1. Introduction

In contrast to normal differentiated cells, cancer cells have been shown to display ten typical cancer hallmarks, among which an emerging hallmark is the metabolic reprogramming [1]. Cancer cells take up and utilize much more glucose than normal cells and switch to glycolysis metabolism even in the presence of adequate oxygen, a phenomenon well known as the Warburg effect, or aerobic glycolysis [2]. Generally, glucose metabolism via aerobic glycolysis provides biosynthetic precursors of nucleic acids, lipids and proteins, as well as reducing power NADPH in rapidly proliferating tumor cells [3,4].

Tumor cell metabolism is sophisticatedly regulated by signal transduction pathways that are affected by genetic mutations or the alterations in tumor microenvironment [5]. PI3K/Akt, HIF-1, c-Myc, p53 and AMPK pathways are intertwined with glucose metabolism through their effects on activities and expression of metabolic enzymes and metabolite transporters [5,6]. The M2 isoform of pyruvate kinase (PKM2), an enzyme which catalyzes the final reaction in glycolysis, is a key regulator of the Warburg effect [7]. PKM2 is frequently highly expressed in several types of tumors [8,9], and is transcriptionally regulated by c-Myc/hnRNPs, PI3K/AKT/mTOR and HIF-1α signaling pathways [10–13]. Maintaining the aerobic glycolysis metabolism should be matched with an increased uptake of glucose by elevating the glucose transporter proteins. Glucose transporter 1 (Glut-1) is the most common glucose transporter in humans and is aberrantly expressed in a variety of malignancies [14].

Nutrient deficiencies, such as glucose deprivation, are important phenomena in solid tumors. How tumor cells adapt to the microenvironment alterations has been far from being elucidated in detail. Glucose regulated protein 78 (GRP78) is a glucose-sensing protein [5,6], which causes inactivation of NF-κB pathway [17]. In the present study, we are focusing on whether GRP78 is involved in the modulation of tumor glucose metabolism and tumor microenvironment alterations.
metabolism. We demonstrate that upon glucose deficiency, the induced GRP78 facilitates the expression and membrane translocation of Glut-1 but suppresses the expression of PKM2, resulting in the enhancement of glucose transportation and the metabolic switch from glycolysis to mitochondrial TCA cycle. These effects of GRP78 are attributed to the inhibition of the NF-κB signaling pathway. Specifically, GRP78 overexpression induces the autophagy-mediated degradation of IKKβ, which results in the decrease of NF-κBp65 levels. Our results thus present a novel manner in which tumor cells coordinate the glucose-starvation stress in the microenvironment. It may hold important implications for strategies aiming at targeting tumor glucose metabolism.

2. Materials and methods

2.1. Materials

Culture media and fetal bovine serum (FBS) were from GIBCO (Grand Island, NY). Trizol, PrimeScript RT Master Mix and SYBR green PCR master mix were from Takara (Shiga, Japan). Antibodies for Glut-1, HIF-1α and β-actin were from Bioworld Technology (Minneapolis, MN). GRP78 antibody was from Abcam (Cambridge, UK). Antibodies for LC-3, NF-κBp65 and IKKβ were obtained from Proteintech (Chicago, USA). PKM2 and VPS34 antibodies were obtained from Cell Signaling Technology (Danvers, MA). FITC-, TRITC- and HRP-conjugated secondary antibodies were obtained from Invitrogen (Carlsbad, CA). BAY 11-7082 (NF-κB inhibitor), Wortmannin (PI3K/Akt inhibitor), Triciribine (Akt inhibitor), 2-methoxyestradiol (HIF-1α inhibitor) and 3-methyladenine (autophagy inhibitor) were from Cayman Chemical (MI, USA). Leupeptin (lysosome protease inhibitor) and MG-132 (proteasome inhibitor) were obtained from Sigma (St. Louis, USA).

2.2. Cell culture and glucose starvation treatment

Human colon carcinoma HT-29, DLD1 and 293 T cell lines were obtained from the American Type Culture Collection and cultured in RPMI-1640 or DMEM medium containing 10% FBS at 37 °C in a humidified tissue culture incubator containing 5% CO2. For glucose starvation stimulation, cells were cultured in medium with low concentration (0.5 mM) of glucose, while the control cells were cultured in medium with standard concentration (25 mM) of glucose [18].

2.3. Lentivirus generation and infection

For stable cell line selection, GRP78 overexpression, GRP78 shRNA or NF-κB-driven GFP plasmid was co-transfected with both pCMVdR8.91 and pCMV-VSV-G into 293 T cells using the Calcium Phosphate method at 15:10,5 μg (for a 10-cm dish). Media containing virus was collected and concentrated using 100-kDa ultrafiltration membranes (Millipore). Colon cancer cells were infected with the viruses and subjected to puromycin selection.

Table 1

<table>
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<tr>
<th>Primers</th>
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<th>Rev Sequence</th>
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Fig. 1. Glucose deficiency induces Glut-1 expression via stimulation of GRP78. (A) DLD1 cells were exposed to glucose deprivation (Gd, 0.5 mM glucose) for the indicated time intervals. The relative mRNA levels of Glut-1 were examined by Real-time PCR. *p < 0.05, **p < 0.01 vs 0 h. (B) Representative Western blots of Glut-1 in DLD1 cells exposed to Gd for 0, 12, 24 and 48 h. Fold changes of Glut-1 were normalized to the expression of β-actin. (C) Western blots of GRP78 in DLD1 cells exposed to Gd for 0, 12, 24 and 36 h. Fold changes were normalized to the expression of β-actin. (D) Relative mRNA levels of GRP78 and Glut-1 in GFP and GRP78 overexpressing DLD1 cells. *p < 0.01 vs GFP. (E) Western blots of Glut-1 in GFP- and GRP78-overexpressing cells. (F) DLD1 cells were transfected with either Ctrl-shRNA or GRP78-shRNA, and the relative mRNA levels of Glut-1 were examined by Real-time PCR. *p < 0.05, **p < 0.01 vs Ctrl-shRNA. The shRNA sequences used in this study were previously described [17].
2.4. RNA extraction and Real-time PCR analysis

Total RNA extraction, reverse transcription and Real-time PCR were performed as previously described [19]. The primers used in this study are shown in Table 1.

2.5. Membrane protein extraction, co-immunoprecipitation, GST-pulldown and Western blotting analysis

The cellular membrane proteins were extracted as previously described [19]. Changes in Glut-1 on cell membrane were determined by Western blotting. Co-immunoprecipitation and GST-pulldown assays were also performed as previously described [17]. For total protein expression analyses, lysates of stimulated cells were prepared and the protein concentrations were determined using the BCA protein assay. Equal amounts of protein per lane were determined then mixed with 5× SDS sample buffer, boiled for 5 min and separated by 10–15% SDS-PAGE before transferring the proteins onto PVDF membranes (Millipore). After blocking in 5% skimmed milk for 1 h, the membranes were rinsed and incubated overnight at 4 °C with the appropriate diluted primary antibody. The membranes were subsequently incubated with HRP-conjugated secondary antibody for 1 h at room temperature. The bands were visualized using an enhanced chemiluminescence detection kit and X-ray film.

2.6. Immunofluorescence analysis

Cells were plated on 12-well glass slides. After the required treatments, the cells were then fixed in 4% paraformaldehyde in PBS for 30 min, or alternatively permeabilized with 0.3% Triton X-100 in PBS for 10 min in the process of cytoplasmic protein staining. Next, the slides were blocked in 2% goat serum for 1 h and incubated with the primary antibodies at 4 °C overnight. The slides were then washed and incubated with the corresponding secondary antibodies. After three PBS washes, the slides were mounted in gelvatol for confocal immunofluorescence analysis.

2.7. Detection of intracellular LDH activity and extracellular lactate release

The GRP78–GFP and GFP expressing DLD1 cells were cultured in serum free medium for 24 h. After that the cells and medium were collected separately to perform assays in order to detect intracellular LDH activity and extracellular lactate release, as per manufacturer’s instructions (Nanjing Jiancheng Bioengineering Institute, China).

2.8. In vitro PI3P ELISA assays in GFP- and GRP78-expressing cells

PI3P content was measured in a quantitative and competitive ELISA assay according to the manufacturer’s instruction (Echelon Biosciences). Briefly, after lipid extraction, the lipid fraction obtained from the cells were incubated with the PI3P detector, then added to a PI3P-precoated microplate for competitive binding. The peroxidase-conjugated antibody against PI3P detector protein was added and colorimetric detection was performed to detect PI3P detector protein binding on the plate. The quantities of PI3P were calculated by the standard curve from non-linear fitting of PI3P standards.

2.9. Statistical analysis

Data are expressed as the mean ± SEM. Differences among groups were tested by one-way analysis of variance (ANOVA). Comparisons between two groups were evaluated using Student’s t-test. A value of p < 0.05 was considered statistically significant.
3. Results

3.1. Glucose deprivation induces Glut-1 expression via stimulation of GRP78

Nutritional deficiency, such as glucose deprivation is an important phenomenon in solid tumors. To investigate the effects of glucose deprivation on glucose metabolism, DLD1 colon cancer cells were exposed to low concentrations of glucose (0.5 mM) and the kinetic Glut-1 expression was subsequently determined. As shown in Fig. 1A and B, upon glucose deprivation (Gd), Glut-1 expression was increased at both mRNA and protein levels but then decreased to the basal levels, suggesting that induction of Glut-1 may be a pro-survival stress response. GRP78, an important glucose-regulated protein [20], was consistently up-regulated throughout the time course of glucose deprivation (Fig. 1C). Furthermore, overexpression of GRP78 in DLD1 cells by lentivirus gene transfer significantly raised the Glut-1 mRNA and protein levels (Fig. 1D and E). Consistently, knockdown of GRP78 expression by RNAi diminished the Glut-1 mRNA level (Fig. 1F). These results suggest that glucose deficiency-induced Glut-1 expression is at least partially mediated by GRP78.

3.2. GRP78 facilitates membrane translocation of Glut-1

Glut-1 is a glucose transporter located in the cell membrane. To investigate whether GRP78 expression could elevate the surface abundance of Glut-1, the membrane proteins from GRP78–GFP and GFP expressing DLD1 cells were extracted and detected by Western blotting. As shown in Fig. 2A, cell-surface Glut-1 was increased in GRP78-overexpressing cells. Interestingly, immunocytochemical staining showed the co-localization of Glut-1 with GRP78–GFP protein but not with GFP in transfected DLD1 cells (Fig. 2B). Co-IP assays further showed that GRP78 could co-immunoprecipitate with Glut-1 in DLD1 cells (Fig. 2C). We have demonstrated that sodium butyrate (SB), a differentiation inducer, was able to block the membrane translocation of GRP78 in colon cancer cells [19]. Here, we showed that the membrane location of Glut-1 was also severely affected by SB treatment (Fig. 2D), while the total expression of Glut-1 was significantly induced by SB treatment at both mRNA and protein levels (Fig. 2E and F). The results above suggest that GRP78 may facilitate cell surface translocation of Glut-1 via protein–protein interaction.

3.3. GRP78 promotes Glut-1 expression via stimulation of HIF-1α pathway

Glut-1 is an important target gene of the HIF-1α pathway [21,22]. Thus, the kinetic expression of HIF-1α was examined in Gd-treated and GRP78-overexpressing DLD1 cells. As shown in Fig. 3A and B, HIF-1α expression was significantly up-regulated at both mRNA and protein levels in Gd-treated DLD1 cells, as it was in GRP78-overexpressing DLD1 cells (Fig. 2C and D). Conversely, knockdown of endogenous GRP78 expression in DLD1 cells by shRNA (GRP78-shRNA) reduced the mRNA levels of HIF-1α (Fig. 3E). The above results suggest that glucose deprivation-induced HIF-1α expression is also mediated by GRP78. Next, to further confirm the regulation of Glut-1 expression by HIF-1α, 2-methoxyestradiol (2-ME2), an HIF-1α

![Figure 3. GRP78 promotes Glut-1 expression via stimulation of HIF-1α pathway. (A) DLD1 cells were exposed to glucose deprivation (Gd) for 0, 6, 12, and 24 h. The relative mRNA levels of HIF-1α were examined by Real-time PCR. **p < 0.01 vs 0 h. (B) Western blots of HIF-1α in DLD1 cells exposed to Gd for 0, 12, 24 and 48 h. Fold changes of HIF-1α were normalized to the expression of β-actin. (C) Relative mRNA levels of HIF-1α in GFP and GRP78 stably expressing DLD1 cells. **p < 0.01 vs GFP. (D) Western blots of HIF-1α in GFP and GRP78 stably expressing DLD1 cells. Fold changes of HIF-1α were normalized to the expression of β-tubulin. (E) Relative mRNA levels of HIF-1α in GRP78-knockdown DLD1 cells. *p < 0.05, **p < 0.01 vs Cont-shRNA. (F) Relative mRNA levels of Glut-1 in GRP78 stably expressing DLD1 cells in the presence of Triciribine (TCN) or 2-methoxyestradiol (2-ME2). ***p < 0.001 vs GFP, **p < 0.01 vs GFP–GRP78. (G) Western blotting analysis of Glut-1 expression in GRP78 expressing DLD1 cells in the presence of Triciribine (TCN, 10 μM) or 2-methoxyestradiol (2-ME2, 10 μM). Fold changes of Glut-1 were normalized to the expression of β-actin. (H) DLD1 cells were exposed to glucose deprivation in the presence or absence of 2-ME2 for 12 h. The images of morphological changes were produced using phase-contrast inverted microscopy. (I) Cell viabilities were also quantitatively evaluated by MTT assay (mean ± SEM). **p < 0.01 vs Cont.](1240)
inhibitor, was used to block the HIF-1α pathway in GRP78-overexpressing DLD1 cells. As shown in Fig. 3F and G, 5 μM or 10 μM 2-ME2 could significantly reverse the inducing effect of GRP78 on Glut-1 expression. Though Akt signaling is also the downstream target of GRP78 and implicated in regulation of Glut-1 expression [23,24], the Akt inhibitor (TCN) failed to obviously affect the induction of Glut-1 by GRP78. Furthermore, inhibition of HIF-1α by 2-ME2 for 12 h accelerated Gd-induced DLD1 cell death as demonstrated by morphological and MTT assays (Fig. 3H and I), suggesting that activation of GRP78/HIF-1α/Glut-1 axis is a pro-survival stress response to glucose deprivation.

3.4. GRP78 promotes HIF-1α expression via inhibition of NF-κB pathway

Several lines of evidence have shown that HIF-1α transcription can be regulated by the NF-κB pathway [25,26]. The levels of NF-κBp65 were examined to check the status of NF-κB pathway in Gd-treated and GRP78-overexpressing DLD1 cells. Although HIF-1α expression was up-regulated (Fig. 3B and D), the levels of NF-κBp65 were significantly suppressed in both Gd-treated and GRP78-overexpressing DLD1 cells, as demonstrated by Western blotting and immunofluorescence staining assays (Fig. 4A, B and D). To further characterize the alterations of NF-κB transcriptional activity, HT-29 colon cancer cells were transfected with the NF-κB-driven GFP reporter and cultured under the conditions of low glucose (0.5 mM) or standard conditions (25 mM glucose). As shown in Fig. 4C, the fluorescence intensity of GFP was weakened by Gd treatment, indicating that glucose deficiency causes a reduction of NF-κB signaling activity. Furthermore, inhibition of NF-κB pathway with its inhibitor BAY 11-7082 caused an increased expression of HIF-1α, which mirrored the effects of glucose deprivation (Fig. 4E). These results indicate that glucose deficiency induces HIF-1α expression by blocking the NF-κB signaling pathway.

3.5. GRP78 inhibits NF-κB pathway through autophagic degradation of IKKβ

The proteasomal and lysosomal pathways are the two main routes of protein and organelle clearance in eukaryotic cells [27]. To further investigate the mechanism by which glucose deficiency or GRP78 overexpression inhibits NF-κB signaling pathway, the proteasome inhibitor MG-132 and the lysosomal protease inhibitor leupeptin were used to inhibit proteasomal and lysosomal pathways, respectively. As shown in Fig. 5A and B, the inhibition of NF-κBp65 by glucose deficiency and GRP78 was abrogated by leupeptin but not MG-132, suggesting that these inhibitory effects were dependent on the lysosomal degradation pathway.

Autophagy is a degradative mechanism that is part of the lysosomal system and large amounts of proteins are degraded in the lysosome through the autophagic pathway [28]. Glucose deficiency can induce autophagy in different types of cells [29–31]. GRP78 has been reported to be required for ER integrity and stress-induced autophagy in mammalian cells [32]. We observed that GRP78 overexpression also induced autophagy, as characterized by an increase in LC3-II protein and a decrease in p62 protein (Fig. 5C). Consistent with the above observations,
down-regulation of NF-κBp65 by GRP78 was found to be reversed by the autophagy inhibitor 3-methyladenine (3-MA) (Fig. 5D), confirming that the inhibition of NF-κBp65 by GRP78 is dependent on autophagy. 3-MA is an inhibitor of the Class III PI3K (VPS34), an essential initiator of autophagy [33], and GRP78 can form complex with and activate PI3K [34]. To test the possibility of GRP78–VPS34 interaction, GST-pulldown assay was performed and demonstrated that purified recombinant GST–GRP78 protein bound to VPS34 protein from DLD1 cell extracts (Fig. 5E). Co-immunoprecipitation (co-IP) assay further verified that GRP78 formed a complex with VPS34 in 293 T cells which were co-transfected with VPS34- and GRP78-expressing plasmids (Fig. 5F). By deletion mutant analysis, the C-terminal 154 amino acids of GRP78 were found to mediate its interaction with VPS34 (Fig. 5G). Importantly, in contrast to the GFP-expressing cells, there was a notable increase of total levels of PI3P, a product of VPS34, in GRP78-expressing cells, indicative of the activation of VPS34 (Fig. 5H). The results above suggest that GRP78 is likely to induce autophagic degradation of IKKβ, through associating with and activating VPS34.

3.6. Glucose deprivation-induced GRP78 results in a reduction of PKM2 expression

Tumor cells undergo metabolic rewiring from oxidative phosphorylation to aerobic glycolysis to maintain the increased anabolic requirements for cell proliferation. It is widely accepted that a shift from the M1 type to the M2 type pyruvate kinase (PKM2) in tumor cells contributes to this aerobic glycolysis phenotype [35]. To examine the relative expression of PKM1 to PKM2 in colon cancer cells, both PKM1 and PKM2 transcripts were amplified by a PCR-based assay. The PCR products were then digested by PstI to distinguish the cleavable PKM2 amplicon from the uncleavable PKM1 product [36]. As shown in Fig. 6A, PKM2 was the predominant isoform over PKM1 in DLD1 cells. Surprisingly, PKM2 mRNA level was diminished in GRP78-overexpressing DLD1 cells. To verify the above results, PKM2 expression was further examined by qPCR. We showed that GRP78 overexpression resulted in the impaired expression of PKM2 at both mRNA and protein levels (Fig. 6B and C), while knockdown of GRP78 leads to the increased PKM2 expression (Fig. 6F). Low concentration (0.5 mM) of glucose also leads to a reduction of PKM2 expression in DLD1 cells in a time-dependent manner (Fig. 6D and E). Interestingly, we observed that the expression of GRP78 and PKM2 varied according to the supply of glucose. GRP78 expression was induced at low concentrations of glucose, but retained to a relatively low level when the glucose supply was restored. During this process, PKM2 expression was suppressed or restored, corresponding to the alterations of GRP78 expression (Fig. 6G and H). These results indicate that the modulation of PKM2 expression by GRP78 is affected by the glucose levels in the tumor microenvironment.

3.7. GRP78 inhibits PKM2 expression and lactate production through inhibiting NF-κB pathway

Next, NF-κB inhibitor (BAY 11-7082, 10 μM) and PI3K/Akt inhibitor (Wortmannin, 1 μM) were used to characterize the mechanism of

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**Fig. 5.** GRP78 inhibits NF-κB pathway through autophagic degradation of IKKβ. (A) Western blotting analysis of NF-κBp65 expression in DLD1 cells after growth in high glucose or glucose deprivation for 24 h in the presence and absence of MG-132 or leupeptin. Fold changes of NF-κBp65 were normalized to the expression of β-actin. (B) Western blotting analysis of NF-κBp65 expression in GFP and GRP78 expressing DLD1 cells in the presence and absence of MG-132 or leupeptin. Fold changes of NF-κBp65 were normalized to the expression of β-actin. (C) Western blotting analysis of LC3-I, LC3-II and p62 in GFP and GRP78 expressing DLD1 cells. Fold changes of LC3-II and p62 were normalized to the expression of β-actin. (D) Western blotting analysis of NF-κBp65 and IKKβ in GFP and GRP78 expressing DLD1 cells in the presence and absence of 3-methyladenine (3-MA). Fold changes of NF-κBp65 and IKKβ were normalized to the expression of β-actin. (E) Cell lysates from DLD1 cells were incubated with GST or GST–GRP78 glutathione beads. The precipitates were separated by SDS-PAGE and immunoblotted with anti-VPS34 antibody. Bands of VPS34 were normalized to the input level of VPS34. (F) Analysis of the interaction of GRP78 with VPS34. Immunoprecipitation experiments were carried out on the lysates of 293 T cells transfected with VPS34- and GRP78-expressing plasmids. (G) Identification of the domain of GRP78 mediating its interaction with VPS34. GFP-tagged GRP78 deletion mutants were generated as diagrammed. 293 T cells were co-transfected with mutant GRP78 constructs and Flag-tag VPS34 plasmid. The lysates were immunoprecipitated with anti-flag antibody, followed by immunoblotting with anti-GFP antibody. (H) Determination of PKM contents in GFP- and GRP78-expressing cells by a quantitative PI3P ELISA assay. The PI3P level was normalized by the amount of proteins used in the assay, *p < 0.05.
Western blotting (H). continued to be exposed to glucose deprivation for another 12 h (Gd12 + Gd12). Relative mRNA levels were examined in these cells with different treatments using Real-time PCR (G).

Glut-1, which is frequently overexpressed in a variety of malignancies, promotes entry of glucose into the cell. This is achieved by a family of glucose transporters (Gluts), especially Glut-1, which is overexpressed in many malignancies and associated with tumor progression [14].

Enhancement of Glut-1 expression and membrane translocation by insulin-like growth factor is able to promote cell survival under glucose deprivation conditions [38].

4. Discussion

Glucose is one of the most critical carbon sources for tumor cells, and is required in abundance for generating ATP and metabolic intermediates to support cancer cell growth [37]. Efficient transport of glucose across the plasma membrane is a necessary prerequisite for the acquisition of an aerobic glycolysis phenotype by tumor cells. Glucose transport is achieved by a family of glucose transporters (Gluts), especially Glut-1, which is frequently overexpressed in a variety of malignancies and associated with tumor progression [14].
dependent manner. Consistently, we further observed that GRP78 can interact with the critical autophagy initiator VPS34 and their association may result in activation of autophagy.

Cancer cells preferentially take advantage of aerobic glycolysis for glucose metabolism and energy supply. This metabolic characteristic is largely controlled by the specific expression of PKM2 in tumor cells. Under conditions of adequate glucose supply, PKM2 allows tumor cells to divert glucose into anabolic pathways branching from glycolysis, meeting the biosynthetic demands for rapid proliferation [7]. While under glucose starvation conditions, cellular SAICAR (an intermediate of the de novo purine nucleotide synthesis pathway) is increased in an oscillatory manner and stimulates PKM2 activity via direct binding, thereby promoting cancer cell survival [43]. Our results reveal that glucose deficiency can also impair the expression of PKM2 via induction of GRP78. Interestingly, the level of PKM2 expression changes according to the glucose concentration and the induced GRP78 level. Consistently, inhibition of PKM2 by GRP78 is accompanied with a decreased lactate dehydrogenase activity and lactate production, but with enhanced expression of mitochondrial pyruvate dehydrogenase A (PDHA) and PDHB. These findings suggest that under glucose deprivation conditions, induction of GRP78 expression results in the shift of glucose metabolism from glycolysis to TCA cycle, which helps to overcome the harsh condition of glucose scarcity.

In summary, our study establishes GRP78 as a crucial molecular link between cancer cell metabolism and tumor microenvironment alterations. High expression of GRP78 not only fuels cell growth and proliferation under adequate glucose conditions, but also supports cell survival via induction of GRP78 expression results in the shift of glucose metabolism from glycolysis to TCA cycle, which helps to overcome the harsh condition of glucose scarcity.

In summary, our study establishes GRP78 as a crucial molecular link between cancer cell metabolism and tumor microenvironment alterations. High expression of GRP78 not only fuels cell growth and proliferation under adequate glucose conditions, but also supports cell survival via induction of GRP78 expression results in the shift of glucose metabolism from glycolysis to TCA cycle, which helps to overcome the harsh condition of glucose scarcity.

**Fig. 7.** GRP78 inhibits PKM2 expression and lactate production through inhibiting NF-κB pathway. (A) Relative mRNA levels of PKM2 in GFP and GRP78 stably expressing DLD1 cells in the presence and absence of BAY 11-7082 (10 μM) and Wortmannin (1 μM). *p < 0.05 vs GFP–GRP78. (B) Western blots of NF-κBp65 and PKM2 in DLD1 cells exposed to BAY 11-7082 for 0, 18, and 36 h. Fold changes of NF-κBp65 and PKM2 were normalized to the expression of β-actin. (C) The relative contents of lactate release from GFP and GFP–GRP78 expressing DLD1 cells. *p < 0.05 vs GFP. (D) The relative lactate dehydrogenase (LDH) activity in GFP and GFP–GRP78 expressing DLD1 cells. *p < 0.05 vs GFP. (E) Relative mRNA levels of LDHA, LDHB, PDHA, and PDHB in GFP and GRP78 stably expressing DLD1 cells. *p < 0.05, **p < 0.01 vs GFP.
of GRP78 and aerobic glycolysis may be a rational strategy to interfere with tumor cell metabolism and survival for therapeutic purposes.

Conflict of Interest

The authors declare no competing interests.

Acknowledgments

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