Expression of NM23 and tenascin in invasive ductal carcinomas of the breast

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Summary

Objective: In this series of ductal carcinoma of the breast, immunoexpression of antimetastasis gene nm23 and tenascin was examined and the role in prognosis was investigated by correlation with the tumour grade and stage, and ER/PR immunoexpression.

Material and Methods: In this study 27 ductal carcinomas of the breast were analysed for expression of tenascin and nm23 antimetastasis genes by immunohistochemistry.

Results: The results of our study revealed a statistically significant correlation between nm23-H1 immunoexpression and lymph node metastasis. We also found a statistically significant correlation between tenascin and nm23-H1 immunoexpression. Our results suggest that tenascin limits tumour spread.

Conclusion(s): Antimetastasis gene expression can be used in predicting lymph node metastasis in ductal carcinomas of the breast.

Key Words: Tenascin; nm23 expression; Ductal carcinoma; Breast.

Introduction

Breast carcinoma is the second common cause of death in women. Every year more than 40,000 women die from this disease. Distant metastases are the major causes of morbidity and mortality in women with breast cancer [1].The prediction of this metastatic proclivity is important in determining prognosis and to assess effective therapy protocols.

Estrogen/progesterone receptors and c-erbB-2 amplification are some well-known factors in this aspect [2]. Some recent studies have implicated other factors such as mutations of c-myc or p53 [3]. Expression of nm23-H1 or tenascin has been found to be correlated with metastasis, invasion or recurrence [4, 5]. In this study, nm23-H1 and tenascin were examined in ductal carcinomas of the breast.

Tenascin is a large glycoprotein of the extracellular matrix (ECM). Interacting with the other ECM proteins, it is prominently expressed during embryogenesis of tissues where the development of epithelial structures are induced by mesenchymes. It is found to decrease or disappear from normal adult breasts of different species [5]. The presence of tenascin in the stroma around tumour cells, the proliferating or normal ducts near the tumour cell nests and in the ductal carcinoma in situ component of invasive carcinoma may suggest a role of tenascin in tumour cell migration [6-8].

nm23, an antimetastasis gene, was identified by differential screening of a murine melanoma cell line; cDNA library with RNA from cell lines of differing metastatic potentials [9]. nm23RNA levels are found to be differently expressed in human breast tumours and low nm23RNA levels are associated with histopathological indication of high metastatic potential [4].

The aim of this study was to investigate *i)* immunoexpression of tenascin, *ii)* immunoexpression of the antimetastasis gene (nm23-H1), *iii)* correlation of the immunoexpression of tenascin and the antimetatastasis gene with tumour grade and stage iv) their role in the prognosis of ductal carcinoma of the breast.

Material and Methods

Cases: We studied 27 patients who had had a mastectomy and axillary dissection with a diagnosis of invasive ductal carcinoma. The mean age of the patients was 54 years (range 29-78 years).

Patients received no radiation therapy or chemotherapy before the radical mastectomy. Clinical data was collected from patient records, tumours were staged according to AJCC (American Joint Committee on Cancer, Philadelphia, 1992) classification and histological grade was determined according to the Scarff-Bloom-Richardson grading system (10). Lymphatic and/or vascular invasion was found in three cases of the ductal carcinomas. Lymph node metastases were present in 13 cases. Fourteen of the cases in the study group were non-metastatic. The differentiation of tumours was grade 3 in six cases, grade 2 in 20 cases and grade 1 in one case.

A cut-off point of > 30% intranuclear immunoexpression of the tumour was used to assess estrogen and progesterone immunoexpression [11].

Clinical, histomorphological and immunohistochemical data are presented in Table 1.

Immunohistochemistry: Immunohistochemical detection was performed by the StreptAvidin-Biotin complex method on 5 μm sections from archival representative paraffin-embedded blocks. Each case was observed and scaled by three pathologists independently.

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Table 1. — *Clinical, histomorphologic and immunohistochemical features of the cases*

Cases	Age	Stage&Grade	E/P	mm 23	Tenascin
1	48	T4b N2 G3	-	+	+++
2	39	T2 N1b G2	+	+++	+++
3	49	T2 N0 G3	-	++	+++
4	71	T2 N1 G2	+	++	+++
5	64	T2 N1 G2	+	++	++
6	29	T1c N2 G2	-	+++	+++
7	48	T1 N1b G2	-	-	+++
8	54	T2 N1 G2	-	+	+++
9	64	T1 N1 G2	-	+	+
10	73	T1c N1 G2	-	++	+++
11	55	T2 N1 G3	+	++	++
12	50	T2 N0 G3	-	++	+++
13	63	T3 N1 G3	-	++	++
14	71	T1 N0 G2	+	+	++
15	52	T1 N1 G2	+	+	++
16	42	T1 N0 G2	+	+++	+++
17	45	T2 N0 G2	+	+++	+++
18	61	T2 N1 G1	+	+	+++
19	37	T3 N0 G2	+	+++	+++
20	43	T1 N0 G2	-	+++	+++
21	78	T1 N0 G3	+	+++	+++
22	56	T2 N0 G2	+	+++	+++
23	63	T2 N0 G2	+	++	++
24	43	T2 N0 G2	+	++	+++
25	35	T2 N0 G2	+	++	+++
26	50	T2 N0 G2	+	+++	+++
27	64	T3 N0 G2	+	+	++

E/P : Estrogen and progestrone immunoexpression

The nm23-H1 expression was determined according to the intensity of the intracytoplasmic immunoreactivity as negative (0), weak (1+), moderate (2+) or strong (3+). Tenascin immunoreactivity was observed as thick bundles around tumour nests and reported according to the intensity of the immunoreactivity as either negative (0), focal or diffuse weak (1+), focal strong (2+) or diffuse strong (3+).

Statistical Analysis

Statistical analysis was based on a two-sided Chi-Square test. The computations were performed using GraphPad InStat version 2.04 (GraphPad software U.S.A). The statistical difference was considered significant if the p value was less than 0.05 ($p < 0.05$).

Results

Intracytoplasmic immunoexpression of the antimetastasis gene nm23-H1 was detected (1+) in eight cases, (2+) in ten cases, (3+) in nine cases as intracytoplasmic immunoreactivity (Figure 1). A statistically significant correlation between lymph node metastasis and nm23 immunoexpression was found ($p < 0.05$). No statistical correlation between nm23 and tumour grade, estrogen/progesterone immunoexpression was found.

Immunoexpression of tenascin was demonstrated as thick bands around tumour nests in peritumoral areas (Figure 2). Diffuse strong (3+) peritumoural immunoexpression was demonstrated with tenascin in 19 cases, focal weak (2+) immunoexpression in eight cases. Statistically no correlation between estrogen/progesterone immunoexpression, tumour grade, stage and tenascin immunoexpression was revealed.

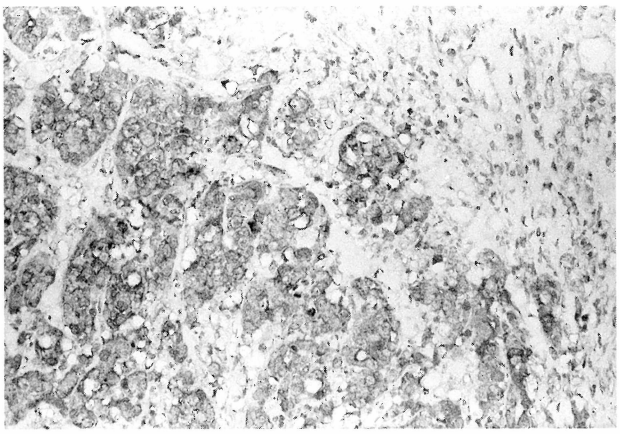

Figure 1. — Strong (3+) intracytoplasmic immunoexpression of nm23-H1 (x 400).

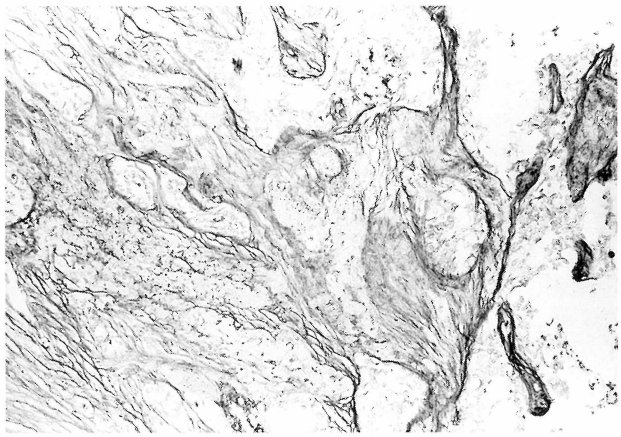

Figure 2. — Diffuse strong (3+) peritumoral immunoexpression with tenascin (x 200).

The results of this study showed statistically significant correlation between tenascin and nm23 immunoexpression ($p < 0.05$).

Discussion

The prediction of accurate biological behaviour and the metastatic potential of invasive breast carcinoma is essential in determining prognosis. However, the well-known ER/PR or bcl-2, HER-2/*neu* expression status is still not sufficient to predict the exact outcome.

Tenascin is a large glycoprotein of the extracellular matrix (ECM). The presence of tenascin in tumour cells in the proliferating and some normal ducts, near the tumour cell nests, in the stroma and in the ductal carcinoma in situ component of invasive carcinoma may suggest a role of tenascin in tumor cell migration [4-6, 8]. A correlation between tenascin expression and prognostic factors such as tumour size, lymph node metastasis and

tumour necrosis could not be demonstrated [7]. Tenascin immunoexpression was found to be more prominent in tumours of high histological grade [6]. The results of our study demonstrated that tenascin could be a stromal marker of breast carcinomas. Statistically no correlation between estrogen/progesterone immunoexpression, tumour grade, stage and tenascin immunoexpression was found.

nm23 is an antimetastasis gene that was identified by differential screening of a murine melanoma cell line [9]. nm23-H1 has also been shown to restore normal phenotype in cultured metastatic breast cancer cells [12]. Inverse/diverse correlations between nm23 expression in tumour stage and/or the outcome of patients with breast cancer have been demonstrated [13]. Charpin *et al.*, showed that immunexpression of nm23-H1 correlates with longer metastasis-free survival [14]. In this study we could not reveal any correlation with histological grade and nm23 immunoexpression. Yoshida *et al.*, observed that nm23 expression was focally positive/negative in lymph node metastasis of invasive ductal breast carcinoma compared to diffusely positive in benign and noninvasive carcinomas [15]. However Hahnel *et al.* could not demonstrate any correlation with antimetastasis gene immunoexpression and tumour metastasis in breast carcinomas [13]. Midulla *et al.*, demonstrated that positive staining for nm23 is significantly correlated with histological grade (16). In a recent study we demonstrated that nm23 immunoexpression of lymph node metastasis cases were either (1+) or (2+). The results of our study also revealed a statistically significant correlation between nm23-H1 immunoexpression and lymph node metastasis.

Conclusion

Our results suggest that tenascin limits tumour spread as proposed by other studies and that antimetastasis gene immunoexpression correlates with lymph node metastasis in ductal carcinomas of the breast. Antimetastasis gene expression and tenascin can be used as a predictive marker for prognosis in ductal carcinomas of the breast.

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References

[1] Gilewski T., Norton L.: "The molecular basis of cancer". In: "Cytogenetics of Neoplasia-Growth and Spread of Cancer". Mendelsohn J., Howley P. M., Israel M. A., Liotta L. A. (eds). Philadelphia, WB Saunders Company, 1995, 143.

[2] Bertheau P., Steinberg S. M., Merino M. J.: "C-erbB-2, p53, and nm23 gene product expression in breast cancer in young women: immunohistochemical analysis and clinicopathologic correlation". *Hum. Pathol.*, 1998, *4*, 323.

[3] Bland K. I., Konstadoulakis M. M., Vezeridis M. P., Wanebo H. J.: "Oncogene protein co-expression. Value of Ha-ras, c-myc, c-fos, and p53 as discriminants for breast carcinoma". *Ann. Surg.*, 1995, *221(6)*, 706.

[4] Bevilacqua G., Sobel M. E., Liotta L. A., Steeg P. S.: "Association of low nm23 RNA levels in human primary infiltrating ductal breast carcinomas with lymph node involvement and other histopathological high metastatic potential". *Cancer Res.*, 1989, *49*, 5185.

[5] Mackie E. J., Chiquet-Ehrisman R., Pearson C. A., Inaguma Y., Taya K., Kawarada Y., *et al.*,: "Tenascin is a stromal marker for epithelial malignancy in the mammary gland". *Proc. Natl. Acad. Sci.*, 1987, *84*, 4621.

[6] Iskaros B. F., Hu X., Sparano J. A., Fineberg S. A.: "Tenascin pattern of expression and established prognostic factors in invasive breast carcinoma". *J. Surg. Oncol.*, 1998, *68(2)*,107.

[7] Tokes A. M., Paku S., Toth S., Paal S., Kulka J., Toth J., *et al.*: "Tenascin expression in primary and reccurent breast carcinomas and the effect on breast tumor cell cultures". *Pathol. Oncol. Res.*, 2000, *6(3)*, 202.

[8] Melis M., Baiocchini A., Soda G., Bosco D.: "Tenascin expression in elastotic cuffs of invasive ductal carcinoma of the breast". *Pathol. Res. Prac.*, 1997, *193*, 479.

[9] Steeg P., Bevilacqua G., Kopper L., Thorgeirsson U. P., Talmadge J. E., Liotta L. A., Sobel M. E.: "Evidence for a novel gene associated with low tumor metastatic potential". *J. Natl. Cancer İnst.*, 1988, *80*, 200.

[10] Elston C. W., Ellis I. O.: "The breast". In: Elston C. W., Ellis I. O., (eds). "Assessment of Histological Grade". Edinburgh: Churchill Livingstone, 1998, 365.

[11] Elston C. W., Ellis I. O., Goulding H., Pinder S. E.: "The breast". In: Elston C. W., Ellis I. O., (eds). "Role of Pathology in the Prognosis and Management of Breast Cancer". Edinburgh, Churchill Livingstone, 1998, 385.

[12] Howlett A. R., Petersen O. W., Steeg P. S., Bissel M. J.: "A novel function for the nm-23H1 gene: overexpression in human breast carcinoma cells leads to the formation of basement membrane and growth arrest". *J. Natl. Cancer Inst.*, 1994, *86*, 1833.

[13] Hahnel E., Dawkins H., Robbins P., Hahnel R.: "Expression of stromelysin-3 and nm 23 in breast carcinoma and related tissues". *Int J.Cancer*, 1994, *58*, 157.

[14] Charpin C., Garcia S., Bonnier P., Martini F., Andrac L., Horschowski N., *et al.*: "Prognostic significance of Nm23/NDPK expression in breast carcinoma, assessed on 10 year follow-up by automated and quantitative immunocytochemical assays". *J. Pathol.*, 1998, *184(9)*, 401.

[15] Yoshida H., Kijima H., Terasaki Y., Suto A., Omiya H., Shimojima K., *et al.*: "Heterogeneous expression of nm23 gene product as a predictor of lymph nodal status in human breast cancer". *Int. J. Oncol.*, 1998, *13(6)*, 1141.

[16] Midulla C., De Iorio P., Nagar C., Pisani T., Cenci M., Valli C., *et al.*: "Immunohistochemical expression of nm23-H1,Ki67 and DNA ploidy: correlation with lymph node status and other clinical pathologic parameters in breast cancer". *Anticancer Res.*, 1999, *19(5B)*, 4033.

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