EGFR and MMP7 are important targets for gastric cancer metastasis

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Abstract

The invasion and metastasis of gastric cancer pose frequent clinical challenges following standard treatment. Investigating the molecular mechanisms underpinning gastric cancer invasion and metastasis constitutes a critical research area. This study aims to pinpoint potential target molecules involved in gastric cancer metastasis. After analyzing the TCGA database, we identified overexpression of EGFR and MMP7 in gastric adenocarcinoma, which correlates with unfavorable patient outcomes. Notably, MMP7 expression is closely linked to gastric adenocarcinoma metastasis. Immunohistochemical analysis of clinical gastric adenocarcinoma tissue samples confirmed the association of both EGFR and MMP7 with metastasis, aligning with the findings from bioinformatics analysis. Moreover, our immunohistochemical results revealed a positive correlation between EGFR and MMP7 expression, providing a foundational basis for future endeavors in searching for drug targets to prevent and treat gastric cancer invasion and metastasis.

Introduction

GC (Gastric cancer) is the fifth most common cancer and the fourth most common cause of cancer-related deaths, accounting for nearly 800.000 deaths worldwide[12]. Asia has the highest reported incidence rate and mortality in the world, with 820000 new cases of gastric cancer and 576000 deaths in 2020[3]. Due to the atypical clinical symptoms and hidden onset of gastric cancer, and the limited role of tumor markers in tumor screening, most patients are already in the advanced stage of gastric cancer at the time of diagnosis, losing the opportunity for surgical resection. At present, the treatment of gastric cancer still relies on chemotherapy, targeted therapy, and combination chemotherapy regimens (including 5-FU based regimens, platinum based regimens, and new chemotherapy drug combinations), which have made continuous progress in the past 40 years. However, due to issues such as recurrence and invasion, these methods cannot completely cure them, affecting the quality of life of patients[4–6]. However, little is known about the specific mechanism by which the invasiveness of cancer cells increases during the progression of gastric cancer. Therefore, further research is needed on the mechanisms of metastasis and invasion in gastric cancer.

The invasion and metastasis of tumors are considered the main causes of cancer-related deaths. More and more researchers have shown that the enhancement of gastric cancer invasion ability is often accompanied by abnormal activation of signaling pathways such as EGFR, PI3K/AKT/mTOR, Ras/Raf/ERK, JAK/STAT, and the occurrence of EMT[7–11]. Abnormal activation of signaling pathways promotes tumor progression by regulating downstream effector targets. Epidermal growth factor receptor (EGFR) is widely distributed on the surface of mammalian epithelial cells, fibroblasts, glial cells, keratinocytes, and other cells. The EGFR signaling pathway plays an important role in the processes of cell growth, proliferation, and migration[12]. Some studies have reported that EGFR can be used as a marker to predict the progression and prognosis of breast cancer[13]. Some studies have reported that EGFR is associated with metastasis of gallbladder cancer, bladder cancer and lung cancer[14–16]. Research

reports have shown that EGFR is associated with metastasis of gastric cancer[17, 18]. Recent reports have shown that EGFR can promote the metastasis of colon cancer by regulating the expression of MMP7[19].

Gastric cancer patients are prone to recurrence, invasion, and metastasis after surgery, which seriously endangers their quality of life and survival. Matrix metalloproteinases (MMPs) play an undeniable role in the invasion and metastasis of gastric cancer. MMP7 is one of the most important members of the MMPs family. Although the structure of MMP7 is the simplest in the MMPs family, with a size of only 19KDA after activation, it degrades a wide range of substrates, including extracellular matrix and non-extracellular matrix, which is conducive to tumor metastasis and invasion. With the deepening of research on MMP7 by researchers, it has been found that MMP7 is overexpressed in gastric cancer, liver cancer, and colon cancer[20–22]. More importantly, MMP7 is secreted by tumor cells themselves, while other members of the MMPs family are secreted by tumor stromal cells. This lays the foundation that MMP7 can serve as a tumor marker and a target for subsequent tumor treatment[23]. This suggests the important role of MMP7 in the recurrence, invasion, and migration of gastric cancer.

In this study, we analyzed the results through the TCGA database and found that both EGFR and MMP7 are overexpressed in gastric cancer, and their overexpression is not conducive to the survival and prognosis of patients. We conducted immunohistochemical analysis on clinical gastric cancer samples collected, and the results showed that EGFR and MMP7 expression were higher in gastric cancer patients with metastasis, and there was a positive correlation between EGFR and MMP7 expression. In summary, our research results indicate that EGFR and MMP7 are valuable targets in the process of gastric cancer metastasis, providing a theoretical basis for solving clinical problems such as GC metastasis and invasion.

Results

Bioinformatics analysis of MMP7 expression in gastric cancer

Gastric adenocarcinoma represents the primary form of gastric cancer, with metastasis in gastric adenocarcinoma patients being a critical determinant of their quality of life and survival. Our analysis of gene expression profiles in gastric adenocarcinoma, utilizing data from the TCGA database, revealed elevated expression levels of MMP11 and MMP7, members of the MMPs family (as depicted in Fig. 1). MMP7, specifically, is secreted by cancer cells and possesses the ability to degrade the extracellular matrix, facilitating the breach of the initial defensive barrier during cancer cell metastasis.

Subsequent to this, we conducted an analysis of MMP7 RNA expression levels in different types of tumors. As illustrated in Fig. 2A-B, the results indicate a significant elevation in MMP7 expression within gastric adenocarcinoma. When compared to normal tissue samples (n = 34), the transcription levels of MMP7 in gastric adenocarcinoma tissue samples (n = 415) were notably higher, as demonstrated in

Fig. 2C. Furthermore, the survival prognosis curve reveals that heightened levels of MMP7 expression are associated with unfavorable survival outcomes for patients, as depicted in Fig. 2D.

Next, we delved into an analysis of MMP7 expression levels in gastric adenocarcinoma tissues across various stages. In comparison to normal tissues (n = 34), MMP7 expression exhibited a significant increase in Grade 1 (n = 12), Grade 2 (n = 148), and Grade 3 (n = 246) with strong statistical significance. Nevertheless, there were no statistically significant disparities in MMP7 expression levels among gastric adenocarcinoma tissues at different stages, as depicted in Fig. 3A.

Furthermore, we investigated MMP7 expression levels in gastric adenocarcinoma tissues across different grades. Contrasting with normal tissues (n = 34), MMP7 expression displayed a marked rise in Stage 1 (n = 18), Stage 2 (n = 123), Stage 3 (n = 169), and Stage 4 (n = 41), all exhibiting significant statistical significance. Notably, there were no discernible distinctions in MMP7 expression levels among gastric adenocarcinoma tissues of various grades, as illustrated in Fig. 3B.

Subsequent to these analyses, we examined the disparities in MMP7 levels within gastric adenocarcinoma tissues of male and female patients. The results indicated that in comparison to normal tissues (n = 34), MMP7 expression was elevated in both male (n = 268) and female (n = 147) patients, but no notable differences were observed in MMP7 expression levels between male and female patients, as shown in Fig. 3C.

Crucially, we also compared MMP7 expression differences in gastric adenocarcinoma patients with lymph node metastasis. The findings highlighted that, when compared to normal tissues (n = 34), MMP7 expression increased significantly in N0 (n = 123), N1 (n = 112), N2 (n = 79), and N3 (n = 82), without any statistically significant disparities in MMP7 expression levels between gastric adenocarcinoma tissues from patients with varying degrees of lymph node metastasis, as depicted in Fig. 3D.

These observations underscore the pivotal role of MMP7 in the metastasis process of gastric adenocarcinoma, emphasizing the clinical significance of selecting MMP7 as a target for further exploration and potential intervention.

Bioinformatics analysis of EGFR expression in gastric cancer

The transcription level expression of MMP7 is regulated by upstream signaling pathways. Research reports have shown that EGFR can regulate the expression of MMP7 in diabetic kidney disease[24]. We analyzed the differential expression of EGFR in normal and gastric adenocarcinoma tissues using the TCGA database, and the results showed that EGFR was highly expressed in gastric adenocarcinoma (Fig. 4A). More importantly, we analyzed the impact of EGFR on survival and prognosis in gastric adenocarcinoma, and the results showed that overexpression of EGFR in gastric adenocarcinoma is not conducive to the survival and prognosis of patients (Fig. 4B).

The expression of MMP7 and EGFR is higher in metastatic gastric adenocarcinoma tissues

A total of 32 clinical samples, comprising gastric adenocarcinoma and corresponding normal tissue samples, were collected. Among these, 17 samples were derived from metastatic cases, and 15 from non-metastatic cases. Immunohistochemical staining for EGFR and MMP7, along with HE staining, was performed on these samples. Here, we present the representative findings from two non-metastatic cases (Patients 1 and 2) and two metastatic cases (Patients 3 and 4).

Upon analyzing the correlation between EGFR and MMP7 in gastric adenocarcinoma tissues with and without metastasis using Graphpad Prism, the results unveiled higher expression levels of both EGFR and MMP7 in metastatic tissues when compared to non-metastatic tissues. These findings align with the results obtained from our prior bioinformatics analysis, underscoring the crucial role of EGFR and MMP7 in the metastatic progression of gastric adenocarcinoma (Fig. 5A-C). This collective evidence reaffirms the significance of these proteins in the metastasis process of gastric adenocarcinoma.

EGFR and MMP7 are positively correlated in gastric adenocarcinoma tissue

Subsequently, we examined the expression levels of EGFR and MMP7 in 17 metastatic patients, categorized into low to moderately high MMP7 expression levels, as illustrated in Fig. 6A. Patient 5 exhibited low immunohistochemical staining of MMP7, Patient 6 displayed moderate immunohistochemical staining of MMP7, and Patient 7 showcased high immunohistochemical staining of MMP7 (Fig. 6A).

We further scrutinized the immunohistochemical staining levels of EGFR and MMP7 in the 32 gastric adenocarcinoma tissue samples and their corresponding normal tissue counterparts. The results demonstrated elevated expression levels of both EGFR and MMP7 in gastric adenocarcinoma tissue samples compared to normal tissue samples, as delineated in Fig. 6B-C.

Notably, from Fig. 6A, it becomes evident that as the MMP7 expression level decreases (as seen in Patient 5), the EGFR expression is also lower. Conversely, when MMP7 expression is high (as observed in Patient 7), EGFR expression levels increase correspondingly. Consequently, we performed a correlation analysis between EGFR and MMP7 in the 32 gastric adenocarcinoma tissue samples, revealing a positive correlation between EGFR and MMP7 expression ($r^2 = 0.6219$, $P < 0.0001$), as represented in Fig. 6D. This correlation underscores the association between EGFR and MMP7 in gastric adenocarcinoma, reinforcing their potential significance in the disease process.

Discussion

According to the data reported by the International Agency for Research on Cancer (IARC) in 2020, gastric cancer ranks sixth in the global cancer incidence rate and fourth in the mortality rate; It is estimated that there were approximately 1.1 million new cancer cases and 770000 deaths in 2020; It is expected that by 2040, the annual burden of cancer will increase to approximately 1.8 million new cases and approximately 1.3 million deaths[25]. The incidence rate of gastric cancer is the highest in Southeast Asia, and it is the most common type of cancer in China. In the past decade, significant progress has been made in the treatment of gastric cancer after recognizing its severity and harmfulness. Due to recurrence and metastasis after routine treatment, the prognosis is still unsatisfactory. Finding target molecules in the process of gastric cancer metastasis and improving postoperative survival and quality of life has always been a challenge in this field.

MMPs have attracted the attention of researchers due to their association with metastasis. Matrix metalloproteinases (MMPs), as a whole, are a group of proteolytic enzymes containing active Zn^{2+} (hence called metalloproteinases). MMPs can target many extracellular proteins, including proteases, growth factors, cell surface receptors, and adhesion molecules. MMPs are considered important factors in normal tissue remodeling during embryonic development, damage repair, cancer invasion, angiogenesis, carcinogenesis, and cell apoptosis[26, 27]. MMPs can degrade the basement membrane and extracellular matrix, which are closely related to tumor growth, invasion, and metastasis[28]. Research has shown that the activity and expression level of MMPs such as MMP2, MMP3, MMP7, and MMP9 are increased in gastric cancer patients, which can reduce the survival period of gastric cancer patients, promote the metastasis and recurrence of cancer, and is associated with poor prognosis of gastric cancer[29–31]. MMP7 is secreted by tumor cells themselves, which is better as a marker of tumor progression compared to other members of the MMPs family. Therefore, it has been widely concerned by researchers. Studies have reported that MMP7 is overexpressed in prostate cancer and breast cancer, and is related to metastasis[32, 33]. Research has reported that MMP7 can be used as a marker to measure the prognosis of colon and esophageal cancer[34 35]. We found that MMP7 is overexpressed in gastric adenocarcinoma through bioinformatics analysis and is not conducive to patient prognosis, indicating that MMP7 is a valuable target molecule in gastric adenocarcinoma. More importantly, our immunohistochemical results showed that MMP7 is highly expressed in gastric adenocarcinoma tissues with lymph node metastasis, which fully demonstrates the correlation between MMP7 and metastasis in gastric adenocarcinoma. MMP7 is associated with cancer progression and is a tumor marker for invasion, metastasis, and poor prognosis of gastric cancer. It is a potential therapeutic target for gastric cancer treatment.

The transcription level of MMP7 gene is regulated by signaling pathways. EGFR is a member of the human epidermal growth factor receptor family. After binding to ligands, EGFR can undergo phosphorylation, activating downstream signaling pathways, including PI3K/AKT, STAT, and MAPK, thereby regulating biological effects such as tumor cell survival, apoptosis, invasion, and metastasis[36–39]. Research reports have shown that abnormal activation of the human epidermal growth factor receptor EGFR signaling pathway plays an important role in the metastasis and invasion of gastric cancer[18, 40].

Recent research reports have shown that EGFR can regulate the transcription of MMP7 and promote the progression of lung cancer[41, 42]. We found through TCGA database analysis that overexpression of EGFR in gastric cancer is not conducive to the prognosis of patients, which is consistent with the report. More importantly, it is important to collect clinical gastric adenocarcinoma samples. Immunohistochemical results suggest that EGFR and MMP7 are both expressed in gastric adenocarcinoma. Through correlation analysis with whether metastasis occurs in gastric cancer samples, it is found that patients with high levels of EGFR and MMP7 expression experience metastasis, indicating the correlation between EGFR and MMP7 and gastric cancer metastasis. In addition, we also found that tissues with high EGFR expression also had higher MMP7 expression, while tissues with low EGFR expression also had lower MMP7 expression. This finding suggests that there seems to be a regulatory relationship between EGFR and MMP7 expression in gastric cancer. We further conducted correlation analysis between EGFR and MMP7, and the results showed that EGFR and MMP7 are positively correlated in gastric adenocarcinoma. However, due to limited platform resources, we did not further explore the regulatory mechanism of EGFR and MMP7 in gastric adenocarcinoma.

In summary, overexpression of EGFR and MMP7 in gastric adenocarcinoma is not conducive to the prognosis of patients, and both EGFR and MMP7 are associated with metastasis of gastric adenocarcinoma. More importantly, we found a positive correlation between the expression levels of EGFR and MMP7 in clinical gastric adenocarcinoma tissues through immunohistochemical staining analysis. This study is conducive to revealing the important roles of EGFR and MMP7 in gastric cancer metastasis, and is expected to provide theoretical and experimental basis for finding effective new targets for the treatment of gastric cancer.

Materials and Methods

Tissue samples

32 gastric adenocarcinoma tissue samples and their corresponding normal tissue samples (samples taken at a distance of 2.5cm or more from the cancer tissue) were obtained from gastric tumor patients undergoing surgery at The First Affiliated Hospital of Anhui Medical University south district. These patients have not experienced chemotherapy or radiation therapy before. All tumor samples include 17 metastatic samples and 15 non metastatic samples. This study was approved by the Ethics Committee of the First Affiliated Hospital of Anhui Medical University (Approval No. 20231337) and conducted in accordance with the principles of the Helsinki Declaration. Obtain written informed consent from all patients.

Reagents

EGFR (phonor Y1068, ab40815) and MMP7 (ab207299) were purchased from the Abcam Plc.PV-9000 histochemical reagent kit and DAB staining solution from Beijing Zhong Shan Golden Bridge Biological Technology Co., Ltd.

Bioinformatics analysis

UALCAN(http://ualcan.path.uab.edu/)is a comprehensive database for analyzing genomics, providing users with differential gene expression analysis and patient survival and recovery data through the analysis of the TCGA database. Firstly, we analyzed the gene profile of overexpression in gastric adenocarcinoma and found that MMP7 was overexpressed in gastric adenocarcinoma. Next, we analyzed the expression differences of MMP7 in various tumor tissues, as well as the differences in expression between healthy and gastric adenocarcinoma tissues, and conducted survival prognosis analysis. MMP7 was found to be highly expressed in gastric adenocarcinoma and not conducive to patient prognosis. Next, analyze the expression of MMP7 in the staging, grading, and metastasis of gastric adenocarcinoma. Finally, analyze the differential expression of EGFR in gastric adenocarcinoma and its normal tissues, and analyze the survival prognosis of EGFR expression in gastric adenocarcinoma.

Immunohistochemistry

Cut paraffin tissue blocks into 5 μ and attached to anti detachment tissue slide. Dewaxing and hydration are carried out in xylene and ethanol. Antigen repair involves soaking tissue slices in citrate buffer and boiling for 15 minutes before natural cooling. H_2O_2 (reagent 1) treatment for 20 minutes eliminates the impact of endogenous enzymes on the results. After 20 minutes of serum blockade, incubate the first antibody at 4 ℃ overnight. Incubate at room temperature for 20 minutes with reaction enhancing solution (reagent 2) and secondary antibody (reagent 3) in sequence. Reagents 1, 2, and 3 are all from the PV-9000 histochemical kit. Join DAB (Shanghai Biyuntian Biotechnology Co., Ltd.) for 5 minutes of color rendering. Hematoxylin(Shanghai Biyuntian Biotechnology Co., Ltd.) stained the nucleus and sealed it with neutral resin. The panoramic scanner(Pannoramic MIDI)captures images and scores tissue staining using Jetta JD801 (Jiangsu Jetta Technology Development Co., Ltd.). Use Graphpad Prism8 to calculate the difference in staining scores between EGFR and MMP7, and perform correlation analysis between EGFR and MMP7.

HE

The tissue slices were dehydrated in xylene dewaxing and ethanol. Stain the nucleus with hematoxylin for 3 minutes and the cytoplasm(Shanghai Biyuntian Biotechnology Co., Ltd.) with eosin for 1 minute. After sealing with neutral resin, use a panoramic scanner(Pannoramic MIDI)to take pictures.

Statistical analysis

The statistical significance of the differences between the two groups was tested using paired or unpaired two tailed student t-tests. Use linear regression to analyze the correlation between EGFR and MMP7. All statistics are completed by Graphpad Prism8. $P < 0.05$ is considered to indicate a statistically significant difference.

Declarations

Conflicts of interest

There are no conflicts to declare including any competing financial interest.

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Authors' Contributions

Biran Ding and Yiqiu Wan cconceived the study and participated in the data analysis and drafting, and wrote the manuscriptits. Yao Wu, Zhang Zhang and Ying Ma collected clinical tissue samples and conducted immunohistochemical experiments. Zuo Wang and Runqiu Jiang provided revisions and guidance to the manuscript. All authors read and approved the final manuscript.

Data availability

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

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Figures

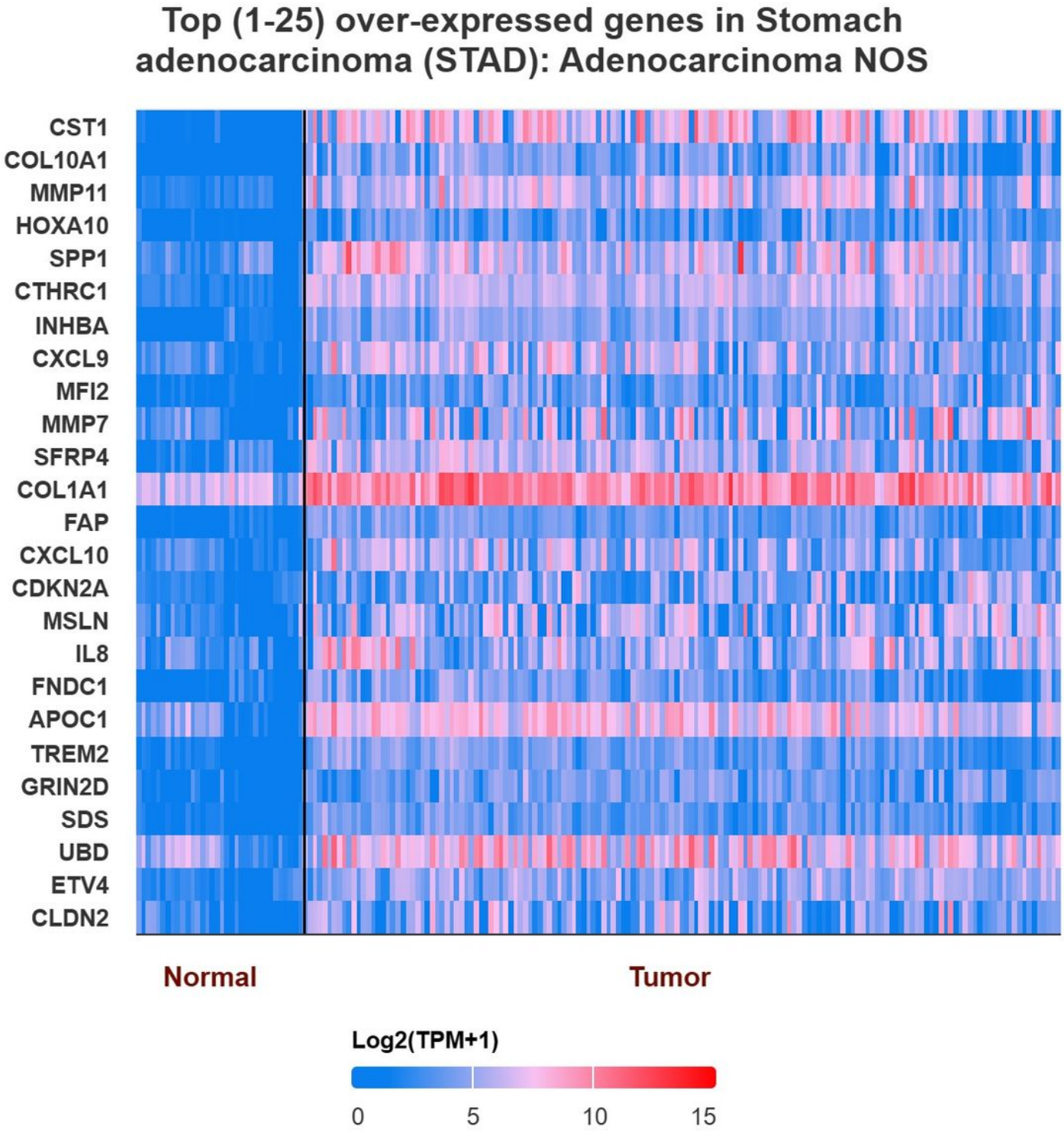

Figure 1

shows the gene profile of overexpression in gastric adenocarcinoma, showing the top 25 overexpressed genes.

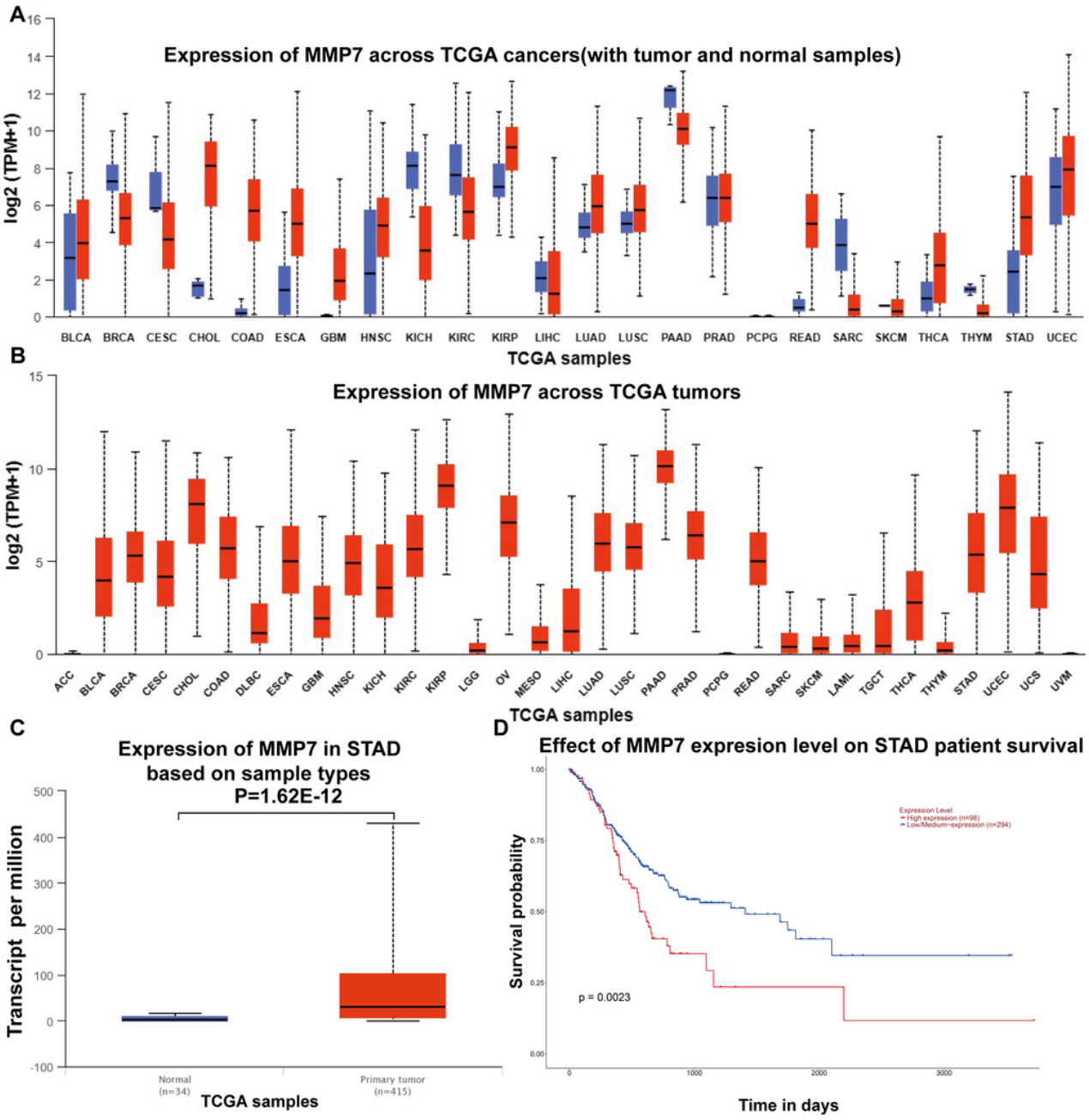

Figure 2

Figure 2A shows the expression differences of MMP7 in various tumors and their normal tissues, with red representing cancer tissue and blue representing normal tissue. Figure 2B shows the differential expression of MMP7 in various tumor tissues. The differential expression of MMP7 in gastric adenocarcinoma and its normal tissues (Figure 2C). The survival prognosis analysis of MMP7 in gastric adenocarcinoma is shown in Figure 2D.

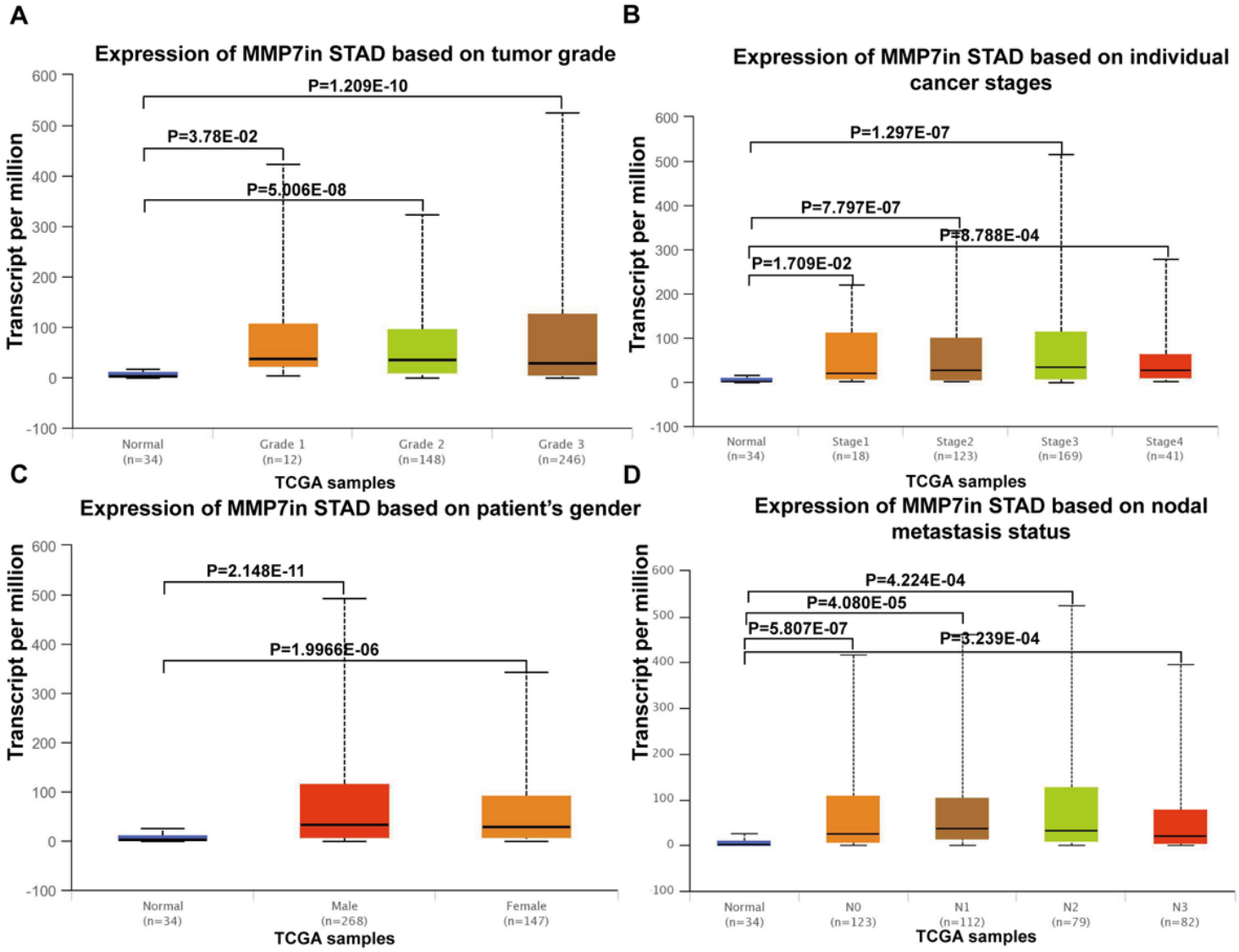

Figure 3

Differential expression of MMP7 in different stages of gastric adenocarcinoma (Figure 3A). Figure 3B shows the expression differences of MMP7 in different grades of gastric adenocarcinoma and normal tissues. Figure 3C shows the differential expression of MMP7 in male and female patients with gastric adenocarcinoma. Figure 3D shows the expression differences of MMP7 in different lymph node states of gastric adenocarcinoma.

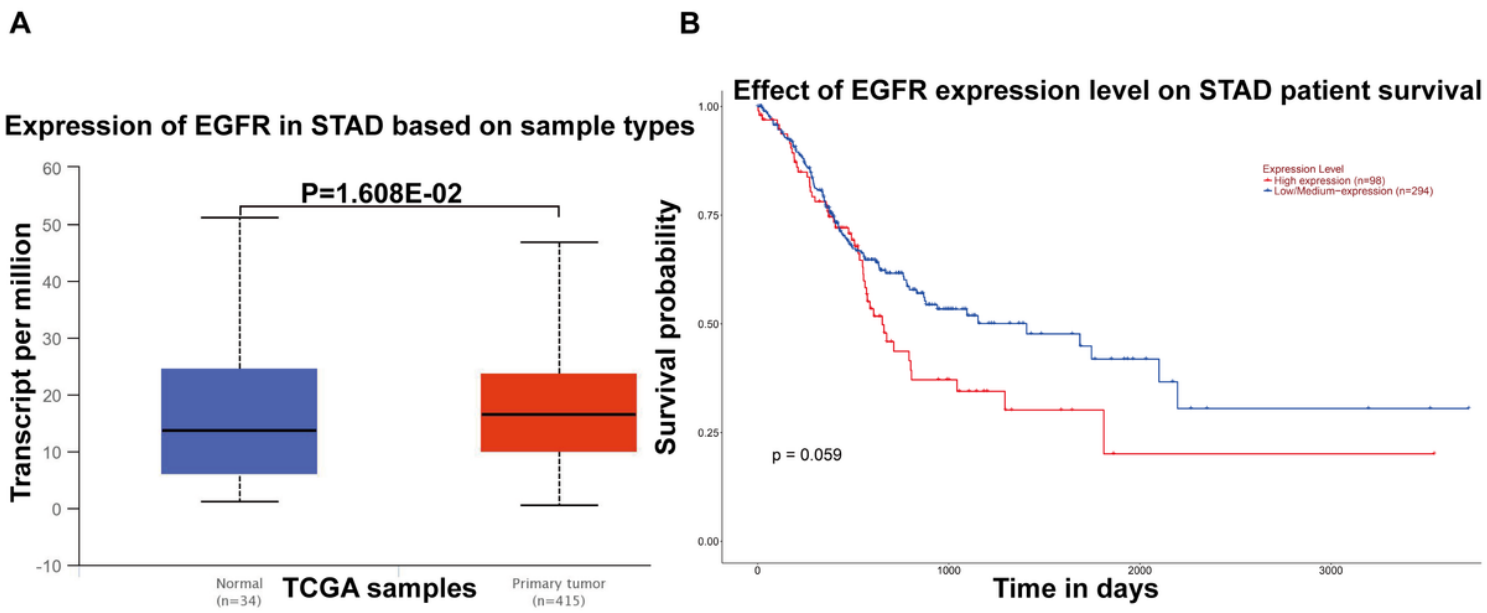

Figure 4

Overexpression of EGFR in gastric adenocarcinoma is not conducive to patient prognosis. Figure 4A shows the differential expression of EGFR between normal and gastric adenocarcinoma samples. Analysis of survival prognosis of EGFR in gastric adenocarcinoma(Figure 4B).

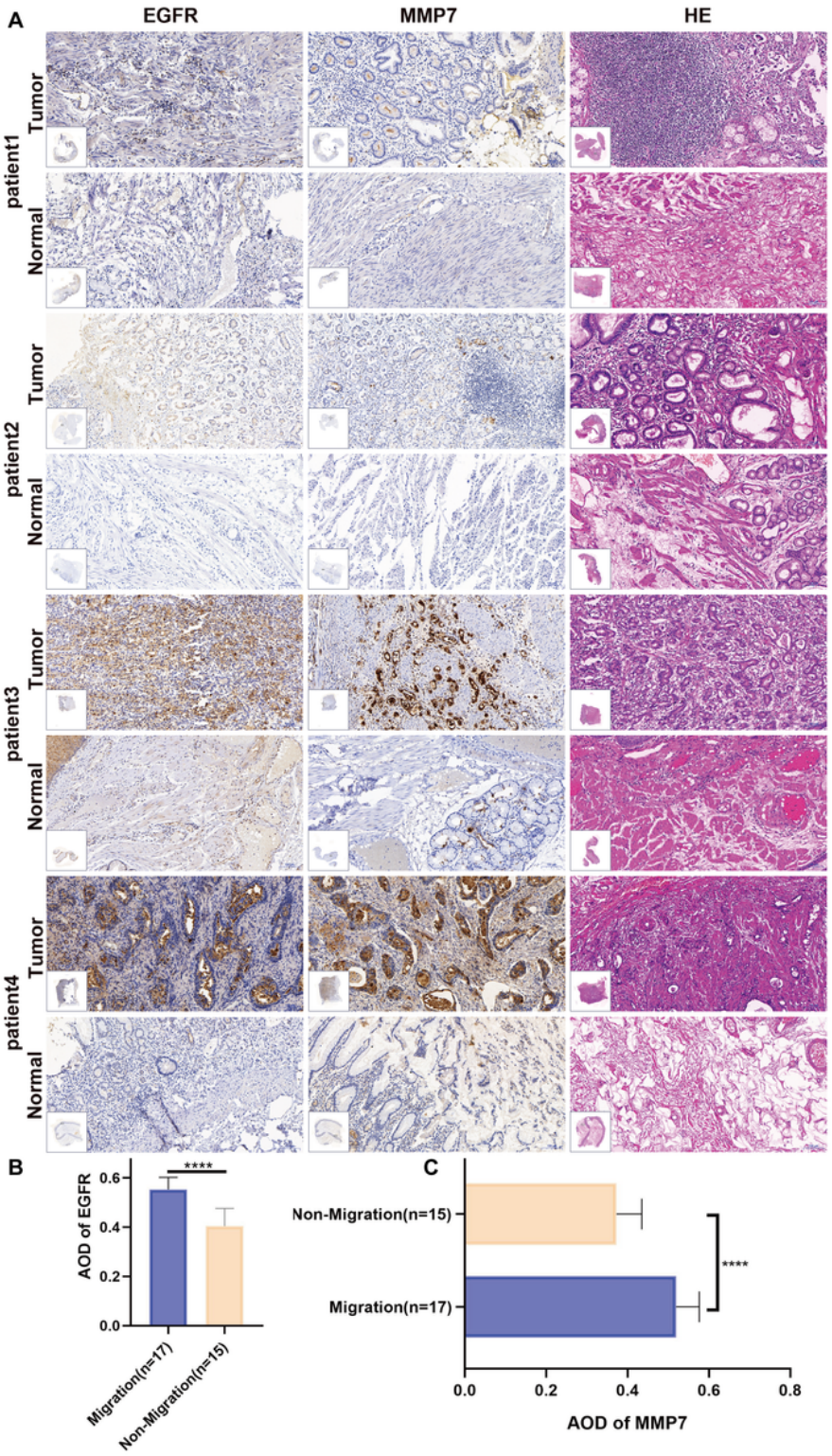

Figure 5

Figure 5A shows a representative image of immunohistochemical staining and HE staining of EGFR and MMP7 in gastric adenocarcinoma. Figure 5B shows the difference in optical density of EGFR between metastatic and non metastatic gastric adenocarcinoma tissues. The optical density difference of MMP7 between metastatic and non metastatic gastric adenocarcinoma tissues (Figure 5C).**** Represents $P<0.0001$.

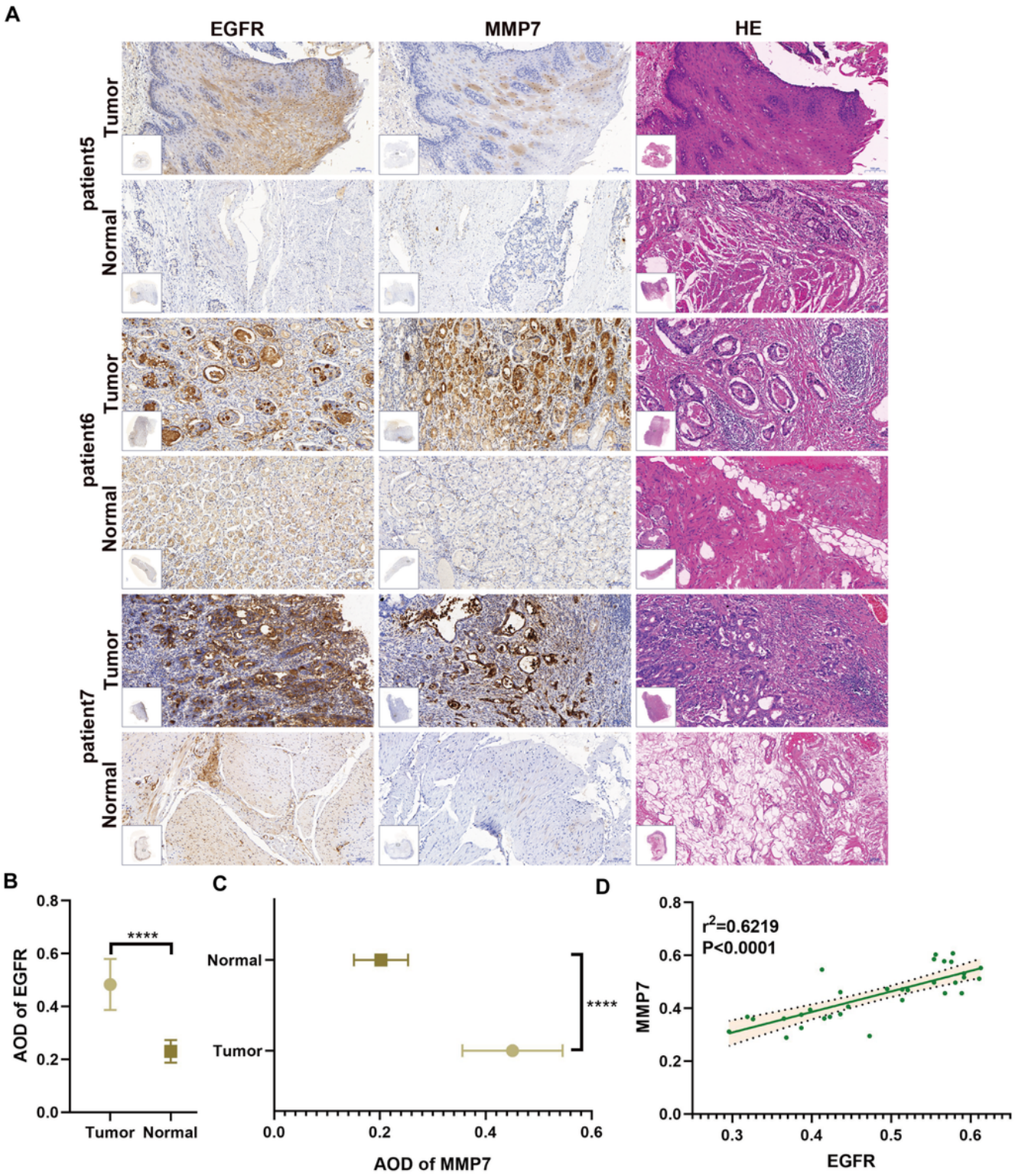

Figure 6

Representative images of low expression, moderate expression, and high expression of MMP7 (Figure 6A). Analysis of optical density differences in cancer tissues and their corresponding normal tissues using EGFR staining (Figure 6B). Figure 6C shows the difference in MMP7 staining scores between cancer tissue and normal tissue. Figure 6D shows the correlation analysis between EGFR and MMP7 in

gastric adenocarcinoma, with the orange part representing a 95% confidence interval. **** Represents $P<0.0001$.