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ORIGINAL RESEARCH

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Ketamine inhibits endometrial cancer cell growth and motility by inducing ferroptosis

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Abstract

Endometrial cancer (EC) is a prevalent gynecological malignancy with an escalating incidence and mortality rate, notably in China. Surgical options are limited, and drug resistance development poses significant challenges to EC treatment. Ketamine, a rapid-acting anesthetic, exhibits anti-inflammatory, analgesic, and antidepressant properties, yet its potential in cancer therapy remains largely unexplored. Here, we investigate ketamine's effects on EC cell growth, motility and ferroptosis, alongside its impact on the phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt)/the mammalian target of rapamycin (mTOR) axis. Ketamine significantly inhibits EC cell growth and motility while promoting ferroptosis. Mechanistically, ketamine's anti-tumor effects correlate with suppression of the PI3K/Akt/mTOR axis crucial for cell survival and growth. In summary, our findings highlight ketamine's potential therapeutic application in EC treatment by impeding cell growth and motility, fostering ferroptosis, and suppressing the PI3K/Akt/mTOR axis. These insights offer novel avenues for EC therapy.

Keywords

Ketamine; Endometrial cancer; Ferroptosis; PI3K/Akt/mTOR; Cell growth; Cell motility

1. Introduction

Endometrial cancer (EC) is one of the most prevalent gynecological malignancies, with its incidence rate rapidly ascending worldwide [1]. In China, its mortality rate is reported to be surging, positioning it as the second most prevalent gynecological malignancy following cervical cancer [2]. While surgery remains the cornerstone of treatment for nearly all patients with EC, its suitability is compromised for young and obese patients due to concerns regarding fertility loss, suboptimal surgical outcomes, and the substantial financial burden of surgical interventions [3]. Chemotherapy, radiotherapy and pharmacotherapy represent other commonly employed modalities for EC management [3]. Nonetheless, the efficacy of EC treatment is impeded by the emergence of drug resistance in EC cells.

Since its identification in 1970, ketamine has been commercially utilized as a rapid-acting anesthetic [4]. Its short half-life makes it an optimal choice in clinical settings [5]. In addition to its anesthetic properties, ketamine has been shown to possess anti-inflammatory, analgesic, and antidepressant effects [6]. Despite being widely recommended as an adjunctive medication for managing cancer-related pain, its impact on cancer treatment and underlying mechanisms remains largely elusive. Notably, ketamine has been found to induce ferroptosis in liver cancer cells through modulation of the Glutathione Peroxidase 4 (GPX4) pathway [7]. It also exerts inhibitory effects on the malignant behavior of colorectal cancer cells by blocking N-Methyl-D-aspartic acid (NMDA) receptors. Moreover, ketamine demonstrates anti-gastric tumor activity by promoting apoptosis and suppressing the PI3K/Akt/mTOR axis [8].

The PI3K/Akt pathway is pivotal in various cellular physiological processes as it can activate downstream effector molecules [9], and its aberrant activation can lead to uncontrolled cell growth and anti-apoptosis, contributing to tumorigenesis [10]. However, although ketamine, an anesthetic, shows promise in inhibiting tumor growth and metastasis, its underlying molecular mechanisms remain unclear.

In this study, we hypothesize that ketamine can attenuate the growth and motility of EC cells by inducing ferroptosis *via* modulation of the PI3K/Akt/mTOR signaling pathway. To investigate this, we conducted this study to investigate the therapeutic potential of ketamine for EC, and the findings offer fresh perspectives on the anti-tumor properties of ketamine and could help in the formulation of innovative treatment approaches for EC.

2. Materials and methods

2.1 Cell culture and treatment

The human EC cell lines HEC-1A and Ishikawa were purchased from American Type Culture Collection (ATCC) and maintained in Dulbecco's Modified Eagle Medium

(DMEM) complete medium (11965092, Gbico, Grand Island, NY, USA) at 37 °C with 5% Carbon dioxide Ketamine (Y0000450, Sigma, DE, USA) was administered to EC cells at concentrations of 0, 5, 10 and 20 μg/mL for 24 hours.

2.2 Cell viability assays

HEC-1A and Ishikawa cells were seeded into 96-well plates and incubated at 37 °C. Following the designated treatment period of 24 hours, the cells were exposed to cell counting kit-8 (CCK-8) reagent at 37 °C for 4 hours. Then, the relative cell growth was evaluated using a spectrophotometer at a wavelength of 450 nm (iMark, Bio-Rad, Hercules, CA, USA).

2.3 Colony formation assay

HEC-1A and Ishikawa cells were seeded at a density of 400 cells per well in a 6-well plate and incubated for approximately two weeks. Subsequently, the cells were fixed and stained with crystal violet. The colonies were counted using a light microscope based on colony size and clarity.

2.4 Transwell-motility or invasion assay

BD Falcon cell culture inserts were utilized as upper chambers, while 24-well plates were used as the lower chambers. The cell culture inserts were coated with 100 μL of Matrigel (diluted 1:3 with serum-free media) for invasion assays, while motility assays were conducted without Matrigel coating. A total of 1.05×10^5 HEC-1A and Ishikawa cells were seeded into the upper chamber. The cells that invaded the underside of the insert were fixed with 4% paraformaldehyde, stained with 0.2% crystal violet, and imaged. Subsequently, the stained cells were manually counted under a microscope to quantify the number of migrated cells.

2.5 Fe level detection

The Fe detection kit (A039-1-1) from Nanjing Jiancheng Bioengineering Institute (Nanjing, China) was utilized to measure iron levels in EC cells treated with ketamine. This kit employs a colorimetric assay, where a chromogenic reagent forms a colored complex with iron ions. The intensity of the resulting color, measured spectrophotometrically, is directly proportional to the iron concentration in the sample. Following the designated treatment of ECs, cells were collected and subjected to iron detection using the relevant commercial kits based on the manufacturer's instructions.

2.6 Immunoblot assay

Proteins were extracted from EC cells using a lysis buffer (P0013K, Beyotime, Shanghai, China), and their concentrations were determined using a BCA Protein Assay Kit (P0011, Beyotime, Shanghai, China). The separated proteins were then transferred onto Polyvinylidene Fluoride (PVDF) membranes after sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) separation. Following this, the membranes were blocked with 5% milk, and primary antibodies including Matrix Metalloproteinase 2 (MMP2) (ab92536; 1:500, Abcam), MMP9 (ab76003; 1:500, Abcam), GPX4 (ab125066; 1:500, Abcam), achaete-scute complex homolog 4 (ASCL4) (ab303504; 1:500, Abcam), PI3K (ab302958; 1:1000, Abcam), p-PI3K (ab278545; 1:500, Abcam), Akt (ab8805; 1:1000, Abcam), p-Akt (ab38449; 1:1000, Abcam), mTOR (ab134903; 1:1000, Abcam), p-mTOR (ab109268; 1:1000, Abcam), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (ab8245; 1:3000, Abcam) were added. Subsequently, the membranes were incubated with secondary antibodies for an additional hour. The signals were then detected, and the intensity of the bands was quantified using ImageJ software to analyze the immunoblot images.

2.7 Statistics

Statistical analyses were conducted using GraphPad Prism 8.0 software (Graphpad. San Diego, CA, USA). Data are presented as mean $\pm$ standard deviation (SD). Group differences were evaluated using appropriate statistical tests, including Student's *t*-test for comparing two groups or one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test for multiple group comparisons. Each experiment was independently replicated three times to ensure result reliability and reproducibility. Results were considered statistically significant at $p < 0.05$.

3. Results

3.1 Ketamine suppresses the growth of EC cells

To investigate the potential impact of Ketamine on EC progression, we initially established a cellular model using HEC-1A and Ishikawa EC cells. The chemical structure of Ketamine is shown in Fig. 1A. Ketamine was administered to HEC-1A and Ishikawa cells at concentrations of 0, 5, 10 and 20 μg/mL for 24 hours. The CCK-8 assay results revealed a decrease in the Optical density (OD)450 value of both HEC-1A and Ishikawa cells upon Ketamine treatment, indicating suppression of cell growth (Fig. 1B). Subsequently, colony formation assays were conducted to assess the effects of Ketamine on cell growth. Notably, Ketamine treatment led to a reduction in colony numbers of both HEC-1A and Ishikawa cells, indicating growth inhibition (Fig. 1C). Thus, these findings indicate that ketamine can effectively impede the growth of EC cells.

3.2 Ketamine inhibits EC cell motility

Subsequently, we investigated the effects of ketamine on the motility of HEC-1A and Ishikawa cells. Transwell assays revealed that ketamine treatment significantly inhibited the motility of both HEC-1A and Ishikawa cells, as evidenced by the decreased number of migrating cells (Fig. 2A). Similarly, ketamine treatment also attenuated the invasion of both HEC-1A and Ishikawa cells, as observed in transwell invasion assays (Fig. 2B). Furthermore, we examined the expression levels of motility-related factors, such as MMP2 and MMP9, *via* Immunoblot analysis. Interestingly, ketamine treatment resulted in reduced expression levels of these factors, indicating suppression of motility (Fig. 2C). Thus, ketamine effectively

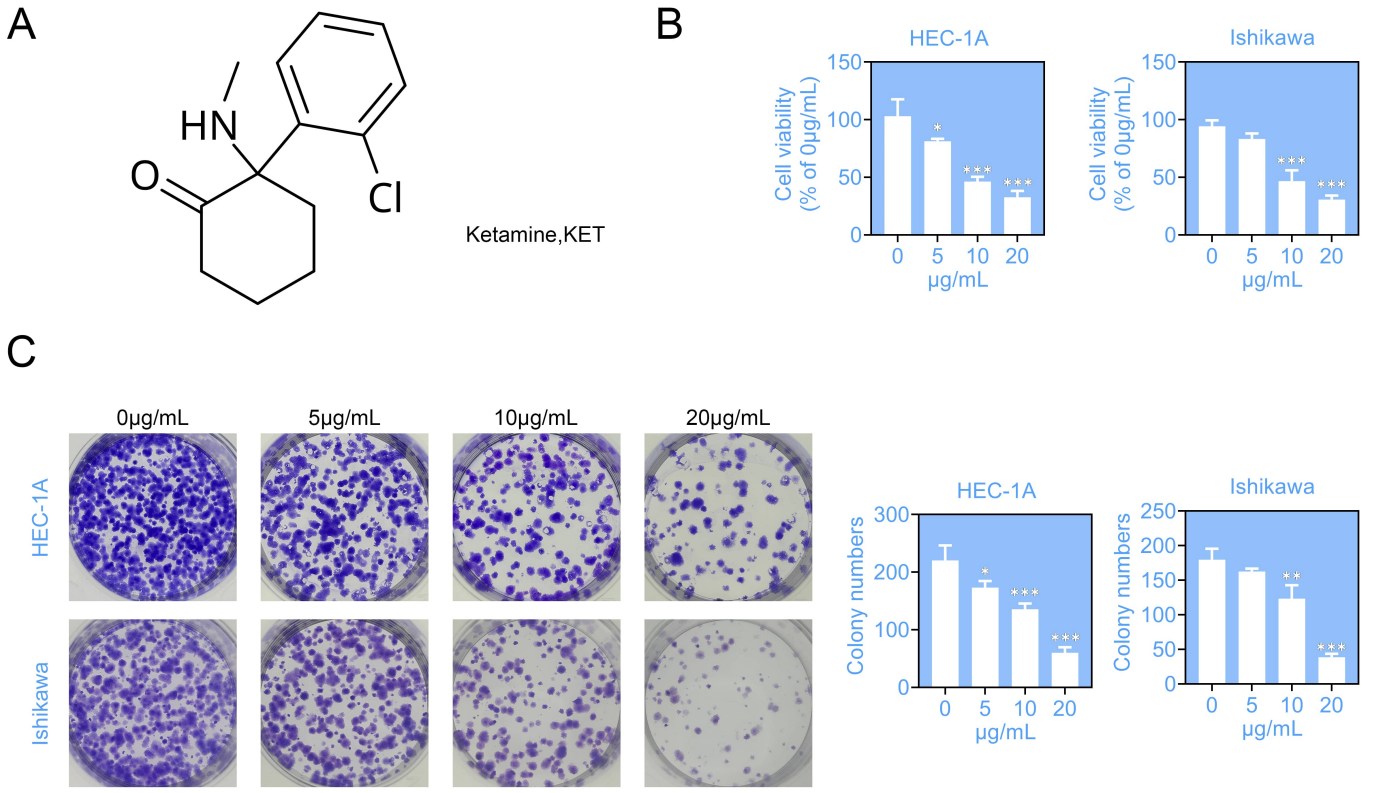

FIGURE 1. Ketamine suppresses the growth of EC cells. (A) The chemical formula of ketamine. (B) CCK-8 assays depicting the growth of HEC-1A and Ishikawa cells following treatment with ketamine concentrations of 0, 5, 10 and 20 μg/mL for 24 hours. Optical density (OD450) values were measured. (C) Colony formation assays illustrating the growth of HEC-1A and Ishikawa cells after treatment with ketamine concentrations of 0, 5, 10 and 20 μg/mL for 24 hours. The colony numbers of cells were quantified. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. KET: Ketamine.

suppressed the motility of both HEC-1A and Ishikawa cells.

3.3 Ketamine promotes ferroptosis in EC cells

Furthermore, we investigated the effects of Ketamine on the ferroptosis of EC cells. By assessing Fe levels using specific kits, we observed alterations in Fe levels in both HEC-1A and Ishikawa cells upon ketamine treatment, indicative of ferroptosis inhibition (Fig. 3A). Concurrently, we evaluated the expression levels of two ferroptosis markers, GPX4 and ASCL4. Remarkably, ketamine treatment led to decreased expression of GPX4 and increased levels of ASCL4 in both HEC-1A and Ishikawa cells, suggesting a blockade of ferroptosis (Fig. 3B). Thus, ketamine may effectively suppress ferroptosis in both HEC-1A and Ishikawa cells.

3.4 Ketamine inhibits the expression of PI3K/Akt/mTOR in EC cells

Here, we investigated the potential mechanism underlying ketamine's suppression of EC progression *in vitro*. Specifically, we examined the effects of ketamine on the PI3K/Akt/mTOR axis, a key pathway implicated in mediating cell ferroptosis and motility, *via* Immunoblot analysis. The results showed that ketamine treatment led to a decrease in the phosphorylation levels of PI3K, Akt and mTOR in both HEC-1A and Ishikawa cells, indicating inhibition of the PI3K/Akt/mTOR axis (Fig. 4). Thus, ketamine demonstrated effectiveness in suppressing the PI3K/Akt/mTOR axis in EC cells.

4. Discussion

EC poses a substantial health burden due to its escalating incidence and the constraints of existing treatment modalities. Despite progress in surgical approaches and adjunctive therapies, the prognosis for advanced-stage or recurrent EC remains not good [11]. Furthermore, the adverse effects associated with conventional treatments, such as chemotherapy and radiotherapy, often detrimentally impact patients' quality of life. Therefore, exploring alternative therapeutic strategies capable of effectively targeting EC cells while minimizing adverse effects is imperative. Our study furnishes compelling evidence that ketamine exerts significant anti-tumor effects on EC cells by impeding their growth and motility, inducing ferroptosis, and suppressing the PI3K/Akt/mTOR axis. These findings offer novel insights into the potential therapeutic utility of ketamine in EC treatment, which holds particular significance given the escalating incidence and mortality rate of this malignancy, notably in China.

The observed inhibition of EC cell growth and motility by ketamine constitutes a pivotal finding in our study. EC is recognized for its aggressive behavior, characterized by the infiltration of surrounding tissues and metastasis to distant organs [12]. The ability of ketamine to mitigate these

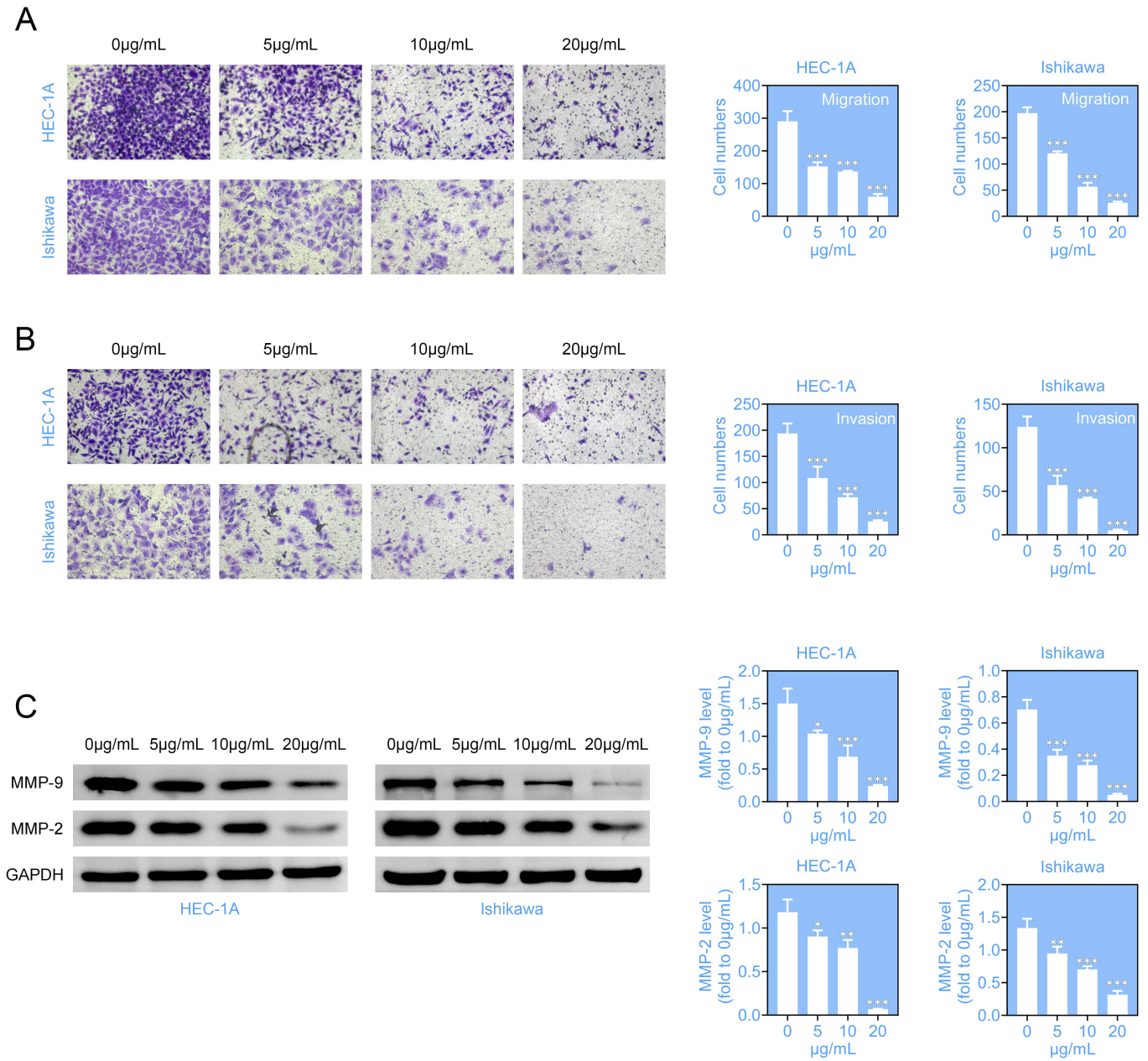

FIGURE 2. Ketamine inhibits EC cell motility. (A) Transwell assays demonstrating the motility of HEC-1A and Ishikawa cells following treatment with Ketamine at concentrations of 0, 5, 10 and 20 μg/mL for 24 hours. The number of migrating cells was quantified. (B) Transwell assays illustrating the invasion of HEC-1A and Ishikawa cells upon treatment with Ketamine at concentrations of 0, 5, 10 and 20 μg/mL for 24 hours. The number of invasive cells was quantified. (C) Immunoblot assays depicting the expression of MMP2 and MMP9 in HEC-1A and Ishikawa cells following treatment with Ketamine at concentrations of 0, 5, 10 and 20 μg/mL for 24 hours. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. KET: Ketamine; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; MMP2: Matrix Metalloproteinase 2.

oncogenic traits implies its potential efficacy as a therapeutic agent in halting the dissemination of EC and enhancing patient outcomes.

The induction of ferroptosis by ketamine in EC cells represents another important finding in our study. Ferroptosis, characterized by iron accumulation and lipid peroxide buildup, has emerged as a promising therapeutic avenue in cancer treatment, particularly for cancers resistant to traditional cell death mechanisms [13]. Ketamine's ability to trigger ferroptosis in EC cells suggests its potential to overcome drug resistance and enhance the effectiveness of current therapies [14, 15].

Suppression of the PI3K/Akt/mTOR axis by ketamine represents an important mechanistic insight from our study. This pathway holds paramount importance in cell survival, growth, and metabolism, with its dysregulated activation implicated in the onset and advancement of diverse cancers, including EC [16–18]. By modulating this pathway, ketamine may exert its anti-tumor effects by curtailing the survival signals that promote cancer cell proliferation and resistance to therapy.

Our study presents significant clinical implications for the

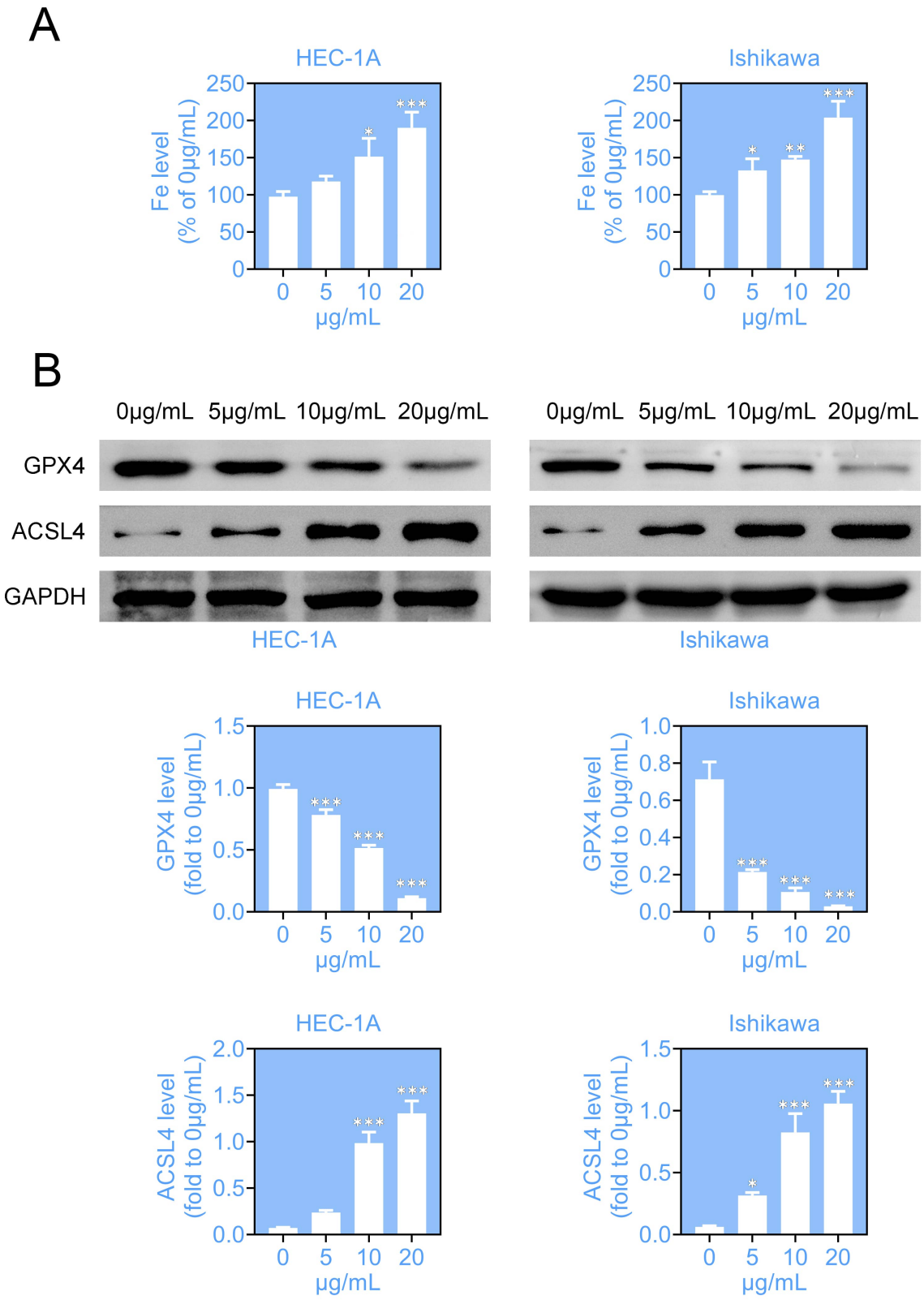

FIGURE 3. Ketamine promotes ferroptosis in EC cells. (A) Iron detection assays depicting the levels of Fe in HEC-1A and Ishikawa cells following treatment with Ketamine at concentrations of 0, 5, 10 and 20 μg/mL for 24 hours. (B) Immunoblot assays illustrating the expression of GPX4 and ASCL4 in HEC-1A and Ishikawa cells after treatment with Ketamine at concentrations of 0, 5, 10 and 20 μg/mL for 24 hours. The relative expression of the indicated proteins was quantified. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. KET: Ketamine; GPX4: Glutathione Peroxidase 4; ASCL4: achaete-scute complex homolog 4; GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

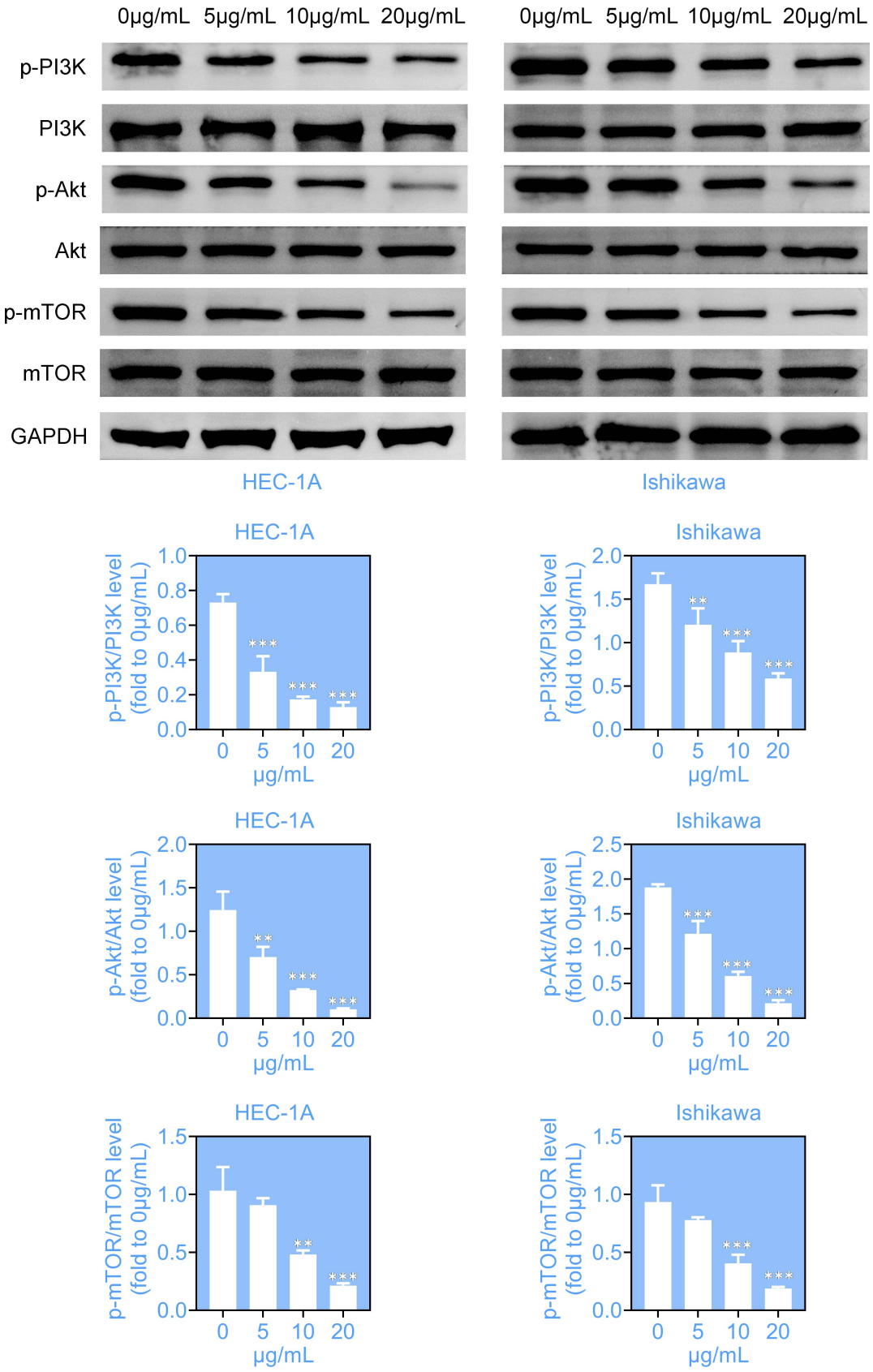

FIGURE 4. Ketamine inhibits the expression of PI3K/Akt/mTOR in EC cells. Immunoblot assays illustrating the expression and phosphorylation levels of PI3K, Akt and mTOR in HEC-1A and Ishikawa cells following treatment with Ketamine at concentrations of 0, 5, 10 and 20 μg/mL for 24 hours. The relative phosphorylation levels of the indicated proteins were quantified. $^{**}p < 0.01$, $^{***}p < 0.001$. KET: Ketamine. PI3K: phosphoinositide 3-kinase; Akt: phosphoinositide 3-kinase; mTOR: the mammalian target of rapamycin; GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

management of EC. Firstly, ketamine could be considered a potential adjunctive therapy in EC treatment, particularly in cases where traditional therapies have failed due to drug resistance. Secondly, the ability of ketamine to induce ferroptosis suggests a promising avenue for further research into developing ferroptosis-inducing agents as a novel therapeutic strategy for EC. Lastly, the suppression of the PI3K/Akt/mTOR axis by ketamine highlights the importance of targeting this pathway in combating EC and other cancers.

In addition to the studies above, recent research has further elucidated the anticancer properties of Ketamine. He *et al*. [7] demonstrated that Ketamine induces ferroptosis in liver cancer cells by targeting the lncRNA plasmacytoma variant translocation 1 (PVT1)/miR-214-3p/GPX4 axis, revealing a novel mechanism underlying its anticancer activity. Similarly, Zhao *et al*. [8] reported that Ketamine exhibits antigastric tumor activity by inducing apoptosis and inhibiting the PI3K/Akt/mTOR axis, thereby reinforcing its potential in cancer therapy. These findings underscore the multifaceted nature of ketamine's anticancer effects and offer valuable insights for future research in this field.

While our study offers valuable insights into the potential therapeutic effects of ketamine on EC cells, it is important to acknowledge several limitations. Firstly, our research was conducted *in vitro* using EC cell lines. Although these models serve as valuable tools for initial screening and mechanistic studies, they may not fully capture the complexity of the tumor microenvironment. Thus, the observed efficacy and safety of ketamine warrant validation in animal models and clinical trials to ascertain its translational potential for EC treatment. Secondly, our study primarily focused on investigating the effects of ketamine on cell growth, motility, ferroptosis induction, and its impact on the PI3K/Akt/mTOR axis. Further investigations are warranted to delve into the intricate pathways and interactions underlying the anti-tumor activity of ketamine in EC cells.

Ferroptosis is characterized by iron-dependent reactive oxygen species (ROS) accumulation and lipid peroxidation, intricately tied to the cellular oxidative milieu. In our study, the modulation of ferroptosis markers, such as GPX4, by ketamine suggests its potential influence on oxidative stress pathways. However, a comprehensive understanding of how ketamine induces oxidative stress and ferroptosis in cancer cells necessitates further investigation, with a particular focus on iron metabolism, lipid peroxidation, and antioxidant defense systems. Delving into these mechanisms will enrich our comprehension of the therapeutic potential of ferroptosis-inducing agents in cancer treatment.

5. Conclusions

In conclusion, our study highlights the potent anti-tumor effects of ketamine on EC cells, operating through various mechanisms such as the inhibition of cell growth and motility, induction of ferroptosis, and suppression of the PI3K/Akt/mTOR axis. These findings underscore the significance of further exploring the therapeutic potential of ketamine in EC treatment and emphasize the importance of investigating novel therapeutic targets and strategies in combatting this malignancy.

AVAILABILITY OF DATA AND MATERIALS

The authors declare that all data supporting the findings of this study are available within the paper and any raw data can be obtained from the corresponding author upon request.

AUTHOR CONTRIBUTIONS

HH—designed the study and carried them out; prepared the manuscript for publication and reviewed the draft of the manuscript. HH, JBZ, YZ—supervised the data collection. HH, JBZ—analyzed the data. JBZ—interpreted the data. All authors have read and approved the manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This article does not contain any studies with human participants or animals performed by any of the authors.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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