Investigation of Clinical Potential Roles of *JS-2* Gene in Pathogenesis of Colorectal Cancer

Negin Akbarieh [1], Saeid Ghorbian [1, *] and Shahriar Hashemzadeh [2]

[1]Department of Molecular Genetics, Ahar Branch, Islamic Azad University, Ahar, Iran
[2]Department of Generals and Vascular Surgery, Tabriz University of Medical Sciences, Tabriz, Iran

Corresponding author: Department of Molecular Genetics, Ahar Branch, Islamic Azad University, Ahar, Iran. Email: ghorbian20@yahoo.com

Abstract

Background: Colorectal cancer (CRC) is the third prevalent adenocarcinoma type with a high mortality rate worldwide. The *JS-2* is a novel gene, which first was described in esophageal squamous cell carcinoma (ESCC). To date, several studies have investigated the role of *JS-2* in the pathogenesis of CRC.
Objectives: The purpose of our survey was to assess the *JS-2* gene expression level in subjects with CRC in the Iranian population.
Methods: The current case-control study was conducted on one hundred fresh tissues, consisting of 50 cancerous colorectal adenocarcinomas and 50 adjacent non-cancerous tissues. The relative *JS-2* gene expression level was assessed using quantitative real-time polymerase chain reaction (qReal-Time PCR) method.
Results: Our findings showed that the *JS-2* gene was significantly over-expressed in cancerous tissues compared with the adjacent non-cancerous tissues (P = 0.037). In addition, overexpression of *JS-2* was significantly related to the tumor differentiation (P = 0.047) and tumor stage (P = 0.036).
Conclusions: The results suggested that the *JS-2* gene played an important role in the progression of CRC and may be applied as a novel prognostic biomarker in patients with CRC.

Keywords: CRC, Gene Expression, *JS-2* Gene, Colorectal Cancer

1. Background

Colorectal cancer (CRC) is the third adenocarcinoma type, which has a high prevalence and mortality rate worldwide. Fifty percent of patients with CRC are diagnosed in the last stage of disease (1). The molecular pathogenesis of CRC is complicated and comprised of several regulatory processes such as gene rearrangements, chromatin remodeling, epigenetic, genetic alterations, and gene expression alterations (2-6). It is believed that oncogenes and tumor suppressor genes (TSG) are related to the molecular pathogenesis of CRC (7-9). Nevertheless, the exact molecular genetic mechanism of CRC tumorigenesis process remained unclear (10). A novel biomolecular prognostic and diagnostic marker is required to verify the molecular carcinogenic mechanisms and improvement of medicinal protocols in the CRC management (11). A previous investigation performed on the esophageal squamous cell carcinoma (ESCC) tissues showed that a gene copy number was increased in the 5p15.2 region (12). Two novel mRNAs have been investigated in the ESCC tissues and other types of malignancies, which was located in the

5' upstream of the Delta-catenin gene (2). The *JS-2* is one of the novel genes, which was analyzed using qReal-Time PCR method. The findings revealed that the *JS-2* gene was overexpressed in 18% of ESCC tissues (11).

The *JS-2* encodes a special protein containing 233 amino acids, which does not indicate the amino acid sequence compared with other recognized protein sequences in Swiss-Prot (http://au.expasy.org/sprot/). Although the overexpression of the *JS-2* gene was observed in the ESCC and CRC cancerous tissues, future studies are required to reveal the potential clinical roles of *JS-2* gene expression level in other human disorders (11, 12).

2. Objectives

Given the *JS-2* gene expression pattern has not been effectively explored in different human malignancies, we aimed to investigate the potential role of the *JS-2* in patients with CRC. This is the first study to investigate the *JS-2* gene expression level in North Western Iranian patients with CRC.

3. Methods

3.1. Specimens Selection

In this case-control study, we evaluated 100 fresh colorectal tissue samples, including 50 cancerous adenocarcinoma and 50 adjacent non-neoplastic tissues, which were obtained from of the patients with CRC undergoing colorectal surgery or colonoscopy, who had been referred to the Imam Reza Hospital, Tabriz University of Medical Sciences, from April 2015 to March 2016. The inclusion criteria consisted of the patients that their malignancy was diagnosed and confirmed by the histopathological tests. The pathological board re-assessed all specimens and determined grades and classification according to the World Health Organization (WHO) criteria (13). The exclusion criteria were the subjects who underwent chemotherapy and radiotherapy regimen before the surgery or colonoscopy. In addition, we excluded the patients with CRC having other malignancies, systemic diseases, and diabetes disorders. Ethics and Human Rights Committee in the pathology center approved the current research, and all subjects signed written informed consents.

3.2. RNA Isolation and Complementary DNA Synthesis

All tissue specimens transferred into the RNA later solution (Qiagen, Germany) and stored at -80°C before RNA isolation. Total RNA was purified from tissues using TRIzol reagent (Molecular Research Center Inc., Cincinnati, OH, USA) and treated with DNase I enzyme (Thermo Scientific™, USA), according to the guideline. To confirm the quality and quantity of separated RNA, we have used 1% agarose gel electrophoresis and NanoDrop instruments (Thermo Scientific™, Wilmington, Denmark). Reverse transcription reaction was performed using 1 μg of total RNA in a 20 μL final reaction volume. RNA was converted to cDNA using cDNA Synthesis Kit (Fermentas, Burlington, Canada) with random hexamer primer, according to the manufacturer's protocol. All cDNA samples were separately diluted to 50 ng/μL in order to obtain a uniform concentration specimen for qReal-Time PCR experiments. Conventional PCR was done with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) primers to ensure the proper synthesis of cDNA samples.

3.3. Quantitative Real-Time PCR

Amplification was carried out in the triplicate form using specific primer pairs (11) (Table 1) and SYBR Green-I dye Master Mix (Bioneer, Korea) by the Rotor-Gene™ 6000 systems (Corbett Research, Australia) according to the manufacturer's guidance. The GAPDH gene was used as the housekeeping gene to normalize gene expression. In all reaction, we included 150 ng cDNA, SYBR Green-I dye Master Mix, and 10 pM of each primer for JS-2 and GAPDH in a 25 μL final volume. Real-time PCR conditions were started with an initial denaturation step (94°C, 5 minutes), followed by 35 cycles of denaturation (94°C, 10 seconds), annealing temperatures (59°C for JS-2 and 62°C for GAPDH) for 10 seconds, followed by extension cycle at 72°C for the 20 seconds. The $2^{-\triangle\triangle ct}$ method was used to measure the relative quantification of gene expression changes.

3.4. Statistical Analysis

The statistical analysis was performed using SPSS version 25.0 (SPSS Inc., Chicago, IL, USA). To compare the JS-2 gene expression change between cancerous and adjacent non-cancerous tissues in subjects with CRC and clinical features, the student t-test and chi-square test were used. The P < 0.05 was considered statistically significant.

4. Results

The mean age of the patients with CRC was 50.38 $\pm$ 6.12 years (ranging between 45 and 74 years), consisting of 33 (66%) males and 17 (34%) females. We evaluated the JS-2 gene expression change whether significant differences are found between cancerous and non-cancerous tissues of patients with CRC. Our findings showed that the JS-2 gene was over-expressed in cancerous tissues compared with the adjacent non-cancerous ones (P = 0.037), (Figure 1).

In addition, we evaluated the correlation between JS-2 gene expression and clinical parameters in patients with CRC. To assess the correlation between JS-2 gene expression and CRC clinicopathological characteristics, we divided 50 CRC samples into high and low expression according to median $\triangle$ct (Table 2). The median amount $\geq$ 0.93 of JS-2 gene expression was considered to be the over-expression group and the median amount < 0.93 considered to be the low-expression group. Overexpression of JS-2 gene was associated with the tumor differentiation (P = 0.047) and tumor stage (P = 0.036), (Table 2). However, the JS-2 expression did not correlate with other characteristics such as age (P = 0.774), sex (P = 0.520), tumor site (P = 0.696), and tumor size (P = 0.851), (Table 2).

**Table 1.** Primer Sequences of JS-2 and GAPDH Genes Used in qReal-Time PCR

Genes/Status		Primers Sequence	Amplicon Size, bp
JS-2			136
	Forward	5'-ATGCACGAGTTATTGCTTGC-3'	
	Reverse	5'-ATCGATGTACAGGGCCTCTT-3'	
GAPDH			152
	Forward	5'-AGGGCTGCTTTTAACTCTGGT-3'	
	Reverse	5'-CCCCACTTGATTTTGGAGGGA-3'	

Table 2. The Correlation Between of *JS-2* Gene Expression Change and Clinicopathological Features of CRC Patients[a]

Clinical Parameters	JS-2 Gene Expression Change		P Value
	Low Expression Median Amount < 0.93	High Expression Median Amount ≥ 0.93	
Gender			0.520
Male	7 (14)	26 (52)	
Female	5 (10)	12 (24)	
Age			0.774
< 50	11 (22)	10 (20)	
> 50	14 (28)	15 (30)	
Tumor site			0.696
Colon	15 (30)	13 (26)	
Rectum	13 (26)	9 (18)	
Tumor size, mm			0.851
< 50	15 (30)	20 (40)	
> 50	6 (12)	9 (18)	
Histological grade			0.047
Well	2 (4)	36 (72)	
Moderate + poor	3 (6)	9 (18)	
T staging			0.036
T1 + T2	6 (12)	25 (50)	
T3 + T4	9 (18)	10 (20)	

[a]Values are expressed as No. (%).
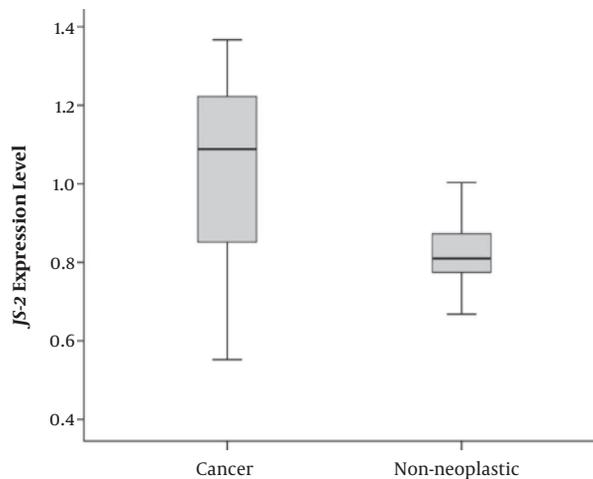
Figure 1. The relative gene expression change of *JS-2* in cancerous and adjacent non-cancerous colorectal tissues

5. Discussion

This is the first study to evaluate the *JS-2* gene expressions change in Iranian patients with CRC. Consistent with other studies, the results of the current study revealed that the *JS-2* gene expression alteration may be implicated in the pathogenesis of CRC (11). The findings showed a significant difference in the *JS-2* gene expression pattern between cancerous tissues compared with the adjacent non-cancerous tissues (11).

Previous studies reported a new section at the 5p15.33 cytogenetic region, which contains *JS-2* gene, involving in the different malignancies such as bladder, thyroid, ESCC, and lung cancer (14-17). Fatima et al. investigated the mRNA expression levels of *JS-2* in 20 cases of ESCC and revealed that it was upregulated and downregulated in 14% and 9% of cases, respectively (18). Lam et al. (11) investigated the *JS-2* gene expression in 176 colorectal tissues consisting of 116 Adenocarcinomas, 30 adenomas, and 30 non-neoplastic tissues as well as two colorectal cancer cell and benign cell lines, including SW480 and SW48.

The findings revealed that the relative gene expression levels of *JS-2* were increased in mucinous adenocarcinoma compared with the non-mucinous adenocarcinoma. In addition, colorectal cancer cell line showed a lower expression of *JS-2* compared with the benign cell line (11). The results suggested that the *JS-2* gene was upregulated and may

have a profound role in the pathogenesis of CRC (11, 18). In addition, Bohm et al. described several TSGs located in 5p13-12 region, which were implicated in carcinogenesis of the solid tumors and were inactivated during tumorigenesis (19). Although the findings of several investigations revealed that the *JS-2* gene expression changes involved in the development of different malignancies, the precise roles remained unclear in CRC carcinogenesis (14-17).

These data proposed that the *JS-2* gene was overexpressed in the higher advanced tumors without affecting the healthy tissue. However, since *JS-2* plays an anti-growth activity, it is conceivable that the *JS-2* gene overexpression in higher advanced carcinomas may be a feedback to intensify signaling for TSG arising from gene control processes recognizing the growing malignant cells. In the present investigation, *JS-2* gene expression change correlated with the histological grade and T staging of clinical features. However, our findings are compatible with the other results reported and may be implicated in CRC pathogenesis (11).

In conclusion, our findings revealed that the *JS-2* gene may be involved in the progression of CRC. In addition, we suggested that the *JS-2* gene may be implemented as an important potential prognostic biomarker for CRC patients.

Acknowledgments

Footnotes

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2017. *CA Cancer J Clin*. 2017;**67**(1):7–30. doi: 10.3322/caac.21387. [PubMed: 28055103].

2. Fearon ER. Molecular genetics of colorectal cancer. *Annu Rev Pathol*. 2011;**6**:479–507. doi: 10.1146/annurev-pathol-011110-130235. [PubMed: 21090969].

3. Chen W, Swanson BJ, Frankel WL. Molecular genetics of microsatellite-unstable colorectal cancer for pathologists. *Diagn Pathol*. 2017;**12**(1):24. doi: 10.1186/s13000-017-0613-8. [PubMed: 28259170]. [PubMed Central: PMC5336657].

4. Carethers JM, Jung BH. Genetics and genetic biomarkers in sporadic colorectal cancer. *Gastroenterology*. 2015;**149**(5):1177–1190 e3. doi: 10.1053/j.gastro.2015.06.047. [PubMed: 26216840]. [PubMed Central: PMC4589489].

5. Ballester V, Rashtak S, Boardman L. Clinical and molecular features of young-onset colorectal cancer. *World J Gastroenterol*. 2016;**22**(5):1736–44. doi: 10.3748/wjg.v22.i5.1736. [PubMed: 26855533]. [PubMed Central: PMC4724605].

6. Weisenberger DJ, Liang G, Lenz HJ. DNA methylation aberrancies delineate clinically distinct subsets of colorectal cancer and provide novel targets for epigenetic therapies. *Oncogene*. 2018;**37**(5):566–77. doi: 10.1038/onc.2017.374. [PubMed: 28991233].

7. Song K, Su W, Liu Y, Zhang J, Liang Q, Li N, et al. Identification of genes with universally upregulated or downregulated expressions in colorectal cancer. *J Gastroenterol Hepatol*. 2018. doi: 10.1111/jgh.14529. [PubMed: 30395690].

8. Slattery ML, Herrick JS, Mullany LE, Samowitz WS, Sevens JR, Sakoda L, et al. The co-regulatory networks of tumor suppressor genes, oncogenes, and miRNAs in colorectal cancer. *Genes Chromosomes Cancer*. 2017;**56**(11):769–87. doi: 10.1002/gcc.22481. [PubMed: 28675510]. [PubMed Central: PMC5597468].

9. Ayani V, Ghorbian S, Abolghasem Fakhri M. [Evaluating the diagnostic value of Argonaute 2 gene expression changes in patients with colorectal cancer]. *Ebnesina J Med*. 2018;**20**(3):47–54. Persian.

10. Hahn MM, de Voer RM, Hoogerbrugge N, Ligtenberg MJ, Kuiper RP, van Kessel AG. The genetic heterogeneity of colorectal cancer predisposition - guidelines for gene discovery. *Cell Oncol (Dordr)*. 2016;**39**(6):491–510. doi: 10.1007/s13402-016-0284-6. [PubMed: 27279102]. [PubMed Central: PMC5121185].

11. Lam AK, Gopalan V, Nassiri MR, Kasim K, Dissanayake J, Tang JC, et al. Altered JS-2 expression in colorectal cancers and its clinical pathological relevance. *Mol Oncol*. 2011;**5**(5):475–81. doi: 10.1016/j.molonc.2011.06.003. [PubMed: 21802380]. [PubMed Central: PMC5528300].

12. Kwong D, Lam A, Guan X, Law S, Tai A, Wong J, et al. Chromosomal aberrations in esophageal squamous cell carcinoma among Chinese: Gain of 12p predicts poor prognosis after surgery. *Hum Pathol*. 2004;**35**(3):309–16. doi: 10.1016/j.humpath.2003.10.020. [PubMed: 15017586].

13. Li ZS, Li Q. [The latest 2010 WHO classification of tumors of digestive system]. *Zhonghua Bing Li Xue Za Zhi*. 2011;**40**(5):351–4. Chinese. [PubMed: 21756837].

14. Smallridge RC, Marlow LA, Copland JA. Anaplastic thyroid cancer: Molecular pathogenesis and emerging therapies. *Endocr Relat Cancer*. 2009;**16**(1):17–44. doi: 10.1677/ERC-08-0154. [PubMed: 18987168]. [PubMed Central: PMC2829440].

15. Kang JU, Koo SH, Kwon KC, Park JW, Kim JM. Gain at chromosomal region 5p15.33, containing TERT, is the most frequent genetic event in early stages of non-small cell lung cancer. *Cancer Genet Cytogenet*. 2008;**182**(1):1–11. doi: 10.1016/j.cancergencyto.2007.12.004. [PubMed: 18328944].

16. Nymark P, Wikman H, Ruosaari S, Hollmen J, Vanhala E, Karjalainen A, et al. Identification of specific gene copy number changes in asbestos-related lung cancer. *Cancer Res*. 2006;**66**(11):5737–43. doi: 10.1158/0008-5472.CAN-06-0199. [PubMed: 16740712].

17. Zheng M, Simon R, Mirlacher M, Maurer R, Gasser T, Forster T, et al. TRIO amplification and abundant mRNA expression is associated with invasive tumor growth and rapid tumor cell proliferation in urinary bladder cancer. *Am J Pathol*. 2004;**165**(1):63–9. doi: 10.1016/S0002-9440(10)63275-0. [PubMed: 15215162]. [PubMed Central: PMC1618551].

18. Fatima S, Chui CH, Tang WK, Hui KS, Au HW, Li WY, et al. Transforming capacity of two novel genes JS-1 and JS-2 located in chromosome 5p and their overexpression in human esophageal squamous cell carcinoma. *Int J Mol Med*. 2006;**17**(1):159–70. doi: 10.3892/ijmm.17.1.159. [PubMed: 16328025].

19. Bohm M, Kleine-Besten R, Wieland I. Loss of heterozygosity analysis on chromosome 5p defines 5p13-12 as the critical region involved in tumor progression of bladder carcinomas. *Int J Cancer*. 2000;**89**(2):194–7. [PubMed: 10754499].