

Prostate Specific Antigen Production by Breast Tumors After Induction with Oral Contraceptives

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Introduction

The majority of breast tumors contain relatively low levels of prostate specific antigen (PSA) (1,2). PSA appears to be a favorable prognostic marker of the disease (3). *In vivo* and tissue culture experiments have suggested that PSA production in breast tumors is regulated by steroid hormones and their receptors (4–7). The biological role of PSA in the breast is unknown (8), but it is certain that the molecule is produced by normal, hyperplastic, as well as cancerous breast tissue (9). We hypothesized that if PSA regulation is under the influence of steroid hormones and their receptors, then, exogenous administration of steroid hormones would induce PSA production, at least in a subset of tumors, which possess an operational steroid hormone receptor system. If this prediction is correct, it may be possible to assess the functional integrity of the steroid hormone receptor system with a simple external stimulation procedure and possibly use this information to predict the success of therapy with drugs that target this system. In this study, we have examined the levels of PSA in tumor tissue and serum in patients who received progestin-containing oral contraceptives 1 week preceding surgery.

Materials and Methods

PATIENT POPULATION

We studied 20 patients with primary breast cancer. Patients 1, 2, and 3 were control subjects who did not receive any treatment before surgery and they never received oral contraceptives in the past.

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In addition to these three patients, we have randomly selected another seven breast tumor tissues from women who did not receive oral contraceptives and used them as additional controls. No matched serum was available for these latter seven patients (patients 4–10).

Another 10 patients (patients 11–20) were given oral contraceptives on informed consent as follows. Patients were given one pill of Mercilon (combined oral contraceptive containing 20 µg of the synthetic estrogen ethinylestradiol and 150 µg of the synthetic progestin desogestrel) per day for five consecutive days before surgery. On day 5 (the day before surgery) a serum sample was obtained (presurgical serum). Tumor tissue was excised during surgery on day 6 and stored at –70° C until extraction of PSA was performed. Two days after surgery, another serum sample was obtained (postsurgical serum).

BIOCHEMICAL ANALYSIS

Breast tumor cytosolic extracts were prepared as previously described (1,9). The total protein of the tumor extracts was approximately 0.8–1.4 mg/mL. Serum and cytosols were measured for PSA with an ultrasensitive assay as described previously (2). The detection limit of this assay is 1 ng/L.

Results

Table 1 summarizes the clinicopathological features of the patients studied. In Table 2, we present data on tumor extract PSA and presurgical and postsurgical serum PSA. Tumor extract PSA concentration was <80 ng/L in all samples from patients who received oral contraceptives or not. A notable exception was patient 19 whose tumor cytosol PSA concentration reached 1934 ng/L. This was the only patient who received oral contraceptive and had serum PSA of 26 ng/L, which then declined after tumor removal and withdrawal of the contraceptive to 2 ng/L. Patient 19, who responded to oral contra-

TABLE 1
 Clinicopathological Features of the Patients

Patients	Age (Years)	Menopausal Status	Tumor Type	Tumor Stage	Tumor Grade	Lymph Node Status (\pm) ^b	ER (\pm) ^c	PR (\pm) ^c
Control Group								
1	45	Pre	Ductal	I	III	+	—	—
2	65	Post	Ductal	I	I	—	—	+
3	46	Pre	Ductal	II	II	+	—	—
4	51	Pre	ND ^a	ND	ND	ND	+	+
5	47	Pre	ND	ND	ND	ND	—	—
6	66	Post	ND	ND	ND	ND	+	+
7	62	Post	ND	ND	ND	ND	+	+
8	60	Post	ND	ND	ND	ND	—	—
9	69	Post	ND	ND	ND	ND	+	+
10	92	Post	ND	ND	ND	ND	+	+
Oral Contraceptive Group								
11	37	Pre	Ductal	II	III	—	—	—
12	35	Pre	Ductal	I	II	+	—	—
13	34	Pre	Ductal	II	III	—	—	—
14	54	Post	Ductal	II	III	+	+	—
15	67	Post	Ductal	II	I	+	+	—
16	64	Post	Ductal	I	I	—	+	+
17	69	Post	Ductal	II	I	+	—	—
18	59	Post	Ductal	II	II	+	—	—
19	51	Pre	Ductal	II	III	+	—	+
20	76	Post	Ductal	II	III	—	+	—

^aND = not determined.

^bPositivity established if at least one lymph node was found positive.

^cCutoff for positivity 10 fmol/mg of cytosolic protein.

ceptive stimulation with PSA production, was one of only two patients in the oral contraceptive group who were progesterone receptor positive [PR(+)] and the only patient who was ER (–) and PR (+).

Discussion

The prostate specific antigen gene is regulated by steroid hormones through their steroid hormone receptors. The structure of the 5' region of the PSA gene was characterized and three functional androgen response elements (AREs) were identified in the promoter and enhancer region (10,11). It is now well-established that androgens upregulate the PSA gene in the prostate and in prostate cancer cell lines. We have provided strong evidence that PSA gene regulation in the breast is controlled by steroid hormones (4–6). *In vivo*, we have shown that PSA can be upregulated by progestin containing oral contraceptives (7). In breast cancer cell lines, androgens and progestins strongly upregulate the PSA gene while glucocorticoids and mineralocorticoids have much weaker activity (6). Estrogens not only do not upregulate the PSA gene but may block the activity of androgens and progestins (6). Interestingly, among about 20 breast carcinoma cell lines tested, both steroid hormone receptor positive and negative, only two were able to upregulate the PSA gene (T-47D and BT-474). From our tissue culture studies, we have concluded that steroid hormone

receptors are necessary but not sufficient for PSA gene upregulation (6). We speculate that the ability of these two cell lines to produce and secrete PSA upon stimulation indicates an intact steroid hormone receptor pathway beginning from the binding of the hormone to the receptor and ending at the level of translation of PSA mRNA. The inability of many steroid hormone receptor-positive cell lines to regulate PSA may represent either a functional deficiency of the genomic DNA (*e.g.*, mutation of PSA gene sequences in the 5'-regulatory region as described elsewhere [12]) or a functional deficiency of the receptors or the transcriptional/translational machinery. Although these possibilities are now under investigation, we here chose to examine clinically, and without any focus on the actual mechanism, as to how many breast tumors have the ability to upregulate the PSA gene *in vivo* after stimulation by an oral contraceptive containing a progestin. We previously found that progestin-containing oral contraceptives are potent stimulators of PSA protein production in breast cancer cell lines (5,6).

Our data can be summarized as follows: relatively low levels of PSA are found in all breast tumor extracts tested, irrespective of steroid hormone receptor status, tumor stage or grade, lymph node status, patient age, or menopausal status. These data are similar to those of already published larger series (1,2,4). With exception of patient 19, there does not seem to be any major

TABLE 2
PSA Levels in Tumor Extracts and Serum of Breast Cancer Patients

Patients	Tumor Extract PSA (ng/L)	Serum PSA Presurgical (ng/L) ^a	Serum PSA Postsurgical (ng/L) ^b
Control Group			
1	12	4	3
2	15	20	15
3	79	1	2
4	6	—	—
5	4	—	—
6	21	—	—
7	1	—	—
8	12	—	—
9	3	—	—
10	1	—	—
Oral Contraceptive Group			
11	1	<1	<1
12	2	1	<1
13	1	1	<1
14	7	<1	<1
15	2	<1	<1
16	9	5	4
17	7	<1	<1
18	2	<1	<1
19	1934	26	2
20	11	<1	<1

^aSerum taken after 5 days of stimulation with the oral contraceptive.

^bSerum taken 2 to 3 days postsurgery.

difference in tumor PSA levels between the patients who received oral contraceptives or not. The spectacular PSA levels in tumor tissue from patient 19 prompt us to speculate the following: (a) that the oral contraceptive stimulated PSA production in this tumor, (b) that PSA secreted by the tumor diffused into the general circulation and increased serum PSA from ≤ 2 ng/L to 26 ng/L within 5 days, (c) serum PSA declined to ~ 2 ng/L within 2 to 3 days after tumor removal.

These preliminary data, which need confirmation with a larger series, suggest that some tumors are able to produce PSA after *in vivo* stimulation with exogenous hormones. The reasons for the failure to stimulate more tumors may be: (a) PSA gene mutation in the promoter region, (b) steroid hormone receptor absence or presence of nonfunctional receptors, (c) absence of critical elements of either the transcriptional or translational machinery. These possibilities are now under investigation. Our data additionally suggest that the functional integrity of the steroid hormone receptor pathway can be assessed with this procedure, which involves stimulation of the tumor with an oral contraceptive 1 week before surgery and measurement of PSA in the tissue extract after surgical removal. It remains to be seen if this information is useful in assessing the response of these patients to hormone receptor-targeting therapies.

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