



BREAST PATHOLOGY



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Tumor induced angiogenesis

ABSTRACT

Angiogenesis is the formation of a new vascular network. The tumor growth and formation of metastasis are dependent on angiogenesis. We investigated how tumor angiogenesis correlates with metastases. Using monoclonal antibodies we stained microvasculature of the breast carcinoma. The microvessels count and density was greater in breast carcinomas with metastases. The number of microvessels may be a prognostic indicator for the rate of metastases.

KEYWORDS: Neovascularization, Pathologic; Breast Neoplasms; Neoplasm Metastasis

INTRODUCTION

Angiogenesis is the formation of a new vascular network out of preexisting vessels. The term „angiogenesis“ was coined in 1935 to describe the formation of new blood vessels in the placenta (1). The microvasculature is an important component of the tumor stroma, it mediates transport of nutrients to the tumor cells and therefore is a crucial element in cancer growth. Specific angiogenic molecules released by the tumor mediate the induction of angiogenesis. We investigated how tumor angiogenesis correlates with metastases in breast carcinoma.

MATERIALS AND METHODS

We studied 12 patients with primary breast carcinoma. Of these patients, seven had axillary lymph nodes metastases.

Frozen sections were processed by immunoalkaline phosphatase (APAAP), using monoclonal antibody to von Willebrand factor (factor VII-related antigen) and antibody to collagen type IV. Microvessels were counted per 100-x field. The area of the highest neovascularization was identified and subjectively graded on a scale of 1-4.

RESULTS

With antibody to von Willebrand factor, cytoplasmic staining in endothelial cells was observed. Tumors were heterogeneous in their microvessels density. The carcinomas of the patients with metastases had a mean microvessels density grade 1, whereas the carcinomas of patients without metastases had a grade 2.

DISCUSSION

The microvasculature consists of endothelial cells and pericytes surrounded by basal lamina (2). Therefore, the antibodies to components of any of these constituents may theoretically be used to visualize both the degree of vascularization and vascular invasion by the tumor cells. There is considerable evidence to indicate that the tumor growth is dependent on angiogenesis. As soon as a new tumor has attained a small size of a few millimeters in diameter, further expansion of the tumor cell population requires the induction of new capillary blood vessels (3). The induction of angiogenesis is mediated by specific angiogenic molecules (growth factors) as: endothelial, fibroblast and platelet derived endothelial growth factor, which are thought to stimulate angiogenesis directly by stimulating vascular endothelial cell growth. On the other hand, growth factors, such as epidermal growth factor and tumor necrosis growth factor are thought to regulate angiogenesis indirectly, possibly by inducing the release of direct acting angiogenic inducers from inflammatory cells (1). New, proliferating capillaries have fragmented basement membranes and are more penetrable by the tumor cells than are mature vessels. We found a significant correlation between the density of microvessels of an invasive breast carcinoma and the occurrence of metastases. These results are consistent with the known role of angiogenesis in the metastatic process.

CONCLUSION

The number of microvessels may be a prognostic indicator for the rate of metastases.

REFERENCES

1. Folkman J, Klagsbrun M. Angiogenic factors. *Science* **1987**;235:442-7.
2. Schlingemann RO et al. Differential expression of markers for endothelial cells, pericytes and basal lamina in the microvasculature of tumors and granulation tissue. *Am J Pathol* **1991**;138:1325-47.
3. Griffioen AW et al. Angiogenesis a target for tumor therapy. *J Lab Clin Med* **1998**;132:363-8.
4. Weidner N et al. Tumor angiogenesis and metastasis correlation in invasive breast carcinoma. *N Engl J Med* **1991**;324:1-8.

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Expression of p53 and Ki-67 immunoreactivity in breast cancer patients

ABSTRACT

The aim of this study is to investigate the association between p53, Ki-67 expression and hormone receptor status as well as clinicopathological parameters in breast cancer patients. The study group consists of 146 breast cancer patients (stage I to stage III, according to the postoperative TNM classification of UICC, 1997 guideline system) who underwent radical mastectomy with axillary lymphadenectomy between January 1998 and March 2000. The expression of p53, Ki-67, estrogen receptor (ER) and progesterone receptor (PgR) has been evaluated by using standard immunoperoxidase technique. The scoring system has been performed for determination the results of p53 and Ki-67 immunoreactivity. The semiquantitative ER-ICA and PR-ICA scoring system has been used for assessment of staining for ER and PgR. The results from p53 and Ki-67 expression were correlated to hormone receptor status, and to clinicopathological parameters. Statistical significance was determined with χ^2 test. Strong correlation was found between the values of p53 and Ki-67 ($p < 0.00001$), p53 and ER ($p = 0.0004$), Ki-67 and ER ($p = 0.0006$) as well as ER and PgR ($p < 0.0001$). p53 and Ki-67 expressed additional correlation to the age of the patients ($p = 0.01$). No correlation was found between p53, Ki-67, ER as well as PgR and lymph node involvement or the stage of the disease. The preliminary results of our study suggest that determination of p53 and Ki-67 expression associated to hormone receptor status and some clinicopathological parameters could be helpful in standardizing the protocols for further treatment of breast cancer patients. However, additional investigations with long term follow-up of the patients are needed to clarify the prognostic significance of p53 and Ki-67 immunoreactivity.

KEYWORDS: Breast Neoplasms; Ki-67 Antigen; Protein p53; Receptors, Estrogen; Receptors, Progesterone

INTRODUCTION

Breast cancer is a common disease with a variable clinical course. Numerous prognostic factors have been proposed in different studies in order to help the selection of patients who should receive additional therapy. Proliferative rate determined by Ki-67 antibody expression has been frequently reported as an important prognostic marker in breast cancer patients (1). The prognostic value of p53 immunohistochemical expression in breast cancer is still controversial, although many reports have shown that it may be an

indicator of poor prognosis (2,3). The aim of this study is to investigate the association between p53, Ki-67 expression and hormone receptor status as well as clinicopathological parameters in breast cancer patients.

PATIENTS, MATERIALS AND METHODS

The study group consists of 146 breast cancer patients (stage I to stage III, according to the postoperative TNM classification of UICC, 1997 guideline system) who underwent radical mastectomy with axillary lymphadenectomy between January 1998 and March 2000. Routinely-processed, formalin-fixed, paraffin-embedded samples have been examined and treated with standard streptavidin-biotin-peroxidase complex technique for assessment of p53 and Ki-67 immunoreactivity. Standard peroxidase-antiperoxidase (PAP) method has been used for immunohistochemical localization of estrogen receptor (ER) and progesterone receptor (PgR). p53 and Ki-67 expression was determined by counting the positively stained nuclei in at least 100 cells of primary tumor tissue samples. Tumors were scored as 0 (fewer than 10% positive cells in 10X HPF), 1 (11-20%), 2 (21-50%) or 3 (51-100%). Tumors were classified as p53 positive if at least 10% of tumor cells exhibited distinct nuclear immunostaining (cut-off point, score 1-3), (5,6) and Ki-67 positive if at least 20% of tumor cells were stained intensively (cut-off point, score 2-3) (1,6). ER and PgR were scored in a semiquantitative fashion (ER-ICA and PR-ICA), incorporating the intensity and the percent of positively stained cells designated as HSCORE (4). The results from p53 and Ki-67 expression were correlated to hormone receptor status and clinicopathological parameters (age, tumor size, histopathologic grade, lymph node involvement, histologic type of the tumor and the stage of the disease). Statistical significance was determined with χ^2 test.

RESULTS

The mean age of the patients was 46.93 years (range, 29-85). Half of the patients were in the age group under 50 years and the other half in the age group over 50 years old. 16 (11%) patients were in stage I, 95 (65%) in stage II, and 35 (24%) in stage III of the disease. Ductal carcinoma was diagnosed in 91 (62%) and lobular carcinoma was found in 55 (38%) patients. Lymph nodes involvement was present in 90 (62%) patients. p53 was positive in 58 (39.7%) breast carcinomas, and strongly correlated to tumor size ($p = 0.0002$) and to age of the patients ($p = 0.01$), (Table 1). Ki-67 was positive in 46 (31.5%) breast carcinomas and strongly correlated to p53 expression ($p < 0.00001$), tumor size ($p = 0.001$), histological type of tumor ($p = 0.007$) and to age of the patients ($p = 0.01$). The ER was positive in 93 (63.7%) and PgR was positive in 72 (49.3%) breast carcinomas. Expression of ER was in strong correlation to p53 ($p = 0.0004$), Ki-67 ($p = 0.0006$), PgR ($p < 0.00001$), as well as to histologic grade ($p = 0.019$) and histologic type of the tumor (ductal vs. lobular carcinoma, $p = 0.00002$), while PgR was strongly correlated only to histologic type of the tumor ($p = 0.0007$).

No correlation was found between p53, Ki-67, ER as well as PgR and lymph node involvement or the stage of the disease.

DISCUSSION

The preliminary results of our investigation of the prognostic significance of p53 and Ki-67 expression in breast carcinomas are consistent to the observations of other authors reported in similar studies (1-3). We have determined an association of p53 and Ki-67 expression to hormone receptor status as well as to tumor size, histopathologic grade and the age of the patients, that is in concordance to the results of other studies (6,7). Nevertheless, the lack of association between p53 or Ki-67 and lymph node involvement or the stage of the disease was reported only in few other studies (7). However, additional investigations with long term follow-up of the breast cancer patients are needed to clarify the prognostic significance of p53 and Ki-67 immunoreac-

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Table 1. Correlation between p53, Ki-67 and hormone receptor status

	p53		Ki-67		ER		PgR		
	+	-	+	-	+	-	+	-	
p53	+		32	26	27	31	26	32	
	-		14	74	66	22	46	42	
Ki-67	+				22	24	21	25	
	-	p<0.00001			71	29	51	49	
ER	+						66	27	
	-	p=0.0004	p=0.0006				6	47	
PgR	+								
	-	NS	NS		p<0.00001				
		58	88	46	100	93	53	72	74

tivity.

CONCLUSION

The preliminary results of our study suggest that determination of p53 and Ki-67 expression associated to hormone receptor status and some clinico-pathological parameters could be helpful in standardizing the protocols for further treatment of breast cancer patients. However, additional investigations with long term follow-up of the patients are needed to clarify the prognostic significance of p53 and Ki-67 immunoreactivity.

REFERENCES

1. Keshgegian AA, Cnaan A. Proliferation markers in breast carcinoma. *Am J Clin Pathol* **1995**;104:42-9.
2. Berteau P, Steinberg SM, Merino MJ. c-erbB-2, p53 and nm23 gene product expression in breast cancer in young woman. *Hum Pathol* **1998**;29:323-9.
3. Barnes DM, Dublin EA, Fisher CJ et al. Immunohistochemical detection of p53 protein in mammary carcinoma. *Hum Pathol* **1993**;24:469-76.
4. Crusel LB, Szabo E et al. Immunocytochemical analyses of estrogen receptor as a predictor of prognosis in breast cancer patients: Comparison with quantitative biochemical methods. *J Cancer Res* **1989**;49:1052-6.
5. Nakopoulou L, Michailopoulou A et al. bcl-2 protein expression is associated with a prognostically favorable phenotype in breast cancer irrespective of p53 immunostaining. *Histopathology* **1999**;34:310-9.
6. Haerslev T, Jacobsen GK. An immunohistochemical study of p53 with correlation to histopathological parameters, c-erbB-2, PCNA and prognosis. *Hum Pathol* **1995**;26:295-301.
7. Esteban JM, Ahn CI, Battifora H, Felder B. Predictive value of ER evaluated by quantitative immunohistochemical analyses in breast cancer. *Am J Clin Pathol* **1994**;102(Suppl):S9-S12.

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Immunocytochemical staining of cells in pleural effusions from patients with breast carcinomas

ABSTRACT

In order to assess the value of immunocytochemical staining as a method of discriminating between reactive mesothelial cells and malignant epithelial cells in pleural fluids, we have studied the reactions of a few markers on cells harvested from pleural effusions due to breast carcinomas. A total of 20 specimens of malignant pleural effusions formed the basis of this study. The antibodies used were raised against epithelial membrane antigen (EMA), carcino-embryonic antigen (CEA) and cytokeratin (CAM 52). Malignant epithelial cells in all cases demonstrated a strong peripheral membrane-type reaction for EMA and CEA, while mesothelial cells were negative. In only 40% neoplastic cells exhibited moderate or strong diffuse cytoplasmic reaction with CAM 52, while in all cases mesothelial cells demonstrated strong positive reaction for cytokeratin. EMA and CEA, in contrast to CAM 52, are specific markers for differentiating carcinoma cells from mesothelial cells in pleural effusions due to breast carcinoma.

KEYWORDS: Breast Neoplasms; Pleural Effusion, Malignant; Immunohistochemistry

INTRODUCTION

The diagnosis of serous effusions remains one of the most difficult tasks in diagnostic cytology. The main problem is the differentiation of neoplastic cells from reactive mesothelial cells. The latter, which occur either singly or arranged in small clusters, have an epithelial appearance and often show large and hyperchromatic nuclei, related to their hyperplastic nature. Neoplastic cells, especially breast cancer cells, can look benign and do not always show highly atypical nuclei. This study was carried out in order to determine the usefulness of immunocytochemical analysis of pleural effusions due to breast carcinomas in discriminating between reactive mesothelial and malignant epithelial cells.

MATERIALS AND METHODS

A total of 20 specimens of malignant pleural effusions, which had been sent to the Institute of Pathology in Niš, formed the basis of this study. All samples in this study came from patients who had been documented as having primary breast carcinoma. After cytocentrifugation of the fluids, slides were fixed in acetone for 10 min. Immunocytochemical staining was per-

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formed by the avidin/biotin/complex (ABC) method, using the following markers: epithelial membrane antigen (EMA), carcino-embryonic antigen (CEA) and cytokeratin (CAM 5.2).

RESULTS

Malignant epithelial cells of the breast carcinoma in all investigated cases demonstrated a strong peripheral membrane-type staining with EMA and CEA. The negative EMA and CEA reactions were observed in reactive mesothelial cells. In 8 of 20 cases (40%) neoplastic cells exhibited moderate or strong diffuse cytoplasmic staining with CAM 5.2, while mesothelial cells in all pleural effusions demonstrated strong positive reaction for cytokeratin.

DISCUSSION

One reason for the low diagnostic yield of conventional cytology can be sometimes difficult morphologic distinction between mesothelial cells and tumor cells, particularly in cases with severely hemorrhagic effusions or with severe inflammation in which the percentage of tumor cells among the total cells in the pleural spaces may be very low. In this study carcinoma cells in pleural effusions consistently gave strong reactions with EMA, while, in contrast, mesothelial cells were negative (1). Although EMA positive mesothelial cells have been reported by several authors, their reaction pattern was clearly distinguishable from that of the tumor cells (2,4). We found that CEA is a very specific marker for adenocarcinoma including breast carcinoma cells; and mesothelial cells never reacted with CEA (3). As has been reported previously, cytokeratin CAM 5.2 is not a specific marker in distinguishing a reactive mesothelial cells from carcinoma cells in pleural effusions (5).

CONCLUSION

To summarize, immunocytochemistry is advocated as very helpful, especially in solving doubtful cases. It could be recommended as a routine procedure to achieve a significant increase in the diagnostic yield of cytology in pleural effusions due to breast cancer. As it is a relatively expensive procedure the technique should be used with a selected panel of markers. EMA and CEA, in contrast to CAM 5.2, are specific markers proposed to identify carcinoma cells and to differentiate them from reactive mesothelial cells in pleural effusions due to breast carcinoma.

REFERENCES

1. Daste G, Serre G, Mauduyt MA et al. Immunophenotyping of mesothelial cells and carcinoma cells with monoclonal antibodies to cytokeratin, vimentin, CEA and EMA improves the cytodagnosis of serous effusions. *Cytopathology* 1991;2:19-28.
2. Li CY, Ziesmer SC, Wong YC et al. Diagnostic accuracy of the immunocytochemical study of body fluids. *Acta Cytologica* 1989;33:5:667-73.
3. Nafussi AA, Carder PJ. Monoclonal antibodies in the cytodagnosis of serous effusions. *Cytopathology* 1990;1:119-28.
4. Tickman RJ, Cohen C, Varma VA et al. Distinction between carcinoma cells and mesothelial cells in serous effusions. *Acta Cytologica* 1990;34:491-6.
5. Wazir JF, Martin-Bates E et al. Evaluation of immunocytochemical staining as a method of improving diagnostic accuracy in a routine cytopathology laboratory. *Cytopathology* 1991;2:75-82.
7. Ilić R. *Patologija dojke*. Vol I. Niš: Prosveta, 1997.

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Diagnosis of sclerosing adenosis

KEYWORDS: Breast Neoplasms; Proto-Oncogene Proteins c-bcl-2

Sclerosing adenosis is a histopathological term referring to an entity of varying complexity which can be observed and recognized on microscopic examination of a section of the human breast tissue. Characteristically, the proliferating epithelial cells of the breast lobules are surrounded by a mass of fibers which the early observers regarded as myoepithelium. Classically, the proliferating fibrous or myoepithelial tissue is disposed in whorls which distort the normal architecture of the lobules, the epithelium of which often shows hyperplasia. Of 20 patients with histopathological diagnosis of sclerosing adenosis, 7 patients had mastalgia with discrete palpable masses and 13 patients had mammographic features suggestive or diagnostic of cancer. Surgically specimens were fixed in 10% formaldehyde, routinely processed and embedded in paraffin. Laboratory sections were stained with HE, Van Gieson and Gomory methods. The average age of our affected patients was 42 years. In a prospective study of 7 patients presented with breast pain and of 13 patients with mammographic features suggestive of cancer, sclerosing adenosis was pathohistologically found. Pathological criteria we used to define sclerosing adenosis were: nodular epithelial lesions in which fibrosis or myoepithelial proliferation was associated with a stellate or whorled distortion of the normal type of the lobular pattern. Staining of the ductal basement membranes showed: Contrast to infiltrating cells of ductal carcinoma that have disrupted basement membranes, sclerosing adenosis do not have this. Mammographic signs which indicate the presence of sclerosing adenosis were: distortion of the breast architecture of a type very similar to that caused by a small cancer, fine smooth calcification scattered widely throughout the breast tissue, usually bilaterally, the presence of a few microcalcifications, up to 10 in number, arranged in a small localized group and a combination of architectural distortion and microcalcification. The radiological difficulty of distinguishing between this benign lesion and cancer is emphasized by the present study. The calcification accompanying sclerosing adenosis is characteristically of the fine type, very much as it is seen in cancer. This study has shown that mastalgia was a symptom in 7 patients in whom sclerosing adenosis was identified in the symptomatic breast. Some authors found sclerosing adenosis to be the one most frequently found to contain neural invasion by mammary epithelial cells. Because that, they ask themselves „is the direct invasion („pseudoinfiltration“) of the nerve tissue the cause of pain in patients with sclerosing adenosis?“ The benign lesion, sclerosing adenosis, mimics carcinoma on microscopy, and is often indistinguishable from cancer on mammograms. However, paraffin rather than frozen section histology is more certain.



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Inverse association of bcl-2 protein and microvessel density in breast cancer

KEYWORDS: Breast Neoplasms; Proto-Oncogene Proteins c-bcl-2

Bcl-2 is well known as anti-apoptotic protein. However, bcl-2 expression has been correlated with a better survival in a variety of human malignancies, including breast cancer. On the other hand, high intratumoural microvessel density (MVD) is a potent independent prognostic factor in breast carcinomas. In the present study we investigated comparatively the immunohistochemical expression of the bcl-2 protein with the MVD in a series of 110 breast cancer cases. Bcl-2 cytoplasmic /peri-nuclear expression in more than 10% of cancer cells was noted in 35% of cases analyzed, which were considered as positive for bcl-2. The MVD ranged from 10-89 microvessels per x 200 optical field and were divided into three groups: low MVD (< 25), medium MVD (25-44) and high MVD (>45). The mean MVD in cases with positive bcl-2 expression was 40.15 vs 25.12 in cases with absence of bcl-2 expression. This was statistically significant ($p=0.04$) showing a potential role of bcl-2 protein as a suppressor of angiogenesis in breast cancer. The paradoxical correlation of bcl-2 expression with better prognosis in breast cancer may therefore be explained by the association of bcl-2 with poor tumor vascularisation, which prevents tumor invasion and dissemination.