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Clinicopathological and Prognostic Significance of SPOCK1 Expression in Gastric Adenocarcinoma: An Immunohistochemical Study

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Abstract

Adenocarcinoma of the stomach is a malignant epithelial neoplasm of gastric mucosa with glandular differentiation. In 2020, gastric adenocarcinoma (GC) accounted for over 1.1 million new cases worldwide, making it the 5th most frequent malignant tumor. With a variety of genotypes and histological traits (phenotypes), GC is considered a heterogeneous illness. Furthermore, it is a complex illness, as it has not yet been fully understood. The SPARC family, comprising SPARC, SPOCK1, SPOCK2 and SPOCK3. It is supposed that they are regulators in the development of cancer. SPOCK1 may be involved in a variety of malignancies, including prostate and gallbladder tumors, according to mounting data. Evaluation of immunohistochemical expression of SPOCK1 in gastric adenocarcinoma cases, and its association with various clinic-pathological data. The current study included 46 randomly selected paraffin-embedded tissue specimens of gastric adenocarcinoma. Tissue sections were stained immunohistochemically with SPOCK1 antibody. In the present study, SPOCK1 positive expression was detected in 16 (34.78%) cases. SPOCK1 was shown to be strongly expressed in GC tissues relative to their nontumor counterparts ($p = 0.048$). Additionally, high statistically significant association was detected between SPOCK1 expression and tumor grade of differentiation ($p<0.0001$). Although, there was no statistically significant association between SPOCK1 expression and lymph node metastases. SPOCK1 expression concordance rate of 52.17% (n=12) was found between primary tumors and their metastatic lymph nodes. Furthermore 39.13% (n=9) of cases showed higher SPOCK1 score in lymph node metastases, while only two cases showed a lower SPOCK1 score in lymph node metastasis. No statistically significant association between SPOCK1 and other variables such as patient age, sex, tumor size, site, stage, lympho-vascular invasion, pre-operative chemotherapy. SPOCK1 is obviously highly expressed in GC tissue in comparison to its expression in normal adjacent gastric tissue, which may be evidence that it contributes to GC oncogenesis. SPOCK1 has direct association with tumor grade of differentiation. Additionally, SPOCK1 can play a role in acquiring the metastatic ability in tumor cells.

Keywords: SPOCK1, immunohistochemistry, Gastric adenocarcinoma

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1. Introduction

With almost 1.1 million new cases, gastric adenocarcinoma (GC) ranked as the fifth most prevalent malignant tumor worldwide in 2020 [1]. It accounts for 723,000 deaths annually and is the third most common cause of cancer-related mortality [2]. East Asia had the highest estimated death rates, while North America had the lowest. In the United States and Europe during the first half of the 20th century, stomach cancer was the most common cause of death from malignant tumors [1]. In Egypt, GC ranks as the 12th most frequent cancer among Egyptians, accounting for 1.6% of all cancer cases [3]. Numerous factors most likely influence the risk of gastric cancer. Due to genetic vulnerability, the impact of dietary and environmental exposures may be altered, contributing to the wide variation in the prevalence of stomach cancer observed worldwide [4]. Additionally, one of the most important risk factors is a family history of GC. However, while GCs are generally sporadic, 10% show evidence of a familial aggregation [5]. SPOCK1, a proteoglycan that is mainly ignored, is a member of the Sparc protein family. Only 106 publications on SPOCK1 have been published since it was isolated and identified as a chondroitin sulfate-heparan sulfate (CS-HS) proteoglycan from human seminal plasma in 1992. Early research found that endothelial cells and the extracellular matrix (ECM) of both mice and humans had high levels of SPOCK1 in their central nervous systems (CNS) [6]. An extracellular matrix (ECM) regulator plays a significant role in the remodeling and breakdown of the ECM, which is essential for both carcinogenesis and ECM homeostasis. The control of mediators, including growth

factors and hormones, complicates the course of epithelial mesenchymal transition (EMT) [7]. It is proposed that SPOCK1-mediated EMT is connected to many signaling pathways [8]. SPOCK1-mediated epithelial-mesenchymal transition (EMT) controls invasion and proliferation by activating the PI3K/Akt signaling pathway in many cancers such as gliomas, pancreatic ductal adenocarcinomas, colorectal cancers, and gallbladder cancers [9]. It is important to remember that in gastric cancer, SPOCK1 regulates invasion and metastasis through Slug-mediated EMT [10]. It is interesting to notice that SPOCK1 may affects cancer cells resistance to chemotherapy drugs. This occurs through the Akt and Wnt/β-catenin signaling pathways, where SPOCK1 causes lapatinib resistance in HER2-positive gastric cancer and temozolomide (TMZ) resistance in glioblastoma multiforme (GBM) cells, respectively. In lung cancer, SPOCK1 is linked to osimertinib resistance, which is coupled with MMP11 and TGF-β1 [11]. The aim of the current study is to assess the immunohistochemical expression SPOCK1 in gastric adenocarcinoma cases and its association with various clinicopathologic variables.

2. Materials and Methods

2.1. Study design and patient characteristics

This is a retrospective cross-sectional study, comprised of 46 randomly selected tissue blocks of formalin-fixed and paraffin embedded gastric adenocarcinoma cases. Patients' data were obtained from the clinical and medical reports including, patient age, sex, size of lesion (greatest dimension), site of lesion, histological grade, pathological tumor stage, lympho-vascular invasion, TNM staging and history of pre-operative chemotherapy (Table 1). Patient's age was classified in two age groups according to median as cutoff point: the first group <62 years and the second age group ≥62 years. Regarding tumour size in GC cases, it was classified in two groups according to median: the first group <6 cm and the second group ≥6 cm.

2.2. Histopathological evaluation

Hematoxylin and eosin (H&E) stained sections were prepared and evaluated for the histological type of the tumor according to World Health Organization (WHO) histological classification of tumors of stomach; 2019 .Tumor grade was evaluated by two-tiered system (low grade and high grade). Tumor pathological stage was estimated by AJCC/UICC TNM staging system, 5th edition; 2019 (12). Other features were assessed as, extent of invasion, presence of vascular and lymph node metastases. Among the 46 collected cases, 32/46 cases were positive for lymph node metastasis, only 23 case were available to be stained for SPOK1 expression in both the primary tumor and the corresponding metastatic lymph node (N=23).

2.3. SPOCK1 immunostaining

Five µm sections were prepared on positive charged slides for immunohistochemistry of SPOCK1 primary antibody utilizing the avidin biotin-peroxidase complex method with diaminobenzidine (DAB) chromogen detection system. Initially tissue sections on the positive charged slides were deparaffinized and rehydrated. Then the endogenous peroxidase was blocked by immersion in a 3% solution of hydrogen peroxide and incubated for 30 minutes. Antigen retrieval was performed by immersing the slides in Tris-EDTA solution (pH 9) for 4 times (5 minutes each) at 750-W. To block nonspecific background staining, the slides were treated by UV block. Primary antibody SPOCK1 (polyclonal rabbit antibody, 7ml, Ready to use, Medaysis Laboratories, USA) was then added and tissue sections were incubated for 1 hour at room temperature. Excess reagent was thrown off and the slides were then rinsed gently with buffer solution for 5 minutes. After that Secondary biotinylated antibody was added for each slide for 30 minutes. DAB substrate and chromogen solutions were added to each slide and following that tissue sections were counter stained by Mayer's hematoxylin. As a positive control for SPOCK1, tissue sections of normal human prostatic tissue were used. Negative control tissue sections were processed by omitting the specific primary antibody from the staining procedure and replaced with PBS. Evaluation of SPOCK1 expression was assessed as follows: SPOCK1 was expressed in cytoplasm of tumor cells. The immunohistochemical scores were obtained by light microscopy (Olympus, Tokyo, Japan). Cellular positivity was assigned as 0: 0% immunoreactive cells, 1: 50% immunoreactive cells, 2: 5–50% immunoreactive cells; and 3: >50% immunoreactive cells. Next, staining intensity was graded as follows: 0= (negative), 1= (weak), 2= (intermediate) and 3= (strong). The overall immunoreaction score was defined as the sum of both parameters, and the samples were grouped as negative (0), weak (1–2), moderate (3), and strong (4–6) staining. The cases were then categorized into two groups: negative cases that include negative and weak groups, while positive cases that include moderate and strong groups [13].

2.4. Statistical analysis

The analysis of the data was carried out using the IBM SPSS 28.0 statistical package software (IBM; Armonk, New York, USA). Data were tested for normal distribution using Shapiro-Wilk test. Data were presented as mean ± SD where normally distributed and as median (interquartile range) where data were skewed. Data were expressed both number and percentage for qualitative data and were analyzed by the Chi-square test or Fisher's exact test. A p-value less than 0.05 was considered significant.

3. Results and discussion

In the present study, SPOCK1 was expressed in the cytoplasm of malignant cells. Evaluation of SPOCK1 expression was performed in tumor cells and adjacent normal gastric tissue. Expression in tumor cells was correlated with different clinic-pathological variables for all cases. Regarding SPOCK1 intensity of staining, 18/46 (39.1%) cases were scored negative, 13/46 (28.3%) cases were scored weak, 9/46 (9.6%) cases were scored intermediate, and 6/46 (13%) cases were scored strong. Regarding SPOCK1 percentage of staining, 18/46 (39.1%) cases were scored negative, 12/46 (26.1%) cases were scored showed <5% of cellular staining, 2/46 (4.3%) cases showed 6-50 % cellular staining, and 14/46 (30.4%) cases showed >50% cellular staining. Final scoring revealed that SPOCK1 was positive in 16/46 (34.8%) cases

and was negative in 30/46 (65.2%) cases. Regarding expression in normal adjacent gastric tissue, statistically significant difference was found between SPOCK1 expression in tumor cells and its expression in adjacent normal gastric tissue (p = 0.048), as only 2/46 (4.34%) of cases showed SPOCK1 expression while most of cases 44/46 (95.65%) showed negative SPOCK1 expression. In this study, 32/46 cases were positive for lymph node metastasis, only 23 case were available to be stained for SPOK1 expression in both the primary tumor and the corresponding metastatic lymph node (N=23). Regarding expression of SPOK1 in primary tumor, 9/23 (39.13%) case showed positive SPOCK1 expression and 14/23 (60.86%) showed negative SPOCK1 expression. In corresponding lymph nodes; SPOCK1 was localized in cytoplasm of metastatic tumor cells. SPOCK1 expression was positive in 16/23 (69.6%) metastatic LN cases and was negative in 7/23 (30.4%) metastatic LN cases. Concerning the change in SPOK1 expression pattern between primary tumors and their corresponding lymph nodes, no significant difference was found between primary tumor and their corresponding lymph node (p = 0.06). However, it was clearly noticed that twelve cases (52.17%)) maintained the same expression score. Nine cases (39.13%) showed higher SPOK1 expression in corresponding metastatic lymph nodes, while only two cases showed lower expression of SPOK1 in corresponding metastatic lymph nodes (Table 3). Regarding the association between SPOCK1 expression in tumor cells and various clinicopathological data; high statistically significant association was detected between SPOCK1 expression and tumor grade of differentiation (p <0.0001), in which 13/18 (72.22%) of high-grade cases were SPOCK1 positive, while 25/28 (89.29%) of low-grade cases were SPOCK1 negative. No significant association was found between SPOCK1 expression and patient's age, patient's sex, size of tumor, site of tumor, stage of tumor, lymph node metastasis, lympho-vascular invasion, presence of pre-operative chemotherapy (p = 0.515, 0.208, 0.1, 0.1, 0.572, 0.930, 0.549, 0.665 respectively). Despite being identified in 1997, SPOCK1's carcinogenic potential was only documented in publications that described the role of the proteoglycan in stomach cancer around 2010. Many more reports have highlighted SPOCK1's carcinogenic potential in the last ten years. SPOCK1's physiological roles outside of the central nervous system and its in vivo location are still unknown, nevertheless. It is still unknown whether it is an ECM protein or not? [14]. A growing evidence of research has revealed that SPOCK1 may have an important role in the development of numerous cancer types, including colorectal, ovarian, prostate, gallbladder, breast, urothelial, glioblastoma, lung, and hepatocellular carcinomas [15] meanwhile previous research showed that SPOCK1 had no influence on the proliferation of gastric cancer cells both in vitro and in vivo, others found the opposite [8]. It is interesting to notice that SPOCK1 affects cancer cells drug resistance to chemotherapy drugs [11]. Because of the possibility of poor prognosis that is associated with SPOCK1 expression, SPOCK1 could be a useful prognostic factor in cancer. The fact that SPOCK1 expression is substantially higher in metastatic tumor tissues than in nonmetastatic tumor tissues has clinical implications. It is noteworthy that a variety of SPOCK1 signaling pathways contribute to the development and carcinogenesis of tumors. SPOCK1 might be a vital target for cancer therapy. Above all, SPOCK1 might be a desirable therapeutic target and prognostic biomarker for the treatment of cancer [16]. In our study, we analyzed the association between SPOCK1 immunohistochemical expression and various clinicopathological data of gastric adenocarcinoma cases. Regarding SPOCK1 expression among gastric adenocarcinoma cases, we found that it was localized at cytoplasm of tumor cells. Negative Expression was found in 65.2% cases, while 34.8% of cases were positive for SPOCK1 expression. SPOCK1 was shown to be strongly expressed in GC tissues in contrast to negative expression that noticed in nontumor counterparts in our study, suggesting that SPOCK1 may be involved in the formation of GC. This was in agreement with only one study that evaluated immunohistochemical expression of SPOCK1 in normal gastric tissue in comparison to gastric adenocarcinoma tissue [17]. Similarly, another study reported that, SPOCK1 expression was significantly higher in tumor tissues of gall bladder adenocarcinoma compared with that in cholelithiasis tissues [13]. To our knowledge, this study was the first to evaluate relation between SPOCK1 expression in gastric adenocarcinoma and grade of tumor differentiation. In this study, a significant association between SPOCK1 expression in GC cases and tumor grade of differentiation was observed. This was in accordance to results of previous studies, including gall bladder cancer cases and breast cancer cases that demonstrated significant association between SPOCK1 expression and tumor grade of differentiation [13,18]. Regarding other clinicopathologic data, including patient age, sex and tumor size, we found no statistical significance between these clinicopathological data and SPOCK1 expression. This was in concordance with previous studies who tested SPOCK1 expression in GC and gall bladder cancer respectively [13, 17]. Additionally, no statistical significance association was detected between SPOCK1 expression in GC cases with either tumor site or history of pre-operative chemotherapy. To our knowledge, our study was the first to evaluate these associations. Regarding tumor stage, in this study, no significant association between SPOCK1 expression and pathological tumor stage was detected, this was in accordance with previous study who demonstrated a similar finding in gallbladder cancer cases [13]. In contrast, this finding was in discordance with another study that demonstrated a significant association between tumor stage and SPOCK1 expression in GC cases [17]. This could be explained due to the difference in tumor stage distribution among cases in both studies, as in our study there was no T1 stage cases while T2 cases were only two cases while most of the cases were T3&T4. For Lymph node metastasis, There is no significant association between SPOCK1 expression in GC cases and lymph node metastasis could be detected. A previous study reported similar findings regarding no association between SPOCK1 expression and lymph node metastasis [19].

Table 1. Clinicopathological characteristics for all studied patients (N=46)

Clinico-pathological features	No. (%)
Age	
<62	20 (43.5%)
≥62	26 (56.5%)
Sex	
Male	32 (%69.56)
Female	14 (%30.34)
Tumor size	
<6	22 (47.8%)
≥6	24 (52.2%)
Tumor site	
Gastro-esophageal junction	6 (13%)
Cardia	4 (8.7%)
Fundus	4 (8.7%)
Body	15 (32.6%)
Greater curvature	1 (2.2%)
Lesser curvature	7 (15.2%)
Pylorus	9 (19.6%)
Tumor grade	
Low grade	28 (60.87%)
High grade	18 (39.13%)
TNM stage	
T2	2 (4.34%)
T3	25 (54.34%)
T4	19 (41.3%)
Tumor with Lymph Node Metastasis	
Positive	32 (69.6 %)
Negative	14 (30.4 %)
Lympho-vascular Invasion	
Present	11 (23.91%)
Absent	35 (76.08%)
Pre-operative chemotherapy	
No Pre-operative Chemotherapy	32 (69.6%)
Received Pre-operative chemotherapy	12 (26.1%)
No available data	2 (4.3%)

Table 2. Association between SPOCK1 expression and various clinicopathological variables for all studied cases (N=46)

Variables		SPOCK1 Expression		*P*-value
		Negative Expression N= 30 (65.2%)	Positive Expression N=16 (34.8%)	
Normal Gastric Tissue	Negative	30 (100%)	0 (0%)	**0.048***
	Positive	14 (87.5%)	2 (12.5%)	
Age groups	< 62	12 (60%)	8 (40%)	0.515
	≥ 62	18 (69.23%)	8 (30.77%)	
Sex	Male	19 (59.37%)	13 (40.63%)	0.208
	Female	11 (78.57%)	3 (21.43%)	
Size groups	<6 cm	17(77.27%)	5(22.73%)	0.1
	≥6 cm	13(54.17%)	11(45.83%)	
Site of lesion	GOJ	3(50%)	3(50%)	0.1
	Cardia	1 (25%)	3 (75%)	
	Fundus	1 (25%)	3(75%)	
	Body	10(66.67%)	5(33.33%)	
	Greater Curvature	1(100%)	0(0%)	
	Lesser Curvature	6(85.71%)	1(14.29%)	
	Pylorus	8(88.89%)	1(11.11%)	
Tumor Grade	Low Grade	25 (89.29%)	3(10.71%)	**<0.0001***
	High Grade	5 (27.78%)	13(72.22%)	
TNM Stage	T2	2 (100%)	0 (0%)	0.572
	T3	16 (64%)	9 (36%)	
	T4	12 (63.16%)	7 (36.84%)	
Lymph Node Metastasis	Positive	21 (65.63%)	11 (34.37%)	0.930
	Negative	9 (64.29%)	5 (35.71%)	
Lympho-vascular invasion	Present	8 (72.72%)	3 (27.27%)	0.549
	Absent	22 (62.86%)	13 (37.14%)	
Pre-operative Chemotherapy	No Pre-operative Chemotherapy	20 (62.5%)	12(37.5%)	0.665
	Received Pre-operative chemotherapy	9 (75%)	3(25%)	
	No available data	1(50%)	1(50%) (%)	

* P - value <0.05 are considered statistically significant according to Chi-Square test and Fisher's exact test.

Table 3. The change in SPOCK1 expression pattern in 23 pairs of primary tumour and their corresponding lymph node metastases

	Changes in Expression Patterns			p =0.065
	P=M[a]	M>P[b]	P>M[c]	
SPOCK1 Expression	12/23 (52.17%)	9/23 (39.13%)	2/23 (8.7%)	

P: Primary gastric carcinoma. M: metastatic malignant LN.

a: Equal expression in primary tumour and lymph node metastasis.

b: Higher expression in lymph node metastasis compared to primary tumour.

c: Higher expression in primary tumour compared to lymph node metastasis.

Test of significance: McNemar

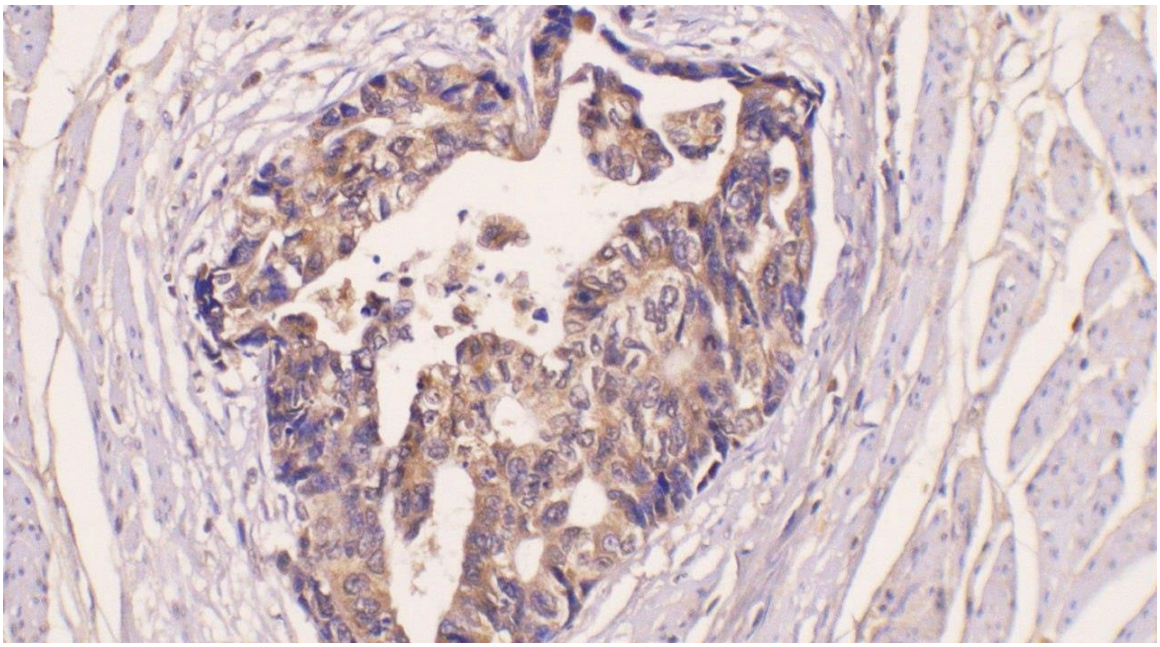

Figure 1. High cytoplasmic SPOCK1 expression in Gastric Adenocarcinoma, Scored as positive (IHC, X200).

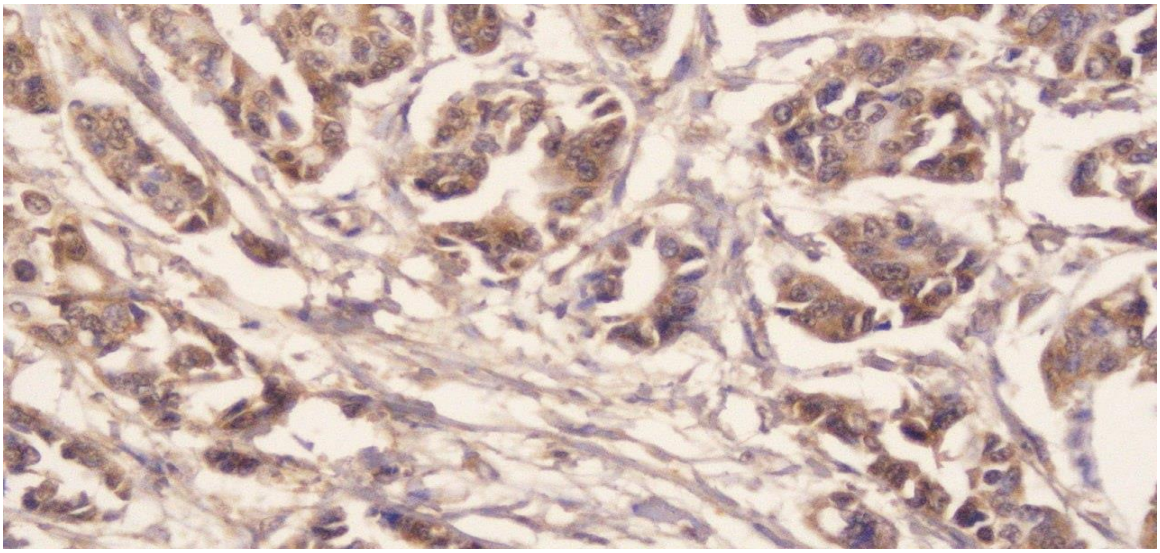

Figure 2. High cytoplasmic SPOCK1 expression in Gastric Adenocarcinoma, Scored as positive (IHC, X100)

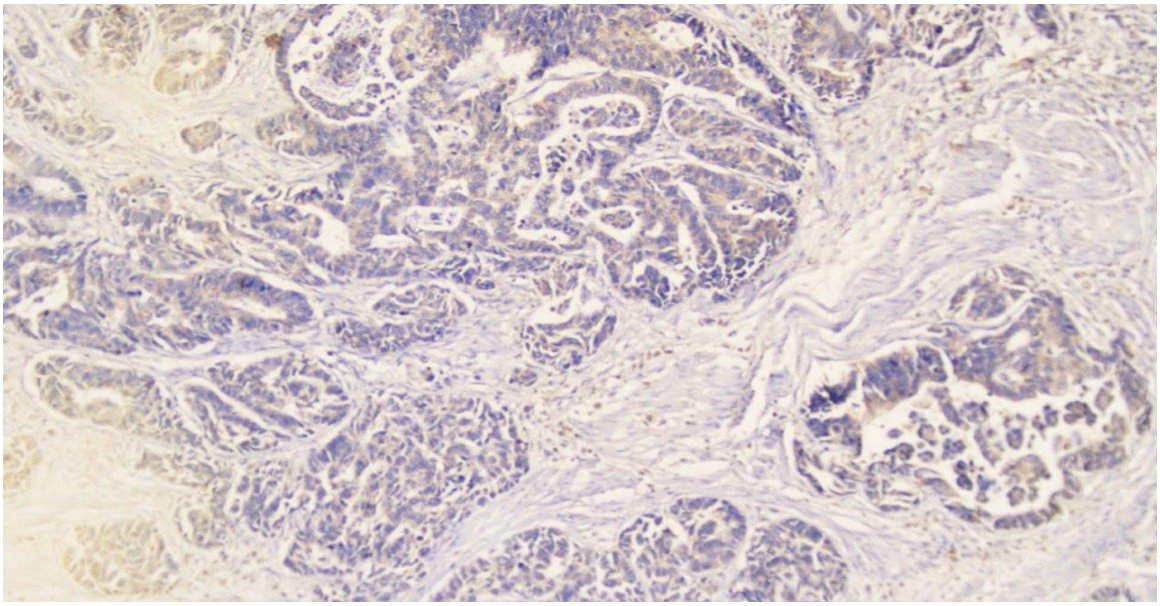

Figure 3. Low cytoplasmic SPOCK1 expression in Gastric adenocarcinoma, Scored as negative (IHC, X100)

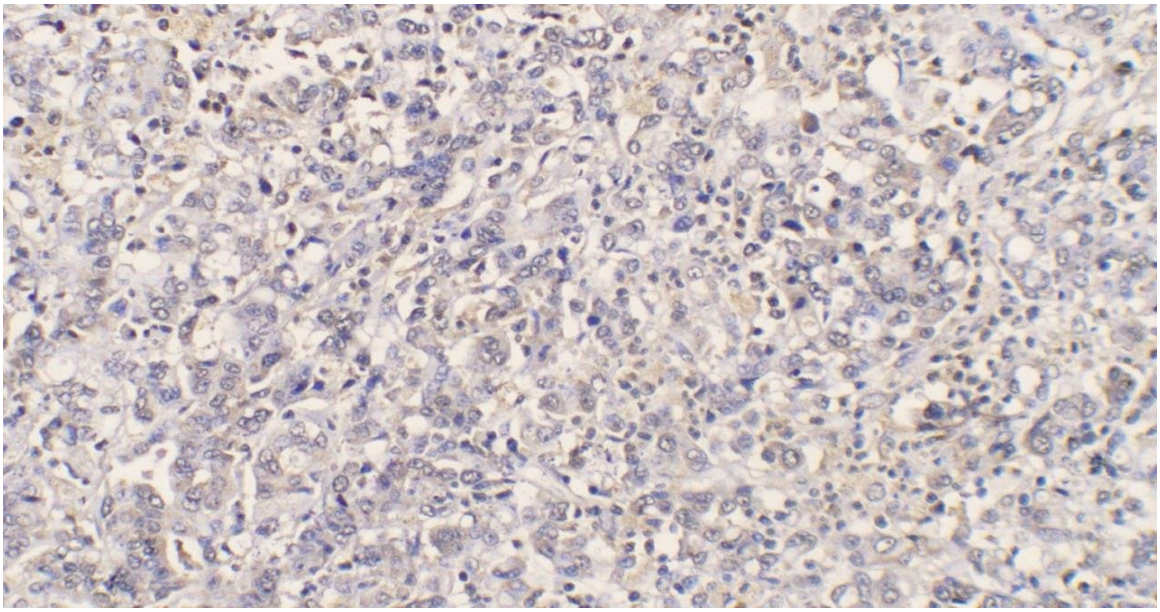

Figure 4. Low cytoplasmic SPOCK1 expression in Gastric adenocarcinoma, scored as negative (IHC, X200)

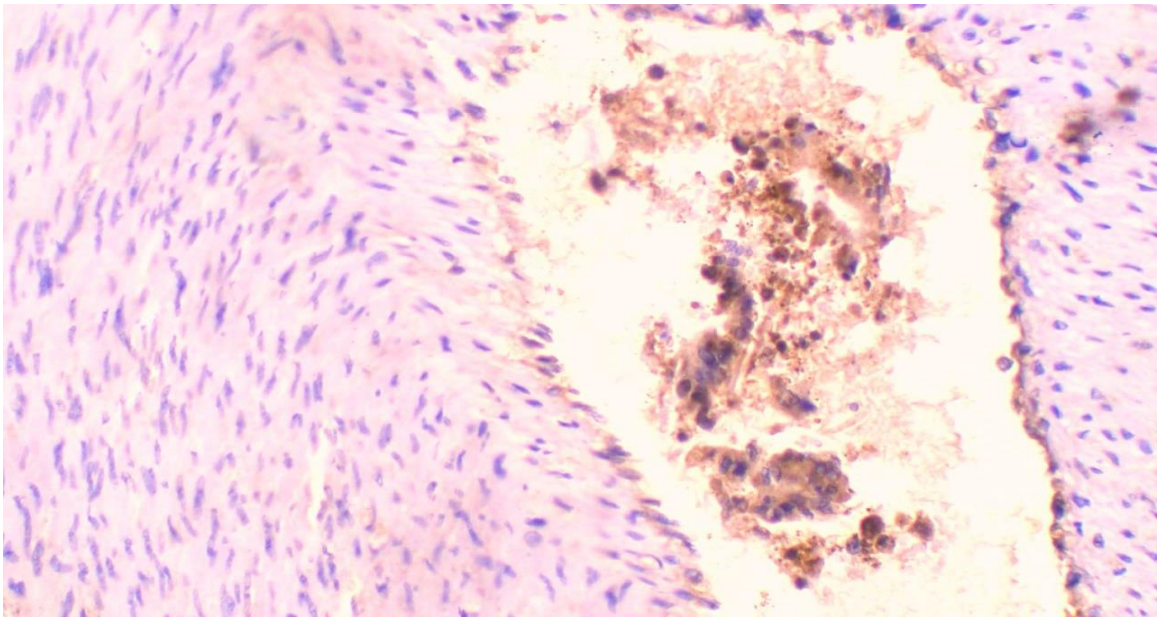

Figure 5. High cytoplasmic SPOCK1 expression in Gastric adenocarcinoma with Lympho-vascular Invasion (IHC, X200)

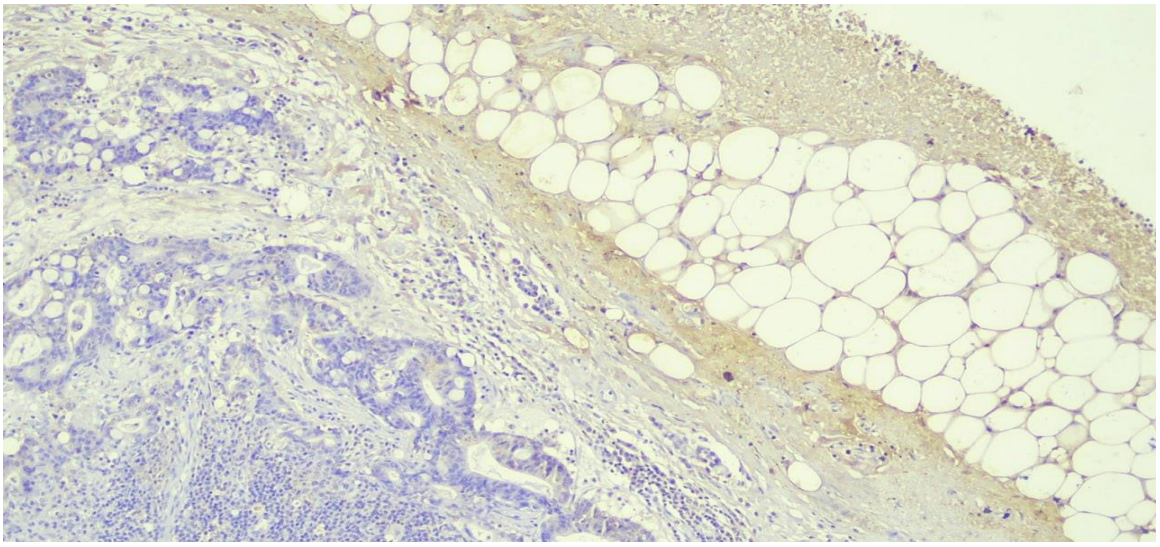

Figure 6. Low cytoplasmic SPOCK1 expression in Gastric adenocarcinoma in Lymph Node Metastasis, scored as negative (IHC, X100)

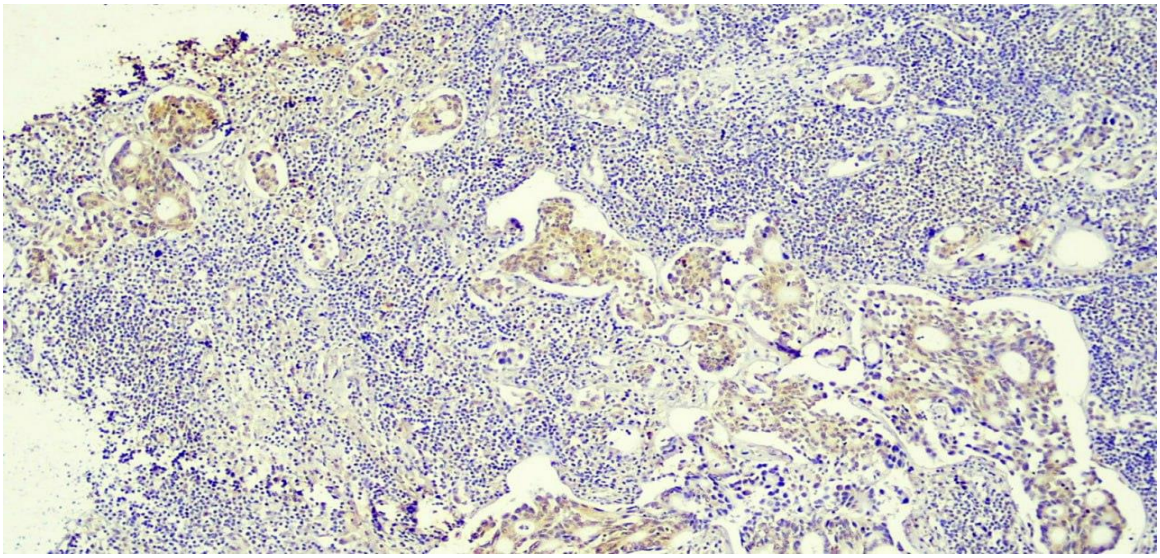

Figure 7. High cytoplasmic SPOCK1 expression in Gastric adenocarcinoma in Lymph Node Metastasis, scored as positive (IHC, X100)

Although in our study, there is no significant association was detected between SPOCK1 and Lymph node metastasis, upon comparing SPOCK1 expression between primary tumor and their lymph node metastases, higher SPOCK1 scores were noticed in a considerable proportion of cases suggesting the role of SPOK1 in acquiring the metastatic ability in tumour cells. On the contrary, two reported previous studies demonstrated significant association between SPOCK1 expression and lymph node metastasis in GC cases and breast cancer cases respectively [17-18]. Regarding lympho-vascular invasion, no significant association was detected between lympho-vascular invasion and SPOCK1 in this study, this was in accordance with previously reported study [17]. Lastly, in this context, the current study demonstrated the presence of a significant association between SPOK1 expression and one of poor prognostic factors of GC which is tumor grade differentiation. Also, higher SPOK1 scores were obviously noticed in a considerable proportion of metastatic lymph nodes as compared to its primary tumor, suggesting the role of SPOCK1 in acquiring the metastatic ability in tumor cells. Hence these findings highlight the important role of SPOCK1 in tumorigenesis, progression and spread.

4. Conclusions

SPOCK1 is obviously highly expressed in GC tissue in comparison to its expression in normal adjacent gastric tissue, which may be an evidence that it contributes to GC oncogenesis. SPOCK1 has direct association with tumor grade of differentiation, and this could be a reflection of its poor prognostic significance. Additionally, SPOCK1 can play a role in acquiring the metastatic ability in tumor cells.

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