Scutellarin combined with Lidocaine: a new combination of anti-glioma drugs

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Abstract

Background

Glioma is the most common primary intracranial tumors. Although great achievements in the treatment have been made, the efficacy is still not satisfactory, which imposes a great burden on patients and society. Therefore, the exploration of new and effective anti-glioma drugs is urgent.

Methods

Human glioma cells U251 and LN229 cells were included in the study. The proliferation was detected by cell counting kit-8, plate clone formation assay, EdU incorporation assay and xCELLigence real-time cell analyzer. The cell apoptosis was evaluated by TUNEL assay and flow cytometry. The transwell assay was for assessing the migration. Moreover, Western blot was performed to detect the protein level of Epidermal growth factor receptor (EGFR).

Results

In present study, we found that Scutellarin(SCU) and Lidocaine suppressed the proliferation and migration, and induced the apoptosis of human glioma cells, including U251 and LN229 cells, in a dose-dependent manner. Moreover, the combination of Scutellarin and Lidocaine further restrained the proliferation and migration ability of U251 and LN229 cells, while induced their apoptosis. Mechanistically, the effect of Scutellarin and its combination with Lidocaine on glioma cells was partially associated with the downregulation of EGFR protein.

Conclusions

Scutellarin and Lidocaine exert a synergistic effect on suppressing the proliferation and migration and induce the apoptosis of glioma cells partly via repressing the EGFR expression.

Introduction

Glioma is one of the most common primary intracranial tumors, which is formed by a special type of brain cells (astrocytes, oligodendrocytes, ependymal cells, etc)[1–4]. The symptoms and signs caused by glioma mainly depend on the space occupying effect and the function of the affected brain regions[5–7]. Due to its space occupying effect, glioma can lead to headache, nausea and vomiting, epilepsy, blurred vision and other symptoms. In addition, because of its influence on the function of local brain regions, the affected patients can also experience other features. For example, gliomas in the language area can cause difficulties in language expression and understanding. Of course, the malignant degree of glioma determines the speed of the symptom progression. In most cases, the exact etiology is unknown[5–7].

However, some known genetic diseases, such as neurofibromatosis (type I) and tuberculous sclerosis, are genetic predisposing factors for glioma[5–7]. More importantly, there is currently no cure for glioma. Treatment is palliative and may include surgery, radiotherapy and/or chemotherapy[5–7]. However, the operation is traumatic and radiotherapy is often insensitive. Therefore, the exploration of new and effective anti-glioma drugs is urgent.

Scutellarin (4',5,6-trihydroxyflavone-7-glucuronide) belongs to the active ingredient of flavonoids isolated from Erigeron breviscapus, which hold broad pharmacological effects and has been used to treat cardiac ischemic and cerebral ischemic diseases[8–11]. Among them, Scutellarin now has been attracted increasing attention for its anti-tumor effect on most tumors. In colorectal cancer, Scutellarin sensitized RSV- and 5-FU-triggered apoptosis by promoting caspase-6 activation in a P53 dependent manner[12]. In hepatocellular carcinoma, Scutellarin down-regulated STAT3, BCL-XL and Mcl-1 through STAT3 signaling pathway to inhibit cell proliferation and induce apoptosis[13]. In human tongue squamous cell carcinoma, Scutellarin restrained the proliferation and migration of tumor cells by inhibiting matrix metalloproteinases-2 and 9 (MMP-2 and MMP-9) and αVβ6 integrin[14]. However, the role of Scutellarin in gliomas remains to be further illustrated.

Lidocaine is a commonly used local anesthetic and antiarrhythmic drug[15]. In recent years, studies have found that Lidocaine exerts anti-tumor effect. Firstly, Lidocaine repressed colon cancer cells proliferation, and induced their cell-cycle arrest and apoptosis by activation of apoptosis protein pathway[16, 17]. In addition, Lidocaine inhibited the growth of HepG2 cells in a dose- and time-dependent manner[18]. What's more, Lidocaine suppressed tumor development and enhanced the sensitivity of cisplatin, so combining Lidocaine with cisplatin may be a novel treatment option for hepatocellular carcinoma and breast cancer[18, 19]. Mechanistically, Lidocaine and ropivacaine exerted demethylating effects on breast cancer cell at their clinically relevant doses[20]. Furthermore, clinical studies demonstrated that intraoperative intravenous Lidocaine infusion was associated with improved overall survival in patients undergoing pancreatectomy[21]. Therefore, Lidocaine is a potential and useful antitumor agent. Nevertheless, the effect of Lidocaine on glioma is still not well-understood.

The aim of this study was to determine the potential effects of Scutellarin and its combination with Lidocaine on the human glioma cells. Moreover, we also assessed the molecular mechanisms involved in Scutellarin- and Lidocaine-induced anti-glioma effect. The results will provide experimental and theoretical basis for the clinical treatment of glioma.

Materials And Methods

Cell culture

Glioma cell lines U251 and LN229 cells were obtained from American Type Culture Collection (ATCC, Manassas, Virginia, USA). Both cells were cultured at 37 °C in a humidified incubator with 5% CO_2 in

Dulbecco's modified Eagle's medium (DMEM) (Hyclone) supplemented with 10% foetal bovine serum (FBS) (HyClone, Logan, UT, USA), 100 U/ml penicillin and 100 μg/ml streptomycin (Hyclone).

Cell viability analysis

Cell count kit 8 (CCK-8 kit; DOJINDO, Japan) was used for cell viability analysis. Briefly, 3000~5000 cells were seeded in one well of 96-well plates(Corning). After incubation for 24h, the drugs(SCU and Lidocaine) were administrated. For 48h of intervention, 10μl of CCK-8 reagent was added into each well and incubated at 37°C for 4 hours. The absorbance (OD value) was acquired by Multiskan Spectrum Microplate Spectrophotometer (Thermo) at a wavelength of 450 nm. The inhibition rate was calculated by this formula: inhibition rate=(Ac-As)/(Ac-Ab)*100%, in which As was absorption of drug-added wells, Ac was absorption of solvent-added wells, and Ab was absorption of blank control wells.

Real-time analysis of cell proliferation

The xCELLigence Real Time Cell Analyzer (ACEA), which could be placed in a incubator containing 5% CO_2, 95% humidity and at 37℃, was applied to continuously monitor the proliferation of U251 cells. Moreover, this apparatus could integrate the relative impedance change of microelectronic sensors on E-plate 16 (Roche Diagnostics GmbH) bottom, whose output was the cell index used for evaluating the cell proliferation ability. Breifly, U251 cells were seeded at E-plate 16 at 5000 cells/well. At about 24h, the drugs were added into the medium. From the time of cell inoculation, the cells were monitored every 15 minutes for a total of 3 days. Data analysis was carried out by xCELLigence Real Time Cell Analyzer software 1.2.

Plate clone formation assay

As described previously[1], 1000 cells were seeded per well in 6-well plates (Corning). After the cells were attached overnight, the drugs was added into the medium and the cells were cultured in a 37 °C incubator for 14 days. On the 14th day, the cells were fixed with 4% paraformaldehyde for 15 min and then stained with 0.5% crystal violet (Beyotime). Finally, the digital camera (Nikon) was used to take a picture of the formed clones and the clones were counted.

EdU incorporation assay

EdU incorporation assay were performed by EdU cell proliferation detection kit (RiboBio) according to the manufacturer's instructions. In brief, the cells were inoculated into 96-well plates with 5000/well. After 48h of drug administration, 100μl 50μM EdU medium was added into each well and the plates were incubated at 37 °C for 2h. Then the cells were fixed for 30 minutes by 4% paraformaldehyde at room temperature and perforated with 0.5% TritonX-100 solution for 10 minutes. After rinsing with 0.01M PBS, the cells were incubated with 1× Apollo® dyeing solution for 30 minutes at room temperature and in the dark. Finally, the cell nucleus was stained with 1× Hoechst 33342 reaction solution. Images were acquired

by inverted fluorescence microscopy camera system (Leica). The proliferation rate was equal to the ratio of EdU positive cells (red) to Hoechst positive cells (blue).

TUNEL assay

The Terminal-deoxynucleoitidyl Transferase Mediated Nick End Labeling (TUNEL) detection kit (Roche) was applied for apoptosis analysis. Following the instructions, the cells were fixed in 4% paraformaldehyde for 15 min at room temperature and then permeabilizeed by 0.1% Triton X-100 on ice (2-8°C) for 2 minute. Then the prepared 50μl TUNEL reaction mixture, consisting of 50μl TdT and 450μl fluorescein labelled dUTP solution, was added into each well and the reaction was conducted at 37°C for 1 hour in the dark. After rinsing, the cell nuclei were stained with 5μg/ml DAPI (Beyotime). The images were captured by inverted fluorescence microscopy camera system (Leica). The apoptotic rate was equal to the ratio of TUNEL positive cells (red) to DAPI positive cells (blue).

Apoptosis analysis by flow cytometry

Cell apoptosis analysis was conducted with Annexin-V cell apoptosis detection kit (BD Bioscience) and Flow cytometry. As described previously[1], the cells intervened for 48h were stained with the 20ug/ml Annexin-V labeled with FITC for 30min and 50μg/ml PI for 5min at room temperature and in the dark, respectively. After adding 400μl of combined buffer, the samples were tested by flow cytometry immediately according to the standards. The living cells were not marked with both Annexin-V and PI, the early apoptotic cells were stained with Annexin-V only, the late apoptotic cells were stained with both Annexin-V and PI, and the mechanically injured cells were only labelled with PI.

Transwell assay

The migration ability was detected by transwell assay. Briefly, 5×10^4 cells intervened by drugs for 48h were resuspend with serum-free medium and seeded into Transwell Chamber (Millipore), this was the upper chamber, while the DMEM medium containing 10% FBS was added into the lower chamber. After 48h of migration, the non-invasive cells in the upper chamber were gently removed, and the invasive cells on the bottom of the upper chamber were fixed with 4% paraformaldehyde and stained with 0.5% crystal violet. After rinsing, the images were photographed by a microscope. 15 fields were captured in each group, and the invasive cells were counted and statistically analyzed.

Western blotting

Referring to the previous description[1], cells (5×106) were lysed for 20 min with lysis buffer (Beyotime Biotechnology) containing protease inhibitors (Roche, Indianapolis, IN, USA). After centrifugation at 12000×g for 15 min at 4 °C, the protein concentrations were determined by the BCA method (Beyotime) and the samples were resolved by SDS/PAGE, transferred to PVDF membranes (Immobilon-P membrane, Millipore, Massachusetts, USA), and analysed by immune blotting using HRP-conjugated secondary antibodies(1:5000, GeneTex, USA). The membranes were blocked with 5% (wt/vol) skimmed milk in TBS

for 1 hour at room temperature and then incubated with primary antibodies-anti-EGFR(Rabbit, 1:1000, Proteintech) and β-actin(Mouse, 1:5000, Proteintech) at 4 °C overnight. An enhanced chemiluminescent (ECL) chromogenic substrate(Biosharp) was used to visualize the bands. Blotting was captured by Molecular Imager ChemiDocTM XSR+ Gel Imaging System (BIO-RAD) and analyzed using ImageJ software (NIH). In semi-quantitative analysis of the target protein, each sample was normalized to β-actin.

Statistical analysis

In this study, the data were expressed as Mean±SD. One-way ANOVA was performed on continuous data from three independent groups and above, and analysis of variance of factorial design was for the factorial design data. All the analyses were performed using SPSS 16.0 software. As long as P□0.05, the difference was statistically significant.

Results

1. **Lidocaine inhibited the proliferation of glioma cells in a dose-dependent manner.**

We detected the cell viability of U251 and LN229 cells intervened with Lidocaine for 48h by cell-count kit 8, and found that Lidocaine inhibited the proliferation of these cells in a dose-dependent manner (Fig 1. A, B). Moreover, the IC50 (the concentration when the inhibitory efficiency reaches 50%) of Lidocaine in U251 and LN229 cells was 2.531mM and 1.211mM, respectively (Fig 1. C, D). The 95% confidence interval for them was separately 2.255mM~2.841mM and 1.022mM~1.435mM.

In addition, the effect of Lidocaine on the proliferation of U251 cells was also continuously monitored by xCELLigence Real Time Cell Analyzer. Our results showed that the cell index curves of proliferation in all groups basically coincided before Lidocaine administration; but after administration, the cell index of U251 decreased as the Lidocaine dose increased (Fig 1. E, F). At 24h of Lidocaine intervention, compared with control group (0.133% DMSO), the cell index was going down, and the difference was significant ($P < 0.01$) (Fig 1. E, F). With the extension of intervention time, such as at 36h, 48h and 60h, the difference of cell index between Lidocaine groups and control group was all statistically significant ($P < 0.01$) (Fig 1. E, F). Furthermore, the inhibitory effect of Lidocaine 3mM was better than that of Lidocaine 1.5mM (Fig 1. E, F).

2. **Scutellarin and its combination with Lidocaine suppressed the proliferation of glioma cells.**

Many studies have reported that Scutellarin(SCU) has anti-tumor effect. Here we studied the anti-glioma effect of SCU combined with Lidocaine. Firstly, the cell viability of U251 and LN229 cells intervened by SCU and its combination with Lidocaine 1mM for 24, 48, and 72 h was detected by CCK8. The results demonstrated that SCU possessed a concentration-dependent effect of inhibiting cell viability on glioma cells (Fig 2. A, B, C). For U251 cells, it suppressed the cell viability only at high concentration (400μM), but at low concentration (100μM) it held a certain effect on the proliferation. However, the proliferation effect

of SCU at low concentration could be reversed by Lidocaine 1mM (Fig 2. A, B, C). For LN229 cells, the inhibition rate was increased with the rise of SCU dose, and the inhibitory effect of SCU on LN229 was enhanced by Lidocaine 1mM (Fig 3. B, D).

Secondly, the cell cloning was detected by clone formation assay. After the cells adhered to the wall overnight, Scutellarin and its combination with Lidocaine 1mM were administrated for 2 weeks and the medium was changed once every three days (Fig 2.D). We found that with the increase of Scutellarin dose, the clone number of U251 and LN229 cells decreased significantly in comparison with the control group($P \leq 0.05$) (Fig 2.D, E, F). Moreover, Lidocaine 1mM also inhibited the colony formation of U251 and LN229 cells ($P \leq 0.05$) (Fig 2.D, E, F). In addition, the inhibitory effect of Scutellarin was time-dependent and reached the peak at SCU 200 μM. Therefore, the addition of Lidocaine 1mM did not showed the further inhibitory effect on clone formation (Fig 2. D, E, F). When Lidocaine 1mM was added on the basis of Scutellarin, the promoting effect by Lidocaine was only showed at Scutellarin 100 μM, but $p > 0.05$ (Fig 2. D, E, F).

Thirdly, the proliferation of LN229 cells intervened with the combination of Scutellarin and Lidocaine for 48h was also assessed by EdU incorporation assay. We found that with the rise of Scutellarin dose, the proliferation rate of LN229 cells was significantly lower than that of control group ($P \leq 0.05$) (Fig 3. A, B). Additionally, Lidocaine 1mM inhibited the proliferation of LN229 cells ($P \leq 0.05$) (Fig 3. A, B). Moreover, on the basis of Scutellarin, Lidocaine 1mM could further inhibit the proliferation of LN229 cells(Fig 3. A, B).

3. **Scutellarin and its combination with Lidocaine induced the apoptosis of glioma cells.**

The apoptosis of U251 and LN229 cells, induced by SCU and its combination with Lidocaine for 48h, was evaluated by both terminal-deoxynucleoitidyl Transferase Mediated Nick End Labeling (TUNEL) staining and flow cytometry. In TUNEL assay, we found that SCU promotes the apoptosis of U251 and LN229 cells in a concentration-dependent manner (Fig 4. A, B, C). Moreover, compared with control group, the apoptosis rate of U251 and LN229 cells were also increased by Lidocaine 1mM (Fig 4. A, B, C), and Lidocaine 1mM could further improve the effect of inducing apoptosis by SCU on U251 and LN229 cells (Fig 4. A, B, C).

Additionally, the result of flow cytometry were consistent with that of TUNEL assay. With the increase of SCU dose, the early apoptosis rate and late apoptosis rate (i.e. necrosis) of U251 and LN229 cells were increased after 48 h of intervention (Fig. 4. D, E, F). Moreover, Lidocaine 1.5 mM raised the early and late apoptosis rates of U251 and LN229 cells compared with control group (Fig. 4. D, E, F). Furthermore, the early and late apoptosis rates of U251 and LN229 cells intervened by the combination of SCU and Lidocaine 1.5mM was higher than that in the groups with SCU or Lidocaine alone ($P \leq 0.05$) (Fig 4. D, E, F).

4. **Scutellarin and its combination with Lidocaine repressed the migration of glioma cells.**

The migration ability of U251 and LN229 cells intervened by SCU and its combination with Lidocaine for 48h was determined by Transwell assay. The results indicated that with the increase of SCU dose, the number of U251 and LN229 cells migrating to the bottom of the chamber was significantly declined ($P \leq 0.05$) compared with the control group (Fig. 5. A, B, C). Lidocaine 1 mM also inhibited the migration of U251 to the bottom of the chamber ($P \leq 0.05$) (Fig. 5. A, B). Furthermore, on the basis of SCU, Lidocaine 1mM could further repress the migration of U251 and LN229 cells, and the difference was statistically significant ($P \leq 0.05$) (Fig 5. A, B, C).

5. **EGFR was downregulated by Scutellarin and its combination with Lidocaine.**

In order to explore the possible molecular mechanism of the anti-glioma effect of Scutellarin combined with Lidocaine, we used malacards (the human disease database, https://www.malacards.org/) to find the genes related to glioblastoma (see Supplementary table 1). The results revealed that EGFR was the gene related to glioblastoma with the highest score after PTEN (see Supplementary table 1). Further analysis demonstrated that EGFR was involved in many terms of biological processes and pathways related to Glioblastoma according to GeneCards Suite gene sharing, such as negative regulation of apoptotic process, positive regulation of ERK1 and ERK2 cascade, epidermal growth factor receptor signaling pathway, ERBB2 signaling pathway, cellular response to drug and MAPK cascade in the top 10 biological processes, and almost all the top 20 pathways (Fig 6. A, B). These results indicated that EGFR and its signaling pathway were closely related to the occurrence and development of glioma. Furthermore, through the UALCAN database(http://ualcan.path.uab.edu/index.html), we found that the mRNA expression of EGFR was up-regulated in primary glioblastoma in comparison with normal tissues, and the difference was statistically significant (P=1.13220000041991E-08) (Fig 6. C).

Therefore, we speculated whether Scutellarin combined with Lidocaine was down-regulated EGFR to inhibit the proliferation and migration of glioma cells and induce the apoptosis. Interestingly and dramatically, the results of western blot demonstrated that Scutellarin downregulated the EGFR protein level of U251 and LN229 cells in dose-dependent manner, and the difference between SCU groups and Control group was statistically significant ($P \leq 0.05$) (Fig 6. D, E and Supplementary figure 1). Moreover, the EGFR protein expression of U251 and LN229 cells in the Lidocaine 1.5mM group was also declined ($P \leq 0.05$) (Fig 6. D, E and Supplementary figure 1). More importantly, the EGFR protein level of U251 and LN229 cells intervened by the combination of SCU and Lidocaine 1.5mM was less than that in the groups with SCU or Lidocaine alone ($P \leq 0.05$) (Fig 6. D, E and Supplementary figure 1).

Discussion

In this study, Scutellarin combined with Lidocaine was first applied in human glioma cells for a new therapeutic strategy. We found that single-agent SCU and Lidocaine suppressed the proliferation and migration, and induced the apoptosis of U251 and LN229 cells in a dose-dependent manner. Under administrated with the combination of SCU and Lidocaine, the proliferation and migration ability of U251 and LN229 cells were further reduced, and the apoptosis was also higher, that is, the two drugs exerted a

synergistic effect. Mechanistically, the effect of Scutellarin and its combination with Lidocaine on glioma cells was partially associated with the downregulation of EGFR. These results could provide a reference for the treatment of glioma and the combination of Scutellarin and Lidocaine may become a new chemotherapy method in future.

Scutellarin, an extractant of the Chinese herbal medicine Erigeron breviscapus, exerts the anti-tumor effect in many types of tumors[1–7]. The previous studies have showed that Scutellarin at low dose (10 μM) could induce cell cycle arrest at G0/G1 transition by down regulating the expression of cyclin D1 and CDK4, and at high dose (15μM or higher) could promote apoptosis by promoting caspases activation[8]. For example, Feng Y and his colleagues found Scutellarin could regulate the cell cycle of cancer cells and induce apoptosis in lymphohematological tumors[9]. In addition, Scutellarin held a strong PKM2 activation effect and could inhibit cell growth[10, 11]. Consistent with the previous studies, we also found that Scutellarin suppressed the proliferation and migration, and induced the apoptosis of glioma cells in a concentration-dependent manner in present study.

Many studies have reported that Lidocaine hold anti-cancer effect. In clinically relevant concentrations, Lidocaine had significant antiproliferative effects on human hepatocarcinoma cells by modifying the P53 expression level, and these effects were time and dose-dependent[12]. In addition, Lidocaine inhibited the viability and migration of breast cancer cell[13]. Moreover, intraperitoneal lidocaine improved survival of mice with MDA-MB-231 peritoneal carcinomatosis using doses that are consistent with the current clinical settings for analgesia[13]. Although the anti-tumor effect of local anesthetic drug-Lidocaine was not the first discovery in this study, but it was first used for human glioma cell. Similar to previous results, we found that Lidocaine also suppressed the proliferation and migration, and induced the apoptosis of U251 and LN229 cells in a dose-dependent manner. In agreement with the notion that local anesthesia may be beneficial for cancer therapy.

As is known to all, the combination of multi-drugs at low-dose can not only reduce the drug resistance of tumor, but also reduce the toxicity of high-dose single drug[14]. Therefore, we studied the combined effect of Scutellarin and Lidocaine on glioma cells, and found that these two drugs synergistically reduced the cell viability, clone formation and proliferation, induced the apoptosis, and suppressed the migration of U251 and LN229 cells. Studies have reported that SCU and Lidocaine not only possessed anti-tumor effect, but also could improve the sensitivity of cancer cells to chemotherapeutic drugs. For example, they could be used as a sensitizer for cisplatin. In ovarian cancer[15], non-small cell lung cancer (NSCLC)[16] and prostate cancer[17], Scutellarin interacted with cisplatin to sensitize the response of cancer cells to cisplatin and declined its toxicity. Moreover, Lidocaine suppressed tumor development and enhanced the sensitivity of cisplatin in hepatocellular carcinoma and breast cancer[18, 19]. Therefore, it's not difficult to understand that the combination of Scutellarin and Lidocaine could exert more effective anti-glioma effect.

Epidermal growth factor receptor (EGFR) is a transmembrane glycoprotein and a member of the tyrosine kinase superfamily receptor[20]. Many EGFR gene alterations have been identified in gliomas, including

amplifications, deletions and single nucleotide polymorphisms (SNPs)[21–23], so it has served as a clinical marker in gliomas[21, 23]. More importantly, Lidocaine suppressed the viability, migration, and invasion of lung cancer cells while induced apoptotic death via up-regulation of miR-539, which blocked EGFR signaling by directly binding with EGFR[24]. Moreover, Lidocaine reduced the proliferation and induced the apoptosis of Retinoblastoma cells by decreasing EGFR expression[25]. Based on this and the result from malacards database(https://www.malacards.org/) (see Supplementary table 1), we detected the EGFR protein expression in U251 and LN229 cells intervened by Scutellarin and its combination with Lidocaine. Our results demonstrated that Scutellarin and Lidocaine also separately downregulated the EGFR protein level of U251 and LN229 cells in dose-dependent manner. Furthermore, the combination of SCU and Lidocaine could further declined the EGFR protein expression of U251 and LN229 cells.

In conclusion, Scutellarin combined with Lidocaine suppressed the proliferation and migration, and induced the apoptosis of glioma cells, which was partly associated with the repression of EGFR expression.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Author contributions

THW and LZ participated in the guidance and design of the study and the revise of the paper. XYH was responsible for the design of the study, participated in all the tests and data analysis and description, and was a major contributor in writing and revising the manuscript. QJX performed qPCR test and supervised

the experiments. XMZ performed cell culture, cck8 assay, wound healing assay and the data analysis. All authors have read and approved the final version of the manuscript.

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Figures

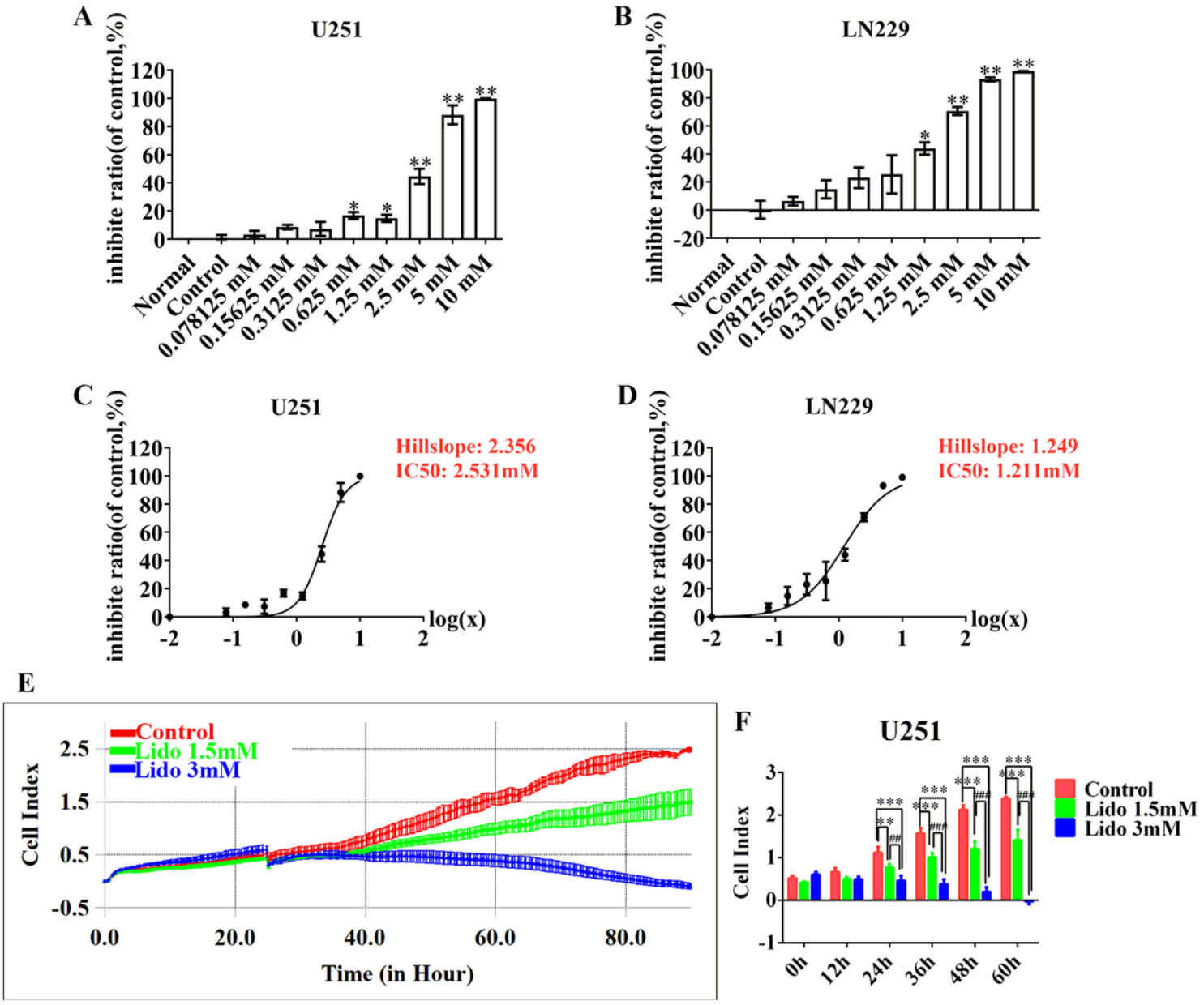

Figure 1

Effect of Lidocaine on the proliferation of glioma cells A, B: the inhibitory rate of different concentrations of Lidocaine on U251 and LN229 cells. C, D: IC50 curve and IC50 of Lidocaine in U251 and LN229 cells. IC50: U251 and LN229 cells are 2.531mM and 1.211mM, respectively, and 95% confidence intervals are 2.255mM~2.841mM and 1.022mM~1.435mM, respectively. E, F: Lidocaine suppressed the proliferation of U251 cells. E) Cell index was recorded at 15 minutes interval after inoculation. F) Cell index was recorded after intervened by Lidocaine for 0h, 12h, 24h, 36h, 48h and 60h. IC50, the concentration of lidocaine on which the cell inhibition rate was 50%; Lido: Lidocaine. Data are shown as mean + SD(n=3). * vs control (0.133% DMSO). * P≤0.05, ** P≤0.01, ***P≤0.001.

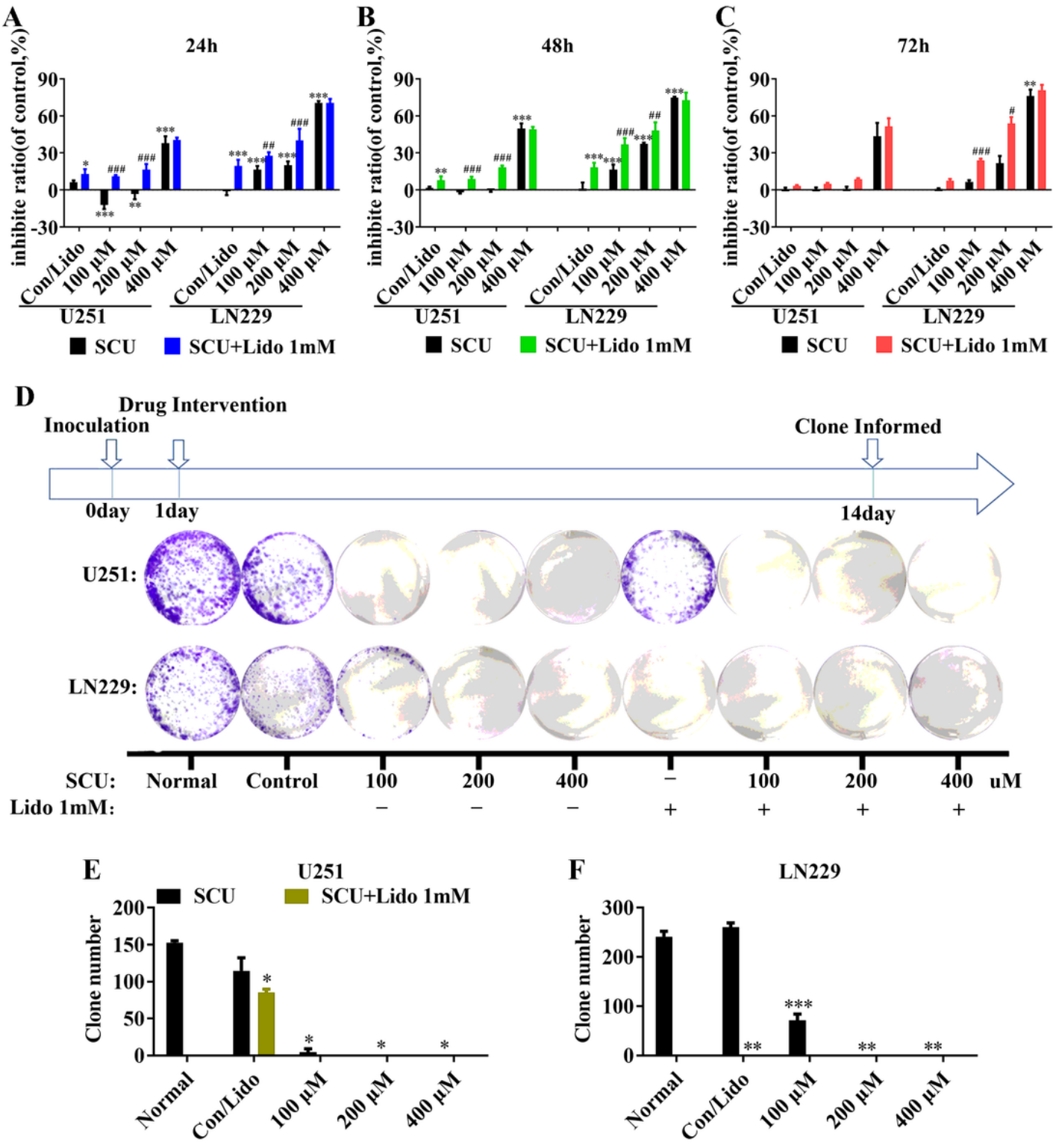

Figure 2

Scutellarin and its combination with Lidocaine inhibited the viability and colony formation of glioma cells. A, B, C: Inhibition rate of SCU and its combination with Lidocaine 1mM on U251 and LN229 cells after intervention for 24, 48 and 72h. D: The images showed the effect of SCU and its combination with Lidocaine on the clone formation of U251 and LN229 cells. E, F: The clone number of U251 and LN229

cells in D. The number of cells in one clone was more than 50. *: vs control group (Con), #: SCU x vs SCU x + Lidocaine 1mM. */# P < 0.05, **/## P < 0.01, ***/### P < 0.001 (n=3).

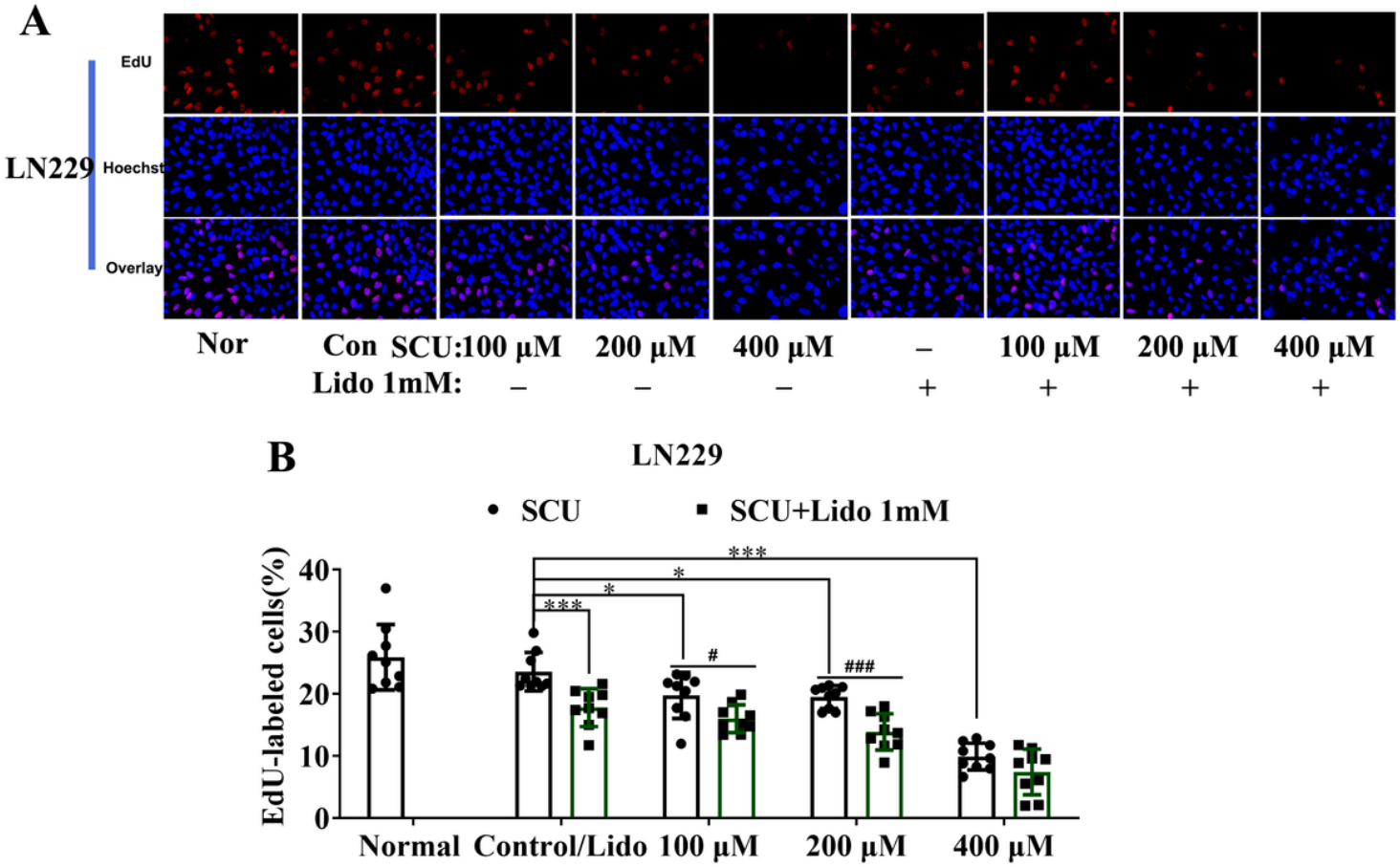

Figure 3

Scutellarin and its combination with Lidocaine suppressed the proliferation of glioma cells. A: The effect of SCU and its combination with Lidocaine on the proliferation of LN229 cells. B: Quantitative analysis of A. That is the proliferation rate of LN229 cells. */** vs control, * P≤0.05, ** P≤0.01，***P≤0.001; #/## SCU x vs SCU x + Lidocaine 1mM.

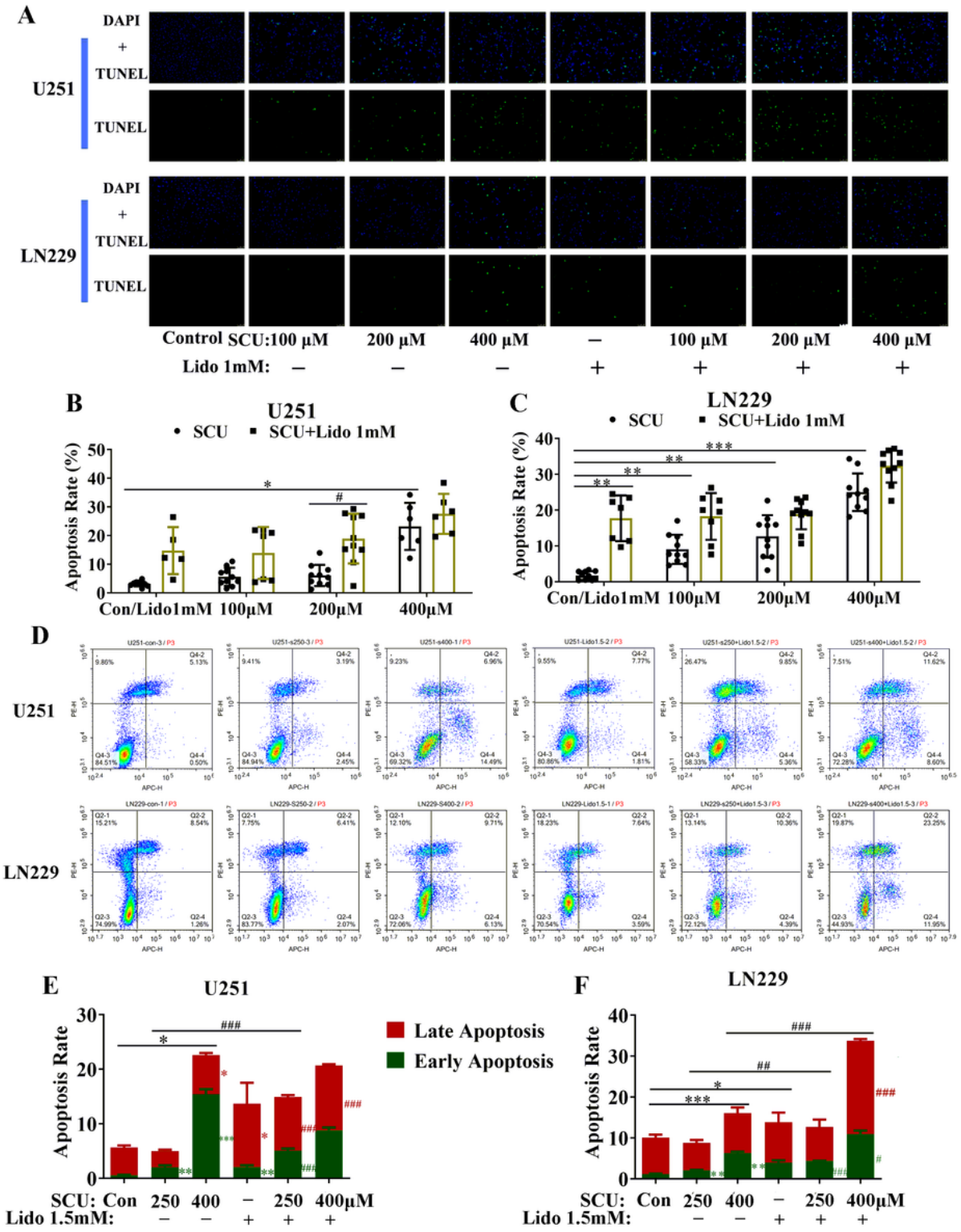

Figure 4

Scutellarin and its combination with Lidocaine induced the apoptosis of glioma cells. A: Fluorescence images by TUNEL assay showed the apoptosis of U251 and LN229 cells induced by SCU and its combination with Lidocaine 1mM. B, C: Quantification of apoptosis rate of U251 and LN229 cells induced by SCU and its combination with Lidocaine 1mM (n=10). D: The apoptosis diagrams of U251 and LN229 cells induced by SCU and its combination with Lidocaine 1mM. E, F: Quantification of the early, late and

the sum apoptosis rate of U251 and LN229 cells (n=3). *: vs Control, #: SCU x vs SCU x+Lido 1/1.5mM. */# P < 0.05, **/## P < 0.01, ***/### P < 0.001.

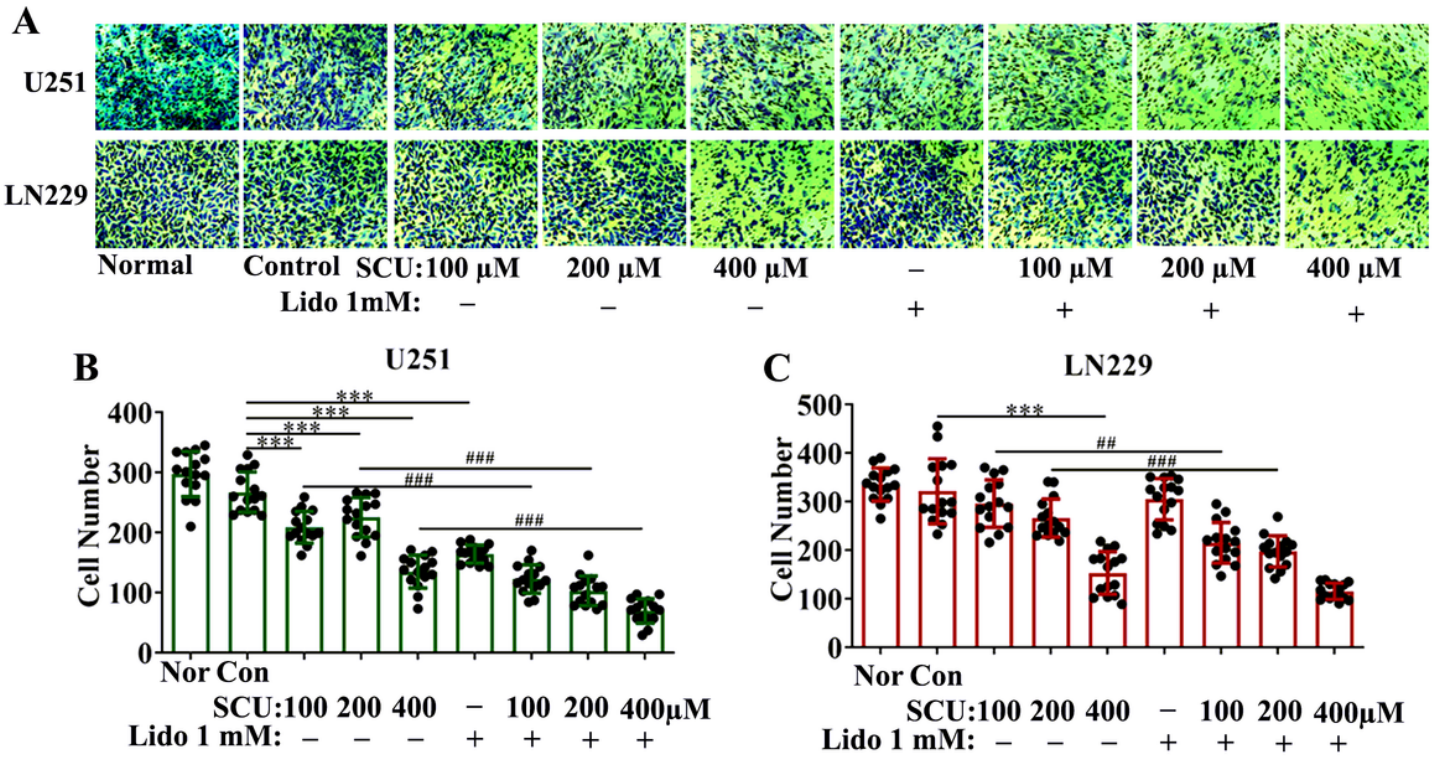

Figure 5

Scutellarin and its combination with Lidocaine suppressed the migration of glioma cells. A: Images show the U251 and LN229 cells that vertically migrated to the bottom of the chamber after intervention by SCU and its combination with Lidocaine 1mM for 48h. B, C: Quantification of the U251 and LN229 cells that vertically migrated to the bottom of the chamber. Nor: Normal group (cells) that didn't receive any intervention. *: vs Control, #: SCU x vs SCU x+Lido 1mM. */# P < 0.05, **/## P < 0.01, ***/### P < 0.001 (n=15).

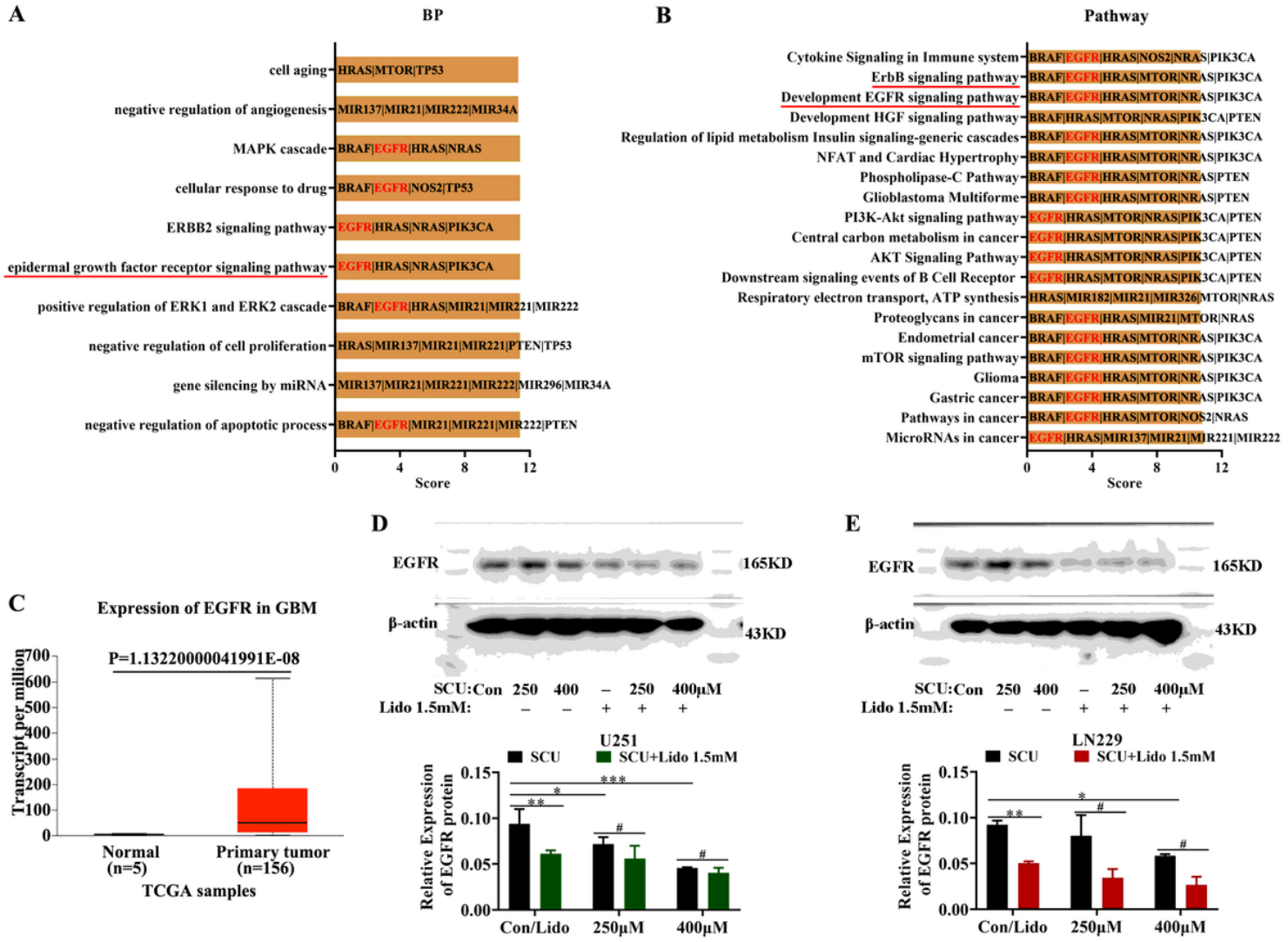

Figure 6

The EGFR expression in Glioma cells after intervention by SCU and its combination of Lidocaine were down-regulated. A: The top 10 biological processes related to Glioblastoma according to GeneCards Suite gene sharing. B: The top 20 pathways related to Glioblastoma according to GeneCards Suite gene sharing. C: The mRNA expression of EGFR in normal tissue and primary glioblastoma multiforme(GBM). D: The relative protein level of EGFR in U251 cell intervened by SCU and its combination of Lidocaine for 48h. E: The relative protein level of EGFR in LN229 cell intervened by SCU and its combination of Lidocaine for 48h. *: vs Control, #: SCU x vs SCU x+Lidocaine 1.5mM. */# $P < 0.05$, **/## $P < 0.01$, ***/### $P < 0.001$ (n=3). The bolting bands from three repeats were shown in Supplementary figure 1.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementaryfigure1.tif

- Supplementarytable1.xlsx