Clinical Significance and Bioinformatics Analysis of SMC Family in Pancreatic Adenocarcinoma

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Research Article

Keywords: bioinformatics analysis, SMC family, immune infiltration, pancreatic adenocarcinoma, biomarker, prognosis

Posted Date: March 7th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1415881/v1>

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Abstract

Objective: Pancreatic adenocarcinoma (PAAD) is a malignant digestive tract tumor with a dismal prognosis. The structural maintenance of chromosomes (SMC) gene family plays a role in various of human cancers. However, the function of SMC family members in PAAD remains unclear. This study explores the potential clinical significance of SMC family members in PAAD.

Methods: We analyzed the differential expression, prognostic value and methylation levels of SMCs in PAAD patients using R language, Gene Expression Profile Interactive Analysis (GEPIA), University of Alabama Cancer Database (UALCAN). We used Tumor Immune Estimation Resource (TIMER) to assess the correlation between SMC2 and six major tumor-infiltrating immune cells. Immunohistochemistry (IHC) was used to detect the expression of SMC2 in 72 PAAD tissues and 65 adjacent tissues. The relationship between SMC2 and the clinicopathological characteristics and prognosis of PAAD was further analyzed according to the clinical data of the patients.

Result: In TCGA (The Cancer Genome Atlas) and GSE16515, GSE15471 datasets, compared to normal tissue, the mRNA and protein expression levels of SMC1A, SMC2, SMC3, SMC4, and SMC6 were higher in PAAD. In addition, increased expression of SMC2 led to a poor prognosis, which could be a potential prognostic biomarker for PAAD patients in TCGA, GSE28735 and GSE21501. Cox univariate and multivariate analysis showed that SMC2 was a prognostic risk factor for PAAD patients in TCGA and GSE21501 dataset. In the 72 PAAD patients of our hospital, the expression level of SMC2 in PAAD tissues was significantly higher than in adjacent tissue, and patients with high expression had a shorter Overall Survival (OS); the expression of SMC2 was related to N stage ($P=0.028$) and Pathologic stage ($P=0.048$) of PAAD. Cox univariate and multivariate analysis suggested that SMC2 and N stage were closely related to the prognosis of PAAD patients. Using KEGG (Kyoto Encyclopedia of Genes and Genomes) and GO (Gene Ontology) analysis, we analyzed the gene expression enrichment of SMC2 in biological functions and pathways. Immune infiltration analysis showed that SMC2 was closely associated with B cells, CD8+ T cells, macrophages, neutrophils and dendritic cells.

Conclusions: Our study provides a new approach for selecting SMC family prognostic biomarkers in pancreatic cancer. These findings may provide a new direction for diagnosing and treating PAAD in the future.

Background

PAAD is one of the most aggressive digestive tract tumors and the seventh leading cause of cancer mortality worldwide, with a 5-year relative survival rate of less than 10%[1, 2]. Several factors contribute to the dismal success rate of pancreatic cancer therapies. These include lack of appropriate screening and diagnostic methods in the early stage, the deep position of the organ, difficulty in tissue diagnosis, and low response rate to both radiotherapy and chemotherapy [3]. Surgical resection is the only realistic curative strategy against PAAD. However, its high recurrence rate significantly hinders the improvement of

postoperative prognosis [4]. Therefore, revealing the molecular mechanism of rapid proliferation and high invasion of PAAD is pivotal for improved prognosis.

SMC proteins play an essential role in maintaining the stability of chromosome structure during cell division[5]. They are involved in chromosome condensation, sister chromatid cohesion, sex chromosome dosage compensation, genetic recombination and DNA repair and transcription[6]. The SMC family has six members, which form the core of three distinct multi-subunit protein complexes. The SMC1/SMC3 complex is linked with the kleisin subunit to create a tripartite ring, the cohesin complex's core. The most critical role of the cohesin complex is to bind the sister chromatids together[7]. Both SMC2 and SMC4 are core subunits of human condensin complexes, which belong to a large family of DNA-binding ATPase[8]. SMC5/ SMC6 complexes promote homologous recombination repair and thus play an essential role in DNA damage response[9].

SMCs play complex roles in various tumors. Several reports indicate their tumor-promoting potential. Phosphorylated SMC1A is overexpressed in hepatocellular carcinoma (HCC) and promotes its progression[10]. SMC2 hyperactivity is tumorigenic in human bladder cancer tissue[11]. SMC4 is overexpressed in liver cancer, glioma, and colorectal cancer, and its high expression is associated with poor prognosis in glioma[12]. Similarly, SMC5 and SMC6 expression is higher in sarcomas than in normal tissues and is associated with inferior OS and Disease-free Survival (DFS) [13]. Contrastingly, SMC3 may play an anti-tumor role in acute myeloid leukemia, where its protein level is elevated, and high expression is associated with improved prognosis[14].

Multiple studies have demonstrated that SMC family members are dysregulated at the gene expression level in tumor cells. However, the expression and role of the SMC family in PAAD is still unclear. In this study, we utilize a series of bioinformatics analysis techniques to explore the expression of the SMC family in PAAD and its relationship with prognosis and immune infiltration. Our study may shed new insights into the prognostic and biological role of the SMC family in PAAD.

Materials And Methods

1.1 Patients

72 PAAD patients who underwent surgical resection in the First People's Hospital of Lianyungang (from 2017 to 2021) and had complete clinicopathology were selected. All specimens were pathologically confirmed to be pancreatic ductal carcinoma and were graded according to the Union for International Cancer Control (UICC) TNM (Tumor (T), Nodes (N), Metastases (M)) staging criteria. None of the enrolled patients received any tumor-specific treatment before surgery. The study was approved by the Institutional Ethics Committee, and informed consent was obtained from each patient.

1.2 Immunohistochemistry (IHC)

The surgically resected pancreatic cancer and adjacent tissue specimens were fixed and embedded in 4% paraffin and cut into 4 µm thick sections. The sections were de-paraffinized in xylene and rehydrated in gradient ethanol. They were then fixed and covered with a coverslip. Next, samples were incubated with a rabbit polyclonal antibody against SMC2 (Affinity Biosciences Cat# DF13315, RRID: AB_2846334). The cell staining intensity was classified into 4 levels (0–3) (0, negative; 1, weak staining; 2, moderate staining; 3, intense staining). The percentage of stained cells was classified into 4 grades (1, < 25%; 2, 25–50%; 3, 51–75%; 4, > 75%). The two values were multiplied to obtain the IHC score and evaluate IHC staining intensity[15].

1.3 Gene Expression Analysis

PAAD patients' gene expression profiles, along with their clinical data (such as age, gender, TNM classification, survival status), were downloaded from Genomic Data Commons (GDC) database (<https://portal.gdc.cancer.gov/>). A total of 178 PAAD samples were included in our study. Patients lacking clinical information were excluded. Simultaneously, we downloaded and analyzed pancreatic cancer cohorts GSE28735, GSE16515, GSE21501 and GSE15471 in the GEO database. Datasets GSE28735 and GSE21501 were further analyzed with clinical prognostic data.

Gene Expression Profiling Interactive Analysis (GEPIA) is a newly developed interactive web server for analyzing the RNA sequencing expression data of thousands of tumors and normal samples from the TCGA and the GTEx projects [16]. GEPIA was used to provide tumor/normal differential expression analysis.

The Human Protein Atlas (HPA) database contains IHC and immunofluorescence expression data in normal and cancer tissues[17]. In this study, HPA provided IHC staining images of SMC gene family proteins in normal and cancerous pancreatic tissue.

1.4 Survival Analysis

We used "survival" and "survminer" in the R package to determine the optimal cut-off value of the survival curve. We plotted the Kaplan-Meier (KM) survival curve in the TCGA, GSE28735, and GSE21501 datasets and the 72 PAAD patients in our hospital. We also used Cox univariate and multivariate analyses to compare the effect of SMCs on the pancreatic cancer prognosis.

1.5 Methylation Analysis and SMC2- related Gene Enrichment Analysis

We used the UALCAN database (<http://ualcan.path.uab.edu>), an interactive web resource for analyzing cancer omics data[18]. We predicted DNA methylation changes of SMC family members in 184 PAAD

samples and 10 normal pancreatic samples using the UALCAN database. We obtained 50 SMC2 binding proteins based on the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) website (<https://string-db.org/>). We then used the R packages: "tidyr", "ggplot2", and "ClusterProfiler", for Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) analysis for mapping.

1.6 Immune Infiltration Analysis

We used the TIMER database to evaluate the correlation between SMC2 mRNA levels and immune infiltration in PAAD (B cells, CD8 + T cells, CD4 + T cells, macrophages, neutrophils and dendritic cells). In addition, the correlation between SMC2 expression and molecular biomarkers of tumor-infiltrating immune cells was analyzed by correlation module. The Spearman algorithm calculated the correlation R-value and corrected it according to tumor purity and age.

1.7 Statistical Analysis

R4.0.1 and Statistical Package for the Social Sciences (SPSS) 23.0 were used for statistical analyses. A two-sided Student's t-test for unpaired samples was used to evaluate the significance of differences in experiments. Pearson correlation coefficients were calculated to measure associations among the mRNA expression level of various genes. The prognostic impact of SMCs in pancreatic cancer patients was analyzed using Cox univariate and multivariate analyses. $p < 0.05$ was considered to be statistically significant.

Results

2.1 Differential Expression of SMCs in PAAD

This study analyzed the mRNA expression of SMCs in tumor tissue and normal tissue using the TCGA and GEO databases. In the GSE16515 and GSE15471 datasets, the expression of SMC family genes in PAAD tissues was significantly higher than in normal tissues (Fig. 1A, B). Moreover, we used GEPIA datasets (<http://gepia.cancer-pku.cn/>) to compare the mRNA levels of SMCs in PAAD and normal tissues. It was shown that SMC1A, SMC, SMC3, SMC4 and SMC6 expression was higher in PAAD than in normal pancreatic tissues (Fig. 1C). To investigate the protein level of the SMC family in pancreatic cancer tissues, we further explored the HPA database. SMC1A, SMC2, SMC3, SMC4 and SMC6 protein levels were elevated in PAAD tissues compared to normal pancreatic tissues (Supplementary Fig. 1). In contrast, SMC1B and SMC5 showed no significant changes between PAAD and normal tissues.

2.2 The Prognostic Value of SMCs Expression in PAAD

To further clarify the relationship between SMC expression and PAAD prognosis in patients, clinically relevant parameters about SMCs were extracted from the GSE28735, GSE21501 and TCGA datasets, and the best cut-off values were selected for analysis. In the GSE28735 dataset, low expression of SMC1A and SMC2 was associated with improved prognosis (Fig. 2A). Similarly, low expression of SMC2 and SMC6 in the GSE21501 dataset was also associated with good prognosis (Fig. 2B). In the TCGA dataset, the high expression of SMC1A, SMC2, SMC3, SMC4, SMC5 and SMC6 was negatively correlated with the OS of patients, and the higher the expression level, the shorter the OS of patients (Fig. 2C). In summary, PAAD patients with high transcriptional levels of SMC2 were significantly associated with short OS in all three cohorts (GSE28735, GSE21501, TCGA). Univariate analysis in the TCGA cohort showed that the expression levels of SMC1A, SMC2, SMC3, SMC5 and SMC6 were statistically significant (Supplementary Fig. 2). Multivariate analysis showed that only SMC2 had significant prognostic effect in PAAD in two cohorts (TCGA, GSE21501) (Supplementary Fig. 3).

2.3 The Methylation Levels of SMC Genes in PAAD

It is well known that genetic alterations play a key role in cancer development. Using the UALCAN database, we determined the methylation levels of SMC genes in PAAD patients. Compared with healthy people, the DNA methylation levels of SMC4 were significantly reduced in PAAD samples, while the DNA methylation levels of SMC2, SMC3, SMC5 and SMC6 were significantly increased in PAAD tissues. Promoter methylation of SMC1A and SMC1B were no significant different in normal and tumors (Fig. 3). Changes in DNA methylation levels of SMC family members were closely related to their differences in expression levels, which may provide new targets for DNA methylation targeting drugs for cancer patients.

2.4 Correlation of SMC2 Expression with Clinicopathology and Prognosis of PAAD

As reported above, SMC2 was highly expressed in PAAD, and its elevated expression was correlated with shorter OS. Therefore, we proceeded to perform clinical data validation. Among 72 PAAD patients, SMC2 was highly expressed in PAAD tissues (72.2%, 52/72) compared with adjacent normal tissues (15.4%, 10/65) ($P < 0.001$; Fig. 4A, B). KM (Kaplan-Meier) survival analysis showed that patients with high expression of SMC2 had a poor prognosis (Fig. 4C). The analysis of the relationship between the SMC2 expression and PAAD clinicopathologic variables showed that SMC2 is significantly correlated with N stage ($P = 0.028$) and Pathologic stage ($P = 0.048$) (Table 1). Cox univariate analysis showed that SMC2 expression, N stage, pathologic stage, and distant metastasis were correlated with prognosis ($P = 0.002$, $P = 0.006$, $P = 0.009$, $P = 0.001$). Cox multivariate analysis showed that high SMC2 expression and N stage were independent risk factors for pancreatic cancer prognosis (Table 2). Contrastingly, patient age, gender, tumor size, histological differentiation, perineural invasion, and vascular invasion were not associated with prognosis (according to Cox univariate analysis).

Table 1
Correlation between SMC2 expression and
clinicopathological variables in patients with
PAAD

Characteristics	SMC2		
	Low	High	P-value
Age(y)			
< 66	11	26	0.875
≥ 66	11	24	
Gender			
Female	8	23	0.447
Male	14	27	
T classification			
T1 + T2	14	30	0.771
T3 + T4	8	20	
N classification			
N0	20	33	0.028
N1	2	17	
M classification			
M0	22	46	0.175
M1	0	4	
Pathologic stage			
Stage I + II	22	42	0.048
Stage III + IV	0	8	
Histological differentiation			
Well-moderate	4	20	0.072
Poor	18	30	
Perineural invasion			
Yes	16	40	0.494
No	6	10	
Vascular invasion			

Characteristics	SMC2		
Yes	19	45	0.653
No	3	5	

Table 2
Univariate and Multivariate analyses of overall survival in patients with PAAD

Parameters	Univariate analysis			Multivariate analysis		
	P-value	HR	95%CI	P-value	HR	95%CI
SMC2	0.002	9.24	2.212–38.602	0.017	6.158	1.383–27.421
Gender	0.751	0.896	0.454–1.768	0.579	1.241	0.579–2.662
Age	0.77	0.904	0.461–1.775	0.244	1.635	0.715–3.743
T stage	0.343	0.714	0.355–1.433	0.499	0.747	0.32–1.741
N stage	0.006	2.714	1.334–5.523	0.023	3.222	1.175–8.833
M stage	0.001	6.554	2.222–19.326	0.052	9.305	0.984–27.962
Pathologic stage	0.009	3.045	1.316–7.043	0.873	0.885	0.198–3.954
Histological differentiation	0.615	0.831	0.403–1.712	0.634	0.801	0.321–1.996
Perineural invasion	0.404	0.666	0.257–1.728	0.537	0.703	0.23–2.149
Vascular invasion	0.886	1.091	0.331–3.6	0.96	0.966	0.253–3.687

2.5 SMC2 Co-expression Networks in PAAD

Next, we investigated the molecular mechanism of SMC2 in tumor development. We screened out the SMC2-binding proteins for pathway enrichment analysis. Based on the STRING database, we obtained 50 proteins that bind to SMC2 (Fig. 5A). This set of data was used for KEGG and GO analysis. Results (Fig. 5B) showed that the SMC2 pathogenesis in tumors might be related to the cell cycle and DNA replication pathways. In GO analysis, the biological process was enriched in chromosome segregation and nuclear division, the cellular component was enriched in chromosomal region and condensed chromosome, and molecular function was enriched in ATPase activity and tubulin binding (Fig. 5C).

2.6 Association Between the Expression of SMC2 and Immune Infiltration in PAAD

As an essential part of the tumor microenvironment (TME), tumor-infiltrating immune cells are closely related to tumor occurrence, development, proliferation, and progression. Therefore, to further understand the role of SMC2 in PAAD, we used the TIMER database to explore the molecular characteristics of tumor-immune interactions. As shown in Fig. 6, SMC2 transcription expression was positively correlated with B cells, CD8 + T cells, macrophages, neutrophils and dendritic cells in the PAAD tissues ($r > 0.2$, $P < 0.05$). These results suggest a potential role of SMC2 in the immune response of the pancreatic cancer TME. Next, we also investigated the potential correlation of SMC2 expression with various immune markers of immune cells in PAAD using the TIMER database. The results showed that SMC2 in PAAD was strongly correlated with SIGLEC5 and FPR1 in neutrophils, CD163 and MS4A4A in M2 macrophages; CCR8, STAT5B in regulatory T cells (Tregs); C3AR1, CD86 and CSF1R in monocytes. These correlations remained unchanged after adjustment for tumor purity and age (Table 3). This further confirms that the expression of SMC2 in PAAD is associated with immune infiltration.

Table 3
Correlation analysis between SMC2 and immune cell type markers in TIMER database

		None		Purity		Age	
		COR	P	COR	P	COR	P
B cells	FCRL2	0.054	4.77E-01	0.053	4.92E-01	-0.051	4.98E-01
	CD19	0.065	3.90E-01	0.076	3.26E-01	0.064	3.94E-01
	CD79A	0.092	2.19E-01	0.104	1.74E-01	0.093	2.18E-01
	MS4A1	0.045	5.49E-01	0.052	4.97E-01	0.044	5.61E-01
CD8 + T cells	CD8A	0.184	1.37E-02	0.204	7.37E-03	0.182	1.48E-02
	CD8B	0.182	1.49E-02	0.199	9.13E-03	0.181	1.58E-02
T cells(general)	CD3D	0.07	3.54E-01	0.082	2.85E-01	0.066	3.84E-01
	CD3E	0.126	9.31E-02	0.144	6.08E-02	0.122	1.05E-01
	CD2	0.123	1.00E-01	0.145	5.89E-02	0.119	1.12E-01
Neutrophils	FCGR3B	0.155	3.83E-02	0.183	1.64E-02	0.154	4.04E-02
	CEACAM3	0.012	8.70E-01	0.015	8.48E-01	0.01	8.93E-01
	SIGLEC5	0.215	3.96E-03	0.241	1.48E-03	0.211	4.64E-03
	FPR1	0.207	5.59E-03	0.248	1.05E-03	0.203	6.63E-03
	CSF3R	-0.065	3.84E-01	-0.049	5.23E-01	-0.068	3.70E-01
	S100A12	0.166	2.64E-02	0.173	2.37E-02	0.162	3.11E-02
TAM	CCL2	0.004	9.59E-01	0.015	8.46E-01	-0.003	9.66E-01
	CD68	0.188	1.16E-02	0.225	3.16E-03	0.189	1.17E-02
	IL10	0.141	5.92E-02	0.15	4.97E-02	0.143	5.68E-02
M1 Macrophages	NOS2	0.256	5.66E-04	0.25	9.77E-04	0.258	5.20E-04
	IRF5	0.09	2.30E-01	0.078	3.09E-01	0.087	2.47E-01
	PTGS2	0.26	4.71E-04	0.27	3.54E-04	0.258	4.97E-04
M2 Macrophages	VSIG4	0.19	1.11E-02	0.247	1.15E-03	0.19	1.30E-02
	CD163	0.301	4.48E-05	0.352	2.27E-06	0.299	4.90E-05
	MS4A4A	0.224	2.64E-03	0.267	4.18E-04	0.223	2.76E-03
Dendritic cells	CD209	0.369	4.41E-07	0.389	1.48E-07	0.368	4.33E-07

		None		Purity		Age	
NK cells	KIR2DL1	0.069	3.61E-01	0.058	4.52E-01	0.068	3.68E-01
	KIR2DL3	0.134	7.40E-02	0.12	1.19E-01	0.14	6.21E-02
	KIR2DL4	0.278	1.63E-04	0.277	2.43E-04	0.277	1.86E-04
	KIR3DL1	0.113	1.33E-01	0.122	1.11E-01	0.114	1.29E-01
	KIR3DL2	0.139	6.40E-02	0.144	6.03E-02	0.14	6.28E-02
	KIR3DL3	0.13	8.31E-02	0.13	8.94E-02	0.126	9.49E-02
	KIR2DS4	0.071	3.46E-01	0.048	5.34E-01	0.075	3.20E-01
Th1	TBX21	0.135	7.07E-02	0.152	4.68E-02	0.135	7.21E-02
	STAT4	0.19	1.08E-02	0.209	6.08E-03	0.184	1.39E-02
	STAT1	0.401	3.68E-08	0.432	3.56E-09	0.399	3.45E-08
Th2	GATA3	0.15	4.47E-02	0.16	3.69E-02	0.146	5.18E-02
	STAT6	0.135	7.17E-02	0.129	9.36E-02	0.132	7.84E-02
	STAT5A	0.195	9.10E-03	0.199	8.90E-03	0.193	9.95E-03
	IL13	0.033	6.58E-01	0.042	5.90E-01	0.03	6.93E-01
Tfh	BCL6	0.235	1.58E-03	0.238	1.74E-03	0.229	2.11E-03
	IL21	0.23	1.95E-03	0.232	2.27E-03	0.236	1.49E-03
Th17	STAT3	0.438	1.25E-09	0.458	3.03E-10	0.44	8.18E-10
	IL17A	0.172	2.13E-02	0.185	1.52E-02	0.176	1.90E-02
Treg	FOXP3	0.139	6.32E-02	0.162	3.40E-02	0.134	7.47E-02
	CCR8	0.26	4.34E-04	0.279	2.14E-04	0.256	5.67E-04
Monocyte	C3AR1	0.227	2.27E-03	0.284	1.66E-04	0.226	2.38E-03
	CD86	0.236	1.52E-03	0.271	3.38E-04	0.232	1.85E-03
	CSF1R	0.226	2.36E-03	0.279	2.23E-04	0.223	2.78E-03

Discussion

Chromosomal instability is a cancer hallmark. It is an early event in cancer pathogenesis, increases with age, and contributes to tumor malignancy[19, 20]. Defects in different SMC complexes and their regulatory factors are important causes of cancer. This is due to the central role of SMCs in chromosome separation and gene regulation[21]. Abnormal expression of SMC1A and SMC3 genes, important

components of the cohesin complex, has been found in various human malignancies. SMC1A is highly expressed in multiple malignancies, including colorectal cancer[22] and triple-negative breast cancer[23]. It stimulates the release of inflammatory mediators and recruits downstream targeted molecules, thereby promoting the tumor formation and development. SMC2 is responsible for tightly linking genomic DNA together before it separates into daughter cells[24]. SMC2 participates in cell mitosis and promotes the occurrence and development of cancer[25]. In lung cancer cell lines, SMC3 overexpression abrogated H₂-induced apoptosis and inhibited cancer cell proliferation, migration and invasion[26]. SMC4 promotes the growth of HCC[27] and colon cancer[28]. In addition, the OS and DFS rates of patients with strong positive expression of SMC4 are significantly lower than those of patients with negative expression of SMC4. Previous studies have shown that SMC5/6 is involved in the alternative lengthening of telomeres (ALT) maintenance pathway, and some tumors rely on ALT to lengthen telomeres and gain unlimited proliferative potential[29]. These findings demonstrate the diverse, crucial, and often contrasting role of SMCs in cancer.

This study analyzed clinical data from TCGA and the high-throughput GEO databases. We used KM survival curve and Cox risk model to investigate the relationship between expression of SMC family members and PAAD prognosis. In the TCGA, GSE16515, and GSE15471 datasets, SMC1A, SMC2, SMC3, SMC4, and SMC6 were overexpressed (mRNA) in PAAD compared to matched normal tissues. Moreover, patients with high expression of SMC2 in TCGA, GSE28735, and GSE21501 had a poor prognosis. Univariate and multivariate Cox analyses indicate that SMC2 is an independent risk factor for the pancreatic cancer prognosis. We validated these results using 72 PAAD samples from our hospital, and the results were consistent with those in the above database.

These findings underline the importance of SMC2 in cancer. Cell cycle deregulation is found in many types of cancer[30], and SMC2 is part of the condensin complex that plays a central role in chromosome biology. SMC2/Condensin complexes are involved in the segregation of sister chromatids, chromosomes compaction during cell division, and the regulation of intercellular gene expression[31]. In colon cancer cell line HCT116, silencing of SMC2 inhibited cell proliferation, resulted in a significant cell cycle arrest in the G1 phase and promoted apoptosis [32]. In conclusion, SMC2 has excellent potential in predicting patient prognosis and as a therapeutic target for PAAD patients.

The treatment of PAAD remains a considerable challenge to date. In addition to traditional surgical intervention, chemotherapy, radiotherapy, and other treatment modalities, immunotherapy has become the hope of PAAD therapy[33]. Tumor immunotherapy reshapes the tumor immune microenvironment and transforms tumor cells from "cold" to "hot"[34]. Cancer vaccines are a form of active immunotherapy aimed at enhancing tumor antigen presentation to activate the immune response and immune memory. One study found that GVAX vaccination therapy is safe in patients with localized pancreatic cancer after resection, and high-dose GVAX can effectively promote the development of anti-tumor immunity[35]. The immune checkpoint inhibitor combined with the vaccine regimen effectively prolonged the OS while increasing the mesothelin-specific CD8 + T cells in the patients' peripheral blood[36].

Currently, only a handful of studies have linked SMC family members to immune cells in different types of diseases. SMC complexes have been associated with the development and function of T cells[37]. Therefore, we explored the relationship between SMC2 and immune infiltration and immune signaling. Our results show that SMC2 is associated with five types of immune cells, including B cells, CD8 + T cells, macrophages, neutrophils, and dendritic cells. The immune infiltration analysis we performed indicated that SMC2 is related to the PAAD microenvironment. SMC2 in PAAD was positively correlated with CD163 and MS4A4A in M2 Macrophages; CCR8, STAT5B in Tregs. CD163 is an M2 tagged protein with high uptake of apoptotic cells[38]. M1 macrophages promote inflammation and suppress tumors. Conversely activated M2 macrophages have immunosuppressive and tumor-promoting effects[39]. In addition, Regulatory T cells (Tregs) regulate sustained immune responses and suppress immune function through multiple mechanisms, leading to tumor progression[40]. Our results suggest that SMC2 is positively correlated with M2 macrophage and Treg cells, we speculate that the immune propensity of SMC2 in pancreatic cancer is a tumor-promoting effect.

Conclusions

In summary, we systematically analyzed the expression of SMC family members in PAAD and assessed their prognostic value. We found that SMC2 was strongly correlated with immune markers in PAAD, suggesting that it may play an important role in tumor progression and immune response. This can enable us to find a better PAAD diagnosis strategy to improve prognosis. However, our study also has certain limitations, most of the results of this study are based on databases and lack the support of in vitro and in vivo experimental results. So we need to continue to study the mechanism of action, molecular interactions and clinical application of SMCs in PAAD.

Abbreviations

PAAD: Pancreatic adenocarcinoma; SMC: Structural maintenance of chromosomes; GEPIA: Gene expression profile interactive analysis; UALCAN: University of alabama cancer database; TIMER: Tumor immune estimation resource; IHC: Immunohistochemistry; TCGA: The cancer genome atlas; KEGG: Kyoto encyclopedia of genes and genomes; GO analysis: Gene ontology analysis; DFS: Disease-free Survival.

Declarations

Ethics approval and consent to participate

Approval from Ethics committees of the Affiliated Lianyungang Hospital of Xuzhou Medical University (Lianyungang, China) (approval no. KY20190924002) were obtained before enrollment into the study. Informed consent was obtained from all individual participants included in the study. The study was performed in accordance with government policies and the Helsinki Declaration.

Consent for publication

Written informed consent for publication was obtained from all patients enrolled in the study.

Availability of data and materials

Publicly available datasets were analyzed in this study. These data can be found here: TCGA: <https://portal.gdc.cancer.gov/>; GEO: <https://www.ncbi.nlm.nih.gov/geo/>; UALCAN <http://ualcan.path.uab.edu/> TIMER <https://cistrome.shinyapps.io/timer/>.

Competing interests

The authors declare that they have no competing interests.

Funding

This research was supported by the National Natural Science Foundation of China (81900772), the Lianyungang City Science and Technology Plan Funding Project (SF2119), the Foundation of Health and Family planning Commission of Lianyungang (201906 and 202102) and the Startup Fund for Doctoral Research of the Affiliated Lianyungang Hospital of Xuzhou Medical University (BS202003).

Authors' contributions

ML and WH conceived and designed the experiments. ML and YW performed the histological examination of pancreatic cancer. ML, CZ, DS and HF analyzed the data and wrote the paper. YW and ZW revised the manuscript and supervised the work. All authors read and approved the final manuscript.

Acknowledgements

Not applicable.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A: Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018, 68:394-424.
2. Siegel RL, Miller KD, Jemal A: Cancer statistics, 2018. *CA Cancer J Clin* 2018, 68:7-30.
3. Maisonneuve P, Lowenfels AB: Epidemiology of pancreatic cancer: an update. *Dig Dis* 2010, 28:645-656.
4. Zhang L, Sanagapalli S, Stoita A: Challenges in diagnosis of pancreatic cancer. *World J Gastroenterol* 2018, 24:2047-2060.
5. Hirano T: At the heart of the chromosome: SMC proteins in action. *Nat Rev Mol Cell Biol* 2006, 7:311-322.
6. Nishiwaki T, Daigo Y, Kawasoe T, Nagasawa Y, Ishiguro H, Fujita M, Furukawa Y, Nakamura Y: Isolation and characterization of a human cDNA homologous to the *Xenopus laevis* XCAP-C gene

- belonging to the structural maintenance of chromosomes (SMC) family. *J Hum Genet* 1999, 44:197-202.
7. Yatskevich S, Rhodes J, Nasmyth K: Organization of Chromosomal DNA by SMC Complexes. *Annu Rev Genet* 2019, 53:445-482.
 8. Pandey R, Abel S, Boucher M, Wall RJ, Zeeshan M, Rea E, Freville A, Lu XM, Brady D, Daniel E, et al: Plasmodium Condensin Core Subunits SMC2/SMC4 Mediate Atypical Mitosis and Are Essential for Parasite Proliferation and Transmission. *Cell Rep* 2020, 30:1883-1897.
 9. Hassler M, Shaltiel IA, Haering CH: Towards a Unified Model of SMC Complex Function. *Curr Biol* 2018, 28:R1266-R1281.
 10. Zhang Y, Yi F, Wang L, Wang Z, Zhang N, Wang Z, Li Z, Song X, Wei S, Cao L: Phosphorylation of SMC1A promotes hepatocellular carcinoma cell proliferation and migration. *Int J Biol Sci* 2018, 14:1081-1089.
 11. Han YH, Wan Y, Xiong H, Sun GL: Structural maintenance of chromosomes 2 is identified as an oncogene in bladder cancer in vitro and in vivo. *Neoplasia* 2020, 67:364-370.
 12. Jiang L, Zhou J, Zhong D, Zhou Y, Zhang W, Wu W, Zhao Z, Wang W, Xu W, He L, et al: Overexpression of SMC4 activates TGFbeta/Smad signaling and promotes aggressive phenotype in glioma cells. *Oncogenesis* 2017, 6:e301.
 13. Zhou J, Wu G, Tong Z, Sun J, Su J, Cao Z, Luo Y, Wang W: Prognostic relevance of SMC family gene expression in human sarcoma. *Aging (Albany NY)* 2020, 13:1473-1487.
 14. Kraft B, Lombard J, Kirsch M, Wuchter P, Bugert P, Hielscher T, Blank N, Kramer A: SMC3 protein levels impact on karyotype and outcome in acute myeloid leukemia. *Leukemia* 2019, 33:795-799.
 15. Liu XH, Yang YF, Fang HY, Wang XH, Zhang MF, Wu DC: CEP131 indicates poor prognosis and promotes cell proliferation and migration in hepatocellular carcinoma. *Int J Biochem Cell Biol* 2017, 90:1-8.
 16. Tang Z, Li C, Kang B, Gao G, Li C, Zhang Z: GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. *Nucleic Acids Res* 2017, 45:W98-W102.
 17. Navani S: *Manual evaluation of tissue microarrays in a high-throughput research project: The contribution of Indian surgical pathology to the Human Protein Atlas (HPA) project.*; 2016.
 18. Chandrashekar DS, Bashel B, Balasubramanya S, Creighton CJ, Ponce-Rodriguez I, Chakravarthi B, Varambally S: UALCAN: A Portal for Facilitating Tumor Subgroup Gene Expression and Survival Analyses. *Neoplasia* 2017, 19:649-658.
 19. Bakhoun SF, Cantley LC: The Multifaceted Role of Chromosomal Instability in Cancer and Its Microenvironment. *Cell* 2018, 174:1347-1360.
 20. Laurie CC, Laurie CA, Rice K, Doheny KF, Zelnick LR, McHugh CP, Ling H, Hetrick KN, Pugh EW, Amos C, et al: Detectable clonal mosaicism from birth to old age and its relationship to cancer. *Nat Genet* 2012, 44:642-650.
 21. Uhlmann F: SMC complexes: from DNA to chromosomes. *Nat Rev Mol Cell Biol* 2016, 17:399-412.

22. Zhou P, Xiao N, Wang J, Wang Z, Zheng S, Shan S, Wang J, Du J, Wang J: SMC1A recruits tumor-associated-fibroblasts (TAFs) and promotes colorectal cancer metastasis. *Cancer Lett* 2017, 385:39-45.
23. Li K, Ying M, Feng D, Chen Y, Wang J, Wang Y: SMC1 promotes epithelial-mesenchymal transition in triple-negative breast cancer through upregulating Brachyury. *Oncol Rep* 2016, 35:2405-2412.
24. Li X, Song G, Zhao Y, Ren J, Li Q, Cui Z: Functions of SMC2 in the Development of Zebrafish Liver. *Biomedicines* 2021, 9.
25. Dávalos V, Suárez-López L, Castaño J, Messent A, Abasolo I, Fernandez Y, Guerra-Moreno A, Espín E, Armengol M, Musulen E, et al: Human SMC2 protein, a core subunit of human condensin complex, is a novel transcriptional target of the WNT signaling pathway and a new therapeutic target. *The Journal of biological chemistry* 2012, 287.
26. Wang D, Wang L, Zhang Y, Zhao Y, Chen G: Hydrogen gas inhibits lung cancer progression through targeting SMC3. *Biomed Pharmacother* 2018, 104:788-797.
27. Zhou B, Chen H, Wei D, Kuang Y, Zhao X, Li G, Xie J, Chen P: A novel miR-219-SMC4-JAK2/Stat3 regulatory pathway in human hepatocellular carcinoma. *J Exp Clin Cancer Res* 2014, 33:55.
28. Feng X, Song Q, Li C, Chen J, Tang H, Peng Z, Wang X: Structural maintenance of chromosomes 4 is a predictor of survival and a novel therapeutic target in colorectal cancer. *Asian Pacific journal of cancer prevention : APJCP* 2014, 15.
29. Potts PR, Yu H: The SMC5/6 complex maintains telomere length in ALT cancer cells through SUMOylation of telomere-binding proteins. *Nat Struct Mol Biol* 2007, 14:581-590.
30. Williams GH, Stoeber K: The cell cycle and cancer. *J Pathol* 2012, 226:352-364.
31. Fazio TG, Panning B: Condensin complexes regulate mitotic progression and interphase chromatin structure in embryonic stem cells. *J Cell Biol* 2010, 188:491-503.
32. Montero S, Seras-Franzoso J, Andrade F, Martinez-Trucharte F, Vilar-Hernandez M, Quesada M, Xandri H, Arango D, Abasolo I, Rafael D, Schwartz SJ: Intracellular Delivery of Anti-SMC2 Antibodies against Cancer Stem Cells. *Pharmaceutics* 2020, 12.
33. Kiaie SH, Sanaei MJ, Heshmati M, Asadzadeh Z, Azimi I, Hadidi S, Jafari R, Baradaran B: Immune checkpoints in targeted-immunotherapy of pancreatic cancer: New hope for clinical development. *Acta Pharm Sin B* 2021, 11:1083-1097.
34. Torphy RJ, Zhu Y, Schulick RD: Immunotherapy for pancreatic cancer: Barriers and breakthroughs. *Ann Gastroenterol Surg* 2018, 2:274-281.
35. Jaffee EM, Hruban RH, Biedrzycki B, Laheru D, Schepers K, Sauter PR, Goemann M, Coleman J, Grochow L, Donehower RC, et al: Novel allogeneic granulocyte-macrophage colony-stimulating factor-secreting tumor vaccine for pancreatic cancer: a phase I trial of safety and immune activation. *J Clin Oncol* 2001, 19:145-156.
36. Le DT, Lutz E, Uram JN, Sugar EA, Onners B, Solt S, Zheng L, Diaz LJ, Donehower RC, Jaffee EM, Laheru DA: Evaluation of ipilimumab in combination with allogeneic pancreatic tumor cells

- transfected with a GM-CSF gene in previously treated pancreatic cancer. *J Immunother* 2013, 36:382-389.
37. Rawlings JS: Roles of SMC Complexes During T Lymphocyte Development and Function. *Adv Protein Chem Str* 2017, 106:17-42.
38. Roszer T: Understanding the Mysterious M2 Macrophage through Activation Markers and Effector Mechanisms. *Mediators Inflamm* 2015, 2015:816460.
39. Lee HW, Choi HJ, Ha SJ, Lee KT, Kwon YG: Recruitment of monocytes/macrophages in different tumor microenvironments. *Biochim Biophys Acta* 2013, 1835:170-179.
40. Wei T, Zhong W, Li Q: Role of heterogeneous regulatory T cells in the tumor microenvironment. *Pharmacol Res* 2020, 153:104659.

Figures

Figure 1

Expression levels of SMC family members in PAAD. (A, B) Expression heatmap of SMCs mRNA expression in tumor and normal samples of GSE16515 and GSE15471. C. SMC mRNA expression comparison between tumor and normal tissues in the TCGA.

Figure 2

Prognostic value of SMC family in PAAD (GSE28735, GSE21501, TCGA). A. High expression of SMC1A and SMC2 was associated with poor prognosis in GSE28735. B. Low expression of SMC2 and SMC6 in the GSE21501 dataset was also associated with good prognosis. C. High expression of SMC1A, SMC2, SMC3, SMC4, SMC5 and SMC6 was negatively correlated with short OS of patients in TCGA.

Figure 3

DNA methylation levels of SMC family genes in PAAD. (A-G) Results from UALCAN showed the promoter methylation level of SMC2, SMC3, SMC5 and SMC6 were significantly increased in tumor tissues, the methylation level of SMC4 was significantly reduced in tumor tissues. Promoter methylation of SMC1A and SMC1B were no significant different in normal and tumors.

Figure 4

Immunohistochemical detection of SMC2 expression and the survival curve of SMC2 in our patients. (A, B) Expression of SMC2 in PAAD and peritumor tissues was detected by IHC. SMC2 was highly expressed in PAAD tissues (72.20%, 52/72) compared with adjacent normal tissues (15.4%, 10/65). C. Survival curves of cases with different SMC2 expression levels in the 72 PAAD patients.

Figure 5

pathway enrichment analysis of SMC2 in PAAD. A. SMC2 interaction analysis was evaluated by STRING database. B. All KEGG pathway enrichment results of SMC2 and 50 interaction proteins. C. All GO enrichment analysis results of SMC2 and 50 interaction proteins. BP: Biological Process CC: Cellular Component; MF: Molecular Function.

Figure 6

Analysis of correlations involving the expression of SMC2 and immune cell infiltration in PAAD (TIMER).

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