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RESEARCH ARTICLE

Clinicopathological Significance of Mesothelin Expression In Colorectal Cancer

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Abstract

Background:Colorectal cancer is one of the leading causes of cancer associated morbidity and mortality in the world. Mesothelin, a plasma membrane differentiation antigen, is expressed at varying degrees in several human cancers. Its expression is associated with unfavorable patient outcome.

Aim:To determine the expression profile of mesothelin gene in colorectal cancer and to correlate its expression with other established prognostic parameters of colorectal carcinoma including patient survival.

Patients and Methods:The subjects of the present study were 40 patients who underwent elective surgical resection for primary colorectal carcinoma.To evaluate prognostic significance of mesothelin, immunohistochemistry was used to assess the expression pattern of mesothelin protein in surgically resected, formalin-fixed, paraffin-embedded colorectal carcinoma specimens.

Results:Mesothelin protein was seen mainly expressed in the cell membrane of colorectal carcinoma cells. Immunohistochemically, luminal membrane positive for mesothelin expression was identified in 40% (16 out of 40) of cases. No significant association was found between mesothelin immunoreactivity and patient's age, gender, tumor location and tumor size ($P> 0.05$). A statistically significant correlation was observed between mesothelin expression and histopathologic grade ($P\leq 0.05$). High level of mesothelin expression was noted in mesothelin-expressing colorectal tumors of high grade malignancy. Luminal membrane expression of mesothelin was significantly associated with lymph node metastasis. No significant correlation was detected between mesothelin expression and tumor stage. Positive mesothelin expression was significantly associated with poor disease free survival. Patients with mesothelin +ve tumors had a shorter overall survival as compared to patients with mesothelin –ve tumors, but the difference was not statistically significant ($P>0.05$).

Conclusion:Our results suggest that immunohistochemical evaluation of luminal membrane expression of mesothelin in colorectal carcinoma would be of clinical benefit not only as a prognostic factor, but also, as a predictive factor for the eligibility to mesothelin-targeting therapies in the future.

INTRODUCTION

Colorectal cancer is one of the most common types of cancer in the world. Worldwide it affects more than one million people every year and is responsible for more than 0.5 million cancer related deaths annually.(1) Although surgery is a common treatment option for colorectal cancer, the recurrence rate after curative surgery is 17.3% and an overall-5 year survival rate of surgically treated colorectal cancer patients is 65%.(2,3) In about 20% of patients distant metastasis are present at the time of diagnosis.(3) The profile of molecular markers may provide valuable insights into the underlying mechanisms of disease progression, thereby aiding intervention strategies. Chemotherapy has improved the clinical outcome and survival rate in unresectable and recurrent colorectal cancer cases, however there is still a need to identify patient subgroups with high or low risks of tumor recurrence and to tailor individual therapeutic interventions.(4,5) .

Mesothelin (MSLN) is a cell surface glycoprotein present on normal mesothelial cells that line the pleural, peritoneum and pericardium. The mesothelin gene encodes a precursor protein of 71KDa that is processed to a 31KDa shedded protein called megakaryocyte potentiating factor and a 40 KDa membrane- bound protein, mesothelin.(6) Mesothelin is the antigen recognized by the monoclonal antibody K1 which was generated by the immunization of BALB/c mice with the human ovarian carcinoma cell line OVCAR-3.(7) The biological function of mesothelin is unknown, although some studies have suggested that it might have a role in the spread of tumours.(8) It has been shown that expression of MSLN gene is limited in normal tissues to the mesothelial cells lining the pleura, pericardium and peritoneum.(6,7) As a result of its limited expression in normal tissues, MSLN has been reported as an ideal tumor-associated marker for the development of targeted therapy.(9) However overexpression of mesothelin has been identified in various cancers including mesothelioma, ovarian, biliary and pancreatic adenocarcinoma, and lung cancer by using immunohistochemistry (IHC) staining.(10)

Expression of the MSLN gene in colorectal cancer has not been fully explored. Thus, the present study was conducted to determine the expression profile of mesothelin gene in colorectal carcinoma and to correlate its expression with other established prognostic parameters of colorectal cancer.

Patients and methods:

The study population comprised a series of 40 patients undergoing elective surgical resection of a histologically proven primary colorectal cancer at the Department of Surgery, Medical Research Institute Hospital, Alex. University, Egypt. These patients were treated between July 2011 to October 2014. Patients included in this study were 25 males and the remaining 15 were females with a male to female ratio (5:3). The mean age of the studied patients was 52.32±11.96 years, range (26-74) years. Preoperatively patients were subjected to full clinical examination, routine lab. investigations, radiological investigations (Abd. US& CT abdomen) and colonoscopy for accurate localization and biopsy from the tumor for histopathological examination to confirm malignancy. All patients included in this study didn't receive radiation treatment or chemotherapy prior to surgery. The tumor location was the cecum in 4(10%), ascending colon in 14(35%), descending colon in7(17.5%), and rectosigmoid in 15(37.5%) patients. Patients with right sided tumors (cecum &ascending colon) were subjected to right hemicolectomy while those with left sided tumors (descending& rectosigmoid colon) underwent left hemicolectomy and anterior resection respectively.

After surgery patients were referred to the Oncology Department for adjuvant treatment and were followed up for up to 2 years. Colorectal carcinoma specimens were sent to the Pathology Department for pathological evaluation and immunohistochemical staining

Tumor samples and pathological evaluation:

Colorectal carcinoma specimens obtained from 40 patients were examined at the Pathology Department. Regarding tumor size 14 cases were ≤5cm in size, while the other 26 cases were >5cm in size. According to tumor type and grading, the tumors were subdivided as regard tumor type into 2 cases mucoid adenocarcinoma, 3 cases signet ring carcinoma, and 35 cases were adenocarcinomas with varying degrees of differentiation (7 cases well differentiated, 19 cases moderately differentiated, and 9 cases poorly differentiated adenocarcinoma). Tumors were classified according to the modified Dukes staging into 6 cases stage B1, 12 cases stage B2, 8 cases stage C1and 14 cases were stage C2.(11)

Immunohistochemistry:

Formalin-fixed, paraffin-embedded tissue blocks were prepared from surgical specimens and adjacent non cancerous mucosa. Sections were sliced and stained with Hematoxylin and Eosin (H&E) for routine histopathological examination. All specimens were diagnosed as colorectal carcinomas. The H&E stained slides of tumor specimens were reviewed and the tissue blocks that included the tumor area for formalin-fixing and paraffin-embedding were selected. Slides were cut at 4- 5 µm and were processed using a hot citric acid antigen retrieval method, for 30 min. according to the manufacturer's recommendations and then incubated for 2 hrs with Mesothelin monoclonal antibodies. The immunohistochemical staining was performed by labeled Strept-Avidin-Biotin method with staining kit of Zymed Lab,Inc. USA.(12) Following the instructions of manufacturer, the bound antibody complex was visualized by reaction in 3,3' Diaminobenzidine substrate and sections were counterstained with Meyer's Hematoxylin. The staining intensities of Mesothelin in tumor cells were evaluated. Sections from a block of pancreatic adenocarcinoma were stained as a positive control for Mesothelin . A negative control slide was prepared by omission of the primary antibody. Mesothelin immunostaining was measured as the percentage of the number of the positively stained cells over the total number of the cells. Five high resolution fields from each slide were randomly chosen for the measurement.

Interpretation of immunohistochemical results:

Immunostaining for Mesothelin was evaluated for both the proportion and staining intensity of tumor cells in each case. The proportion of Mesothelin expression was assessed using the scoring system described by Einama et al. (13). For the immunostained slides, the proportion of stained cancer cells was scored as (+1) for 1-10%, (+2) for >10-50% and (+3) for >50%. The intensity of the staining was scored as (+1) for weak intensity and (+2) for moderate to strong intensity, and the location of the staining was also recorded either in the luminal membrane or the cytoplasm. The term " luminal membrane positive " was given when the luminal membrane of cancer cell was stained partially or faintly or the entire circumference of the luminal membrane was clearly stained. Cases without membrane staining and those in which only cytoplasmic staining was observed in any intensity level were defined as "luminal membrane negative". The final evaluation of C-ERC/ Mesothelin expression was based on the following scoring system:' positive- staining' was defined as a proportion score of ≥(+3)and ⁄ or an intensity score of(+ 2), while ' negative staining' was defined as a total score of < (+3) except in cases involving a proportion score of(+1) and an intensity score of (+2) (Figure 1).

Adjuvant treatment and follow up:

All patients included in this study received 12 cycles of chemotherapy. Each cycle consisted of Oxaliplatin 85 mg/m^2 intravenous on day 1 and Leucoverin 200 mg/m^2 on days 1&2 with 5-Fluro-Uracil (5FU) 400 mg/m^2 bolus intravenous injection and 5FU 600 mg/m^2 continuous infusion on days 1&2 (FolFox regimen). Each cycle was repeated every 21 days for 12 cycles. All patients were followed up for 2 years.During the follow up period patients underwent complete physical examination, laboratory investigations (CBC, liver &kidney functions) and tumor markers (CA 19-9,CEA) to monitor treatment response and to detect early recurrence. Radiological investigations including chest x-ray& abdominal US were done every 3 months. Abdominal &pelvic CT scan was performed to detect relapse. Patients underwent colonoscopy 6 months after surgery and every 6 months till the end of the follow up period.

Statistical analysis:

All statistical analyses were performed using SPSS program statistical software, version 15.0 (SPSS Inc. Chicago, IL, USA) for Windows. Associations between mesothelin protein immunoreactivity score and clinicopathological variables were analysed using Chi square test and Fisher exact test. Results were expressed in Odd's ratios (OR) and 95% confidence intervals (CI). Survival curves for patients were drawn by the Kaplan-Meier method. Disease-free survival (DFS) was measured from the date of surgery to the date of first documented local or distant recurrence or lost follow-up. Overall survival (OS) was measured from the date of surgery to the date of death or lost to follow-up. All differences were considered statistically significant at a P value of less than 0.05 ($P \leq 0.05$).

Results

A total of 40 patients with colorectal cancer were included in the present work. The colorectal carcinoma cases were 25 males and 15 females with a male to female ratio (5:3). Their mean age was 52.32±11.96, range (26-74) years, 27 patients were ≤60 years and 13 patients were >60 years.

In the current study, Mesothelin (MSLN) expression was seen mainly in the cell membrane of the colorectal carcinoma (CRC) cells. Representative examples of the different immunohistochemical staining pattern for MSLN protein in CRC tissue are shown in (Fig.1).

Immunohistochemically, detectable MSLN expression has been found in 16 out 40 cases (40%) of CRC specimens analysed. In all positive cases, MSLN immunoreactivity pattern was located mainly in the cell membrane of the tumor cell (Luminal membrane +ve) for MSLN expression.Weak positivity for MSLN expression (+1) was found in 4 out of 16 cases (25%), while moderate to strong positivity (+2) was observed in 12 out of 16 cases (75%). Negative expression of MSLN was recorded in 24 out of 40 cases (60%) of CRC (Luminal membrane –ve). The correlation between MSLN expression and various clinicopathological features is summarized in table (1).

In the present work , there was no significant correlation between MSLN immunoreactivity and patient's age or sex (P>0.05).

As regards tumor location, positive immunoreactivity for MSLN expression was found in 6 out of 18 cases (33.3%) in right sided tumors, while in left sided tumors ,it was expressed in 10 out of 22 cases (45.5%). However, no significant association has been found between MSLN expression and tumor location.

Concerning tumor size, positive MSLN expression was found in 4 out of 14 cases (28.6%) of CRC specimens having tumors ≤ 5cm in size , but in tumors more than > 5 cm in size, MSLN expression was detected in 12 out of 26 cases (46.2%). Although the level of MSLN expression was higher in large sized tumors as compared to their smaller sized counterparts, the difference was not statistically significant (P>0.05).

According to histological type and grade, the histopathological examination of CRC tissue revealed that immunoreactive MSLN expression was observed in 12 out of 35 differentiated tumors accounting for (34.3%). Differentiated tumors were graded as, GI well differentiated adenocarcinoma in one case out of 7 cases(14.3%), GII moderately differentiated adenocarcinoma in 4 out of 19 cases (21.05%), and GIII poorly differentiated adenocarcinoma in 7 out of 9 cases (77.8%) positive for MSLN expression. Positive MSLN expression was also detected in one case out of 2 cases (50%) mucoid adenocarcinoma, and in 3 cases of signet ring carcinoma (100%). The results of statistical analysis showed that there was a statistically significant association between high tumor grade and MSLN expression (P≤ 0.05). MSLN positive cases were higher in moderately than well differentiated adenocarcinoma and in poorly than moderately differentiated tumors. Also positive MSLN expression was significantly correlated with signet ring carcinoma tumors (P≤ 0.05).

Regarding lymph node status, +ve luminal membrane expression of MSLN was observed in 4 out of 18 cases (22.2%) of tumors with no lymph node metastases, while in tumors with positive lymph nodes, MSLN expression was detected in 12 out of 22 cases (54.5%). The results of statistical analysis revealed that there was a significant association between positive lymph node status and MSLN expression (P≤ 0.05). MSLN positivity was more frequently expressed in tumors with positive lymph nodes.

Furthermore , and according to Modified Duke's Staging , 6 patients (15%) were stage B1, 12 patients (30%) were stage B2, 8 patients (20%) were stage C1 and 14 patients (35%) were stage C2. The immunohistochemical expression of MSLN protein showed that MSLN was positively expressed in 2 out of 6 cases (33.3%), in 3out of 12 cases (25%), in 5 out of 8 cases (62.5%), and in 6 out of 14cases (42.9%) in stages B1,B2,C1,C2 respectively. Statistical analysis revealed that MSLN protein was more frequently expressed in CRC tumors of stage C2 than stage C1, more in C1 than B2, and more in B2 than B1stage. However, there was no statistically significant association between MSLN expression and tumor stage (P>0.05).

In the present study, all patients with CRC were followed –up for up to 2 years. By the end of this period, the disease free surviva l(DFS) was 87.5% (21 out of 24 patients) and 43.8% (7 out of 16 patients) in patients with MSLN negative and MSLN positive tumors respectively. A statistically significant association was found between positive MSLN expression and DFS (P≤ 0.05). Poor disease free survival was more frequently noted in MSLN positive tumors than MSLN negative tumors.

The overall survival (OS) was 87.5% (21out of 24 patients) and 75% (12 out of 16 patients) in patients with MSLN negative and MSLN positive tumors respectively. Patients with MSLN positive tumors had a shorter survival time as compared to those with MSLN negative tumors although the difference was not statistically significant (P> 0.05).

Kaplan-Meier survival curves for DFS and OS by mesothelin expression are illustrated in (Fig. 2 &3).

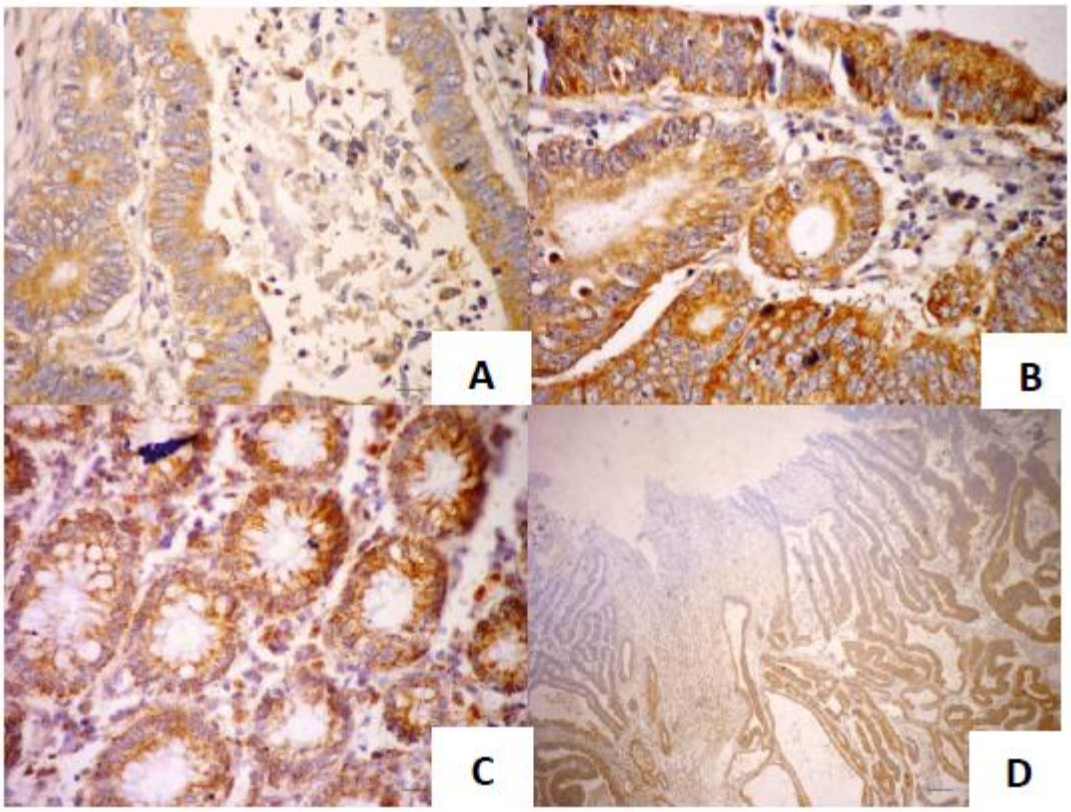

Figure (1): Different immunohistochemical staining pattern for MSLN protein in CRC tissue. (A) Moderately differentiated adenocarcinoma showing strong luminal membrane mesothelin immunoreactivity. (B) Moderately differentiated adenocarcinoma showing granular cytoplasmic mesothelin expression (luminal membrane –ve). (C) Well differentiated adenocarcinoma showing entire luminal membrane expression for mesothelin. (D) Moderately differentiated adenocarcinoma showing partial luminal membrane mesothelin immunoreactivity.

Table (1): CORRELATION BETWEEN MESOTHELIN EXPRESSION AND CLINICOPATHOLOGICAL PARAMETERS OF COLORECTAL CANCER

CLINICOPATHOLOGICAL VARIABLES	MESOTHELIN IMMUNOREACTIVITY				χ^2	p	OR	95% CI
	Positive (n=16)		Negative (n =24)					
	No.	%	No.	%				
Age								
- ≤60	11	68.8	16	66.7	0.019	0.890	0.909	0.234 – 3.527
->60	5	31.3	8	33.3			1.100	0.284 – 4.267
SEX								
- Men	9	56.3	16	66.7	0.444	0.505	1.556	0.423 – 5.721
- Women	7	43.8	8	33.3			0.643	0.175 – 2.364
Site of the tumor:								
-Right side (ascending colon and cecum)	6	37.5	12	50.0	0.606	0.436	1.667	0.459 – 6.056
-Left side (descending colon &rectosigmoid)	10	62.5	12	50.0			0.600	0.165 – 2.180
Size of the Tumor:								
≤5 cms	4	25.0	10	41.7	1.172	0.279	2.143	0.532 – 8.625
>5 cms	12	75.0	14	58.3				
Histological type & grade:								
-Mucoid adenocarcinoma	1	6.3	1	4.1	0.088	$^{FE}p = 0.767$	0.652	0.038 – 11.242
-Adenocarcinoma:								
-Well Differentiated	1	6.3	6	25.0	2.338	$^{FE}p = 0.210$	5.00	0.540 – 46.274
-Moderately Differentiated	4	25.0	15	62.5	5.414*	0.020*	5.00*	1.231-20.301
- Poorly Differentiated	7	43.8	2	8.3	6.906*	$^{FE}p=0.018$*	0.117*	0.020 – 0.674
-Signet ring carcinoma	3	18.8	0	0.0	4.865*	0.027*	-	-
LN:								
- No LNs involved(-ve)	4	25.0	14	58.3	4.310*	0.038*	4.200*	1.004 – 16.904
- Presence of LN involvement (+ve)	12	75.0	10	41.7			0.238*	0.059-0.958
Stage:								
Stage B1	2	12.5	4	16.7	0.131	$^{FE}p = 1.000$	1.400	0.225 – 8.724
Stage B2	3	18.8	9	37.5	1.607	$^{FE}p = 0.297$	2.600	0.578 – 11.687
Stage C1	5	31.3	3	12.5	2.109	$^{FE}p = 0.229$	0.314	0.063 – 1.567
Stage C2	6	37.5	8	33.3	0.073	0.787	0.833	0.222 – 3.122

χ^2: Chi square test.
FE: Fisher Exact test.
OR: Odd's ratio.
CI: Confidence interval.
*: Statistically significant at $p \leq 0.05$.

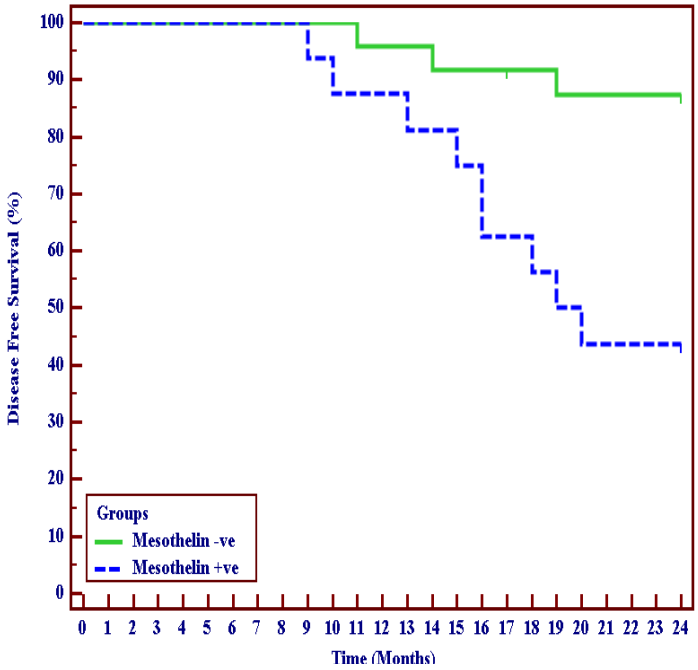

Figure (2): Kaplan - Meier survival curve for disease free survival

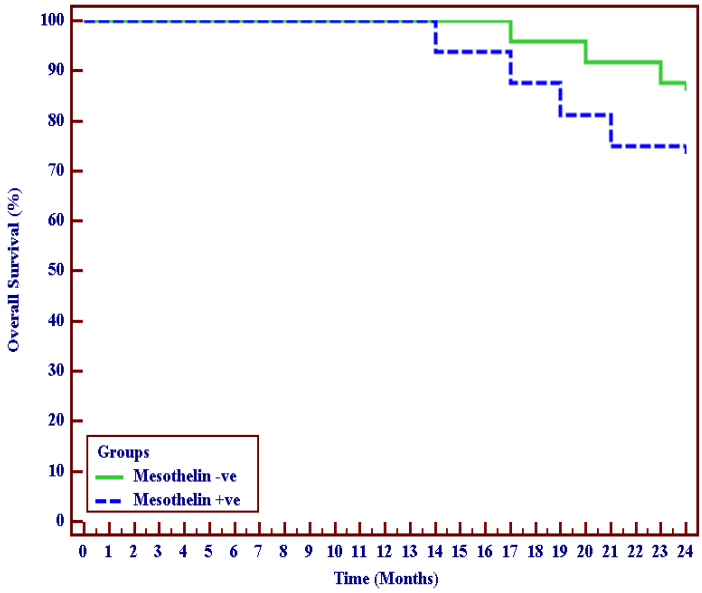

Figure (3): Kaplan- Meier survival curve for overall survival

Discussion

Colorectal cancer is one of the leading causes of cancer associated morbidity and mortality in the world. Rapid advances in our understanding about the molecular and biologic characteristics of CRC have provided useful knowledge into the pathogenesis of CRC.Therefore,biomarkers have been developed to identifying individuals who will benefit most from cancer surveillance and management.(14) Identifying biomarkers that can detect CRC earlier or monitor cancer progression would enable personalization of medicine and improve survival rates of patients with cancer.

The underlying mechanisms of action in cancer progression are beginning to be unraveled. The reported molecular and biochemical mechanisms that may contribute to the phenotypic changes in favor of carcinogenesis include, inhibited apoptosis, enhanced tumor cell proliferation, increased invasiveness, perturbtion of cell adhesion, promotion of angiogenesis and inhibited immune surveillance. These events may contribute to the development and progression of cancer.(15)

Apoptosis plays an important role in many biological events, including morphogenesis, cell turnover, and elimination of harmful cells. A disturbance in apoptosis may confer a survival advantage on malignant cells harboring genetic alterations and thus promote cancer progression.(16) Therefore a better understanding of the molecular biology of CRC may assist in leading the path to improve and customize drug-development with the goal of improve outcomes.

The biological functions of MSLN remains largely unknown. Many researchers have investigated the role of MSLN expression in tumor biology and demonstrated the importance of MSLN gene expression for tumor progression in Vitro (17,18) and in Vivo.(19,20) However, the critical biological role of MSLN in cancer progression resulting in patients' poor prognosis has not yet been clarified.

The human MSLN gene codes the primary product, which is a 71-KDa precursor protein. This protein can be physiologically cleaved by some Furin-like proteases into a 40-KDa C-terminal fragment (C-ERC/mesothelin) that remains membrane-bound and a 31-KDa N-terminal fragment (N-ERC/ mesothelin) that is secreted into the blood.(6) The C-terminal 40-KDa fragment (C-ERC/ mesothelin) is referred to as mesothelin (MSLN) and is attached to the cell membrane through a glycosyl-phosphatidylinositol (GPI) anchor.(21) The membrane bound form of MSLN is the active form which promotes aggressive cellular characteristics including increase in cell motility, invasive or migratory ability and growth of metastatic tumors.(22)

In the current study, immunohistochemically the frequency of luminal membrane expression of MSLN in CRC specimens analysed in our series was found to be 40% (16 out of 40) cases. Previous publications reported that MSLN ,a plasma membrane differentiation antigen, was expressed at significantly high levels in several human cancers,including nearly all mesotheliomas (6) and pancreatic carcinomas (23,24), as well as about 70% of ovarian cancers (8,25) , 50% of lung carcinomas (26,27) and 46% of oesophageal adenocarcinomas.(28)

As for CRC, Liebig et al reported that mesothelin was expressed in 28 out of 46 adenocarcinomas (58%) and MSLN-positive tumor cells were restricted to the invasive front.(29) Ryohei et al evaluated MSLN expression in 25 patients with cholangiocarcinoma using MSLN monoclonal antibody and demonstrated that 8 out of 25 patients (32%) showed MSLN immunoreactivity.(30) Chang et al reported MSLN expression in 66.7% (10 out of 15) non mucinous carcinoma of the ovary using the K1 antibody.(31)

In the literature, MSLN gene was expressed to varying degrees by other tumors including, head and neck cancer, gastric carcinoma, gall bladder cancer and tumors of the common bile duct.(32) Our results were in line with these previous studies.

In accordance with other publications, no significant association was found in this study between MSLN immunoreactivity and many clinicopathological parameters including age, gender, tumor size and tumor location.(33)

The present series, demonstrated that luminal membrane expression of MSLN was observed in 12 out of 35 differentiated tumors accounting for 34.3%.We found a statistically significant correlation between MSLN expression and the degree of tumor differentiation. High level of MSLN expression was observed in mesothelin-expressing colorectal tumors of high grade malignancy. Our findings were in agreement with previous reports as in the study of Hassan et al (10), Argani et al (23) and others.(34,35) In contrast to our data, Wang et al reported insignificant association between MSLN expression and histopathologic grade.(36)

In our study, luminal membrane expression of MSLN was significantly associated with lymph node metastasis. Lymph node metastasis is widely accepted as one of the most important prognostic factors in CRC patients.(37) Luminal membrane positive of MSLN revealed significant unfavorable patients'outcome as compared to luminal membrane negative of MSLN among the patients with lymph node metastasis. Moreover, in a recent report on gastric cancer, luminal membrane expression of MSLN was correlated with histological grade, lymph node metastasis, stage , recurrence and poor patient outcome.(35) Another study evaluated mesothelin expression in all breast cancer subtypes and stated that high MSLN levels in the tumor cells were significantly associated with tumor infiltration of the lymph nodes.(36) Our results were consistent with the previously published reports. The current study demonstrated that MSLN protein was more frequently expressed in higher tumor stages, although, no significant correlation was found between MSLN expression and tumor stage confirming the results obtained by previous publications.(38,39) Other investigators, reported that MSLN expression had a significant correlation with clinical stage.(35)

Regarding survival analysis in our series, we found that luminal membrane expression of MSLN was significantly associated with poor disease free survival. On the other hand, patients with MSLN positive tumors had a shorter overall survival as compared to patients with MSLN negative tumors but the difference was not statistically significant. Previous authors reported that higher MSLN expression was associated with chemoresistance and shorter patient survival in patients with ovarian cancer.(39) A recent report demonstrated that MSLN expression was correlated with disease recurrence and poor patient outcome.(35) Other workers, stated that raised MSLN level was associated with poor and decreased overall survival.(36,40) Our findings were in accordance with previous studies. Meanwhile, contrary to our obtained data, a significant correlation between raised MSLN level and prolonged patients'survival has also been reported in ovarian and gastric cancers.(41,42)

The correlation between MSLN level and poor disease free survival, shorter overall survival and tumor infiltration of lymph nodes suggests that MSLN may promote tumor invasion and could function as a potential predictive biomarker of disease free and overall survival in colorectal cancer.

The immunohistochemical examination of MSLN expression in surgically resected tumor specimens is clinically useful for assessing the prognosis and for deciding on the necessity of additional treatment modalities following surgical procedures in patients with colorectal cancer. Furthermore, treatment that suppresses MSLN expression, either immunologically, or genetically, in MSLN-expressing malignant tumors might in the future become an important therapeutic alternative for cases in which surgical treatment is insufficient.

In conclusion, our results suggest that the immunohistochemical evaluation of luminal membrane expression of MSLN in colorectal carcinoma would be of clinical benefit not only as a prognostic factor, but also as a predictive factor for the eligibility to MSLN- targeting therapies in the future.

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