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Receptors for Retinoic Acid and Retinol in Human Mammary Carcinomas*

LAMBERT SKOOG,†‡ SOLVEIG HUMLA,† CLAES KLINTENBERG,§ MARIA PASQUAL|| and ARNE WALLGREN ¶

†*Department of Tumor Pathology, Karolinska sjukhuset, 104 01 Stockholm Sweden,* §*Department of Oncology, Regionsjukhuset, 581 83 Linköping, Sweden,* ||*Department of Biochemistry, INOR, Havanna, Cuba and* ¶ *Department of Oncology, Karolinska sjukhuset, 104 01 Stockholm, Sweden*

Abstract—*The cellular content of receptors for retinol (CRBP) and retinoic acid (CRABP) was measured in 148 human mammary carcinomas. High levels of CRABP were found in lobular carcinomas while those of the papillary subgroup had low levels of the receptor. Intermediate values for CRABP were observed for ductal, colloid and medullar carcinomas. The cellular levels of CRBP were high in ductal carcinomas and low in papillar carcinomas. A positive correlation was observed between the receptor for estradiol and CRABP. However, no significant correlation was found between the tumor cell DNA pattern and the content of CRABP and CRBP respectively. Recurrence of disease could be predicted by the nodal status and the level of estradiol receptor while the DNA pattern and the content of receptors for retinoic acid and retinol failed.*

INTRODUCTION

VITAMIN A and retinoids are of importance in the growth and differentiation of epithelial tissues [1]. Thus vitamin A deficiency results in squamous cell metaplasia in secretory epithelium of the respiratory tract [1]. In tissue culture retinoids can induce differentiation of embryonal cancer cells [2, 3].

Vitamin A-deprived tissues are prone to malignant transformation when exposed to radiation, and viral- and chemical carcinogens [4, 5]. In line with these findings it has been demonstrated that vitamin A can prevent the induction of experimental cancers of skin, lung, bladder and breast [5]. The exact molecular mechanism involved in the action of vitamin A is unknown. However, in the cell there exist two specific receptor proteins for retinoic acid (CRABP) and retinol (CRBP) [6]. It is likely that the effect of vitamin A is mediated by these receptors in an analogous way to what has been described for steroid receptors [6].

CRABP and CRBP have been detected in both normal and neoplastic epithelium of different origin [6]. Moreover, some data suggest that there is a difference in the cellular content of CRABP and CRBP between normal and malignant cells [6]. In human mammary tumors the level of CRABP has been found to be higher in histologically well differentiated than in poorly differentiated carcinomas [7].

The present study was undertaken to analyze the tumor cell content of CRABP and CRBP in a large series of human mammary carcinomas. Correlations between CRABP and CRBP and markers for tumor cell differentiation such as estrogen receptor content and DNA pattern were studied. Moreover, the potential value of CRABP and CRBP in predicting the prognosis in breast cancer was also investigated.

MATERIALS AND METHODS

Patients

The patients in this study were operated on for primary breast cancer in 1978. No preoperative treatment was given and all patients were in clinical stage I or II at diagnosis. The median age was 61 yr, ranging from 26 to 86 yr. The mean follow-up time was 60 months and 40 patients had experienced local or distant recurrence of the disease.

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‡To whom requests for reprints should be addressed.

Tumors

All specimens were collected from fresh surgical resections and stored at -80°C until analyzed for DNA pattern, estrogen and retinoid receptors.

Receptor analysis

Cytosol receptors for retinoic acid and retinol were analyzed by sucrose gradient centrifugation as described by Ong and Chytil [8]. The amount of specific binding was calculated as difference between the amount of the [^{3}H]vitamin A derivative sedimenting in the 2S region in the absence and presence of a 200-fold excess of unlabeled ligand.

The cytosol receptor for estradiol was measured as described by Wrange *et al.* [9]. In short, tumor tissue was thawed at 4°C in 1 ml of buffer (5 mM Tris-HCl, pH 7.4, + 1 mM DTT). After homogenization by 5 bursts each of 10 sec in a Polytron homogenizer, the pellet and the cytosol fraction were isolated by centrifugation at 25,000 *g* for 20 min at 4°C. The receptor-steroid complex was isolated by isoelectric focusing on polyacrylamide gels, pH 3.5-10.0 (LKB-produkter AB), and the complex focused at pH 6.2.

All receptor values were normalized to the amount of DNA in the tissue sample.

DNA analysis

Quantitative DNA measurements were performed as described by Burton [10].

Single-cell DNA measurements were carried out on imprint specimens from thawed tumor material as outlined by Bjelkenkrantz *et al.* [11].

Cases which had diploid or tetraploid DNA values were designated group A. Group B consists of cases with a sizeable number of cells outside the diploid-tetraploid region [12].

Histological classification

The classification proposed by McDivitt *et al.* was used [13].

Materials

[^{3}H]Retinoic acid (sp. act. 30 Ci/mmol) and cold retinoic acid were generously supplied by Hoffman La Roche, Basel, Switzerland. [^{3}H]-Retinol (sp. act. 45 Ci/mmol) and [^{3}H]estradiol (sp. act. 137 Ci/mmol) were purchased from New England Nuclear Corp., Massachussetts, USA. Unlabeled estradiol and retinol were obtained from Sigman, St Louis, MO, U.S.A.

Statistical analysis

Correlations between the variables were analyzed by means of contingency table analyses. For the multifactorial analyses of the prognostic information of the variables the proportional hazards linear model procedure according to Cox [14] using Breslow's modification [15].

RESULTS

The cellular levels of CRABP and CRBP

The receptor for retinoic acid was detected in 95% of the tumors and Fig. 1 shows the distribution of the receptor in the 148 mammary carcinomas. From this figure it can be seen that the cellular content of CRABP varied markedly. Thus 5% of the tumors contained no detectable CRABP while 5.5% had levels above 2.5 fmol/μg DNA. The mean value was 1.0 fmol/μg DNA.

The cellular levels of CRBP also showed large variations between the tumors as shown in Fig. 2. Forty-four percent of the carcinomas lacked measurable receptor and the mean value was 0.2 fmol/μg DNA.

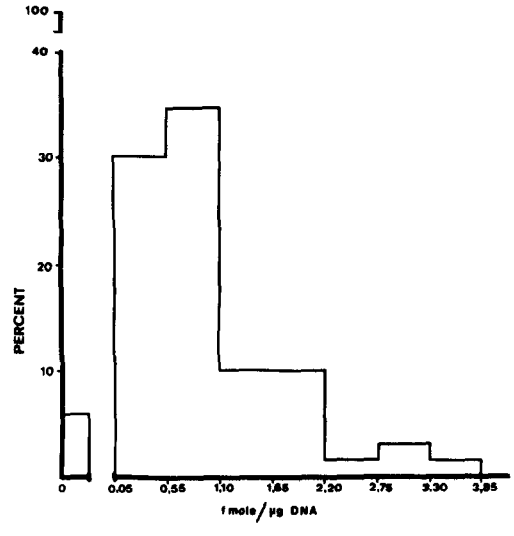

Fig. 1. Frequency distribution of CRABP values.

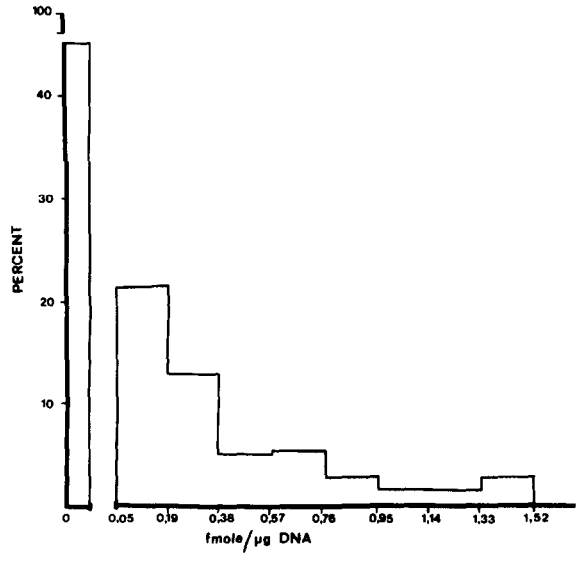

Fig. 2. Frequency distribution of CRBP values.

There was no correlation between the levels of CRABP and CRBP (Table 1).

The CRABP and CRBP content in different histological subtypes of mammary carcinomas

Analysis of the CRABP and CRBP content in different histological subclasses showed that lobular carcinomas had a mean CRABP level of 1.40 fmol/μg DNA (Table 2). This figure is higher than that found for ductal carcinomas (1.0 fmol/μg DNA). On the other hand lobular carcinomas contained less CRBP than ductal carcinomas, 0.13 fmol/μg as compared to 0.25 fmol/μg DNA.

Papillary carcinomas showed low values for CRABP and CRBP compared with both lobular and ductal carcinomas (Table 2). From this table it can also be seen that colloid and medullary carcinomas contained CRABP and CRBP levels which were similar to those observed for ductal carcinomas.

Receptors for estrogen, retinoic acid and retinol

The cellular level of ER has been suggested to reflect the differentiation of mammary carcinomas [16]. Since vitamin A has been shown to be involved in the differentiation of epithelial cells it was considered of interest to analyze if there existed any correlation between the levels of the different receptors. It can be seen from Table 3 that there was a positive correlation between ER and CRABP. This correlation is statistically significant ($P = 0.03$). No such correlation was observed between ER and the CRBP.

The cellular pattern of DNA and content of retinoid receptors

Quantitative DNA analysis has shown that mammary carcinomas consist of cells which are either diploid or aneuploid. Tumors with a diploid-tetraploid DNA content tend to have a better prognosis than those with an aneuploid pattern [17]. When the DNA pattern of the 148 tumors in the present series was analyzed 52% of the tumors were diploid-tetraploid as shown in Table 4. The CRABP content tended to be higher in the tumors with a diploid DNA pattern. Thus of the 50 tumors with a CRABP level above 1 fmol/μg DNA, 64% were diploid-tetraploid.

A comparison between DNA pattern and CRBP content showed no correlation (Table 5).

Results of the Cox proportional hazard model procedure

When introduced as a single predictor into the Cox model, nodal status (no metastasis vs metastatis) significantly contributed to the prediction of recurrence ($P = 0.0008$) as did ER ($P = 0.03$), while CRABP, CRBP and DNA pattern failed ($P = 0.948$, $P = 0.800$ and $P = 0.06$ respectively). When all predictors were simultaneously introduced into the model only nodal

Table 1. Contingency table analysis of correlation between CRBP and CRABP levels

CRABP \ CRBP	0	0.01-0.23	0.24-2.73	Observ. (No.) Line (%)
0-0.51	19	14	13	46
	13.8	10.1	9.4	33.3
0.52-0.97	19	11	17	47
	13.8	8.0	12.3	34.1
>0.97	24	13	8	45
	17.4	9.4	5.8	32.6
Observ. (No.)	62	38	38	138
Column (%)	44.9	27.5	27.5	100

$\chi^2 = 4.3532$; d.f. = 4.

Table 2. Cellular receptor levels for retinoic acid, retinol and estrogen in different subclasses of mammary carcinoma

Type of carcinoma	No.	Receptor content (mean value fmol/μg DNA) CRABP	CRBP	ER
Ductal	119	1.00 (0-9.12)	0.25 (0-2.73)	1.38 (0-11.50)
Lobular	17	1.40 (0.18-3.70)	0.13 (0-0.60)	0.81 (0-3.10)
Pappilar	4	0.50 (0.18-1.05)	0.04 (0-0.18)	2.40 (1.00-3.70)
Kolloid	8	0.89 (0-1.69)	0.13 (0-0.58)	3.00 (0.72-13.70)
Medullar	4	0.91 (0.44-1.90)	0.20 (0-0.50)	0.44 (0.00-1.60)

status ($P = 0.0001$) and ER ($P = 0.01$) were significant predictors. CRABP gave some additional information although not at a statistically significant level ($P = 0.08$).

DISCUSSION

One hundred and forty-eight human mammary carcinomas were analyzed for CRABP and CRBP.

Table 3. Contingency table analyses of correlation between the ER and CRABP levels

ER \ CRABP	0–0.87	>0.97	Observ. (No.) Line (%) Column (%)
	54	18	72
0–0.70	75.0	25.0	100
	58.1	40.0	52.2
	39	27	66
>0.70	59.1	40.9	100
	41.9	60.0	47.8
Observ. (No.)	93	45	138
Line (%)	67.4	32.6	100
Column (%)	100	100	100

$\chi^2 = 3.9660$; d.f. = 1.

The levels of both receptors were found to vary markedly, with 95 and 54.7% of the tumors being positive for CRABP and CRBP, respectively. It has been speculated that CRABP is present in malignant tissue while CRBP is found only in normal tissue [6]. Our results do not support this since a majority of the tumors were shown to have detectable levels of both CRABP and CRBP. Moreover, it is possible that our figures underestimate the receptor level since some of the receptor might be blocked by endogenous ligand. This question can, however, only be settled by the use of immunohistochemistry with antibodies specific to the receptor.

It has previously been described that the cellular levels of CRABP are positively correlated to the degree of differentiation as determined by histopathological classification [7]. The histopathological malignancy grading of mammary carcinomas often correlates poorly with the outcome of the disease. We therefore decided to correlate our CRABP and CRBP levels to more objective parameters of malignancy such as DNA pattern and ER content [12]. It is of interest to note

Table 4. Contingency table analyses of correlation between cellular DNA pattern and CRABP level

DNA \ CRABP	0–0.51	0.52–0.97	>0.97	Observ. (No.) Line (%) Column (%)
	22	23	32	77
Group A	28.6	29.9	41.6	100
	44.9	46.9	64.0	52.0
	27	26	18	71
Group B	38.0	36.6	25.4	100
	55.1	53.1	36.0	48.0
Observ. (No.)	49	49	50	148
Line (%)	33.1	33.1	33.8	100
Column (%)	100	100	100	100

$\chi^2 = 4.8778$; d.f. = 2.

Table 5. Contingency table analyses of correlation between the cellular DNA pattern and CRBP level

DNA \ CRBP	0	0.01–0.23	0.24–2.73	Observ. (No.) Line (%) Column (%)
	37	22	18	77
Group A	48.1	28.6	23.4	100
	55.2	55.0	43.9	52.0
	30	18	23	71
Group B	42.3	25.4	32.4	100
	44.8	45.0	56.1	48.0
Observ. (No.)	67	40	41	148
Line (%)	45.3	27.7	27.0	100
Column (%)	100	100	100	100

$\chi^2 = 1.5003$; d.f. = 2.

that a high CRABP content was found in tumors with a diploid DNA pattern which is suggestive of a relatively low malignancy grade [17]. In addition there was a statistically significant correlation between ER and CRABP. It therefore seems likely that CRABP to a certain extent reflects the degree of tumor cell differentiation. Moreover CRABP level provides limited prognostic information when introduced into the Cox proportional hazard model together with nodal status and ER.

The observation that the CRABP and the CRBP levels varied between the different histological subclasses is to our knowledge novel. It should be pointed out that the number of cases in some subclasses is small and the findings should be interpreted cautiously. Lobular carcinomas had high CRABP and low CRBP levels as compared to ductal carcinomas. It is even more interesting to note that papillary carcinomas had low levels of CRABP. This is unexpected since this group of tumors has a low degree of malignancy which is reflected by a high ER content, a diploid DNA pattern and a good prognosis. We are at present unable to explain this contradictory finding but hope that immunohistochemistry will add further information. Such studies are now being planned in our group.

Retinoids have been shown to have a preventive effect on induction of breast cancer [18-20]. Moreover, it has been shown that ovariectomy in combination with dietary retinoids effectively prevented mammary tumor recurrence in rat [21]. A majority of the human mammary tumors in this study contained the cellular factors, ER, CRBP and CRABP, which appear to be necessary for hormonal manipulation and retinoid action. Thus there seems to exist a biochemical basis for a trial of retinoids for the prevention of human mammary carcinoma recurrence.

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