LAMP2A overexpression in colorectal cancer promotes cell growth and glycolysis via chaperone-mediated autophagy

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Abstract. Lysosome-associated membrane protein type 2A (LAMP2A) is a key protein in the chaperone-mediated autophagy (CMA) pathway and has been demonstrated to be involved in the pathogenesis of a number of tumors. However, the role of CMA in colorectal cancer cell proliferation, metastasis and cell survival during oxidative stress and oxaliplatin resistance remains to be elucidated. In the present study, elevated expression of LAMP2A was observed in colon cancer tissues. Then, CMA activity was increased in SW480 and HT29 colorectal cancer cells with a LAMP2A overexpression vector and CMA activity was decreased using a LAMP2A short interfering RNA vector. MTT and colony formation assays showed that the colorectal cancer cell proliferation ability and cell viability following treatment with H_2O_2 or oxaliplatin were decreased significantly after LAMP2A knockdown and increased significantly after LAMP2A overexpression. Wound healing assays and Transwell invasion assays demonstrated that downregulation of LAMP2A expression inhibited the cell migration and invasion abilities of colorectal cancer and that upregulation of LAMP2A expression promoted cell migration and invasion. Extracellular acidification rate (ECAR) assay and lactate determination assay showed that glycolysis in colorectal cancer cells was significantly downregulated after LAMP2A knockdown and significantly upregulated after LAMP2A overexpression. Inhibition of glycolysis by 2-DG markedly attenuated LAMP2A-induced chemoresistance in colorectal cancer cells. Collectively, these data indicated that CMA can promote colorectal cancer cell proliferation, metastasis and cell survival during oxidative stress and oxaliplatin resistance and that the mechanism is related to the glycolytic pathway, which may provide a new therapeutic target for colorectal cancer patients.

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Introduction

Due to its malignant growth and high invasive ability, colorectal cancer is one of the leading cancers and recurrence and metastasis are usually the main causes of mortality in affected patients (1-3). Unfortunately, a number of patients are diagnosed with advanced-stage colon cancer, leading to a high rate of mortality (4). At present, the standard treatment for colorectal cancer includes surgery and chemotherapy. Despite significant progress in the treatment of colon cancer patients, the overall prognosis and long-term survival rate of patients have not been significantly improved in the past decade (5,6). Therefore, elucidating its pathological process is of great significance in finding new targets for the treatment of colon cancer.

Chaperone-mediated autophagy (CMA) is a selective form of autophagy in which the substrate protein contains a peptide sequence motif (KFERQ) (7,8), which can be recognized by the molecular chaperone heat shock cognate 70 (HSC70), targeted to the lysosome surface (9) and then combined with lysosome-associated membrane protein type 2A (LAMP2A) to enter lysosomes for degradation (10). The activity of CMA is determined by the level of LAMP2A on the lysosomal membrane (11,12) and the efficiency of LAMP2A assembly and disassembly (13). Studies have shown that CMA leads to significant changes in glucose and lipid metabolism, as well as whole body energy metabolism (14,15), which is closely related to the occurrence and development of tumors. CMA has been demonstrated to be abnormally activated in a variety of tumor tissues (16-21), which can promote the survival, migration and drug resistance of tumor cells. It has been confirmed that down-regulation of CMA reduces cell proliferation by regulating glucose metabolism in lung cancer cells and melanoma (17). Our research also found that CMA can promote glycolysis in

breast cancer (22). A recent study found that LAMP2A expression levels are elevated in colon cancer tissues and mouse models and LAMP2A knockdown can inhibit the proliferation of colon cancer cells (16). However, the role of CMA in colorectal cancer cells remains largely unknown and exploring the undefined relationship between CMA and LAMP2A may provide valuable insight into the pathogenesis of colorectal cancer. The present study hypothesized that LAMP2A can regulate colorectal cancer growth via CMA.

Materials and methods

Tissue array and immunohistochemistry. A human colon cancer tissue array (CO808) was purchased from Alenabio Biotechnology and written informed consent was obtained from all subjects before collecting the samples. All procedures were approved by the Ethics Committee of the General Hospital of Tibet Area Military Command with the ethical approval number 2023-KD004-01. All experiments were performed in accordance with the relevant guidelines and regulations of the Declaration of Helsinki. Immunohistochemistry was conducted as described previously (20). Briefly, the slide was deparaffinized in xylene, rehydrated in alcohol and water, antigen retrieved with an improved citrate antigen retrieval solution (Beyotime Institute of Biotechnology) at 100˚C for 20 min, blocked using an immunohistochemical blocking buffer (Beyotime Institute of Biotechnology) at 37˚C for 60 min, incubated with LAMP2A antibody (Abcam, 1 mg/ml; cat. no. ab18528; 1:100) at 4˚C overnight, treated with a horseradish peroxidase-labeled polymer at room temperature 25˚C for 60 min, then incubated with DAB (Beyotime Institute of Biotechnology) and counterstained with hematoxylin (Beyotime Institute of Biotechnology) at room temperature 25˚C for 5 min. Each sample was assigned an immunoreactivity score that calculated the sum of the intensity of positive tumor cells (0=none; 1=weak; 2=intermediate, 3=strong) and the estimated fraction of positive staining tumor cells (0=none, 1 ≤10%, 2=10-50% and 3 ≥50%).

Cell culture and cell infection. SW480 and HT29 human colorectal cancer cell lines were purchased from Cobioer Biosciences and cultured in DMEM supplemented with 10% fetal bovine serum (all from Gibco; Thermo Fisher Scientific, Inc.), incubated in an atmosphere of 5% CO_2 at 37˚C and STR profiling given by Cobioer Biosciences was used for authentication of the HT29 cell line. The short interfering (sh)RNA sequences targeting the coding region of LAMP2A gene and negative control were 5'-GCAGTGCAGATGACGACAA-3' (1283) and 5'-TTCTCCGAACGTGTCACGT-3', respectively. The full shRNA sequences used for inserting into the *Bam*HI and *Eco*RI sites of the pGLV-EGFP vector (GenePharma) were synthesized as follows: LAMP2A sense: 5'-GATCCG CAGTGCAGATGACGACAATTCAAGAGATTGTCGTCA TCTGCACTGCTTTTTTG-3', LAMP2A antisense: 5'-AAT TCAAAAAAGCAGTGCAGATGACGACAATCTCTTGAA TTGTCGTCATCTGCACTGCG-3'; negative shRNA control sense: 5'-GATCCGTTCTCCGAACGTGTCACGTTTCAA GAGAACGTGACACGTTCGGAGAACTTTTTTG-3', negative shRNA control antisense: 5'-AATTCAAAAAAGTTC TCCGAACGTGTCACGTTCTCTTGAAACGTGACACGT TCGGAGAACG-3'. The primers used for LAMP2A overexpression were as follows: forward:5'-GATATGGCGGCC GCGCCACCATGGTGTGCTTCCGCCTCTTCC-3', reverse: 5'-GTATGGGATCCCTAAAATTGCTCATATCCAGCA TGATG-3' and then the LAMP2A sequences were cloned into the *Not*I and *Bam*HI sites of EF1α-LV5-EGFP vector (GenePharma). The LAMP2A shRNA and overexpressing vector were used for lentivirus packing and then the relative lentivirus suspension was obtained (all performed by GenePharma). SW480 and HT29 cells ($3x10^5$ cells/well) were infected with lentivirus-mediated LAMP2A shRNA and overexpressing vector at a multiplicity of infection of 30 for 12 h at 37˚C, and then cells were stably selected with 1 μg/ml puromycin (Invitrogen; Thermo Fisher Scientific, Inc.) after 3 days.

Western blotting. Western blotting analysis was conducted as described previously (20). Anti-LAMP2A antibody was from Abcam (1 mg/ml; cat. no. ab18528, 1:1,000), and anti-β-actin antibody (500 μg/ml; cat. no. BM3873, 1:10,000) and anti-GAPDH antibody (500 μg/ml; cat. no. BA2913, 1:10,000) were from Wuhan Boster Biological Technology, Ltd. Briefly, the cells were lysed in RIPA buffer (Beyotime Institute of Biotechnology) and protein levels were tested using a BCA kit (Beyotime Institute of Biotechnology). A total of 20 μg protein was loaded per lane, then 10% SDS-PAGE was performed and the proteins were transferred to polyvinylidene difluoride (PVDF) membranes, blocked with blocking buffer (Beyotime Institute of Biotechnology) at room temperature 25˚C for 60 min and incubated with the corresponding primary antibodies in primary antibody dilution buffer (Beyotime Institute of Biotechnology) at 4˚C overnight, washed and incubated with secondary antibody (Wuhan Boster Biological Technology, Ltd.; 1 mg/ml; cat. no. BA1082, 1:5,000) in secondary antibody dilution buffer at room temperature 25˚C for 60 min and then detected by an ECL kit (MilliporeSigma). Densitometry was analyzed using ImageJ v1.53e software (National Institutes of Health).

Degradation of human recombinant his-GAPDH by lysosomal association. Degradation of human recombinant his-GAPDH by lysosomal association was conducted as described previously (20). Briefly, 250 mg pellets of colorectal cancer cells with different LAMP2A expression were harvested. Intact lysosomes (100 μg protein) were isolated using a Lysosome Enrichment Kit (Merck KGaA), incubated with protease inhibitor on ice for 10 min, mixed with 6X energy regenerating system (60 mM ATP, 12 mM phosphocreatine, 0.3 mg/ml creatine phosphokinase, 60 mM $MgCl_2$; pH 7.3), purified recombinant protein of 50 μg his-GAPDH and 2 μg his-HSC70 (all obtained from Clinical Biochemistry Laboratory, Army Medical University) and then collected by centrifugation at 18,000 x g for 30 min at 4˚C, washed and immunoblotted with GAPDH antibody (incubated with primary antibody at 4˚C overnight, and incubated with secondary antibody at 25˚C for 60 min).

Colony formation assay. Colony formation assays were performed as described previously (20). Cells were seeded (300 cells/well) onto 6-well plates and cultured for 14 days. Then, 4% paraformaldehyde was used to fix the colonies for 15 min and 1% crystal violet was used to stain the cells for

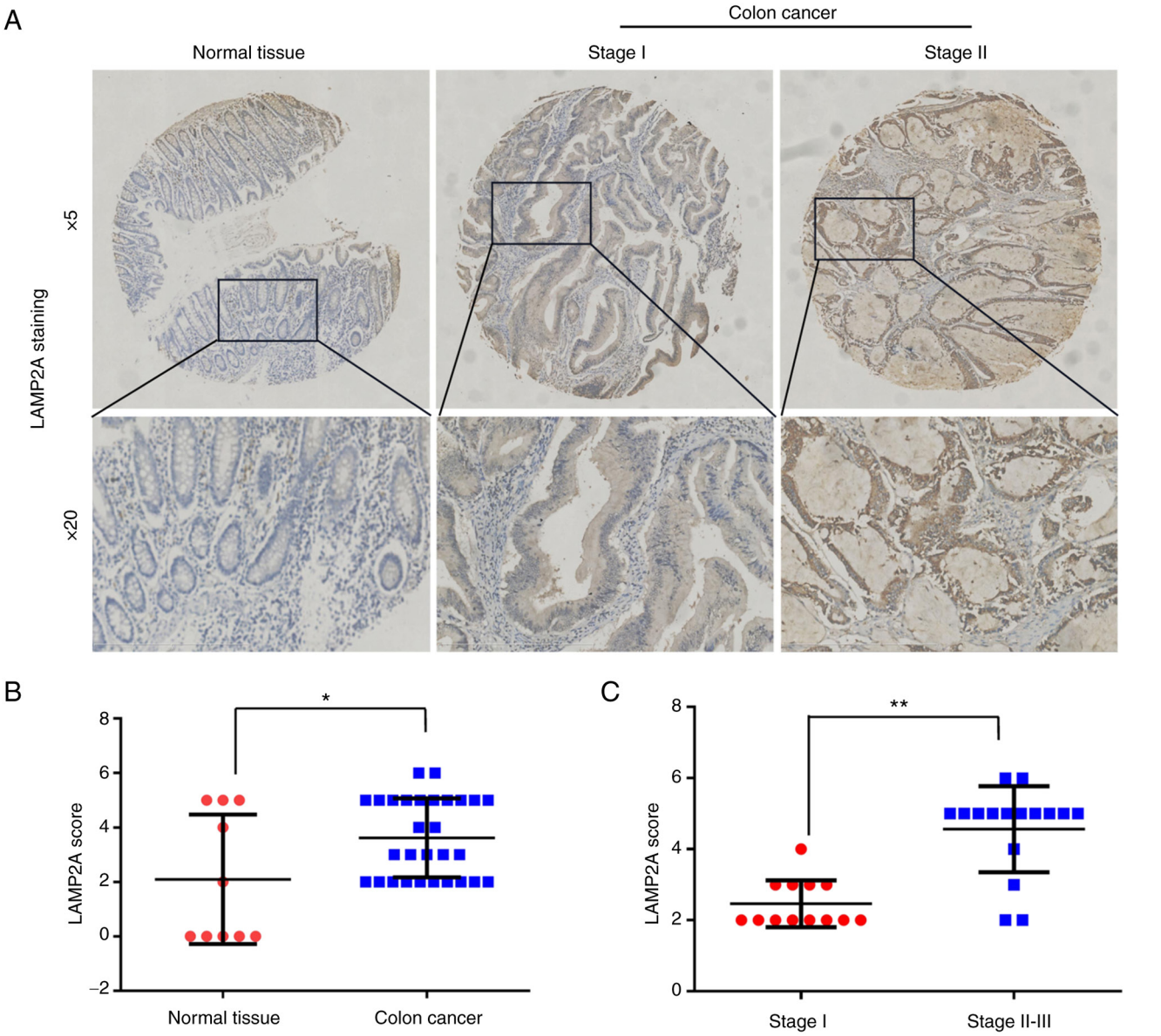

Figure 1. LAMP2A expression is elevated in colon cancer. (A) Immunostaining for LAMP2A in normal tissues and colon cancer tissues. (B) Immunoreactivity scores of LAMP2A expression levels in normal tissues and colon cancer tissues. (C) Immunoreactivity scores of LAMP2A in colon cancer tissues at different stages. A total of 29 colon cancer tissues and 10 normal tissues (five normal tissues and five normal adjacent tissues 1.5 cm from tumor) were assessed by tissue array-based immunohistochemistry. Among all the colon cancer tissues, 13 specimens were in stage I, 15 specimens were in stage II and 1 specimen was in stage III. Each sample was assigned an immunoreactivity score that calculated the sum of the intensity of positive tumor cells (0=none; 1=weak; 2=intermediate, 3=strong) and the estimated fraction of positive staining tumor cells (0=none, 1 ≤10%, 2=10-50% and 3 ≥50%) ranging from 0-6. *P<0.05, **P<0.01. LAMP2A, lysosome-associated membrane protein type 2A.

30 min at room temperature, 25˚C. The colonies were counted under a light microscope at room temperature, 25˚C. A clone was defined as containing at ≥50 cells.

MTT assay. For the cell growth curve, SW480 and HT29 cells (1x10^3/well) were seeded onto a 96-well plate. The number of viable cells was determined using the MTT assay on days 1, 2, 3, 4, 5, 6 and 7. Briefly, 20 μl of 5 mg/ml MTT (MilliporeSigma) was added to each well and the cells were incubated at 37˚C for 5 h. DMSO (200 μl per well) was added to the plates and the optical density (OD) at 490 nm was detected. For the oxaliplatin, H_2O_2 or 2-deoxy-D-glucose (2-DG) treatment assay, SW480 and HT29 cells (1x10^4/well) were seeded onto a 96-well plate and treated with oxaliplatin (Selleck Chemicals; 0, 1, 2 and 3 μmol/l), H_2O_2 (0, 0.2, 0.4 and 0.8 μM) or 5 mmol/l 2-DG for 36 h.

Wound healing assay. SW480 and HT29 cells (2x10^4/well) were inoculated onto a 24-well plate and wounds were made using a 200 μl pipette tip upon reaching 90-100% confluence. After washing with PBS three times, the cells were cultured in serum-free medium and the wound widths were recorded under a light microscope at 0 and 48 h, at a magnification of x40. The wound healing rate (%) was equal to (width at 48 h/width at 0 h) x100%.

Transwell invasion assay. The extracellular matrix gel (Matrigel; MilliporeSigma) was diluted with cold DMEM at

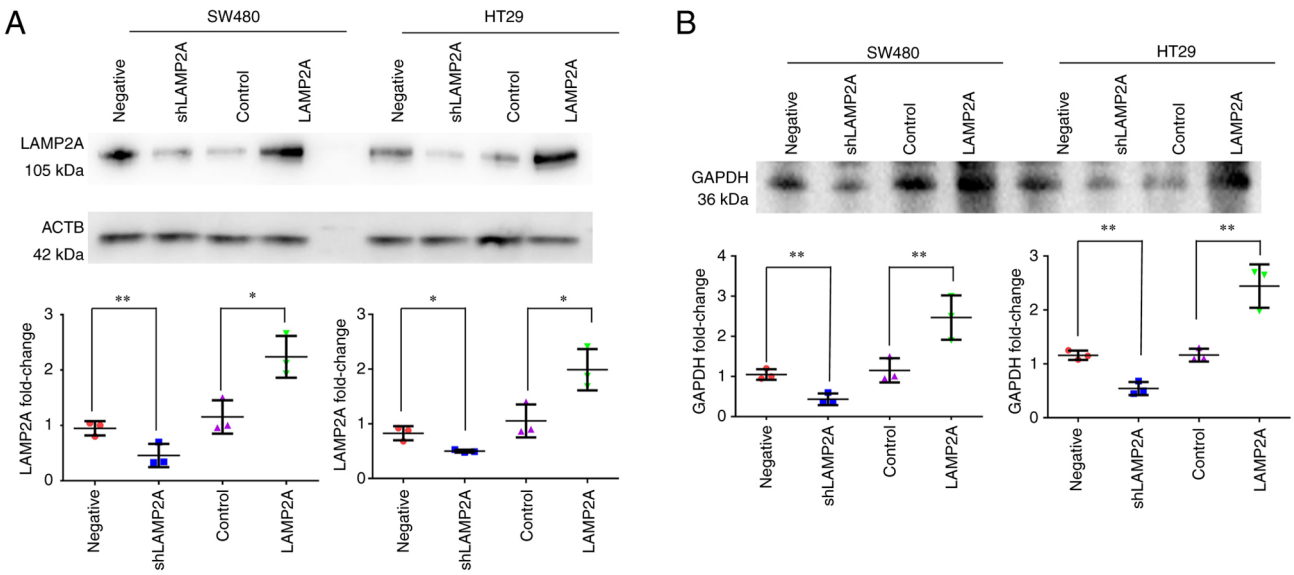

Figure 2. Elevated LAMP2A expression in colorectal cancer cells is related to elevated CMA activity. (A) LAMP2A expression levels in SW480 and HT29 colorectal cancer cells were detected by western blotting after cells were infected with LAMP2A shRNA or overexpressing vector (left) and quantification of the detected proteins after normalization to β-actin (right). (B) Intact lysosomes isolated from colorectal cancer cells with different LAMP2A expression levels were incubated with purified GAPDH and HSC70 protein and the energy regenerating system and then harvested. The samples were fractionated and used to make western blots with GAPDH antibody (left). GAPDH fold change was quantified (right). All values are the means ± SDs of three different experiments; *P<0.05 and **P<0.01. LAMP2A, lysosome-associated membrane protein type 2A.

a ratio of 1:2, incubated at 37˚C for 30 min to form a gel and inoculated into the upper chamber of a Transwell chamber (MilliporeSigma) at 40 μl. SW480 and HT29 cells (1x10^4/well) were resuspended in serum-free medium and inoculated into the upper chamber for 48 h to allow the cells to invade into the lower chamber. The cells in the upper chamber were wiped off with a cotton swab, fixed with 4% paraformaldehyde for 15 min, stained with 1% crystal violet for 30 min and then counted in five randomly selected areas under a light microscope at room temperature 25˚C, and magnification, x400.

Extracellular acidification rate (ECAR) assay. The ECAR assay was performed as described previously (22). Cells (0.8x10^4/well) were seeded onto XF 96-well plates, incubated for 16 h, incubated with 200 μl of Seahorse basic culture medium (Agilent Technologies, Inc.) and then placed in a CO_2-free incubator at 37˚C for 60 min. After calibration with the hydration plate, the XF 96-well plate with cells was supplemented with 25 mmol/l glucose, 1 μmol/l oligomycin and 50 mmol/l 2-DG, all from Seahorse and then detected by a Seahorse energy metabolism analyzer (Agilent Technologies, Inc.).

Lactate determination assay. The level of lactic acid in the culture medium of colorectal cancer cells was detected by the L-Lactate Assay Kit (Merck KGaA). The supernatant from cells (1x10^6/well) was seeded onto a 6-well plate for 36 h and collected and centrifuged at a speed of 3,350 x g for 10 min at room temperature, 25˚C. A standard curve was prepared and the level of lactic acid was detected according to the manufacturer's instructions.

Statistical analysis. Statistical analysis was conducted using the statistical software SPSS 21.0 (IBM Corp.). The results of the experiments were analyzed by unpaired Student's t-test and P<0.05 was considered to indicate a statistically significant difference.

Results

LAMP2A expression is elevated in colon cancer. To understand the physiological significance of LAMP2A in colon cancer, LAMP2A expression was assessed in 29 colon cancer tissues and 10 normal tissues by tissue array-based immunohistochemistry. The LAMP2A expression level and immunoreactivity score (calculated as the sum of the LAMP2A intensity and percentage scores) were higher in colon cancer tissues than in normal tissues (Fig. 1A and B), indirectly suggesting higher CMA activity in colon cancer tissues. In addition, the LAMP2A expression level was significantly higher in stage II-III colon cancer than in stage I colon cancer, indicating that LAMP2A expression is positively associated with the stage of colon cancer (Fig. 1C).

Elevated LAMP2A expression in colorectal cancer cells is related to elevated CMA activity. The most efficient way to regulate CMA activity is by modifying the expression level of LAMP2A (23). The present study manipulated CMA activity in SW480 and HT29 cells by cell infection with LAMP2A shRNA and overexpression vector to illustrate the role of CMA in colorectal cancer more fully. The results showed that SW480 and HT29 colorectal cancer cells with different levels of LAMP2A expression were successfully constructed (Fig. 2A). A direct method was performed to measure CMA activity by lysosome association of purified GAPDH with active intact lysosomes under the condition of an energy regenerating system, his-HSC70 and protease inhibitor (21). Lysosomes isolated from SW480 and HT29 cells with

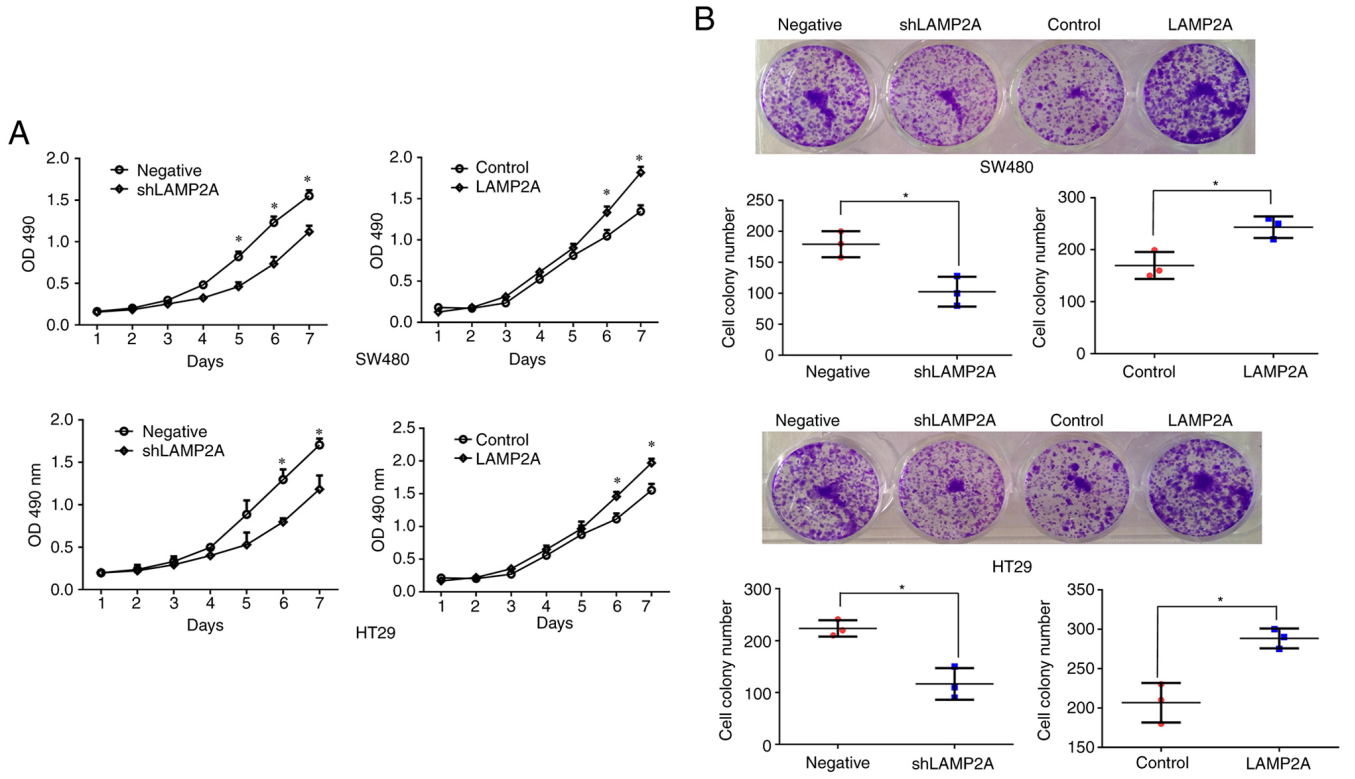

Figure 3. LAMP2A promotes colorectal cancer cell proliferation. (A) Colorectal cancer cell proliferation ability was determined by a 7-day MTT growth curve after LAMP2A knockdown or overexpression. (B) Colorectal cancer cell proliferation ability was analyzed by cell colony formation assay after downregulation and upregulation of LAMP2A; n=3, *P<0.05. LAMP2A, lysosome-associated membrane protein type 2A.

LAMP2A knockdown showed decreased lysosomal association of GAPDH, suggesting lower CMA activity, in these cells compared with the control cells, while lysosomes from SW480 and HT29 cells with LAMP2A overexpression demonstrated increased lysosomal association of GAPDH, indicating higher CMA activity (Fig. 2B).

LAMP2A promotes colorectal cancer cell proliferation. CMA has been demonstrated to be involved in tumor proliferation and is required for tumor growth (17). The 7-day MTT growth curve was used to observe the effect of LAMP2A on cell proliferation and cell proliferation was inhibited after LAMP2A knockdown and promoted after LAMP2A overexpression in SW480 and HT29 cells (Fig. 3A). Colony formation assays also demonstrated that LAMP2A knockdown significantly suppressed the proliferation of SW480 and HT29 cells and that LAMP2A overexpression significantly promoted the proliferation of SW480 and HT29 cells (Fig. 3B).

LAMP2A protects colorectal cancer cells from oxidative damage and promotes colorectal cancer cell drug resistance. Tumors are always under the influence of oxidative stress mediated by reactive oxygen species (ROS), which might be one of the causes of cancer progression (21). To induce oxidative stress, colorectal cancer cells were treated with different concentrations of H_2O_2. The cell viability rate was decreased after LAMP2A knockdown and increased after LAMP2A overexpression (Fig. 4A). Oxaliplatin is a chemotherapeutic drug that is widely used to treat colon cancer (24). Colorectal cancer cells were treated with different concentrations of

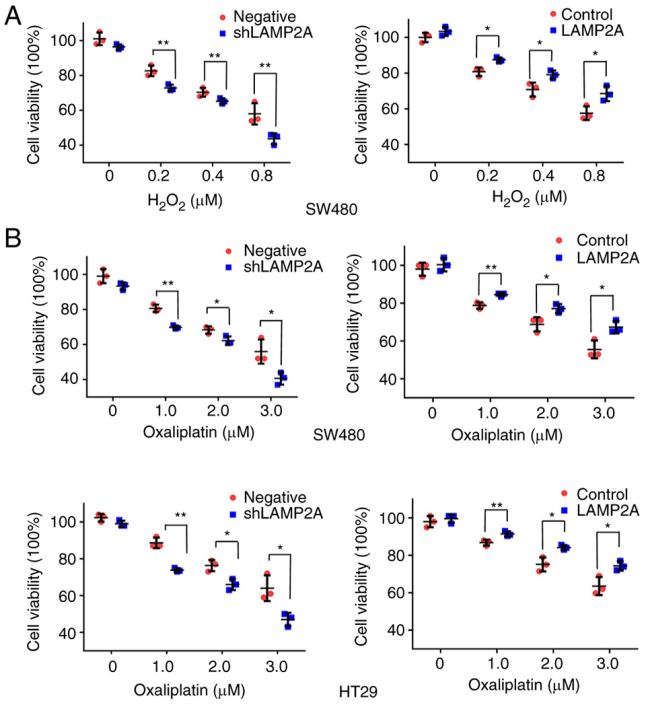

Figure 4. LAMP2A protects colorectal cancer cells from oxidative damage and promotes colorectal cancer cell drug resistance. (A) Colorectal cancer cells with different LAMP2A expression levels were treated with H_2O_2 (0, 0.2, 0.4 and 0.8 μM) for 36 h and cell viability was measured by MTT assay. (B) Colorectal cancer cells with different LAMP2A expression levels were treated with oxaliplatin (0, 1, 2 and 3 μmol/l) for 36 h and cell viability was measured by MTT assay. All values are the means ± SDs of three different experiments; *P<0.05, **P<0.01. LAMP2A, lysosome-associated membrane protein type 2A.

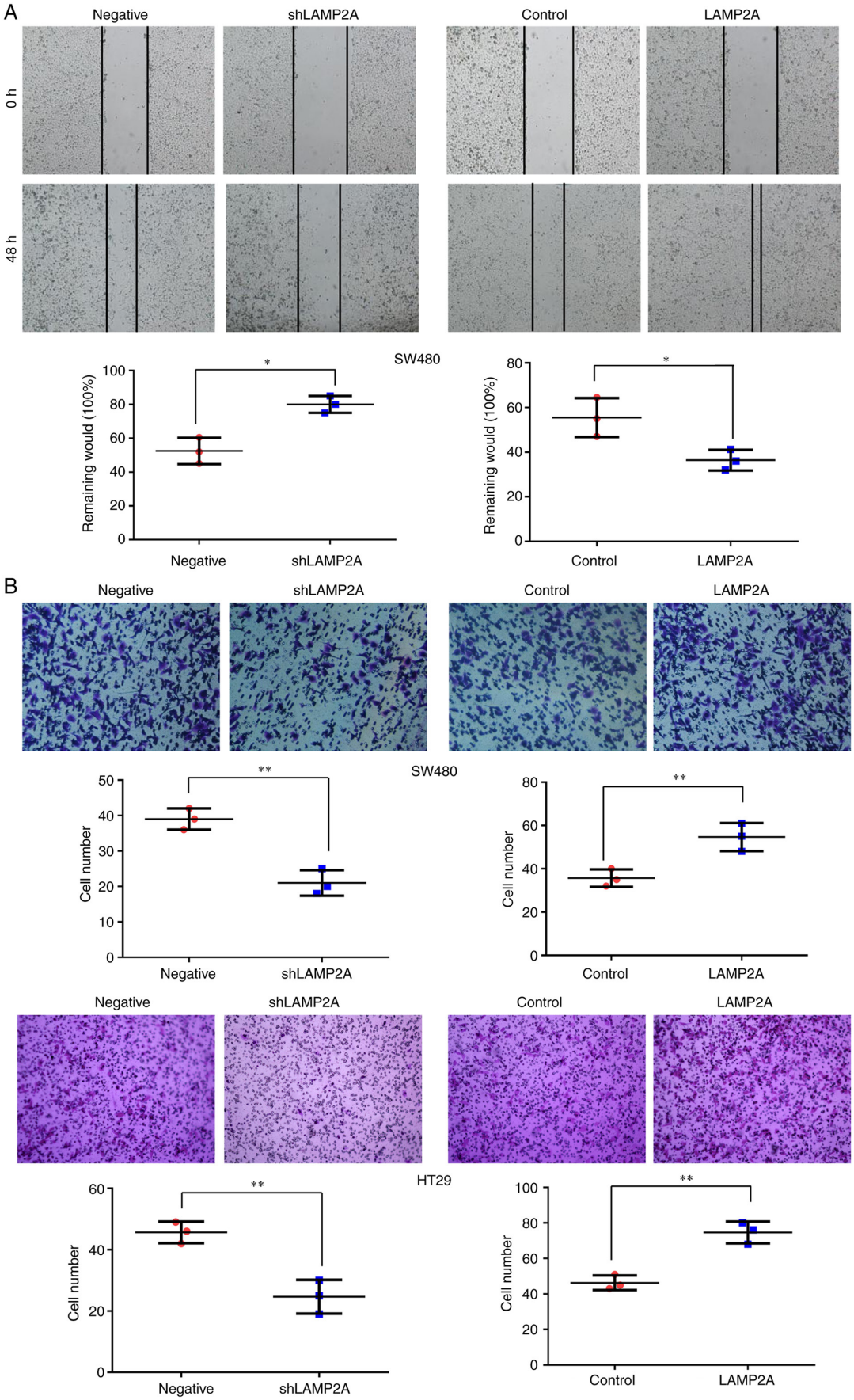

Figure 5. LAMP2A promotes colorectal cancer cell migration and invasion. (A) The migration ability of colorectal cancer cells was detected by wound healing assay. The cells were cultured for another 48 h after the wound was made and the remaining cells were detected by a microscope at 0 and 48 h. Then, the size of the wound was quantified (magnification, x40). (B) The invasion ability of colorectal cancer cells was measured by Transwell invasion assay. Cells with different LAMP2A expression levels were cultured for 48 h and cells that invaded into the lower chamber were counted under a microscope following staining with crystal violet (magnification, x400); n=3, *P<0.05, **P<0.01. LAMP2A, lysosome-associated membrane protein type 2A.

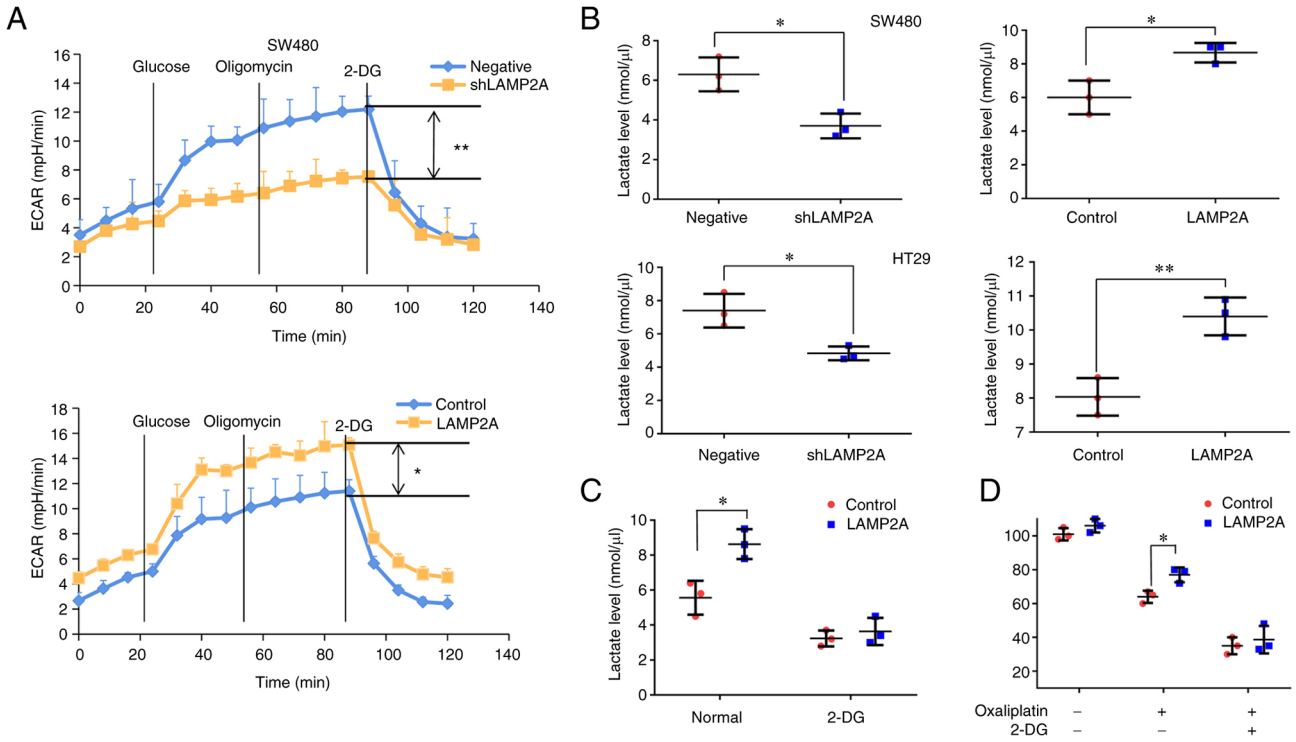

Figure 6. LAMP2A promotes glycolysis in colorectal cancer cells. (A) ECAR was detected in colorectal cancer cells after LAMP2A knockdown or overexpression, followed by injection of (vertical line) glucose (25 mM), oligomycin (1 μM) and 2-DG (50 mM). (B) Lactate levels in the culture medium of colorectal cancer cells with different LAMP2A expression levels were determined using an L-lactate assay kit. (C) Lactate production and (D) cell viability were measured after SW480 cells were treated with 2-DG (5 mM) and oxaliplatin (2 μmol/l) for 36 h. n=3, *P<0.05, **P<0.01. ECAR, extracellular acidification rate; LAMP2A, lysosome-associated membrane protein type 2A.

oxaliplatin and it was found that oxaliplatin resistance was attenuated after LAMP2A knockdown and enhanced after LAMP2A overexpression (Fig. 4B).

LAMP2A promotes colorectal cancer cell migration and invasion. Metastasis is the most complex and deadly process related to cancer and metastasis relies on tumor cell migration and invasion (25). The effect of LAMP2A on colorectal cell migration was measured by wound healing assay. LAMP2A knockdown significantly inhibited the migration of SW480 cells, while LAMP2A overexpression significantly promoted the migration of SW480 cells (Fig. 5A). To further ascertain the role of CMA in colorectal cancer cell invasion, a Transwell invasion assay was conducted. The number of invasive cells was significantly reduced after LAMP2A knockdown and increased after LAMP2A overexpression in SW480 and HT29 cells (Fig. 5B).

LAMP2A promotes glycolysis in colorectal cancer cells. Energy metabolism is required to maintain tumor growth (26). The ECAR assay was used to detect the effect of LAMP2A on the extracellular acidification rate of colorectal cancer cells. The results showed that the maximum glycolytic capacity (MPH/min) was significantly reduced after LAMP2A knockdown and the maximum glycolytic capacity (MPH/min) was significantly increased after LAMP2A overexpression (Fig. 6A). The extracellular lactate level was decreased after LAMP2A knockdown and increased after LAMP2A overexpression (Fig. 6B). To further explore the effect of glycolysis on LAMP2A-induced oxaliplatin resistance in colorectal cancer cells, SW480 cells were treated with 2-DG (a glycolysis inhibitor). Lactate production was significantly suppressed in SW480 cells overexpressing LAMP2A (Fig. 6C) after 2-DG treatment. In addition, treatment with 2-DG also markedly attenuated the effect of LAMP2A overexpression on the viability of SW480 cells treated with oxaliplatin (Fig. 6D), which suggests that inhibition of glycolysis could abate LAMP2A-induced chemoresistance in SW480 cells.

Discussion

Colon cancer is characterized by a high degree of malignancy and rapid progression; 20-30% of colon cancer patients present with metastasis at initial diagnosis (27) and ~50% of colon cancer patients will experience recurrence within 3 years of initial surgery (28,29). Combination chemotherapy is usually used in patients with colon cancer, especially in advanced stages. The main conventional chemotherapy drugs are 5-fluorouracil, oxaliplatin, irinotecan and capecitabine (30) and combination chemotherapy is initially effective for most patients. However, with the emergence of drug resistance, ~50% of colon cancer patients experience recurrence, leading to a decrease in the 5-year survival rate of patients with distant metastasis to ~14% (31).

The latest cancer research indicates that CMA is involved in the growth and proliferation of tumors and is expected to become a new target for tumor treatment (32). LAMP2A, as the key protein of the CMA pathway, has been confirmed

to exhibit abnormal expression in various tumor tissues and is generally considered to promote tumor proliferation, metastasis and drug resistance (33). Previous studies have shown that CMA can reduce apoptosis and promote the proliferation of colon carcinoma cells and they monitored CMA activity by measurement of changes in LAMP2A expression levels (16,34). However, accurate measurement of CMA is only achieved by directly measuring the turnover of CMA substrate proteins into lysosomes in the presence of protease inhibitor. The present study performed a direct method of measuring CMA activity by GAPDH degradation in colon cancer cells to explore the effect of CMA on colorectal cancer more fully. Lysosomal association of GAPDH was reduced in LAMP2A knockdown cells and upregulated in cells with LAMP2A overexpression, indicating the corresponding CMA activity had been directly observed. Pathologic importance of CMA in cancer cell proliferation has been extensively studied, whereas a link between CMA and cell viability under the treatment of H_2O_2 or oxaliplatin has not been thoroughly explored, especially in colorectal cancer. ROS have been regarded as a series of destructive molecules that have a detrimental effect on cell homoeostasis and H_2O_2 appears to be the main ROS (35). The cell viability rate was decreased after LAMP2A knockdown and increased after LAMP2A overexpression, indicating that LAMP2A can protect colorectal cancer cells from oxidative damage. Oxaliplatin resistance is a challenge in the treatment of colorectal cancer patients (36). The present study found that oxaliplatin resistance was significantly inhibited following LAMP2A knockdown and enhanced following LAMP2A overexpression. The data obtained from the previous studies (16,34) demonstrated the significance of CMA in colorectal cancer, which prompted the present study to further explore the other effects of CMA on different colorectal cancer cells due to the cell heterogeneity. Metastasis causes most cancer-related deaths and is a multistep biological process that involves the dissemination of cancer cells to anatomically distant organs/sites (37). In present study, the migration and invasion of colorectal cancer cells was significantly inhibited after silencing LAMP2A and significantly increased after upregulation of LAMP2A.

Abnormal glucose metabolism is considered an important marker of cancer cells and this characteristic of tumor cells has attracted widespread interest (38). Mitochondrial oxidative phosphorylation is the main energy source for normal cells. Conversely, aerobic glycolysis is usually the main energy source of cancer cells. This phenomenon is called the 'Warburg effect' (39). Metabolic reprogramming has become a new center of activity in cancer research and is closely related to tumor proliferation, metastasis, drug resistance and a poor prognosis (40). CMA has been shown to regulate glycolysis in lung cancer, melanoma and breast cancer (17,22). However, the modulation between CMA and glycolysis in colorectal cancer cells remains to be elucidated. The present study demonstrated that LAMP2A could promote the production of lactic acid, increase the extracellular acidification rate and increase glycolysis levels in colorectal cancer cells. In addition, inhibition of glycolysis by 2-DG markedly attenuated LAMP2A-induced chemoresistance in colorectal cancer cells, which might provide a new strategy to treat colorectal cancer by combination of downregulation of CMA activity and glycolysis inhibitor. However, the exact mechanism by which LAMP2A regulates chemoresistance and glycolysis in colorectal cancer requires further study.

In summary, the present study demonstrates that CMA could promote cell proliferation, cell metastasis, cell survival during oxidative stress, oxaliplatin resistance and glycolysis in colorectal cancer and thus could have remarkable diagnostic and therapeutic implications for colorectal cancer patients.

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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

RC, SL and QH conceived and designed the experiments, JW, CH, JX, XZ, MT, ZW, LP, YZ and MH performed the experiments, ZW, JY and YG analyzed the data, SL and QH supervised the study and wrote the manuscript. SL and QH confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Written informed consent was obtained from all patients before collecting the samples. All the procedures were approved by the Ethics Committee of General Hospital of Tibet Area Military Command and the ethical approval number was 2023-KD004-01. All experiments were performed in accordance with relevant guidelines and regulations of Declaration of Helsinki.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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