miR-3188 Regulates proliferation and apoptosis of granulosa cells by targeting KCNA5 in the polycystic ovary syndrome

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Introduction

Abnormal proliferation of granulosa cells is implicated in ovarian dysfunction and dysregulated folliculogenesis in the polycystic ovary syndrome (PCOS). Aberrant microRNA (miRNA) expression might contribute to disordered folliculogenesis and granulosa cell proliferation in PCOS. This study aimed to investigate the roles of miR-3188 in ovarian dysfunction, as well as the mechanism involved in granulosa cell proliferation in PCOS. Firstly, peripheral blood samples were isolated from PCOS patients and healthy controls, and qRT-PCR analysis demonstrated a dramatic increase in miR-3188 in PCOS patients when compared to the healthy controls. Secondly, miR-3188 overexpression increased cell viability of the granulosa-like tumor cell line (KGN). However, cell viability of KGN was repressed by interference with miR-3188. MiR-3188 promoted cell cycle of KGN through increasing cyclinD1 and decreasing p21 levels. Moreover, cell apoptosis was suppressed by miR-3188 in KGN, indicated by enhanced Bcl-2, and reduced Bax and cleaved caspase-3 levels, whereas knockdown of miR-3188 resulted in opposite effects. Lastly, potassium voltage-gated channel subfamily A member 5 (KCNA5) was verified as a target of miR-3188. KCNA5 expression was decreased and displayed negative correlation with miR-3188 levels in PCOS patients. Overexpression of KCNA5 attenuated the promotive effects of miR-3188 on cell viability and cell cycle in KGN. In conclusion, miR-3188, a key miRNA enhanced in PCOS, promoted granulosa cell proliferation through down-regulation of KCNA5, providing a new therapeutic target for PCOS.

Keywords: miR-3188, KCNA5, proliferation, granulosa cell, PCOS

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Abbreviations: KGN, granulosa-like tumor cell line; KCNA5, potassium voltage-gated channel subfamily A member 5; PCOS, polycystic ovary syndrome

MATERIALS AND METHODS

Patient samples

This study was approved by the Ethics Committee of the Hwa Mei Hospital, University of Chinese Academy of Sciences. A total of 28 PCOS patients and 36 healthy controls with written informed consents were recruited from the Hwa Mei Hospital, University of Chinese Academy of Sciences. Peripheral blood samples were collected from the patients and controls into tubes (Pre-
Enhanced miR-3188 level in PCOS increases granulosa cell viability

To determine the expression of miR-3188 in PCOS, blood samples were collected from PCOS patients and healthy controls. qRT-PCR analysis demonstrated a significant increase in miR-3188 level in PCOS patients when compared to the controls (p<0.01) (Fig. 1A). In order to establish the functional roles of miR-3188 in PCOS in vitro, KGN was transfected with miR-3188 mimic or inhibitor (Fig. 1B). The results of the MTT assay showed that miR-3188 overexpression increased cell viability of KGN, while miR-3188 knockdown led to opposite effects (Fig. 1C), suggesting the pro-proliferative role of miR-3188 in PCOS granulosa cells.

Effect of miR-3188 on KGN cell cycle

Cell cycles of KGN transfected with miR-3188 mimic or inhibitor were evaluated to assess the role of miR-3188 in PCOS progression. Results demonstrated that miR-3188 promoted cell cycle of KGN, with a decreased cell number in the G1 phase and increased cell number in the G2 and S phases (Fig. 2A). In contrast, in KGN cells transfected with miR-3188 inhibitor, the KGN cell number in the G1 phase was increased, whereas there was a lower number of cells in the G2 and S phase (Fig. 2A). This indicates that miR-3188 knockdown leads to cell cycle arrest in KGN. The levels of cell cycle proteins were also assessed by western blotting, which revealed that miR-3188 overexpression led to increased levels of cyclinD1 and reduced p21 to promote cell cycle progression (Fig. 2B). On the contrary, cyclin D1 level was reduced and that of p21 was increased in KGN transfected with miR-3188 inhibitor, consistent with cell cycle arrest (Fig. 2B). These results confirmed the pro-proliferative role of miR-3188 in PCOS granulosa cells.

Effect of miR-3188 on KGN cell apoptosis

Next, the effect of miR-3188 on cell apoptosis was investigated in KGN, and the result indicated that miR-3188 suppressed cell apoptosis of KGN (Fig. 3A). Moreover, there was an increase in the number of apoptotic cells in KGN transfected with miR-3188 inhibitor (Fig. 3A), demonstrating the anti-apoptotic
The levels of apoptotic proteins were also evaluated by western blotting. Results revealed that miR-3188 overexpression enhanced protein expression of Bcl-2, but reduced the levels of Bax and cleaved caspase-3 (Fig. 3B). In contrast, Bax and cleaved caspase-3 levels were enhanced, and Bcl-2 level was reduced in KGN transfected with miR-3188 inhibitor (Fig. 3B). These results confirmed the anti-apoptotic role of miR-3188 in PCOS granulosa cells.

miR-3188 negatively regulates KCNA5 in PCOS

In order to uncover the underlying mechanisms of miR-3188 in the pathogenesis of PCOS, prediction of potential target genes of miR-3188 was performed using TargetScan (http://www.targetscan.org/vert_72/), followed by subsequent validation by luciferase reporter assays. The predicted binding sites between miR-3188 and KCNA5, as well as mutated KCNA5 binding site, which disrupted the binding ability of KCNA5 with miR-3188 are shown in Fig. 4A. Results from the lucif-
erase activity assays showed that transfection with miR-3188 inhibitor increased luciferase activities of KCNA5 wt compared to NC inhibitor (Fig. 4B), while KCNA5 mut demonstrated no response to miR-3188 inhibitor or NC inhibitor (Fig. 4B). Furthermore, the protein expression of KCNA5 was increased in KGN transfected with miR-3188 inhibitor, while it was decreased by miR-3188 mimic (Fig. 4C), confirming that KCNA5 was regulated
miR-3188/KCNA5 in polycystic ovary syndrome

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by miR-3188 in granulosa cells. A significant decrease in KCNA5 was found in PCOS patients when compared to controls (p<0.01) (Fig. 4D), and the expression of KCNA5 and miR-3188 revealed a negative correlation in PCOS patients (Fig. 4D). These results demonstrated that miR-3188 negatively regulates KCNA5 in PCOS.

Over-expression of KCNA5 counteracts the promotive effects of miR-3188 on PCOS progression

To explore the role of miR-3188/KCNA5 axis in PCOS granulosa cells, KGN was cotransfected with pcDNA-KCNA5 and miR-3188 mimic. KCNA5 overexpression attenuated miR-3188 mimic-mediated cell viability increase (Fig. 5A). Of note, miR-3188 mimic transfection failed to decrease the protein expression of KCNA5 in KCNA5-overexpressed cells (Fig. 5B). In addition, KCNA5 overexpression also lessened the miR-3188 overexpression-induced increase in cyclin D1 and Bcl-2 levels, and decrease in p21, Bax and cleaved caspase levels in KGN (Fig. 5B), further suggesting that miR-3188 could promote cell viability of granulosa cells in PCOS through down-regulation of KCNA5.

DISCUSSION

PCOS, characterized by such inconvenient symptoms as irregular menstrual bleeding and infertility, has been regarded as a “nuisance disease” devoid of effective therapeutic strategies (Conway et al., 2014; Peng et al., 2020). Inhibition of granulosa cell survival provides a novel insight into PCOS treatment (Zhong et al., 2018; Cox et al., 2020). Considering the important regulatory role of miRNAs in granulosa cell functions, miRNAs have been regarded as critical mediators in physiological and pathological conditions of PCOS (Tu et al., 2019). To date, miR-3188 has been shown as either suppressor or promoter in various tumors to regulate cell proliferation and apoptosis. The study presented here was performed to uncover the role of miR-3188 in granulosa cell proliferation and apoptosis in PCOS.

Biochemical markers, such as the luteinizing hormone or follicle-stimulating hormone levels, circulating androgens and hyperinsulinemia, are diagnostic criteria for PCOS (Barthelmess & Naz 2014; EBERSOLE & BONY 2020). MiRNAs are also considered as diagnostic or prognostic biomarkers for PCOS (Wang et al., 2019). Here, in line with previous reports (Hou et al., 2019; Wang et al., 2019), miR-3188 was found to be increased in the peripheral blood samples of PCOS patients when compared to the healthy controls. However, to further validate the potential of miR-3188 as a diagnostic or prognostic marker for PCOS, the correlation between clinical features of PCOS patients and miR-3188 levels should be investigated in a future study.

Aberrant profiling of miRNAs that participated in steroidogenesis or the cell apoptosis process was detected in follicular fluid or granulosa cells in PCOS patients (Sorensen et al., 2014; Lionett et al., 2020). MiR-93, which has the ability to promote granulosa cell
proliferation, could be a therapeutic target for PCOS (Jiang et al., 2015). Here, based on MTT, flow cytometry and western blotting analyses, our findings demonstrated that miR-3188 could increase granulosa cell viability, promote cell cycle progression through increasing cyclin D1 and decreasing p21 levels, and suppress cell apoptosis via promoting expression of Bcl-2 and lowering Bax and cleaved caspase-3 levels. Collectively, our results demonstrated that miR-3188 is a novel key miRNA that promotes granulosa cell proliferation in PCOS. This is in contrast to previous finding, which proposed that miR-3188 suppresses cell proliferation and cell cycle progression in non-small cell lung cancer cells through targeting mammalian target of rapamycin (Wang et al., 2018). This prompted the identification of cell type-specific downstream target of miR-3188 in PCOS granulosa cells.

Cation channels have been implicated in the apoptosis of granulosa cells, indicating their role in the etiology of PCOS (Köse & Nazıroğlu 2015). Antagonists of potassium channel regulate granulosa cell proliferation and apoptosis (Manikkam et al., 2002; Traut et al., 2009). Previous study has shown that KCNA in granulosa cells binds to an antagonist, 4-aminopyridine, and decreases progesterone production through the steroidogenic pathway (Li et al., 2003). Here, potassium channel KCNA5 level was found to be reduced in granulosa cells of PCOS, and was validated to be the downstream target gene of miR-3188. Gao et al. (Gao et al., 2020) has reported that miR-3188 levels in PCOS patients increased miR-3188 levels in PCOS patients were found to be reduced in granulosa cells of PCOS, and was validated to be the downstream target gene of miR-3188. Gao et al. (Gao et al., 2020) has reported that miR-3188 levels in PCOS patients increased miR-3188 levels in PCOS patients.

In conclusion, as shown in Fig. 6, our results indicated that increased miR-3188 levels in PCOS patients could promote cell viability and cell cycle progression, and suppress cell apoptosis through down-regulation of KCNA5. These observations provide novel insights into the potential development of miR-3188 as a therapeutic approach for the treatment of PCOS.

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Competing interests

The authors state that there are no conflicts of interest to disclose.

Ethics approval

Ethical approval was obtained from the Ethics Committee of the Hwa Mei Hospital, University of Chinese Academy of Sciences.

Statement of Informed Consent

Written informed consent was obtained from a legally authorized representative(s) for anonymized patient information to be published in this article.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors’ contributions

Shan Zhou and Liang Xia designed the study, supervised the data collection, analyzed the data, Yuanyuan Chen interpreted the data and prepared the manuscript for publication, Weiying Guo and Jinxing Hu supervised the data collection, analyzed the data and reviewed the draft of the manuscript. All authors have read and approved the manuscript.

REFERENCES


