Int J Hum Genet, 21(3): 137-143 (2021)
PRINT: ISSN 0972-3757 ONLINE: ISSN 2456-6330
DOI: 10.31901/24566330.2021/21.03.777

Investigation of *SP1* and *SP3* Expressions in Colorectal Cancer Carcinogenesis

Turkan Gurer[1,*], Ibrahim Al-Doori[1], Alper Aytekin[2] and Deniz Mihcioglu[3]

[1]Department of Biology, Faculty of Art and Science, Gaziantep University, 27310, Gaziantep, Turkey
[2]Department of General Surgery, School of Medicine, Gaziantep University, 27310, Gaziantep, Turkey
[3]Department of Nutrition and Dietetics, Faculty of Health Science, SANKO University, 27090, Gaziantep, Turkey

KEYWORDS Colorectal Tumour. Gene Expression. *Specificity Protein 1*. *Specificity Protein 3*. Transcription Factors

ABSTRACT The study aimed to determine the expressions of *SP1* and *SP3* and the clinical-pathological characteristics of patients, and the correlation between the expressions of *Specificity Protein 1 (SP1)* and *Specificity Protein 3 (SP3)* in colorectal cancer (CRC). In this study, tumour and adjacent non-tumour tissue samples were obtained from 41 individuals with CRC. *SP1* and *SP3* expressions were performed using Quantitative Real-Time Polymerase Chain Reaction (RT-qPCR). According to the results of our study, there was no statistically significant difference in *SP1* and *SP3* expression levels between tumour tissues and non-tumour tissues ($p>0.05$), as well as no association with clinical-pathological features of patients. In addition, a high positive correlation was found between the expressions of *SP1* and *SP3* genes in CRC ($p=0.01$). Consequently, it can be said that there is a correlation between *SP1* and *SP3* expressions, but *SP1* and *SP3* expressions are not related to CRC carcinogenesis.

INTRODUCTION

According to the data of global cancer statistics, over 19 million new cancer cases and 10 million cancer-associated deaths were detected in 2020 and approximately 1.9 million of these cases were colorectal cancer (CRC) cases (Sung et al. 2021). It has been reported that the incidence of CRC has doubled worldwide in the last 27 years (Ye et al. 2020). It was estimated that the incidence of colon and rectum cancer might increase to over ninety percent in young ages by 2030. Although the reason for the increase in the incidence of the disease is unknown, it is thought that these young patients with CRC are related to hereditary CRC syndromes. The progression of CRC is influenced by various factors related to age, environmental and genetic factors. Familial colon cancer in first-degree relatives doubles the incidence of colon cancer compared to the general population. Besides these, sex, obesity, dietary habits (for example, processed meat and a diet lacking in fibre), tobacco use, Crohn's disease, diabetes mellitus, insulin resistance, ulcerative colitis are the other associated factors with CRC (Thanikachalam and Khan 2019). Transcription factors that are in protein structure bind to specific DNA sequences, and play a major role in modulating of gene expression and important functions at cellular mechanisms (Lambert et al. 2018). There are many transcription families with different DNA binding domains (Inukai et al. 2017). One of them is the Specificity Protein (SP) transcription factor family and SP factors are divided into two groups of SP1-SP4 and SP5-SP9. While SP1-SP4 is quite similar, SP5-SP9 is significantly different. There are N-terminal transactivation domains in SP1-4. Unlike other SP family members, SP includes C terminal multimerisation domains and these domains provide high activation of promoters and have multiple SP regions (Sankpal et al. 2011; Beishline and Azizkhan-Clifford 2015). *SP1*, which is similar to Cys2His2 type zinc finger and Kruppel-like factors, is among the basic members of the SP transcription family (O'Connor et al. 2016). In humans, the *SP1* gene is located in the 12th chromosome (Vellingiri et al. 2020). The SP1 family transcription factors bind to the GGGGCGGGG consensus sequence known as GC boxes (Huang et al. 2020). SP1 as-

[*]*Address for correspondence:*
Department of Biology,
Faculty of Art and Science,
Gaziantep University,
27310, Gaziantep, Turkey
Telephone: +90 532 505 13 92,
*E-mail:*turkanayte@hotmail.com,
taytekin@gantep.edu.tr

sists with the transcription and regulation of many cellular genes as well as sustaining the continuity of basal transcription (Beishline and Azizkhan-Clifford 2015). Post-translational modifications, for example, acetylation, phosphorylation, sumoylation, O-linked glycosylation, ubiquitylation and proteolytic cutting, moderate the activity of SP1 and accordingly SP1 can act as an activator or a repressor (Tummala et al. 2020; Vellingiri et al. 2020). Despite SP1 and SP3 proteins being recognised to regulate transcription, both positively and negatively, it suggests that the content of chromatin is a significant element in the distinctive connection of SP1 and SP3 to the regulator regions of genes (Cheng et al. 2015). SP3 is quite similar to SP1 compared to other family members. The *SP3* gene is located in the 2nd chromosome (Tapias et al. 2005). SP3 includes a domain located at the N-terminal, which in some cases has a suppressive effect (Beishline and Azizkhan-Clifford 2015). SP1 and SP3 both accelerate and inhibit the promoters of genes taking place in differentiation, progression of cell cycle and tumorigenesis (Freiman and Tjian 2002). Studies have shown that *SP1* and *SP3* levels are mainly more than in cancer cells than in normal cells. SP1 was highly expressed in many tumours and an unfavourable prognostic factors for patient survival (Hedrick et al. 2016). In previous studies, SP1 levels were higher in thyroid, gastric, breast, hepatocellular, pancreatic, and lung cancers than normal cells (Davie et al. 2008; Chuang et al. 2009; Li and Davie 2010; Kong et al. 2010).

Objectives

The aim of the study was to determine *SP1* and *SP3* gene expression levels in CRC tissues and normal tissues. In addition, to evaluate the clinicopathological features of the patients in terms of *SP1* and *SP3* activity, and the correlation between the expressions of *SP1* and *SP3* genes in CRC tissues.

MATERIAL AND METHODS

Study Group

The study included a total of 41 patients diagnosed with CRC. Patients who did not take chemotherapy and radiotherapy were included in the study. Fresh tumour and adjacent non-tumour colon or rectum tissue samples were collected from the same patients at the Department of General Surgery, Faculty of Medicine, Gaziantep University, and tissue samples were stocked in RNA later (Thermo Fisher Scientific) at -80 °C until RNA isolation. The research verification was received from the local ethics commission of Gaziantep University and the study was followed out in suitability with the Declaration of Helsinki (ethical approval number: 2017/191). Confirmation of participants have been taken by informed consent form in the study.

RNA Isolation

The total RNA was extracted from tumour and adjacent non-tumour tissues using the Pure Link RNA Mini Kit (Thermo Fisher Scientific, 12183018A) and following the instructions of the supplier. The quality and purity of extracted RNAs were measured by a nanodrop spectrophotometer (NanoDrop, Maestrogen) based on absorbance density in 260 nm/280 nm and extracted RNAs were stocked at -80 °C.

cDNA Synthesis

Single-stranded cDNA synthesis was performed from 30 ng/µl of RNA with a High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific, 4368814) conforming to the supplier's instructions. Reverse transcriptase random primers were used to amplify all RNA. RNA samples (10 µl) were performed in 20 µl reaction mixtures containing 2 µl 10 X RT Buffer, 2 µl 10 X RT Random Primers, 0.8 µl 25 X dNTP Mix (100 mM), 1 µl RNaz Inhibitor, 1 µl Reverse Transcriptase, and 3.2 µl Nuclease-free H2O, using a thermal cycler (ProFlex PCR System, Applied Biosystems, USA). Reaction settings were as follows, that is, 25 °C for 10 minutes, 37 °C for 120 minutes, and 85 °C for 5 minutes. The produced cDNAs were kept at -20°C until Quantitative Real-Time Polymerase Chain Reaction (RT-qPCR).

Quantitative Real-time Polymerase Chain Reaction (RT-qPCR)

RT-qPCR is an effective method that provides sensitive and specific quantification of nucleic

acids (Chapman and Waldenström 2015). The general steps performed in the RT-qPCR can be listed as RNA isolation, cDNA Synthesis, and RT-qPCR (Nestorov et al. 2013). RT-qPCR was performed by Applied Biosystems (StepOne& StepOnePlus Real-Time PCR Systems) using the TaqMan Primer Probe (Thermo Fisher Scientific) for *SP1*, *SP3* and *GAPDH* (as housekeeping) genes. Final concentration 20 µl reaction mixtures included cDNA samples (2 µl), 10 µl 20X TaqMan Gene Expression master mix (Thermo Fisher Scientific, 4369016), 1 µl 2X TaqMan Gene Expression Assay for *SP1*, *SP3* and *GAPDH* genes, and 7 µl RNase-free water. The primer sequences required performing RT-qPCR are shown in Table 1. Reaction settings were 50 °C for 2 minutes, 95 °C for 10 minutes, 40 cycles of 95 °C for 15 seconds, and 60 °C for 1 minute. RT-qPCR was applied in duplicate and changes in levels of *SP1* and *SP3* expression detected making use of the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001).

Statistical Analysis

The data were exhibited as the mean ± standard deviation. Paired t-test was used to compare the *SP1* and *SP3* expression levels between tumour and non-tumour tissues. The relationship between clinicopathological factors and *SP1* and *SP3* gene expression levels were determined using the Chi-square test. The Spearman correlation test was performed to analyze the expression levels among *SP1* and *SP3* genes. Statistical significance was accepted at the $p<0.05$ level. The SPSS statistical software package version 22.0 (IBM Corporation, Armonk, NY, USA) was performed for analyses.

RESULTS

A total of 41 patients with CRC were studied to analyze the expression levels of *SP1* and *SP3*. 58.5 percent of the samples in the study were localised in the colon, and the remaining 41.5 percent in the rectum. Demographic and clinicopathological information of patients with the CRC are given in Table 2. The mean age of study group was 54.4 (with minimum of 26 years and maximum of 84 years). While 65.9 percent of these patients were men, 34.1 percent of the patients were women. Metastasis was observed in 24.4 percent of the patients, while lymph node involvement was detected in 43.9 percent of the cases. The mucosal and submucosal invasion was identified in 53.7 percent of these individuals, while the invasion of serosa and subserosa was detected in 46.3 percent of them.

Table 2: Demographic, clinical and pathological data of patients with CRC

Demographic, clinical and pathological parameters	*Variables*	*N*	*Percent*
Age	50≤	15	36.6
	50>	26	63.4
Gender	Female	14	34.1
	Male	27	65.9
Smoking	Yes	16	39
	No	25	61
Origin of Tissue	Colon	24	58.5
	Rectum	17	41.5
Stage of Cancer	I-II	22	53.7
	III-IV	19	46.3
Lymph node involvement	Positive	18	43.9
	Negative	23	56.1
Metastasis	Positive	10	24.4
	Negative	31	75.6

RT-qPCR were performed for determining the *SP1* and *SP3* gene expression levels in both tumour and non-tumour tissues. ΔCt values were calculated for each patient's tumour and non-tumour samples, and these values were compared with using the paired t test. In *SP1* and *SP3*, the values of ΔCt (means ± SD) were 3.173 ± 3.158 and 4.362 ± 2.947 in tumour tissues, and 3.103 ± 3.608 and 4.474 ± 3.479 in non-tumour

Table 1: List of primers in the RT-qPCR

Gene	*Forward primer*	*Reverse primer*
SP1	5'-GCGAGAGGCCATTTATGTGT-3'	5'-GGCCTCCCTTCTTATTCTGG-3'
SP3	5' -ATTCTGGAGAACGCCCTTTT-3'	5' -TATGTTTGGCAAGGTGGTCA-3'
GAPDH	5'-AATCCCATCACCATCTTC-3'	5'- AGGCTGTTGTCATACTTC-3'

tissues, respectively (Fig. 1). There was not any significant difference between tumour and adjacent non-tumour tissues for both *SP1* and *SP3* genes statistically (p=0.39 and p=0.223, respectively).

The expression levels of *SP1* and *SP3* genes were categorised according to $2^{-\Delta\Delta CT}$ values ($2^{-\Delta\Delta CT}$> 1; high, $2^{-\Delta\Delta CT}$<1; low) (Gurer et al. 2020) and compared with clinicopathological features of the study group. There were no statistically significant differences between both *SP1* and *SP3* expression levels and clinicopathological features such as age, gender, smoking status, origin of tissue, stage of cancer, lymph node involvement and metastasis (p>0.05) (Table 3).

The correlation between *SP1* and *SP3* genes expression levels was determined by Spearman correlation test. Results showed that there was a high positive correlation between *SP1* and *SP3*

Table 3: Association between the expression levels of *SP1* and *SP3* and clinicopathological parameters of patients with CRC

Clinicopathological parameters		*SP1 gene expression*			*SP3 gene expression*		
		Low	*High*	*p value*	*Low*	*High*	*p value*
Age	50≤	5	10	0.133	6	9	0.183
	50>	15	11		16	10	
Gender	Female	5	9	0.228	7	7	0.735
	Male	15	12		15	12	
Smoking	Yes	8	8	0.901	8	8	0.707
	No	12	13		14	11	
Origin of Tissue	Colon	13	11	0.412	14	10	0.476
	Rectum	7	10		8	9	
Stage of Cancer	I-II	9	13	0.278	11	11	0.613
	III-IV	11	8		11	8	
Lymph Node Involvement	Positive	10	8	0.443	10	8	0.829
	Negative	10	13		12	11	
Metastasis	Positive	5	5	1.0	4	6	0.469
	Negative	15	16		18	13	

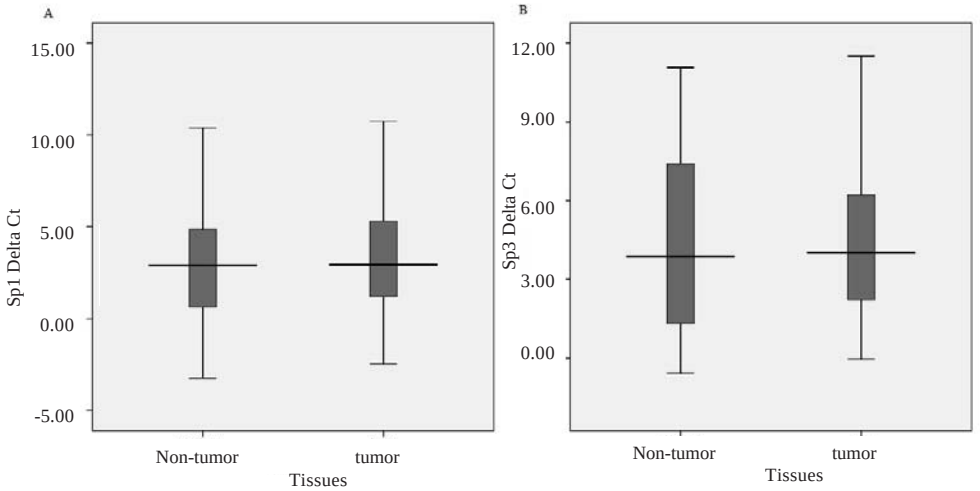

Fig. 1. The expression levels of *SP1* (A) and *SP3* (B) genes in tumour and non-tumour tissues of CRC patients. There was not any significant difference between tumour and non-tumour tissues for both *SP1* and *SP3* genes statistically
(p>0.05, Paired t test). Tumour: colorectal cancer tissue, Non-tumour: Adjacent healthy colorectal tissue
Source: Authors

gene expression levels in CRC patients (r=0.827, p=0.01) (Fig. 2).

DISCUSSION

CRC is the second-highest deadly cancer type over the world and the ranking as at the third place most widespread kind of cancer. Over nineteen million new cancer cases and 10 million cancer-related deaths were detected in 2020 (Sung et al. 2021). Environmental factors are as important as genetic factors that are effective in the development of CRC. Members of the SP family play an important role in many biological processes such as development, differentiation, carcinogenesis and apoptosis. SP1 and SP3 proteins interact with sequences in the promoter regions of genes (Vizcaíno et al. 2015). SP1 and SP3 is a transcription factor, which belongs to Kruppel-like family, one of the frequently seen one, and has many functions (Vellingiri et al. 2020). In literature, it was exhibited that *SP1* and *SP3* were overexpressed in various types of cancers and were prognostic biomarkers for cancer patients (Mansour 2020). Due to the important roles of *SP1* and *SP3* in biological processes, the relationship between *SP1* and *SP3* expressions and CRC were investigated in the present study. The researchers analysed expression levels of *SP1* and *SP3* genes in tumour and adjacent non-tumour tissues of 41 CRC patients while performing the RT-qPCR technique. As shown in Figure 1, both *SP1* and *SP3* gene expression levels did not have statistically significant differences between tumour and non-tumour tissues (p=0.39 and p=0.223, respectively). Besides, the researchers found that there was no correlation between the *SP1* and *SP3* expression levels and clinicopathological factors. Many different studies demonstrated that both up-regulation and down-regulation of *SP1* affects the transcription of several oncogenes

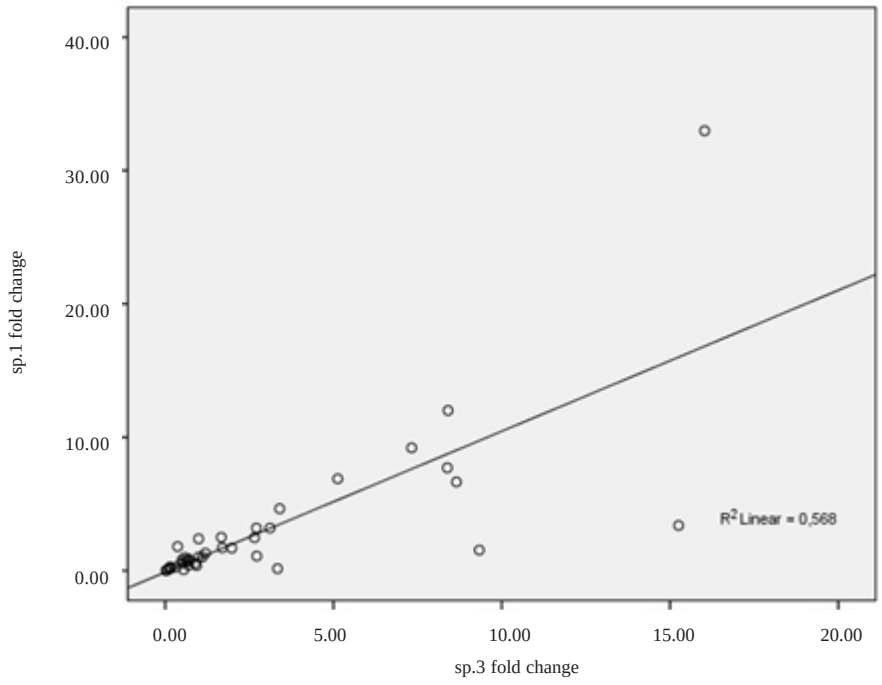

Fig. 2. Correlation between *SP1* and *SP3* genes expression levels in CRC patients. Data are presented as fold change of *SP1* and *SP3*. *SP1* and *SP3* expression levels were found to be highly positive in terms of correlation in CRC patients (p<0.05, Spearman correlation test)
Source: Authors

(Vellingiri et al. 2020) and the SP1 protein level was abnormal in different types of cancer (Vizcaíno et al. 2015). In a previous study, it was exhibited that the *SP1* gene was over expressed in human glioma cells and was also related with the prognosis of the disease (Guan et al. 2012). Yao et al. reported that unregulated level of *SP1* gene expression was related with high expression of vascular endothelial growth factor and also caused growth and progression of gastric carcinoma (Yao et al. 2004). It has been shown in a study on human gastric cancer that increased *SP1* gene expression may affect development and spread of cancer (Wang et al. 2003). In pancreatic carcinoma, it was presented that *SP1* increased the activity of NFATc2 and the interaction between *SP1* and NFATc2 was regulated by *TNF-alpha*, *integrin beta-3* and *c-FOS* genes (Malsy et al. 2019). Ye et al. showed that decreased *SP1* in gastric cell lines affect the SHIp2 expression, which has pro- or anti-tumour effects in different cancers, which may play a role in the development of the cancer (Ye et al. 2017). Additionally, in another research it was reported that SP1 protein level was overexpressed in ovarian cancer (Vellingiri et al. 2020). Huang et al. studied both *SP1* and *SP3* expressions in hepatocellular carcinoma and found that both transcription factors were upregulated (Huang et al. 2015). It was reported that transcriptional regulation of the *β4GalT3* gene is affected by different DNA binding cites of *SP1* and *SP3* in lung carcinoma and neuroblastoma (Tange et al. 2019). In another research, it was demonstrated that *SP3* had high expression in the breast carcinoma cells (Mansour 2020). Besides, in the present study, the researchers determined that *SP1* and *SP3* expression levels were found to be highly positive in terms of correlation in CRC patients (r=0.827, p=0.01). *SP1* and *SP3* genes have a highly similar affinity, as both recognise and bind to the same DNA sites. It was reported that close grades of *SP1* and *SP3* are modulated expression of genes. *SP1* and *SP3* genes were regulated by post-transcriptional modifications and by interaction with different protein molecules (Li and Davie 2010; Vizcaíno et al. 2015; Malsy et al. 2019).

In this study, the researchers have found no significant association between *SP1* and *SP3* with CRC. *SP1* and *SP3* expressions may be modulated genetic and epigenetic factors (Wang et al. 2003). New studies are required to exactly exhibit the effect of *SP1* and *SP3* on CRC carcinogenesis.

CONCLUSION

Consequently, in the present study, it was concluded that there is no relationship between *SP1* and *SP3* expression levels and CRC ($p>0.05$). Also, no correlation was found between *SP1* and *SP3* expression levels and the clinicopathological features of patients with CRC ($p>0.05$). Moreover, a high positive correlation between *SP1* and *SP3* gene expression levels was observed in CRC ($r=0.827$, $p=0.01$).

RECOMMENDATIONS

Considering that the activity of *SP1* and *SP3* is regulated by post-transcriptional mechanisms, in the future, it will be beneficial to carry out studies, which reveal the roles of these mechanisms, *SP1* and *SP3* protein levels, and as well as post-translational modifications with epigenetic factors on CRC.

ACKNOWLEDGEMENT

Research was funded by the Scientific Research Projects Managements Unit of Gaziantep University (Grant number: FEF.YLT.17.20).

ABBREVIATIONS

- β4GalT3: β4-galactosyltransferase 3
- CRC: colorectal cancer
- GAPDH: glyceraldehyde-3-phosphate dehydrogenase
- NFATc2: nuclear factor of activated T cells 2
- RT-qPCR: Quantitative Real-Time Polymerase Chain Reaction
- SP1: Specificity Protein 1
- SP3: Specificity Protein 3
- TNF-alpha: Tumour Necrosis factor-alpha

REFERENCES

Beishline K, Azizkhan Clifford J 2015. SP1 and the 'hallmarks of cancer'. *FEBS J*, 282: 224-258.

Chapman JR, Waldenström J 2015. With reference to reference genes: A systematic review of endogenous controls in gene expression studies. *PloS One*, 10: e0141853.
Cheng D, Zhao Y, Wang S et al. 2015. Human telomerase reverse transcriptase (hTERT) transcription requires Sp1/Sp3 binding to the promoter and a permissive chromatin environment. *J BiolChem*, 290: 30193-30203.
Chuang JY, Wu CH, Lai MD et al. 2009. Overexpression of Sp1 leads to p53 dependent apoptosis in cancer cells. *Int J Cancer*, 125: 2066-2076.
Davie JR, He S, Li L et al. 2008. Nuclear organization and chromatin dynamics–Sp1, Sp3 and histone deacetylases. *Adv Enzyme Regul*, 48: 189-208.
Freiman RN, Tjian R 2002. A glutamine-rich trail leads to transcription factors (Perspectives: neurodegeneration). *Science*, 296: 2149-2151.
Guan H, Cai J, Zhang N et al. 2012. Sp1 is upregulated in human glioma, promotes MMP 2 mediated cell invasion and predicts poor clinical outcome. *Int J Cancer*, 130: 593-601.
Gurer T, Aytekin A, Alahdab Y 2020. *Arf6* expression in the tissues of patients with colorectal cancer. *Int J Hum Genet*, 20: 132-137.
Hedrick E, Cheng Y, Jin U-H et al. 2016. Specificity protein (Sp) transcription factors Sp1, Sp3 and Sp4 are non-oncogene addiction genes in cancer cells. *Oncotarget*, 7: 22245.
Huang C, Zhao J, Zhu Y 2020. Drug eluting stent targeting Sp1 Attenuated restenosis by engaging YAP mediated vascular smooth muscle cell phenotypic modulation. *J Am Heart Assoc*, 9: e014103.
Huang Z, Huang L, Shen S et al. 2015. Sp1 cooperates with SP3 to upregulate MALAT1 expression in human hepatocellular carcinoma. *Oncol Rep*, 34: 2403-2412.
Inukai S, Kock KH, Bulyk ML 2017. Transcription factor–DNA binding: Beyond binding site motifs. *Curr Opin Genet Dev*, 43: 110-119.
Kong LM, Liao CG, Fei F et al. 2010. Transcription factor Sp1 regulates expression of cancer associated molecule CD147 in human lung cancer. *Cancer Sci*, 101: 1463-1470.
Lambert SA, Jolma A, Campitelli LF et al. 2018.The human transcription factors. *Cell*, 172: 650-665.
Li L, Davie JR 2010. The role of Sp1 and Sp3 in normal and cancer cell biology. *Ann Anat*, 192: 275-283.
Livak KJ, Schmittgen TD 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods*, 25: 402-408.
Malsy M, Graf B, Almstedt K 2019. The active role of the transcription factor Sp1 in NFATc2-mediated gene regulation in pancreatic cancer. *BMC Biochem*, 20: 1-11.
Mansour MA 2020. Sp3 is associated with migration, invasion, and Akt/PKB signalling in MDA MB 231 breast cancer cells. *J Biochem Mol Toxicol*, e22657.
Nestorov J, Matic G, Elakovic I et al. 2013. Gene expression studies: How to obtain accurate and reliable data by quantitative real-time RT PCR. *J Med Biochem*, 32: 325-338.
O'Connor L, Gilmour J, Bonifer C 2016. Focus: Epigenetics: The role of the ubiquitously expressed transcription factor Sp1 in tissue-specific transcriptional regulation and in disease. *Yale J Biol Med*, 89: 513.
Sankpal TU, Goodison S, Abdelrahim M et al. 2011. Targeting Sp1 transcription factor in prostate cancer therapy. *Med Chem*, 7: 518-525.
Sung H, Farley J, Siegel RL et al. 2021. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*, 1-41.
Tange R, Tomatsu T, Sato T 2019. Transcription of human β4-galactosyltransferase 3 is regulated by differential DNA binding of Sp1/Sp3 in SH-SY5Y human neuroblastoma and A549 human lung cancer cell lines. *Glycobiology*, 29: 211-221.
Tapias A, Monasterio P, Ciudad CJ et al. 2005. Characterization of the 52 -flanking region of the human transcription factor Sp3 gene. *BBA-Gene Structure and Expression*, 1730: 126-136.
Thanikachalam Ki, Khan G 2019. Colorectal cancer and nutrition. *Nutrients*, 11: 164.
Tummala H, Walne AJ, Bewicke-Copley F et al. 2020. A frameshift variant in specificity protein 1 triggers superactivation of Sp1-mediated transcription in familial bone marrow failure. *PNAS*, 117: 17151-17155.
Vellingiri B, Iyer M, Devi Subramaniam M et al. 2020. Understanding the role of the transcription factor Sp1 in ovarian cancer: From theory to practice. *Int J Mol Sci*, 21: 1153.
Vizcaíno C, Mansilla S, Portugal J 2015. Sp1 transcription factor: A long-standing target in cancer chemotherapy. *Pharmacology and Therapeutics*, 152: 111-124.
Wang L, Wei D, Huang S et al. 2003. Transcription factor Sp1 expression is a significant predictor of survival in human gastric cancer. *Clin Cancer Res*, 9: 6371-6380.
Yao JC, Wang L, Wei D et al. 2004. Association between expression of transcription factor Sp1 and increased vascular endothelial growth factor expression, advanced stage, and poor survival in patients with resected gastric cancer. *Clin Cancer Res*, 10: 4109-4117.
Ye P, Xi Y, Huang Z et al. 2020. Linking obesity with colorectal cancer: Epidemiology and mechanistic insights. *Cancers*, 12: 1408.
Ye Y, Qian XY, Xiao MM et al. 2017. Decreased Sp1 expression mediates downregulation of SHIP2 in gastric cancer cells. *Int J Mol Sci*, 18: 220.

Paper received for publication in February, 2021
Paper accepted for publication in March, 2021