Original Research

DKK1 as a robust predictor for adjuvant platinum chemotherapy benefit in resectable pStage II-III gastric cancer

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ABSTRACT

Background: Adjuvant chemotherapy (ACT) with 5-FU alone or 5-FU plus platinum after curative surgery improves the prognosis of pStage II-III gastric cancer (GC). However, only a subset of patients benefits from adjuvant platinum. To avoid the side effects of platinum, it is significant to accurately screen the patients who would benefit maximally with this treatment. The present study aimed to assess the value of DKK1 in predicting the benefit of adjuvant platinum chemotherapy in patients with pStage II -III GC.
Methods: Platinum sensitivity-related genes were screened by bioinformatics. DKK1 expression in 380 GC specimens was detected by immunohistochemistry (IHC) staining, and the correlation with adjuvant platinum-specific benefits were analyzed.
Results: DKK1 was screened as the most significant platinum sensitivity-related gene. In patients with DKK1^{high} GC, the estimated absolute 5-year overall survival (OS) benefits from adjuvant platinum for pStage II-III, II, IIIA, IIIB, and IIIC were 25.5%, 17.3%, 36.4%, 29.2% and 31.1%, respectively, and the estimated absolute 5-year disease-free survival (DFS) benefits in the corresponding stages were 27.4%, 17.5%, 36.7%, 29.7% and 31.5%, respectively. These benefits were significantly higher than those in the same TNM stage without adjusting for DKK1 status. The performance of DKK1 was independent of the TNM stage and other clinicopathological variables. Similar results were obtained in the TCGA and ACRG cohorts. Furthermore, nomograms were constructed to predict the survival benefits in DKK1 subgroups.
Conclusions: The stratification strategy based on DKK1 status is more precise than the TNM staging system for the selection of pStage II-III GC patients suitable for platinum-containing ACT.

Introduction

Gastric cancer (GC) is the sixth most common cancer and the third leading cause of cancer-related deaths worldwide [1], and has been ranked second in both the incidence and mortality of cancers in China [2]. Most patients have advanced disease at diagnosis. Adjuvant chemotherapy (ACT) after curative D2 gastrectomy prevents relapse and improves survival compared to curative surgery alone for patients with pStage II-III GC according to two phase III trials: ACTS-GC and CLASSIC [3–6]. According to the two studies, ACT with S-1 alone or the capecitabine plus oxaliplatin (XELOX) has been recommended as the standard of care for patients with pStage II-III GC. However, the survival benefits

in both studies are similar but limited. The absolute 5-year overall survival (OS) benefit from ACT in the ACTS-GC and CLASSIC trials is 10.6% and 9%, respectively [4,6], suggesting that only a few patients benefit from these approaches. The current standard ACT regimen can be summarized as the single-drug (5-FU or its derivatives alone) and double-drug (5-FU or its derivatives plus platinum) regimens. The double-drug regimen increases the adverse events due to the addition of platinum [3,5,7]. To date, high-quality phase III clinical trials to directly compare the single- and double-drug regimens are lacking, but the consensus on ACT regimens has not been reached. Recently, Kim *et al.* [8] compared the efficacy of adjuvant S-1 *vs.* XELOX among 1088 patients with GC after D2 surgery and found that adjuvant XELOX was
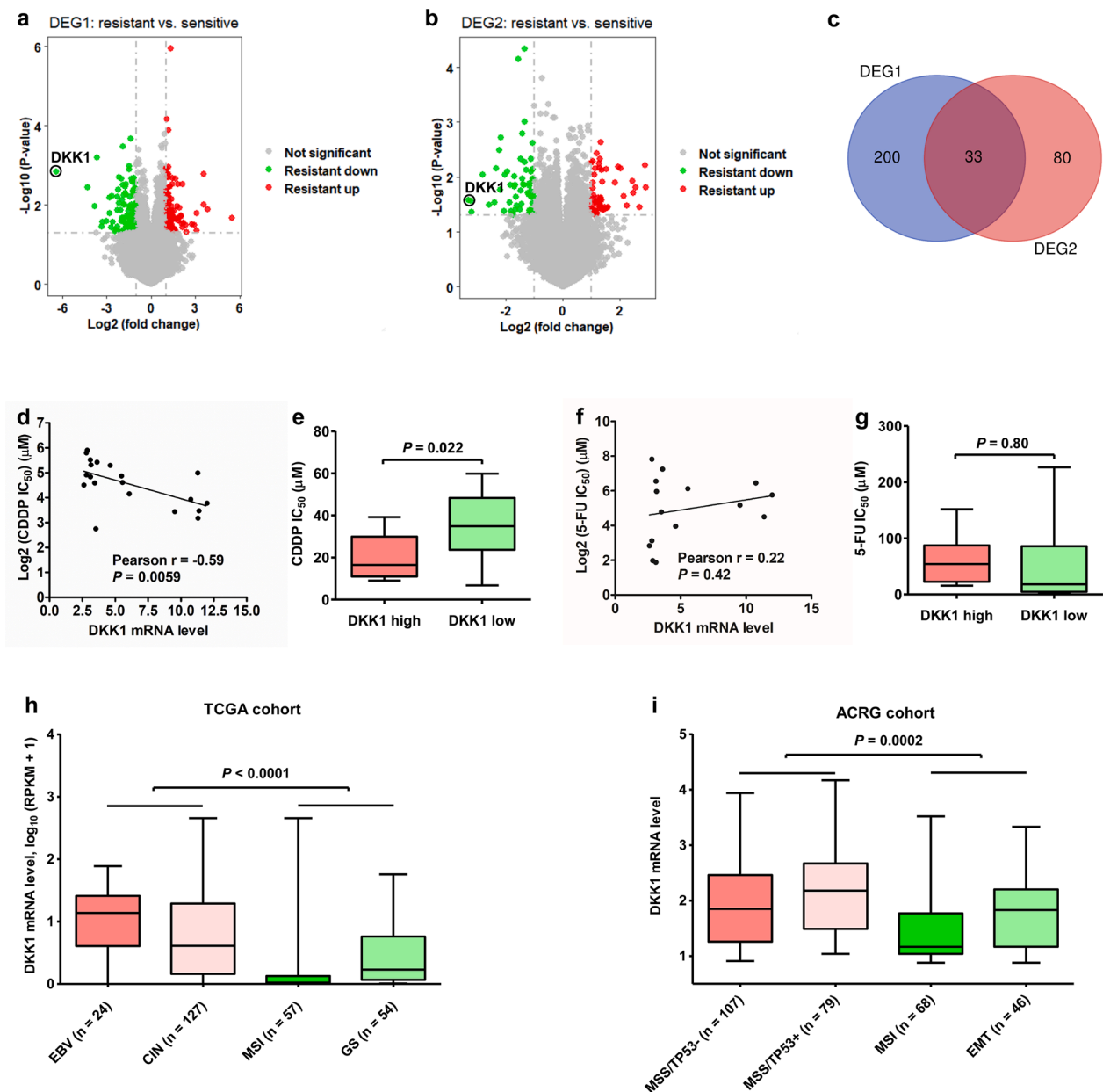

Fig. 1. DKK1 is a candidate indicator for the sensitivity of GC cells to platinum. **a,b** The volcano plot illustrates that DKK1 is the most significantly downregulated gene in the comparison of the top 5 CDDP-resistant *vs.* top 5 CDDP-sensitive GC cell lines (**a**), and in the comparison of the top 10 CDDP-resistant *vs.* top 10 CDDP-sensitive GC cell lines (**b**). **c** Venn diagram showing the overlap of the significantly (FC ≥ 2 and $P < 0.05$) DEGs. **d,f** Correlation analyses of DKK1 mRNA levels and $\log_2$ (IC$_{50}$s) of the 20 GC cell lines to CDDP (**d**) and 5-FU (**f**). **e,g** GC cell lines were classified into DKK1^{high} and DKK1^{low} groups according to the median mRNA expression level of DKK1. The IC$_{50}$s of the two groups of GC cell lines to CDDP (**e**) and 5-FU (**g**) were compared using two-tailed unpaired Student's *t* test. Due to the absence of the IC$_{50}$ data of five GC cell lines against 5-FU, the number of GC cell lines included for analysis in (**f**) and (**g**) was 15. **h** Comparison of DKK1 mRNA levels in the four GC molecular subtypes of TCGA cohort. **i** Comparison of DKK1 mRNA levels in the four GC molecular subtypes of ACRG cohort.

more effective than S-1 in pStage IIIB and IIIC but not in pStage II-IIIA. Also, in pStage IIIB and IIIC, the benefit of oxaliplatin is limited. Therefore, screening patients suitable for ACT and further determining whether these patients should receive platinum-containing regimen is essential. Recent studies have developed some patient stratification methods to predict the benefits of general ACT, according to either biomarkers [9–13] or clinical characteristic-based models [14]. However, efficient methods to direct chemotherapy regimen selection are lacking.

In this retrospective study, we aimed to evaluate the power of the platinum sensitivity-associated gene for predicting the survival benefit of adjuvant platinum in patients with resectable pStage II -III GC. Therefore, we identified Dickkopf-1 (DKK1) as a biomarker with a robust prediction power and developed nomograms to predict the survival benefits in DKK1 subgroups.

Materials and methods

Ethics approval and consent to participate

This study was approved by an institutional review board of the Human Research Ethics Committee of Ruijin Hospital, Shanghai Jiao Tong University School of Medicine (Shanghai, China) and conducted in accordance with ethical guidelines (Declaration of Helsinki). All the sample studies were conducted after obtaining written consent from all patients.

Analysis of platinum sensitivity-related genes in GC cell lines

The gene expression profile data of 20 GC cell lines and their IC_{50} data to cisplatin (CDDP) and 5-FU were downloaded from the GDSC database (https://www.cancerrxgene.org/) [15] and differentially expressed gene (DEG) analysis between CDDP-resistant and -sensitive GC cell lines was performed using the the limma package [16]. The standard for DEG was fold change (FC) $\geq$ 2 (or $\leq$ 0.5) and unpaired t-test P-value < 0.05.

Analysis of DKK1 expression in TCGA and ACRG molecular subtypes of GC

DKK1 expression data in the TCGA and ACRG GC cohorts were downloaded from the TCGA (http://cancergenome.nih.gov/) and the GEO database (http://www.ncbi.nlm.nih.gov/geo/) (accession number: GSE62254), respectively. The molecular subtype information was obtained from the original articles [17,18].

Patients and tumors

A retrospective study was conducted on 380 gastric adenocarcinoma patients who underwent curative D2 gastrectomy between September 2008 and 2012 at Shanghai Ruijin Hospital. All patients, $\geq$ 18-years-old, had a pathological TNM stage of II-III (pStage II-III), and had not received preoperative radiotherapy/chemotherapy or combined malignant neoplasm. All patients had received ACT; 114 were treated with 5-FU or its derivatives alone (the single-drug group), 266 were treated with 5-FU or its derivatives plus platinum (the double-drug group). The FFPE specimens for the primary tumors of all patients were collected for immunohistochemical (IHC) staining to assess DKK1 expression. The patient characteristics included age, gender, tumor site, tumor size, tumor differentiation, nerve invasion status and TNM stage. All patients were staged according to the American Joint Committee on Cancer (AJCC) cancer staging manual (seventh edition) [19] and followed up routinely. Overall survival (OS) was defined as the time from the date of first pathological diagnosis to the date of death from any cause or the last date of follow-up, while relapse-free survival (RFS) was defined as the time from the date of radical surgery to the time of recurrence, development of a new GC, death from any cause or the last date of
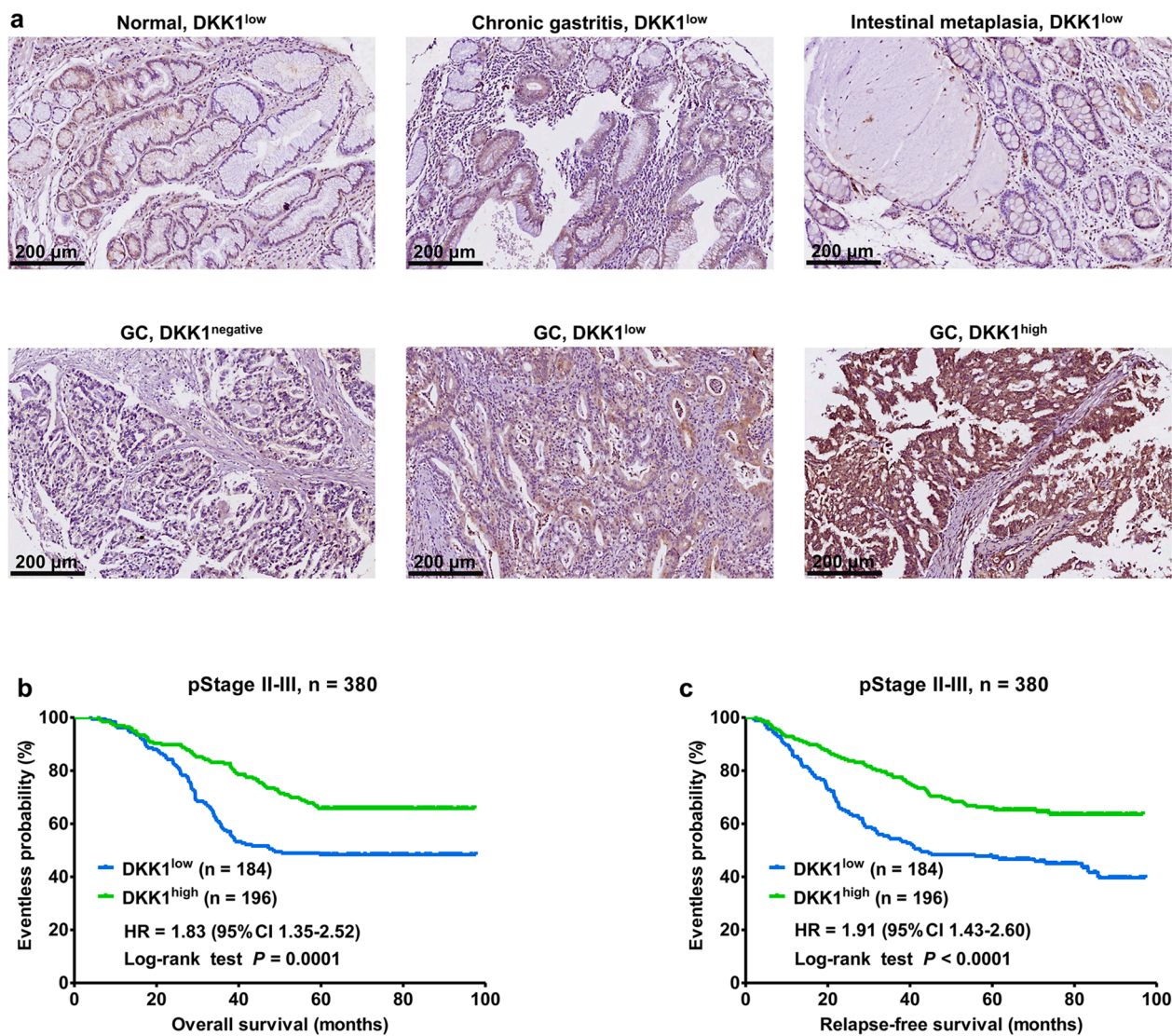

Fig. 2. Kaplan–Meier analysis reveals the correlation between patient prognosis and DKK1 levels in the IHC cohort. **a** Representative images of DKK1 protein levels (original magnification, $\times$ 200 for all images). **b,c** Comparison of the Kaplan–Meier plots for the OS (**b**) and RFS (**c**) between patients with DKK1^{high} and DKK1^{low} GC. For all Kaplan-Meier plots, the HRs and P-values were obtained using the log-rank test.

Table 1

Multivariate analysis of adjuvant platinum-specific OS and RFS benefits in patient subgroups in the IHC cohort ($n = 380$).

Characteristics	OS Double-drug vs. single-drugHR (95% CI)	Log-rank P	$P_{interaction}$	RFS Double-drug vs. single-drugHR (95% CI)	Log-rank P	$P_{interaction}$
Age (years)			0.15			0.14
< 60	0.38 (0.18-0.79)	0.007		0.40 (0.20-0.80)	0.0094	
≥ 60	0.41 (0.26-0.64)	0.00012		0.39 (0.25-0.61)	3.27e-05	
Gender			0.59			0.62
Male	0.42 (0.27-0.67)	0.00022		0.44 (0.29-0.67)	0.00017	
Female	0.37 (0.19-0.70)	0.0024		0.40 (0.22-0.74)	0.0034	
Tumor size (cm)			0.031			0.030
< 5	0.54 (0.33-0.89)	0.014		0.52 (0.33-0.82)	0.0048	
≥ 5	0.27 (0.15-0.47)	4.95e-06		0.28 (0.16-0.49)	5.51e-06	
Tumor differentiation			0.0011			0.0030
Well-differentiated	0.46 (0.16-1.35)	0.16		0.32 (0.12-0.86)	0.0247	
Poor-differentiated	0.41 (0.28-0.61)	6.07e-6		0.44 (0.30-0.63)	1.13e-05	
Tumor site			0.67			0.67
Antral	0.37 (0.24-0.58)	1.57e-05		0.36 (0.23-0.55)	2.69e-06	
GEJ*	NA	NA		NA	NA	
Body	0.31 (0.14-0.68)	0.0036		0.35 (0.17-0.71)	0.0042	
Multisite	0.76 (0.095-5.99)	0.79		0.76 (0.095-5.99)	0.79	
Nerve invasion			0.052			0.011
No	0.50 (0.31-0.79)	0.0033		0.47 (0.30-0.73)	0.00072	
Yes	0.31 (0.17-0.56)	0.00011		0.33 (0.18-0.58)	0.00012	
T stage			0.054			0.048
T1~2#	0.45 (0.12-1.69)	0.24		0.46 (0.12-1.75)	0.26	
T3~4	0.43 (0.29-0.62)	8.78e-06		0.44 (0.31-0.62)	5.74e-06	
N stage			4.35e-06			1.60e-06
N0	0.47 (0.17-1.28)	0.14		0.47 (0.18-1.19)	0.11	
N1	0.80 (0.30-2.16)	0.67		0.66 (0.26-1.68)	0.38	
N2	0.20 (0.096-0.44)	3.92e-05		0.21 (0.10-0.44)	3.10e-05	
N3	0.43 (0.23-0.79)	0.0068		0.49 (0.27-0.89)	0.018	
TNM stage			6.02e-09			3.00e-11
II	0.38 (0.15-1.00)	0.050		0.45 (0.18-1.13)	0.088	
IIIA	0.61 (0.21-1.73)	0.35		0.48 (0.18-1.28)	0.14	
IIIB	0.40 (0.20-0.81)	0.011		0.35 (0.18-0.71)	0.0037	
IIIC	0.35 (0.19-0.64)	0.00069		0.40 (0.23-0.70)	0.0014	
DKK1 expression			3.08e-11			4.41e-8
Low	0.89 (0.54-1.48)	0.66		0.86 (0.54-1.39)	0.54	
High	0.19 (0.11-0.33)	9.13e-09		0.18 (0.11-0.32)	2.24e-09	
Overall	0.43 (0.30-0.61)	3.52e-06		0.43 (0.31-0.61)	1.44e-06	

* GEJ, gastroesophageal junction

There are 14 T1b (early gastric cancer) cases, all of whom have a pN stage N2-3.

follow-up. The median follow-up for OS and RFS were 75.0 and 74.3 months, respectively.

IHC staining

For IHC staining, all the steps were performed according to a standard LSAB protocol (Dako). The primary antibody was rabbit antibody against human DKK1 (Abcam, Cat#ab109416). The secondary antibodies were biotinylated swine anti-rabbit antibody (Dako). The protein level of DKK1 was detected mainly in the plasma membrane and cytoplasma. Medium to strong positivity for > 20% of the cancer cells was defined as DKK1high. The omission of the primary antibody served as the negative control.

Statistical analysis

All statistical analyses were performed using the R 3.6.1 software. A two-sided $P < 0.05$ was considered statistically significant. Chi-square test, Fisher's exact test, Cochran–Mantel–Haenszel test, and linear trend test was applied to compare enumeration variables. The OS and RFS were evaluated using the Kaplan-Meier survival curves and the log-rank test. Variables that achieved statistical significance at $P < 0.05$ in univariate analyses were included into the multivariate analyses. Multivariate analyses based on the Cox proportional hazards (Coxph) regression model determined the interactions among variables and whether a variable was an independent predictor for survival. Variables that met the following two criteria at the same time were defined as the independent predictors for adjuvant platinum benefits: (i) log-rank test $P < 0.05$ (double-drug vs single-drug) of multivariate analysis in at least one subgroup of a variate, (ii) $P_{interaction} < 0.05$ (interaction between this variable and ACT regimen) in multivariate analysis.

Variables that were defined as the independent predictors in multivariate analyses were used to construct nomograms. Nomograms were constructed using the package of rms in R version R 3.6.1 (http://www.r-project.org/) as described in a previous study [20]. A backward step-down selection process with the Akaike information criterion was performed for a final model selection. The performance of the nomogram was measured by concordance index (C-index) and the calibration curves were drawn to compare the nomogram-predicted *vs.* the Kaplan-Meier curve-estimated actual survival probability using bootstraps with 1000 resampling [14,20,21]. A larger C-index means more accurate for the prediction of patient prognosis. The and calibration curves and C-indexes were derived based on the regression analysis and $P < 0.05$ was considered statistically significant.

Results

DKK1 expression is associated with platinum sensitivity in GC cell lines

To screen the platinum sensitivity-related genes, two DEG strategies were applied. The comparison of the top 5 CDDP-resistant *vs.* -sensitive GC cell lines identified 233 DEGs (DEG1) (Fig. 1a; Table S1, supporting information), while the comparison of the top 10 CDDP-resistant versus -sensitive GC cell lines identified 113 DEGs (DEG2) (Fig. 1b; Table S1,
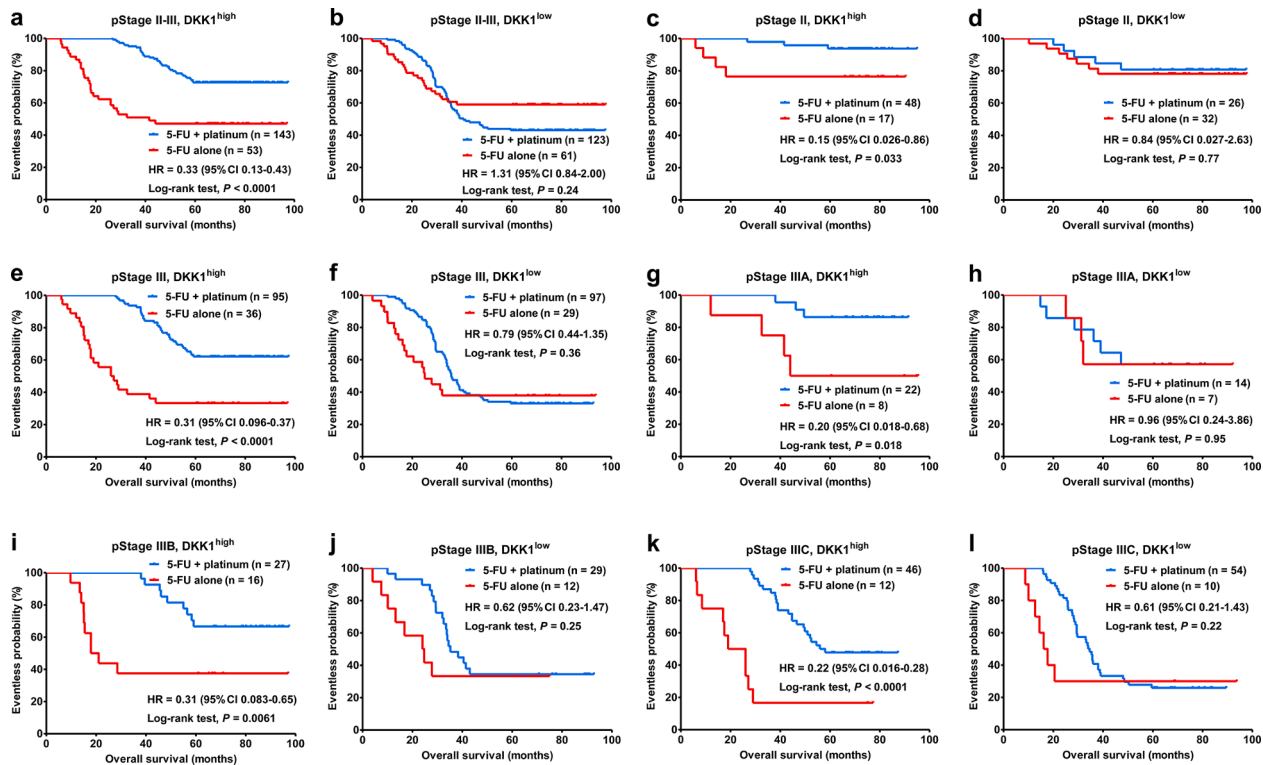
Fig. 3. Kaplan–Meier analysis of OS benefits from adjuvant platinum in patients with pStage II-III GC in the IHC cohort according to DKK1 levels. **a** DKK1^{high} pStage II-III GC. **b** DKK1^{low} pStage II-III GC. **c** DKK1^{high} pStage II GC. **d** DKK1^{low} pStage II GC. **e** DKK1^{high} pStage III GC. **f** DKK1^{low} pStage III GC. **g** DKK1^{high} pStage IIIA GC. **h** DKK1^{low} pStage IIIA GC. **i** DKK1^{high} pStage IIIB GC. **j** DKK1^{low} pStage IIIB GC. **k** DKK1^{high} pStage IIIC GC. **l** DKK1^{low} pStage IIIC. For all Kaplan–Meier plots, the HRs and *P* values were obtained using the log-rank test.

supporting information). A total of 33 genes were common DEGs (Fig. 1c; Table S2, supporting information); 23/33 (69.7%) were down-regulated in CDDP-resistant GC cells and DKK1 was the most significant gene identified in both strategies (86.23-fold down-regulated, *P* = 0.0014 in DEG1; 10.04-fold down-regulated, *P* = 0.027 in DEG2) (Fig. 1a and 1b; Table S2, supporting information). The FC of the 10 CDDP-resistant up-regulated common DEGs was < 4-fold. Therefore, DKK1 was considered the candidate gene for platinum sensitivity. DKK1 mRNA level was negatively associated with CDDP IC_{50} in GC cells (Fig. 1d; Pearson's *r* = -0.59, *P* = 0.0059) and not related to 5-FU sensitivity (Fig. 1f; Pearson's *r* = 0.22, *P* = 0.42). The CDDP IC_{50}s in DKK1^{high} GC cells were significantly lower than those in DKK1^{low} GC cells (Fig. 1e; *P* = 0.022), and the 5-FU IC_{50}s were similar between the two groups (Fig. 1g; *P* = 0.80). These data suggested that DKK1 is associated with platinum sensitivity in GC cells.

DKK1 expression is associated with TCGA and ACRG molecular subtypes of GC

To evaluate the association between DKK1 expression and GC molecular subtypes, we analyzed the TCGA and ACRG cohorts and found that the expression level of DKK1 in the TCGA EBV and CIN subtypes was significantly higher than that in the MSI and GS subtypes (Fig. 1h; *P* < 0.0001). Similarly, the expression level of DKK1 in ACRG MSS/TP53^{−} and MSS/TP53^{+} subtypes was significantly higher than that in MSI and EMT subtypes (Fig. 1i; *P* = 0.0002).

Low expression of DKK1 is positively associated with poor prognosis of pStage II-III patients

To evaluate the DKK1 expression pattern in primary GC lesions of 380 patients receiving ACT after radical surgery, the protein levels in specimens were detected by IHC. DKK1^{low} and DKK1^{high} were observed

in 184 (48.4%) and 196 (51.6%) GC, respectively. The typical DKK1 staining in normal and different stages of GC is shown in Fig. 2a. We did not observe an attribution difference in the clinicopathological parameters between the DKK1^{low} and DKK1^{high} groups (Table S3, supporting information). The DKK1^{low} group had a significantly poorer OS and RFS than the DKK1^{high} group (Fig. 2b and c). Furthermore, DKK1^{low} was an independent predictor for poor OS and patients' RFS (Tables S4 and S5, supporting information).

DKK1 expression is a robust predictor for adjuvant platinum chemotherapy benefit in pStage II-III patients after radical D2 surgery

In the IHC cohort, patients in the double-drug group were younger and had later TNM stages than those in the single-drug group, and the remaining baseline characteristics, including the usage of fluorouracil drugs between the two groups were similar (Table S6, supporting information). Essentially, the platinum drug used in the double-drug group was oxaliplatin (256/266, 96.2%) (Table S6, supporting information). In the overall pStage II-III GC patients, the OS and RFS benefit from adjuvant platinum were significant in both univariate and multivariate analyses (Figs. S1a and S2a, Tables 1, S7 and S8, supporting information). However, these survival benefits were very limited. The estimated absolute 5-year OS and RFS benefits from adjuvant platinum were only 5.5% and 6.9%, respectively (Tables S7 and S8, supporting information). We also observed that the OS and RFS benefit from adjuvant platinum were significant in pStage III patients rather than in pStage II patients in both univariate and multivariate analyses (Figs. S1b, S1c, S2b and S2c, Tables S7 and S8, supporting information; Table 1). Next, we observed that pStage IIIB and IIIC patients obtained significant OS and RFS benefits from adjuvant platinum in both the univariate and multivariate analysis (all *P* < 0.05), while pStage IIIA patients did not obtain significant OS and RFS benefits (both *P* > 0.05 in multivariate analysis) (Figs. S1b, S1c, S2b and S2c, Tables S7 and S8,
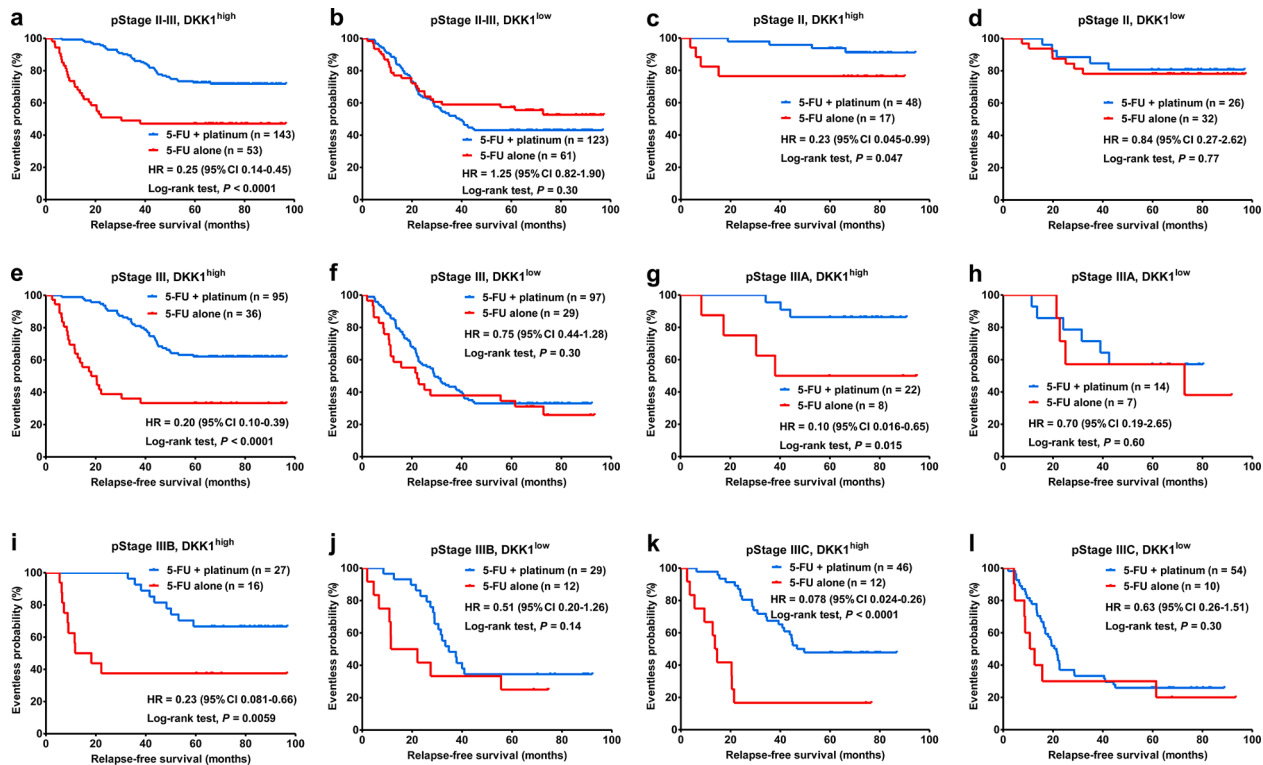
Fig. 4. Kaplan–Meier analysis of RFS benefits from adjuvant platinum in patients with pStage II-III GC in the IHC cohort according to DKK1 levels. **a** DKK1^{high} pStage II-III GC. **b** DKK1^{low} pStage II-III GC. **c** DKK1^{high} pStage II GC. **d** DKK1^{low} pStage II GC. **e** DKK1^{high} pStage III GC. **f** DKK1^{low} pStage III GC. **g** DKK1^{high} pStage IIIA GC. **h** DKK1^{low} pStage IIIA GC. **i** DKK1^{high} pStage IIIB GC. **j** DKK1^{low} pStage IIIB GC. **k** DKK1^{high} pStage IIIC GC. **l** DKK1^{low} pStage IIIC. For all Kaplan–Meier plots, the HRs and *P* values were obtained using the log-rank test.

supporting information; Table 1). The estimated absolute 5-year OS and RFS benefits from adjuvant platinum chemotherapy were 14.3% and 21.4% in pStage IIIB patients, and 13.3% and 16.6% in pStage IIIC patients, respectively. In summary, the OS benefit from adjuvant platinum in pStage II-III GC patients is limited, especially in pStage II-IIIA patients; the benefit may limit to only a few patients. Thus, more efficient strategies other than the TNM staging system are essential.

To evaluate the clinical validity of DKK1 expression in predicting adjuvant platinum benefit, the predictive power of DKK1 status was investigated. We found that adjuvant platinum significantly improved the OS and RFS in patients with DKK1^{high} tumors (Figs. 3 and 4; Tables S7 and S8, supporting information; double-drug *vs* single-drug, hazard ratio (HR) 0.33, confidence interval (CI) 0.13-0.43, *P* < 0.0001 for OS of overall pStage II-III and HR 0.25, 95% CI 0.14-0.45, *P* < 0.0001 for RFS of overall pStage II-III; HR 0.15, 95% CI 0.026-0.86, *P* = 0.033 for OS of pStage II and HR 0.23, 95% CI 0.045-0.99, *P* = 0.047 for RFS of pStage II; HR 0.31, 95% CI 0.096-0.37, *P* < 0.0001 for OS of pStage III and HR 0.20, 95% CI 0.10-0.39, *P* < 0.0001 for RFS of pStage III; HR 0.20, 95% CI 0.018-0.68, *P* = 0.018 for OS of pStage IIIA and HR 0.10, 95% CI 0.016-0.65, *P* = 0.015 for RFS of pStage IIIA; HR 0.31, 95% CI 0.083-0.65, *P* = 0.0061 for OS of pStage IIIB and HR 0.23, 95% CI 0.081-0.66, *P* = 0.0059 for RFS of pStage IIIB; HR 0.22, 95% CI 0.016-0.28, *P* < 0.0001 for OS of pStage IIIC and HR 0.078, 95% CI 0.024-0.28, *P* < 0.0001 for RFS of pStage IIIC) but not in patients with DKK1^{low} tumors (Figs. 3 and 4; Tables S7 and S8, supporting information; double-drug *vs* single-drug, HR 1.31, 95% CI 0.84-2.00, *P* = 0.24 for OS of overall pStage II-III and HR 1.25, 95% CI 0.82-1.90, *P* = 0.30 for RFS of overall pStage II-III; HR 0.84, 95% CI 0.027-2.63, *P* = 0.77 for OS of pStage II and HR 0.84, 95% CI 0.27-2.62, *P* = 0.77 for RFS of pStage II; HR 0.79, 95% CI 0.44-1.35, *P* = 0.36 for OS of pStage III and HR 0.75, 95% CI 0.44-1.28, *P* = 0.30 for RFS of pStage III; HR 0.96, 95% CI 0.24-3.86, *P* = 0.95 for OS of pStage IIIA and HR 0.70, 95% CI 0.19-2.65, *P* = 0.60 for RFS of pStage IIIA; HR 0.62, 95% CI 0.23-1.47, *P* = 0.025 for OS of

pStage IIIB and HR 0.51, 95% CI 0.20-1.26, *P* = 0.14 for RFS of pStage IIIB; HR 0.61, 95% CI 0.21-1.43, *P* = 0.22 for OS of pStage IIIC and HR 0.63, 95% CI 0.26-1.51, *P* = 0.030 for RFS of pStage IIIC) in univariate analysis. In patients with DKK1^{high} tumors, the estimated absolute 5-year OS and RFS benefits from adjuvant platinum were 25.5% and 27.4% in the overall pStage II-III, 17.3% and 17.5% in pStage II, 36.4% and 36.7% in pStage IIIA, 29.2% and 29.7% in pStage IIIB, and 31.1% and 31.5% in pStage IIIC in univariate analysis (Figs. 3 and 4; Tables S7 and S8, supporting information), and were significantly higher than those in the same TNM stage without adjusting for DKK1 status (Figs. S1 and S2, Tables S7 and S8, supporting information). The survival benefits from adjuvant platinum in patients with pStage II-III DKK1^{high} tumors were significant in multivariate analysis (Table 1; HR 0.19, 95% CI 0.11-0.33, *P* = 9.13e-09 for OS of pStage IIIC and HR 0.18, 95% CI 0.11-0.32, *P* = 2.24e-09 for RFS). Also, the interaction between DKK1 status and adjuvant platinum benefits was significant (Tables S7 and S8, supporting information, in univariate analysis, $P_{interaction}$= 0.00048 for OS benefit and $P_{interaction}$= 0.00014 for RFS benefit; Table 1, in multivariate analysis, $P_{interaction}$= 3.08e-11 for OS benefit and $P_{interaction}$= 4.41e-8 for RFS benefit). We further validated the ability of DKK1 status to determine the survival benefits from adjuvant platinum in the TCGA and ACRG cohorts (Fig. S3, supporting information). In summary, high expression of DKK1 is an independent and robust predictor of survival benefits of adjuvant platinum in pStage II-III patients.

Development of nomogram prediction models for adjuvant platinum chemotherapy benefits in DKK1 subgroups

In multivariate analysis, we identified tumor size, tumor differentiation, N stage, TNM stage and DKK1 status as the independent predictors for long-term OS benefit from adjuvant platinum, and tumor size, tumor differentiation, nerve invasion, T stage, N stage, TNM stage and DKK1 status as independent predictors for long-term RFS benefit from
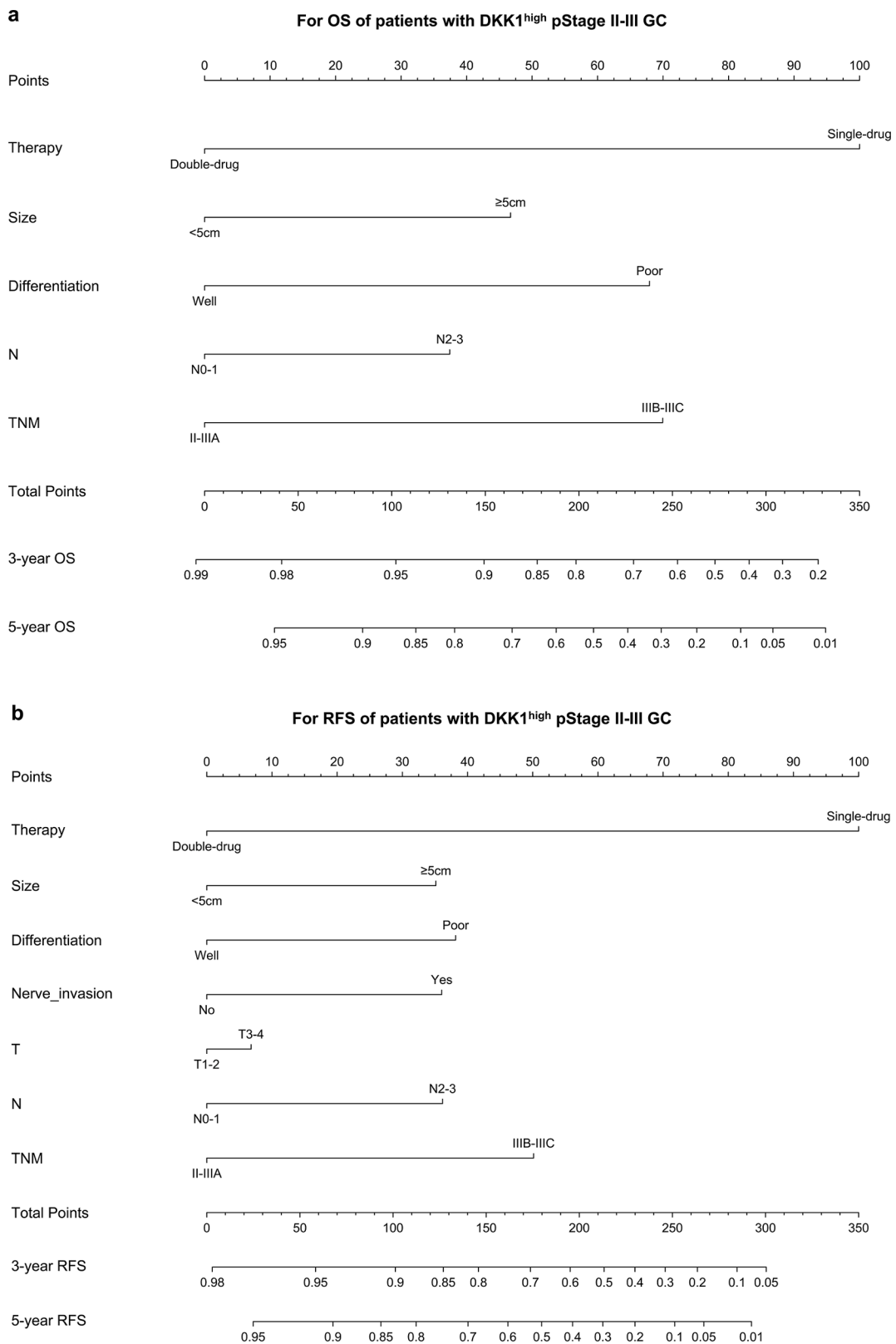
a

For OS of patients with DKK1^high pStage II-III GC

Points

Therapy

Size

Differentiation

N

TNM

Total Points

3-year OS

5-year OS

b

For RFS of patients with DKK1^high pStage II-III GC

Points

Therapy

Size

Differentiation

Nerve_invasion

T

N

TNM

Total Points

3-year RFS

5-year RFS

Fig. 5. Survival nomograms for OS and RFS of patients with DKK1^high pStage II-III GC in the IHC cohort. **a** For OS. **b** For RFS. For an individual patient, nomogram **a** was used to calculate the estimated 3- and 5-year OS with double-drug or with single-drug ACT, and nomogram **b** was used to calculate the estimated 3- and 5-year RFS with double-drug or with single-drug ACT. The difference between the two predicted values is the estimated absolute 3- and 5-year OS and RFS improvements as a result of adjuvant platinum chemotherapy.

adjuvant platinum (Table 1). We did not observe a significant association between DKK1 status and any of these variates (*Table* S3, supporting information). To precisely predict the survival benefits in the DKK1 subgroups, we constructed four nomograms for OS and RFS of patients with DKK1^{high} and DKK1^{low} pStage II-III tumors based on these variables together with ACT regimen (Figs. 5; S4, supporting information). We also found that ACT with 5-FU plus platinum was the most significant prognostic factor for both OS and RFS of patients with DKK1^{high} pStage II-III tumors but a weak prognostic factor for both OS and RFS of patients with DKK1^{low} pStage II-III tumors (Figs. 5; S4, supporting information). The C-indexes for OS and RFS prediction in patients with DKK1^{high} pStage II-III GC were 0.79 (95% CI 0.73-0.85) and 0.78 (95% CI 0.74-0.82), respectively. The C-indexes for OS and RFS prediction in patients with DKK1^{low} pStage II-III GC were 0.69 (95% CI 0.65-0.73) and 0.71 (95% CI 0.66-0.76), respectively. The calibration curve for the probability of OS and RFS at 3 or 5 year showed an optimal agreement between the prediction by nomogram and the actual observation in the DKK1^{high} group (Fig. S5, supporting information) and in the DKK1^{low} group (Fig. S6, supporting information). According to these nomograms, we could predict the absolute 5-year OS and RFS improvements from adjuvant platinum in the clinical subgroups of patients with DKK1^{high} and DKK1^{low} tumors. This finding further confirmed that the predicted absolute 5-year OS and RFS improvements from adjuvant platinum in the DKK1^{high} group were significantly greater than those in the DKK1^{low} group when patients were stratified by the variates in the nomograms (Tables S9 and S10, supporting information). Regarding the OS of patients in the DKK1^{high} group with a specific TNM stage, those with tumors $\geq$ 5 cm and poor differentiation might benefit maximally, while those with small tumors (< 5cm) and well differentiation might benefit the least from adjuvant platinum. For the RFS of patients in the DKK1^{high} group with a given TNM stage, those with large tumors ($\geq$ 5 cm), poor differentiation, and nerve invasion might benefit maximally most from adjuvant platinum, while those with small tumors (< 5cm), well differentiation and without nerve invasion might benefit the least from adjuvant platinum (Tables S9 and S10, supporting information).

Discussion

Accurate stratification of patients with pStage II-III GC to receive curative surgery only, curative surgery plus single-drug ACT, or plus double-drug ACT is clinically significant because it allows the patients to avoid huge side effects. The TNM staging system is a major determinant in selecting the appropriate ACT regimen. The subgroup analysis of the ACTS-GC trial showed that patients with pStage II and IIIA rather than pStage IIIB GC benefitted significantly from adjuvant S-1 monotherapy [3,4]. The subgroup analysis of the CLASSIC trial showed that patients with pStage II, IIIA, and IIIB GC benefit from adjuvant XELOX [5,6]. Two recent retrospective studies [7,8] showed that the significant survival benefit advantage of adjuvant XELOX *vs.* S-1 alone was observed only in pStage IIIC. Moreover, the results of pStage IIIB were inconsistent in the two studies. The current study showed that pStage IIIB and IIIC rather than pStage II-IIIA patients benefitted significantly from adjuvant platinum, which was consistent with the above-mentioned two studies. The estimated absolute 5-year OS benefit from adjuvant platinum in pStage IIIB and IIIC patients was only moderate. These findings suggested a limited value of TNM staging system in directing the selection of platinum-containing ACT. However, other efficient methods are yet lacking. Therefore, a precise method for predicting adjuvant platinum benefit, especially in patients with pStage II-IIIA GC is an urgent requirement. Herein, we identified DKK1 as a robust predictor for benefits from adjuvant platinum in patients with both pStage IIB -IIIC and II-IIIA GC. In the DKK1^{high} group, patients with pStage II and IIIA GC showed an improvement in the 5-year OS improvements of 17.3% and 36.4%, respectively. The performance of DKK1 was independent of the TNM stage and other variables; similar results were observed in the TCGA and ACRG cohorts. Furthermore, we developed nomograms to predict the survival benefits in DKK1 subgroups. Thus, our study demonstrated a more robust method than the TNM staging system to determine whether a patient should receive platinum-containing ACT.

DKK1 is an antagonist of the canonical Wnt/β-catenin signaling [22,23]. Both oncogene-like [23–25] and tumor suppressor-like [23,26,27] functions of DKK1 have been reported in other cancers, while opposite results have been reported on the correlation between DKK1 expression and GC patient prognosis [28–33]. High level of DKK1 was associated with poor prognosis in most studies [31–33]. However, Jia *et al.* [29] reported that low expression of DKK1 was associated with poor prognosis, and silencing DKK1 in GC cells led to resistance to cisplatin and promoted cell proliferation and invasion. Cai *et al.* [34] and Wan *et al.* [30] showed that DKK1 reduces self-renewal of cancer stem-like cells and tumorigenicity in GC cells by attenuating Wnt signaling. These pieces of evidence indicated that DKK1 plays a tumor suppressor role in GC. The current study showed that the low expression of DKK1 was an independent predictor for the poor prognosis of pStage II-III GC patients who received ACT after radical surgery, which was consistent with the findings of Jia *et al.* [29] but not the other studies. Notably, all previous studies did not consider ACT. Interestingly, the high expression of DKK1 may be associated with poor prognosis because we observed this trend in patients who received adjuvant 5-FU alone. Similar observations were reported in lung cancer [35], which showed that poor prognoses associated with low RPS could be negated by chemotherapy because low-RPS tumors are sensitive to platinum. Therefore, the converse prognostic impact of DKK1 can be explained by heterogeneous patient cohorts, especially the heterogeneity of ACT. In addition, the mechanisms underlying the association between DKK1 expression and platinum sensitivity are yet to be clarified. Herein, we inferred that Wnt signaling might be involved in platinum resistance and its inhibition of the Wnt signaling might reverse the platinum resistance, showing a synergistic anti-tumor effect in DKK1^{low} GC.

Conclusions

In summary, we provide a robust patient stratification strategy to select pStage II-III suitable GC patients who would receive platinum-containing ACT according to DKK1 status. However, these results need to be substantiated further in subsequent prospective, larger, multi-centered randomized clinical trials.

Availability of data and materials

The data that support the findings of this study are available upon request from the corresponding author.

CRediT authorship contribution statement

Zhiyuan Fan: Conceptualization, Data curation, Writing – original draft. **Beiqin Yu:** Methodology, Software, Writing – review & editing, Funding acquisition. **Tao Pan:** Formal analysis. **Fangyuan Li:** Investigation. **Jianfang Li:** Resources. **Junyi Hou:** Validation. **Wentao Liu:** Visualization, Supervision. **Liping Su:** Visualization, Supervision. **Zhenggang Zhu:** Supervision, Funding acquisition. **Chao Yan:** Conceptualization, Supervision. **Bingya Liu:** Conceptualization, Supervision, Writing – review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare no conflict of interests.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.tranon.2022.101577.

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