SIK2 Promotes Cisplatin Resistance Induced by Aerobic Glycolysis in Breast Cancer Cells through PI3K/AKT/mTOR Signaling Pathway

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Research article

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Abstract

Objective

This study aimed to investigate the effect of SIK2 on cisplatin resistance induced by aerobic glycolysis in breast cancer cells and its potential mechanism.

Methods

qRT-PCR and Western blot were used to detect SIK2 mRNA and protein levels. Cisplatin (DDP) resistant cell lines of breast cancer cells were established, CCK-8 was used to measure and evaluate the viability, and Transwell was used to evaluate the cell invasion capability. Flow cytometry was adopted to evaluate the apoptosis rate. The glycolysis level was evaluated by measuring glucose consumption and lactic acid production. The protein levels of p-PI3K, p- protein kinase B (Akt) and p-mTOR were determined by western blot.

Results

SIK2 is highly expressed in breast cancer tissues and cells compared with adjacent tissues and normal human breast epithelial cells, and has higher diagnostic value for breast cancer. Silencing SIK2 expression can inhibit proliferation and invasion of breast cancer cells and induce their apoptosis. In addition, SIK2 knockdown inhibits glycolysis, reverses the resistance of drug-resistant cells to cisplatin, and inhibits PI3K/AKT/mTOR signaling pathway. When LY294002 is used to inhibit PI3K/AKT/mTOR signaling pathway, the effect of Sh-SIK2 on aerobic glycolysis of breast cancer cells can be reversed.

Conclusion

SIK2 can promote cisplatin resistance caused by aerobic glycolysis of breast cancer cells through PI3K/AKT/mTOR signaling pathway, which may be a new target to improve cisplatin resistance of breast cancer cells.

Background

Breast cancer (BC), as one of the general malignant tumors in related female, is also one of the primary causes of their cancer-related death [1]. In recent years, with the changes of social environment and living habits, the morbidity of breast cancer is also getting higher and higher, and showing a younger trend [2]. Although great progress has been made in BC' diagnosis and treatment with the improvement of medical methods, there are still some patients with poor prognosis, and chemotherapy resistance is one of the main reasons [3, 4]. Therefore, how to help breast cancer patients overcome cisplatin resistance is also one of the most urgent problems in breast cancer treatment.

Metabolic reprogramming is one of the main factors of tumor progression, and aerobic glycolysis is the most typical marker of metabolic changes [5]. During the occurrence and development of tumors, their

growth and development are supported by enhancing the function of aerobic glycolysis, that is, increasing glucose uptake and lactic acid production of tumor cells. Therefore, many researches believe that aerobic glycolysis has a major part to play in treating tumors [6, 7]. Previous studies [8] have pointed out that increasing aerobic glycolysis of tumors can promote chemical resistance of tumor cells. Another study [9] has indicated that inhibition of tumor metabolic abnormalities may be a way to overcome drug resistance, but its mechanism has not been well explained. Salt-inducible kinase (SIK2) pertains to the AMP-activated protein kinase family, and it regulates many biological functions, including glucose metabolism reprogramming [10]. In addition, research [11] showed that the chemosensitivity of colorectal cancer can be enhanced by silencing the expression of SIK2. Although the downstream mechanism of SIK2 affecting chemoresistance is not further elaborated, it is sufficient to show that SIK2 is closely related to it. In the past, some studies [12] have also revealed that inhibiting the expression of SIK2 in breast cancer can promote tumor growth, but the mechanism has not been further explored.

Based on the past literature, we speculate whether SIK2 can regulate the metabolism of breast cancer to affect its chemotherapy resistance, so we have explored this and its related mechanisms in order to provide possible solutions to the chemotherapy resistance of breast cancer.

Methods

Clinical specimen

Seventy-six patients who underwent breast cancer resection in hospital from March 2015 to May 2017 were collected. They were (52.87 ± 6.39) years old on average. Seventy-six breast cancer tissues and 76 paracancerous tissues were obtained during the operation with the consent of the patients and stored in a liquid nitrogen tank. Inclusion criteria were as follows: patients diagnosed with breast cancer by pathological diagnosis; patients diagnosed with breast cancer for the first time. Exclusion criteria were as follows: patients who have received radiotherapy and chemotherapy; patients with other malignant tumors; patients with severe renal dysfunction; patients with serious infectious diseases; patients who refused to provide experimental specimens. This study was reviewed and approved by the Ethics Committee of People's Hospital of Rizhao. All patients and their families agreed to participate in the experiment and sign an informed consent form.

Cell Culture And Transfection

Human breast cancer cell strains MDA-MB-231, MCF-7, MDA-MB-468, MCF-10A along with human normal breast epithelial cells Hs 278Bst (purchased from ATCC) were placed in DMEM medium containing 10%PBS, 2 mm penicillin as well as streptomycin, and cultured at 37℃, 5% CO_2. When the adherent growth and fusion of the cells were observed to reach 85%, 25% pancreatin was added for digestion; after that, the cells were placed into the medium for continuous culture and passage was completed. Subsequently, the drug-resistant cell strain was cultured, and the MDA-MB-231/DDP cell strain resistant to DDP was obtained by the method of Reference [13]. The cell was placed in a medium containing 0.5 μg/mL cisplatin to maintain its drug resistance and cultured at 37℃, 5% CO_2. Then SIK2 expression

in each cell line was detected. MDA-MB-231, MCF-7, and MDA-MB-231/DDP cells are selected for transfection. SIK2 RNA (si-SIK2) was targetedly inhibited, RNA (NC) was negatively controlled, and SIK2 RNA (sh-SIK2) was targetedly overexpressed; cells were respectively transfected with Lipofectamine™ 2000 kit, and our operation procedures were rigidly carried out on the basis of the kit instructions.

Evaluation Of Cisplatin IC50 In Breast Cancer Cells

Cells were inoculated into 96-well plates at a density of 1×10^5 per well, 100 μL of cisplatin with different concentrations was added respectively, fresh culture medium was changed 48 h after incubation, and 10 μl of CCK-8 solution was added into each well; then, they were put into an incubator to continue culturing for 2 h, the absorbance value of each well was determined at 450 nm wavelength by SpectraMax M5 elisa reader to detect cell proliferation, and this test was duplicated 3 times. Afterwards, the cisplatin IC50 on MDA-MB-231/DDP cells was calculated according to the cell survival rate.

Real-time Quantitative PCR

Firstly, the total RNA in tissues and cells was drawn with Trizol reagent, 5 μg total RNA was taken respectively for transcribing cDNA reversely according to the illustrations of the kit, and 1 μL of synthesized cDNA was taken for amplification after transcription. The PCR reactivation conditions were as follows: pre-denaturation at 95℃ for 10 s, denaturation at 94℃ for 10 s, annealing at 60℃ for 30 s, totaling 40 cycles. We set up three repeated wells for each sample and carried out the test 3 times. SIK2 used GAPDH as internal reference and data were analyzed by $2^{-\triangle\triangle ct}$. The primer sequences were shown in Table I.

Western Blot Test

RIPA lysis method was used to lyse cells and extract total protein, and its concentration was detected by the BCA method and then adjusted to 4 μg/μL. The proteins were segregated by 12% SDS-PAGE and then transferred to a PVDF membrane. Then, the membrane was sealed with 5% defatted milk powder for 2 h. SIK2 (1: 500), p-Akt (1: 500), p-PI3K (1: 500), p-mTOR (1:500), Caspase-3 (1:500), Bax (1:500), Bcl-2 (1: 500), Glut1 (1:500), HK2 (1:500), LDH-A (1:500) and β-Actin (1: 1000) primary antibody were added and sealed all night long at 4℃. It was washed to remove primary antibody, horseradish peroxidase labeled goat anti-rabbit secondary antibody (1: 1000) was replenished, and then it was hatched at 37℃ for 1 h, rinsed in PBS 3 times, each time for 5 min. The protein bands were developed in a darkroom via the enhanced chemiluminescence reagent, and the membrane's excess liquid was removed with a filter paper.

Cell Proliferation Detected By CCK-8

The proliferation ability of cells was evaluated by CCK-8 kit. Cells 48 h after transfection were collected and diluted to 3×10^4 cell/ml. Then, they were inoculated into 96-well plates. Each well was inoculated with 100 μl of cells and cultured at 37℃ with 5% CO_2. Each well was added with 10 μl of CCK8 solution at 0 h, 24 h, 48 h and 72 h after the cells adhered to the wall. After adding reagents, they were

continuously cultured in an incubator at 37°C, 5% CO_2 for 2 h, and then OD values were measured at 450 nm using an enzyme reader to detect cell proliferation and draw a growth curve. The experiment was repeated 3 times.

Apoptosis Test

Transfected cells were digested by 0.25% trypsin, washed two times with PBS after digestion, added with 100 μL of binding buffer, prepared into 1*106/mL suspension, sequentially added with AnnexinV-FITC and PI, incubated at room temperature in dark for 5 min, detected with FACSVerse flow cytometer system, and averaged over 3 repetitions.

Cell Invasion Test

The invasion ability of cells was evaluated by Transwell test. Firstly, we added 200 μL DMEM culture solution containing 1×10^5 cells to the upper chamber, and 500 mL DMEM containing 20% FBS to the lower chamber. After culture at 37°C for 48 h, the matrix and cells failed to cross over the film surface in the upper chamber were cleaned, washed by PBS 3 times, fastened with paraformaldehyde for 10 min, cleaned by double distilled water 3 times, dyed with 0.1% crystal violet for 10 min after drying, and the cell invasion was observed with a microscope.

Glucose Consumption And Lactic Acid Content

Collected cells were inoculated in a 6-well plate at a density of 3×10^5 per well and cultured at 37°C with 5% CO_2 for 48 h. Then their culture medium was used to measure glucose consumption and lactic acid production. The glucose and lactic acid levels were strictly described in accordance with the operation instructions of the glucose and lactic acid determination kits.

Statistical Methods

In our research, we used SPSS19.0 software package to analyze the collected data statistically, and adopted GraphPad 7 software package to draw the required pictures; besides, independent-samples T test was used for inter-group comparison, one-way analysis of variance (ANOVA) was used for multi-group comparison, LSD-t test was used for post hoc pairwise comparison, repeated measures ANOVA was used for multi-time point expression, and Bonferroni was used for back testing. A p value lower than 0.05 was regarded as markedly different difference.

Results

SIK2 was highly expressed in BC tissues

SIK2 mRNA expression in BC tissues was quantitatively detected by qRT-PCR. It showed that SIK2 mRNA expression in BC tissues was markedly up-regulated compared with adjacent normal tissues, SIK2 mRNA expression in BC cells was also obviously up-regulated compared with normal BC epithelial cells, and

SIK2 mRNA expression in BC drug-resistant cells was markedly higher than that in non-drug-resistant BC cells ($P < 0.05$). ROC analysis of the subjects found that the area under the SIK2 curve was 0.912. According to the median expression (1.69) of SIK2 mRNA, the patients were divided into 41 cases of SIK2 mRNA high expression group and 35 cases of low expression group. The experimental results indicated that the expression of SIK2 mRNA was related to the pathological staging, differentiation degree, lymph node metastasis and drug resistance of breast cancer patients; patients' 3-year survival rate of SIK2 high expression group was substantially lower than that of low expression group ($P < 0.05$) (Table II, Fig. 1).

Effect of SIK2 on biological function of breast cancer cells

SIK2 expression in MDA-MB-231 and MCF-7 transfected cells was substantially lower than that in NC transfected cells, and it in Sh-SIK2 transfected cells was dramatically higher than that in NC transfected cells ($P < 0.05$). Examining their biological functions in both groups, it was found that compared with NC group, the proliferation and invasion ability of transfected Si-SIK2 cells reduced significantly, the apoptosis rate increased dramatically, the Bcl-2 expression decreased memorably, and the Caspase-3 expression levels and Bax protein raised substantially ($P < 0.05$). Compared with NC group, the proliferation and invasion ability of transfected Sh-SIK2 cells increased markedly, the apoptosis rate reduced remarkablely ($P < 0.05$), the Bcl-2 expression raised dramatically, and the Caspase-3 expression levels and Bax protein reduced substantially ($P < 0.05$). More details were shown in Fig. 2.

SIK2 Enhances DDP Resistance Of BC Cells

The results showed that DDP significantly inhibited MDA-MB-231 and MCF-7 cells' proliferation ($P < 0.05$) and was dose-dependent. The IC50 of MDA-MB-231/DDP cells to DDP was remarkablely higher than that of MDA-MB-231 and MCF-7 cells ($P < 0.05$). Furthermore, down-regulation of SIK2 expression can significantly increase the sensitivity of MDA-MB-231 and MCF-7 cells to paclitaxel, reverse the resistance of MDA-MB-231/DDP cells to DDP, and cut down the IC50 of cells to DDP ($P < 0.05$), while up-regulation of SIK2 can reduce the sensitivity of these two cells to DDP, enhance the resistance of the aforesaid cells to DDP, and up-regulate the IC50 of cells to DDP ($P < 0.05$). More details were shown in Fig. 3.

SIK2 Enhances DDP Resistance By Promoting Glycolysis

Compared with NC group, the glycolysis of MDA-MB-231 and MCF-7 cells transfected with Si-SIK2 were significantly inhibited ($P < 0.05$), the expression levels of Glut1, HK2 and LDH-A proteins also decreased substantially ($P < 0.05$), and the glycolysis of the two cells transfected with Sh-SIK2 enhanced obviously ($P < 0.05$). The expression levels of the three proteins were also significantly up-regulated ($P < 0.05$). In order to verify whether SIK2 affects DDP resistance through glycolysis, these two cells were cured by 0.5 mg/mL oligomycin (glycolytic inducer, oligomycin) and cultured in the DDP concentration of IC50. The results showed that the cell proliferation ability after oligomycin treatment was dramatically higher than that of cells without oligomycin treatment ($P < 0.05$). More details were shown in Fig. 4.

Effect Of SIK2 On PI3K/AKT/mTOR Signaling Pathway In BC

After transfecting cells, it was found that compared with NC group, MDA-MB-231 and MCF-7 cells transfected with Si-SIK2 had significantly inhibited PI3K/AKT/mTOR signaling pathway, and the p-Akt, p-PI3K and p-mTOR expression levels decreased dramatically ($P < 0.05$). In the two cells transfected with Sh-SIK2, the PI3K/AKT/mTOR signaling pathway was significantly further activated, and the p-Akt, p-PI3K and p-mTOR expression levels increased markedly ($P < 0.05$). More details were shown in Fig. 5.

SIK2 regulates aerobic glycolysis of BC through PI3K/AKT/mTOR signaling pathway

In order to verify that SIK2 regulates aerobic glycolysis of BC through PI3K/AKT/mTOR signaling pathway, MDA-MB-231 as well as MCF-7 cells were cured with 5 μL LY294002 (PI3K specific inhibitor) for 48 h. Then it was found that the p-Akt, p-PI3K, p-mTOR expression levels in the two cells treated with LY294002 were remarkablely lower than those without LY294002 treatment ($P < 0.05$). Subsequently, the cells transfected with Sh-SIK2 were exposed to 5 μL LY294002 for 48 h. The observation results showed that compared with untreated cells, the proliferation, invasion and aerobic glycolysis ability of BC cells treated with LY294002 were markedly inhibited, and the apoptosis rate increased significantly ($P < 0.05$). Inhibition of PI3K/AKT/mTOR signaling pathway could reverse the effect of Sh-SIK2 on BC cells. More details were shown in Fig. 6.

Discussion

At present, BC is still one of the major diseases leading to female death. It occurrence poses a severe threat to the health of women all over the world [14, 15]. Although the medical profession has made great progress in treating BC in recent years, chemotherapy resistance is still the main reason leading to unsatisfactory efficacy of breast cancer patients [16]. Therefore, for the purpose of improving the chemotherapy efficacy of BC patients and their prognosis further, the study of drug resistance mechanism is essential.

During the growth of tumor cells, aerobic glycolysis can provide necessary energy for cell growth by decomposing glucose and producing lactic acid, thus enhancing its ability of proliferation and invasion [5, 17]. In the past, some studies [18, 19]found that aerobic glycolysis could enhance the chemosensitivity of tumor cells through a variety of different mechanisms, which made us see a possibility to improve the chemotherapy effect of cisplatin for breast cancer. As a salt-induced kinase, SIK2 has been found in previous studies [20] that when SIK protein is deleted in mice, glucose and lipid metabolism can be changed. This suggests that SIK2 may react on aerobic glycolysis. In our study, SIK2 was found to be significantly up-regulated in BC tissues and cells, and its expression level was also related to the presence of chemotherapy resistance in those patients. This further leads us to wonder whether SIK2 can affect the aerobic glycolysis and chemoresistance of breast cancer cells. To verify this, we have carried out in vitro cell experiments. Our research found that when the SIK2 expression in BC cells was silenced, their proliferation, invasion and aerobic glycolysis ability enhanced significantly, while the opposite phenomenon was observed after the SIK2 expression increased further. Previous studies [21] have found

that inhibiting the expression of SIK2 can inhibit the growth of ovarian cancer cells, suggesting that SIK2 was effective as a oncogene, which is similar to our conclusion.

Subsequently, to investigate the effect of SIK2 on DDP resistance of BC cells, we established a cisplatin-resistant cell line and analyzed the sensitivity of SIK2 to DDP in cells after intervention. The results signified that downregulation of SIK2 expression could significantly increase the sensitivity of BC cells to DDP, reverse the resistance of MDA-MB-231/DDP cells to cisplatin, reduce the IC50 of cells to cisplatin, while upregulation of SIK2 has the opposite effect. Previously, there were relatively few studies on SIK2 in tumor drug resistance; but there were reports in colorectal cancer studies [11] that miR-203 could enhance the drug resistance of colorectal cancer cells to paclitaxel by targeting SIK2; there was also research [22] which found that inhibiting the expression of SIK2 with a specific inhibitor could enhance the sensitivity of ovarian cancer cells to paclitaxel. All indicate that SIK2 plays a vital part in the chemotherapy resistance of tumors, but this is the first time that we have proved that downregulation of SIK2 expression in BC cells can reverse their resistance to cisplatin. In order to prove that SIK2 induces cisplatin resistance through aerobic glycolysis, we used oligomycin to induce and stimulate aerobic glycolysis in BC cells, and observed its biological function in cisplatin. The results showed that when aerobic glycolysis was induced and stimulated, the proliferation and invasion ability of BC cells were significantly higher than those of uninduced BC cells, and the resistance to cisplatin enhanced significantly. This also proves that SIK2 does induce cisplatin resistance of BC cells by promoting aerobic glycolysis. In past studies [23]when analyzing chemical resistance of ovarian cancer, it was clearly pointed out that glycolysis was an inherent chemical resistance promoting factor, and it was believed that the enhancement of chemical resistance of ovarian cancer cells was induced by aerobic glycolysis. This also confirms our conclusion. However, the mechanism of SIK2 regulating aerobic glycolysis is still unclear, so we have carried out the next experiment.

PI3K/AKT/mTOR is a signaling pathway that plays vital regulatory roles in cell growth and differentiation, and it also works in the discovery and development of tumors [24]. Moreover, the pathway of PI3K signal transmission has always been one of the focuses in BC research [25]. Previous studies have clearly pointed out that the signaling pathway involves a large number of biological co-occurrence of tumor cells, including their cycle and metabolism [26, 27]. In our research, we also found that when SIK2 was inhibited, the signaling pathway was also significantly inhibited, which indicated that the former could regulate the latter. In order to verify that SIK2 affects the aerobic glycolysis of BC cells through the PI3K/AKT/mTOR signaling pathway, we inhibited that in BC cells. The results showed that inhibiting this pathway could effectively inhibit the proliferation, invasion and aerobic glycolysis of BC cells, promote their apoptosis, and reverse the influence of Sh-SIK2. This confirms that SIK2 regulates aerobic glycolysis of BC cells through this signaling pathway. In the past, many studies found that it played an momentous part in the regulation of aerobic glycolysis. For example, research [28] found that immunomodulatory protein B7-H3 could promote aerobic glycolysis of oral squamous cell carcinoma cells by inducing activation of that signaling pathway. There were also studies [29] that could reverse aerobic glycolysis of primary lymphoma cells by targeting that signaling pathway. All of these are similar to some of our

conclusions, but this is the first time that SIK2 can affect aerobic glycolysis of BC cells by regulating that signaling pathway.

Conclusions

To sum up, SIK2 can enhance cisplatin resistance of BC cells by promoting aerobic glycolysis. The mechanism may be realized by regulating PI3K/AKT/mTOR signaling pathway, which may be a potential target for improving cisplatin resistance of BC cells. Nevertheless, in our study, there are still some limitations. For example, on the one hand, we have not carried out tumor formation in nude mice, so it is not clear whether SIK2 has effect on tumor size after cisplatin intervention in them. On the other hand, we have not explored the upstream regulatory mechanism of SIK2, but in the future we will carry out more basic experiments to continuously improve our conclusions.

List Of Abbreviations

Breast cancer (BC)

Salt-inducible kinase (SIK2)

Declarations

Ethics approval and consent to participate

This study was reviewed and approved by the Ethics Committee of People's Hospital of Rizhao. All patients and their families agreed to participate in the experiment and sign an informed consent form.

Consent for publication

Not applicable

Availability of data and materials

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

Competing interests

The authors declare no conflict of interest.

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Authors' contributions

SZ and WD conducted the experiments; WF, KW and XG designed the experiments and wrote the paper.

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Not applicable

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Tables

Table I Primer sequence table

Factor	Upstream primer	Downstream primer
SIK2	5'-CTGGACATCTGGAGTATGGG-3'	5'-GAATCTTCCTTCCAGAACCCT-3'
GAPDH	5’-GCCCAATACGACCAAATCC-3'	5’-AGCCACATCGCTCAGACAC-3'

Table II Relationship between SIK2 and pathological data of breast cancer patients

Factor		SIK2		x^2 value	P value
		High expression (n=41)	Low expression (n=35)		
Age				0.038	0.846
	≥52 years old (n=40)	22 (53.66)	18 (51.43)		
	< 52 years old (n=36)	19 (46.34)	17 (48.57)		
Drug resistance				9.572	0.002
	Yes (n=27)	21 (51.22)	6 (17.14)		
	No (n=49)	20 (48.79)	29 (82.86)		
Tumor size				0.008	<0.092
	≥2cm (n=33)	18 (43.90)	15 (42.86)		
	2cm (n=43)	23 (56.10)	20 (57.14)		
TNM staging				9.496	<0.001
	Phase I-II (n=42)	16 (39.02)	26 (74.29)		
	Phase III (n=34)	25 (60.98)	9 (25.71)		
Differentiation				7.521	<0.001
	Moderately and lowly differentiated (n=21)	6 (14.63)	15 (42.86)		
	Highly differentiated (n=55)	35 (85.37)	20 (57.14)		
Lymphatic metastasis				6.258	0.012
	Transfer (n=24)	18 (43.90)	6 (17.14)		
	Not transferred (n=52)	23 (56.10)	29 (82.86)		

Figures

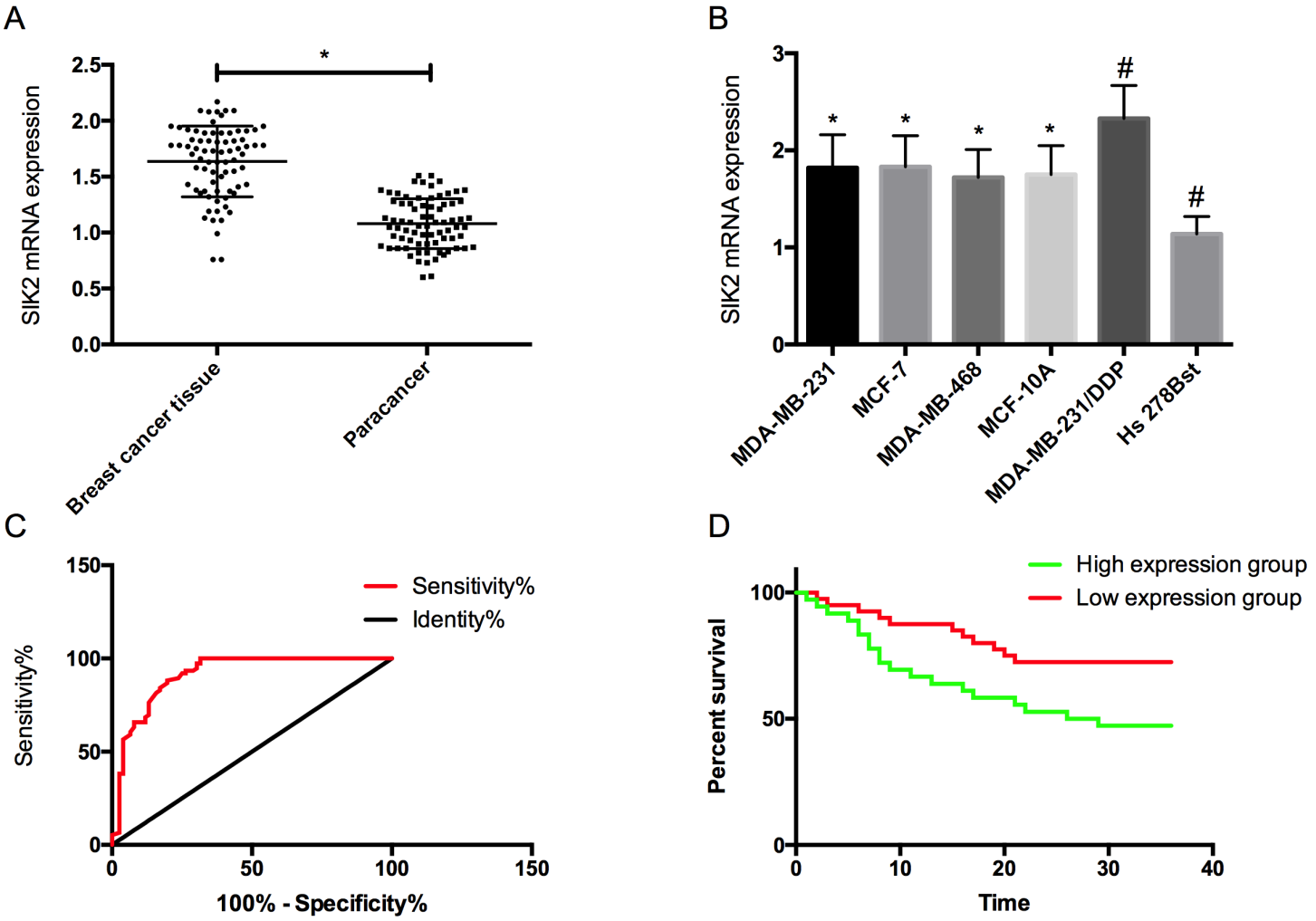

Figure 1

Expression and clinical significance of SIK2 in breast cancer Figure A: SIK2 expression in breast cancer tissue Figure B: SIK2 expression in breast cancer cells Figure C: ROC curve analysis of SIK2 for breast cancer Figure D: effect of SIK2 on survival rate of breast cancer patients. * indicates P<0.05; * was compared with # (P<0.05).

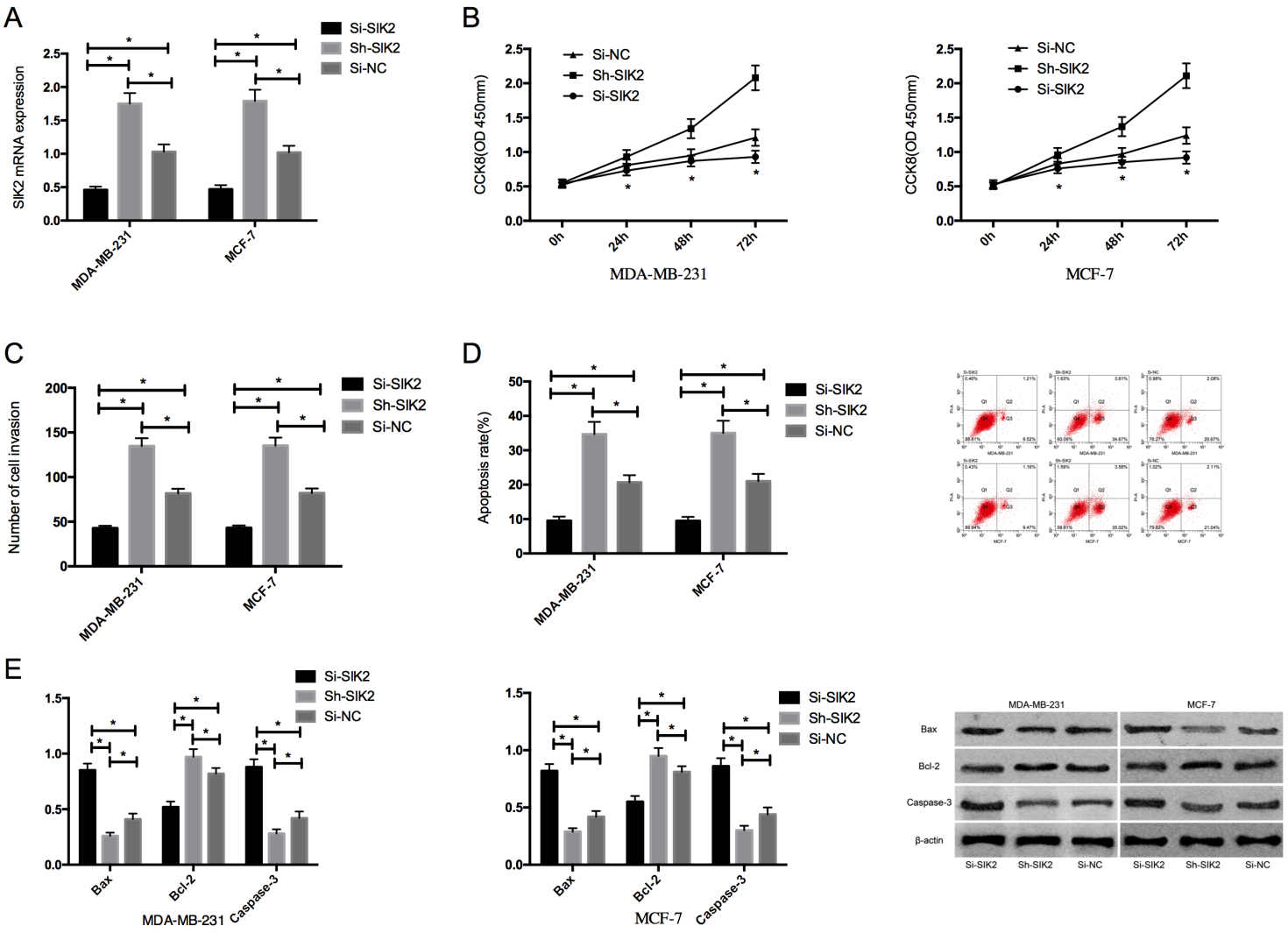

Figure 2

2 Effect of SIK2 on biological function of breast cancer cells Figure A: expression of SIK2 in breast cancer cells after transfection Figure B: effect of SIK2 on proliferation of breast cancer cells Figure C: effect of SIK2 on invasive ability of breast cancer cells Figure D: effect of SIK2 on apoptosis rate of breast cancer cells Figure E: effect of SIK2 on apoptosis-related proteins in breast cancer cells. * indicates P<0.05.

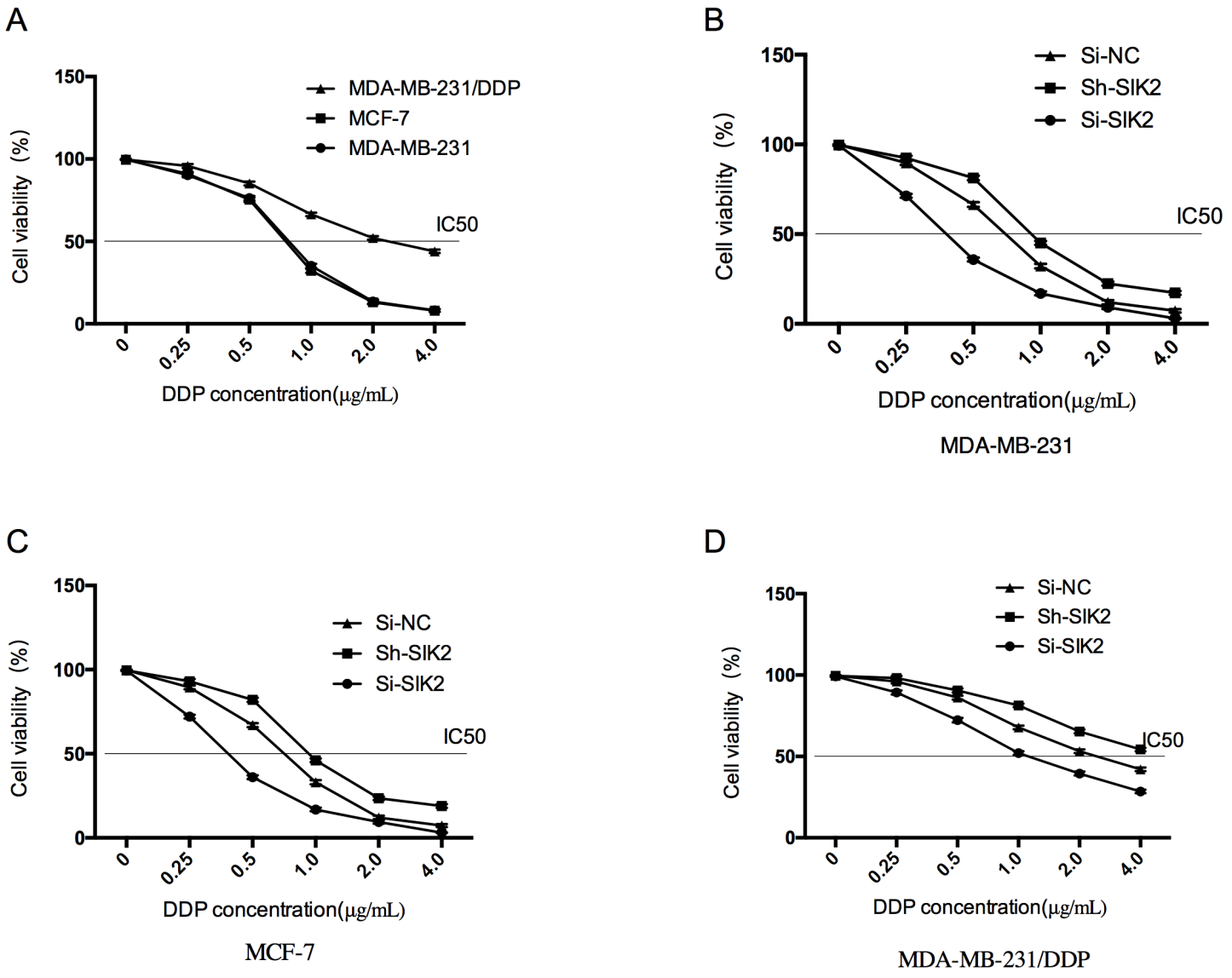

Figure 3

Effect of SIK2 on DDP resistance Figure A: IC50 of DDP on MDA-MB-231, MCF-7 and MDA-MB-231/DDP cells Figure B: effect of SIK2 regulation on DDP resistance of MDA-MB-231 cells Figure C: effect of regulating SIK2 on DDP resistance of MCF-7 cells Figure D: effect of SIK2 regulation on DDP resistance of MDA-MB-231/DDP cells

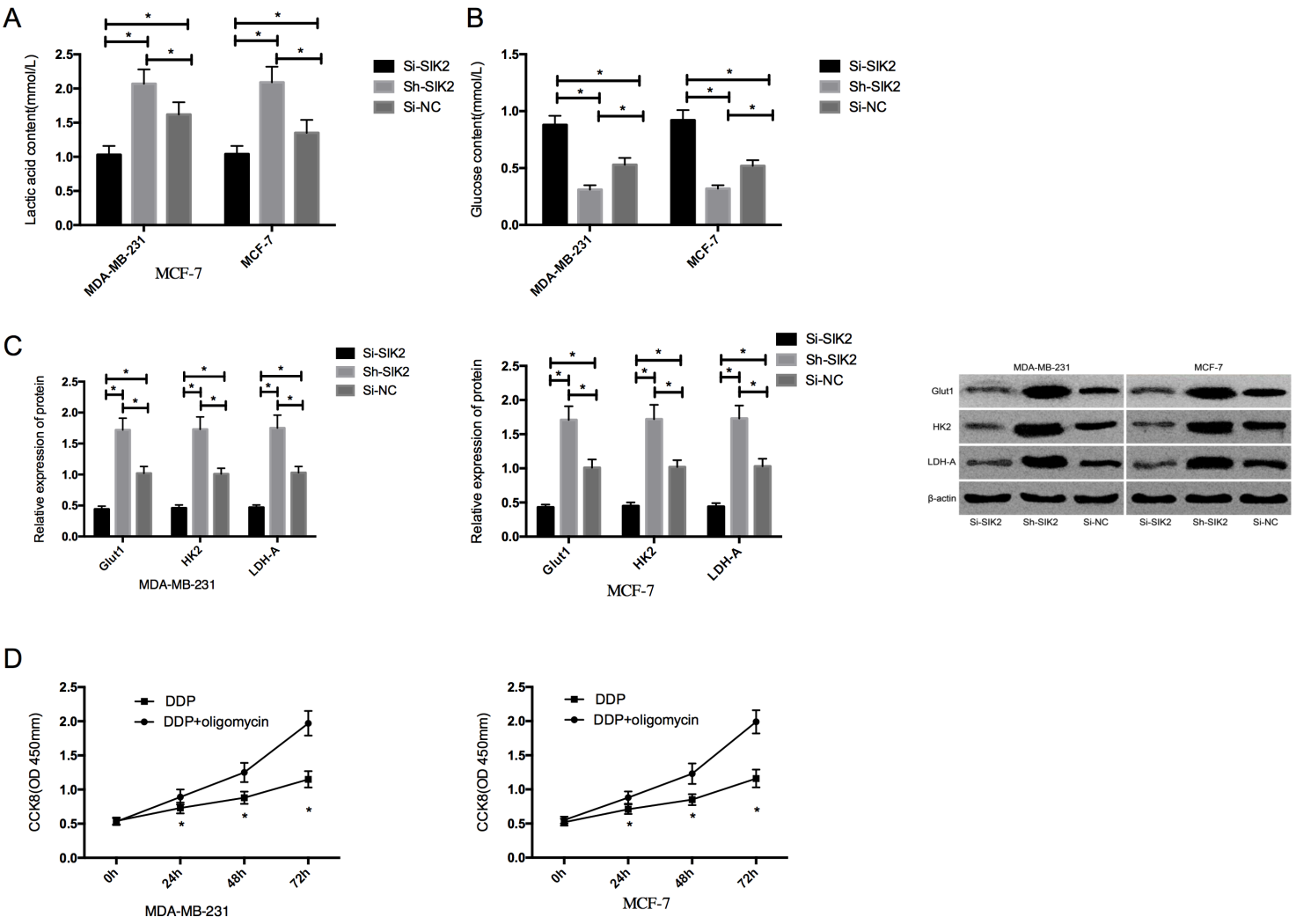

Figure 4

SIK2 enhances DDP drug resistance by promoting glycolysis Figure A: effect of SIK2 on glucose content in breast cancer cell culture medium Figure B: effect of SIK2 on lactic acid content in breast cancer cell culture medium Figure C: effect of HSIK2 on expression of glycolysis related proteins in breast cancer cells Figure D: effect of activated aerobic glycolysis on DDP resistance of breast cancer cells * indicates P<0.05.

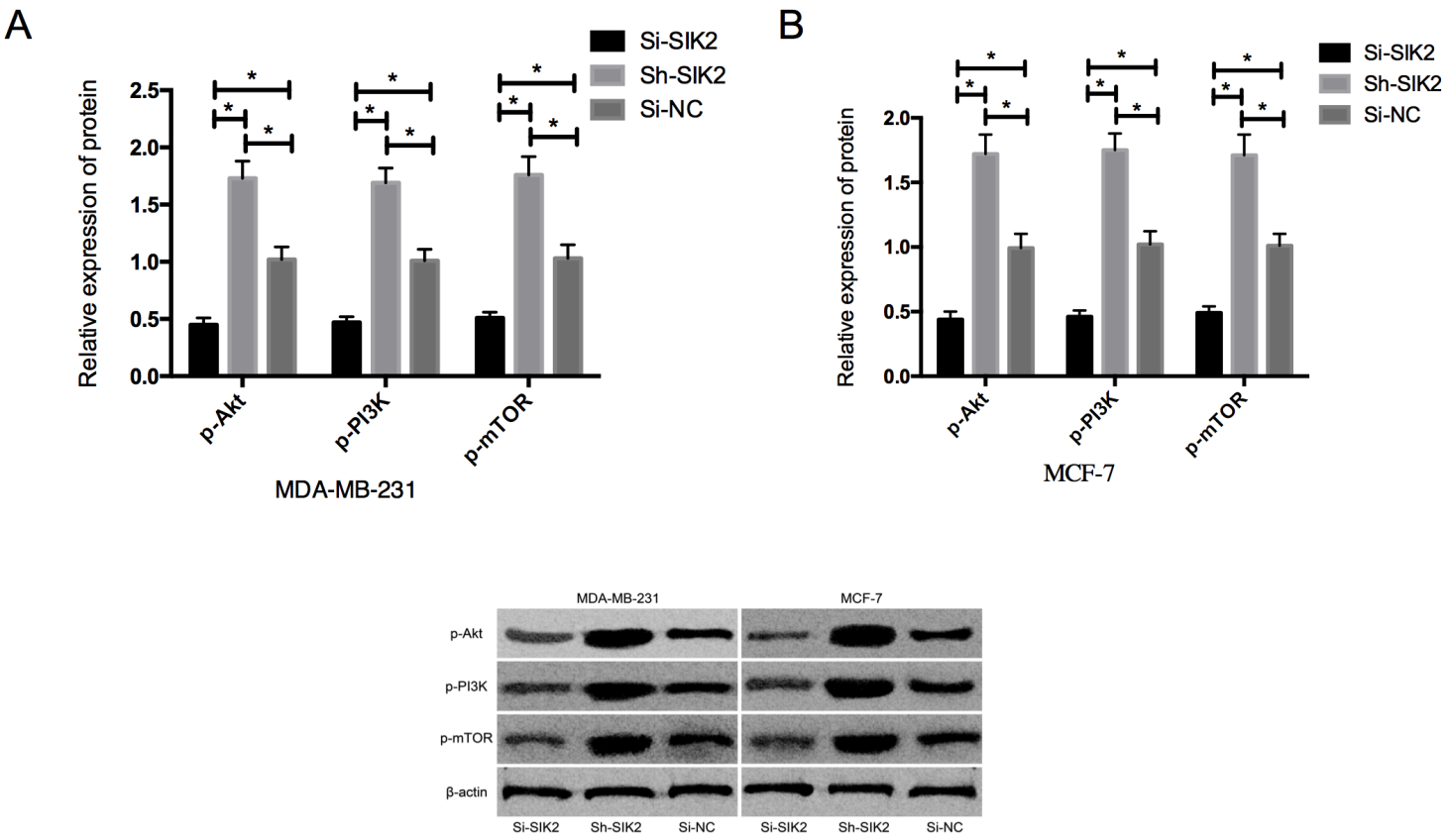

Figure 5

Effect of SIK2 on PI3K/AKT/mTOR signaling pathway in breast cancer Figure A: effect of SIK2 on PI3K/AKT/mTOR signaling pathway in breast cancer MDA-MB-231 cells Figure B: effect of SIK2 on PI3K/AKT/mTOR signaling pathway in breast cancer MCF-7 cells. * indicates P<0.05.

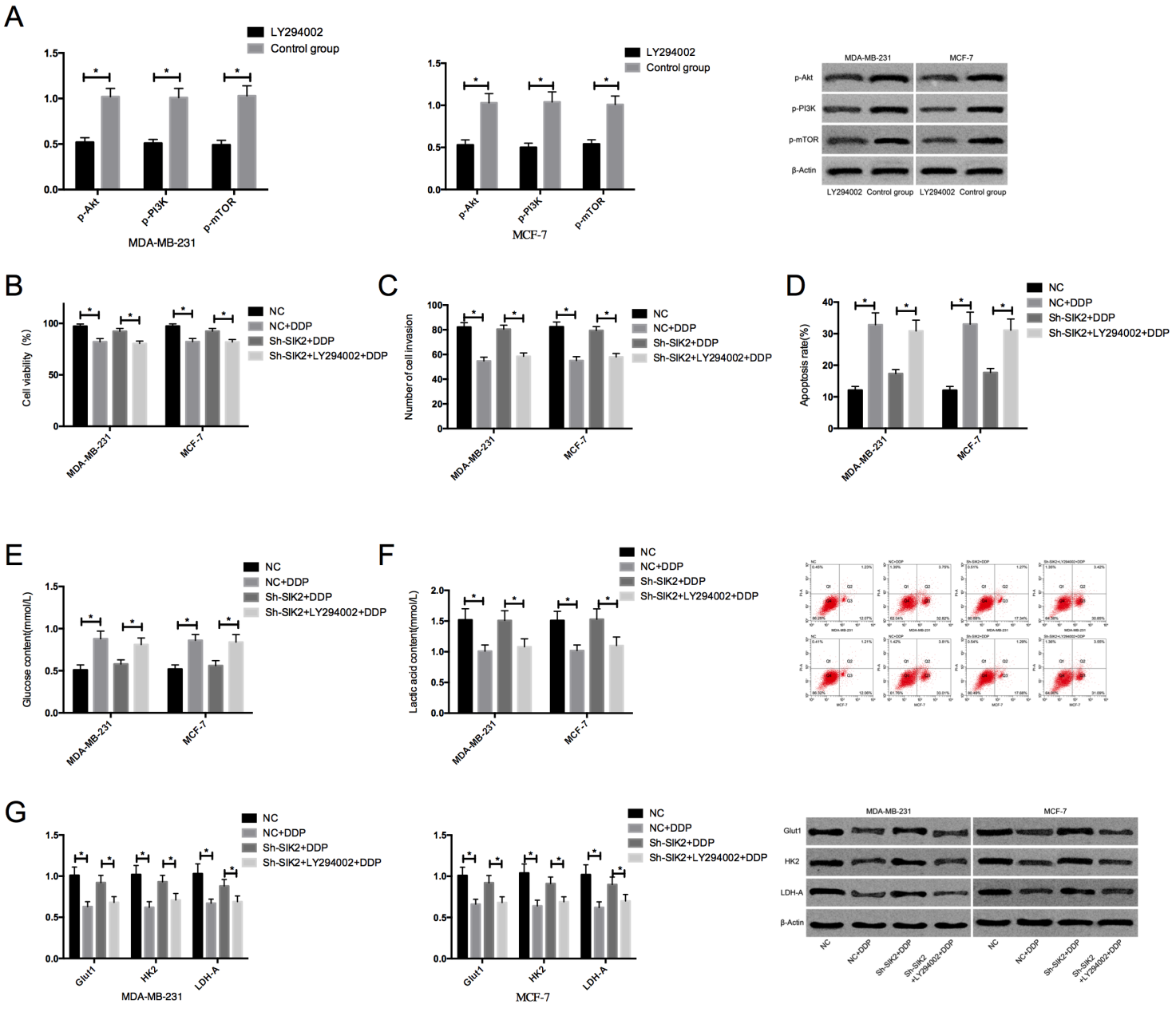

Figure 6

SIK2 regulates aerobic glycolysis of breast cancer through PI3K/AKT/mTOR signaling pathway Figure A: expression of p-Akt, p-PI3K, and p-mTOR protein in breast cancer cells after inhibiting PI3K/AKT/mTOR signaling pathway Figure B: comparison of cell survival rate Figure C: comparison of cell invasion ability Figure D: comparison of apoptosis rate Figure E: comparison of glucose content in culture medium Figure F: comparison of lactic acid content in culture medium Figure G: expression of glycolysis related proteins in breast cancer cells. * indicates P<0.05.