Original Research Article

Assessment of Fascin by immunohistochemistry in colorectal carcinoma

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Abstract

Background: The increased incidence of colon cancer may be attributed to an increase in the awareness of the disease, accessible screening modalities through widespread availability of preventive health check-ups & affordable newer imaging techniques. One of the essential components of clinical management of colon cancer is histopathological diagnosis and the role of Fascin-1 expression in colon cancers remains unexplored. This study was carried out to evaluate the fascin-1 expression in colon cancer specimens. **Methods:** This retrospective study was carried out on 60 paraffin blocks of colorectal carcinoma specimens received for a period of four years. IHC for Fascin was done on the sections along with controls. Clinical data regarding the participant's demographics and medical history were captured from the hospital medical records. Gross findings were recorded from the blocks. Blocks with section containing normal epithelium and tumour were chosen for immunohistochemistry. **Results:** There was a significant association between pT1/pT2 staging and grade 0 fascin expression (80%) compared to those with pT3/pT4 staging (50%) ($p<0.05$). Similarly, grade 1 had strong positive correlation with fascin expression (70%) compared to other grades of the tumor ($p<0.05$). **Conclusion:** Presence of fascin expression significantly indicates the metastatic potential of colon carcinomas. Further research is required to delineate the mechanism of fascin expression at the cellular level in determining the prognosis of colon cancers.

Keywords: Colorectal cancers, Fascin-1 expression, Immunohistochemistry, Metastasis

Introduction

Cancers involving systemic organs is often associated with increased rates of morbidity, mortality and disability and is often associated with poor quality of life. Among the several cancers involving systemic organs, colon cancer is the most morbid one. Colon cancers in general are associated with diet devoid of fiber and therefore it is more prevalent in the western countries compared to Asian countries. According to the Bombay registry data, the incidence of colon cancer in India is relatively less [1]. This can be attributed to the increase in the fiber content of Indian diets.

Although the incidence of colon cancer is lesser in India, the consequences of the cancers remain higher. Among the various sites involved, large bowel is the most common site of the colon cancer compared to the small bowel. The incidence is predominantly higher among the males compared to females and this was substantiated by the gender wise incidence in the 2013 based registry [2]. In recent years, there has been an increase in the awareness regarding colon cancer and the dietary pattern which predisposes the risk for colon cancer. Likewise, there are several screening modalites that have been developed in recent times as a part of preventive health program for early detection of colon cancers [3].

The management of colon cancer largely remains surgical. The type of surgery depends on the stage of disease, lymph node involvement and also distant metastasis. Although surgery is cardinal in treatment, it is essential to diagnose and grade the disease for the effective clinical management. The ideal method for diagnosis has been histopathological examination for many decades till now. The key aspect of

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histopathological examination involves microscopic examination to look for cellular invasion and cytoskeletal organisation. Among the various aspects of histopathology, detection of fascin has been identified to play a key role in pathogenesis of colonic cancers. Fasin is a 55 KD, actin bundling globular protein which plays a major role in cell protrusion and motility of mesenchymal and neuronal cell [4].

Fasin has been implicated in cyto skeletal organisation and cell migration in colon carcinoma. Therefore, there is an increased scope for clinical significance in using fascin for not only detecting the infiltration of colonic cancer cells, but also a prospective as therapeutic target in aggressive forms of colorectal adeno carcinoma.

Studies have shown that fascin is absent or minimally expressed in the epithelium. When presence of fascin is detected, it is indicative of neoplasm, often of invasive phenotype [5]. In addition, the studies done on the western population have a demonstrated strong and diffuse expression of fascin in colorectal carcinoma and this has been associated with shorter survival rates [6].

There are very few studies done among the Asian population to examine the prognostic role of fascin in colorectal adeno carcinoma.

An evaluation of the role of fascin expression as bio markers will help in categorising the risk level and also risk assessment and this will also be a useful prognostic marker which will help the physicians to plan the treatment for colorectal carcinoma.

Objectives

This study was carried out

- To evaluate expression of Fascin in primary colorectal adenocarcinoma.
- To correlate the Fascin expression with other clinicopathological prognostic parameter
- To assess the role of Fascin expression as a prognostic and theranostic marker in patients with carcinoma of colon.

Methodology

Study settings and study participants- This retrospective study was carried out on paraffin blocks of colorectal carcinoma specimens in the Department of Pathology in a tertiary care hospital. All the paraffin blocks of specimens received for a period of four years between 2010 and 2014 were included in the study. A total of 60 specimens were studied.

Ethical approval- Approval was obtained from the Institutional Ethics Committee prior to the commencement of the study (Ref no: CSP-MED/13/ AUG/ 08/50).

Data collection- The paraffin blocks were made on samples from tumor areas along with adjacent normal areas from colectomy specimens received in the department. IHC for Fascin was done on the sections along with controls. Clinical data regarding the participant's demographics and medical history were captured from the hospital medical records. Gross findings were recorded from the blocks. Blocks with section containing normal epithelium and tumour were chosen for immunohistochemistry.

Hematoxylin and Eosin staining was done for microscopic analysis, for resected lymph nodes and surgical margins. Other histopathological features observed were intratumoral and peritumoral inflammatory response, adjacent dysplasia and adenomas. Immunohistochemical staining for Fascin was performed on all 60 cases by submitting the blocks with prediluted anti-Fascin primary antibody. The staining of Fascin (IHC) in the cytoplasm and grade 3+ intense staining were considered as strong positive and accounted for positivity while the rest with less intensity was considered as negative.

Data analysis- Data was entered and 771nalysed using SPSS ver 21. Percentages were computed for the demographic data. Independent sample t test was used to test statistical significance between fascin expression and patient characteristics. A p value <0.05 was considered statistically significant.

Results

In the present study the age of the patients ranged from 31 to 87 years. Highest incidence was noted in the age group 51 to 60 years (32%), followed by the age group 61 to 70 years (27%). In the present study it was also observed that colon cancer occurred predominantly in men as compared to women with a male to female ratio of 2.1:1 with 68% males and 32% females (Figure 1). Right colon was the most common site involved (56%) and second most common site was rectum (22%). The most common histological variants as analysed in the present study were in the order of frequency as adenocarcinoma (78%), mucinous (12%), signet ring (5%) and undifferentiated carcinoma (5%). In the present study most of the tumors (60%) belong to pT2 (Tumor invades muscularis propria) of the TNM (primary tumor, regional

lymph nodes, and distant metastasis) classification, followed by pT3 -34%. Majority of the participants belonged to N0 (47%) and clinically distant metastasis cMo was the most common metastasis stage (69%) (Table 1).

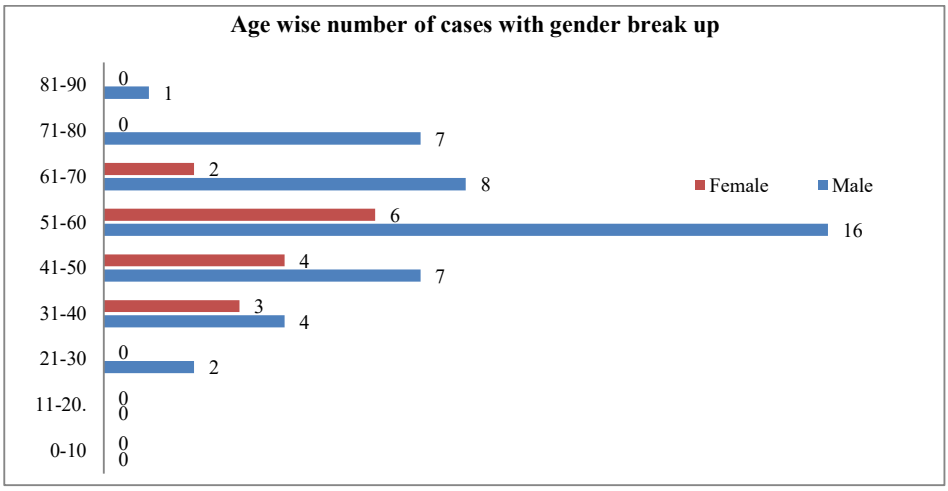

Figure 1: Age distribution of the participants:

Table-1: Tumor characteristics of the study participants.

Characteristics	Frequency (100)	Percentage
Site		
Right	56	56.0
Left	22	22.0
Rectum	22	22.0
Size of Tumor		
<5Cm	53	53.0
>5Cm	47	47.0
Types of Carcinoma		
Adenocarcinoma	78	78.0
Mucinous	12	12.0
Signet ring	5	5.0
Un differentiated	5	5.0
Grade		
G1	18	18.0
G2	60	60.0
G3	15	15.0
G4	7	7.0
Colon cancer-pT staging		
pT2	60	60.0
pT3	34	34.0
pT4	6	6.0
N break up		
N0	47	47.0
N1	32	32.0
N2	18	18.0
Nx	3	3.0
Colon cancer and metastasis		
cM0	69	69.0
cM1	31	31.0

The grading of intensity of fascin-1 expression showed that majority of tumors in grade 0 were positive (70%) while only 15% and 10% in grade II and III were positive for fascin expression (Table 2).

Table-2: Fascin expression among the study participants.

Grade	N (60)	Positive (%)	Negative (%)
0	60	42 (70.0)	18 (30.0)
1	60	0 (0.0)	0 (0.0)
2	60	6 (10.0)	54 (90.0)
3	60	9 (15.0)	51 (85.0)

There was a significant association between pT1/pT2 staging and grade 0 fascin expression (80%) compared to those with pT3/pT4 staging (50%) ($p<0.05$). Similarly, grade 1 had strong positive correlation with fascin expression (70%) compared to other grades of the tumor ($p<0.05$) (Table 3).

Table-3: Association between fascin expression and tumor characteristics.

Characteristic	Grade 0 fascin expression		N (60)	P value
	Positive (%)	Negative (%)		
pT staging				
pT1/pT2	32 (80.0)	8 (20.0)	40	0.017
pT3/pT4	10 (50.0)	10 (50.0)	20	
cM staging				
CM0	41 (80.4)	10 (19.6)	51	0.000
CM1	1 (11.1)	8 (88.9)	9	
Tumor grade				
1	8 (72.7)	3 (27.3)	11	0.005
2	31 (81.6)	7 (18.4)	38	
3	3 (33.3)	6 (66.7)	9	
4	0 (0.0)	2 (100)	2	

The present study observed a statistically significant association between the tumor grade with grade 2+ fascin expression. Grade 3 tumor had highest positivity (33.3%) compared to other grades ($p<0.05$) (Table 4).

Table-4: Association between fascin expression and tumor grade.

Tumor grading	Grade 2+ fascin expression		N (60)	P value
	Positive	Negative		
1	2 (18.1)	9 (81.9)	11	0.032
2	1 (2.6)	37 (97.4)	38	
3	3 (33.3)	6 (66.7)	9	
4	0 (0.0)	2 (100.0)	2	

There was a significant association between cM staging and grade 3+ positivity of fascin expression. Participants with cM1 stage had higher positivity (55.6%) compared to those in stage cM0 (7.8%). This association was statistically significant ($p<0.001$) (Table 5).

Table-5: Association between fascin expression and tumor characteristics.

Characteristic	3		N (60)	P value
	Positive	Negative		
Correlation of cM with expression of Fascin				
CM0	4 (7.8)	47 (92.2)	51	0.000
CM1	5 (55.6)	4 (44.4)	9	
Correlation of grade with expression of Fascin				
1	1 (9.1)	10 (90.9)	11	0.005
2	4 (10.5)	34 (89.5)	38	
3	2 (22.2)	7 (77.8)	9	
4	2 (100.0)	0 (0.0)	2	

Discussion

Colorectal carcinoma is one of the most frequent causes of cancer death of worldwide and is often associated with poor survival rates, especially in developing countries. They are a group of heterogenous cancers involving multiple tumorigenic pathways. It is essential to provide accurate histopathological diagnosis, including staging and evaluation of prognostic parameters including evaluation of lymphovascular and perineural invasion. In addition, evaluation of immunohistochemical expression of certain markers help in determining the progression of the disease and ascertainment of the metastatic component of colorectal malignancies.

According to Nelson H et al, adeno carcinoma was most frequent variant of colorectal cancers (92%) and this finding is similar to the present study [7]. In the present study, majority of the participants were around 58 years of age similar to studies done by Choks KS et al [8]. The present study was carried out with an objective of exploring the role of fascin expression in colorectal adeno carcinoma. It was observed that there was no association between fascin expression and age or gender. This observation was similar to the study by Hashimoto Y et al, which evaluated clinically annotated tumors, where fascin immuno reactivity was not associated with age or gender [9]. In a study done by Puppa G et al, however fascin was significantly associated with females compare to males [10].

In the present study, it was observed that right sided lesions were increasingly prevalent, compared to the left sided ones. Moreover, right sided lesions were likely to present at an older age while left sided lesion have a greater chance of presenting with bleeding per rectum and changes in the bowel habits. Similar observation was seen in studies done by Hashimoto Y, and Puppa G et al, while in a study done by Marley et al, majority of the lesions were on the left side (54%) [9-11]. Studies have shown the size of the tumor has limited role in determining the prognosis. In the present study it was observed that tumor of varying sizes had the same stage, and tumor extent was more important than the size. As far as the grading was concerned, degree of gland formation has been the most important feature of grading. We graded colorectal carcinoma as well differentiated (grade I), moderately differentiated (grade II) and poorly differentiated (grade III). In this study grade II was predominantly present (69%) followed by grade 1 (18%). However, higher grades were present in males and among older adults beyond 60 years.

Fascin, a globular protein is aggregation of F-actin into parallel bundles which rearranges the cytoskeleton and promotes cellular motility [12]. In human beings, the gene encoding fascin-1 is located on chromosome 7q22 [13]. Fascin is often completely negative are scattered in epithelia of intestinal organs including biliary duct, colon, ovary, pancreas, stomach and breast [14]. Fascin is highly expressed in cancers associated with lung, gastric and easophagus and is often associated with poor prognosis and reduced survival outcomes [15-19].

The pathogenesis of expression of fascin in metastasis of colorectal carcinoma has been attributed to the promotion of migratory and invasive phenotype through filopodia formation. Fasicn is expressed in human colon carcinoma in a grade and stage dependent manner but it is absent in normal colonic epithelium. Fascin up-regulation in many other cancers was associated with poor prognosis [10,11-18]. Studies have shown that fascin is up regulated in more aggressive and metastatic epithelial cancers and it is an independent prognostic

indicator of poor outcome. In many studies fascin staining was high in poorly differentiated and advanced tumors and presence of fascin is indirectly indicative of invasive metastasis [18]. Further evaluation of this mechanism was demonstrated by Hashimoto et al, who attenuated the expression of fascin using short hairpin RNA (shRNA) in primary tumor of colon. As a result, the depletion of fascin resulted in decrease in the filopodia and altered morphology of cell protrusions.

This further reduced the number Rac-dependent migration of laminin and decreased xenograft tumor development and metastasis [20]. This phenomenon clearly elucidated that fascin mRNA and protein expression were increased in primary tumors and transient upregulation promotes migratory and invasive phenotypes leading to metastasis.

Several recent advances have explored the role of beta catenin in tumorigenesis. It has been documented that presence of beta catenin in the nuclei of the cells showing fascin1 expression is indicative of activity in beta catenin-TCF signalling pathways. In early carcinogenesis of primary tumors, beta catenin aids in the activation of proliferation-associated genes. In the presence of fascin expression, the beta-catenin-TCF target genes are activated, further aggravating the metastatic process [21].

Conclusion

Expression of Fascin-1 in colonic cancer aids substantially in the diagnosis and staging of the tumors. In the present study, a significant correlation was observed between the fascin expression and tumor staging, thereby indicating its role in determining the prognosis of these patients. The basis for the correlation between high fascin expression and poor prognosis of human carcinomas resides in undermining the role of fascin in one or more steps of metastasis. Metastasis, which is a complex, multistage process is characterized by cell migration at various steps resulting in tumor dissemination. Presence of fascin expression significantly indicates the metastatic potential of colon carcinomas. Further research is required to delineate the mechanism of fascin expression at the cellular level in determining the prognosis of colon cancers.

What the study adds to the existing knowledge?

In the present study it has been elucidated the significance of fascin expression in metastatic tumors of colon and rectum. This study has provided scope for further exploration of associated signaling pathways at molecular level linking fascin expression and metastasis.

Author's contribution

Dr. Rajesh H.: Conceptualization

Dr. Rajendiran Swaminathan: Literature review

Dr. Leena Dennis Joseph: Data collection

Dr. Jamuna Srirangaramasamy: Data analysis

Dr. Sangeetha BS: Manuscript writing and editing

Declaration

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