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Molecular characteristics of gastric cancer with *ERBB2* amplification

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ABSTRACT

Gastric cancer is a prevalent malignancy with a high degree of heterogeneity, which has led to a poor therapeutic response. Though there are numerous HER2-targeted medicines for HER2+ gastric cancer, many trials have not indicated an improvement in overall survival. Here 29 *ERBB2* amplification (*ERBB2*-Amp) type gastric cancer samples with WES and RNA-seq data were selected for investigation, which copy-number aberration (CNA) was +2. Initially, the somatic mutation and copy number variant (CNV) of them, which might cause resistance to HER2-targeted therapies, were systematically investigated evaluated, as well as their mutation signatures. Moreover, 37 modules were identified using weighted gene co-expression network analysis (WGCNA), including the blue module related to DFS status and lightcyan module correlated with *ARHGAP26_ARHGAP6_CLDN18* rearrangement. In addition, focal adhesion and ECM-receptor interaction pathways were considerably enriched in the turquoise module with *ERBB2* gene. ExportNetworkToCytoscape determined that *MIEN1* and *GRB7* are tightly connected to *ERBB2*., Finally, 14 single-cell intestinal gastric cancer samples were investigated, and it was shown that the *TFAP2A* transcription factor regulon was highly expressed in *ERBB2*^{high} group, as was the EMT score. Overall, our data provide comprehensive molecular characteristics of *ERBB2*-Amp type gastric cancer, which offers additional information to improve HER2-targeted gastric cancer treatment.

1. Introduction

Gastric cancer was the fifth most frequently diagnosed cancer and the fourth leading cause of cancer-related mortality worldwide [1]. Infection with *Helicobacter pylori* (*Hp*) or Epstein-Barr virus (EBV) increases the risk of developing gastric cancer. Furthermore, heritable pathogenic mutations, such as CDH1 mutations, might cause familial gastric cancer [2]. According to histopathological or molecular feature, there were numerous categories of gastric cancer based on histological or genetic characteristics to inform clinical prognosis and tailor treatment. For example, gastric cancer was separated into diffuse, intestinal and mixed types in Lauren

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classification [2]. The publication of a comprehensive single-cell atlas of gastric cancer provided a high-resolution molecular resource spanning diverse subtypes of gastric cancer, including intestinal, diffuse, and mixed kinds [3]. Additionally, the Cancer Genome Atlas (TCGA) revealed four tumor subgroups, including positive for Epstein-Barr virus (EBV), microsatellite instability (MSI), genomically stable (GS) and chromosomal instability (CIN) [4]. Interestingly, immunotherapy could be beneficial for EBV and MSI types.

Human epidermal growth factor receptor 2 (HER2, which was encoded by *ERBB2* gene) is a transmembrane receptor tyrosine kinase that belongs to the human epidermal growth factor receptor (EGFR) family. HER2 overexpression may result in homodimerize and heterodimerize with other ERBB family members [5–7], which activates the RAS-RAF-MEK-ERK and PI3K/Akt pathways [8–10], thereby promoting differentiation, apoptosis regulation, cellular proliferation, invasion and driving tumorigenesis [11,12].

The introduction of HER2 as a molecular biomarker was motivated by its ability to predict for gastric cancer was response to targeted therapies [12–14], including small-molecule kinase inhibitors (lapatinib) and anti-HER2 monoclonal antibody therapies (pertuzumab and trastuzumab) [15,16]. The ToGA trial showed that trastuzumab plus fluoropyrimidine and cisplatin significantly improved the OS of patients with HER2-positive advanced gastric or gastro-oesophageal junction cancer [15]. However, the following several trials of HER2-targeted treatments, such as lapatinib plus oxaliplatin and capecitabine (TRIO-013/LOGiC) pertuzuma [17] and cisplatin plus fluoropyrimidine and trastuzumab (JACOB) [18] in the first-line therapy, lapatinib plus paclitaxel (TyTAN) [19] and T-DM1 (GATSBY) [20] in the second-line therapy, had not demonstrated the efficacy of an OS benefit for HER2+ gastric cancer, which was not completely understood [21].

According to the statistics, 12%–20% of gastric cancer were HER2 positive (HER2+) [22,23], which were defined by immunohistochemistry (IHC) 2+ and fluorescence in-situ hybridisation (FISH)-positive or IHC 3+ [15,24,25]. Niu et al. [26] and Nakamura et al. [27] tried to use gene copy number (CN) to identify HER2+ type by next-generation sequencing (NGS) in breast cancer and gatric cancer, but the accurate *ERBB2* CN therapeutic cutoff value has not been established, so we tried to only focus on copy-number aberration (CNA) generated by GISTIC. According to cBioPortal (https://docs.cbioportal.org/user-guide/faq/), CNA = +2 indicated a high-level amplification.

In general, HER2+ gastric cancer was produced by overexpression of the HER2 protein, amplification of the *ERBB2* gene, or both. HER2 protein overexpression stands for the protein level of HER2, while *ERBB2*-Amp refer to the DNA level of *ERBB2*. In this study we selected samples with amplified *ERBB2* that contained both WES and RNA-seq data. We systematically analyzed the somatic mutation, copy number variants (CNV) and mutation signature on WES data. Using WGCNA with RNA-seq data, we also identified significant modules associated with clinical traits and the hub genes within in the same module. As we know, WGCNA may play similarly expressed genes in the same module [28], therefore the turquoise module containing ERBB2 was investigated to identify the related genes and pathways. The published findings on single-cell gastric cancer data [3], revealed that epithelial cells with high expression *ERBB2* were and those cells had high EMT score posed a greater risk for tumor spread. Overall, we determined the molecular characteristics of *ERBB2*-Amp type gastric cancer and discovered modules with a strong association to certain characteristics. Taken together, these data could provide further information on the pathways and genes and contribute to understand the mechanisms of resistance to HER2-targeted therapies and metastasis.

2. Materials and methods

2.1. Data collection and screening

WES mutation, copy-number aberration (CNA) and clinical traits data were downloaded from cBioProtal (Stomach Adenocarcinoma (TCGA, Nature 2014), https://www.cbioportal.org/); RNA-seq FPKM data was collected from Xena (GDC TCGA Stomach Cancer (STAD), https://xenabrowser.net/). In this study, we chose 29 samples with *ERBB2*-Amp (CNA = +2) and 18 samples with *ERBB2* deletion (*ERBB2*-Del, CNA ≤ -1), which both has RNA-seq and WES data. Besides, gene-cell matrix data of 14 single-cell intestinal gastric cancer samples (Tumor) were obtained from https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE183904 [3].

2.2. Systematic analysis of somatic mutations and CNV and mutation signature in ERBB2-Amp type gastric cancer

Somatic nucleotide variants (SNVs), INDELs, and CNVs were plotted by maftools [29] and ComplexHeatmap package [30]. Pathway genelists of RTK-RAS and PIK3CA were obtained from the paper of Sanchez-Vega et al. [31]. Mutation signature was analyzed by SomaticSignatures package [32], and was compared with 30 mutation signatures in Catalogue of Somatic Mutations in Cancer (COSMIC) database (https://cancer.sanger.ac.uk/signatures/signatures_v2) [33].

2.3. WGCNA construction and hub gene validation

Genes with FPKM less than 3 in any sample were filtered. Then those top 10000 coefficient of variation genes were kept for further analysis. In order to find the gene modules, the power of $\beta = 8$ was used to build the co-expression network by WGCNA [28]. The module eigengenes (ME), which was the first principal component of a given module, represents the gene expression of each module. Modules related to clinical traits were identified by correlation between traits and the ME with cutoff of 0.8 ($p < 0.05$). In the corresponding module, hub genes had the highest correlation with the clinical trait. Here hub genes were identified based on gene significance (GS) > 0.9 and module membership (MM) > 0.85 [34]. Moreover, the *ERBB2* related genes in modules were found by the function exportNetworkToCytoscape in the WGCNA package with threshold 0.02.

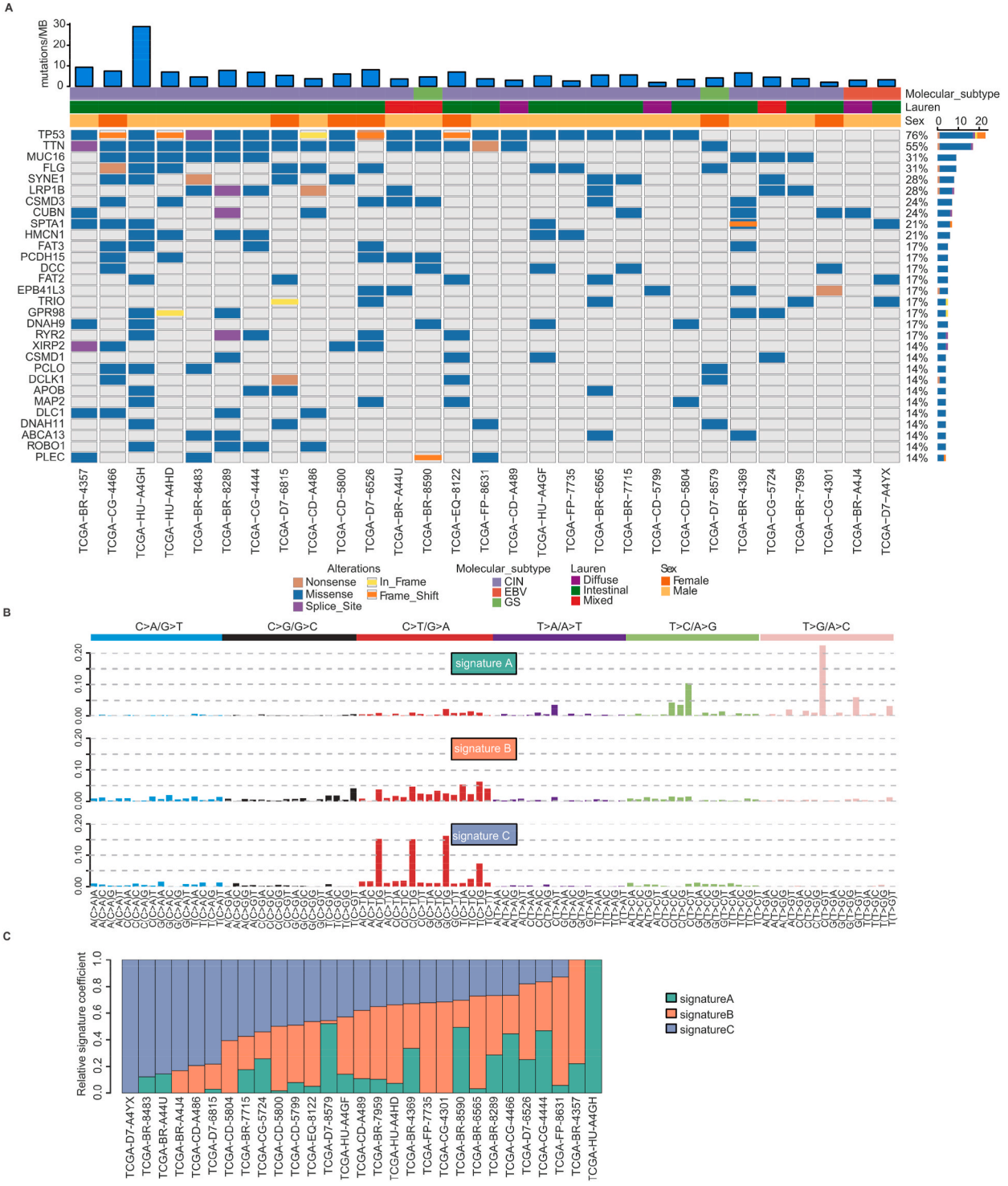

Fig. 1. The landscape of somatic mutations and mutational signatures of *ERBB2*-Amp type gastric cancer. **(A)** Somatic mutations of 29 *ERBB2*-Amp type gastric cancer samples. The matrix showed somatic mutations. The top histogram showed the frequency of mutation in each sample. The top tracks showed clinical trait, including Molecular_subtype, Lauren types, and Sex. The right histogram showed the number of somatic mutations of the gene. **(B)** Mutation signatures derived from WES data of 29 *ERBB2*-Amp type gastric cancer sample. **(C)** The distribution of mutation signatures in *ERBB2*-Amp type gastric cancer.

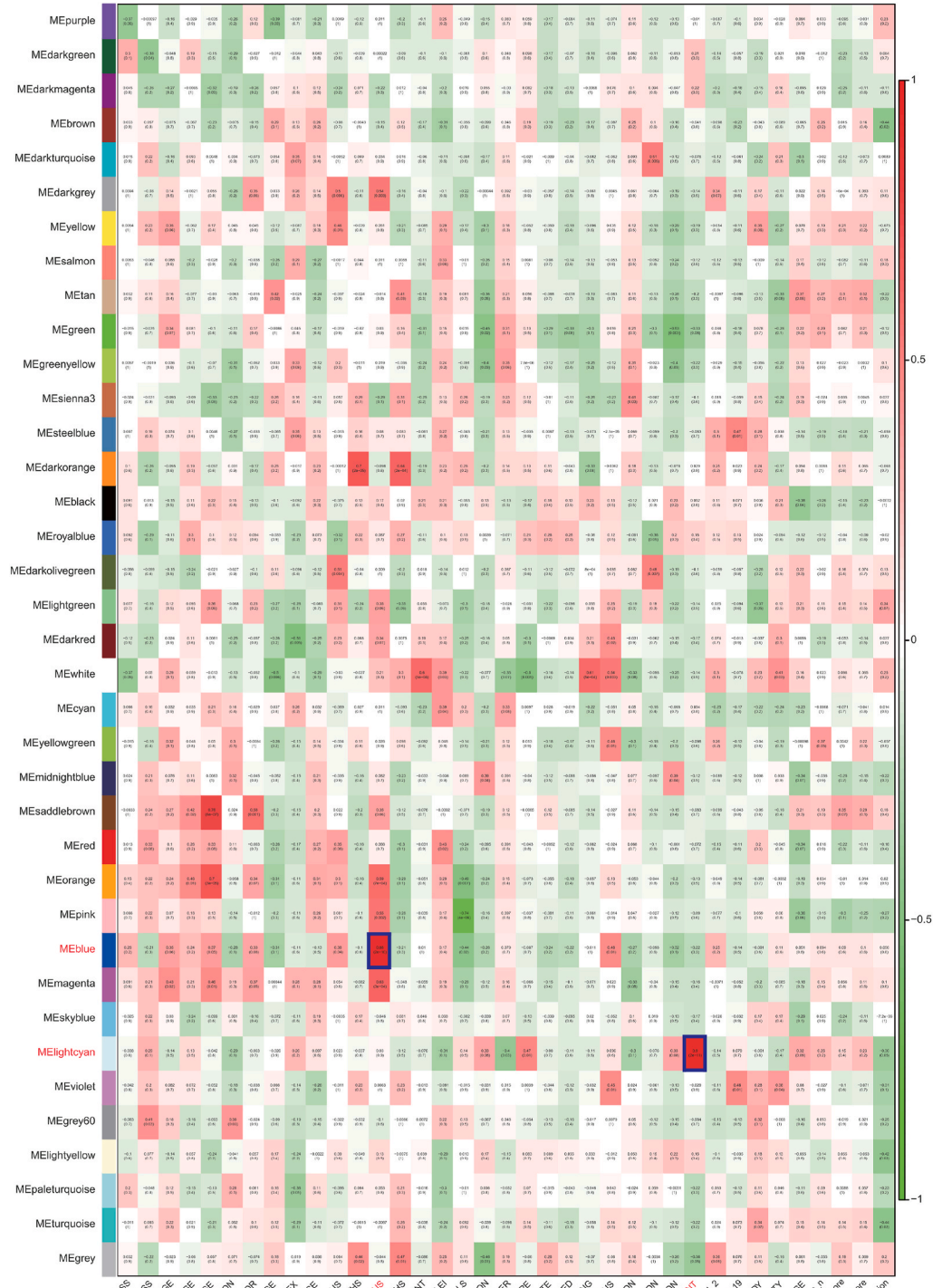

Fig. 2. Module-trait relationship. Correlation value of ME and clinical trait and P-value are shown in each cell.

2.4. Enrichment analysis

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment were performed by the clusterProfiler package [35]. The p value was adjusted by 'BH' method, and the cutoff value of padj was 0.05.

2.5. Single cell data process and analysis

Using Seurat R package [36] (v4.0.3; https://satijalab.org/seurat), cells containing more than 6000 or fewer than 500 detected genes, or more than 20% mitochondrial genes were removed; genes that were detected in fewer than 3 cells were also removed. Besides, DoubletFinder [37] (version 2.0.2; https://github.com/chris-mcginnis-ucsf/DoubletFinder) was used to remove doublets. Finally, a total of 69,524 high quality cells were used for downstream analysis.

In the subsequent analysis, 2000 highest variable genes were used in the principal component analysis by Seurat. Besides, harmony [38] was used to correct batch effect among samples. Using RunHarmony, RunUMAP, FindNeighbors, FindClusters (resolution = 0.5) of Seurat, cell clusters were identified. Then, clusters were annotated based on the expression level of canonical markers.

Moreover, regulons of transcription factors (TFs) were analyzed by pySCENIC [39] (v0.10.0, https://github.com/aertslab/pySCENIC), and EMT score was calculated by AddModuleScore of Seurat using the genes list from Ref. [3].

3. Results

3.1. Genome molecular characteristics of ERBB2-Amp type gastric cancer

To increase understanding of the genome molecular characteristic of *ERBB2*-Amp type gastric cancer, we chose the 29 gastric cancer samples with *ERBB2* amplification (CNA = +2), which both had WES data and RNA-seq data. Landscape showed the top 30 mutation genes, including TP53 (76%), TTN (55%), MUC16 (31%), FLG (31%), SYNE1 (28%), CSMD3 (28%) (Fig. 1A). In our study, there were more patients with CIN type than other molecular subtype in the *ERBB2*-Amp type gastric cancer. As reported by TCGA, CIN type gastric cancer had more TP53 mutation, more intestinal histology and RTK-RAS activation [4]. We also found the high ratio of TP53 mutation and intestinal histology in our study (Fig. 1A). Besides, there were more men than women in this cohort. Here, we also found some somatic SNVs, INDELs and CNV amplification and deletion in pathways of RTK-RAS and PIK3CA (Figure S1). Additionally, those samples could be divided into three mutation signatures (Fig. 1B and C). Generally, different mutation processes usually generate different combinations of mutation types, termed "signatures". Based on the analysis of 10,952 exomes and 1048 whole-genomes across 40 distinct types of human cancer, the version 2 of mutational signature contains 30 signatures showed in COSMIC (https://cancer.sanger.ac.uk/signatures/signatures_v2). Compared with those 30 mutation signatures previously identified [40], Signature A of *ERBB2*-Amp is similar to Signature 17, which has been found in gastric cancer; Signature C of *ERBB2*-Amp is similar to Signature 1, which was correlated with age and were found in all cancer types; while Signature B of *ERBB2*-Amp is weakly correlated with other signatures (Figure S2A).

Here, the characters of 18 gastric cancer samples with *ERBB2* deletion were also analyzed, and were compared with *ERBB2*-Amp. According to the RNA-seq data, the expression level of *ERBB2* in *ERBB2*-Amp was significantly higher than *ERBB2*-Del (Figure S3A). Moreover, the top 30 mutation genes of *ERBB2*-Amp were also explored in *ERBB2*-Del type cancer, and most of them had higher mutation ratio in *ERBB2*-Amp (Fig. 1A and Figure S3B). In addition, the mutation signatures of *ERBB2*-Del were obviously different with *ERBB2*-Amp. For example, Signature A of *ERBB2*-Del is similar to Signature 6, which is associated with defective DNA mismatch repair (Figure S2B, Figure S3C and S3D).

3.2. Construction of WGCNA and identification of key modules in ERBB2-Amp type gastric cancer

To comprehensively understand the gene co-expression network of *ERBB2*-Amp type gastric cancer, the top 10000 coefficient of variation genes were kept if the FPKM was more than 3 in any samples. Those genes were divided into 37 modules by WGCNA with the power of $\beta = 8$ as the soft threshold (Figure S4A and S4B). In Fig. 2, it shown genes associated with clinical traits including Lauren class, WHO class, Path T stage, Path N stage, Path M stage, anatomic region, residual tumor, age, sex, race, OS status, OS months, DFS status, DFS months, EBV present, percent tumor nuclei, percent tumor cells, percent lymphocyte infiltration, copy number cluster, molecular subtype, mutation rate, hypermutated, *CDKN2A* silencing, MSI status, *TP53* mutation, *PIK3CA* mutation, *ARID1A* mutation, *ARHGAP26_ARHGAP6_CLDN18* rearrangement, *MET* skipped exon 2, *MET* skipped exons 18 and 19, absolute extract ploidy, absolute extract purity, estimated leukocyte percentage, EstimateScore_n, ImmuneScore, ESTIMATEScore. Interestingly, the blue module ($R = 0.88$, $p = 2e{-}10$) was significantly correlated with DFS status while lightcyan module ($R = 0.90$, $p = 2e{-}11$) was highly related to *ARHGAP26_ARHGAP6_CLDN18* rearrangement (Fig. 2).

To identify significant biological process associated with traits in the modules, we performed GO and KEGG enrichment analysis. In the blue module, KEGG pathway analysis was significantly enriched in pathway of systemic lupus erythematosus ($p < 0.05$) and GO analysis was enriched in terms of DNA replication-dependent nucleosome assembly, nucleosome organization, chromatin assembly, chromatin assembly or disassembly, DNA conformation change. In the lightcyan module, GO terms were enriched in cytokine activity, SH2 domain binding, signaling receptor activator activity, receptor ligand activity, phosphatidylinositol 3-kinase binding (Figure S5). Moreover, hub genes were also identified with GS > 0.9 and MM > 0.85, which were shown in Table 1.

3.3. Identification of related genes and pathways in the turquoise module with ERBB2 gene

Modules were defined as densely interconnected gene clusters [28], herein we performed GO and KEGG enrichment analysis of the turquoise module which had 1217 genes including *ERBB2*. As shown in Fig. 3 (A, B) and Figure S6, KEGG pathways of the turquoise module mainly enriched in focal adhesion, ECM−receptor interaction, gap junction, regulation of actin cytoskeleton, Hedgehog signaling pathway, Calcium signaling pathway, Wnt signaling pathway, Pathways in cancer Basal cell carcinoma, while GO enrichment analysis showed terms of glycosaminoglycan binding, actin binding, growth factor. We also found that *ERBB2* was related to *MIEN1*, *GRB7* used exportNetworkToCytoscape with threshold 0.02.

3.4. High expression level of ERBB2 epithelial cells has high EMT score

According to Seurat analysis and cell annotation using marker genes, 69,524 cells were defined as eight cell types, including Epithelial, Endothelial, Fibroblast, T_NK, B, Plasma, Myeloid, Mast (Fig. 4A and B). As shown in Fig. 4C, the cell ratio of each sample has obvious heterogeneity. In downstream analysis, Epithelial was chosen as the research object. Based on the expression level of *ERBB2* gene, cells were classified as Positive and Negative group in each sample, and the cell ratios of those two groups were presented in Fig. 4D. Moreover, Epithelial of four samples (sample29, sample16, sample33, sample15) with more Positive cells (>35%) were subsequently studied. Using pySCENIC analysis, *TFAP2A* transcription factor regulon was found as the specific regulon in the $ERBB2^{high}$ group (expression >1, Fig. 4E). In both mouse and human systems, *TFAP2A* was a core positive transcription factor in the EMT process [41]. Compared to Negative group of those four samples, $ERBB2^{high}$ group all has higher EMT score (Fig. 4F), which indicated *ERBB2* was a facilitator in gastric cancer.

4. Discussion

Trastuzumab plus chemotherapy is now considered as the standard first-line therapy for patients with HER2+ advanced gastric cancer [15]. The DESTINY-Gastric01 trial confirmed that therapy with trastuzumab deruxtecan led to significant improvements in response and overall survival among patients with HER2+ gastric cancer [42]. The phase II trial demonstrated that pembrolizumab (the anti-programmed death 1 antibody) could be safely combined with trastuzumab and chemotherapy and had promising activity in HER2+ metastatic esophagogastric cancer [43]. The phase III KEYNOTE-811 study found trastuzumab plus pembrolizumab and chemotherapy could reduce tumor size of unresectable or metastatic, HER2+ gastric or gastro-oesophageal junction adenocarcinoma [44]. However, many other trials such as first-line TRIO-013/LOGiC [17] and JACOB [18], second-line TyTAN [19] and GATSBY [20], had not demonstrated the efficacy of an OS benefit for HER2+ gastric cancer, which might be due to heterogeneous HER2 expression, amplification or mutation of certain genes. Therefore, to further investigate molecular characteristics of *ERBB2*-Amp type gastric cancer to improve the targeted therapy, we analyzed mutations including SNVs, INDELs and CNV, mutation signatures, gene-trait correlations and *ERBB2* related module.

HER2+ type gastric cancer was defined as HER2 positive by IHC or FISH, which was usually accompanied with HER2 protein overexpression, *ERBB2* gene amplification or both. Here, we found the expression level of *ERBB2* in *ERBB2*-Amp was much greater than in *ERBB2*-Del (Figure S3A). *ERBB2* amplification could be as a potential therapeutic target in certain malignancies, including lung, ovary, bladder, bile duct cancers, endometrial, oesophageal and colorectal [45–52], although gastric cancer as a heterogeneous illness exhibited little OS advantage in some trails. Genomic alterations could acquire resistance of HER2-targeted therapies, such as baseline *CCNE1* amplifications [53], newly emergent amplifications of *MYC*, *EGFR*, *FGFR2* and *MET* with disease progression. Mutations in *ERBB2*/4, *NF1* and *PIK3CA*/R1/C3*1* could also affect the effectiveness of HER2-targeting [54,55]. In *ERBB2*-Amp type gastric cancer, we also found *CCNE1*, *EGFR*, *MET*, *MYC* amplification and *PIK3CA*, *ERBB2*/4, *NF1*, *PTEN* mutations (Fig. 1 and Figure S1). Moreover, there were many other amplification and mutation in pathways of PI3K, RTK-RAS, which may lead to primary drug resistance and affect the effect of targeted therapy (Figure S1).

WGCNA could make similarly expressed genes in the same module, identify the most key genes in every module and explore the connections between gene modules and clinical traits [28,56,57]. In turquoise module, which had *ERBB2* in it, microenvironment related pathways were enriched, such as extracellular matrix (ECM) and focal adhesion, which might be related to cancer metastasis and intestinal. As the main component of the extracellular microenvironment [58], the ECM is composed of a variety of insoluble extracellular macromolecules that provide cells with connectivity, support, pressure resistance, water retention and protection. ECM remodeling including synthesis, degradation and distribution [59], may greatly increase the propensity for tumor invasive and

Table 1
The hub genes in blue and lightcyan module.

Trait	Module	Hub genes
DFS status	blue	*RNU6-777P, AC004522.1, RNU6-927P, RNA5SP25, CLEC19A, Y_RNA, RPS26P42, AC096775.1, RPS26P41, RNU6-883P, RNU6-198P, LATS2-AS1, RANP8, AL513185.2, RPL26P29, AL512656.1, AL355001.1, MPRIP-AS1, RAB5CP1, AC022400.2, AL731563.3, AL596247.1, KRR1P1, ZDHHC20-IT1, AC073842.2, AC004951.3, AC004951.1, STAG3L5P-PVRIG2P-PILRB, AC004951.2, STAG3L5P*
ARHGAP26_ARHGAP6_CLDN18 rearrangement	lightcyan	*AC002066.1, A2ML1, LINC00973, UNC93B4, RPSAP52*

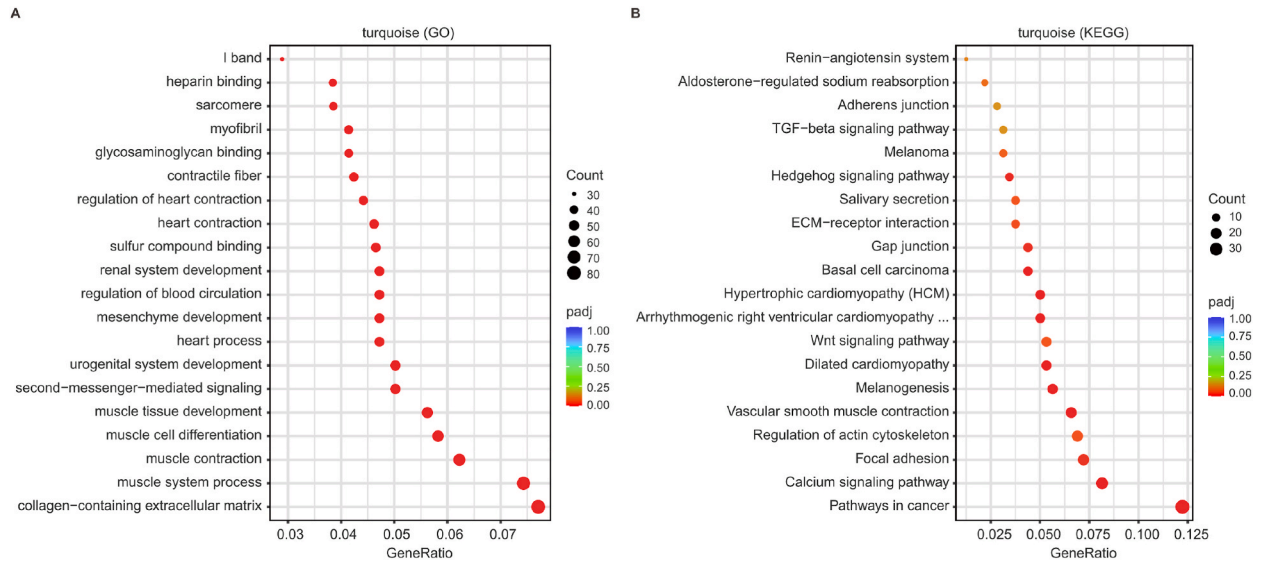

Fig. 3. Dot plot of GO and KEGG enrichment of turquoise module. **(A)** Top 20 GO terms of turquoise module (padj <0.05) were shown in dot plot. **(B)** Top 20 KEGG pathways of turquoise module (padj <0.05) were shown in dot plot.

metastatic spread [60–62]. In the recent studies of breast cancer and ovarian cancer, ECM remodeling, similar to embryonic development, was occurred during tumor progression and formed a loose microenvironment for tumor cells, making tumor cells highly proliferative, poorly differentiated, invasive and metastatic [63]. In gastric cancer, ECM compositions contributed to the tumorigenesis, progression, and poor survival [59]. Focal adhesion could anchor the cell to the substratum and mediate biochemical and mechanical and signaling. As one of types adhesive connecting the ECM and cell, formation of focal adhesion could promote tumor metastasis, the tumor microenvironment change, and cancer cell resistance [64]. In addition, the blue module was significantly correlated with DFS status (Fig. 2). DFS, namaly disease-free survival, was an important indicator to evaluate the clinical efficacy of treatment in cancer. GO analysis found the genes in blue module enriched in some terms about DNA replication (Figure S5A), which is a promising strategy for cancer treatment [65]. Moreover, the lightcyan module was highly related to *ARHGAP26_ARHGAP6_CLDN18* rearrangement (Fig. 2), which was a common fusion in gastric cancer [4], and impaired the epithelial integrity and wound healing [66]. In gastric signet-ring cell carcinoma, the frequent of *CLDN18-ARHGAP26/6* fusion was 25%, and *CLDN18-ARHGAP26/6* fusion was associated with signet-ring cell content, age at diagnosis, female/male ratio, and TNM stage [67].

ERBB2, *MIEN1* and *GRB7* are located the adjacent position to the chromosomal region 17q12-21 [68,69]. *MIEN1* (also known as RDX12, C17orf37, C35, and MGC14832) was highly expressed in breast tumor tissues and promote systemic metastasis as a key regulator of cancer cell invasion and migration [70,71]. Additionally, *MIEN1* was frequently amplified in gastric cancer [72]. MiR-124-5p could change the progression of gastric cancer by targeting *MIEN1* to inhibit cell proliferation and metastasis phenotype [73]. CircRNA_100876 sponges miR-136 could upregulate *MIEN1* expression, which could promote metastasis of gastric cancer [74]. *GRB7* was a key mediator of *EGFR*/ERBB signaling, which could be as a potential therapeutic target [75]. *ERBB2*, *MIEN1* and *GRB7* had similar CNV amplification and expression (Fig. 5), implying that *MIEN1* and *GRB7* might also play an important role in metamorphosis.

This study provides a systematic investigation of molecular features of *ERBB2*-Amp type gastric cancer. Moreover, the list of candidate genes and pathways may provide further information on the clinical trait of DFS status and *ARHGAP26_ARHGAP6_CLDN18* rearrangement. In addition, we identified pathways of focal adhesion, ECM-receptor interaction and *MIEN1*, *GRB7* amplification might influence cancer metabolism, and *TFAP2A* might affect EMT process, which deserved further study. *MIEN1*, *GRB7* and *TFAP2A* may serve as prospective therapeutic targets for the treatment of anti-HER2 drug-resistant individuals. In addition, medications that suppress the EMT process may be therapeutic for patients whose HER2-targeting treatment is ineffective.

However, the work also has some shortcomings. Although several intriguing genes, including *MIEN1*, *GRB7*, and *TFAP2A*, were found in *ERBB2*-Amp-type gastric cancer, little is known about their underlying mechanism. Whether or not those genes support a benefit of immunotherapy for patients with *ERBB2*-Amp-type gastric cancer remains unknown. In addition, HER2+ type gastric cancer samples are not identified in the TCGA gastric cancer data by FISH or IHC, therefore we are unable to assess the distinction between HER2+ type gastric cancer and *ERBB2*-Amp type gastric cancer.

Author contribution statement

Dongyan Cao: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.
Hongping Xu: Analyzed and interpreted the data; Wrote the paper.
Longteng Li: Analyzed and interpreted the data.
Zheng Ju: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

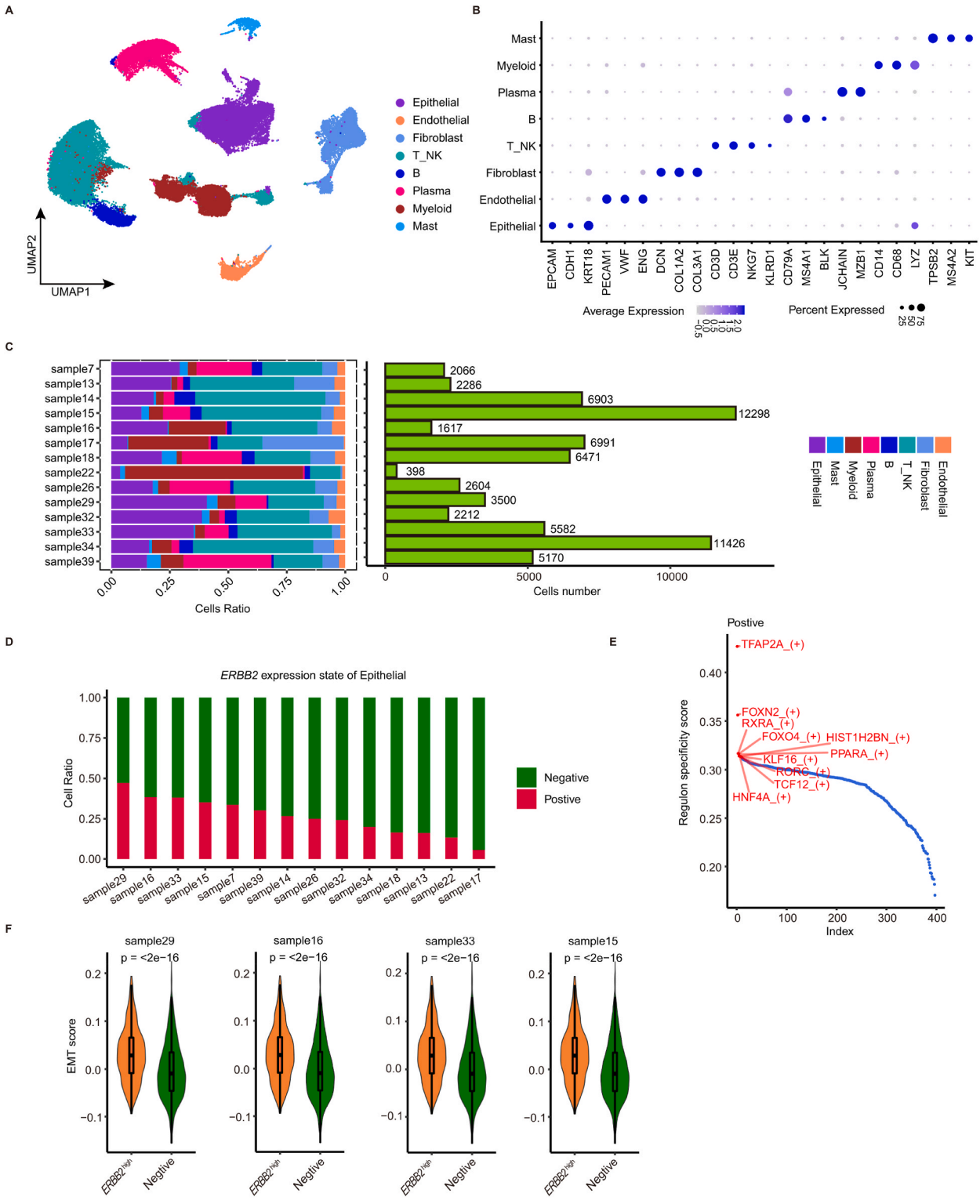

Fig. 4. Analysis of single-cell data of intestinal gastric cancer. **(A)** Uniform Manifold Approximation and Projection (UMAP) of 69,524 cells. Each dot in the UMAP represents a single cell. **(B)** Dot plot of marker genes in eight cell types. **(C)** The cells ratio of each cell type and cell number of 14 samples. **(D)** The cell ratio of Positive and Negative group in each sample. **(E)** Point plot of Regulon specificity score in *ERBB*^{high} group obtained from the pySCENIC. (F) Volin plot of the EMT score in *ERBB*^{high} and Negative group.

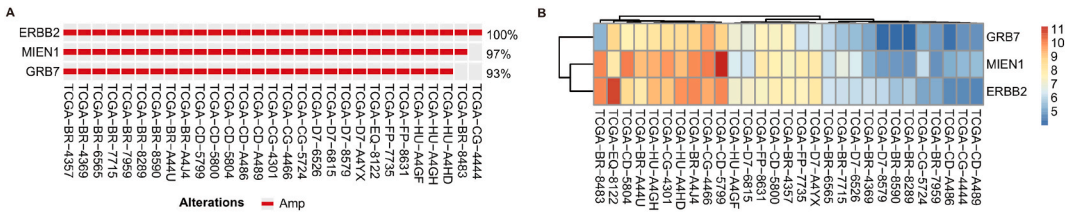

Fig. 5. Amplification and expression of *ERBB2*, *MIEN1* and *GRB7*. **(A)** The landscape of *ERBB2*, *MIEN1* and *GRB7* (CNA = +2). **(B)** Heatmap of *ERBB2*, *MIEN1* and *GRB7* expression based on log2(FPKM+1).

Baiqiang Zhai: Conceived and designed the experiments.

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Data availability statement

Data associated with this study has been deposited at: WES mutation, copy-number aberration (CNA) and clinical traits data were downloaded from cBioProtal (Stomach Adenocarcinoma (TCGA, Nature 2014), https://www.cbioportal.org/); RNA-seq FPKM data was collected from Xena (GDC TCGA Stomach Cancer (STAD), https://xenabrowser.net/);gene-cell matrix data of 14 single-cell intestinal gastric cancer samples (Tumor) were obtained from https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE183904.

Declaration of competing interest

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

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.heliyon.2023.e18654.

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