Phosphorylated-EGFR and MMP7 upregulation in gastric cancer: Association with metastasis and poor prognosis

BIRAN DING[1], YIQIU WAN[1], YAO WU[2], ZHAN ZHANG[3], YING MA[3], ZUO WANG[4], RUNQIU JIANG[1] and TAO LI[1]

[1]Department of Clinical Laboratory, The First Affiliated Hospital of Anhui Medical University, Hefei, Anhui 230011, P.R. China; [2]Department of Clinical Laboratory, Anhui Feixi County Traditional Chinese Medicine Hospital, Feixi, Anhui 231200, P.R. China; [3]Department of Pathology, The First Affiliated Hospital of Anhui Medical University, Hefei, Anhui 230011, P.R. China; [4]Department of Critical Care Medicine, The First Affiliated Hospital of Anhui Medical University, Hefei, Anhui 230011, P.R. China

Received July 17, 2025; Accepted December 15, 2025

DOI: 10.3892/ol.2026.15460

Abstract. Aggressive invasion and metastatic dissemination of gastric cancer (GC) are two major clinical challenges that frequently arise following standard treatments, markedly compromising patient outcomes. Elucidating the molecular drivers of GC progression would be key to developing effective therapeutic strategies. The present study employed an integrated approach combining bioinformatics analysis and immunohistochemical (IHC) validation to identify the key molecular players in GC metastasis. The Cancer Genome Atlas (TCGA) database analysis demonstrated marked upregulation of both epidermal growth factor receptor (EGFR) and matrix metalloproteinase 7 (MMP7) in gastric adenocarcinoma and elevated expression levels notably associated with poor patient prognosis. MMP7 expression exhibited a particularly robust association with metastatic progression, highlighting its potential role in facilitating tumor dissemination and experimental validation using IHC analysis of clinical specimens confirmed the coordinated involvement of both phosphorylated (p)-EGFR and MMP7 in metastatic processes. Notably, the present study identified a positive correlation between p-EGFR and MMP7 expression, suggesting a potential mechanistic interplay between these molecules in driving GC metastasis. These findings provide notable evidence that p-EGFR and MMP7 collectively contribute to GC progression and metastasis. The correlation between these markers offered novel insights into potential cooperative signaling pathways and presented a rational basis for the development of dual-targeted therapeutic approaches. The present study established a key foundation for future research aimed at disrupting the metastatic pathways in GC through targeted inhibition of p-EGFR and MMP7.

Correspondence to: Dr Runqiu Jiang or Dr Tao Li, Department of Clinical Laboratory, The First Affiliated Hospital of Anhui Medical University, 218 Jixi Road, Hefei, Anhui 230011, P.R. China
E-mail: jiangrq@nju.edu.cn
E-mail: limedical1974@126.com

Abbreviations: GC, gastric cancer; IHC, immunohistochemical; EGFR, epidermal growth factor receptor; MMPs, matrix metalloproteinases; ECM, extracellular matrix; H&E, hematoxylin-eosin

Key words: phosphorylated-EGFR, MMP7, gastric cancer, metastasis

Introduction

Gastric cancer (GC) is the fifth most common malignancy and the fourth leading cause of cancer-related mortality worldwide, accounting for ~800,000 mortalities annually (1,2). Asia has the highest global burden, with 820,000 novel cases and 576,000 mortalities reported in 2020 alone (3). Due to non-specific early symptoms (such as epigastric discomfort or dull pain, loss of appetite, early satiety, belching, acid reflux and nausea) (4,5), delayed clinical presentation and limited sensitivity of current tumor markers such as CD101 and Tim3 (6,7), the majority of patients with GC are diagnosed at advanced stages, precluding curative surgical resection. Current treatment strategies, including chemotherapy, targeted therapy and combination regimens (for example, 5-fluorouracil-based, platinum-based and newer drug combinations), have markedly improved over the past four decades. Nevertheless, persistent challenges such as tumor invasion, metastasis and recurrence often lead to treatment failure, markedly impairing patient survival and quality of life (8-10). Therefore, elucidating the molecular mechanisms underlying GC invasion and metastasis remains a key research priority to improve therapeutic outcomes in the future.

Increasing evidence indicates that dysregulated activation of key signaling pathways, including epithelial-mesenchymal transition (EMT), PI3K/AKT/mTOR, Ras/Raf/ERK, Janus kinase/STAT and epidermal growth factor receptor (EGFR)-mediated signaling, serves a key role in promoting malignant progression (11-14). The aberrantly activated pathways facilitate tumor aggressiveness by modulating downstream effector molecules. EGFR is a transmembrane tyrosine kinase receptor that is extensively expressed in mammalian epithelial cells, fibroblasts, glial cells and keratinocytes. As a key regulator of cell proliferation, survival and migration, the

EGFR pathway is implicated in the pathogenesis of multiple cancer types. EGFR upregulation has been validated as a prognostic biomarker in breast cancer progression (15). Previous studies have reported that EGFR can be used as a marker in predicting breast cancer progression and prognosis (16-18). Furthermore, EGFR has been reported to be associated with the metastasis of gallbladder, bladder, lung and colon cancer (19-22). Notably, emerging studies have associated EGFR dysregulation with the invasion and metastasis of GC, highlighting its potential as a therapeutic target (23,24).

Postoperative recurrence, invasion and metastasis are major challenges in GC management that markedly compromise patient survival and quality of life (25,26). Among the molecular mediators of these processes, matrix metalloproteinases (MMPs) serve a well-established role in facilitating GC progression (27,28). MMP7, a key member of the MMP family, exhibits unique characteristics despite its relatively simple structure. With a molecular weight of only 19 kDa upon activation, MMP7 demonstrates notably broad substrate specificity, degrading both extracellular matrix (ECM) and non-ECM components to promote tumor invasion and metastasis. Emerging evidence indicates that MMP7 is upregulated in multiple malignancies, including GC, hepatocellular carcinoma and colorectal cancer (29-31). Notably, MMP7 differs from other MMP family members in its tumor cell-specific secretion pattern (vs. stromal cell-derived production), making it a potential diagnostic biomarker as well as a promising therapeutic target (32). The aforementioned findings underscore the key involvement of MMP7 in GC recurrence, invasion and metastasis.

Although the individual roles of EGFR and MMP7 in cancer are well-recognized, their direct association and functional interdependence in GC remain insufficiently explored. Phosphorylation of EGFR at tyrosine 1068 (p-EGFR) is a well-established indicator of its activation and downstream signaling, with significant implications in tumor progression (33-35). To clarify the clinical relevance of this modification in the context of MMP7 co-expression, p-EGFR was specifically assessed in the present study. The present study integrated The Cancer Genome Atlas (TCGA) data with clinical immunohistochemical (IHC) validation to assess their co-expression as a hallmark of metastatic GC. Through comprehensive analysis of the TCGA database, the expression profiles of EGFR and MMP7 and their association with clinical outcomes in patients with GC were examined. Furthermore, IHC analysis of clinical GC specimens was conducted to explore the correlation between p-EGFR and MMP7 expression. Collectively, the present study aimed to establish whether p-EGFR and MMP7 function as key mediators in GC metastasis, thereby providing insights that could inform future therapeutic strategies targeting GC invasion and metastasis.

Materials and methods

Tissue samples. This was a retrospective cohort study. GC tissue samples (n=32) and their corresponding normal tissue samples (samples taken at a distance of ≥2.5 cm from the cancer tissue) were obtained from patients with GC who underwent surgery at The First Affiliated Hospital of Anhui Medical University (Hefei, China) from November 2022 to December 2023. Tumor classification was based on the World Health Organization (WHO) Classification of Tumors, 5th edition (36). The inclusion criteria were as follows: i) Underwent a definitive surgical resection for primary GC between November 2022 to December 2023; ii) had a confirmed histopathological diagnosis of GC according to the WHO Classification of Tumors, 5th edition; iii) formalin-fixed, paraffin-embedded tumor tissue blocks with sufficient quality and quantity were available for subsequent molecular and IHC analyses; and iv) had complete clinicopathological data and follow-up records that were accessible from the institutional database. The exclusion criteria were as follows: i) Received any form of neoadjuvant chemotherapy or radiotherapy prior to surgical resection; ii) had a history of other synchronous or metachronous active malignancies within 5 years prior to the diagnosis of the index tumor; iii) presented with distant metastasis (Stage 4 disease) at the initial diagnosis; iv) had insufficient clinical follow-up data (defined as <12 months post-surgery for surviving patients); and v) the available tumor specimen was deemed inadequate for analysis due to extensive necrosis or poor preservation upon central pathological review. The patients had not received chemotherapy or radiotherapy previously. Metastasis was defined by the histological confirmation of tumors in the regional lymph nodes or distant organs at surgery. The tumor samples included 17 metastatic and 15 non-metastatic samples. The present study was approved by the Ethics Committee of the First Affiliated Hospital of Anhui Medical University (approval no. 20231337; Hefei, China) and was conducted according to the principles of the Declaration of Helsinki. Written informed consent was obtained from all patients.

Reagents. p-EGFR (phospho-Y1068; cat. no. ab40815; 1:500) and MMP7 (cat. no. ab207299; 1:1,000) were purchased from Abcam. PV-9000 histochemical reagent kit (cat. no. PV-9000) was obtained from Beijing Zhongshan Jinqiao Biotechnology Co., Ltd. and DAB (cat. no. P0201S) staining solution was obtained from Beyotime Biotechnology.

Bioinformatics analysis. Publicly available databases and analytical tools were employed to investigate gene expression correlations and association with survival. The profile of gene upregulation in GC was obtained from the University of Alabama at Birmingham Cancer database (UALCAN; https://ualcan.path.uab.edu/cgi-bin/TCGAExHeatMap2.pl?size=25&cancer=STAD). The transcript levels of MMP7 and EGFR were compared between tumor and adjacent normal tissues using TCGA-stomach adenocarcinoma (STAD) dataset within the UALCAN platform. The specific analysis can be replicated using the following direct links: MMP7 (https://ualcan.path.uab.edu/cgi-bin/TCGAExResultNew2.pl?genenam=MMP7&ctype=STAD) and EGFR (https://ualcan.path.uab.edu/cgi-bin/TCGAExResultNew2.pl?genenam=EGFR&ctype=STAD). To ensure full reproducibility of the present analyses, a comprehensive step-by-step protocol is provided in Data S1. Kaplan-Meier plotter (https://kmplot.com/analysis/) was used to examine the associations between MMP7 or EGFR expression and overall survival. All available datasets in the platform were included without any

dataset-specific restrictions. Cut-off values for gene expression were determined automatically based on percentiles. Specifically, the threshold corresponding to the 70th percentile was used for MMP7 (absolute expression value, 1,486; range, 5-37,183), and the threshold corresponding to the 75th percentile was used for EGFR (absolute expression value, 53; range, 1-880). These thresholds were applied to stratify patients into high and low expression groups for subsequent survival analysis.

IHC analysis. Paraffin-embedded tissue sections were used for immunohistochemistry. The source tissues had been previously fixed in 10% neutral buffered formalin at room temperature for 24-48 h during routine pathological processing. For IHC, 5 μm sections were mounted on positively charged slides and dried in an oven at 60˚C for 1 h prior to staining. Tissue slides were deparaffinized using xylene (100%, 15 min, 25˚C, twice) and rehydrated through a graded ethanol series (100% ethanol, 3 min, 25˚C, twice; 95% ethanol, 3 min, 25˚C; 90, 80 and 70% ethanol, 1 min each, 25˚C). The tissue slices were soaked in citrate buffer (0.01 M, 100˚C) for antigen retrieval. After boiling for 15 min, the samples were allowed to cool. The effects of endogenous enzymes were eliminated after 20 min of treatment with H_2O_2 (reagent 1, 25˚C). Serum (10%) blocking was performed at 25˚C for 20 min, followed by incubation with p-EGFR or MMP7 primary antibodies at 4˚C for 16 h. Reaction-boosting solution (reagent 2) and secondary antibody (reagent 3) were added sequentially and incubated for 20 min at 25˚C. Reagents 1, 2 and 3 were included in the PV-9000 histochemical kit. DAB was used for 5 min for color development. Nuclei were stained with hematoxylin (Beyotime Biotechnology) for 3 min at 25˚C and sealed with neutral glue. Staining was independently assessed by two experienced pathologists who were blinded to the clinical data. Inter-observer variability was quantitatively evaluated using Cohen's κ coefficient. A light Panoramic MIDI scanner with Jetta JD801 (Jiangsu Jetta Technology Development Co., Ltd.) was used to capture the images. A combined score of staining intensity and distribution was used to semi-quantitatively evaluate p-EGFR and MMP7 expression (37). For comparative analysis, patients were stratified into low-(lowest 30%), moderate- and high-(highest 30%) expression cohorts. The average optical density was analyzed using ImageJ software (version 1.44p; National Institutes of Health).

Hematoxylin-eosin (H&E) staining. Paraffin-embedded tissues were cut into 5 μm sections and mounted on positively charged slides and dried in an oven at 60˚C for 1 h prior to staining. Tissue slides were deparaffinized using xylene (100%, 15 min, 25˚C, twice) and rehydrated through a graded ethanol series (100% ethanol, 3 min, 25˚C, twice; 95% ethanol, 3 min, 25˚C; 90, 80 and 70% ethanol, 1 min each, 25˚C). The nuclei were stained with hematoxylin for 3 min at 25˚C and the cytoplasm was stained with eosin for 1 min at 25˚C (Beyotime Biotechnology). Images were captured using a panoramic scanner (Panoramic MIDI).

Statistical analysis. IHC staining for MMP7 or EGFR was performed on sequential sections from 32 tissue samples, with each staining procedure repeated in triplicate to ensure reproducibility. The average optical density values were quantified using ImageJ software. All data are presented as the mean ± SD. The primary statistical analyses, including between two groups comparisons using paired or unpaired two-tailed Student's t-tests and correlation analysis using Pearson's correlation coefficient, were performed using GraphPad Prism (version 8; Dotmatics), whereas the results for Figs. 1-3 were obtained directly from TCGA analysis portal of the UALCAN database without further modification by the authors. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Bioinformatics analysis of MMP7 expression in GC. Gastric adenocarcinoma represents the primary form of GC, with metastasis in patients with gastric adenocarcinoma being a key determinant of their quality of life and survival. The present study analyzed the top 25 upregulated genes in gastric adenocarcinoma (Normal, n=34; Tumor, n=415) using data from TCGA database and identified the expression levels of MMP11 and MMP7 elevated (both MMP7 and MMP11 were members of the MMPs family; Fig. 1A). MMP7, specifically, is secreted by cancer cells and possesses the ability to degrade the ECM, facilitating the breach of the initial defensive barrier during cancer cell metastasis.

The transcript levels of MMP7 in gastric adenocarcinoma tissue samples (n=415) were significantly higher compared with those in normal tissue samples (n=34; $P=1.620x10^{-12}$; Fig. 1B). Furthermore, the survival prognosis curve revealed that increased MMP7 expression was significantly associated with unfavorable survival outcomes in patients (Fig. 1C; $P=2.000x10^{-2}$).

Next, the stage-specific analysis of MMP7 expression in gastric adenocarcinoma revealed significantly elevated levels across all tumor grades compared with that in normal tissues (Grade 1, $P=3.780x10^{-2}$, n=12; Grade 2, $P=5.006x10^{-8}$, n=148; Grade 3, $P=1.209x10^{-10}$, n=246), demonstrating progressively increasing expression from well-differentiated to poorly-differentiated tumors, although inter-grade differences did not reach statistical significance (Fig. 2A). The present study findings revealed that MMP7 is consistently upregulated in gastric adenocarcinoma regardless of tumor grade, with a non-significant increase accompanying loss of differentiation. The pattern supports its involvement in both tumor initiation and progression. The findings suggest that MMP7 upregulation occurs early in gastric adenocarcinoma development and persists throughout tumor progression, supporting its potential role as a consistent molecular marker across different disease stages.

Furthermore, the present comprehensive analysis revealed significant MMP7 upregulation across all clinical stages of gastric adenocarcinoma compared with that in normal tissues (n=34), with stage-specific elevations observed in Stage 1 (n=18; $P=1.709x10^{-2}$), Stage 2 (n=123; $P=7.797x10^{-7}$), Stage 3 (n=169; $P=1.297x10^{-7}$) and Stage 4 (n=41; $P=8.788x10^{-4}$) tumors. Notably, while MMP7 expression progressively increased from the early to advanced stages, the inter-stage comparisons did not reach statistical significance (Fig. 2B). The significant upregulation of MMP7 across all clinical stages, compared

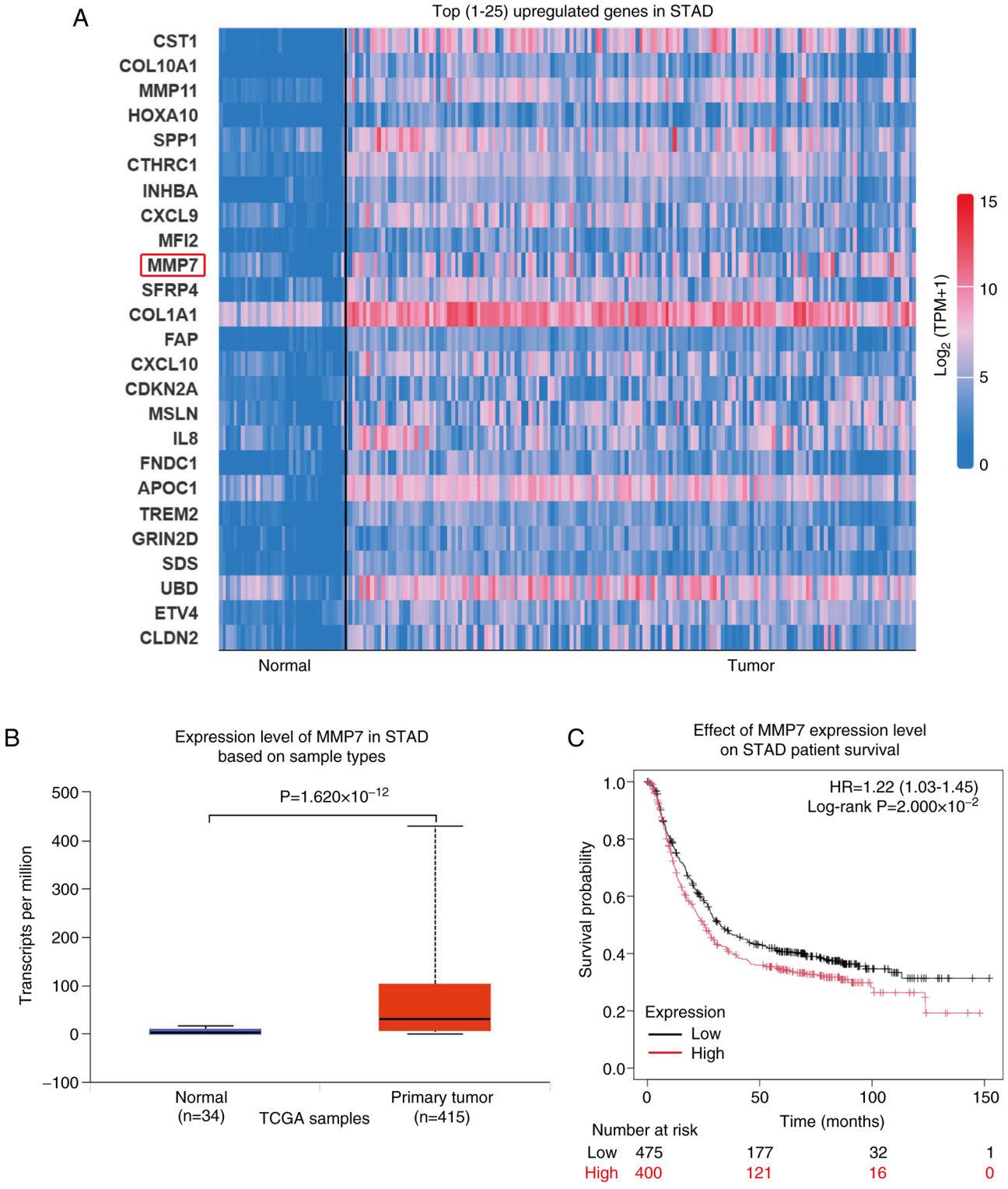

Figure 1. Bioinformatics analysis of MMP7 expression in gastric cancer. (A) Gene profile of upregulation in GC, showing the top 25 upregulated genes. (B) TCGA analysis: MMP7 expression in GC and normal tissue. The error bars indicate the non-outlier data range, the box shows the interquartile range and the internal line marks the median. Individual dots represent outliers. (C) Survival prognosis of patients with GC based on MMP7 expression: An analysis of TCGA data. MMP7, matrix metalloproteinase 7; GC, gastric cancer; TCGA, The Cancer Genome Atlas; STAD, stomach adenocarcinoma; HR, hazard ratio; TPM, transcripts per million.

with that in normal tissue, establishes its broad association with gastric tumorigenesis. The non-significant increasing trend with disease progression possibly reflects the function of MMP7 as a sustained driver of tumor aggressiveness. The consistent increase across all stages underscores the potential utility of MMP7 as a reliable biomarker and therapeutic target throughout the disease continuum.

After these analyses, the present study examined the disparities in MMP7 levels in the gastric adenocarcinoma tissues of male and female patients. The results indicated

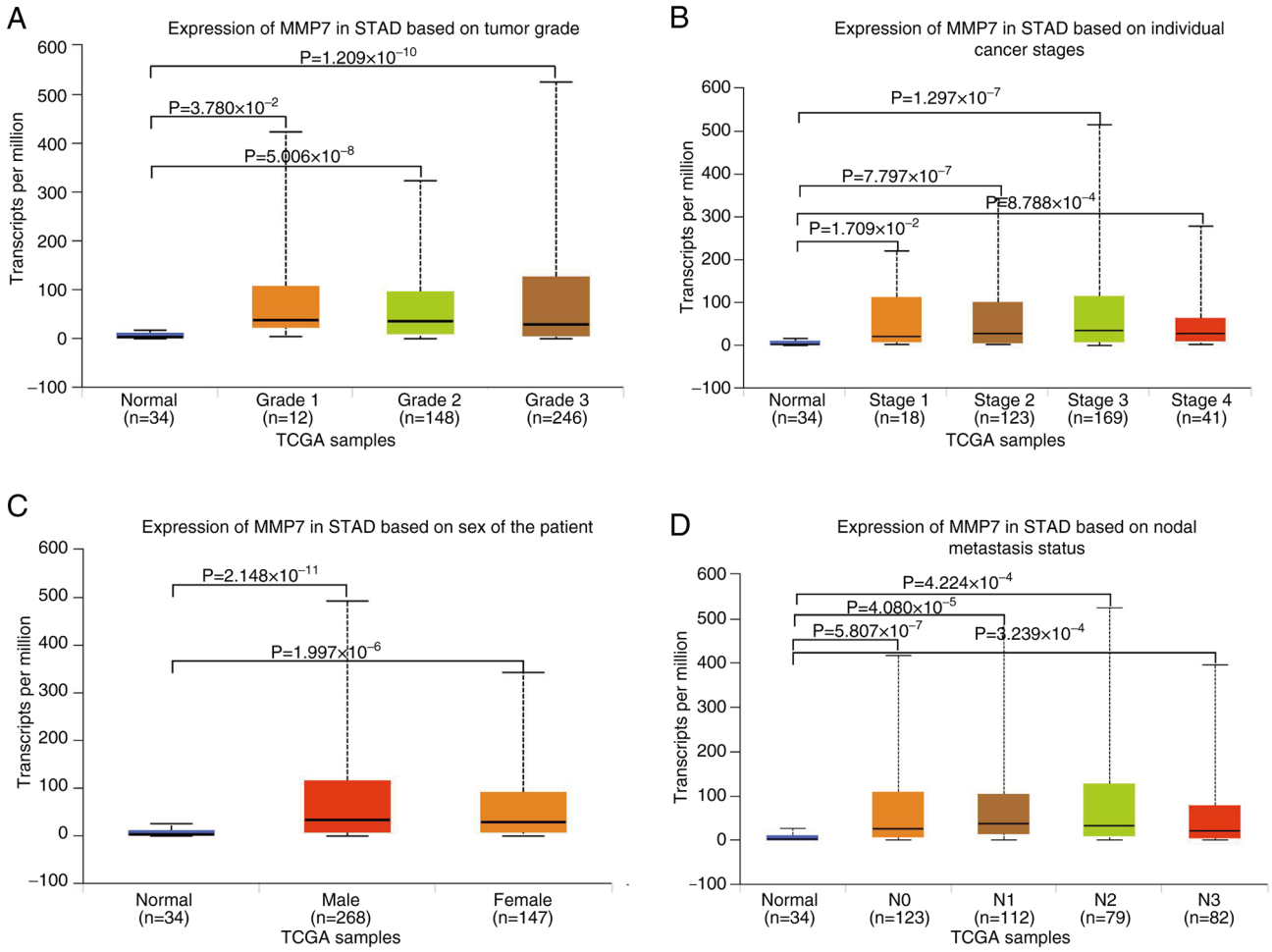

Figure 2. Bioinformatics analysis of MMP7 expression in gastric cancer. (A) Expression level of MMP7 in different stages of GC. (B) Expression level of MMP7 in different grades of GC and normal tissues. (C) Expression level of MMP7 in male and female patients with GC. (D) Expression level of MMP7 in different lymph node states of GC. For all box plots, error bars depict the variability of the data outside the interquartile range, extending to the farthest non-outlier data points. The box shows the interquartile range and the internal line marks the median. MMP7, matrix metalloproteinase 7; GC, gastric cancer; STAD, stomach adenocarcinoma.

that compared with normal tissues (n=34), MMP7 expression was elevated in both male (n=268, P=2.148x10^{-11}) and female (n=147, P=1.996x10^{-6}) patients, although no notable differences were observed in the expression levels between male and female patients, as shown in Fig. 2C.

The present study compared the MMP7 expression differences in patients with gastric adenocarcinoma and lymph node metastasis. The findings highlighted that compared with normal tissues (n=34), MMP7 expression increased significantly in N0 (n=123; P=5.807x10^{-7}), N1 (n=112; P=4.080x10^{-5}), N2 (n=79; P=4.224x10^{-4}) and N3 (n=82; P=3.239x10^{-4}), with no statistically significant differences in the MMP7 expression levels between lymph node metastasis grades (N0-N3), as depicted in Fig. 2D. The observations underscored the key role of MMP7 in the metastasis of gastric adenocarcinoma and emphasized the clinical significance of selecting MMP7 as a target for further exploration and potential intervention.

Bioinformatics analysis of EGFR expression in GC. Emerging evidence indicates that MMP7 expression is modulated by upstream regulatory pathways (38,39). Notably, EGFR-mediated regulation of MMP7 has been reported not only in diabetic kidney disease (40), but also in the context of GC metastasis (41), suggesting a potentially conserved mechanism across inflammatory and malignant conditions. The present bioinformatic analysis of TCGA dataset revealed significant differential expression level of EGFR between normal gastric tissue and gastric adenocarcinoma, with notable upregulation observed in malignant tissues (P=1.608x10^{-2}; Fig. 3A). Survival analysis demonstrated that elevated EGFR expression is significantly associated with worse clinical outcomes in patients with gastric adenocarcinoma (P=2.300x10^{-3}; Fig. 3B), highlighting its prognostic relevance in GC progression.

Expression levels of MMP7 and p-EGFR are higher in metastatic gastric adenocarcinoma tissues. The present study utilized 32 matched pairs of gastric adenocarcinoma and adjacent normal tissue samples, including 17 metastatic and 15 non-metastatic cases. All specimens underwent comprehensive histological evaluation using H&E staining, coupled with IHC analysis of p-EGFR and MMP7 expression. Fig. 4A displays representative IHC results from 2 non-metastatic (patients 1 and 2) and 2 metastatic (patients 3 and 4) cases.

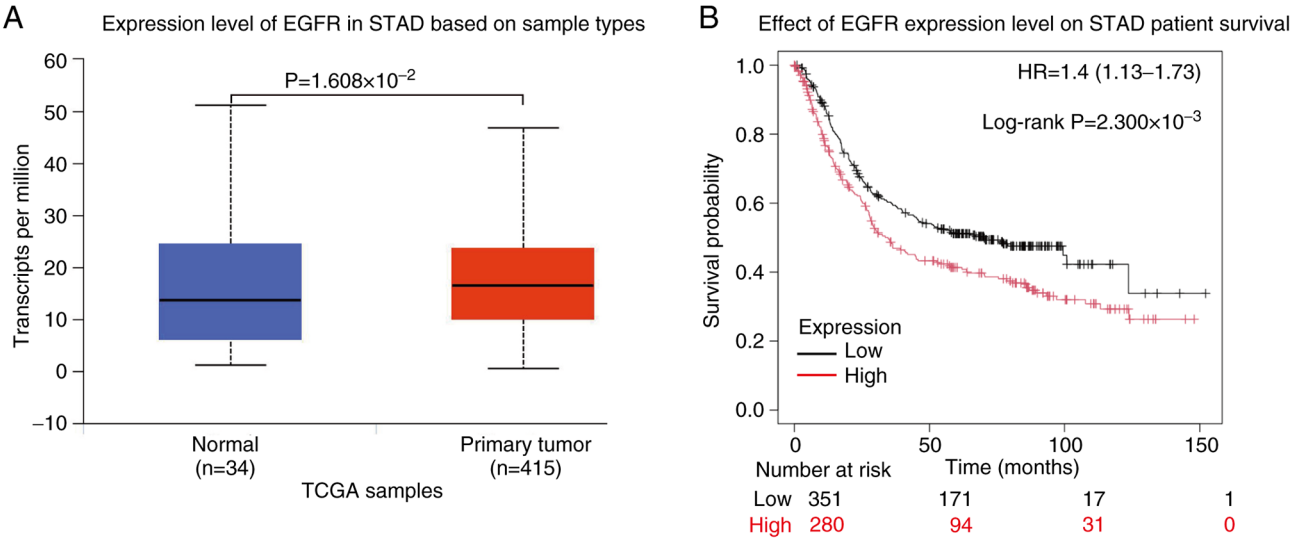

Figure 3. Bioinformatics analysis of EGFR expression in gastric cancer. Upregulation of EGFR in gastric adenocarcinoma is associated with worse patient prognosis. (A) Expression level of EGFR between normal and GC samples. The box plot shows the median, interquartile range and full data range (error bars). Individual data points are overlaid. (B) Analysis of survival prognosis of EGFR in GC. EGFR, epidermal growth factor receptor; TCGA, The Cancer Genome Atlas; STAD, stomach adenocarcinoma; HR, hazard ratio.

Using GraphPad Prism, the present study performed a comparative analysis of the protein expression patterns between the metastatic and non-metastatic groups. The results demonstrated a significantly elevated expression levels of both p-EGFR (P<0.0001) and MMP7 (P<0.0001) in metastatic tissues compared with that in the non-metastatic counterparts (Fig. 4B and C). The experimental findings provided notable evidence for the involvement of p-EGFR and MMP7 in gastric adenocarcinoma metastasis.

p-EGFR and MMP7 are positively correlated in gastric adenocarcinoma tissue. IHC analysis of 17 metastatic GC cases revealed differential MMP7 expression levels, ranging from low (patient 5), moderate (patient 6) to high (patient 7) staining intensity (Fig. 5A). Comparative evaluation of all 32 matched tumor-normal tissue pairs demonstrated significantly elevated expression levels of both p-EGFR (P<0.0001) and MMP7 (P<0.0001) in gastric adenocarcinoma tissues compared with that in their normal counterparts (Fig. 5B and C).

Notably, as shown in Fig. 5A, as the MMP7 expression level decreased (as seen in patient 5), p-EGFR expression decreased. By contrast, when MMP7 expression was high (as observed in patient 7), p-EGFR expression levels increased. Therefore, the present study performed a correlation analysis between p-EGFR and MMP7 expression in 32 gastric adenocarcinoma tissue samples and revealed a positive correlation between p-EGFR and MMP7 expression (r^2=0.6219; P<0.0001), as shown in Fig. 5D. Furthermore, a correlational analysis of EGFR and MMP7 expression, conducted via the Gene Expression Profiling Interaction Analysis platform (https://gepia3.bioinfoliu.com/), identified a statistically significant yet weak positive relationship (r=0.27; P=4.4x10^{-12}; Fig. S1). The correlation underscores the association between p-EGFR and MMP7 in gastric adenocarcinoma, reinforcing their potential relevance in disease progression.

Discussion

GC is one of the most lethal malignancies worldwide and ranks among the leading causes of cancer-related mortality (42). Its aggressive nature, characterized by its pronounced invasive and metastatic potential, poses notable clinical challenges. Although therapeutic advances over the past decade have improved patient outcomes, persistent issues of post-treatment recurrence and metastasis continue to compromise patient prognosis. The unresolved clinical challenges underscore the key need to identify the molecular targets involved in GC metastasis, a key research priority that could markedly enhance postoperative survival rates and quality of life in affected individuals.

MMPs are key mediators of tumor metastasis, drawing notable research interest due to their multifaceted roles in both physiological and pathological processes. They were initially characterized for their functions in embryonic development and tissue remodeling (43,44). However, they are now recognized as key contributors to cancer pathogenesis due to their ability to degrade basement membrane components and ECM proteins (45). This proteolytic activity facilitates key oncogenic processes, including tumor invasion, angiogenesis and metastatic dissemination. Previous studies have reported that the activity and expression levels of MMPs, such as MMP2, MMP3, MMP7 and MMP9, are increased in patients with GC and can reduce their survival period, promote the metastasis and recurrence of cancer and render a poor prognosis (46-48).

MMP7 exhibits unique clinical value as a tumor-derived protease, thereby being distinct from other MMP family members that are primarily secreted by stromal cells. This tumor cell-specific expression pattern makes MMP7 an ideal biomarker in monitoring cancer progression. Increasing evidence demonstrates MMP7 upregulation in multiple malignancies, including prostate and breast cancer, and its

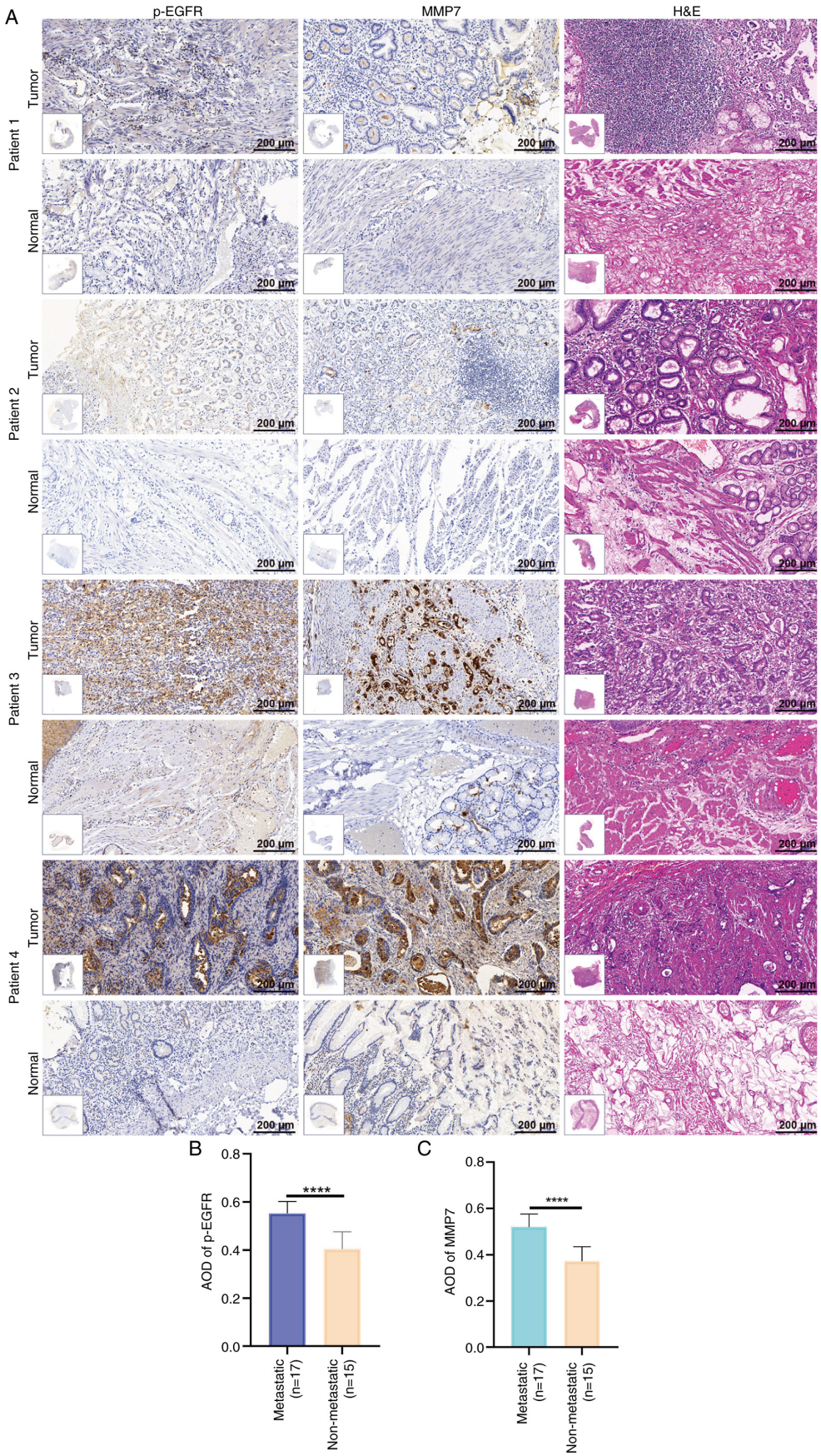

Figure 4. Expression levels of MMP7 and p-EGFR are higher in metastatic gastric adenocarcinoma tissues. (A) IHC and H&E analysis for the differential expression levels of p-EGFR and MMP7 in metastatic and non-metastatic GC and normal tissues. Patients 1-2 were classified as non-metastatic, while patients 3-4 were categorized as metastatic (scale bar, 200 μm). (B) Quantitative analysis of p-EGFR expression (AOD) using IHC staining between metastatic (n=17) and non-metastatic (n=15). ****P<0.0001. (C) Quantitative analysis of MMP7 expression (AOD) using IHC staining between metastatic (n=17) and non-metastatic (n=15). ****P<0.0001. Error bars represent the SD from the mean of three independent experiments. MMP7, matrix metalloproteinase 7; p-EGFR, phosphorylated-epidermal growth factor receptor; GC, gastric cancer; AOD, average optical density; IHC, immunohistochemical; H&E, hematoxylin-eosin.

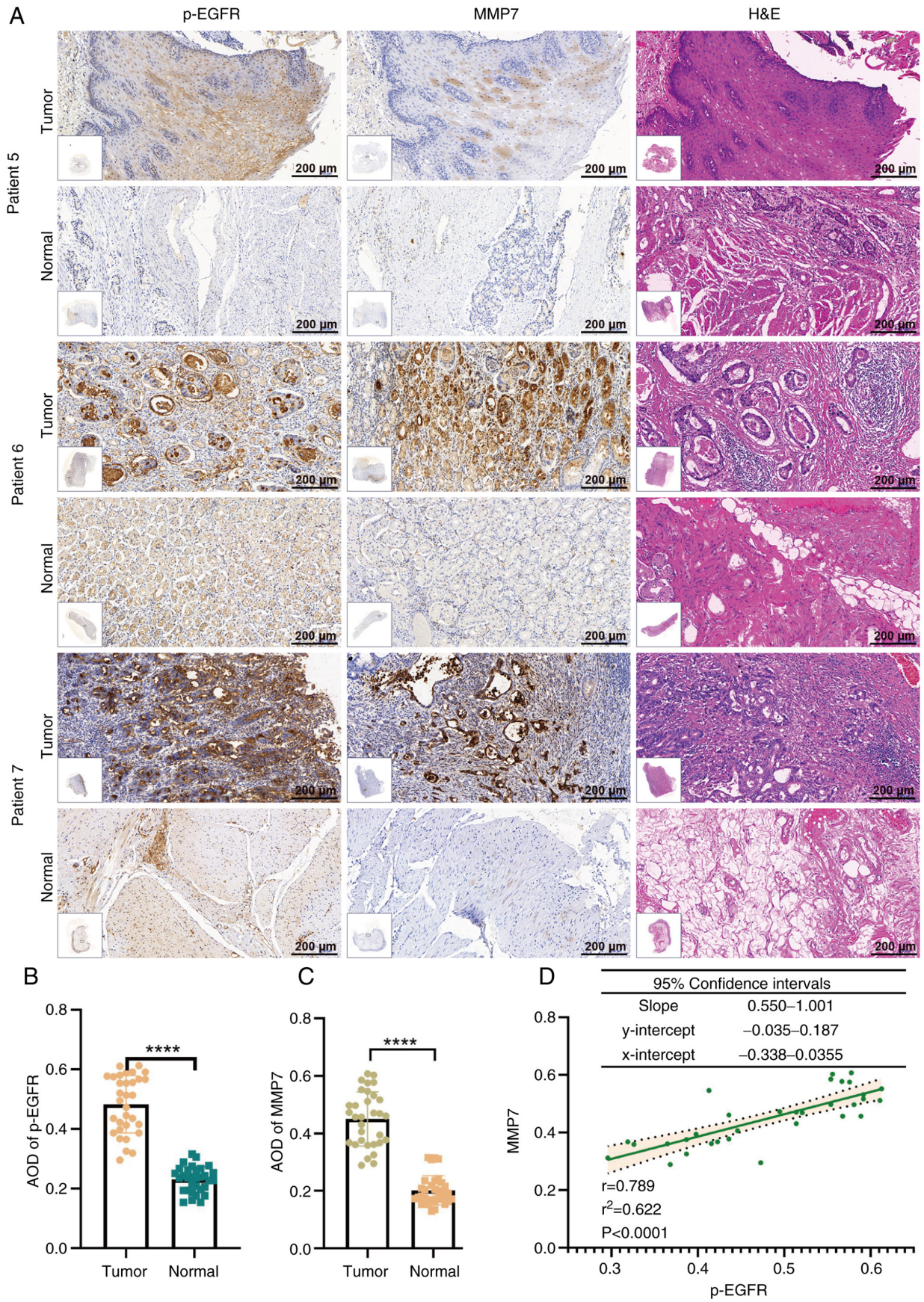

Figure 5. p-EGFR and MMP7 are positively correlated in gastric adenocarcinoma tissue. (A) IHC analysis for p-EGFR and MMP7 expression and H&E staining in GC tissues and normal tissues (scale bar, 200 μm). (B) Quantitative analysis of p-EGFR expression (AOD) using IHC staining between GC and corresponding normal tissues. (C) Quantitative analysis of MMP7 expression (AOD) using IHC staining between GC and corresponding normal tissues. (D) Correlation between p-EGFR and MMP7 expression in GC tissues, with the orange part representing a 95% confidence interval. ****P<0.0001. Error bars represent the SD from the mean of three independent experiments. MMP7, matrix metalloproteinase 7; p-EGFR, phosphorylated-epidermal growth factor receptor; GC, gastric cancer; AOD, average optical density; IHC, immunohistochemical; HE, hematoxylin-eosin.

association with metastasis (49,50). MMP7 can be used as a marker to measure the prognosis of colon and esophageal cancer (51). Using bioinformatics analysis, the present study demonstrated that MMP7 is upregulated in gastric adenocarcinoma and is associated with a worse patient prognosis. Notably, the IHC results of the present study revealed that MMP7 was highly expressed in gastric adenocarcinoma tissues with lymph node metastasis, which demonstrated that MMP7 is associated with cancer progression and is a tumor marker for the invasion, metastasis and poor prognosis of GC. Therefore, it could be a potential therapeutic target for the treatment of GC.

EGFR, a key member of the human EGFR family, initiates downstream signaling cascades (for example, the PI3K/AKT, STAT and MAPK pathways) upon ligand binding and phosphorylation. These pathways notably regulate tumor cell survival, apoptosis, invasion and metastasis (52-55). Aberrant EGFR activation in GC is strongly implicated in the promotion of metastatic progression (24,56). Emerging evidence indicates that EGFR can directly modulate MMP7 transcription, as in lung cancer progression (57,58). Using TCGA database analysis, the present study identified that the upregulation of EGFR in GC is associated with a worse prognosis of patients, consistent with a previous report. In addition, IHC results suggested that p-EGFR and MMP7 were both expressed in clinical GC samples. Notably, the present study identified that p-EGFR and MMP7 are positively correlated in GC. To definitively establish causality and elucidate the mechanism associating p-EGFR with MMP7, a rigorous experimental follow-up would be required. This would include genetic manipulation (small interfering RNA/CRISPR) to confirm the functional necessity and sufficiency, pharmacological inhibition to delineate the key downstream signaling cascades (for example, MEK/ERK and PI3K/AKT) and chromatin immunoprecipitation assays to identify the transcription factors (for example, activator protien-1 and E26 transformation-specific) that directly bind to the MMP7 promoter. The current clinical evidence in the present study serves as a key foundation for such targeted mechanistic studies.

Although the present study established an association between p-EGFR and MMP7 in GC, the precise regulatory mechanisms remain to be elucidated due to platform constraints. Further investigation is warranted to elucidate a few points. First, the association between p-EGFR and MMP7, although significant in the present study, would require mechanistic validation; whether p-EGFR directly regulates MMP7 transcription and which specific signaling intermediates are involved remain to be elucidated. Second, the prognostic and therapeutic potential of this axis has not yet been fully defined. Future efforts should focus on prospectively validating the combined p-EGFR/MMP7 profile as a clinical biomarker for patient stratification and evaluation of the efficacy of targeting this pathway. Lastly, the relatively short follow-up period of the present study cohort limited a robust overall survival analysis. Therefore, expanding the present study to a larger, independent validation cohort with longer follow-up period is a priority. Furthermore, investigating the potential interplay among MMP7, p-EGFR and other upregulated proteins (for example, collagen type I α 1 chain, ubiquitin D and cystatin 1) would be a compelling avenue for future studies.

In summary, the present study systematically elucidated the synergistic mechanism of p-EGFR and MMP7 in GC using an integrated analysis of TCGA database and IHC detection of clinical samples. The key findings were as follows: i) Significant upregulation of both p-EGFR and MMP7 in GC tissues, which were significantly associated with poor patient prognosis and metastatic progression; and ii) to the best of our knowledge, IHC results demonstrated for the first time a significant positive correlation between p-EGFR and MMP7 expression in GC tissues. The findings not only provide novel experimental evidence in understanding the molecular mechanisms of GC metastasis, but, more notably, establish the p-EGFR/MMP7 signaling axis as a potential dual target for metastatic GC treatment, laying a key theoretical foundation for the development of novel targeted therapies in the future.

Acknowledgements

Not applicable.

Funding

The present study was supported by The Hefei Medical Research Project (grant no. Hwk2023zc015) and The National Natural Science Foundation of China (grant no. 81972266).

Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

BD and YWa conceived the present study, participated in data analysis and drafted and wrote the manuscript. YWu, ZZ and YM collected clinical tissue samples and conducted immunohistochemical experiments. ZW, RJ and TL conceived the study, led its design and contributed to the revision of the manuscript. All authors read and approved the final version of the manuscript. BD, YWa, YWu, ZZ, YM, ZW, RJ and TL confirm the authenticity of all the raw data.

Ethics approval and consent to participate

The present study was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board of First Affiliated Hospital of Anhui Medical University (Hefei, China; approval no. 20231337). Written informed consent was obtained from all participants involved in the study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A and Bray F: Global Cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 71: 209-249, 2021.
2. Smyth EC, Nilsson M, Grabsch HI, van Grieken NC and Lordick F: Gastric cancer. Lancet 396: 635-648, 2020.
3. Ferlay J, Colombet M, Soerjomataram I, Parkin DM, Pineros M, Znaor A and Bray F: Cancer statistics for the year 2020: An overview. Int J Cancer: Apr 5, 2021 doi: 10.1002/ijc.33588.
4. Shi AN, Zhou YB and Wang GH: Immunotherapy: Progress and challenges of a revolutionary treatment for gastric cancer. Zhonghua Wai Ke Za Zhi 63: 563-567, 2025 (In Chinese).
5. Chen J, Ji Y, Liu Y, Cen Z, Chen Y, Zhang Y, Li X and Li X: Exhaled volatolomics profiling facilitates personalized screening for gastric cancer. Cancer Lett 590: 216881, 2024.
6. Cheng Z, Lu J, Chen Y, Cao W and Shao Q: The role of CD101 and Tim3 in the immune microenvironment of gastric cancer and their potential as prognostic biomarkers. Int Immunopharmacol 146: 113835, 2025.
7. Komekbay Z, Shirazi R, Yessultanova G, Garifollin A, Tulyayeva A, Kereyeva N, Akhmetova S and Kaliev A: Bibliometric analysis of tumor marker application in gastric cancer diagnosis from 2019 to 2024. Front Med (Lausanne) 12: 1547850, 2025.
8. Lordick F, Carneiro F, Cascinu S, Fleitas T, Haustermans K, Piessen G, Vogel A and Smyth EC; ESMO Guidelines Committee. Electronic address: clinicalguidelines@esmo.org: Gastric cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up. Ann Oncol 33: 1005-1120, 2022.
9. Ajani JA, D'Amico TA, Bentrem DJ, Chao J, Cooke D, Corvera C, Das P, Enzinger PC, Enzler T, Fanta P, *et al*: Gastric cancer, version 2.2022, NCCN clinical practice guidelines in oncology. J Natl Compr Canc Netw 20: 167-192, 2022.
10. Bae SH, Kim DW, Kim MS, Shin MH, Park HC and Lim DH: Radiotherapy for gastric mucosa-associated lymphoid tissue lymphoma: Dosimetric comparison and risk assessment of solid secondary cancer. Radiat Oncol J 35: 78-89, 2017.
11. Yang T, Jia L, Bian S, Chang X, Zhang Q, Tang Q, Zhu J, Yang Z and Feng Z: TROP2 Down-regulated DSG2 to promote gastric cancer cell invasion and migration by EGFR/AKT and DSG2/PG/β-catenin pathways. Curr Cancer Drug Targets 22: 691-702, 2022.
12. Rong L, Li Z, Leng X, Li H, Ma Y, Chen Y and Song F: Salidroside induces apoptosis and protective autophagy in human gastric cancer AGS cells through the PI3K/Akt/mTOR pathway. Biomed Pharmacother 122: 109726, 2020.
13. Kang X, Xu E, Wang X, Qian L, Yang Z, Yu H, Wang C, Ren C, Wang Y, Lu X, *et al*: Tenascin-c knockdown suppresses vasculogenic mimicry of gastric cancer by inhibiting ERK-triggered EMT. Cell Death Dis 12: 890, 2021.
14. Yang YL, Liu P, Li D, Yang Q, Li B and Jiang XJ: Stat-3 signaling promotes cell proliferation and metastasis of gastric cancer through PDCD4 downregulation. Kaohsiung J Med Sci 36: 244-249, 2020.
15. Schlessinger J: Receptor tyrosine kinases: Legacy of the first two decades. Cold Spring Harb Perspect Biol 6: a008912, 2014.
16. Masuda H, Zhang D, Bartholomeusz C, Doihara H, Hortobagyi GN and Ueno NT: Role of epidermal growth factor receptor in breast cancer. Breast Cancer Res Treat 136: 331-345, 2012.
17. Luo D, Liu Y, Lu Z and Huang L: Targeted therapy and immunotherapy for gastric cancer: Rational strategies, novel advancements, challenges, and future perspectives. Mol Med 31: 52, 2025.
18. Li S, Sun M, Cui Y, Guo D, Yang F, Sun Q, Ding Y, Li M, Liu Y, Ou G, *et al*: Ephrin A1 functions as a ligand of EGFR to promote EMT and metastasis in gastric cancer. EMBO J 44: 1464-1487, 2025.
19. Ametller E, Garcia-Recio S, Pastor-Arroyo EM, Callejo G, Carbo N, Gascon P and Almendro V: Differential regulation of MMP7 in colon cancer cells resistant and sensitive to oxaliplatin-induced cell death. Cancer Biol Ther 11: 4-13, 2011.
20. Shen H, He M, Lin R, Zhan M, Xu S, Huang X, Xu C, Chen W, Yao Y, Mohan M and Wang J: PLEK2 promotes gallbladder cancer invasion and metastasis through EGFR/CCL2 pathway. J Exp Clin Cancer Res 38: 247, 2019.
21. Zhou Z, Zhang Z, Chen H, Bao W, Kuang X, Zhou P, Gao Z, Li D, Xie X, Yang C, *et al*: SBSN drives bladder cancer metastasis via EGFR/SRC/STAT3 signalling. Br J Cancer 127: 211-222, 2022.
22. Mittal S, Kamath A, Joseph AM and Rajala MS: PLCgamma1-dependent invasion and migration of cells expressing NSCLC-associated EGFR mutants. Int J Oncol 57: 989-1000, 2020.
23. Lei ZN, Teng QX, Tian Q, Chen W, Xie Y, Wu K, Zeng Q, Zeng L, Pan Y, Chen ZS and He Y: Signaling pathways and therapeutic interventions in gastric cancer. Signal Transduct Target Ther 7: 358, 2022.
24. Cao T, Lu Y, Wang Q, Qin H, Li H, Guo H, Ge M, Glass SE, Singh B, Zhang W, *et al*: A CGA/EGFR/GATA2 positive feedback circuit confers chemoresistance in gastric cancer. J Clin Invest 132: e154074, 2022.
25. Li Y, Huang L, Li L, Chen L, Chen P and Chen X: The evaluation of gastric cancer lymphovascular invasion using CT volume perfusion. Discov Med 36: 2037-2045, 2024.
26. Hu C, Xu J, Zhang Y, Zhang R, Pan S, Chen J, Wang Y, Zhao Q, Wang Y, Zhu W, *et al*: Inhibition of glutathione peroxidase 4 suppresses gastric cancer peritoneal metastasis via regulation of RCC2 homeostasis. Redox Biol 80: 103519, 2025.
27. Baghbanzadeh A, Rahmani S, Eslami S, Ahmadpour Youshanlui M, Shafiee N, Shafiee A, Khalaji A and Baradaran B: miR-146a-5p suppresses migration and downregulates vimentin and MMP-9 expression in gastric cancer cells. Discov Oncol 16: 1075, 2025.
28. El-Sayed SF, Mahmoud SM, Samy W, Wahid RM, Talaat A and Seada SG: Vitamin D3 mitigates aspirin-induced gastric injury by modulating gastrokines, E-cadherin, and inhibiting NLRP3 and NF-κB/MMP-9 signaling pathway. Tissue Cell 93: 102724, 2025.
29. Wattanawongdon W, Bartpho TS and Tongtawee T: Expression of matrix Metalloproteinase-7 predicts poor prognosis in gastric cancer. Biomed Res Int 2022: 2300979, 2022.
30. Yueh TC, Tsao HY, Chien WC, Tsai CW, Pei JS, Wu MH, Chen CP, Chen CC, Wang ZH, Mong MC, *et al*: The contribution of matrix Metalloproteinase-7 promoter genotypes to hepatocellular carcinoma susceptibility. Anticancer Res 42: 5275-5282, 2022.
31. Chen L and Ke X: MMP7 as a potential biomarker of colon cancer and its prognostic value by bioinformatics analysis. Medicine (Baltimore) 100: e24953, 2021.
32. Lu L, Ma GQ, Liu XD, Sun RR, Wang Q, Liu M and Zhang PY: Correlation between GDF15, MMP7 and gastric cancer and its prognosis. Eur Rev Med Pharmacol Sci 21: 535-541, 2017.
33. Nakatani K, Yamaoka T, Ohba M, Fujita KI, Arata S, Kusumoto S, Taki-Takemoto I, Kamei D, Iwai S, Tsurutani J, *et al*: KRAS and EGFR amplifications mediate resistance to rociletinib and osimertinib in acquired Afatinib-resistant NSCLC harboring exon 19 deletion/T790M in EGFR. Mol Cancer Ther 18: 112-126, 2019.
34. Gao S, Luan Y, Yu X, Wang L, Huang X, Yang J and Liu W: TAIII suppresses the growth of T790M-mutant non-small-cell lung cancer by targeting the EGFR/ERK signaling pathway. Pharmaceuticals (Basel) 18: 1431, 2025.
35. Wang L, Lu YF, Wang CS, Xie YX, Zhao YQ, Qian YC, Liu WT, Wang M and Jiang BH: HB-EGF activates the EGFR/HIF-1α pathway to induce proliferation of Arsenic-transformed cells and tumor growth. Front Oncol 10: 1019, 2020.
36. Daum O, Daumova M and Svajdler M: Comments on the 5th edition of WHO classification of digestive system tumors-Part 1. Gastrointestinal tract. Cesk Patol 56: 194-206, 2020.
37. Qiu S, Wang Q, Jiang H and Feng L: Immunohistochemistry staining of Eag1 and p16/Ki-67 can help improve the management of patients with cervical intraepithelial Neoplasia after cold knife conversion. Diagn Pathol 19: 97, 2024.
38. Wang T, Du A, Peng Y, Yin J, Sun G, Yu Y, Sun Z, Chang Q, Gong K, Han S, *et al*: DSG3 promotes bladder cancer growth and metastasis via AKT/GSK3β/β-catenin pathway. J Transl Med 23: 729, 2025.
39. Bai T, Li P, Liu Y, Cai B, Li G, Wang W, Yan R, Zheng X and Du S: Knockdown of miR-411-3p induces M2 macrophage polarization and promotes colorectal cancer progression by regulation of MMP7. Eur J Histochem 69: 4178, 2025.
40. Hirohama D, Abedini A, Moon S, Surapaneni A, Dillon ST, Vassalotti A, Liu H, Doke T, Martinez V, Md Dom Z, *et al*: Unbiased human kidney tissue proteomics identifies matrix metalloproteinase 7 as a Kidney disease biomarker. J Am Soc Nephrol 34: 1279-1291, 2023.
41. Liu G, Jiang C, Li D, Wang R and Wang W: MiRNA-34a inhibits EGFR-signaling-dependent MMP7 activation in gastric cancer. Tumour Biol 35: 9801-9806, 2014.
42. Morgan E, Arnold M, Camargo MC, Gini A, Kunzmann AT, Matsuda T, Meheus F, Verhoeven RHA, Vignat J, Laversanne M, *et al*: The current and future incidence and mortality of gastric cancer in 185 countries, 2020-40: A population-based modelling study. EClinicalMedicine 47: 101404, 2022.

43. Ge X, Lin F, Wu Z, Lin Y, Tang W, McKay MJ, Sahu A, Lino-Silva LS, Tseng J and Li J: Role of ROR2 in promoting gastric cancer metastasis by enhancing c-JUN-mediated MMP3 transcription. Ann Transl Med 10: 1117, 2022.
44. Liu HQ, Song S, Wang JH and Zhang SL: Expression of MMP-3 and TIMP-3 in gastric cancer tissue and its clinical significance. Oncol Lett 2: 1319-1322, 2011.
45. Dong Z, Guo S, Wang Y, Zhang J, Luo H, Zheng G, Yang D, Zhang T, Yan L, Song L, *et al*: USP19 Enhances MMP2/MMP9-mediated tumorigenesis in gastric cancer. Onco Targets Ther 13: 8495-8510, 2020.
46. Wang HL, Zhou PY, Zhang Y and Liu P: Relationships between abnormal MMP2 expression and prognosis in gastric cancer: A meta-analysis of cohort studies. Cancer Biother Radiopharm 29: 166-172, 2014.
47. Choi EK, Kim HD, Park EJ, Song SY, Phan TT, Nam M, Kim M, Kim DU and Hoe KL: 8-Methoxypsoralen induces apoptosis by upregulating p53 and inhibits metastasis by downregulating MMP-2 and MMP-9 in human gastric cancer cells. Biomol Ther (Seoul) 31: 219-226, 2023.
48. Li T, Cao H, Wu S, Zhong P, Ding J, Wang J, Wang F, He Z and Huang GL: Phosphorylated ATF1 at Thr184 promotes metastasis and regulates MMP2 expression in gastric cancer. J Transl Med 20: 169, 2022.
49. Tregunna R: Serum MMP7 levels could guide metastatic therapy for prostate cancer. Nat Rev Urol 17: 658, 2020.
50. Sizemore ST, Sizemore GM, Booth CN, Thompson CL, Silverman P, Bebek G, Abdul-Karim FW, Avril S and Keri RA: Hypomethylation of the MMP7 promoter and increased expression of MMP7 distinguishes the basal-like breast cancer subtype from other triple-negative tumors. Breast Cancer Res Treat 146: 25-40, 2014.
51. Gao Y, Nan X, Shi X, Mu X, Liu B, Zhu H, Yao B, Liu X, Yang T, Hu Y, *et al:* SREBP1 promotes the invasion of colorectal cancer accompanied upregulation of MMP7 expression and NF-κB pathway activation. BMC Cancer 19: 685, 2019.
52. Janecka-Widla A, Majchrzyk K, Mucha-Malecka A and Biesaga B: EGFR/PI3K/Akt/mTOR pathway in head and neck squamous cell carcinoma patients with different HPV status. Pol J Pathol 72: 296-314, 2021.
53. Lu X, An L, Fan G, Zang L, Huang W, Li J, Liu J, Ge W, Huang Y, Xu J, *et al*: EGFR signaling promotes nuclear translocation of plasma membrane protein TSPAN8 to enhance tumor progression via STAT3-mediated transcription. Cell Res 32: 359-374, 2022.
54. Greenspan LJ, de Cuevas M, Le KH, Viveiros JM and Matunis EL: Activation of the EGFR/MAPK pathway drives transdifferentiation of quiescent niche cells to stem cells in the Drosophila testis niche. Elife 11: e70810, 2022.
55. Liang N, Bing Z, Wang Y, Liu X, Guo C, Cao L, Xu Y, Song Y, Gao C, Tian Z, *et al*: Clinical implications of EGFR-associated MAPK/ERK pathway in multiple primary lung cancer. Clin Transl Med 12: e847, 2022.
56. Yu J, Fang T, Yun C, Liu X and Cai X: Antibody-drug conjugates targeting the human epidermal growth factor receptor family in cancers. Front Mol Biosci 9: 847835, 2022.
57. Hu DD, Chen HL, Lou LM, Zhang H and Yang GL: SKA3 promotes lung adenocarcinoma metastasis through the EGFR-PI3K-Akt axis. Biosci Rep 40: BSR20194335, 2020.
58. Chang CH, Chen MC, Chiu TH, Li YH, Yu WC, Liao WL, Oner M, Yu CR, Wu CC, Yang TY, *et al*: Arecoline promotes migration of A549 lung cancer cells through activating the EGFR/Src/FAK pathway. Toxins (Basel) 11: 185, 2019.