High expression of SHP2 predicts a promising prognosis in colorectal cancer

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Research Article

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Abstract

Background
Src homology 2 domain-containing phosphatase 2 (SHP2) is hyper-activated in some solid tumors. Previous findings suggest that the expression of SHP2 in colorectal cancer (CRC) may be associated with prognosis. However, validation with large sample data is lacking.

Methods
Tissue microarrays containing 860 CRCs and 197 mucosal tissues adjacent to the tumors were constructed. Immunohistochemistry was used to evaluate the expression of SHP2. Differences between SHP2 expression and clinicopathological parameters were evaluated. Kaplan-Meier survival curves and log-rank tests were used to analyze the relationships between SHP2 expression and overall survival of patients. A Cox proportional hazard regression model was used for univariate and multivariate analyses of prognostic factors.

Results
SHP2 expression levels in CRCs tissues were significantly higher than those in adjacent mucosal tissues ($P < 0.001$). SHP2 expression was related to tumor differentiation, depth of invasion, distant metastasis, vascular tumor thrombus, lymph node metastasis, and TNM classification ($P < 0.05$). The prognosis of the high expression group of SHP2 was significantly better than that of the low expression group ($P = 0.008$). Univariate analysis showed that the expression level of SHP2 was a prognostic factor for CRC ($P = 0.008$). Multivariate analysis demonstrated that SHP2 remained an independent prognostic factor for CRC ($P = 0.033$).

Conclusion
The expression levels of SHP2 were significantly higher in CRC tissues than in adjacent normal tissues. High expression levels of SHP2 were associated with a promising outcome, suggesting that SHP2 may be a favorable prognostic indicator of CRC.

Introduction
Colorectal cancer (CRC) is one of the most common and fatal malignancies worldwide. It is the fourth deadliest cancer in the world, killing nearly 900,000 people annually \(^1\). In recent years, the incidence and mortality of CRC has been continually increasing in China \(^2\), with an age-standardized cancer mortality rate of 12 per 100,000 people \(^3\). Despite great strides in the diagnosis and adjuvant treatment of CRC, the
overall survival for patients with CRC has not significantly improved. Therefore, a better understanding of the pathogenesis of CRC is urgently required to develop more effective treatment strategies. Identifying novel molecular biomarkers to predict the prognosis of patients with CRC can provide important information for clinical management.

Tyrosine phosphorylation plays a critical role in the manipulation of cellular activities associated with cancer initiation and progression. Cellular signal transduction pathways are regulated by protein tyrosine kinases and protein tyrosine phosphatases (PTP). The PTP Src homology 2 domain-containing phosphatase 2 (SHP2), encoded by PTPN11, is a ubiquitously expressed non-receptor PTP. Aberrant expression of SHP2 can lead to dysregulation of multiple signaling pathways, causing different diseases and tumorigenesis. Acting as both an oncogenic factor and tumor suppressor in different diseases, the conserved catalytic dephosphorylation mechanisms of SHP2 as well as its unique allosteric regulatory mechanisms provide opportunities for the development of SHP2 inhibitors and activators.

In recent years, SHP2 has become a popular topic in the study of tumorigenesis, progression, and treatment strategies. Studies on SHP2 in solid tumors have mainly focused on lymphoma, breast cancer, cervical cancer, gastric cancer, CRC, non-small cell lung cancer, liver cancer, pancreatic ductal adenocarcinoma, and thyroid carcinoma. However, SHP2 plays unequal roles in various types of cancer, which may lead to different therapeutic strategies and interventions. Currently, there are few studies on the prognostic value of SHP2 expression in patients with solid tumors. The prognostic value of SHP2 may vary in different tumors because the prognostic role of SHP2 is strongly influenced by tumor location. In a previous exploratory study, we used several biomarkers to screen the prognostic factors of CRC and found that SHP2 may be related to the prognosis of colorectal cancer. In vitro experiments suggest that SHP2 can inhibit the proliferation and migration of CRC cells. However, data on the clinical significance of SHP2 in CRC appear to be scarce. Therefore, it is necessary for us to further test the previously proposed hypothesis. In the current study, we have increased the sample size to further analyze the clinicopathological significance of SHP2 expression in CRC and confirmed its prognostic value with follow-up data.

**Patients And Methods**

**Patients and specimens**

Surgically resected specimens were collected from the database based on the available tissues of patients with CRC who had undergone radical resection at the Second Affiliated Hospital of Zhejiang University School of Medicine between 2002 and 2011. This study was approved by the Institutional Ethics Committee, and patients signed informed consent when receiving treatment. The patient had not received chemotherapy or radiotherapy before surgery. There were 860 valid cases, including 513 males and 347 females, with an age range of 18–92 years. Normal mucosal tissue specimens were collected from paracancerous mucosal tissues of surgically resected specimens. The presence of tumors was
confirmed in CRC tissue specimens by histopathological examination and then diagnosed by pathologists. According to the 4th edition of the 2010 World Health Organization Classification of Tumors of the Digestive System, the degree of differentiation of the cases was as follows: well, moderate, and poor. Tumor-specific clinicopathological parameters were collected, including patient age and sex, tumor site, maximum diameter, distant metastasis, lymph node metastasis, number of metastatic lymph nodes, differentiation, depth of invasion, blood CEA level, and TNM stage. There were 432 patients with TNM stages 1–2 and 312 patients with 3–4 stages. Some patients with incomplete data were not included in the TNM staging. Because some surviving patients were unable to correctly state whether the disease was still present during telephone follow-up, the overall survival was recorded and follow-up data were up to 106 months.

**Tissue microarrays**

Tissue samples were fixed in 10% formalin, dehydrated in ethanol, and embedded in paraffin wax. Areas representing tumor and normal mucosal tissues were marked on hematoxylin and eosin (H&E)-stained sections. A 1.00 mm diameter tumor tissue core was extracted from a representative tissue block of each sample, and normal mucosal tissue was taken from the paracancerous tissue block and confirmed histologically, and then mounted into a tissue microarray using a manual array device (TMA). In total, 860 CRC tumor tissues and 197 mucosal tissues adjacent to the tumor were evenly distributed in the TMAs. All specimens were sliced continuously into 4µm-thick sections and either stained with H&E or prepared for immunohistochemistry.

**Immunohistochemistry**

Immunohistochemical staining for SHP2 was performed using an Envision immunohistochemistry kit according to the manufacturer’s instructions. The sections were deparaffinized using xylene and rehydrated in decreasing concentrations of ethanol. Following antigen retrieval with citrate buffer, the endogenous peroxidase activity was blocked by incubation with 3% hydrogen peroxide for 10 min. The slides were incubated with rabbit anti-human SHP2 [Y478] (1:200, ab32083, Abcam, Waltham, MA, USA) at 37°C for 1 h and then washed twice with 0.01 mM phosphate buffer saline. After a two-step immunostaining kit standard procedure (Envision kit-0015; Fuzhou Maixin Biological Technology Ltd., Fujian, China), slides were stained with DAB and mounted with neutral balsam.

The immunohistochemistry results were evaluated by two pathologists. When the results were inconsistent, the two pathologists discussed until an agreement was reached. The staining intensity of glandular epithelial and tumor cells was recorded for each case, regardless of the percentage of positive cells. The results were marked as negative, weak, moderate, or strong. Negative and weak staining were regarded as low expression whereas moderate and strong staining were considered high expression.

**Statistics**

Statistical calculations were performed using IBM SPSS ver. 26 (SPSS Inc., Chicago, IL, USA). Chi-square tests were performed for categorical variables in the differences between SHP2 expression levels and
clinicopathological parameters, such as sex and age of patients, tumor location, vascular tumor thrombus, differentiation, depth of invasion, distant metastasis, lymph node metastasis, and TNM stage. The number of metastatic lymph nodes, maximum tumor diameter, and CEA levels were presented in the form of quantitative data. If the quantitative data conformed to normal distribution and homogeneity of variance, the Student’s t-test was used; otherwise, the Mann-Whitney U test was used. Survival curves were calculated according to the Kaplan-Meier method with log-rank tests. A Cox proportional hazard regression model was used for univariate and multivariate analyses of prognostic factors in patients with CRC using the forward stepwise (likelihood ratio) method. All statistical tests were two sided. $P < 0.05$ was considered significant.

Results

Increased SHP2 expression in CRC

To evaluate the prevalence and clinical manifestations of SHP2 in CRC, immunohistochemistry was performed on the TMAs of 860 and 197 mucosal tissues adjacent to the tumor CRC specimens. SHP2 expression was observed in epithelial and tumor cells, as described below. The expression levels of SHP2 in mucosal epithelial tissues and CRCs were divided into four levels according to the staining intensity: negative, weak, moderate, and strong. Representative photomicrographs are shown in Fig. 1. The immunoreactivity of SHP2 expression was graded into two groups: low expression (negative and weak) and high expression (moderate and strong).

The expression feature of SHP2 in mucosal epithelial cells was obviously different from that in cancer cells. SHP2 was positively expressed in the nucleus and perinuclear cytoplasm of mucosal epithelial cells, while the regions near the luminal surface were unstained (Fig. 2A). In contrast, SHP2 showed diffuse and uniform staining in the cytoplasm and nuclei of the tumor cells (Fig. 2B). Stromal cell staining was observed in some cases.

There were significant differences in the expression levels of SHP2 between the tumor tissues and mucosal epithelial tissues adjacent to the tumor. The expression level of SHP2 was significantly higher in cancer tissues (641/860, 74.5%) than that in adjacent mucosal tissues (27/197, 13.7%) ($\chi^2 = 254.999, P < 0.001$). In most cases, the staining intensity of SHP2 was stronger in tumors than in adjacent mucosal tissues, dysplasia, or intraepithelial neoplasia. (Fig. 2C and 2D).

Relationship between SHP2 expression and clinical pathological parameters

We used statistical analyses to assess the potential association between SHP2 expression and tumor characteristics, including sex and age, tumor location, maximum tumor diameter, vascular tumor thrombus, blood CEA level, differentiation, depth of invasion, distant metastasis, lymph node metastasis, number of metastatic lymph nodes, and TNM stage. The results indicated that the expression levels of SHP2 were related to differentiation ($P < 0.001$), depth of invasion ($P = 0.001$), vascular tumor thrombus...
(P = 0.013), lymph node metastasis (P < 0.001), and TNM stage (P < 0.001). However, SHP2 expression levels were not associated with the sex and age of the patients, tumor location, or distant metastasis (Table 1). As the maximum diameter of the tumor, number of lymph node metastases, and CEA levels did not conform to the normal distribution, the Mann-Whitney U test was used. The results suggested that the expression level of SHP2 did not correlate with the maximum diameter of the tumor (P = 0.625), number of metastatic lymph nodes (P = 0.092), or CEA levels (P = 0.983).
Table 1
The differences between expression levels of SHP2 and clinical pathological parameters

| Parameters                      | N   | SHP2 expression, N (%) | $\chi^2$ | $P$ value |
|---------------------------------|-----|------------------------|----------|-----------|
|                                 |     | Low | High                |          |           |
| Gender                          | 860 |     |                      |          |           |
| Male                            | 513 | 127(24.8) | 386(75.2) | 0.337    | 0.562     |
| Female                          | 347 | 92(26.5)  | 255(73.5) |           |           |
| Age                             | 860 |     |                      | 0.007    | 0.933     |
| ≤ 60                            | 422 | 108(25.6) | 314(74.4) |           |           |
| > 60                            | 438 | 111(25.3) | 327(74.7) |           |           |
| Disease location                | 838 |     |                      | 0.147    | 0.701     |
| Colon                           | 490 | 121(24.7) | 369(75.3) |           |           |
| Rectum                          | 348 | 90(25.9)  | 258(74.1) |           |           |
| Vascular tumor thrombus         | 200 |     |                      | 6.147    | 0.013     |
| No                              | 149 | 23(15.4)  | 126(84.6) |           |           |
| Yes                             | 51  | 16(31.4)   | 35(68.6)  |           |           |
| Differentiation                 | 811 |     |                      | 15.121   | < 0.001   |
| Well-moderate                   | 653 | 146(22.4) | 507(77.6) |           |           |
| Poor                            | 158 | 59(37.3)   | 99(62.7)  |           |           |
| Depth of invasion               | 831 |     |                      | 10.650   | 0.001     |
| Within subserosa                | 417 | 84(20.1)  | 333(79.9) |           |           |
| Beyond serosa                   | 414 | 124(30)    | 290(70)   |           |           |
| Distant metastasis              | 837 |     |                      | 0.032    | 0.859     |
| No                              | 752 | 188(25)   | 564(75)   |           |           |
| Yes                             | 85  | 22(25.9)   | 63(74.1)  |           |           |
| Lymph nodes metastasis          | 838 |     |                      | 25.107   | < 0.001   |
| No                              | 454 | 83(18.3)  | 371(81.7) |           |           |
| Yes                             | 384 | 128(33.3) | 256(66.7) |           |           |
| TNM stage                       | 744 |     |                      | 13.038   | < 0.001   |
| Parameters | N  | SHP2 expression, N (%) | $\chi^2$ | $P$ value |
|------------|----|------------------------|---------|----------|
|            |    | Low | High |         |          |
| Low        | 432 | 81(18.8) | 351(81.3) |       |          |
| High       | 312 | 94(30.1) | 218(69.9) |       |          |

**Feature of SHP2 expression in CRC**

The expression of SHP2 were observed in different histological subtypes of CRC. SHP2 is expressed at low levels in invasive micropapillary carcinoma, which is considered a subtype of poorly differentiated cancer (Fig. 3A), compared with its high expression in well-moderately differentiated cancer (Fig. 3B). Moreover, SHP2 was negatively expressed in all cases of mucinous adenocarcinoma and signet ring cell carcinoma (Fig. 3C). In addition, SHP2 was negatively expressed in mucous cells in non-mucinous tumors (Fig. 3D).

**High expression of SHP2 was associated with long OS**

Survival curve analysis was performed using the Kaplan-Meier method with log-rank test. The results showed that the expression levels of SHP2 in tumor tissues are closely related to the prognosis of patients with CRC. Patients with strong expression of SHP2 had the best outcomes, while those with negative expression had the worst outcomes ($\chi^2 = 15.372, P = 0.002$, Fig. 4A). However, the expression levels of SHP2 in the mucosal tissues adjacent to the tumors were not correlated with the prognosis of patients.

The intensity of SHP2 immunostaining was reclassified into low (negative and weak) versus high (moderate and strong) groups. The prognosis of the high expression group of SHP2 was significantly better than that of the low expression group ($\chi^2 = 7.045, P = 0.008$, Fig. 4B). In addition, vascular tumor thrombus, differentiation, depth of invasion lymph nodes metastasis, distant metastasis and TNM stage are all related to the prognosis of patients. ($P$-values all $< 0.001$, Table 2, Figure S1).
Table 2
Univariate survival analysis using the Kaplan-Meier method

| Parameters                        | $\chi^2$ | $P$ value |
|-----------------------------------|----------|-----------|
| Age                               | 1.85     | 0.174     |
| Gendar                            | 0.007    | 0.935     |
| Site                              | 0.08     | 0.778     |
| Vascular tumor thrombus           | 22.005   | < 0.001   |
| Differentiation                   | 18.336   | < 0.001   |
| Depth of invasion                 | 34.254   | < 0.001   |
| Lymph nodes metastasis            | 69.406   | < 0.001   |
| Distant metastasis                | 243.088  | < 0.001   |
| TNM stage                         | 105.35   | < 0.001   |
| SHP2 expression                   | 7.045    | 0.008     |

Increase in SHP2 expression was an independent prognostic marker in CRC

A Cox proportional hazard regression analysis was performed. Considering that the three factors represented by the depth of invasion, lymph node metastasis and distant metastasis were consistent with the TNM staging, they were not included in the regression model. A multivariate analysis with SHP2 expression level and clinicopathological characteristics as independent variables and prognosis of colorectal cancer patients as dependent variables demonstrated that SHP2 remained an independent prognostic factor for CRC (hazard ration 0.699, 95% CI 0.503–0.972, $P = 0.033$). High expression of SHP2 indicated a better prognosis of patients with CRC, while poor differentiation and high TNM grade indicated poor prognoses. Therefore, high SHP2 expression was an independent factor for good prognosis of patients with CRC, while low differentiation and high TNM stage were independent risk factors for poor prognoses. (Table 3).
Table 3
Cox proportional hazards regression for a multivariate analysis of prognostic factors in patients with CRC by Forward Stepwise (Likelihood Ratio) method

| Variables       | HR  | 95.0% CI for HR | P value |
|-----------------|-----|-----------------|---------|
| SHP2            |     |                 |         |
| Low expression  | 1.000 |                 |         |
| High expression | 0.699 | 0.503–0.972     | 0.033   |
| differentiaion  |     |                 |         |
| Well-moderate   | 1.000 |                 |         |
| Poorly          | 1.516 | 1.069–2.150     | 0.020   |
| TNM stage       |     |                 |         |
| TNM stage 1     | 1.000 |                 |         |
| TNM stage 2     | 1.950 | 0.917–4.145     | 0.083   |
| TNM stage 3     | 4.299 | 2.053–9.001     | < 0.001 |
| TNM stage 4     | 17.876 | 8.538–37.430   | < 0.001 |

Discussion

We detected the expression levels of several biomarkers in CRC in previous exploratory studies and found that SHP2 may be associated with patient prognosis. To confirm the prognostic value of SHP2 in CRC, we investigated SHP2 expression levels in 860 CRC tumors and 197 tumor-adjacent normal tissues and analyzed the relationship between SHP2 expression levels and clinicopathological parameters. We found that SHP2 expression was significantly higher in CRC tissues than in adjacent noncancerous tissues. In the same case, SHP2 was moderately to strongly expressed in cancer tissue and negatively to weakly expressed in normal mucosa adjacent to the tumor. Although there are some missing data when collecting clinical data, we still found that the expression level of SHP2 is related to the depth of invasion, lymph node metastasis and TNM stage according to the existing data, suggesting that SHP2 is related to the invasion and metastasis of CRC. Survival curves using the Kaplan-Meier method revealed that CRC patients with higher SHP2 expression had better prognoses. Cox proportional hazards regression for univariate analysis showed that the expression level of SHP2 was a prognostic factor for CRC. Moreover, multivariate analysis indicated that SHP2 is an independent prognostic indicator of CRC. Therefore, SHP2 is considered a useful factor for predicting the prognosis of patients with CRC. However, our results did not show a correlation between SHP2 expression and distant metastasis, which requires further confirmation from multicenter clinical samples.

By observing the immunohistochemical staining, we found that SHP2 was expressed in a “none” or “all” manner except in tumors containing mucinous adenocarcinoma, which means that nearly all the tumor cells were stained in the SHP2 positive cases, while virtually no tumor cells were stained in SHP2 negative cases. In the same tumor, SHP2-positive cells exhibited a uniform staining intensity; therefore, we did not consider the percentage of positive cells in the immunohistochemical staining results.
SHP2 was expressed diffusely and uniformly in the cytoplasm and nucleus of tumor cells, whereas only the nucleus and perinuclear cytoplasm were stained in mucosal epithelial cells adjacent to the tumor, yet the regions near the luminal surface were not. The specificity of cell type may be another feature of SHP2 expression. SHP2 is always negatively expressed in mucinous cells, both in the normal mucosal epithelium and in mucinous adenocarcinoma. This phenomenon has not been previously reported in the literature; however, the reasons for this are still unknown.

SHP2 plays important physiological roles in organism development and homeostasis maintenance by regulating fundamental intracellular signaling pathways in response to a variety of growth factors and hormones. SHP2/ERK signaling plays an anti-infection role in colonic mucosal erosion and colitis. Furthermore, SHP2 expression levels are upregulated in many solid tumors. Our findings demonstrate that SHP2 is upregulated in colorectal cancer, which is similar to that reported in the literature on pancreatic ductal adenocarcinoma, gastric cancer, and oral cancer.

However, contrary results have been reported regarding the prognostic significance of SHP2 expression, which has been associated with poor prognosis. Thus, the prognostic effect of SHP2 may not be equivalent in different cancers; it is highly influenced by the tumor site. SHP2 may possibly play a duplicitous role in tumorigenesis. Even in the same type of cancer, SHP2 has been reported to play different roles as a suppressor in hepatocellular carcinogenesis and as an enhancer in liver cancer progression.

Inhibitors of the tyrosine phosphatase SHP2 have been extensively studied for their broad roles in tumors. SHP2 is not only a useful predictor of prognosis, but is also a promising target for pro-senescence cancer therapies. SHP2 deletion reduces tumor microvascular density and leads to tumor vascular normalization, which is why SHP2 in endothelial tumors is a promising antiangiogenic target for cancer therapy. SHP2 plays an important role in regulating immune cell functions in the tumor microenvironment; therefore, SHP2 inhibitors may be a promising strategy for cancer immunotherapy. In addition, SHP2 inhibition has been shown to enhance responses to anti-PD-1 blockade, further suggesting the therapeutic effects of targeting SHP2.

Although great progress has been made in studies focusing on SHP2-related mechanisms, the specific processes of SHP2 involvement in the molecular mechanism remain unclear in CRC. Therefore, the molecular mechanism by which CRC patients with high SHP2 expression achieve longer survival warrants further study.

**Declarations**

**Competing interests**

The authors declare that they have no competing interests.
Authors' contributions

Xibo Liu and Lirong Chen judged the immunohistochemical results. Xibo Liu drafted the manuscript. Mengyao Li analyzed the data. Fei Wen collected the data and performed the experiment. Weiting Ge revised the manuscript. Shu Zheng and Weiting Ge designed this project. Both authors have read and approved the final manuscript.

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Figures

![Figure 1](image)

The expression levels of SHP2 in mucosal epithelial tissues and CRCs were divided into four levels according to the staining intensity: negative, weak, moderate, and strong.
Figure 2

Expression of SHP2 of mucosal and tumor tissues in CRC. (A) SHP2 was positively expressed in the nucleus and perinuclear cytoplasm of mucosal epithelial cells, while the regions near the luminal surface were unstained. (B) SHP2 showed diffuse and uniform staining in the cytoplasm and nuclei of the tumor cells. (C, D) the staining intensity of SHP2 was stronger in tumors (right) than in adjacent mucosal tissues, dysplasia, or intraepithelial neoplasia (lift).
Figure 3

Expression feature of SHP2 in different differentiation and subtype of CRC. (A) SHP2 was expressed at low levels in invasive micropapillary carcinoma. (B) SHP2 was highly expressed in well-moderate differentiated cancer. (C) SHP2 was negatively expressed in mucinous adenocarcinoma. (D) SHP2 was negatively expressed in mucous cells in non-mucinous tumors.
Figure 4

Survival curve analysis using Kaplan-Meier method with log-rank test. (A) Patients with strong expression of SHP2 had the best outcomes, while those with negative expression had the worst outcomes ($\chi^2 = 15.372, P = 0.002$). (B) The prognosis of the high expression group of SHP2 was significantly better than that of the low expression group ($\chi^2 = 7.045, P = 0.008$).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- FigS1.jpg