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Original Article

Aldolase A promotes cell proliferation and cisplatin resistance via the EGFR pathway in gastric cancer

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Abstract: Background: Gastric cancer is the third leading cause of cancer-related mortality worldwide, and the 5-year survival rate remains poor, globally. Overexpression of Aldolase A (ALDOA) has been linked to tumor cell proliferation and metastasis in numerous cancer types, including pancreatic, colorectal, hepatocellular carcinoma, and lung cancer. Although the significance of ALDOA as a potential biomarker in GC prognosis has been reported, its potential role and possible mechanism of ALDOA in GC cell sensitivity to chemotherapy remains to be elucidated. Methods: The GEPIA platform and clinical samples were used to investigate ALDOA expression in GC tumors and neighboring normal tissues. The CCK8 and colony formation tests were used to examine whether ALDOA increased GC cell proliferation and decreased resistance to the chemotherapy drug cisplatin. Furthermore, the underlying molecular mechanisms were elucidated. Results: Overexpression of ADOLA was seen in GC tumors and GC cells. Prognostic markers, i.e., invasion depth, tumor size, and metastasis of lymph node were all negatively impacted by ADOLA overexpression. Following ADOLA knockdown, *in vitro* proliferation of AGS cells was decreased and drug resistance was reduced. Conversely, ADOLA overexpression exhibited an inverse effect in MKN45 cells. ALDOA knockdown dramatically slowed the development of GC tumors in *in vivo* experiments. Mechanistically, ADOLA regulated the activity of epidermal growth factor receptor (EGFR), its downstream molecue the extracellular signal-regulated kinase 1/2 (ERK1/2) and protein kinase B (AKT) signaling pathway in GC cells. Moreover, in the absence of EGFR, ALDOA overexpression had no effect on GC cell growth. Conclusion: In the EGFR signaling pathway, ADOLA boosted the proliferation and cisplatin resistance of GC cells, making it a viable GC therapeutic target.

Keywords: ALDOA, EGFR, cisplatin resistance, gastric cancer, proliferation

Introduction

On a worldwide scale, the annual number of new cancer diagnoses is expected to rise to 19.3 million, with the death toll from cancer-related causes expected to be 10 million by the year 2020 [1]. Globally, gastric cancer (GC) is the third leading cause of cancer-related mortality and the fifth most common type of cancer found during a cancer screening [1]. Despite the introduction of multimodality therapy, as well as advancements in surgical procedures, systemic chemotherapy, targeted treatments, and immunotherapies for GC, for patients with stage IA and IB cancers, 5-year survival chances are still quite poor after surgery, ranging between 60% and 80%, and the median survival of patients with stage III is between 18% and 50% [2].

Altered metabolism is considered a hallmark of cancer, and glycolysis represents an important factor promoting tumor development and progression, which involves a series of enzymatic reactions. Aldolase A (ALDOA) catalyzes the reversible conversion of fructose 1,6-diphosphate into dihydroxy-acetone phosphate and glyceraldehyde 3-phosphatex, which is involved in biological processes associated with the maintenance of the actin-containing filament of the cytoskeleton, striated muscle contraction, and ATP biosynthetic process [3, 4]. ALDOA is highly expressed in embryonic tissues, adult muscles, and blood cells [5]. Elevated expression of ALDOA is primarily associated with myotonic muscle diseases and muscle injury. Moreover, previous investigations have shown that ALDOA expression is altered in a variety of malignancies, including pancreatic, hepatocel-

lular, colorectal, and lung tumors. Although ALDOA has been identified as a possible biomarker for GC progression, the role of ALDOA in GC cell chemosensitivity remains to be investigated.

Overexpression and knockdown of ALDOA on GC cell growth and cisplatin resistance were investigated in this work, as were the underlying processes. GC researchers have looked at the biomarker or potential pharmacological target of ALDOA.

Methods

Human GC tissues and cell lines

The Gene Expressing Profiling Interactive Analysis [GEPIA; http://gepia.cancer-pku.cn/] platform provided the clinical data used in this study. 68 histopathologically verified GC tissues and matched adjacent normal stomach tissue samples were collected from patients who had radical surgery at the First Affiliated Hospital of Wannan Medical College (Wuhu, Anhui) between 2018 and 2019. Neither radiation nor chemotherapy had been administered to any of the patients before surgery. Detailed demographic and clinical characteristics data were available for every patient in the trial. The First Affiliated Hospital of Wannan Medical College's Independent Ethics Committee authorized this research, and signed informed consent was acquired from all patients. All tests were carried out in accordance with all applicable standards and regulations.

Normal gastric epithelial cell line GES-1 and human GC cell lines (MGC803, AGS, SGC7901, and MKN45) were bought by the Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences in Shanghai, China. All cells were cultured in DME medium with 1% penicillin-streptomycin and 10% FBS in a humidified environment with 5% CO_2, at 37°C.

Immunohistochemistry (IHC)

IHC was employed to compare the ALDOA levels between 68 GC tissue specimens and normal gastric tissue samples. Tissues were embedded in paraffin and sectioned at a thickness of 4 μm after being fixed in 10% paraformaldehyde. After deparaffinization in xylene, tissue slices were rehydrated for IHC staining using a graded series of ethanol. In a microwave oven, tissue slices were heated for 30 minutes at 100°C in sodium citrate buffer to remove antigens. Hydrogen peroxide and serum were used to inhibit the activity of endogenous peroxidase (5% goat serum). ALDOA monoclonal antibody was used to treat sections overnight at 4°C after they had been blocked for 30 minutes. Finally, PBS was used to clean the sections before the chromogens were applied to stain them with the three 3'-diaminobenzidine (DAB; Shanghai, China). Finally, hematoxylin counterstaining was carried out for 60 seconds at room temperature after drying, mounting, and counterstaining the slices. A microscope was used to examine the slices at the end.

Two expert pathologists blinded to the patients' clinicopathological features and treatment outcomes independently rated the immunostaining data.

Evaluation of IHC staining

Cells in GC tissues mostly expressed ALDOA in their cytoplasm. The immunological staining of ALDOA was semi-quantitatively analyzed in five high-power fields, and the proportion of cells that stained positive and the staining intensity were assessed. Each region's percentage of positive cells was scored as follows: 0 for ≤5%, 1 for 6% to 25%, 2 for 26% to 50%, 3 for 51% to 75%, and 4 for ≥ 76% positive cells. Additionally, staining intensity was rated as follows: 0, 1, 2, and 3 for no staining, faintly positive, moderately positive, and strongly positive, respectively [11]. The percentage score was multiplied with intensity score (staining score = % x intensity) to arrive at the overall score.

SiRNA transfection and stable cell line construction

Stable knockdown and overexpression in GC cell lines were achieved using lentiviral transduction (GeneChem, Shanghai, China) of human ALDOA-specific short hairpin RNA (shRNA) and protein expression plasmids. The manufacturer's instructions for lentiviral transduction were followed. qRT-PCR and Western blot assays validated the transfection efficiency. 5'-CCATGCTTGCACTCAGAAGTT-3' was employed as the shRNA target sequence for human ALDOA. Lipofectamine RNAiMax (Invitrogen)

was used to transfect siRNA at a final dose of 20 nM, as per the manufacturer's recommendations. 5'-CCUAUGCCUUAGCAGUCUUTT-3' was the siRNA sequence for targeting human EGFR.

Protein extraction and western blot assay

The transfected cells were removed from the culture and washed in PBS. A protease inhibitor cocktail and RIPA buffer (Beyotime Inc., NanTong, China) were used to extract the total protein for 25 minutes on ice. The sample's protein concentration was determined using a BCA protein assay kit in accordance with the manufacturer's instructions. On nitrocellulose membranes, an equivalent quantity of protein was electrophoretically transferred from 10% SDS-PAGE. After that, the blots were cut depending on the protein's molecular weight and blocked for an hour with 5% nonfat milk, followed by incubation with primary antibodies at 4°C overnight. Three TBST washes were performed at room temperature, and then membranes were incubated with secondary antibodies for one hour. Enhanced chemiluminescence was used to see the immunoreactive bands, and ImageJ was used to quantify them. Antibodies against p21 (dilution 1:1000; #10355-1-AP, proteintech), cyclin D1 (dilution 1:1000; #26939-1-AP, proteintech), p-EGFR (dilution 1:1000; #3777, CST), ALDOA (dilution 1:1000; #11217-1-AP, proteintech), EGFR (dilution 1:1000; #18986-1-AP, proteintech), AKT (dilution 1:1000; #9272, CST), p-AKT (dilution 1:1000; #4058, CST), GAPDH (dilution 1:1000; #AF0006, Beyotime), ERK (dilution 1:1000; #4695, CST), and p-ERK (dilution 1:1000; #4370, CST) were used in this study.

RNA extraction and quantitative real-time PCR (qRT-PCR)

The cells' total RNA was extracted using TRIzol (Invitrogen, USA) according to the manufacturer's instructions. qRT-PCR was done on an ABI 7500 system using a TaKaRa SYBR Green RT-PCR kit (Applied Biosystems, Waltham, MA, UK). 2-ΔΔCt was employed to assess target gene fold change using human 18s as an endogenous control. Every experiment was run at least three times. ATG CCC TAT CCA GCA TAT CCA GCA, human-ALDOA-R: GCCC AGT GGA CTC ATCTG, human-18s-R: 5C TCC AAT CGG AAT CGG TAGCG-3, and human-18s-F: GCTA ACC CGT TGA TGA ACC CCATT-3 were employed as the primer sequences for the samples.

Cell counting kit-8 (CCK8) assay

According to the instructions provided by the manufacturer, the CCK8 was used to test cell viability (CCK8; Ameresco, USA). 2000 cells per well were plated in 96-well plates. After 24 hours, each well received a 10% solution of CCK-8. The plates were kept at 37°C for an additional two hours. Whenever a fresh medium and 10% CCK8 were needed, the old medium was discarded and a new one added. The absorbance of samples was measured at 450 nm using a microplate reader.

Colony formation assay

1000 cells were placed in each well of six-well plates. The colonies were fixed and stained with 0.1% crystal violet for ten days. Under a light microscope (Olympus, Tokyo, Japan), cells in clumps of more than 50 cells were counted.

In vivo xenograft tumor model

SPF grade BALB/c nude mice were randomly assigned into two groups. Each group was implanted with 5×10^6 gastric cancer cells subcutaneously. After four weeks, the tumors were weighed and the mice's necks were severed. The Nanjing Medical University Committee on the Ethics of Animal Experiments approved all animal experiments as long as they met the established standards. The study was in accordance with the ARRIVE standards.

Statistical analysis

The results of three separate trials are shown as mean ± S.E.M. The statistical significance was determined using a student's t-test or a chi-square test. $P < 0.05$ was used as the significant threshold. The statistical evaluation was done using IBM SPSS Statistics (20.0; IBM, Chicago, IL USA).

Results

GC tumor tissues and cell lines had increased ALDOA expression

Initially, The GEPIA platform was first used to examine the expression levels of ALDOA mRNA in stomach adenocarcinoma (STAD). In compar-

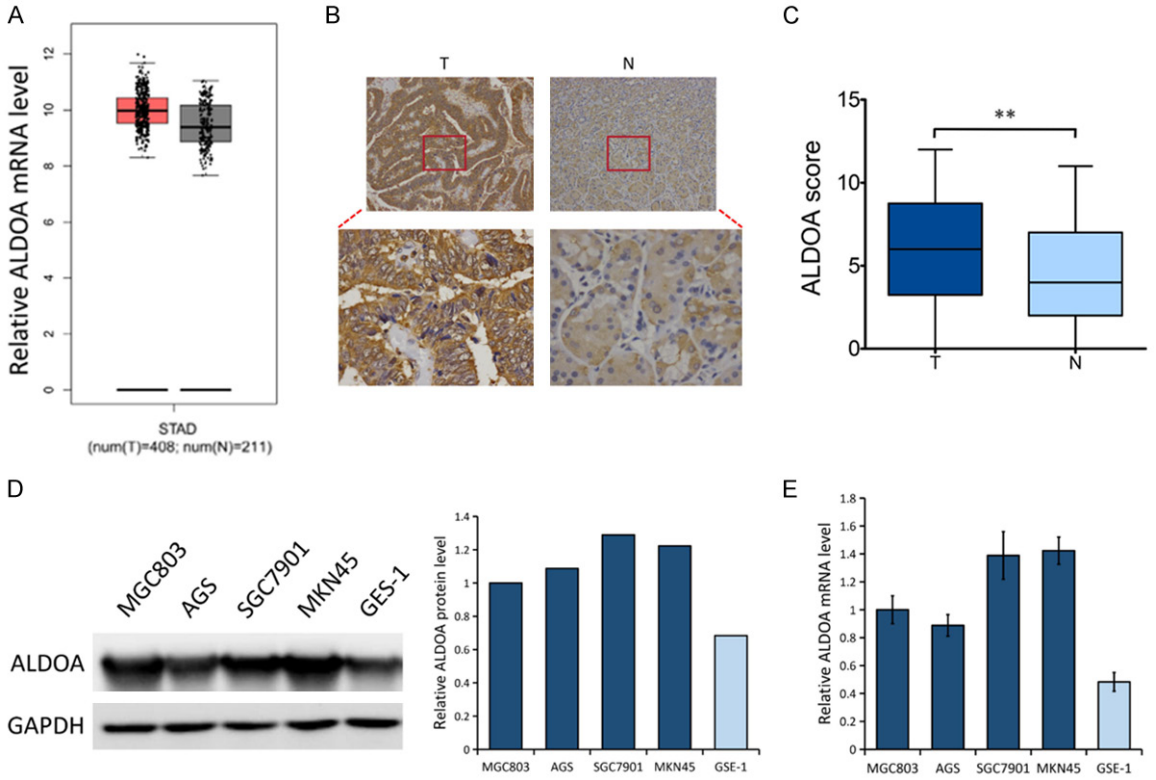

Figure 1. ALDOA expression in human GC tissues and cell lines. (A) Relative ALDOA mRNA expression in stomach adenocarcinoma (STAD) in the TCGA datasheet from the GEPIA. (B, C) IHC staining (B) and scores (C) of ALDOA in GC tumor tissues (T) and normal tissues (N). (D, E) The protein (D) and mRNA (E) expression of ALDOA in four GC (MGC803, AGS, SGC7901 and MKN45) and GES-1 cell lines. $^{**}P < 0.01$. The original Western blots are included in the Supplementary Figure 2.

ison to para-tumor tissues, GC tissues had higher levels of ALDOA (**Figure 1A**). GC tissues and nearby normal tissues were utilized for IHC labelling to exam the protein expression of ALDOA. When GC tissues were compared to their corresponding nearby normal tissues, the ALDOA expression was found to be considerably greater (P < 0.01, **Figure 1B** and **1C**). Additionally, cytological studies showed that GC cell lines (MKN45, MGC803, SGC7901, and AGS) expressed more ALDOA mRNA and protein than the normal stomach epithelium (GES-1) cells (**Figure 1D** and **1E**).

ALDOA and GC clinicopathological features

ALDOA expression was found to be greater in GC patients with tumors less than 5 cm in diameter than those with tumors larger than 5 cm in diameter (P < 0.01; **Figure 2A**). ALDOA expression was greater in GC tumor tissues with lymph node metastasis (LNM), deeper invasion (T3-4), and a more advanced tumor-node-metastasis (TNM) stage (III-IV) as compared to T1-2 (**Figure 2B**) and TNM stage I-II (**Figure 2C**), respectively (**Figure 2D**, P < 0.01; **Figure 2D**). Age, gender, or tumor site had no effect on ALDOA expression levels (P > 0.05; **Figure 2E-G**). According to GEPIA results (**Figure 2H**), ALDOA mRNA levels were positively linked with the TNM stage (P = 0.0345).

ALDOA expression was then compared to the clinical and pathological characteristics of GC patients. Increased ALDOA expression was strongly correlated with tumor size (P = 0.003), LNM (P < 0.001), invasion depth (P = 0.022), and TNM stage (P = 0.002). According to **Table 1**, there was no statistically significant relationship between the expression of ALDOA and the tumor's age, gender, or location (P > 0.05).

GC cell ALDOA overexpression and knockdown

Stable transfectants were obtained when ALDOA was knocked out of AGS and MKN45 cells. When compared to cells transfected with control shRNA but not ALDOA knockdown (KD),

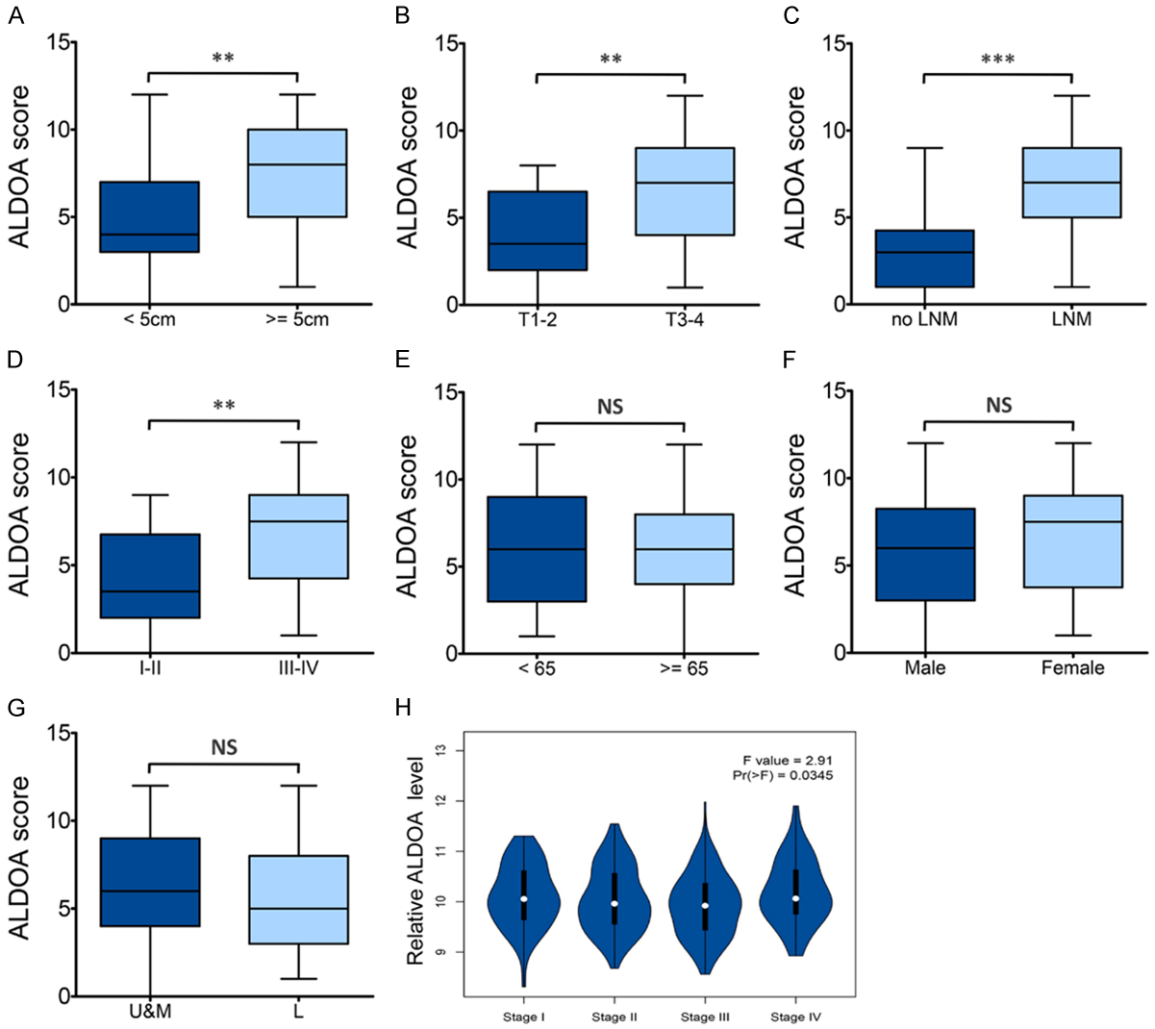

Figure 2. ALDOA expression in different subgroup of human GC tumor tissues. (A) IHC scores of ALDOA in GC tumors with tumor size < 5 cm or ≥ 5 cm. (B) IHC scores of ALDOA in GC tumors with depth of invasion T1-2 or T3-4. (C) IHC scores of ALDOA in GC tumors with or without LNM. (D) IHC scores of ALDOA in GC tumors with TNM stage I-II or stage III-IV. (E) IHC scores of ALDOA in GC tumors with age < 65 or ≥ 65 years. (F) IHC scores of ALDOA in GC tumors of male or female. (G) IHC scores of ALDOA in GC tumors of different locations. Or (H) ALDOA mRNA expression in different TNM stages in the TCGA datasheet from the GEPIA. NS, nonsignificant; ***P* < 0.01; ****P* < 0.001.

KD cells have less ALDOA protein and mRNA (NC; P < 0.001; Supplementary Figure 1A and 1B). GC cells were transfected with plasmids expressing human ALDOA (OE) or an empty vector, and the transfection efficiency was assessed using Western blot and qRT-PCR tests. In cells transfected with human ALDOA plasmids, protein and mRNA levels were higher than those in empty vector cells (VEC; P < 0.01; Supplementary Figure 1C and 1D).

ALDOA stimulates GC cells in vitro and in vivo

The impact of ALDOA on cell proliferation was assessed using the CCK8 and colony formation assays. When ALDOA was knocked down in AGS cells, both viability and colony-forming capacity were drastically reduced (P < 0.00; **Figure 3A** and **3B**). In contrast, overexpression of ALDOA significantly increased MKN45 cells' ability to proliferate (P < 0.01, **Figure 3C**) and form colonies (P < 0.01; **Figure 3D**). In addition, xenograft models of GC carcinogenesis were employed to test the effects of ALDOA in vivo. ALDOA knockdown AGS cells were implanted into nude mice and studied. In comparison to the control group, the results demonstrated that ALDOA knockdown dramatically reduced tumor development (P< 0.001, **Figure 3E** and

Table 1. Relationships between the expression of ALDOA and clinicopathological features in GC

Characteristics	Total	ALDOA expression		χ^2	*P* value
		None or low	High		
Total	68	26	42		
Age					
< 65	33	13	20	0.036	0.849
≥ 65	35	13	22		
Gender					
Male	46	19	27	0.567	0.451
Female	22	7	15		
Tumor size					
< 5 cm	34	19	15	8.967	0.003**
≥ 5 cm	34	7	27		
Tumor location					
U&M	41	14	27	0.731	0.393
L	27	12	15		
Depth of invasion					
T1-2	16	10	6	5.216	0.022*
T3-4	52	16	36		
Lymph node metastasis					
No	21	15	6	14.175	< 0.001***
Yes	47	11	36		
TNM Stage					
I/II	24	15	9	9.247	0.002**
III/IV	44	11	33		

Abbreviations: TNM, tumor-lymph node-metastasis. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

3F), confirming ALDOA's pro-growth effect on GC cells.

In addition, we examined the expression of cyclin D1 and the G1 gatekeeper p21 protein in GC cells. As shown in **Figure 3G**, ALDOA knockdown inhibited cyclin D1 and boosted p21 production, whereas ALDOA overexpression had the opposite effect, confirming the onco-genic function of ALDOA in GC cell.

ALDOA contributes to cisplatin resistance in GC cells

Cell viability was evaluated using the CCK8 assay in AGS cells (NC vs. KD) that were exposed to cisplatin for 48 hours. ALDOA reduction resulted in AGS cells being more sensitive to cisplatin, as shown by the lowered IC_{50} value (**Figure 4A**, **4B**). Overexpression of ALDOA in MKN45 cells resulted in an increase in cisplatin resistance, with an increased IC_{50} value in comparison to the control group (**Figure 4C** and **4D**).

ALDOA stimulates GC cell proliferation through EGFR signaling

Many signalling pathways, including the EGFR pathway, were examined in an effort to better understand how ALDOA regulates cell proliferation and cisplatin resistance. There was a reduced phosphorylation of EGFR and its downstream signaling components, such as, p-ERK1/2, and p-AKT (**Figure 5A** and **5B**). ALDOA overexpression inhibited EGFR signaling (**Figure 5C** and **5D**). EGFR was suppressed in ALDOA-overexpressed MKN45 cells to investigate its role in GC cell effects. Cells treated with an EGFR-specific siRNA (siE) showed decreased EGFR expression compared to cells transfected with control siRNA (si-Con; **Figure 5E**). ALDOA overexpression didn't boost cell proliferation without EGFR (**Figure 5F**). Our results showed that ALDOA increased GC cell proliferation through EGFR.

Discussion

Cancer claimed the lives of approximately 19.3 million people globally in the year 2020, and an additional 10 million people will die as a result of the disease [12]. Among all malignancies, GC was the third most frequent cause of cancer-related mortality and the fifth most prevalent malignancy [13]. Although there have been significant advances in current treatment approaches, including radiotherapy, chemotherapy, surgery, and targeted immunotherapy, the long-term prognosis of GC remains poor. Because of the tumor's genetic and clinical heterogeneity, increasing overall survival rates for patients with GC is a major hurdle to overcome. The prognosis, survival, and responsiveness to treatment of GC patients can only be improved by identifying new therapeutic targets.

Tumor cells have specific aberrations in glucose metabolism to enable growth and metastasis, making altered glucose metabolism a hallmark of cancer formation and development [14, 15]. Therefore, metabolic manipulation of crucial glycolytic enzymes represents a promis-

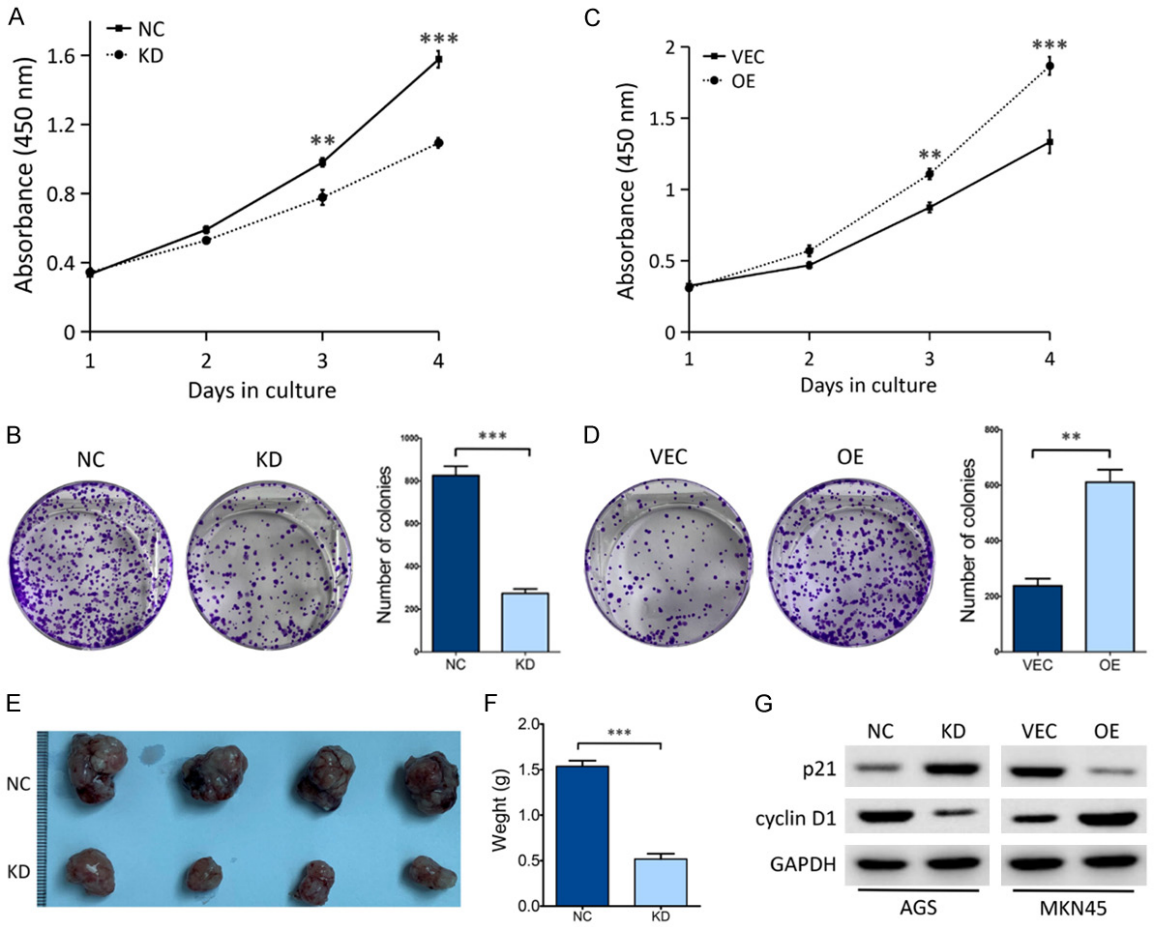

Figure 3. ALDOA promotes GC cell proliferation and growth of GC cells. A. The growth curves of AGS cells stably transfected with either control shRNA (NC) or ALDOA shRNA (KD). B. The growth curves of MKN45 cells stably transfected with either empty vector (VEC) or plasmids encoding human ALDOA (OE). C. Colony formation assay was performed to determine the growth of AGS cells (NC vs. KD). D. Colony formation assay was performed to determine the growth of MKN45 cells (VEC vs. OE). The bands were quantified and presented as the mean ± SEM. E. Image of subcutaneous tumors derived from AGS cells (NC vs. KD) in nude mice (n = 4). F. The weight of subcutaneous tumors was shown. G. Western blot analysis of the indicated proteins in GC cell. $^{**}P < 0.01$, $^{***}P < 0.001$. The original Western blots are included in the Supplementary Figure 4.

ing method for metabolic interventions. The Warburg effect, or aerobic glycolysis, promotes tumor cell proliferation by reprogramming the metabolic profile of tumor cells to increase glucose uptake and induce lactate production even at normal oxygen concentrations. It is considered a hallmark of tumorigenesis and progression [15]. The therapeutic potentials of glycolytic enzymes in the fight against cancer have been investigated. Cancers have a high concentration of a glycolytic enzyme called ALDOA, which is an essential member of this family. It is becoming clearer that ALDOA is upregulated in cancerous tissues, patients, and cancer development, according to a growing number of research. The survival and prognosis of patients with various malignancies, including pancreatic cancer, renal carcinoma, cervical cancer, and GC, were shown to be directly linked to the overexpression of ALDOA. Overexpression of ALDOA in GC cells increased cell proliferation and cisplatin resistance via the EGFR pathway, as this research showed.

According to the results of the IHC staining, GC cells express considerably more ALDOA than nearby normal cells. ALDOA expression was considerably higher with tumor diameters more than 5 cm, invasion depths of T3-4 stage, lymph node metastases, and at the TNM III-IV stage, when examining the link between ALDOA expression and clinicopathological features in

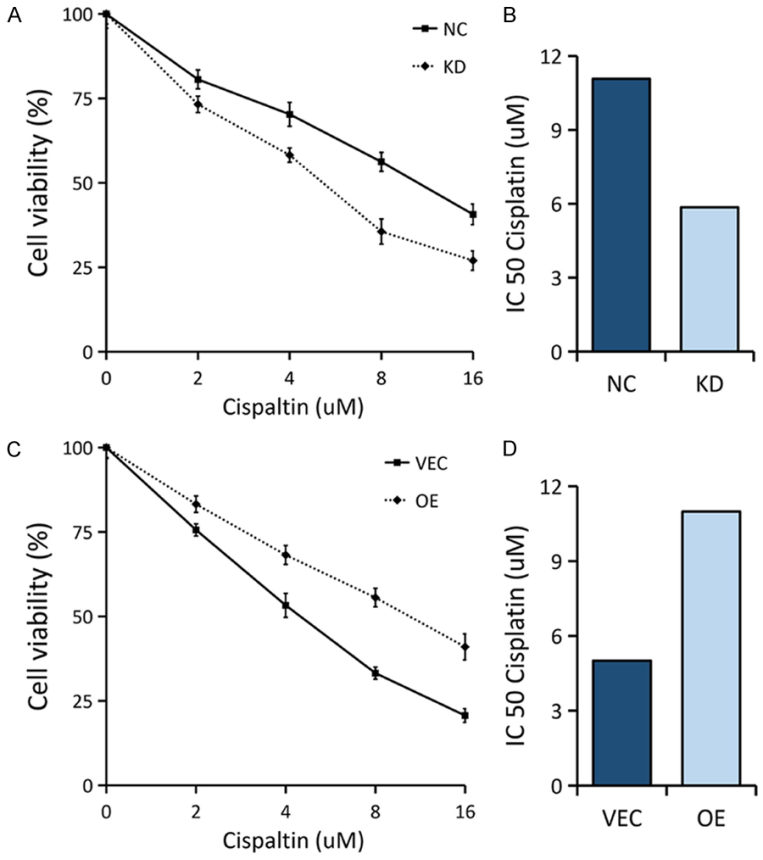

Figure 4. ALDOA contributes to cisplatin resistance in GC cells. A. AGS cells (NC vs. KD) were treated with cisplatin at the indicated concentrations for 48 hours, and the cell viabilities were measured by CCK8 assay (left). B. IC_{50} of cisplatin for AGS cells was presented (right). C. MKN45 cells (VEC vs. OE) were treated with cisplatin at the indicated concentrations for 48 hours, and the cell viabilities were measured by CCK8 assay (left). D. IC_{50} of cisplatin for MKN45 cells was presented (right).

GC patients. In addition, ALDOA's significance in illness development was shown to be evident from these findings. Furthermore, the findings revealed that knockdown of ALDOA significantly suppressed proliferation, whereas overexpression of ALDOA significantly increased it, indicating that ALDOA expression contributed to the malignant potential of GC cells. According to these findings, ALDOA may be a useful GC therapeutic target and biomarker.

Cisplatin, a platinum-based drug, is the most widely used first-line chemotherapeutic drug to treat GC patients, particularly for those with advanced GC. However, cisplatin-resistant cancer cells have become a major problem in the treatment of GC patients. This study identified that ALDOA-depleted cells became more sensitive to cisplatin, whereas GC cells with ALDOA overexpression exhibited significantly higher resistance to cisplatin.

The ErbB receptor family is increasingly recognized to be connected to human malignant tumors [16]. EGFR stimulates several downstream signaling pathways as a member of the ErbB family of receptor tyrosine kinases, including ERK1/2 and PI3K/AKT, to promote tumor cell proliferation, invasion, and metastasis [17]. Excessive EGFR expression has been linked to resistance to cytotoxic chemotherapy, hormone treatment, and radiation, according to previous research [18, 19]. Furthermore, the majority of previous studies have linked the EGFR pathway to cisplatin resistance in a variety of tumor cells [20-24]. The findings here demonstrated that ALDOA regulated the activity of EGFR and its downstream targets, AKT and ERK1/2, which may be implicated in the ALDOA-induced effects in GC cells. In order to determine this, EGFR was silenced in ALDOA-overexpressed MKN-45 cells. It seems that EGFR is required for ALDOA control of the growth of GC cells, since ALDOA overexpression failed to stimulate cell proliferation when it was not combined with EGFR.

Many types of cancer, particularly breast cancer, have been linked to abnormalities in the EGFR signaling pathway, which ALDOA controls. There is evidence that ALDOA is a factor in non-small cell lung cancer's stimulation of the EGFR signaling pathway, which in turn promotes cell proliferation and the transition from G1 to S. In another investigation, ALDOA promotes bladder cancer cell invasion by activating the E-cadherin/EGFR pathway [26], demonstrating that E-cadherin is a direct and indirect EGFR negative regulator. We hypothesized that the ALDOA-mediated Warburg effect drives cell pro-

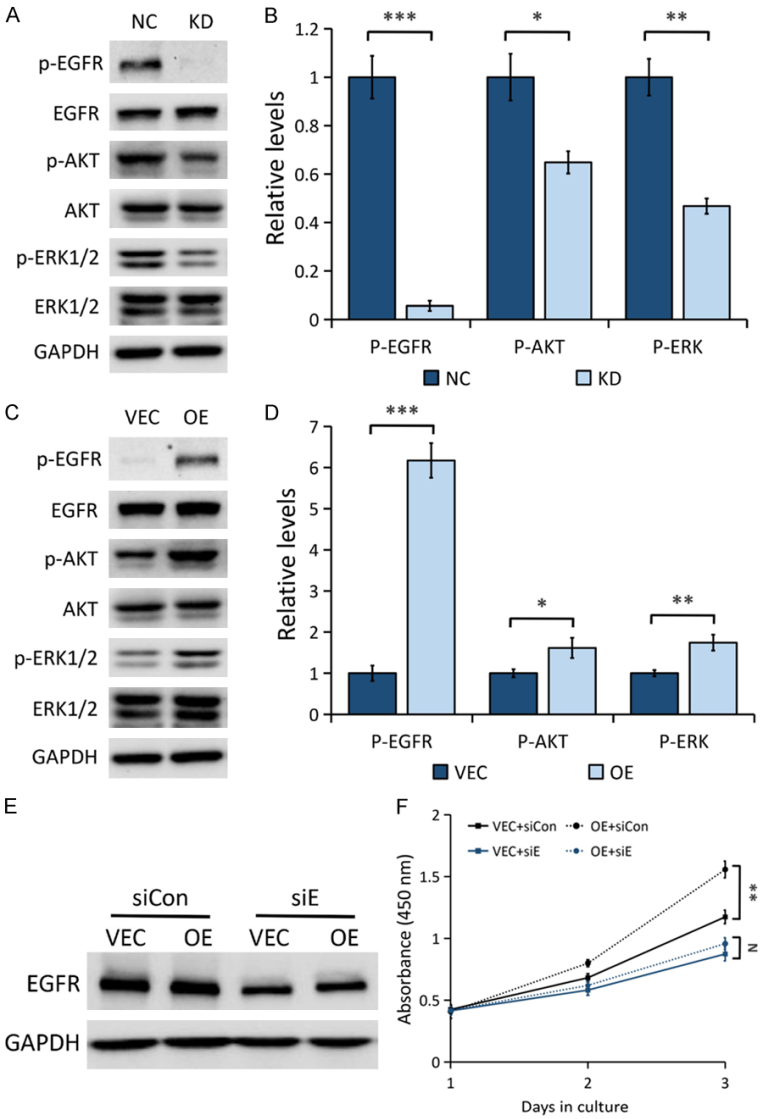

Figure 5. ALDOA regulates EGFR signaling pathway in GC cells. A, B. Western blot analysis of the indicated proteins in AGS cells (NC vs. KD). The bands were quantified and presented as the mean ± SEM. C, D. Western blot analysis of the indicated proteins in MKN45 cells (VEC vs. OE). The bands were quantified and presented as the mean ± SEM. E. Western blot analysis of the EGFR in MKN45 cells (VEC vs. OE) transfected with EGFR-specific siRNA (siE) or control-siRNA-transfected cells (siCon). GAPDH was used as a loading control. F. The growth curves of MKN45 cells (VEC vs. OE) transfected with EGFR-specific siRNA (siE) or control-siRNA-transfected cells (siCon). N, nonsignificant; *P < 0.05, **P < 0.01, ***P < 0.001. The original Western blots are included in the Supplementary Figures 5 and 6.

liferation, chemoresistance, and tumor formation in GC, but additional research is needed.

Conclusions

In conclusion, according to the results of this research, ADOLA enhanced the proliferation and re-susceptibility of prostate cancer cells to the cisplatin chemotherapy, perhaps via the EGFR signaling pathway.

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Disclosure of conflict of interest

None.

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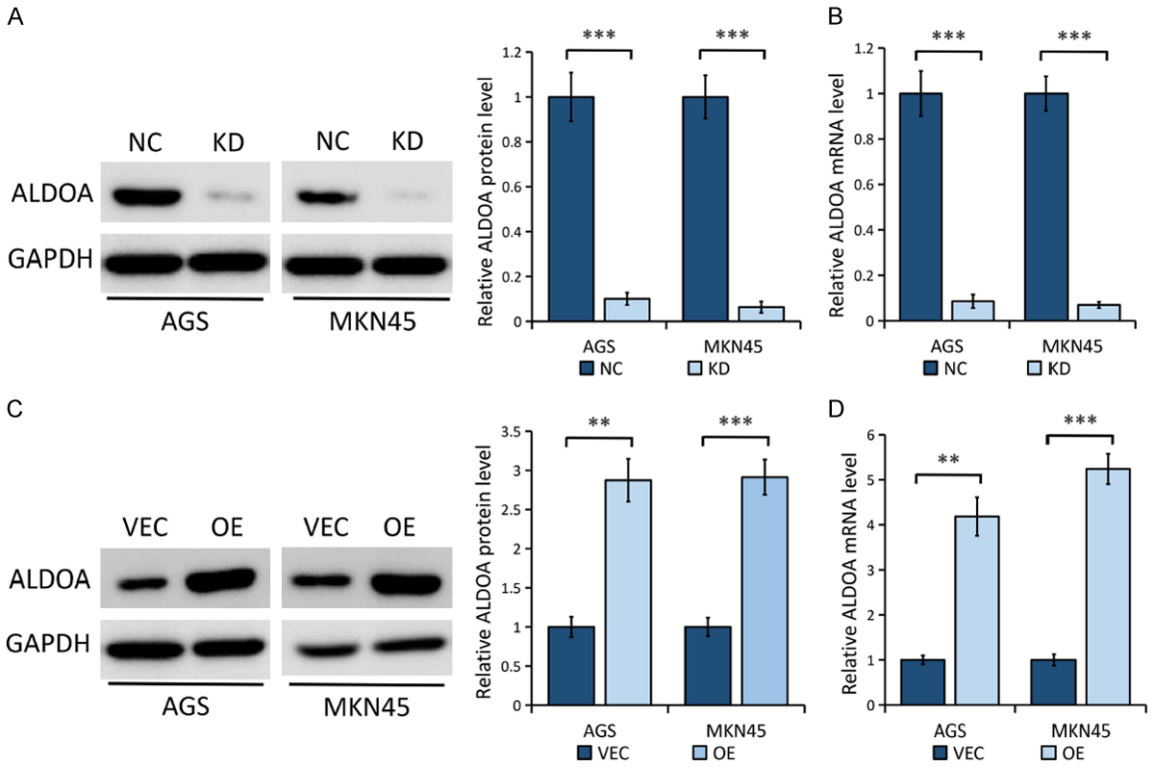
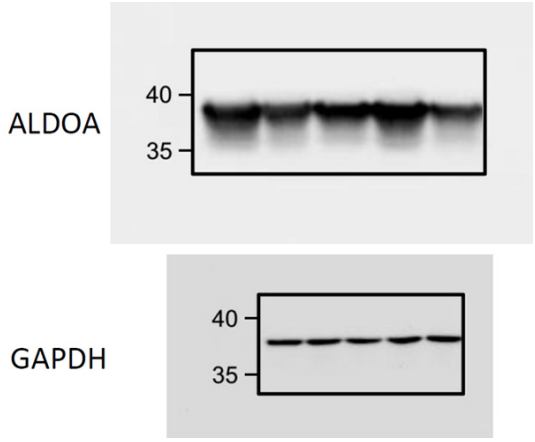
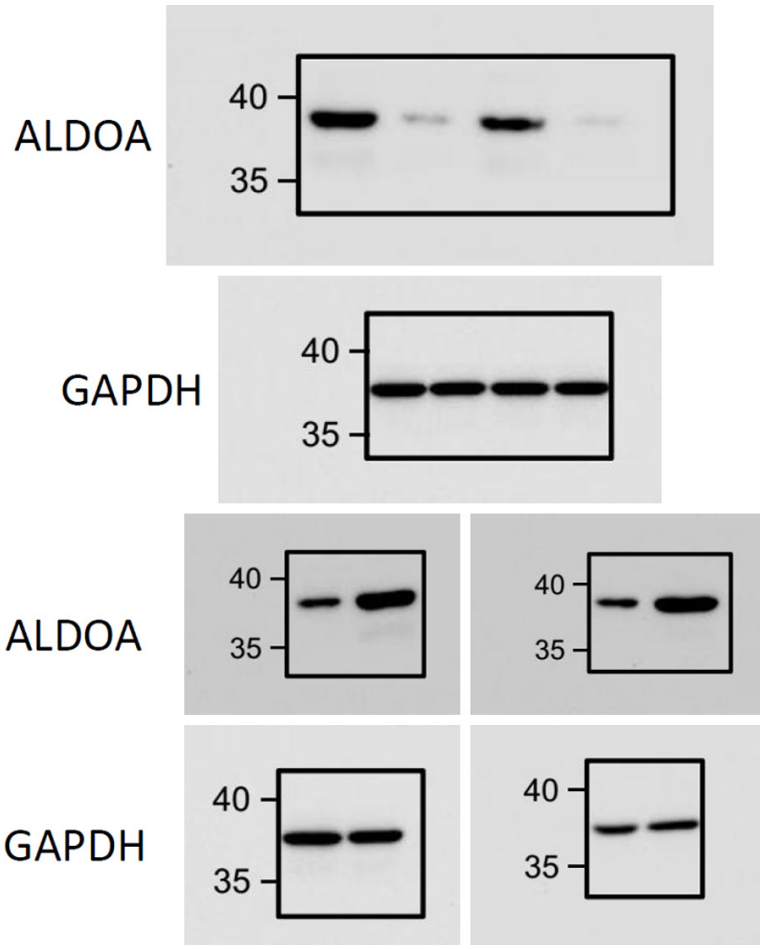


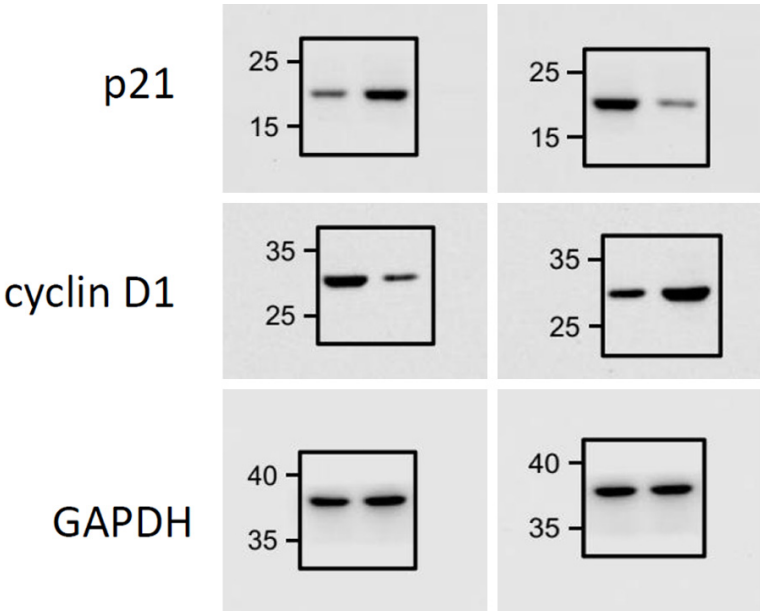
Supplementary Figure 1. Knockdown and overexpression efficiency of ALDOA in GC cells. (A and B) The protein (A) and mRNA (B) expression of ALDOA in AGS and MKN45 cells stably transfected with either control shRNA (NC) or ALDOA shRNA (KD). (C and D) The protein (C) and mRNA (D) expression of ALDOA in AGS and MKN45 cells stably transfected with either empty vector (VEC) or plasmids encoding human ALDOA (OE). GAPDH or human18s as the loading control. $^{**}P < 0.01$; $^{***}P < 0.001$. The original Western blots are included in the Supplementary Figure 3.



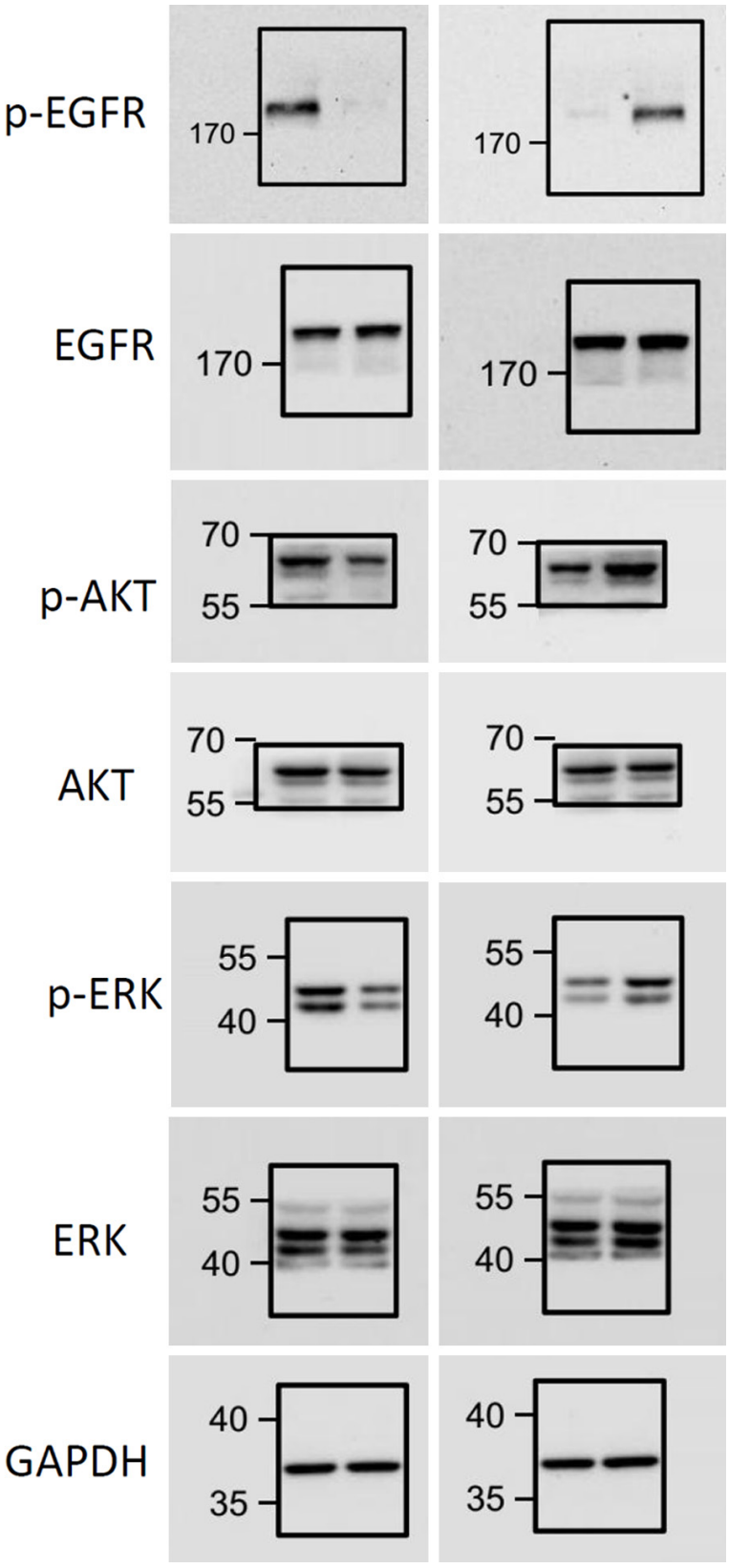
Supplementary Figure 2. Full scans of original blots for data in **Figure 1D**.



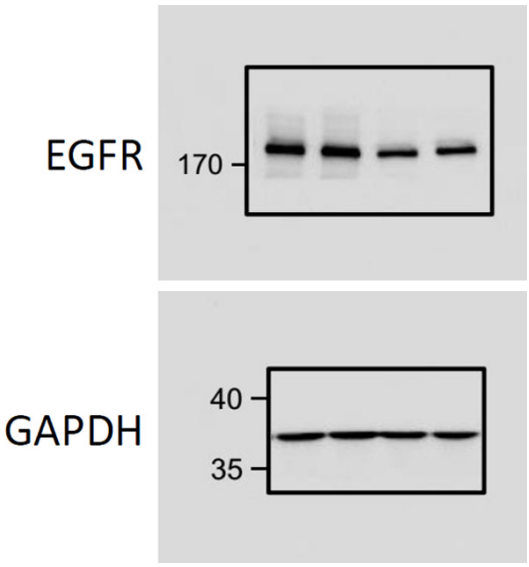
Supplementary Figure 3. Full scans of original blots for data in Supplementary Figure 2.



Supplementary Figure 4. Full scans of original blots for data in **Figure 3**.



Supplementary Figure 5. Full scans of original blots for data in **Figure 5A, 5C**.



Supplementary Figure 6. Full scans of original blots for data in **Figure 5E**.