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Mesothelin expression and its prognostic role according to microsatellite instability status in colorectal adenocarcinoma

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Abstract

The cell-surface glycoprotein, mesothelin, is normally present on mesothelial cells. Overexpression of mesothelin has been reported in many tumors and is correlated with poor outcome. We investigated the clinicopathologic significance of mesothelin expression in colorectal adenocarcinoma with microsatellites instability (MSI) status.

Mesothelin expression was evaluated immunohistochemically in tissue microarray blocks from 390 colorectal adenocarcinoma samples. Mesothelin expression was interpreted according to the intensity and extent. A score of 2 was considered high expression. We analyzed the correlation between mesothelin expression and clinicopathologic characteristics.

High mesothelin expression was observed in 177 (45.4%) out of 390 colorectal adenocarcinoma samples and was significantly associated with high histologic grade ($P = .037$), lymphatic invasion ($P = .028$), lymph node metastasis ($P = .028$), and high AJCC stage ($P = .026$). Kaplan–Meier survival curves revealed no significant difference between patients with high mesothelin expression and patients with low mesothelin expression in both recurrence-free survival (RFS) and cancer-specific survival ($P = .609$ and $P = .167$, respectively). In subgroup survival analyses, high mesothelin expression was associated with poor RFS in the MSI-High group of colorectal adenocarcinoma ($P = .004$).

High mesothelin expression was significantly associated with aggressive phenotypes and poor patient outcome in MSI-High colorectal adenocarcinoma.

Abbreviations: CSS = cancer-specific survival, H&E = hematoxylin and eosin, HR = hazard ratio, MSI = microsatellites instability, MSS = microsatellite stable, PD-1 = programmed cell death 1, RFS = recurrence-free survival, SD = standard deviation.

Keywords: colorectal adenocarcinoma, immunohistochemistry, mesothelin, prognosis

1. Introduction

Colorectal adenocarcinoma is one of the most common cancers in the world with over 1.8 million new colorectal adenocarcinomas estimated to have occurred in 2018.[1] Colorectal adenocarcinoma is the second most frequently diagnosed cancer and the fourth leading cause of cancer death in Korea.[2] Surgical excision is the first choice for curing colorectal adenocarcinoma, and adjuvant systemic therapy including immunotherapy can improve survival.[3,4] Although the mortality is decreasing, the prognosis of some colorectal adenocarcinoma is still poor. New prognostic factors that can stratify patients into different risk groups are needed.[4]

Mesothelin is a cell-surface glycoprotein that is normally present on the mesothelial cells lining human body cavities.[5] Many studies have investigated the role of mesothelin in tumor biology and suggested its role in proliferation, local invasion, and metastasis. Overexpression of mesothelin has been reported in many solid tumors including mesothelioma, pancreatic cancer, gastric cancer, ovarian cancer, non-small cell lung cancer, and triple-negative breast cancer.[6–11] Due to its limited expression rate in normal tissue and high expression rate in cancer, therapeutic agents targeting mesothelin have been developed and are currently undergoing clinical trials.[12] Several studies have shown that mesothelin expression is correlated with poor prognosis in stomach cancer and esophageal adenocarcinoma.[8,13–16]

In colorectal adenocarcinoma, mesothelin expression was first described in 2003, and its overexpression was reported in about 50% of colorectal adenocarcinoma cases.[17–19] Still, studies on the prognostic value of mesothelin in colorectal adenocarcinoma are limited. Kawamata et al showed that luminal staining of mesothelin correlated with lymphatic invasion and lymph node metastasis.[19] Moreover, Shiraishi et al reported that non-luminal staining of mesothelin was associated with shorter cancer-specific survival (CSS) in stage II/III, while luminal staining was not associated with CSS.[20] Molecular heterogeneity in colorectal adenocarcinoma influences patient outcome and response to treatment, especially depending on the state of the microsatellite instability (MSI).[21] However, there are no studies on the prognostic value of mesothelin in colorectal cancer according to MSI status.

In this study, we investigated the clinicopathologic significance of mesothelin expression and its association with survival rates according to MSI molecular subgroups in colorectal adenocarcinoma.

Editor: Yan Li.

This work was supported by the research fund of Hanyang University (HY-2016).

The authors report no conflicts of interest.

Supplemental Digital Content is available for this article.

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Medicine (2019) 98:26(e16207)

Received: 30 January 2019 / Received in final form: 15 May 2019 / Accepted: 3 June 2019

http://dx.doi.org/10.1097/MD.0000000000016207

2. Materials and methods

2.1. Patients

We retrospectively collected the data of 390 patients who had undergone curative resection for primary colorectal adenocarcinoma at Hanyang University Hospital from January 2005 to December 2010. Patients who had received neoadjuvant chemotherapy and/or neoadjuvant radiotherapy, or those who had recurrent colorectal cancer were excluded. We reviewed medical records to determine the following clinical characteristics: age, sex, follow-up interval, survival, recurrence status, and adjuvant treatment application status and type. Two surgical pathologists (H. K. and Y. C.) reviewed pathology reports and hematoxylin and eosin (H&E)-stained slides to define the clinicopathologic characteristics and confirm the diagnosis. Pathologic parameters including TNM stage (8th AJCC) were determined according to a protocol for examining specimens from patients with primary adenocarcinoma of the colon and rectum.[22,23] Pathologic characteristics included tumor size, histologic grade, lymphatic invasion, vascular invasion, perineural invasion, tumor deposit, tumor budding, TNM stage, and MSI status.[22,24] This study was approved by the Institutional Review Board of the Hanyang University Hospital (HYUH 2016–12–030–001), and the requirement for informed consent was waived.

2.2. Tissue microarray construction

We used a manual tissue microarrayer (Unitma, Seoul, Korea) for tissue microarray construction from archival formalin-fixed, paraffin-embedded tissue blocks. Representative tumor area was selected by light microscopy of H&E-stained sections. Tissue cylinders (diameter: 2 mm) were punched from a previously marked lesion on each donor block and transferred to the recipient block (Unitma).

2.3. Microsatellite analysis

Microsatellite analysis was performed as previously described using a panel of five National Cancer Institute workshop-recommended consensus microsatellite markers (BAT25, BAT26, D2S123, D17S250, and D5S346).[25] Cases showing a shifting of microsatellites at 2 or more markers were classified as MSI-High. A shifting of microsatellites at one marker was classified as MSI-Low. Microsatellite stable (MSS) and MSI-L were classified as non-MSI-High.

2.4. Immunohistochemical study and interpretation

The immunohistochemical study for mesothelin expression was performed with 4-μm-thick sections from TMA blocks using the Ventana Benchmark XT automated staining system (Ventana Medical Systems, Tucson, AZ) according to the manufacturer's protocol. The rabbit monoclonal anti-mesothelin (1:50; SP74; Abcam, Cambridge, UK) antibody was used for immunohistochemical staining. We evaluated the mesothelin expression according to the staining intensity and extent of the tumor cells. A luminal staining pattern without membranous and/or cytoplasmic staining was observed only in sections with weakly positive staining intensity. In moderately and strongly positive staining sections, both luminal and non-luminal staining patterns (membranous and/or cytoplasmic) were observed simultaneously. The intensity of staining (any pattern, luminal and non-luminal) was graded as negative, weakly positive, moderately positive, or strongly positive (Fig. 1). The staining was

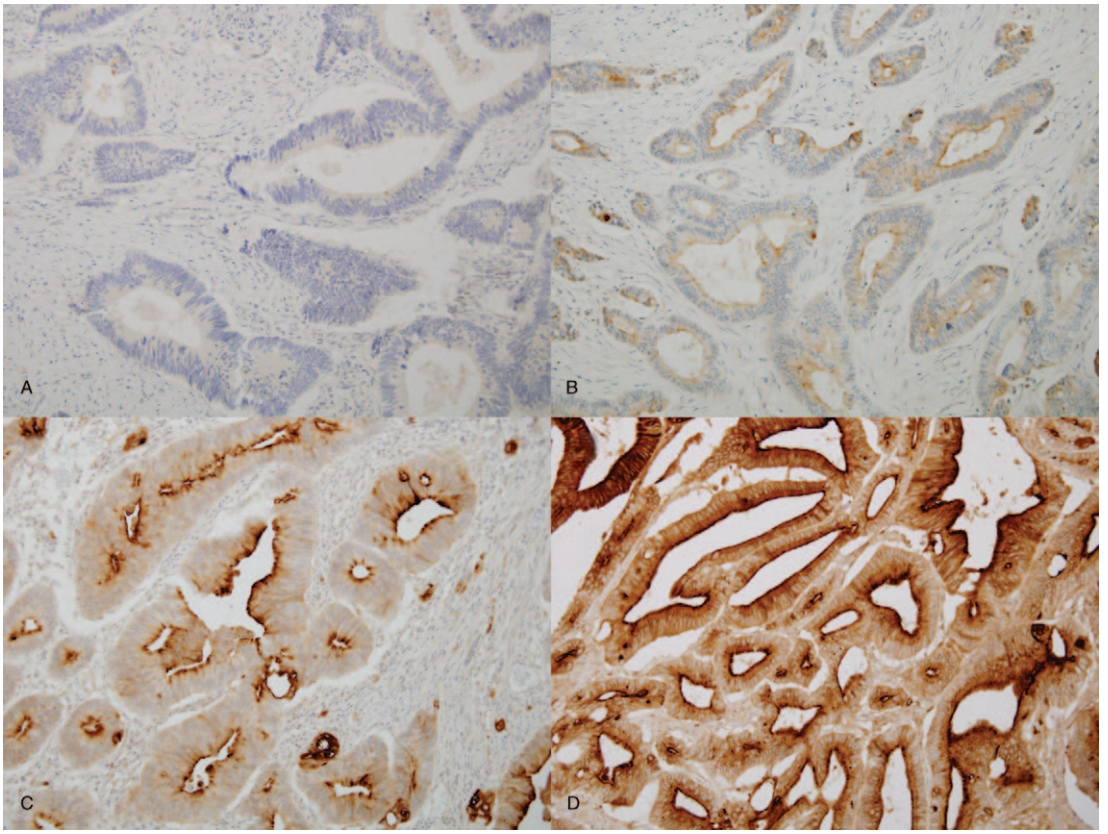

Figure 1. Immunohistochemical staining of mesothelin in colorectal adenocarcinoma. The intensity of membranous and/or cytoplasmic staining was graded as negative (A), weakly positive (B), moderately positive (C), or strongly positive (D) (A–D, ×200).

scored as follows: 0, no expression or any intensity expression less than 5% of tumor cells; 1, weak expression in ≥5% of tumor cells or moderate to strong expression in ≥5% and <30% of tumor cells; and 2, moderate to strong expression in ≥30% of tumor cells. The cases were subdivided into low expression (score 0–1) and high expression groups (score 2).

2.5. Statistical analysis

Statistical analysis was performed using SPSS software version 21 (IBM Corp., Armonk, NY). The chi-square and Mann–Whitney *U* tests were used to evaluate the association between mesothelin expression and clinicopathologic characteristics including tumor size, histologic grade, lymphatic invasion, vascular invasion, perineural invasion, tumor deposit, tumor budding, TNM stage, and MSI status. CSS was defined as the time interval between the date of curative resection and the date of death due to colorectal adenocarcinoma. Recurrence-free survival (RFS) was defined as the time interval between curative resection and the date of any recurrence. Kaplan–Meier survival curves, the log-rank test, and the Cox proportional hazard regression model were used for survival analysis. Two-sided *P* values <.05 were considered statistically significant.

3. Results

3.1. Baseline characteristics of patients

The clinicopathologic characteristics of patients are summarized in Supplemental Table 1, http://links.lww.com/MD/D62. Median follow-up period was 109.5 months (range 1–166), mean age at surgery was 63.4 years (range 27–89), and 237 (60.8%) patients were men with a male-to-female ratio of 1.54:1. Pathological evaluation revealed that 29 tumors (7.4%) were histologic grade 1, 183 (46.9%) were grade 2, 157 (40.2%) were grade 3, and 21 (5.4%) were grade 4. According to 8th AJCC staging system, 55 (14.1%) were stage I, 121 (31.0%) were stage II, 185 (47.4%) were stage III, and 29 (7.4%) were stage IV. Among 390 cases, 363 (93.1%) were non-MSI-High and 27 (6.9%) were MSI-High. Distant metastasis was identified in 29 (7.4%) patients at the time of initial diagnosis, 82 (21.0%) had experienced metastasis or relapse during the follow-up period, and 138 (35.4%) had died due to colorectal adenocarcinoma.

For treatment, 207 (53.1%) patients received curative surgical resection only, and 183 (46.9%) patients received curative surgical resection plus adjuvant chemotherapy (158 cases) or adjuvant combined chemo-radiotherapy (25 cases). The detailed adjuvant chemotherapy regimens are summarized in supplemental Table 1, http://links.lww.com/MD/D62.

3.2. Correlations between mesothelin expression and clinicopathologic characteristics

Overall, high mesothelin expression was observed in 177 (45.4%) of 390 colorectal cancers. Mesothelin expression was significantly correlated with high histologic grade (*P*=.037), lymphatic invasion (*P*=.028), lymph node metastasis (*P*=.028), and high AJCC stage (*P*=.026). Moreover, mesothelin expression was more frequent in cases with high-grade tumor budding (Table 1). There was no statistically significant correlation between mesothelin expression and other clinicopathologic parameters. In subgroup analysis, high mesothelin expression was also more frequently observed in cases with lymphatic invasion (*P*=.057), high-grade tumor budding (*P*=.081), lymph node metastasis (*P*=.057), and high AJCC stage (*P*=.055) in the non-MSI-High group. In the MSI-High group, mesothelin expression was significantly correlated with high histologic grade (*P*=.018) and tumor deposit (*P*=.047; Table 2).

Table 1

Correlation between mesothelin expression and clinicopathologic features of colorectal adenocarcinoma patients (n=390).

Variables	Low expression (n=213)	High expression (n=177)	*P* value
Age, yr (Mean ± SD)	63.58±11.96	63.11±10.74	.492*
Sex			.172
Male	136 (57.4%)	101 (42.6%)	
Female	77 (50.3%)	76 (49.7%)	
Tumor size, cm (Mean±SD)	5.03±2.12	4.91±2.19	.411*
Histologic grade			.037
Grade1 and Grade2	126 (59.4%)	86 (40.6%)	
Grade3 and Grade 4	87 (48.9%)	91 (51.1%)	
Lymphatic invasion			.028
Absent	108 (60.7%)	70 (39.3%)	
Present	105 (49.5%)	107 (50.5%)	
Vascular invasion			.970
Absent	170 (54.7%)	141 (45.3%)	
Present	43 (54.4%)	36 (45.6%)	
Perineural invasion			.271
Absent	113 (57.4%)	84 (42.6%)	
Present	100 (51.8%)	93 (48.2%)	
Tumor deposit			.368
Absent	175 (55.7%)	139 (44.3%)	
Present	38 (50.0%)	38 (50.0%)	
Tumor budding			.070
Absent/ low/ intermediate-grade (0∼9 buds/x200)	136 (58.4%)	97 (41.6%)	
High-grade (≥10 buds/x200)	77 (49.0%)	80 (51.0%)	
pT category			.163
pT1–2	34 (47.2%)	38 (52.8%)	
pT3–4	179 (56.3%)	139 (43.7%)	
pN category			.028
pN0	108 (60.7%)	70 (39.3%)	
pN1–2	105 (49.5%)	107 (50.5%)	
Distant metastasis			.402
Absent	195 (54.0%)	166 (46.0%)	
Present	18 (62.1%)	11 (37.9%)	
AJCC stage†			.026
I and II	107 (60.8%)	69 (39.2%)	
III and IV	106 (49.5%)	108 (50.5%)	
MSI status			.367
Non-MSI-High (MSS, MSI-Low)	196 (54.0%)	167 (46.0%)	
MSI-High	17 (63.0%)	10 (37.0%)	
Treatment			.157
Surgery	120 (58.0%)	87 (42.0%)	
Surgery + Adjuvant therapy	93 (50.8%)	90 (49.2%)	

* Mann–Whitney test.
† AJCC 8th edition.

3.3. Prognostic significance of mesothelin expression

There were no significant differences between the high mesothelin expression and low mesothelin expression groups for both RFS and CSS (*P*=.609 and *P*=.167, respectively; Fig. 2A–B). In subgroup analyses, there were no significant differences in both the RFS and CSS in the non-MSI-High group (*P*=.870 and *P*=.156, respectively; Fig. 2 C–D); however, high mesothelin expression was associated with shorter RFS in the MSI-High

Table 2

Correlation between mesothelin expression and clinicopathologic features of non-MSI-High (n = 363) and MSI-High (n = 27) colorectal adenocarcinoma patients.

	Non-MSI-High (n = 363)			MSI-High (n = 27)		
Variables	**Low expression (n = 196)**	**High expression (n = 167)**	***P* value**	**Low expression (n = 17)**	**High expression (n = 10)**	***P* value**
Age, yr (Mean ± SD)	63.88 ± 11.70	63.24 ± 10.49	.364*	60.06 ± 14.54	61.00 ± 14.94	.980*
Sex			.306			.219
Male	123 (56.2%)	96 (43.8%)		13 (72.2%)	5 (27.8%)	
Female	73 (50.7%)	71 (49.3%)		4 (44.4%)	5 (55.6%)	
Tumor size, cm (Mean ± SD)	4.83 ± 1.95	4.73 ± 2.05	.429*	7.24 ± 2.82	7.80 ± 2.62	.711*
Histologic grade			.134			.018
Grade1 and Grade2	114 (57.6%)	84 (42.4%)		12 (85.7%)	2 (14.3%)	
Grade3 and Grade 4	82 (49.7%)	83 (50.3%)		5 (38.5%)	8 (61.5%)	
Lymphatic invasion			.057			.212
Absent	97 (59.5%)	66 (40.5%)		11 (73.3%)	4 (26.7%)	
Present	99 (49.5%)	101 (50.5%)		6 (50.0%)	6 (50.0%)	
Vascular invasion			.896			.561
Absent	155 (53.8%)	133 (46.2%)		15 (65.2%)	8 (34.8%)	
Present	41 (54.7%)	34 (45.3%)		2 (50.0%)	2 (50.0%)	
Perineural invasion			.360			.573
Absent	101 (56.4%)	78 (43.6%)		12 (66.7%)	6 (33.3%)	
Present	95 (51.6%)	89 (48.4%)		5 (55.6%)	4 (44.4%)	
Tumor deposit			.723			.047
Absent	159 (54.5%)	133 (45.5%)		16 (72.7%)	6 (27.3%)	
Present	37 (52.1%)	34 (47.9%)		1 (20.0%)	4 (80.0%)	
Tumor budding			.081			.406
Absent/ low/ intermediate-grade (0∼9 buds/x200)	129 (57.6%)	95 (42.4%)		7 (77.8%)	2 (22.2%)	
High-grade (≥10 buds/x200)	67 (48.2%)	72 (51.8%)		10 (55.6%)	8 (44.4%)	
pT category			.122			.260
pT1–2	32 (45.7%)	38 (54.3%)		2 (100.0%)	0 (0.0%)	
pT3–4	164 (56.0%)	129 (44.0%)		15 (60.0%)	10 (40.0%)	
pN category			.057			.257
pN0	97 (59.5%)	66 (40.5%)		11 (73.3%)	4 (26.7%)	
pN1–2	99 (49.5%)	101 (50.5%)		6 (50.0%)	6 (50.0%)	
Distant metastasis			.363			
Absent	178 (53.3%)	156 (46.7%)		17 (63.0%)	10 (37.0%)	
Present	18 (62.1%)	11 (37.9%)				
AJCC stage†			.055			.257
I and II	96 (59.6%)	65 (40.4%)		11 (73.3%)	4 (26.7%)	
III and IV	100 (49.5%)	102 (50.5%)		6 (50.0%)	6 (50.0%)	
Treatment			.534			.002
Surgery	105 (55.6%)	84 (44.4%)		15 (83.3%)	3 (16.7%)	
Surgery + Adjuvant therapy	91 (52.3%)	83 (47.7%)		2 (22.2%)	7 (77.8%)	

* Mann–Whitney test.
† AJCC 8th edition.

group ($P = .004$; Fig. 2E). We additionally divided the groups according to treatment. Mesothelin expression had no prognostic implication for RFS and CSS in both colorectal cancer groups with adjuvant therapy (n = 183; $P = .944$ and $P = .480$, respectively) and without adjuvant therapy (n = 207; $P = .763$ and $P = .291$, respectively).

4. Discussion

In this study, we revealed that mesothelin expression was correlated with aggressive phenotypes in colorectal adenocarcinoma, such as high histologic grade, frequent lymphatic invasion, high-grade tumor budding, lymph node metastasis, and high AJCC stage. Mesothelin was not a prognostic indicator of CSS and RFS in all patients but served as a significant prognostic factor of RFS in the MSI-High group of colorectal adenocarcinoma. Although the number of MSI-high cases was small, this finding is interesting. The survival discrepancy between groups might be explained by their distinct genomic background. Recent studies revealed that MSS/MSI-Low and MSI-High groups of colorectal adenocarcinoma exhibit distinct genomic profiles and show different phenotypes and prognoses. The MSI-High group of colorectal adenocarcinoma patients usually show proximal colon predominance, poor histologic differentiation and/or mucin production, and increased tumor-infiltrating lymphocytes.[26] Several studies have shown that MSI-High colorectal adenocarcinoma has a better prognosis than MSS/MSI-Low colorectal adenocarcinoma.[27] A recent study has revealed that advanced stage MSI-High colorectal adenocarcinoma has

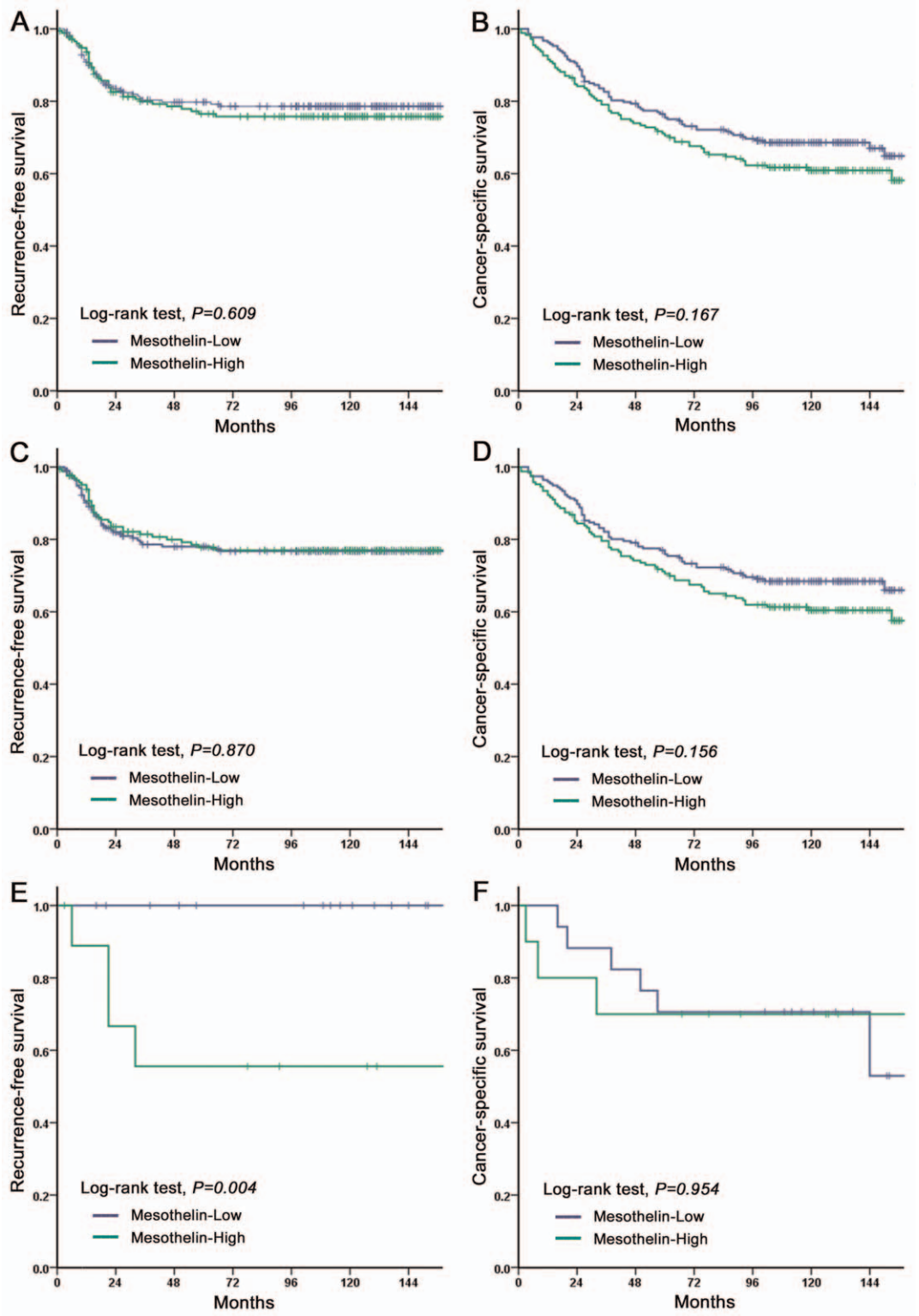

Figure 2. Kaplan–Meier survival analysis of recurrence-free survival (RFS) and cancer-specific survival (CSS) in colorectal adenocarcinoma patients. No significant difference in RFS or CSS was observed in all colorectal adenocarcinoma (A–B) and non-MSI-High subgroup (C–D). High mesothelin expression was associated with shorter RFS (E), and no significant difference in CSS (F) was observed in MSI-High subgroup.

higher rates of treatment response and progression-free survival to pembrolizumab, an immunomodulatory drug targeting programmed cell death 1 (PD-1) protein, than MSS/MSI-Low colorectal adenocarcinoma.[21]

Mesothelin overexpression in malignancies is associated with tumor cell migration and invasion.[6,28] These findings correspond with our results that mesothelin overexpression correlated with frequent lymphatic invasion and high-grade tumor budding.

A previous study demonstrated that mesothelin overexpression promotes tumor cell motility and invasion via the activation of MMP-7 and MMP-9.[6,29]

In gastrointestinal tumors, mesothelin expression is a poor prognostic factor.[30] Co-expression of mesothelin and CA125 was associated with poor patient prognosis in pancreatic cancer.[7] Luminal membrane expression of mesothelin was one of the poor prognostic factors in gastric cancer.[8] Intracellular mesothelin expression correlated with poor patient prognosis in extrahepatic bile duct cancer.[31] In several studies, the expression pattern of mesothelin is divided into luminal and non-luminal patterns. In a 2014 study, luminal staining of mesothelin was correlated with poor prognostic factors in colorectal adenocarcinoma.[19] In another recent study, a luminal pattern of mesothelin expression was not associated with CSS and poor prognostic factors; however, a non-luminal pattern of mesothelin expression was associated with CSS and poor prognostic factors, such as tumor budding, high histologic grading, and lymph node metastasis.[20] In the present study, a completely luminal pattern of staining was observed only in cases with weak intensity, while both luminal and non-luminal patterns were observed simultaneously in cases with moderate to strong positive staining. A score of 2 (moderate to strong expression in ≥30% of tumor cells) was considered as high expression. As a result, cases with only a luminal pattern were considered to have low expression, and non-luminal pattern was associated with poor prognostic factors. These findings are consistent with a previous study; although not statistically significant, our study also showed shorter CSS in patients with mesothelin overexpression.[20]

Clinical trials of mesothelin-targeting therapy are currently under way for malignant mesothelioma and pancreatic cancer.[12] Mesothelin expression was found in a large number of cases (45.4%) and was associated with poor prognosis. Mesothelin-targeted therapy may be a new treatment candidate for colorectal adenocarcinoma.

5. Conclusion

We revealed that mesothelin expression was significantly associated with aggressive phenotypes in colorectal adenocarcinoma and shorter RFS in the MSI-High group. Further studies are warranted to assess larger cohorts with a large number of MSI-High cases.

Author contributions

Conceptualization: Su-Jin Shin.
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Methodology: Kiseok Jang.
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Writing – original draft: Hyunsung Kim.
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