Fibroblast growth factor 21 is related to cisplatin resistance in ovarian cancer

Qian Zhong[1,2], Lifei wang[1,2], Meng Cheng[1,2], Hanshuo Yang[3]

[1]department of Gynecology and Obstetrics, West China Second University Hospital of Sichuan University, Chengdu, Sichuan 610041, China;
[2]Key Laboratory of Birth Defects and Related Diseases of Women and Children, Sichuan University, Ministry of Education, Chengdu, Sichuan 610041, China;
[3]State Key Laboratory of Biotherapy and Cancer Center, West China Hospital, Sichuan University, and Collaborative Innovation Center for Biotherapy, Chengdu, Sichuan 610041, Chaina.

To the Editor: Epithelial ovarian cancer has the highest mortality rate among all gynecologic cancers. Cisplatin and carboplatin are the primary first-line therapies for the treatment of ovarian cancer. Although most ovarian cancer patients are initially sensitive to platinum, many eventually develop drug-resistant diseases.[1] However, the exact mechanism of platinum resistance is still unclear.[2] Through DNA microarray analyses of a cisplatin-resistant human ovarian cancer cell line (A2780CP) and the parental cell line (A2780), we found that the fibroblast growth factor 21 (*FGF21*) gene was extremely upregulated by 18.74-fold in A2780cp cells compared with that in A2780 cells. The upregulated and downregulated genes identified in the microarray analyses were involved in various biological processes including the regulation of metabolic process and response to growth factors [Supplementary Figure 1, http://links.lww.com/CM9/B12]. Pathway analysis revealed that the mitogen-activated protein kinase (MAPK) signaling pathway was involved in cisplatin resistance [Supplementary Figure 2, http://links.lww.com/CM9/B12].

FGF21 is a member of an atypical FGF subfamily that sometimes circulates as hormones.[3] FGF21 is involved in the above-mentioned biological processes and is one of the key factors in the MAPK signaling pathway.[4-7] FGF21 is a promising new treatment in diabetes and obesity and has also been demonstrated to have a therapeutic role in nonalcoholic fatty liver disease.[3] The safety of chronic FGF21 administration has not yet been fully studied. Recent studies have suggested that FGF21 may have a potential influence on cancer pathogenesis and treatment.[8-10] However, little is known about the relationship between FGF21 and ovarian cancer. This study examined the association between FGF21 and cisplatin sensitivity in ovarian cancer.

Access this article online

Quick Response Code:

Website: www.cmj.org

DOI: 10.1097/CM9.0000000000002095

We constructed a pCDH-CMV-MCS-EF1-copGFP plasmid encoding FGF21 (pCDH-copGFP-FGF21) and three small interfering RNAs (siRNAs) targeting FGF21 to upregulate and downregulate FGF21, respectively. A2780 ovarian cancer cells transfected with FGF21-overexpressing plasmid or FGF21 siRNA demonstrated enhanced or reduced expression of FGF21 compared with control pCDH-copGFP plasmid or control siRNA-treated cells. siRNA2 was most potent in downregulating FGF21 among all three siRNAs and was selected for subsequent experiments [Supplementary Figure 3, http://links.lww.com/CM9/B12].

To evaluate the effect of FGF21 on cisplatin sensitivity in ovarian cancer, A2780 cells were transfected with FGF21-overexpressing, control plasmid, FGF21 siRNA, or negative control (NC) siRNA, and after 12 h, cells were incubated with cisplatin at different concentrations (0, 1, 2, 3, and 4 μg/mL) for 2 days. The results showed that FGF21-overexpressing A2780 cells gained resistance to cisplatin compared with control cells [Figure 1A]. In contrast, A2780 cells transfected with FGF21 siRNA showed enhanced sensitivity to cisplatin compared with control cells [Figure 1B].

Next, we investigated whether FGF21 influenced the cell apoptosis caused by cisplatin. A2780 cells were transfected with FGF21-overexpressing, control plasmid, FGF21 siRNA, or NC siRNA overnight and treated with 1 μg/ mL cisplatin for 2 days. Quantitative evaluation of cellular apoptosis was performed by Annexin V/propidium iodide (PI) staining and flow cytometric analysis. The percentage of apoptotic cells was significantly reduced in the FGF21-overexpressing cells treated with cisplatin

Correspondence to: Dr. Qian Zhong, Department of Gynecology and Obstetrics, West China Second University Hospital of Sichuan University, No.20 Section 3, Renmin South Road, Chengdu, Sichuan 610041, China
E-Mail: cdcd91761@163.com
Dr. Hanshuo Yang, State Key Laboratory of Biotherapy and Cancer Center of Sichuan University, Chengdu, Sichuan 610041, China
E-Mail: yhansh@126.com

Chinese Medical Journal 2022;135(12)
Received: 21-12-2021; **Online:** 21-07-2022 **Edited by:** Yanjie Yin

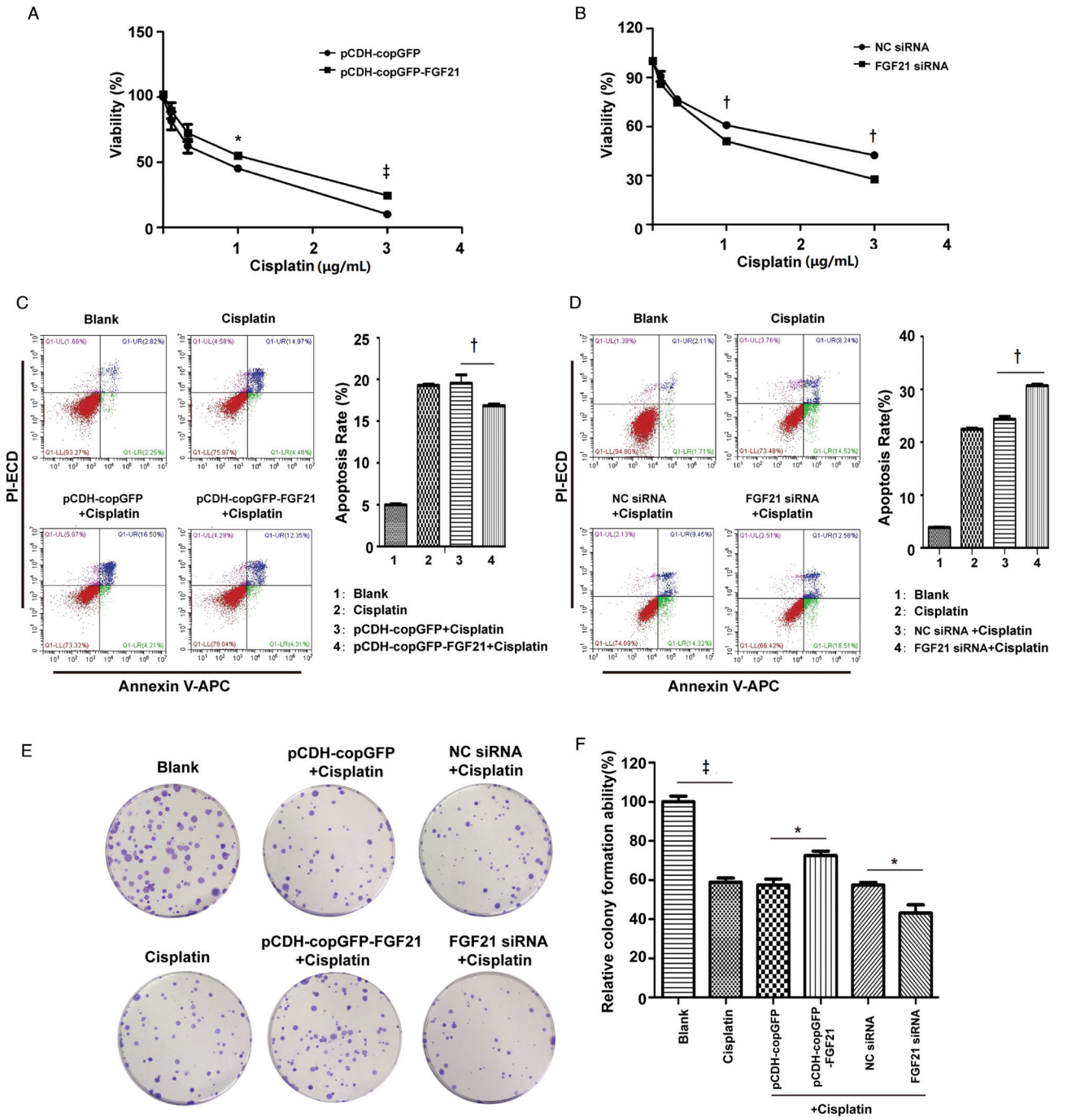

Figure 1: Effect of overexpression or downregulation of FGF21 on cisplatin sensitivity, cisplatin induced apoptosis, and growth ability of cisplatin-treated ovarian cancer cells. A2780 cells were transfected with pCDH-copGFP, pCDH-copGFP-FGF21, FGF21 siRNA, or NC siRNA, and 12 h later incubated with cisplatin for 2 days at different concentrations (0, 1, 2, 3, and 4 μ,g/mL). MTT assay was used to validate the changes in cisplatin sensitivity (A, B). A2780 cells were transfected with pCDH-copGFP, pCDH-copGFP-FGF21, FGF21 siRNA, or NC siRNA, and 12 h later incubated with cisplatin (1 μ.g/mL) for 2 days; apoptosis was examined by flow cytometry via Annexin/PI staining (C, D). A2780 cells were transfected with pCDH-copGFP, pCDH-copGFP-FGF21, FGF21 siRNA, or NC siRNA overnight and then incubated with 0.5 μ,g/mL cisplatin for 4 h, followed by cell culture for another 2 weeks. The colony formation assay showed that upregulation of FGF21 resulted in increased colony numbers in cisplatin-treated A2780 cells. FGF21 siRNA significantly inhibited the colony formation of cisplatin-treated A2780 cells (E, F). Results were mean ± SD of three independent experiments. $^{*}P < 0.05$. $^{\dagger}P < 0.01$. $^{\ddagger}P < 0.001$. FGF21: Fibroblast growth factor 21; NC: Negative control.

compared with cisplatin-treated control cells, $P < 0.05$ [Figure 1C], and increased in cells transfected with FGF21 siRNA and treated with cisplatin compared with cisplatin-treated control cells, $P < 0.05$ [Figure 1D].

We then evaluated whether FGF21 affected the growth ability of cisplatin-treated A2780 cells. Cells were transfected with FGF21-overexpressing, control plasmid, FGF21 siRNA, or NC siRNA overnight and then incubated with 0.5 μg/mL cisplatin for 4 h, followed by cell culture for another 2 weeks. Colony-formation assay indicated that the growth ability of cisplatin-treated A2780 cells was significantly improved by the upregulation of FGF21 and reduced by the downregulation of FGF21 [Figure 1E, F].

As a whole-body metabolic regulator, FGF21 has gained much attention in recent years. Many clinical trials have tested the effectiveness of long-acting FGF21 analogs in treating type 2 diabetes, obesity, and non-alcoholic fatty liver disease. However, it remains unknown whether longterm FGF21 exposure causes side effects in humans, such as promoting tumors or adversely impacting cancer chemotherapy. Circulating FGF21 was implicated in renal and colorectal cancer pathogenesis,[8,9] and serum FGF21 level was elevated in response to hepatocarcinogenesis.[10,11] An elevated level of FGF21 was also found in breast cancer.[12] However, little is known about the role of FGF21 in ovarian cancer, let alone the relationship between FGF21 and cisplatin resistance. In this study, we demonstrated that upregulated FGF21 reduced cisplatin sensitivity in ovarian cancer and decreased the apoptosis induced by cisplatin. The growth ability of cisplatin-treated ovarian cancer cells was also significantly improved by the upregulation of FGF21. Our data suggested that elevated FGF21 level may impair cisplatin sensitivity in ovarian cancer. Therefore, doctors should be aware of the potential side effects on cancer cells when using FGF21 analogs to treat diseases. This study also showed that downregulation of FGF21 increased cisplatin sensitivity, enhanced cisplatin-induced apoptosis, and decreased the growth ability of cisplatin-treated ovarian cancer cells. Our findings indicate that FGF21 may serve as a potent mediator for cisplatin sensitivity in ovarian cancer, and FGF21 targeted therapy combined with platinum compounds may have clinical implication in ovarian cancer therapy.

Our next step is to explore the clinical data on the correlation between FGF21 and cisplatin sensitivity and conduct preclinical experiments on the combined therapy of cisplatin and FGF21-targeted therapy in ovarian cancer.

Funding

This work was supported by grants from the Medical Association Foundation of Sichuan Province of China (No. S19061) and the Fundamental Research Funds for the Central Universities (No. SCU2020D4132).

References

1. Zhong Q, Hu Z, Li Q, Yi T, Li J, Yang H. Cyclin D1 silencing impairs DNA double strand break repair, sensitizes BRCA1 wildtype ovarian cancer cells to olaparib. Gynecol Oncol 2019;152:157–165. doi: 10.1016/j.ygyno.2018.10.027.
2. Liu D, Zhang XX, Li MC, Cao CH, Wan DY, Xi BX, *et al*. C/EBPbeta enhances platinum resistance of ovarian cancer cells by reprogramming H3K79 methylation. Nat Commun 2018;9:1739. doi: 10.1038/s41467-018-03590-5.
3. Kliewer SA, Mangelsdorf DJ. A dozen years of discovery: insights into the physiology and pharmacology of FGF21. Cell Metab 2019;29:246–253. doi: 10.1016/j.cmet.2019.01.004.
4. Geller S, Arribat Y, Netzahualcoyotzi C, Lagarrigue S, Carneiro L, Zhang L, *et al*. Tanycytes regulate lipid homeostasis by sensing free fatty acids and signaling to key hypothalamic neuronal populations via FGF21 secretion. Cell Metab 2019;30. 833-844.e837. doi: 10.1016/j.cmet.2019.08.004.
5. Degirolamo C, Sabba C, Moschetta A. Therapeutic potential of the endocrine fibroblast growth factors FGF19, FGF21 and FGF23. Nat Rev Drug Discov 2016;15:51–69. doi: 10.1038/nrd.2015.9.
6. Cornu M, Oppliger W, Albert V, Robitaille AM, Trapani F, Quagliata L, *et al*. Hepatic mTORC1 controls locomotor activity, body temperature, and lipid metabolism through FGF21. Proc Natl Acad Sci U S A 2014;111:11592–11599. doi: 10.1073/pnas.1412047111.
7. Zhang Y, Jiang K, Xie G, Ding J, Peng S, Liu X, *et al*. FGF21 impedes peripheral myelin development by stimulating p38 MAPK/c-Jun axis. J Cell Physiol 2021;236:1345–1361. doi: 10.1002/jcp.29942.
8. Knott ME, Minatta JN, Roulet L, Gueglio G, Pasik L, Ranuncolo SM, *et al*. Circulating fibroblast growth factor 21 (Fgf21) as diagnostic and prognostic biomarker in renal cancer. J Mol Biomark Diagn 2016;1 (Suppl 2):015. doi: 10.4172/2155-9929.S2-015.
9. Qian J, Tikk K, Weigl K, Balavarca Y, Brenner H. Fibroblast growth factor 21 as a circulating biomarker at various stages of colorectal carcinogenesis. Br J Cancer 2018;119:1374–1382. doi: 10.1038/s41416-018-0280-x.
10. Finn RS, Kudo M, Cheng AL, Wyrwicz L, Ngan RK, Blanc JF, *et al*. Pharmacodynamic biomarkers predictive of survival benefit with lenvatinib in unresectable hepatocellular carcinoma: from the phase 3 REFLECT Study. Clin Cancer Res 2021;27:4848–4858. doi: 10.1158/1078-0432.CCR-20-4219.
11. Yang C, Lu W, Lin T, You P, Ye M, Huang Y, *et al*. Activation of liver FGF21 in hepatocarcinogenesis and during hepatic stress. BMC Gastroenterol 2013;13:67. doi: 10.1186/1471-230X-13-67.
12. Akyol M, Alacacioglu A, Demir L, Kucukzeybek Y, Yildiz Y, Gumus Z, *et al*. The alterations of serum FGF-21 levels, metabolic and body composition in early breast cancer patients receiving adjuvant endocrine therapy. Cancer Biomark 2017;18:441–449. doi: 10.3233/CBM-161507.

How to cite this article: Zhong Q, wang L, Cheng M, Yang H. Fibroblast growth factor 21 is related to cisplatin resistance in ovarian cancer. Chin Med J 2022;135:1500–1502. doi: 10.1097/CM9.0000000000002095