Received: 8 May 2019 | Accepted: 8 October 2019

DOI: 10.1002/jcb.29533

Journal of Cellular Biochemistry WILEY

RESEARCH ARTICLE

The new role of riluzole in the treatment of pancreatic cancer through the apoptosis and autophagy pathways

Rulin Sun[1,2,3,4] | Xujun He[2,4] | Xiaoting Jiang[1,2,4] | Houquan Tao[2,4]

[1]Clinical Laboratory Center, Zhejiang Provincial People's Hospital, Zhejiang, P. R. China

[2]People's Hospital of Hangzhou Medical College, Hangzhou, P. R. China

[3]Key Laboratory of Tumor Molecular Diagnosis and Individualized Medicine of Zhejiang Province, Zhejiang Provincial People's Hospital, Zhejiang, P. R. China

[4]Key Laboratory of Gastroenterology of Zhejiang Province, Zhejiang Provincial People's Hospital, Zhejiang, P. R. China

Correspondence
Rulin Sun, Clinical Laboratory Center, Zhejiang Provincial People's Hospital, Shangtang Road 158, 310014, Hangzhou, Zhejiang, P. R. China.
Email: sunrulin@hmc.edu.cn

Funding information
Rulin Sun, Grant/Award Numbers: C, Foundation of Zhejiang Provincial Health Bureau (N), National Natural Science Foundation of China (81803087) Natural Science Foundation of Zhejiang Province, Outstanding Young Personnel Research Fund of Zheji

Abstract

Pancreatic cancer is always diagnosed at an advanced stage. Hence, chemotherapy becomes the best choice for patients. Therefore, new anticancer drugs for pancreatic cancer are needed. Riluzole (RIL) is mainly used to treat amyotrophic lateral sclerosis clinically, but many previous studies have shown that RIL could inhibit tumors. However, no report has explored the association between RIL and pancreatic cancer. To validate this association, we performed this study. Our data showed that RIL could induce cytotoxicity, block the cell cycle, and inhibit clone formation, apoptosis, and migration in pancreatic cancer cells. Moreover, we demonstrated that RIL could suppress autophagy. However, more experiments will be needed to validate the reliability of our conclusions. In summary, our data suggest that RIL might provide clues for the development of a treatment for human pancreatic cancer in the future.

KEYWORDS

apoptosis, autophagy, chemotherapy, pancreatic cancer, riluzole

1 | INTRODUCTION

Pancreatic cancer is a global health problem.[1] According to the statistics, only eight percent of patients with pancreatic cancer can be cured, leading to the lowest rate among various tumors.[2] To date, few new approaches have affected the 5-year survival rate of pancreatic cancer.[3,4] Pancreatic cancer cells always hide deeply inside the body. Almost all pancreatic cancer patients are diagnosed at a late stage.[5,6] Therefore, many patients suffering from pancreatic cancer do not have the opportunity for operative treatment, and chemotherapy becomes the best choice. As an effective chemotherapy drug,[7] gemcitabine has been widely used in clinical practice, but an increasing number of patients are resistant to the drug.[8] Therefore, research on novel agents targeting pancreatic cancer cells has become essential in clinical work.

To date, riluzole (RIL) is mainly used to treat amyotrophic lateral sclerosis (ALS) clinically.[9] Although the number of in vivo studies on RIL antitumor activity, such as in liver cancer, glioma, and renal cancer, has increased in recent years,[10,11] there have been few investigations into the effect of RIL on pancreatic cancer. Hence, we carried out this study to evaluate the potential anticancer roles of RIL in pancreatic cancer cells.

Apoptosis, necrosis, and autophagy are the three primary modes of cell death.[12] During the process of

apoptosis, caspases control a group of kinases that affect cell survival.[13] Therefore, the relevant caspase proteins, including caspase3, are involved in apoptotic pathways.[14] Among these modes of cell death, autophagy is a relatively new type. Autophagy leads to nonapoptotic death and suppresses the progression of tumorigenesis by inhibiting tumorigenesis.[15] Notably, autophagy has been suggested to play dual roles in different kinds of cells, promoting or preventing the survival of cells.[15] However, for pancreatic cancer, autophagy always favors cell survival.[16]

In this current study, RIL-induced cytotoxic effects were extensively investigated in four types of pancreatic cell lines: PANC1, SW1990, BXPC3, and ASPC1. It has been reported that RIL could inhibit tumor progression through caspase pathways in different tumors, such as liver cancer, glioma, and renal carcinoma. According to the known information, our results are the first to prove that RIL not only affected the process of apoptosis but also inhibited autophagy in pancreatic cancer. The related mechanism study showed that RIL targeted human pancreatic cancer cells by caspase3-associated apoptosis induction and autophagy suppression. These data suggest that RIL might provide insight for the development of a new anticancer agent for the clinical treatment of human pancreatic cancer.

2 | MATERIALS AND METHODS

2.1 | Reagents

RIL was purchased from Selleck Chemicals (Shanghai, China). The final solution was dissolved in dimethyl sulfoxide at a concentration of 200 mM and then stored at −80°C. The working concentrations of 0, 25, 50, 100, 200, 400, and 1000 μM RIL, which were diluted in media, were used fresh in each experiment. All of the reagents used in cell culture were purchased from Gibco (Grand Island, NY). The CCK-8 reagent, crystal violet solution, and Annexin V-FITC/PI apoptosis detection kit were purchased from Beyotime Company (Jiangsu, China). The bicinchoninic acid protein assay kits were purchased from Life Technologies (Grand Island, NY). All indicated specific antibodies used in the current study were purchased from Abcam (Cambridge, MA).

2.2 | Cell culture

The human pancreatic cancer cell lines PANC1, SW1990, BXPC3, and ASPC1 were purchased from the Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences. These cells were maintained in Dulbecco modified Eagle medium (Gibco) containing 10% fetal bovine serum and 1% penicillin/streptomycin at 37°C with 5% CO_2 and 95% humidity.

2.3 | Cell viability assay

The PANC1, SW1990, BXPC3, and ASPC1 pancreatic cancer cells were seeded onto cell plates. Five thousand cells in 100 μL of culture medium were placed in every well. The next day, these cells were incubated with the indicated concentrations (0, 25, 50, 100, 200, 400, and 1000 μM) of RIL for 48 hours. Then, the CCK-8 reagent was applied to these cells. The absorbance was measured in an enzyme-linked immunosorbent assay microplate reader (450 nm).

2.4 | Cell-cycle analysis

PANC1 and ASPC1 cells were placed in cell plates. These cells were maintained with the corresponding concentrations (0, 100, 200, and 400 μM) of RIL. The cells were collected, washed, and fixed in 70% ethyl alcohol. The next day, the cells were stained with PI and analyzed by flow cytometry (BD). Representative flow cytometry histograms showing PI staining for cell-cycle analysis are displayed.

2.5 | Colony-forming assay

PANC1 and ASPC1 cells were seeded onto cell plates and incubated with corresponding concentrations (0, 100, 200, and 400 μM) of RIL. After 2 weeks, the cells were harvested and stained with crystal violet (0.1%). Representative photographs are shown for colony-forming analysis.

2.6 | Apoptosis assay

PANC1 and ASPC1 cells were seeded in cell plates and treated with the indicated concentrations (0, 100, 200, and 400 μM) of RIL. Then, these cells were harvested. Apoptotic cells were labeled with the annexin V/PI apoptosis detection kit according to the manufacturer's instructions. The stained cells were immediately examined by flow cytometry (BD). These cells were divided into three groups: nonapoptotic dead cells (the upper left area), late apoptotic cells (the upper right area), and early apoptotic cells (the lower right area). Representative graphs of different treatments are displayed.

2.7 | Wound-healing assay

PANC1 and ASPC1 cells were seeded onto cell plates. These cells were cultured overnight. A tip was used to

scratch the confluent monolayers. Cells were then maintained in medium with or without RIL. The cells were photographed at time points (0, 18, and 36 hours) under an inverted phase-contrast microscope.

2.8 | Western blot analysis

PANC1 and ASPC1 cells were seeded on cell plates with or without the indicated concentrations (0, 100, 200, and 400 μM) of RIL. Then, these cells were harvested and lysed. The concentrations of protein in each group were detected by the BCA method. The lysates were subjected to immunoblotting with specific antibodies against caspase3, CDK1, LC3B, p62, ubiquitinated proteins, GAPDH, and α-tubulin. The results were imaged using Imaging System (Bio-Rad).

2.9 | Statistical analysis

These data were compiled and analyzed with the GraphPad Prism software. All data are presented as the mean ± *SEM* and analyzed with one-way ANOVA in this study. When $P < .05$, the difference between groups was statistically significant. Furthermore, * means $P < .05$, ** means $P < .01$, and *** means $P < .001$.

3 | RESULTS

3.1 | Riluzole induces cytotoxicity in pancreatic cancer cells

In this study, we analyzed the cytotoxicity effect of RIL in vitro using four kinds of human pancreatic cancer cells. To check the effect of RIL on these cells, we treated PANC1, SW1990, BXPC3, and ASPC1 cells with corresponding concentrations (0, 25, 50, 100, 200, 400, and 1000 μM) of RIL. Cell viability was evaluated using a cell counting kit-8 assay (Figure 1A-D). On the one hand, these results indicated that RIL reduced pancreatic cancer cell viability as the concentration increased. On the other hand, the low concentrations (25 and 50 μM) of RIL were not significantly involved in the growth of BXPC3 cells. Moreover, the SW1990 cells were obviously affected by these low concentrations. Thus, different cells might have different sensitivities under certain conditions. Therefore, PANC1 and ASPC1 cells were used in the following experiments. Obviously, our results indicated that RIL possessed an antiproliferative effect on pancreatic cancer cells. However, to comprehensively understand the effect of RIL on pancreatic cells, we carried out further experiments.

In addition, the half-maximal inhibitory concentrations (IC50) of PANC1, SW1990, BXPC3, and ASPC1 cells were calculated and were 91.07, 44.40, 122.00, and 51.07 μM, respectively. The cell lines with the highest or lowest IC50 were not included in the cell cycle, apoptosis induction, and colony formation assays to reflect the most representative effects. Hence, the PANC1 and ASPC1 cells were used for the aforementioned experiments.

3.2 | RIL blocks the cell cycle

To examine the change in the cell cycle of these pancreatic cancer cells when treated with RIL, we analyzed the cell cycle in PANC1 and ASPC1 cells. As shown in Figure 1E and 1F, RIL blocked the cell cycle of PANC1 cells in the G2/M phase. RIL upregulated the percentage of cells in the G2/M phase in a dose-dependent manner. Similar data emerged for ASPC1 cells (Figure 1G and 1H). In summary, the data indicated that RIL might suppress pancreatic cancer by blocking the cell cycle in these cells.

3.3 | RIL inhibits the colony-forming ability of pancreatic cancer cells

In addition to the above experiments, we performed a colony formation assay to evaluate the long term antiproliferative effect of RIL. As we showed, the visible colonies of PANC1 and ASPC1 cells (Figure 2A and 2B) were obvious. However, the number of colonies in groups treated with high concentrations of RIL decreased significantly.

3.4 | RIL induces apoptosis in pancreatic cancer cells

To clarify whether RIL-induced cell death involved the apoptosis signaling pathway, we determined the number of apoptotic cells via an apoptosis detection kit using annexin V and propidium iodide. The number of apoptotic cells was analyzed by flow cytometry. The data indicated that a dose-dependent increase existed in the RIL treatment group. As shown in Figure 2C and 2D, the proportion of live PANC1 cells decreased as the concentration of RIL increased. However, the numbers of early apoptotic cells, late apoptotic cells, and total apoptotic cells increased. These results showed that RIL-treated PANC1 cells elevated the number of apoptotic cells compared with that of the control group, in a dose-dependent manner, especially at 200 and 400 μM. Similar results were observed in ASPC1 cells (Figure 2E and 2F). Briefly, our data demonstrated that RIL induces apoptosis in pancreatic cancer cells.

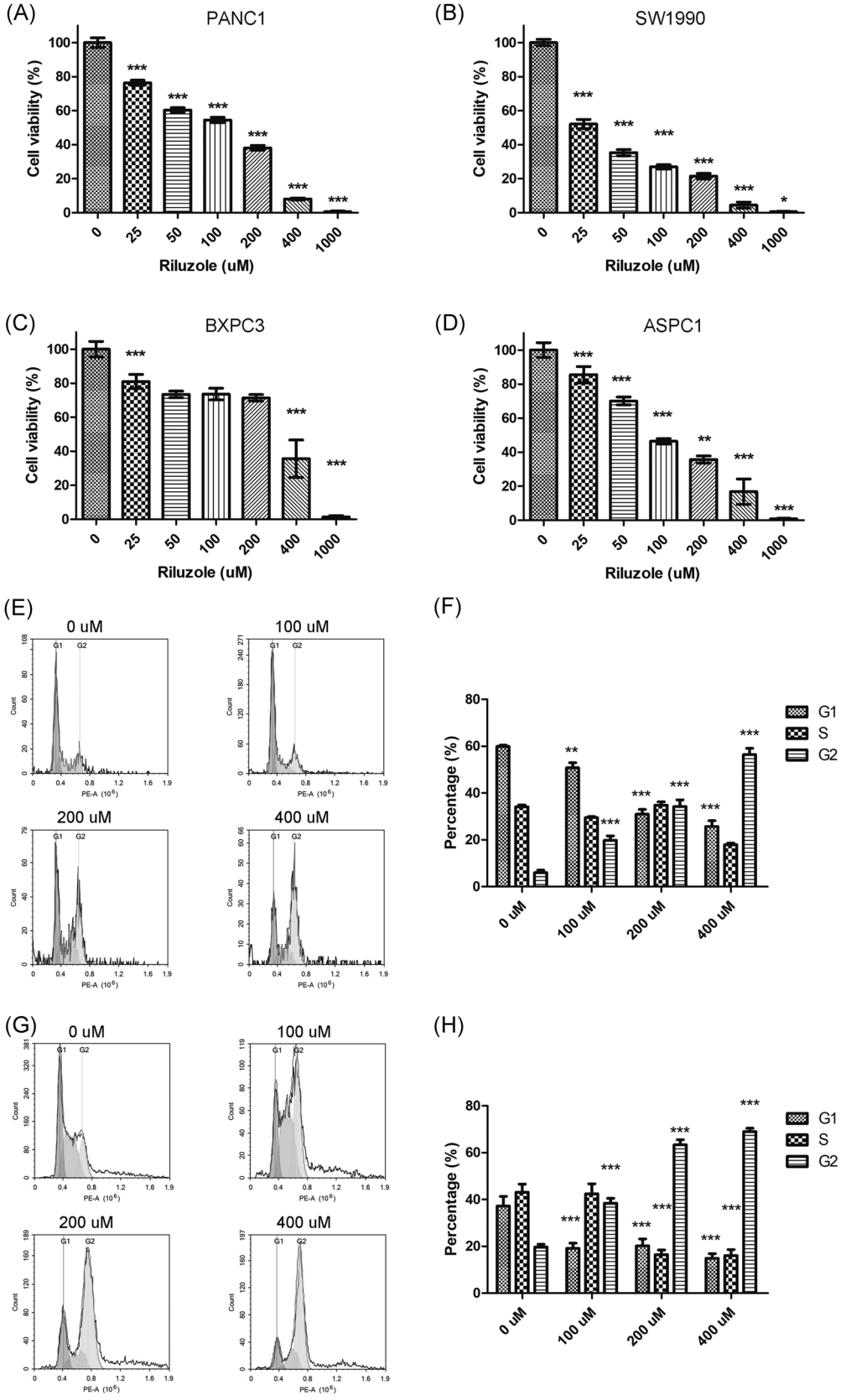

FIGURE 1 Continued.

3.5 | RIL inhibits migration in both PANC1 and ASPC1 cells

To determine whether RIL affected the migratory ability of human pancreatic cancer cells, we performed wound-healing assays. The results indicated that RIL could significantly weaken the cell migration capacity of both PANC1 and ASPC1 cells with increasing concentrations of RIL (Figure 3). Therefore, our data support that RIL could suppress pancreatic cancer cell metastasis in vitro.

3.6 | RIL promotes apoptosis and suppresses autophagy in pancreatic cancer

Apoptosis and autophagy are two major modes of cell death. To confirm whether the inhibition of cell activity involved RIL-induced apoptosis, we detected the protein levels of pro-caspase3 and caspase3 (Figure 4A and 4B), which are effector enzymes related to a cascade of apoptotic pathway events, in our study. Our data indicated that the level of activated apoptotic proteins, such as cleaved-caspase3, increased with RIL treatment. Otherwise, the protein level of CDK1, which is involved in the G2/M phase, decreased significantly as the concentration of RIL was increased (Figure 4C and 4D). Taken together, our Western blot data showed that RIL-induced apoptosis through the activation of caspase3.

Moreover, LC3B is well known as an autophagy marker and was upregulated in PANC1 and ASPC1 cells treated with RIL (Figure 4E). These results suggest that RIL also induced autophagy in these cells. To evaluate the change in these cells, we examined the protein level of p62. However, our data showed that the expression of p62 also increased with increased RIL concentration. These results further demonstrated that RIL inhibited the complete autophagy flow in pancreatic cancer cells (Figure 4E). Therefore, we concluded that RIL promoted apoptosis but inhibited autophagy in a dose-dependent manner in pancreatic cancer cells.

Furthermore, we detected the protein level of ubiquitinated proteins that might also cause protein degradation in our system. However, no significant difference was found between cells treated with or without RIL. This result suggested that the process of ubiquitination was not involved in cell death in our experiments (Figure 4F).

3.7 | The cytotoxicity of RIL is enhanced by chloroquine and inhibited by rapamycin

To further address the inhibitory effect of RIL on autophagy, we treated PANC1 and ASPC1 cells using the autophagy enhancer rapamycin (RP) or inhibitor chloroquine (CQ). Then, the cell viability was examined using CCK-8 reagent. Our data showed that the cells treated with CQ showed decreased viability (Figure 4G and 4I). Meanwhile, the cells treated with RP showed rescued viability (Figure 4H and 4J). The results demonstrated that the autophagy induced by RIL might be protective in pancreatic cancer and that autophagy suppression contributed to the anticancer role of RIL.

4 | DISCUSSION

In the current study, the cytotoxicity of RIL was first shown in four types of pancreatic cancer cells. During RIL-mediated suppression of cell proliferation, RIL not only promoted apoptosis (which was consistent with the published studies) but also inhibited autophagy (which was first validated in our system). Moreover, RIL could inhibit migration and block the cell cycle in PANC1 and ASPC1 cells.

Currently, surgery and chemotherapy remain the most common methods used to treat cancers. The late diagnosis and resistance to current chemotherapies lead to the poor survival rate of pancreatic cancer patients. Most pancreatic cancer patients are not suitable for surgery therapy.[17] Chemotherapy that inhibits the growth of cancer cells via directly destroying them becomes the best choice.[18] However, the therapy has some limitations and adverse effects.[19] Therefore, new anticancer drugs for pancreatic cancer are needed.

The research on mechanisms demonstrated that RIL was a noncompetitive antagonist of metabotropic glutamate receptors (mGluR). RIL inhibited the release of glutamate at neural synapses.[20] Currently, RIL is used to treat ALS in clinical work.[9] However, an increasing number of studies have shown that mGluR plays important roles not only in the nervous system but also in tumorigenesis, such as in melanoma.[21,22] Therefore, as an antagonist of mGluR1, RIL might have an antitumor

FIGURE 1 Riluzole induces concentration-dependent cytotoxicity and blocks the cell cycle in pancreatic cancer cells. PANC1(A), SW1990(B), BXPC3(C), and ASPC1(D) cells were treated with the indicated concentrations (0, 25, 50, 100, 200, 400, and 1000 μM) of riluzole for 48 hours, respectively. The cell viability of pancreatic cancer cells was detected by CCK-8 assay (n = 6/group). Cell cycle in PANC1 and ASPC1 cells were determined after treatment with indicated concentrations (0, 100, 200, and 400 μM) of riluzole for 24 hours. Representative flow cell graphics of PANC1 (E) and ASPC1 (G) cells were followed by PI staining for cell-cycle analysis. Quantification of cell-cycle analysis on PANC1 (F) and ASPC1 (H) cells were showed. (*$P < .05$, **$P < .05$, and ***$P < .001$)

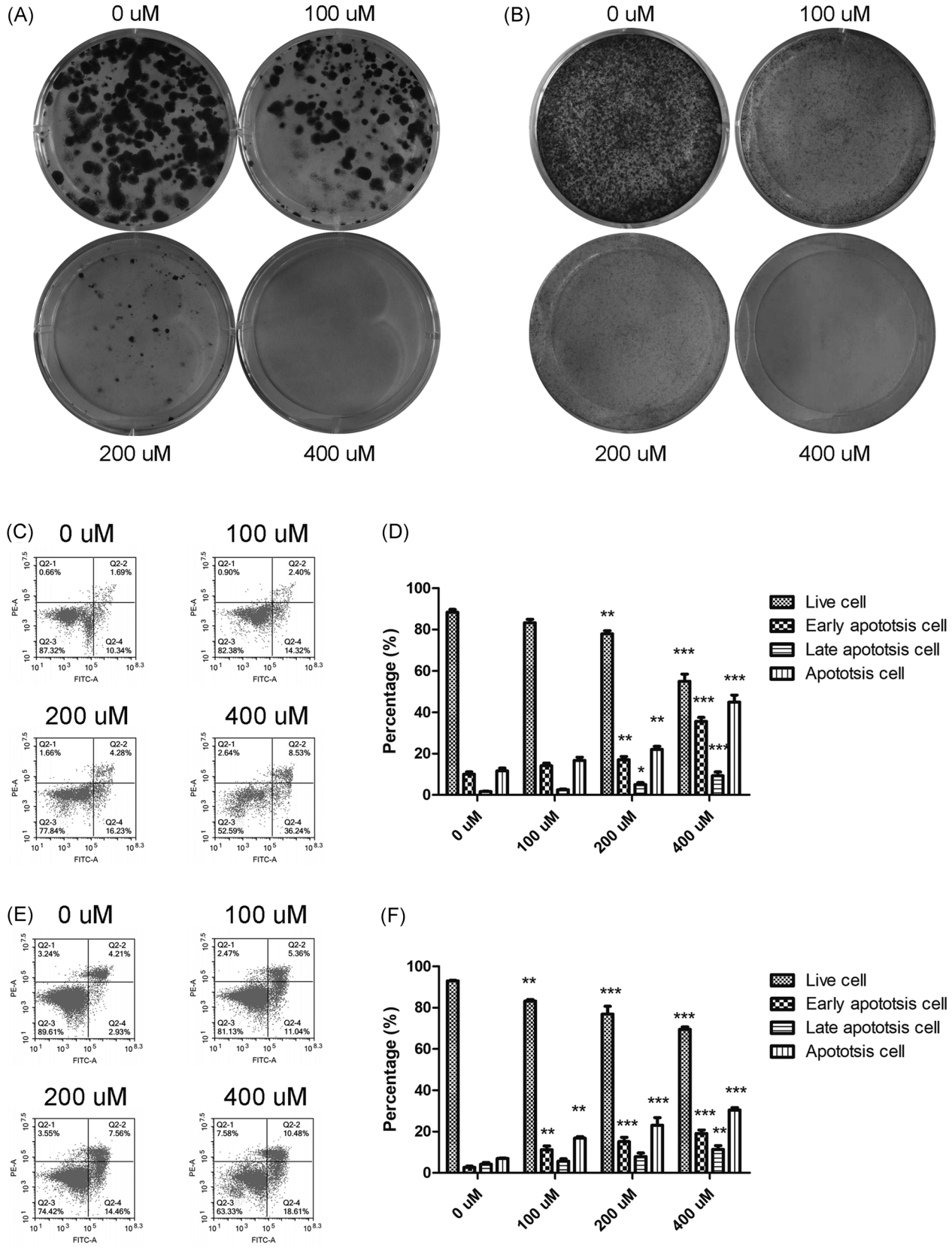

FIGURE 2 Continued.

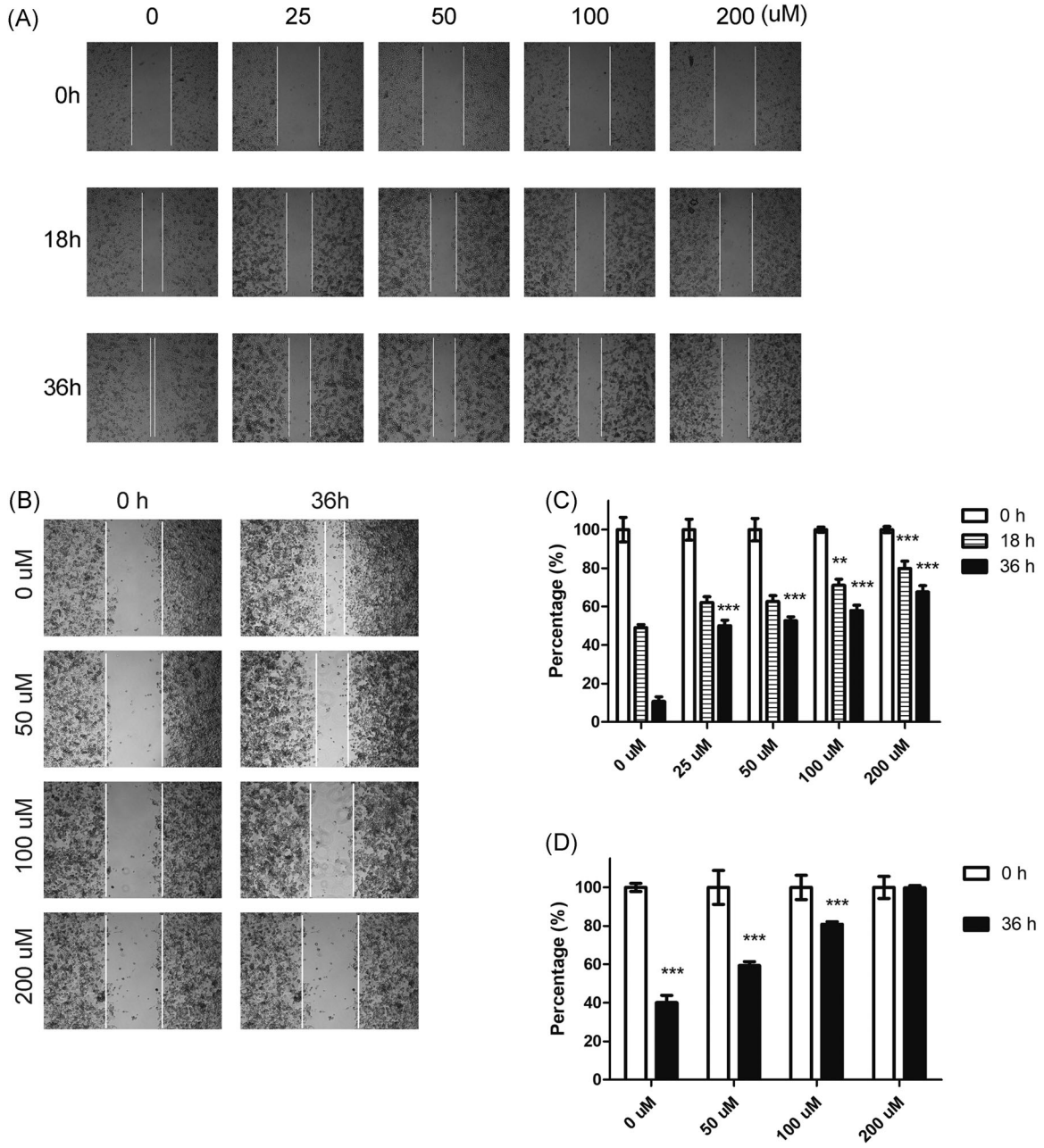

FIGURE 3 Inhibition of Riluzole on migration in both PANC1 and ASPC1 cells. PANC1 and ASPC1 cells were treated with indicated concentrations of riluzole (0, 25, 50, 100, and 200 μM) for 0 to 36 hours. Wound-healing assays were carried out to assess cell migration inhibition. Representative images of PANC1 (A) and ASPC1 (B) cells were displayed. Bar chart indicating wound healing in PANC1 (C) and ASPC1 (D) cells. (**$P < .05$ and ***$P < .001$)

FIGURE 2 The colony-forming ability and apoptosis in pancreatic cancer cells are affected by riluzole. Riluzole showed concentration-dependent (0, 100, 200, and 400 μM) inhibition on the cell colony formation of pancreatic cancer cells. Representative photographs of PANC1 (A) and ASPC1 (B) cells with different treatments, followed by crystal violet staining, were displayed. Cell apoptosis in PANC1 and ASPC1 cells were detected after treatment with indicated concentrations (0, 100, 200, and 400 μM) of Riluzole for 24 hours. Representative graphics of PANC1 (C) and ASPC1 (E) cells were followed by annexin-V/PI staining and flow cytometry analysis. Quantification of apoptosis analysis on PANC1 (D) and ASPC1 (F) cells were showed. (*$P < .05$, **$P < .05$, and ***$P < .001$)

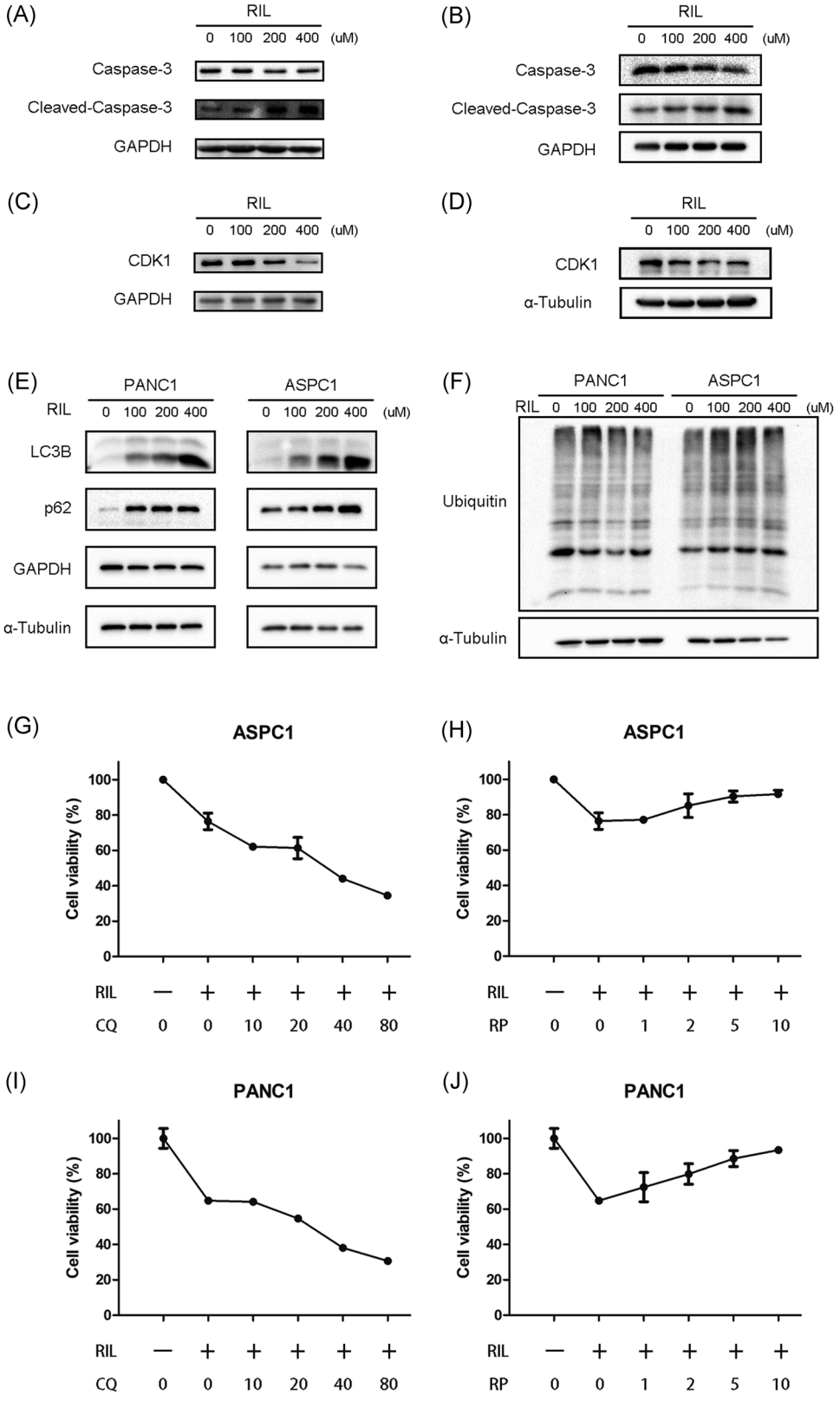

FIGURE 4 Continued.

effect.[23] A published study indicated that RIL inhibited liver cancer.[10] During the process, RIL affected the mGluR1 distribution on the cell surface. The release of glutamate in cells was decreased. More glutamate with cysteine inhibited the synthesis of GSH, leading to the eventual apoptosis of cells.[24]

Several recent studies demonstrated that RIL exhibited antitumor activities in many kinds of cancer cells by affecting the processes of cell-cycle arrest and apoptosis.[10,11,21,25] The promising anticancer roles of RIL have been shown to be regulated by activating caspase-mediated apoptotic signaling pathways in various tumors. Several previous studies demonstrated that RIL promoted the apoptosis of other cancer cells through caspase3 signaling pathways.[10,21] However, the other antiproliferative effects and molecular mechanism of RIL on pancreatic cells remain unclear.

A large number of studies have shown that RIL induces caspase3 activation-dependent apoptosis in various tumor cells.[25] Our results provided supporting evidence that RIL-induced antiproliferation effects on pancreatic cancer cells were associated with the activation of caspase3 in time- and dose-dependent manners.

Caspases are well known to be involved in apoptosis are composed of many kinases.[13] Therefore, apoptosis induced by caspase3 contributes to the cytotoxicity of pancreatic cancer. However, accumulating evidence has shown that the process of tumor cell death includes apoptosis and autophagy in chemotherapy.

Autophagy is an important process, during which cells dispose of unnecessary proteins by sending them to the lysosome.[26] They are degraded and recycled in this organelle. Autophagy may have dual roles, including an antitumor effect via promoting cell death in specific conditions and a function that promotes survival in starved cells.[15] As a marker of autophagy, LC3 has two members, LC3-I and LC3-II. The number of autophagic vacuoles always correlates with the expression of LC3-II.[27] Moreover, p62 has been proven to be a mediator in the formation of autophagic vacuoles.[28] According to published studies, autophagy affects cell viability and death in pancreatic cancer cells.[29] To date, no studies have reported on autophagy in RIL-treated cancer cells. Thus, autophagy in pancreatic cancer cells needs to be studied.

In the current study, our data demonstrated that RIL affected autophagy in pancreatic cancer cells and induced the expression of autophagy-related proteins, such as p62, LC3-I, and LC3-II. The RIL-induced inhibition of autophagy led to a cancer-suppressing process and promoted death in PANC1 and ASPC1 cells. To further validate the effect of autophagy in PANC1 and ASPC1 cells treated with RIL, we treated cells with either the autophagy inhibitor CQ or the autophagy enhancer RP.[30] Our data indicated that the cells treated with RIL and CQ had lower survival than the cells treated with RIL alone, which also had a decreased viability. Thus, the autophagy inhibitor CQ could augment the antitumor role of RIL in PANC1 and ASPC1 cells. Furthermore, the autophagy enhancer RP had the opposite effect. Therefore, we inferred that autophagy in pancreatic cancer cells might be cytoprotective and that RIL might function as an autophagy inhibitor similar to CQ. Autophagic flux could be blocked by RIL, inhibiting autophagy in pancreatic cancer cells. However, more experiments are needed to validate the molecular mechanism.

Although apoptosis and autophagy are various types of cell death that have different purposes, a common point may exist in their regulation. For instance, caspase8, which is a key molecule in apoptosis signaling pathways, has been reported to cause autophagy in recent years.[31] Therefore, a fine balance between apoptosis and autophagy is important for the fate of cells.[32,33] Overall, the interaction between autophagy and apoptosis is complex and important for cytotoxicity.

In the future, we will further explore whether apoptosis is associated with autophagy in pancreatic cancer cell death. Otherwise, the corresponding animal experiments will be carried out in our system to validate the reliability of our conclusions.

5 | CONCLUSION

In conclusion, our results demonstrated that riluzole (RIL) affected the viability of pancreatic cancer cells. The

FIGURE 4 The cytotoxicity of riluzole, involved in caspase and autophagy pathways, is enhanced by chloroquine (CQ) and inhibited by rapamycin (RP) in pancreatic cancer cells. PANC1 and ASPC1 cells were treated without or with indicated concentrations of riluzole (0, 100, 200, and 400 μM) for 24 hours. Detection of apoptosis-associated proteins (caspase3 and cleaved-caspase3) was executed by Western blot analysis in PANC1 (A) and ASPC1 (B) cells. Investigation of cell cycle-associated proteins (CDK1) was carried out by Western blot analysis in PANC1 (C) and ASPC1 (D) cells. The expression levels of autophage-associated proteins (LC3B and p62) and ubiquitinated protein in PANC1 and ASPC1 cells were detected by Western blot analysis (E,F). PANC1 and ASPC1 cells were treated without or with riluzole (200 μM) for 24 hours. Meanwhile, ASPC1 and PANC1 cells were treated with indicated concentrations of autophagy inhibitor (CQ) (G and I) or enhancer (RP) (H,J). Cell viability of PANC1 and ASPC1 cells was checked using CCK-8 assay

role of RIL in suppressing autophagy was proven in our study. Previous studies have demonstrated that the promotion of apoptotic cell death and the suppression of the cell-protective effects induced by autophagy could be used in the development of new therapies for tumors. Hence, our results suggest that RIL could provide clues for the development of treatments for pancreatic cancer.

ACKNOWLEDGMENTS

This work was supported by the Outstanding Young Personnel Research Fund of Zhejiang Provincial People's Hospital (No. ZRY2016C004), the Natural Science Foundation of Zhejiang Province, China (No. LQ18H160021), the National Natural Science Foundation of China (No. 81803087), and the Foundation of Zhejiang Provincial Health Bureau (No. 2019RC098).

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

ORCID

Rulin Sun http://orcid.org/0000-0002-8525-8929

REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. *CA Cancer J Clin*. 2018;68(1):7-30. https://doi.org/10.3322/caac.21442
2. Kindler HL. A glimmer of hope for pancreatic cancer. *N Engl J Med*. 2018;379(25):2463-2464. https://doi.org/10.1056/NEJMe1813684
3. Reiter JG, Iacobuzio-Donahue CA. Pancreatic cancer: pancreatic carcinogenesis - several small steps or one giant leap? *Nat Rev Gastroenterol Hepatol*. 2016;14(1):7-8. https://doi.org/10.1038/nrgastro.2016.190
4. Vakoc CR, Tuveson DA. Soils and seeds that initiate pancreatic cancer metastasis. *Cancer Discov*. 2017;7(10):1067-1068. https://doi.org/10.1158/2159-8290.CD-17-0887
5. Lin QJ, Yang F, Jin C, Fu DL. Current status and progress of pancreatic cancer in China. *World J Gastroenterol*. 2015;21(26):7988-8003. https://doi.org/10.3748/wjg.v21.i26.7988
6. Hackeng WM, Hruban RH, Offerhaus GJ, Brosens LA. Surgical and molecular pathology of pancreatic neoplasms. *Diagn Pathol*. 2016;11(1):47. https://doi.org/10.1186/s13000-016-0497-z
7. Zhang D, Wu J, Liu S, Zhang X, Zhang B. Network meta-analysis of Chinese herbal injections combined with the chemotherapy for the treatment of pancreatic cancer. *Medicine (Baltimore)*. 2017;96(21):e7005. https://doi.org/10.1097/MD.0000000000007005
8. Chen R, Lai LA, Sullivan Y, et al. Disrupting glutamine metabolic pathways to sensitize gemcitabine-resistant pancreatic cancer. *Sci Rep*. 2017;7(1):7950. https://doi.org/10.1038/s41598-017-08436-6
9. Jaiswal MK. Riluzole and edaravone: a tale of two amyotrophic lateral sclerosis drugs. *Med Res Rev*. 2019;39(2):733-748. https://doi.org/10.1002/med.21528
10. Seol HS, Lee SE, Song JS, et al. Glutamate release inhibitor, riluzole, inhibited proliferation of human hepatocellular carcinoma cells by elevated ROS production. *Cancer Lett*. 2016;382(2):157-165. https://doi.org/10.1016/j.canlet.2016.08.028
11. Zhang C, Yuan XR, Li HY, et al. Anti-cancer effect of metabotropic glutamate receptor 1 inhibition in human glioma U87 cells: involvement of PI3K/Akt/mTOR pathway. *Cell Physiol Biochem*. 2015;35(2):419-432. https://doi.org/10.1159/000369707
12. Ou L, Lin S, Song B, Liu J, Lai R, Shao L. The mechanisms of graphene-based materials-induced programmed cell death: a review of apoptosis, autophagy, and programmed necrosis. *Int J Nanomedicine*. 2017;12:6633-6646. https://doi.org/10.2147/IJN.S140526
13. Neves SP, de Carvalho NC, da Silva MM, et al. Ruthenium complexes containing heterocyclic thioamidates trigger caspase-mediated apoptosis through MAPK signaling in human hepatocellular carcinoma cells. *Front Oncol*. 2019;9:562. https://doi.org/10.3389/fonc.2019.00562
14. Milani R, Brognara E, Fabbri E, et al. Targeting miR1555p and miR2213p by peptide nucleic acids induces caspase3 activation and apoptosis in temozolomide resistant T98G glioma cells. *Int J Oncol*. 2019;55(1):59-68. https://doi.org/10.3892/ijo.2019.4810
15. Lorente J, Velandia C, Leal JA, et al. The interplay between autophagy and tumorigenesis: exploiting autophagy as a means of anticancer therapy. *Biol Rev Camb Philos Soc*. 2018;93(1):152-165. https://doi.org/10.1111/brv.12337
16. Yang A, Herter-Sprie G, Zhang H, et al. Autophagy sustains pancreatic cancer growth through both cell-autonomous and nonautonomous mechanisms. *Cancer Discov*. 2018;8(3):276-287. https://doi.org/10.1158/2159-8290.CD-17-0952
17. Goral V. Pancreatic cancer: pathogenesis and diagnosis. *Asian Pac J Cancer Prev*. 2015;16(14):5619-5624.
18. Ahmad A, Khan F, Mishra RK, Khan R. Precision cancer nanotherapy: evolving role of multifunctional nanoparticles for cancer active targeting. *J Med Chem*. 2019. https://doi.org/10.1021/acs.jmedchem.9b00511
19. Fahmy SA, Brussler J, Alawak M, El-Sayed MMH, Bakowsky U, Shoeib T. Chemotherapy based on supramolecular chemistry: a promising strategy in cancer therapy. *Pharmaceutics*. 2019;11(6):E292. https://doi.org/10.3390/pharmaceutics11060292
20. Satkunendrarajah K, Nassiri F, Karadimas SK, Lip A, Yao G, Fehlings MG. Riluzole promotes motor and respiratory recovery associated with enhanced neuronal survival and function following high cervical spinal hemisection. *Exp Neurol*. 2016;276:59-71. https://doi.org/10.1016/j.expneurol.2015.09.011
21. Wen Y, Li J, Koo J, et al. Activation of the glutamate receptor GRM1 enhances angiogenic signaling to drive melanoma progression. *Cancer Res*. 2014;74(9):2499-2509. https://doi.org/10.1158/0008-5472.CAN-13-1531
22. Yu LJ, Wall BA, Wangari-Talbot J, Chen S. Metabotropic glutamate receptors in cancer. *Neuropharmacology*. 2017;115:193-202. https://doi.org/10.1016/j.neuropharm.2016.02.011

23. Martino JJ, Wall BA, Mastrantoni E, et al. Metabotropic glutamate receptor 1 (Grm1) is an oncogene in epithelial cells. *Oncogene*. 2013;32(37):4366-4376. https://doi.org/10.1038/onc.2012.471
24. Lv C, Yuan X, Zeng HW, Liu RH, Zhang WD. Protective effect of cinnamaldehyde against glutamate-induced oxidative stress and apoptosis in PC12 cells. *Eur J Pharmacol*. 2017;815:487-494. https://doi.org/10.1016/j.ejphar.2017.09.009
25. Lemieszek MK, Stepulak A, Sawa-Wejksza K, Czerwonka A, Ikonomidou C, Rzeski W. Riluzole Inhibits Proliferation, Migration and Cell Cycle Progression and Induces Apoptosis in Tumor Cells of Various Origins. *Anticancer Agents Med Chem*. 2018;18(4):565-572. https://doi.org/10.2174/1871520618666180228152713
26. Tait SW, Ichim G, Green DR. Die another way--non-apoptotic mechanisms of cell death. *J Cell Sci*. 2014;127(Pt 10):2135-2144. https://doi.org/10.1242/jcs.093575
27. Jiang TX, Zou JB, Zhu QQ, et al. SIP/CacyBP promotes autophagy by regulating levels of BRUCE/Apollon, which stimulates LC3-I degradation. *Proc Natl Acad Sci USA*. 2019;116(27):13404-13413. https://doi.org/10.1073/pnas.1901039116
28. Luhr M, Torgersen ML, Szalai P, et al. The kinase PERK and the transcription factor ATF4 play distinct and essential roles in autophagy resulting from tunicamycin-induced ER stress. *J Biol Chem*. 2019. https://doi.org/10.1074/jbc.RA118.002829
29. Bryant KL, Der CJ. Blocking autophagy to starve pancreatic cancer. *Nat Rev Mol Cell Biol*. 2019;20(5):265. https://doi.org/10.1038/s41580-019-0120-8
30. Fu ZP, Cheng X, Kuang J, et al. CQ sensitizes human pancreatic cancer cells to gemcitabine through the lysosomal apoptotic pathway via reactive oxygen species. *Mol Oncol*. 2018;12(4):529-544. https://doi.org/10.1002/1878-0261.12179
31. Zhang YB, Gong JL, Xing TY, Zheng SP, Ding W. Autophagy protein p62/SQSTM1 is involved in HAMLET-induced cell death by modulating apotosis in U87MG cells. *Cell Death Dis*. 2013;4:e550. https://doi.org/10.1038/cddis.2013.77
32. Marino G, Niso-Santano M, Baehrecke EH, Kroemer G. Self-consumption: the interplay of autophagy and apoptosis. *Nat Rev Mol Cell Biol*. 2014;15(2):81-94. https://doi.org/10.1038/nrm3735
33. Xue R, Yang J, Jia L, et al. Mitofusin2, as a protective target in the liver, controls the balance of apoptosis, and autophagy in acute-on-chronic liver failure. *Front Pharmacol*. 2019;10:601. https://doi.org/10.3389/fphar.2019.00601

How to cite this article: Sun R, He X, Jiang X, Tao H. The new role of riluzole in the treatment of pancreatic cancer through the apoptosis and autophagy pathways. *J Cell Biochem*. 2019;1–11. https://doi.org/10.1002/jcb.29533