Clinicopathological profiling of small heterodimer partner, as a negative regulator of inflammatory responses in rectal cancer patients

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Abstract

Background: Small heterodimer partner (SHP) is an orphan nuclear receptor family member that plays an essential role in the regulation of innate immune and inflammatory responses. However, the expression of SHP in rectal cancer and its prognostic significance have not been investigated. The aim of this study was to identify whether SHP levels are associated with cancer-related inflammation, treatment response and prognosis in rectal cancer patients. **Methods:** First, we performed classic gene set enrichment analysis (GSEA) to detect the association between high and low SHP expression using TCGA data sets. Then, the correlation between the expression of SHP and the prognosis of rectal cancer patients was evaluated using online OncoLnc database. Finally, we performed immunohistochemistry and analysed the correlation between SHP expression and clinicopathological / haematologic features or treatment response of rectal cancer patients treated with radiochemotherapy. **Results:** Bioinformatics analysis indicated that low SHP mRNA expression was significantly associated with inflammatory response (NES = -1.67507) and poor prognosis ($p = 0.0319$). Nuclear expression of SHP was associated with cN stage ($p = 0.003$), Neutrophil ($p = 0.023$), Lymphocyte ($p = 0.024$), NLR ($p = 0.023$) and complete pathologic response after radiochemotherapy ($p = 0.031$). Low nuclear expression of SHP was associated with poor overall and distant metastasis-free survival for rectal cancer ($p = 0.029$ and $p = 0.008$) and retained significance as an independent prognostic factor for rectal cancer ($p = 0.087$ and $p = 0.037$). **Conclusions:** These results indicate that SHP may act as an antiinflammatory mediator by regulating systemic and local immune responses in rectal cancer. Moreover, SHP might be used a candidate markers or therapeutic target in rectal cancer.

Background

The prognosis of locally advanced rectal cancer depends largely on the tumour stage at diagnosis. In the preoperative setting, post-treatment TNM stage and pathological complete response to neoadjuvant treatment have been reported to be correlated with disease-free survival [1]. However, these factors cannot be determined before surgery. Predictors of tumour response and long-term outcome that are identifiable before surgery are necessary for designing a customized management plan for each patient to increase the percentage of cure. It is therefore important to identify additional biomarkers to predict and select patients who will respond favourably to therapy. In addition to improving outcomes, such studies may also provide novel insight into the molecular mechanism of LARC.

Small heterodimer partner (SHP; also known as NR0B2) is an orphan member of the nuclear receptor (NR) superfamily and contains a putative ligand-binding domain without the classical DNA-binding domain [2, 3]. SHP has been implicated in binding specific activating molecules through ligand-binding domains (LBDs)- and interacting with other coactivators and corepressors to mediate transcriptional regulation. Due to this ability, SHP signaling dysfunction leads to a wide variety of metabolic, reproductive and proliferative disorders [4, 5]. The role of SHP in cancer has been extensively studied in liver and breast cancer [6]. In liver cancer, previous studies have reported that SHP exhibits potent tumour suppressive activity by inhibiting cellular growth and increasing the sensitivity of tumour cells to

apoptotic stimuli [7-9]. In addition, a study of in SHP -/- mice suggested that SHP plays a critical role in tumour suppression by repressing the transcription of cell proliferation related genes [8]. Furthermore, another mechanism may be that SHP-induced inhibition of ER α results in the interruption of notch3 signalling through the activation of miR-206 [10]. In breast cancer, it has been consistently reported that there are close associations between SHP and estrogen-related signalling [11-13]. SHP blocks estrogen action by inhibiting ER-mediated transcriptional activation, and enhancing PPAR α which is effective inhibitors on aromatase expression [11, 12, 14, 15]. Recently, induction of the FXR-SHP-LRH1 pathway has been identified as a potential new therapeutic approach to repress tumour growth and induce apoptosis in breast cancer [16]

The role of inflammation in cancer is well established. Cancer-related inflammation can increase the risk of cancer and impact the progression and treatment responses of patients with various cancers, including colorectal, prostate, bladder and other cancer. A previous meta-analysis reported that markers of the systemic inflammatory response, such as the C-reactive protein level, NLR, LMR and PLR could be useful in predicting treatment response and monitoring progression in colorectal cancer patients. Additionally, local inflammatory markers such as the Tumour-to-stroma ratio, Klintrup-Makinen score and Galon immunoscore can predict the prognosis of colorectal cancer patients [17, 18]. Therefore, there is a growing interest that potential biochemical mediators of linking systemic and tumour inflammatory responses. Recently, SHP has been shown to plays an intricate role in preventing excessive inflammation by regulating the innate immune system. However, the role of SHP expression in cancer-related inflammation and the clinical outcome of rectal cancer is not investigated yet. In this study, we first investigated SHP expression and its correlation with systemic inflammatory markers, treatment response and survival in rectal cancer patients using combined methods of bioinformatics and immunohistochemistry.

Methods

Bioinformatics analysis

GSEA was performed as previously described [19]. Briefly the mRNA-Seq profiles (illuminahisep_rnaseqv2-RSEM_genes_normalized) and clinical data of rectum adenocarcinoma patients were obtained from Firehose (<https://gdac.broadinstitute.org/>). TCGA RNA-Seq data were cross-referenced with the clinical information recorded for the patients. Patients with missing clinical/expression values were excluded from further analyses. Data from 95 samples were included in the study. The mRNA-Seq data were normalized using the Rank Normalize module in GenePattern (<http://broadinstitute.org/cancer/software/genepattern>). Phenotype labels, defined as SHP with high 30% or low 70% expression according to the mRNA expression of the SHP gene, were determined. Phenotype labels were permuted 1,000 times and the normalised $p \leq 0.05$ and false discovery rate (FDR) ≤ 0.25 were selected as statistically significant enrichments.

The OncoLnc database (<http://www.oncolnc.org/>) was used to determine whether the expression of SHP was correlated with the overall survival of rectal adenocarcinoma. We specifically queried SHP gene expression and plotted kaplan with “READ (rectal adenocarcinoma)”.

Patients and pretreatment evaluation

Between March 2003 and December 2011, 89 LARC patients who had been treated with preoperative radiochemotherapy (RCT) at Chungnam National University Hospital, Daejeon, Republic of Korea, with the available pre-treatment tissue blocks were enrolled in this study. Eligibility for the study was determined based on the following criteria: (1) histologic proof of rectal adenocarcinoma, (2) tumour extension through the bowel wall (T3–T4) or pelvic lymph node involvement without evidence of distant metastasis, and (3) presence of a resectable tumour. Patient and tumour characteristics are listed in Table 1. Essential pre-treatment workups included a complete history, physical examination, complete blood count, serum chemistry, carcinoembryonic antigen (CEA) level analysis, chest radiography, abdominal/pelvic computed tomography (CT), and colonoscopy with biopsy. The pretreatment clinical TNM stage was determined mainly by CT imaging. This study was approved by the institutional review board of Chungnam National University Hospital (IRB No. 2017-07-037).

Radiotherapy treatment and evaluation of tumour response

Radiation was delivered via 6- and 10-MV photons using a three-field technique (posterior and laterals) in most patients. Treatment was planned via computerized dosimetry, and a dose of 1.8 Gy per fraction was prescribed to cover the planning target volume. Radiotherapy was administered 5 days per week, once per day, at 1.8 Gy/d. Pelvic radiotherapy consisted of 45 Gy in 25 fractions over a period of 5 weeks, which was followed by a boost dose of 5.4 Gy administered in three fractions to the primary tumour using two lateral fields. The clinical target volume contained the primary tumour, the mesorectum, the presacral space, and lymph nodes, including the perirectal, presacral, internal iliac, and/or external iliac nodes as indicated. For the whole pelvic field, the superior border was located at the L5–S1 interspace, and the inferior border was located 3 to 4 cm below the primary tumour. The lateral border was located 1.5 cm outside of the true bony pelvis. For the lateral fields, the posterior margin was 1.5 cm behind the anterior bony sacral margin, and the anterior border generally comprised the anterior acetabulum. Preoperative chemotherapy was administered concurrently with radiation therapy. Patients received oral chemotherapy consisting of two cycles of capecitabine and leucovorin according to our institutional chemotherapy protocol. Approximately 6 weeks after the completion of RCT the patients underwent definitive surgery. Surgical management included a sphincter-preservation approach whenever possible through the total mesorectal excision technique. Pathologic evaluation of surgical specimens, including the primary tumour and resected nodes, was performed by a specialist pathologist. The complete absence of residual tumour cells in the primary tumor was designated pathologic complete response.

Immunohistochemistry

The expression of SHP was analysed by immunohistochemistry on paraffin-embedded tissue sections from rectal cancer patients. Sections from paraffin blocks with a thickness of 3 mm were used for immunohistochemistry. A polyclonal rabbit antibody directed against NR0B2 (ab186874, Abcam, Cambridge, MA) was diluted 1:400 with a background-reducing diluent (Dako, Carpinteria, CA), and tissue sections were incubated in the mixture overnight at 4 °C. Without any access to clinical information, two experienced pathologists (MKY and JMK) examined slides and assigned scores. The immunostaining of the tumour was divided into four grades based on the staining intensity and scored: score 0, no staining; score 1, weak; score 2, intermediate; and score 3, strong. In the cases of heterogeneous staining within samples, the higher score was chosen if >50% of the cells showed a higher staining intensity. Cases with no staining and a score of 1 were categorized into a low-expression group (LEG), whereas those with a score of 2 and 3 were categorized into a high-expression group (HEG).

Statistical analysis

Relationships between clinicopathologic / haematologic factors and SHP levels were analysed by use of the chisquare test, Survival curves were determined with the Kaplan-Meier method. The prognostic value of SHP expression was evaluated using the log-rank test for univariate analyses and the Cox proportional hazards model for multivariate analyses. A backward stepwise selection of covariates was used for the Cox proportional hazards model, and $p < 0.1$ was defined as the threshold for covariate inclusion. We considered $p < 0.05$ to indicate statistical significance. All statistical analyses were conducted using PASW statistics software (version 17.0; SPSS, Chicago, IL)

Results

Bioinformatics analysis of SHP expression in rectal adenocarcinoma

Numerous published studies have shown that SHP prevents or controls acute inflammatory responses in innate immune cells [20-23]. These studies led us to hypothesize that SHP may play a role in regulating cancer-associated inflammation. We reasoned that if this hypothesis is correct, low SHP expression should block an adequate immune response in tumours. Therefore, to test this hypothesis, we performed GSEA comparing the high and low SHP mRNA expression group for hallmarks using TCGA mRNA-Seq data (Fig 1). GSEA revealed a significant difference ($FDR < 0.25$, $NOM\ P\text{-value} < 0.05$) in the enrichment of MSigDB Collection (h.all.v6.2.symbols.gmt) and details are shown in Fig. 1a. Fig. 1a shows that inflammatory responses ($NES = -1.67507$), NOTCH ($NES = -1.70074$), IL2-STAT5 ($NES = -1.58612$) and KRAS signaling ($NES = -1.5011$) are differentially enriched in the SHP low-expression group.

Our GSEA results suggest that several cancer progression-related pathways are upregulated in the low SHP expression group of rectal adenocarcinoma patients. Therefore, the OncoLnc database was used to investigate whether the mRNA expression level of SHP was associated with the prognosis of rectal adenocarcinoma patients. We analysed the data from the OncoLnc database which has gene expression data and clinical information for colorectal cancer patients. Rectal cancer patients with low SHP mRNA expression levels had a worse prognosis than those with high SHP mRNA level ($P = 0.0319$) (Fig. 1b).

Association between SHP expression and patient characteristics

Because both GSEA and survival analysis rely on the expression profile of mRNA instead of proteins, we examined the SHP protein level in clinical samples derived from rectal adenocarcinoma patients treated with RCT. Immunohistochemistry showed that SHP was expressed mainly in the nuclei but also in the cytoplasm. Representative SHP staining results are shown in Fig 2. Based on SHP expression, 37 of 89 patients with rectal adenocarcinoma expressed SHP in tumour tissues, with a high expression rate of 41.6%, whereas 53 patients could be categorized into the LEG (Table 1).

The clinicopathological characteristics of the 89 rectal adenocarcinoma patients associated with SHP expression are presented in Table 1. The median age of the patients was 62 years (range, 33–81 years); low SHP expression was positively correlated with cN stage (N (-) vs N (+)) ($P = 0.003$). No significant correlation was found between SHP expression and other clinicopathologic variables, including age, sex, tumour distance from anal verge and cT stage.

Because the aforementioned GSEA results show that SHP expression is negatively correlated with inflammatory responses, we estimated the relationship between the SHP protein expression and systemic inflammatory markers. The correlation between SHP expression and haematologic characteristics of patients are depicted in Table 2. Among the various inflammatory markers, SHP negativity was associated with hematologic parameters, including neutrophil counts ($p=0.023$), lymphocyte counts ($p=0.024$) and NLR values ($p=0.023$). In addition, no association was detected between SHP and other factors including platelet counts and PLR values..

Association of SHP expression with pathologic complete response to radiochemotherapy

We then focused on correlations between treatment response and patient characteristics using a chi-squared test. After preoperative RCT, a pathologic complete response (pCR) was observed in 19 patients (21.1%). The association between pCR and patient characteristics including SHP expression and clinical/haematologic factors are listed in table 3. Nuclear expression of SHP tended to be significantly higher in the pCR patients than in those without pCR (13.2 vs 32.4%, $p = 0.028$). Additionally, a significant association was detected between pathologic tumour response and other factors including the NLR (33.3 vs 8.9%, $p=0.005$) and PLR (33.3 vs 8.9%, $p=0.005$). No significant correlation was found between pCR and age, sex, tumour distance from the anal verge, cT or cN stage, neutrophil counts, lymphocyte counts and platelet counts.

Association of SHP expression with survival

The median follow-up time was 54.0 months (range, 16 to 88 months) for all the patients and 58.5 months (range, 16 to 88 months) for the surviving patients. Local and distant failure were observed 13 (14.4%) and 24 (26.7%) cases, respectively. The 5-year overall survival, and distant metastasis-free survival rates were 81.3% and 73.3%, respectively (Fig. 3a).

Because SHP expression was significantly associated with overall survival and distant metastasis-free survival, prognostic factors were analysed for the effect on overall survival and distant metastasis-free survival. Prognostic factors were analysed for the effect on overall survival and distant metastasis-free survival. Table 4 shows the associations of potential prognostic factors with overall survival outcomes assessed by univariate and multivariate analyses. In the univariate analysis, pCR and SHP expression were significantly associated with overall survival. Moreover, 53 patients with low nuclear SHP expression exhibited poorer overall survival rates than 37 patients with high nuclear expression (72.3% vs. 94.4%) (Fig. 3b). Furthermore, the CEA level before RCT and the NLR were marginally significant prognostic factors for overall survival. Variables with $p < 0.1$ based on the univariate analysis were entered into a Cox proportional hazards model for multivariate analysis of overall survival. Multivariate analysis confirmed that SHP and CEA level before RCT were marginally independent prognostic factors for OS. Many parameters were associated with distant metastasis-free survival (Table 5). In the univariate analysis, pCR and SHP expression were significant prognostic factors for distant metastasis-free survival. In the multivariate analysis, SHP expression (hazard ratio = 0.315, 95% CI = 0.107–0.932; $p = 0.037$) remained significant.

As shown in Table 2, there were significant correlations between SHP protein expression and various haematologic parameters. Among the correlated haematologic parameters, NLR was a significant predictor of pCR and OS. Therefore, 89 cases with rectal adenocarcinoma were classified into 3 groups according to SHP expression and the NLR, as follows: Group 1 had high SHP expression and low NLR ($n=24$); group 2 had high SHP expression/NLR or low SHP expression/NLR ($n=31$); group 3 had low SHP and high NLR ($n=21$). Kaplan–Meier analysis (Fig. 4) indicated that patients with a high NLR and a low SHP expression had the shortest overall and distant metastasis free-survival and patients with a low NLR and a high SHP expression had the longest survival ($P=0.009$ and $P=0.021$, respectively, Fig. 4).

Discussion

In this study, we first used a data-driven approach to investigate the role of SHP in rectal adenocarcinoma using GSEA. Our GSEA results revealed the potential underlying mechanism; low SHP mRNA expression in rectal adenocarcinoma was associated with the upregulation of inflammatory responses, NOTCH, IL2-STAT5 and KRAS signaling. Because these signalling pathways are related to treatment resistance and poor prognosis, we also checked the clinical significance of SHP expression in rectal cancer by using the OncoLnc database (<http://www.oncolnc.org/>). Kaplan-Meier survival curves showed that low SHP mRNA expression was a poor prognostic factor in rectal adenocarcinoma. We then investigated the prognostic significance of SHP expression in LARC using IHC data in our hospital. Similarly, IHC data and clinical data from our hospital confirmed the results from public databases, suggesting that SHP expression is a favourable prognostic factor in rectal cancer. Furthermore, our results showed that nuclear SHP expression is associated with pCR and DMFS in LARC patients treated with RCT. These findings suggest that nuclear expression of SHP may be used as an indicator for adverse prognosis for LARC patients who receive preoperative RCT. To date, no clinical studies have evaluated the prognostic role of SHP in rectal

cancer. To the best of our knowledge, this study is the first to show the predictive and prognostic significance of SHP expression in LARC.

Another important finding of this study is that SHP expression is correlated with systemic inflammatory markers in rectal cancer patients. Our results show that there are significant associations between SHP expression levels and neutrophil counts, lymphocyte counts and NLR. These results reveal the potential regulatory role of SHP in local and systemic inflammation of rectal cancer patients. In addition, our results indicated that rectal cancer patients who have a high NLR in blood samples and low SHP expression status in tumour tissues had extremely poor 5-year OS and DMFS.

Our previous studies have demonstrated the importance of SHP in the regulation of the innate immune response and inflammatory response against pathogen invasion [20-23]. SHP regulates innate immunity in two ways. First, SHP has a dual regulatory functions in the canonical transcription factor NF- κ B signalling pathway, acting as both a repressor of transactivation of the NF- κ B subunit p65 and an inhibitor of the polyubiquitination of the adaptor TRAF6 [20]. These results indicate that the orphan nuclear receptor SHP acts as a negative regulator in inflammatory signalling triggered by Toll-like receptors. Second, SHP interacts with the NLRP3 and negatively regulates activation of the NLRP3 inflammasome [22]. SHP deficiency results in an increased secretion of the proinflammatory cytokines IL-1 β and IL-18, and excessive pathologic responses typically observed in mouse models of kidney tubular necrosis and peritoneal gout [22].

Thus far, Cancer-associated local and systemic inflammation has been identified as a key player in tumour invasion and metastasis [18, 24, 25]. It has been demonstrated that several biomarkers and haematologic indices are representative of the local immune response, including tumour necrosis, inflammasomes, cytokines, chemokines, and transcription factors, and systemic inflammatory markers, such as acute-phase proteins and circulating immune cells [18]. Among these, the NLR, PLR, LMR, albumin level and CRP level in cancer patients are frequently employed prognostic factors for their ease of use in clinical practice. A systemic review and meta-analysis demonstrated that an elevated pre-treatment NLR predicts poor OS (HR: 1.813, 95% CI: 1.499–2.193) and PFS (HR: 2.102, 95% CI: 1.554–2.843) in patients with CRC [26].

In particular, previous studies have reported that inflammation plays a key role in treatment failure following radiotherapy. In colorectal cancer, the NLR and PLR are associated with pCR or primary tumour downstaging after preoperative RCT [27-30]. Similarly, our results showed that haematologic parameters including NLR and PLR are significant predictors of pCR after preoperative RCT for rectal cancer. Due to the detrimental effect of cancer-related inflammation on radiotherapy responses, there is substantial interest in therapeutic strategies manipulating the inflammatory response. Thus, there is growing interest in novel approaches for targeting cancer-related inflammatory pathways in combination with radiation therapy. A large variety of natural and synthetic compounds have been reported to interfere with cancer-related inflammation thorough regulating various molecular pathways including NF- κ B, STAT3, HIF-1, and PGHS-2, and are regarded as putative radiosensitizing agents [31].

Many studies over the past 20 years have shown that GW4064, androsterone, bile acids (BA) and chenodeoxycholic acid (CDCA), AHPN, 3-Cl-AHPC and metformin are potent inducers of SHP gene expression [6, 32, 33]. Of these, a few SHP ligands showed anticancer effects by promoting upregulating the apoptotic pathway [6, 34]. For example, GW4064 inhibits aromatase activation and causes apoptosis in breast cancer[6]. A recent interesting study reported that AHPN and 3-Cl-AHPC induce apoptosis by promoting SHP expression [6]. In contrast to the aforementioned compounds, metformin, a biguanide oral anti-diabetic agent, is involved in various aspects of cancer such as cell growth, cell death, therapy response and inflammation by regulating metabolic reprogramming [35-37]. Metformin, with regard to cancer-related inflammation has the ability to block Src-mediated-nuclear factor kappa B (NF- κ B) signalling pathways [38]. This ability is especially associated with the prophylactic effect of metformin on cancer progression. Furthermore, accumulating evidence indicates that metformin can modify radiotherapy response by specifically regulating underlying mechanisms including DNA damage/repair response and ROS generation [39]. Additionally, experimental studies reported that metformin enhances tumour response to radiotherapy in experimental models and clinical studies have shown that metformin use was associated with treatment outcomes in cancer patients treated with radiotherapy [39]. However,, there are no studies investigating the anti-inflammatory ability of metformin in tumour responses to radiotherapy. Our results indirectly explain possible radiosensitizing mechanisms by which metformin-induced SHP induction may attenuate the proinflammatory response in cancer patients.

In conclusion, this is a pilot study to assess the role of SHP expression in rectal cancer. Our immunohistochemical results suggested that the expression level of SHP is associated with systemic inflammation, treatment outcome and prognosis in LARC. The enrichment and survival data from bioinformatics analysis support our results that the low expression of SHP was connected with cancer-related inflammation and poor prognosis. Therefore, these findings provide valuable insights for identifying potential therapeutic targets and promising prognostic markers in rectal cancer.

Abbreviations

SHP: Small heterodimer partner; **READ:** rectum adenocarcinoma; **TCGA:** The Cancer Genome Atlas; **GSEA:** gene set enrichment analysis; **RCT:** radiochemotherapy **pCR:** pathologic complete response

Declarations

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Authors' contributions

SK, EKJ and JMK designed the study. SK and MNJ collected the data. SK and JMK analysed the data. SK, EKJ and JMK organized the manuscript. SK, MKY, MJC, JSK, EKJ and JMK reviewed the papers and revised the manuscript. All the authors have read and approved the final manuscript. All authors

contributed to data analysis, drafting of the paper and manuscript revisions and agree to be accountable for all aspects of the work.

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Availability of data and materials

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

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Chungnam National University Hospital. Written informed consent was obtained from all patients.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1. Association of SHP with clinicopathological features in locally advanced rectal cancer patients.

Characteristics	Total No.(%)	SHP expression		
		Low (%)	High (%)	<i>P</i>
		52 (100)	37 (100.0)	
Age (y)				0.755
<60	57 (64.0)	34 (65.4)	23 (62.2)	
≥60	32 (36.0)	18 (34.6)	14 (37.8)	
Sex				0.636
Male	65 (73.0)	37 (71.2)	28 (75.7)	
Female	24 (27.0)	15 (28.8)	9 (24.3)	
Tumor distance from anal verge				0.737
<6cm	58 (65.2)	33 (63.5)	25 (67.6)	
≥6cm	31 (34.8)	19 (36.5)	12 (32.4)	
CEA before RCT				0.689
≤5 ng/mL	58 (65.2)	33 (63.5)	25 (67.6)	
>5 ng/mL	31 (34.8)	19 (36.5)	12 (32.4)	
cT stage				0.143
T2-3	76 (85.4)	42 (80.8)	34 (91.9)	
T4	13 (14.6)	10 (19.2)	3 (8.1)	
cN stage				0.003
N (-)	6 (6.7)	0 (0.0)	6 (16.2)	
N (+)	83(93.3)	53 (100.0)	31 (83.8)	

Table 2. Association of SHP with hematologic parameters in locally advanced rectal cancer patients.

Characteristics	Total No.(%)	SHP expression		
		Low (%)	High (%)	<i>P</i>
		52 (100)	37 (100.0)	
Neutrophil				0.023
Below the median	45(50.6)	21 (40.4)	24 (64.9)	
Above the median	44(49.4)	31 (59.6)	13 (35.1)	
Lymphocyte				0.024
Below the median	44(49.4)	23 (44.2)	21 (56.8)	
Above the median	45(50.6)	29 (55.8)	16 (43.2)	
Platelet				0.761
Below the median	45(50.6)	27 (51.9)	18 (48.6)	
Above the median	44(49.4)	25 (48.1)	19 (51.4)	
NLR				0.023
Below the median	45(50.6)	21 (40.4)	24 (64.9)	
Above the median	44(49.4)	31 (59.6)	13 (35.1)	
PLR				0.900
Below the median	45(50.6)	26 (50.0)	19 (51.4)	
Above the median	44(49.4)	26 (50.)	18 (48.6)	

Table 3. Analysis of predictive factors associated with pathologic complete response.

Characteristics	Pathologic complete response		
	[No. of patients (%)]		
	No	Yes	P
	70	19	
Age (y)			0.654
<60 vs. ≥60	44 (62.9) vs. 26 (37.1)	13 (68.4) vs. 6 (31.6)	
Sex			0.513
Male vs. Female	50 (71.4) vs. 20 (28.6)	15 (78.9) vs. 4 (21.1)	
Tumor distance from anal verge			0.453
<6cm vs. ≥6cm	47 (67.1) vs. 23 (32.9)	11 (57.9) vs. 8 (42.1)	
CEA before RCT			0.380
≤5 ng/mL vs. >5 ng/mL	44 (62.9) vs. 26 (37.1)	14 (73.7) vs. 5 (26.3)	
cT stage			0.570
T2-3 vs. T4	59 (84.3) vs. 11 (15.7)	17 (89.5) vs. 2 (10.5)	
cN stage			0.772
N (-) vs. N (+)	5 (7.1) vs. 65 (92.9)	1 (5.3) vs. 18 (94.7)	
SHP			0.031
Low vs. High	45 (64.3) vs. 25 (35.7)	7 (36.8) vs. 12 (63.2)	
Neutrophil			0.471
Below vs. Above the median	34 (48.6) vs. 36 (51.4)	11 (57.9) vs. 8 (42.1)	
Lymphocyte			0.216
Below vs. Above the median	37 (52.9) vs. 33 (47.1)	7 (36.8) vs. 12 (63.2)	
Platelet			0.471
Below vs. Above the median	34 (48.6) vs. 36 (51.4)	11 (57.9) vs. 8 (42.1)	
NLR			0.005
Below vs. Above the median	30 (42.9) vs. 40 (57.1)	15 (78.9) vs. 4 (21.1)	
PLR			0.005
Below vs. Above the median	30 (42.9) vs. 40 (57.1)	15 (78.9) vs. 4 (21.1)	

Table 4. Prognostic factor analysis for overall survival.

Prognostic factor	5-year overall survival rate (%)	p-value	
		Univariate analysis	Multivariate analysis
Age (y)		0.266	
<60 vs. ≥60	77.8 vs. 90.4		
Sex		0.480	
Male vs. Female	82.3 vs. 81.5		
Tumor distance from anal verge		0.510	
<6cm vs. ≥6cm	83.3 vs. 80.3		
CEA before CRT		0.053	0.093
≤5 ng/mL vs. >5 ng/mL	87.3 vs. 72.8		
cT stage		0.673	
T2-3 vs. T4	82.0 vs. 80.9		
cN stage		0.217	
N (-) vs. N (+)	100.0 vs. 79.9		
Pathologic complete response		0.025	0.957
no vs. yes	77.4 vs. 100		
SHP		0.029	0.087
Low vs. High	73.7 vs. 94.4		
Neutrophil	-	0.686	-
Below vs. Above the median	83.3 vs. 81.2	-	-
Lymphocyte		0.543	
Below vs. Above the median	81.6 vs. 82.6		
NLR		0.059	0.357
Below vs. Above the median	87.4 vs. 77.1		
Platelet		0.247	
Below vs. Above the median	79.0 vs. 85.6		
PLR		0.838	
Below vs. Above the median	80.8 vs. 83.5		

Table 5. Prognostic factor analysis for distant metastasis-free survival.

Prognostic factor	5-year distant metastasis-free survival rate (%)	p-value	
		<i>Univariate analysis</i>	<i>Multivariate analysis</i>
Age (y)		0.257	
<60 vs. ≥60	70.6 vs. 80.4		
Sex		0.950	
Male vs. Female	74.8 vs. 70.8		
Tumor distance from anal verge		0.355	
<6cm vs. ≥6cm	76.9 vs. 69.2		
cT stage		0.821	
T2-3 vs. T4	72.3 vs. 70.5		
cN stage		0.542	
N (-) vs. N (+)	83.3 vs. 73.3		
CEA before CRT		0.438	
≤5 ng/mL vs. >5 ng/mL	76.5 vs. 69.9		
Pathologic complete response		0.024	0.099
No vs. Yes	68.3 vs. 94.7		
SHP		0.008	0.037
Low vs. High	63.4 vs. 89.0		
Neutrophil		0.661	
Below vs. Above the median	71.9 vs. 75.8		
Lymphocyte		0.821	
Below vs. Above the median	74.1 vs. 74.1		
NLR		0.348	
Below vs. Above the median	78.5 vs. 69.3		
Platelet		0.735	
Below vs. Above the median	75.6 vs. 72.3		
PLR		0.794	
Below vs. Above the median	76.8 vs. 71.7		

Figures

A

B

Figure 1

Prognostic role of SHP mRNA expression in rectal adenocarcinoma. (A) GSEA enrichment analysis of coexpression genes. (B) Kaplan-Meier plots from OncoLnc database (<http://www.oncolnc.org/>) were used to assess correlations between SHP gene expression and patient survival of rectal adenocarcinoma.

Figure 2

Representative photomicrographs of SHP immunohistochemical staining in human rectal cancer tissues. (A) No staining intensity. (B) Weak staining intensity. (C) Moderate staining intensity. (D) Strong staining intensity (original magnification $\times 400$).

Figure 3

Survival curves for patients with locally advanced rectal cancer (LARC) according to SHP expression. (A) OS and DMFS for the entire patient cohort. (B) OS and DMFS curves for the patients with LARC as stratified by SHP expression. Patients with low SHP expression had shorter overall and distant metastasis-free survival times than those with high SHP expression

Figure 4

Survival curves of patients with high SHP and low NLR (n=24), high SHP/NLR or low SHP/NLR (n=31) and low SHP and high NLR (n=21). Patients with low SHP and high NLR had poorest overall and distant

metastasis-free survival times than two other groups