Heliyon 10 (2024) e37896

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Integrated bioinformatics analysis and validation identify KIR2DL4 as a novel biomarker for predicting chemotherapy resistance and prognosis in colorectal cancer

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ARTICLE INFO

Keywords:
Colorectal cancer
Prognosis
KIR2DL4
Immunotherapy
Bioinformatics

ABSTRACT

Background: Chemotherapy and immunotherapy have improved the cure rate and survival period for colorectal cancer (CRC), but genetic heterogeneity among patients leads to chemotherapy resistance and disease progression. Identifying new molecular markers is crucial for improving prognosis for CRC patients. KIR2DL4, a transmembrane glycoprotein expressed by immune cells, has shown potential therapeutic and prognostic value in other cancers, but in CRC remains unclear.

Methods: This study validated the expression levels of KIR2DL4 in CRC by integrating multiple public databases and assessed through immunohistochemistry (IHC). We further evaluated the diagnostic and prognostic value of KIR2DL4 and explored correlation with immune cell infiltration and chemotherapy sensitivity. The role of KIR2DL4 was further validated through functional enrichment analysis. Cellular assays were conducted using CCK8, colony-formation assay and scratch wound assay.

Results: The study found that KIR2DL4 is significantly downregulated in CRC and closely associated with poor prognosis. The low expression of KIR2DL4 is associated with decreased immune cell infiltration and reduced chemotherapy sensitivity. Functional enrichment analysis suggests that KIR2DL4 may inhibit development of CRC by affecting immune cell infiltration and modulating chemotherapy sensitivity. Cellular assays have confirmed that inhibiting KIR2DL4 significantly promotes the proliferation and migration of CRC. Inhibition of KIR2DL4 expression significantly decreased the chemosensitivity of CRC cells to oxaliplatin and 5-FU.

Conclusion: The significant downregulation of KIR2DL4 in CRC, associated with CRC metastasis and poor prognosis, highlights its importance as a potential new biomarker for treatment and prognosis assessment of CRC. Future research should delve into the molecular mechanisms of KIR2DL4 and potential applications in regulating immunotherapy and chemotherapy sensitivity.

1. Background

The incidence and mortality rates of colorectal cancer (CRC) have been steadily increasing [1]. For advanced or recurrent CRC

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https://doi.org/10.1016/j.heliyon.2024.e37896
Received 3 September 2024; Accepted 12 September 2024
Available online 13 September 2024

patients, chemotherapy has been shown to improve cure rates and extend survival [2,3]. Combined immunotherapy has also demonstrated comprehensive therapeutic effects [4]. However, genetic heterogeneity among patients often leads to differences in treatment response, commonly resulting in chemotherapy resistance and disease progression [5,6]. Therefore, finding new and reliable molecular markers is crucial.

In CRC, immunotherapy and the immune cells have become significant directions for treatment. PD-1/PD-L1 inhibitors, by activating immune cells, have shown significant therapeutic effects against CRC [7,8]. The degree of immune cell infiltration can influence the immune response of the tumor. Tumors with high immune infiltration are often more sensitive to immunotherapy [9,10]. Exploring new targets gene related to immune cells promises to improve the treatment efficiency and survival rates of CRC patients.

KIR2DL4 is a transmembrane glycoprotein found on natural killer (NK) cells and specific subsets of T cells [11]. Studies have found that inhibiting the interaction between KIR2DL4 and HLA-G can resensitize breast cancer to trastuzumab [12,13]. KIR2DL4 is also expressed in human renal cell carcinoma cells. KIR2DL4 could promote the occurrence of renal cell carcinoma through the activation of PI3K/AKT [11]. However, research on the role of KIR2DL4 in prognosis, chemotherapy resistance and immune infiltration in CRC is currently lacking.

Therefore, this study integrates biological assays and bioinformatics to explore the application value of KIR2DL4 in CRC. Firstly, we cross-validated the RNA expression of KIR2DL4 in CRC using the public database (the Gene Expression Omnibus (GEO), the Cancer Genome Atlas (TCGA)). The protein expression of KIR2DL4 was determined through immunohistochemistry (IHC) analysis. We detected the diagnostic and prognostic value of KIR2DL4 in public databases and validated with patients from the Fujian Provincial Hospital. Furthermore, we explored the relationship between KIR2DL4 expression levels, immune cell infiltration, and chemotherapy sensitivity. Through functional enrichment analysis, we investigated the potential significance of KIR2DL4 in CRC. Finally, cellular assays further validated the function of KIR2DL4 in CRC.

2. Materials and methods

2.1. CRC samples and data collection

We obtained relevant CRC datasets from the GEO database [14]. Standardized expression data and relevant clinical information from TCGA were analyzed and cross-validated with GEO (GSE20842, GSE31737, GSE44076, GSE73360, GSE87211, GSE89076, GSE90627, GSE106582, GSE146009, and GSE221925) data [15]. Information on CRC patients from the Fujian Provincial Hospital was used to validate the results from public databases. The flowchart of this study is detailed in Fig. 1.

2.2. Cell counting Kit-8 (CCK8) assay

CRC cells were seeded into 96-well plates. Each assay group had five replicates. 10 μl of CCK-8 solution was added. Using the microplate reader, we measured the optical density (OD) at 450 nm. For the detection of chemotherapy resistance, the effect of si-

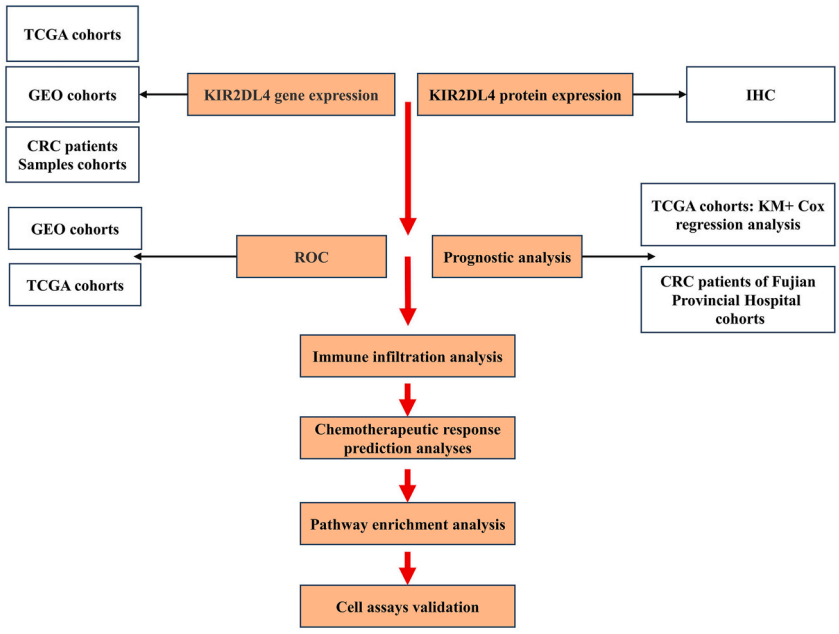

Fig. 1. Study flowchart
This illustrates the design and related assays of this study.

KIR2DL4 on the response to oxaliplatin and 5FU was also tested by CCK8 assay.

2.3. Colony-formation assay

CRC cells were seeded into six-well plates. The plates underwent incubation for 7–10 days until colonies became visible. Subsequently, cells were fixed and stained with crystal violet. The stained colonies were photographed and counted, with three replicates per assay group.

2.4. Scratch wound assay

CRC cells were seeded into six-well plates. A sterile pipette tip was employed to generate a "scratch" in the cell layer. The scratch wound was made uniform and straight. Cell debris was gently removed with PBS. Initial photographs of the scratch were taken immediately (0 h) using an inverted microscope. Cells were returned to the incubator and photographed at specific time points. The width of scratch wound was quantified using ImageJ to assess migration ability of cell.

2.5. RT–qPCR

Traditional Trizol method was used. PCR amplification and cycle threshold (CT) value were performed using the Roche Light Cycler 480 system. The primer sequences of KIR2DL4: forward 5'- TCATCATCCTGGCATGTCTTG-3', reverse 5'- ACGATAGTGACACCGAAGAGT-3'.

2.6. RNA interference (RNAi)

CRC cells were seeded into six-well plates. Cell was transfected with small interfering RNAs (siRNA) when cell growth reached 60%–70 % of density. 150 μl of diluted Lipofectamine 3000 was mixed with 150 μl of diluted plasmid DNA, shaken well to ensure the plasmid DNA was fully encapsulated by liposomes (room temperature for 15 min). The liposome-DNA complex was then gently added to the cells and mixed well. The culture medium for transfected cells was changed after 8–10 h of incubation. The sequence for si-KIR2DL4 is CAGUGGCCAUCAUCCUCUUUAdTd.

2.7. Immunohistochemistry (IHC)

Fixing tissue samples on slides, followed by dehydration, paraffin infiltration, and embedding. The embedded paraffin sections were placed in a 60 °C oven (12h), followed by a series of dewaxing and hydration processes. Antigen retrieval steps were performed, and then Anti-KIR2DL4 antibody (Lot number DF13591, Affinity, USA) was added to the specimens (4 °C for 12). Rabbit antibodies were then added. Diaminobenzidine was used as a substrate for color development. Photographs were taken.

2.8. Diagnostic and prognostic value of KIR2DL4 in CRC

The diagnostic and prognostic value of KIR2DL4 in CRC was calculated and visualized using the pROC package (version 1.18.0) and ggplot2 package (version 3.3.6) in R software (version 4.2.1). Survival data from the TCGA database were analyzed using the survival package (version 3.3.1), survminer package (version 0.4.9), and ggplot2 package (version 3.3.6).

2.9. Analysis of immune cell infiltration

Analysis of immune cell infiltration used the GSVA package (version 1.46.0) and the ssGSEA algorithm with 24 immune cell markers provided by previous research in TCGA database [16].

2.10. Chemotherapy sensitivity

We determined the expression levels of KIR2DL4 to chemotherapy response using the Genomics of Drug Sensitivity in Cancer (GDSC) [17]. The prediction process utilized the pRRophetic package in R, estimating the half-maximal inhibitory concentration (IC50) of the samples via ridge regression.

2.11. Pathway enrichment analysis

Pathway enrichment analysis involved analyzing and selecting differentially expressed genes (DEGs) related to KIR2DL4. The DEGs were annotated and subjected to pathway enrichment analysis using the cluster Profiler package (version 4.4.4). The outcomes of the analysis were visualized with the ggplot2 package (version 3.3.6).

2.12. Statistical analysis

We performed analysis using R software, GraphPad Prism (version 9.5.1), and online analysis platforms. Wilcoxon rank-sum test and Student' s *t*-test was chosen for comparisons based on data distribution characteristics. Kaplan-Meier (KM) survival analysis was used to assess differences in survival times between groups.

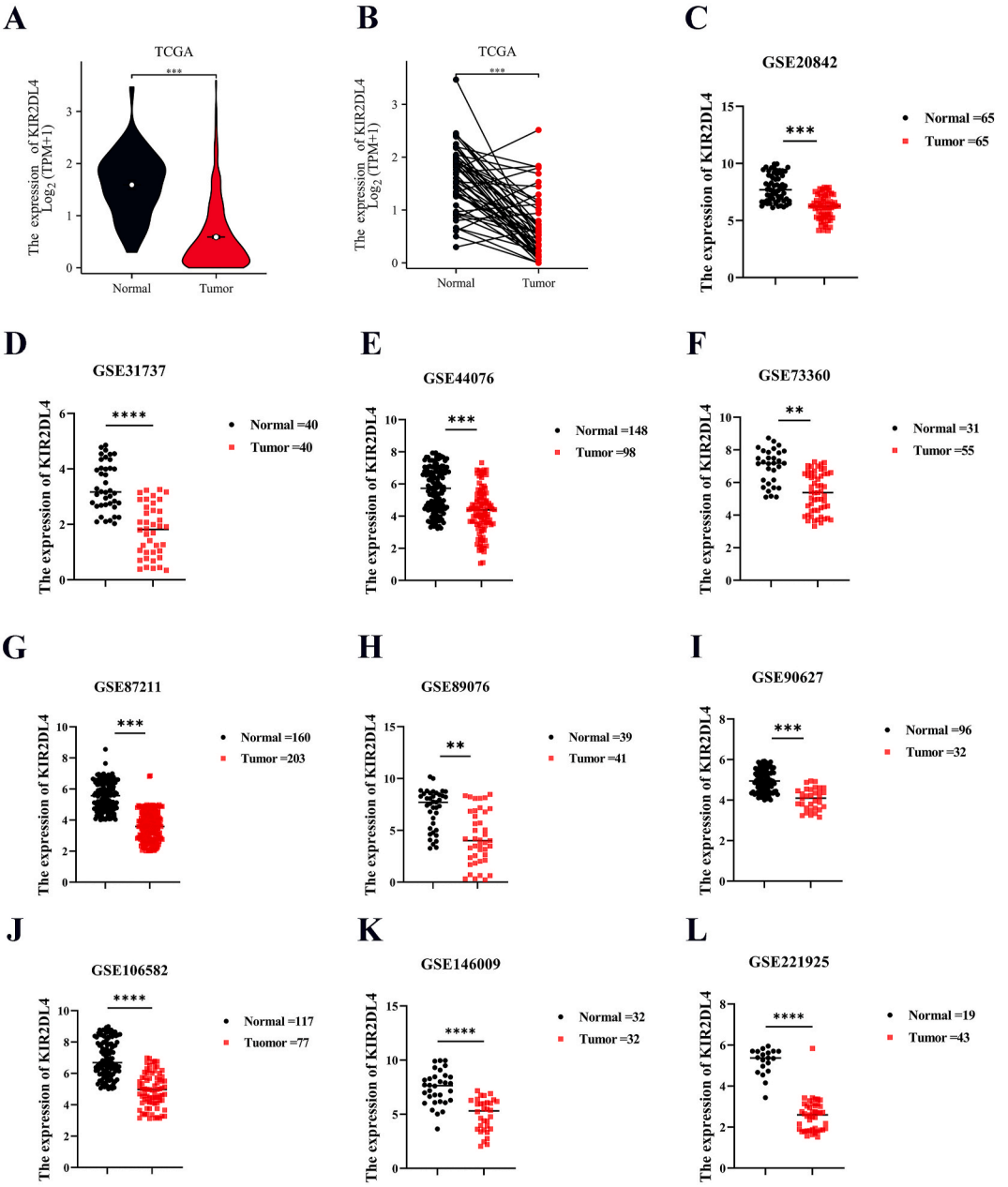

Fig. 2. The RNA levels of KIR2DL4 in CRC
(A) Analyzing the RNA levels of KIR2DL4 in CRC tissues and normal intestinal epithelial tissues based on TCGA. (B) Analyzing the RNA levels of KIR2DL4 in CRC tissues and matched normal intestinal epithelial tissues based on TCGA. Analyzing the RNA levels of KIR2DL4 in CRC tissues and normal intestinal epithelial tissues based on GSE20842 (C), GSE31737 (D), GSE44076 (E), GSE73360 (F), GSE87211 (G), GSE89076 (H), GSE90627 (I), GSE106582 (J), GSE146009 (K), and GSE221925 (L). ($P > 0.05$, ns. nonsignificant; $P < 0.05$ *; $P < 0.01$ **; $P < 0.001$ ***; $P < 0.0001$ ****; analyses were performed using Student' s *t*-test or Wilcoxon rank-sum test, respectively).

3. Results

3.1. KIR2DL4 is significantly downregulated in CRC and associated with CRC metastasis

We extracted and analyzed data on KIR2DL4 from the TCGA database. The findings indicated that KIR2DL4 expression was markedly reduced in CRC tissues when contrasted with normal tissues (Fig. 2A, $p < 0.05$). Additionally, analysis of paired CRC and normal tissues also shown that KIR2DL4 levels were significantly reduced in CRC tissues (Fig. 2B, $p < 0.05$). To further validate the low expression of KIR2DL4 in CRC, we mined multiple datasets from the GEO database. The results cross-validated that KIR2DL4 levels were significantly reduced in CRC tissues in comparison to normal tissues (GSE20842, GSE31737, GSE44076, GSE73360, GSE87211, GSE89076, GSE90627, GSE106582, GSE146009, and GSE221925; Fig. 2C-L, all $p < 0.05$). It is worth exploring whether KIR2DL4 shows differential expression in other cancers. Through pan-cancer data from TCGA, we found that KIR2DL4 is differentially expressed in 25 types of cancers, with increased expression in 15 types of cancer and decreased expression in 10 types of cancer (Figure Supplementary1A, all $p < 0.05$). Results from paired samples in pan-cancer showed differential expression of KIR2DL4 in 7 types of cancer (Fig. S1B, all $p < 0.05$). These observations imply that KIR2DL4 holds a significant role across a broad spectrum of cancers. In conclusion, the mRNA expression level of KIR2DL4 is notably decreased in CRC.

In addition to mRNA levels, we further validated the protein expression level of KIR2DL4. Through IHC of patient tumor samples, our findings revealed that KIR2DL4 levels were lower in CRC tissues in comparison to normal tissues (Fig. 3A). Having established that the expression of KIR2DL4 is markedly reduced in CRC tissues compared to normal intestinal epithelial tissues, we were curious about the relationship between KIR2DL4 and clinical information of CRC. Utilizing the TCGA database, it was discovered that the decreased KIR2DL4 is associated with advanced N, M and TNM stages (Fig. 3B-D, all $p < 0.05$). This suggests a potential correlation between KIR2DL4 and invasion, metastasis in CRC. CEA is a serum biomarker used clinically for diagnosis of CRC [18], and is associated with staging and prognosis. We found that KIR2DL4 is negatively correlated with CEA (Fig. 3E, $p < 0.05$). This supports KIR2DL4 as a protective factor involved in inhibiting development of CRC, although the diagnostic capability of KIR2DL4 for CRC needs further validation. To further validate the findings from TCGA, we cross-validated the results using patient information from the Second Affiliated Hospital of Fujian Medical University (Fig. 3F-I, all $p < 0.05$). In summary, KIR2DL4 is significantly higher in normal

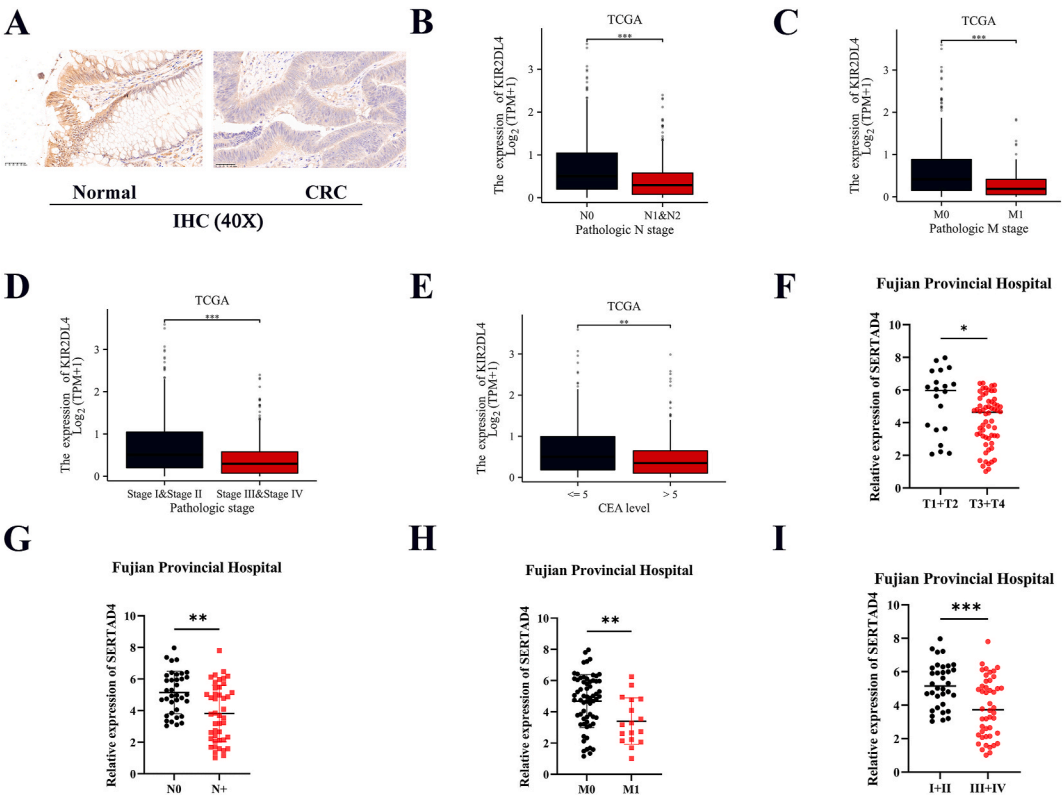

Fig. 3. The protein levels of KIR2DL4 in CRC and association with clinical information
(A) The protein expression of KIR2DL4 in CRC tissues and normal tissues using IHC based on patient samples. Based on TCGA, the expression difference of KIR2DL4 among CRC patients in different N (B), M (C), TNM stages (D), and CEA (E), respectively. Based on data from Fujian Provincial Hospital patients (n = 80 CRC patients), the expression difference of KIR2DL4 among CRC patients in different T (F), N (G), M (H), and TNM stages (I), respectively. ($P > 0.05$, ns. nonsignificant; $P < 0.05$ *; $P < 0.01$ **; $P < 0.001$ ***; $P < 0.0001$ ****; analyses were performed using Student' s *t*-test or Wilcoxon rank-sum test, respectively).

intestinal epithelial tissues compared to CRC tissues.

3.2. KIR2DL4 as a new diagnostic biomarker in CRC

We further explored whether KIR2DL4 can distinguish CRC from healthy individuals. Using patient data from TCGA, we found that KIR2DL4 can effectively diagnose CRC (Fig. 4A, area under curve (AUC) = 0.880). We cross-validate the diagnostic capability of KIR2DL4 for CRC. We retrieved several classic CRC GEO datasets. The results confirmed that KIR2DL4 can effectively distinguish normal intestinal epithelial tissues from CRC (Fig. 4B-I, AUCs are 0.846, 0.881, 0.835, 0.930, 0.871, 0.890, 0.879, and 0.978, respectively). These findings suggest that KIR2DL4 is a new biomarker for predicting CRC.

3.3. KIR2DL4 as a new prognostic biomarker in CRC

Having established the diagnostic capability of KIR2DL4 for CRC, we further explored prognostic capability. We plotted KM curves using TCGA patient data. The results from TCGA suggested that the expression of KIR2DL4 is linked to an extended progression-free survival (PFS) period. (Fig. 5A, $p < 0.001$). By analyzing overall survival (OS), we found that KIR2DL4 expression is associated with better OS (Fig. 5B, $p = 0.017$). After excluding non-tumor death factors, the protective effect of KIR2DL4 on OS was validated (Fig. 5C, $p = 0.021$). These findings were cross-validated using prognosis data from the Second Affiliated Hospital of Fujian Medical University

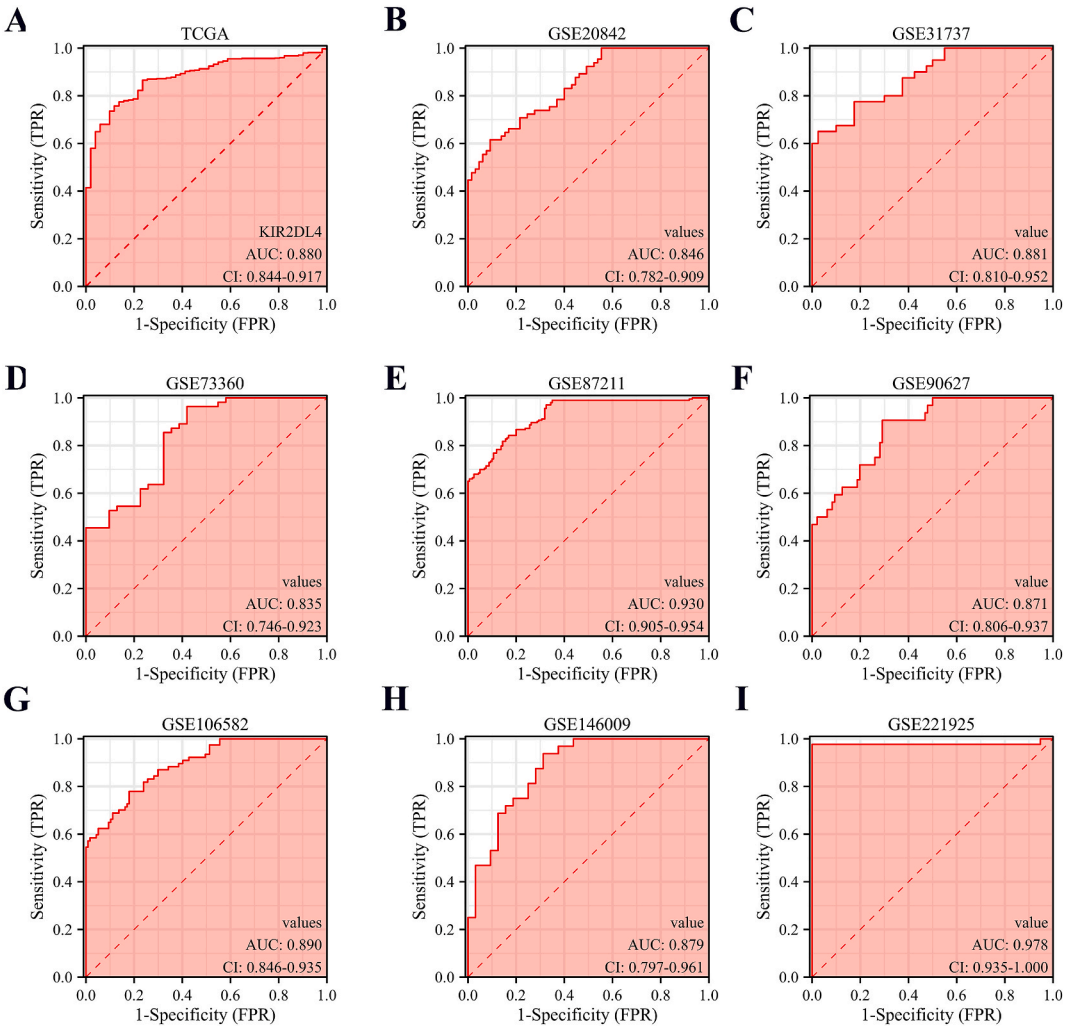

Fig. 4. The diagnostic potential of KIR2DL4 for CRC
(A) Based on TCGA, the diagnostic capability of KIR2DL4 for distinguishing CRC patients from normal individuals. Based on GSE20842 (B), GSE31737 (C), GSE73360 (D), GSE87211 (E), GSE90627 (F), GSE106582 (G), GSE146009 (H), and GSE221925 (I), the diagnostic capability of KIR2DL4 for distinguishing CRC patients from normal individuals. (P > 0.05, ns. nonsignificant; P < 0.05 *; P < 0.01 **; P < 0.001 ***; P < 0.0001 ****; analyses were performed using Student's *t*-test or Wilcoxon rank-sum test, respectively).

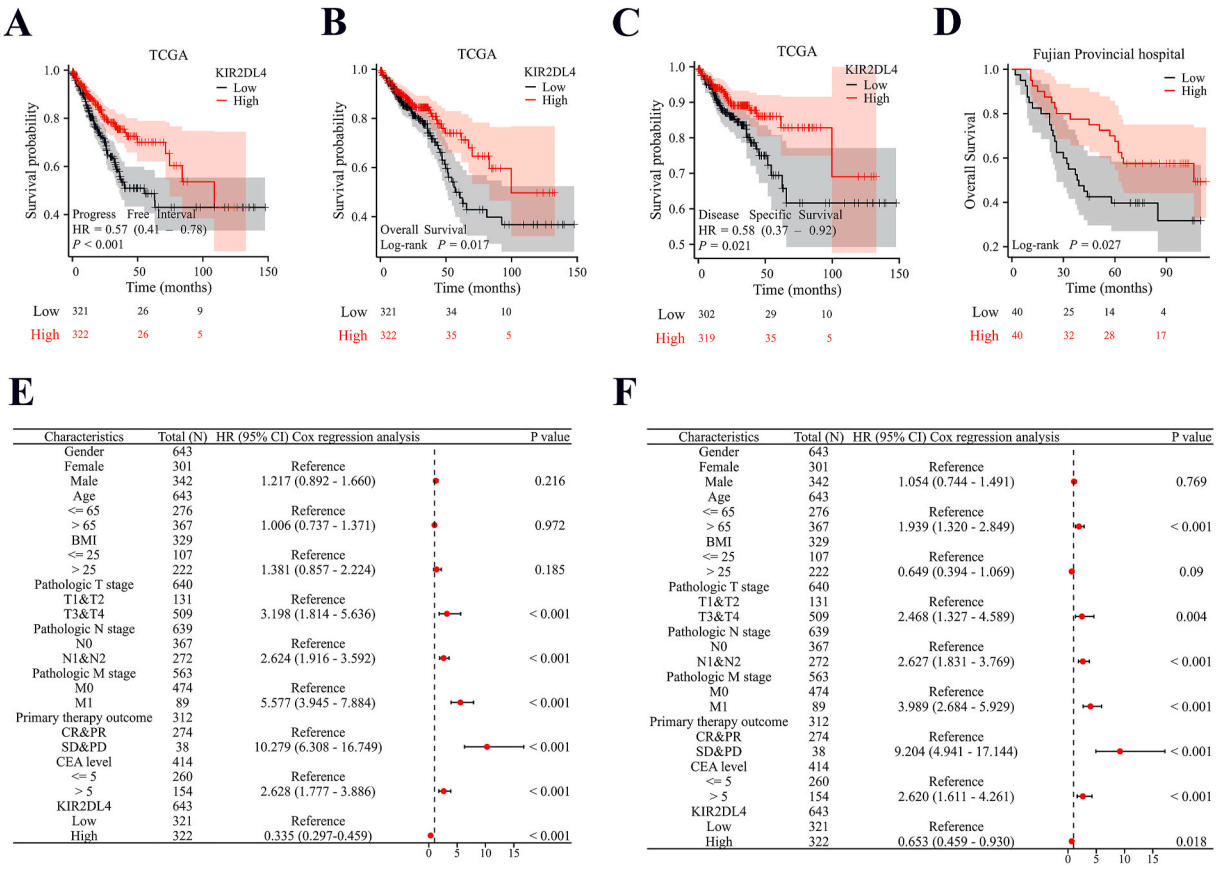

Fig. 5. Low expression of KIR2DL4 indicates poor prognosis in CRC patients
(A) Based on TCGA, KM survival curve analysis of the impact of KIR2DL4 expression on PFS in CRC patients. (B) Based on TCGA, KM survival curve analysis of the impact of KIR2DL4 expression on OS in CRC patients. (C) Based on TCGA, KM survival curve analysis of the impact of KIR2DL4 expression on DSS in CRC patients. (D) Based on the data from Fujian Provincial Hospital patients, KM survival curve analysis of the impact of KIR2DL4 expression on OS in CRC patients. (E) A forest plot showing cox regression analysis of the impact of KIR2DL4 on PFS in CRC. (F) A forest plot showing cox regression analysis of the impact of KIR2DL4 on OS in CRC. (P > 0.05, ns. nonsignificant; P < 0.05 *; P < 0.01 **; P < 0.001 ***; P < 0.0001 ****; analyses were performed using Student's *t*-test or Wilcoxon rank-sum test, respectively).

patients, confirming that KIR2DL4 is associated with better OS (Fig. 5D, p = 0.027). To address confounding factors, we conducted cox regression analysis. The forest plot confirmed that KIR2DL4 is associated with better PFS and OS (Fig. 5E-F, all p < 0.05). In summary, the low expression of KIR2DL4 is closely associated with a poor prognosis in CRC.

3.4. Correlation between KIR2DL4 and immune cell infiltration in CRC

The immune system plays an important role in monitoring, detecting, eliminating abnormal cells. Additionally, immune cells, as an important component of the tumor microenvironment (TME) in CRC [19,20]. Studying the role of immune cell infiltration is crucial, and we investigated the relationship between KIR2DL4 expression and various immune cell subgroups in CRC. Correlation analysis showed that only pDC and TH17 cells were not correlated with KIR2DL4 (p > 0.05), while the remaining 22 immune cells were positively correlated with the expression of KIR2DL4 (Fig. 6A-P, Figs. S2A–S2H, all p < 0.05). Additionally, we examined the relationship between KIR2DL4 expression and immune cell infiltration across various cancers (Fig. S2I), showing that KIR2DL4 has significant immune infiltration functions in pan-cancer. These results indicate that KIR2DL4 inhibits tumors in CRC by inducing immune cell infiltration. However, the interpretation of immune infiltration data is not sufficiently detailed. We do not distinguish between different types of immune cells, which may have varying roles in tumor progression and response to therapy. This oversimplification undermines the study's conclusions about the immune microenvironment in CRC. Future studies should pay more attention to the effect of KIR2DL4 on the infiltration of different types of immune cells.

3.5. Correlation between KIR2DL4 and chemotherapy sensitivity in CRC

Besides the role in immunity, we were interested in whether KIR2DL4 can enhance the sensitivity of CRC patients to chemotherapy

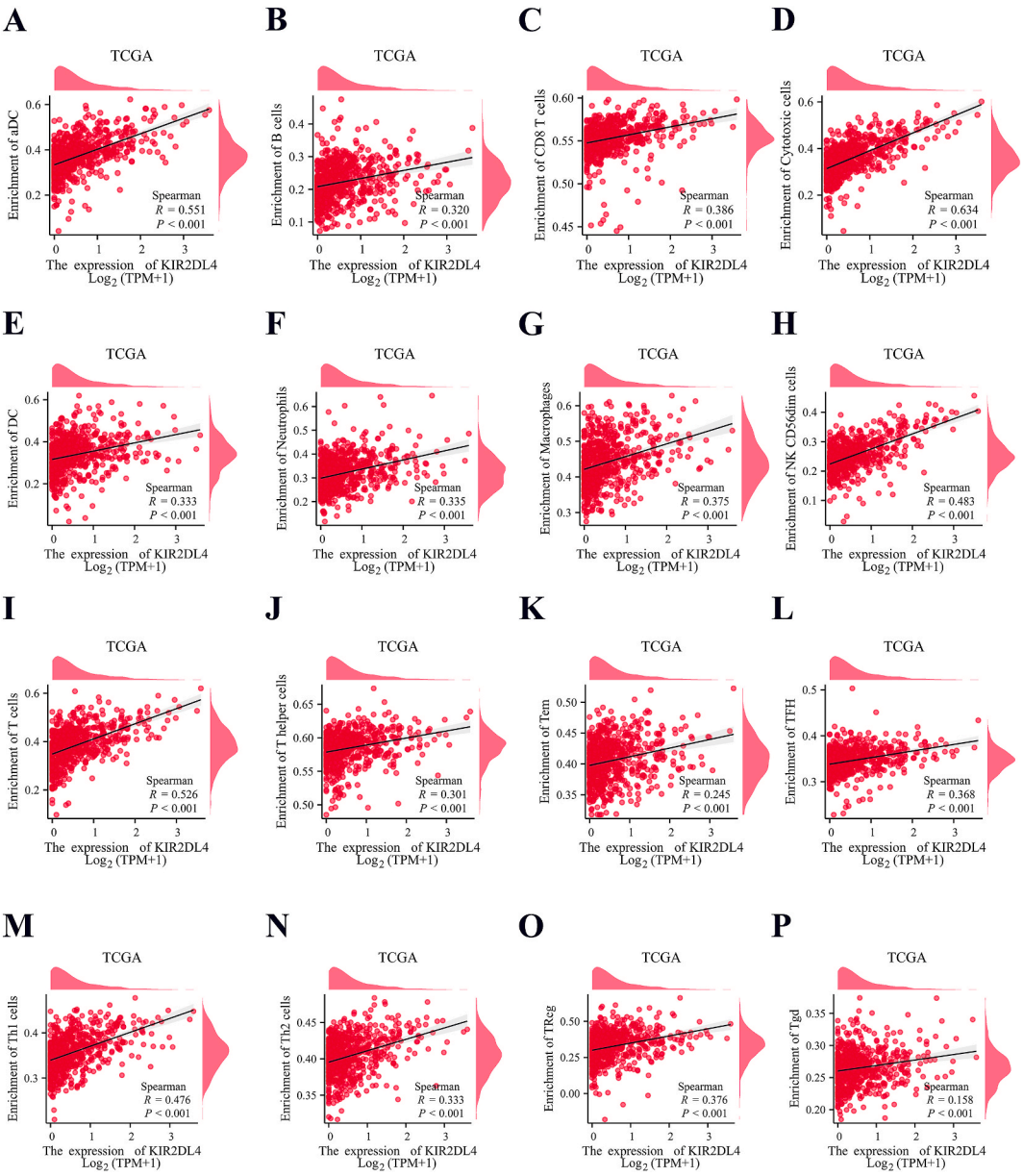

Fig. 6. The relationship between expression levels of KIR2DL4 and immune cell infiltration
The correlation of expression levels of KIR2DL4 with aDC (A), B cell (B), CD8T cell (C), cytotoxic cell (D), DC (E), neutrophils (F), macrophages (G), cd56dim cell (H), T cell (I), T helper (J), Tem (K), TFH (L), TH1 cells (M), TH2 cells (N), Treg (O), and Tgd (P). (P > 0.05, ns. nonsignificant; P < 0.05 *; P < 0.01 **; P < 0.001 ***; P < 0.0001 ****; analyses were performed using Student' s *t*-test or Wilcoxon rank-sum test, respectively).

drugs. Based on the TCGA database, we discovered that elevated expression of KIR2DL4 is linked to an increased objective response rate (ORR) in CRC, suggesting that KIR2DL4 may enhance chemotherapy sensitivity (Fig. 7A, $p < 0.05$). Therefore, we combined data from the GDSC database, and the analysis suggested that high levels of KIR2DL4 are associated with lower IC50 values for Cisplatin, Gemcitabine, Rapamycin, Paclitaxel, and Methotrexate (Fig. 7B–5F, all $p < 0.05$). This suggests that the high expression of KIR2DL4 can enhance the sensitivity of CRC patients to these chemotherapy drugs. However, study fails to demonstrate how KIR2DL4 modulation directly impacts chemotherapy efficacy, and the proposed mechanisms are speculative. This aspect of the study requires more thorough investigation, including functional assays that specifically measure changes in chemotherapy response due to KIR2DL4 modulation.

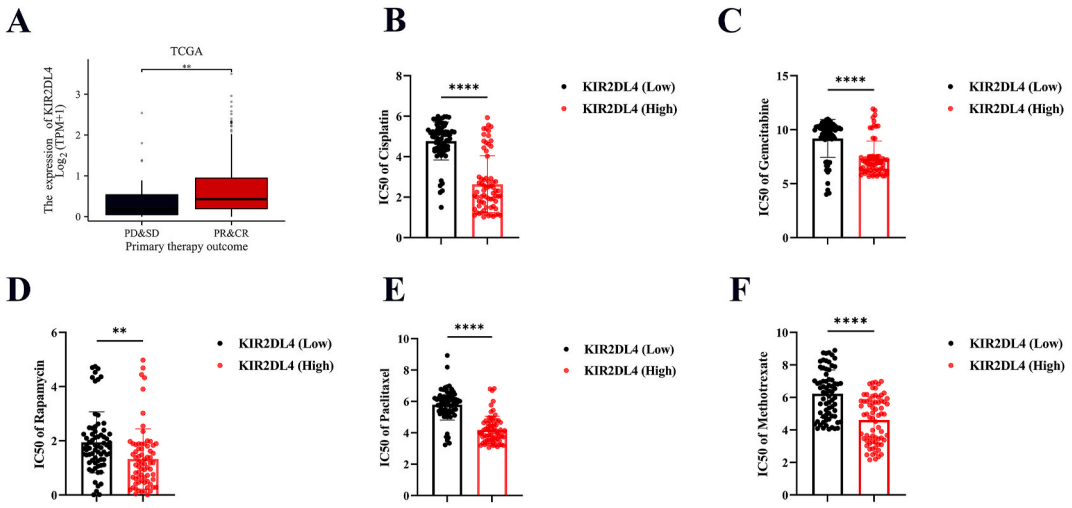

Fig. 7. The correlation between KIR2DL4 and chemotherapy sensitivity in CRC
(A) Based on TCGA, the correlation between the expression level of KIR2DL4 and the response of CRC to chemotherapy. IC50 of response to Cisplatin (B), Gemcitabine (C), Rapamycin (D), Paclitaxel (E), and Methotrexate (F) between the high and low expression of KIR2DL4 groups. (P > 0.05, ns. nonsignificant; P < 0.05 *; P < 0.01 **; P < 0.001 ***; P < 0.0001 ****; analyses were performed using Student' s *t*-test or Wilcoxon rank-sum test, respectively).

3.6. Functional enrichment analysis of KIR2DL4 in CRC

Having established the abnormal expression of KIR2DL4 in CRC and related functions, we became interested in the potential mechanisms of KIR2DL4. Using TCGA data, we inferred molecules associated with KIR2DL4. We detected 2554 significantly correlated DEGs caused by differential expression of KIR2DL4 (|FC| > 1, p.adj <0.05) (Fig. 8A). We annotated and enriched these DEGs, finding that KIR2DL4 is associated with leukocyte mediated immunity, regulation of T cell activation, lymphocyte mediated immunity, and so on (Fig. 8B). KEGG analysis showed that the functions of KIR2DL4 are related to Cytokine-cytokine receptor interaction, Cell adhesion molecules and so on (Fig. 8C). These findings demonstrate that KIR2DL4 exerts anti-cancer effects in CRC through immune-related functions. We further validated these findings through GSEA analysis (Fig. 8D). To further explore genes interacting with KIR2DL4, based on TCGA data, we found that the expression of KIR2DL4 is negatively correlated with SLC39A5, QPRT, IHH, TDGF1, RNF43, LRRC36, TNNC2, AXIN2, GNG4, and SGK2 (Fig. 8E). The expression of KIR2DL4 is positively correlated with NCR1, GNLY, GZMA, CCL5, KIR3DL2, CTSW, TBX21, FASLG, ZNF683, and CD8A (Fig. 8F). These genes may be downstream genes through which KIR2DL4 exerts functions, requiring further study of their relationships.

3.7. Knockdown of KIR2DL4 promoted the viability of CRC

The role of migration and stemness in immune cell infiltration and chemotherapy sensitivity highlights the complexity of the TME and the challenges in treatment strategies [21,22]. High migratory capacity of tumor cells allows to effectively evade immune surveillance, reducing the efficiency of attack and clearance from immune cell. Additionally, the presence of cancer stem cells increases tumor heterogeneity and complexity. These cells can regulate the immune microenvironment by secreting specific signaling molecules, inducing an immunosuppressive state, which further diminishes immune cell infiltration and chemotherapy sensitivity [23,24]. Therefore, after identifying the potential role of KIR2DL4 in CRC, we experimentally verified whether KIR2DL4 can inhibit migration of CRC. SW48 cells were transfected with siRNA (Fig. 9A). The CCK8 assay confirmed that inhibiting KIR2DL4 promoted proliferation of CRC (Fig. 9B, $p < 0.05$). Further colony formation assays revealed that inhibiting KIR2DL4 n significantly promoted stemness of CRC (Fig. 9C, $p < 0.05$). The scratch wound indicated that silencing KIR2DL4 significantly enhanced migration of CRC (Fig. 9D, $p < 0.05$). Oxaliplatin and 5-FU are classic chemotherapeutic agents for CRC. We found that inhibition of KIR2DL4 expression significantly decreased the chemosensitivity of CRC cells to oxaliplatin and 5-FU (Fig. 9E-F, $p < 0.05$). In summary, in CRC, KIR2DL4 demonstrates anti-cancer properties by suppressing cell proliferation, stemness, and the ability to migrate.

4. Discussion

Immunotherapy enhances the immune system to identify and destroy cancer cells, supplementing traditional treatments like surgery, chemotherapy, and radiotherapy [25,26]. Antibodies targeting PD-1/PD-L1 and CTLA-4, have shown significant efficacy in certain subtypes of CRC [27,28]. Nevertheless, the application of immunotherapy in CRC still faces challenges, including how to overcome immunosuppression in the TME, and how to combine immunotherapy with other treatments to enhance efficacy [29,30]. Therefore, we aim to identify new immune-related targets to provide more effective treatment options for patients.

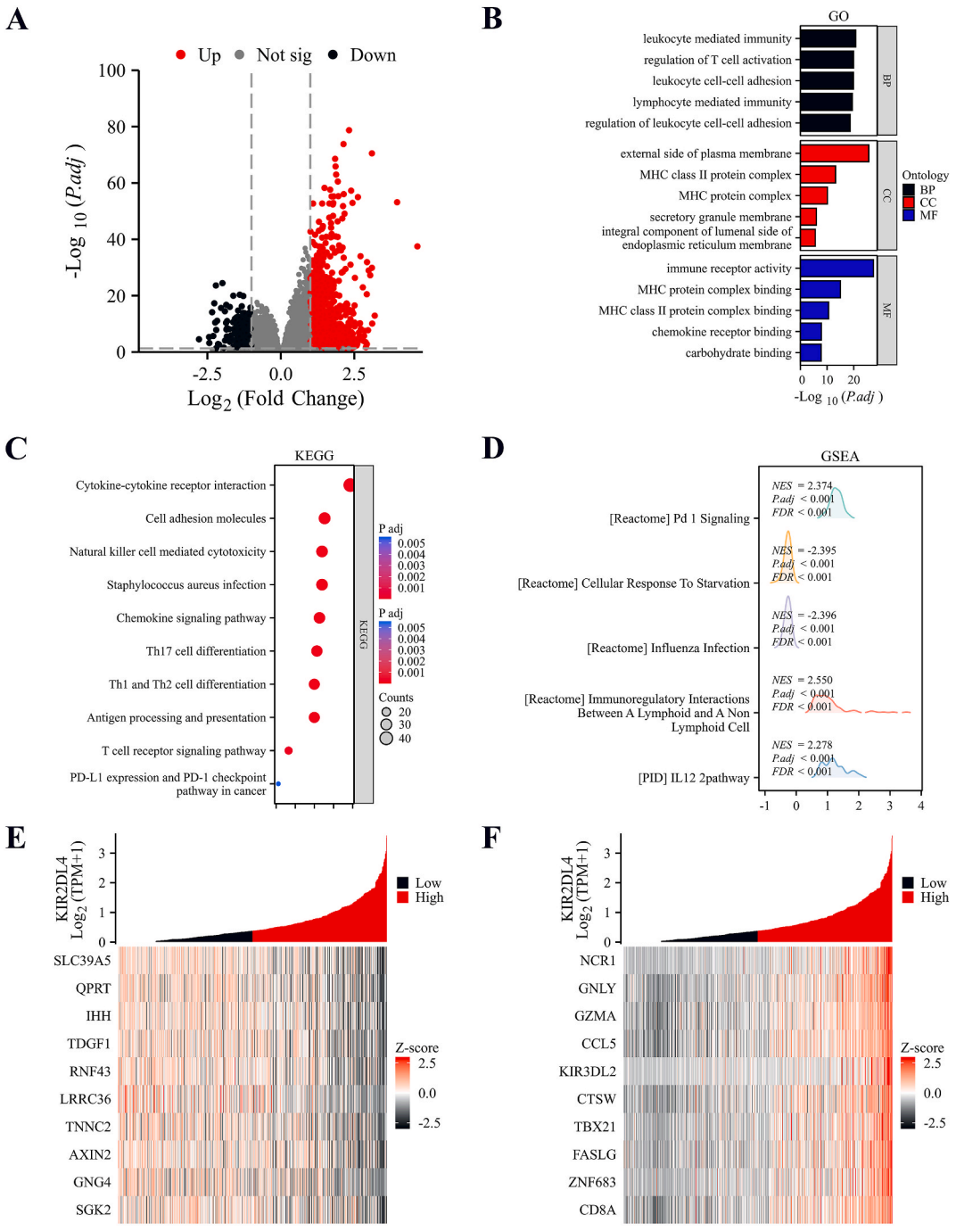

Fig. 8. Pathway enrichment analysis of KIR2DL4 in CRC
(A) Volcano plot of KIR2DL4-related DEGs. (B) GO analysis of BP by KIR2DL4-related DEGs. (C) KEGG analysis of pathways by KIR2DL4-related DEGs. (D) GSEA analysis of functions affected by expression differences of KIR2DL4. (E–F) Analysis of co expressed genes related to KIR2DL4. (P > 0.05, ns. nonsignificant; P < 0.05 *; P < 0.01 **; P < 0.001 ***; P < 0.0001 ****; analyses were performed using Student' s *t*-test or Wilcoxon rank-sum test, respectively).

HLA-G can regulate immune cells through KIR2DL4 to maintain pregnancy and immune escape in endometrial diseases [31]. KIR2DL4 has the capability to activate the cytotoxic functions of natural killer (NK) cells [32]. This highlights the significant role of KIR2DL4 in human immunity. Moreover, the immune-related functions of KIR2DL4 have been preliminarily demonstrated in cancer [11,13,33]. However, there are currently no study on KIR2DL4 in CRC, indicating that the specific functions and mechanisms of KIR2DL4 in CRC have research potential. Utilizing bioinformatics to analyze public databases is an indispensable part of basic

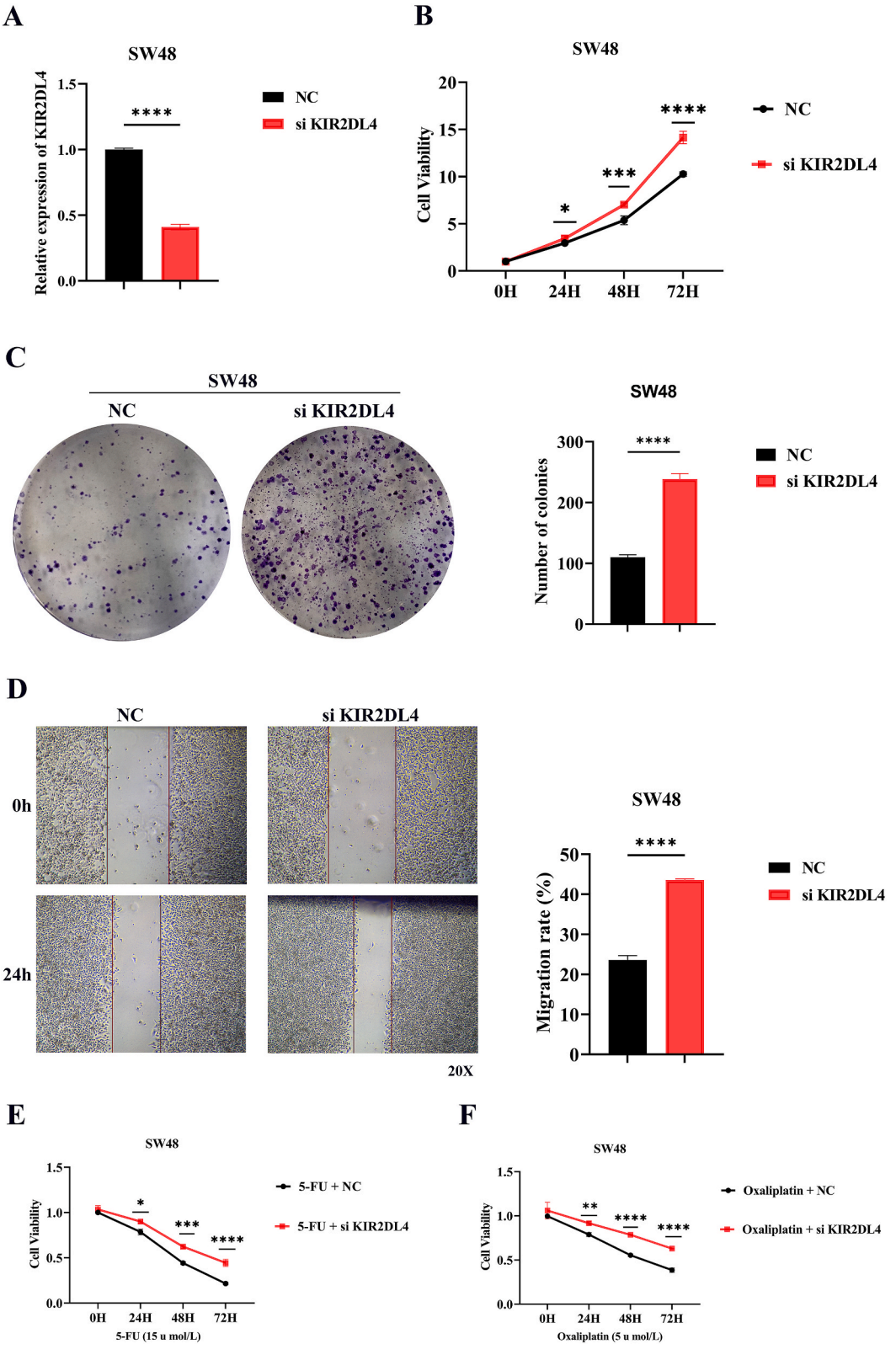

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Fig. 9. Knockdown of KIR2DL4 promoted the viability of CRC
(A) RT-qPCR confirmed the knockdown of KIR2DL4. (B) CCK8 assay detected the effect of siKIR2DL4 on proliferation of CRC. (C) Colony formation assay detected the effect of siKIR2DL4 on stemness capability of CRC. (D) The scratch wound assay confirmed the effect of siKIR2DL4 on migration ability of CRC. (E–F) Chemosensitivity test based on oxaliplatin and 5-FU ($P > 0.05$, ns. nonsignificant; $P < 0.05$ *; $P < 0.01$ **; $P < 0.001$ ***; $P < 0.0001$ ****; analyses were performed using Student's *t*-test or Wilcoxon rank-sum test, respectively).

research, and this trend is increasing annually [34,35]. To compensate for the lack of rigor in single-database studies, this research combines multiple databases and patient data from the Second Affiliated Hospital of Fujian Medical University for cross-validation. This study found that the immune-related molecule KIR2DL4 has predictive diagnostic and prognostic roles in CRC. This study revealed that the differential expression of KIR2DL4 exerts effects by activating immune cell infiltration and enhancing chemotherapy sensitivity in CRC for the first time. This has been validated by pathway enrichment analysis and cell assays.

Tumor suppressor genes play a pivotal role in the initiation and progression of tumors, including inhibiting tumor formation and negatively regulating cell proliferation, promoting cell senescence and apoptosis, activating DNA repair mechanisms, and inhibiting angiogenesis [36,37]. Exploring how to utilize tumor suppressor genes as potential therapeutic targets or prognostic biomarkers is a current research trend. This study, through comprehensive database analysis and IHC, found that KIR2DL4 is significantly lower in CRC than normal tissues. Correspondingly, different expression levels of KIR2DL4 can diagnose CRC and predict prognosis. These findings have been cross-validated with multiple independent datasets and clinical patient information. Future research should focus more on the combined application of multiple targets for screening CRC patients and predicting prognosis. It is evident that KIR2DL4 can serve as a new biomarker for screening and predicting prognosis in CRC patients.

The immune microenvironment, especially the presence and status of immune cells within the tumor, has a substantial influence on the development, prognosis, and treatment response in CRC [38]. High immune cell infiltration is usually associated with better prognosis in CRC patients [39,40]. These cells can recognize and kill cancer cells, preventing further spread of cancer. Additionally, the extent and type of immune cell infiltration are also used as biomarkers to evaluate response to immunotherapy. Research on immune cell infiltration in CRC not only reveals the key role of the immune system in inhibiting development but also provides important evidence for developing new treatment strategies, especially in personalized medicine and immunotherapy. We found that KIR2DL4 is closely related to high immune cell infiltration in CRC. This has been validated by pathway enrichment analysis. This indicates that KIR2DL4 has potential value in enhancing the immune response of CRC patients by regulating the balance of immune cells in the TME.

The importance of chemotherapy in treatment of CRC cannot be overlooked, which is one of the key weapons against lethal tumors [41,42]. For advanced or metastatic CRC patients, chemotherapy is one of the main treatment methods, effectively controlling disease progression and extending survival [43,44]. By comprehensively applying different chemotherapy regimens, the OS of CRC patients can be significantly improved. We found through TCGA combined with GDSC that KIR2DL4 can enhance the sensitivity to chemotherapy. Understanding the levels of KIR2DL4 in patients can help doctors formulate more personalized treatment plans and select the most suitable drugs for patients. This can maximize treatment efficiency and avoid ineffective treatments for patients who are not sensitive to specific drugs. This fully demonstrates that selecting chemotherapy drugs based on specific genetic backgrounds and tumor characteristics can significantly improve success rates of treatment, reduce unnecessary side effects, and alleviate economic burdens. Future research should focus on the role of KIR2DL4 in chemotherapy sensitivity testing, which can predict responses to specific chemotherapy drugs before treatment.

We conducted a comprehensive bioinformatics analysis and validation of the role of KIR2DL4 in CRC. However, some limitations need to be considered. Although we cross-validated with GEO datasets, some CRC-related GEO datasets may have been omitted. Secondly, the specific molecular mechanisms by which KIR2DL4 inhibits proliferation, stemness, and migration capabilities of CRC remain unclear. The study does not adequately address the issue of tumor heterogeneity in CRC. CRC is known for its genetic and phenotypic diversity, which can significantly impact the effectiveness of biomarkers like KIR2DL4. The study fails to stratify patients based on different CRC subtypes or genetic backgrounds.

5. Conclusion

This study discovered that KIR2DL4 is significantly downregulated in CRC, closely associated with metastasis and poor prognosis. As a potential regulator of immunotherapy and chemotherapy sensitivity, KIR2DL4 holds significant importance in treatment of CRC. Future research will focus on thoroughly investigating the molecular mechanisms of KIR2DL4.

Funding information

The authors reported there is no funding associated with the work featured in this article.

Ethics approval and consent to participate

Approval of the research protocol was obtained by the Institutional Reviewer Board of Fujian Provincial Hospital. This study has been examined by the ethics committee and have therefore been performed in accordance with the ethical standards laid down in the Declaration of Helsinki. For patients admitted to the hospital, we provided the informed consent. The content included whether the patient agreed to consent for the researchers to extract information and data for scientific research. All patients enrolled in the study

signed this informed consent form.

Availability of data and materials

Data for this study may be requested from the corresponding author where appropriate.

Consent for publication

Not applicable.

CRediT authorship contribution statement

HuiE. Zhuang: Methodology, Project administration, Supervision, Validation, Visualization, Writing – review & editing. **Yizhen Chen:** Formal analysis, Methodology, Project administration, Software, Writing – original draft.

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.

Acknowledgments

Not applicable.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e37896.

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