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STAT5A reprograms fatty acid metabolism and promotes tumorigenesis of gastric cancer cells

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Abstract. – OBJECTIVE: The aim of this study was to determine the underlying effect of STAT5A-mediated fatty acid metabolism on the tumorigenesis of gastric cancer cells.

MATERIALS AND METHODS: The expression patterns of STAT5A and FASN in gastric cancer were investigated based on the Cancer Genome Atlas (TCGA) database and compared between 40 pairs of cancer samples and adjacent tissues. The pathological significance of STAT5A in gastric cancer was explored by GESA assay, and the molecular mechanism of STAT5A-mediated FASN expression was investigated by Luciferase assay and ChIP-qPCR. Fatty acid metabolic change was explored by detecting the content of neutral lipid, triglycerides and phospholipids in STAT5A silenced MKN28 and NCI-N87 cells. Furthermore, Cell Counting Kit-8 (CCK-8) assay, colony formation, and Mice xenograft were used to detect the function of STAT5A-mediated fatty acid metabolism in tumorigenic ability of gastric cancer cells.

RESULTS: Upregulated STAT5A in gastric cancer was found to be not only an unconventional risk for poor survival of gastric cancer patients, but also associated with fatty acid metabolism signaling. Furthermore, STAT5A can regulate the expression of the fatty acid binding protein 5 (FABP5) by binding to the promoter of FABP5 in MKN28 and NCI-N87 cells. Functional studies have shown that STAT5A-dependent FABP5 expression promoted cell proliferation and tumorigenesis of gastric cancer cells by reprogramming intracellular fatty acid metabolism.

CONCLUSIONS: Our results indicate that STAT5A-dependent FABP5 expression plays a carcinogenic role in the tumorigenesis of gastric cancer cells via reprogramming intracellular fatty acid metabolism, which establishes a new mechanism for the tumorigenesis of gastric cancer cells.

Key words: STAT5A, FABP5, Fatty acid metabolism, Tumorigenesis, Gastric cancer.

Introduction

Although in recent years its global incidence has largely declined, gastric cancer is still one of the main reasons for tumor-related death, due to its poor prognosis[1]. Recent epidemiological data[2,3] have shown that obesity was significantly correlative with the risk of malignant tumors in the population. Clinical evidence-based medical research[4-6] found that excessive body mass index (BMI) or excessive waist circumference was one of the important risks of gastric or esophageal tumors. Although histological staining showed the fat infiltration in gastric cancer tissues[5,7,8], the status of fatty acid metabolism in gastric cancer and its molecular mechanism is still confusing, and there is no study to explore the role of lipid metabolism - on clinical prognosis in gastric cancer.

In mammalian cells, lipid metabolism is strongly associated with fatty acid binding proteins (FABPs), belonging to the intracellular lipid-binding proteins (iLBPs) family and regulating fatty acid uptake, transport, and metabolism[9-11]. Studies[10-12] have shown that FABP5 was a major member of the long-chain fatty acid binding proteins in cells and involved in the activation of the non-classical retinol nuclear receptor PPARβ/δ. Although the mechanism was still unclear, abnormal expression of FABP5 resulted in diseases such as atherosclerosis of the carotid artery and coronary arteries, reflecting its important role in

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the cellular fatty acids metabolism[13,14]. Interestingly, FABP5 has been indicated as an oncogene in mammals and was highly expressed in many human cancers[15]. In the MMTV-neu mouse model of breast cancer, tumorigenesis was often accompanied with up-regulation of FABP5[16]. However, the development of tumors can be significantly inhibited by down-regulating FABP5 in these mice[17]. However, the molecular mechanism of FABP5 in tumorigenesis and pathological processes in gastric cancer remains unclear.

Signal transduction and transcriptional activator 5A (known as STAT5A) acts as a nuclear transcription factor that can be activated by inflammatory cytokines or growth factor receptors, not only functioning in many cellular physiological processes, such as immune response and inflammation, but also playing a key role in human cancers[18,19]. Excessive activation of STAT5A caused by self-mutation or hyper-phosphorylation is a key mechanism for malignant proliferation of tumor cells in breast cancer or cervical cancer[20]. Studies[21] have shown that STAT5A was involved in tumor progression by intermediating epithelial-mesenchymal transition. Also, other rese[illegible]es[22] have found that high STAT5A expressi[illegible] usually associated with poor prognosis in o[illegible]n cancer. Although those results reveal the sig[illegible]cance of STAT5A in the pathology of tumors, [illegible] role of STAT5A in gastric can[illegible] not bee[illegible] studied.

The aim of our study [illegible]to inve[illegible]gate the expression pattern of ST[illegible]A and [illegible] functions in gastric c[illegible]er. [illegible] that [illegible]-T5A can participat[illegible] the cel[illegible]lipid metabolism process b[illegible]ecting the [illegible]ession of FABP5, which [illegible]ffects the t[illegible]origenesis of gastric [illegible]ncer ce[illegible]. These findings define the new p[illegible]ological fu[illegible]s of STAT5A and provid[illegible] theoretical basis [illegible] understanding the r[illegible]onshi[illegible]tween tumorigenesis of gastri[illegible]er a[illegible]ellular fatty acid metabolism.

M[illegible] and Methods

C[illegible]s, Reagents, and Antibodies

[illegible]e f[illegible] gastric cancer cell lines used in this [illegible]udy (MKN28, MKN45, KATOIII, and AGS) [illegible]a normal human gastric mucosal epithelial c[illegible]e (GSE1) were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA) and cultured in Roswell Park Memorial Institute-1640 (RPMI-1640) medium supplemented with 10% fetal bovine serum (FBS; Gibco, Grand Island, NY, USA), and 1% penicillin-streptomycin (Gibco, Grand Island, NY, USA). All cells were negative for mycoplasma tested wi[illegible]the MycoProbe Detection Kit (R&D Syst[illegible] neapolis, MN, USA), and all expe[illegible]nts were repeated at least 3 times with two [illegible]ines.

The plasmids were transfected w[illegible]posomal Neofect (Neofect Biotech, Be[illegible]g, Chi[illegible]cord-ing to the supplier's instr[illegible]ns. A spec[illegible] mary antibody recogniz[illegible]ABP5 [illegible]b84028, cam, Cambridge, MA[illegible]A), ST[illegible]A (ab320[illegible]; Abcam, Cambrid[illegible], and [illegible]eral-dehyde 3-phosp[illegible] de[illegible]enase [illegible]APDH; ab181602; Ab[illegible] Cambrid[illegible]SA) was purchased f[illegible]m (Cambri[illegible]MA, USA).

Clinical Specimen[illegible]

A[illegible]aining the i[illegible]ed consent from 40 ra[illegible]nly selected gastric c[illegible]ncer patients who did [illegible] receive chem[illegible]herapy or radiotherapy, their g[illegible]ic cancer [illegible]es and para-cancer tissues w[illegible]ollected b[illegible]rgery. This investigation was app[illegible]by [illegible]Research Ethics Committee of Taizhou[illegible]'s Hospital (Jiangsu, China).

[illegible]mohistochemical Staining

T[illegible]uman gastric cancer tissue fixed in formalin was made into - section (3-5 μm) in a paraffin-embedded state. Then, the deparaffinized and rehydrated sections were boiled in 10 mM citrate buffer (pH 6.0) for 5 min for antigen retrieval. 3% hydrogen peroxide in methanol was used to inactivate endogenous peroxidase for 10 min at room temperature, followed by blocking with 5% bovine serum albumin (BSA). After incubation with primary antibody for 1 h at 37°C, protein expression in tissues was detected using diaminobenzidine (DAB) labeled secondary antibodies according to the manufacturer's protocol. The staining results were imaged using a Leica microscope and the expression levels of STAT5A in 40 gastric cancer tissues were scored.

Virus Packaging and Infection

The packaging plasmid pMDLg/pRRE, pCMV-VSVG, and the pRSV-REV plasmid containing specific shRNA targeting STAT5A or FABP5 were co-transfected into 293T cells. The supernatant of the 293T cells containing the virus was collected and centrifuged at 32000 g to prepare a concentrated virus suspension. Once infected with indicated virus for 24 h, MKN28 and AGS cells were screened with G418 (Geneticin, 800 ng/

mL) for 1 week to obtain a cell line stably knocking down the targeted gene.

Dual-Luciferase Reporter Gene Assay

Plasmids carrying firefly Luciferase and different-fragments of the FABP5 promoter or pGL3 vectors were co-transfected into MNK28 and AGS cells with plasmid expressing STAT5A and the plasmid pRL-TK carrying *Renilla* Luciferase. After 48 h of transfection, Luciferase activity was detected using a Dual-Luciferase reporter assay kit (Promega, Madison, WI, USA) according to the manufacturer's instructions. All experiments were performed in triplicate and the data are expressed as mean ± SD (Standard Deviation).

Quantitative Real-Time PCR Analysis

Total RNA in the tissues freeze in liquid nitrogen or indicated cells was extracted with TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. After detecting the quality and concentration of the extracted RNA by Nano-Drop 2000 system, 2 μg total RNA was reverse-transcribed into cDNA with a reverse transcriptase kit (TO BO, Osaka, Japan). The mRNA levels targeted genes were detected with the R (Synergy Brands) green method (TOYO Osaka, Japan). The data represents two in pendent experiments with thre ical rep cates for each set of experi GAPDH gene mRNA transcription el was d as an internal reference for ea mple used in this study we sho le 1.

Quantification eutral Lip

To detect ne in gastric er cells, the neutral lip content KN28 and AGS cells was meas with the lip ic fluorescent dye BODIP 3/503 (Invitrogen, sbad, CA, USA) acco to the ufacturer's instructions.

Quantification of Triglycerides and Phospholipids

According to the manufacturer's instructions, cellular phospholipid assay kit (Invitrogen, C bad, CA, USA) and triglyceride assay rogen, Carlsbad, CA, USA) were u o quanti fy the content of triglyceride or holipid in MKN28 and AGS cells. All exp ts were performed in triplicate and th ata ar ssed as mean ± SD.

Clonal Formation

Cells (1000 cell/we eded i well plates. After the es we ugh to visible, the medium discarded the nes were fixed with 4 ldehyde so for 10 min, following ning 0.2% crystal violet for 15 min. Three replicate were set for each experi group.

Viability A ay

ell viability different cell lines was detected by c counting kit-8 (CCK-8) kit (Bi Kir rg, Switzerland). Equal numbers of t genotype cells were incubated in 96-well plates for 24 h. The medium was then d with medium containing 10% CCK-8 and ubation continued for 1 h. OD450 was measured using a microplate reader [Multiskan FC, Thermo Fisher Scientific (Waltham, MA, USA)]. The measurement was continued for 3 days, and each set of experiments was repeated 3 times.

Mouse Xenograft

5.0×10^6 stable FABP5-KD or FABP5-KD/STAT5A-OE cell lines and control cells were resuspended in phosphate-buffered saline (PBS), and Matrigel (BD Biosciences, San Jose, CA, USA) was mixed in samples in a 1:1 ratio (v/v). The indicated cells were injected subcutaneously

e 1. P used f CR.

G	Primers (5′ to 3′, forward/reverse)
	GCAGAGTCCGTGACAGAGG/CCACAGGTAGGGACAGAGTCT
ABP5 m A	TGAAGGAGCTAGGAGTGGGAA/TGCACCATCTGTAAAGTTGCAG
ASN mRNA	AAGGACCTGTCTAGGTTTGATGC/TGGCTTCATAGGTGACTTCCA
C1 mRNA	GCTCCTTGTCACCTGCTTCT/CAAGGCCAAGCCATCCTGTA
X1 mRNA	GGCGCATACATGAAGGAGACCT/AGGTGAAAGCCTTCAGTCCAGC
A mRNA	CTGGACAATACCTCGGAGCC/AACGTCACAAAGAACGCTGC
GAPDH mRNA	GGAGCGAGATCCCTCCAAAAT/GGCTGTTGTCATACTTCTCATGG
FABP5 promoter	GAGGAGCAGAAGGTCAGGGAG/CGGGCGAGTCCCTGCTTGCAG

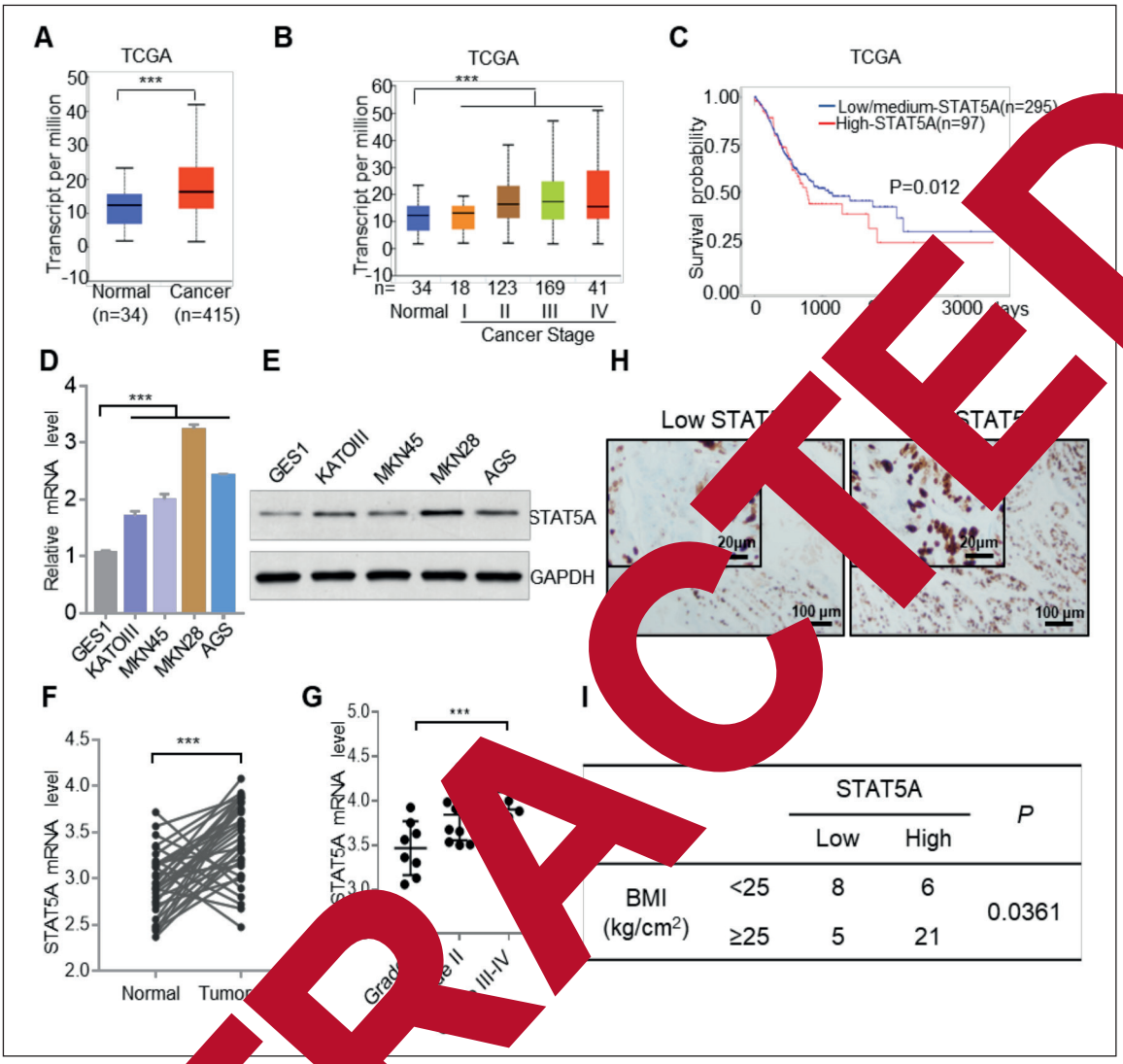

Figure 1. Expressio ern of STAT astric cancer. **A,** Expression lever of STAT5A in primary gastric cancer (Cancer) compared to n ue (Normal) A dataset. **B,** STAT5A mRNA expression levers in four different stages of gastric cancer b n dataset. **C,** Ov ll survival of patients with gastric cancer was calculated using Kaplan-Meier analysis acco ding to the S mRNA expression lever. **D-E,** Relative mRNA and protein expression level of STAT5A in four gastric er cell lines co d to GES1 cells. GAPDH served as endogenous control. **F,** STAT5A mRNA expression pattern i pairs of human gast cer tissues (Cancer) and adjacent tissues (Normal). **G,** STAT5A mRNA expression lever i differe grades of 40 ca cer samples. **H,** Representative IHC image show STAT5A expression pattern in gastric can sues (m ication 100X and 600X). I, Correlation of STAT5A expression and BMI was statistically analyzed with Chi- tes

in ck of 5 weeks old male BALB/C nude growth size was measured every days when palpable tumor-like products were uced (about 4 mm in diameter) and tumor g curves were plotted. Four weeks later, the mice were sacrificed and tumors were harvested for further analysis. All protocols were approved by the Institutional Animal Ethics Committee.

Statistical Analysis

Statistical analysis of the data in this study was performed using SPSS 21.0 software (Version X; IBM, Armonk, NY, USA), and plots were drawn with GraphPad prism 7.0 (Version X; San Diego, CA, USA). The distribution differences of quantitative data were analyzed with one-way ANOVA or two-sided *t*-test, and Bonferroni test was used

to validate ANOVA for pairwise comparisons. The relationship between STAT5A expression and BMI was detected by χ^2-test. $p < 0.05$ was considered as statistically significant.

Results

Expression Pattern of STAT5A in Gastric Cancer

To explore the potential role of STAT5A in the pathogenesis of gastric cancer, the expression pattern of STAT5A was first analyzed. The TCGA database showed a raising mRNA expression level of STAT5A in gastric cancer compared to adjacent tissues (Figure 1A). After classifying these gastric cancers, it was found that STAT5A expression increased with the pathological stages (Figure 1B). Patients with high STAT5A expression showed worse survival than those in low STAT5A expressed patients (Figure 1C). To further validate the expression pattern of STAT5A in gastric cancer, STAT5A expression was compared in four gastric cancer cell lines and normal gastric cell lines (G[illegible]). The results showed that STAT5A exp[illegible] in gastric cancer cells was higher than t[illegible] in GSE1, and STAT5A expressing in MKN28 [illegible] AGS cells was the most evident.

Further, the expression of ST[illegible] 40 clin[illegible] cally collected cancer tissues [illegible] adj[illegible] t tissues was compared. In parallel[illegible] AT5A [illegible] pressing in gastric cancer tissues [illegible] signifi[illegible] compared to adjacent [illegible] F, **Supplementary Table SI**). [illegible] nsideri[illegible] ical grading of gastric cancer [illegible] verage exp[illegible] n level of STAT5A in hig[illegible] ades (grade [illegible] VI) was found signific[illegible] dy hig[illegible] han that in lower tumor grad[illegible] rade I and I[illegible] re 1G).

To f[illegible] er clarify STAT[illegible] protein expression [illegible] rn in [illegible] cal gastric cancer tissues, we pe[illegible] d IH[illegible] taining of STAT5A in 40 gastric c[illegible] ues, a[illegible] und that STAT5A was [illegible] ly e[illegible] ed i[illegible] nucleus of gastric cancer [illegible] Figur[illegible] analyze the relationship between STAT5[illegible] pression and clinical characte[illegible] patients with gastric cancer, we found [illegible] elationship between STAT5A expression and body mass index (BMI) in gastric [illegible] er patients (χ^2-test, p=0.0361; Figure 1H). To be [illegible] e specific, 52.5% (21/40) of patients were overweight (BMI>25) and high expression of STAT5A in gastric cancer tissues, while 72.5% (29/40) patients showed consistent feature of BMI and STAT5A expression (Figure 1I). These results demonstrated that STAT5A expression in gastric cancer tissues was associated with patient BMI.

STAT5A Function on Fatty Acid Metabolism in Gastric Cancer [illegible]s

Numerous studies[23-25] have in[illegible]ed the key function of fatty acid metabolism i[illegible] rigenesis, and the above results sh[illegible]d that [illegible] pression pattern of STAT5A i[illegible] tric cance[illegible] is associated with BMI[illegible] erefor[illegible]e sou[illegible] further verify wheth[illegible] AT5A[illegible] s involved in fatty acid metaboli[illegible]m [illegible] [illegible] ncer. [illegible]d on data from GSE6[illegible], ST[illegible] was si[illegible]cantly associated wi[illegible] tty acid m[illegible] li[illegible] pathways (NES valu[illegible] < 0.001; [illegible] 2A). Moreover, Kap[illegible] Mei[illegible] vival plot showed gastric cancer patients with [illegible] ≥ 25 kg/m^2 showed a poo[illegible] al prognosi[illegible] ure 2B). It is specu[illegible] that STAT5A was involved in the regu[illegible]n of fatty a[illegible]d metabolism and combined fatty acid m[illegible]olic signaling pathway with S[illegible]5A may f[illegible]ion in the development and prog[illegible] tric cancer.

To a[illegible] hether STAT5A was involved in reprogramming fatty acid metabolism in gastric [illegible] cell lines, we examined the expression [illegible] l key fatty acid metabolic enzymes enriched in the fatty acid metabolism pathway of GSE69306 in STAT5A knockdown cells, and the results showed that FASN, ACOX1, CPT1A, ACC1, and FABP5 mRNA expression levels were down-regulated in STAT5A knockdown MKN28 and AGS cells. Surprisingly, FABP5 mRNA levels showed the greatest change, and exogenous overexpression of STAT5A can significantly rescue FABP5 expression (Figure 2C).

Further, we measured the effect of STAT5A expression on the content of fatty acids and their metabolic intermediates in gastric cancer cells. Our data showed that knockdown STAT5A in MKN28 and AGS cells resulted in a significant decrease in intracellular triglycerides and phospholipids levels, whereas restoring STAT5A expression increased the levels of triglycerides and phospholipids in MKN28 and AGS cells (Figure 2D, 2E). Moreover, cellular staining confirmed the content of neutral lipids in MKN28 and AGS cells were significantly reduced by silencing STAT5A, while exogenous expression STAT5A restored the cellular content of neutral lipids to a certain degree (Figure 2F). These results indicated that STAT5A had a potential effect on the expression of fatty acid metabolism-related enzymes, which

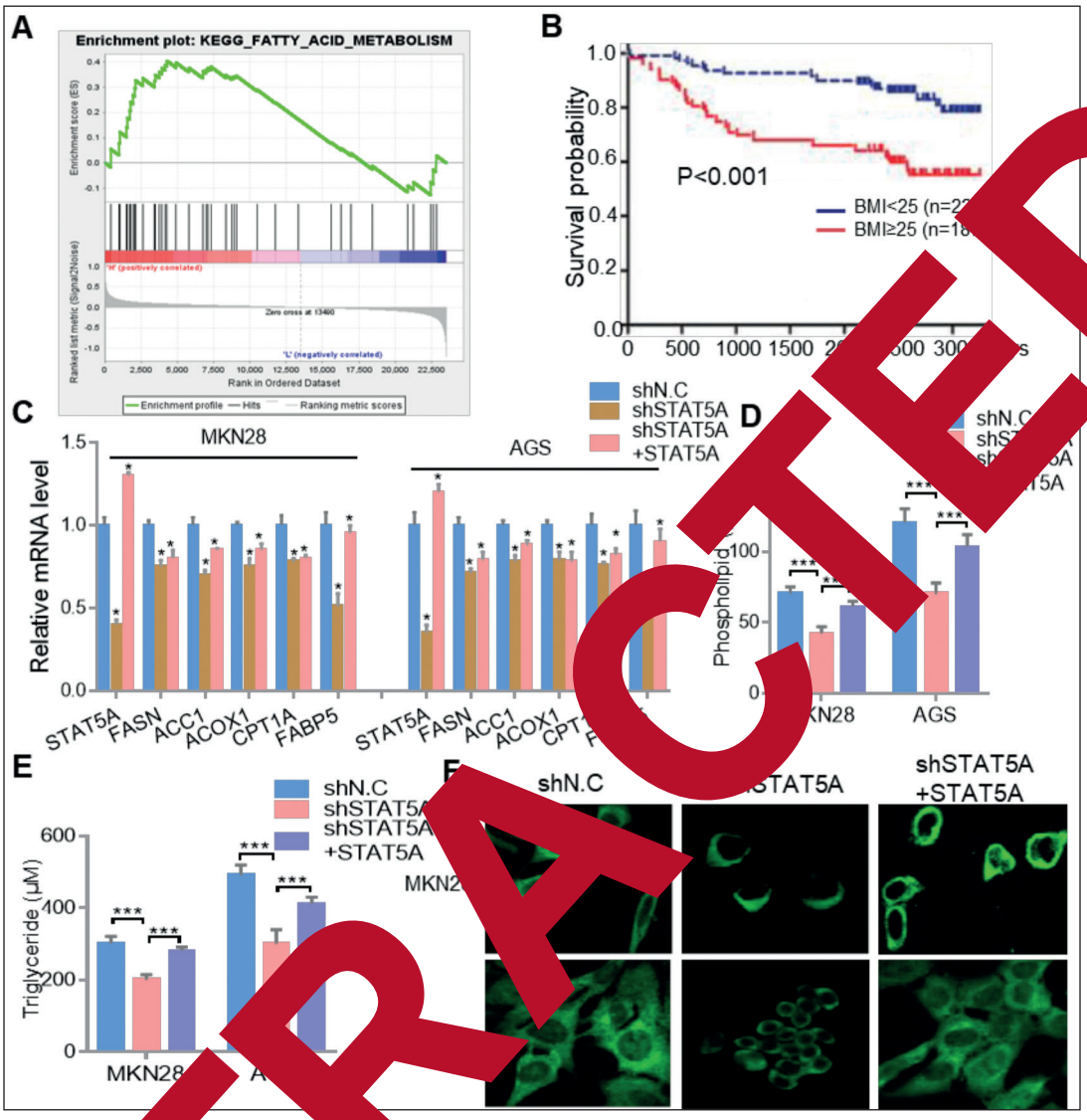

Figure 2. STAT5 on fatty acid lism in gastric cancer cells. **A,** Identification of gene sets enriched in phenotypes correlat th S by GSEA wi GSE69306 data. **B,** Kaplan-Meier survival curves showed poor over survival with BMI≥25 kg/m²). **C,** R P analysis for mRNA levels of the key lipid metabolic enzymes FASN, ACC1, ACOX1, CPT1A, an BP5 in the indi ells. **D-E,** Cellular content of phospholipids **(D)** and triglycerides **(E)** was detected in the indic cells. F. The neutral content was detected by double staining with BODIPY 493/503 dye in the indicated cells (ification 0X).

her a d the ls of fatty acids and their olic i tes in gastric cancer cells.

ST Dependent FABP5 Expression tty Acid Metabolism Gastric Cancer Cells

confirm that STAT5A has a potential effect o expression of FABP5 in gastric cancer cells, we first explored the relationship of FABP5 and STAT5A expressed in gastric cancer. The results showed that the mRNA expression level of FABP5 in gastric cancer tissues was significantly higher than that in normal tissues (Figure 3A, **Supplementary Table SII**). Moreover, the expression level of FABP5 in gastric cancer had a significant positive correlation with STAT5A (Figure 3B). FABP5 expression was significantly downregulated in the stable STAT5A knockdown MKN28 and AGS cells, but FABP5 level was also up-regulated after exogenous overexpression of STAT5A (Figure 3C). Those disclosed that STAT5A can regulate FABP5 expression in gastric cancer cells.

To explore how STAT5A regulated FABP5 expression in gastric cancer, we tested whether STAT5A bind to the promoter of the FABP5 gene. Truncates of the FABP5 promoter region were cloned into vectors containing the Luciferase reporter gene (Figure 3D). By measuring the

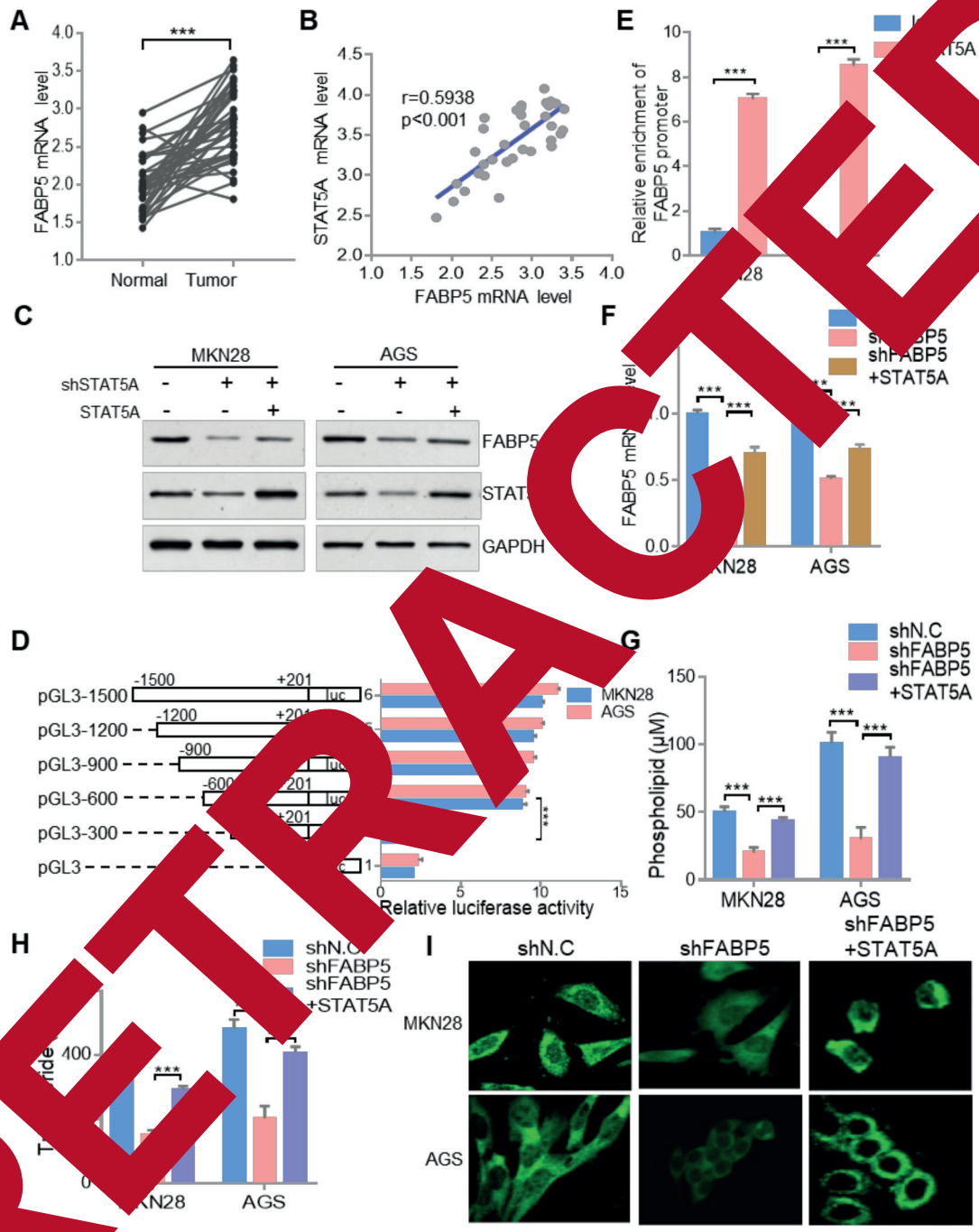

Figure 3. STAT5A dependent FABP5 expression mediates fatty acid metabolism in gastric cancer cells. **A,** Expression lever of FABP5 mRNA in gastric cancer contract to normal tissues. **B,** FABP5 co-expressed with STAT5A in gastric cancer based on gastric cancer tissues. **C,** Relative protein expression of FABP5 in the indicated cells. **D,** FABP5 promoter deletions fused to the luciferase reporter gene were transfected with STAT5A in MKN28 and AGS cell lines. **E,** ChIP assay was used to examine the interaction of FABP5 promoter with STST5A in MKN28 and AGS cell lines. **F,** Relative mRNA expression of FABP5 in the indicated cells. **G-H,** The levels of phospholipids (**G**) and triglycerides (**H**) were measured in the indicated cells. **I,** The neutral lipid content was detected by double staining with BODIPY 493/503 dye in the indicated cells (magnification 600 ×).

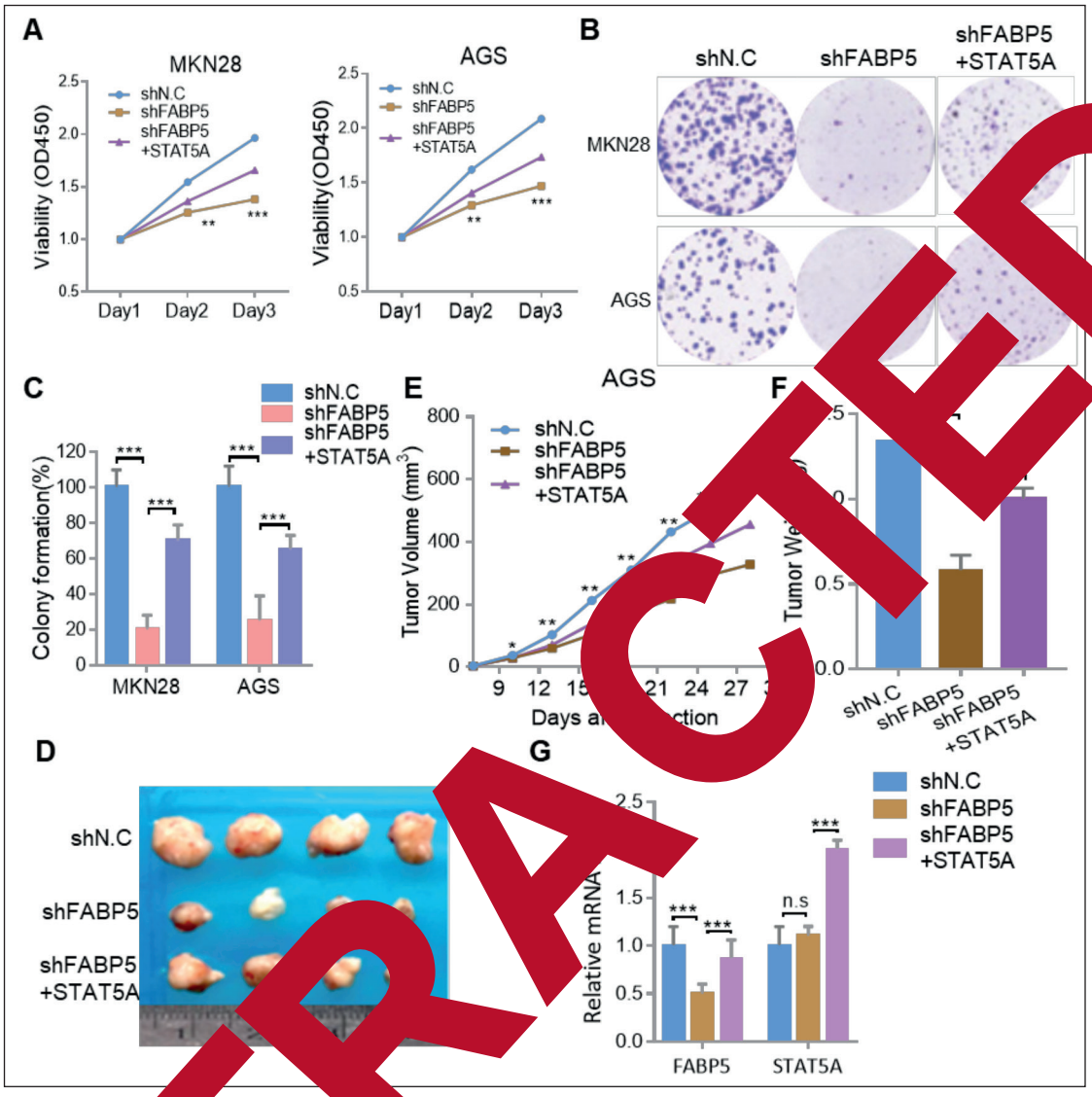

Figure 4. STAT5A [illegible] d fatty acid m[illegible]ism promotes tumorigenic ability of gastric cancer cells *in vitro* and *in vivo*. **A,** CCK8 assay s[illegible]d [illegible]ability in the [illegible]icated cells. **B,** Colony formation assay showed cell growth of the indicated cells (magnificat[illegible]20X). [illegible]lification of the colony formation shown in B (n=3). **D,** The representative pictures of dissected tumo[illegible]m nude mice [illegible]lanted with stable FABP5-KD or FABP5-KD/STAT5A-OE AGS cells. **E,** Subcutaneous tumor gr[illegible]curves of mice in [illegible]t treatment groups was presented. **F,** The average weight of tumors at the time the animal[illegible]e sacrif[illegible]d in the indic[illegible]ed groups. **G,** Relative mRNA expression pattern of associated gene in tumor tissue acqu[illegible]rom nu[illegible]ce.

[illegible]ucif[illegible]rter gene expression, overexp[illegible]sion of [illegible]T5A was found to increase the re[illegible]ne expression, and when we intercept[illegible]m of the FABP5 promoter by approximately 300 bp, the reporter gene expression [illegible]sity was significantly reduced (Figure 3D). T[illegible]ore, we hypothesized that approximately 300 bp upstream of the FABP5 promoter are sufficient as a binding site for STAT5A.In addition, the ChIP-qPCR assay indicated that STAT5A was able to significantly enrich the 300 bp upstream of the FABP5 gene promoter (Figure 3E). Those results indicated that STAT5A was a transcription factor of FABP5 in gastric cancer cells.

To further investigate the effect of STAT5A-dependent FABP5 transcription on fatty acid metabolism in gastric cancer cells, we constructed a stably knock down FABP5 MKN28 and AGS cell line, and exogenously expressed STAT5A in this cell line can restore the expres-

sion level of FABP5 to some extent (Figure 3F). By measuring the levels of fatty acids and their metabolites in those cell lines, we found that knockdown FABP5 significantly down-regulated the levels of triglycerides and phospholipids in MKN28 and AGS cells, while restoring STAT5A rescued intracellular levels of triglycerides and phospholipids (Figure 3G, 3H). This result was consistent with the staining result of neutral lipids in MKN28 and AGS cells (Figure 3I). These results indicated that STAT5A can affect fatty acid metabolism by regulating the expression of FABP5 in gastric cancer cells.

STAT5A-Regulated Fatty Acid Metabolism Promotes Tumorigenic Ability of Gastric Cancer Cells

To assess whether STAT5A-mediated FABP5 expression has an effect on the tumorigenic capacity of gastric cancer cells via regulating fatty acid metabolism, we next investigated the effect of STAT5A-regulated FABP5 expression on the proliferation of gastric cancer cells *in vitro*. In the MKN28 and AGS cell lines that have been constructed stably knock down FABP5, the C[illegible]8 assay showed that silencing FABP5 sign[illegible]ly down-regulated cell viability, but cell [illegible]il-ity increased significantly after overexpres[illegible] of STAT5A (Figure 4A). Consistent with t[illegible] knockdown FABP5 significantl[illegible]d the co[illegible]ony formation of gastric ca[illegible]ce[illegible] whereas more clones were observe[illegible]er ove[illegible]pression of STAT5A (Figure 4B, 4[illegible]herefo[illegible]e results can be inferr[illegible] tha[illegible]X-regu[illegible]d FABP5 expression [illegible]the pos[illegible]effort on the proliferation of g[illegible] cancer cel[illegible]mediating fatty acid meta[illegible]s[illegible].

Next, we e[illegible]lored t[illegible]ffect of STAT5A-mediated fa[illegible]acid metab[illegible] on the tumorigenesis[illegible]gastric cancer ce[illegible]*in vivo*. A stable FAB[illegible]ilence[illegible]GS cell line was selected as a [illegible]enta[illegible] to ex[illegible]lore the tumorigenesis in a [illegible] mous[illegible]del. Interestingly, the [illegible]lt o[illegible]graft[illegible]or model displayed that s[illegible]ing F[illegible]t only significantly down-reg[illegible]ed tum[illegible]rowth rate, but also weakened th[illegible]nd weight of the tumor formation, [illegible]pression of STAT5A significantly up-regulated tumor growth rate, size, and [illegible]ht (Figure 4D, 4E, 4G). Therefore, the a[illegible] results indicated that STAT5A-regulated fatty acid metabolism promotes tumorigenic ability of gastric cancer cells *in vivo* via raising the expression of FABP5.

Discussion

The association between metabolism and the development and treatment of cancer ha[illegible]n described in a number of cancer studi[illegible] Aleman et al[26] showed that control[illegible] the incidence of obesity is increasingly r[illegible]ized as an important measure to reduce can[illegible]k. With the progress in cancer resea[illegible] the r[illegible]ship between fatty acid metabo[illegible] and cance[illegible] opment and treatment [illegible] also b[illegible] intens[illegible] explored, which fu[illegible] incre[illegible] our understanding of their c[illegible][27][illegible]ty aci[illegible]stabolism was also [illegible]gnize[illegible]lay a l[illegible]ole in the initiation, [illegible]ression, a[illegible] stance of cancer[28]. To[illegible]ny obesity-r[illegible] drugs [lipid-loweri[illegible]statin[illegible]nti-diabetic (metformin)] have been found to [illegible]etter therapeutic effect on [illegible][30]. Collecti[illegible] these observations f[illegible]r suggested that stu[illegible]es[31] about lipid me[illegible]lism in tumo[illegible]ells may be a viable strategy [illegible]ldress cance[illegible]evelopment, progression, and d[illegible]esistance.

C[illegible]earc[illegible]und that fatty acid metabolism in gast[illegible]r cells can also significantly affect the tumorigenic ability of gastric cancer cells. [illegible]n-regulation of fatty acid binding protein [illegible]ca[illegible]nificantly reduce the tumorigenic ability of gastric cancer cells *in vivo* accompanying with the reducing content of fatty acids in the cells. Therefore, in the future, further exploration of lipid metabolism in gastric cancer will provide a new dawn for the treatment of gastric cancer.

STAT5A was widely recognized as a tumor-promoting gene in tumors[32]. STAT5A has been recognized as transcription factor that promotes the proliferation, differentiation, and the pathogenesis of tumors, and this has been emphasized in some breast cancer models[33]. In the WAP-TAg mouse model of breast cancer, the STAT5A hemizygous mice sufficiently resulted with a less, smaller, and delayed tumor formation than the wild type mice[34]. Furthermore, in a mouse model overexpressing STAT5A, mice were more susceptible to tumors[35]. These studies indicated that abnormal expression of STAT5A was an important factor in the progression of tumor pathology.

In this research, we sought to clarify the specific function of STAT5A in the pathogenesis of gastric cancer and its molecular mechanism. We not only found that STAT5A was up-regulated in gastric cancer, but STAT5A can significantly affect the fatty acid metabolism pathway in gastric cancer cells, which was a new discovery of

STAT5A in the field of cancer research. In addition, except for fatty acid metabolism pathways, we also found that STAT5A significantly enrich glucose-related pathways, such as "fructose and mannose metabolism", "pentose and glucuronate interconversions", and "starch and sucrose metabolism" pathway. This evidence fully demonstrated the important role of STAT5A in the field of tumor metabolism. In conclusion, a more in-depth understanding of how STAT5A activity is regulated in gastric cancer will bring new dawn to the treatment of gastric cancer.

Conclusions

In summary, our study described a novel mechanism of STAT5A on the fatty acid metabolism of gastric cancer. In addition, we demonstrated for the first time that STAT5A can significantly affect the tumorigenic ability of gastric cancer cells by regulating the expression of FABP5 in gastric cancer, and confirmed that the up-regulated STAT5A plays a cancer-promoting function in the pathological process of g[illegible] cancer. Besides, it provides a new pers[illegible] for STAT5A in cancer research.

Conflicts of interest

The authors declare no conflicts [illegible]

Ref[illegible]

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