CDK5RAP3 as a Novel Biomarker Signature Predicting Survival and Adjuvant Chemotherapeutic benefit in Gastric Cancer

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Abstract

Background: We previously demonstrated that CDK5RAP3 acts as a tumour suppressor in gastric cancer through negative regulation of the Wnt/β-catenin signalling pathway, but its function in chemotherapeutic responsiveness of gastric cancer has not been investigated. In this study, we aimed to examine the clinical significance of CDK5RAP3 to predict chemotherapeutic responsiveness in gastric cancer.

Methods: A collection of 188 pairs of tumour tissue microarray specimens from Fujian Medical University were employed for the discovery set, and 310 tumour tissue samples of gastric cancer patients were employed for the internal validation set. Eight-five tumour tissue samples from Qinghai University Hospital were used as the external validation set 1. Transcriptomic and clinical data of 299 gastric cancer patients from TCGA were used as the external validation set 2. CDK5RAP3 expression, microsatellite instability (MSI) status, and tumour-infiltrating lymphocytes (TIL) were examined with immunohistochemistry. Clinical outcomes of patients were compared with Kaplan-Meier curves and the Cox model.

Results: In a multi-centre evaluation, increased CDK5RAP3 indication of better prognosis depends mainly on MSI-L/MSS status or TIL^{high}. High CDK5RAP3 expression predicts sensitive therapeutic responsiveness to postoperative adjuvant chemotherapy in gastric cancer. In a stratification analysis based on CDK5RAP3 combined with TIL or MSI status, patients with $CKD5RAP3^{low}$ TIL^{low} showed no significant difference in prognosis after receiving chemotherapy, whereas patients with $CKD5RAP3^{low}$ TIL^{high}, $CKD5RAP3^{high}$ TIL^{low}, and $CKD5RAP3^{high}$ TIL^{high} had better responsiveness to chemotherapy. In addition, patients with $CKD5RAP3^{high}$ MSI-L/MSS status benefitted the most from adjuvant chemotherapy among all patients evaluated.

Conclusions: CKD5RAP3 can be used as an effective marker to evaluate individualized chemotherapy regimens in gastric cancer patients dependent on their TIL and MSI status.

Background

Postoperative adjuvant chemotherapy is a standard treatment for locally advanced gastric cancer after radical resection, and it improves patient outcomes.[1, 2] Currently, the staging system is insufficient at defining prognosis and distinguishing between patients who will benefit significantly from chemotherapy in gastric cancer.[3, 4] Some predictive biomarkers for the responsiveness to chemotherapy have been identified; however, most of the proposed biomarkers lack reproducibility or standardization.[4, 5] Thus, new molecular markers that predict patients sensitivity to chemotherapy are warranted for treatment selection.

Cyclin-dependent kinase 5 (CDK5) regulatory subunit-associated protein 3 (CDK5RAP3, also called C53/LZAP) was reported in our preliminary studies to be a tumour suppressor in gastric cancer through inhibition of Wnt/β-catenin signalling.[6, 7] We previously demonstrated that CDK5RAP3 affects the dephosphorylation of GSK-3β (Ser9) by inhibiting AKT phosphorylation and degrades β-catenin through phosphorylation, thereby downregulating the Wnt/β-catenin signalling pathway.[7] Additionally, among the subtypes of CDK5RAP3, we found that the C53d subtype is a tumour suppressor and affects the Wnt pathway in gastric cancer.[8]

The Wnt/β-catenin signalling pathway plays a vital role in resistance to therapeutics, malignant tumour progression, and modulating tumour immune escape.[9] A study on melanoma indicated that inherent activation of the Wnt/β-catenin signalling pathway is correlated with a lack of T cell infiltration, and this may be a mechanism for primary resistance to some immunotherapies.[10] Preclinical data indicate that WNT/β-catenin signalling subverts the tumour microenvironment (TME) through promoting immune cell development, activating the immune rejection of tumour cells and providing cancer immune surveillance. [11] Deregulated WNT signalling has been shown to favour recruitment of tumour-infiltrating lymphocytes (TIL) into the TME. [12, 13]

TIL play a key role in the TME and reflect the anti-tumour immune contexture.[14] The literature suggests that patients with gastric cancer who have high TIL have superior survival benefit from adjuvant chemotherapy.[15, 16] Further elucidating the interaction between TIL and chemotherapy can help reverse tumour resistance to immunosuppression.[17] In our study, we collected multi-centre data to explore the role of CDK5RAP3 in the chemotherapy responsiveness of gastric cancer patients through the immune microenvironment to evaluate individualized chemotherapy regimens in gastric cancer patients.

Methods

Patients and gastric tissue sample

The study was primarily based on 4 independent patient cohorts. Cohort 1 included 201 gastric cancer tissue specimens collected from January 2010 to April 2014 at Fujian Medical University Union Hospital; thirteen samples were excluded due to data missing. Gastric tissue specimens included tumour tissue of the stomach and adjacent non-tumour tissue. To screen for valuable indicators, tissue microarray specimens (TMAs) consisting of the remaining 188 patients who underwent radical gastrectomy and standard D2 lymphadenectomy were used. Cohort 2 included 315 gastric cancer tissue specimens collected from January 2010 to April 2014 at Fujian Medical University Union Hospital; five samples were excluded due to data missing. The remaining 310 patients were employed for the internal validation set. Cohort 3 included 95 gastric cancer samples collected from January 2010 to April 2014 at Qinghai University Hospital; ten samples were excluded due to missing data. The remaining 85 patients were employed for external validation set 1. Samples of Cohort 2 and Cohort 3 were embedded in paraffin for immunohistochemistry. The inclusion criteria were as follows: (a) histological identification of gastric cancer; (b) availability of follow-up data and clinicopathological characteristics; and (c) TNM staging of gastric cancer tumours according to the 2010 International Union Against Cancer (UICC) guidelines. The exclusion criteria were as follows: (1) patients with no formalin-fixed paraffin-embedded (FFPE) tumour sample, including the centre of the tumour (CT) and the invasive margin (IM), from initial diagnosis; and (2) patients who received chemotherapy or radiotherapy before surgery. All participating patients with advanced GC routinely received fluorine-based chemotherapy. Cohort 4 was derived from the Cancer Genome Atlas (TCGA) with 343 gastric cancer patients in all; forty-four patients were excluded due to data missing. The remaining 299 patients were employed for external validation set

2. Comprehensive information of the 4 recruited cohorts is listed (Fig. 1A and Table 1). The study was approved by the Ethics Committees of Fujian Medical University Union Hospital and the Affiliated Hospital of Qinghai University. This study has been approved by the Ethics Committee of Union Hospital Affiliated to Fujian Medical University (Ethics approval number of scientific research project: 2019KTCX012). Informed consent was obtained from all participants.

Table 1
Relationship Between CDK5RAP3 Expression and Baseline Characteristics of Patients

variables	Discovery Set			Internal Validation Set			External Validation Set 1			Ext 2
	$CDK5RAP3^{low}$	$CDK5RAP3^{high}$	P	$CDK5RAP3^{low}$	$CDK5RAP3^{high}$	P	$CDK5RAP3^{low}$	$CDK5RAP3^{high}$	P	CD
All patients	87	101		187	123		34	51		15
Gender			0.323			0.324			0.136	
Female	17	27		50	26		14	12		58
Male	70	74		137	97		20	39		95
Age at surgery(yr)			0.301			0.522			0.794	
< 65	48	47		113	69		25	40		76
≥ 65	39	54		74	54		9	11		77
BMI			0.649			0.850			1.000	
< 25	71	86		160	107		30	44		No
≥ 25	16	15		27	16		4	7		No
T classification			0.186			**0.007**			0.321	
T1	5	5		12	18		7	5		5
T2	5	16		17	19		6	15		37
T3	38	40		63	44		4	9		65
T4	39	40		94	42		17	22		46
N classification			0.204			**0.027**			0.468	
N0	7	11		0	2		16	22		48
N1	17	29		72	61		4	12		39
N2	21	27		33	24		6	5		32
N3	42	34		81	36		8	12		34
M classification			1.000			NA			NA	
M0	85	98		187	123		34	51		14
M1	2	3		0	0		0	0		9
TNM stage			0.243			**0.005**			0.797	
I	4	12		15	23		8	10		20
II	22	29		60	45		11	20		54
III	59	57		112	55		15	21		73
IV	2	3		0	0		0	0		6
Chemotherapy*			0.737			0.430			**0.001**	
No	40	50		89	65		26	13		87
Yes	47	51		98	58		8	38		66
Surgery type			NA			0.572			NA	
Laparoscopic	None	None		184	119		0	0		No
open	None	None		3	4		34	51		No
Tumor size (mm)			0.054			0.048			0.098	

P < 0.05 marked in bold font shows statistical significance.

*Adjuvant chemotherapy after surgery, no radiotherapy was administered to anyone of the patients enrolled.

CDK5RAP3 indicates cyclin dependent kinase 5 regulatory subunit-associated protein 3; MSI, Microsatellite instability; TIL, Tumor-infiltrating lymphocytes; TN available.

variables	Discovery Set			Internal Validation Set			External Validation Set 1			Ext 2
	CDK5RAP3^{low}	CDK5RAP3^{high}	P	CDK5RAP3^{low}	CDK5RAP3^{high}	P	CDK5RAP3^{low}	CDK5RAP3^{high}	P	CD
≤40	28	48		63	56		15	33		No
>40	58	53		124	67		19	18		No
Resection type			0.646			0.807			0.257	
Part gastrectomy	29	38		86	54		26	45		No
Total gastrectomy	58	63		101	69		8	6		No
Pathological type			0.835			0.949			0.163	
Adenocarcinoma	63	76		155	103		25	46		15
mix	16	18		25	26		5	4		0
non Adenocarcinoma	8	7		7	5		4	1		0
MSI status			**0.001**			0.570			0.468	
MSI-L/MSS	62	93		141	97		28	46		13
MSI-H	25	8		46	26		6	5		18
TIL			0.500			0.239			1.000	
TIL low	50	52		126	74		19	29		No
TIL high	37	49		61	49		15	22		No
P < 0.05 marked in bold font shows statistical significance.										
*Adjuvant chemotherapy after surgery, no radiotherapy was administered to anyone of the patients enrolled.										
CDK5RAP3 indicates cyclin dependent kinase 5 regulatory subunit-associated protein 3; MSI, Microsatellite instability; TIL, Tumor-infiltrating lymphocytes; TN available.										

Construction of tissue microarray (TMA)

From January 2010 to April 2014, a total of 201 gastric cancer tissue samples were selected. Briefly, the pathologist examined all gastric cancer tissues and marked the paraffin blocks based on the tumour position of the HE stained section and immunohistochemical slides; the pathologist selected more areas of the tumour tissue without representation of necrosis and haemorrhagic material areas to prepare tissue chips for experiments. Paraffin wax was mixed with an equal amount of beeswax to make 2 blank wax blocks. A puncture hole with a diameter of 1 mm was made in the blank paraffin to separate the two holes, and 80 tissue punches were made. For each sample, a 1.5 mm core was punched from the donor block using a tissue microarray instrument. A tissue analyser was used to sample the tumour-marked wax block, the sampled tissue was placed into the corresponding channel of the blank wax block, and the determined array position was transferred to the recipient paraffin block. Several serial sections (4 μm thick) were cut from all TMAs, and one section of each TMA was stained with haematoxylin-eosin to ensure that the TMA was constructed correctly. The intratumour dot was harvested from the centre of the tumour, while the peritumoural dot was punched out from the area ≥2 cm from the tumour margin. The prepared TMA slides were used for immunohistochemistry (IHC).

IHC and evaluation

The serial sections of the FFPE sample were 4 μm and were mounted on a glass slide for IHC analysis. The sections were deparaffinized with xylene and rehydrated with alcohol. We blocked the endogenous peroxidase by immersing the slices in a 3% H_2O_2 aqueous solution for 10 minutes and microwaved them in 0.01 mol/L sodium citrate buffer (pH 6.0) for 10 minutes for antigen retrieval. The slides were then washed in phosphate-buffered saline (PBS) and incubated with 10% normal goat serum (Zhongshan Biotechnology Co., Ltd., China) to eliminate non-specific reactions. Subsequently, the primary antibody was incubated with the sections overnight at 4°C. The negative control was processed with the same methods, but the primary antibody was omitted. After rinsing 3 times with PBS, the secondary antibody was diluted and incubated on the slide for 30 minutes at room temperature, and the staining was developed with diaminobenzidine (DAB) solution. Finally, the slides were counter-stained with haematoxylin, dehydrated, and fixed with a cover glass and neutral resin.

We performed CDK5RAP3 (ab157203, Abcam, 1:100) immunohistochemical staining on tumour tissue of gastric cancer patients. The staining intensity and average percentage of positive cells in 5 randomly selected regions were evaluated to represent the protein expression level. The scoring criteria (Supplementary Figure 2) were as follows: staining intensity was divided into 0 (negative staining), 1 (weak staining, light yellow), 2 (medium staining, yellow-brown), or 3 (strong staining, brown). The proportion of positive staining tumour cells was categorized as the following thresholds: 0 (≤5% positive cells), 1

(6%-25% positive cells), 2 (26%-50% positive cells), and 3 (≥51% positive cells). The final expression was calculated by multiplying the staining intensity score by the proportional staining score (total 0 to 9). Patients with final scores of 0, 1, 2, and 3 were classified as the low expression group, and patients with scores 4, 6, and 9 were classified as the high expression group.

We performed CD3 (ab16669, Abcam, 1:150) and CD8 (ab4055, Abcam, 1:200) immunohistochemical staining on tumour tissue of gastric cancer patients to assess TIL. To date, there has been no consensus on evaluating TIL infiltration in gastric cancer through IHC, so we adopted and modified the GALON score which uses CD3 and CD8 as markers to reflect the status of TIL.[18] The tumour area was divided into the invasive margin (IM) and the centre of the tumour (CT). We assessed the infiltration of CD3 and CD8 cells and analysed the positively stained cells in each area (CT or IM) at x400 magnification (determining the average percentage of positive cells in 5 fields). As shown in Supplementary Figure 4, the scoring standard was 0 points for <5% CD3CT-positive cells, 1 point for 5%-25% CD3CT-positive cells, 2 points for 26%-50% CD3CT-positive cells, and 3 points for >50% CD3CT-positive cells. A score of 0 or 1 was defined as "low", and a score of 2 or 3 was defined as "high". The same scoring standards were used for CD3IM, CD8CT, and CD8IM. The total TIL score (Immunoscore) was equal to CD3CT + CD3IM + CD8CT + CD8IM. All gastric cancer patients were divided into the TIL^{high} group and TIL^{low} group according to the median of the total TIL score.

We performed MLH1 (ab92312, Abcam, 1:800), MSH2 (ab52266, Abcam, 1:800), MSH6 (ab92471, Abcam, 1:250), and PMS2 (ab110638, Abcam, 1:250) immunohistochemical staining on tumour tissue of gastric cancer patients to evaluate microsatellite instability (MSI) status.[19] The scoring criteria (Supplementary Figure 3) are as follows: deficiency in at least one protein related to mismatch repair genes was interpreted as deficient Mismatch Repair (dMMR) which manifests as MSI-H; no deficiencies in mismatch repair gene-related proteins was interpreted as proficient MMR (pMMR) which manifests as MSI-L/MSS.

The IHC results were evaluated by two independent gastroenterology pathologists who were blinded to the clinical prognosis of the patients. Approximately 90% of the scoring results were the same. When the scores of the two independent pathologists diverged, another pathologist checked the results again and selected one of the scores proposed by the first two doctors, or the three pathologists discussed the decision together.

Processing and analysis of genomic data

We used publicly available level 3 data from TCGA which was downloaded from the Genomic Data Commons (https://portal.gdc.cancer.gov) on June 15, 2020, and this download included clinical information and mRNA expression data. MSI status was downloaded from the UCSC Cancer Browser (http://xena.ucsc.edu) on June 15, 2020 for stomach adenocarcinoma (STAD) samples. The mRNA expression data were presented as counts and were normalized with R software (version 4.0.0) and the “limma” package.

Gene set enrichment analysis

Gene set enrichment analysis (GSEA) performed by the Molecular Signature Database (MSigDB) was used to identify the pathways that were significantly enriched in $CDK5RAP3^{low}$ tumour samples. If a gene set had a positive enrichment score, the majority of its members had higher expression accompanied with higher risk score, and the set was considered “enriched”.

Statistical analysis

All data were processed using SPSS 25.0 (SPSS Inc. Chicago, IL) and R software (version 4.0.0). The cut-off value for CDK5RAP3 expression was the median value. The results are expressed as the mean±SEM. Student’s t-test or Wilcoxon rank-sum test was used for continuous variables. We used the χ^2 test or Fisher exact test to compare categorical variables of clinical characteristics. The Kaplan-Meier method was used to estimate median survival. The log-rank test was used to compare survival between two groups. The association of relevant clinicopathological variables with OS was assessed using the Cox proportional hazard model. Interactions between the clinicopathological parameters and responsiveness to chemotherapy were tested with the Cox model. Clustering charts based on the Z-score normalization method were used to describe the level of protein expression in each case. We defined the survival time of patients who were lost to follow-up as the time from surgery to the last follow-up time, and the survival time of patients who were still alive at the end of the study was defined as the time from surgery to the database deadline. Two-tailed P values < 0.05 were indicated significant differences.

Results

Downregulation of CDK5RAP3 expression correlates with poor prognosis in gastric cancer patients.

Since our previous studies at a single-centre demonstrated the role of CDK5RAP3 in gastric cancer, we extended our investigation to 882 gastric cancer samples from 3 different centres to confirm the clinical significance of CDK5RAP3 in gastric cancer patients. The study was designed as shown in Fig. 1A. The expression level of CKD5RAP3 in tissue microarray specimens (TMAs) from 188 sample pairs of tumour and adjacent tissue from patients was detected by IHC (Fig. 1B). We used Kaplan-Meier curves to compare the overall survival rate of $CKD5RAP3^{low}$ and $CKD5RAP3^{high}$ patients. $CKD5RAP3^{low}$ patients had significantly poorer overall survival rates than $CKD5RAP3^{high}$ patients in the discovery set (Fig. 1C). Similarly, in the internal validation set, external validation set 1, and external validation set 2, the overall survival rate for $CKD5RAP3^{low}$ patients was significantly lower than that of $CKD5RAP3^{high}$ patients (Fig. 1D-I).

We paired adjacent tissue samples (n = 188) in the discovery set to analyse the expression levels of CKD5RAP3 in gastric cancer tissue and adjacent tissue. The results showed that CKD5RAP3 expression was lower in gastric cancer tissue (Supplementary Fig. S1A). We also collected normal gastric tissue samples from the GETX database combined with the TCGA database (n = 207), and the same results were observed in external validation set 2 (Supplementary Fig. S1B).

Additionally, we used GSEA to confirm pathways that were activated in CKD5RAP3^{low} tumours compared with CKD5RAP3^{high} tumours (Supplementary Fig. S1C). Wnt/β-catenin signalling was significantly upregulated in CKD5RAP3^{low} tumours (FDR q-value = 0.000).

Taken together, we used a multi-centre evaluation to confirm the conclusions drawn in our previous studies.

The association of CKD5RAP3 expression with the prognosis of gastric cancer patients depends on MSI status or TIL

We evaluated whether the immune microenvironment affects the prognostic value of CKD5RAP3. The MSI status of each case was determined first. Kaplan-Meier analysis and stratification analysis were performed to evaluate the prognostic value of CKD5RAP3 after stratification by MSI status in the discovery set. As shown in Fig. 2A, the expression of CKD5RAP3 was significantly correlated with the prognosis of gastric cancer patients in the MSIL/MSS status compared with the indistinct result in MSI-H status (Fig. 2B). Furthermore, TIL were detected by IHC in the discovery set. The stratification analysis based on TIL showed that patients with CKD5RAP3^{high} had a significantly better prognosis than those with CKD5RAP3^{low} in the TIL^{high} subgroups, whereas no such prognostic difference was evident in the TIL^{low} subgroups (Fig. 2C,2D). The same results were observed in the internal validation set, external validation set 1, and external validation set 2 (Fig. 2E-N). Hence, the better prognosis in CKD5RAP3^{high} gastric cancer patients may depend on MSIL/MSS status or TIL^{high}.

High expression of CKD5RAP3 indicates better responsiveness of gastric cancer patients to chemotherapy

To investigate the prognosis of gastric cancer patients who received postoperative adjuvant chemotherapy with different CKD5RAP3 expression levels, we performed stratification analysis and Kaplan-Meier analysis for all patients. In the discovery set, the patients with CKD5RAP3^{low} had no difference in overall survival rate with or without postoperative adjuvant chemotherapy, whereas CKD5RAP3^{high} patients had better chemotherapeutic responsiveness (Fig. 3A). A similar result was observed in the internal validation set, external validation set 1, and external validation set 2 (Fig. 3B-D). We calculated the proportional hazard model based on chemotherapy factors and the interaction of combined CKD5RAP3 expression with responsiveness to chemotherapy. In four cohorts, CKD5RAP3^{high} patients receiving chemotherapy had significantly better prognosis (Fig. 3E, 3F). Notably, the test for interaction between CKD5RAP3 expression and adjuvant chemotherapy responsiveness revealed that CKD5RAP3^{high} patients had better therapeutic responsiveness to chemotherapy than CKD5RAP3^{low} patients. Accordingly, these results suggest that CKD5RAP3 high expression indicates enhanced responsiveness to postoperative adjuvant chemotherapy in gastric cancer.

The expression of CKD5RAP3 in combination with TIL can affect the responsiveness of patients to chemotherapy

Wnt/β-catenin signalling is associated with mechanistic development of immunity evasion,[20] and we previously showed that CDK5RAP3 negatively regulates the Wnt/β-catenin signalling pathway. We first explored the responsiveness of patients to chemotherapy after inclusion of TIL. The heat map shows the standardized immunohistochemical score data between CKD5RAP3, CD3, CD8, and TIL in 3 cohorts (Supplementary Fig. S5A-C). In the discovery set, the Kaplan-Meier survival analysis showed a significantly better prognosis of patients who underwent chemotherapy and had TIL^{high} compared to those with TIL^{low} (Supplementary Fig. S6A, 7D).

We further classified the gastric patients into 4 groups based on their expression of CKD5RAP3 and TIL in the discovery set (Fig. 4E). Interestingly, the stratification analysis showed that patients with CKD5RAP3^{low} TIL^{low} had no significant difference in prognosis after receiving chemotherapy, whereas the patients with CKD5RAP3^{low} TIL^{high}, CKD5RAP3^{high} TIL^{low}, or CKD5RAP3^{high} TIL^{high} had better prognosis than those without chemotherapy (Fig. 4A-D). These three subgroups also showed significant responsiveness to adjuvant chemotherapy (Fig. 4F). Similar results were shown in both the internal validation set (Supplementary Fig. S6B, D; Fig. 5A-F) and external validation set 1 (Supplementary Fig. S6C, D; Supplementary Fig. S7A-F).

Conclusively, these data indicate that TIL can be used to screen patients who may benefit from adjuvant chemotherapy from patients with CKD5RAP3^{low} and provide additional pathological characterization for gastric cancer patients receiving chemotherapy.

CKD5RAP3 influences the responsiveness of gastric cancer patients to chemotherapy depending on MSI status

To discover the clinical significance of CKD5RAP3 and MSI status for patients, we first explored the responsiveness to adjuvant chemotherapy for patients with different MSI states. In the discovery set, the Kaplan-Meier curves of patients with MSI-H status revealed no additional significant benefit from chemotherapy, while patients with MSI-L/MSS status showed significantly better prognosis when they received chemotherapy (Supplementary Fig. S8A, E).

We next combined CKD5RAP3 expression and MSI status to perform survival analysis (Fig. 6A). Receiving adjuvant chemotherapy predicted a better prognosis than not receiving chemotherapy only in the CKD5RAP3^{high} MSI-L/MSS status subgroup (Fig. 6E); compared with the other three subgroups (CKD5RAP3^{low} MSI-H status, CKD5RAP3^{low} MSI-L/MSS status, and CKD5RAP3^{high} MSI-H status), patients did not benefit from adjuvant chemotherapy (Fig. 6I) and had no significant difference in prognosis regardless of whether they received chemotherapy (Supplementary Fig. S9A-C). The same results were observed in the internal validation set, external validation set 1, and external validation set 2 (Supplementary Fig. S8B-F; Fig. 6B-D, F-J; and Supplementary Fig. S9D-L).

Consequently, MSI-H status could distinguish patients who may benefit most from adjuvant chemotherapy among the patients with CKD5RAP3^{high}. The improved chemotherapeutic responsiveness in CKD5RAP3^{high} patients may possibly be related to MSI status.

Discussion

Perioperative chemotherapy helps reduce recurrence and improve survival for respectable advanced gastric cancer compared with surgery alone.[1, 2] The methods for predicting the effect of chemotherapy in gastric cancer have limitations, including the TNM staging system and gastric cancer molecular subtypes proposed by The Cancer Genome Atlas Research Network.[21, 22] Nevertheless, selection of chemotherapy for gastric cancer based on clinical and molecular characteristics remains inconclusive.[23] A new molecular marker is urgently needed to determine whether chemotherapy is suitable for certain gastric cancer patients.

CDK5RAP3 was originally identified as a binding protein of Cdk5 activator p35 and p39 via yeast two-hybrid.[24, 25] In recent years, a large body of literature has explored the role of CDK5RAP3 in different cancers. Mak et al.[26] found that CDK5RAP3 promotes metastasis of human hepatocellular carcinoma through activating p21-activated protease 4 (PAK4). Egusquiaguirre et al.[27] reported that CDK5RAP3 acts as an enhancer of STAT3-dependent gene expression in progression of primary human breast cancers. Our previous study showed that CDK5RAP3 negatively regulates the Wnt/β-catenin signalling pathway by blocking GSK-3β phosphorylation of Ser9 to act as a tumour suppressor in gastric cancer.[6] Here, high expression of CDK5RAP3 is associated with superior survival benefit for adjuvant chemotherapy in gastric cancer patients. Furthermore, assessment of the interaction between CDK5RAP3 expression and chemotherapy responsiveness revealed that patients with CDK5RAP3^{high} have far better therapeutic responsiveness than patients with CDK5RAP3^{low}. To our knowledge, this is the first report to demonstrate that CDK5RAP3 is related to chemotherapy responsiveness in gastric cancer.

Previous studies have reported that Wnt/β-catenin signalling is a well-known target in cancer therapy and plays key roles in gastrointestinal tumourigenesis.[28–31] Wnt/β-catenin signalling is essential for both tumour progression and the anti-tumour immune system.[9, 20] The activation of β-catenin inhibits the transcription of chemokines C-C motif chemokine ligand 4 (CCL4) and reduces the recruitment and activation of basic leucine zipper ATF-like transcription factor 3 (BATF3)lineage dendritic cells (DC) in the tumour microenvironment, thereby reducing the level of DC-derived C-X-C motif chemokine ligand 10 (CXCL10) and ultimately affecting the recruitment of T cells into the TME.[11, 32] The Wnt/β-catenin signal activates T cell factor 1 (TCF-1) and blocks the transcriptional activity of forkhead box P3 (FoxP3) to affect the infiltration of regulatory T cells (Treg) into TME and dampen immunosuppressive activity of Treg cells.[11, 12]

TILs are the major type of infiltrating immune cells and are essential in host protection and tumour surveillance.[22, 33] Recently, studies have shown that high levels of TIL are closely correlated with better responsiveness to adjuvant chemotherapy in gastric cancer patients. [15, 16, 34] Our results also revealed that patients with TIL^{high} have better survival from adjuvant chemotherapy. As suggested in the literature, multi-regional tumour analysis should be performed to define how to best incorporate the assessment of tumour heterogeneity into biomarker-based predictions.[35] However, the assessment of TIL in gastric cancer patients still remains inconclusive. [36] Thus, we performed quantitative TIL immune scoring based on the lymphocytes at the invasive margin (IM) and the centre of the tumour (CT) by IHC. Our results, together with findings from previous studies, can be summarized in four main points. First, CDK5RAP3 is known to regulate the Wnt/β-catenin signalling pathway and its key oncogenes in gastric cancer. Second, Wnt/β-catenin signalling plays a critical role in recruitment of TIL into the tumour microenvironment. Third, stratification analysis revealed that high expression of CKD5RAP3 predicts a better prognosis of gastric cancer patients depends on TIL^{high}. Finally, among the CKD5RAP3^{high} subgroups, both TIL^{low} and TIL^{high} patients have significant benefit from adjuvant chemotherapy. Based on these results, we speculate that CKD5RAP3 may affect the sensitivity of gastric cancer patients to chemotherapy through TIL regulation. Therefore, even in the case of TIL^{low}, these patients still show significant sensitivity to chemotherapy when CKD5RAP3 is highly expressed. The relatively worse benefit of patients with low CKD5RAP3 expression to chemotherapy may be caused by the lack of T cell infiltration.

The Cancer Genome Atlas Research Network described four subtypes of gastric cancers, and the ACRG recently provided a new classification of gastric cancer identifying four subtypes.[22, 37] Despite this evidence, gastric cancer is still widely treated as one disease. In this study, we found that patients with MSI-H status show no significant benefit from perioperative adjuvant chemotherapy. Such results are consistent with the phenomenon observed in previous literature.[38–40] Additionally, the stratification analysis revealed that the high expression of CDK5RAP3 predicts both better prognosis and superior benefit from adjuvant chemotherapy in a MSI-L/MSS status-dependent manner. In the case of low CDK5RAP3 expression, no difference of therapeutic responsiveness was observed for MSI-L/MSS status. This finding suggests that combining CKD5RAP3 expression with MSI status might enable identification of patients who could most benefit from adjuvant chemotherapy among all gastric cancer patients.

Our research has some limitations. First, this is a retrospective study with limited generalization. These results need further verification. In addition, the specific mechanisms of how CKD5RAP3 affects the function of TILs is still unclear, and more work is necessary to clarify the exact molecular mechanisms. Finally, the sample size from external validation set 1 is insufficient. Therefore, a prospective, international multi-centre clinical trial is needed to further verify our findings.

Conclusions

A multi-centre evaluation shows that CKD5RAP3 distinguishes gastric cancer patients who could benefit from chemotherapy. Additionally, high CKD5RAP3 expression predicts survival prognosis and chemotherapy benefits in an MSI status-dependent manner. This phenomenon may result from CKD5RAP3 affecting TILs. CKD5RAP3 might be a potential immunotherapeutic target and to evaluate individualized chemotherapy regimens in gastric cancer patients.

Abbreviations

TIL: Tumor-infiltrating lymphocyte; MSI: Microsatellite instability; IHC: immunohistochemistry; CDK5RAP3: Cyclin dependent kinase 5 regulatory subunit-associated protein 3; TME: Tumour microenvironment; TMA: Tissue microarray specimen; FFPE: Formalin-fixed paraffin-embedded; CT: Centre of the tumour; IM: Invasive margin; MMR: Mismatch repair

Declarations

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Founding

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Availability of data and materials

The dataset analyzed for this study is available from the corresponding author upon reasonable request.

Authors' contributions

JB Wang, YX Gao and LZ Lian conceived and designed the project. YB Ma, P Li, JX Lin and J Lu performed patient collection and clinical data interpretation. QY Chen, LL Cao and M Lin participated performed the statistical analysis. LC Liu, RH Tu, JL Lin, ZN Huang and HL Zheng performed the experiments and wrote the manuscript. JW Xie, CH Zheng and CM Huang contributed to the writing and to the critical reading of the paper. All authors read and approved the final manuscript. All authors read and gave their approval for the final version of the manuscript.

Ethics approval and consent to participate

All procedures performed in studies involving human participants were in accordance with the Helsinki declaration. And all patients whose tissue samples were used in this research provided written informed consent. The study was approved by the Ethics Committees of Fujian Medical University Union Hospital and the Affiliated Hospital of Qinghai University. This study has been approved by the Ethics Committee of Union Hospital Affiliated to Fujian Medical University (Ethics approval number of scientific research project: 2019KTCX012).

Consent for publication

Informed consent was obtained from all individual participants included in the study.

Competing interests

The authors declare that they have no competing interests.

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