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Utility of SPC25 as a Biological Biomarker in Colorectal Adenocarcinoma

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Abstract

Spindle pole body component 25(SPC25) is involved in a variety of biological processes, including carcinogenesis. However, no research has been done on the clinical importance of SPC25 in colorectal adenocarcinoma (COAD). The goal of this work was to investigate the mRNA level of SPC25 in COAD and normal tissues, as well as its diagnostic and prognostic usefulness. The mRNA level of SPC25 was examined in colorectal adenocarcinoma (COAD) using Gene Expression Profiling Interactive Analysis (GEPIA), Firebrowse, Gene Expression Omnibus (GEO), Oncomine and The Cancer Genome Atlas (TCGA) databases. To examine the diagnostic significance of SPC25, we use receiver operating characteristic (ROC) plotter. SPC25 was upregulated in several type of cancers, and the highest expression was in COAD cohort study. SPC25 was higher in tumor COAD tissues in comparison to normal tissues (p value <0.001). High level of SPC25 at mRNA level was associated with tumor stage (p value= 0.045) and lymph node (p value= 0.019). Patients with high SPC25 had poor overall survival and disease free survival. In ROC analysis, SPC25 was shown to be a highly diagnostic biomarker in COAD with Area value =0.548 and p value= 4.5e-2. SPC25 was elevated in COAD and was found to be an independent predictor of worse Overall Survival and Disease free survival in patients with COAD. Therefore, SPC25 is a useful biomarker for diagnostic and prognostic COAD patients.

Keywords: SPC25, COAD, Overall survival, TCGA

1. Introduction

Colorectal adenocarcinoma cancer (COAD) continues to be the leading cause of cancer-related death worldwide, with a high annual incidence of cancer per population (Jemal et al., 2011; Sung et al., 2021). In general, liver metastasis affects roughly 30 % of COAD patients, while more than half of individuals acquire metastasis at particular sites after identification of a tumor stage (Cardona et al., 2013; Mayo et al., 2011). Genetic mutations, epigenetic alterations, and environmental variables all have a role in development of COAD. COAD is more common in those between the ages of 40 and 50; however, it is becoming more common among younger people (Karunanithi & Levi, 2018). Surgery, chemotherapy, and radiotherapy are the most common treatments for COAD today; nonetheless, in metastatic cases, the 5-year overall survival rate is less than 10%(Jiang, Yang, Yuan, & Liu, 2018). As a result, it is necessary to discover a critical gene involved in the development of COAD in order to design appropriate diagnostics and successful treatment strategies for COAD patients.

Spindle pole body component 25 (SPC25) is a part of a nuclear division cycle 80 complex (Ndc80) controls the interaction of kinetochore protein with microtubules.; it plays an important function in chromosomal segregation (F. Chen et al., 2020; Tooley & Stukenberg, 2011; Ustinov, Korshunova, & Gudimchuk, 2020). Several theories have been postulated that fault in chromosomal segregation causes genetic instability, which links to cancer development (Raaijmakers & Medema, 2014). Recent studies have reported the role of SPC25 in cancer progression in several type of malignancies; prostate cancer, hepatocellular carcinoma, breast cancer, gastric cancer and lung cancer (J. Chen, Chen, Yang, & Dai, 2018; Cui, Hu, Fan, Tan, & Tang, 2018; Wang et al., 2019; Yang, Sun, Song, Yang, & Liu, 2020; B. Zhang et al., 2020). Blood samples from those with mild cognitive impairment showed considerably increased SPC25 expression, indicating that it may serve as a biomarker for alzheimer's disease, according to a cell-based assay (X. Zhang, Li, Shen, & Lau, 2018). However, no definitive research has been done on the link between SPC25 gene expression and CROAD clinical diagnosis and disease prognosis.

In this study, the potential role of SPC25 as a diagnostic and prognostic biomarker was verified by using several bioinformatics tools; TCGA xena browser, GEPIA, Oncomine and GEO databases. For the first time, we report on the differential expression of SPC25 in COAD and its relationship to prognosis.

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2. Material and Methods

2.1. Expression profile of SPC25 in colorectal adenocarcinoma cancer (COAD) using GEPIA.

GEPIA **(**http://gepia.cancer-pku.com**)** is an interactive web-based program that delivers RNA sequencing data based on TCGA and GTEx, with roughly 10,000 tumor samples and 9,000 normal samples. We used the Expression DIY functionality to find the SPC25 gene, then selected Boxplot from the Expression DIY menu, with Colorectal cancer (COAD) added to the Datasets list. Then, automatically, Log scale, Jitter size, and Matched normal data were all established.

2.2. Expression profile of SPC25 in COAD using Oncomine and TCGA databases

Oncomine — a cancer microarray dataset-providing server that grants access to about 800 datasets from numerous study cohorts. (http://www.oncomine.org). In addition, TCGA-BC cohort by UCSC (University of California at Santa Cruz, CA, USA) Cancer Genomics Browser (https://xenabrowser.net/) assisted the collection of data of 300 COAD tissue samples in link to clinicopathological parameters.

We searched for "TCGA colon cancer (COAD) in S*earch for a study*, then *Genomic* was selected for the D*ata Type*, and the desired SPC25 gene was added; finally, G*ene Expression* was checked as a dataset.

2.3. Gene expression Omnibus database

We used GEO datasets website (https://www.ncbi.nlm.nih.gov.com) looking for a study on profile of mRNA in colorectal cancer. We then download the GSE156720 dataset which examines the expression of mRNAs between 3 pairs of colorectal tumor tissues and normal tissues. This study reported significantly up-regulated as well as down-regulated mRNAs and lncRNAs in CRC tissues compared with their matched non-tumoral tissues.

2.4. Receiver operating characteristic (ROC) Plotter

By using the RNA sequencing data of over 1500 COAD patients, the ROC plotter (http://www.rocplot.org/) was able to expect the relationship between gene expression and therapeutic response.

2.5. Statistical analysis

SPSS version 18.0 was used to analyze the data (SPSS, Inc., Chicago, IL, USA). One-way ANOVA, as well as chi-squared tests, were used to analyze the association between STARD3 and BC clinicopathological parameters. Statistical significance was defined as * $p <$ 0.05 and ** $p < 0.001$.

3. Results

3.1. SPC25 is overexpressed in colorectal cancer than in other types of cancer

The GEPIA web-based RNA expression tool was used to determine the SPC25 mRNA expression in different types of human malignancies (n = 5) in contrast to neighboring normal tissues (Figure 1 A). When compared to other kinds of human cancer, colorectal cancer (COAD) had the greatest level of SPC25 expression. Furthermore, based on firebrowse when compared to nearby normal colorectal tissues, SPC25 was upregulated in colorectal carcinoma tissues (Figure 1 B). SPC25 mRNA levels were elevated in primary colorectal tissues (n=286) more than normal tissues (n=41) when we used the UALCAN website. Altogether, SPC25 mRNA expression was shown to be greater in COAD tissues than in normal tissues.

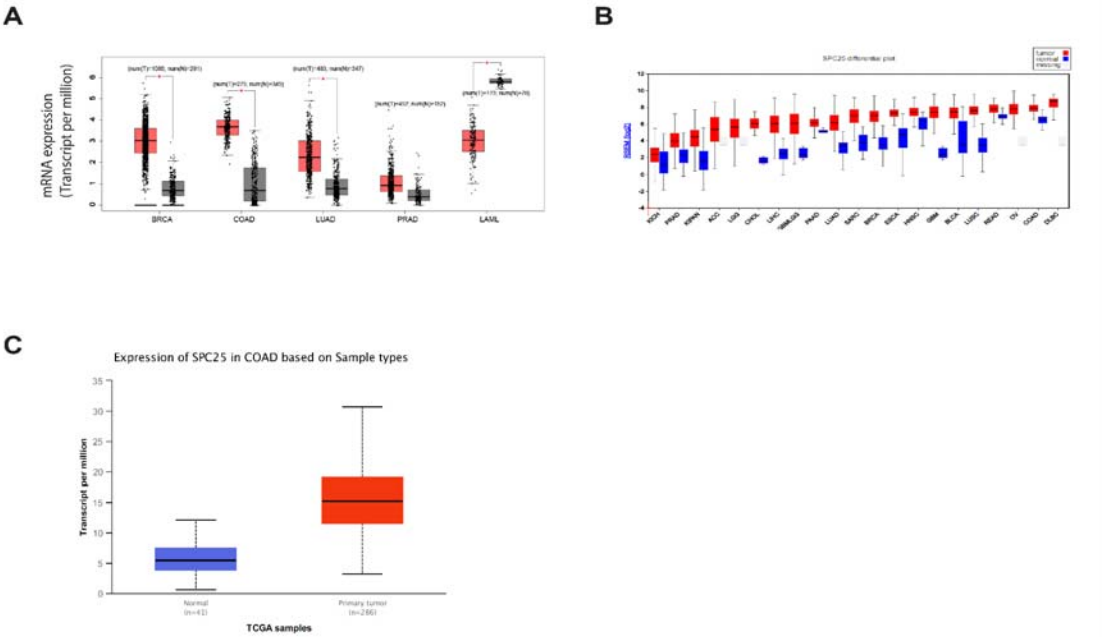

Figure 1 Expression level of SPC25 in different types of cancers. A) The graph was created from the GEPIA, **B)** Firebrowse webpages, which indicates the expression of SPC25 at the mRNA level in several types of malignancies. The red box indicates the expression of SPC25 in tumor tissues, and the gray box indicates the expression of SPC25 in normal tissues. **C)** SPC25 expression in COAD using UALCAN database, the blue box indicates the normal tissues (n=41) and the red box indicates the primary COAD tissues (n=286) the p value <1E-12.

3.2. SPC25 expression based on Oncomine and GEO databases

With solid, peer-reviewed analytic methodologies and a rich collection of analysis functions that compute gene expression signatures, the Oncomine Platform delivers solutions for individual researchers and multinational corporations. We analyzed the mRNA level of SPC25 in different types of colorectal cancer. Overexpression of SPC25 in COAD has been found in eight studies (Figure 2 A/B). 1; Rectal Adenocarcinoma vs. Normal *Gaedcke*/ 2; Colon Adenoma vs. Normal *Sabates-Bellver* Colon/ 3; Colorectal Carcinoma vs. Normal *Skrzypczak* / 4; Cecum Adenocarcinoma vs. Normal *TCGA* Colorectal / 5; Colon Adenocarcinoma vs. Normal *TCGA* Colorectal/ 6; Colon Mucinous Adenocarcinoma vs. Normal *TCGA* Colorectal / 7; Rectal Adenocarcinoma vs. Normal *TCGA* Colorectal /8; Rectal Mucinous Adenocarcinoma vs. Normal *TCGA* Colorectal.

To confirm these results, we downloaded RNA seq data from GEO microarray datasets based on transcriptome analysis to identify the expression level of SPC25 in colorectal cancer (GSE156720) was performed on 3 pairs of colorectal tumor tissues and normal tissues. Total RNA was extracted and Samples labeling and array hybridization were done based on the Agilent One-Color Microarray-Based Gene Expression Analysis protocol (Agilent Technology). All the three tumor tissues had high level of SPC25 in comparison to the adjacent normal tissues, the (**Figure 2 C**), shows the average mRNA level for the three tumor tissues and the three normal tissues.

3.3. Association of SPC25 with COAD clinicopathological features

Next, relationship between SPC25 and COAD clinicopathological parameters have been analyzed from COAD-TCGA dataset (Table 1). High level of SPC25 was significantly associated with tumor stage (p=0.045) and lymph node status (*p*=0.019). No significant association had been found with tumor size or radiation therapy or height or weight of the patients.

Patients with high level of SPC25 mRNA had poor overall survival (OS) and disease-free survival (DFS); however, better overall survival and disease-free survival are predicted by low levels of SPC25. (Figure 2 D/E).

We created a ROC curve utilizing SPC25 expression data from colorectal adenocarcinoma tissues using ROC plotter to explore the diagnostic utility of SPC25 gene. SPC25 was found to have a high clinical utility in separating COAD normal from tumor tissues, as shown in (Figure 2 F), with an AUC of 0.548, a true positive rate of 0.56, and a false positive rate of 0.53, and a p value of 4.5e-2.

Clinicopathological characteristics	Variables	SPC25 mRNA low	SPC25 mRNA high	P-value
Height	≤168 cm	48	54	0.339
	vs	46	44	
	≥169cm			
Weight	≤82kg	52	56	0.172
	vs	53	42	
	≥83kg			
Tumor size	T1 &T2	25	125	0.197
	vs	26	95	
	T3&T4			
Tumor stage	Stage I & II	74	73	0.045*
	vs	72	45	
	Stage III& IV			
Node	N0	73	72	0.019*
	N1	41	16	
	N2	27	21	
Radiation therapy	No	126	4	0.285
	Yes	97	1	

Table 1: Clinicopathological features of colorectal cancer with high vs low SPC25 mRNA expression based on TCGA database.

Clinicopathological parameters were assessed using Chi-square analysis. * $p < 0.05$, ** $p < 0.001$.

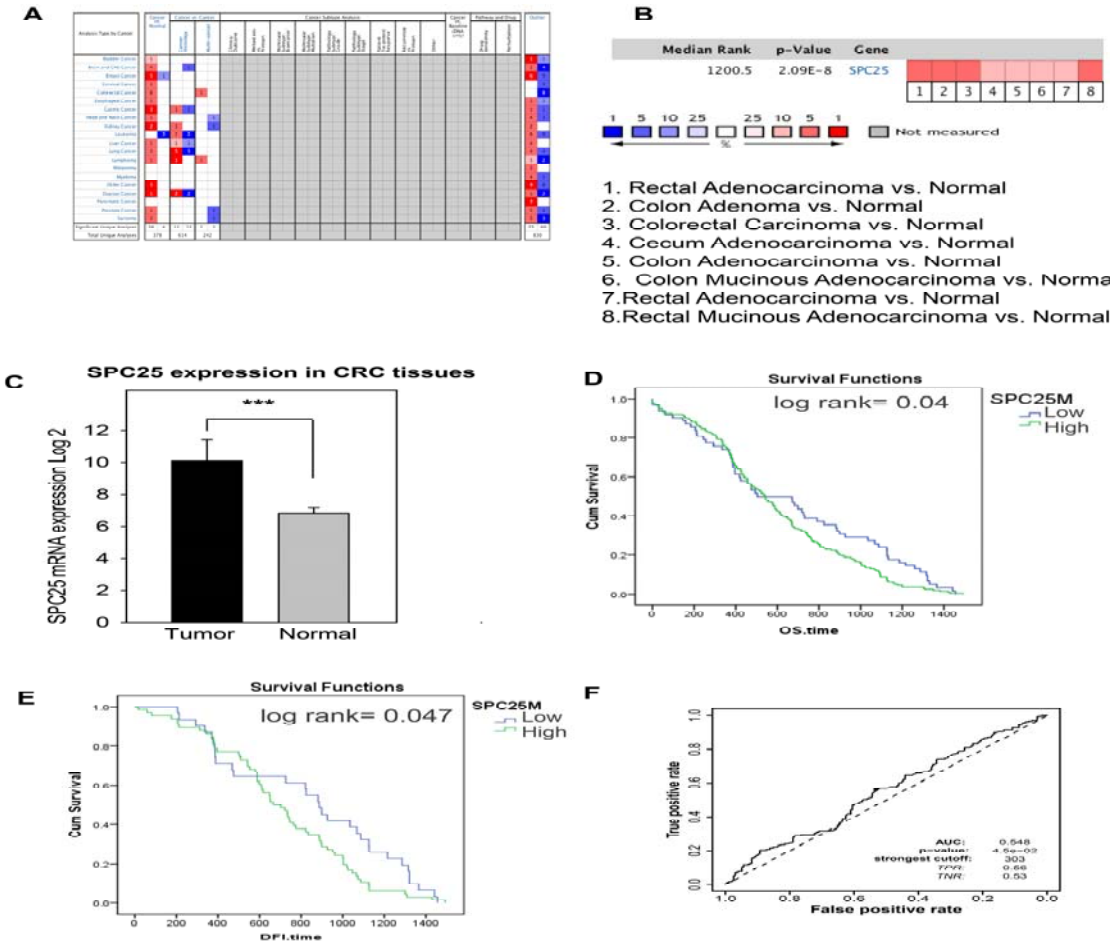

Figure 2 Oncomine, GEO analysis and the prognostic significance of SPC25 in CAOD. A) The SPC25 gene expression in different types of malignancies based on Oncomine database. **B)** SPC25 mRNA expression in COAD subtypes is compared; high expression is indicated by a red, whereas low expression is indicated by a blue. **C)** SPC25 in colorectal adenocarcinoma based on GEO analysis. **D&E)** Survival analysis of SPC25 in COAD patients. **F)** ROC analysis for true positive rare vs false positive rate.

4. Discussion

SPC25 is a component of the Ndc80 complex that regulates the segregation of mitotic chromosomes during mitosis (Tooley & Stukenberg, 2011). During the mitotic process, SPC25, can form a dimer with SPC24 to control microtubule-kinetochore binding, chromosomal arrangement, and spindle checkpoint initiation (Sun, Lee, Xu, & Kim, 2010). Ndc80 was found to be overexpressed in several type of human cancers (Liu et al., 2016; Xing, Wu, Chen, & Feng, 2016). SPC25 was observed to play an important role in thyroid cancer development, and it has been discovered to be a new prognostic biomarker for lung cancer and hepatocellular carcinoma (Yin, Meng, Zhou, Chen, & Song, 2017; Zhou et al., 2017; Zhu et al., 2015). In the current study, we focused on SPC25 and investigate the prognostic significant and expression pattern in patients with colorectal adenocarcinoma. Per our information, this is the first research to examine the SPC25 expression at mRNA in COAD.

Recently, SPC25 was upregulated in hepatocellular carcinoma and breast cancer, and was associated with poor overall survival (Wang et al., 2019; Yang et al., 2020). Furthermore, SPC25 was discovered to be an effective diagnostic marker for distinguishing between normal and HCC tissues. Upregulation of SPC25 in lung adenocarcinoma can increase cancer stem cell characteristics and indicate poor survival in patients with lung adenocarcinoma. In lung adenocarcinoma, knocking down SPC25 harmed cancer stem cell characteristics (J. Chen et al., 2018). SPC25 knockdown was also found to increase prostate cancer cell death in a new study (Cui et al., 2018).

The expression pattern and prognostic value of SPC25 in COAD were investigated using the GEPIA and TCGA databases in this study. We found that SPC25 at mRNA level was upregulated in several types of cancers using GEPIA database and firebrowse webpage in comparison to the normal tissues. The highest expression was found to be in COAD. These findings could point to SPC25's oncogenic function in colorectal cancer. More research is needed to determine the molecular involvement of SPC25 in CRC.

Using TCGA RNA sequencing data, the potential prognostic significance of SPC25 was assessed in this work. According to the results, SPC25 expression was observed to be higher in stages III&IV than in stages I&II. SPC25 levels were similarly higher in N2 stage COAD samples than in N0 stage COAD samples, according to

these findings. Patients with COAD who had low SPC25 expression had a longer OS free survival time than those who had high SPC25 expression, according to a Kaplan Meier analysis. To the best of our knowledge, this is the first study to show that SPC25 is implicated in COAD prediction.

Previously, SPC25 knockdown decreased cell proliferation and resulted in a decrease in the number of PCa cells in the S phase and an increase in the number of cells in the G2/M phase, according to the results of the SPC25 loss of function experiment. Furthermore, knocking down SPC25 enhanced PCa apoptosis. SPC25 has several functional roles in regulating cell proliferation, apoptosis, invasion, the involvement of tissue factor in cancer, transforming growth factor signaling, and the Sumoylation pathway in PCa, according to bioinformatics studies (Cui et al., 2018).

5. Conclusion

In conclusion, the current study's findings imply that SPC25 is a predictive and diagnostic biological marker for COAD patients, and that it may have a unique oncogenic role in colon cancer.

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