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Original Articles

Histological Differentiation in Human Breast Cancer is Related to Steroid Receptors and Stromal Elastosis

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Summary. *In 503 cases of human breast cancer, the stroma reaction of elastosis was investigated with respect to histological differentiation, pathological and biochemical prognostic factors, and steroid receptor (SR) content. Unlike perivascular elastosis, gland-related (ductal + interstistial) elastosis was not related to the age, menopausal status, and number of pregnancies of each patient, and could thus be considered a histological feature characteristic of mammary cells. Elastosis was encountered most frequently in histologically differentiated lesions and in lesions of histoprognostic grades I and II (low degree of malignancy). Elastosis-positive lesions thus seem to constitute a good prognosis. Elastosis was related to the presence of estrogen and progestin receptors (ER and PR), and in menopausal patients it was observed mostly when both ER and PR were present concurrently, i.e., under conditions reflecting the hormone-dependence of neoplastic cells better than the presence of ER alone. Taken together, these results suggest that the presence of hormone-dependent cells in breast carcinomas can be demonstrated by both biochemical and morphological features. Since each of these factors has its own prognostic value, prognosis could probably be by the assessed more accurately if all these parameters were examined at the same time.*

Introduction

Elastosis arises from stromal modifications accompanying the development of human breast cancer [2, 5, 8, 14, 19]. In mammary carcinomas, elastosis has been related to the presence of estrogen receptor [15], the menopausal status of patients [6, 16], prognosis, and response to endocrine therapy [17, 18]. Since the combined presence of progestin (P) and estrogen (E) receptors (R) reflects hormone-dependence better than ER alone and since hormone-dependence is associated with a type of differentiated lesion and a moderate degree of malignancy of neoplastic cells [11–13], in the present paper we have attempted to correlate these biochemical and pathological features with elastosis. To investigate the role of elastosis in human breast cancer, we therefore recorded for a series of lesions their ER and PR content, their histological differentiation (typing), their histoprognostic grade, and finally, the age and menopausal status of the patients.

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Materials and Methods

From September 1978 to November 1979, 503 cases of breast cancer were routinely investigated simultaneously for ER and PR content and pathologic features in relation to elastosis. Subcutaneous lesions were excluded because of the presence of cutaneous elastic fibers.

Pathological Features

For each sample, a minimum of three sections along the lesion's two main axes were stained with hematein-eosin-saffran (HES). Histological type and histoprognostic grade (Scarff and Bloom) were recorded as previously described [12, 13]. Lesional elastosis was assessed according to a previously published technique [4, 10, 14] as follows: First, the presence of hyalin and eosinophilic deposits in the stroma was investigated, since they are thought to reflect the presence of elastin [4, 10]. Such deposits (defining HES-positive cases) were in fact seen in 113 of our 503 lesions. Since this procedure does not, however, discriminate between elastosis and fibrohyalin and/or fibrinoid deposits that are not elastic fibers, elastosis was assessed on additional slides treated with Weigert's stain, which specifically stains elastin and elastic fibers. With this technique only 91 of the 113 lesions containing eosinophilic deposits were found to show true elastosis (defining Weigert-positive cases). The reliability of the technique, which is used routinely, was tested on a series of 50 sections, with or without

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eosinophilic stromal changes, stained with Weigert's stain. Elastosis was graded as follows: Grade 0, no evidence of elastic fibers; grade 1, presence of some thin elastic fibers; grade 2, moderate elastosis, and grade 3, marked evidence of elastosis. Five groups of lesions were considered according to the location of the elastosis: ductal, interstitial, ductal + interstitial, vascular, and vascular + ductal.

Steroid Receptor Analysis

The ER and PR content of lesions was determined, as previously described in detail [11], by a Dextran-coated charcoal adsorption technique in which Moxestrol and Promegestone, respectively, are used as radioligands. Results are expressed in fmoles per milligram of cytosol protein and are considered positive if they are above 10 fmoles. Menopausal status was assessed on the basis of plasma hormone levels and clinical examination (a minimum period of 2 years without menstruation was used as a criterion for attainment of menopause). In this particular series, as well as in our general population of patients now totalling over 2,000 cases [12, 13], menopause occurs on average at the age of 50 years.

Statistical Analysis

The statistical analysis was based on Student's *t*-test and the Chi-square test, with Yate's correction for small populations. To investigate the possibility of bias in the composition of the HES-positive population (113 cases), a paired control population (also 113 cases) was randomly chosen from 450 human breast cancer patients not included in the 503 cases of this series, and was used to further validate the statistical analysis.

Results

Elastosis and Menopausal Status

Changes suggesting elastosis in routinely stained sections led to the selection of an HES-positive population that was found to be representative of the overall population since (1) the distribution of patients, according to either their menopausal status (Table 1) or the histological type of lesions (Table 4), was statistically identical with the distribution found in both the overall and the control populations; and (2) the same distribution of menopausal and non-menopausal patients was observed in both Weigert-negative and Weigert-positive populations (Table 1). With regard to menopausal status, neither grade of elastosis (Table 2) nor ductal and interstitial topography of the elastosis (Table 3) led to any discrimination among patients. However, vascular elastosis was only encountered in menopausal patients (Table 3). Neither was the mean number of pregnancies related to grade of elastosis (Table 2).

These results demonstrate that the presence of gland-related elastosis, i.e., that with a ductal and interstitial topography, is not related to the menstrual status, whereas vascular elastosis does seem to be. However, a definite relationship between vascular elastosis and menopause cannot be ascertained, since both phenomena are also related to the aging process.

Table 1. Relationship between elastosis and menopausal status

	Overall population	Control population	HES-positive population	Weigert's stain	
				Negative	Positive
Premenopausal patients	144 (28.6%)	25 (22.2%)	35 (30.9%)	7 (31.8%)	28 (30.7%)
Menopausal patients	359 (71.8%)	88 (77.8%)	78 (69.1%)	15 (68.2%)	63 (69.3%)
Total	503	113	113	22	91

Results are expressed as numbers of cases and, in parentheses, as percentages of their respective populations

Table 2. Relationship between grade of elastosis, menopausal status, and number of pregnancies

	Elastosis, grade			
	0	1	2	3
Premenopausal patients	7 (31.8%)	10 (43.5%)	9 (23.1%)	9 (31.0%)
Menopausal patients	15 (68.2%)	13 (56.5%)	30 (76.9%)	20 (69.0%)
Pregnancies (mean)	2.4	2.6	2.1	3.1

Results are expressed as numbers of cases and, in parentheses, as percentages of their respective populations

Elastosis and Histopathological Differentiation and Prognosis

The relationship between elastosis and histological type is presented in Table 4. Elastosis was preferentially encountered in histologically differentiated lesions ($P < 0.01$); the grade of elastosis was not related to this feature (data not shown). Furthermore, as shown in Table 5, elastosis was significantly more frequent in histoprognostic grade I and II lesions than in grade III lesions. This relationship was found to be more marked in menopausal ($P < 0.02$) than in premenopausal patients ($P < 0.05$).

Elastosis and Steroid Receptors

The presence of an elastosis stroma reaction was associated with the presence of steroid receptors (Table 6). In premenopausal patients whose lesions

Table 3. Relationship between elastosis topography and menopausal status

Topography	Ductal	Interstitial	Ductal and interstitial	Vascular	Vascular and ductal
Premenopausal patients	7 (30.4%)	11 (34.4%)	10 (30.3%)	–	1 (12.5%)
Menopausal patients	16 (69.6%)	21 (65.6%)	23 (69.7%)	3	7 (87.5%)
Total	23	32	33	3	8

Results are expressed as numbers of cases and, in parentheses, as percentages of their respective populations

Table 4. Relationship between elastosis and histological type of lesions

Histological type	Overall population	Control population	HES-positive population	Weigert's stain	
				Negative	Positive
Well-differentiated	30	5	7	0	7
Polymorphic	258	58	76	11	65
Atypical	156	38	17	6	11
Lobular infiltrate	22	7	8	1	7
Isolated cells	4	1	1	0	1
Ductal	14	2	2	2	0
Medullary	11	1	1	1	0
Colloid	8	1	1	1	0
Total	503	113	113	22	91

Results are expressed as numbers of cases

Table 5. Relationship between elastosis and histoprognostic grade of lesions

Histoprognostic grade	Overall population	Control population	HES-positive population	Weigert's stain	
				Negative	Positive
I	53 (10.8%)	10 (8.9%)	13 (11.5%)	1 (4.5%)	12 (13.2%)
II	228 (45.3%)	60 (53.1%)	60 (53.1%)	6 (27.3%)	54 (59.4%)
III	163 (32.4%)	31 (17.4%)	28 (24.8%)	11 (50.0%)	17 (18.7%)
Lesions of no prognostic value	59 (11.7%)	12 (10.6%)	12 (10.6%)	4 (18.0%)	8 (8.8%)
Total	503	113	113	22	91

Results are expressed as numbers of cases and, in parentheses, as percentages of their respective populations. For definition of lesions of no prognostic value, see reference 13

Table 6. Relationship between elastosis and steroid receptors

Menopausal status	Receptor status	Overall population	Control population	HES-positive population	Weigert's stain	
					Negative	Positive
Premenopausal patients	ER +	86	17	25	2	23
	ER −	38	8	10	5	5
	PR +	37	9	7	3	4
	PR −	87	16	22	20	2
Menopausal patients	ER +	272	67	57	9	48
	ER −	107	21	21	6	15
	PR +	116	32	34	6	28
	PR −	263	56	17	7	10

Results are expressed as numbers of cases with (+) or without (−) ER or PR

showed signs of elastosis, an increased incidence of both ER and PR was observed ($P < 0.05$). In these patients, elastosis-positive lesions showed a higher ER content (177 ± 187 fmoles) than either the overall population (83 ± 63 fmoles) or control (76 ± 70 fmoles) populations ($P < 0.01$). Similarly, in menopausal patients, there was a relationship between the presence of elastosis and either ER ($P < 0.05$) or PR ($P < 0.02$). In these patients, elastosis-positive lesions apparently had higher ER contents (213 ± 177 fmoles) than did elastosis-negative specimens (170 ± 220 fmoles), but this relationship was not statistically significant.

Discussion

This report deals with a further attempt to characterize hormone-dependent cancer cells at the primary tumor site in which, to date, the presence of steroid receptor appears to be the major determinant [7, 13]. Our attention has been focused on the stromal changes leading to elastosis in breast carcinomas. Histological type and differentiation and histoprognostic grade were routinely determined on HES-stained slides in which the presence of stromal changes suggestive of elastosis was first checked. Elastosis was then assessed on specifically stained additional slides from HES-positive lesions. This procedure could have excluded lesions containing very low amounts of elastic fibers, but in these the elastosis stromal reaction is presumably of minor importance.

Our results indicate that interstitial + ductal elastosis is related neither to the menopausal status nor to the age of the patient. Furthermore, although perivascular elastosis was preferentially encountered in menopausal women, it could not be definitely related to the menopause since, at this time, other factors such as vascular aging have to be taken into account. Owing to its topography, interstitial + ductal elastosis reflects a gland-related process and thus, whatever the cell-type secreting elastic fibers, can be considered a histological feature characteristic of at least some of the mammary cells undergoing a specific stroma reaction.

The stroma reaction of elastosis can be related to acinoductal differentiation of carcinomas. This relationship is supported by in vitro findings showing that the onset of extracellular matrix protein synthesis, including elastin synthesis, is associated with cell differentiation processes [9]. Thus elastosis in lesions could be related to the presence of cells that have retained, at least in part, both their physiological differentiation and their tissular dependence.

Histoprognostic grade (HPG) reflects the degree of malignancy of lesions and is a histological factor of prognosis for the survival rate of patients [13]. We report here that elastosis is related to HPG I + II carcinomas, i.e., to lesions exhibiting a moderate degree of malignancy. As a tentative explanation for these findings, it has been suggested that the elastic fibers wall-in the cells and constitute a mechanical barrier to tumor spread [3]. An increased 5-year survival rate has been reported for patients having elastosis-positive lesions [18], supporting the idea of a relationship between elastosis and HPG I + II lesions.

Elastosis is related to the presence of ER and PR in human breast carcinomas, the statistical relationship depending upon menopausal status and receptor hormone class. In premenopausal women, the relationship between elastosis and ER was found to be more significant than in menopausal patients, whereas the reverse was true for PR. These results suggest that before the menopause, the frequency of elastosis is lower in cases where ER and PR are both present. In postmenopausal patients, the frequency of ER is

higher than in premenopausal women [12]. Since the combination of ER + PR probably reflects hormone-dependence better than ER alone, some of the ER may, for some reason, be non-functional and unable to induce PR [7, 13]. The combination of elastosis and ER status has been shown to give a better prediction of clinical response to endocrine therapy than either ER or elastosis alone [17]. These findings strongly suggest that elastosis results from the presence of hormone-dependent neoplastic cells in human breast carcinomas.

In conclusion, the presence of a stromal reaction of elastosis in the human breast is associated with a histologically differentiated state, a moderate degree of malignancy, and the presence of steroid receptors. We have previously demonstrated that these factors are all related [13, 15]. Although the underlying mechanisms regulating elastosis in human breast cancer remain to be established, these results suggest that the presence of hormone-dependent cells in breast carcinomas can be demonstrated by both their biochemical and their pathologic features. Since these factors each have their own prognostic value [8, 15, 18], the evaluation of prognosis can probably be enhanced by the concurrent analysis of all of them.

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