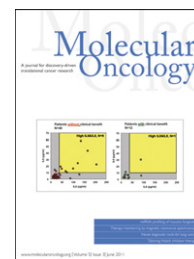


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Altered *JS-2* expression in colorectal cancers and its clinical pathological relevance

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ABSTRACT

JS-2 is a novel gene located at 5p15.2 and originally detected in primary oesophageal cancer. There is no study on the role of *JS-2* in colorectal cancer. The aim of this study is to determine the gene copy number and expression of *JS-2* in a large cohort of patients with colorectal tumours and correlate these to the clinicopathological features of the cancer patients. We evaluated the DNA copy number and mRNA expression of *JS-2* in 176 colorectal tissues (116 adenocarcinomas, 30 adenomas and 30 non-neoplastic tissues) using real-time polymerase chain reaction. *JS-2* expression was also evaluated in two colorectal cancer cell lines and a benign colorectal cell line. *JS-2* amplification was noted in 35% of the colorectal adenocarcinomas. Significant differences in relative expression levels for *JS-2* mRNA between different colorectal tissues were noted ($p = 0.05$). Distal colorectal adenocarcinoma had significantly higher copy number than proximal adenocarcinoma ($p = 0.005$). The relative expression level of *JS-2* was different between colonic and rectal adenocarcinoma ($p = 0.007$). Mucinous adenocarcinoma showed higher *JS-2* expression than non-mucinous adenocarcinoma ($p = 0.02$). Early T-stage cancers appear to have higher *JS-2* copy number and lower expression of *JS-2* mRNA than later stage cancers ($p = 0.001$ and 0.03 respectively). Colorectal cancer cell lines showed lower expression of *JS-2* than the benign colorectal cell line. *JS-2* copy number change and expression were shown for the first time to be altered in the carcinogenesis of colorectal cancer. In addition, genetic alteration of *JS-2* was found to be related to location, pathological subtypes and staging of colorectal cancer.

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1. Introduction

JS-2 was identified as a novel gene of 1871 base pairs (bp) at 5p15.2 by comparative DNA fingerprinting using inter-simple sequence repeat PCR in 2001 (Tang et al., 2001). *JS-2* has a 702 bp coding sequence and at discovery was predicted to encode a novel protein of 233 amino acids that could not be

matched with any known protein sequences in SwissProt (<http://au.expasy.org/sprot/>).

Using semi-quantitative real-time polymerase chain reaction, overexpression of *JS-2* was noted in 18% (2/11) of oesophageal squamous cell carcinoma cell lines and in 14% (3/22) of tissues from patients with oesophageal squamous cell carcinoma (Fatima et al., 2006). Also, expression of *JS-2* was noted

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in 4% (1/22) of squamous dysplasia of oesophagus. In non-cancer tissue, JS-2 was not found to be expressed in oesophagus but was noted in the stomach, ileocaecum, jejunum and rectum. In addition, JS-2 did not show any cellular transforming properties when transfected into NIH 3T3 cells. However, these results were obtained in a limited number of cases (Fatima et al., 2006). Larger studies are required to fully identify the prevalence of expression for JS-2 in human cancer. Furthermore, despite the discovery of the gene in a region known for amplification in cancer, the role of copy number changes to JS-2 has not been studied in human cancer.

Colorectal cancer is one of the commonest cancers in the world and usually ranks high in incidence and mortality (Parkin et al., 2005). There is evidence that research in the molecular pathway of cancers contributes directly to improve the care of patients with colorectal cancer by more accurately refining prognosis and selecting the most appropriate adjuvant therapy for individual patients with colorectal cancer (Alvarado et al., 2006). In this study, we examined the DNA copy number and RNA expression of JS-2 in a large cohort of patients with pre-invasive and invasive colorectal tumours. The relationship of the JS-2 genetic changes and clinicopathological features in patients with colorectal cancer were also analysed. It is hoped that the findings can give clues to the roles of the gene in colorectal cancer.

2. Materials and methods

2.1. Tissue samples

The patients who were chosen for this study had resection for primary colorectal adenocarcinomas, adenomas and non-neoplastic colorectal tissue between January 2004 and December 2006 in Queensland, Australia. The patients were consecutively chosen and with no selection bias. The resected colorectal tissues were fixed in 10 percent formalin and embedded in paraffin wax. Histological sections were cut and stained for haematoxylin and eosin for light microscopic examination. Non-neoplastic colorectal tissue was also recruited to act as a control population. Ethical approval of this study has been obtained from the Griffith University Human Research Ethics Committee (GU Ref No: MED/05/06/HREC).

The pathological features of patients with these colorectal tumours were analysed by reviewing the histological sections of the surgical specimen. The colorectal tumours were graded and classified according to the World Health Organization (WHO) criteria (Hamilton et al., 2000). Both conventional and mucinous adenocarcinomas were included in the study. The definition of colorectal mucinous adenocarcinoma is an adenocarcinoma with >50 percent of the tumour composing of extracellular mucin. Lymph node metastases were also recorded at the time of surgery. The carcinomas were staged according to TNM classification (Cserni, 2003).

After review, 116 patients (62 men; 54 women) with colorectal adenocarcinomas were selected for the analysis. The mean age of the patients with adenocarcinomas was 70 years (range, 36 to 91). The carcinoma was located in proximal colon

(caecum, ascending colon, transverse colon) in 36% ($n = 42$) and in distal colorectum (descending colon, sigmoid colon and rectum) in 64% ($n = 74$) of samples. Among the 116 cases studied, 34% ($n = 39$) of the colorectal adenocarcinomas had lymph node metastasis and 66% ($n = 77$) had no lymph node metastasis. There were 22% ($n = 27$) stage I carcinoma, 44% ($n = 50$) stage II carcinoma, 31% ($n = 36$) stage III carcinoma and 3% ($n = 3$) stage IV carcinoma. One tissue block from each of these samples were chosen for DNA and RNA extraction. The tissue block was checked to ensure that it contained a representative cancer area.

In addition, a tissue block with representative features was selected from each of the 30 patients with colorectal adenomas (17 men; 13 women) and 30 patients (20 men; 10 women) with non-neoplastic colorectal lesions. The level of dysplasia for colorectal adenomas has noted as high or low grade dysplasia. Non-neoplastic samples including diverticular disease, polyps and volvulus were recruited to act as control tissue.

2.2. Cell culture

The human colon cancer cell lines SW480 and SW48 were obtained from American Type Culture Collection (ATCC) and cultured in RPMI 1640 medium (containing 10% foetal bovine serum and 1% penicillin/streptomycin) and Leibovitz's medium (containing 1% 1% penicillin/streptomycin) respectively. A normal colon epithelial cell line FHC (obtained from ATCC) was used as a control and the cells were cultured in 1:1 mixture of Ham F-12 medium and DMEM medium supplemented with 25 mmol/L HEPES (N-[2-hydroxyethyl] piperazine-N'[2-ethanesulfonic acid]) 10 ng/mL cholera toxin, 0.005 mg/mL insulin, 0.005 mg/mL transferrin, 100 ng/mL hydrocortisone and 10% foetal bovine serum. SW480 and FHC cell lines were cultured at 37 °C in a humidified atmosphere containing 50 mL/L CO₂. SW48 cell lines were cultured in 37 °C in a humidified atmosphere without CO₂.

2.3. Extraction of DNA and RNA

Haematoxylin and eosin sections were taken from the selected tissue paraffin blocks to choose the area for DNA and RNA extraction. The cancers were separated from the surrounding morphologically benign tissue by micro-dissection. For each selected block, ten 10-micron sections were cut for micro-dissection prior to DNA and RNA extraction. DNA was extracted and purified with Qiagen DNeasy Blood & Tissue kit according to the manufacturer's protocol (Qiagen Pty. Ltd., Hilden, NRW, Germany). RNA was purified with Qiagen RNeasy FFPE Kits (Qiagen Pty. Ltd.), which were specially designed for purifying total RNA from formalin-fixed, paraffin-embedded tissue sections. RNA was purified from cell lines using miRNeasy Mini kit (Qiagen Pty. Ltd.) which were specifically designed for purifying both mRNA and miRNAs.

RNA quality was assessed by using a Bio-Rad Experion electrophoretogram instrument (Bio-Rad, Hercules, CA, USA). Purity of DNA and RNA was obtained by checking the optical density (OD) 260/280 ratio by using a nanodrop

spectrophotometer. Concentration of DNA and RNA was also noted in ng/ μ L.

2.4. cDNA preparation from RNA extracted

Reverse transcription reactions were performed using 6.5 μ g total RNA in a final reaction volume of 20 μ L. RNA was converted to cDNA using Superscript III according to the manufacturer's instructions (Invitrogen, Carlsbad, CA, USA). Each cDNA sample was diluted to 30 ng/ μ L. This was to provide uniformly concentrated sample for real-time PCR experiments.

2.5. Primers

Primers were designed for the target gene – JS-2, a ubiquitous control gene- haemoglobin delta (HBD) for DNA analysis and another ubiquitous control gene- glyceraldehyde 3-phosphate dehydrogenase (GAPDH) for the mRNA study. The primer sets for amplification of JS-2, HBD and GAPDH genes were selected using Primer3plus interface (<http://frodo.wi.mit.edu/>).

2.6. Quantitative real-time polymerase chain reaction (qRT-PCR)

Real-time quantitative polymerase chain reaction (PCR) was performed for detecting JS-2 gene copy number changes and expression. An IQ5 Multicolour Real-Time PCR Detection system (Bio-Rad) was used to run real-time quantification PCR.

PCR was performed in a total volume of 20 μ L reaction mixture containing 10 μ L iQ SYBR green supermix (Bio-Rad), 1 μ L of each 5 μ mol/L primer, 2 μ L of DNA or cDNA at 30 ng/ μ L, and 6 μ L of 0.1% diethylpyrocarbonate (DEPC) treated water. In the last tube, 2 μ L of DEPC treated water was added instead of DNA as a non template control. The primers used in the PCR reactions and PCR protocols are summarized in Table 1. A melting curve was run after each PCR to verify amplification specificity. For each tissue sample, the PCR reactions were performed in triplicate and accompanied by a non template control to increase the reliability of the results. Amplified PCR products were electrophoresed in 2% agarose gels and visualized by CyberSafe DNA gel stain (Invitrogen, Carlsbad, CA, USA) using UV transilluminator (Bio-Rad) to confirm melt-curve results.

2.7. Data analysis

JS-2 copy number changes and mRNA expression levels were analysed according to the methods published by Livak and

Schmittgen (Livak and Schmittgen, 2001). Normalised values for each duplicate sample were then averaged to give the final data used. $2^{-[\Delta\Delta Ct]}$ (fold change) was chosen to represent the level of copy number variation and mRNA expression changes. Inverse ratio of JS-2 (target gene) versus GAPDH/HBD (housekeeping gene) was used to represent the copy number variations and mRNA expression levels in different colorectal tissues. A fold change of more than 1.5 was considered as gain of JS-2 copy number (amplification) or high JS-2 mRNA expression (overexpression) and a fold change of less than 1 was considered as loss of JS-2 copies (deletion) or under expression. A baseline of 1.5 fold change was used to raise the threshold for high expression.

All clinical, pathological, DNA copy number variations and mRNA expression changes were computerized. Statistical analysis was performed using the Statistical Package for Social Sciences for Windows (version 19.0, SPSS Inc., Chicago, IL, USA). Chi-square test or likelihood ratio was used for categorical variables. Pearson correlation test was used for continuous variables. Independent t-test and ANOVA was performed for the analysis of continuous variables in categories. Significance level of the tests was taken at $p < 0.05$.

3. Results

3.1. JS-2 copy number changes in CRC tissues

For colorectal adenocarcinomas, JS-2 amplification was noted in 35% (41 of 116), JS-2 deletion was noted in 43% (50 of 116) and 22% (25 of 116) were in normal range. On the other hand, JS-2 amplification was noted in one third (33%, 10 of 30) of the colorectal adenomas and deletion in 53% (16 of 30) of colorectal adenomas. The rest of the adenoma tissues showed JS-2 gene copy number within normal range.

The copy number variations of JS-2 among different colorectal tissues was not statistically significant ($P = 0.94$).

Within the colorectal adenocarcinomas, however, some significant differences in copy number were observed. The copy number for JS-2 DNA for proximal colonic adenocarcinoma was significantly lower than distal colorectal adenocarcinoma ($p = 0.005$). Also, 24% (10 of 42) proximal colonic adenocarcinoma showed JS-2 amplification whereas 42% (31 of 74) distal colorectal adenocarcinoma showed amplification. The level of JS-2 copy numbers was significantly different with the depth of cancer invasion, as determined by tumour T staging ($P = 0.001$). JS-2 was highly amplified in cancers with early

Table 1 – Primer sequences and PCR protocol used for the qRT-PCR assay.

Target Genes	Primers	PCR Protocol	Amplicon size (base pairs)
JS-2	5'-ATGCACGAGTTATTGCTTGC-3' 5'-ATCGATGTACAGGGCCTCTT-3'	95 °C for 3 min for denaturation (1 cycle) 95 °C for 10 s, 60 °C for 30 s and 72 °C for 30 s (40 cycles)	136
HBD	5'-TGGATGAAGTTGGTGGTGAG-3' 5'-CAGCATCAGGAGTGGACAGA-3'	Melt-curve analysis performed	229
GAPDH	5'-TGGATGAAGTTGGTGGTGAG-3' 5'-CAGCATCAGGAGTGGACAGA-3'	at 55 °C for 30 s (81 cycles)	87

tumour stage (T1) compared to advanced tumour stages (T2, T3 and T4). There was no relationship identified between the pattern of *JS-2* DNA copy change and patients' age, gender, histological subtypes, grades and size of the cancer (Table 2).

3.2. *JS-2* mRNA expression in CRC tissues

The *JS-2* mRNA expression levels were different between non-cancer, adenoma and adenocarcinoma ($P = 0.05$) (Figure 1). In colorectal adenocarcinomas, 14% (16 of 116) of cases revealed increased level of *JS-2* mRNA, 84% (98 of 116) showed reduced/no expression and 2% (2 of 116) were in normal range. In colorectal adenomas, 7% (2 of 30) of cases showed increased level of *JS-2* mRNA, 86% (26 of 30) showed decreased expression and 7% (2 of 30) were in normal range.

JS-2 mRNA expression appears to be higher in males than females (mean fold change compared to normal tissue = 0.95 versus 0.86 respectively, $p = 0.03$). Colorectal mucinous adenocarcinomas showed higher *JS-2* mRNA expression compare to conventional adenocarcinomas (mean fold change = 3.64 versus 0.79 respectively, $p = 0.02$). There was also a difference in the pattern of *JS-2* expression in adenocarcinoma from

Table 2 – Copy number variation of *JS-2* and its correlation with clinicopathological features of colorectal adenocarcinoma.

Type	No.	Amplification	Deletion	No Change	<i>p</i> value
<i>Gender</i>					
Male	62	23 (37.1%)	25 (40.3%)	14 (22.6%)	0.34
Female	54	18 (33.3%)	25 (46.3%)	11 (20.4%)	
<i>Age</i>					
≤60	22	8 (36.4%)	14 (63.6%)	0 (0.0%)	0.69
>60	94	33 (35.1%)	36 (38.3%)	25 (26.6%)	
<i>Site</i>					
Colon	78	25 (32.1%)	37 (47.4%)	16 (20.5%)	0.63
Rectum	38	16 (42.1%)	13 (34.2%)	9 (23.7%)	
PC	42	10 (23.8%)	23 (54.8%)	9 (21.4%)	0.005
DC	74	31 (41.9%)	27 (36.5%)	16 (21.6%)	
<i>Size</i>					
≤50 mm	85	28 (32.9%)	37 (43.5%)	20 (23.5%)	0.62
>50 mm	31	13 (41.9%)	13 (41.9%)	5 (16.1%)	
<i>Histological grade</i>					
Well	21	6 (28.6%)	9 (42.9%)	6 (28.6%)	0.07
Moderate	75	28 (37.3%)	31 (41.3%)	16 (21.3%)	
Poor	20	7 (35.0%)	10 (50.0%)	3 (15.0%)	
<i>Histological subtypes</i>					
CA	111	40 (36.0%)	46 (41.4%)	25 (22.5%)	0.78
MA	5	1 (20.0%)	4 (80.0%)	0 (0.0%)	
<i>T staging</i>					
T1	7	3 (42.9%)	3 (42.9%)	1 (14.3%)	0.001
T2	26	10 (38.5%)	9 (34.6%)	7 (26.9%)	
T3	76	26 (34.2%)	33 (43.4%)	17 (22.4%)	
T4	7	2 (28.6%)	5 (71.4%)	0 (0.0%)	
<i>Pathological staging</i>					
I	27	12 (44.4%)	9 (33.3%)	6 (22.2%)	0.35
II	50	15 (30.0%)	24 (48.0%)	11 (22.0%)	
III	36	12 (33.3%)	16 (44.4%)	8 (22.2%)	
IV	3	2 (66.7%)	1 (33.3%)	0 (0.0%)	

Abbreviations: PC, proximal colon; DC, distal colon; CA, conventional adenocarcinoma; MA, mucinous adenocarcinoma. Bold values indicate a clinical significance of $P < 0.05$.

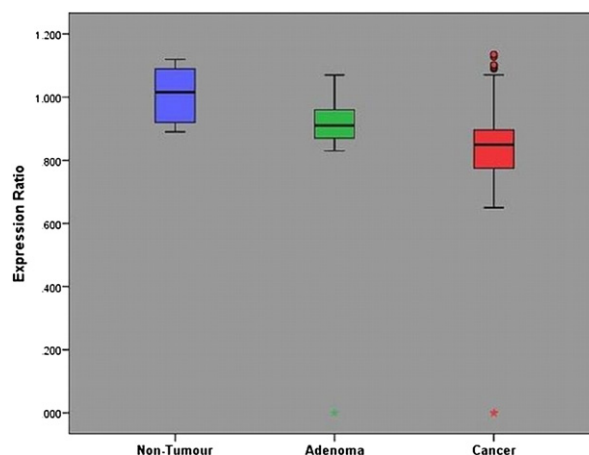


Figure 1 – The mRNA expression levels of *JS-2* in different colorectal tissues. The difference in mRNA expression was significantly altered in non tumour, adenomas and cancer tissues from colorectum ($p = 0.05$).

different sites ($P = 0.007$). Adenocarcinomas located in rectum showed lower *JS-2* mRNA compared to adenocarcinoma arising in the colon. *JS-2* mRNA expression was higher in adenocarcinomas with advanced T pathological stages (T3 and T4) compared with adenocarcinomas with early T pathological stages (T1 and T2) ($P = 0.03$). There was no relationship between the *JS-2* mRNA expression patterns and patients' age, gender, pathological grade and size of the tumour (Table 3).

3.3. *JS-2* mRNA expression in CRC cell lines

JS-2 mRNA expression was lower in both CRC cell lines (SW480 and SW48) compared to the normal colon epithelial cell line (FHC). SW48 cell line showed slightly lower *JS-2* mRNA expression than the SW480 cell line (fold change = 0.69 versus 0.84 compared to FHC) (Figure 2).

4. Discussion

This is the first study to quantitatively examine the DNA copy number change and mRNA expression of *JS-2* in colorectal cancers and adenomas. Our findings indicated that there are significant changes to the expression of the *JS-2* gene in colorectal adenocarcinoma and colorectal adenoma in comparison to non-neoplastic colorectal tissue. This implies that *JS-2* is involved in the pathogenesis of colorectal tumours.

Studies have reported a gain of 5p15, the region containing *JS-2*, in cancers from bladder, thyroid, lung and oesophagus (Smallridge et al., 2009; Kang et al., 2008; Nymark et al., 2006; Zheng et al., 2004). As a result, regional amplification of 5p and the production of extra copies of *JS-2* or other genes in cells may lead to either tumour initiation or further progression of cancer. In the present study, the cancer and adenoma tissue samples showed amplification of *JS-2* in approximately one third of the cases. In addition, deletion of *JS-2* was also common in both colorectal lesions and with almost half of

Table 3 – JS-2 mRNA expression pattern and its correlation with clinicopathological features of colorectal adenocarcinoma.

Type	No.	High Expression	Low Expression	No Change	p value
Gender					
Male	62	9 (14.5%)	53 (85.5%)	0 (0.0%)	0.03
Female	54	7 (13.0%)	45 (83.3%)	2 (3.7%)	
Age					
≤60	22	3 (13.6%)	18 (81.8%)	1 (4.5%)	0.69
>60	94	13 (13.8%)	80 (85.1%)	1 (1.1%)	
Site					
Colon	78	11 (14.1%)	66 (84.6%)	1 (1.3%)	0.007
Rectum	38	5 (13.2%)	32 (84.2%)	1 (2.6%)	
PC	42	5 (11.1%)	31 (88.1%)	0 (0.0%)	0.08
DC	74	11 (14.9%)	61 (82.4%)	2(2.7%)	
Size					
≤50 mm	85	11 (12.9%)	72 (84.7%)	2 (2.4%)	0.62
>50 mm	31	5 (16.1%)	26 (83.9%)	0 (0.0%)	
Histological grade					
Well	21	4 (19.0%)	16 (76.2%)	1 (4.8%)	0.86
Moderate	75	10 (13.3%)	64 (85.3%)	1 (1.3%)	
Poor	20	2 (10.0%)	18 (90.0%)	0 (0.0%)	
Histological subtypes					
CA	111	13 (11.7%)	96 (86.5%)	2 (1.8%)	0.02
MA	5	3 (60.0%)	2 (40.0%)	0 (0.0%)	
T staging					
T1	7	1 (14.3%)	6 (85.7%)	0 (0.0%)	0.03
T2	26	5 (19.2%)	20 (76.9%)	1 (3.8%)	
T3	76	10 (13.2%)	65 (85.5%)	1 (1.3%)	
T4	7	0 (0.0%)	7 (100%)	0(0.0%)	
Pathological staging					
I	27	5 (18.5%)	22 (81.5%)	0 (0.0%)	0.05
II	50	9 (18.0%)	41 (82.0%)	0 (0.0%)	
III	36	2 (5.6%)	32 (88.9%)	2 (5.6%)	
IV	3	0 (0.0%)	3 (100%)	0 (0.0%)	

Abbreviations: PC, proximal colon; DC, distal colon; CA, conventional adenocarcinoma; MA, mucinous adenocarcinoma. Bold values indicate a clinical significance of $P \leq 0.05$.

each showing deletion. It is worth noting that previous work on JS-2 has shown that deliberate overexpression of JS-2 by transfection into NIH 3T3 cells caused no change to the ability of those cells to form colonies (Fatima et al., 2006). While JS-2 may have some other role to play in tumour biology, it seems

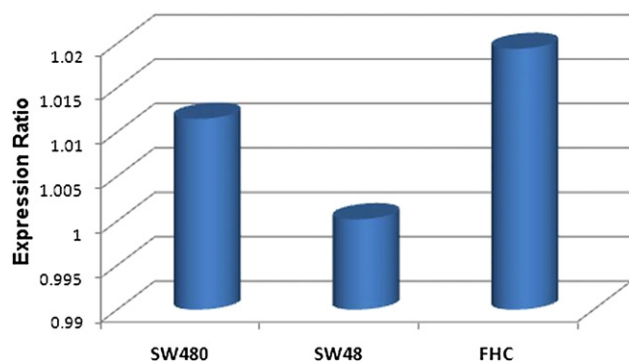


Figure 2 – The mRNA expression levels of JS-2 in colorectal cancer and normal epithelial cell lines. Cancer cell lines showed low expression of JS-2 mRNA compared to normal epithelial cell line.

likely that the amplification or deletion of the gene seen in this study is coincidental to the generalised amplification/deletion of 5p in these cancers. Interestingly, reports of 5p amplification in colorectal cancer seem to be rare, at approximately 14% in one review of the data (Kwong et al., 2004).

In this study we first reported the mRNA expression of JS-2 in colorectal cell lines. Data on cell lines have been included for confirming the changes of JS-2 mRNA levels on tissues are reproducible in viable cancer cells. Reduced JS-2 mRNA levels in colorectal cancer cells coincide with our findings of JS-2 mRNA levels in colorectal cancer tissues. Lower JS-2 mRNA levels in SW48 cell line from advanced pathological stage (Stage 3) compared to SW480 cell line from low pathological stage (Stage 2) may indicate significance of JS-2 expression in determining the tumour progression.

In our previous study on oesophageal squamous cell carcinoma, JS-2 mRNA expression was studied in only 22 cases. Of these, 14% (3/22) and 9% (2/22) showed overexpression and decreased expression of JS-2 mRNA respectively (Fatima et al., 2006). The majority of the cases showed no alterations of JS-2 mRNA expression. In this study, we performed the study in 116 samples of colorectal cancer. The expression of JS-2 is reduced in a majority of colorectal carcinomas and adenomas compared to non-neoplastic colorectal tissue. Similar results were obtained for 5 genes (namely, OSMR, BASP1, NNT, PAIP1 and FASTKD3) located in the 5p region in a genomic analysis of cervical cancer (Scotto et al., 2008).

In this study, it appears that mRNA and DNA data gave inverse results. The reduced expression may be due to the down regulation of JS-2 mRNA by other genes or could be reduced exposure to transcription factors. This change may also include feedback mechanisms, where initial increases in JS-2 activity following amplification lead to a reduction in mRNA production.

The results obtained by this study would indicate that a similar regulatory mechanism is at work in the tested colorectal cancers, as 84% of adenocarcinomas and 86% of adenomas showed under-expression of JS-2. This implies that JS-2 down regulation may have some role in the carcinogenesis or may be regulated in concert with other genes. The methodology of our previous study on the JS-2 gene was attempting to determine its oncogenicity and would not have detected a possible function as a tumour suppressor (Fatima et al., 2006).

The JS-2 gene localizes to chromosome 5p15.2 (Smallridge et al., 2009; Kang et al., 2008; Nymark et al., 2006), a site that has been implicated in the development of various cancers. The JS-2 gene is also located upstream to δ -catenin, a gene which has been reported to act as an oncogene (Zeng et al., 2009). It is also interesting to note that inactivation of several tumour suppressor genes on 5p13-12 has been reported to be involved in carcinogenesis of solid cancers (Böhm et al., 2000). JS-2 could be part of a block of tumour suppressors inactivated or downregulated during carcinogenesis in colorectal cancer. Also, given its proximity to delta catenin, it could simply be caught in some large scale modification to inactivate an amplified tumour suppressor, such as methylation or chromatin remodelling.

There are some alternate possibilities to JS-2 having tumour suppressor function. Expression of JS-2 in normal rectal tissue was found to be quite low in comparison with other

tissues in the gastrointestinal tract (Fatima et al., 2006). Thus, it is possible that the down regulation observed is simply an overcompensation of feedback mechanisms, resulting in a reduced expression for a protein that is not required or lowly required in the tissue in the first place. There are some more location specific factors at work, however.

Researchers have demonstrated that cancers located in the right (proximal) and left (distal) colorectum can be distinguished by clinical criteria (Iacopetta, 2002; Kapiteijn et al., 2001; Birkenkamp-Demtroder et al., 2005; Jass, 2007). Proximal colorectal cancers, when compared to distal cancers, are more often found in older age and females. The works of our group and others have demonstrated a few molecular markers that express differently in these two types of colorectal cancers (Iacopetta, 2002; Kapiteijn et al., 2001; Birkenkamp-Demtroder et al., 2005; Jass, 2007; Lam et al., 2006; Saleh et al., 2008; Lam et al., 2008a,b). In the present study, both the gene copy number change and mRNA expression of *JS-2* was demonstrated to be different in cancers from these sites. The differences in molecular profiles of cancers in different locations in colorectum may have important implications regarding specifically targeted therapeutic regimens in the future.

Colorectal mucinous adenocarcinoma has distinctive clinicopathological features (Lam et al., 2006). In addition, our previous studies have shown that this type of cancer had different molecular profiles when compared to colorectal non-mucinous adenocarcinoma. For instance, mucinous adenocarcinoma had higher p53, higher p21 and lower aurora kinase expressions than non-mucinous adenocarcinoma (Lam et al., 2008a,b). In this study, we noted that mucinous adenocarcinoma showed higher *JS-2* expression than non-mucinous adenocarcinoma, and indeed, of benign colorectal cancer tissue. *JS-2* may be directly involved of regulating genes that are involved in the development of the mucinous phenotype, or be a downstream component of the same genetic pathways and may also contribute directly to the phenotype. Thus, differential *JS-2* expression in these 2 pathological subtypes of colorectal cancer supports the notion that these 2 subtypes of cancers are different entities and should be managed differently.

In this study, *JS-2* alterations were highly correlated with pathological staging of colorectal cancers. Early T-stage cancers appear to have higher *JS-2* copy number and lower expression of *JS-2* mRNA. This may indicate that reduced *JS-2* expression is involved in the early stage of cancer progression as T-stage is based on the extent of the tumour invasion. The increased *JS-2* expression noted in the more advanced cancers did not reach the same level as normal tissue. If *JS-2* does have some anti-growth functions, it is possible that the increased expression in more advanced tumours may be a response to increased signalling for tumour suppression resulting from intact or semi-intact gene regulation mechanisms recognising the increasing malignancy of the cells. In addition, these results may suggest that *JS-2* alterations may be a marker for aggressive of the colorectal cancer.

To conclude, we have identified changes in the copy number and expression of *JS-2* in progression of colorectal neoplasm. In addition, genetic alteration and differential regulation of *JS-2* was found to be related to location, pathological subtypes and staging of colorectal cancer.

Conflict of interest

None declared.

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