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ORIGINAL ARTICLE

Prognostic significance of the immunohistochemical localization and serological detection of CA19-9 tumor antigen in colon carcinoma

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Summary

Purpose: *This study was carried out in order to evaluate the expression of CA19-9 antigen in serum and tissue samples of colon cancer patients in relation with other pathological prognostic factors.*

Materials and methods: *99 patients diagnosed with colon cancer entered the study. The CA19-9 antigen was measured in the serum by ELISA (cut-off value 37 IU/L) and in malignant tissue samples by immunohistochemistry using Mab 192. Seventy-five tissue samples of normal colon served as controls.*

Results: *In cancer cells the CA19-9 antigen was expressed on the cell membrane, in the lumen and in the cytoplasm. The expression of the antigen in the cytoplasm and also the high serum values of the tumor marker correlated well with more differentiated tumors (grade I and II) and with positive lymph nodes cases. There was also a positive correlation between cytoplasmic localization of CA19-9 and high levels in the serum.*

Conclusion: *The presence of CA19-9 in the cytoplasm reflects the malignant potential of the colon cancer cell. In less differentiated tumors (grade III) loss of antigenicity is observed. The two methodologies correlate well with each other and their simultaneous application confers to the better use and understanding of this tumor-associated antigen.*

Key words: CA19-9 tumor antigen, CA19-9 serum tumor marker, colon cancer, immunohistochemistry

Introduction

Mucins are high-molecular weight glucoproteins consisted of a backbone protein to which oligosacharides are attached. The linkage of carbohydrate to the peptide is termed O-glucosidic bond and involves the hydroxylic groups of serine or threonine with N-acetyloglucosamine. Only the backbone proteins are genetically determined (*myc* genes). Gangliosides are proteins similar to Lewis antigen or its derivatives [1-5].

CA19-9 is an epitope present on a mucinous antigen and plays an important role in the process of tumor progression as an adhesion molecule. CA19-9 along with CEA is the second most commonly used tumor marker for colorectal cancer detection in clinical practice [6-8].

CA19-9 is detected in the serum of healthy individuals at low concentrations (<37 IU/L) [9,10]. The fact that serum levels of CA19-9 show transient elevations in benign hepatobiliary diseases and high levels in a variety of carcinomas limits its value as a diagnostic marker, but elevated serum levels prior to any treatment do carry an independent poor prognosis which may reflect not just the presence of tumor itself but also an aggressive tumor biology [11-14]. CA19-9 correlates well with the clinical course of the disease and reflects the treatment results exhibiting a) a quick normalization within 2-4 weeks after

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complete surgical resection of the tumor and b) a transient decrease after successful palliative anticancer therapy. In cases of relapse or progressive disease, CA19-9 increases sometimes before any clinico-laboratory sign of such a disease evolution [15].

The evaluation of the prognostic significance of tumor markers and their ability to predict response to chemotherapy allow better scheduling of palliative treatment in advanced colon cancer [16]. In this study we set out to preoperatively compare the circulating serum CA19-9 tumor marker in colon cancer patients with the immunohistochemical expression of CA19-9 tumor antigen in colon cancer tissues and their relationship with other prognostic factors.

Materials and methods

A total of 99 patients with non-metastatic colon adenocarcinoma operated on with curative intent were studied. Forty-eight of them had stage B2 and 51 stage C (5 C1 and 46 C2) according to the Astler-Coller classification. Tumor specimens were obtained from the Surgical Pathology Department and included 45 cases of grade I, 36 cases of grade II, and 18 cases of grade III colon adenocarcinoma.

Sera were preoperatively collected and stored at –20° C for further analysis.

The immunohistochemical study was performed in buffered formalin-fixed, paraffin-embedded tissues. The sections were treated with trypsin for 10 min at 37° C and then incubated with the primary mouse antibody Mab 192 (Zymed Lab Inc., San Francisco, CA 94080, USA). The detection was performed by the streptavidin-biotin- alkaline phosphatase complex (Super Sensitive kit, Biogenex, San Ramon CA, USA). The chromogen was fast red. Seventy-five tissue samples of normal colon served as controls.

The results were evaluated visually by two observers and the detection of CA19-9 was considered positive when a distinct staining was observed in the cytoplasm, on the membrane and in the lumen of the cells.

The serum CA19-9 was determined by the AXSYM CA19-9 assay kit (Abbott Lab. Diagn. Div, USA) using the automatic analyser AXSYM. The serum samples were analysed in duplicate in 3 consecutive runs along with Low, Medium and High control sera. The reproducibility of the assay was satisfactory. The upper limit of normal was set at 37 IU/L.

Correlations were made using Spearman's rank correlation coefficients and their significance was determined by the Student's t-test.

Table 1. Immunohistochemical pattern of CA19-9 antigen in normal colon tissues and colon carcinomas

	Normal tissue n (%)	*Malignant tissue n (%)*	*p-value*
Cytoplasm	–	51 (51.5)	0.0001
Membrane	75 (75.7)	70 (70.7)	0.52
Lumen	75 (75.7)	89 (89.9)	0.012

Results

In the 75 tissue samples from normal colon the CA19-9 tissue antigen was localized mainly on the membrane of the epithelial cells, on the apical border of the glandular cells and in the lumen of the glands (Table 1).

In all 99 colon carcinomas the CA19-9 tissue antigen was distributed either on the membrane, or in the cytoplasm of the tumor cell, or both, or in the lumen (Table 1).

In studying the correlation between the cytoplasmic localization of CA19-9 antigen and the grading of the carcinomas a trend for positivity from grade I to grade II, but surprisingly a loss of positivity in grade III tumors was found (Table 2a). The localization of CA19-9 on the membrane revealed a rather inverse correlation between positive staining and grading (Table 2b). The CA19-9 antigen immunoreactivity in both the cytoplasm and the membrane correlated strongly with the lymph node status ($p < 0.01$ and $p < 0.05$, respectively) (Tables 3a and 3b).

The serum levels of CA19-9 tumor marker displayed a strong correlation ($p < 0.0001$) with the immunoreactivity in the cytoplasm but no correlation at all

Table 2. Correlation of CA19-9 antigen staining distribution and grading of colon carcinomas

a. Cytoplasm *Staining*	*Grade I n (%)*	*Grade II n (%)*	*Grade III n (%)*	*p-value*
Positive (n=51)	19 (37.2)	25 (49.0)	7 (13.7)	0.007
Negative (n=48)	26 (54.1)	11 (22.8)	11 (22.8)	
b. Membrane *Staining*	*Grade I n (%)*	*Grade II n (%)*	*Grade III n (%)*	*p-value*
Positive (n=70)	33 (47.1)	29 (41.4)	8 (11.4)	0.03
Negative (n=29)	12 (41.3)	7 (24.1)	10 (34.5)	

Table 3. Correlation of CA19-9 antigen staining distribution and lymph node status

a. Cytoplasm

Staining	*Negative lymph nodes n (%)*	*Positive lymph nodes n (%)*	*p-value*
Positive (n=51)	18 (37.5)	33 (64.7)	< 0.01
Negative (n=48)	30 (62.5)	18 (35.3)	

b. Membrane

Staining	*Negative lymph nodes n (%)*	*Positive lymph nodes n (%)*	*p-value*
Positive (n=70)	29 (41.4)	41 (58.6)	0.049
Negative (n=29)	19 (65.5)	10 (34.5)	

with the immunoreactivity on the membrane (p =0.45, Tables 4a and 4b).

The investigation of the serum levels of CA19-9 tumor marker and the histological parameters of the carcinomas showed a strong correlation between the level of the circulating tumor marker and the lymph node status (p <0.0001, Table 5) and also a trend for correlation from grade I to grade II and a loss of correlation in grade III tumors (Table 6).

Discussion

The gastrointestinal tract is the most common site of malignancies of any anatomic system in the body. Early detection of primary tumors is difficult in asymptomatic patients and these tumors are often

Table 4. Correlation of CA19-9 antigen staining distribution and serum CA19-9 values

a. Cytoplasm

Staining	*CA19-9 ≤37 IU/L n (%)*	*CA19-9 >37IU/L n (%)*	*p-value*
Positive (n=51)	18 (33.3)	33 (73.3)	< 0.0001
Negative (n=49)	36 (66.6)	12 (26.6)	

b. Membrane

Staining	*CA19-9 ≤37 IU/L n (%)*	*CA19-9 >37IU/L n (%)*	*p-value*
Positive (n=70)	36 (51.4)	34 (80.0)	0.45
Negative (n=29)	18 (62.0)	11 (38.0)	

Table 5. Correlation of serum CA19-9 and grading of colon carcinomas

Serum CA19-9	*Grade I n (%)*	*Grade II n (%)*	*Grade III n (%)*	*p-value*
≤37IU/L (n=54)	33 (61.1)	9 (16.7)	12 (22.2)	< 0.0001
>37IU/L (n=45)	12 (26.7)	27 (60.0)	6 (13.3)	

detected at an advanced stage. The improvement of survival in patients operated on for colorectal cancer might be the consequence of early diagnosis rather than improvements in the treatment and the follow up. The primary goal of tumor marker research is detection of a cancer in early stages. Positive expression of CA 19-9 in tumor tissues and high preoperative serum levels are predictive of increased cancer mortality.

CA19-9 cancer antigen detection in tumor tissues and serum identifies patients at high risk of cancer recurrence and selects patients for adjuvant therapy. Furthermore, it allows better scheduling of palliative treatment in advanced colorectal cancer through its ability to predict response to chemotherapy.

In our study, CA19-9 cancer antigen was analysed in cancer tissues and in patients' sera, and the relationship between immunohistochemical evaluation, serum values and some other histopathological prognostic factors was investigated. In normal tissues CA19-9 cancer antigen is expressed on the membrane and the lumen of the cells. In cancer tissues the antigen is also expressed in the cytoplasm. These findings agree with those of other authors [17-19]. The positive reactivity of CA19-9 antigen in the cytoplasm correlates well with grade I and grade II of the tumor samples, but this correlation disappears in grade III tumors. The reactivity on the membrane exhibits a rather inverse correlation with grading. It is possible that a transformation of some antigenic sites in less differentiated tumors may occur, which probably have lost the integrity of their structure [20,21]. We

Table 6. Correlation of serum CA19-9 and lymph node status

Serum CA19-9	*Negative lymph nodes n (%)*	*Positive lymph nodes n (%)*	*p-value*
≤37IU/L (n=54)	36 (66.6)	18 (33.3)	< 0.0001
>37IU/L (n=45)	12 (26.6)	33 (73.3)	

also found a strong positive correlation between lymph node involvement and the expression of the antigen either on the membrane or in the cytoplasm [22].

High CA19-9 serum values (>37 IU/L) were found in 45% of the patients studied and in 73% of the patients with CA19-9 expression in the cytoplasm of the tumor cells. On the contrary, the antigen expression on the membrane did not correlate with the high serum values. This may reflect the fact that the carbohydrate structure of glucoconjugates of cancer cells may change markedly, and these structural changes may facilitate the passage and the accumulation of CA19-9 antigen in the cytoplasm.

The behavior of the CA19-9 circulating tumor marker follows the pattern of most circulating tumor markers. Its concentration in the blood varies with the grade of the tumor (I,II) and is a measure of the state of cellular differentiation. In grade III tumors a loss of antigenicity of this tumor marker was noticed. There was also a strong correlation between high serum marker values and lymph node status, which is in agreement with the reports of some investigators [23].

We believe that the diagnosis of cancer itself and the estimation of the malignant potential of cancer cells with the serum marker values and other histopathological prognostic factors, such as tumor grade and nodal invasion, will help provide better treatment for colon cancer patients.

References

1. Magnani JL, Brockhaus M, Smith DF et al. A monosialoganglioside is a monoclonal antibody-defined antigen of colon carcinoma. Science 1981; 212: 55-56.
2. Magnani JL, Nilsson B, Brockhans M et al. A monoclonal antibody defined associated with gastrointestinal cancer is a ganglioside containing sialylated lacto-N-fucopentose II. J Biol Chem 1982; 257: 14365-14369.
3. Magnani JL, Steplewski Z, Kortowski H et al. Identification of the gastrointestinal and pancreatic cancer-associated antigen detected by monoclonal antibody CA19-9 in the sera of patients as mucin. Cancer Res 1983; 43:5489-5492.
4. Marc DR, Chenp PW, Perini F. Altered expression of sialylated carbohydrate antigens in colonic carcinoma cells. Glycoconj J 1998; 15:1155-1163.
5. Rhodes TM. Usefulness of novel tumor markers. Ann Oncol 1999; 10 (Suppl 4): 118-121.
6. Paraskeva C, Corfield AP, Harper S et al. Colorectal carcinogenesis: sequential steps in the *in vitro* immortalization and transformation of human colonic epithelial cells. Anticancer Res 1990; 10: 1189-1200.
7. Nakaga T, Sawai T, Tsuji T et al. Circulating sialyl Lewis(x), sialyl Lewis(a) and sialyl Tn antigens in colorectal cancer patients: multivariate analysis of predictive factors for serum antigen levels. J Gastroenterol 2001; 36:166-172.
8. Narimatsu H, Iwasaki H, Nishihara S et al. Genetic evidence for the Lewis enzyme which synthesizes type I Lewis antigens in colon tissue and intracellular localization of the enzyme. Cancer Res 1996; 56:330-338.
9. Del Villano BC, Zurawinski VR. The carbohydrate antigenic determinant 19.9. A monoclonal antibody defined tumor marker. In: Aloisi RM, Hyxn J (eds). Immunodiagnostics. New York, AR Liss Inc, 1983, pp 269-282.
10. Lamerz R. Role of tumor markers, cytogenetics. Ann Oncol 1999; 10 (Suppl 4): 145-149.
11. Thomas WM, Robertson JF, Price MR et al. Failure of CA19-9 to detect asymptomatic colorectal carcinoma. Br J Cancer 1991; 63: 975-976.
12. Webb A, Scottt-Mackie P, Cunnigham LD et al. The prognostic value of CEA, b-HCG, AFP, CA125, CA19-9 and cERB2 immunohistochemistry in advanced colorectal cancer. Ann Oncol 1995; 6: 581-587.
13. Behbehani AI, Al Sayer H, Farghaly M et al. Prognostic significance of CEA and CA19-9 in colorectal cancer in Kuwait. Int J Biol Markers 2000; 15:51-55.
14. Morales-Gutierrez C, Vegh I, Colina F et al. Survival of patients with colorectal carcinoma: possible prognostic value of tissue carbohydrate antigen 19-9 determination. Cancer 1999 ; 86: 1675-1681.
15. Nakayama T, Watanaba M, Teramoto T et al. CA19-9 as a predictor of recurrence in patients with colorectal cancer. J Surg Oncol 1997; 66:238-243.
16. Halubec L Jr, Topolcan O, Pikner R et al. The significance of CEA, CA19-9 and CA72-4 in the detection of colorectal carcinoma recurrence. Anticancer Res 2000; 20: 5237-5441.
17. Brown RW, Campagna LB, Dunn JK et al. Immunohistochemical identification of tumor markers in metastatic adenocarcinoma. A diagnostic adjunct in the determination of primary site. Am J Clin Path 1997;107:12-19.
18. Alvarez JA, Marin J, Jover JM et al. Sensitivity of monoclonal antibodies to carcinoembryonic tissue polypeptide antigen, alfa-fetoprotein, carbohydrate antigen 50 and CA19-9 in the diagnosis of colorectal adenocarcinoma. Dis Colon Rectum 1995; 38: 535-542.
19. Tucci GF, Grande M, Stopper I et al. Tissue expression of carbohydrate antigen CA19-9 in adenomatous lesions of the colorectum. Clin Ital 1999;51:165-172.
20. Gebaner G, Muller-Ruchholtz W. Carcinoembryonic antigen and CA19-9: implications of quantitative marker measurement in tissues for prognosis of colorectal cancer. Cancer Detect Prev 2001; 25: 344-351.
21. Lorenzi M, Vindigni C, Minacci C et al. Histopathological and prognostic evaluation at immunohistochemical findings in colorectal cancer. Int J Biol Markers 1997;12:68-74.
22. Seregni E, Ferrari L, Martinelli A et al. Diagnostic and prognostic tumor markers in gastrointestinal tract. Semin Surg Oncol 2001; 20: 147-166.
23. Duraker N, Celik A. The prognostic significance of preoperative serum CA19-9 in patients with resectable gastric carcinoma: comparison with CEA. J Surg Oncol 2001; 76:226-271.

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1. Magnani JL, Brockhaus M, Smith DF et al. A monosialoganglioside is a monoclonal antibody-defined antigen of colon carcinoma. Science 1981; 212: 55-56.
2. Magnani JL, Nilsson B, Brockhans M et al. A monoclonal antibody defined associated with gastrointestinal cancer is a ganglioside containing sialylated lacto-N-fucopentose II. J Biol Chem 1982; 257: 14365-14369.
3. Magnani JL, Steplewski Z, Kortowski H et al. Identification of the gastrointestinal and pancreatic cancer-associated antigen detected by monoclonal antibody CA19-9 in the sera of patients as mucin. Cancer Res 1983; 43:5489-5492.
4. Marc DR, Chenp PW, Perini F. Altered expression of sialylated carbohydrate antigens in colonic carcinoma cells. Glycoconj J 1998; 15:1155-1163.
5. Rhodes TM. Usefulness of novel tumor markers. Ann Oncol 1999; 10 (Suppl 4): 118-121.
6. Paraskeva C, Corfield AP, Harper S et al. Colorectal carcinogenesis: sequential steps in the *in vitro* immortalization and transformation of human colonic epithelial cells. Anticancer Res 1990; 10: 1189-1200.
7. Nakaga T, Sawai T, Tsuji T et al. Circulating sialyl Lewis(x), sialyl Lewis(a) and sialyl Tn antigens in colorectal cancer patients: multivariate analysis of predictive factors for serum antigen levels. J Gastroenterol 2001; 36:166-172.
8. Narimatsu H, Iwasaki H, Nishihara S et al. Genetic evidence for the Lewis enzyme which synthesizes type I Lewis antigens in colon tissue and intracellular localization of the enzyme. Cancer Res 1996; 56:330-338.
9. Del Villano BC, Zurawinski VR. The carbohydrate antigenic determinant 19.9. A monoclonal antibody defined tumor marker. In: Aloisi RM, Hyxn J (eds). Immunodiagnostics. New York, AR Liss Inc, 1983, pp 269-282.
10. Lamerz R. Role of tumor markers, cytogenetics. Ann Oncol 1999; 10 (Suppl 4): 145-149.
11. Thomas WM, Robertson JF, Price MR et al. Failure of CA19-9 to detect asymptomatic colorectal carcinoma. Br J Cancer 1991; 63: 975-976.
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13. Behbehani AI, Al Sayer H, Farghaly M et al. Prognostic significance of CEA and CA19-9 in colorectal cancer in Kuwait. Int J Biol Markers 2000; 15:51-55.
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15. Nakayama T, Watanaba M, Teramoto T et al. CA19-9 as a predictor of recurrence in patients with colorectal cancer. J Surg Oncol 1997; 66:238-243.
16. Halubec L Jr, Topolcan O, Pikner R et al. The significance of CEA, CA19-9 and CA72-4 in the detection of colorectal carcinoma recurrence. Anticancer Res 2000; 20: 5237-5441.
17. Brown RW, Campagna LB, Dunn JK et al. Immunohistochemical identification of tumor markers in metastatic adenocarcinoma. A diagnostic adjunct in the determination of primary site. Am J Clin Path 1997;107:12-19.
18. Alvarez JA, Marin J, Jover JM et al. Sensitivity of monoclonal antibodies to carcinoembryonic tissue polypeptide antigen, alfa-fetoprotein, carbohydrate antigen 50 and CA19-9 in the diagnosis of colorectal adenocarcinoma. Dis Colon Rectum 1995; 38: 535-542.
19. Tucci GF, Grande M, Stopper I et al. Tissue expression of carbohydrate antigen CA19-9 in adenomatous lesions of the colorectum. Clin Ital 1999;51:165-172.
20. Gebaner G, Muller-Ruchholtz W. Carcinoembryonic antigen and CA19-9: implications of quantitative marker measurement in tissues for prognosis of colorectal cancer. Cancer Detect Prev 2001; 25: 344-351.
21. Lorenzi M, Vindigni C, Minacci C et al. Histopathological and prognostic evaluation at immunohistochemical findings in colorectal cancer. Int J Biol Markers 1997;12:68-74.
22. Seregni E, Ferrari L, Martinelli A et al. Diagnostic and prognostic tumor markers in gastrointestinal tract. Semin Surg Oncol 2001; 20: 147-166.
23. Duraker N, Celik A. The prognostic significance of preoperative serum CA19-9 in patients with resectable gastric carcinoma: comparison with CEA. J Surg Oncol 2001; 76:226-271.