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ORIGINAL ARTICLE

Fucoidan inhibits ovarian cancer growth *via* the miR-20a-5p/PKD2 axis

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Summary

Purpose: *To elucidate the influence of Fucoidan on changing ovarian cancer cell functions through the miR-20a-5p/PKD2 axis.*

Methods: *Ovarian cancer cell lines SKOV3 and CAOV3 were induced with DMSO (negative control), 20 ng/ml Fucoidan, 50 ng/ml Fucoidan, 100 ng/ml Fucoidan or 200 ng/ml Fucoidan for 24 h, followed by fresh medium replacement and cell culture for another 48 h. Proliferation inhibition rate was detected by CCK-8 assay. Subsequently, proliferative and migratory rates in SKOV3 and CAOV3 cells induced with 100 ng/ml Fucoidan for 24 h were determined by CCK-8, EdU and Transwell assay, respectively. Relative levels of miR-20a-5p and PKD2 in Fucoidan-induced cells were detected. Subsequently, regulatory effects of miR-20a-5p on phenotypes of Fucoidan-induced ovarian cancer cells were examined. The binding interaction between miR-20a-p and PKD2 was finally assessed by dual-luciferase reporter assay and rescue experiments.*

Results: *Fucoidan induction enhanced dose-dependently proliferation inhibition rate in SKOV3 and CAOV3 cells. It remarkably inhibited proliferative and migratory functions in ovarian cancer cells. Fucoidan induction upregulated miR-20a-5p and downregulated PKD2. The binding relationship between miR-20a-5p and PKD2 was ascertained. Overexpression of miR-20a-5p reduced proliferative and migratory functions in Fucoidan-induced ovarian cancer cells, which were abolished by overexpressed PKD2.*

Conclusions: *Fucoidan inhibits ovarian cancer cells to proliferate and migrate via mediating the miR-20a-5p/PKD2 axis.*

Key words: *fucoidan, miR-20a-5p, PKD2, ovarian cancer, proliferation, migration*

Introduction

Among all gynecologic malignancies, epithelial ovarian cancer has the highest mortality [1-3]. Over time the therapeutic strategies for ovarian cancer achieved great progress [3,4]. Tumor cytoreductive surgery combined postoperative paclitaxel and platinum-based chemotherapy is the standard treatment for ovarian cancer at present [4,5]. It effectively improves prognosis of initially diagnosed ovarian cancer cases. Nevertheless, advanced patients have a poor response to this standard treatment. The 5-year survival of stage III and IV ovarian cancer is only 29% and 13%, respectively, which is largely attributed to chemotherapy resistance and metastasis [5,6], so it is urgent to search more effective treatments for ovarian cancer [7-9].

Fucoidan is a water-soluble polysaccharide mainly containing the structure of L-fucose and sulfate groups [10,11]. Fucoidan can effectively inhibit the proliferative potential and angiogenesis of solid tumor cells [12,13]. As a potential translational medicine, Fucoidan is of significance in adjuvant cancer therapy [13,14]. However, how ovarian cancer cells develop drug resistance that escapes from chemotherapy effect remains unclear.

Accumulating miRNAs/miRs have been emerged that participate in human cancer process

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[15-18]. They are either oncogenes or tumor-suppressor genes involved in nearly every aspect of cancer cell function [19,20]. In the present study, we aimed to uncover the role of Fucoidan in inhibiting malignant phenotypes of ovarian cancer cells and the molecular mechanism.

Methods

Patients and ovarian cancer samples

Thirty ovarian cancer tissues and paired non-tumor ones were surgically resected. TNM staging was determined based on the 8th edition of UICC/AJCC guideline. Patients did not receive preoperative anti-cancer treatment. This study was approved by the research ethics committee of The First Hospital of Hangzhou Fuyang and complied with the Helsinki Declaration. Informed consent was obtained from subjects and their parents.

Cell lines and reagents

Ovarian cancer cell lines SKOV3 and CAOV3 were purchased from American Type Culture Collection (ATCC) (Manassas, VA, USA). They were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Gibco, Rockville, MD, USA) with 10% fetal bovine serum (FBS) (Gibco, Rockville, MD, USA) at 37°C, 5% CO_2.

Transfection

Transfection plasmids were synthesized by GenePharma (Shanghai, China). They were transfected in cells that were cultured to 30-50% density using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). At 48 h, cells were collected for testing transfection efficacy.

Cell counting kit-8 (CCK-8)

Cells were inoculated in 96-well plates with 2.5×10^3 cells/well. On days 1, 2, 3 and 4, optical density (OD) at 450 nm of each sample was recorded using the CCK-8 kit (Dojindo Laboratories, Kumamoto, Japan) for plotting the viability curves.

5-Ethynyl-2'-deoxyuridine (EdU) assay

Cells were induced with 50 μM EdU (RiboBio, Guangzhou, China) for 2 h, and dyed using AdoLo and 4',6-diamidino-2-phenylindole (DAPI) in the dark. EdU-labeled cells were captured for calculating the percentage of EdU incorporation.

Transwell migration assay

200 μl of suspension containing 4×10^4 SKOV3 cells or 2×10^4 CAOV3 cells were applied on the top of a Transwell insert, which was placed in each well containing 500 μl of medium and 10% FBS. Cells were allowed to migrate for 48 h, and they were fixed, dyed and captured for counting in five random fields per sample.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Samples were processed for isolating RNAs using TRIzol method (Invitrogen, Carlsbad, CA, USA). After purification of cellular RNAs, they were reversely transcribed to complementary DNAs (cDNAs) and subjected to qRT-PCR using SYBR®Premix Ex Taq™ (TaKaRa, Dalian, China). Relative levels of PCR products were calculated by $2^{-\Delta\Delta Ct}$ and normalized to that of glyceraldheyde 3-phosphate dehydrogenase (GAPDH) or U6. Primers sequences were as follows: MiR-20a-5p: Forward: 5'-TAAAGTGCTTATAGTGCAGGTAG-3', Reverse: 5'-GTGGCAAAATGGCGGACTTT-3'; U6: Forward: 5'-TCCGGGTGATGCTTTTCCTAG-3', Reverse: 5'-CGCTTCACGAATTTGCGTGTCAT-3'; PKD2: Forward: 5'-GGCTTGGGGGCTACCAC-3', Reverse: 5'-CTTGTTCCCCAGAGACCTCG-3'; GAPDH: Forward: 5'-CGCTCTCTGCTCCTCCTGTTC-3', Reverse: 5'-ATCCGTTGACTCCGACCTTCAC-3'.

Western blot

Radioimmunoprecipitation assay (RIPA) (Beyotime, Shanghai, China) was used to lyse cells on ice, and the lysate was subjected to 14000×g centrifugation at 4°C. Fifteen min later, the precipitant was resuspended for measuring protein concentration. Prepared protein samples were loaded on sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and polyvinylidene fluoride (PVDF) membranes (Millipore, Billerica, MA, USA), which were blocked for non-specific antigens in 5% skim milk for 2 h. Later, membranes were immunoblotted with primary and secondary antibodies (Cell Signaling Technology, Danvers, MA, USA) for grey value analysis.

Dual-luciferase reporter assay

HEK293T cells were seeded in 24-well plates, and co-transfected with NC/miR-20a-5p mimic and PKD2-WT/PKD2-MUT, respectively using Lipofectamine 2000. Luciferase activity was measured at 48 h in lysed cell mixture using the commercial kit (Promega Corp., Madison, WI, USA).

Statistics

SPSS 22.0 software package (IBM, Armonk, NY, USA) was used for statistical analyses. Data were expressed as mean±standard deviation. Differences between groups were compared by the t-test. Correlation between expression levels of two genes was assessed by Pearson correlation test. A significant difference was set at $p<0.05$.

Results

Fucoidan inhibited proliferative and migratory potentials of ovarian cancer

SKOV3 and CAOV3 cells were induced with DMSO (negative control), 20 ng/ml Fucoidan, 50 ng/ml Fucoidan, 100 ng/ml Fucoidan or 200 ng/ml Fucoidan for 24 h. Proliferation inhibition rate was dose-dependently declined, which achieved the point of inflection at 100 ng/ml (Figure 1A). In the following experiments, SKOV3 and CAOV3 were pre-induced with 100 ng/ml Fucoidan for 24 h. Cell viability was much lower in Fucoidan-induced ovarian cancer cells than those of controls (Figure 1B). EdU incorporation was reduced by Fucoidan induction as well (Figure 1C). Transwell assay results

showed that Fucoidan induction suppressed the migratory potential of ovarian cancer (Figure 1D).

Fucoidan regulated miR-20a-5p/PKD2 axis in ovarian cancer

QRT-PCR data showed that Fucoidan induction upregulated mRNA level of miR-20a-5p and downregulated protein level of PKD2 in SKOV3 and CAOV3 cells (Figure 2A, 2B). Compared with non-tumor tissues, downregulated miR-20a-3p and upregulated PKD2 were detected in ovarian cancer tissues (Figure 2C, 2D). Based on the predicted binding site in PKD2 3'UTR that paired to miR-20a-3p promoter sequence, dual-luciferase reporter assay verified their binding relationship (Figure 2E, 2F). In addition, a negative correlation was discovered between expression levels of miR-20a-3p and PKD2 in ovarian cancer tissues (Figure 2G).

MiR-20a-5p abolished Fucoidan-induced inhibited proliferative and migratory potentials of ovarian cancer

Transfection efficacy of miR-20a-5p mimic was tested by qRT-PCR (Figure 3A). Overexpression of miR-20a-5p reduced the viability in ovarian cancer cells either induced with DMSO or Fucoidan (Figure 3B). In Fucoidan-induced SKOV3 and CAOV3 cells, overexpression of miR-20a-5p markedly downregulated the protein level of PKD2 (Figure 3C). Moreo-

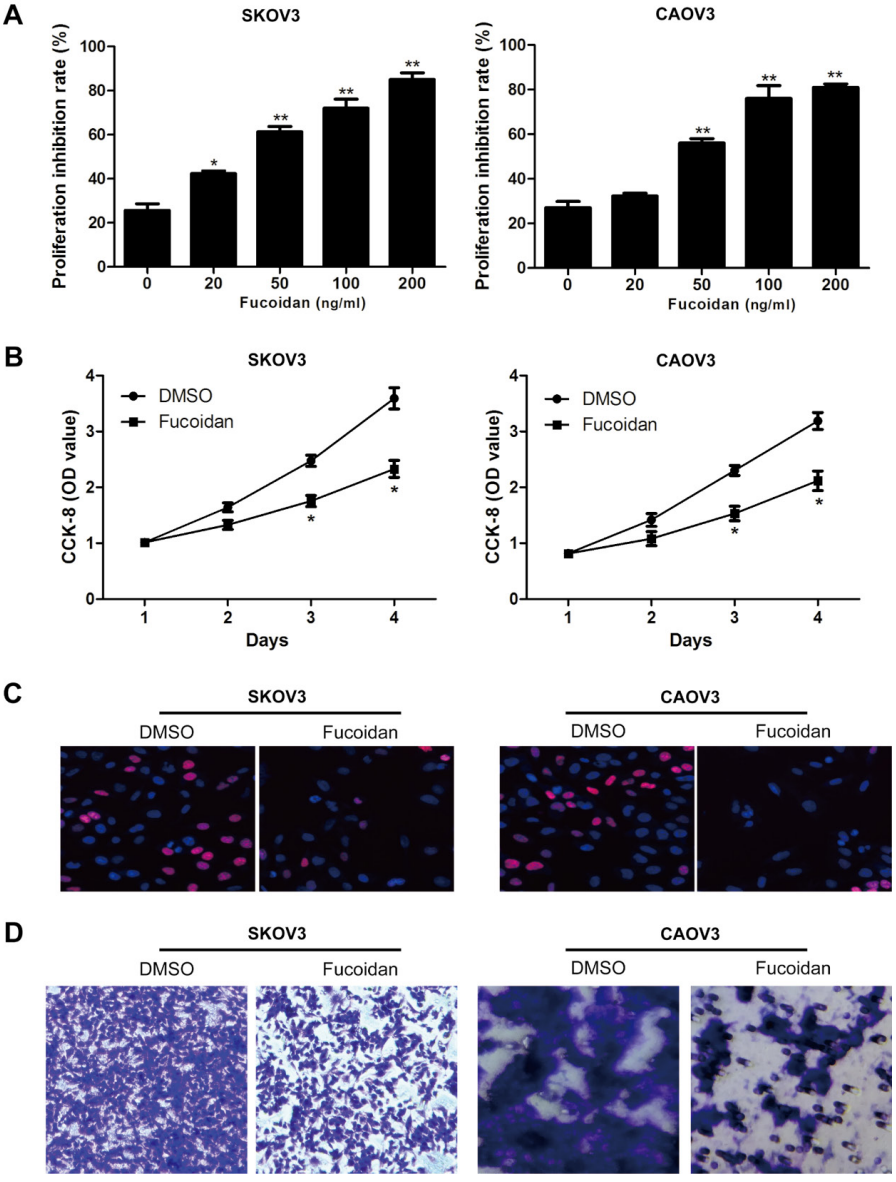

Figure 1. Fucoidan inhibited the proliferative and migratory potentials of ovarian cancer. **A:** Proliferation inhibition rate in SKOV3 and CAOV3 cells induced with DMSO, 20 ng/ml, 50 ng/ml, 100 ng/ml or 200 ng/ml Fucoidan for 24 h. **B:** Cell viability in SKOV3 and CAOV3 cells induced with 100 ng/ml Fucoidan or DMSO. **C:** EdU incorporation in SKOV3 and CAOV3 cells induced with 100 ng/ml Fucoidan or DMSO (40×). **D:** Migration in SKOV3 and CAOV3 cells induced with 100 ng/ml Fucoidan or DMSO (40×). *p<0.05, **p<0.01.

ver, viability and EdU incorporation were both declined (Figure 3D, 3E). Transfection of miR-20a-5p mimic also reduced the migratory cell number in Fucoidan-induced ovarian cancer cells (Figure 3F).

Overexpression of PKD2 abolished the role of miR-20a-5p in regulating Fucoidan-induced ovarian cancer cell functions

To further explore the co-regulation of miR-20a-5p and PKD2 in Fucoidan-induced ovarian cancer cells, cells were co-transfected with miR-20a-5p mimic and pcDNA-PKD2. Transfection efficacy of pcDNA-PKD2 was tested by Western blot (Figure 4A). Compared with Fucoidan-induced ovarian cancer cells only overexpressing miR-20a-5p, those co-overexpressing miR-20a-5p and PKD2 had higher viability, EdU incorporation and migratory cell number (Figure 4B-4D).

Discussion

Ovarian cancer is a common malignancy of the female reproductive organs. Its morbidity and mortality are on the rise, with an annual growth

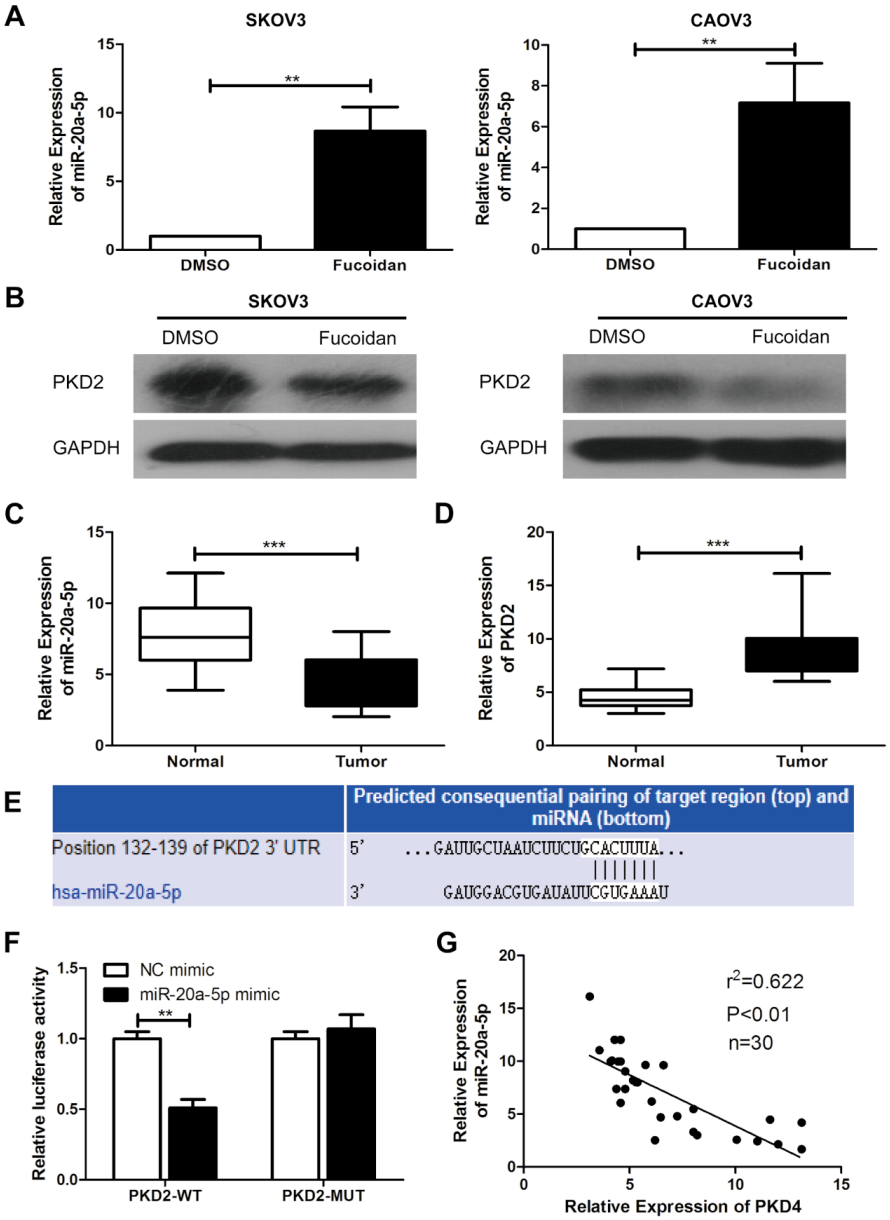

Figure 2. Fucoidan regulated the miR-20a-5p/PKD2 axis in ovarian cancer. **A:** MiR-20a-5p level in SKOV3 and CAOV3 cells induced with 100 ng/ml Fucoidan or DMSO. **B:** Protein level of PKD2 in SKOV3 and CAOV3 cells induced with 100 ng/ml Fucoidan or DMSO. **C:** MiR-20a-5p level in ovarian cancer tissues and normal ones. **D:** PKD2 level in ovarian cancer tissues and normal ones. E: Predicted binding sites in 3'UTR of miR-20a-5p and PKD2. **F:** Luciferase activity in PKD2 vectors regulated by miR-20a-5p. **G:** A negative correlation between expression levels of PKD2 and miR-20a-5p in ovarian cancer tissues. **p<0.01, ***p<0.001.

rate of 1.8% [1-3]. About 75% of ovarian cancer patients are diagnosed in advanced stage at the time of diagnosis. They are usually comprehensively treated by surgery and postoperative chemotherapy [4-7]. Clinical outcomes of ovarian cancer treatment are not ideal. Hence, great efforts should be made on analyzing the pathogenesis of ovarian cancer and developing first-line chemotherapy drugs [7,8]. It is well known that cancer development is closely linked to genetic mutations of many types of cells, and the tumor interstitial microenvironment [7-9].

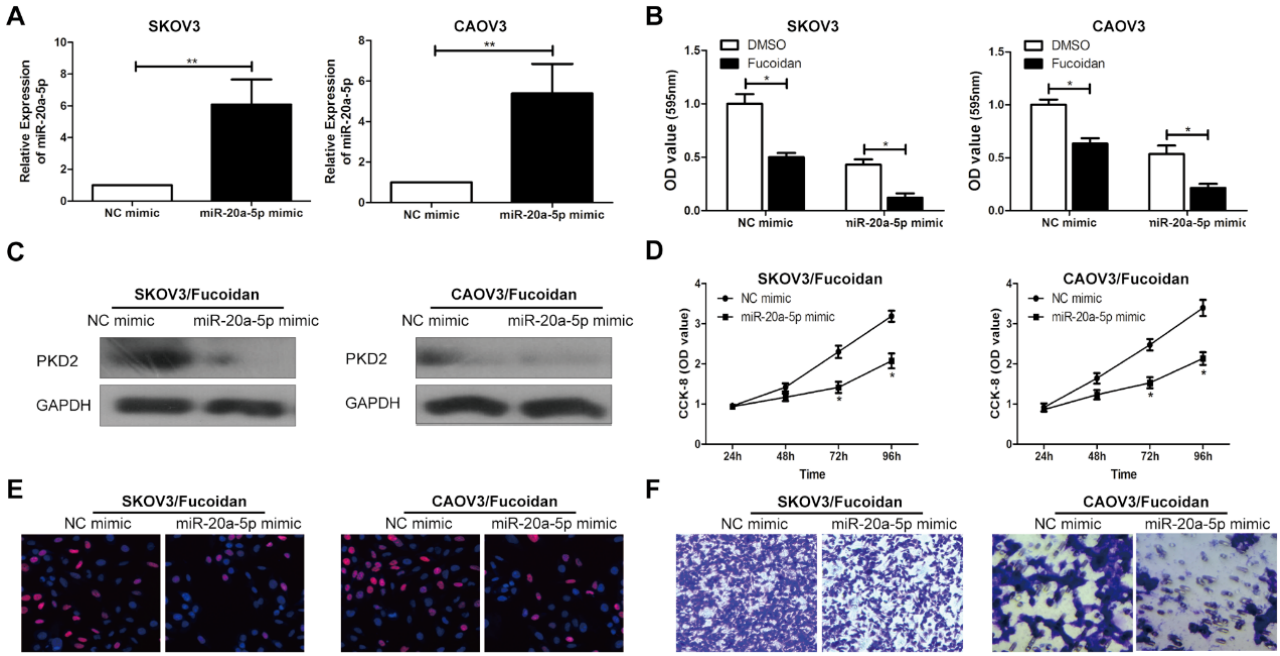

Figure 3. MiR-20a-5p abolished Fucoidan-induced inhibited proliferative and migratory potentials of ovarian cancer. **A:** Transfection efficacy of miR-20a-5p mimic in SKOV3 and CAOV3 cells. **B:** Viability in DMSO or Fucoidan-induced SKOV3 and CAOV3 cells overexpressing miR-20a-5p or not. **C:** Protein level of PKD2 in Fucoidan-induced SKOV3 and CAOV3 cells overexpressing miR-20a-5p or not. **D:** Viability in Fucoidan-induced SKOV3 and CAOV3 cells overexpressing miR-20a-5p or not. **E:** EdU incorporation in Fucoidan-induced SKOV3 and CAOV3 cells overexpressing miR-20a-5p or not (40×). **F:** Migration in Fucoidan-induced SKOV3 and CAOV3 cells overexpressing miR-20a-5p or not (40×). *p<0.05, **p<0.01.

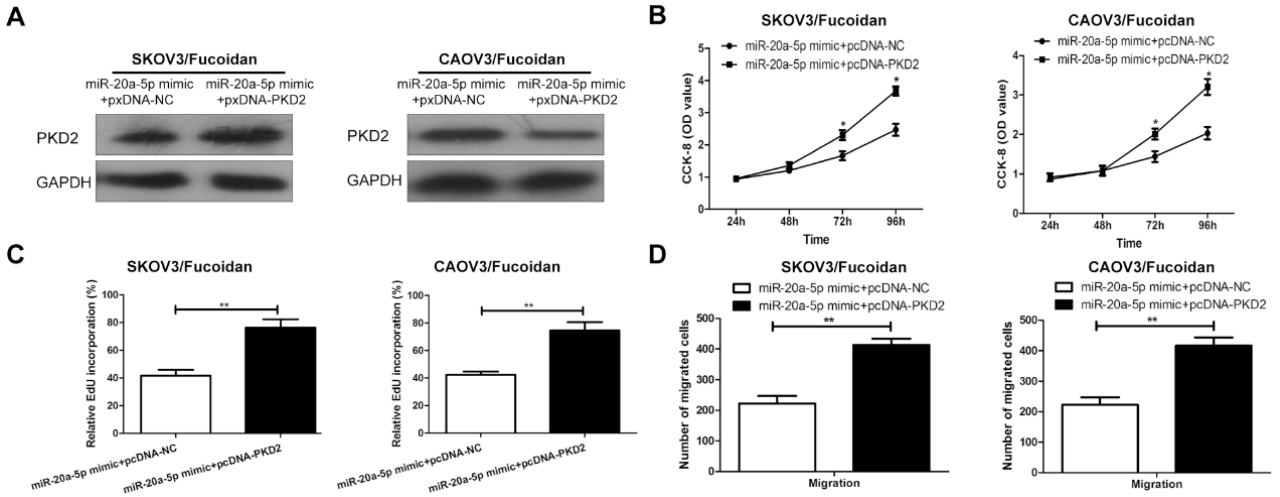

Figure 4. Overexpression of PKD2 abolished the role of miR-20a-5p in regulating Fucoidan-induced ovarian cancer cell functions. **A:** Transfection efficacy of pcDNA-PKD2. **B:** Viability in Fucoidan-induced SKOV3 and CAOV3 cells overexpressing miR-20a-5p or co-overexpressing miR-20a-5p and PKD2. **C:** EdU incorporation in Fucoidan-induced SKOV3 and CAOV3 cells overexpressing miR-20a-5p or co-overexpressing miR-20a-5p and PKD2. **D:** Migration in Fucoidan-induced SKOV3 and CAOV3 cells overexpressing miR-20a-5p or co-overexpressing miR-20a-5p and PKD2. *p<0.05, **p<0.01.

Fucoidan is an intercellular polysaccharide inherent in brown algae, which is distributed in cell wall matrix. Fucoidan is necessary for brown algae to absorb light and nutrients. The content of Fucoidan in kelp is about 0.3%-1.5% [10-12]. Fucoidan is a functional substance for inducing cancer cell apoptosis without toxic side effects on normal cells [13]. However, the detailed mechanism underlying the anti-cancer role of Fucoidan is inconsistent and unclear [13,14]. This study aimed to explore the inhibitory effect of Fucoidan on ovarian cancer development. Our study first uncovered that Fucoidan induction enhanced dose-dependently the proliferation inhibition rate in SKOV3 and CAOV3 cells. It also attenuated the proliferative and migratory potentials of ovarian cancer cells.

As a vital component of gene regulatory network, miRs are of significance in cancer development and progression [16-18]. In ovarian cancer cells, Fucoidan induction upregulated miR-20a-5p. Moreover, overexpression of miR-20a-5p remarkably inhibited the stimulated proliferative and migratory potentials in Fucoidan-induced ovarian cancer cells.

MiRs exert their biological functions through post-transcriptionally regulating gene expressions in a manner of complementary base pairing to target mRNAs [21,22]. In human genomes, there are thousands of functional miRs that are capable of regulating nearly 33% of protein-encoding genes [23-26]. A binding site in PKD2 3'UTR was predicted using online software, which was within miR-20a-3p 3'UTR. PKD2 was downregulated by Fucoidan induction in ovarian cancer cells, which had a negative correlation to miR-20a-3p level. Interestingly, overexpression of PKD2 could partially abolish the regulatory effects of miR-20a-5p on the proliferative and migratory potentials in Fucoidan-induced ovarian cancer cells. Taken together, the anti-cancer role of Fucoidan in ovarian cancer could be mediated by the miR-20a-5p/PKD2 axis. In our future analysis, how Fucoidan inhibits miR-20a-5p level and thus exerts its anti-cancer role will be explored.

Conclusions

Fucoidan inhibits ovarian cancer cells to proliferate and migrate *via* mediating the miR-20a-5p/PKD2 axis.

Conflict of interests

The authors declare no conflict of interests.

References

1. Lheureux S, Gourley C, Vergote I, Oza AM. Epithelial ovarian cancer. Lancet 2019;393:1240-53.
2. Lheureux S, Braunstein M, Oza AM. Epithelial ovarian cancer: Evolution of management in the era of precision medicine. CA Cancer J Clin 2019;69:280-304.
3. Antonijevic A, Rancic N, Ilic M, Tiodorovic B, Stojanovic M, Stevanovic J. Incidence and mortality trends of ovarian cancer in central Serbia. JBUON 2017;22:508-12.
4. Corrado G, Salutari V, Palluzzi E, Distefano MG, Scambia G, Ferrandina G. Optimizing treatment in recurrent epithelial ovarian cancer. Expert Rev Anticancer Ther 2017;17:1147-58.
5. Orr B, Edwards RP. Diagnosis and Treatment of Ovarian Cancer. Hematol Oncol Clin North Am 2018;32: 943-64.
6. Reavis HD, Drapkin R. The tubal epigenome - An emerging target for ovarian cancer. Pharmacol Ther 2020;210:107524.
7. Trinidad CV, Tetlow AL, Bantis LE, Godwin AK. Reducing Ovarian Cancer Mortality Through Early Detection: Approaches Using Circulating Biomarkers. Cancer Prev Res (Phila) 2020;13:241-52.
8. Kubelac P, Vlad C, Berindan NI, Irimie A, Achimas CP. The clinical features associated with mutated BRCA1 and 2 genes in ovarian cancer patients. JBUON 2019;24:1538-43.
9. Pignata S, Pisano C, Di Napoli M, Cecere SC, Tambaro R, Attademo L. Treatment of recurrent epithelial ovarian cancer. Cancer 2019;125 (Suppl 24):4609-15.
10. Fitton HJ, Stringer DS, Park AY, Karpiniec SN. Therapies from Fucoidan: New Developments. Mar Drugs 2019;17:571.
11. Wang Y, Xing M, Cao Q, Ji A, Liang H, Song S. Biological Activities of Fucoidan and the Factors Mediating Its Therapeutic Effects: A Review of Recent Studies. Mar Drugs 2019;17:183.
12. van Weelden G, Bobinski M, Okla K, van Weelden WJ, Romano A, Pijnenborg J. Fucoidan Structure and Activity in Relation to Anti-Cancer Mechanisms. Mar Drugs 2019;17:32.
13. Zayed A, Ulber R. Fucoidans: Downstream Processes and Recent Applications. Mar Drugs 2020;18:170.
14. Hsu HY, Hwang PA. Clinical applications of fucoidan in translational medicine for adjuvant cancer therapy. Clin Transl Med 2019;8:15.
15. Hrovatin K, Kunej T. Classification of miRNA-relat-

ed sequence variations. Epigenomics-Uk 2018;10: 463-81.

16. Roy B, Yoshino Y, Allen L, Prall K, Schell G, Dwivedi Y. Exploiting Circulating MicroRNAs as Biomarkers in Psychiatric Disorders. Mol Diagn Ther 2020;24:279-98.
17. Tutar Y. miRNA and cancer; computational and experimental approaches. Curr Pharm Biotechnol 2014;15:429.
18. Sun Z, Shi K, Yang S et al. Effect of exosomal miRNA on cancer biology and clinical applications. Mol Cancer 2018;17:147.
19. Labbe M, Hoey C, Ray J et al. microRNAs identified in prostate cancer: Correlative studies on response to ionizing radiation. Mol Cancer 2020;19:63.
20. SiamiGorji S, Jorjani I, Tahamtan A, Moradi A. Effects of microRNAs polymorphism in cancer progression. Med J Islam Repub Iran 2020;34:3.
21. Ha M, Kim VN. Regulation of microRNA biogenesis. Nat Rev Mol Cell Biol 2014;15:509-24.
22. Tang X, Deng R, Sun Y, Ren X, Zhou M, Li J. Amplified Tandem Spinach-Based Aptamer Transcription Enables Low Background miRNA Detection. Anal Chem 2018;90:10001-8.
23. Vaschetto LM. miRNA activation is an endogenous gene expression pathway. Rna Biol 2018;15:826-8.
24. Younger ST, Corey DR. Identification and validation of miRNA target sites within nontraditional miRNA targets. Methods Mol Biol 2015;1206:53-67.
25. Mellis D, Caporali A. MicroRNA-based therapeutics in cardiovascular disease: screening and delivery to the target. Biochem Soc Trans 2018;46:11-21.
26. Schafer M, Ciaudo C. Prediction of the miRNA interactome - Established methods and upcoming perspectives. Comput Struct Biotechnol J 2020;18:548-57.