Clinical significance and prospective molecular mechanism of C-C motif chemokine receptors in patients with early-stage pancreatic ductal adenocarcinoma after pancreaticoduodenectomy

XIN ZHOU[1], XIWEN LIAO[1], XIANGKUN WANG[1], KETUAN HUANG[1], CHENGKUN YANG[1], TINGDONG YU[1], JUNQI LIU[1], CHUANGYE HAN[1], GUANGZHI ZHU[1], HAO SU[1], WEI QIN[1], QUANFA HAN[1], ZHENGQIAN LIU[1], JIANLV HUANG[1,2], YIZHEN GONG[3], XINPING YE[1] and TAO PENG[1]

[1]Department of Hepatobiliary Surgery, The First Affiliated Hospital of Guangxi Medical University, Nanning, Guangxi Zhuang Autonomous Region 530021; [2]Department of Hepatobiliary Surgery, The Third Affiliated Hospital of Guangxi Medical University, Nanning, Guangxi Zhuang Autonomous Region 530031; [3]Department of Colorectal and Anal Surgery, The First Affiliated Hospital of Guangxi Medical University, Nanning, Guangxi Zhuang Autonomous Region 530021, P.R China

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Abstract. The present study aimed to determine the clinical significance and potential molecular mechanisms of C-C motif chemokine receptor (CCR) genes in patients with early-stage pancreatic ductal adenocarcinoma (PDAC). The transcriptomic, survival and clinical data of 112 patients with early-stage PDAC who underwent pancreaticoduodenectomy were obtained from The Cancer Genome Atlas. The prognostic values of the CCR genes involved in early-stage PDAC were evaluated using Kaplan-Meier analysis and the multivariate Cox proportional risk regression model, and the potential molecular mechanisms were determined using bioinformatics tools. The identified CCRs closely interacted with each other at both the gene and protein levels. High expression levels of CCR5 [adjusted P=0.012; adjusted hazard ration (HR)=0.478, 95% confidence interval (CI)=0.269-0.852], CCR6 (adjusted P=0.026; adjusted HR=0.527, 95% CI=0.299-0.927) and CCR9 (adjusted P=0.001; adjusted HR=0.374, 95% CI=0.209-0.670) were significantly associated with longer overall survival times in patients with early-stage PDAC. The contribution of CCR5, CCR6 and CCR9 to the outcome of early-stage PDAC was also demonstrated. Combined survival analysis of CCR5, CCR6 and CCR9 suggested that patients with high expression levels of these CCRs exhibited the most favorable outcomes. A prognostic signature was constructed in terms of the expression level of CC5, CCR6 and CCR9, and time-dependent receiver operating characteristic curves indicated that this signature was able to effectively predict the outcome of patients with early-stage PDAC. The potential molecular mechanisms of CCR5, CC6 and CCR9 in PDAC include its intersection of the P53, nuclear factor (NF)-κB, generic transcription, mitogen-activated protein kinase and STAT signaling pathways. Collectively, this highlights that CCR5, CCR6 and CCR9 are potential prognostic biomarkers for early-stage PDAC.

Correspondence to: Professor Tao Peng, Department of Hepatobiliary Surgery, The First Affiliated Hospital of Guangxi Medical University, 6 Shuang Yong Road, Nanning, Guangxi Zhuang Autonomous Region 530021, P.R. China
E-mail: pengtaogmu@163.com

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Introduction

In 2018, ~458 million new cases of pancreatic cancer were diagnosed worldwide, resulting in 432 million mortalities, the seventh highest among all cancer-associated deaths (1). Pancreatic cancer is a lethal malignancy with a <5% five-year survival rate, indicating a mortality rate almost equal to its occurrence (1-4). Pancreatic ductal adenocarcinoma (PDAC), which accounts for ~90% of all pancreatic cancer cases (5) is the third and sixth leading cause of cancer-associated death in the United States and China, respectively (6-8). As 80-90% of patients with PDAC are diagnosed at an advanced stage, when the tumor has usually metastasized, radical resection is not possible (6,9-11). Conventional treatments such as chemotherapy and radiotherapy, as well as targeted therapies, have largely failed to prolong the overall survival (OS) time of patients with PDAC (12), and only a few novel anti-PDAC strategies are currently in use (13,14). Therefore, the identification of early diagnostic markers and therapeutic targets for PDAC is critical. Taking into account the encouraging results of immuno- and gene therapies against PDAC (15-17), the present study aimed to identify novel immunological targets associated with its prognosis.

Chemokines and their receptors are critical mediators of the inflammatory and immune responses (18-20), and have

recently been implicated in tumorigenesis (21,22). Chemokine receptors promote tumorigenesis via numerous mechanisms (23), including inflammation, that is known to play a significant role in the pathogenesis and progression of pancreatic cancer (24-26). In addition, the poor prognosis and frequent distant metastasis of pancreatic cancer are also associated with immune surveillance escape (12,27,28). The chemokine receptors are classified into four subfamilies-CCR, CXCR, XCR and CX3CR-based on variations within the cysteine motif. Although several studies have elucidated the potential roles of these CCRs (C-C motif chemokine receptors) in pancreatic cancer, using cell lines or murine models (29-35), the association of CCRs with OS remains ambiguous. Therefore, the aim of the present study was to investigate the association between CCRs and the prognosis of patients with PDAC.

Materials and methods

Data mining and processing. The transcriptome profiles of patients with PDAC were obtained from TCGA database (https://cancergenome.nih.gov/, accessed at April 20, 2017), and normalized using the *DESeq* Package in R (36,37). The corresponding clinical data were acquired from the University of California, Santa Cruz Xena (UCSC Xena; http://xena.ucsc.edu/, accessed at April 20, 2017). In order to eliminate interference from unrelated factors, the patients were selected based on the following inclusion criteria: i) Histological validation; ii) pathological stage I or II according to the 7th American Joint Committee on Cancer (AJCC); iii) availability of complete survival data; and iv) having undergone pancreaticoduodenectomy.

Bioinformatics and correlation analysis of CCR genes. The CCR genes were functionally annotated using Gene Ontology (GO) terms, and the associated pathways were determined by Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis using the Database for Annotation, Visualization and Integrated Discovery (https://david.ncifcrf.gov/home.jsp version 6.8, accessed at January 19, 2018) (38). The correlation between CCR genes was analyzed by Pearson's correlation coefficient using the *corrplot* package in R (version 1.2.1335; www.r-project.org). A protein-protein interaction (PPI) map was constructed using the Search Tool for the Retrieval of Interacting Genes/Proteins (https://string-db.org/, version 11, accessed at march 30, 2019) (39). All CCR gene symbols (CCR1-CCR10) were entered into the platform for *Homo sapiens* and an interaction score >0.4 was considered to be significant. Finally, a gene-gene interaction network was constructed using GeneMANIA (http://genemania.org/, accessed at March 30, 2019) (40).

Survival analysis. The association between different clinical factors and the prognosis of patients with early-stage PDAC was determined using Kaplan-Meier analysis with the log-rank test; the relevant factors were then included in the multivariate Cox proportional risk regression model to identify the CCR genes significantly associated with OS. Based on the results of the survival analysis of individual genes, combined effect survival analysis was performed and a nomogram was constructed. The CCR genes associated with PDAC prognosis were assessed using combined effect survival analysis (Kaplan-Meier analysis with log-rank test) and the multivariate Cox proportional risk regression model. The nomogram was constructed in R (version 3.5.2; www.r-project.org) using the *rms* package, based on clinical variables and the expression levels of CCR genes. The scale marked on the line indicates the value range of each variable, and the length of the line segment reflects the contribution of this factor to the outcome event.

Prognostic signature construction. According to the results of the survival analysis, the CCR genes associated with PDAC prognosis were combined to construct a prognostic model based on gene expression level. The risk score formula was as follows: Risk score=expression of $gene_1$ x β_1 + expression of $gene_2$ x β_2+… expression of $Gene_n$ x β_n (41,42), where β_n is the regression coefficient derived from the result of multivariate Cox proportional hazards regression analysis for the corresponding gene. Based on the median risk score value, the patients were divided into a high- and low risk group. To assess the predictive value of the prognostic signature, a time-dependent ROC curve was constructed using the *survivalROC* package in R (43). Survival analysis was performed to compare prognoses between the high- and low-risk groups.

Gene set enrichment analysis (GSEA). In order to identify the pathways in which the CCR gene is enriched, and to determine whether the CCR genes in each gene set are enriched in the upper or lower part of the phenotype-related sorted gene list, genome-wide expression profile datasets and corresponding grouping files determined by the expression of CCR genes were uploaded to GSEA (44) for enrichment analysis with database c2 and c5 of the Molecular Signatures Database (MSigDB) (45). A set of genes with both false discovery rate (FDR) <0.25 and P<0.05 was considered to be statistically significant.

Statistical analysis. Statistical analysis was conducted using SPSS 22.0 (IBM Corporation) or R 3.52 (https://www.r-project.org/). Hazard ratios (HRs) and 95% confidence intervals (CI) were used to indicate the relative risk between the high-C and low-CCR expression groups. Pearson's correlation coefficient was used to determine the correlation between CCR genes, where P<0.05 was considered to indicate a statistically significant result. The FDR control in GSEA was achieved using the Benjamini-Hochberg procedure and adjusted for multiple testing (46-48), where an FDR<0.25 was considered to indicate a statistically significant difference.

Results

Data collection and arrangement. The expression profiles of patients with PDAC were acquired from TCGA database. After screening based on the inclusion criteria, patients that fell outside of these parameters were eliminated, and the profiles of the remaining 112 patients were further analyzed.

Functional annotation and correlation analysis of CCR genes. As shown in Fig. 1A and Table SI, the results of GO and KEGG pathway analysis indicated that CCR genes are primarily involved in pathways related to immunity and

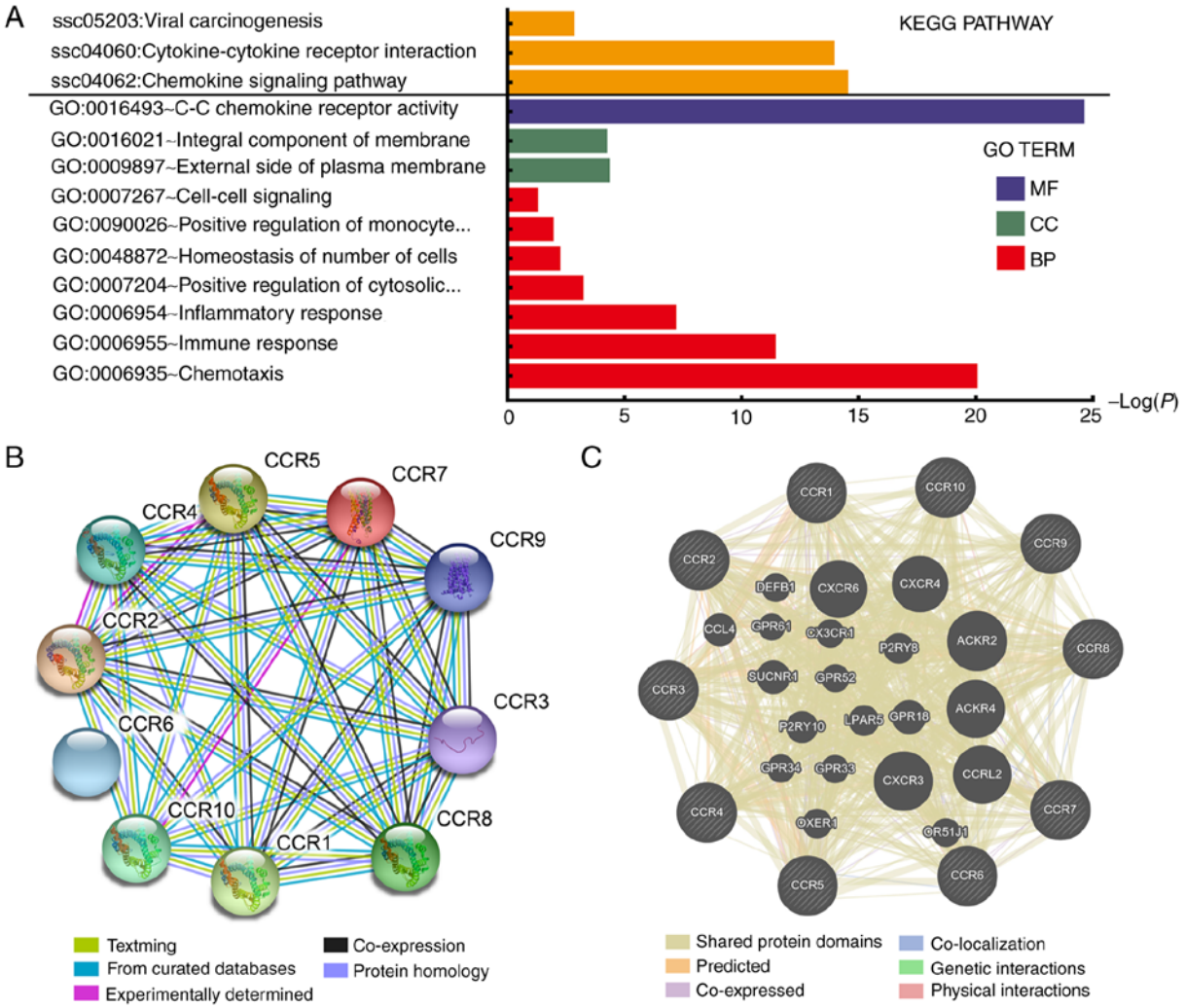

Figure 1. KEGG pathway and GO term analysis of CCR genes and gene interactions. (A) KEGG pathway and GO term analysis of CCR genes. (B) STRING and (C) GeneMANIA protein-protein association networks of CCR genes. KEGG, Kyoto Encyclopedia of Genes and Genomes; GO, Gene Ontology; CCR, C-C motif chemokine receptor; STRING, Search Tool for the Retrieval of Interacting Genes/Proteins; MF, Molecular Function; BP, Biological Process; CC, Cellular Component.

inflammation, and that the JAK/STAT, mitogen-activated protein kinase (MAPK) and nuclear factor (NF)-κB signaling pathways were associated with chemokine signaling (Fig. S1). PPI and gene-gene interaction network analyses revealed that CCRs interact closely with each other (Fig. 1B and C). A matrix graph of Pearson's correlation analysis indicated that CCR1, CCR2, CCR4, CCR5 and CCR8 are closely related to each other with a correlation coefficient ≥0.5. In addition, a higher degree of correlation was observed among CCR4, CCR6 and CCR7 (correlation coefficient ≥0.7) (Fig. 2). The numbers in each grid represent the correlations between the corresponding genes.

Survival analysis of CCR genes. The clinical data of all patients are summarized in Table SII. Histological grade, targeted molecular therapy, radiation therapy and residual resection were all significantly associated with OS. Patients harboring tumors of a higher histological grade, and those who did not receive either targeted or radiation therapies or undergo residual resection were at a higher risk of poor prognosis. In addition, high expression levels of CCR5 (adjusted P=0.012; adjusted HR=0.478, 95% CI=0.269-0.852), CCR6 (adjusted P=0.026; adjusted HR=0.527, 95% CI=0.299-0.927) and CCR9 (adjusted P=0.001; adjusted HR=0.374, 95% CI=0.209-0.670) were significantly associated with lower mortality rates (Table I and Fig. 3). The nomogram also indicated that CCR5, CCR6 and CCR9 may contribute to the prognosis of PDAC, with low expression corresponding to a high point (Fig. 4E).

Combined effect survival analysis of CCR genes. The patients were stratified into groups based on the expression levels of different CCR genes. The expression levels of CCR genes in different groups are summarized in Table II. Favorable overall survival was observed in Group D (compared with Groups A, B and C; adjusted P=0.012; adjusted HR=0.434, 95% CI=0.226-0.833), Group IV (compared with Group I, II and III; adjusted P<0.001; adjusted HR=0.236, 95% CI=0.107-0.520), Group d (compared with Group a, b and c; adjusted P=0.001; adjusted HR=0.284, 95% CI=0.136-0.595) and Group 4 (compared with Group 1, 2 and 3; adjusted P=0.001; adjusted HR=0.253, 95% CI=0.112-0.574) (Table II and Fig. 4A-D).

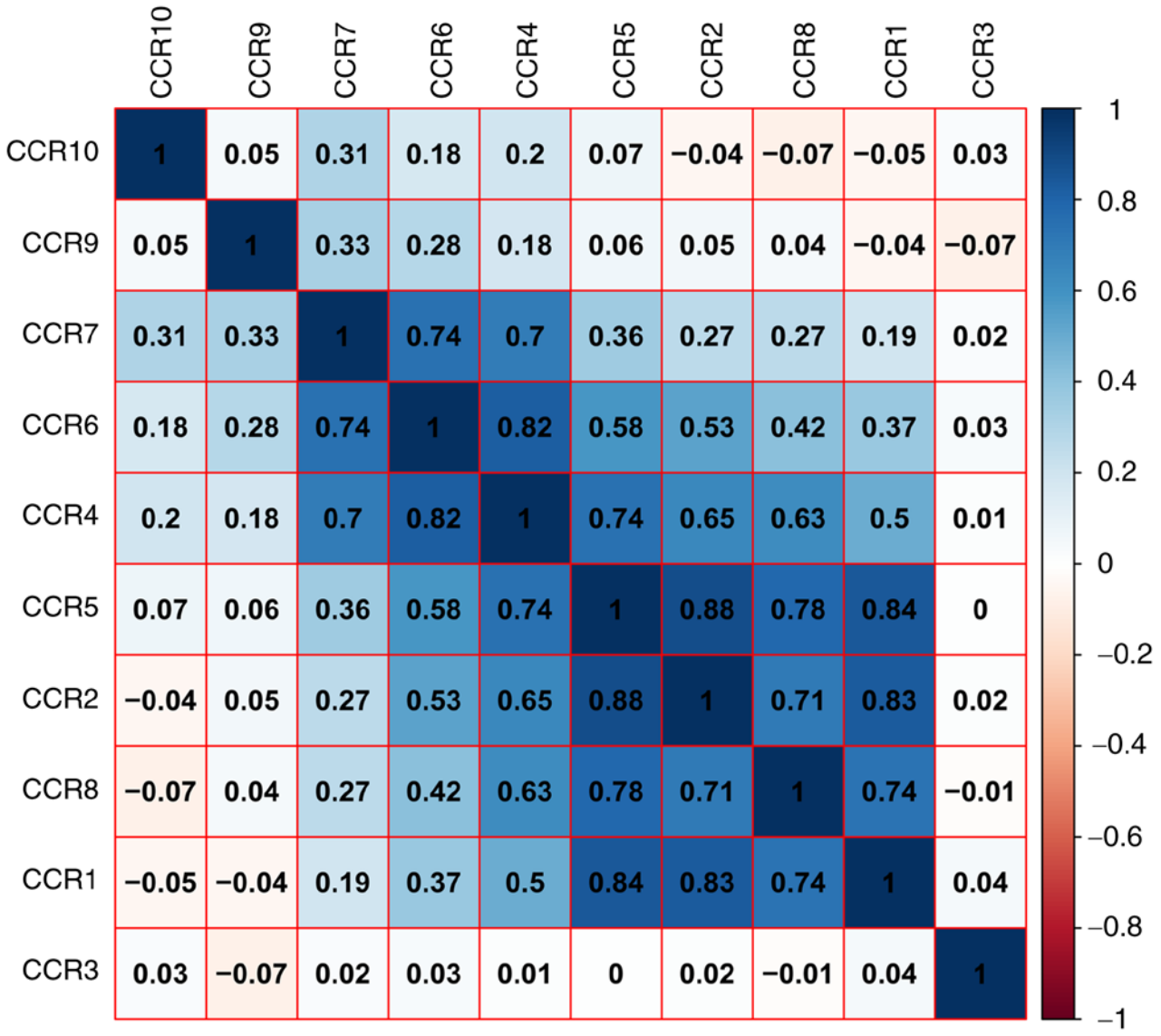

Figure 2. Matrix graphs of Pearson's correlation analysis of CCR genes.

Prognostic signature construction. Using both single gene survival analysis and combined effect survival analysis, CCR5, CCR6 and CCR9 were demonstrated to be associated the prognosis of patients with early-stage PDAC; therefore, these three genes were selected for the construction of the prognostic signature. The regression coefficient of CCR5, CCR6 and CCR9 from the multivariate Cox proportional hazards regression model was -0.836, -0.618 and -0.476 respectively. Because all β-values in this investigation were <0, and to make the result easier to interpret, a constant was added to the end of the following risk score formula: Risk score=expression of CCR5 x -0.836 + expression of CCR x -0.618 + expression of CCR x -0.476 + 4[constant]. The effect of the constant is to ensure that the risk score output is >0. Survival analysis between the high and low risk score groups indicated that a high risk score was significantly associated with the poor outcome of patients with early-stage PDAC (adjusted P=0.018; adjusted HR=1.988, 95% CI=1.125-3.513) (Fig. 4F and G). Time-dependent ROC analysis demonstrated that the prognostic signature effectively predicted the outcome of patients with early-stage PDAC (1-year AUC=0.674; 2-year AUC=0.649; 3-year AUC=0.673; Fig. 4H).

GSEA. Since CCR5, CCR6 and CCR9 were favorably associated with OS, the patients were stratified according to their respective median expression values. GSEA results are displayed in Figs. 5-7. Analysis of the C2 (curated) gene sets revealed that CCR5 was enriched in TP53 target, TP63 target, MAPK signaling pathway, generic transcription pathway, DNA damage and STAT5A target (Table SIII, Fig. 5A-F); in the C5 (GO) gene sets, CCR5 was enriched in inflammatory response, STAT cascade, MAPK cascade, regulation of NF-κB and endothelial proliferation (Table SIII, Fig. 5G-P); CCR6 was enriched in TGF-β1 signaling pathway, TP53 and TP63 targets, KEGG MAPK signaling pathway, MAPK14 targets and NF-κB signaling in the C2 set (Table SIV and Fig. 6A-G), and in STAT cascade, MAPK cascade, NF-κB import into nucleus, NF-κB signaling and transcription factor importing into nucleus in the C5 set (Table SV, Fig. 6H-M); CCR9 was enriched in the IL-2/STAT5 pathway, proliferation, NF-κB

Table I. Prognostic value of the expression levels of CCR genes in early-stage pancreatic ductal adenocarcinoma using The Cancer Genome Atlas database.

		Overall survival					
Gene expression level	Patients (n=112)	Number of events	Median survival time (days)	Crude HR (95% CI)	Crude Log-rank P-value	Adjusted HR (95% CI)	Adjusted P-value[a]
CCR1							
Low	56	34	485	1		1	
High	56	35	592	0.820 (0.508-1.326)	0.418	0.812 (0.474-1.393)	0.450
CCR2							
Low	56	38	458	1		1	
High	56	31	603	0.518 (0.317-0.846)	0.007	0.668 (0.379-1.178)	0.163
CCR3							
Low	56	33	511	1		1	
High	56	36	518	0.854 (0.527-1.385)	0.521	0.947 (0.556-1.613)	0.841
CCR4							
Low	56	40	470	1		1	
High	56	29	607	0.490 (0.299-0.804)	0.004	0.601 (0.343-1.054)	0.076
CCR5							
Low	56	39	393	1		1	
High	56	30	603	0.433 (0.263-0.714)	0.001	0.478 (0.269-0.852)	0.012
CCR6							
Low	56	39	458	1		1	
High	56	30	596	0.539 (0.329-0.882)	0.013	0.527 (0.299-0.927)	0.026
CCR7							
Low	56	38	473	1		1	
High	56	31	592	0.562 (0.344-0.918)	0.020	0.630 (0.360-1.102)	0.105
CCR8							
Low	56	38	470	1		1	
High	56	31	603	0.588 (0.362-0.955)	0.030	0.642 (0.372-1.107)	0.111
CCR9							
Low	56	37	485	1		1	
High	56	32	568	0.621 (0.381-1.014)	0.054	0.374 (0.209-0.670)	0.001
CCR10							
Low	56	40	481	1		1	
High	56	29	634	0.750 (0.462-1.217)	0.242	0.835 (0.491-1.422)	0.507

[a]Adjusted for histological grade, radiation therapy, radical resection and targeted molecular therapy using the multivariate Cox proportional hazards regression model. CCR, C-C motif chemokine receptor; HR, hazard ratio; CI, confidence interval.

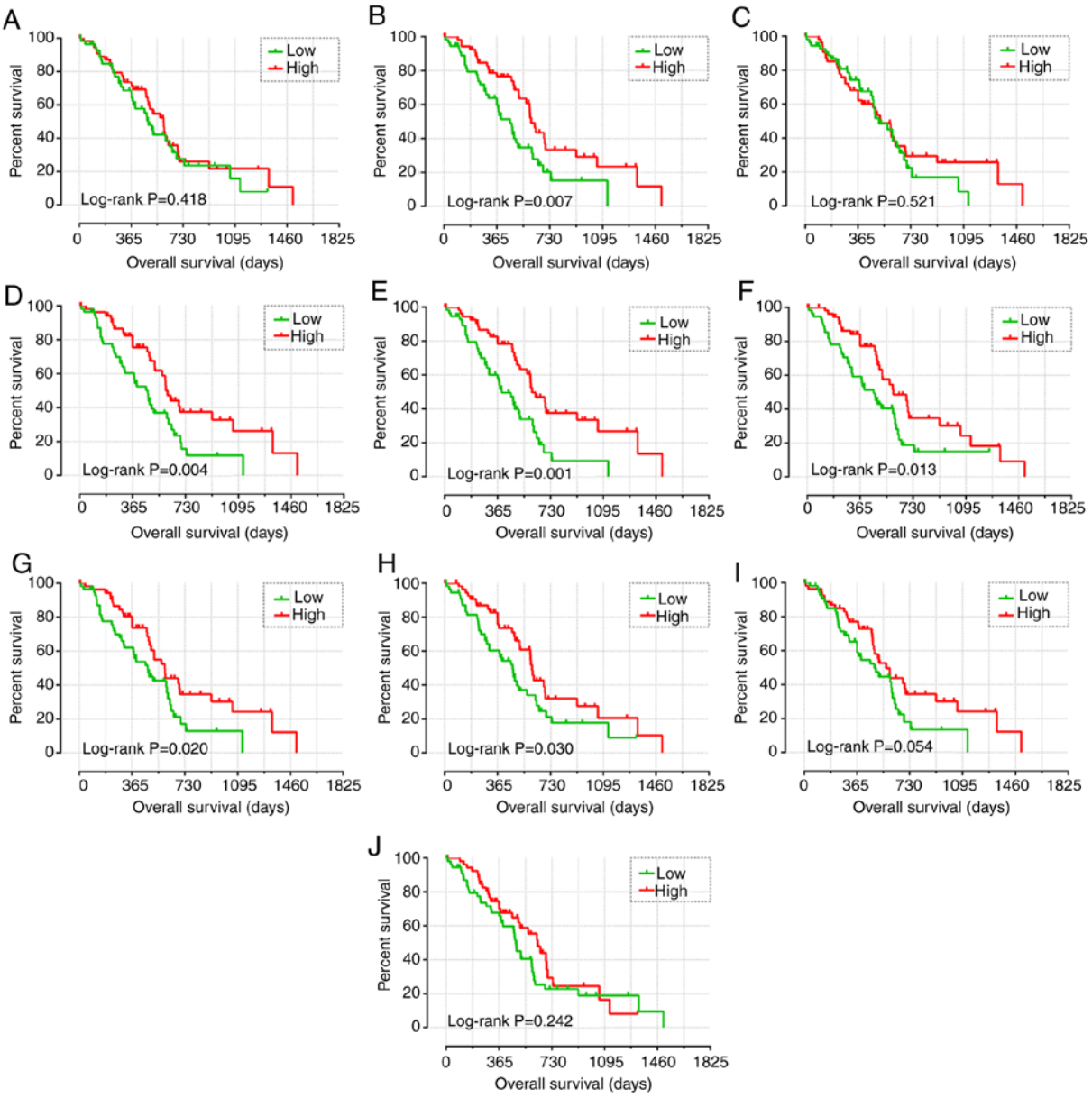

Figure 3. Kaplan-Meier survival curve analysis of the association between the high and low expression levels of CCR genes and overall survival in patients with early-stage PDAC, generated using The Cancer Genome Atlas. Overall survival curves for (A) CCR1, (B) CCR2, (C) CCR3, (D) CCR4, (E) CCR5, (F) CCR6, (G) CCR7, (H) CCR8, (I) CCR9 and (J) CCR10. CCR, C-C motif chemokine receptor; PDAC, pancreatic ductal adenocarcinoma.

atypical pathway, STAT 5 targets and PTEN pathway in the C2 set (Table SVI and Fig. 7A-G).

Discussion

Chemokines and chemokine receptors serve critical roles in oncogenesis and cancer progression via a number of complex mechanisms. It was reported that the chemokines secreted by the tumor, immune and stromal cells were able to initiate the uncontrolled proliferation and metastasis of tumor cells in an autocrine and paracrine manner, by binding to their cognate receptors (49). To date, CXCR4 is the most widely studied and clearly understood chemokine receptor associated with cancer, and was revealed to be involved in the development, growth, invasion, angiogenesis and metastasis of pancreatic cancer in a number of previous studies (50-57). However, a limited number of studies have investigated the role of the CCR gene in PDAC; therefore, the present study primarily focused on the CCR gene family in PDAC.

Chemokine receptors have been reported to impact tumor progression by regulating the MAPK/ERK, JAK/STAT and NF-κB signaling pathways (49,58-60); CCR5 upregulated c-Fos in tumor cells by stimulating the JAK/STAT pathway (60), and CCR5 stimulation by CCL5 restricted the proliferation of breast cancer cells by increasing p53 transcription via the JAK2 and p38-MAPK pathways (61). Further studies have also illustrated the anti-invasive and anti-metastatic roles of CCR5 in mouse models of breast cancer (62,63). However, a contradictory study revealed that the CCR5-Δ53 polymorphism was associated with a greater risk of developing gallbladder cancer (64), highlighting that the role of CCR5 may be cancer type-dependent. In the present

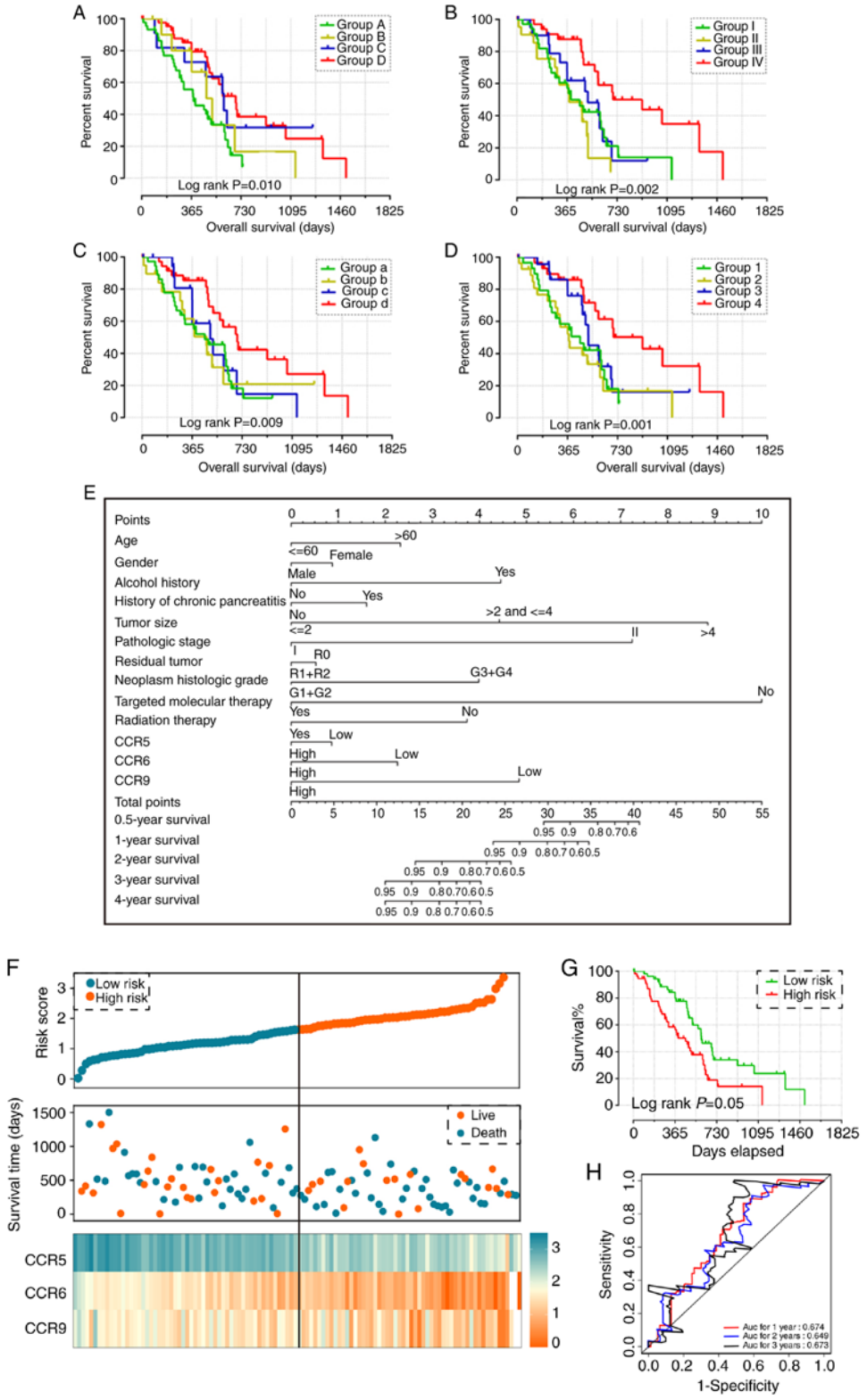

Figure 4. Combined effect of CCR5, CCR6 and CCR9 on the overall survival of patients with early-stage PDAC. Nomogram for predicting 1-, 2- and 3-year events and a prognostic model with risk score, in terms of CCR5, CCR6 and CCR9 expression in early-stage PDAC. (A) Overall survival curves for the combined effect of CCR5 and CCR6. Group A, Low CCR5 + Low CCR6; Group B, Low CCR5 + High CCR6; Group C, High CCR5 + Low CCR6; Group D, High CCR5 + High CCR6. (B) Overall survival curves for the combined effect of CCR5 and CCR9. Group I, Low CCR5 + Low CCR9; Group II, Low CCR5 + High CCR9; Group III, High CCR5 + Low CCR9; Group IV, High CCR5 + High CCR9. (C) Overall survival curves for the combined effect of CCR6 and CCR9. Group a, Low CCR5 + Low CCR9; Group b, Low CCR5 + High CCR9; Group c, High CCR5 + Low CCR9; Group d, High CCR5 + High CCR9. (D) Overall survival curves for the combined effect of CCR5, CCR6 and CCR9. Group 1, Low CCR5 + Low CCR6 + Low CCR9; Group 2, Low CCR5 + Low CCR6 + High CCR9, High CCR5 + Low CCR6 + Low CCR9, and Low CCR5 + High CCR6 + Low CCR9; Group 3, Low CCR5 + High CCR6 + High CCR9, High CCR5 + High CCR6 + Low CCR9, and High CCR5 + Low CCR6 + High CCR9; Group 4, High CCR5 + High CCR6 + High CCR9. (E) Nomogram for predicting 1-, 2- and 3-year events (mortalities) that combine clinical data with CCR5, CCR6 and CCR7 expression. (F) From top to bottom; risk score plot, survival status scatter plot and heat map of the expression levels of CCR5, CCR6 and CCR9 in low- and high-risk groups. (G) Kaplan-Meier curves for low- and high-risk groups. (H) Receiver operating characteristic curve for predicting 1-, 2- and 3-year survival in patients with early-stage PDAC by risk score. CCR, C-C motif chemokine receptor; PDAC, pancreatic ductal adenocarcinoma.

Table II. Joint effects survival analysis of CCR gene expression levels with overall survival in patients with early-stage pancreatic ductal adenocarcinoma derived from The Cancer Genome Atlas database.

Group	CCR5	CCR6	CCR9	Patients (n)	Number of events	Median survival time (days)	Crude HR (95% CI)	Crude P-value	Adjusted HR (95% CI)	Adjusted P-value[a]
A	Low	Low		45	32	381	1		1	
B	Low	High		11	7	476	0.703 (0.307-1.612)	0.406	0.996 (0.374-2.651)	0.994
C	High	Low		11	7	603	0.451 (0.196-1.038)	0.061	0.803 (0.283-2.280)	0.680
D	High	High		45	32	591	0.386 (0.218-0.681)	0.010	0.434 (0.226-0.833)	0.012
I	Low		Low	35	24	393	1		1	
II	Low		High	21	15	381	1.469 (0.759-2.841)	0.253	0.780 (0.367-1.657)	0.518
III	High		Low	21	13	517	0.846 (0.430-1.667)	0.630	1.158 (0.550-2.437)	0.699
IV	High		High	35	17	702	0.345 (0.178-0.667)	0.002	0.236 (0.107-0.520)	<0.001
A		Low	Low	37	26	458	1		1	
B		Low	High	19	13	470	1.020 (0.521-1.998)	0.954	0.568 (0.256-1.261)	0.165
C		High	Low	19	11	695	0.828 (0.412-1.702)	0.624	0.931 (0.446-1.943)	0.849
D		High	High	37	19	518	0.438 (0.235-0.817)	0.009	0.284 (0.136-0.595)	0.001
1	Low	Low	Low	30	21	458	1		1	
2	Low	Low	High	27	19	375	1.094 (0.585-2.047)	0.778	0.874 (0.451-1.692)	0.689
	High	Low	Low	-	-	-				
	Low	High	Low	-	-	-				
3	Low	High	High	24	14	518	0.667(0.338-1.316)	0.243	0.747(0.347-1.609)	0.457
	High	High	Low	-	-	-				
	High	Low	High	-	-	-				
4	High	High	High	31	15	913	0.350(0.172-0.711)	0.004	0.253(0.112-0.574)	0.001

[a]Adjusted for histological grade, radiation therapy, radical resection and targeted molecular therapy using the multivariate Cox proportional hazards regression model. CCR, C-C motif chemokine receptor; HR, hazard ratio; CI, confidence interval.

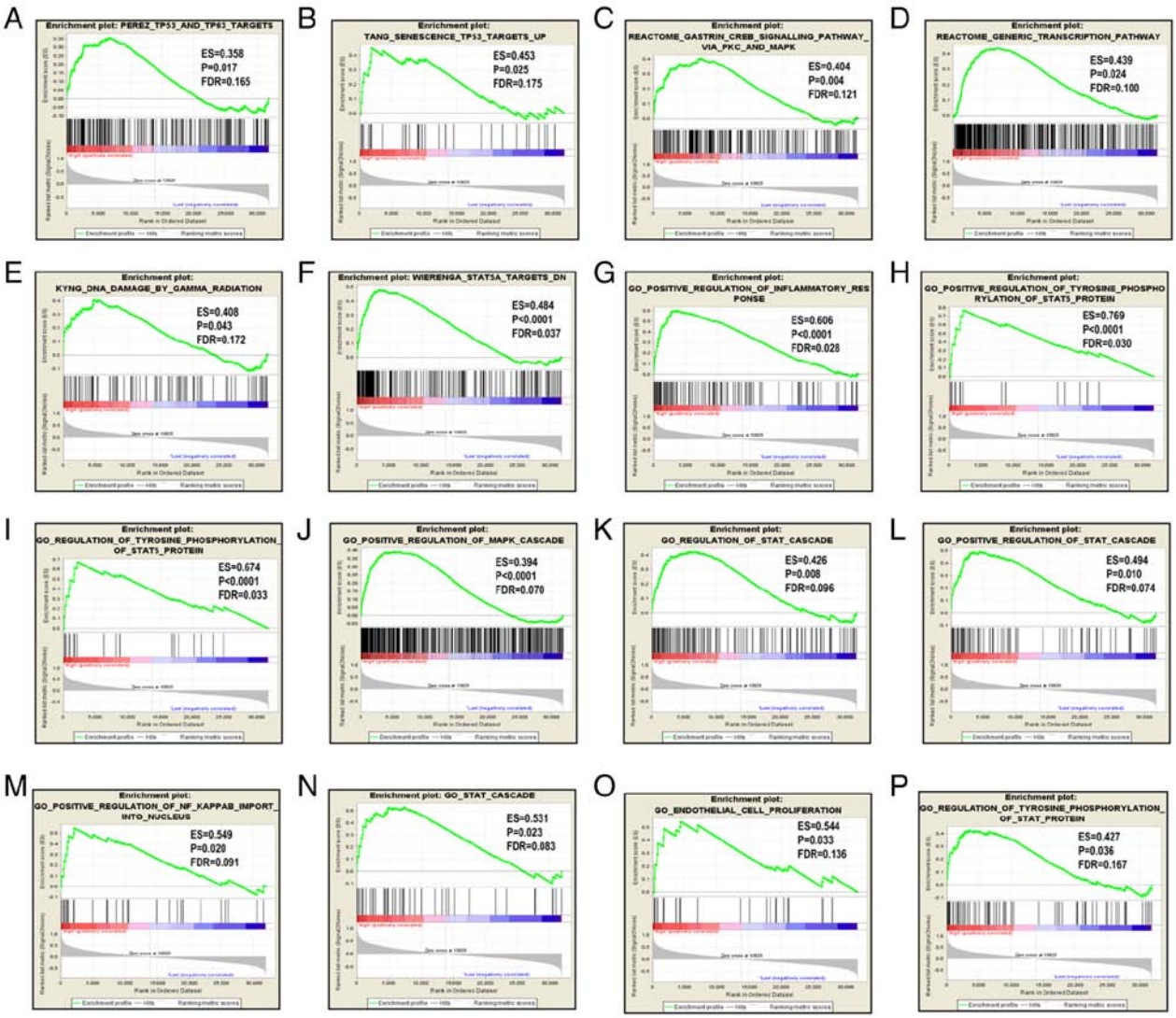

Figure 5. GSEA results for CCR5 in patients with pancreatic ductal adenocarcinoma. GSEA results of (A-F) c2-reference and (G-P) c5-reference gene sets for groups with increased CCR5 expression levels. GSEA, gene set enrichment analysis; CCR, C-C motif chemokine receptor; ES, enrichment score; FDR, false discovery rate.

study, bioinformatics analysis suggested that CCR genes were primarily involved in immune and inflammatory responses, and also revealed that the JAK/STAT, MAPK and NF-κB signaling pathways are involved in chemokine signaling. These pathways are consistent with the downstream pathways regulated by p53, therefore, it was hypothesized that CCR5 may also serve a role in PDAC by activating p53. Furthermore, CCR3, CCR4, CCR5 and CCR8 were also found to be associated with viral carcinogenesis, a greater number of CCR genes than those identified to be concerned with carcinogenesis in previous studies (23,65-68). Since various studies have shown its close association with inflammation and immunity (69-75), this is highly relevant to PDAC. Also, considering the function of CCR genes in mediating inflammation, and that CCR5, CCR6 and CCR9 were significantly associated with the overall survival of patients with early-stage PDAC (as indicated by the results of survival analysis), it was concluded that CCR5, CCR6 and CCR9 serve important roles in the development and progression of PDAC. Furthermore, the results of the bioinformatics and survival analysis of CCR genes in PDAC also verified previous findings of the role of CCR genes in cancer (23,35,61,76,77).

The present study is believed to be the first to show that high expression levels of CCR5, CCR6 and CCR9 are associated with prolonged overall survival in patients with early-stage PDAC. The role of CCR5 as a protective factor in PDAC is in agreement with previous studies; it was reported that knocking out CCR5 in pancreatic tumor-bearing mice reduced the infiltration and subsequent cytotoxicity of NK cell in tumors (78). Furthermore, smokers carrying a CCR5 mutant allele have a significantly higher risk of developing pancreatic cancer (76). Moreover, the protective role of CCR5 has also been reported in other malignancies. The CCR5 superagonist 1P7 was found to act as an adjuvant to anti-tumor DNA vaccination by inducing specific CD8+ T-cell responses (77), and the CCR5-Δ53 polymorphism was discovered to be associated with susceptibility to breast cancer in the Indian population (35). It has also been noted that in breast cancer, the absence of CCR5 on the tumor cell surface may promote the proliferation of tumor cells which carry wild-type p53,

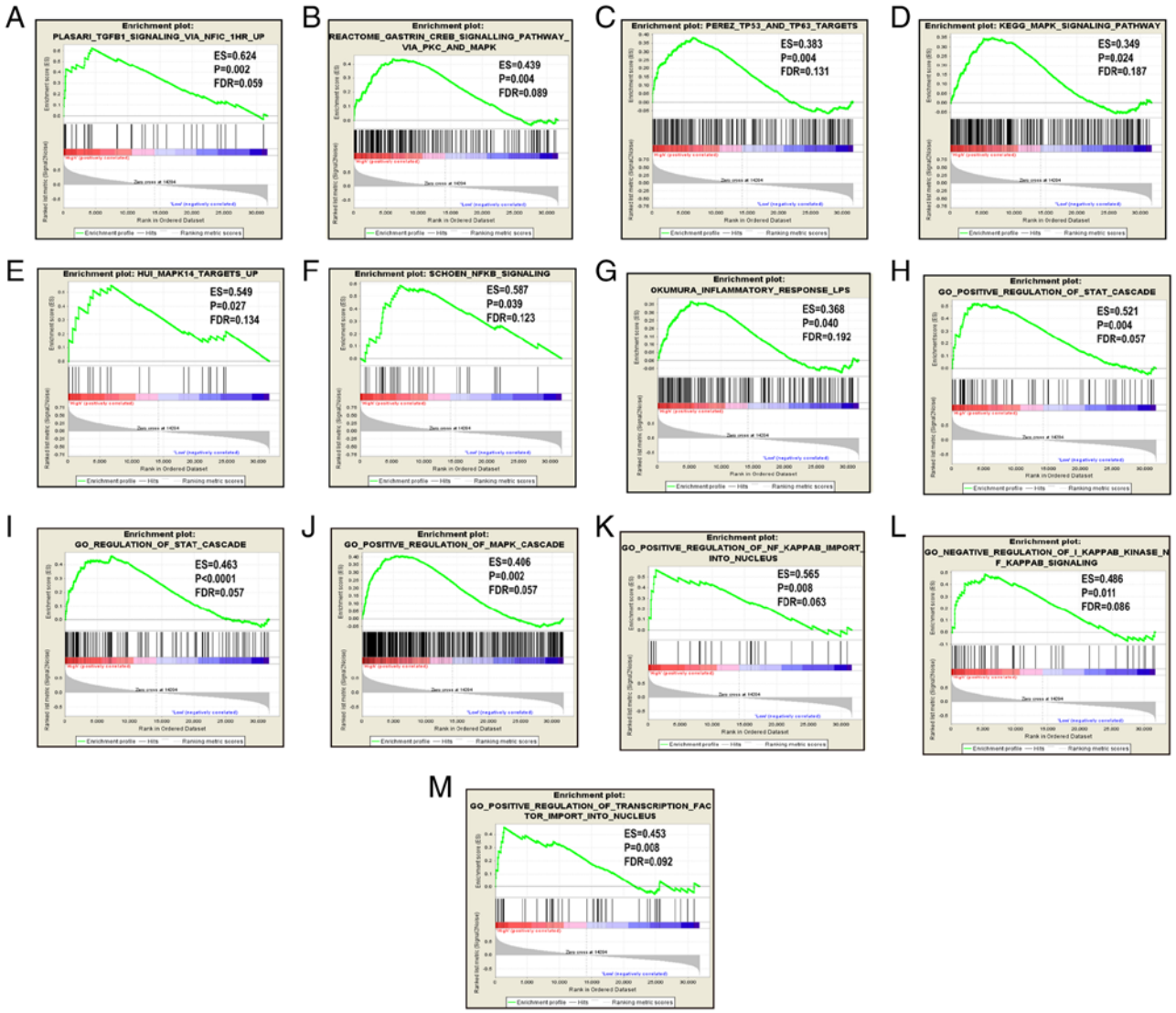

Figure 6. GSEA results for CCR5 and CCR6 in patients with pancreatic ductal adenocarcinoma. GSEA results of (A-G) c2-reference and (H-M) c5-reference gene sets for groups with increased CCR6 expression. GSEA, gene set enrichment analysis; CCR, C-C motif chemokine receptor; ES, enrichment score; FDR, false discovery rate.

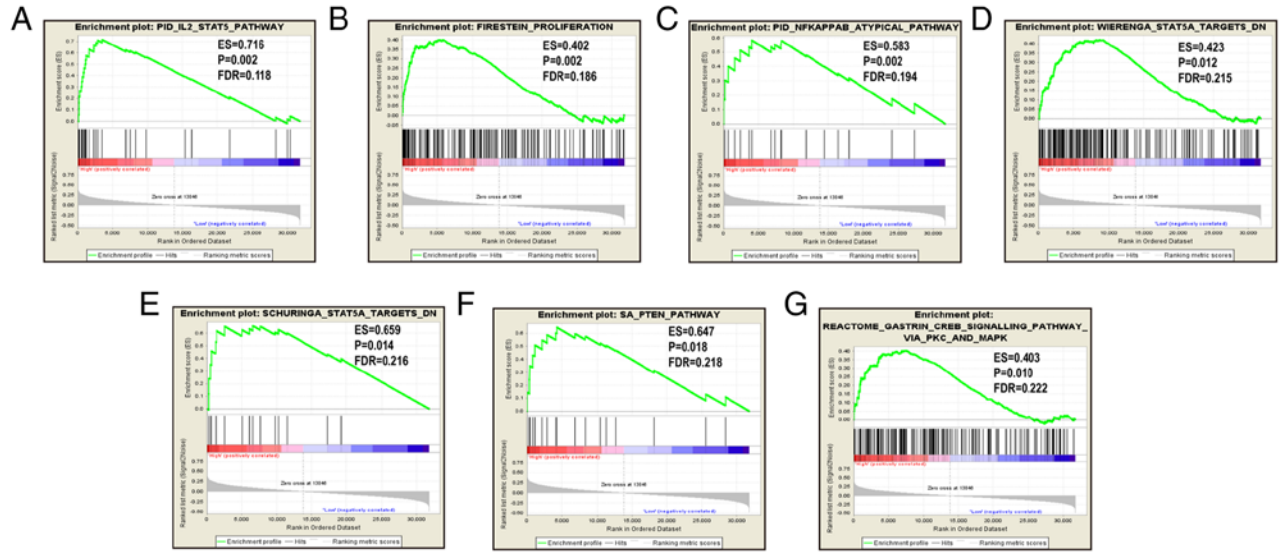

Figure 7. GSEA results of CCR6 and CCR9 in patients with pancreatic ductal adenocarcinoma. (A-G) GSEA results of c2-reference gene sets for groups with increased CCR9 expression. GSEA, gene set enrichment analysis; CCR, C-C motif chemokine receptor; ES, enrichment score; FDR, false discovery rate.

but not those with mutated p53 (61). CCR5 may also serve a role in PDAC via its indirect impact on tumor cells, such as regulating the anti-tumor immune response. CCR5 is also involved in the chemotaxis of activated naive T cells and T-cells homing (79,80).

Existing studies of CCR6 expression in pancreatic cancer are ambiguous; while one study reported higher levels of CCR6 expression in the pancreatic tumor relative to the adjacent healthy tissues (81), another showed lower expression levels in pancreatic cancer cell lines than normal pancreatic cells (82). Due to the limited number of studies surrounding CCR6 and CCR9 in pancreatic cancer, it was not possible to support the present findings of these CCRs. Therefore, it was surmised that CCR6 and CCR9 may modulate other tumor suppressor genes to inhibit tumor progression, in the same manner as the CCR5-mediated activation of TP53 (61,83,84). This was supported by the GSEA results of the present study, which suggested that CCR6 was enriched in the p53 and STAT cascade, and that CCR9 was enriched in the STAT cascade and NF-κB signaling pathway. However, further studies are required to elucidate the exact mechanisms involved.

The present study possessed various limitations. Firstly, the sample size was relatively small, which may have led to false negative results. Secondly, since the clinical information of a number of patients was incomplete, the clinical variables used for adjustment were not comprehensive. Thirdly, the relationship between CCR and prognosis was only explored at the transcriptional level. Nevertheless, not only was a novel association between the CCR genes and the prognosis of early-stage PDAC discovered, but also the potential molecular mechanisms. Further studies are required to validate these findings and to establish CCRs as therapeutic targets for PDAC.

Though there were several limitations to this investigation, the present study was the first to reveal the association between the CCR genes and the prognosis of early-stage PDAC. In addition, GSEA was used to identify the potential molecular mechanisms of CCR genes that may impact the prognosis of patients with early-stage PDAC. With subsequent studies to verify these findings, CCR genes may become novel targets for the treatment of PDAC.

In conclusion, CCR5, CCR6 and CCR9 represent potential prognostic biomarkers for patients with early-stage PDAC, and are involved in signaling pathways such as those of p53, NF-κB, generic transcription, MAPK and the STAT cascade.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

XZ and TP conceived and designed the study; XZ, XL, XW and KH acquired and processed the raw data. CY, TY, JL, CH, GZ, HS, WQ, QH, ZL, JH, YG, XY, ZC and TP performed the data analysis. XZ wrote the manuscript, and Tp guided and supervised the manuscript writing. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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