ANTICANCER RESEARCH *25*: 1755-1760 (2005)

Immunohistochemical Studies of Mucinous Mammary Carcinomas and their Metastases

LIVI GUNKEL[1], IOANNIS MYLONAS[2], DAGMAR U. RICHTER[1] and JOSEF MAKOVITZKY[1]

[1]*Department of Obstetrics and Gynaecology, University of Rostock, Rostock;*
[2]*1st Department of Obstetrics and Gynaecology, Ludwig-Maximilians-University Munich, Munich, Germany*

Abstract. *In cancer cells the expression of mucins is variable in amount and cellular localization. Alteration in glycosylation occurs and leads to the appearance of novel structures. The aim of this study was to investigate the expression of epitopes known as CA19-9, CA50, CA242, MUC1, MUC1-Core and Thomsen-Friedenreich (TF) in mucinous carcinomas. The formalin-fixed and paraffin-embedded tissue samples of the breast (n=29) and their metastases in axillary lymph nodes (n=6) were analysed using immunohistochemical methods. The mucinous breast tumours expressed MUC1, MUC1-Core and TF, while CA19-9, CA50 and CA242 showed a negative staining reaction. The examined metastases showed similar expression patterns as the corresponding primary tumours. In the case of an unknown metastatic primary tumour, such immunohistochemical analysis in axillary lymph nodes might be helpful. A positive reaction of CA19-9, CA50 and CA242 might suggest the presence of a gastrointestinal tumour, especially a pancreatic carcinoma, whereas positive findings of MUC1, MUC1-Core and TF could indicate the presence of a mucinous mammary carcinoma.*

Mucins are a family of large, highly glycosylated proteins, which can be divided into membrane-bound and secretory forms. The most important membrane-bound mucin is named MUC1, an integral component of the cell membrane in normal breast tissue. In cancer cells, the expression of MUC1 is increased and its glycosylation is altered. Therefore, epitopes like MUC1-Core protein, Thomsen-Friedenreich (TF) epitope, CA19-9, CA50 and CA242, which are masked in healthy breast tissue due to the numerous branched carbohydrate chains (1-5), can be demonstrated.

Breast cancer is the most common malignancy in the female population. Invasive ductal carcinoma accounts for approximately 90% of all invasive breast carcinomas. Mucinous cancer of the breast, with an approximate incidence of 2% of all breast cancers, differs from infiltrating ductal carcinoma in its histology and extracellular mucin production. It is thought to be a variant of invasive ductal carcinoma, though with a better prognosis. However, only a few studies have addressed the immunohistochemical analysis of mucinous mammary tumours. Therefore, the aim of this study was to assess the expression of the epitopes MUC1, MUC1-Core, TF, CA19-9, CA50 and CA242 in mucinous mammary carcinomas and in their metastases in axillary lymph nodes by immunohistochemical means.

MUC1, MUC1-Core and TF have been shown to have prognostic significance in breast cancer, whereas the significance of CA19-9, CA50 and CA242 lies mainly in the diagnosis and monitoring of gastrointestinal, especially pancreatic, malignancies (6-12).

Materials and Methods

Tissue samples. Archival samples of 29 mucinous mammary carcinomas were obtained from the files of the Department of Obstetrics and Gynaecology of the University of Rostock, Germany. The tumour material consisted of formalin-fixed and paraffin-embedded tissue from the years 1990 to 2003. Additionally, metastatic axillary lymph nodes were also examined in 6 cases.

Immunohistochemical staining. Two-4 μm slices of the paraffin-embedded tissue were cut. The specimens were dewaxed and deparaffined with xylene and rehydrated in a descending alcohol concentration row. Immunohistochemical staining was done using the antibodies listed in Table I.

CA19-9, CA50 and CA242 were analysed according to the APAAP method. Briefly, the antigens were demasked by heating

Correspondence to: Josef Makovitzky, MD, PhD, University of Rostock, Department of Obstetrics and Gynaecology, Doberaner Str. 142, 18057 Rostock, Germany. Tel: ++49-381 494 8193, Fax: ++49-381 494 8102, e-mail: josef.makovitzky@med.uni-rostock.de

Key Words: Mucinous carcinoma, breast cancer, MUC1, MUC1-Core, Thomsen-Friedenreich, CA19-9, CA50, CA242, immunohistochemistry.

Table I. *Antibodies used in this study.*

Antigen	Antibody Clone	Source	Dilution
CA19-9	C241: 5:1:4 mouse IgG	Novocastra Laboratories Ltd, Newcastle-upon-Tyne, UK	1:200
CA50	C50: 8:2:4 mouse IgM	Novocastra Laboratories Ltd, Newcastle-upon-Tyne, UK	1:20
CA242	C242:II mouse IgG	Novocastra Laboratories Ltd, Newcastle-upon-Tyne, UK	1:20
MUC1	Ma695 mouse IgG	Novocastra Laboratories Ltd, Newcastle-upon-Tyne, UK	1:100
MUC1-Core	Ma552 mouse IgG	Novocastra Laboratories Ltd, Newcastle-upon-Tyne, UK	1:50
TF	A78-G/A7 mouse IgM	Serotec Ltd, Kidlington, Oxford, UK	1:3

in Tec buffer (pH 7.8) in a microwave (3 x 5 min at 900 watts). The sections were incubated with dilutions of the primary antibodies for 30 min. Further incubations with the secondary antibody (rabbit-anti-mouse IgG diluted 1:50, DAKO Diagnostika, Hamburg, Germany) for 30 min and the APAAP complex (alkaline-phosphatase-anti-alkaline-phosphatase, diluted 1:50, DAKO) for 30 min were subsequently performed. Each step was followed by rinsing in distilled water. The development of the alkaline phosphatase was performed with naphthol AS-BI phosphate as substrate and neufuchsine as coupling salt. Counterstaining was performed with haemalaun.

The immunohistochemical staining of MUC1, MUC1-Core and TF was performed using the avidin-biotin-complex method with the NexEs® IHC Autostainer (Ventana, Tucson, AZ, USA) and a protocol for staining with DAB and the use of a microwave (3 x 10 min, 700 watts), as described by the manufacturer (Ventana Medical System). Briefly, slides were incubated with inhibitor serum (4 min) and protease solution (8 min). Incubations with dilutions of the primary antibodies were performed for 32 min. Further incubations with biotinylated secondary antibodies (8 min) and avidin-peroxidase (8 min) were subsequently performed. Each step was followed by several washing steps with phosphate-buffered saline (PBS), as described by the manufacturer. Visualisation of peroxidase activity was performed with DAB (8 min) and copper-sulphate solution (4 min). Counterstaining was performed with haematoxylin reagent (2 min).

Positive cells showed a brownish colour and negative controls as well as unstained cells were blue. Parallel slides incubated with PBS instead of the primary antibody served as negative controls and did not show positive staining.

Immunohistochemical evaluation. A semi-quantitative approach was used to score the staining: 0 = no reaction, 1+ = positive reaction of single cells or small cell groups in only one area, 2+ = positive reaction in single cells or small cell groups in several visual fields, 3+ = positive reaction in large cell groups. Results between 0 and 1 were regarded as negative, while immunohistochemical results between 2 and 3 were regarded as positive.

Results

The immunohistochemical reaction with anti-CA19-9, anti-CA50 and anti-CA242 was of low intensity and the pattern was sialylepitope-associated. The mucinous carcinomas showed negative findings for antigen CA19-9 in 86.2%, for CA50 in 100% and for CA242 in 92.6%, respectively (Table II). The localization of these three antigens is in accordance with similar reaction patterns in gastrointestinal tumours, *i.e.* pancreatic carcinomas.

MUC1, MUC1-Core and Thomsen-Friedenreich showed a high immunohistochemical intensity (Figures 1, 2, 3). The expression of MUC1 was observed in 91.7% of the examined specimens, while MUC1-Core could be demonstrated in all mucinous carcinomas. The TF antigen was positive in 84.6% of the cases.

In six analysed axillary lymph node metastases, no expression of the antigens CA19-9, CA50 and CA242 was observed. All the six examined metastases showed strong staining of MUC1, MUC1-Core and, although heterogeneous, TF epitope (Table III). Primary tumours and corresponding lymph node metastases showed identical reaction patterns and intensity.

Discussion

In this study, the expression of MUC1, MUC1-Core and TF was demonstrated in mucinous breast carcinomas. MUC1 is an integral component of the cell membrane in normal breast tissue, primarily located in the apical surface of epithelial cells. In breast cancer cells, an underglycosylated form of MUC1 is overexpressed. Due to its altered structure with simpler and fewer carbohydrate chains, epitopes like MUC1-Core and Thomsen-Friedenreich, which are normally masked, can be assessed with the use of specific antibodies (1-5).

The polarity loss in malignant transformed cells leads to the identification of MUC1 of the whole cell surface as well as in the cytoplasm. This is normally seen in non-mucinous carcinomas of the breast, while in mucinous carcinomas the expression of MUC1 is predominantly seen on the cell surface

Table II. *Immunohistochemical results of the examined mucinous breast carcinomas.*

Result	CA19-9 (%)	CA50 (%)	CA242 (%)	MUC1 (%)	MUC1-Core (%)	TF (%)
0	18 (62.1)	20 (74.1)	19 (70.4)	0	0	0
1+	7 (24.1)	7 (25.9)	6 (22.2)	2 (8.3)	0	4 (15.4)
2+	2 (6.9)	0	0	3 (12.5)	1 (4.3)	3 (11.5)
3+	2 (6.9)	0	2 (7.4)	19 (79.2)	22 (95.7)	19 (73.1)

Table III. *Results of examined axillary lymph nodes metastases of mucinous breast carcinomas.*

Case-number	CA19 9	CA50	CA242	MUC1	MUC1-Core	TF
1	-	-	-	3+	3+	3+
2	-	-	-	3+	3+	3+
3	1+	-	-	3+	3+	3+
4	-	-	-	3+	3+	1+
5	1+	1+	1+	3+	3+	2+
6	1+	-	-	3+	3+	3+

(13, 14). Several trials showed that increased cytoplasmatic expression is correlated with a higher metastatic potential and a less favourable prognosis, while increased membranous, especially apical, staining generally correlates with increased functional differentiation and a better prognosis (1, 15).

The membrane-bound mucin can be released from cells in soluble form. Therefore, MUC1 and MUC1-Core as well as Thomsen-Friedenreich were detected not only in the tumour cells, but also in the mucin.

Furthermore, mucinous cancers did not express the antigens CA19-9, CA50 and CA242, confirming previous results (14). A limited number of studies have investigated their expression in association with breast cancer (16, 17). Recently, the presence of CA19-9 and CA50 were demonstrated in invasive ductal breast carcinomas, while mucinous mammary tissues did not express these antigens (14).

Additionally, we examined, in 6 of the 29 mucinous carcinomas, metastases in the axillary lymph nodes. The lymph node metastases showed similar expression patterns as the corresponding primary tumours, being positive for MUC1 and MUC1-Core and negative for CA19-9, CA50 and CA242. TF presented a heterogeneous staining pattern, being mostly positive.

In the case of a unknown metastatic primary tumour, such immunohistochemical analysis in axillary lymph nodes might be helpful. The significance of CA19-9, CA50 and CA242 lies in their association with gastrointestinal and especially pancreatic malignancies (6-12). In these tumours, the three carbohydrate antigens are overexpressed and, after being released from the cell surface, their serum levels are used for monitoring. In axillary metastases of an unknown primary therefore, positive reaction of CA19-9, CA50 and CA242, together with elevated serum levels, might suggest the presence of gastrointestinal tumours, especially pancreatic carcinomas, whereas positive staining of MUC1, MUC1-Core and TF may point to mucinous mammary carcinomas.

References

1 McGuckin MA, Walsh MD, Hohn BG, Ward BG and Wright RG: Prognostic significance of MUC1 epithelial mucin expression in breast cancer. Hum Pathol *26(4)*: 432-439, 1995.

2 Burchell JM, Mungul A and Taylor-Papadimitriou J: O-linked glycosylation in the mammary gland: changes that occur during malignancy. J Mammary Gland Biol Neoplasia *6(3)*: 355-364, 2001.

3 Hanisch FG, Uhlenbruck G, Peter-Katalinic J, Egge H, Dabrowski J and Dabrowski U: Structures of neutral O-linked polylactosaminoglycans on human skim milk mucins. A novel type of lineraly extended poly-N-acetyllactosamine backbones with Gal ß(1- 4)GlcNAc ß(1-6) repeating units. J Biol Chem *264(2)*: 872-883, 1989.

4 Lloyd KO, Burchell J, Kudryashov V, Yin BWT and Taylor-Papadimitriou J: Comparison of O-linked carbohydrate chains in MUC-1 mucin from normal breast epithelial cell lines and breast carcinoma cell lines. Demonstration of simpler and fewer glycan chains in tumour cells. J Biol Chem *271(52)*: 33325-33334, 1996.

5 Fung PY, Madej M, Koganty RR and Longenecker BM: Active specific immunotherapy of murine mammary adenocarcinomas using a synthetic tumour-associated glycoconjugate. Cancer Res *50(14)*: 4308-4314, 1990.

6 Haglund C, Lindgren J, Roberts PJ, Kuusela P and Nordling S: Tissue expression of the tumour associated antigen CA242 in benign and malignant pancreatic lesions: a comparison with CA50 and CA19-9. Br J Cancer *60(6)*: 845-851, 1989.

7 Haglund C, Roberts PJ, Jalanko H and Kuusela P: Tumour markers CA19-9 and CA50 in digestive tract malignancies. Scand J Gastroenterol *27(3)*: 169-174, 1992.

8 Kuusela P, Haglund C and Roberts PJ: Comparison of a new tumour marker CA242 with CA19-9, CA50 and carcinoembryonic antigen (CEA) in digestive tract diseases. Br J Cancer *63(4)*: 636-640, 1991.

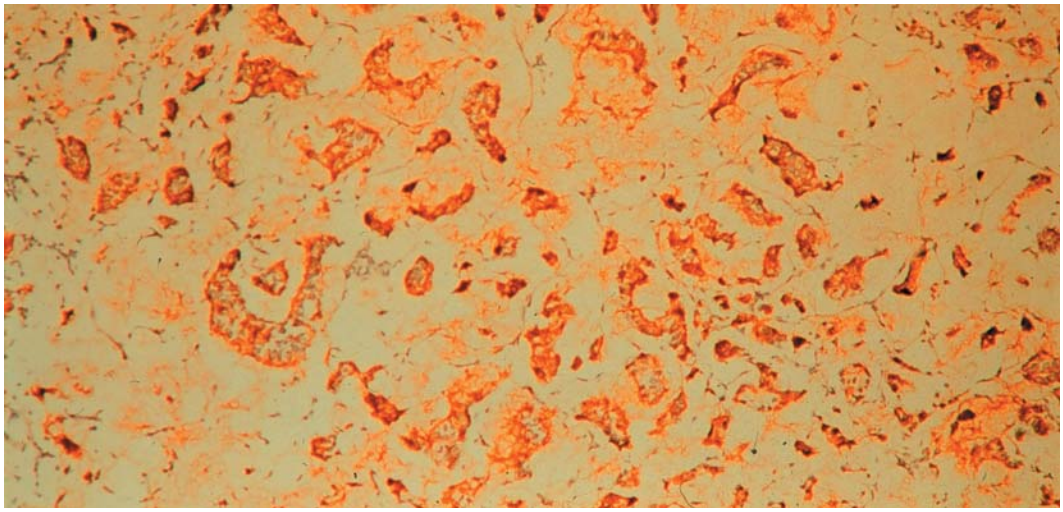

Figure 1. *Expression of MUC1 in a lymph node metastasis (40x).*

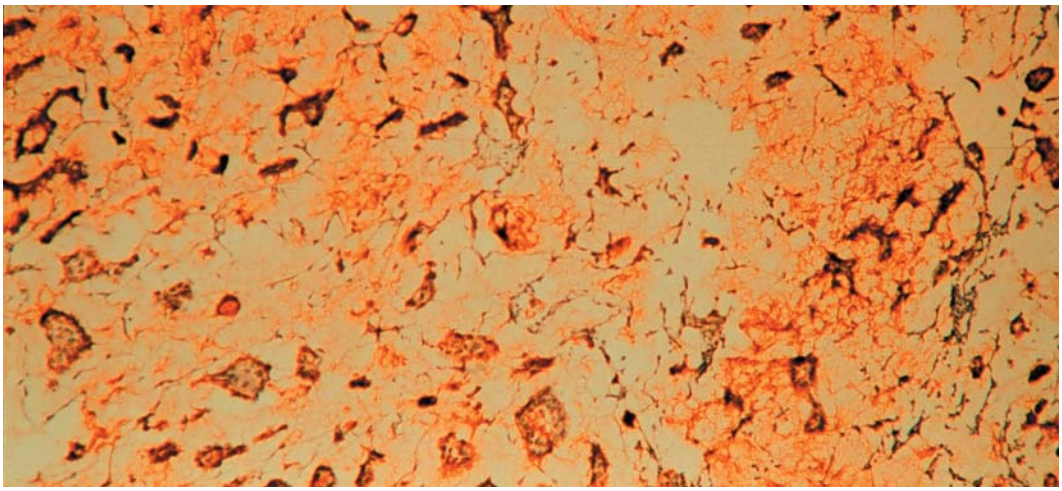

Figure 2. *Expression of MUC1-Core in a lymph node metastasis (40x).*

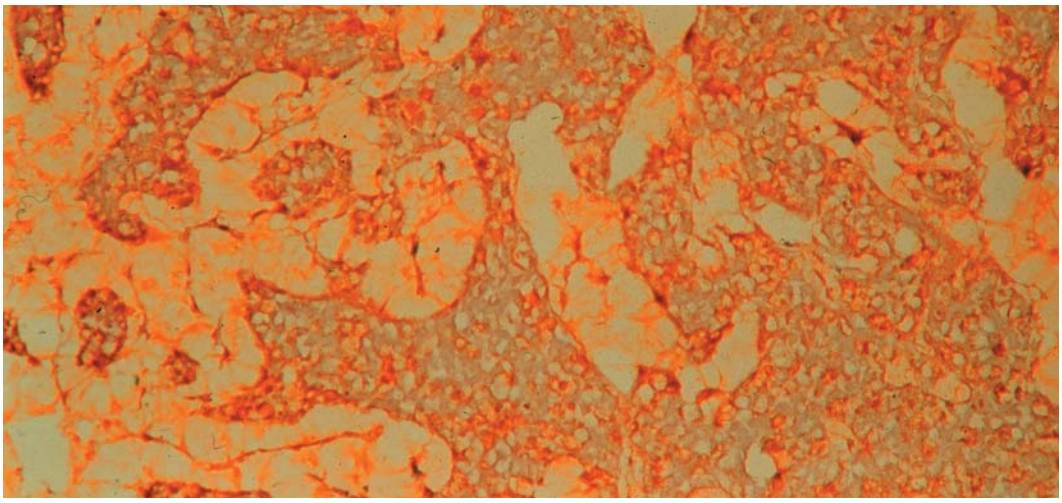

Figure 3. *Expression of Thomsen-Friedenreich in a primary tumour (80x).*

9 Reiter W, Stieber P, Reuter C, Nagel D, Lau-Werner U and Lamerz R: Multivariate analysis of the prognostic value of CEA and CA19-9 serum levels in colorectal cancer. Anticancer Res *20(6D)*: 5195-5198, 2000.
10 Spila A, Ferroni P, Cosimelli M, D'Alessandro R, Abbolito MR, Mariotti S, Aloe S, Carone MD, Graziano F, Tedesco M, Martini F, Mancini R, Stigliano V, Roselli M and Guadagni F: Comparative analysis of CA242 and CA19-9 serum tumor markers in colorectal cancer patients. A longitudinal evaluation. Anticancer Res *21(2B)*: 1263-1270, 2001.
11 Schwenk J and Makovitzky J: Tissue expression of the cancer-associated antigens CA19-9 and CA50 in chronic pancreatitis and pancreatic carcinoma. Int J Pancreatol *5(1)*: 85-98, 1989.
12 Gattani AM, Mandeli J and Bruckner HW: Tumor markers in patients with pancreatic carcinoma. Cancer *78(1)*: 57-62, 1996.
13 O'Connell JT, Shao ZM, Drori E, Basbaum CB and Barsky SH: Altered mucin expression is a field change that accompanies mucinous (colloid) breast carcinoma histogenesis. Human Pathol *29(12)*: 1517-1523, 1998.
14 Chu JS and Chang KJ: Mucin expression in mucinous carcinoma and other invasive carcinomas of the breast. Cancer Letters *142(1)*: 121-127, 1999.
15 Rahn JJ, Dabbagh L, Pasdar M and Hugh JC: The importance of MUC1 cellular localization in patients with breast carcinoma: an immunohistologic study of 71 patients and review of the literature. Cancer *91(11)*: 1973-1982, 2001.
16 Walker RA and Day SJ: Expression of the antigen detected by the monoclonal antibody Ca19.9 in human breast tissues. Virchows Arch A Pathol Anat Histopathol *409(3)*: 375-383, 1986.
17 Eskelinen M, Lipponen P and Syrjänen K: Expression of tumour markers CA50, CEA and TPA in female breast carcinoma as related to histopathological findings and survival. Anticancer Res *12(1)*: 91-96, 1992.

Received December 24, 2003
Accepted January 31, 2005