (Fig. 3C, $P < 0.05$). Thus, it can be inferred that IL-8 secreted by CAFs was involved in human gastric cancer chemoresistance.

3.3. IL-8 mediates chemoresistance to cispatin in human gastric cancer cells via NF-κB activation and ABCB1 up-regulation

To probe the mechanism of IL-8 mediating chemoresistance, we evaluated the *in vitro* effects of IL-8 on response to cisplatin (CDDP) in gastric cancer cell lines, AGS and MGC-803. As shown in Fig. 4A, IL-8 had little effects on cell proliferation at the concentration of 100 ng/ml; however, IL-8 at this concentration increased the IC50 of CDDP in both AGS (8.14 ± 0.40 μg/ml *vs* 11.67 ± 1.48 μg/ml, $P = 0.032$) and MGC-803 cells (4.64 ± 0.23 μg/ml *vs* 6.48 ± 0.20 μg/ml, $P = 0.001$), which meant that IL-8 promotes chemoresistance in human gastric cancer cells.

IL-8 is one of proinflammatory cytokine, and we assayed the expression of nuclear factor kappa B (NF-κB) pathway-associated proteins. We found that the expression of PI3K, p-AKT, p-IKb and p-p65 was enhanced after IL-8 treatment (Fig. 4B), indicating IL-8 mediates chemoresistance via NF-κB activation. Importantly, ATP-binding cassette subfamily B member 1 (ABCB1) expression was increased accordingly. It has been well established that ABCB1 acts as drug pump to reduce the intracellular drug concentration, and leads to chemoresistance in several kinds of human cells [26]. We further examined the expression of ABCB1 and p-p65 in human gastric cancer tissues, and showed that ABCB1 and p-p65 were overexpressed in tumor tissues of chemoresistant patients rather than tissues of chemosensitive patients ($P = 0.046$ and $P = 0.033$, respectively) (Fig. 4C). Thus, it can be concluded that CAFs derived IL-8 promotes chemoresistance in human gastric cancer via NF-κB activation and ABCB1 up-regulation.

4. Discussion

Recurrence and metastasis after radical gastrectomy results in dismal prognosis in advanced gastric cancer patients, and cancer's growing chemoresistance continues to be a challenge for the overall survival of patients with advanced gastric cancer undergoing chemotherapy [13,27,28]. Resistance to chemotherapy involves several mechanisms concerning both genetic characteristics of cancer cells and the interaction with their tumor microenvironment (TME), which consists of the extracellular matrix (ECM), growth factors, cytokines, and a variety of cell types, such as CAFs, immune cells, endothelial cells, and inflammatory cells [29]. CAFs are the main cell type in TME, and originate from normal fibroblasts, mesenchymal stem cells (MSCs), and even the perivascular cells in the vicinity of tumor sites during tumourgenesis [19,30]. CAFs specifically play crucial roles in promoting tumor development and progression of many cancer types, including gastric cancer [21,27,31,32]. However, the exact molecular mechanism underlying the interaction between gastric cancer cells and CAFs remains poorly understood [29].

Alpha-smooth muscle actin (α-SMA), fibroblast-specific protein-1 (FSP-1), and fibroblast-activated protein (FAP) are specific markers of CAFs, and α-SMA was used in our study. Intensive researches have revealed that CAFs mediates chemoresistance through producing IL-6 [17,20,21,33], IL-11 [23], Lumican [27], and vascular endothelial growth factor (VEGF) [34], and that the expression of tumor endothelial marker 1 (TEM1) [20] or CXCL12 [35] in CAFs is correlated with a poor prognosis in patients with gastric cancer. Our study investigated the clinicopathological significance of IL-6 and IL-8 in advanced gastric cancer patients, and IL-8 derived from CAFs was revealed to be associated with gastric cancer chemoresistance, which may be due to the organ specificity of fobroblasts. We speculated that a set of factors are involved in the mechanism of CAFs in chemoresistance, and that its roles in chemoresistance may be tumor-specific, which needs to be further investigated.

Our study showed that the pretherapeutic serum IL-8 level of advanced gastric cancer patients was associated the response to platinum-based NAC negatively. Furthermore, the serum IL-8 level increased with NAC implementation in the chemoresistant patients, while it decreased gradually in the chemosensitive patients. These results suggested that the IL-8 level may portend both intrinsic and acquired chemoresistance, and the serum IL-8 level may be a new potential marker to evaluate the chemotherapy response in gastric cancer. Importantly, the IL-8 level dropped dramatically one month post-operation in the chemoresistant patients. We inferred that the IL-8 may be produced by tumor tissues, and the immunohistochemisrty study confirmed this speculation. IL-8 was exclusively and highly expressed in CAFs in chemoresistant tumor, but there was no IL-8 immunoactivity in CAFs in nearly CR tumor although there were CAFs in tissues. The primary CAFs from gastric cancer tissues produced more IL-8 than the primary normal fibroblasts.

The *in vitro* cell proliferation study showed that IL-8 treatment increased the IC50 of cisplatin in human gastric cancer cell lines. To investigate the mechanisms underlying of IL-8 in gastric cancer chemoresistance, the human stomach fibroblast line Hs738 was introduced in this study. As anticipated, the conditioned media from human gastric cancer cells increased the α-SMA level in Hs738 cells, namely activated the fibroblasts [31]. The activated Hs738 cells also secreted more IL-8.

Investigations into the mechanisms of IL-8 in regulating therapeutic response and resistance indicated that IL-8 activated AKT, IKb and p65 through phosphrylation in gastric cancer cells, namely activated NF-κB pathway. Furthermore, IL-8 up-regulated the expression of ABCB1, an important drug pump [26], in gastric cancer cells. We confirmed the overexpression of ABCB1 and p-p65 in the tumor tissues from chemoreisitant patients. Taken together, it could be concluded that CAFs derived IL-8 promotes chemoresistance in human gastric cancer via NF-κB activation and up-regulating ABCB1 (Fig. 4D).

IL-8 usually functions in different biologic processes by binding its receptors, CXCR1 and CXCR2 [23]. CXCR1 has been demonstrated to promote gastric cancer cell proliferation, migration and invasion [36], and intratumoral CXCR2 expression is associated with the survival in gastric cancer patients [37]. Repertaxin, an inhibitor of CXCR1/2, has been demonstrated to inhibit malignant behavior of human gastric cancer cells *in vitro* and in vivo and to enhance efficacy of 5-FU [38]. However, whether CAFs-derived IL-8 mediates chemoresistance via its receptors remains unknown. We will continue to investigate the roles of the CXCR1/2 in IL-8 mediated chemoresistance in the tumor microenvironment.

Certainly, there were some limits in this study. We just probed IL-6 and IL-8 in the serum of gastric cancer patients. Other cytokines derived from CAFs in the chemoresistance of gastric cancer, the dynamic IL-8 level variation and its functions in the gastric cancer progression, the other mechanisms involved in IL-8-mediated resistance to cisplatin, as well as whether IL-8 derived from CAFs leads to the circumvention of other cytotoxic drugs beside ciaplatin, remained to be intensively studied. There was no survival data for these patients due to short follow-up time.

In short, this study highlights the importance and its underlying mechanisms of CAFs in gastric cancer chemoresistance, and provides serum IL-8 level as a new prognostic indicator for evaluating the response to chemotherapy in gastric cancer, as well as a novel strategy targeting IL-8 or CAFs to improve the chemotherapeutical efficacy and the overall survival of gastric cancer patients.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgments

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