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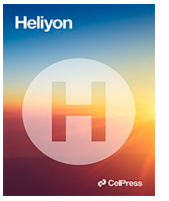

Research article

SQSTM1/p62 promotes the progression of gastric cancer through epithelial-mesenchymal transition

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ABSTRACT

Background: SQSTM1/p62 is an autophagy-related receptor protein that participates in regulating tumorigenesis and multiple signaling pathways. Gastric cancer (GC) is a common tumor in the digestive tract and continues to pose a significant threat to human health. Therefore, this study aims to investigate the impact of p62 on gastric cancer.

Methods: Immunohistochemistry and Western blotting were employed to assess the expression level of the p62 protein in gastric cancer tissues and its correlation with prognosis. Subsequently, in vitro cell experiments were conducted to determine the role of p62 in gastric cancer cell proliferation, migration, and metastasis.

Result: The expression of p62 in gastric cancer tissues was significantly higher than in normal tissues. The expression of p62 was positively correlated with poor prognosis in gastric cancer patients. In vitro cell experiments indicated that p62 promotes gastric cancer cell proliferation and migration. Mechanistically, elevated p62 expression induced epithelial-mesenchymal transition (EMT), leading to upregulation of E-cadherin and downregulation of N-cadherin and vimentin.

Conclusion: This study provides novel and robust evidence for the mechanism by which elevated p62 expression promotes the progression of gastric cancer. It offers promising therapeutic targets for anti-tumor treatment strategies in gastric cancer patients.

1. Introduction

As a multifunctional receptor protein, p62 plays a pivotal role in transporting damaged organelles and redundant proteins within cells to the autophagic lysosomes for degradation [1]. The relationship between p62 and autophagy has been well-established, and subsequent research has utilized p62 as a hallmark protein to determine autophagic activity. With deeper investigations, it has become evident that the relationship between autophagy and tumorigenesis is complex. Notably, in a majority of gastrointestinal tumors such

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as hepatocellular carcinoma, colorectal carcinoma, intrahepatic cholangiocarcinoma, esophageal squamous cell carcinoma, and pancreatic carcinoma, p62 has been found to exert pro-oncogenic effects [2–7]. Elevated p62 expression can expedite the progression of various tumors, including hepatocellular carcinoma, colorectal carcinoma, and pancreatic carcinoma, through pathways such as p62-Keap 1-Nrf2, MEK/ERK signaling, NF-κB, among others [8–10]. Currently, existing research suggests elevated p62 expression in Gastric cancer (GC) tissues, potentially implicating its involvement in GC development. However, the precise mechanisms underlying p62's role in GC initiation remain an area of significant research exploration.

Epithelial-Mesenchymal Transition (EMT) is a process wherein epithelial cells undergo transformation into mesenchymal cells under specific circumstances. EMT is involved in regulating embryonic stem cell differentiation, inducing pluripotency, and influencing the behavior of tumor stem cells [11]. During EMT, epithelial cells experience reduced intercellular adhesion and loss of cellular polarity, gaining the ability to invade the extracellular matrix. Through the transition to mesenchymal cells, these cells then migrate into the bloodstream and subsequently regain an epithelial phenotype, facilitating their colonization in new organs [12]. In the context of tumor progression, the conversion between epithelial and mesenchymal cell phenotypes becomes a critical factor driving tumor cell invasion and metastasis.

GC is the most common malignant tumor in the digestive system, and its incidence continues to rise each year. In 2020, there were 1.08 million newly diagnosed cases of GC globally, with 768,000 deaths. Due to the similarity of early symptoms with common benign gastric diseases, there is an increased likelihood of missed diagnoses and misdiagnoses, often leading to advanced disease presentation when severe symptoms arise [13]. Despite the extension of patient survival through surgical treatment, radiotherapy, and chemotherapy in recent years, the side effects during treatment significantly impact patients' quality of life. In 2018, immunotherapy introduced a new approach to cancer treatment in China. Unfortunately, the emergence of hyperprogression during tumor treatment has affected its efficacy, and the successes achieved in the treatment process have been limited [14]. Therefore, a thorough understanding of the pathogenesis of GC is imperative to achieve precise targeted therapies. As a result, we investigated the expression levels of p62 in GC tissues and adjacent non-cancerous tissues. We discovered that p62 may participate in GC development through the EMT pathway. In our cellular experiments, by inhibiting p62 expression, we observed a potential reduction in EMT functionality, leading to suppressed proliferation and migration of GC cells.

2. Methods

2.1. Patients and specimens

The specimens were collected from 41 patients who received surgical treatment at the Department of Oncology, Gansu Provincial People's Hospital, from 2019 to 2020, and were pathologically diagnosed with gastric adenocarcinoma. The inclusion criteria for patients were as follows: ① Pathologically diagnosed with gastric adenocarcinoma; ② No prior anti-tumor treatment before surgery; ③ No concomitant other tumorous diseases; ④ Possess complete clinical and pathological data. Immunohistochemical staining (IHC) was performed on specimens from 41 gastric adenocarcinoma patients. This research protocol was approved by the Ethics Review Committee of Gansu Provincial People's Hospital.

2.2. Cell culture

Human GC cell lines AGS, MKN-45, HGC-27, and MGC-803, as well as the normal gastric mucosal cell line GES-1, were purchased from the Cell Bank of Type Culture Collection of Chinese Academy of Sciences and cultured in low glucose RPMI-1640 complete medium supplemented with 10% serum and 1%Penicillin-streptomycin mixture. The cells were maintained in logarithmic growth phase and passaged for continued cultivation for future use.

2.3. Western blotting analysis

Cellular and tissue proteins were extracted using RIPA cell lysis buffer containing a protease inhibitor cocktail. The protein content of cells and tissues was quantified using the BCA assay method. Equal amounts of protein (20μg) were loaded for SDS-PAGE separation, followed by transfer onto PVDF membranes. After blocking with a rapid antibody blocking solution, the membranes were incubated with primary and secondary antibodies. Protein bands were visualized using an ECL super-enhanced chemiluminescence reagent and analyzed using ImageJ software.The following antibodies were used: Anti-GAPDH (Abcam,ab181602),Anti-SQSTM1 (Zenbio, R25788),Anti-E-cadherin (Abcam,ab40772),Anti-N-cadherin (Abcam,ab76011),Anti-vimentin (Abcam,ab92547).

2.4. Quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR)

Total RNA was extracted using the M5 HiPer Universal RNA Mini Kit (Mei5bio, FM036) following the manufacturer's instructions. The extracted total RNA was reverse transcribed into cDNA using the SweScript RT I First Strand cDNA Synthesis Kit (Servicebio, G3330). For quantitative real-time PCR (qRT-PCR), the Hieff qPCR SYBR Green Master Mix (Yeasen 11201ES03) from Yeasen was utilized. GAPDH was used as the internal reference gene. The relative expression of genes was assessed using the $2^{\hat{}-\Delta\Delta Ct}$ method. The primer sequences for qRT-PCR are detailed as follows: GAPDH: forward: 5'–GGAAGCTTGTCATCAATGGAAATC-3′, reverse: 5'–TGATGACCCTTTTGGCTCCC-3′; p62: forward: 5′–GACTACGACTTGTGTAGCGTC-3′, reverse:5′–AGTGTCCGTGTTTCACCTTCC-3′; E-cadherin: forward: 5′–GTA GGAAGGCACAGCCTGTC-3′, reverse: 5′–CAGCAAGAGCAGCAGAATCA-3′; vimentin: forward: 5′–

CTGCAGGACTCGGTGGACTT-3′, reverse: 5′–GAAGCGGTCATTCAGCT CCT-3’.

2.5. Cell colony formation

In the cell cloning experiment, 1000 cells were seeded into the wells of a 6-well plate containing 10% FBS culture medium. After 24–48h of incubation, the adherent cells were fixed using 4% paraformaldehyde. Crystal violet staining was performed, followed by capturing images and conducting analysis using ImageJ software.

2.6. Wound healing assay

For the wound healing assay, cells were first cultured in a 6-well plate until they reached confluence. A vertical scratch was made at the center of each well using a 200μl pipette tip. Images of the scratch area were captured under a microscope at 0 and 48h after the scratch was made. The migration rate was calculated as follows: migration cell surface area after healing/total scratch area × 100%.

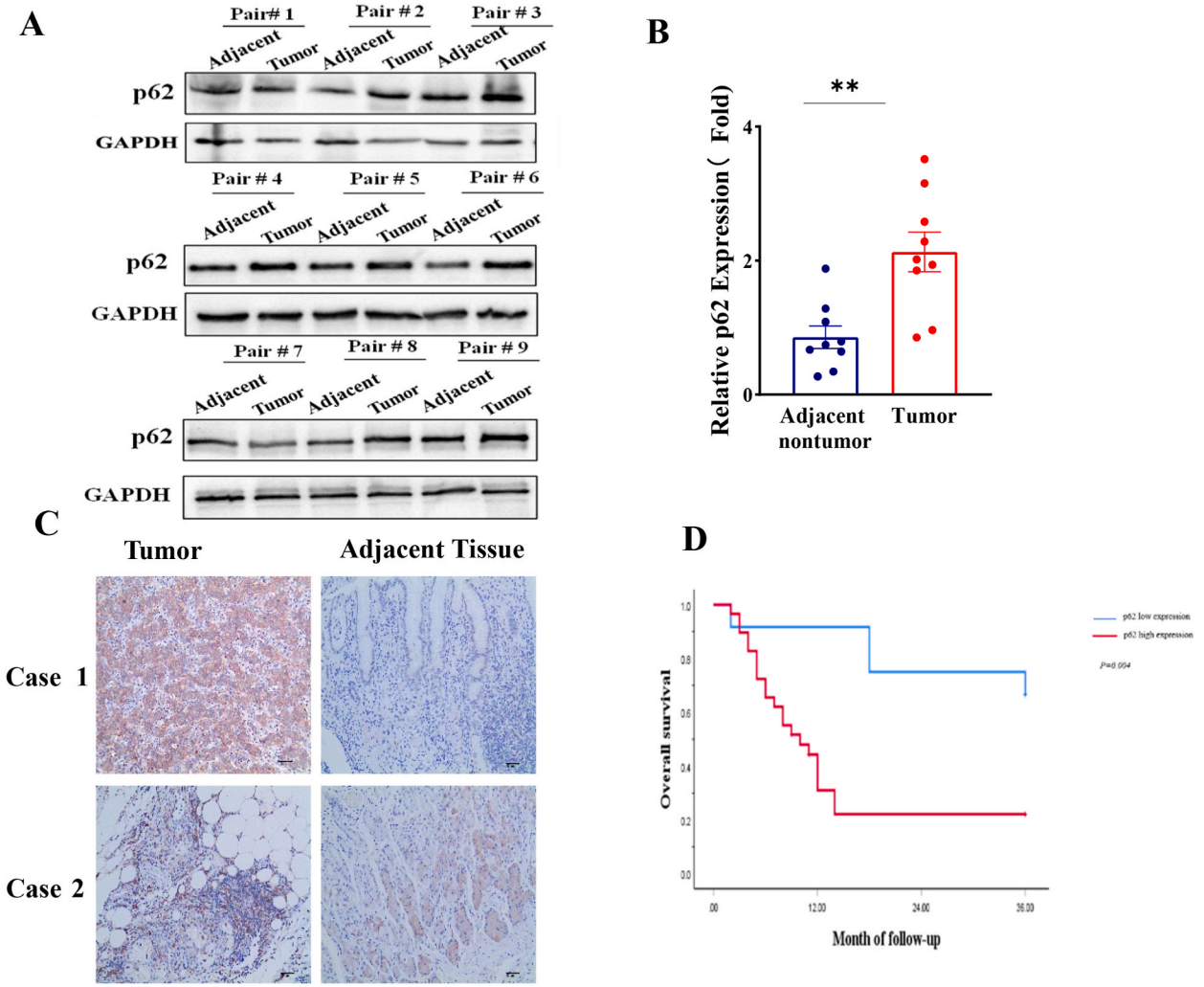

Fig. 1. Expression of p62 in GC Tissues and its Impact on Prognosis
(A) Western blotting images showing p62 expression in 9 pairs of GC tissues and adjacent tissues.(B) Statistical analysis of Western blotting images showing p62 expression in 9 pairs of GC tissues and adjacent tissues.(C) Immunohistochemical staining (IHC) images depicting p62 expression in tumor tissues from 41 GC patients and adjacent tissues. (D) Kaplan–Meier showed overall survival time of 41 patients with different p62 expression. Scale bar = 40 μm. Data are presented as mean ± SD or median (range). Statistical significance was assessed using Student’s t-test, log-rank test, or Mann-Whitney *U* test as appropriate. *p<0.05, **p<0.01, ***p<0.001.

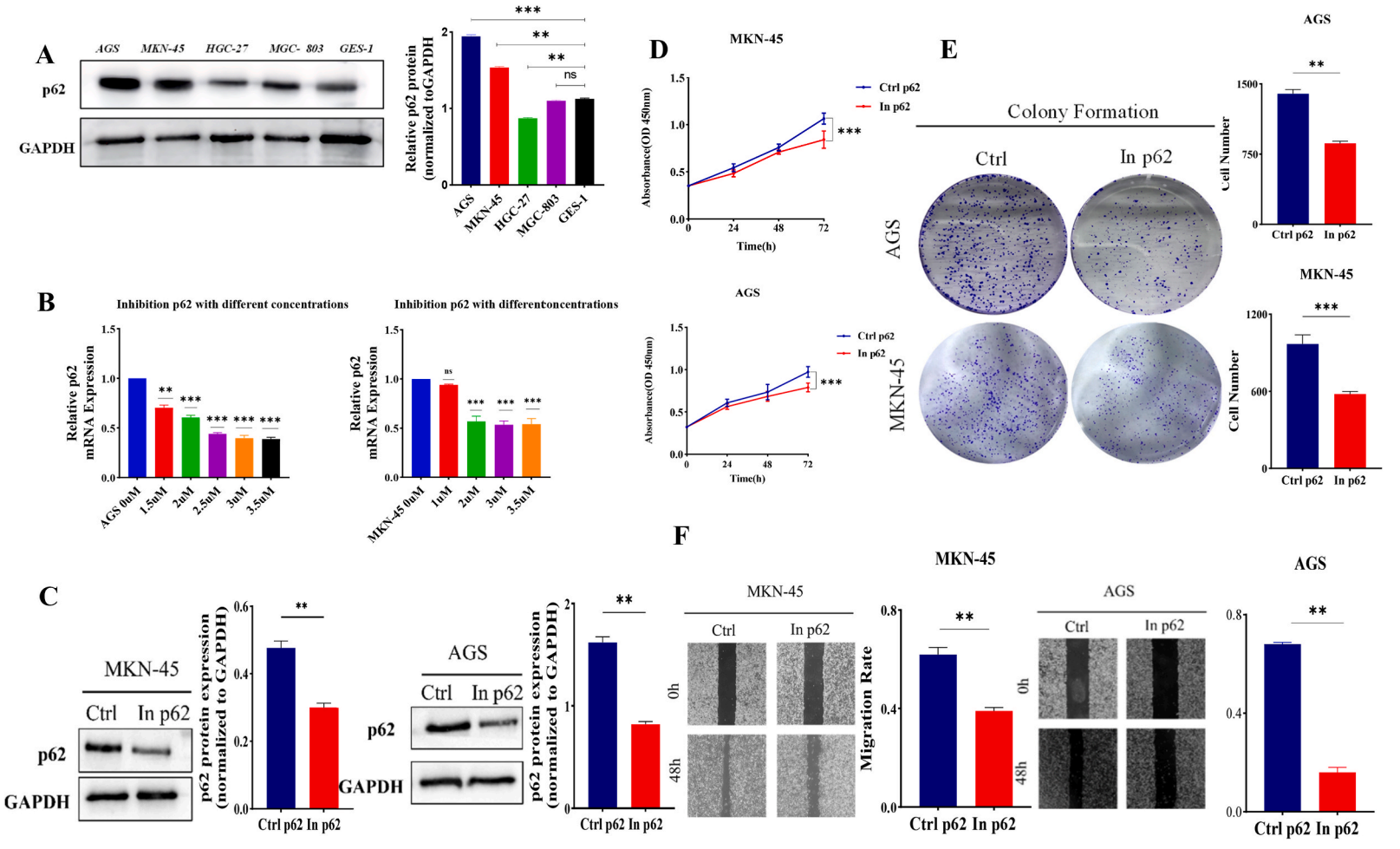

Fig. 2. Inhibition of p62 Attenuates GC Progression in Vitro

(A) Western blotting images and statistical analysis of p62 expression in different GC cell lines.(B) Validation of p62 inhibition in GC cells using qRT-PCR after treatment with different concentrations of p62 inhibitor. (C) Western blotting images of p62 inhibition in AGS (3.0μM) and MKN-45 (3.5μM) cells using p62 inhibitor. Quantitative analysis of normalized band intensities relative to GAPDH in the right panels.(D) Cell proliferation quantification using the Counting Kit-8 assay (CCK8) in AGS and MKN-45 cells treated with p62 inhibitor (In p62) compared to the control (Ctrl).(E) Colony formation assay images and histogram showing the number of proliferating cells on the right.(F) Representative images of wound healing assays and histogram analysis of cell migration distance in the right panel. All data are presented as mean ± standard deviation. Statistical significance was evaluated using Student's t-test. **p<0.01, ***p<0.001.

2.7. Cell proliferation experiment

In the cell proliferation assay, the cell suspension was inoculated in a 96-well plate and pre-placed in an incubator with 37°Celsius and 5%CO2 saturation humidity. 10μl of CCK8 reagent was added to each well, and the culture plate was placed in the incubator for 1–4h. The absorbance was measured at the 450 nm wavelength.

2.8. Immunohistochemistry staining

Anti-SQSTM1 were utilized for immunohistochemistry (IHC) based on a two-step protocol as previously described [15]. The staining intensity was categorized into levels 0, 1, 2, and 3. The percentage of positive staining cells was scored as 0 (0%), 1(1–25%), 2 (26–50%), 3 (51–75%), and 4 (76–100%). The product of intensity and percentage score was used as a final score.

2.9. Statistical analysis

GraphPad Prism 9.0 (GraphPad Software, Inc., La Jolla, CA, USA) was used for statistical analyses. All data are given as the (mean ± SD). Statistical analyses were performed using a two-tailed Student's t-test for two-group comparisons, one-way ANOVA for multi group comparisons, and a Kaplan-Meier nonparametric log-rank test for survival rate comparisons. $P \leq 0.05$ was considered significant. * indicates $P<0.05$, ** indicates $P<0.01$, *** indicates $P<0.001$, **** indicates $P<0.0001$, and ns indicates nonsignificant.

3. Results

3.1. p62 expression in GC tissues

The expression level of p62 was found to be higher in GC tissues compared to adjacent non-cancerous tissues, suggesting that patients with elevated p62 expression levels may exhibit poorer prognosis and a correlation with tumor infiltration depth, lymph node metastasis, clinical stage, and tumor differentiation. To elucidate the expression level of p62 in GC tissues, we compared the expression of p62 protein in cancerous and adjacent non-cancerous tissues from 9 GC patients (Fig. 1A). It was observed that p62 expression was significantly higher in GC tissues compared to adjacent non-cancerous tissues (Fig. 1B). We further validated the expression level of

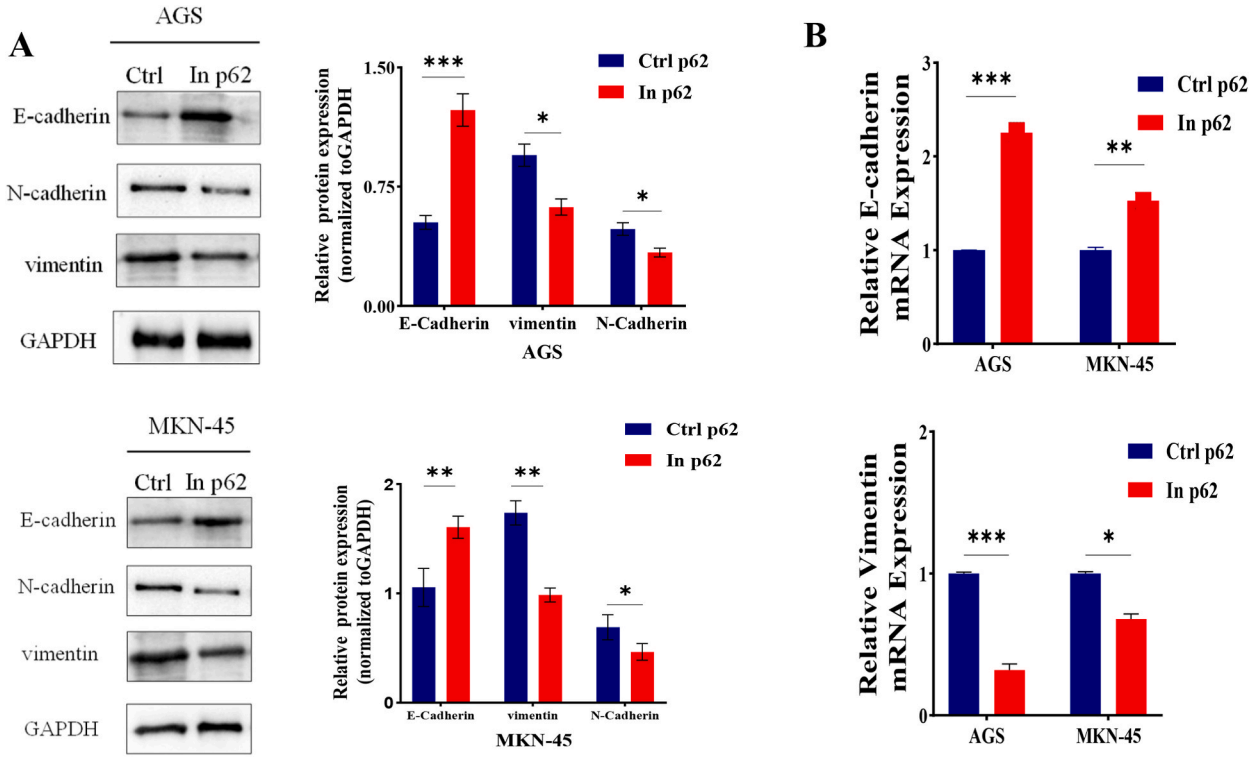

Fig. 3. Elevated p62 Promotes GC Progression through Induction of EMT.
(A) Western blotting assessed the expression of E-cadherin, N-cadherin, and vimentin proteins in AGS and MKN-45 cells treated with p62 inhibitor (In p62) compared to control (Ctrl). Band intensities were quantified and normalized to GAPDH in the right panels.(B) qRT-PCR analysis of E-cadherin and vimentin mRNA levels in GC cells with different p62 expressions. *p<0.05, **p<0.01, ***p<0.001.

p62 in GC tissues and adjacent non-cancerous tissues using immunohistochemistry. Through immunohistochemical staining of tumor tissues and their corresponding adjacent non-cancerous tissues from 41 patients, we observed a significant increase in p62 protein expression in tumor tissues (Fig. 1C). Moreover, patients with higher p62 expression levels exhibited poorer prognosis (Fig. 1D). The clinical parameters of the 41 patients are presented in Table S1, along with their correlation to p62 expression.

3.2. Inhibition of p62 expression affects GC cell proliferation and migration ability

We compared the expression levels of p62 protein in GC cell lines MKN-45、AGS、HGC-27、MGC-803 and normal gastric mucosal cell line GES-1, revealing prominent overexpression of p62 in MKN-45 and AGS cells (Fig. 2A). XRK3F2 acts as a domain inhibitor, XRK3F2 Inhibition of p62-ZZ Domain and it is are purchased from Topscience (Product number:T001-100030,600) [16,17].To further investigate the impact of p62 on the proliferation and migration ability of GC cells, First of all, by consulting the literature, we found that the IC50 of XRK3F2 is 4.5μM [17], and we set different concentrations between 0 and 5μM. We treated the cells with different concentrations of p62 inhibitor and verified the inhibitory effect by qRT-PCR after 48h. We found that the highest inhibition efficiency was achieved when adding a concentration of 3μM p62 inhibitor to AGS cells and 3.5μM to MKN-45 cells (Fig. 2B). We then confirmed the inhibition efficiency at these concentrations using western blotting (Fig. 2C). Next we examined the effect of p62 expression on the proliferation ability of GC cells. The results indicated a significant reduction in cell proliferation after inhibiting p62 expression (Fig. 2D), which was further validated through colony formation assays as described above (Fig. 2E). Considering that the migratory ability of cancer cells is closely related to tumor progression, we evaluated the impact of inhibiting p62 expression on the migration ability of GC cells using a wound healing assay. The results showed delayed wound closure in the experimental group treated with a p62 inhibitor for 48h compared to the control group, indicating decreased migration ability (Fig. 2F). In summary, the in vitro cell experiments demonstrated that inhibiting p62 expression led to decreased proliferation and migration ability in GC cells.

3.3. p62 promoted the tumor progression of GC cells through induction of EMT

EMT plays a crucial role in tumor invasion and metastasis. In GC tissues, the low expression of E-cadherin and high expression of vimentin are consistent with the characteristics of epithelial-to-mesenchymal transition (EMT). Therefore, we conducted western blotting to assess the expression of EMT-related markers, including E-cadherin, N-cadherin, and vimentin, in GC cells with different p62 expression levels. The results revealed that inhibiting p62 expression led to an increase in E-cadherin expression and a decrease in vimentin expression (Fig. 3A). Validation through qRT-PCR further confirmed that inhibiting p62 expression upregulated E-cadherin mRNA.

Expression and downregulated vimentin mRNA expression (Fig. 3B). In conclusion, these data suggest that inhibition of p62 expression may inhibit the occurrence of gastric cancer by inhibiting the occurrence of EMT.

4. Discussion

The concept of autophagy, or self-digestion, was first introduced by de Duve in 1963 as a cellular self-consumption phenomenon [18]. In recent years, the relationship between autophagy and cancer has garnered significant attention. The association between autophagy and tumor development is dual-faceted. On one hand, in the early stages of tumorigenesis, autophagy suppresses cancer initiation through its cytoprotective functions. On the other hand, autophagy is regulated by various signaling pathways, such as PI3K/AKT/mTOR, Beclin-1, and JAK/STAT, which can promote cancer cell proliferation and tumor growth, thereby facilitating tumorigenesis [19]. As an autophagy-related protein, p62 is not only involved in multiple signaling pathways but also regulates the occurrence of inflammation, obesity, aging, and neurodegeneration [20,21]. Growing evidence suggests that p62 is intricately linked to the development of various cancers, including those of the digestive tract, leading to its consideration as a therapeutic target for cancer treatment [22]. Accumulation of p62 has been observed in liver tissues infected with HCV and HBV, as well as in hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma. Ectopic expression of p62 in mouse liver tissues has been shown to significantly induce liver cancer development [23,24]. Aberrant p62 expression in liver tissues accelerates the progression of HCC, leading to poorer patient survival [25]. In HCC, p62 can activate Nrf2 to redirect glucose into the hexosamine pathway and guide glutamine into glutathione synthesis, resulting in resistance to anticancer drugs and enhanced proliferation of HCC cells [26].

It has been identified that as a tumor oncogene, SQSTM1/p62 is often abnormally up-regulated and involved in the invasion of gastrointestinal tumors, including gastric, colorectal and pancreatic cancer [27]. Many studies have shown that the specific expression of SQSTM1/p62 in tumor cells is closely related to tumor invasion and metastasis, and can predict the prognosis. It is worth noting that the punctate expression of SQSTM1/p62 in the cytoplasm and/or nucleus may be an independent prognostic factor for esophageal adenocarcinoma, especially in early esophageal adenocarcinoma, in which the expression of SQSTM1/p62 is usually abnormally up-regulated. However, patients with higher expression of SQSTM1/p62 in cytoplasm and nucleus of tumor cells had a better prognosis, while patients with lower expression of SQSTM1/p62 or combined expression of LC3 and SQSTM1/p62 in tumor cells had higher invasive esophageal adenocarcinoma [6]. The overexpression of SQSTM1/p62 in gastric cancer is related to blood metastasis and liver metastasis, especially in early gastric cancer, the prognosis is poor. However, in colorectal cancer treated with 5-fluorouracil, the expression of SQSTM1/p62 did not show a significant correlation with prognosis [28,29]. All these data suggest that SQSTM1/p62 disorder may be an early and important event in tumorigenesis. The expression of SQSTM1/p62 in different gastrointestinal tumors has different prognostic significance, which may be due to the location of cancer lesions, immune response to cancer cells expressing SQSTM1/p62 and immune response to tumor microenvironment, which shows high density of regulatory forkbox P3T cells [30]. In

addition, inhibition of SQSTM1/p62 may inhibit autophagy and block tumor invasion through MEK/ERK signaling pathways in colorectal cancer cells involved in KRAS and BRAFV600E mutations. This evidence comes from previous studies that found that positive cytoplasmic SQSTM1/p62 staining was significantly present in most colorectal cancer tissues. Although there is no significant correlation between SQSTM1/p62 positive staining and KRAS mutation, patients with SQSTM1/p62 positive staining in the cytoplasm, especially those with KRAS mutation, may have better overall survival [31]. However, when the phosphatidylinositol-3 kinase (PI3K)/mTOR signal pathway is inhibited, KRAS and BRAFV600E mutations have been shown to activate MEK/ERK signal pathway to promote autophagy and down-regulate SQSTM1/p62 expression in colorectal cancer cells [32]. Therefore, the activation of autophagy and the down-regulation of SQSTM1/p62 may indirectly reflect the effects of KRAS and BRAF mutations in cells. However, autophagy can be inhibited by SQSTM1/p62 inhibition, leading to the cessation of cancer cell growth and tumorigenesis [33].Previous research in GC has demonstrated elevated p62 protein expression in cancer tissues compared to adjacent tissues through immunohistochemical staining. Furthermore, high p62 expression has been correlated with advanced clinical pathological stages and poor prognosis [34]. However, this research was limited to histological analysis, indicating p62's potential involvement in GC development, without delving into cellular phenotypes and mechanisms underlying its contribution to cancer initiation. Our experiments further corroborate the high expression of p62 in GC tissues and lower expression in adjacent tissues. Through clinical pathological analysis of patient data, we found that elevated p62 expression in GC correlates with increased tumor invasion depth, lymph node metastasis, and advanced clinical staging (Detailed data can be found in Table S1). Additionally, our analysis reveals that patients with higher p62 expression levels tend to have poorer prognoses. These findings reinforce the idea that p62, acting as an oncogene, likely participates in GC progression. The abnormal expression of p62 in GC could be associated with dysregulated autophagy. When autophagy dysregulation occurs, p62 accumulation in cells contributes to genomic instability and promotes tumor development [35].The reason why cancer cannot be cured is due to the fact that tumor cells not only possess the ability of limitless proliferation, but also have a migration capacity far exceeding that of normal cells. In order to validate the potential role of p62 as an oncogene in promoting GC development, and to elucidate the mechanisms by which p62 promotes the proliferation and migration of tumor cells, we conducted experiments to inhibit the expression of the p62 gene. The results showed a significant reduction in the proliferation and migration abilities of GC cells. Over the past several decades of unveiling the mysteries of tumor metastasis, EMT has gradually been discovered by researchers. EMT bestows epithelial cancer cells with strong motility and invasiveness [36]. Many studies have shown that there is a close relationship between p62 and EMT. In intrahepatic cholangiocarcinoma, it has been found that p62 promotes tumor progression through EMT [7]. In metastatic prostate cancer cells, high expression of p62 promotes EMT, resulting in enhanced abilities for proliferation, invasion, and migration [37].

In another study, p62 accumulation was found in both normal and tumor cells during growth factor-induced EMT [38]. In RAS mutant cancer cells, inhibition of autophagy leads to SQSTM1 accumulation and activation of NF- κ B signal pathway to promote EMT [39]. Other literature studies have shown that p62-mediated Twist 1 stabilization promotes EMT in vitro and promotes tumor growth and metastasis in mice [40]. In cells, p62 can affect the activity of EMT-related factors [41]. p62 has been identified as a regulator of EMT induction, invasion and metastasis in esophageal squamous cell carcinoma [42].In previous studies by our research group, the expression levels of EMT markers in GC with lymph node metastasis were investigated, revealing lower levels of E-cadherin expression and relatively higher levels of vimentin expression [43]. Through our experiments, we have also validated the role of p62 in GC EMT. Experimental data indicate that inhibition of p62 expression significantly suppresses the expression of mesenchymal markers N-cadherin and vimentin, while prominently increasing the expression of the epithelial marker protein E-cadherin. p62 is a stress-induced cellular protein with multiple domains, including the PB1 domain, ZZ-type zinc finger domain, and the TB domain involved in binding to Tumor Necrosis Factor Receptor-Associated Factor 6 (TRAF6), among others [21]. Research has revealed that p62's ZZ domain binds to Receptor-Interacting Protein 1 (RIP1) and, through its PB1 domain, collaborates with atypical Protein Kinase C (aPKC) to activate NF-κB [44]. The small molecule compound XRK3F2 acts as a domain inhibitor, binding to p62's- ZZ domain. XRK3F2 inhibits p62's role as a signaling hub for NF-κB, p38 MAPK and JNK pathways, thereby suppressing osteoclastogenesis and the growth of multiple myeloma cells, which is a critical factor in multiple myeloma bone disease [45]. In this experiment, we treated GC cells with the ZZ domain inhibitor XRK3F2 at various concentrations, based on previous literature, to suppress p62 expression. After experimental validation, we selected the optimal concentration for EMT-related experiments, where the expression of EMT-related proteins changed upon inhibiting p62's function. Research has shown that p62 upregulates Snail through NF-κB, thereby promoting EMT [46]. Thus we hypothesized that inhibiting p62's ZZ domain might prevent proper NF-κB activation, subsequently inhibiting EMT. Through reverse deduction, we propose that p62 might participate in NF-κB activation, thus promoting EMT and accelerating tumor migration. Unfortunately, this experiment did not directly investigate the relationship between p62 and NF-κB, thus lacking direct evidence to confirm whether p62 promotes EMT through NF-κB activation in GC. Therefore, for future experiments, we can delve deeper into the relationship between p62 and NF-κB, aiming to elucidate the precise mechanism of p62's involvement in GC metastasis.In summary, this study initially investigated the expression levels of p62 in cancerous and adjacent tissues at the tissue level. The findings suggested that p62 might be involved in the development of GC. Subsequently, the study delved into the impact of p62 on the biological behavior of GC cells at the cellular level. Following this, the relationship between p62 and EMT was explored. While the experiment still has limitations, as it did not thoroughly examine the specific signaling pathways through which p62 contributes to GC development, the combined results from these three parts of the study indicate that p62 can promote EMT in GC. Therefore, targeting p62 with pharmaceutical interventions might emerge as a potential focal approach for the treatment of GC.

5. Conclusions

In conclusion, the elevated expression of p62 signifies an unfavorable prognosis for patients. Our in vitro experiments have

unequivocally demonstrated that p62 promotes the proliferation and migration of GC cells through EMT. Therefore, the findings from our experimental research suggest that p62 could potentially serve as a biomarker for GC prognosis and a therapeutic target. Moreover, these findings could offer a solid foundation for the development of targeted drug therapies for GC.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Ethical statement

Our study was approved by The Medical Ethics Committee of Gansu Provincial Hospital (approval no. 2022–191). All GC patients provided informed consent to participate in the study.

Data availability statement

No data was used for the research described in the article.

CRediT authorship contribution statement

Yan Xu: Writing – original draft, Data curation. **Ciba Zhu:** Writing – review & editing. **Chenglou Zhu:** Writing – review & editing, Supervision, Data curation, Conceptualization. **Lingzhi Peng:** Writing – review & editing, Methodology, Conceptualization. **Dandan Ji:** Writing – review & editing, Writing – original draft, Investigation. **Qiong Wu:** Software, Methodology. **Pengwei Bai:** Resources, Formal analysis, Data curation. **Zhaozhao Bai:** Writing – review & editing, Data curation. **Mingxu Da:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e24409.

References

[1] J.M. Mulcahy Levy, A. Thorburn, Autophagy in cancer: moving from understanding mechanism to improving therapy responses in patients, Cell Death Differ. 27 (3) (2020) 843–857.
[2] T. Saito, Y. Ichimura, K. Taguchi, et al., p62/Sqstm1 promotes malignancy of HCV-positive hepatocellular carcinoma through Nrf2-dependent metabolic reprogramming, Nat. Commun. 7 (2016) 12030.
[3] J. Zhang, S. Yang, B. Xu, et al., p62 functions as an oncogene in colorectal cancer through inhibiting apoptosis and promoting cell proliferation by interacting with the vitamin D receptor, Cell Prolif. 52 (3) (2019) e12585.
[4] M. Komatsu, H. Kurokawa, S. Waguri, et al., The selective autophagy substrate p62 activates the stress responsive transcription factor Nrf2 through inactivation of Keap 1, Nat. Cell Biol. 12 (3) (2010) 213–223.
[5] M.S. Gilardini Montani, N. Cecere, M. Granato, et al., Mutant p53, stabilized by its Interplay with HSP90, activates a positive Feed-Back loop between NRF2 and p62 that induces chemo-resistance to apigenin in pancreatic cancer cells, Cancers 11 (5) (2019) 703.
[6] O. Adams, B. Dislich, S. Berezowska, et al., Prognostic relevance of autophagy markers LC3B and p62 in esophageal adenocarcinomas, Oncotarget 7 (26) (2016) 39241–39255.
[7] J. Chen, Z. Gao, X. Li, et al., SQSTM1/p62 in intrahepatic cholangiocarcinoma promotes tumor progression via epithelial-mesenchymal transition and mitochondrial function maintenance, Cancer Med. 12 (1) (2023) 459–471.
[8] J. Tang, Y. Li, S. Xia, et al., Sequestosome 1/p62: a multitasker in the regulation of malignant tumor aggression, Int. J. Oncol. 59 (4) (2021) 77 (Review).
[9] X. Chao, H.M. Ni, W.X. Ding, An unexpected tumor suppressor role of SQSTM1/p62 in liver tumorigenesis, Autophagy 18 (2) (2022) 459–461.
[10] J. Cuyler, P. Murthy, N.G. Spada, T.F. McGuire, M.T. Lotze, X.Q. Xie, Sequestsome-1/p62-targeted small molecules for pancreatic cancer therapy, Drug Discov. Today 27 (1) (2022) 362–370.
[11] C. Lachat, P. Peixoto, E. Hervouet, Epithelial to mesenchymal transition history: from embryonic development to cancers, Biomolecules 11 (6) (2021) 782.

[12] M. Fedele, R. Sgarra, S. Battista, L. Cerchia, G. Manfioletti, The epithelial-mesenchymal transition at the crossroads between metabolism and tumor progression, Int. J. Mol. Sci. 23 (2) (2022), 800. Published 2022 Jan 12.
[13] H. Sung, J. Ferlay, R.L. Siegel, et al., Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, CA Cancer J Clin 71 (3) (2021) 209–249.
[14] S. Camelliti, V. Le Noci, F. Bianchi, et al., Mechanisms of hyperprogressive disease after immune checkpoint inhibitor therapy: what we (don't) know, J. Exp. Clin. Cancer Res. 39 (1) (2020) 236. Published 2020 Nov 9.
[15] X.T. Fu, K. Song, J. Zhou, et al., Tumor-associated macrophages modulate resistance to oxaliplatin via inducing autophagy in hepatocellular carcinoma, Cancer Cell Int. 19 (2019) 71.
[16] J. Adamik, R. Silbermann, S. Marino, et al., XRK3F2 inhibition of p62-ZZ domain signaling rescues myeloma-induced GFI1-driven epigenetic repression of the Runx 2 gene in pre-osteoblasts to overcome differentiation suppression, Front. Endocrinol. 9 (2018) 344.
[17] Rebecca Silbermann, et al., The p62-ZZ domain inhibitor XRK3F2 alters myeloma-induced suppression of osteoblast differentiation and is highly cytotoxic to myeloma cells in combination with bortezomib, Blood 124 (2014) 2083, 2083.
[18] L. Galluzzi, E.H. Baehrecke, A. Ballabio, et al., Molecular definitions of autophagy and related processes, EMBO J. 36 (13) (2017) 1811–1836.
[19] A.V. Onorati, M. Dyczynski, R. Ojha, R.K. Amaravadi, Targeting autophagy in cancer, Cancer 124 (16) (2018) 3307–3318.
[20] A.V. Kumar, J. Mills, L.R. Lapierre, Selective autophagy receptor p62/SQSTM1, a pivotal player in stress and aging, Front. Cell Dev. Biol. 10 (2022) 793328.
[21] M. Tao, T. Liu, Q. You, Z. Jiang, p62 as a therapeutic target for tumor, Eur. J. Med. Chem. 193 (2020) 112231.
[22] M. Long, X. Li, L. Li, M. Dodson, D.D. Zhang, H. Zheng, Multifunctional p62 effects underlie diverse metabolic diseases, Trends Endocrinol. Metabol. 28 (11) (2017) 818–830.
[23] W. Yang, J. Wei, L. Lv, et al., p62 promotes malignancy of hepatocellular carcinoma by regulating the secretion of exosomes and the localization of β-catenin, Front. Biosci. 27 (3) (2022) 89.
[24] X. Chao, S. Wang, S. Fulte, et al., Hepatocytic p62 suppresses ductular reaction and tumorigenesis in mouse livers with mTORC1 activation and defective autophagy, J. Hepatol. 76 (3) (2022) 639–651.
[25] C.T. Tan, N.J.H. Soh, H.C. Chang, V.C. Yu, p62/SQSTM1 in liver diseases: the usual suspect with multifarious identities, FEBS J. 290 (4) (2023) 892–912.
[26] X. Yan, R. Tian, J. Sun, et al., Sorafenib-induced autophagy promotes glycolysis by upregulating the p62/HDAC6/HSP90 Axis in hepatocellular carcinoma cells, Front. Pharmacol. 12 (2022) 788667.
[27] A. Mohamed, A. Ayman, J. Deniece, et al., P62/Ubiquitin IHC expression correlated with clinicopathologic parameters and outcome in gastrointestinal carcinomas, Front. Oncol. 5 (2015) 70.
[28] G.O. Masuda, M. Yashiro, K. Kitayama, et al., Clinicopathological correlations of autophagy-related proteins LC3, Beclin 1 and p62 in gastric cancer, Anticancer Res. 36 (1) (2016) 129–136.
[29] J.M. Park, S. Huang, T.T. Wu, N.R. Foster, F.A. Sinicrope, Prognostic impact of Beclin 1, p62/sequestosome 1 and LC3 protein expression in colon carcinomas from patients receiving 5-fluorouracil as adjuvant chemotherapy, Cancer Biol. Ther. 14 (2) (2013) 100–107.
[30] K. Kosumi, Y. Masugi, J. Yang, et al., Tumor SQSTM1 (p62) expression and T cells in colorectal cancer, OncoImmunology 6 (3) (2017) e1284720.
[31] K.J. Schmitz, C. Ademi, S. Bertram, et al., Prognostic relevance of autophagy-related markers LC3, p62/sequestosome 1, Beclin-1 and ULK1 in colorectal cancer patients with respect to KRAS mutational status, World J. Surg. Oncol. 14 (1) (2016) 189.
[32] M. Goulielmaki, E. Koustas, E. Moysidou, et al., BRAF associated autophagy exploitation: BRAF and autophagy inhibitors synergise to efficiently overcome resistance of BRAF mutant colorectal cancer cells, Oncotarget 7 (8) (2016) 9188–9221.
[33] F. Ren, G. Shu, G. Liu, et al., Knockdown of p62/sequestosome 1 attenuates autophagy and inhibits colorectal cancer cell growth, Mol. Cell. Biochem. 385 (1–2) (2014) 95–102, https://doi.org/10.1007/s11010-013-1818-0.
[34] J.S. Kim, G.E. Bae, K.H. Kim, et al., Prognostic significance of LC3B and p62/SQSTM1 expression in gastric adenocarcinoma, Anticancer Res. 39 (12) (2019) 6711–6722.
[35] M. Komatsu, Potential role of p62 in tumor development, Autophagy 7 (2011), 1088e1090.
[36] A. Dongre, R.A. Weinberg, New insights into the mechanisms of epithelial-mesenchymal transition and implications for cancer, Nat. Rev. Mol. Cell Biol. 20 (2) (2019) 69–84.
[37] X. Jiang, Y. Huang, X. Liang, et al., Metastatic prostate cancer-associated P62 inhibits autophagy flux and promotes epithelial to mesenchymal transition by sustaining the level of HDAC6, Prostate 78 (6) (2018) 426–434.
[38] M. Bertrand, V. Petit, A. Jain, et al., SQSTM1/p62 regulates the expression of junctional proteins through epithelial-mesenchymal transition factors, Cell Cycle 14 (3) (2015) 364–374.
[39] Y. Wang, H. Xiong, D. Liu, et al., Autophagy inhibition specifically promotes epithelial-mesenchymal transition and invasion in RAS-mutated cancer cells, Autophagy 15 (5) (2019) 886–899.
[40] L. Qiang, Y.Y. He, Autophagy deficiency stabilizes TWIST1 to promote epithelial-mesenchymal transition, Autophagy 10 (10) (2014) 1864–1865.
[41] X. Liu, L. Meng, X. Li, et al., Regulation of FN1 degradation by the p62/SQSTM1-dependent autophagy-lysosome pathway in HNSCC [published correction appears in Int J Oral Sci. 2021 Nov 2;13(1):34], Int. J. Oral Sci. 12 (1) (2020) 34.
[42] X. Chen, L. Yu, J. Zhou, et al., Inhibition of p62 and/or NFE2L2 induced autophagy impaires esophageal squamous cell cancer metastasis by reversing EMT, Gene 858 (2023) 147194.
[43] J. Yao, Y. Zhang, Y. Xia, et al., PRRX1 promotes lymph node metastasis of gastric cancer by regulating epithelial-mesenchymal transition, Medicine (Baltim.) 100 (6) (2021) e24674.
[44] P. Sanchez, G. De Carcer, I.V. Sandoval, et al., Localization of atypical protein kinase C isoforms into lysosome-targeted endosomes through interaction with p62, Mol. Cell Biol. 18 (5) (1998) 3069–3080.
[45] J. Teramachi, R. Silbermann, P. Yang, et al., Blocking the ZZdomain of sequestosome 1/p62 suppresses myeloma growth and osteoclast formation in vitro and induces dramatic bone formation in myeloma-bearing bonesin vivo, Leukemia 30 (2016) 390–398.
[46] Y. Wang, H. Xiong, D. Liu, et al., Autophagy inhibition specifically promotes epithelial-mesenchymal transition and invasion in RAS-mutated cancer cells, Autophagy 15 (5) (2019) 886–899.