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ORIGINAL ARTICLE

Correlation of steroid hormone receptor status with histological and nuclear grading in breast carcinoma

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Summary

Purpose: *The present study was performed to evaluate the immunohistochemical analysis of estrogen receptor (ER) and progesterone receptor (PR) in invasive breast carcinomas of various histological subtypes and grades. In this paper an attempt was made to establish a correlation between hormone receptor status and histological and nuclear grading of breast carcinoma.*

Materials and methods*: Immunohistochemistry was performed on paraffin sections of 80 invasive breast carcinomas (38 ductal, 18 lobular, 18 ducto-lobular, 2 medullary, 2 mucinous, 1 tubular and 1 papillary). The same scoring system was used for immunohistochemically stained ER and PR. The results were compared with the histological and nuclear grade and analyzed by the chi-square test.*

Results: *Positive immunoreactivity for ER and PR were seen in 71.25% and 60.00% cases, respectively. Both ER and PR positive immunostaining was observed in all (100%) well-differentiated (grade I) breast carcinomas, while in grade II tumors ER and PR-positive cancer cells were 76.36% and 61.62%, respectively. The corresponding figures for grade III carcinomas were 41.18% and 35.29%. A significant association ($p < 0.05$) between different histological grades of breast carcinomas and ER and PR immunoreactivity was found. No significant association was found between nuclear grade of breast carcinoma and ER and PR immunoreactivity.*

Conclusion: *The results presented herein suggest that histological grade of invasive breast carcinoma was significantly associated with ER and PR immunoreactivity, while nuclear grade alone showed no correlation. Moreover, our findings showed that ER and PR positivity declined with increasing tumor grade.*

Key words: breast carcinoma, histological grade, hormone receptor status, immunohistochemistry, nuclear grade

Introduction

Knowledge of steroid hormone receptor status is important, both as a prognostic indicator and as a guide to the choice of systemic treatment in patients with breast carcinoma. Treatment decisions should be based not only on the standard histological features of breast carcinoma, such as its type and grade, but also on the phenotypic characteristics of the tumor, such as the presence or absence of hormone receptors [1]. The assessment of ER and PR status in breast cancer by immunohistochemistry has become standard of care, and is rapidly being incorporated as a biomarker for other tumors as well [2-7]. Breast cancer patients whose lesions contain both ER and PR have the best probability of remission following hormonal therapy (approaching 70%) [8,9]. It has been shown that tumors expressing ER and PR tend to be better differentiated and low grade tumors, but this is not always the case [10].

The present study was performed to evaluate the ER and PR status in invasive breast carcinomas of various histological subtypes and grades and to search for possible correlation between hormone receptor status and histological and nuclear grading.

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Materials and methods

Patients

The material comprised 80 primary breast carcinomas, collected for immunohistochemical hormonal receptor analysis at the Institute of Pathology, Medical Faculty of Nis. The specimens were obtained from women with a mean age of 56 years (range 30 -77 years). Consecutive sections were used for routine hematoxylin-eosin (HE) staining and for immunostaining. According to histopathological features, invasive breast carcinomas were of various histological subtypes: 38 ductal, 18 lobular, 18 ducto-lobular, 2 medullary, 2 mucinous, 1 tubular and 1 papillary. A modified Bloom and Richardson system described by Elston and Ellis [11] was used for histological grading of breast carcinomas. The follow-up of these cases was short; consequently, adequate recurrence and survival information is not available.

Immunostaining

The previously described microwave (MW) pretreatment technique [12] was used. In brief, the dewaxed and rehydrated samples were placed in a Coplin jar containing 0.01M sodium citrate buffer (pH 6.0) for MW pretreatment. Samples were heated in a 700 W microwave oven for 3 min at full power (the solution comes to a rapid boil), followed by a variable period at 40% of full power (this power setting adjusts the oven cycles on and off every 12-19 sec and the solution is maintained near boiling point). After heating, sections were let stand for 20 min in the MW, then rinsed in PBS (pH 7.5) at room temperature for 10 min. Immunohistochemical analysis for ER and PR were performed with reagents supplied by DAKO-Citomation, Copenhagen, Denmark. Labelled streptavidin-biotin (LSAB) method for determination ER and PR in cancer cells was used. The procedure for immunostaining was supplied by the manufacturer. Positive staining reaction was seen in nuclei of the target tissue cells. In breast carcinoma, epithelial cells of both ductal and lobular origin displayed nuclear staining. Normal epithelial cell nuclei served as an internal control. The same scoring system, previously described, was used for ER and PR [13]. In brief, a score was given to the proportion of cells staining positive: 0%=0; <1%=1; 1-10%=2; 11-30%=3; 31-66%=4 and >66%=5. An intensity score also was given: no staining=0; weak staining=1; moderate staining=2 and strong staining=3. Intensity and proportion scores were totaled. A tumor with a total score of 0-2 was classified as negative. If the score was 3 or 4, the tumor was characterized low-positive, and a score of 5-8 indicated that the tumor was positive.

Statistical analysis

ER and PR status were compared with histological and nuclear grade of the tumor and the results were analyzed by the chi-squared test. The significance level was set to $p < 0.05$.

Results

The distribution of ER and PR immunoreactivity according to the histological subtype of the carcinoma is shown in Table 1.

Positive immunoreactivity for ER and PR (low-positive and positive cases were considered positive) were seen in 71.25% and 60.00% cases, respectively. Both ER and PR positive immunostaining was observed in all (100%) well-differentiated (grade I) breast carcinomas, while in grade II tumors ER and

Table 1. Histological subtypes of 80 invasive breast carcinomas and estrogen (ER) and progesterone receptor (PR) status

Tumor subtype	*Number*	*ER(+) PR(+) n (%)*	*ER(–) PR(–) n (%)*	*ER(+) PR(–) n (%)*	*ER(–) PR(+) n (%)*
Invasive ductal	38	17 (44.74)	11 (28.95)	7 (18.42)	3 (7.89)
Invasive lobular	18	15 (83.33)	3 (16.67)	–	–
Invasive ducto-lobular	18	9 (50)	3 (16.67)	5 (27.78)	1 (5.55)
Medullary	2	–	1 (50)	1 (50)	–
Mucinous	2	–	–	1 (50)	1 (50)
Tubular	1	1 (100)	–	–	–
Invasive papillary	1	1 (100)	–	–	–

PR-positive cancer cells were 76.36% and 61.62%, respectively. The corresponding figures for grade III carcinomas were 41.18% and 35.29% (Table 2).

The relationship of the ER and PR immunostaining to histological and nuclear grade of breast carcinomas is shown in Tables 3 to 6.

A significant association ($p < 0.05$) between different histological grades of breast carcinomas and ER and PR immunoreactivity (negative, low-positive and positive) was found (Tables 3 and 5).

No significant association was found between nuclear grade and ER and PR immunoreactivity (Tables 4 and 6).

Table 2. Percent of estrogen (ER) and progesterone receptor (PR) positivity in different histological grades of invasive breast carcinoma

Histological grade	*Number*	*ER(+) (%)*	*PR(+) (%)*
I	8	100	100
II	55	76.36	61.62
III	17	41.18	35.29

Table 3. Estrogen receptor (ER) immunostaining (N-negative, LP-low positive and P- positive) of invasive breast carcinomas in relation to histological grade

	ER immunostaining			
Histological grade	*N (0-2)*	*LP (3-4)*	*P (5-8)*	*Total*
I	0	1	7	8
II	13	6	36	55
III	10	1	6	17
Total	23	8	49	80

$x^2 = 11.46$; p=0.022

Table 4. Estrogen receptor (ER) immunostaining (N-negative, LP-low positive and P- positive) of invasive breast carcinomas in relation to nuclear grade

	ER immunostaining			
Nuclear grade	*N (0-2)*	*LP (3-4)*	*P (5-8)*	*Total*
I	0	0	5	5
II	9	3	30	42
III	14	2	17	33
Total	23	5	52	80

$x^2 = 6.87$; p=nonsignificant

Table 5. Progesterone receptor (PR) immunostaining (N-negative, LP-low positive and P-positive) of invasive breast carcinomas in relation to histological grade

	PR immunostaining			
Histological grade	*N (0-2)*	*LP (3-4)*	*P (5-8)*	*Total*
I	0	1	7	8
II	21	2	32	55
III	11	1	5	17
Total	32	4	44	80

$x^2 = 10.63$; p=0.031

Table 6. Progesterone receptor (PR) immunostaining (N-negative, LP-low positive and P- positive) of invasive breast carcinomas in relation to nuclear grade

	PR immunostaining			
Nuclear grade	*N (0-2)*	*LP (3-4)*	*P (5-8)*	*Total*
I	0	0	5	5
II	14	2	26	42
III	18	0	15	33
Total	32	2	46	80

$x^2 = 8.57$; p=nonsignificant

Discussion

The prognostic significance of morphologic grading of breast carcinomas was first emphasized by Greenough [14]. Interest in histological grading was later revived by the work of Bloom and Richardson [15]. These investigators utilized a combination of architectural and cytological features to separate breast carcinomas into prognostic categories, and they established principles that were ultimately incorporated into the classification system. This system of histological grading, later modified by Elston and Ellis [11], relies on the assessment of 3 features: tubule formation, nuclear pleomorphism and mitotic rate. Moreover, ER and PR analysis is now an integral part of the assessment of breast cancer. Quantification of ER and PR expression in tumor tissue is important in the clinical management of breast cancer patients. Steroid receptor expression has been established as an independent prognostic factor and is also a predictor of response to hormonal therapy [16]. Estrogens are known to stimulate cell proliferation in both normal and neoplastic breast tissue. This biological effect is exerted when they bind to the ER. Estrogens, through ER, may regulate the synthesis of PR, and in breast carcinoma a positive correlation was found between ER and PR concentration [13]. The role of

progesterone on breast tumor cell proliferation is controversial; it may be stimulatory, or inhibitory [10]. The mechanisms that regulate growth may be reflected in the pattern of growth, and the possible presence of such mechanisms might therefore be assessed by a careful appraisal of morphological features [17]. We observed (Table 1) that invasive lobular carcinomas were more frequently ER/PR positive and medullary carcinomas were less frequently positive than were typical ductal carcinomas. In addition, ductolobular carcinomas were more frequently ER/PR positive than ductal carcinomas (no special type). We also noted that both tubular and invasive papillary carcinomas were strongly ER and PR-positive. In a few previous studies, no correlation could be demonstrated between the presence of ER and specific histological features [18,19]. However, Rosen et al. reported that the mean cellular diameter of ER-rich tumors was less than the mean cellular diameter of ER-poor tumors [18]. The cells of infiltrating lobular carcinoma are smaller than those of infiltrating ductal carcinoma; they are also more frequently ER-positive. Similarly, the cells of medullary carcinoma are usually larger than cells of typical ductal carcinoma, and are frequently ER-negative [17]. Consideration of the tumor alone in prognostication is somewhat artificial because it fails to recognize the importance of possible host factors. For example, by most classification methods, medullary carcinoma would be considered a highly malignant (grade III) lesion, yet its favorable prognosis has been repeatedly emphasized. Nevertheless, close correlation between histological grade and survival has been demonstrated [11,15].

The correlation between the presence of ER/PR and histological grading of breast carcinoma has already been documented, although some studies have found no such correlation [18,19]. In our study we observed an apparent correlation between histological grade and ER/PR expression. Our results showed that both ER and PR positivity declined with increasing tumor grade (Table 2). This is in agreement with findings previously reported [10]. Although histological grade III carcinomas are usually ER and PR negative, we observed that some of them do display significant ER and PR expression. However, the results presented in this paper showed a significant association ($p < 0.05$) between ER and PR immunoreactivity (negative, low-positive and positive) and different histological grades of invasive breast carcinomas (Tables 3 and 5). These findings are in agreement with the results of other relevant studies [20-22].

The method of histological grading of breast carcinomas is partially based on nuclear features. It is, therefore, not surprising that previous studies suggested that nuclear morphology may be a better predictor of the presence of ER and PR than other histological features [10,19]. However, no significant association between nuclear grade of breast carcinoma and ER/PR immunoreactivity was found in our study (Tables 4 and 6). The reason for this discrepancy is not clear. Despite the importance of nuclear morphology in the evaluation of patients with breast cancer, a paucity of reports on this topic exists in the relevant literature. Comparison of histological grading and quantitative assessment of the nuclear area strongly suggests that the presence of ER can be predicted better by measurement of the nuclear area of cells in properly prepared cytological preparations than by nuclear grading in tissue sections [17]. Anyway, larger studies are required to determine whether pure nuclear grade of breast carcinoma can be a good predictor of ER and PR expression in tissue sections.

In conclusion, the results presented herein suggest that histological grade of invasive breast carcinoma was significantly associated with ER and PR immunoreactivity, while nuclear grade alone showed no correlation. In addition, ER and PR positivity declined with increasing tumor grade.

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