c-Kit and Musashi-1 expression in colorectal carcinoma and its association with etiological factors

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INTRODUCTION

Colorectal cancer is the third most commonly diagnosed cancer in males and the second in females, with a global estimate of over 1.2 million new cancer cases and 608,700 deaths in 2008.1 Although many molecular therapeutic targets have been identified in treating cancer, it still remains a threat due to the recurrence of therapy resistant forms2 which has been mostly attributed to the presence of cancer stem cells. Colorectal cancer has been identified to follow the classical normal – dysplastic aberrant crypt foci – early adenoma – intermediate adenoma – late adenoma – carcinoma sequence.3 The colorectal tumors contain numerous inflammatory leukocytes, and chronic inflammation leads to a functional link between chronic inflammation and cancer.4 Though neoplastic transformation in inflammatory bowel disease (IBD) is similar to sporadic colorectal cancer (CRC), some differences exist, like loss of APC function which is an early event in sporadic CRC but it is late and less frequent in colitis-associated

ABSTRACT

Introduction: The proto-oncogene c-Kit encodes a tyrosine kinase receptor which is essential during embryonic and postnatal life but its expression in precancerous lesions has not been studied. Musashi-1 was initially identified as a neuronal stem cell marker and recently as an intestinal stem cell marker and is aberrantly expressed in several cancers including colorectal cancer, but its role in neoplastic tissues has not been elucidated. The present study aims to study the expression of c-Kit and Musashi-1 in precancerous lesions and colorectal carcinoma in correlation with etiological factors. Methods: Immunohistochemical analysis, western blot and RT-PCR were done to observe the expression of c-Kit and Musashi-1 in normal, ulcerative colitis, adenoma and adenocarcinoma of colorectal tissues. Results: c-Kit expression was observed to decrease from normal to adenocarcinoma. In contrast, Musashi-1 showed increased expression from adenoma to carcinoma stages. Furthermore, c-Kit showed statistically significant association with alcoholic and non-alcoholic patients (p < 0.03*) and Musashi-1 had statistically significant association with age (≥ 60 and < 60) (p<0.005*), gender (p<0.02*) and alcoholics (p<0.001**). However, statistically significant negative correlation was found between the expression of c-Kit and Musashi-1 in colorectal carcinoma. Conclusions: Overall, these studies indicate that the decreased or loss of c-Kit and increase in the Musashi-1 expression in carcinoma can be attributed to the disease progression and malignant transformation of colorectal epithelium. A statistically significant negative correlation was found between c-Kit and Musashi-1 in colorectal carcinoma.

Keywords: c-Kit, Musashi-1, Ulcerative colitis, Adenoma.

Abbreviations: IBD, inflammatory bowel disease; CRC, Colorectal cancer; GIST, gastrointestinal stromal tumors.
dysplasia-carcinoma sequence and p53 mutation, which is an early event in colitis-associated cancer but is late in sporadic CRC. [8] Colorectal cancer originates from epithelial cells of gastrointestinal tract, which undergoes sequential mutations, thus disrupting the normal mechanism of proliferation and self-renewal of stem cells. The inborn genetic aberration, tobacco, smoking, alcohol, environmental carcinogens, and chronic inflammation are some of the factors that drive the transformation of normal colonic mucosa to dysplastic adenoma and to colorectal cancer. [9] Several stem cell markers independently or in combination have a potential role in the malignant transformation of colorectal epithelium.

The proto-oncogene c-Kit (CD117) encodes a transmembrane tyrosine kinase receptor related to the platelet-derived growth factor PDGF/CSF-1 (c-fms) receptor subfamily. [7] Its pathogenic role has been reported in a number of malignancies including breast carcinomas, germ cell tumors, colon carcinoma, some subtypes of sarcoma, melanoma, ovarian and small cell lung carcinoma, in addition to gastrointestinal stromal tumors (GIST). [8–10] It has been observed that binding of SCF to KIT activates multiple signal transduction pathways, including phosphatidylinositol 3-kinase (PI3K)/Akt, p44/42 mitogen-activated protein kinase (MAPK) and signal transducers and activators of transcription (STAT). [11,12] The SCF–KIT signaling system emphasizes its importance in the proliferation, differentiation, and its expression was observed in several cell types such as hematopoietic progenitors, mast cells, melanocytes and cells of Cajal. [13,14] It plays an important role in the development of multiple cell types including hematopoietic cells, germ cells, and melanocytes. [13,14] Some reports have also shown that c-Kit expression was a rare event in colorectal cancer tissue. [17–19] From these previous reports, we sought to analyse the expression of c-Kit in precancerous and colorectal carcinoma tissues.

Cancer stem cells are a very small unique population of cells within the tumor with the ability to self-renew and give rise to the rest of the tumor cells. Although the origin of the cancer stem cells is not fully known, it is widely believed that, they arise from the normal stem cells or progenitors upon mutation(s). [20] Identification and characterization of cancer stem cells is still in its infancy and the combination of molecules used to identify cancer stem cells varies according to the tissue type.

Intestinal stem cells can be identified and isolated by using reliable intestinal markers, such as Musashi-1, a RNA-binding protein primarily identified as a neuronal stem cell marker and more recently as a putative intestinal stem cell and early lineage marker and was found to be up regulated in tumors of APC<sup>+/−</sup> mice. [21] Musashi-1 expression was also observed in several other tumors including colorectal adenoma, glioma, retinoblastoma, hepatoma and cervical carcinoma. [22] Musashi-1 was up regulated in brain tumors, including medulloblastoma and gliomas. [27,28] Nishimura et al. [29] showed that Musashi-1 expression confined mostly to the base of the crypt of human colon. In neural stem cells, Musashi-1 was found to activate Hes1 expression, a notch-1 downstream target and also found to suppress m-Numb, an inhibitor of Notch-1 signaling. [28,30] Musashi-1 was mostly expressed within the cytoplasm but was also detected in the nucleus of some tumor cells and was located mainly within the base of the crypts in the normal colorectal mucosa [31] suggesting that, Musashi-1 might have the crucial role in maintaining the proliferative notion in normal intestinal mucosa and up regulated proliferation in colorectal adenocarcinoma. However, its clear regulatory mechanism needs to be addressed.

Therefore, the aim of this study was to investigate the expression pattern of c-Kit and Musashi-1 in normal, inflamed mucosa from patients with ulcerative colitis, adenoma and adenocarcinoma of colorectal tissues and their association with etiological factors and to correlate the expression of c-Kit and Musashi-1 in colorectal adenocarcinoma.

**MATERIALS AND METHODS**

**Patients and tissues**

Human colon and rectal biopsy and surgical tissue samples for the study were obtained from 152 patients, including 24 (9 biopsy &15 resected) normal tissues obtained from biopsy and adjacent tissues from surgery, 37 ulcerative colitis, 29 adenoma and 62 adenocarcinoma who underwent Colonoscopical examination and surgery from the medical and surgical gastroenterology Departments at Stanley Medical Hospital, Chennai after getting prior proper informed consent from each patients. The study was conducted in accordance with the principles outlined in the declaration of Helsinki and the study protocol was approved by the Research Advisory Committees, Institutional Review Board and Ethical Committees of Stanley Medical College and Hospital, Chennai, India. All histopathological grading were examined independently by two pathologists who were unaware of the patient’s details.

**Immunohistochemistry**

Serial sections from paraffin-embedded colorectal biopsy specimens were deparaffinized in xylene and dehydrated through graded concentrations of isopropanol. The
slides were blocked with 3% BSA in 1x PBS for 1 hour at room temperature. The sections were then incubated overnight at 4 °C with primary antibodies for c-Kit and Musashi-1 (1:200), (Santa Cruz Biotechnology-USA). The slides were washed with 1x PBS for 5 min after which, they were incubated with the corresponding secondary antibody conjugated with HRP and washed in 1x PBS with 0.05% Tween 20 at room temperature. Then, slides were developed with DAB solution containing 0.05% DAB, 10 µL H₂O₂ in 1x PBS in a dark room for 5 min at room temperature and counter stained with haematoxylin. Finally, the sections were dehydrated and mounted with DPX.

Western blotting

For Western blot analysis, we have analysed 32 biopsy specimens including 8 samples from each stage. Protein lysates were prepared with extraction buffer and protein concentrations were determined by Lowry et al.[34]. 40 µg of protein was separated by SDS-PAGE and transferred to a polyvinylidene fluoride (PVDF) membrane (Immobilon-P, Millipore, USA) and probed with anti-c-Kit and anti-Musashi-1 primary antibodies, followed by corresponding HRP-conjugated secondary antibody incubated at room temperature. The specific signals were detected by chemiluminescence using luminogen substrate (Amersham, ECL advance western blotty detection kit-RPN 2135,UK). The relative intensity was normalized with β-actin using Image J software.

Reverse transcriptase PCR

RNA was extracted using Isol-RNA Lysis Reagent (5PRIME Inc, Gaithersburg, USA) according to the manufacturer’s protocol. The RNA concentration was determined by Biophotometer (Eppendorf, Germany) and total RNA (1 mg) was used to generate cDNA using standard method. Specific primer sequences for c-Kit and Musashi-1 were used to amplify gene transcripts. The reactions were cycled after an initial 5 min denaturation at 94 °C followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 60 °C for 30 s, and polymerization at 72 °C for 2 min. β-actin, was used as a positive loading control. The PCR products were electrophoresed on 1% agarose gels with ethidium bromide and visualized in UV light (Vilber Lourmat, France).

### Primer sequence of β-Actin, c-Kit and Musashi-1

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Gene</th>
<th>Primer pair</th>
<th>Product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>β-Actin</td>
<td>F- 5’ CCA CCC ATG GCA AAT TTC 3’&lt;br&gt;R- 5’ GCC CAG GAT GCC CTT GA 3’</td>
<td>125</td>
</tr>
<tr>
<td>2</td>
<td>c-Kit</td>
<td>F-5’GGGCCACCCCTGGTTCATTACAGA-3’&lt;br&gt;R-5’ AGGATCTGCTGATCAGACATCGTGGTGGCACAAG-3’</td>
<td>944</td>
</tr>
<tr>
<td>3</td>
<td>Musashi-1</td>
<td>F- 5’ GCG ACA CTG CTC GAC AGG AAT TA 3’&lt;br&gt;R - 5’ AGA GGG ACA CAC AGA AGG GGG AT 3’</td>
<td>257</td>
</tr>
</tbody>
</table>

Statistical analysis

The χ² test was used for the percentage of samples with positive staining among lesions of different histological grades using StatCalc 5.0.4 (AcaStat software).The independent student’s t-test was used for statistical significant in immunoblot analysis, p <0.05* was considered statistically significant and p <0.01** was considered as highly significant.

RESULTS

Immunohistochemical localization of c-Kit and Musashi-1 in study population

The immunoreactivity evaluation of c-Kit and Musashi-1 was carried out in the study population. c-Kit was mainly localized in the cytoplasm and cell membrane of stromal region of normal specimens and inflammatory cells. However, modest expression was observed in epithelial cells. There was no significant level of c-Kit expression in colorectal carcinoma tissues, with only some weak staining of c-Kit (Figure 1). Musashi-1 expression was mainly confined to the lower part of the crypts in the normal colorectal mucosa. In normal tissues, cytoplasmic and rarely nuclear staining of Musashi-1 was observed in some tumor cells. Intense staining was observed in adenoma and colorectal carcinoma tissues than normal mucosa.

Relationship between c-Kit & Musashi-1 expression and Clinicopathological Characteristics

c-Kit expression showed statistically significant association with alcoholics and non-alcoholics (p <0.003*) and tumor site (p <0.02*) (Table 2A). However, no significant association between c-Kit expression and age, gender, smoking was observed. c-Kit positive expression was observed as a distinct membranous and cytoplasmic staining. In normal cases 11 (45.8%) were positive for c-Kit, 9 (37.5%) mild, 2 (8.3%) moderate and no intense staining was observed. In ulcerative colitis 15 (40.5%) cases were positive, 11 (29.7%)
mild, 3 (8.1%) moderate and 1 (2.7%) was intensely stained for c-Kit. In adenoma tissues 11 (37.9%) cases were positive with 6 (20.6%) mild, 4 (13.7%) moderate and 1 (3.4%) was intensely stained. In adenocarcinoma tissues, 15 (24.1%) cases were positive with 11 (17.7%) mild, 3 (4.8%) moderate and 1 (1.6%) was intensely stained (Table 1A).

Musashi-1 had statistically significant association with age (≥60 and <60) (p < 0.005*) and gender (p < 0.02*). Further, it showed significant association with respect to alcoholics and non-alcoholics (p < 0.001**) (Table 2A), however no significant association between expression and smokers or tumor site was observed. Musashi-1 expression showed cytoplasmic and rarely nuclear staining. In normal cases, 10 (41.6%) were positive, 8 (33.3%) mild, 2 (8.3%) moderate and no intense staining was observed. In ulcerative colitis 25 (67.5%) cases were positive, 14 (37.8%) mild, 9 (24.3%) moderate, and 2 (5.4%) stained intensely. In adenoma, 20 (68.9%) cases were positive, 12 (41.3%) mild, 4 (13.7%) moderate and 5 (17.2%) stained intensely. In carcinoma stages, 45 cases (72.5%) with 12 (19.3%) mild, 14 (22.5%) moderate and 19 (30.6%) showed intense expression (Table 1B).

**Figure 1. Immunolocalization of c-Kit and Musashi-1 in different colorectal tissues.** c-Kit was mainly localized in the cytoplasm and cell membrane in stromal region of normal specimens (A), only some weak staining in neoplastic tissues (B-C) and scarce expression in colorectal adenocarcinoma tissues (D), the cytoplasmic expression of Musashi-1 was observed exclusively in the base of crypt in normal mucosa (E) and its expression in neoplastic tissues (F-G), diffused expression and rare nuclear staining was observed in adenocarcinoma tissues (H). The tissue sections were counterstained with hematoxylin.

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**Table 1A c-Kit expression in the various stages**

<table>
<thead>
<tr>
<th>Histological Grade</th>
<th>–</th>
<th>+</th>
<th>++</th>
<th>+++</th>
<th>n</th>
<th>Positive rate%</th>
<th>p-value ¥</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>13</td>
<td>9</td>
<td>2</td>
<td>0</td>
<td>24</td>
<td>45.8</td>
<td></td>
</tr>
<tr>
<td>Ulcerative Colitis</td>
<td>22</td>
<td>11</td>
<td>3</td>
<td>1</td>
<td>37</td>
<td>40.5</td>
<td>p &lt; .68</td>
</tr>
<tr>
<td>Adenoma</td>
<td>18</td>
<td>6</td>
<td>4</td>
<td>1</td>
<td>29</td>
<td>37.9</td>
<td>p &lt; .56</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>47</td>
<td>11</td>
<td>3</td>
<td>1</td>
<td>62</td>
<td>24.1</td>
<td>p &lt; .05*</td>
</tr>
</tbody>
</table>

¥ calculated using $\chi^2$.
Expression of c-Kit shows significant difference between groups (p < .05*).

**Table 1B Musashi-1 expression in the various stages**

<table>
<thead>
<tr>
<th>Histological Grade</th>
<th>–</th>
<th>+</th>
<th>++</th>
<th>+++</th>
<th>n</th>
<th>Positive rate%</th>
<th>p-value ¥</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>14</td>
<td>8</td>
<td>2</td>
<td>0</td>
<td>24</td>
<td>41.6</td>
<td></td>
</tr>
<tr>
<td>Ulcerative Colitis</td>
<td>12</td>
<td>14</td>
<td>9</td>
<td>2</td>
<td>37</td>
<td>67.5</td>
<td>p &lt; .089</td>
</tr>
<tr>
<td>Adenoma</td>
<td>9</td>
<td>11</td>
<td>4</td>
<td>5</td>
<td>29</td>
<td>68.9</td>
<td>p &lt; .087</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>17</td>
<td>12</td>
<td>14</td>
<td>19</td>
<td>62</td>
<td>72.5</td>
<td>p &lt; .018*</td>
</tr>
</tbody>
</table>

¥ calculated using $\chi^2$.
Expression of Musashi-1 shows significant difference between groups (p < .05*).
0, no staining or <5% positive cells; 1+, 5% to 25% positive cells; 2+, 26% to 50% positive cells; 3+, 50% to 75% positive cells.
Table 2A Demographic characteristics of study groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>n</th>
<th>c-Kit+</th>
<th>c-Kit-</th>
<th>p-value</th>
<th>Msi1+</th>
<th>Msi1-</th>
<th>p-value ¥</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age ≥ 60</td>
<td>96</td>
<td>31</td>
<td>65</td>
<td>p &lt; .51</td>
<td>71</td>
<td>25</td>
<td>p &lt; .005*</td>
</tr>
<tr>
<td>Age &lt; 60</td>
<td>56</td>
<td>21</td>
<td>35</td>
<td></td>
<td>29</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>Gender Male</td>
<td>83</td>
<td>27</td>
<td>56</td>
<td>p &lt; .63</td>
<td>61</td>
<td>22</td>
<td>p &lt; .02*</td>
</tr>
<tr>
<td>Gender Female</td>
<td>69</td>
<td>25</td>
<td>44</td>
<td></td>
<td>39</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Smoking Never</td>
<td>87</td>
<td>29</td>
<td>58</td>
<td>p &lt; .79</td>
<td>53</td>
<td>34</td>
<td>p &lt; .14</td>
</tr>
<tr>
<td>Smoking Smoking</td>
<td>65</td>
<td>23</td>
<td>42</td>
<td></td>
<td>47</td>
<td>18</td>
<td></td>
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<tr>
<td>Alcohol Never</td>
<td>81</td>
<td>19</td>
<td>62</td>
<td>p &lt; .003*</td>
<td>56</td>
<td>15</td>
<td>p &lt; .001**</td>
</tr>
<tr>
<td>Alcohol Alcoholics</td>
<td>71</td>
<td>33</td>
<td>38</td>
<td></td>
<td>44</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>Tumor site Colon</td>
<td>89</td>
<td>24</td>
<td>65</td>
<td>p &lt; .02*</td>
<td>58</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>Tumor site Rectum</td>
<td>63</td>
<td>28</td>
<td>35</td>
<td></td>
<td>42</td>
<td>21</td>
<td>p &lt; .84</td>
</tr>
</tbody>
</table>

¥-Calculated using chi square. *- Significant, **- Highly significant.
c-Kit and Musashi-1 expression was correlated with etiological factors.

Table 2B Relationship between the expression of c-Kit and Musashi-1 in colorectal cancer

<table>
<thead>
<tr>
<th>Expression of c-Kit</th>
<th>n=62</th>
<th>Expression of Musashi-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (17)</td>
<td>47</td>
<td>9</td>
</tr>
<tr>
<td>1’ (12)</td>
<td>11</td>
<td>6</td>
</tr>
<tr>
<td>2’ (14)</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>3’ (19)</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

Significant Negative correlation was found between the expression of c-Kit and Musashi-1 in colorectal cancer samples. (p < .05; r = −0.2)

c-Kit expression was down regulated in adenocarcinoma tissues compared to normal. The reduced c-Kit expression may be associated with the malignant transformation of disease progression. Unlike the western blot analysis, the immunohistochemistry results reveal that its scarce expression which may be due to proliferation of epithelial cells, which tends to reduce the stromal compartments.

Western blot analysis of c-Kit and Musashi-1 protein expression

c-Kit and Musashi-1 expression was confirmed at the protein level by western blot analysis. Western blot result shows that c-Kit expression was significantly decreased in carcinoma tissues (p < .023*) (Figure 2B) than normal tissues.

Musashi-1 expressed in all colorectal progressive lesions showed a faint expression in normal mucosa with a gradual increase and intense from adenoma to carcinoma stages. Musashi-1 expression was significantly increased in adenoma tissues (p < .02*) and carcinoma tissues (p < .01**). β-actin were used as a positive loading control. The relative intensity were normalised with β-actin using Image J software (Figure 2B).

c-Kit and Musashi-1 mRNA expression levels in colorectal tissues

RT-PCR analyses were done to portray the presence of c-Kit and Musashi-1 mRNA transcripts in colorectal tissue samples. c-Kit mRNA expression was observed in normal and neoplastic tissues with mild expression in carcinoma. Musashi-1 mRNA was expressed in all colorectal progressive lesions showed a mild expression in normal mucosa with a gradual increase and intense from adenoma to carcinoma stages. β-actin were used as a positive loading control.

Correlation between c-Kit and Musashi-1 in colorectal carcinoma

The comparison between the expression of c-Kit and Musashi-1 in colorectal carcinoma tissues using Spearman rank correlation are presented in Table 2B. Statistically significant negative correlation was found between c-Kit and Musashi-1 in colorectal carcinoma (p < .005; r = −0.2).

DISCUSSION

c-Kit expression has been studied in germ cell tumors, small cell lung carcinoma, neuroblastoma, meloma, ovarian carcinoma, breast carcinoma in addition to CML and GISTs.13 c-Kit mRNA levels were quantitatively lower in CRC than that of the adenoma or normal.34 Sammarco I et al., reported that c-Kit positive staining in the epithelium of the neoplastic colon showed decreased expression from normal to cancer.35 In concomitant to these reports, our western blot and RT-PCR analysis demonstrates that the c-Kit expression was down regulated in adenocarcinoma tissues compared to normal. The reduced c-Kit expression may be associated with the malignant transformation of disease progression. Unlike the western blot analysis, the immunohistochemistry results reveal that its scarce expression which may be due to proliferation of epithelial cells, which tends to reduce the stromal compartments.
Loss of c-Kit is associated with an advanced stage of breast carcinoma and malignant transformation and disease progression. In the light of these reports, our results also show reduced c-Kit expression in neoplastic and carcinoma tissues and it has been suggested that loss of c-Kit correlates with malignant progression suggesting their definitive role in normal tissues. c-Kit expression showed statistically significant association with alcoholics and non-alcoholics (p < 0.003*) and tumor site (p < 0.02*) (Table 2A) suggests that alcohol along with inflammatory cues may be associated with reduced c-Kit expression in neoplastic transformation. From these observations, it can be concluded that c-Kit expression significantly reduced in adenocarcinoma (p < 0.05*) (Table 1A) and intense staining of 1.6% was observed in carcinoma tissues which was concurrent with other reports, confirming that c-Kit expression was a rare event in colorectal tissues.

The normal colorectal mucosa is lined with simple columnar epithelium and invaginates to form crypts. The colon crypt progenitors rapidly divide and migrate upward during which they differentiate to form functional cell types. In normal state, intestinal epithelial stem cells are involved in continuous and rapid renewal by asymmetric division and differentiate while migrating and are shed into the lumen. Any disruption in the mechanism of stem cell proliferation is thought to initiate tumor growth and produce heterogeneity within the tumor cell population. Growing evidences suggest that, Musashi-1 is associated with different grades of malignancy and proliferating activity. The stem cell marker, Musashi-1 expression was confined to colon crypt similar to the distribution of stem cells and is likely to be involved in regulation of intestinal differentiation. Musashi-1 was found to be over expressed in several cancers.

Musashi-1, a RNA binding protein, initially identified as a neuronal stem cell marker and more recently identified as a presumed intestinal stem cell and early lineage marker, is up-regulated in tumors of APC mice signifying their association with colorectal carcinoma and its association with Musashi-1 expression.
definitive role in the canonical Wnt pathway. Musashi-1 expression was induced by Wnt 3a[38] suggesting their role in proliferation. Nishimura et al.,[29] showed that expression was confined mostly to the base of the crypt of human colon where continuous cell renewal and homeostasis of the intestinal epithelium are regulated by Wnt and notch pathways[43,44]. In agreement with Nishimura et al., our western blot results show that the intense band corresponds to Musashi-1 was observed in adenoma (p < .02*) and adenocarcinoma (p < .001*) tissues. Musashi-1 expression has statistically high significant association with alcoholics and non-alcoholics (p < .0001**) which implies that the alcohol along with persistent inflammation could transform the intestinal stem cells in the disease progression. Immunohistochemistry results demonstrated that the Musashi-1 expression was mainly confined within the lower part of the crypts in the normal colorectal mucosa. In accordance with previous reports and our results, we speculate that it is possible for Musashi-1 expressing cells to be so called colorectal cancer initiating cells hypothesized that tumor arise from intestinal stem cells that are inherently expressing Musashi-1 but this needs further validations. The intense staining of Musashi-1 with cytoplasmic and/or rarely nuclear staining of Musashi-1 was observed in some tumor cells suggesting the aberrant expression of Musashi-1 in colorectal adenoma and carcinoma tissues suggests that the deregulated expression was associated with intestinal tumorigenesis.

CONCLUSIONS

In conclusion, our results supports the previous studies analyzing the c-Kit expression in solid tumors demonstrate that c-Kit expression was rare event in colorectal cancer. The increase in the Musashi-1 expression in adenocarcinoma implies the tumor-inducing potential in colorectal epithelium. Moreover, the negative correlation existing between c-Kit and Musashi-1 in colorectal carcinoma needs extensive studies in large number of samples and regulations governing their expression may reveal their possibilities of using these markers for therapeutic implications.

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REFERENCES

Natarajan Gopalakrishnan, et al.: c-Kit and Musashi-1 expression in colorectal carcinoma and its association