

PCSK1 Overexpression in Rectal Cancer Correlates with Poor Response to Preoperative Chemoradiotherapy and Prognosis

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Background: In a data mining search for potential therapeutic targets to improve the outcome of rectal cancer, we identified *PCSK1* as the cell–cell signaling gene most significantly associated with poor response to concurrent chemoradiotherapy (CCRT). This study aims to investigate the prognostic value of PCSK1 expression in rectal cancer patients who underwent neoadjuvant CCRT.

Methods: Endoscopic biopsy specimens from 172 rectal cancer patients receiving neoadjuvant CCRT followed by curative surgery were assessed immunohistochemically for PCSK1 expression, and H-scores were determined. Expression levels of PCSK1 were further analyzed for correlations with clinicopathologic features, tumor regression grade, metastasis-free survival, disease-specific survival, and recurrence-free survival.

Results: PCSK1 overexpression was significantly associated with pretreatment tumor status (T3–4; $p = 0.009$), pretreatment nodal status (N1–2; $p < 0.001$), posttreatment tumor status (T3–4; $p < 0.001$), posttreatment nodal status (N1–2; $p < 0.001$), vascular invasion ($p = 0.003$), and perineurial invasion ($p = 0.023$). PCSK1 overexpression was also found to be significantly associated with a lower degree of tumor regression ($p < 0.001$). In the univariate analysis, PCSK1 overexpression was significantly associated with lower disease-specific survival, metastasis-free survival, and recurrence-free survival ($p < 0.005$). PCSK1 overexpression remained an independent prognostic factor of lower disease-specific survival ($p = 0.003$; hazard ratio, 5.478) in the multivariate analysis.

Conclusion: Determination of PCSK1 overexpression may be useful for identifying rectal cancer patients at risk for a poor response and worse survival after CCRT.

Keywords: PCSK1, rectal cancer, chemoradiotherapy, response, survival

Introduction

Colorectal cancer remains one of the most common malignancies and the third-highest cause of cancer-related deaths worldwide.¹ Compared to colon cancer, rectal cancer patients often experience worse outcomes because resection is more difficult.² In recent decades, preoperative concurrent chemoradiotherapy (CCRT) has been shown to provide better local control, greater anal preservation, and lower toxicity over surgery alone.³ However, the effect on a given outcome varies greatly between studies, and 10–20% of patients still experience recurrence or metastasis.⁴ Therefore, to choose the most suitable treatment option, prognostic biomarkers may be better predictors of response to CCRT and prognosis than cancer stage.

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To identify potential biomarkers for rectal cancer, we analyzed a public transcriptomic dataset of rectal cancer (GSE35452) from Gene Expression Omnibus, National Center for Biotechnology Information (GEO, NCBI, Bethesda, MD, USA) and identified convertase subtilisin/kexin-type 1 (*PCSK1*) as the most significantly upregulated gene among those associated with cell-cell signaling pathways (GO:0007267). As a member of the proprotein convertase family, the PCSK1 protein (also known as PC1/3) plays a vital role in the proteolytic processing of mature bioactive proteins from large prohormones, including proopiomelanocortin, proinsulin, proglucagon, pro-islet amyloid polypeptide, and prorenin.^{5,6} The resulting end products are widely involved in energy balance, glucose metabolism, and blood pressure regulation. In humans, loss-of-function mutations in the *PCSK1* gene cause monogenic obesity, impaired glucose tolerance, hypertension, cardiac remodeling, and microvascular damage.^{7,8} A variety of studies also report a role for PCSK1 expression in human cancers and tumor cell lines.^{9,10}

This study investigates PCSK1 expression and its association with tumor response to preoperative CCRT in patients with rectal cancer. The level of PCSK1 protein expression was determined in 172 pairs of cancer tissue samples, and the role of PCSK1 was elucidated by analyzing the relationships between clinical and pathological features, including tumor response and survival.

Materials and Methods

Ethics Statement

This study was reviewed and approved by the Institutional Review Board of Chi-Mei Medical Center in Taiwan (IRB: CMFHR10501-008). The requirement for informed consent was waived because all identifying information was removed from the dataset before analysis. This manuscript was also designed according to the guidelines of the Helsinki Declaration as revised in 2013.

Analysis of the Published Transcriptome Dataset

To identify potential genes associated with the response to CCRT, data in a public transcriptome database (GSE35452; Gene Expression Omnibus, National Center for Biotechnology Information, GEO, NCBI, Bethesda, MD, USA) comprising 46 patients with rectal cancer treated with preoperative CCRT were analyzed. Raw CEL files were computerized using the Affymetrix Human Genome

U133 Plus 2.0 microarray platform with Nexus Expression 3 statistical software (BioDiscovery, Hawthorne, CA, USA). All probe sets were analyzed without pre-selection. Under supervision, the statistical significance of each transcript was examined by comparing responders to non-responders, with special attention to genes involved in cell-cell signaling pathways (GO:0007267). We chose those with $p < 0.01$ and a difference in log₂-transformed expression of at least ± 0.1 -fold for further analysis.

Demographic Characteristics and Tumor Specimens

This retrospective study was performed using formalin-fixed, paraffin-embedded tissue specimens from 172 newly diagnosed rectal adenocarcinoma patients treated at Chi Mei Medical Center between 1998 and 2004. The pretreatment staging was determined by endoscopic ultrasound, abdominal computed tomography, or magnetic resonance imaging findings. Patients received 5-fluorouracil-based chemotherapy concomitant with radiotherapy (45–50 Gy) before surgery, and adjuvant chemotherapy was given if the pretreatment or posttreatment tumor or nodal stage was greater than T3 or N1. Tumors from all patients were re-staged and re-graded according to the 7th edition of the AJCC staging system and the World Health Organization classification of Tumors of the Colon and Rectum. All patients were regularly monitored after diagnosis until death or last follow-up.

Histopathologic Assessment of Tumor Specimens

Tumor specimens were evaluated histologically by two independent pathologists (CF Li and YC Wei) who were blinded to all patient clinical information. The assessment of the tumor response to preoperative CCRT was assessed using the standard 5-point tumor regression grading system.¹¹

PCSK1 Immunohistochemical Analysis

As previously described, tumor specimens at initial diagnosis were routinely deparaffinized, rehydrated, heated, quenched, and washed for immunohistochemical staining.^{12,13} After epitope retrieval, tumor specimens were incubated for 1 hour with primary antibody recognizing PCSK1 (Sigma, clone 3D2, 1:50). The immunoexpression levels of PCSK1 in all tumor specimens were scored by two independent pathologists of the addition of secondary antibody and hematoxylin

Table I Summary of Differentially Expressed Genes Associated with Cell–Cell Signaling (GO: 0007267) in Relation to Response to CCRT in Rectal Carcinoma

Probe	Comparison Log Ratio	Comparison p-value	Gene Symbol	Gene Name	Biological Process	Molecular Function
205767_at	-1.339	0.0001	REG	Epiregulin	Anatomical structure morphogenesis, angiogenesis, cell differentiation, cell proliferation, cell–cell signaling, cytokine and chemokine-mediated signaling pathway, epidermal growth factor receptor signaling pathway, female meiosis, keratinocyte differentiation, keratinocyte proliferation, luteinizing hormone signaling pathway, mRNA transcription, multicellular organismal development, negative regulation of cell proliferation, negative regulation of epithelial cell proliferation, negative regulation of smooth muscle cell differentiation, negative regulation of transcription, oocyte maturation, organ morphogenesis, ovarian cumulus expansion, ovulation, positive regulation of DNA replication, positive regulation of cell proliferation, positive regulation of cytokine biosynthetic process, positive regulation of cytokine production, positive regulation of epidermal growth factor receptor activity, positive regulation of fibroblast proliferation, positive regulation of innate immune response, positive regulation of interleukin-6 biosynthetic process, positive regulation of mitosis, positive regulation of phosphorylation, positive regulation of protein kinase activity, positive regulation of smooth muscle cell proliferation, primary follicle stage; oogenesis, regulation of progression through cell cycle, wound healing	Epidermal growth factor receptor binding, growth factor activity, protein binding, protein heterodimerization activity

(Continued)

Table I (Continued).

Probe	Comparison Log Ratio	Comparison p-value	Gene Symbol	Gene Name	Biological Process	Molecular Function
205825_at	1.8447	<0.0001	PCSK1	Proprotein convertase subtilisin/kexin type 1	Cell-cell signaling, metabolic process, proteolysis	Calcium ion binding, hydrolase activity, peptidase activity, proprotein convertase 1 activity, serine-type endopeptidase activity, subtilase activity
210163_at	-1.2994	<0.0001	CXCL11	Chemokine (C-X-C motif) ligand 11	Cell-cell signaling, chemotaxis, immune response, inflammatory response, signal transduction	Chemokine activity, cytokine activity
210861_s_at	1.1635	<0.0001	WISP3	WNT1-inducible-signaling pathway protein 3	Cell-cell signaling, regulation of cell growth, signal transduction	Growth factor activity, insulin-like growth factor binding
211122_s_at	-1.3734	0.0001	CXCL11	Chemokine (C-X-C motif) ligand 11	Cell-cell signaling, chemotaxis, immune response, inflammatory response, signal transduction	Chemokine activity, cytokine activity

staining. For the positive and negative controls, normal bowel tissue treated with or without PCSK1 primary antibody, respectively, were stained in parallel. The immunorepression levels of PCSK1 in tumor cell nuclei were calculated using the H-score method as follows: $H\text{-score} = \sum P_i (i + 1)$, where P_i represents the percentage of tumor cells stained at various intensities (0–100%) and i represents the tumor staining intensity (0 - 3+).¹⁴ H-scores were used to designate high and low PCSK1 expression (high expression, above or equal to the median; low expression, below the median).

Statistical Analysis

All statistical analyses were performed using SPSS for Windows 22.0 (IBM Corporation, Armonk, NY, USA), with $p < 0.05$ considered statistically significant. The primary endpoints comprised 5-year disease-specific survival (DSS), local recurrent-free survival (LRFS), and metastases-free survival (MeFS) rates. Deaths due to cancer were defined as valid events, and deaths secondary to other causes were censored. Associations between PCSK1 expression and clinicopathological features were determined using the chi-square test. The 5-year DSS,

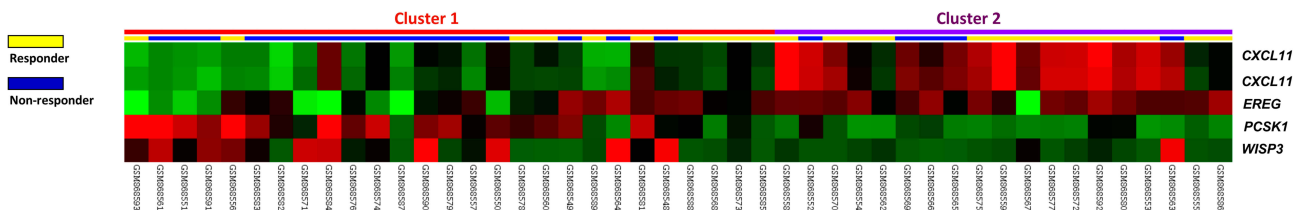


Figure 1 Analysis of gene expression in CCRT responders versus non-responders from a published transcriptomic dataset of rectal cancers (GSE35452). Clustering analysis of genes related to cell-cell signaling indicated PCSK1 is the most significantly upregulated gene in patients responsive to CCRT. Tissue specimens from responders (yellow lines) and non-responders (blue lines) are indicated on top of the heatmap, and expression levels of upregulated and downregulated genes are expressed as a spectrum of brightness of red and green, respectively, with those unaltered in mRNA.

Table 2 Associations and Comparisons Between PCSK1 Expression and Clinicopathological Factors in 172 Rectal Cancer Patients Receiving Preoperative CCRT

Parameters		No.	PCSK1 Expression		p-value
			Low Exp	High Exp	
Gender	Male	108	49	59	0.115
	Female	64	37	27	
Age (Years)	<70	106	50	56	0.347
	≥ 70	66	36	30	
Pre-Tx Tumor Status (Pre-T)	T1–T2	81	49	32	0.009*
	T3–T4	91	37	54	
Pre-Tx Nodal Status (Pre-N)	N0	125	73	52	<0.001*
	N1–N2	47	13	34	
Post-Tx Tumor Status (Post-T)	T1–T2	86	60	26	<0.001*
	T3–T4	86	26	60	
Post-Tx Nodal Status (Post-N)	N0	123	74	49	<0.001*
	N1–N2	49	12	37	
Vascular Invasion	Absent	157	84	73	0.003*
	Present	15	2	13	
Perineurial Invasion	Absent	167	86	81	0.023*
	Present	5	0	5	
Tumor Regression Grade	Grade 0–1	37	10	27	<0.001*
	Grade 2–3	118	62	56	
	Grade 4	17	14	3	

Note: *Statistically significant.

LRFS, and MeFS rates were described using the Kaplan–Meier method, and differences were compared using log-rank statistics. A multivariate Cox regression model was used after adjusting for other confounding variables.

Results

Upregulation of PCSK1 Gene Is Associated with Poor Response to CCRT

As shown in Table 1 and Figure 1, the top-ranking upregulated genes associated with a poor response to CCRT included *PCSK1* and *WISP3*. However, *PCSK1* was the

most significantly upregulated (log 2 ration, 1.8447; $p < 0.0001$). This result motivated us to further investigate the expression status and clinical relevance of PCSK1 in rectal cancers treated with CCRT.

Study Population

A total of 172 patients were enrolled in this study, including 108 (62.8%) males and 64 (37.2%) females (Table 2). The median age was 63 years (range, 22–88 years). The fraction of patients with initial stage I, II, and III disease was 41.9, 29.9, and 28.1%. All stage II and III patients received adjuvant chemotherapy. Fifteen (8.7%) patients presented with vascular invasion, and 5 (2.9%) with perineurial invasion. Of the 172 tumor specimens examined, the response was poor (TRG 0–1, < 25% response) in 37 patients (21.5%), moderate (TRG 2–3) in 118 (68.6%), and complete (no visible tumor in the rectal wall; TRG 4) in 17 (10%) (Table 2).

Immunohistochemical Analysis of PCSK1 Expression and Its Association with Clinicopathologic Features

To determine the relationship between PCSK1 expression level and clinicopathologic characteristics of rectal cancer after neoadjuvant CCRT, immunohistochemical was performed to examine the expression of PCSK1 in 172 rectal cancer specimens. Cytoplasmic expression of PCSK1 was successfully scored in all examined cases, with H-scores varying widely (range, 110–310, Figure 2). High PCSK1 expression was significantly related to pretreatment tumor status (T3–4; $p = 0.009$), pretreatment nodal status (N1–2; $p < 0.001$), posttreatment tumor status (T3–4; $p < 0.001$), posttreatment nodal status (N1–2; $p < 0.001$), vascular invasion ($p = 0.003$), and perineurial invasion ($p = 0.023$) (Table 2). In addition, PCSK1 overexpression was significantly associated with a lower degree of tumor regression ($p < 0.001$). Among patients with PCSK1 overexpression, we observed a tumor regression grade of 0–1 in 27 (15.7%), grade 2–3 in 56 (32.6%), and grade 4 in 3 (1.7%). These findings indicate that PCSK1 plays a role in modulating rectal tumor progression and CCRT sensitivity.

Prognostic Implications of PCSK1 Expression in Rectal Cancer Patients

We further analyzed the prognostic significance of PCSK1 expression in patients with rectal cancer after CCRT. In the univariate analysis (Table 3),

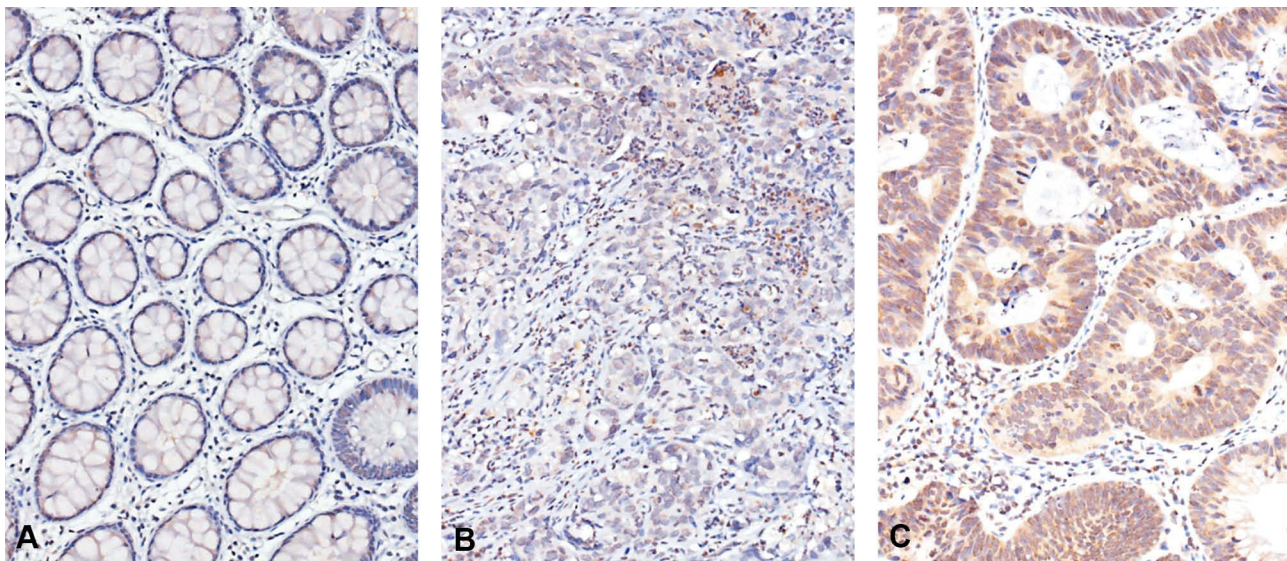


Figure 2 Immunohistochemical staining for proprotein convertase subtilisin/kexin-type 1 (PCSK1) in rectal cancers. The non-neoplastic colonic mucosa (A) reveals no expression of PCSK1 as compared with rectal cancers with low expression (B) and high expression (C) of PCSK1 in pretreatment specimens, respectively.

clinicopathologic parameters including the pretreatment nodal status, posttreatment tumor status, presence of vascular invasion, and TRG were significantly associated with at least one of the three endpoints of our study. Notably, rectal cancer patients with high PCSK1 expression had significantly lower DSS ($p < 0.0001$), LRFS ($p = 0.0063$) and MeFS ($p = 0.0008$) (Table 3; Figure 3). After multivariate analysis (Table 4), TRG and PCSK1 expression remained as independent prognostic factors. Pretreatment nodal status, posttreatment tumor status and presence of vascular invasion were not significantly associated with DSS, LRFS and MeFS. High PCSK1 expression was an independent predictor of DSS ($p = 0.003$; hazard ratio [HR], 5.478; 95% confidence interval [CI], 1.789–16.774).

Discussion

Overexpression of proprotein convertases has been demonstrated in multiple cancer types, and is also related to more aggressive tumor behavior.^{9,15,16} In this study, we observed that high PCSK1 expression is associated with advanced tumor status, poor response to CCRT, and lower survival. To our knowledge, this is the first report describing PCSK1 expression in rectal cancer patients receiving CCRT. More importantly, we also assessed the predisposing clinical and pathological factors influencing survival. These results indicate that PCSK1 may be useful for outcome prediction and may be a potential therapeutic target in rectal cancer.

The proprotein convertase family members convert proproteins into biologically active molecules.^{17,18} To date, at least nine such enzymes have been identified: furin, PC1/PC3, PC2, PC4, PACE4, PC5/PC6, PC7/LPC/PC8, SKI-1/S1P, and NARC-1/PCSK9.¹⁹ In the secretory pathway, proprotein convertases mediate the tissue-specific endoproteolytic activation of precursor proteins, including hormones, neuropeptides, growth factors and their receptors, adhesion molecules, bacterial toxins, and viral glycoproteins. Thus, inhibition of proprotein convertases is associated with many diseases, including Alzheimer's and endocrinopathies.²⁰

In the early 1990s, proprotein convertases were found to be associated with cancer. Since then, numerous studies have established that PCSK1 contributes to tumor development, invasion, and progression in multiple cancer types, including lung, breast, pancreatic, and intestinal cancer.^{16,21-24} Mbikay et al reported that small-cell lung cancers often express more mRNA for PC1 and PC2 and less mRNA for the more ubiquitous furin and PACE4, suggesting inverse roles of these convertases in the development of this neoplasm.²⁵ Cheng et al reported elevated expression of furin and PC1, key enzymes needed to activate vasopressin and other growth factors, in human breast tumors.²² All of this evidence supports our findings that high PCSK1 expression is significantly associated with advanced tumor behavior such as, including pretreatment tumor status, pretreatment nodal status, posttreatment tumor status, posttreatment nodal status, vascular invasion, and perineurial invasion.

Table 3 Univariate Log-Rank Analysis for Important Clinicopathological Variables and PCSK1 Expression

Parameters		No. of Cases	DSS		LRFS		MeFS	
			No. of Events	p-value	No. of Events	p-value	No. of Events	p-value
Gender	Male	108	20	0.9026	7	0.2250	17	0.3520
	Female	64	11		20		14	
Age (Years)	<70	106	19	0.8540	18	0.6615	20	0.7427
	≥ 70	66	12		9		11	
Pre-Tx Tumor Status (Pre-T)	T1–T2	81	10	0.0776	10	0.2261	11	0.1745
	T3–T4	91	21		17		20	
Pre-Tx Nodal Status (Pre-N)	N0	125	19	0.0711	15	0.0070*	19	0.0973
	N1–N2	47	21		12		12	
Post-Tx tumor status (Post-T)	T1–T2	86	7	0.0006*	7	0.0040*	8	0.0033*
	T3–T4	86	24		20		23	
Post-Tx Nodal Status (Post-N)	N0	123	21	0.5998	16	0.1320	20	0.4634
	N1–N2	49	10		11		11	
Vascular Invasion	Absent	157	25	0.0184*	21	0.0028*	27	0.4470
	Present	15	6		6		4	
Perineurial Invasion	Absent	167	29	0.2559	25	0.0940	30	0.9083
	Present	5	2		2		1	
Tumor Regression Grade	Grade 0–1	37	13	0.0038*	10	0.0090*	14	0.0006*
	Grade 2–3	118	17		17		16	
	Grade 4	17	1		0		1	
Down Stage after CCRT	Non-Sig.	150	29	0.1651	24	0.5961	30	0.0853
	Sig. (≥2)	22	2		3		1	
PCSK1 Expression	Low Exp.	86	4	<0.0001*	8	0.0063*	7	0.0008*
	High Exp.	86	27		19		24	

Note: *Statistically significant.

Abbreviations: DSS, disease-specific survival; LRFS, local recurrence-free survival; MeFS, metastasis-free survival.

Thus, altered PCSK1 biology could play a role in human tumor formation and progression resulting from the incomplete or absent processing of its target molecules.

Although the association between PCSK1 expression and colorectal cancer outcomes remains largely unclear, our survival analysis shows that high expression of PCSK1 conferred a negative prognostic impact on rectal cancer in terms of lower DFS. In agreement with our findings, Tzimas et al observed that PC1 and PC2 expression and cleavage are altered in colorectal liver metastases and may be associated with worse clinical outcomes.²⁶ Horsch et al report that PC1 and PC2 are also detected in the human anal canal.²⁷ Collectively, these data indicate that high

PCSK1 expression not only is associated with a more aggressive phenotype but also acts as a predictor of metastasis.

More importantly, we found that high PCSK1 expression also is significantly associated with lower tumor regression grade, indicating a poor response to CCRT. The possible reasons for this association are as follows. First, PCSK1 plays an important role in the regulation of cytokine secretion and, consequently, is also a regulator of the innate immune response. After irradiation, macrophages receive cellular death signals, leading to the recruitment of more immune cells, including monocytes, which differentiate into macrophages at the injury site.²⁸

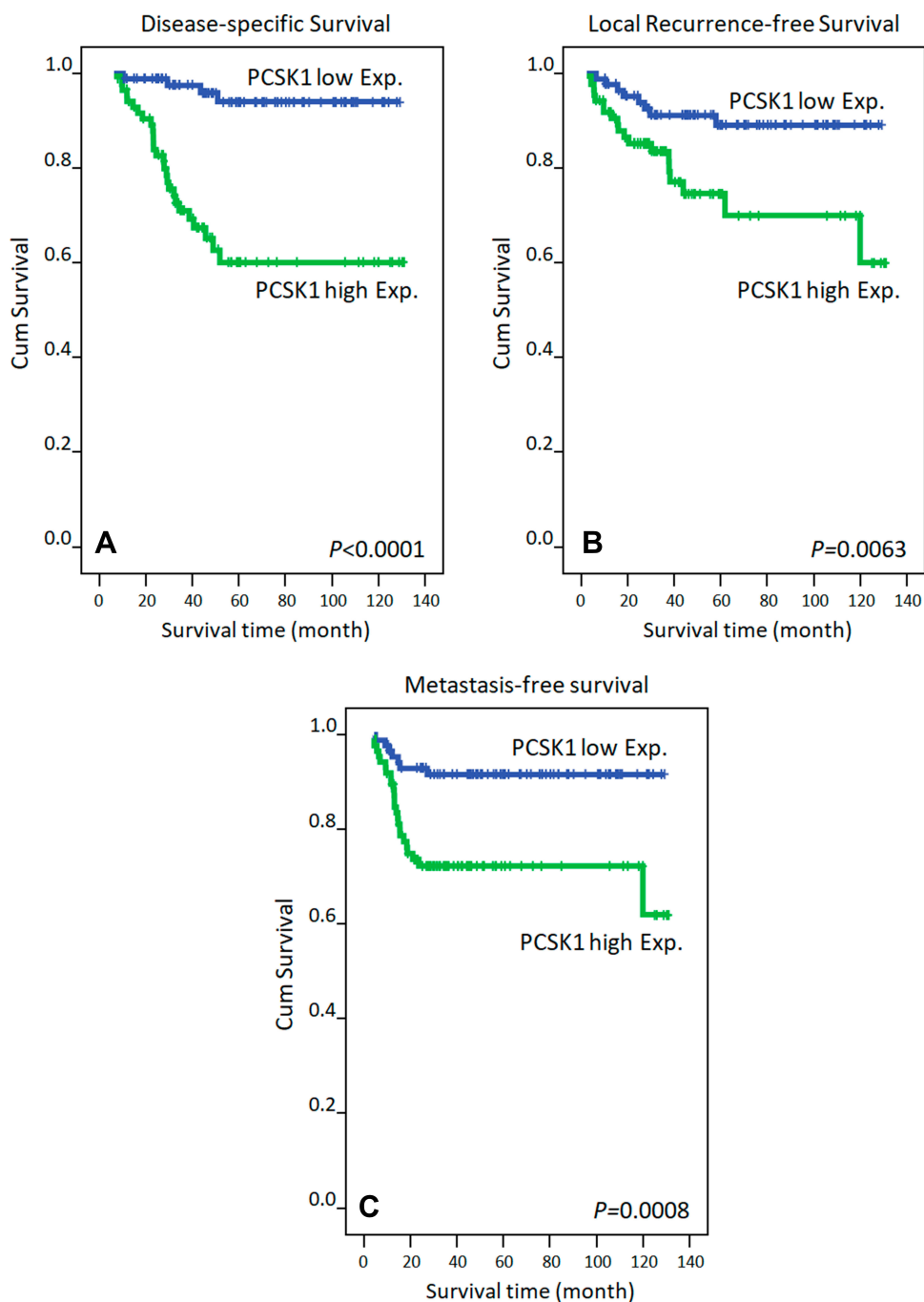


Figure 3 Kaplan–Meier survival curves plotted to predict survival. Using the Log rank test, rectal cancer patients with high expression of PCSK1 had an inferior disease-specific survival (A), local recurrence-free survival (B) and metastasis-free survival (C).

With aberrant PCSK1 expression, macrophage activation would be altered, decreasing the production of profibrotic growth factors and anti-inflammatory cytokines, such as TGF β 1, resulting in a poor response to radiation.²⁹ Second, PCSK1 expression is associated with obesity in humans.^{8,30} Obesity with rectal cancer not only increases the local recurrence rate decreases the possibility of sphincter preservation but also is associated with

inflammation, angiogenesis, and chronic oxidative stress, which may affect the response to radiotherapy.^{31–34} Lee et al recently reported obesity as an independent negative predictive factor for complete response to CCRT in rectal cancer.^{35,36} As we known, tumor response could reflect early response to treatments and related to late response outcomes including recurrence and survival. Many studies also indicate that obesity is a common

Table 4 Multivariate Survival Analysis

Parameters	DSS			LRFS			MeFS		
	HR	95% CI	p-value	HR	95% CI	p-value	HR	95% CI	p-value
Tumor Regression Grade	1.838	0.914–3.690	0.088	2.433	1.120–5.291	0.025*	2.242	1.114–4.525	0.024*
PCSK1 Expression	5.478	1.789–16.774	0.003*	1.299	0.634–4.276	0.594	2.395	0.965–5.945	0.060
Vascular Invasion	1.861	0.737–4.694	0.189	2.427	0.877–6.716	0.088	–	–	–
Post-Tx Tumor Status (Post-T)	1.689	0.693–4.117	0.249	1.924	0.758–4.880	0.168	1.677	0.699–4.024	0.247
Pre-Tx Nodal Status (Pre-N)	–	–	–	1.905	0.793–4.576	0.150	–	–	–

Note: *Statistically significant.

Abbreviations: DSS, disease-specific survival; LRFS, local recurrence-free survival; MeFS, metastasis-free survival; HR, hazard ratio; CI, confidence interval.

risk factor for cardiovascular disease and is an indicator of poor prognosis in multiple cancer types.^{37,38} Thus, the overexpression of PCSK1 in rectal cancers could be used to identify patients at increased risk of poor response and survival after preoperative CCRT.

The current study has several limitations. First, the number of patients was relatively small; thus, the findings should be verified by larger-scale studies. Second, our study cohort included some early-stage rectal cancer patients because they were undergoing preoperative CCRT with the intention of organ preservation. Further studies in a patient cohort limited to locally advanced rectal cancer should be performed.

Conclusion

In summary, the results of this study indicate that the determination of PCSK1 overexpression may help identify and stratify high-risk patients after preoperative CCRT. The strong inverse correlation observed between TRG and DSS suggests that PCSK1 may be a potential prognostic biomarker and that PCSK1 inhibition may be a novel therapeutic strategy for rectal cancer patients.

Abbreviations

CCRT, concurrent chemoradiotherapy; PCSK1, proprotein convertase subtilisin/kexin-type 1; AJCC, American Joint Committee on Cancer; TRG, tumor regression grade; DSS, disease-specific survival; LRFS, local-recurrence-free survival; MeFS, metastases-free survival; HR, hazard ratio; CI, confidence interval.

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Disclosure

The authors have no declarations of interest to report in this work.

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