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RARB associated with MSI, affects progression and prognosis of gastric cancer

Xufan Cai[2†], Wenfa Lin[2,3†], Fang Wu[4], Guangyuan Song[5], Zhenyuan Qian[4*] and Yu Wang[1*]

Abstract
Microsatellite instability (MSI) has been widely acknowledged as an important factor regulating tumor intrinsic biological behavior and affecting the survival of gastric cancer patients. Here, we firstly identified the RARB as a gene associated with MSI gastric cancer. RARB was downregulated in human gastric cancer tissues compared to paired paracancerous tissues, Knockdown of RARB accelerated the proliferation, invasion and migration of cancer cells in vitro. Mechanismly, RARB knockdown promoted epithelial-mesenchymal transition (EMT) process of gastric cancer. However, $RARB^{Low}$ patients exhibited better survival compared to $RARB^{High}$ patients. Further study revealed that RARB expression was inversely correlated with MSI status and immune infiltrates in vivo. Thus, RARB may be a potential target for the treatment of gastric cancer.

Keywords Gastric cancer, MSI, RARB, Tumor immune microenvironment

Background

Gastric cancer is a worldwide life and health threatening issue due to its high morbidity and mortality. In recent years, with the gradual deepening of the knowledge in genetic alterations and molecular features of gastric cancer, many new therapeutic methods have appeared [1], but the 5-year survival rate of gastric cancer is still very poor [2]. Therefore, it is of great significance to continue to study in depth the mechanism of the development of gastric cancer and to search for the factors that are related to the prognosis of gastric cancer.

The retinoic acid receptor (RAR) subfamily, including RARα, RARβ and RARγ, belongs to the nuclear receptor (NR) superfamily of transcription factors. RARs form heterodimers with members of the retinoid X receptor (RXR) subfamily and act as ligand-regulated transcription factors through binding specific RA response elements (RAREs) located in target genes promoters [3]. RARs play a crucial role in many process, including cell proliferation, apoptosis and metabolism [4, 5]. Retinoid acid receptor B (RAR-β), a subtype of RAR, has been demonstrated to be downregulated in different types of

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cancers and participates in the regulation of tumor initiation, development, drug resistance and etc [6–8]. However, the role of RARB in gastric cancer has not been fully elucidated.

A large number of studies have shown that the status of MSI may be a prognostic marker for gastric cancer patients [9, 10]. Compared with microsatellite instability-low (MSI-L) or microsatellite stability (MSS) gastric cancers, microsatellite instability-high (MSI-H) gastric cancer has a better prognosis. Meanwhile, due to its higher mutation loading and neo-antigens exposure, MSI gastric cancer shows more immune infiltrates and greater sensitivity to immunotherapy [11, 12]. A recent study revealed that RXRA SNP, rs12004589, was significantly associated with risk of MSI-high cancers [13]. Nevertheless, the relationship between RARB and MSI status remains an enigma.

Targeting RXR has been established to a promoting strategy for cancer therapy. The use of LG268, a RXR agonist, can improve response to immune checkpoint blockade in HER2+or triple-negative breast cancer [14]. Actually, Bexarotene, the first successful example of an effective therapeutic that molecularly targets RXR, was approved to treat cutaneous T cell lymphoma. Little is known, however, about the crosstalk among RARB in tumor cells, MSI status and immune infiltrates in gastric cancer.

Herein, in this study, we aimed to explore the expression pattern and biological function of RARB in human gastric cancers, as well as to describe the prognostic value of RARB and its relationship with MSI.

Materials and methods

Data sources and availability

All the data used in this study were downloaded from publicly open resource. Differentially expressed genes (DEGs) were defined as p value <0.05 and $|logFC| >0.6$ and screened by MeV software (http://mev.tm4.org). Heatmap and clustering analysis were presented by Wekemo bioincloud (https://www.bioincloud.tech). GO and KEGG pathway enrichment analysis and visualization were performed by NetworkAnalyst (https://www.networkanalyst.ca). Pathway activities were predicted by GSCAlite (http://bioinfo.life.hust.edu.cn/web/GSCALite/). Gene set enrichment analysis (GSEA) was performed to estimate the pathways in gastric cancer using GSEA v4.0.3 (https://www.gsea-msigdb.org/gsea/downloads.jsp). Venn diagrams was drawn by the online tool VENNY2.1 (https://bioinfogp.cnb.csic.es/tools/venny/index.html). The survival analysis module of the GEPIA2 web tool was used for Kaplan-Meier analysis. The expression of genes in different clinical groups was plotted using UALCAN based on data from TCGA.

Sample sources

Clinical tissue samples were obtained from gastric cancer tissues and their paired paracancerous tissues of gastric cancer patients who underwent surgical operations at Zhejiang Provincial People's Hospital from 2020 to 2022 ($n=3$). None of the patients received radiotherapy before surgery. All patients were informed and gave their voluntary, written, informed consent. The study was approved by the Ethics Committee of Zhejiang Provincial People's Hospital (QT2022363).

Bioinformatics analysis

The samples from the TCGA database belonging to the MSI high group should satisfy both the conditions of mantis score>0.6 and sensor score>10. Samples satisfying one of these criteria belong to the disunity group, and those satisfying neither criterion belongs to the MSI low group. By cross-analysis with TOP 500 genes with prognostic value in gastric cancer, candidate genes were subjected to functional enrichment, pathway activity and Kaplan-Meier survival analysis to determine the target gene. Then the target gene's clinical and oncological features were analyzed. Gene set enrichment analysis was also performed for target genes.

Immunohistochemistry

Serial 4-μm-thick sections from 4% paraformaldehyde-fixed and paraffin-embedded tissues were deparaffinized firstly, and then soaked with 3% hydrogen peroxide to inactivate endogenous peroxidase. After antigen retrieval with 10 mM sodium citrate (pH 6) blocking with PBS supplemented with 5% BSA, the sections were incubated with primary antibodies overnight at 4 °C and secondary antibodies at room temperature for 60 min. Finally, the signals were detected with a DAB Detection Kit (MXB, China) and the pictures were taken under a microscope after sealing. The following antibodies were used:

RARB (1:100, ABclonal, Wuhan, China)

CD3 (1:700, ABclonal, Wuhan, China)

CD8 (1:100, ABclonal, Wuhan, China)

CD68 (1:3000, ABclonal, Wuhan, China)

HRP Goat Anti-Rabbit IgG(H+L) (1:200, Servicebio, Wuhan, China)

Cell culture

The gastric cancer cell lines (MNK45, MKN-7, AGS, and HGC-27) and normal gastric epithelial cell line GES-1 obtained from the Shanghai Cell Bank (Chinese Academy of Sciences, Shanghai, China) were cultured in RPMI-1640 (Gibco, Maryland, USA) containing 10% FBS (Excell Bio, Shanghai, China) and antibiotics (NCM biotech, Suzhou, China). All cells were incubated in a humidified environment at 37 °C containing 5% CO2. Cells were

digested and passaged using 0.02% EDTA/0.25% trypsin (Gibco, Maryland, USA) at 80-90% confluence.

RNA extraction and qRT-PCR

Total RNA was extracted from cells using FastPure Cell/Tissue Total RNA Isolation Kit V2 (Vazyme Biotech Co., Ltd, Nanjing, China) and reverse transcribed into cDNA using HiScriptIII RT SuperMix for qPCR (+gDNA wiper) (Vazyme Biotech Co., Ltd, Nanjing, China). Real-time quantitative PCR was performed using SYBR Green. The data were collected using the Applied Biosystems ® 7500 Real-Time PCR System and the relative expression of target genes was calculated using $2^{-\Delta\Delta CT}$. The primers used were designed and produced by Repobio (Hangzhou, China)and the sequences are as follows:

RARB-134-F: CGTGGAGTTTGCTAAACGTCT
RARB-134-R: TGGTGTCTTGTTCTGGGGTAT
GAPDH-101-F: CTGGGCTACACTGAGCACC
GAPDH-101-R: AAGTGGTCGTTGAGGGCAATG.

Cell transfection

Well-grown gastric cancer cells were seeded into a 6 cm dish (3×10^5 cells per dish) one day before transfection. The RARB siRNA and negative control siRNA were transfected into the cells using jetPRIME (Polyplus, Germany) according to the instructions, and the next experiments were carried out in 24–48 h.

The RARB siRNA and negative control siRNA were provided by Ribobio (Guangzhou, China), and the sequences are as follows:

si-RARB-1: AGACGGCCTTACCCTAAAT
si-RARB-2:GATCGTGGAGTTTGCTAAA,
si-RARB-3:GCAGAGCGTGTAATTACCT.

Cell growth and proliferation assays

To draw the cell growth curves, gastric cancer cells after successful transfection were seeded into 96-well plates (2×10^3 cells per well). At 0, 24, 48, 72 and 96 h after seeding, 10% CCK8 solution (Biosharp, Beijing, China) was added to each well and incubated for 2 h. The absorbance was subsequently measured at 450 nm.

To test the colony formation ability, gastric cancer cells after successful transfection were seeded into 6-well plates (1×10^3 cells per well) and cultured for 10–14 days, then fixed with 4% polymethylene and stained with 1% crystal violet. Following washing with PBS, colonies in each well were manually counted and images were captured using a digital camera (Canon DS126211; Canon, Inc., Tokyo, Japan).

Cell migration and invasion assays

Migration experiments were performed in Transwell chambers (Corning, NY, USA). A total of 4×10^4 cells suspended in 100 μL of serum-free medium were added to the upper chamber, and 500 μL of medium containing 10% FBS was added to the lower chamber, and then incubated for 24 h. After wiping off cells in the upper chamber carefully with cotton swabs, the lower chamber of cells was fixed with 4% polyformaldehyde and stained 1% crystalline violet, and then washed and air-dried before being observed and counted under a microscope. For invasion assays, a matrix gel coat was placed in the upper chamber before seeding the cells, and the rest of the steps were the same as above.

Western blot

The extracted proteins concentration was measured by BCA method (Vazyme Biotech Co., Ltd, Nanjing, China). Proteins were separated by SDS-PAGE and then transferred to a 0.45 μm PVDF membrane (Millipore, Billerica, MA, USA). Nonspecific binding was blocked with rapid blocking buffer (ABclonal, Wuhan, China).The membranes were cut prior to hybridisation with antibodies. The membranes were incubated with primary antibody overnight at 4 °C and the corresponding secondary antibody for 1 h at room temperature. Finally, the bands were visualized using the SuperFemto ECL Chemiluminescence Kit (Vazyme Biotech Co.,Ltd, Nanjing, China) in a gel imaging system (BIO-RAD). The images were analyzed with ImageJ. The antibodies used were as follows:

RARB (1:1000, ABclonal, Wuhan, China)
β-actin (1:1000, ABclonal, Wuhan, China)
E-Cadherin (1:1000, proteintech, Chicago, USA)
N-Cadherin (1:1000, ABclonal, Wuhan, China)
Vimentin (1:1000, ABclonal, Wuhan, China)
HRP Goat Anti-Rabbit IgG(H+L)(1:20000, ABclonal, Wuhan, China)

RNA-seq

RARB overexpression plasmid and control plasmid (Repobio, Hangzhou, China)were transfected into gastric cancer cells AGS. The transcriptional differences were analyzed by next-generation high-throughput sequencing. The sequencing and analysis process was completed by LC-BIO TECHNOLOGIES CO., LTD.(Hangzhou, China).

Statistical analysis

One-way analysis of variance and least-significant difference post hoc tests were used to compare datasets containing multiple groups. The log rank test was employed in the analysis of Kaplan-Meier curves. All statistical analyses were conducted with SPSS 17.0 (SPSS, Inc., Chicago, IL, USA) and GraphPad Prism 7 (GraphPad Software, Inc., La Jolla, CA, USA). The results of the in vitro experiments were presented as the mean ± standard error

from three independent experiments. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Screening for DEGs in gastric cancer related to MSI and functional annotation

The mantis score (cutoff 0.6) and sensor score (cutoff 10) were firstly applied to define MSS and MSI gastric cancer patients in TCGA database ($n=410$). In total 3402 and 3497 differentially expressed genes (DEGs) were identified in MSI patients versus MSS ones via mantis score or sensor score respectively (Fig. 1A and B). TOP 500 genes with prognostic value in TCGA gastric cancer patients (http://gepia.cancer-pku.cn/) were also screened. The VENN diagram finally identified 24 downregulated and 1 upregulated genes, which were correlated with MSI gastric cancer (Fig. 1C and D). GO ontology (GO) analysis revealed their main function of positive regulation of

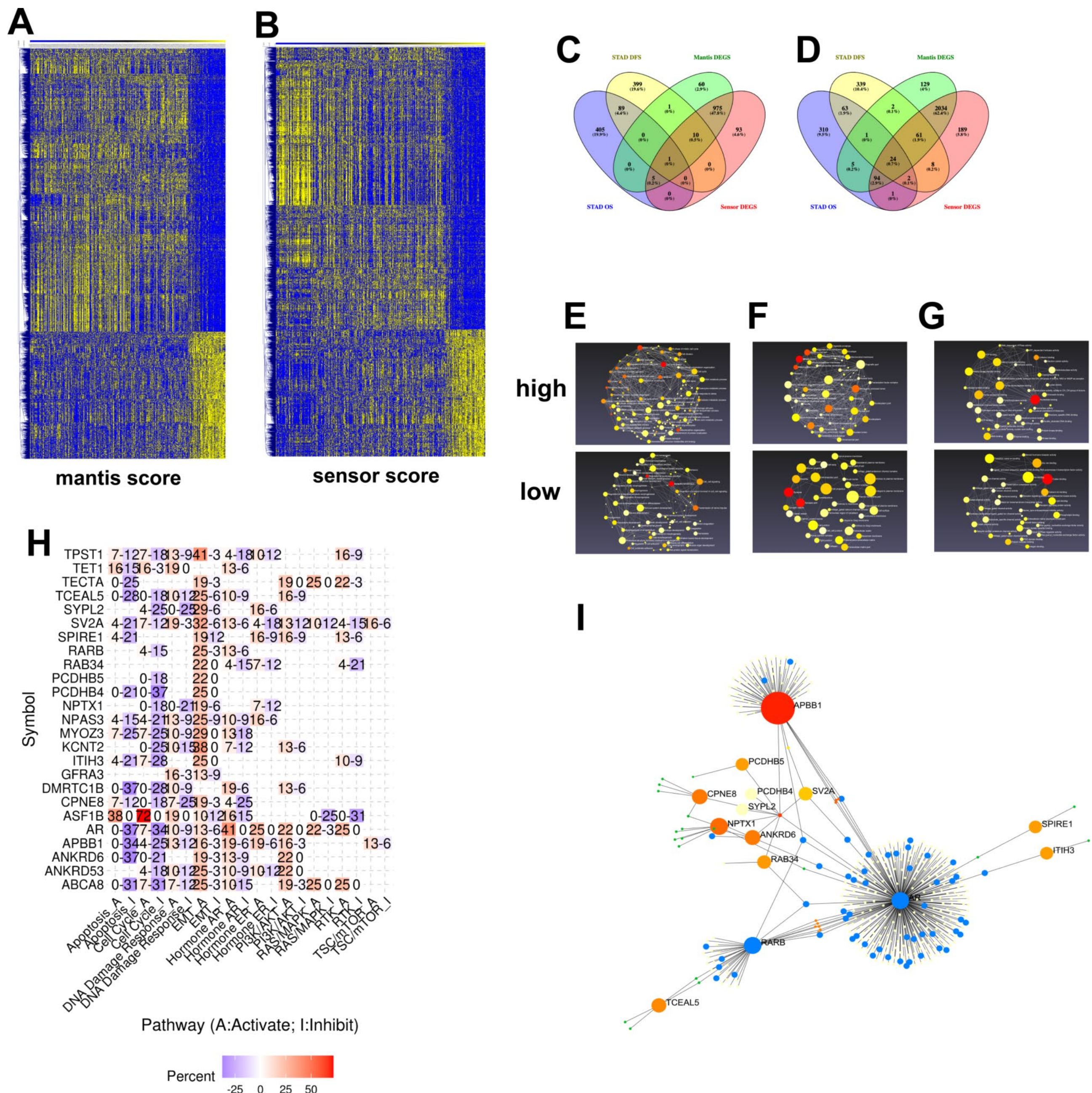

Fig. 1 RARB was identified as an important MSI associated gene (**A**, **B**): Heatmap of mantis score and sensors score; (**C**, **D**): VENN diagram shows 24 downregulated genes and 1 upregulated gene associated with MSI; (**E**, **F**,**G**): GO pathway analysis in terms of biological process, cellular component and molecular function; (**H**): Activity of Candidate Genes in Cancer-Related Pathways. (**I**): RARB and AR were the main genes participating in the cancer pathway

transcription in terms of biological process, cellular component and molecular function (Fig. 1E and G). Pathway activity was analyzed as the composition of 10 common pathways in cancer, including apoptosis, cell cycle, DNA damage response, EMT, hormone AR, hormone ER, PI3K/AKT, RAS/MAPK, RTK, and TSC/mTOR. Most of candidate genes were involved in the activation of the EMT process and inhibition of apoptosis, which indicated the stimulation and acceleration of the cancerous process (Fig. 1H). KEGG pathway analysis was utilized to analyze the protein-protein interactions between these candidate genes, among which, RARB and AR were identified as the core participators in the oncogenic pathway (Fig. 1I). Therefore, RARB gene was chosen for further analysis.

RARB expression pattern in gastric cancer and its clinical relevance

Next, we aimed to verify the relationship between RARB and MSI. The expression of RARB was also found positively correlated to MLH1 ($R=0.44$), MSH6 ($R=0.15$), PMS1 ($R=0.24$), and PMS2 ($R=0.19$), four key molecular in mismatch repair (MMR) in gastric cancer (Fig. 2A and D). Consistently, The correlation analysis of 374 pairs of samples showed that RARB was negatively related to MSI (R=-0.32, $P<0.000$) (Fig. 1E). Further, the relationship between RARB expression and different clinical features was analyzed. As shown in Fig. 2F and N, it varied by race, status of H.pylori infection, histological subtype, nodal metastasis status, TP53 mutation status, tumor grade, individual cancer stage, and patient gender. Notably, RARB was expressed higher in patients with H.pylori infection, adenosignetring histological type, TP53 nonmutation type, tumor grade 3, and cancer stage 3 or 4.

Knockdown of RARB promotes the proliferation, migration, and invasion of gastric cancer cells

It has been reported that RARB is usually downregulated in most cancers, including gastric cancer. We first confirmed that RABR expression was downregulated in human gastric cancer tissues compared to paired paracancerous tissues via IHC assay (Fig. 3A). MKN-7 and HGC-27 cells, with relatively higher RARB expression were chosen for loss-of-function experiments (Fig. 3B). Three different siRNAs were designed to knock down RARB in gastric cancer cell lines. Based on the results of qRT-PCR, R2 and R3, two sequences with higher knockdown efficiency, were selected for subsequent experiments (Fig. 3C and D). The CCK8 assay (Fig. 3E and F) and colony forming assay (Fig. 3G) revealed that RARB inhibition significantly promoted the growth and improved the colony formation ability of HGC-27 and MKN-7 cells in vitro. Furthermore, to explore the potential function of RARB in the mobility of gastric cancer cells, we performed Transwell assay. The results indicated that in HGC-27 and MKN-7 cells, RARB knockdown resulted in increased cell migration and invasion ability (Fig. 3H).

RARB controls different transcriptional programs in gastric cancer

We used RARB overexpression and control plasmids to transfect AGS cells for RNA-seq to characterize the transcriptional changes following RARB overexpression. Compared with the control group, there were 186 upregulated genes and 177 downregulated genes after RARB overexpression (Fig. 4A and C). GO ontology analysis showed that RARB was associated with the molecular function and cellular component composition (e.g., membrane components) of gastric cancer (Fig. 4D), KEGG enrichment analysis showed that RARB was closely related to cellular metabolism, and the differential signaling pathways were mainly focused on the PI3K-Akt, and calcium signaling pathways (Fig. 4E). The above results suggest that RARB plays an important regulatory role in the progression of gastric cancer.

RARB regulates the EMT process of gastric cancer

The GSEA results showed that RARB was associated with advanced cancer processes (NES=2.395, $P=0.000$), lymphatic vessel invasion (NES=1.694, $P=0.006$), and EMT (NES=1.658, $P=0.001$), as presented in Fig. 5A and C. Therefore, we next analyzed if RARB regulates the EMT process. RARB knockdown significantly deceased the expression of epithelial marker E-cadherin, whereas increased the expression of mesenchymal cell marker N-cadherin and Vimentin (Fig. 5D). All these results demonstrated that RARB promoted MSI and suppressed the EMT process of gastric cancer.

RARB expression is inversely correlated with survival of gastric cancer patients

To explore the prognostic value of RARB in gastric cancer, the overall survival (OS) and disease free survival (DFS) curve was drawn with TCGA patients. Kaplan-Meier survival analysis validated that $RARB^{Low}$ patients exhibited better OS and DFS compared to $RARB^{High}$ patients (Fig. 6A and B), demonstrating negative correlation between RARB expression and survival of gastric cancer patients. This is contradictory to the biological function of RARB we observed from in vitro experiment, suggesting other factors might be involved in affecting the survival of gastric cancer patients in vivo. Actually, previous study has observed a significantly higher engraftment rate of MSI tumors (more than two folds) in non-immunocompetent mice compared to MSS tumors [15]. Since the engraftment rate in mice is positively correlated to tumor aggressiveness, these data suggest that,

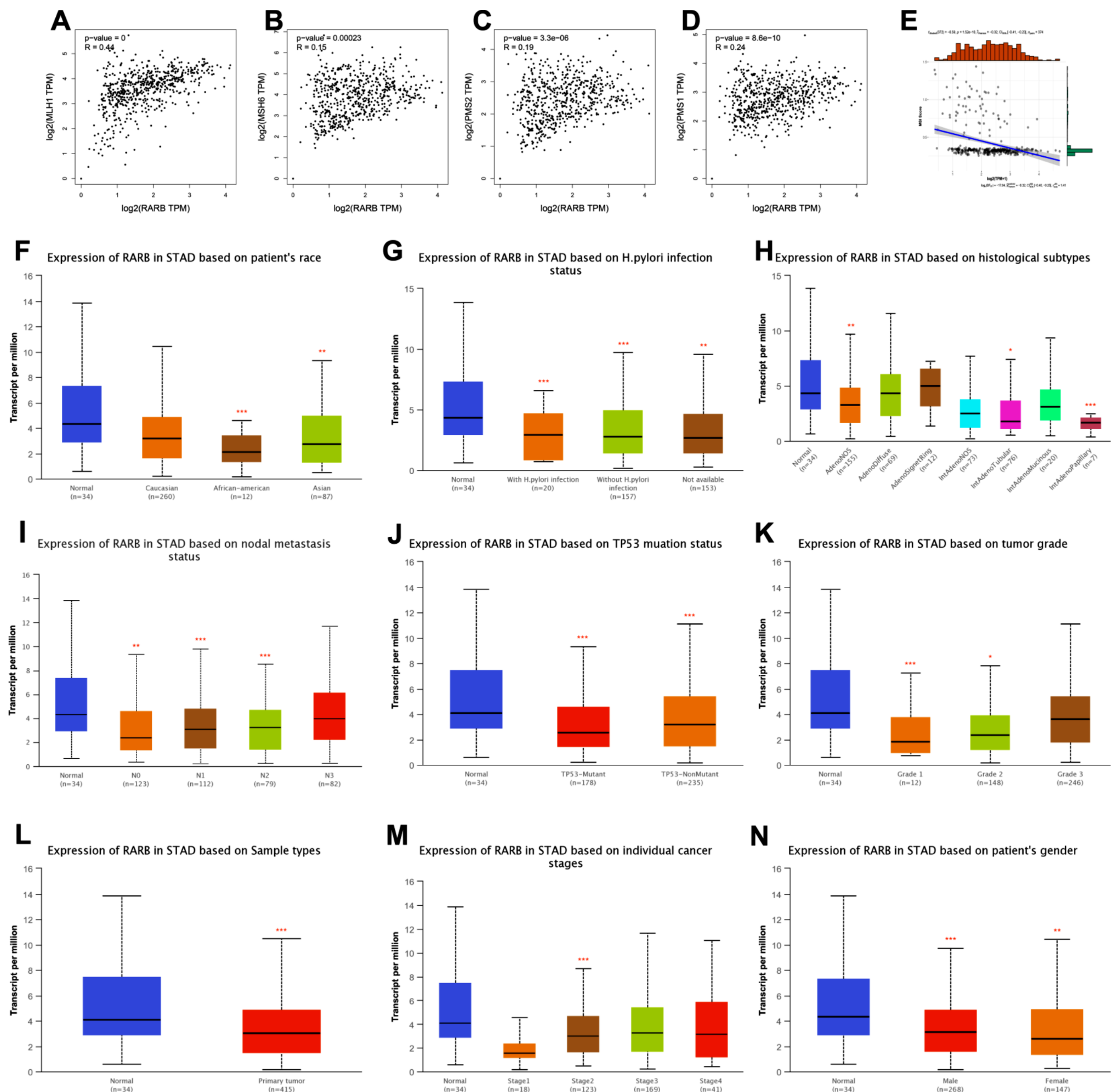

Fig. 2 The Clinical relevance of RARB. (**A**, **B**, **C**, **D**): Relationship between the expression of RARB and MMR system-related proteins; E: Relationship between RARB expression and MSI status in gastric cancer patients; (**F**, **G**, **H**, **I**, **J**, **K**, **L**, **M**, **N**): Relationship between RARB expression and clinical characteristics of RARB. *, $P<0.05$;**, $P<0.01$;***, $P<0.001$

in an immune-deficient environment, MSI GCs behave more aggressively compared to the MSS counterpart. This is consistent with our in vitro data that knockdown of RARB promotes the proliferation, migration, and invasion of gastric cancer cells via facilitating MSI. On the other hand, a body of clinical researches have also found that MSI^{High} status is a favorable prognostic factor in gastric cancer. It is likely that in the presence of a functional immune system, the MSI aggressive behavior is kept under control as it activates the immune system due to the high amount of neo-antigens. Thus, we assumed that RARB might have an impact in gastric cancer immune microenvironment. The IHC staining revealed that $RARB^{Low}$ gastric cancer exhibit higher percentage of $CD3^+$, $CD8^+$ and $CD68^+$ cells, suggesting more immune infiltrates (Fig. 6C). This indicated that $RARB^{Low}$ tumors, which is more likely to be MSI, attracted more immune cells in vivo and exhibited better survival.

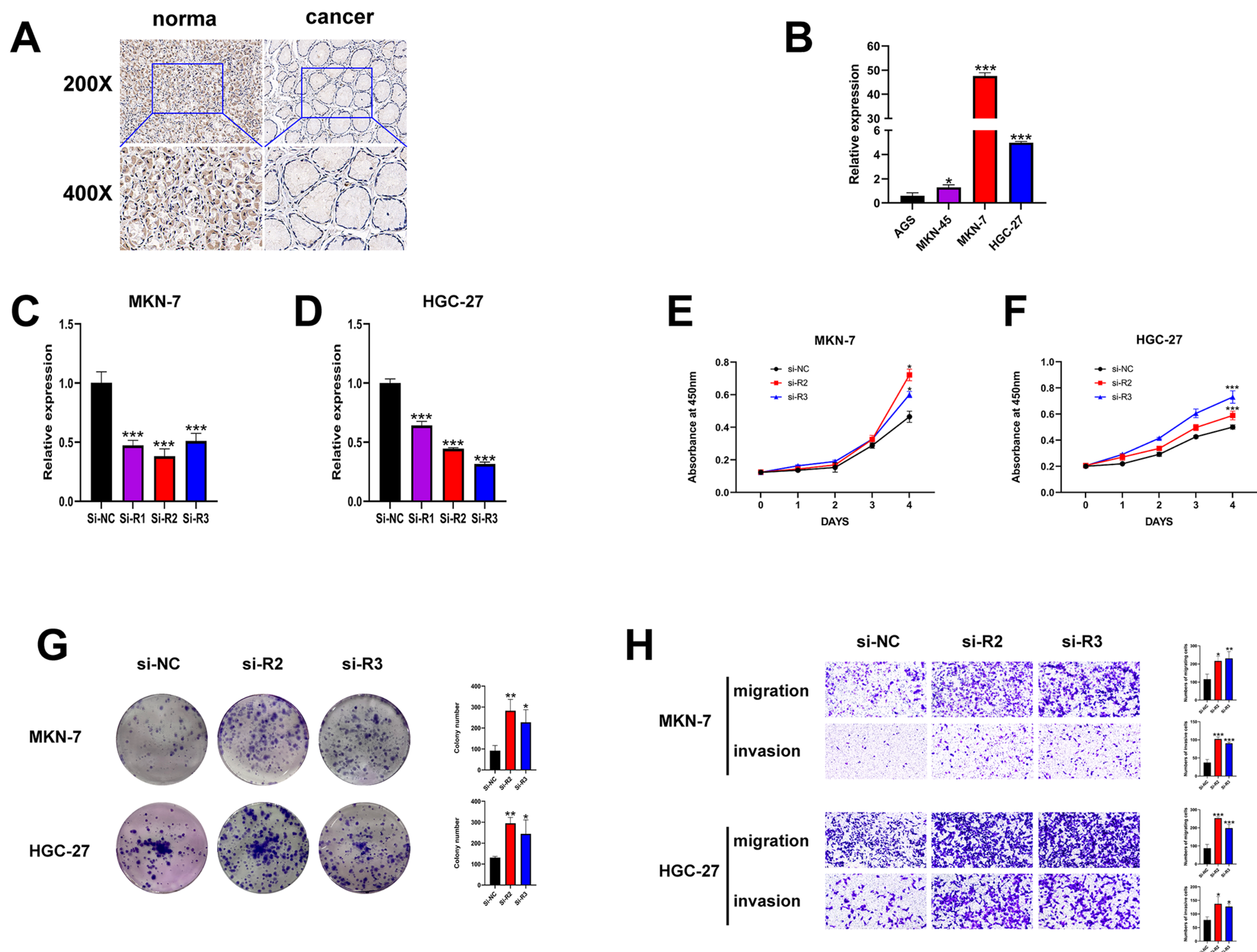

Fig. 3 Knockdown of RARB accelerated the proliferation, invasion and migration of cancer cells in vitro. **A**: Representative immunohistochemical images of RARB expression in normal gastric tissues and gastric cancer tissues; **B**: The mRNA level of RARB in gastric cancer cells detected by qRT-PCR; (**C**, **D**): The expression level of RARB after knockdown by siRNA in two gastric cancer cells, MKN-7 and HGC-27; (**E**, **F**): The CCK-8 assay revealed the growth trend of gastric cancer cells after RARB knockdown; (**G**): The effect of RARB knockdown on the proliferation of gastric cancer cells via colony formation assay; (**H**): The cell migration and invasion capacity of gastric cancer cells detected by transwell assay after RARB knockdown. *,$P<0.05$;**,$P<0.01$;***,$P<0.001$.

Discussion

Retinoid acid receptors (RARs) are a class of receptor proteins that exist in the cell nucleus and function by regulating transcription factor-specific target genes, which are not only involved in cell differentiation and growth and development, but also closely related to differentiation functions [16, 17].There are two known classes of retinoid intranuclear receptors, RARs and retinoid x receptors (RXRs), each with α, β, and γ subtypes [18, 19]. It has been demonstrated that abnormal expression of retinoid acid receptor B (RAR-β) in cells is closely related to tumorigenesis. RARB is highly expressed in normal epithelial cells, and its expression is reduced or absent in a large number of tumor cells, such as breast, lung, esophageal, thyroid, prostate, bladder, endometrial, and cervical cancers [20–27]. Therefore, as a major inducible isoform in retinoic acid, RARB has become a hotspot of research, but the expression, biological function and influence pathway of RARB in gastric cancer have not been studied in more depth.

Here, 410 gastric cancer samples from TCGA database were collected for analysis. 24 downregulated genes and 1 upregulated gene were identified in MSI group compared with MSS group. Functional enrichment analysis revealed their main effect on the positive regulation of transcription. Specifically, RARB participates in the process of gastric cancer progression especially EMT and RARB expression is negatively correlated with MSI.

RABR expression was downregulated in human gastric cancer tissues compared to paired paracancerous tissues, and it is related to some clinical and oncological characteristics of gastric cancer, including patient race, gender, H.pylori infection, tumor grade, type of TP53, histological subtype, nodal metastasis and tumor stage. In vitro experiments showed that knockdown of RARB promoted the proliferation, migration and invasion of gastric cancer

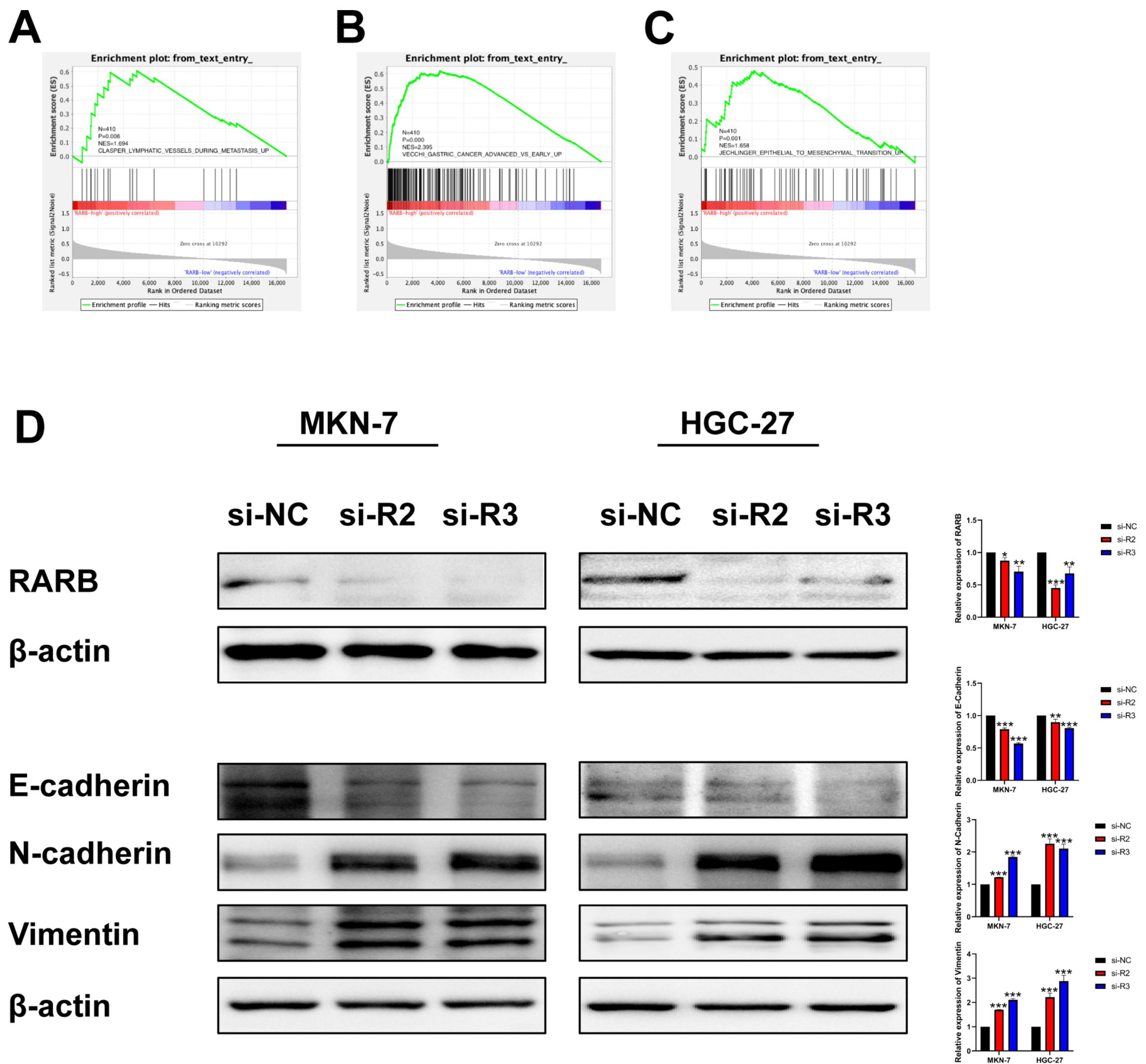

Fig. 4 RARB knockdown promoted epithelial-mesenchymal transition (EMT) process of gastric cancer. (**A**, **B**, **C**): GESA analysis of relationship between RARB and advanced cancer process, lymphatic vessels invasion, and EMT; (**D**): Effect of knockdown of RARB on EMT makers' expression in gastric cancer cells by Western blot. *, $P<0.05$;**, $P<0.01$;***, $P<0.001$

cells. Mechanismly, RARB knockdown promoted EMT process of gastric cancer. However, Kaplan-Meier survival analysis found that RARB expression was inversely correlated with OS and DFS of gastric cancer patients.

This contradiction at the tissue and cellular levels may be related to the immune microenvironment of MSI gastric cancer. MSI tumors have a significantly higher engraftment rate in non-immunodeficient mice than MSS tumors [28], which means that MSI tumors are more aggressive. This is consistent with our in vitro data that knockdown of RARB promotes the proliferation, migration, and invasion of gastric cancer cells via facilitating and EMT process. Actually, a body of clinical research have found that MSI^{High} status is a favorable prognostic factor in gastric cancer. It is likely that in the presence of a functional immune system, the MSI aggressive behavior is kept under control as it activates the immune system due to the high amount of neo-antigens. Many studies have been conducted to show the role of tumor immune microenvironment in predicting tumor behavior [29]. There is a significant correlation between tumor-infiltrating lymphocytes (TILS) as a prognostic marker and MSI [30–32]. Kim et al. reported that a high density of CD8+and FOXP3+cells within the tumor was

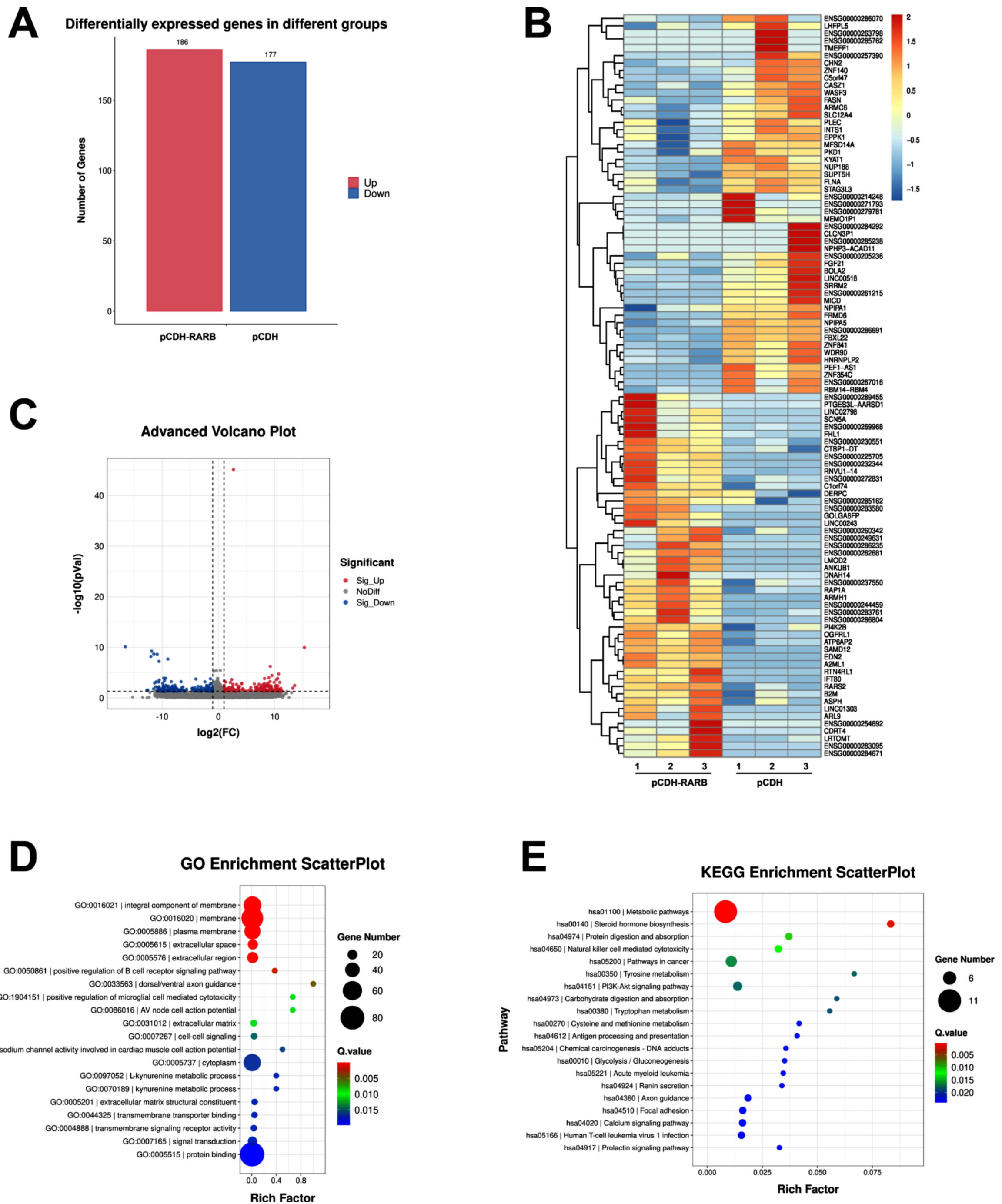

Fig. 5 RARB controls different transcriptional programs in gastric cancer. **A**: DEGs histogram; **B**: DEGs volcano plot; **C**: DEGs heatmap; **D**: GO enrichment analysis; **E**: KEGG enrichment analysis

associated with a favorable prognosis in MSI-H gastric cancer [31]. Similarly, Chiaravalli et al. noted that higher numbers of CD3+and CD8+cells were a positive prognostic factor in MSI and EBV-related gastric cancer [33]. The IHC staining revealed that $RARB^{Low}$ gastric cancer exhibit higher percentage of $CD3^+$, $CD8^+$ and $CD68^+$ cells, suggesting more immune infiltrates. This indicated that $RARB^{Low}$ tumors, which is more likely to be MSI, attracted more immune cells in vivo and exhibited better survival.We acknowledged the limitations of this study like small human sample size and the lack of in vivo data.

Conclusion

We conclude that RARB inhibition is oncogenic at the cellular level, but its aggressive behavior in the human body is reversed due to the immune microenvironment,

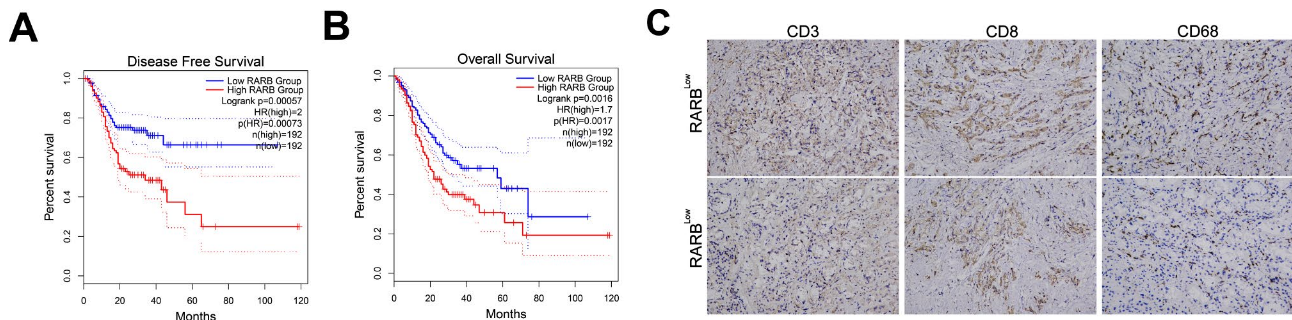

Fig. 6 RARB expression is inversely correlated with survival of gastric cancer patients. **A**: Kaplan-Meier analysis of disease free survival for gastric cancer patients with high ($n = 192$) and low ($n = 192$) RARB expression. **B**: Kaplan-Meier analysis of overall survival for gastric cancer patients with high ($n = 192$) and low ($n = 192$) RARB expression; Representative immunohistochemical images of CD3, CD8 and CD68 expression in RARB RARB^{High} and RARB^{Low} gastric cancer tissues

leading to a poor prognosis. RARB may be a potential target for the effective treatment of MSI gastric cancer.

Abbreviations

MSI Microsatellite Instability
GO Gene Oncology
KEGG Kyoto Encyclopedia of Genes and Genomes
TCGA The Cancer Genome Atlas
IHC Immunohistochemistry

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12876-024-03339-z.

Supplementary Material 1

Acknowledgements

Not applicable.

Author contributions

Conceived and designed the experiments: Yu Wang, Zhenyuan Qian. Performed the experiments: Xufan Cai, Guangyuan Song. Analyzed the data: Fang Wu. Wrote the paper: Xufan Cai, Wenfa Lin.

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Data availability

MeV software (http://mev.tm4.org). Wekemo bioincloud (https://www.bioincloud.tech). NetworkAnalyst (https://www.networkanalyst.ca). GSCAlite (http://bioinfo.life.hust.edu.cn/web/GSCALite/). GSEA v4.0.3 (https://www.gsea-msigdb.org/gsea/downloads.jsp). VENNY2.1 (https://bioinfogp.cnb.csic.es/tools/venny/index.html). GEPIA2 (http://gepia.cancer-pku.cn/). UALCAN(https://ualcan.path.uab.edu/index.html).

Declarations

Ethics and consent to participate

All patients were informed and gave their voluntary, written, informed consent. The study was approved by the Ethics Committee of Zhejiang Provincial People's Hospital (QT2022363).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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