ORIGINAL ARTICLE

Protective effects of lidocaine on polycystic ovary syndrome through modulating ovarian granulosa cell physiology via PI3K/AKT/mTOR pathway

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Abstract Polycystic ovary syndrome (PCOS) is a common endocrine condition in women that causes adverse reproductive and metabolic effects. PCOS is a heterogeneous disorder and its pathogenesis is affected by different factors. Thus, the criteria for diagnosing PCOS, disease and availability of treatment options vary widely across different countries. Lidocaine has been proven to inhibit the proliferation of a variety of cancer cell types, and can be used alone or in combination with other drugs for the treatment of numerous types of disease. The present study aimed to determine whether lidocaine was able to reduce human ovarian granulosa cell tumor cell line KGN cell proliferation and provide a novel insight into potential therapeutic strategies for PCOS. KGN cells were treated alone with lidocaine at different concentrations, or with lidocaine and insulin-like growth factor-1 (IGF-1; a phosphoinositide 3-kinase (PI3K)/Protein kinase B (AKT) signaling pathway agonist) in combination for 48 h. The proliferative ability of KGN cells was detected using an 3-(45)-dimethylthiahiazo (-z-y1)-35-di- phenytetrazoliumromide (MTT) assay, and cell apoptosis was detected using flow cytometry. The expression levels of proteins and mRNAs were measured using western blotting and reverse transcription-quantitative polymerase chain reaction (RT-qPCR), respectively. The results of the present study revealed that lidocaine significantly suppressed KGN cell proliferation and increased apoptosis. Lidocaine significantly downregulated the protein expression levels of phosphorylated (p)-AKT and p-mTOR, but had no effect on their transcriptional levels. Treatment with IGF-1, could reverse the lidocaine-induced abnormal expression of PI3K/AKT signaling pathway-related proteins. Moreover, treatment with IGF-1 could reverse all the effects of lidocaine on KGN cells. In conclusion, the findings of the present study indicated that lidocaine may inhibit KGN cell proliferation and induce apoptosis by inhibiting the activation of the PI3K/AKT/mTOR signaling pathway. These results revealed the potential inhibitory effect of lidocaine on the proliferation of KGN cells and its underlying mechanism of action, providing a novel insight into potential therapeutic strategies for PCOS.

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Introduction

Currently, drug repositioning, which is the process of repurposing old drugs, has become a practical strategy for the development of new drugs (Jourdan et al. 2020). Lidocaine is a local anesthetic commonly used in the clinic (Klein and Jeske 2016). Accumulating evidence has shown that lidocaine exerts numerous other effects, in addition to its anesthetic potential (Slaton et al. 2013; Truesdale and Jurdi 2013; Beaussier et al. 2018; Weibel et al. 2018). In fact, lidocaine has been reported to enhance the therapeutic effect of some drugs; for example, (Hong et al. 2019) demonstrated that the combined formulation of tetrodotoxin and lidocaine exerted a greater anti-arrhythmic effect compared with tetrodotoxin treatment alone. In addition, in BIU-87 human bladder cancer cells, lidocaine significantly enhanced the inhibitory effect of the antitumor drugs, Mitomycin C and pirarubicin, on tumor cell proliferation (Yang et al. 2018). Lidocaine treatment also increased the cisplatin-induced apoptosis and cytotoxicity in breast cancer cell lines (Li et al. 2014a). A large number of studies have reported that lidocaine exerted anti-tumor effects (Jurj et al. 2017; Izdebska et al. 2019; Ye et al. 2019; Zhao et al. 2021; Liu et al. 2021). Moreover, lidocaine was found to affect various different cellular functions and pathways of cancer cells to trigger apoptosis, including regulating the PI3K/AKT and MAPK signaling pathways, inducing cell cycle arrest and inhibiting cell invasion and migration (Wang et al. 2016, 2020; Zhang et al. 2017, 2020). (Zhang et al. 2020) revealed that lidocaine could reduce proliferation and inhibit metabolism by downregulating the PI3K/AKT signaling pathway in hepatocellular carcinoma cells. In addition, (Wang et al. 2020) found that lidocaine could promote autophagy in SH-SY5Y cells by inhibiting the PI3K/AKT/mTOR signaling pathway.

Polycystic ovary syndrome (PCOS) is the most common endocrine condition in women. In total, one in five women of reproductive age are diagnosed with PCOS (Copp et al. 2019) and the condition affects 5–15% of women aged 20–30 years (Ben-Shlomo and Younis 2014; Zheng et al. 2019). PCOS has multiple clinical manifestations, including reproductive, metabolic and psychological features. In addition, as a heterogeneous disease, the phenotype of PCOS varies depending on factors such as life stage (Chang et al. 2016), genotype (Li et al. 2013), ethnicity (Lim et al. 2019) and environmental factors, including lifestyle and weight (Li et al. 2016). At present, there are different hypotheses surrounding the etiology and pathogenesis of PCOS (Hanson and Gluckman 2014; Rosenfield and Ehrmann 2016; Meier 2018; Ruddenklau and Campbell 2019). However, to the best of our knowledge, the exact mechanism underlying the pathogenesis of this condition remains unclear; thus, further research is required. (Li et al. 2016) reported that the overexpression of heat shock protein 90 β family member 1 induced granulosa cell proliferation and promoted the occurrence of PCOS. Other previous studies have also suggested that the abnormal proliferation of granulosa cells may promote the occurrence and development of PCOS (Li et al. 2019; Cai et al. 2020; Yang et al. 2021). Notably, the reduction in the levels of apoptosis of granulosa cells was associated with disordered folliculogenesis in PCOS (Yang et al. 2021). KGN cells express functional follicle-stimulating hormone receptors (FSHRs) and have steroidogenic activities similar to human ovarian granulosa cells (Nishi et al. 2001). As it is difficult to obtain human ovarian granulosa cells in sizable amounts, and the primary culture system of human ovarian granulosa cells is hard to maintain, in recent years, KGN cells have been widely used for in vitro studies of PCOS (Wu et al. 2020; Xia and Zhao 2020).

PI3K/AKT signaling pathway has been reported to play a key role in PCOS (Wu et al. 2018; Cai et al. 2020; Xu et al. 2020; Zhang et al. 2020). (Yang et al. 2021) demonstrated that the inhibition of the PI3K/AKT signaling pathway in KGN cells could repress the pathological progression of PCOS. Furthermore, network pharmacological analysis of potential PCOS treatments also revealed that the PI3K/AKT, insulin receptor, Toll-like receptor, MAPK and hypoxia-inducible factor 1 signaling pathways played important roles in PCOS (Xu et al. 2020). In addition, berberine was proven to decrease insulin resistance in a PCOS rats by activating the PI3K/AKT and inhibiting the MAPK signaling pathways in ovarian tissue (Zhang et al. 2020). In PCOS model rats, the activation of the PI3K/AKT signaling pathway in in skeletal

muscle of rats was discovered to increase insulin sensitivity (Wu et al. 2018).

Previous studies revealed that lidocaine could induce apoptosis in Jurkat cells by damaging mitochondria, and this damage depended on the duration of exposure and dose (Werdehausen et al. 2007; Li et al. 2014b). Thus, the expression levels of Bcl-2 and caspase-3 have been suggested to represent promising biomarkers of lidocaine-induced apoptosis (Werdehausen et al. 2007; Li et al. 2014b). The present study aimed to investigate the effects of lidocaine on the proliferative activity of human ovarian granulosa cell tumor cell line, KGN, and its underlying molecular mechanism of action, with the hope of providing a novel insight into potential therapeutic strategies for PCOS.

Materials and methods

Cell lines and culture

The human ovarian granulosa cell tumor cell line, KGN, was purchased from The Cell Bank of Type Culture Collection of The Chinese Academy of Sciences. The cells were cultured in DMEM (Sigma-Aldrich; Merck KGaA) supplemented with 10% FBS (vol/vol), 100 µg/ml penicillin and 100 µg/ml streptomycin, and maintained in a humidified atmosphere at 37 °C with 5% CO_2. Lidocaine was purchased from Merck KGaA (cat. no. 137-58-6) and insulin-like growth factor-1 (IGF-1) was purchased from Gibco; Thermo Fisher Scientific, Inc. (cat. no. PHG0071).

For cell treatment, KGN cells were treated with 0, 1, 5, 10 mM lidocaine (Ye et al. 2019), or 10 mM lidocaine + 10 µM IGF-1 (Rong et al. 2020), at 37 °C for 48 h.

3-45-dimethylthiahiazo (-z-y1)-35-di-phenytetrazoliumromide (MTT) assay

KGN cells were treated with 0, 1, 5, 10 mM lidocaine, or 10 mM lidocaine + 10 µM IGF-1, at 37 °C for 48 h. Then, a Vybrant® MTT Cell Proliferation assay kit (cat. no. V-13154; Invitrogen) was used to assess cell proliferation. Briefly, cells were seeded at a density of 4×10^3 cells/well into a 96-well flat-bottomed plate in 100 µl medium and incubated at 37 °C for 48 h. After the incubation, 10 µl MTT stock solution (12 mM) was added to each well and the plates were further incubated for 4 h at 37 °C with 5% CO_2. Following the incubation, the cells were treated with MTT solvent for 15 min at room temperature, dispersed with a pipette and the absorbance of each well was measured at a wavelength of 570 nm.

Flow cytometry

KGN cells were treated with 0, 1, 5, 10 mM lidocaine, or 10 mM lidocaine + 10 µM IGF-1, at 37 °C for 48 h. Then, an Annexin V-FITC Apoptosis Detection kit (Beyotime Institute of Biotechnology; cat. no. C1062S) was used, according to the manufacturer's protocol. Briefly, KGN cells were collected, centrifuged ($1000 \times g$, 4 °C, 5 min), and then added with 195 µl Annexin V-FITC binding solution. Then the cells were incubated with 5 µl Annexin V-FITC and 10 µl propidium iodide at room temperature for 10 min in the dark. Finally, cell apoptosis (late or early + late apoptosis) was assessed via flow cytometry (BD Biosciences), and the data were analyzed using Kaluza Analysis (version 2.1.1.20653; Beckman Coulter, Inc.).

Western blotting

KGN cells were treated with 0, 1, 5, 10 mM lidocaine, or 10 mM lidocaine + 10 µM IGF-1, at 37 °C for 48 h. Then, total protein was extracted from cells using RIPA lysis buffer (Beyotime Institute of Biotechnology). Total protein was quantified and 20 µg protein/lane was separated via 12% SDS-PAGE. The separated proteins were subsequently transferred onto a nitrocellulose membrane (MilliporeSigma) and blocked with 5% non-fat dry milk in TBS (10 mM Tris, 150 mM NaCl, pH 7.4) for 1 h at room temperature. The membranes were then incubated overnight at 4 °C with the following primary antibodies diluted in TBS-Tween 20 (TBST) with 5% BSA: Anti-phosphorylated (p)-AKT (1:500; cat. no. ab8933; Abcam), anti-AKT (1:10,000; cat. no. ab179463; Abcam), anti-p-mTOR (1:1,000; cat. no. ab109268; Abcam), anti-mTOR (1:10,000; cat. no. ab134903; Abcam), anti-cleaved caspase-3 (1:200; cat. no. ab2302; Abcam), anti-caspase-3 (1:5,000; cat. no. ab32351; Abcam), anti-Bax (1:1,000; cat. no. ab32503; Abcam), anti-Bcl-2 (1:1,000; cat. no. ab32124; Abcam) and anti-GAPDH (1:500; cat. no. ab8245; Abcam). Following

the primary antibody incubation, the membranes were washed four times for 5 min each in TBST and incubated with an HRP-conjugated secondary antibody (1:2,000; cat. no. 7074; Cell Signaling Technology, Inc.) at room temperature for 2 h. Protein bands were visualized using ECL reagent (Cytiva). The band intensity was semi-quantified using ImageJ version 1.8.0 (National Institutes of Health).

Reverse transcription-quantitative

PCR (RT-qPCR). KGN cells were treated with 0, 1, 5, 10 mM lidocaine, or 10 mM lidocaine + 10 μM IGF-1, at 37 °C for 48 h. Then, total RNA was extracted from cells using TRIzol® reagent (Takara Bio, Inc.; cat. no. 9108). qPCR was performed using a One Step PrimeScript™ III RT-qPCR mix (Takara Bio, Inc.; cat. no. RR600A) on an ABI 7500 Fast Real-Time PCR Detection system (Applied Biosystems; Thermo Fisher Scientific, Inc.). The thermocycling conditions for qPCR were as follows: Initial denaturation at 95 °C for 5 min; followed by 38 cycles of 15 s at 95 °C, 1 min at 60 °C and 30 s at 72 °C and final extension for 10 min at 72 °C. The following primers (synthesized by Sangon Biotech Co., Ltd.) were used for the qPCR: β-actin forward, 5′-CACGATGGAGGGGCC GGACTC-3′ and reverse, 5′-TAAAGACCTCTATGC CAACAC-3′; Bax forward, 5′-TGCTACAGGGTT TCATCCAG-3′ and reverse, 5′-ATCCACATCAGC AATCATCC-3′; Bcl-2 forward, 5′-TGGGATGCC TTTGTGGAAC-3′ and reverse 5′-CATATTTGTTTG GGGCAGGTC-3′; AKT forward, 5′-CATGAGCGA CGTGGCTATTG-3′ and reverse, 5′-GCCTCACGT TGGTCCACATC-3′; and mTOR forward, 5′-GCT AGGTGCATTGACATACAACA-3′ and reverse, 5′-AGTGCTAGTTCACAGATAATGGC-3′. Relative expression levels were quantified using the $2^{-\Delta\Delta Cq}$ method (Livak and Schmittgen 2001). β-actin was used as the internal control for mRNA expression levels, and the expression levels of investigated mRNAs were normalized to β-actin.

Statistical analysis

The data are presented as the mean ± SD from at least three independent experiments. Statistical analysis was performed using GraphPad Prism 6.0 software (GraphPad Software, Inc.). Statistical differences among groups were determined using an unpaired Student's t-test or one-way ANOVA followed by a Tukey's post hoc test. P < 0.05 was considered to indicate a statistically significant difference.
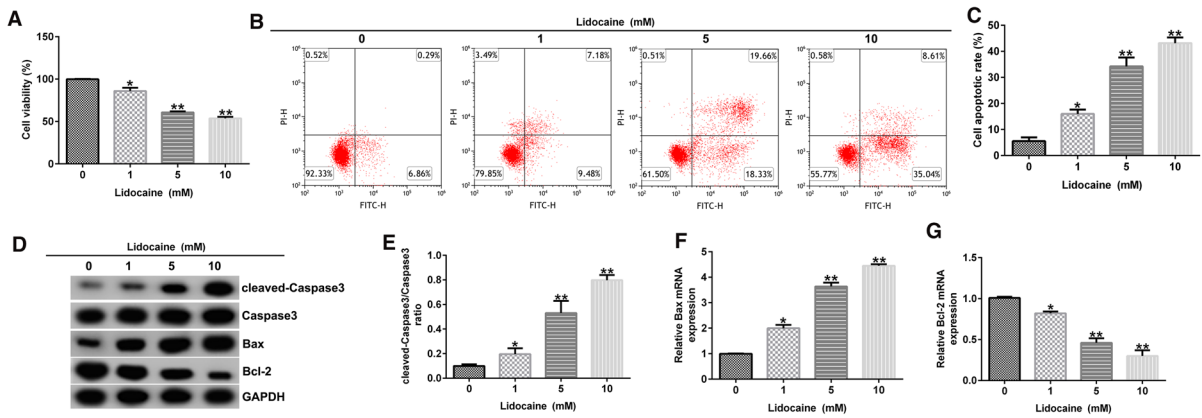
Results

Lidocaine inhibits the proliferation and induces the apoptosis of KGN human ovarian granulosa cell tumor cells

KGN cells were treated with different concentrations of lidocaine for 48 h and an MTT assay was used to detect the proliferative ability. The results revealed that 1, 5, 10 mM lidocaine significantly reduced cell proliferation in a dose-dependent manner (Fig. 1A). Flow cytometric analysis found that 1, 5, 10 mM lidocaine treatment increased KGN cell apoptosis (Fig. 1B and C). In addition, the expression levels of apoptosis-related proteins and mRNAs were detected using western blotting and RT-qPCR, respectively. Western blotting analysis revealed that 1, 5, 10 mM lidocaine upregulated the expression levels of cleaved caspase-3 and increased the ratio of cleaved caspase-3/caspase-3 in a dose-dependent manner (Fig. 1D and E). The anti-apoptotic protein, Bcl-2, and its proapoptotic counterpart, Bax, play an important role in regulating cell apoptosis (Dietrich 1997). The results demonstrated that the protein and mRNA expression levels of Bax were upregulated in KGN cells (Fig. 1D and F), while the protein and mRNA expression levels of Bcl-2 were downregulated by 1, 5, 10 mM lidocaine treatment (Fig. 1D and G). The findings indicated that lidocaine inhibited the proliferation and induces the apoptosis of KGN cells.

Lidocaine inhibits the PI3K/AKT/mTOR signaling pathway in KGN ovarian granulosa cell tumor cells

Accumulating evidence has indicated that the PI3K/AKT/mTOR signaling pathway has an important role in the occurrence and development of PCOS (Liu et al. 2015; Zhao et al. 2017; Cai et al. 2020). Thus, the expression levels of PI3K/AKT/mTOR signaling pathway-related proteins and mRNAs in KGN cells after lidocaine treatment were investigated using western blotting and RT-qPCR, respectively. Western blotting analysis revealed that 1, 5, 10 mM lidocaine significantly downregulated the expression levels
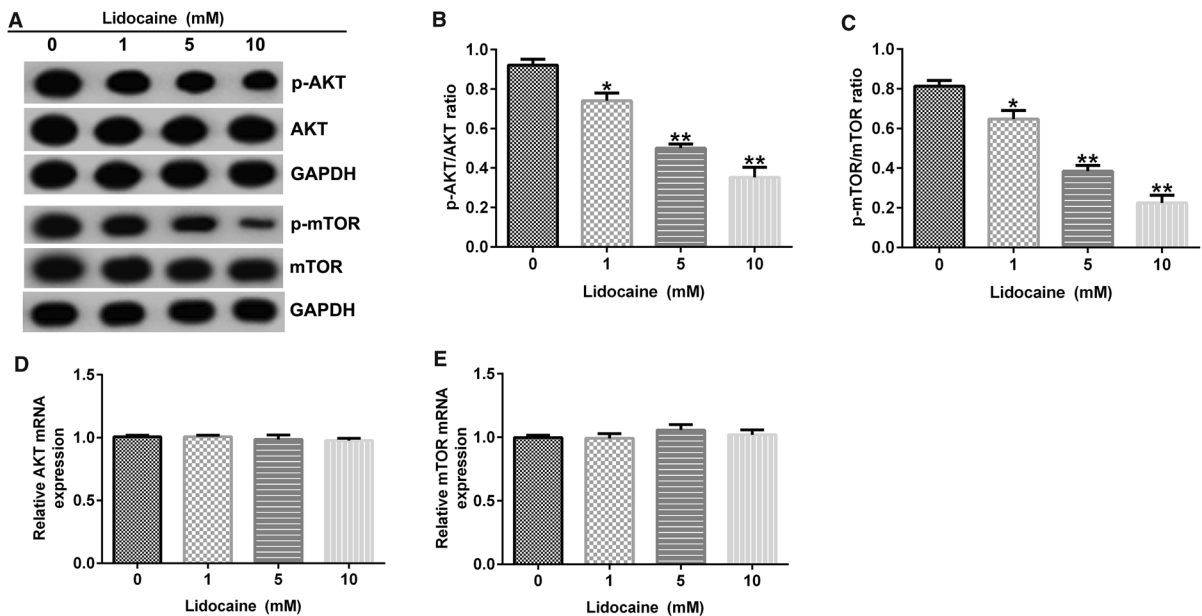
Fig. 1 Lidocaine inhibits the proliferation and induces the apoptosis of the human ovarian granulosa cell-like cell line, KGN. KGN cells were treated with 0, 1, 5 or 10 mM lidocaine for 48 h. **A** MTT assay was used to measure cell proliferation. **B** and **C** Flow cytometry was performed to determine cell apoptosis (Upper left quadrant: necrotic cells; Lower left quadrant: viable cells; Upper right quadrant: late apoptosis; Lower right quadrant: early apoptosis). **D** Western blotting was used to analyze the protein expression levels of cleaved caspase-3, caspase-3, Bax and Bcl-2. **E** Ratio of cleaved caspase-3/caspase-3. **F** and **G** mRNA expression levels of Bax and Bcl-2 were analyzed using reverse transcription-quantitative PCR. $*P < 0.05$, $**P < 0.01$ vs. 0 mM lidocaine group

of p-AKT and p-mTOR (Fig. 2A), and the ratios of p-AKT/AKT and p-mTOR/mTOR were decreased (Fig. 2B and C). However, the results of the RT-qPCR analysis found that the changes in the mRNA expression levels of AKT and mTOR between untreated cells and those treated with 1, 5, 10 mM lidocaine

Fig. 2 Lidocaine inhibits the PI3K/AKT/mTOR signaling pathway in the human ovarian granulosa cell-like cell line, KGN. KGN cells were treated with the indicated concentration of lidocaine for 48 h. **A** Protein expression levels of p-AKT, AKT, p-mTOR and mTOR were measured using western blotting. **B** and **C** Semi-quantitative analysis of p-AKT/AKT and p-mTOR/mTOR expression following lidocaine treatment. **D** and **E** Reverse transcription-quantitative PCR was used to analyze the mRNA expression levels of AKT and mTOR. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. 0 mM lidocaine group. p-, phosphorylated

were not statistically significant (Fig. 2D and E). The data indicated that lidocaine inhibited the PI3K/AKT/mTOR signaling pathway in KGN cells.
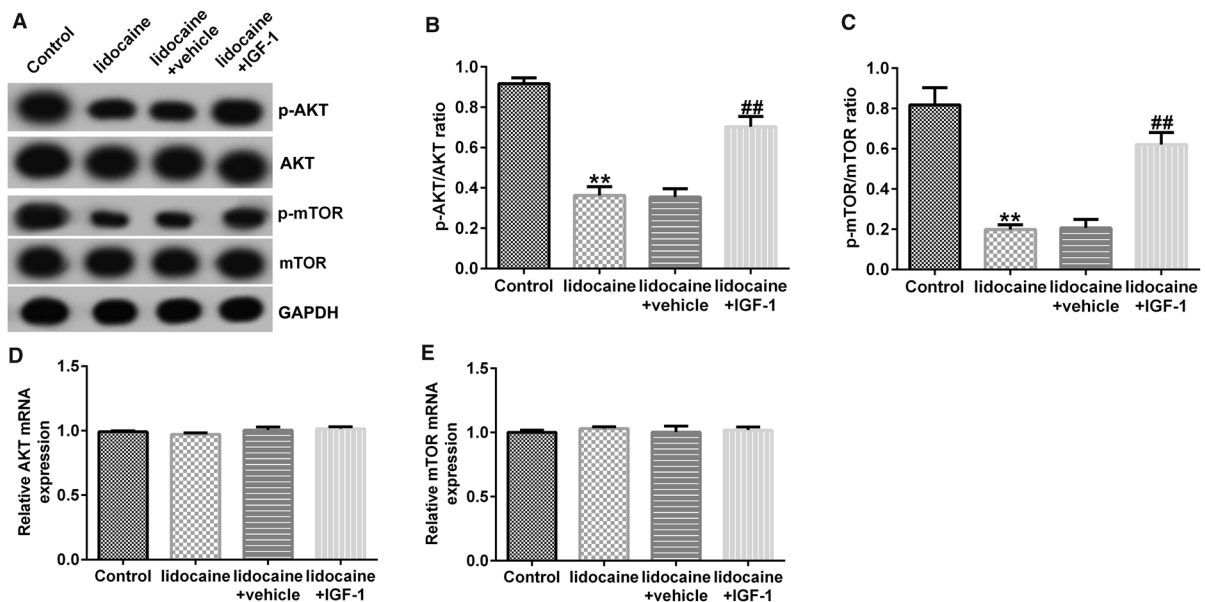
PI3K/AKT agonist, IGF-1, reverses the inhibitory effect of lidocaine on the PI3K/AKT/mTOR signaling pathway

Finally, we investigated the underlying mechanism of action of lidocaine on KGN cells. Western blotting analysis revealed that the expression levels of p-AKT and p-mTOR were downregulated in 10 mM lidocaine-treated KGN cells (Fig. 3A), and the ratios of p-AKT/AKT and p-mTOR/mTOR were decreased (Fig. 3B and C). The expression levels of p-AKT and p-mTOR were found to be upregulated in KGN cells co-treated with 10 mM lidocaine and 10 μM PI3K/AKT agonist, IGF-1, compared with those in cells treated with 10 mM lidocaine only. Next, the mRNA expression levels of AKT and mTOR were analyzed using RT-qPCR. The results revealed that the mRNA expression levels of AKT and mTOR were not affected by lidocaine treatment in KGN cells (Fig. 3D

and E), suggesting that the effects of lidocaine on the PI3K/AKT/mTOR signaling pathway may occur at the protein level. The results suggested that IGF-1 reversed the inhibitory effect of lidocaine on the PI3K/AKT/mTOR signaling pathway in KGN cells.
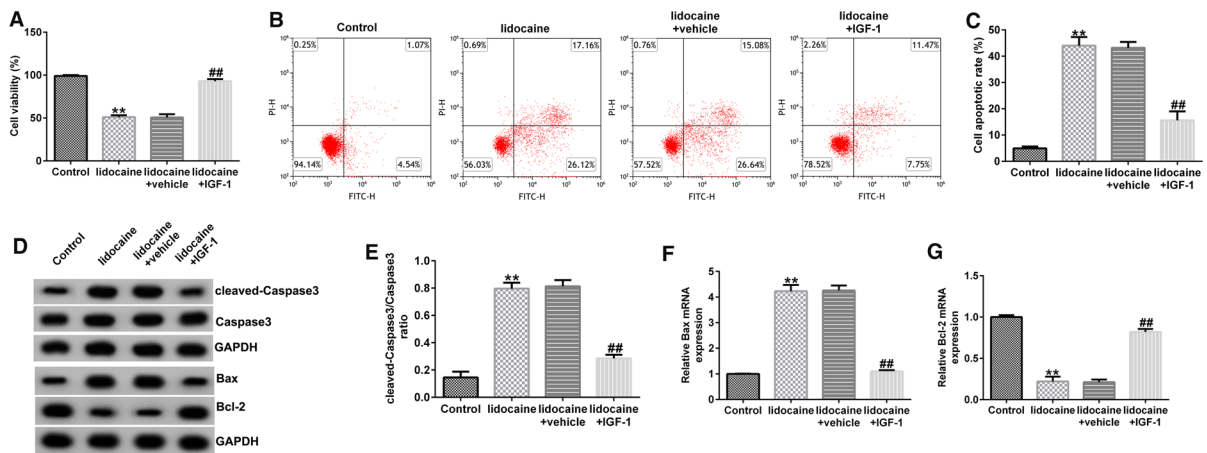
PI3K/AKT agonist, IGF-1, reverses the effect of lidocaine on KGN human ovarian granulosa cell tumor cells

To verify whether lidocaine affects KGN cells by regulating the PI3K/AKT/mTOR signaling pathway, the PI3K/AKT agonist, IGF-1, was used to reverse the effects of lidocaine on the PI3K/AKT/mTOR signaling pathway, and the proliferation and apoptosis of KGN cells were investigated. The results of the MTT assay demonstrated that 10 mM lidocaine significantly reduced cell proliferation, while co-treatment of 10 μM IGF-1 and 10 mM lidocaine reversed the inhibitory effect of lidocaine alone (Fig. 4A). Compared with the lidocaine + vehicle group, 10 mM lidocaine + 10 μM IGF-1 treatment significantly reduced cell apoptosis (Fig. 4B and C), decreased the protein

Fig. 3 PI3K/AKT agonist, IGF-1, reverses the inhibitory effect of lidocaine on the PI3K/AKT/mTOR signaling pathway. KGN cells were treated with 10 mM lidocaine + 10 μM IGF-1, 10 mM lidocaine + vehicle or 10 mM lidocaine for 48 h. **A** Protein expression levels of p-AKT, AKT, p-mTOR and mTOR were measured using western blotting. **B** and **C** Semi-quantitative analysis of p-AKT/AKT and p-mTOR/mTOR expression following lidocaine treatment. **D** and **E** Reverse transcription-quantitative PCR was used to analyze the mRNA expression levels of AKT and mTOR. **P < 0.01 vs. control; ##P < 0.01 vs. lidocaine + vehicle. IGF-1, insulin-like growth factor 1; p-, phosphorylated

Fig. 4 PI3K/AKT agonist IGF-1 reverses the effects of lidocaine on KGN human ovarian granulosa cell-like cells. KGN cells were treated with 10 mM lidocaine + 10 μM IGF-1, 10 mM lidocaine + vehicle or 10 mM lidocaine for 48 h. **A** MTT assay was performed to measure cell proliferation. **B** and **C** Flow cytometry was performed to determine the levels of cell apoptosis (Upper left quadrant: necrotic cells; Lower left quadrant: viable cells; Upper right quadrant: late apoptosis; Lower right quadrant: early apoptosis). **D** Western blotting was used to analyze the protein expression levels of cleaved caspase-3, caspase-3, Bax and Bcl-2. **E** Ratio of cleaved caspase-3/caspase-3. **F** and **G** mRNA expression levels of Bax and Bcl-2 were measured using reverse transcription-quantitative PCR. **P < 0.01 vs. control; ##P < 0.01 vs. lidocaine + vehicle. IGF-1, insulin-like growth factor 1

expression levels of cleaved caspase-3 and the ratio of cleaved caspase-3/caspase-3 (Fig. 4D and E), downregulated the protein and mRNA expression levels of Bax (Fig. 4F and G), as well as up-regulated the protein and mRNA expression levels of the Bcl-2 (Fig. 4F and H) in KGN cells. In summary, IGF-1 reversed the effect of lidocaine on KGN human ovarian granulosa cell tumor cells.

Discussion

PCOS is a common endocrine disease in women; however, the pathogenesis of PCOS is complicated and to date, no effective therapeutic strategy exists for the treatment of the condition (Ben-Shlomo and Younis 2014; Copp et al. 2019). Studies have found that the survival and proliferation of granulosa cells may be associated with PCOS. For example, the apoptotic rate of granulosa cells in patients with PCOS were found to be lower, while the proliferative ability was higher compared with those in healthy women (Li et al. 2016). (Han et al. 2018) indicated that the inhibition of the proliferation of granulosa cells may be a potential method for the treatment of PCOS. The human ovarian granulosa cell tumor cell line, KGN, is commonly used to study the function and underlying mechanism of granulosa cells in PCOS (Wang et al. 2018; Song et al. 2019).

Intra-articular injections of local anesthetics, including lidocaine, are commonly used in the clinic as analgesic agents (Klein and Jeske 2016). Accumulating evidence has shown that lidocaine exerts numerous other effects, in addition to its anesthetic potential (Slaton et al. 2013; Truesdale and Jurdi 2013; Weibel et al. 2018; Beaussier et al. 2018). (Kamiya et al. 2005) found that in U937 histiocytic lymphoma cells, lidocaine induced apoptosis at concentrations of < 12 mM, and induced cell necrosis at concentrations of > 15 mM, indicating that the cytotoxicity of lidocaine may depend on the dose. The results of the present study revealed that 1, 5, 10 mM lidocaine significantly inhibited the proliferation of KGN cells and induced apoptosis in a dose-dependent manner. The anti-apoptotic protein, Bcl-2, and its proapoptotic counterpart, Bax, play key roles in the regulation of apoptosis (Dietrich 1997). Abnormal expressions of Bcl-2, Bax and cleaved-caspase3 have been revealed in PCOS rats in previous syudies (Chi et al. 2018; Jin et al. 2021). The results of the present study demonstrated that lidocaine downregulated the protein and mRNA expression levels of Bcl-2,

significantly upregulated the expression levels of Bax and cleaved-caspase3 in KGN cells.

Previous study has demonstrated that lidocaine could induce apoptosis by affecting the downstream proteins, Bcl-2/Bax, via regulation of the PI3K/AKT signaling pathway (Zhang et al. 2020). The PI3K/AKT signaling pathway plays an important role in cellular metabolism, and has been found to be closely associated with PCOS (Liu et al. 2015; Zhao et al. 2017; Cai et al. 2020). Previous studies have demonstrated that the PI3K/AKT signaling pathway is abnormally regulated in both patients with PCOS and PCOS animal models (Zheng et al. 2012; Li et al. 2017), and the aberrant activation of the PI3K/AKT signaling pathway was discovered to be associated with the occurrence and development of PCOS (Wu et al. 2018; Zhang et al. 2020). Recent PCOS studies have significantly spotted the importance of PI3K/AKT/mTOR in regulating the hormonal, metabolic and proliferative pathways in PCOS (Abuelezz et al. 2020, 2021). And PI3K/AKT/mTOR-dependent apoptosis and autophagy play a key role in PCOS development (Jin et al. 2021; Liu et al. 2021). The findings of current study indicated that lidocaine inhibited the PI3K/AKT/mTOR signaling pathway in KGN cells. It was worth mentioning that, treatment with the PI3K/AKT agonist, IGF-1, could reverse the lidocaine-induced decrease in KGN cell proliferation and increase in apoptosis.

However, there were some limitations of this study. For example, the effects of vehicle alone and IGF-1 alone on KGN cells were not explored in this study. Besides, the effect of more doses of lidocaine on ovarian granulosa cells needs to be studied. Moreover, an ideal model would have been to replicate the results in vivo in PCOS mice to show inhibition of follicles. We will further explored these issues in the future.

In conclusion, the findings of the current study suggested that lidocaine treatment significantly inhibited KGN cell proliferation and induced apoptosis in KGN cells via the PI3K/AKT/mTOR signaling pathway. Therefore, lidocaine may have promise as a potential therapeutic to improve the outcomes of PCOS by exerting anti-apoptotic effects via inhibition of the PI3K/AKT/mTOR signaling pathway. Lidocaine might be a promising agent for PCOS treatment.

Declarations

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