CD38 is involved in cell energy metabolism via activating the PI3K/AKT/mTOR signaling pathway in cervical cancer cells

SHAN LIAO$^{1,2*}$, LIN LIANG$^2$, CHUNXUE YUE$^2$, JUNYU HE$^2$, ZHENGXI HE$^2$, XI JIN$^2$, GENGQIU LUO$^3$ and YANHONG ZHOU$^{1,2}$

$^1$Department of Oncology, Xiangya Hospital, Central South University, Changsha, Hunan 410008; $^2$Cancer Research Institute, Basic School of Medicine, Central South University, Changsha, Hunan 410078; $^3$Department of Pathology, Xiangya Hospital, Basic School of Medicine, Central South University, Changsha, Hunan 410008, P.R. China

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Abstract. In contrast to normal cells, cancer cells typically undergo metabolic reprogramming. Studies have shown that oncogenes play an important role in this metabolic reprogramming. CD38 is a multifunctional transmembrane protein that is expressed abnormally in a variety of tumor types. To investigate the effect and possible mechanism of CD38 in cervical cancer cells and to provide a new therapeutic target for the treatment of cervical cancer, the present study identified that CD38 is involved in regulating cell metabolism in cervical cancer cells. Liquid chromatography-tandem mass spectrometry and bioinformatic analyses revealed that differentially abundant proteins in CD38-overexpressed cervical cancer cells (CaSki-CD38 and HeLa-CD38) are predominantly involved in glycolytic pathways, oxidative phosphorylation and the NAD/NADH metabolic process. Further experiments using an ATP test kit and lactate test kit revealed that CD38 promotes glucose consumption, increases lactate accumulation and increases ATP production. In addition, CD38 increases the phosphorylation of phosphatidylserine/threonine kinase (AKT), mechanistic target of rapamycin (mTOR) and phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K), which play a key role in tumor metabolism. Furthermore, it was found that the energy metabolism of cervical cancer cells was inhibited following treatment with the mTOR inhibitor rapamycin. In conclusion, the results of the present study suggested that CD38 regulates the metabolism of cervical cancer cells by regulating the PI3K/AKT/mTOR pathway, which may be a candidate target for the treatment of cervical cancer.

Introduction

Metabolic reprogramming of cancer cells has become a hot topic in cancer research (1,2). In 1924, Otto Warburg proposed that cancer cells rely predominantly on aerobic glycolysis rather than the more efficient oxidative phosphorylation of mitochondria to produce ATP, even when there is sufficient oxygen supply. The use of aerobic glycolysis leads to an increase in both glucose uptake and lactate production in cancer cells and is termed the Warburg Effect (3,4). Glucose uptake and the demand for metabolic intermediates increases significantly in cancer cells to support rapid cell growth (5). Previously, it was considered that mitochondrial dysfunction in tumor cells leads to a significant decrease of ATP production, thus resulting in a compensatory increase of glycolysis (6-9). However, to the best of our knowledge, the definitive mechanism of the Warburg Effect and how it affects biosynthesis in tumor cells remain unknown. With the development of modern biotechnology, researchers found that in addition to aerobic glycolysis, other metabolic pathways, such as the Krebs cycle (10,11), fatty acid metabolism (12), glutamine metabolism (13-15) and the pentose-phosphate pathway (16), are abnormally regulated in tumor cells. In addition, some studies considered that the aberrant expression of oncogenes and tumor suppressor genes leads to metabolic reprogramming in tumor cells (17-22).

CD38 has dual activities as a ADP-ribosyl cyclase and cyclic ADP-ribose hydrolase (23,24). CD38 is expressed in a variety of cells and regulates diverse activities, such as signal transduction (23), cell adhesion (25), cyclic ADP-ribose synthesis (26), and cell differentiation and activation (27). CD38 is also involved in regulating mitochondria functions (28,29). Our previous study demonstrated that CD38...
could promote cell proliferation and inhibit cell apoptosis, probably by regulating mitochondrial function (30). We also identified that CD38 is highly expressed in cervical cancer and is associated with the phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)/Akt serine/threonine kinase (AKT) signaling pathway. This indicates that CD38 plays an important role in the energy metabolism of cancer cells and is also closely associated with cervical cancer. However, the detailed role and mechanism of CD38 in the carcinogenesis of cervical cancer remains unclear.

The present study aimed to investigate the potential mechanism of CD38 in cervical cancer cells. First, liquid chromatography-tandem mass spectrometry (LC-MS/MS) technology was used to screen for proteins that were differentially abundant in response to CD38 overexpression. Subsequently, the effects of CD38 on key molecules in the PI3K/AKT/mechanistic target of rapamycin (mTOR) signaling pathway was detected. The effects of CD38 on ATP, lactic acid and other metabolites in cervical cancer cells were also analyzed. To clarify whether CD38 could promote cervical cancer through the PI3K/AKT/mTOR signaling pathway, cells were treated with the mTOR inhibitor rapamycin, and the functional changes induced by CD38 after blocking the PI3K/AKT/mTOR signaling pathway were analyzed. The results demonstrated that CD38 is involved in cellular energy metabolism via activating the PI3K/AKT/mTOR signaling pathway in cervical cancer cells.

Materials and methods

Cell lines and cell culture conditions. The cervical cancer cell lines CaSki and HeLa were maintained in the Molecular Genetics Laboratory (Central South University, Changsha, China). Cervical cancer cells were cultured in Roswell Park Memorial Institute (RPMI)-1640 medium containing 10% fetal bovine serum (FBS; both from Gibco; Thermo Fisher Scientific, Inc.) with 5% CO2 at 37°C. The CD38-overexpressing cell lines (CaSki-CD38 and HeLa-CD38) and the control cell lines (CaSki-vector and HeLa-vector) were transfected with the CD38-overexpressing plasmid pEGFP-N1-CD38 and the control plasmid pEGFP-N1 using Lipofectamine 2000 (Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol, as described previously (30,31). The transfected cell lines were grown in RPMI-1640 supplemented with 10% FBS and G418 (500 µg/ml). Following 3 weeks, the stable cells were used for subsequent experiments.

Protein extraction and digestion. Protein extraction and digestion were performed as previously described (32). Briefly, cells were lysed using a protein extraction buffer consisting of 50 nM Tris (pH 7.4), 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDA and sodium orthovanadate, sodium fluoride, EDTA, leupeptin supplemented with 1X halt protease inhibitor cocktail (CWBio) and 1X halt phosphatase inhibitor cocktail (BestBio). The protein concentration was estimated using the bicinchoninic acid (BCA) method using a Micro BCA™ Protein assay kit (Thermo Fisher Scientific, Inc.). Then, cell lysates (50 µg of each sample) were loaded onto 10% SDS-PAGE gels and separated electrophoretically at 80 volts for 40 min and then 120 volts for 60 min (PowerPac Universal; Bio-Rad Laboratories, Inc.). Protein bands were visualized using Coomassie brilliant blue G-250 (Sigma-Aldrich; Merck KGaA) and excised from the gel into eight slices. The proteins were destained using 15 mM KFe(CN)6, and then 50 mM sodium thiosulfate and 1.25 µg trypsin (1:20 enzyme/substrate ratio) were added to each slice and in-gel-digestion was performed at 37°C overnight for ~16 h. The generated peptides were extracted by sonication (15 min, with ice cooling) from the gel pieces in ~20 µl 50% acetonitrile in 0.1% formic acid, twice. Following extraction, the peptides were dried using vacuum centrifugation at room temperature for 30 min to ensure the complete removal of acetonitrile and reconstituted in 20 µl 0.1% formic acid.

LC-MS/MS analysis of peptides. LC-MS/MS analysis was performed as previously described (32). Briefly, peptides were diluted using 0.1% formic acid. Then, peptides were pre-concentrated. The analytes were transferred to the analytical column and separated using a binary system. The mass spectrometer was operated in the data-dependent mode. Normalized collision energy was set to 35% and an isolation width of 2 m/z was chosen.

Protein identification and quantification. Protein identification and quantification were performed as previously described (32,33). Briefly, proteins were identified using Proteome Discoverer 1.4 software (Thermo Fisher Scientific, Inc.). Thermo raw files were imported and used to conduct a search of the UniProt proteomes-Homo sapiens database (UP000005640; https://www.uniprot.org/taxonomy/960). For database searches, mass tolerances were set to 10 ppm and 0.8 Da for precursor and fragment ions, respectively. Peptides identified with false discovery rates <1% (q-value <0.01) were discarded. A common contaminants database was also included for quality control. Proteins that met the following criteria were considered differentially expressed proteins: i) proteins had ≥2 peptides with ≥95% confidence; ii) proteins were considered downregulated when the protein levels demonstrated an average fold-change ≤0.5 in the LC-MS/MS analyses; and iii) proteins were considered upregulated when the protein levels demonstrated an average fold-change ≥2 in the LC-MS/MS analyses (33).

Measurement of glucose concentration. Cells were trypsinized and inoculated into 6-well cell culture plates (1x106 cells/well), and then incubated at 37°C in the presence of 5% CO2. Following 24-48 h, when the density of cells had reached ~80%, the culture medium supernatant was transferred into a new centrifuge tube and centrifuged at 1,000 x g for 5 min at 4°C to remove insoluble materials. The supernatant was transferred into specific tubes to detect the glucose concentration using an ADVIA 1650 automatic biochemical analyzer (Siemens AG). Meanwhile, the cells in the culture plate were trypsinized, washed with cold 1x PBS solution and placed in microcentrifuge tubes. Following ultrasonication on ice for 3 min, the samples were centrifuged at 12,000 x g for 20 min at 4°C. The supernatant was transferred into specific tubes to detect the intracellular glucose concentration using the ADVIA 1650 automatic biochemical analyzer (Siemens AG). All values were normalized on the basis of cell number.
# Table I. Downregulated proteins in CD38-overexpressing HeLa and CaSki cells.

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<th>Unique Peptides</th>
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<th>Ratio (CD38/vector)</th>
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Measurement of the cellular ATP concentration. The cellular ATP concentration was measured using an ATP detection assay kit (catalog no. S0026; Beyotime Institute of Biotechnology), according to the manufacturer's protocol. Cells were trypsinized, seeded into 6-well cell culture plates (1x10^6 cells/well) and incubated at 37˚C in the presence of 5% CO₂. Following 24 h, when the cell density reached ~80%, the culture medium was aspirated from the plate and 200 µl ATP lysis buffer was added into each well. The lysis buffer and cells were pipetted repeatedly to homogenize the cells on ice for 5 min. The cell lysate was transferred into new pre-cooled tubes and centrifuged at 12,000 x g for 15 min at 4˚C. The supernatant was transferred into new pre-cold tubes and kept on ice. The ATP standard buffer and ATP detection buffer were prepared. The ATP detection buffer was added into the wells of a 96-well plate (100 µl/well) and kept at room temperature for 5 min. The samples or ATP standard buffer were then added into the wells (20 µl/well) and incubated in the dark for 5 min at room temperature. The luminescence emitted by the samples was detected using a Paradigm Detection Platform (Beckman Coulter, Inc.). The concentration of cellular ATP (nmol/mg) was calculated based on a standard curve. All values were normalized to the protein concentration.

Measurement of the lactate concentration. The lactate concentration was detected using a Lactate assay kit (catalog no. MAK064; Sigma-Aldrich; Merck KGaA), according to the manufacturer's protocol. Briefly, cells were trypsinized and seeded into 6-well cell culture plates (1x10^5 cells/well). The cells were incubated at 37˚C in the presence of 5% CO₂. Following 24-48 h, when the cell density reached ~80%, the culture medium supernatant was transferred into new centrifuge tubes, and centrifuged at 12,000 x g for 20 min at 4˚C to remove insoluble materials. The supernatant was transferred into 10 kDa cut-off spin filters to deproteinize the samples. The filtrate was transferred into new tubes and kept on ice to detect the lactate concentration in the medium. Meanwhile, cells in the culture plate were digested and washed with cold 1x PBS solution and the resuspended cells were placed in microcentrifuge tubes. Following ultrasonication on ice for 3 min, the samples were centrifuged at 12,000 x g for 20 min at 4˚C. The supernatant was centrifuged at 12,000 x g for 20 min at 4˚C. The supernatant was transferred into 10 kDa cut-off spin filters to deproteinize the samples. The filtrate was transferred into new tubes and kept on ice to detect the intracellular lactate concentration. Then, 50 µl samples were placed into the wells of a 96-well plate, 50 µl Master Reaction mix was added to each sample, and the plate was incubated in the dark for 30 min at room temperature. The absorbance value at 570 nm was detected with a microplate reader and analyzed with SoftMax Pro 6.4 (Beckman Coulter). The lactate concentration was calculated based on a standard curve. All values were normalized on the basis of cell number.

Western blotting analysis. Western blotting was performed as described previously (30,31). The cells were trypsinized and then lysed using RIPA lysate buffer (CWBio). Samples were centrifuged at 12,000 x g for 15 min at 4˚C to remove insoluble materials. The protein concentration estimated using the BCA method, and each protein sample (50 µg) was electrophoresed

Table I. Continued.

<table>
<thead>
<tr>
<th>Gene symbol</th>
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Method, and each protein sample (50 µg) was electrophoresed...
Table II. Upregulated proteins in CD38-overexpressing HeLa and CaSki cells.

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<tr>
<th>Genesymbol</th>
<th>Description</th>
<th>Molecular weight, kDa</th>
<th>Score ( \text{HeLa} )</th>
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using a 10% SDS-PAGE gel at 80 volt for 40 min and then at 120 volt for 60 min (PowerPac Universal; Bio-Rad Laboratories, Inc.). The separated proteins were transferred onto a polyvinylidene fluoride membrane (HyClone; GE Healthcare Life Sciences) at 100 volt for 90 min and then the membranes were blocked for 1-2 h using 5% non-fat milk dissolved in PBS-0.1% Tween 20. The membranes were then incubated overnight with primary antibodies at 4°C. The following primary antibodies were used: Anti-CD38 (catalog no. YM0122; ImmunoWay Biotechnology Company), anti-3-phosphoinositide dependent protein kinase 1 (catalog no. YT3645; ImmunoWay Biotechnology Company), anti-P110 PI10 (catalog no. YT3709; ImmunoWay Biotechnology Company), anti-AKT (catalog no. YT0173; ImmunoWay Biotechnology Company), anti-phosphorylated (p)-AKT T308 (catalog no. YP0590; ImmunoWay Biotechnology Company), anti-mTOR (catalog no. YT2913; ImmunoWay Biotechnology Company), anti-p-mTOR (catalog no. YP0716; ImmunoWay Biotechnology Company), anti-lactate dehydrogenase A (LDH-A; catalog no. 19987-1-AP; ProteinTech Group, Inc.) and anti-ATP synthase peripheral stalk subunit D (ATP5H; catalog no. YT0406; ImmunoWay Biotechnology Company). These antibodies were stored at -20°C, and the dilution used for all antibodies was 1:1,000. The membranes were then washed and incubated with the following horseradish peroxidase-conjugated secondary antibodies for 1 h at 37°C: Anti-rabbit secondary antibody (catalog no. sc-2004; Santa Cruz Biotechnology, Inc.; 1:3,000) and anti-mouse secondary antibody (catalog no. sc-2005; Santa Cruz Biotechnology, Inc.; 1:3,000). ImageJ (v 1.51; National Institutes of Health) was used to quantitatively analyze the results.

**Clonogenic assay.** Cervical cancer cells were transfected with the empty vector and the CD38 overexpression vector and then seeded at a density of 800 cells/well in a 6-well plate. Following adherence, the cells in the wells were treated with 0.1 µM DMSO and 0.1 µM rapamycin (catalog no. S1039; Selleck Chemicals) at 37°C for 24 h. When the clone was visible to the naked eye, the culture was terminated. The culture medium was discarded and the cells were washed three times with PBS buffer. The cells were treated with paraformaldehyde for 30 min at room temperature, washed with 1x PBS, stained with 0.1% crystal violet for 30 min at room temperature and washed with tap water. Images were then obtained and the cells were counted.

**Cell apoptosis experiment.** A Hoechst33342/propidium iodide (PI) double staining kit (catalog no. bb-4131; Bestbio) was used to detect cell apoptosis according to the recommended protocol. The CD38 overexpression vector and the blank vector were transfected into CaSki and HeLa cells. The cells were seeded in 6-well plates and cultured at 37°C with
5% CO₂. After adhering to the wall, the cells were treated with DMSO and rapamycin. Following 24 h, the cells were digested with 0.25% trypsin, collected, and washed twice with PBS. The cells were resuspended in 0.5-1.0 ml staining buffer, and Hoechst33342 staining solution A (5-10 µl) was then added. Following gentle mixing, the cells were incubated at 4°C in
the dark for 10 min, and 5-10 µl PI was then added. Staining solution B was added (5-10 µl), the samples were gently mixed, and then incubated at 4°C in dark for 5-10 min. Finally, the cells were washed with PBS, resuspended in PBS and detected using flow cytometry. Analysis was performed using Summit v5.2 software (Beckman Coulter, Inc.).

Statistical analysis. Data are presented as the mean ± standard deviation of three independent experiments. Differences in parametric variables were analyzed using ANOVA followed by Student-Newman-Keuls post hoc test, and differences in quantitative variables between groups were analyzed by Kruskal-Wallis followed by Bonferroni’s test. Statistical analyses were performed with the EPI Info software (version 3.2.2; www.CDC.gov/epiinfo/). P<0.05 was considered to indicate a statistically significant difference.

Results

Identification of differentially abundant proteins in CD38-overexpressing cervical cancer cells using LC-MS/MS analysis. Our previous study demonstrated that CD38 expression is upregulated in cervical cancer. In vitro experiments revealed that CD38 could inhibit the apoptosis of cervical cancer cells and promote their proliferation. The previous results also demonstrated that CD38 affects the intracellular calcium concentration, mitochondrial membrane potential and intracellular reactive oxygen species in cervical cancer cells (30). Therefore, it was speculated that CD38 may be associated with the metabolism of cervical cancer cells.

To investigate if CD38 affects the metabolism of cervical cancer cells, LC-MS/MS technology was first used to screen and identify differentially abundant proteins between CD38-overexpressing cervical cancer cells and the control groups. Proteins were identified using Proteome Discoverer 1.4 software and analyzed by the UniProtKB/Swiss-Prot database. It was identified that 573 proteins were downregulated in CaSki-CD38 cells compared with the control cells and 274 proteins were downregulated in HeLa-CD38 cells compared with the control cells. Among these proteins, 40 proteins were consistently downregulated in both CaSki-CD38 and HeLa-CD38 cells. These 40 overlapping downregulated proteins are listed in Table 1. By contrast, 357 proteins were found to be upregulated in CaSki-CD38
Figure 2. Detection of glucose uptake ability, intracellular ATP and lactic acid concentrations in cervical cancer cells. (A) Glucose concentration in the culture medium and (B) the intracellular glucose concentration in cultured cervical cancer cells. (C) The lactate concentration in the culture medium and (D) the intracellular lactate concentration in cultured cervical cancer cells. All values were normalized on the basis of cell number.
cells and 359 proteins were upregulated in HeLa-CD38 cells compared with the control cells. Among these, 36 proteins were consistently upregulated both in CaSki-CD38 and HeLa-CD38 cells. These 36 overlapping upregulated proteins are listed in Table II.

Gene Ontology (GO) analysis of biological processes and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis of differentially expressed proteins in CD38-overexpressing cervical cancer cells. The results of the LC-MS/MS analysis revealed that ATP5H-2, cytochrome B5 type B (CYB5B), cytochrome C1 (CYC1) and other protein molecules were upregulated in CD38-overexpressing cervical cancer cells, while BR13 binding protein (BR13BP) and other protein molecules were downregulated. To further analyze the biological processes and signaling pathways that involve these differentially abundant proteins, GO and KEGG analyses were performed. As presented in Fig. 1, the differentially abundant proteins were annotated to be involved in the following biological processes: ‘Glucose catabolic process to pyruvate’, ‘oxidation-reduction process’, ‘glucose metabolic process’, ‘nucleoside monophosphate metabolic process’, ‘glycosyl compound metabolic process’, ‘NAD/NADH metabolic process’ and ‘ATP/ADP metabolic process’. This indicates that these proteins are predominantly involved in glycolytic pathways, oxidative phosphorylation and NAD/NADH metabolic processes. KEGG analysis demonstrated that CD38 overexpression was associated with ‘pyruvate metabolism’, ‘glycolysis/gluconeogenesis’, ‘metabolic pathways’ and ‘fatty acid elongation’. Thus, it can be suggested that CD38 is involved in regulating cellular energy metabolism in cervical cancer cells.

CD38 increases glucose uptake, ATP production, and lactic acid accumulation in cervical cancer cells. To test the hypothesis that CD38 is involved in regulating cellular energy metabolism in cervical cancer cells, the effects of CD38 overexpression on glucose uptake, intracellular ATP production and intracellular lactic acid concentration in cervical cancer cells were investigated. The glucose concentration in the culture medium was significantly decreased in CaSki-CD38 and HeLa-CD38 cells compared with vector-transfected cells (P<0.0002 and P<0.0001, respectively; Fig. 2A). The intracellular glucose concentration showed no significant difference between CD38-overexpressing cervical cancer cells and the control groups (Fig. 2B). It was speculated that, to a certain extent, CD38 promotes glucose consumption in cervical cancer cells. The lactate concentration in the culture medium was significantly increased in CaSki-CD38 and HeLa-CD38 cells compared with vector-transfected cells (P<0.0001 and P<0.0021, respectively; Fig. 2C). The intracellular lactate concentration was also significantly increased in CaSki-CD38 and HeLa-CD38 compared with vector-transfected cells (P<0.0001 and P=0.019; Fig. 2D).

Further research revealed that CD38 overexpression significantly increased the intracellular ATP concentration in CaSki cells (P=0.001; Fig. 2E) and HeLa cells (P=0.003; Fig. 2F). These results suggest that CD38 is involved in regulating the glucose concentration and can lead to an accumulation of lactate in cervical cancer cells.

CD38 affects cellular energy metabolism by activating the PI3K/AKT/mTOR signaling pathway in cervical cancer cells. To investigate the possible effects and mechanism of CD38 on the energy metabolism in cervical cancer cells, key molecules of the PI3K/AKT/mTOR signaling pathway were investigated in CaSki and HeLa cells. In CaSki cells, CD38 overexpression significantly increased the expression levels of PI3K, PDK1, ATP5H and LDHA. CD38 overexpression also significantly increased the ratios of p-AKT/AKT and p-mTOR/mTOR (Fig. 3A). These results suggest that CD38 had a significant effect on the phosphorylation of AKT and mTOR. To confirm that CD38 is involved in the regulation of energy metabolism in cervical cancer cells via the PI3K/AKT/mTOR signaling pathway, the cervical cancer cells were treated with rapamycin. Following treatment with DMSO, the expression levels of LDHA and ATP5H were significantly lower in CaSki-CD38 cells compared with the control CaSki-vector cells. In addition, the p-mTOR/mTOR ratio was significantly higher in CaSki-CD38 cells compared with the control cells following treatment with DMSO. After treatment with rapamycin, the expression levels of LDHA and ATP5H were significantly...
lower in CaSki-CD38 cells compared with the control CaSki-vector cells. In addition, the p-mTOR/mTOR ratio was significantly decreased (Fig. 3B). These results suggest that rapamycin can block the effects of CD38 on LDHA, ATP5H and mTOR. ATP5H and LDH-A affect the concentration of ATP and lactic acid in CaSki cells (34). To determine whether rapamycin alters the effects of CD38 on ATP and lactic acid in CaSki cells, the concentration of ATP and lactic acid were measured in rapamycin-treated cells. The results demonstrated that rapamycin could significantly reverse the effects of CD38 overexpression on ATP and lactic acid levels in CaSki cells (Fig. 3C and D).
Furthermore, the protein levels of mTOR, p-mTOR, LDH-A and ATP5H were detected in HeLa cells. In HeLa cells, CD38 overexpression significantly increased the levels of p-mTOR and LDHA, but no difference was observed for total mTOR or ATP5H. However, CD38 significantly increased the ratio of p-mTOR/total mTOR, indicating that CD38 has a significant effect on the phosphorylation of mTOR (Fig. 4A). Similarly, following treatment with DMSO, the expression levels of LDHA and ATP5H were significantly higher in HeLa-CD38 cells compared with the control HeLa-vector cells. In addition, the ratio of p-mTOR/mTOR was increased in HeLa-CD38 cells. Following treatment with rapamycin, the expression levels of
LDHA and ATP5H in HeLa-CD38 cells were significantly lower compared with the control HeLa-vector cells. Furthermore, the p-mTOR/mTOR ratio was significantly lower in HeLa-CD38 cells compared with HeLa-vector cells following treatment with rapamycin (Fig. 4B). These results were consistent with the results in CaSki cells. The effects of rapamycin on the ATP

Figure 5. Effect of the mammalian target of rapamycin inhibitor, rapamycin, on the proliferation of cervical cancer cells. Colony formation assays were performed to assess the clonogenic capacity of (A) CaSki and (B) HeLa cells following CD38 overexpression and rapamycin treatment. Data are presented as the mean ± standard deviation of three independent experiments. **P<0.01, ***P<0.001.

Figure 6. Effect of the mammalian target of rapamycin inhibitor, rapamycin, on the apoptosis of cervical cancer cells. (A) Flow cytometry analysis of apoptosis in CaSki cells.
and lactic acid levels in HeLa-CD38 cells were consistent with those observed in CaSki-CD38 cells (Fig. 4C and D). Therefore, the mTOR signaling pathway may serve a role in the effects of CD38 on the energy metabolism of cervical cancer cells.

CD38 affects cell proliferation and apoptosis by activating the PI3K/AKT/mTOR signaling pathway in cervical cancer cells. The levels of lactic acid and ATP metabolites are closely associated with cell proliferation. To determine whether CD38 promotes the proliferation of cervical cancer cells by activating the PI3K/AKT/mTOR signaling pathway, cells were treated with rapamycin and the effect of CD38 overexpression on cell proliferation was detected using clone formation experiments. Following CD38 overexpression, the number of clones of CaSki cells treated with DMSO was significantly increased, whereas following treatment with rapamycin the number of clones of CaSki-CD38 cells was significantly decreased compared with those treated with DMSO (Fig. 5A). Similar results were obtained in HeLa cells (Fig. 5B). This indicates that CD38 affects the proliferation of cervical cancer cells via the PI3K/AKT/mTOR signaling pathway.

Furthermore, apoptosis of rapamycin and DMSO-treated CaSki-CD38 and HeLa-CD38 cells was assessed. It was identified that CD38 inhibited the apoptosis of DMSO-treated cervical cancer cells (P<0.01), and compared with the CD38-overexpressed cells treated with DMSO, the apoptosis rate of CD38-overexpressed cells treated with rapamycin increased (P<0.05; Fig. 6). This indicates that the effects of CD38 on the apoptosis of cervical cancer cells may occur via the PI3K/AKT/mTOR signaling pathway (Fig. 7).

Discussion

Cellular biological processes are based on normally regulated metabolism, while metabolism in tumor cells is dysregulated,
which leads to the abnormal survival and growth of malignant cells (1,35). These changes provide sufficient energy, macromolecular precursors and reducing equivalents to support the rapid proliferation of tumor cells (1,2). Cellular metabolism regulation is under precise control and the main molecules and compounds involved in regulating metabolism pathways, such as the tricarboxylic acid cycle, glycolysis, oxidative phosphorylation and the pentose phosphate pathway, have been discovered and have been subjected to intensive research (36-38). However, the exact regulatory mechanisms remain unclear.

The present study found that overexpression of CD38 was associated with changes in the levels of ATP5H-2, CYB5B, CYC1 and BR13BP in cervical cancer cells, as well as changes in metabolic pathways, such as ‘oxidative phosphorylation’, ‘glycosyl compound metabolic process’ and ‘ATP/ADP metabolic process’. Glycolysis is more active in a variety of tumors, including diffuse large B cell lymphoma (39,40), uterine leiomyosarcoma (41) and lung adenocarcinoma (42,43). Cancer cells often exhibit a high rate of aerobic glycolysis, which promotes cancer growth and progression by increasing glucose uptake, increasing lactic acid production and supporting energy requirements (44). Knockdown of keratin 6B significantly inhibits the expression of c-Myc, glucose uptake, lactic acid production, ATP production, extracellular acidification rate, and the protein levels of glucose transporter type 1 and lactate dehydrogenase A. Furthermore, overexpression of c-Myc reverses the decreased glycolysis and malignant phenotype in keratin 6B-knockdown cells (34). It has been proposed that oncogene expression is sufficient to reprogram certain aspects of nutrient utilization, suggesting that cell-autonomous metabolism is driven in part by oncogenes (20,45,46).

PI3K/AKT/mTOR activation is closely associated with the occurrence, development and treatment of malignant tumors. PI3K promotes AKT and mTOR phosphorylation to activate the PI3K/AKT/mTOR signaling pathway (47). The PI3K/AKT/mTOR signaling pathway can mediate the downregulation of E-cadherin levels induced by insulin-like growth factor 1, ultimately leading to the proliferation of ovarian cancer cells (48). Mutations in the genes encoding members of the PI3K/AKT/mTOR pathway are often found in breast cancer (49). Mutations in this pathway are frequently associated with cell transformation, tumorigenesis, tumor progression and multidrug resistance (49).

The results of the present study demonstrated that CD38 overexpression in CaSki cells upregulated the levels of key molecules in the PI3K/AKT/mTOR signaling pathway, such as PI3K, total AKT p-AKT and p-mTOR. CD38 overexpression also upregulated p-TOR expression in HeLa cells. Treatment of cervical cancer cells stably overexpressing CD38 with rapamycin results in downregulation of p-mTOR, and the activity of the PI3K/AKT/mTOR signaling pathway is inhibited accordingly (50). Similarly, treatment with rapamycin resulted in significantly lower levels of ATP and lactate cervical cancer cells compared with those treated with DMSO. Clonogenic assays revealed that rapamycin could reduce the proliferative phenotype of cervical cancer cells induced by CD38 overexpression. Jian et al (47) reported that interleukin-17 activates PI3K/AKT/mTOR signaling pathway and regulates lung cancer cell autophagy by downregulating the expression of Beclin 1 in lung cancer cell lines. In endometrial cancer, the PI3K/AKT/mTOR signaling pathway is often activated (51); however, the results of the clinical trials of PI3K/AKT/mTOR inhibitors remain controversial (52-54). New research on combination therapy using dual inhibitors, multi-channel inhibitors or other targeted drugs may result in more effective treatment (51). T cell immunoglobulin and mucin domain containing 4 can activate angiogenesis via the PI3K/AKT/mTOR signaling pathway, and then recruit tumor-related macrophages, ultimately promoting the growth of colorectal cancer (55). Cellular secretion of epidermal growth factor (EGF) plays a role in M2 polarization of macrophages in colon cancer. EGF can promote macrophage polarization to M2 via the EGF receptor/PI3K/AKT/mTOR pathway (56).

In summary, CD38 regulates glucose uptake, and intracellular ATP and lactic acid levels in cervical cancer cells by regulating the PI3K/AKT/mTOR signaling pathway. Ultimately, this promotes the proliferation of cervical cancer cells. Rapamycin could reverse the effect of CD38. However, to determine the exact mechanism of CD38 in the regulation of cervical cancer cell metabolism requires further experimentation.

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Availability of data and materials

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

Authors' contributions

SL, CY and XJ performed the experiments. LL, JH and ZH analyzed the data. GL and YZ designed the study and wrote the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.
References


