

Comprehensive *in-silico* molecular analysis of early-onset gastric cancer identifies novel genes implicated in disease characterization and progression (Review)

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Abstract. Gastric cancer, a prevalent and fatal form of cancer worldwide, is manifested at different age ranges during the lifespan. Approximately one-third of newly diagnosed gastric cancer cases are early-onset gastric cancer (EO-GC), which affects individuals under the age of 50 years. EO-GC tends to be more aggressive than late-onset gastric cancer (L-GC), with a faster and multifocal disease progression. Furthermore, EO-GC is associated with early metastatic disease. Recent research has underscored the need for a deeper understanding of EO-GC that promotes therapeutic approaches specific to EO-GC. The present study determined the main transcriptomic differences between EO-GC and L-GC. Transcriptomic expression data from The Cancer Genome Atlas-Stomach Adenocarcinoma were explored to elucidate whether age is associated with a specific genomic expression pattern and is associated with gastric cancer. Subsequently, a differential gene expression analysis of the EO-GC vs. L-GC groups was performed, providing new insights into EO-GC gene expression characteristics and their association with survival outcomes. Furthermore, the study focused on whether the influence of representative gene expression in EO-GC cases (*KLHL4*, *MAGEL2*, *CYP8B1*, *RNLS*, *CLDN6*, *MIOX*, *PNMA5* and *ACTL8* genes) may be associated with its aggressive phenotype and methylation profiles of these patients. In this review, the necessity of incorporating age as a crucial element

in understanding the disparities in outcomes for EO-GC cases in public datasets was discussed. Furthermore, this insight may be useful for targeted early personalized clinical interventions to improve patient prognosis and survival rates in EO-GC cases.

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1. Introduction

Gastric cancer (GC) is one of the most prevalent and lethal malignancies worldwide, with significant variations in age of onset (1). Population studies estimate that 30% of newly diagnosed GCs in the USA are early-onset GC (EO-GC), affecting individuals under 50 years (2). A study has highlighted the critical distinctions between EO-GC and late-onset GC (L-GC or traditional GC), emphasizing the need for tailored diagnostic and therapeutic approaches (3). Approximately 10% of EO-GC cases have a family history of the disease, primarily associated with germline mutations in the *CDH1* gene, which encodes the E-cadherin protein (3-5). These and other mutations significantly contribute to the hereditary diffuse GC risk, such as Lynch syndrome and Peutz-Jeghers syndrome, among others (3,6). By contrast, 90% of EO-GC cases lack a family history and are linked to environmental factors such as obesity, heavy alcohol consumption, cigarette smoking,

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Epstein-Barr virus infection and *Helicobacter pylori* infection (7,8).

In general, EO-GC exhibits a more aggressive clinical course than L-GC, with rapid and multifocal disease progression, poorly differentiated histology with a higher prevalence of diffuse histologic types and early metastasis (4,8). Experts agree that these differential clinical behaviors between EO-GC and L-GC may be due to distinctive somatic mutations in each type of GC (4,5,9).

A comprehensive genomic analysis by Han *et al* (4) revealed distinct mutational landscapes between EO-GC and L-GC. EO-GC exhibits higher mutation frequencies in genes such as TP53, CDH1 and MUC6 than L-GC. Triantafyllidis *et al* (8) also summarized the existing data supporting the hypothesis of a series of environmental factors highlighted in recent decades and which are mainly related to dietary habits, intestinal microbiome and an increase in the obese population interacting with genetic factors. All these factors lead to epigenetic changes in DNA and histones that would ultimately favor carcinogenesis at an early age.

A study revealed that EO-GC frequently shows a lower tumor mutation burden (6). However, it has higher mutation rates of genes related to the regulation of cell proliferation (PIK3CA, NOTCH1, ERBB4, CDH1, ATM and APC, among others), which may explain its aggressive nature and poorer prognosis in younger patients (9). The present review aligns with that of Machlowska *et al* (10), who identified several candidate genes with high mutation frequencies in EO-GC, illustrating the unique genetic landscape of EO-GC cases. Despite these advances, optimal screening and treatment strategies for EO-GC remain under investigation. The lack of consensus on the age cutoff for defining EO-GC complicates the establishment of uniform guidelines, as highlighted by Petrillo *et al* (5) and Ugai *et al* (11). Therefore, a differential molecular evaluation of GC cases based on the age of the patients may be essential for new therapeutic approaches to improve outcomes for patients with EO-GC.

A significant limitation currently encountered in data mining of EO-GC cases is that numerous metadata present in publicly available transcriptomic databases do not incorporate age as a variable. Consequently, as an initial approach used in the present study, the transcriptomic profiles of patients with GC were analyzed based on their age, encompassing both EO-GC and L-GC. Using multiple bioinformatics tools, RNA-sequencing (Seq) data from The Cancer Genome Atlas-Stomach Adenocarcinoma (TCGA-STAD) dataset were analyzed.

2. EO-GC exhibits a distinct transcriptomic expression profile

The study of transcriptomics in EO-GC plays a crucial role because it allows the exploration and understanding of the underlying molecular mechanisms and identification of biomarkers and transcription factors involved in the initiation and/or progression of the pathology.

Utilizing the 'TCGAbiolinks' R package (12), RNA-seq data [transcripts per million, (TPM)] and corresponding clinical information of n=377 cases were downloaded from the Genomic Data Commons portal. The data were filtered to

include only cases where the stomach was the primary tumor site with clear and complete clinical information. A total of n=32 cases were classified as EO-GC (≤ 50 years) and the remaining cases (n=345) were classified as L-GC (> 50 years). The clinicopathological characteristics are summarized in Table I.

As a first approach, it was evaluated whether age corresponds to a variable associated with changes in TCGA-STAD transcriptomic expression. To answer this, the EO-GC cases were categorized into two subgroups (21 to 40 years and 41 to 50 years), while the L-GC cases were separated into three groups (51 to 60 years, 61 to 80 years and > 80 years). A principal component analysis (PCA) was then performed to assess the variability of the TPM data matrix concerning these age categories using the 'FactoMineR' R package (13). Of note, the PCA results showed that the age groups of 21-40 and > 80 (81-100 years old) exhibited an evident graphic separation, mainly determined by the second principal component (Fig. 1).

Subsequently, when evaluating the main genes associated with these differences, a strong influence of the genes related to cytoskeleton and cell motility (e.g., ACTB, ACTN4, KRT4, KRT8), immunoglobulins (e.g., IGHA1, IGHA2, IGHG1, IGHG2), metabolism and energy production (e.g., PKM, MT-ATP6, MT-CO1, MT-CO2, MT-CO3) and cell adhesion and communication (e.g., CD24, CD74, EPCAM, CLDN3) was observed, among others. These genes were selected based on their contribution to the second PC (Dim2) from the PCA, as shown in Table SI.

Subsequently, differential gene expression analysis of the EO-GC vs. L-GC groups was conducted utilizing the R package 'DESeq2' (14). To evaluate this parameter, subdivisions by age category were not implemented due to the limited representation of EO-GC in the dataset. Fig. 2 and Table SII illustrate the top 30 genes exhibiting the highest differential expression between EO-GC and L-GCs.

Comprehensive transcriptomic profiling elucidated significant disparities in the gene expression profiles between EO-GC and L-GC (Fig. 2A). KLHL4, MAGEL2, RNLS and CYP8B1 demonstrated upregulation in EO-GC. Concurrently, CLDN6, MIOX, PNMA5 and ACTL8 exhibited downregulation (Fig. 2B). Subsequently, the potential predictive value of these selected genes was assessed utilizing the UALCAN data portal (15). The analysis revealed that elevated expression levels of KLHL4, MAGEL2 and CLDN6 were associated with reduced survival rates in the TCGA-STAD platform dataset when age was not considered as a variable (Fig. 3A). In addition, through the development of receiver operating characteristic (ROC) curves to depict sensitivity and specificity and quantify the area under the curve (AUC) using the 'survivalROC' R package (16), it was observed that the EO-GC upregulated genes (KLHL4, MAGEL2, RNLS and CYP8B1) showed differential survival predictions based on risk scores (Fig. 3B).

Lastly, considering that aging has been incorporated as a crucial variable in the understanding of genomic instability (17) and the multiple efforts to generate early biomarkers based on DNA methylations in GC (18,19), the targeted evaluation of the methylation patterns of these genes according to EO-GC and L-GC classification may be considered. This highlights the necessity of exploring how

Table I. Clinicopathological characteristics of The Cancer Genome Atlas-Stomach Adenocarcinoma cases according to age category.

Item	Early-onset gastric cancer (≤50 years)			Late-onset gastric cancer (>50 years)				P-value (EO-GC vs. L-GC)
	21-40 years	41-50 years	Total	51-60 years	61-80 years	≥81 years	Total	
Age, years	35.0±3.4	46.3±2.7	44.9±4.6	56.3±2.8	70.4±5.2	84.3±3.2	67.8±8.8	<0.001
Sex								0.467
Female	1 (25.0)	21 (75.0)	22 (68.8)	27 (30.7)	89 (38.2)	10 (41.7)	126 (36.5)	
Male	3 (75.0)	7 (25.0)	10 (31.2)	61 (69.3)	144 (61.8)	14 (58.3)	219 (63.5)	
Site of resection or biopsy								0.911
Body of stomach	1 (25)	3 (10.8)	4 (12.5)	21 (23.9)	59 (25.3)	6 (25.0)	86 (24.9)	
Cardia	1 (25)	7 (25.0)	8 (25.0)	20 (22.7)	56 (24.0)	5 (20.8)	81 (23.5)	
Fundus of stomach	-	2 (7.1)	2 (6.3)	14 (15.9)	28 (12.0)	4 (16.7)	46 (13.3)	
Gastric antrum	2 (50)	14 (50.0)	16 (50.0)	31 (35.2)	81 (34.8)	9 (37.5)	121 (35.1)	
Stomach, NOS	-	2 (7.1)	2 (6.3)	2 (2.3)	9 (3.9)	-	11 (3.2)	
Stage								0.502
I	-	4 (14.3)	4 (12.5)	8 (9.1)	35 (15.0)	8 (33.3)	51 (14.8)	
II	-	8 (28.5)	8 (25.0)	31 (35.2)	75 (32.2)	6 (25.0)	112 (32.5)	
III	4 (100)	11 (39.3)	15 (46.9)	38 (43.2)	104 (44.6)	8 (33.3)	150 (43.5)	
IV	-	5 (17.9)	5 (15.6)	11 (12.5)	19 (8.2)	2 (8.4)	32 (9.2)	
Total	4 (12.50)	28 (87.50)	32 (100)	88 (25.5)	233 (67.5)	24 (7.0)	345 (100)	

Values are expressed as the mean ± standard deviation or n (%). NOS, not otherwise specified.

age-related genomic changes contribute to cancer progression.

The following sections will delve into a detailed analysis of their functional implications and potential contributions to the pathogenesis and prognosis of EO-GC.

3. KLHL4, MAGEL2, RNLS and CYP8B1 are upregulated genes in EO-GC

To obtain a deeper understanding of the molecular mechanisms underlying EO-GC, a comprehensive transcriptomic analysis was conducted. This approach aimed to identify differentially expressed genes and pathways that could distinguish EO-GC from L-GC, providing insight into potential drivers of the disease.

KLHL4 gene expression in GC. KLHL4 is part of a family of 42 proteins, each characterized by a BTB/POZ domain at the N-terminus, a BACK domain in the middle and 5-6 Kelch domains at the C-terminus. Most KLHL proteins associate with Cullin 3 to form a Cullin-E3 ubiquitin ligase complex, acting as adapters that recognize target proteins via the Kelch domains during ubiquitination. These proteins are crucial for various cellular processes, including cytoskeletal organization, ion channel gating, transcriptional suppression and protein targeting for ubiquitination (20). Furthermore, the KLHL4 gene has also been linked to the synthesis and transport of long-chain fatty acids (Fig. 4A; pink cluster), a process implicated in diabetes and heart diseases. However, the mechanism of fatty acid entry into cells remains poorly

understood and is thought to involve protein-mediated transport (21). KLHL4 is associated with the kinesin superfamily proteins (KIFs) family (Fig. 4A; light green cluster), which is essential for intracellular transport and fundamental for cellular function, survival and tissue morphogenesis. KIFs act as molecular motors that directionally transport cargo, including organelles, protein complexes and mRNAs, and play crucial roles in tumor suppression (22).

The nuclear factor erythroid 2-related factor 2 (NRF2) is a transcription factor that regulates the cellular antioxidant response and strongly correlates with KLHL4 expression (Fig. 4A; yellow cluster). NRF2 regulates genes that protect cells against oxidative stress, which is significant in cancer. The primary regulator of NRF2 activity is its interaction with Kelch-like ECH-associated protein 1 (Keap1). Under normal conditions, Keap1 binds to NRF2, promoting its degradation, but oxidative stress disrupts this interaction, allowing NRF2 to activate protective genes (23). For instance, during the carcinogenesis of GC (24), oxidative stress promotes the transcription of genes that protect against oxidative and electrophilic stress (25-27). In addition, NRF2 expression is associated with tumor-associated macrophages (TAMs) M2 polarization, which is well-known for exerting a pro-tumorigenic effect. TAMs are critical in the tumor microenvironment (TME) for eliminating tumor cells by creating a toxic environment. Polarization is linked to the NRF2 target protein Cu/Zn-superoxide dismutase, associated with M2 polarization through a redox-sensitive mechanism. Oxidative stress and reactive oxygen species are vital for M2 macrophage activation, promoting an immunosuppressive

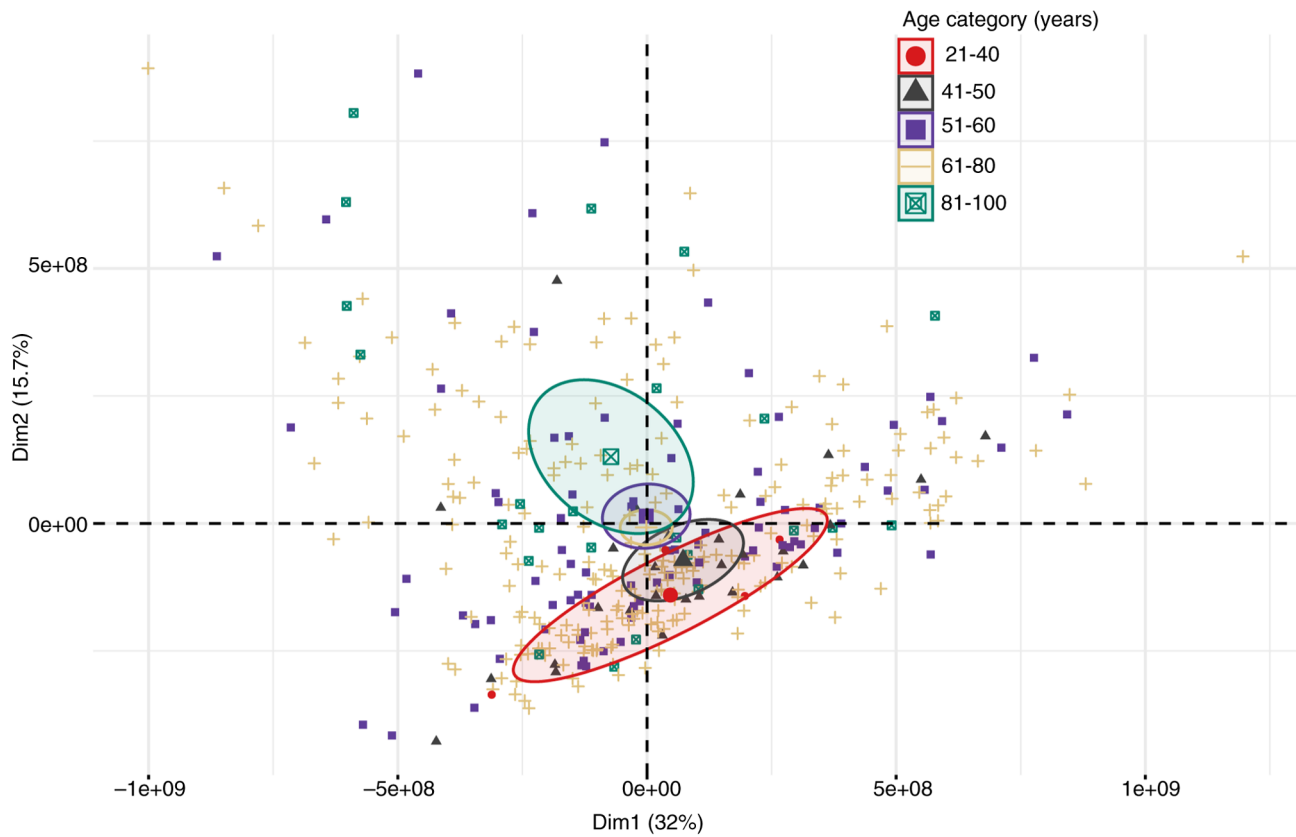


Figure 1. PCA on the TCGA-STAD cohort based on age category. Using the transcriptomic expression matrix (transcripts per million), patients on the TCGA-STAD platform were classified into five groups based on age: Red cluster, 21-40 years; black cluster, 41-50 years; purple cluster, 51-60 years; yellow cluster, 61-80 years; and green cluster, 81-100 years. PCA was performed using the 'FactoMineR' R package. PCA, principal component analysis; TCGA-STAD, The Cancer Genome Atlas-Stomach Adenocarcinoma.

TME in GC (28-30). Therefore, pharmacological activation of NRF2 is a promising therapeutic approach for chronic diseases underlined by oxidative stress and inflammation (31) (Fig. 4A; yellow cluster).

Analysis of the KLHL4 expression in GC. Based on the TCGA-STAD data, EO-GC cases exhibited a higher KLHL4 gene expression than L-GC (Fig. 4Ba), principally in the 21-40-year age range (Fig. 4Bb). Concerning the methylation KLHL4 score, no differences between EO-GC and L-GC were observed (Fig. 4Ca), even when subjects were categorized by age (Fig. 4Cb). Lastly, the Kaplan-Meier analysis, using the median KLHL4 gene expression showed a non-significant tendency where a high expression of KLHL4 would be associated with lower survival of patients with EO-GC from the TCGA-STAD dataset (Fig. 4D). The expression data for KLHL4 in Fig. 4 highlight its significantly higher expression in EO-GC compared to L-GC. This observation aligns with the Kaplan-Meier survival analysis, which, although not statistically significant, suggests a trend where higher KLHL4 expression is associated with poorer survival.

In summary, the upregulation of KLHL4 in EO-GC suggests its potential involvement in enhancing intracellular transport and oxidative stress responses, both of which may contribute to the aggressive clinical phenotype observed in these patients. These processes likely shape the TME by supporting immune

evasion and promoting tumor cell survival, thereby linking increased KLHL4 expression to poorer prognosis.

MAGEL2 gene expression in GC. Following the identification of KLHL4, MAGEL2, another gene found to be upregulated in EO-GC, was next examined. MAGEL2 acts as a tissue-specific regulator of the retromer-dependent endosomal protein recycling pathway, important for secretory granule formation and maturation (32). The retromer complex, composed of VPS26, VPS29 and VPS35, facilitates the recycling of proteins from the endocytic pathway back to the plasma membrane and is critical in secretion regulation (33).

In addition to its role in the endosomal recycling pathway, MAGEL2 is involved in ubiquitination, interacting with and stimulating E3 RING ubiquitin ligases (34,35). This interaction highlights its significant role in ubiquitin processes.

Data mining of the TCGA-STAD platform revealed an overexpression of MAGEL2 in GC, with associations to the retromer multimeric protein complex and ubiquitination system (Fig. 5A; red cluster).

MAGEL2 additionally exhibits interactions with the structural maintenance of chromosome (SMC) protein complexes, which play crucial roles in chromatin structure reorganization, chromosome segregation and DNA repair. The interaction between SMC5-SMC6 proteins and MAGEL2, identified in the TCGA-STAD database, suggests a potential role in DNA double-strand break repair through homologous recombination

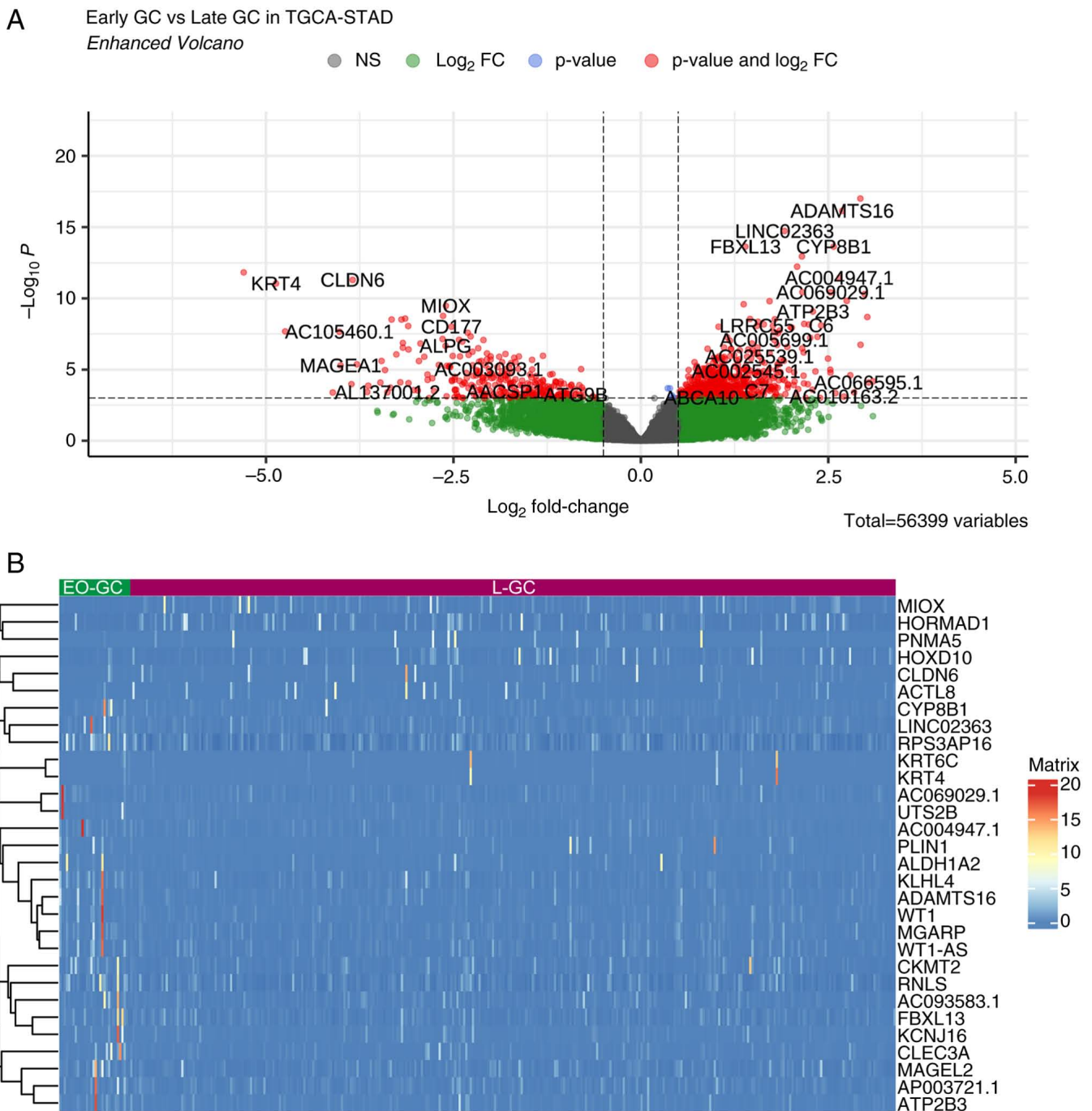


Figure 2. DGEA of EO-GC vs. L-GC groups from the TCGA-STAD dataset. (A) Volcano plot of differentially expressed genes among a total of $n=56,399$ genes. (B) Heatmap of the top 30 more differentiated genes between both groups. The green bar represents the EO-GC and the magenta bar represents the L-GC group. DGEA analysis was performed using the 'DESeq2' R package. Genes with higher expression levels are shown in red, while those with lower expression levels are shown in blue (matrix expression). DGEA, differential gene expression analysis; FC, fold change; EO-GC, early-onset gastric cancer; L-GC, late-onset gastric cancer; NS, no significance; TCGA-STAD, The Cancer Genome Atlas-Stomach Adenocarcinoma.

in patients with GC (36) (Fig. 5A; yellow cluster). Further protein-protein association networks and functional enrichment analyses using the Search Tool for the Retrieval of Interacting Genes and proteins (STRING; <https://string-db.org/>) analysis revealed that MAGEL2 interacts with various transcription factors and RNA-binding proteins, particularly those involved in mRNA metabolic processes. Notably, MAGEL2 co-immunoprecipitates with YTHDF2, reducing its nuclear accumulation after heat shock (37) (Fig. 5A; green cluster).

Analysis of MAGEL2 expression in GC. Based on the TCGA-STAD data, EO-GC cases exhibited significantly higher MAGEL2 gene expression than L-GC (Fig. 5Ba); this difference was not as evident when patients were categorized by age (Fig. 5Bb). Concerning the methylation score of MAGEL2, no statistically significant differences were observed between EO-GC and L-GC (Fig. 5Ca); however, a non-significant decreasing trend in methylation levels was noted with increasing patient age (Fig. 5Cb). Lastly, the Kaplan-Meier survival analysis demonstrated that higher MAGEL2 gene expression is

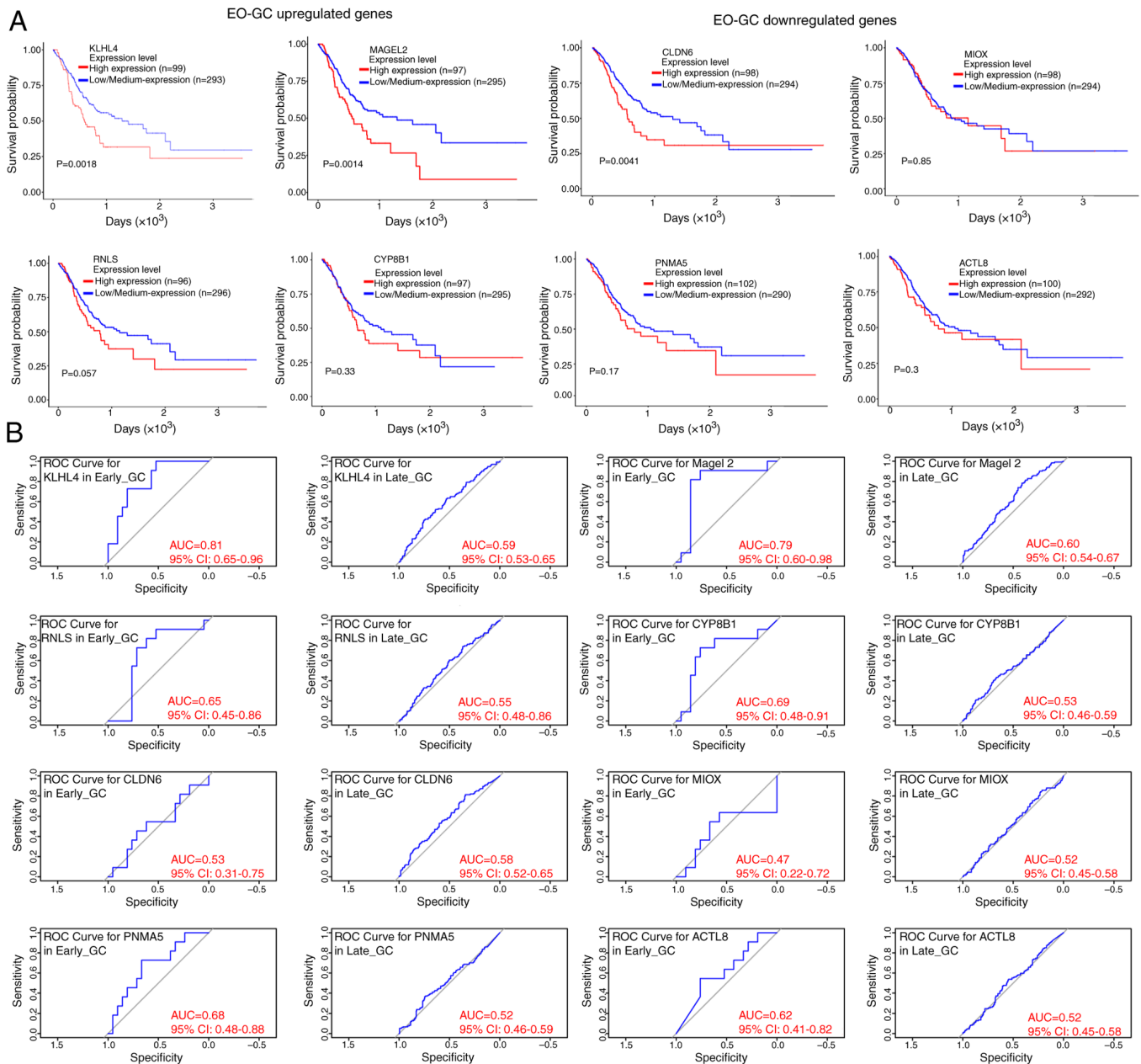


Figure 3. Prognostic value of differentially expressed genes in EO-GC in the TCGA-STAD dataset and ROC curve analysis. (A) Kaplan-Meier survival curves for the TCGA-STAD dataset based on the expression levels of genes upregulated in EO-GC, including KLHL4, MAGEL2, RNLS and CYP8B1, and downregulated genes in EO-GC, namely CLDN6, MIOX, PNMA5 and ACTL8 (based on UALCAN portal data). P-values were calculated to assess statistical significance. (B) ROC curves depicting the sensitivity and specificity of survival predictions based on risk scores for the upregulated and downregulated EO-GC genes and their comparison with L-GC. The AUC was quantified using the 'survivalROC' R package. The optimal cutoff risk score was determined at the turning point of each ROC curve. TCGA-STAD, The Cancer Genome Atlas-Stomach Adenocarcinoma; ROC, receiver operating characteristic; AUC, area under the ROC curve; EO-GC, early-onset gastric cancer; L-GC, late-onset gastric cancer.

associated with low survival in EO-GC (Fig. 5D). This comprehensive analysis underscores the importance of MAGEL2 in EO-GC, highlighting its overexpression and involvement in critical cellular pathways and carcinogenic processes.

The expression patterns of MAGEL2 in Fig. 5 reveal its upregulation in EO-GC, particularly among younger age groups. The Kaplan-Meier analysis indicated that elevated MAGEL2 expression is associated with unfavorable survival outcomes. This suggests that MAGEL2's role in protein recycling and chromatin remodeling may contribute to tumor progression and poor prognosis in patients with EO-GC.

In summary, the overexpression of MAGEL2 in EO-GC may exacerbate disruptions in protein recycling and chromatin structure, leading to cellular dysfunctions that support cancer progression. Its role in endosomal recycling and ubiquitination highlights its potential as a mediator of poor prognosis through tumor-promoting pathways.

RNLS gene expression in GC. Data mining of KLHL4 and MAGEL2 revealed an association with RNLS, a gene involved in oxidative stress and immune regulation. RNLS is an FAD-dependent amine oxidase that metabolizes

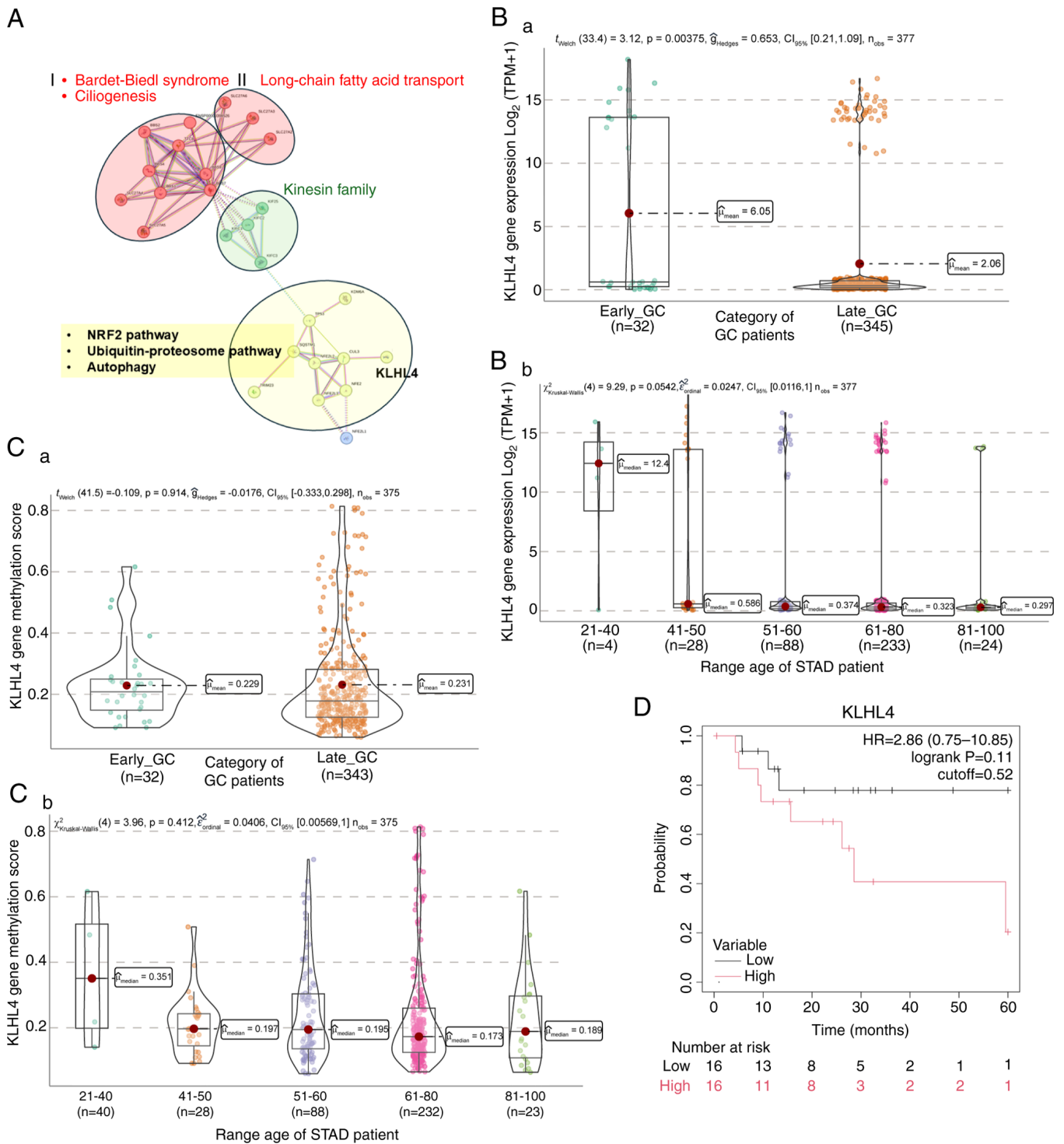


Figure 4. Expression of KLHL4 in patients with EO-GC. (A) The STRING identified four clusters of related proteins. (Ba) Comparison of transcript KLHL4 levels between EO-GC and L-GC (upper graph). (Bb) Transcript expression according to age range (bottom graph). (Ca) Comparison of KLHL4 gene methylation score between EO-GC and L-GC (upper graph). (Cb) Transcript according to age range (bottom graph). (D) Kaplan-Meier analysis of patients with EO-GC based on median KLHL4 gene expression (cutoff=0.52). Welch's t-test and the F-test were performed through the 'ggstatsplot' R package. EO-GC, early-onset gastric cancer; L-GC, late-onset gastric cancer; HR, hazard ratio.

water-soluble vitamins and nicotine. This enzymatic hormone, secreted by the kidneys and circulating in the bloodstream, oxidizes the less abundant forms of 1,2-dihydro-beta-NAD(P) and 1,6-dihydro-beta-NAD(P) to beta-NAD(P) (+) (38,39). RNLS impacts various cell types, suggesting similar roles in cancer. Its transcript levels are increased in pancreatic cancer, melanoma and other malignancies. In these cancers,

higher RNLS levels are associated with shorter survival (40). Treatment with anti-RNLS antibodies at the single-cell level resulted in increased tumor density of macrophages, neutrophils and lymphocytes and increased expression of IFN- γ and granzyme B in natural killer cells and T cells in murine melanoma models (41). The presence of RNLS in both cancer and immune cells suggests that multiple cell

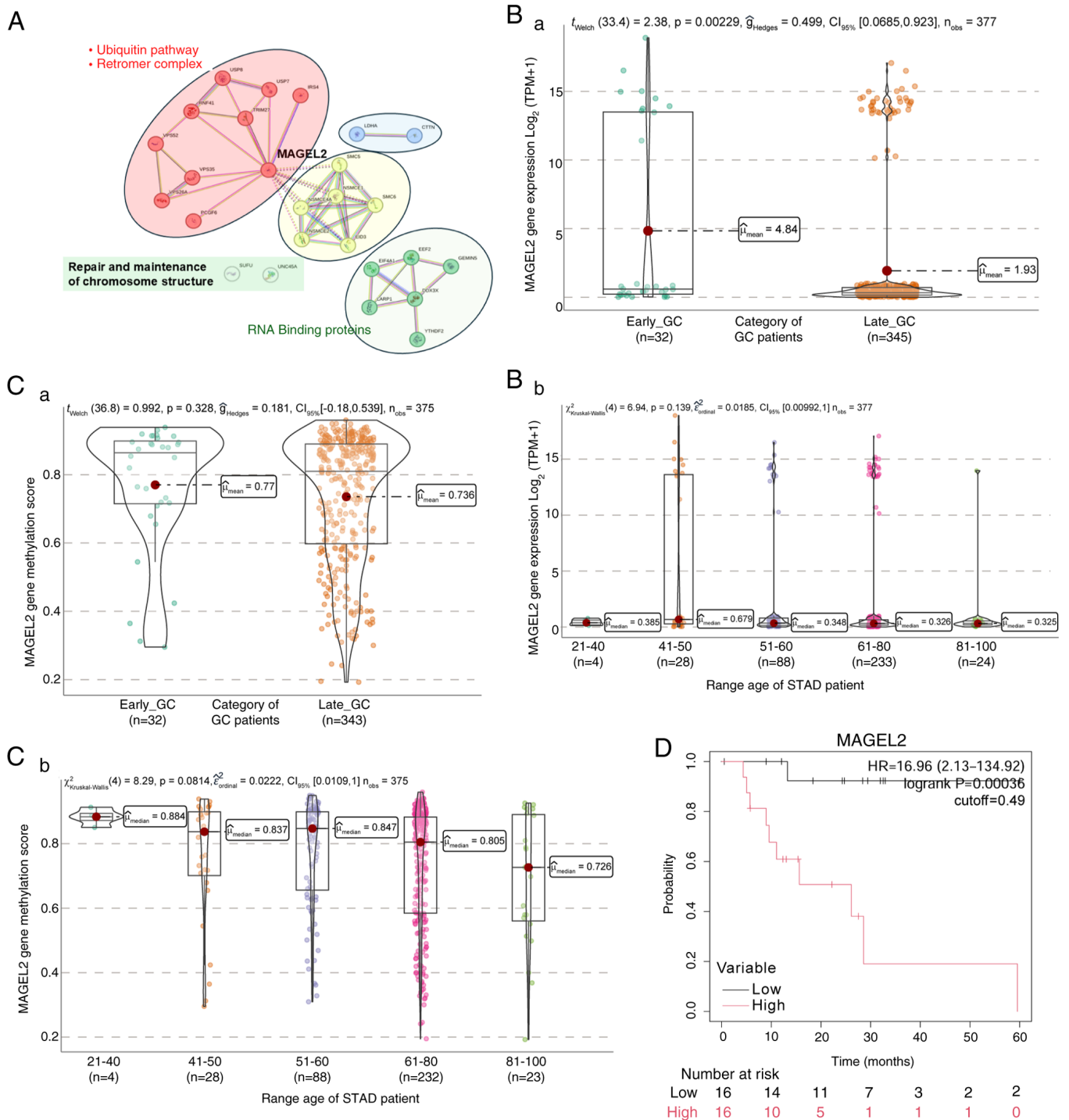


Figure 5. Expression of MAGEL2 in patients with EO-GC. (A) The STRING analysis of the proteins identified four clusters of related proteins. (Ba) Comparison of transcript MAGEL2 levels between EO-GC and L-GC (upper graph). (Bb) Transcript according to age range (bottom graph). (Ca) Comparison of MAGEL2 gene methylation score between EO-GC and L-GC (upper graph). (Cb) Gene methylation levels according to age range (bottom graph). (D) Kaplan-Meier analysis of patients with EO-GC based on median MAGEL2 gene expression (cutoff=0.49). Welch's t-test and the F-test were performed through the 'ggstatsplot' R package. EO-GC, early-onset gastric cancer; L-GC, late-onset gastric cancer; HR, hazard ratio.

types may contribute to the effects of RNLS on cancer cell growth (42,43).

STRING analysis, as a functional protein association network, showed a remarkable association between RNLS and divalent cation transporting channels, such as ATPase plasma membrane Ca^{2+} transporting 4 (ATP2B4) (Fig. 6A; green cluster), which facilitates divalent cation transport, and ATP4B, which is crucial for gastric acid secretion (44). RNLS

also has a significant relationship with zinc finger protein 148 (ZNF148), a member of the Kruppel family of zinc finger DNA-binding proteins (Fig. 6A; green cluster). Increased ZNF148 expression has been linked to lower survival in colorectal cancer. ZNF148 influences the expression of multiple matrix metalloproteinases, which have protective and damaging effects during inflammation and are crucial for health maintenance (45-48).

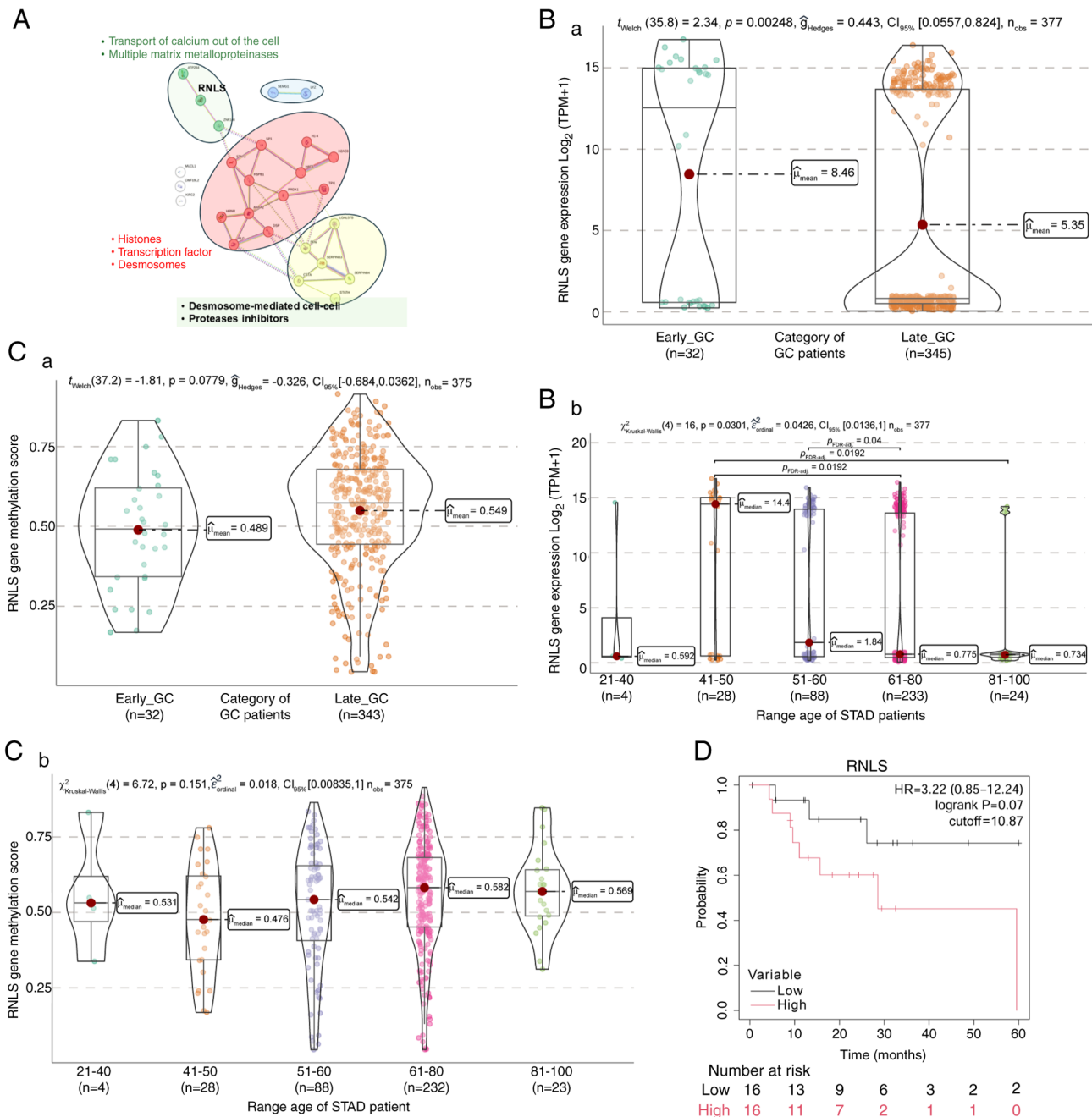


Figure 6. Expression of RNLS in patients with EO-GC. (A) The STRING analysis of the proteins identified three clusters of related proteins. (Ba) Comparison of transcript RNLS levels between EO-GC and L-GC (upper graph). (Bb) Transcript RNLS levels according to age range (bottom graph). (Ca) Comparison of RNLS gene methylation score between EO-GC and L-GC (upper graph). (Cb) Methylation scores according to age range (bottom graph). (D) Kaplan-Meier analysis of patients with EO-GC based on median RNLS gene expression (cutoff=10.87). Welch's t-test and the F-test were performed through the 'ggstatsplot' R package. EO-GC, early-onset gastric cancer; L-GC, late-onset gastric cancer; HR, hazard ratio; FDR, false discovery rate.

ZNF148 protein directly engages with two transcription factors, STAT3 and SP1, which control gene transcription. It also interacts with several histone-coding genes, including H1-4, H2AC8 and H4C6. Furthermore, ZNF148 is associated with genes that contribute to desmosome formation, such as desmoplakin, filaggrin, hornerin and Annexin A2 (ANXA2). ANXA2 encodes a member of the annexin family, which includes calcium-dependent phospholipid-binding proteins that play roles in cellular growth regulation and signal transduction pathways (Fig. 6A; red cluster).

Another group of genes related to RNLS (Fig. 6A; yellow cluster) includes proteins like galectin 7B (LGALS7B), which are involved in cell-cell and cell-matrix interactions necessary for normal growth control. LGALS7B has a tumor-suppressive function, with gene down-regulation in GC (49). Stratifin is an adapter protein regulating general and specialized signaling pathways, playing a significant role in cell proliferation and metastasis in GC (50,51).

Analysis of RNLS expression in GC. Based on the TCGA-STAD data, EO-GC cases exhibited a higher RNLS gene expression than L-GC (Fig. 6Ba), principally in the

41-50-year age range (Fig. 6Bb). Concerning the methylation score of RNLS, an insignificant increase in the methylation score was found in L-GC (Fig. 6Ca), but certain changes in methylation levels were observed in the 41-50-year age range (Fig. 6Cb). Lastly, the Kaplan-Meier survival analysis demonstrated that higher RNLS gene expression showed an insignificant trend toward lower overall survival in patients with EO-GC (Fig. 6D).

Fig. 6 demonstrates the increased expression of RNLS in EO-GC, particularly in the 41-50-year age group. The Kaplan-Meier analysis further shows a trend where higher RNLS expression is linked to reduced survival, emphasizing its potential involvement in oxidative stress regulation and immune evasion, key factors in EO-GC progression.

In summary, the increased expression of RNLS in EO-GC highlights its role in oxidative stress regulation and immune modulation. These functions may contribute to the establishment of an immunosuppressive TME, promoting cancer cell survival and explaining its association with poor prognosis.

CYP8B1 gene expression in GC. In addition to KLHL4, MAGEL2 and RNLS, cytochrome P450 family 8 subfamily B member 1 (CYP8B1) was also identified to be upregulated in EO-GC. This gene's role in bile acid metabolism and steroid hormone synthesis suggests a potential connection between metabolic reprogramming and EO-GC progression.

CYP8B1 plays a crucial role in metabolic pathways such as steroid hormone synthesis, bile acid metabolism, cholesterol metabolism and lipid homeostasis. In steroid hormone synthesis, CYP8B1 collaborates with enzymes such as hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 1,2,7 (HSD3B1, HSB3B2 and HSB3B7) (52,53) (Fig. 7A; yellow cluster). L-GC has been linked to androgens, estrogens, progesterone, their receptors and related signals (54). Cellular responses to steroid hormones, including ESR1, ESR2 and AR, are facilitated by hormone-receptor binding. ESR1 has been implicated in the cancer-promoting effects of estrogen in various cancers, including breast, colon, prostate and gastric cells. However, the association between these receptors and GC has yielded inconsistent results in several studies (55-57).

Additionally, the CYP8B1 pathway produces bile acids, which serve as potent signaling molecules that influence various metabolic processes, such as lipid homeostasis, glucose regulation and microbiota composition (Fig. 7A; red cluster). These bile acids are synthesized from cholesterol in the liver through the action of key enzymes, including CYP8B1 and CYP7A1, which are transcriptionally regulated by NR1H4 (nuclear receptor subfamily 1 group H member 4), a nuclear receptor also known as the bile acid receptor. Lastly, research has demonstrated a correlation between the presence of gastric intestinal metaplasia and an increased risk of gastric cancer, particularly for the intestinal subtype, which follows a well-established carcinogenic cascade (58). A retrospective study showed that high levels of bile acids in the stomach were associated with a higher incidence of GC (59,60).

Analysis with STRING revealed a direct relationship between CYP8B1 and hepatocyte nuclear factor 4 α (HNF4 α) involving bile acid homeostasis (Fig. 7A; blue circle). HNF4 α

is a transcription factor that binds DNA as a homodimer and regulates genes preferentially expressed in the liver. It plays a central role in bile acid homeostasis by controlling genes involved in bile acid biosynthesis, including hydroxylation and beta-oxidation of the cholesterol side chain *in vivo* (61).

Analysis of CYP8B1 expression in GC. Using TCGA-STAD data, it was verified that CYP8B1 transcript expression in EO-GC and L-GC individuals did not exhibit any statistically significant differences (Fig. 7Ba), nor was any change observed when patients were categorized by age (Fig. 7Bb). The methylation levels of the gene did not show any significant differences between both groups of patients (Fig. 7Ca). Subsequently, when subjects were categorized into different age groups, a statistically significant difference in the CYP8B1 methylation score between the 51-60-year age range and the 61-80-year age range was found (Fig. 7Cb). Lastly, Kaplan-Meier survival analysis revealed a non-significant trend toward better overall survival in patients with EO-GC with lower CYP8B1 gene expression (Fig. 7D).

The data in Fig. 7 show no significant differences in CYP8B1 expression between EO-GC and L-GC. However, the Kaplan-Meier analysis indicated a trend where lower CYP8B1 expression is associated with better survival outcomes. This finding highlights its potential role in EO-GC pathogenesis.

In summary, the role of CYP8B1 in bile acid metabolism and lipid homeostasis suggests that its upregulation in EO-GC could contribute to metabolic reprogramming in tumor cells. By influencing bile acid signaling and microbiota composition, CYP8B1 may drive gastric carcinogenesis and affect patient outcomes.

4. CLDN6, MIOX, PNAM5 and ACTL8 are downregulated genes in EO-GC

CLDN6 gene expression in GC. Tight junctions (TJ) are critical for the functioning of epithelial and endothelial cells, maintaining cell polarity, adhesion and permeability. Reduced TJ integrity leads to increased tissue permeability, a characteristic of tumors and inflamed tissues. During the initial stage of tumor metastasis, the disconnection between tumor and endothelial cells makes the TJ the first barrier cancer cells must overcome in metastasis (62).

TJs comprise three essential membrane proteins: Occludin, claudin and junctional adhesion molecules. The CLDN family is vital for TJ functions, including regulating defense and barrier functions, differentiation and polarity in epithelial and endothelial cells (Fig. 8A; blue cluster). The loss of CLDNs contributes to the disruption of cell junctions in a tissue-dependent manner and plays an essential role in cancer cell migration, invasion and metastasis (63). The distribution patterns of various claudins in GC differ between tumor tissue and adjacent tissue (64-66). Specifically, CLDN6 expression is higher in GC tissues than in adjacent tissues (67). However, certain studies suggest that lower levels of CLDN6 expression in GC tissues compared to adjacent tissues are associated with factors such as age, lymph node metastasis, pathological stage and tumor metastasis. Furthermore, several studies have reported that the upregulation of CLDN6 expression is associated with decreased survival rates in GC (68-71).

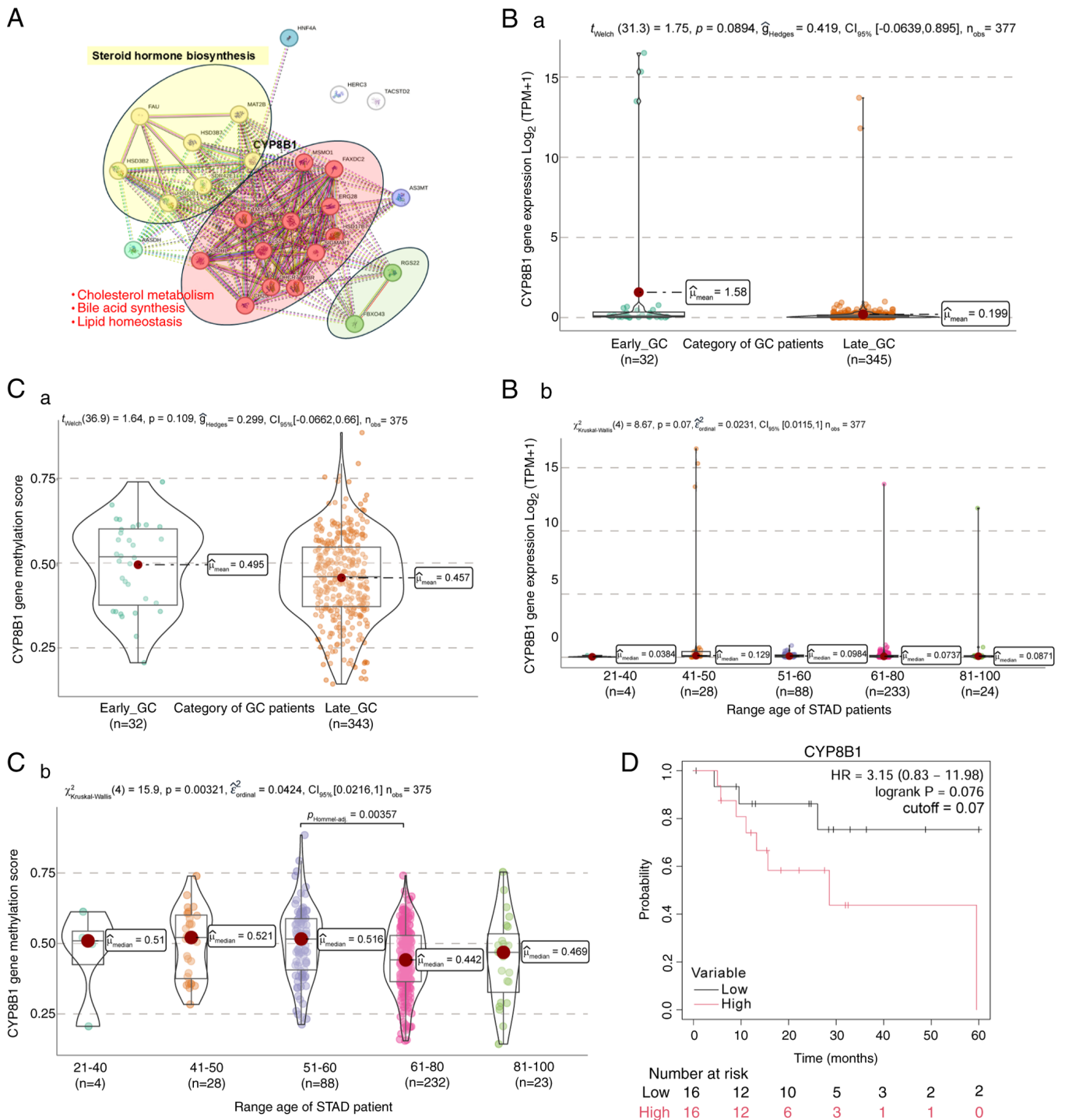


Figure 7. Expression of CYP8B1 in patients with EO-GC. (A) The STRING interaction of the proteins identified three clusters of related proteins. (Ba) Comparison of transcript CYP8B1 levels between EO-GC and L-GC (upper graph). (Bb) Transcript levels according to age range (bottom graph). (Ca) Comparison of CYP8B1 gene methylation score between EO-GC and L-GC (upper graph). (Cb) Methylation genes score according to age range (bottom graph). (D) Kaplan-Meier analysis of patients with EO-GC based on median RNL5 gene expression (cutoff=0.07). Welch's t-test and the F-test were performed through the 'ggstatsplot' R package. EO-GC, early-onset gastric cancer; L-GC, late-onset gastric cancer; HR, hazard ratio.

Known for its role in TJ integrity, its reduced expression may contribute to increased tissue permeability and metastasis in EO-GC.

Analysis of protein interactions using STRING revealed an association between CLDN6 and proteins implicated in mesenchymal cell proliferation (72) (Fig. 8A; green cluster). Another group of proteins interacting with CLDN6 includes mitofusin proteins and mitochondrial outer

membrane GTPases mediating mitochondrial clustering and fusion (Fig. 8A; red cluster). CLDN6 interacted with dynamin (DNMs), which catalyze the hydrolysis of GTP and utilize this energy to mediate vesicle scission (Fig. 8A; green cluster). These proteins participate in various forms of endocytosis, including clathrin-mediated synaptic vesicle and rapid endocytosis (73). Also, DNM2 is part of the machinery responsible for vesicle formation and

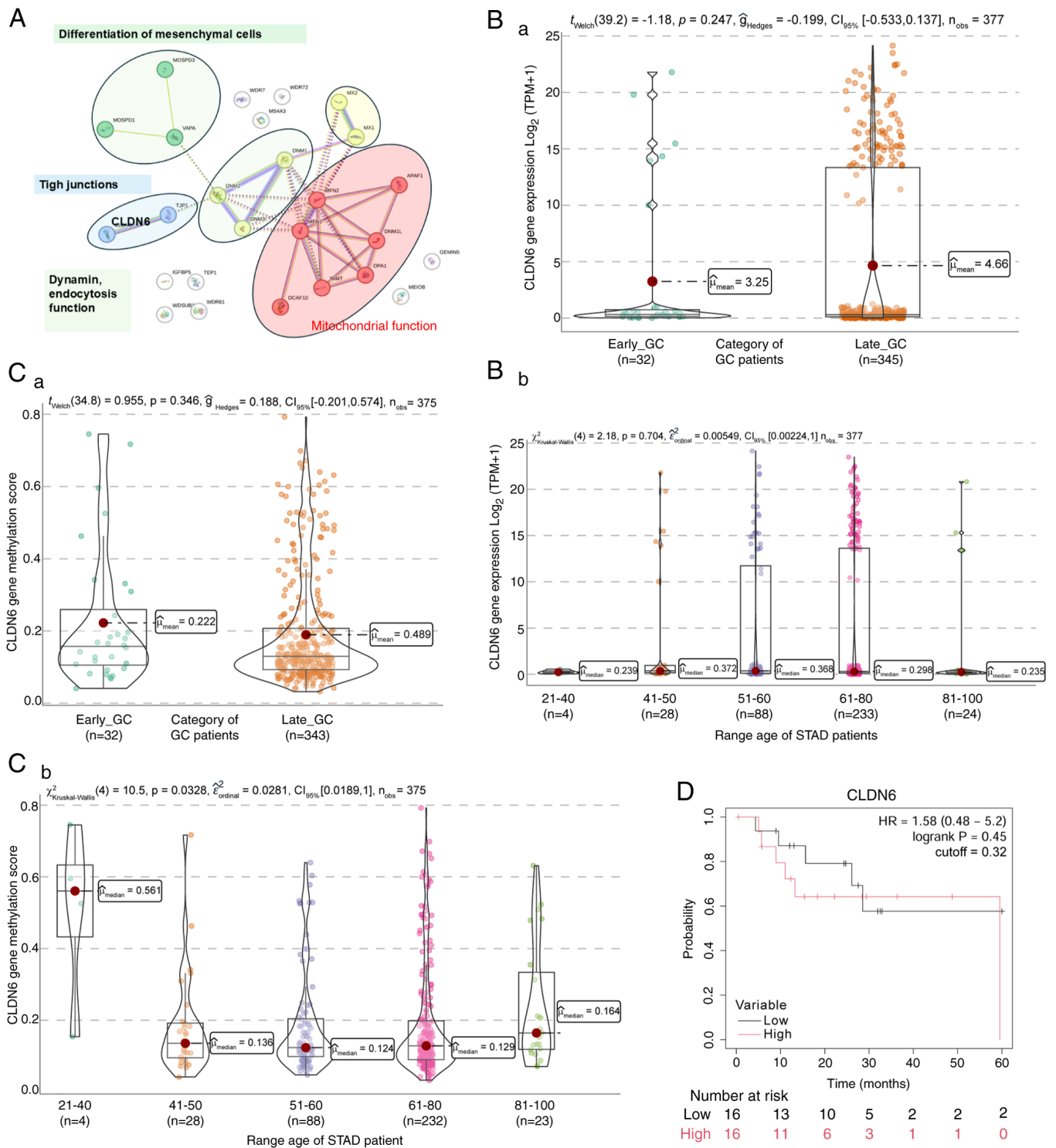


Figure 8. Expression of CLDN6 in patients with EO-GC. (A) The STRING analysis of the proteins identified five clusters of related proteins. (Ba) Comparison of transcript CLDN6 levels between EO-GC and L-GC (upper graph). (Bb) Transcript CLDN6 levels according to age range (bottom graph). (Ca) Comparison of CLDN6 gene methylation score between EO-GC and L-GC (upper graph). (Cb) Transcript levels according to age range (bottom graph). (D) Kaplan-Meier analysis of patients with EO-GC based on median CLDN6 gene expression (cutoff=0.32). Welch's t-test and the F-test were performed through the 'ggstatsplot' R package. EO-GC, early-onset gastric cancer; L-GC, late-onset gastric cancer; HR, hazard ratio.

regulates the cytoskeleton, facilitating intracellular vesicle transport (74).

Analysis of CLDN6 expression in GC. Based on the TCGA-STAD data, no general differences in CLDN6 gene expression were seen between EO-GC and L-GC (Fig. 8Ba); there was also no significant difference in protein levels

when patients were categorized by age (Fig. 8Bb). In terms of the gene methylation levels, no significant changes were observed when comparing both groups of patients (Fig. 8Ca). However, the 21-40-year age range exhibited a significantly higher methylation score than the other age ranges (Fig. 8Cb). Kaplan-Meier survival analysis did not indicate any survival

differences between EO-GC and L-GC according to the median CLDN6 expression (Fig. 8D).

In summary, the downregulation of CLDN6 in EO-GC suggests a weakening of TJ integrity, potentially facilitating cancer cell invasion and metastasis. This highlights the biological importance of CLDN6 in maintaining epithelial barriers and its potential role as a prognostic biomarker in EO-GC.

MIOX gene expression in GC. Following the identification of CLDN6, MIOX gene expression was next examined. The initial committed step in mammalian inositol catabolism is catalyzed by MIOX, which performs the unique four-electron dioxygen-dependent ring cleavage of myo-inositol to D-glucuronate. This enzyme facilitates the binding of ferric iron and inositol oxygenase activity, playing a significant role in the inositol catabolic process, primarily located in the cytoplasm and inclusion bodies (75).

STRING analysis involving the MIOX gene revealed a direct relationship with uridine 5'-diphosphate-glucuronosyltransferase (UGT) genes (Fig. 9A; red cluster), which are membrane proteins of the endoplasmic reticulum expressed in a tissue-specific manner. MIOX has been identified as a regulatory gene of tumor ferroptosis in several cancer types, such as clear cell renal cell carcinoma (ccRCC). In ccRCC, a significant downregulation of MIOX in tumor tissues relative to adjacent renal tissues has been observed, with a negative correlation between MIOX expression levels in ccRCC tissues and the malignant behavior, as well as poor prognosis of ccRCC (76).

Additionally, MIOX has been implicated in bladder (77) and prostate cancer progression (78) and lung squamous cell carcinoma, where it is part of a gene signature indicative of the connection with glycolysis (79). Furthermore, it has been established that certain gene isoforms of the UGT family have differential expressions between normal and tumor stomach tissue, whose expression changes would affect the progression of GC (80). However, no relationship between MIOX and GC, particularly EO-GC, has been reported.

STRING analysis identified a gene cluster, including the ABCG2 transporter and two UGT genes (Fig. 9A; green cluster). Additionally, relationships were observed with genes involved in ATP-dependent ABC-type transporters (Fig. 9A; yellow cluster). A connection was also established between two genes related to E3 ubiquitin ligases (Fig. 9A; blue cluster).

Analysis of MIOX expression in GC. Based on the TCGA-STAD dataset, it was found that EO-GC exhibited a significantly lower MIOX gene expression and a relative increase in gene expression in L-GC (Fig. 9Ba); this was confirmed by an increase in transcripts in older age groups, between 51 and 100 years (Fig. 9Bb). Furthermore, EO-GC displayed a higher methylation score than L-GC (Fig. 9Ca), particularly in the 21-40-year age range (Fig. 9Cb). Kaplan-Meier survival analysis did not indicate any association of MIOX expression with survival in patients with EO-GC using the median MIOX expression level as a cut-off (Fig. 9D).

The lower expression of MIOX in EO-GC (Fig. 9) suggests that an alteration in inositol metabolism may contribute to tumor progression. While Kaplan-Meier

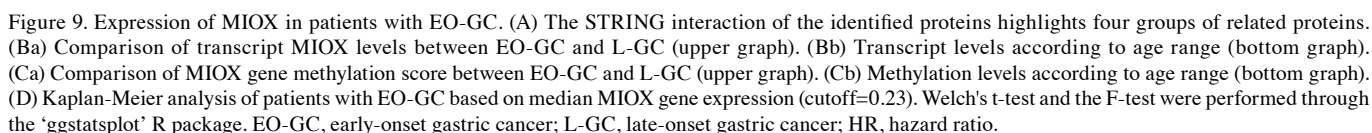
analysis did not show a significant difference in survival, the methylation changes observed in EO-GC warrant further studies on the role of MIOX in metabolic reprogramming and the regulation of ferroptosis. In summary, the significant downregulation of MIOX in EO-GC suggests a disruption in inositol metabolism, potentially impairing ferroptosis-a cell death pathway crucial for tumor suppression. This alteration may create metabolic vulnerabilities that cancer cells exploit for progression.

PNMA5 gene expression in GC. PNMA5 gene, also down-regulated in EO-GC, has been implicated in apoptosis and cancer metastasis. The PNMA family members have been identified as onconeural antigens exhibiting aberrant expression in cancer cells in patients with paraneoplastic disorders. This protein family is closely associated with autoimmunity, neurodegeneration and cancer, with several PNMA family members characterized by their involvement in apoptosis and cancer-related signaling pathways (81). Studies have shown that PNMA5 is deregulated in patients with CRC; it contributes to CRC metastasis by potentially facilitating cancer cell migration and invasion. Cellular markers related to epithelial-mesenchymal transition (EMT) revealed that PNMA5 promotes EMT in CRC, promoting cell migration and invasion (82,83). Given that metastases are more detrimental to cancer-associated mortality than primary tumors, understanding the role of PNMA5 in EMT and metastasis in GC is crucial for developing targeted therapies.

In particular, PNMA5 is associated with ZBTB8A, which facilitates DNA-binding activity specific to RNA polymerase II transcription regulatory regions, potentially playing a role in transcriptional regulation. Another noteworthy gene is ARPC4, identified as a potential biomarker or drug target in metastatic GC (Fig. 10A; green cluster). In addition, a group of PNMA5-related genes is involved in DNA replication, single-strand DNA binding, repair and homologous recombination (Fig. 10A; red cluster). Two smaller clusters relate to genes regulating transcription (Fig. 10A; blue cluster). Furthermore, PNMA5 is related to genes that initiate transcription, such as GTF3C1 (Fig. 10A; yellow cluster), which activates polymerases to initiate gene transcription (84-86).

Analysis of PNMA5 expression in GC. Based on the TCGA-STAD dataset, it was found that EO-GC exhibited a lower PNMA5 gene expression than L-GC (Fig. 10Ba). A tendency toward increased transcript levels was observed in older age groups, particularly between 51 and 80 years of age, although no significant differences were detected across groups (Fig. 10Bb). Gene methylation levels did not show any significant differences when only the two groups of EO-GC vs. L-GC were compared (Fig. 10Ca). Regarding methylation, EO-GC displayed a higher PNMA5 methylation score, especially in the 21-40-year age group. While differences between several age groups were observed (Fig. 10Cb). Kaplan-Meier survival analysis indicated a non-significant trend toward lower survival among EO-GC patients with higher PNMA5 gene expression (Fig. 10D).

Based on the TCGA-STAD dataset, PNMA5 gene expression was significantly lower in EO-GC compared to L-GC.



ACTL8 gene expression in GC. Lastly, ACTL8 was also down-regulated in EO-GC compared to L-GC cases. ACTL8

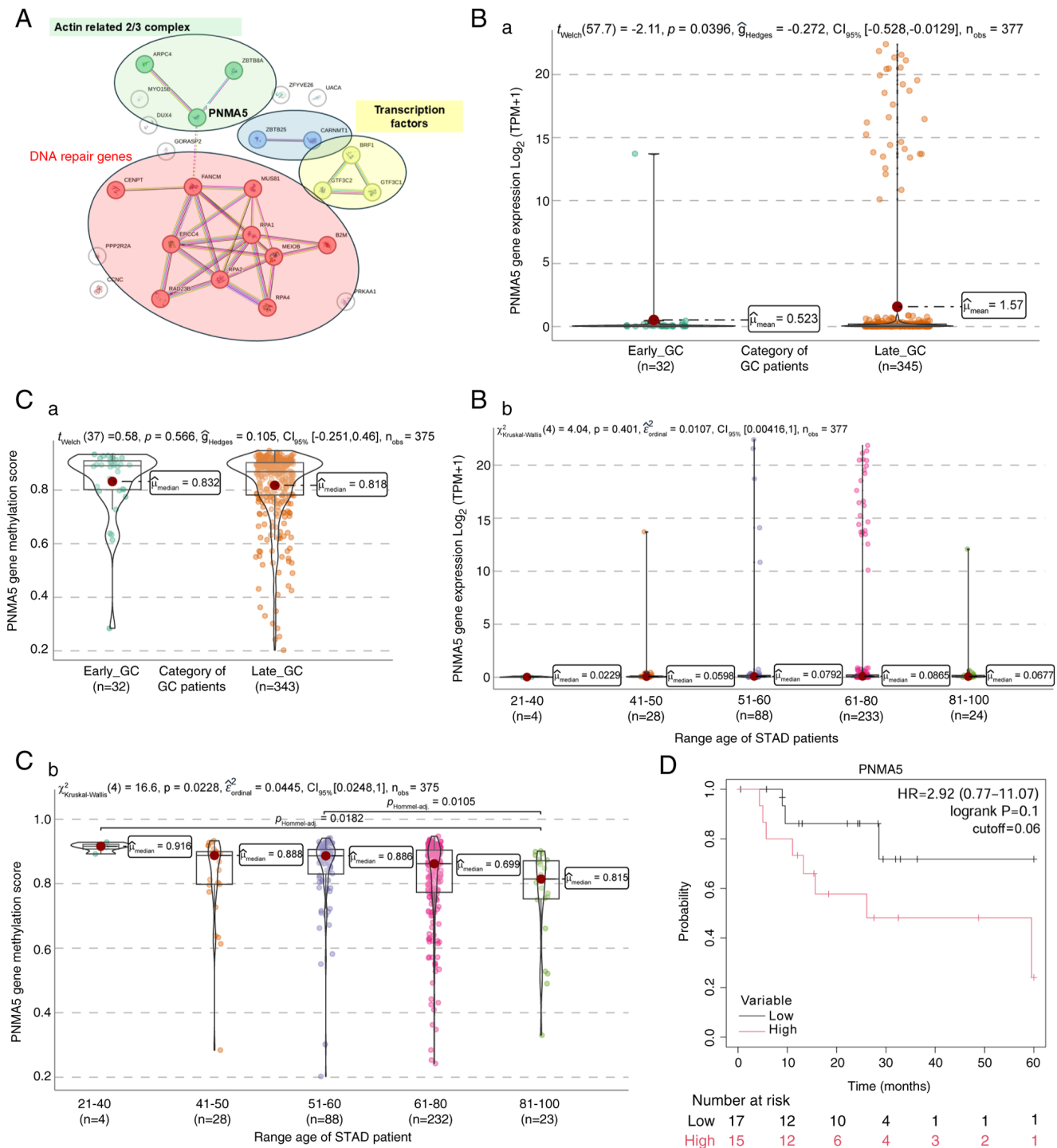


Figure 10. Expression of PNMA5 in patients with EO-GC. (A) The STRING analysis of the proteins identified four clusters of related proteins. (Ba) Comparison of transcript PNMA5 levels between EO-GC and L-GC (upper graph). (Bb) Transcript PNMA5 levels according to age range (bottom graph). (Ca) Comparison of PNMA5 gene methylation score between EO-GC and L-GC (upper graph). (Cb) Comparison of PNMA5 gene methylation score according to age range (bottom graph). (D) Kaplan-Meier analysis of patients with EO-GC based on median PNMA5 gene expression (cutoff=0.06). Welch's t-test and the F-test were performed through the 'ggstatsplot' R package. EO-GC, early-onset gastric cancer; L-GC, late-onset gastric cancer; HR, hazard ratio.

has been implicated in the differentiation of epithelial cells and is thought to be located within the cytoplasm of the dynein complex, facilitating the activation of the dynein molecular motor for ultra-processive transport along microtubules (Fig. 11A; green cluster). The dynein complex, a critical component of the ARP2/3 complex, is crucial for cell shape and movement by forming actin filaments on the lamellipodial cell surface (87). Furthermore, the ARP2/3 complex plays

a role in the cytoplasmic cytoskeleton by promoting actin polymerization in the nucleus, which regulates gene transcription and DNA repair (88).

The relationship between this cluster network and histone-related genes, including EP400, H4C6 and MYSM1, is noteworthy (Fig. 11A; red cluster). These genes involve essential processes, such as chromatin remodeling (89). Furthermore, high ACTL8 expression has been associated

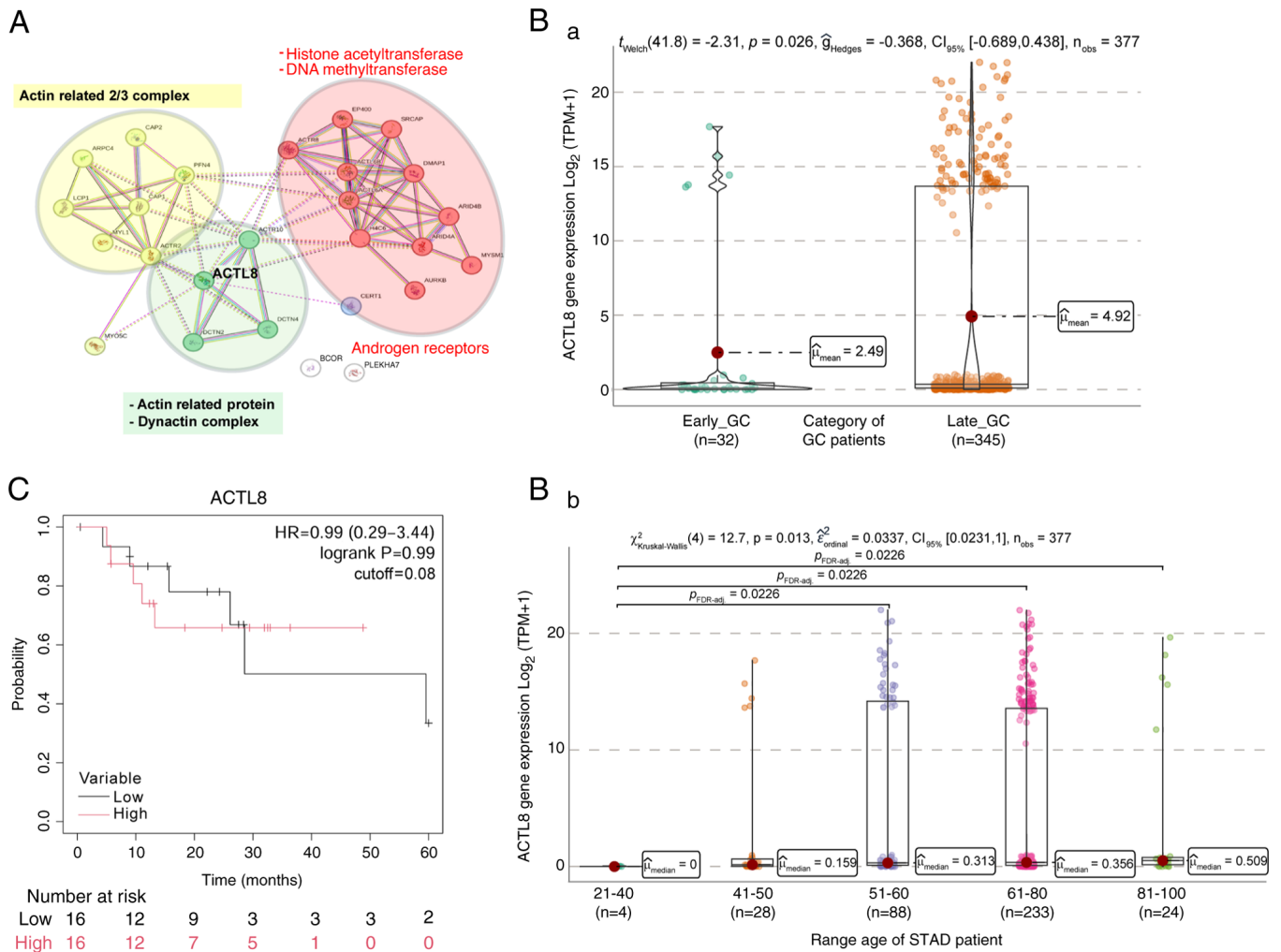


Figure 11. Expression of ACTL8 in patients with EO-GC. (A) The STRING interaction of the proteins identified three clusters of related proteins. (Ba) Comparison of transcript ACTL8 levels between EO-GC and L-GC (upper graph). (Bb) Transcript ACTL8 levels according to age range (bottom graph). (C) Kaplan-Meier analysis of patients with EO-GC based on median ACTL8 gene expression (cutoff=0.06). Welch's t-test and the F-test were performed through the 'ggstatsplot' R package. EO-GC, early-onset gastric cancer; L-GC, late-onset gastric cancer; HR, hazard ratio.

with poor prognosis in head and neck cancer (90) and metastasis in CRC (91).

Analysis of ACTL8 expression in GC. Based on the TCGA-STAD data, it was found that EO-GC exhibited a lower PNMA5 gene expression as compared with L-GC (Fig. 11Ba), particularly in the 21-40-year age range (Fig. 11Bb). Unfortunately, no methylation data for ACTL8 in GC were found. Lastly, Kaplan-Meier survival analysis based on the median ACTL8 gene expression did not indicate any significant influence of ACTL8 on survival outcomes in EO-GC cases (Fig. 11C).

The expression data for ACTL8 in Fig. 11 highlight its downregulation in EO-GC compared to L-GC, particularly in younger patients. Although the Kaplan-Meier analysis did not reveal any significant survival association, ACTL8's role in cytoskeletal organization and chromatin remodeling suggests its potential importance in EO-GC pathogenesis.

In summary, the downregulation of ACTL8 in EO-GC suggests impaired cytoskeletal dynamics and chromatin remodeling. These disruptions may hinder normal cell differentiation while facilitating cancer cell motility, contributing to EO-GC progression.

5. Discussion

Various attempts have been made to characterize the pathological characteristics of EO-GC in different regions, including Colombia (92), China (93) and Japan (94). Additionally, studies have reported on the treatment patterns of patients with EO-GC based on the Surveillance, Epidemiology and End Results database and their impact on patient survival (1,95,96).

Proteogenomics analyses have been published to provide additional information beyond genomic analyses, thereby improving the understanding of cancer biology in patients with EO-GC (97). More epidemiological research and knowledge of the clinicopathological characteristics and mechanisms are urgently required to better understand this emerging situation affecting the young population (5,98). This understanding is crucial for developing preventive strategies and early detection methods tailored to this emerging group.

Considering the context, identifying key genes and pathways involved in EO-GC remains a complex and challenging task. Numerous potential modifications may trigger carcinogenic activity in these genes, with many overlapping

pathways and unclear mutation patterns. Consequently, the current scientific priority is to pinpoint the essential genes and pathways, comprehend the interplay between these modifications and devise strategies to prevent their occurrence (6,99).

The intensive data mining performed in the present study identified KLHL4, MAGEL2, RNLS and CYP8B1 as upregulated genes. These genes are involved in metabolic pathways that pertain to protein ubiquitination and histone regulation. Of note, the particularly significant pathways are very long fatty acid synthesis, cholesterol metabolism, steroid hormone production and their receptors. Furthermore, these pathways contribute to the production of bile acids, which have been implicated in promoting intestinal metaplasia and gastric carcinogenesis (60).

Our comprehensive search for gene expression and methylation data in patients with EO-GC and L-GC revealed several notable findings concerning upregulated genes in EO-GC; first, KLHL4 expression may be particularly high in younger individuals with EO-GC. High KLHL4 expression may also be associated with lower survival in patients with EO-GC. Second, high MAGEL2 expression was found mainly in the 41-50-year age range and was linked to lower survival in patients with EO-GC. Third, RNLS expression was higher in EO-G, particularly in the 41-50-year age range, like MAGEL2. Furthermore, Kaplan-Meier analysis indicated a tendency for higher survival in patients with low RNLS expression. Fourth, CYP8B1 expression showed no significant differences between patients with EO-GC and L-GC. Nevertheless, Kaplan-Meier analysis suggested that lower CYP8B1 expression may be associated with better survival outcomes.

In terms of downregulated genes in EO-GC, the following may be summarized: First, the 21-40-year age range featured significantly lower CLDN6 expression and a tendency towards higher methylation scores. Kaplan-Meier analysis did not indicate any influence of CLDN6 expression on the survival of patients with EO-GC. Studies that have explored the relationship between CLDN6 and prognosis in GC show diverse results. For instance, a study indicated that high transcriptomic expression of CLDN6 was associated with a better survival rate (100), but according to another study, high protein expression was associated with lower survival (70). Furthermore, the CLDN6 gene has even been proposed as a TME prognostic marker in GC (101). Second, EO-GC exhibited significantly lower MIOX expression and higher methylation scores, particularly in the 21-40-year age range. Kaplan-Meier analysis did not correlate MIOX expression with survival outcomes in patients with EO-GC. Third, EO-GC cases had lower PNMA5 expression, particularly in the 21-40-year age range, which also showed higher methylation scores. Kaplan-Meier analysis indicated a trend where high PNMA5 expression may be associated with lower survival in EO-GC cases. Fourth, EO-GC exhibited lower ACTL8 expression than L-GC, particularly in the 21-40-year age range. No methylation data was available for ACTL8. Kaplan-Meier analysis did not link median ACTL8 expression with survival outcomes in EO-GC cases.

A detailed description of the gene expression and methylation status of genes involved in GC highlights the complex interplay between genetic and epigenetic factors and the

effects on the onset and progression of EO-GC. Overall, it was verified that genes overexpressed in EO-GC exhibit bimodal expression patterns and can be overexpressed even in young individuals (aged 21-40 years). In addition, it was discovered that the influences of mutations in EO-GC are mainly described in downregulated genes. Understanding these genetic landscapes is vital for developing targeted therapies and enhancing patient prognosis in GC.

Although numerous unfilled gaps are inherent in data mining approaches, it raises some important questions and sheds light on previously unexplored areas of GC. It is essential to acknowledge certain limitations of the present study, such as the reliance on a single database (TCGA-STAD) and the relatively low representation of EO-GC cases compared to L-GC. Nonetheless, this type of data mining may provide a fertile field for future studies on tumor progression and survival associations of EO-GC in relation to these genes. Furthermore, no *in vitro* assays or preclinical models are currently available that have evaluated the expression of these genes and their methylation profiles in GC.

This study highlights the need for a deeper understanding of the molecular pathways involved in EO-GC to identify novel therapeutic targets and strategies. While the identification of molecular targets, such as KLHL4, MAGEL2 and RNLS, offers exciting prospects, the translational pathway from molecular discovery to therapeutic application requires additional investigation. Existing therapeutic approaches for GC focus on chemotherapeutic agents, immunotherapies and molecularly targeted treatments, such as trastuzumab and immune checkpoint inhibitors, which have shown promise in advanced cases (102,103). In parallel, there is growing interest in exploring alternative therapeutic approaches, including natural compounds with anti-tumor properties (104,105). For instance, *Rabdosia rubescens* has demonstrated potential anti-cancer effects through its phytochemical constituents, offering a complementary avenue for therapy (104).

Although the current study does not focus on therapeutic interventions, the identified genes and pathways provide a foundation for exploring novel therapeutic targets. Integrating these molecular insights with established and emerging treatments, including natural compounds, may lead to more personalized and effective strategies for EO-GC management.

The integration of bioinformatics tools in cancer research allows for the identification of potential biomarkers and therapeutic targets (106). Therefore, based on the present data mining, the development of a gene panel that includes the identified upregulated and downregulated genes may be proposed, along with their methylation profiles focused on the TME of GC categorized according to the age of the patients. This comprehensive panel could enhance EO-GC diagnosis, facilitating prompt and timely clinical management. By integrating methylation data, particularly for the downregulated genes, a more precise and effective approach to understanding and treating EO-GC may be provided.

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Availability of data and materials

Raw counts, upper-quartile normalized fragments per kilo base per million mapped reads and TPM RNA-seq expression, and clinical data related to the STAD-TCGA project can be accessed and downloaded from Genomic Data Commons through the TCGA-biolinks R package.

Authors' contributions

FGV, IS, BGB, JAG and MG were involved in the conceptualization of the study. TdMG and CS were responsible for the methodology. INR, CSM, MMM, MG, FSC, PAM, IC, HB, FP, JME, ACS and AG performed investigations and data acquisition. FSC, PA and JAG were involved in the study's conceptualization, wrote the original draft, and reviewed and edited the manuscript to produce the final version. All authors have read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

MG has been involved as a principal investigator in clinical trials from Merck Sharp & Dohme, Bristol Myers Squibb, Novartis, Roche, Astellas, Deciphera, Thermo Fisher Scientific, IMS Health and Quintiles (IQVIA), Bayer, Principia, Covance, Daiichi-Sankyo, Basilea, PRA-Exelisis, Syneos and Zimeworks. All other authors declare that they have no competing interests.

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