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ORIGINAL ARTICLE

Aberrant Expression of Calretinin, D2–40 and Mesothelin in Mucinous and Non-Mucinous Colorectal Carcinomas and Relation to Clinicopathological Features and Prognosis

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Abstract CRC is a heterogeneous disease in terms of morphology, invasive behavior, metastatic capacity, and clinical outcome. Recently, many so-called mesothelial markers, including calretinin, D2–40, WT1, thrombomodulin, mesothelin, and others, have been certified. The aim of this study was to assess the immunohistochemical expression of calretinin and other mesothelial markers (D2–40 and mesothelin) in colorectal mucinous adenocarcinoma (MA) and non mucinous adenocarcinoma (NMA) specimens and relation to clinicopathological features and prognosis using manual tissue microarray technique. We studied tumor tissue specimens from 150 patients with colorectal MA and NMA who underwent radical surgery from January 2007 to January 2012. High-density manual tissue microarrays were constructed using a modified mechanical pencil tip technique, and paraffin sections were submitted for immunohistochemistry using Calretinin, D2–40 and mesothelin expressions. We found that NMA showed significantly more calretinin and D2–40 expression than MA In contrast, no statistically significant difference between NMA and MA was detected in mesothelin expression. There were no statistically significant relations between any of the clinicopathological or histological parameters and any of the three markers. In a univariate analysis, neither calretinin nor D2–40 expressions showed any significant relations to DFS or OS. However, mesothelin luminal expression was significantly associated with worse DFS. Multivariate Cox regression analysis proved that luminal mesothelin expression was an independent negative prognostic factor in NMA. In conclusion, Calretinin, D2–40 and mesothelin are aberrantly expressed in a proportion of CRC cases with more expression in NMA than MA. Aberrant expression of these mesothelial markers was not associated with clinicopathological or histological features of CRCs. Only mesothelin expression appears to be a strong predictor of adverse prognosis.

Keywords Colorectal · Mucinous · Calretinin · D2–40 · Mesothelin

Introduction

Colorectal carcinoma (CRC) is a major health problem all over the world as it is the third most common cancer in men and the second most common cancer in women worldwide [1]. CRC is a heterogeneous disease in terms of morphology, invasive behavior, metastatic capacity, and clinical outcome [2]. To be distinguished from colorectal non-mucinous adenocarcinoma (NMA), mucinous adenocarcinoma (MA) is a morphologic subtype of CRC that has more than 50 % of the tumor composed of mucin, either extracellular with mucin lakes (colloid carcinoma) or intracellular (signet ring cell adenocarcinoma). If the extracellular mucin is less than 50 % of the tumor, the tumor is called "Ordinary adenocarcinoma with mucinous component" (OAMC) [3]. The importance of these distinct histological types lies in the reported differences between them with regard to clinicopathological characteristics, distinct genetic profiles, and pathogenic pathways [4].

Recently, many so-called mesothelial markers, including calretinin, D2–40, mesothelin, WT1, thrombomodulin, and others have been certified [5, 6]. Calretinin is a calcium-binding protein that has multiple functional roles including intracellular calcium buffering, message targeting [7] and

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neoplastic proliferation of the cells expressing it [8]. However it has been shown to be of good diagnostic value in the distinction between epithelioid malignant mesothelioma and adenocarcinoma, calretinin expression has been described in a wide variety of normal cells, including steroid-producing cells of the testis and ovary, adipocytes, eccrine-glands, keratinizing thymic epithelial cells [9] and numerous tumors involving ovary, testis, adrenal cortex, colon, breast, sinonasal tract, thymus, skin and even soft tissue [9, 10, 11, 12].

In addition to calretinin, D2–40 has been recommended as a marker for malignant mesothelioma [5]. D2–40 was previously described to have selective immunoreactivity for germ cells and lymphatic endothelium and has been shown to react with Kaposi's sarcoma, lymphangioma and Dabska tumor [13].

Mesothelin is a 40 kD cell surface protein that may be involved in cell–cell adhesion. Its expression in normal tissue is limited mainly to mesothelia of peritoneal, pleural, and pericardial cavities [14]. However, overexpression of mesothelin protein was found in several human malignancies, with the highest expression in ovarian, pancreatic, bronchial, gastroesophageal, cervical, endometrial, and biliary carcinomas [15].

It is well known that some genes and proteins are aberrantly expressed in CRC as IGF2 [16], CD133 [17], E-cadherin, and S100 A4 [18] among others, with relations to prognosis. We hypothesized that some mesothelial proteins can be aberrantly expressed in a considerable proportion of CRC cases. To the best of our knowledge, few studies tried to investigate the expression of these mesothelial markers in CRC, but these studies included only small number of cases and focused just on medullary and poorly differentiated cases [12]. Till now, it is not clear whether mesothelial markers are aberrantly expressed in CRC subtypes or not, whether they have a role in adenoma-carcinoma sequence or not, and whether their expressions have true prognostic values or not. So, the aim of this study was to assess the immunohistochemical expression of mesothelial markers (calretinin, D2–40 and mesothelin) in large number of colorectal MA and NMA cases and relation to clinicopathological features and prognosis using manual tissue microarray technique.

Material and Methods

Samples

Samples were collected from the surgical pathology laboratory at Gastroenterology Center, Mansoura, Egypt. Files of all resected CRC cases were revised during the period from 2007 to 2011. Mucinous CRC were selected and revised. Cases with incomplete clinical data and those that were composed completely of pools of mucin with very few epithelial cells were excluded. Seventy five cases with mucinous adenocarcinoma (MA) were fulfilling selection criteria. Another 75 cases of non-mucinous adenocarcinoma (NMA) were chosen randomly for comparison from the same period. The patients did not receive any neoadjuvant therapy. Fourteen normal colorectal tissues and 14 colorectal adenomas were also included in the study.

Clinical Parameters and Histopathological Evaluation

All clinicopathological data of these 150 cases were revised with re-examination of all their slides. This includes: age, gender, location, size, shape, multiplicity, histological type, grade, depth of invasion (T), tumor edges (either pushing or infiltrating microscopically), lymphovascular invasion, perineural invasion, peri- and intra-tumoral lymphocytic infiltration, extent of neutrophilic infiltrate, nearby and distant mucosa, whether the tumor is on top of adenoma or not, number of lymph node metastases (N), distant metastasis (M), TNM staging, state of surgical cut margins (either infiltrated by the tumor or not), associated schistosomiasis and any other findings.

Tissue Microarray (TMA) Construction

Three manual TMA blocks were constructed using modified mechanical pencil tip method as previously described by Foda [19]. Three representative cores of 0.8 mm diameter were punched out from each case of CRC. Cores of normal and adenomatous tissues were included as well as cores of various normal tissues as controls. Sections from TMA blocks were prepared (4 μm thickness) for routine H&E. Other sections were prepared on charged slides for IHC.

Immunohistochemistry

Immunohistochemical staining was performed in the Pathology laboratory at Hammersmith Hospital, Imperial College, London, UK. The slides were deparaffinized in 3 changes of xylene, rehydrated through graded alcohol and brought to water. Endogenous peroxidase was blocked with peroxide block for 15 min. The slides were brought to tap water then subjected to antigen retrieval as required (for 30 min using Epitope retrieval 1 "Leica"). The slides were stained with antibodies to Calretinin (1/200, Leica NCL-L), D2–40 (1/100, Dako) and Mesothelin (1/40, Leica NCL-Meso), using standard procedures on BOND-MAX automated immunohistochemistry system (Leica Microsystems, New Castle, UK). For every run of immunostaining, a case of mesothelioma was used as a positive control. As a negative control, phosphate buffered saline was used to replace the monoclonal antibody whereas normal goat serum was used to replace polyclonal antibody. The detection system of choice was the Super Sensitive™ IHC polymer Detection (Biogenex, California, USA).

Evaluation of IHC

Calretinin, D2–40 and mesothelin expressions were semi-quantitatively assessed for each case. Cytoplasmic/nuclear calretinin expression was considered positive [20], while cytoplasmic/membranous D2–40 and mesothelin expressions were considered positive [21, 22]. Any aberrant expression was also reported. Intensity of staining of each marker was graded as: 0 (negative), 1 (weak), 2 (moderate), and 3 (strong), and the percentage of positive-stained cells was graded as: 0 (<5 %), 1 (5–50 %), 2 (>50 %). For each marker, the final score was determined by the combining intensity and percent scores (0–5). Because expression of these markers is aberrant in CRC and for the purpose of data analysis, expression of each marker was classified as negative (scores 0,1,2) and aberrant positive (scores 3,4,5).

Statistical Analysis

Data were analyzed, applying SPSS, version 16.0 for Windows (SPSS Inc., IBM, Chicago, Illinois). $\chi 2$ (Chi-square) test was used to test significant differences in categorical variables between various groups. Survival data were analyzed using Kaplan-Meier test. A comparison of survival curves was carried out using the log-rank test. For multivariate analysis, Cox proportional hazard models were performed. A 2-tailed $P \leq 0.05$ was considered significant in all tests.

Results

Clinicopathological and Histological Features of CRC Cases

The clinicopathological and histological features cases were previously reported (Foda et al., 2013). In summary, mucinous carcinomas were significantly associated with young age, more depth of invasion, more frequency of lymph node metastasis, less peri- and intra-tumoural neutrophils and more peri-tumoral lymphocytic response (Crohn-like response) than non-mucinous carcinomas.

Calretinin, D2–40 and Mesothelin Expressions in Colorectal Normal, Adenoma and Carcinoma Tissues

Calretinin, D2–40 and mesothelin were totally negative in all colorectal normal and adenomatous tissues. Calretinin was positive in 8 cases (5.3 %) of CRC; 7 cases of NMA and one MA case. All cases showed diffuse weak cytoplasmic calretinin expression with luminal accentuation at two cases. NMA showed significantly more calretinin expression than MA ($P = 0.029$). D2–40 showed aberrant nuclear staining in 14 cases (9.3 %) of CRC; all of NMA group with totally negative MA cases ($P < 0.001$). Diffuse weak cytoplasmic D2–40 was also interpreted in 3 other cases; one of NMA and 2 of MA. Mesothelin was positive in 6 cases (4 %) of CRC; 5 cases of NMA and one MA case. All cases showed luminal cytoplasmic expression, with only one case of NMA that showed aberrant nuclear expression. In contrast to calretinin and D2–40, no statistically significant difference between NMA and MA was detected in mesothelin expression ($P = 0.096$) (Table 1) (Fig. 1).

Calretinin, D2–40 and Mesothelin Expressions in Subtypes of NMA

Calretinin, D2–40 and mesothelin expressions in subtypes of NMA were summarized in Table 2. The 7 cases of NMA that showed calretinin positive staining were distributed as follows: 3 cases of ordinary adenocarcinoma (OA) and 4 cases of ordinary adenocarcinoma with mucinous component (OAMC) with no statistically significant difference ($P = 0.255$). The 14 cases of NMA that showed aberrant D2–40 nuclear staining were distributed as follows: 10 cases of OA and 4 cases of OAMC with no statistically significant difference ($P = 0.334$). The 5 cases of NMA that showed mesothelin luminal staining were distributed as follows: 2 cases of OA and 3 cases of OAMC also with no statistically significant difference ($P = 0.356$).

Interrelation between Calretinin, D2–40 and Mesothelin Expressions in CRC Cases

Of the 14 CRC cases that showed aberrant nuclear D2–40 expression, only 2 cases (14.3 %) showed concomitant cytoplasmic calretinin expression. There were no significant interrelation between nuclear D2–40 and cytoplasmic calretinin

Table 1 Calretinin, D2–40 and mesothelin expressions in NMA and MA

	NMA No. (%)	MA No. (%)	Chi-square ($\chi 2$)	*P* value
Calretinin weak cytoplasmic expression				
-Negative	68 (90.7 %)	74 (98.7 %)		
-Positive	7 (9.3 %)	1 (1.3 %)	4.754	0.029*
D2–40 nuclear expression				
-Negative	61 (81.3 %)	75 100.0 %)		
-Positive	14 (18.7 %)	0 (0.0 %)	15.441	<0.001*
Mesothelin cytoplasmic luminal expression				
-Negative	70 (93.3 %)	74 (98.7 %)		
-Positive	5 (6.7 %)	1 (1.3 %)	2.778	0.096

*$P \leq 0.05$ is significant

NMA Non-mucinous adenocarcinoma, *MA* Mucinous adenocarcinoma

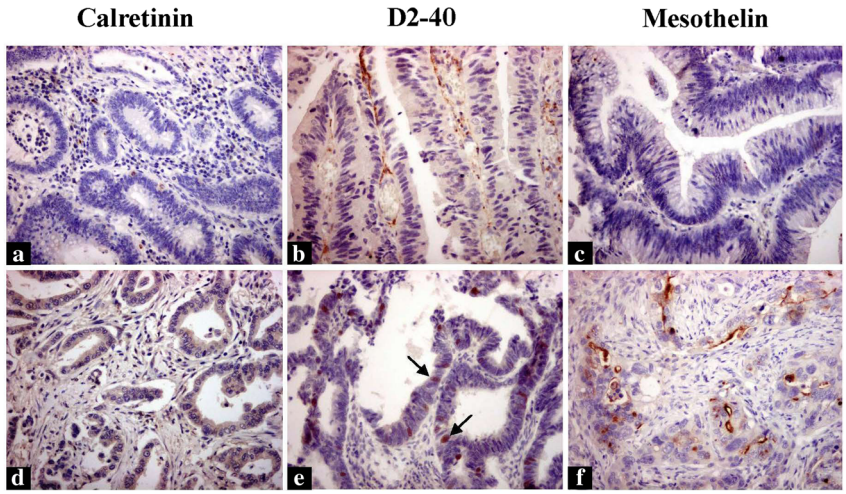

Fig. 1 **a, b, c** Negative calretinin, D2–40 and mesothelin expression in colorectal adenomas respectively. (d): Weak cytoplasmic staining of calretinin in a case of ordinary adenocarcinoma. **e** Aberrant nuclear staining of D2–40 in a case of ordinary adenocarcinoma (arrows). **f** Luminal cytoplasmic staining of mesothelin in a case of ordinary adenocarcinoma (×200).

expressions in CRC ($P = 0.117$). On the other hand, of the 6 cases that showed luminal mesothelin expression, 2 cases (33.3 %) showed concomitant cytoplasmic calretinin expression and 3 cases (50 %) showed concomitant nuclear D2–40 expression. Luminal mesothelin expression was significantly associated with cytoplasmic calretinin ($P = 0.002$) and nuclear D2–40 ($P < 0.001$) expressions in CRC (Table 3).

Relation of Calretinin, D2–40 and Mesothelin Expressions with Clinicopathological and Histological Parameters in CRC Cases

The relation between Calretinin, D2–40 and mesothelin expressions and clinicopathological and histological parameters was tested in CRC. There were no statistically significant relations between any of these parameters and any of the three markers (data not shown).

Table 2 Calretinin, D2–40 and mesothelin expressions in NMA subtypes

	OA No. (%)	OAMC No. (%)	Chi-square ($\chi 2$)	*P* value
Calretinin cytoplasmic expression				
-Negative	44 (93.6 %)	24 (85.7 %)		
-Positive	3 (6.4 %)	4 (14.3 %)	1.295	0.255
D2–40 nuclear expression				
-Negative	37 (78.7 %)	24 (85.7 %)		
-Positive	10 (21.3 %)	4 (14.3 %)	0.565	0.334
Mesothelin luminal expression				
-Negative	45 (95.7 %)	25 (89.3 %)		
-Positive	2 (4.3 %)	3 (10.7 %)	1.176	0.356

OA Ordinary adenocarcinoma, *OAMC* Ordinary adenocarcinoma with mucinous component

Survival of Patients with CRC and Relation of Calretinin, D2–40 and Mesothelin Expressions to Survival

To clarify the prognostic impact of calretinin, D2–40 and mesothelin expressions on survival of NMA cases, univariate and multivariate analyses were carried out. In a univariate analysis, neither calretinin nor D2–40 expressions showed any significant relations to DFS or OS. However, mesothelin luminal expression was significantly associated with worse DFS ($P = 0.010$) (Table 4). Multivariate Cox regression analysis was applied to test the prognostic yield of this finding, and proved that luminal mesothelin expression was an independent negative prognostic factor in NMA (HR: 0.307, 95 % CI 0.118–0.799, $P = 0.016$) (Fig. 2).

Discussion

Calretinin is a widely used immunohistochemical maker for mesothelial cells and malignant mesothelioma. Normal human colon epithelial cells do not express calretinin, but several colon carcinoma cell lines express this protein to various amounts [23]. In harmonay with this, we found that calretinin, and also D2–40 and mesothelin, were totally negative in all colorectal normal and adenomatous tissues while they were positive in small percentage of tumor tissues. These data may suggest that increased expression of mesothelial markers could be a late event in the colorectal adenoma-carcinoma sequence in a minority of cases and might be a molecular marker for adenomas progression to carcinomas.

While relatively sensitive and specific for mesothelioma, calretinin expression has been found in a wide variety of poorly differentiated carcinomas and tumors of mesenchymal origin [24]. In our study, we demonstrated that calretinin was expressed in about 5 % of CRC cases. To the best of our knowldge, only few studies have addressed the expression

Table 3 Interrelation between calretinin, D2–40 and mesothelin expressions in CRC

	D2–40 nuclear expression		Mesothelin luminal expression	
	Negative	Positive	Negative	Positive
Calretinin cytoplasmic expression				
-Negative	130 (95.6 %)	12 (85.7 %)	138 (95.8 %)	4 (66.7 %)
-Positive	6 (4.4 %)	2 (14.3 %)	6 (4.2 %)	2 (33.3 %)
	$\chi2 = 2.451$, $P = 0.117$		$\chi2 = 9.705$, $P = 0.002$*	
D2–40 nuclear expression				
-Negative			133 (92.4 %)	3 (50.0 %)
-Positive			11 (7.6 %)	3 (50.0 %)
			$\chi2 = 12.214$, $P < 0.001$*	

*$P \leq 0.05$ is significant

of calretinin in colon carcinoma. In the study of Lugli et al. [19], they found expression of calretinin in about 9 % of CRC cases which is slightly higher than our study. However, Gotzos et al. [25] found calretinin expression in about 22.5 % of cases and reported that increased calretinin expression coincides with lesser degree of tumor differention and with increased regional lymph nodes and distant metastases. In our study, calretinin expression was not associated with any of these, or other, parameters. This discrepancy may be attributed to the relatively low number of cases in such studies; the largest study that investigated calretinin expression in CRC included 82 cases [25]. Moreover, it is evident that many important histological subtypes were not sufficiently represented in these studies. In addition, different staining protocols/antibodies can reveal relatively low expression level in many cases in some studies that is not detected by others.

To the best of our knowledge, our study is the first to explore the immunohistochemical expression of calretinin in colorectal MA. We found significantly more positive calretinin expression in NMA than MA. Gotzos et al. [25] found that calretinin is expressed by most undifferentiated colorectal adenocarcinomas, but only by a limited number of cells in well-differentiated tumors. Also, Cargnello et al. [23] reported that most of positive calretinin CRC cases were grade III. In contrast, Winn et al. [12] found significantly higher expression of calretinin in medullary carcinoma as opposed to poorly differentiated carcinoma. It is not clear in our study why MA, which is considered poorly differentiated (grade III) [26], showed decreased calretinin expression than NMA. This can be explained by the different genetic profiles of our cases of MA and poorly differentiated NMA than other studied poorly differentiated CRC cases. However previous experiments on colon carcinoma cell lines have shown that downregulation of calretinin leads to the inhibition of the cell cycle with impending apoptotic events [25] and downregulation of calretinin significantly decreased the viability and proliferation of mesothelioma cells in vitro, the condition is so different with mucinous tumors. It is well known that cell lines and tumors that synthesize and secrete large amounts of mucin are characterized by increased proliferation, invasiveness, and tendency for metastasis [27]. Consistent with this fact, our results suggest that mucinous carcinoma cells do not depend on calretinin expression for their aggressiveness, which may be true in NMA, especially in grade III tumors.

D2–40 has selective immunoreactivity for lymphatic endothelium and its clinical uses include the demonstration of lymphatic invasion by primary tumors [28] and in evaluation of lymphovascular microvascular density [29]. In addition, D2–40 is also a marker of cells with a mesothelial phenotype and may be useful to discriminate between mesothelioma and lung adenocarcinoma [13]. D2–40 is expressed in tumor cells of various types of human cancers such as vascular tumors,

Table 4 Univariate analysis of the relation of calretinin, D2–40 and mesothelin expressions to survival of NMA cases

		Median DFS (months)	*P* value	Median OS (months)	*P* value
Calretinin cytoplasmic expression	Negative	61	0.162	61	0.175
	Positive	32		41	
D2–40 nuclear expression	Negative	53	0.553	53	0.388
	Positive	61		63	
Mesothelin luminal expression	Negative	61	0.010*	61	0.317
	Positive	33		41	

*$P \leq 0.05$ is significant

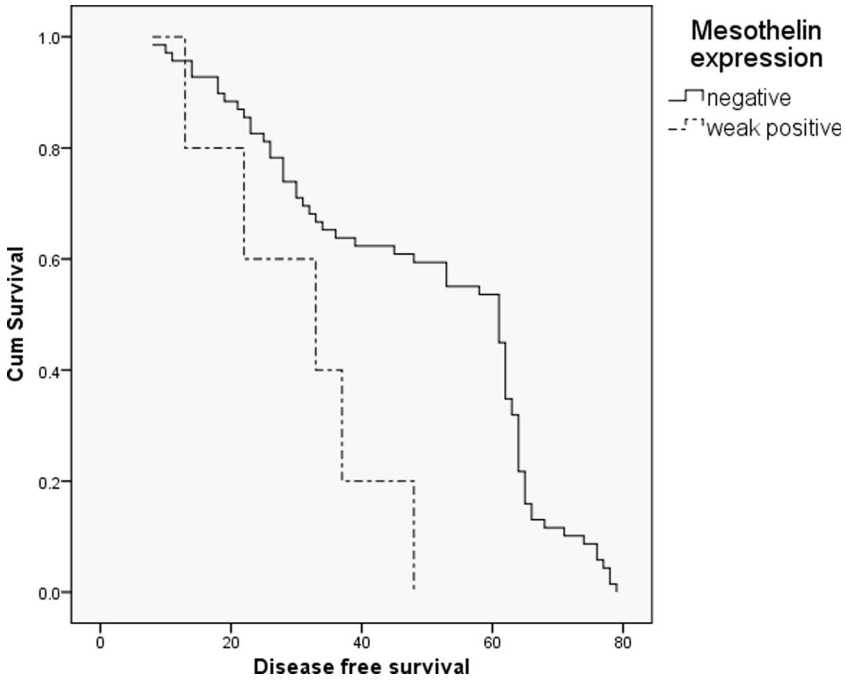

Fig. 2 Relation of luminal D2–40 expression to DFS in NMA

malignant mesothelioma, tumors of the central nervous system (CNS), germ cell tumors and squamous cell carcinomas [29]. To the best of our knowledge, this is the first to study the expression of D2–40 in large number of CRC cases. D2–40 showed aberrant nuclear staining in about 9 % of CRC; all of NMA group with totally negative MA cases. Aberrant nuclear staining of D2–40 was reported only one study that showed strong membranous and focal moderate nuclear staining of D2–40 in malignant mesothelioma [21]. We have no explanation for this nuclear expression, but it is not foreign for mesothelial markers, like calretinin, to show nuclear expression which is considered more specific to mesothelial differentiation [21]. A role for D2–40 in invasion and metastasis has been suggested and its expression seems to be associated with bad prognosis and high risk for lymph node metastases [29]. On the other hand, D2–40 expression was not associated with any clinicopathological parameters or with the prognosis in our study.

Like calretinin and D2–40, the overexpression of mesothelin has been found in several cancer types, including malignant mesothelioma, ovarian cancer, and pancreatic cancer [30]. In our study, mesothelin was luminally expressed in 4 % of CRC. Furthermore, this luminal mesothelin expression was significantly associated with cytoplasmic calretinin and nuclear D2–40 expressions. Kawamata et al. [30] reported that overexpressed membrane bound-mesothelin could enhance the endogenous gene. Expression and could have a biological role in malignancy. These findings may suggest possible interplay between mesothelium-related proteins and/or genes with possible involvement in tumorigenesis. In contrast, Liebig et al. [31], the first who studied mesothelin expression in CRC, reported mesothelin expression in 58 % of CRC cases with restriction of mesothelin-positive tumor cells to the invasive front. In contrast, mesothelin-positive cells in our study were found throughout the tumor. Also, Kawamata et al. [30] found luminal membrane positivity of mesothelin in about 37 % of CRC cases with a relation to lymphatic permeation and they recommended further studies to better define the association between mesothelin and integrin in lymphatic adhesion and invasion in CRC. Again, these discrepancies between our results and other studies regarding percentage of positive cases and relations to clinicopathological factors may be attributed to their relatively low number of cases, limitations of histological subtypes of their cases and different staining protocols/antibodies. To be added here, no statistically significant difference between NMA and MA was detected in mesothelin expression.

In addition to our interesting results regarding mesothelial markers expression in MA, for the first time these markers were studied in OAMC subtype of NMA. Till now, OAMC remains a vague entity that resembles OA in some clinicopathological and molecular respects as well as MA [32]. In our previous study, OAMC showed significantly negative/lower expression of matrix metalloproteinase 13 (MMP-13) and EGFR than OA and significantly higher expression of MMP-13 than MA. On the other hand, there were no significant differences in E-cadherin expressions between AWMC, OA and MA [32]. The same was detected here regarding mesothelial markers expression in OAMC; there were no statistical differences between OA and OAMC in calretinin, D2–40 and mesothelin expressions. Consistent with the rest of our

results, it seems that decreased or increased mesothelial markers expressions have not any role in mucinous activity or aggressiveness of CRC.

The current study was the first to investigate the prognostic impact of mesothelial markers expression in large number and various histological subtypes of CRC. Mesothelin luminal expression was significantly associated with worse DFS in NMA cases. This result was in concordance with Kawamata et al. [30] who reported that luminal membrane expression of mesothelin elucidated the unfavorable prognosis of CRC patients with lymph node metastasis. Conversely, calretinin and D2–40 expressions did not have any significant relations to DFS or OS. In basal-like breast carcinomas, Taliano et al. [24] found a significant association between strong calretinin expression and poor overall survival. Furthermore, a role for calretinin in the epithelial-mesenchymal transition (EMT) may exist in basal-like breast carcinomas [33]. On the other hand, EMT appears to be closely involved in the pathogenesis of colorectal cancer, and analysis referred to it can yield novel targets for therapy [34], although low frequency of calretinin expression in our study does not support a similar relation between both in CRC.

In conclusion, Calretinin, D2–40 and mesothelin are aberrantly expressed in a proportion of CRC cases with more expression in NMA than MA. Aberrant expression of these mesothelial markers was not associated with clinicopathological or histological features of CRCs. Only mesothelin expression appears to be a strong predictor of adverse prognosis.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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References

1. Ferlay J, Soerjomataram I, Ervik M, et al.: GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11 [Internet]. Lyon, France: International Agency for Research on Cancer 2013. http://globocan.iarc.fr. Accessed on 23 May 2015.
2. Foda AA, El-Hawary AK, Abdel-Aziz A (2013) Differential expression of matrix metalloproteinase- 13 in mucinous and nonmucinous colorectal carcinomas. Ann Diagn Pathol 17: 347–353
3. Hamilton SR, Bosman FT, Boffetta P, et al. (2010) Tumors of the colon and rectum. In: Bosman FT, Carneiro F, Hruban RH, et al. (eds) WHO Classification of tumors of the digestive system. IARCPress, Lyon, pp. 132–182
4. Mekenkamp JM, Heesterbeek KJ, Koopman M, et (2012) Mucinous adenocarcinomas: Poor prognosis in metastatic colorectal cancer. Eur J Cancer, 48: 501–509.
5. Ordonez NG (2005) D2–40 and podoplanin are highly specific and sensitive immunohistochemical markers of epithelioid malignant mesothelioma. Hum Pathol 205:372–380
6. Ordonez NG (2006) Value of immunohistochemistry in distinguishing peritoneal mesothelioma from serous carcinoma of the ovary and peritoneum: a review and update. Adv Anat Pathol 13:16–25
7. Rogers JH (1987) Calretinin: a gene for a novel calcium-binding protein expressed principally in neurons. J Cell Biol 105: 1343–1353
8. Gander JC, Gotzos V, Fellay B, et al. (1996) Inhibition of the proliferative cycle and apoptotic events in WiDr cells after down-regulation of the calcium-binding protein calretinin using antisense oligodeoxynucleotides. Exp Cell Res 225:399–410
9. Miettinen M, Limon J, Niezabitowski A, et al. (2001) Calretinin and other mesothelioma markers in synovial sarcoma: analysis of antigenic similarities and differences with malignant mesothelioma. Am J Surg Pathol 25:610–617
10. Lugli A, Forster Y, Haas P, et al. (2003) Calretinin expression in human normal and neoplastic tissues: a tissue microarray analysis on 5233 tissue samples. Hum Pathol. 34:994–1000
11. Dent R, Hanna WM, Trudeau M, et al. (2009) Pattern of metastatic spread in triple-negative breast cancer. Breast Cancer Res Treat 115: 423–428
12. Winn B, Tavares R, Fanion J, et al. (2009) Differentiating the undifferentiated: immunohistochemical profile of medullary carcinoma of the colon with an emphasis on intestinal differentiation. Hum Pathol 40:398–404
13. Chu AU, Litzky LA, Pasha TL, et al. (2005) Utility of D2–40, a novel mesothelial marker, in the diagnosis of malignant mesothelioma. Mod Pathol 18:105–110
14. Chang K, Pastan I (1994) Molecular cloning and expression of a cDNA encoding a protein detected by the K1 antibody from an ovarian carcinoma (OVCAR-3) cell line. Int J Cancer **57**:90–97
15. Ordonez NG (2003) Application of mesothelin immunostaining in tumor diagnosis. Am J Surg Pathol 27:1418–1428
16. Cheng YW, Idrees K, Shattock R, et al. (2010) Loss of imprinting and marked gene elevation are 2 forms of aberrant IGF2 expression in colorectal cancer. Int J Cancer 127:568–577
17. Li H, Zhao P, Lu Y (2012) Correlation of aberrant expression of CD133 with FHIT and malignant phenotype of colorectal adenocarcinoma. Int J Colorectal Dis 27:1015–1020
18. Lee SJ, Choi SY, Kim WJ, et al. (2013) Combined aberrant expression of E-cadherin and S100 A4, but not β-catenin is associated with disease-free survival and overall survival in colorectal cancer patients. Diagn Pathol 19(8):99
19. Foda AA (2013) No-cost manual method for preparation of tissue microarrays having high quality comparable to semiautomated methods. Appl Immunohistochem Mol Morphol 21:271–274
20. Takeshima Y, Amatya VJ, Kushitani K, et al. (2008) A Useful antibody panel for differential diagnosis between peritoneal mesothelioma and ovarian serous carcinoma in japanese cases. Am J Clin Pathol 130:771–779
21. Ordóñez NG (2003) Value of mesothelin immunostaining in the diagnosis of mesothelioma. Mod Pathol 16(3):192–197
22. Hanna A, Pang Y, Bedrossian CW, et al. (2010) Podoplanin is a useful marker for identifying mesothelioma in malignant effusions. Diagn Cytopathol 38(4):264–269
23. Cargnello R, Celio MR, Schwaller B, et al. (1996) Change of calretinin expression in the human colon adenocarcinoma cell line HT29 after differentaition. Biochim Biophys Acta 1313:201–208
24. Taliano RJ, Lu S, Singh K, et al. (2013) Calretinin expression in high-grade invasive ductal carcinoma of the breast is associated with basal-like subtype and unfavorable prognosis. Hum Pathol 44(12):2743–2750

25. Gotzos V, Wintergerst ES, Musy JP, et al. (1999) Selective distribution of calretinin in adenocarcinomas of the human colon and adjacent tissues. Am J Surg Pathol 23:701–711
26. Purdie CA, Piris J (2000) Histopathological grade, mucinous differentiation and DNA ploidy in relation to prognosis in colorectal carcinoma. Histopathol 36:121–126
27. Niv Y (2000) Mucin and colorectal cancer. IMAJ 2:775–777
28. Kahn HJ, Marks A (2002) A new monoclonal antibody, D2–40, for detection of lymphatic invasion in primary tumors. Lab Invest 82: 1255–1257
29. Raica M, Cimpean AM, Ribatti D (2008) The Role of podoplanin in tumor progression and metastasis. Anticancer Res *28*:2997–3006
30. Kawamata F, Homma S, Kamachi H, et al. (2014) C-ERC/mesothelin provokes lymphatic invasion of colorectal adenocarcinoma. J Gastroenterol 49:81–92
31. Liebig B, Brabletz T, Staege MS, et al. (2005) Forced expression of deltaN-TCF-1B in colon cancer derived cell lines is accompanied by the induction of CEACAM5/6 and mesothelin. Cancer Lett 223: 159–167
32. Foda AA, El-Hawary AK, Aziz AA (2015) Colorectal adenocarcinoma with mucinous component: relation of MMP-13, EGFR, and E-cadherin expressions to clinicopathological features and prognosis. APMIS. doi:10.1111/apm.12379
33. Hennessy BT, Gonzalez-Angulo AM, Stemke-Hale K, et al. (2009) Characterization of a naturally occurring breast cancer subset enriched in epithelial-to-mesenchymal transition and stem cell characteristics. Cancer Res 69:4116–4124
34. Zhu QC, Gao RY, Wu W, et al. (2013) Epithelial-mesenchymal Transition and Its Role in the Pathogenesis of Colorectal Cancer. Asian Pacific J Cancer Prev 14(5):2689–2698