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ERBB2 S310F Mutation Independently activates PI3K/AKT And MAPK Pathways through Homo-Dimerization to Contribute Gallbladder Carcinoma Growth

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Abstract

Genomic instability and mutability are a prominent character of tumor. The whole-exosome sequence reveals that ERBB2 mutations are the representative mutations of gallbladder carcinoma, which takes potential targets for gallbladder carcinoma therapy. However, the roles of ERBB2 mutations are unclear in gallbladder carcinoma. We identified S310F mutation is the hottest mutation of ERBB2 mutations from TCGA PanCancer Altas data with 10967samples and our previous study with 157 gallbladder carcinoma samples. S310F mutation located in ERBB2 extracellular domain, promoted ERBB2 homodimerization and consequent auto-phosphorylation to activate the downstream PI3K/AKT and MAPK pathways, which was independent on ERBB1、ERBB3 and ERBB4. ERBB2 S310F mutation up-regulated aerobic glycolysis and promoted gallbladder carcinoma growth. Our study reveals the roles of ERBB2 S310F mutation, which is beneficial to ERBB2 S310F mutant gallbladder carcinoma therapy.

Introduction

Gallbladder carcinoma (GBC) is a highly malignant tumor of the digestive system. Although the morbidity and mortality respectively account for 1.2% and 1.7%[1, 2], which is not the highest compared with other tumors, most of patients are diagnosed at advanced stages with poor prognosis of a median survival of only 6 months[3, 4]. Currently, GBC therapy is mainly dependent on surgery operation and limited chemotherapy drugs. Limited understanding of the molecular characteristics of GBC also hinders the development of new drugs.

The ERBB family members all belong to typical receptor tyrosine kinases (RTK)[5, 6]. The earliest identified ERBB family member is the human epidermal growth factor receptor (EGFR, as known ERBB1)[7]. Since then, the ERBB family has grown to four members including ERBB1/EGFR、ERBB2/HER2、ERBB3/HER3 and ERBB4/HER4[5]. The constructs of ERBB family members are involved in extracellular domain (ECD), transmembrane domain (TMD), juxtamembrane domain (JMD), intracellular tyrosine kinase domain (KD) and C-terminal signalling domain (CSD)[3, 8]. The ligands binding to a monomeric ERBBs promotes receptor dimerization and self-phosphorylation on cytoplasmic tyrosine residues to recruit a number of signal transducers, which activates downstream pathways including the MAPK and PI3K/AKT pathways[5]. Unlike other ERBB family members, ERBB2 lacks the capacity to bind with a ligand, and preferentially heterodimerizes with other ERBB members to initiate cellular signaling[5]. The heterodimeric form of ERBB2 and ERBB3 is often confirmed[5]. ERBB2 is a potent onco-protein and plays key roles in tumor initiation and progression[3, 8, 9]. Overexpression of ERBB2 remains a major mechanism attributing ERBB2-driven tumorigenesis[8, 10]. Three antibodies of trastuzumab、adotrastuzumab、emtansine (T-DM1) and pertuzumab, two small molecule inhibitors of lapatinib and neratinib targeting ERBB2 have been approved by the US Food and Drug Administration (FDA) for use in the clinic for treating ERBB2-driven tumors[8, 11]. Recent large-scale sequencing efforts have identified multiple ERBB2 mutations and their potential tumorigenic effects[8, 9, 12, 13]. However, the roles of ERBB2 mutations in GBC remains unclear.

Our study identifies the prominent S310F mutation of ERBB2, and reveals its roles of activating ERBB2 and downstream pathways in gallbladder carcinoma.

Materials And Methods

Cell culture

The human GBC-SD cells were purchased from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). The human SGC-996 cells were obtained from Professor Yang's Lab of Tongji University School of Medicine. Cells were cultured in DMEM or 1640 medium with 100 U/ml penicillin, 100 μg/ml streptomycin, and 10% fetal bovine serum at 37°C and 5% CO_2.

Antibodies and reagents

Phospho-HER2/ERBB2 (Tyr1221/1222) (Cat. #2243), Phospho-Akt (Ser473) antibody (Cat. #4060), Akt antibody (Cat. #4685), Phospho-p44/42 MAPK (Erk1/2) (Cat. #4370) and p44/42 MAPK (Erk1/2) (Cat. #4695) were purchased form CST (Boston, MA, USA). DDDDK-Tag antibody (Cat. #AE063) and HA antibody (Cat. #AE008) were purchased from Abclonal (Wuhan, China). b-actin antibody (Cat. #GB11001), HRP-conjugated goat anti-mouse IgG (Cat. #GB23301) and HRP-conjugated goat anti-rabbit IgG (Cat. #GB23303) were purchased Servicebio (Wuhan, China)

Western blot

The plasmids or siRNA was transfected to 293T cells for 48h, and EGF treatment was completed at indicated time for western blot detection. Briefly, cells were lysed at 4°C with RIPA lysis, then protein concentration was measured by BCA protein concentration assay kit (Servicebio, Wuhan, China, Cat. #G2026). Samples with same protein mass were separated through SDS-PAGE electrophoresis, and transferred onto NC membrane (nitrocellulose filter membrane), then blocked with 5% non-fat milk and incubated with the primary antibody at 4 °C overnight. Next, the NC membrane was washed with PBST, and incubated with the HRP-conjugated secondary antibody at room temperature for 1h, then washed with PBST. Finally, the target proteins were visualized using an enhanced chemiluminescence system.

Immunoprecipitation

The 293T cells transiently transfected by plasmids for 48h or the overexpressed GBC cells were lysed by 0.3% NP40 for immunoprecipitation. The input samples were prepared, and the rest of lysates were incubated with Flag beads or HA beads overnight at 4°C, then centrifuged by at 20,000 g for 10 min, then protein aggregates were added 1*loading buffer for immunoblotting assay.

ERBB2 dimer assay

Glutaraldehyde cross-linking assay was employed to analyze ERBB2 dimer according to previous description[14]. Firstly, about 10 ml glutaraldehyde solution (Sigma Aldrich, Cat. #340855) was added to

990ml double distilled H_2O as stock solution. Cells were lysed using 0.1% NP-40 lysis buffer at 4℃ for 30min, and centrifuged at 12000rpm for 20min at 4℃, divided into two tubes, one tube as control. The other one was added 0.5% glutaraldehyde stock solution and incubated on ice for 5min, then added 1M glycine for 15min at room temperature. Two tubes were added 1*loading buffer to prepare the protein samples for western blot detection.

Cell proliferation assay

The treated cells were seeded to a 6-well plate with 1000 cells per well to culture for about two weeks, then the number of cell clone (more than 100 cells) was counted through crystal violet staining.

Cell migration and invasion assay

The treated cells were digested and suspended by serum-free medium at a concentration of 10^6 cells/ml. Approximately $5*10^5$ cells (50ml cell suspension) was added into the upper chamber of transwell. The lower chamber was added 600ml DMEM to culture for approximately 12-24h for migration assay. For the invasion assay, the transwell chamber was coated with matrigel in advance. Finally, the chamber was stained with crystal violet, and the number of transferred cells was calculated.

Tumor xenograft growth *in vivo*

Animal experiments were performed with the approval of the animal ethics committee of Zhongshan Hospital of Fudan University. SGC-996 stable cell strains of $ERBB2^{WT}$-Flag and $ERBB2^{S310F}$-Flag were suspended at a concentration of $2*10^7$ cells/ml, then 100ml cell suspension was subcutaneously inoculated to nude mice (4-5 weeks old) abdomen for growth. The tumor xenografts were monitored through measurement of long diameter (length) and short diameter (width) every other day. After about thirty days, tumor xenografts were harvested and tumor volume was calculated using the formation of V (mm^3) = $width^2$ (mm^2) × length (mm)/2.

Statistics

Statistical analysis was carried out for differences with one-way analysis of variance (ANOVA) in more than two groups and two-tailed student's t-test in two groups. The statistical results were shown as the mean±SD from experiments conducted at least in triplicate. Differences were considered to be significant when $p < 0.05$ for all statistical analyses.

Results

ERBB2 S310F was the hot mutation in GBC samples

Our previous study has revealed that ERBB2/3 mutations are characteristic and account for 15% GBC samples[3]. However, few studies are performed to reveal the roles and underlying mechanism of ERBB2/3 mutations in GBC. We analyzed the 32 different projects with 10967 samples from TCGA Pan-

Cancer Atlas database. ERBB2 mutations and ERBB3 mutations are significantly mutually exclusive in these samples (Fig 1A and B, $p<0.001$). The GBC data of MSK (Memorial Sloan Kettering Cancer Center, n=103)[15] and another study (n=32)[16] also indicate the mutual exclusion of ERBB2 mutations and ERBB3 mutations (Fig 1C and D). Moreover, the mutual exclusion of ERBB2 mutations and ERBB3 mutations was confirmed in our previous study (Fig 1E)[3]. These indicated the potentially different roles of ERBB2 mutations and ERBB3 mutations in GBC. We further analyzed the mutations of ERBB2 and ERBB3 in detail. There were identified 376 mutations with 289 missense mutations in ERBB2 (77%), several high frequency mutations included the S310F/Y/A (43 cases)、R678Q (15 cases)、L755S (16 cases)、V777L (13 cases), V842I (14 cases) and S310F (37 cases) was the highest frequency mutation from 10967 samples of TCGA Pan-Cancer Atlas database (Fig 2A). ERBB2 mutations in GBC included E265K (1 case)、G292R(1 case)、S310F/Y(5 cases)、R678Q (1 case)、V842I (1 case)、L869R (1 case), and S310F was consistently the highest frequency mutation (3 cases) from our previous study (Fig 2B)[3]. Moreover, the S310F mutation was located at the ECD, which potentially regulated ERBB2 activity. There were identified 371 mutations with 321 mutations in ERBB3 (86%), several high frequency mutations included V104M/L (30 cases)、F219L/V (5 cases)、D297Y/N/V (8 cases)、E322K/Q (6 cases)、T355I/P (5 cases)、R475W (5 cases) and E928G/K/Q (8 cases), V104M (25 cases) was the highest frequency mutation from 10967 samples of TCGA Pan-Cancer Atlas database (Fig 2C). ERBB3 mutations in GBC included V104L/M (5 cases)、D581N (1 case)、P590H (1 case)、R667S (1 case)、E688Q (1 case)、F965L (1 case)、V1035D (1 case)、S1234C (1 case), but V104L was the highest frequency mutation (4 cases) from our previous study (Fig 2D)[3]. The V104L mutation was located at the ECD of ERBB3, but not the kinase domain, which indicated an unpredictable role on ERBB3. Therefore, we focused on the role of ERBB2 S310F mutation in GBC.

S310F mutation promoted ERBB2 homo-dimerization and self-phosphorylation.

It is well established that ligands induce ERBB receptors dimerization to increase the tyrosine kinase activity of its intracellular domain. Consequently, these phosphotyrosines recruit downstream molecular with SH2 domains to activate signaling pathways[17]. However, ERBB2 has no known ligand and its activation needs help form others ERBB members[17]. Whether S310F mutation has potential effects on ERBB2? Therefore, we transfected the $ERBB2^{WT}$-Flag (wild type), $ERBB2^{S310F}$-Flag (S310F mutation) plasmids and the control vectors to 293T cells for 48h, then detected dimer levels through non-reduced western blot. We found S310F mutant ERBB2 significantly increased the dimer levels compared wild type ERBB2 (Fig 3A). We employed SGC-996 cells to establish the stable cell strains of $ERBB2^{WT}$-Flag, $ERBB2^{S310F}$-Flag and vector (the blank control), and confirmed that ERBB2 S310F mutation obviously increased dimer levels (Fig 3B). Next, we co-transfected the $ERBB2^{S310F}$-Flag and ERBB1-HA, ERBB3-HA, ERBB4-HA to 293T cells and confirmed S310F mutant ERBB2 had no obvious combination with ERBB1, ERBB3 and ERBB4 (Fig 3C). Whether the ERBB2 dimerization induced by S310F mutation contributed the ERBB2 activation? We transfected the $ERBB2^{WT}$-Flag, $ERBB2^{S310F}$-Flag plasmids and the control vectors to 293T cells and further found ERBB2 tyr1221/1222 phosphorylation level was increased by ERBB2 S310F mutation (Fig 3D). Moreover, the increased tyr1221/1222 phosphorylation level of S310F mutant

ERBB2 was identified in SGC-996 stable cell strains (Fig 3E). We co-transfected the ERBB2^{S310F}-Flag and ERBB1-HA, ERBB3-HA, ERBB4-HA to 293T cells and found the transfection of ERBB1, ERBB3 and ERBB4 did not affected the increased phosphorylation level of S310F mutant ERBB2 (Fig 3F). These indicated S310F mutation promoting ERBB2 homo-dimerization and self-phosphorylated activation was independent on ERBB1, ERBB3 and ERBB4.

ERBB2 S310F mutation activated downstream pathway in an independent way.

The MAPK and PI3K/AKT pathways are major downstream pathways of ERBB receptors[18-20]. S310F mutation activating ERBB2 possibly contributed to the activation of downstream pathways. Therefore, we next analyzed the role of ERBB2 S310F mutation on PI3K/AKT and MAPK pathways. We transfected the ERBB2^{WT}-Flag, ERBB2^{S310F}-Flag plasmids and control vectors to 293T cells. Compared the ERBB2^{WT}-Flag transfection, the phosphorylation of AKT and ERK1/2 in ERBB2^{S310F}-Flag transfection was significantly up-regulated (Fig 4A). Consistently, in SGC-996 stable cell strains, the phosphorylation of AKT and ERK1/2 was also higher in ERBB2 S310F mutant cells than that in ERBB2 wild typical cells (Fig 4B). Next, we would like to know whether the activation of PI3K/AKT and MAPK pathways by ERBB2 S310F mutation was involved in other ERBB members. The ERBB1-HA, ERBB3-HA and ERBB4-HA plasmids were co-transfected with ERBB2 ^{S310F}-Flag to 293T cells. The results showed that the increased phosphorylation levels of ERK1/2 and AKT induced by ERBB2 S310F mutation were not significantly changed by the co-transfection of ERBB1-HA, ERBB3-HA and ERBB4-HA plasmids (Fig 4C). These suggested that the activation of PI3K/AKT and MAPK pathways by ERBB2 S310F mutation was independent on other ERBBs.

ERBB2 S310F mutation promoted GBC cell migration and invasion *in vitro*, and tumor xenograft growth *in vivo*.

Next, we detected the roles of ERBB2 S310F mutation in GBC. We first analyzed GBC cell proliferation through clone formation assay. Compared with wild type ERBB2, ERBB2 S310F mutation up-regulated cell proliferation (Fig 5A) in SGC-996 cells. Moreover, ERBB2 S310F mutant GBC-SD cells also showed increased cell proliferation viability compared the ERBB2 wild typical cells (Fig 5A). We next analyzed the GBC cell migration through transwell assay. The results showed that ERBB2 S310F mutation significantly promoted cell migration viability compared with wild type ERBB2 in SGC-996 and GBC-SD cells *in vitro* (Fig 5B). The transwell coated matrigel was employed to detect cell invasion viability. Consistently, the results showed that ERBB2 S310F mutation significantly also promoted cell invasion viability compared with wild type ERBB2 in SGC-996 and GBC-SD cells *in vitro* (Fig 5C). We also checked the role of ERBB2 S310F mutation on GBC proliferation *in vivo*. The SGC-996 stable cell strains expressed wild typical ERBB2 and S310F mutant ERBB2 were subcutaneously injected to nude mice abdomen to establish GBC xenograft model. ERBB2 S310F mutant tumor xenografts showed higher growth viability than wild type ERBB2 *in vivo* (Fig 5D). These indicated that ERBB2 S310F mutation promoted GBC cell migration and invasion *in vitro*, and tumor xenograft growth *in vivo*.

Discusses

Because of the limitation of drugs, surgery operation still is the main therapy for GBC. However, only a few patients are cured through surgery operation. Most patients are diagnosed at advanced stages[3]. New targets are urgent to be explored for GBC. Our previous study has confirmed the gene mutation characters in GBC through whole-exosome sequence, and identified the ERBB2/ERBB3 mutations are the characteristic mutations of GBC[3]. Therefore, it is necessary for GBC target therapy to further explore the role of ERBB2/ERBB3 mutations. We firstly analyzed ERBB2/ERBB3 mutations in TCGA Pan-Cancer Altas data with 10967 samples and confirmed ERBB2 and ERBB3 mutations were mutually exclusive. Moreover, two GBC database and our study also confirmed the mutually exclusion between ERBB2 and ERBB3 mutations[3, 15, 16]. The indicated that the possible different roles of ERBB2 and ERBB3 mutations in tumors. From TCGA Pan-Cancer Altas data, we identified 371 mutations of ERBB2 located in different domains of ECD、TMD、JMD、KD and CSD. These mutations located in different domains potentially plays important roles in tumor initiation and progression[3, 8, 9]. Moreover, TMD/JMD-activating mutations identified in multiple cancer led to allosteric activation of kinases, playing significant roles in oncogenesis[8]. As we known, the ERBB2 could not combine ligands[8]. Therefore, ERBB2 preferentially heterodimerizes with ligand bound HER3 or EGFR to initiate cellular signaling[8, 21, 22]. However, the highest frequency ERBB2 S310F mutation was located at the ECD of ERBB2, which indicated the potential role on ERBB2 activation. By contrast, ERBB3 activation also needs other ERBB members due to the defective kinase activity[5], but the highest frequency ERBB3 V104L mutation was located at the ECD of ERBB3 in GBC, which is not directly related to ERBB3 activation. Therefore, we focus on the ERBB2 S310F mutation. Whether the S310F mutation had a potential effect on ERBB2 activation? Through transfection of $ERBB2^{WT}$-Flag (ERBB2 wild type) and $ERBB2^{S310F}$-Flag (ERBB2 S310F mutation) plasmids to 293T cells, and the detection in SGC-996 stable cell strains, we found the significant dimers of mutant ERBB2, compared with wild type ERBB2. Moreover, the dimers of mutant ERBB2 were independent on ERBB1、ERBB3 and ERBB4. We also further confirmed the self-phosphorylation levels of mutant ERBB2. Through transfection of $ERBB2^{WT}$-Flag and $ERBB2^{S310F}$-Flag plasmids to 293T cells, we confirmed the dimerization of mutant ERBB2 promoted self-phosphorylation. Consistently, the S310F mutant SGC-996 stable cell strains also increased dramatically ERBB2 self-phosphorylation levels, compared with SGC-996 stable cell strains expressed wild type ERBB2. Moreover, the increased self-phosphorylation levels of mutant ERBB2 were independent on ERBB1、ERBB3 and ERBB4. These indicated S310F mutation promoted ERBB2 dimerization and self- phosphorylation levels, and potentially activated ERBB2. The PI3K/AKT and MAPK are important downstream pathways of ERBB2[3]. We next analyzed the PI3K/AKT and MAPK pathways. The increased phosphorylation levels of AKT (473) and ERK1/2 in 293T cells confirmed the activation of downstream pathways through transfection of $ERBB2^{WT}$-Flag and $ERBB2^{S310F}$-Flag plasmids to 293T cells. Consistently, the SGC-996 stable cell strains of ERBB2 S310F mutation showed significant activation of PI3K/AKT and MAPK pathways compared with ERBB2 wild typical cells. The results suggested that ERBB2 S310F mutation activated ERBB2 and the downstream PI3K/AKT and MAPK pathways. Whether ERBB2 S310F mutation activation downstream pathway was

also independent on other ERBBs? Compare with only ERBB2^{S310F}-Flag transfection to 293T cells, the co-transfection of ERBB2^{S310F}-Flag with ERBB1-HA, ERBB3-HA, and ERBB4-HA had no significant effect on phosphorylation of AKT and ERK1/2. Therefore, we considered that S310F mutation induced ERBB2 dimerization and self-phosphorylation to activate the PI3K/AKT and MAPK pathways in an independent way.

We next analyzed the roles of ERBB2 S310F mutation in GBC. We employed clone formation assay to detect the cells proliferation, and confirmed ERBB2 S310F mutation promoted SGC-996 cell proliferation viability *in vitro*. ERBB2 S310F mutation promoted SGC-996 cell migration and invasion viability through the transwell assay and the transwell coated by matrigel. Moreover, ERBB2 S310F mutation also promoted tumor xenograft growth *in vivo*. Altogether, S310F mutation induced ERBB2 dimerization and self-phosphorylation to independently activate the PI3K/AKT and MAPK pathways and promote GBC cell migration and invasion *in vitro* and growth *in vivo*. Our study established the new roles of S310F mutation on ERBB2 in GBC.

Declarations

Compliance with Ethical Standards

Funding: This work was supported by National Natural Science Foundation of China grants (81802751, 82072682). All authors have contributed to this study.

Conflict of Interest: All authors declare no conflicts of interest.

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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Figures

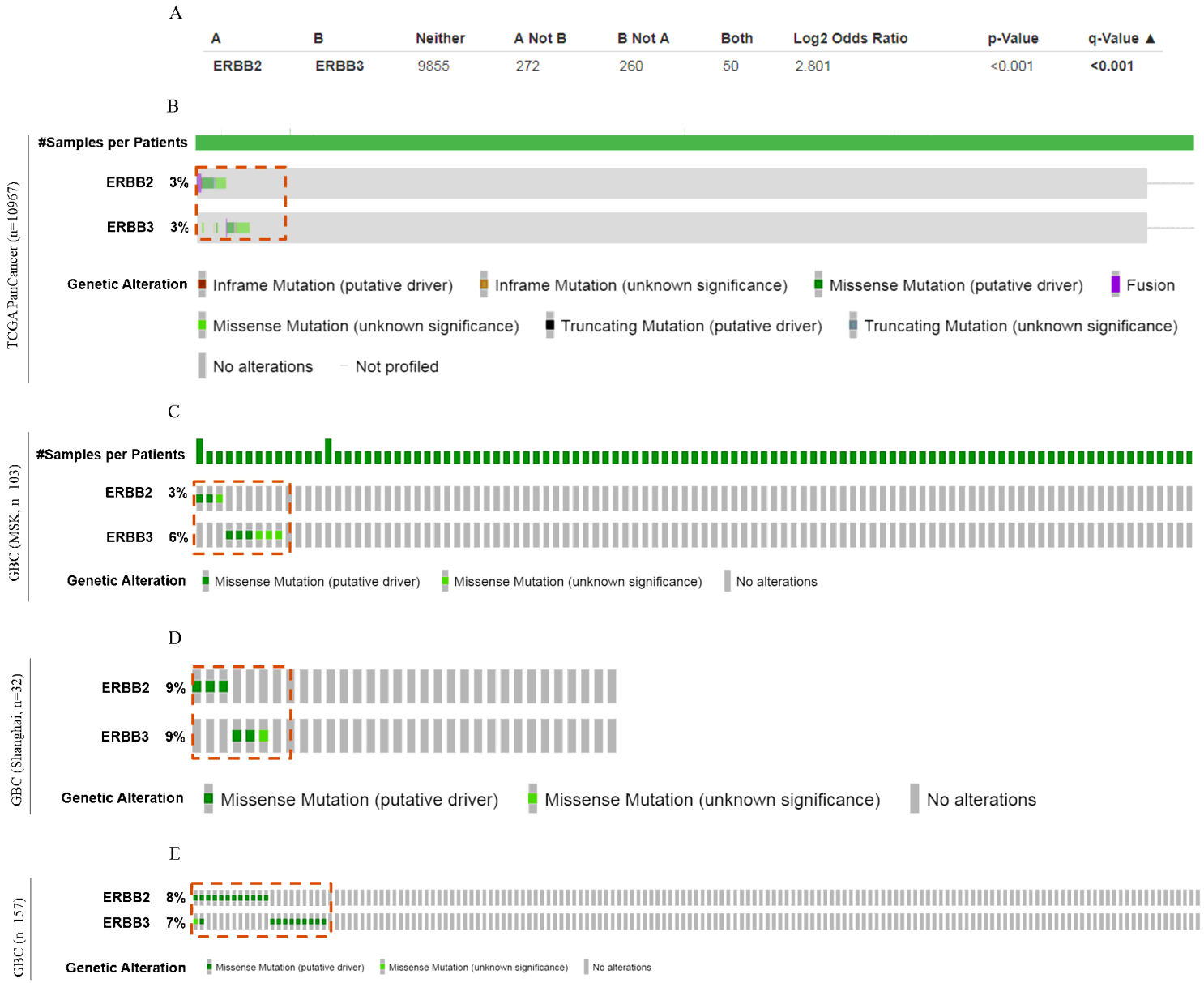

Figure 1

ERBB2 mutations and ERBB3 mutations were mutually exclusive. (A) The analysis of ERBB2 mutations and ERBB3 mutations mutual exclusion in the 32 different projects with 10967 samples from TCGA Pan-Cancer Atlas database. The diagram of ERBB2 mutations and ERBB3 mutations from different data, (B) the 32 different projects with 10967 samples from TCGA Pan-Cancer Atlas database, (C) the GBC data from MSK (Memorial Sloan Kettering Cancer Center, n=103), (D) the GBC data in another study (n=32), (E) the GBC data our previous study (n=157).

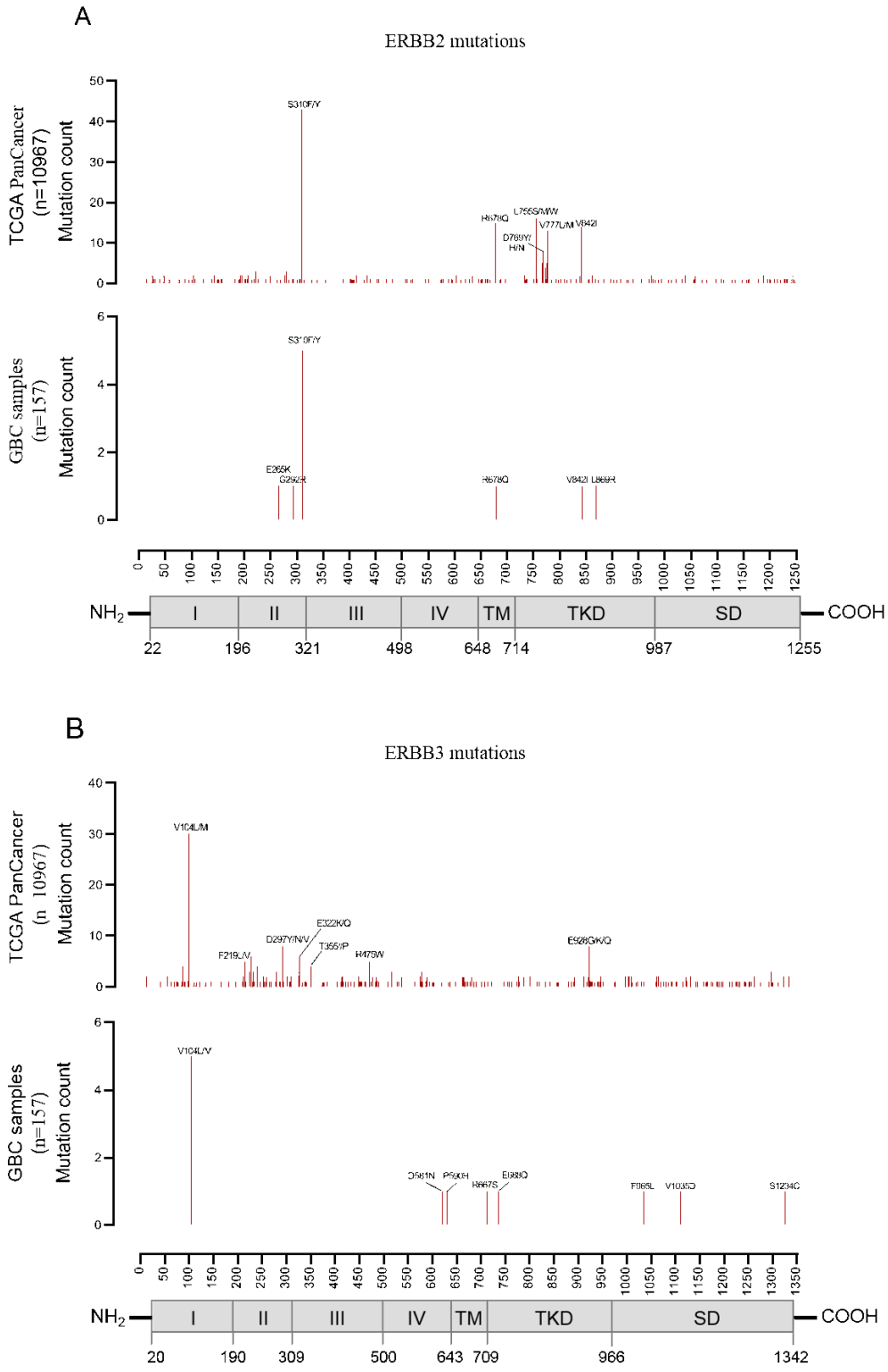

Figure 2

S310F mutation was the high frequency mutation of ERBB2. (A) Frequency of ERBB2 mutations shown with a diagram of the ERBB2 protein domains from the 32 different projects with 10967 samples from TCGA Pan-Cancer Atlas database. (B) Frequency of ERBB2 mutations shown with a diagram of the ERBB2 protein domains from our previous study with 157 GBC samples. (C) Frequency of ERBB3 mutations shown with a diagram of the ERBB3 protein domains from the 32 different projects with 10967

samples from TCGA Pan-Cancer Atlas database. (B) Frequency of ERBB3 mutations shown with a diagram of the ERBB3 protein domains from our previous study with 157 GBC samples.

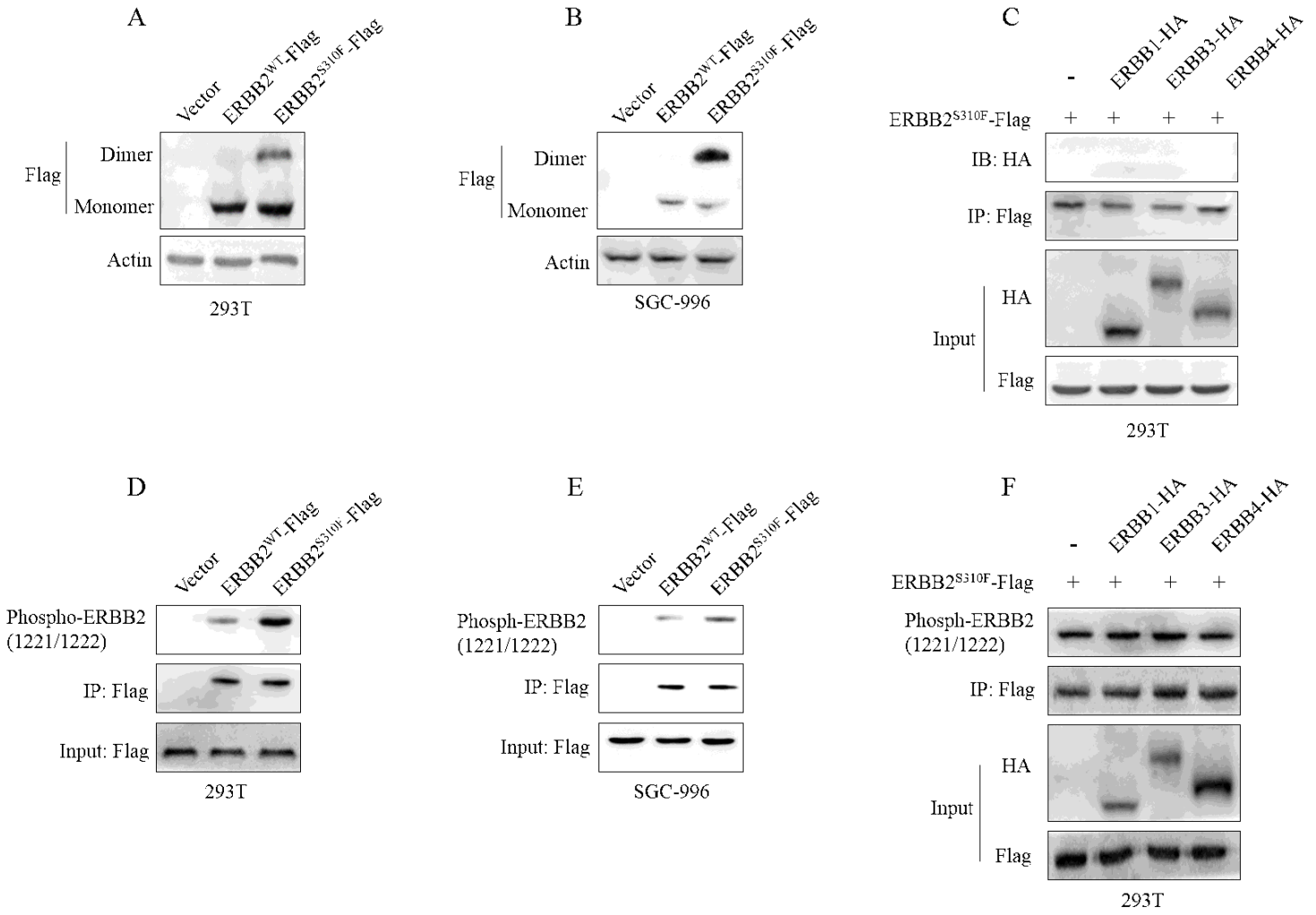

Figure 3

S310F mutation promoted ERBB2 dimerization and self-phosphorylation. (A) The ERBB2WT-Flag, ERBB2S310F-Flag and vector plasmids were transfected to 293T cells for 48h, the ERBB2 dimer was detected through western blot. (B) The SGC-996 stable cell strains expressing ERBB2WT-Flag, ERBB2S310F-Flag were established, the ERBB2 dimer was detected in SGC996 stable cell strains through western blot, the vectors as control. (C) The co-transfection of ERBB2S310F-Flag with ERBB1-HA, ERBB3-HA and ERBB4-HA to 293T cells for 48h, the interaction of ERBB2 with other ERBB members was detected through immunoprecipitation. (D) The ERBB2WT-Flag, ERBB2S310F-Flag and vector plasmids were transfected to 293T cells for 48h, the tyrosine phosphorylation (tyr1221/1222) of ERBB2 was detected. (E) The tyrosine phosphorylation (tyr1221/1222) of ERBB2 was detected in the SGC-996 stable cell strains of ERBB2WT-Flag and ERBB2S310F-Flag, the vectors as control. (F) The co-transfection of ERBB2S310F-Flag with ERBB1-HA, ERBB3-HA and ERBB4-HA to 293T cells for 48h, the tyrosine phosphorylation (Tyr1221/1222) of ERBB2 was detected. Data are represented as the mean±SD from three independent experiments.

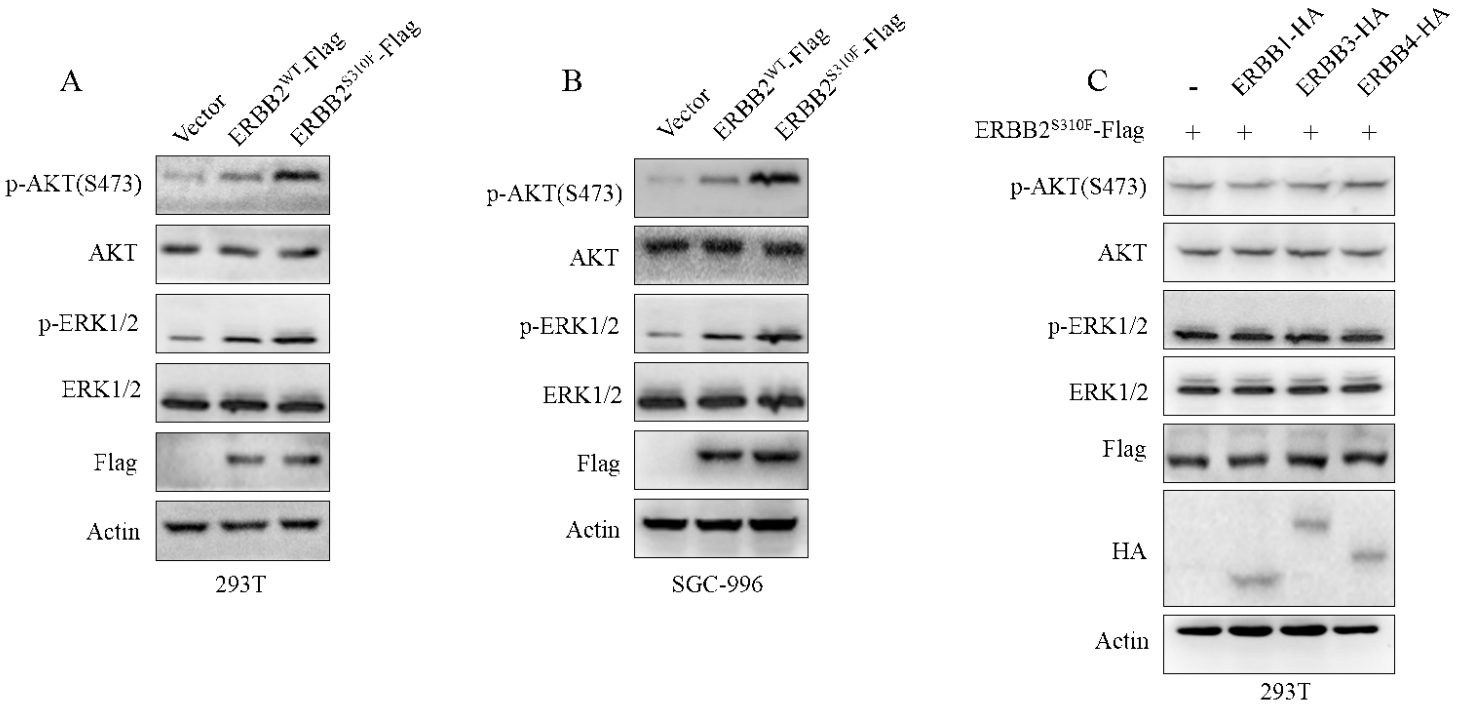

Figure 4

ERBB2 S310F mutation activated downstream PI3K/AKT and MAPK/ERK1/2 pathways. (A) The ERBB2WT-Flag, ERBB2S310F-Flag and vector plasmids were transfected to 293T cells for 48h, then the phosphorylation levels of AKT (Ser473) and ERK1/2 were detected. (B) The phosphorylation levels of AKT (Ser473) and ERK1/2 were detected in SGC-996 stable cell strains of ERBB2WT-Flag and ERBB2S310F-Flag, the vectors as control. (C) The co-transfection of ERBB2S310F-Flag with ERBB1-HA, ERBB3-HA and ERBB4-HA to 293T cells for 48h, then the phosphorylation levels of AKT (Ser473) and ERK1/2 were detected. Data are represented as the mean±SD from three independent experiments.

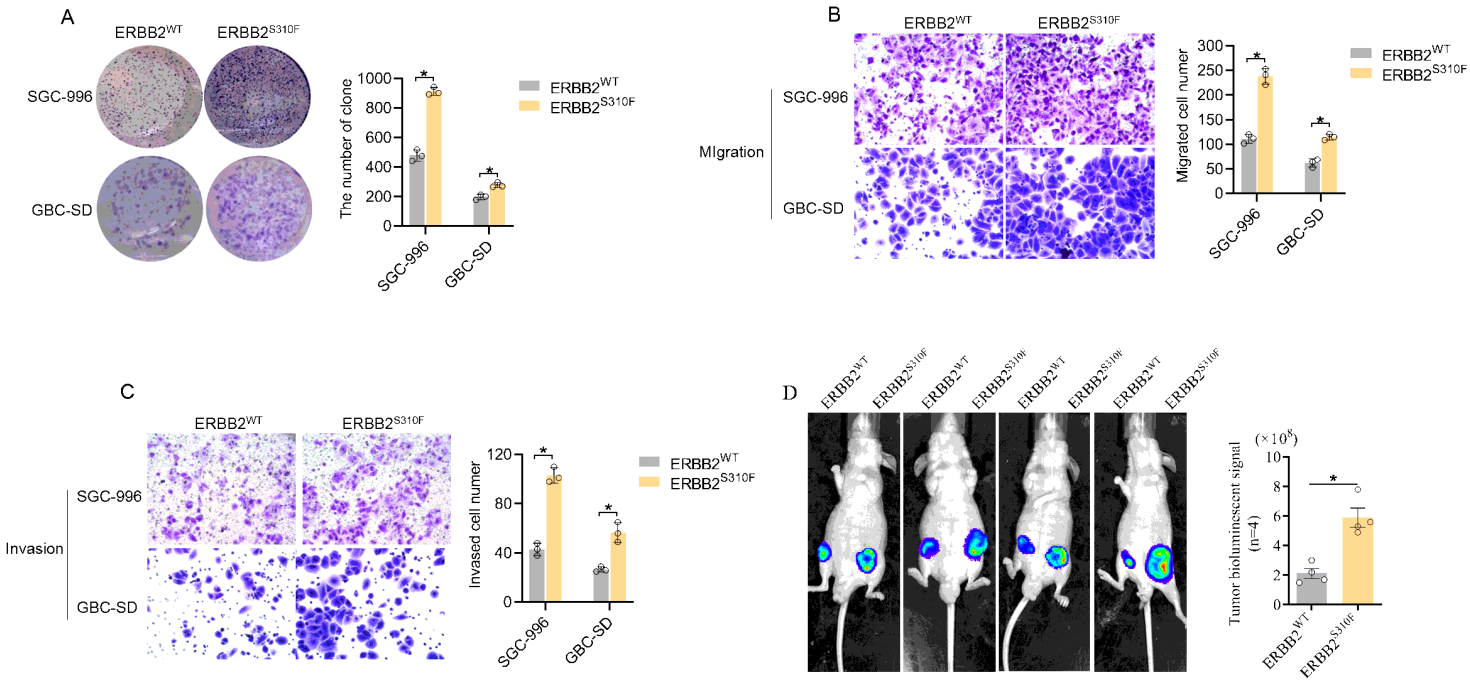

Figure 5

ERBB2 S310F mutation promoted GBC proliferation and metastasis. (A) The clone formation was employed to assay cell proliferation of SGC-996 stable cell strains expressed wild type ERBB2 and S310F mutant ERBB2 in vitro. (B) The transwell was employed to assay cell migration viability of SGC-996 stable cell strains expressed wild type ERBB2 and S310F mutant ERBB2 in vitro. (C) The transwell coated matrigel was employed to assay cell migration of SGC-996 stable cell strains expressed wild type ERBB2 and S310F mutant ERBB2 in vitro. (D) The tumor xenograft model was used to analyze tumor growth of SGC-996 stable cell strains expressed wild type ERBB2 and S310F mutant ERBB2 in vitro. Data are represented as the mean±SD from three independent experiments.