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Secretory component in breast cancer

Analysis of the levels in primary and metastatic disease*

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Summary. *Levels of secretory component (SC) were measured in breast tumors from 95 patients with primary or metastatic cancer. Tumor cytosols were prepared by polytron disruption and high speed centrifugation (105,000 g × 30 min) and SC was measured using a sensitive radioimmunoassay which detects primarily free SC. In frozen samples stored for up to 5 months SC resisted degradation and could be measured quantitatively without interference. In primary tumors from patients between ages 28 and 97, SC positive samples ranged from 4 ng/mg protein to 600 ng/mg protein. In metastatic lesions, SC positive samples ranged no higher than 46 ng/mg protein. The studies indicate that SC can be measured quantitatively in both primary and metastatic tumors and that prolonged storage does not interfere with measurements of SC. The wide variation in SC levels in primary tumor samples may be related to a susceptibility to metastasis. Further, the low levels of SC in metastatic lesions could indicate a potential for SC involvement in immune regulation of tumor growth.*

Introduction

Breast cancer is a pervasive disease which results in 30,000 deaths per year and will strike 1 in 14 American women. High level risk factors include reproductive history and genetic propensity [11]. Since the etiology and the course of this disease are influenced by the hormonal balance of the patient, characterization of breast cancer in terms of hormone dependence was an important step in understanding its development and control [10]. The need for improved treatment has led to the characterization of breast cancer cell surface markers [13]. Such markers are being explored in a variety of tumors as avenues for targeted therapy (administration of tumor-specific antitumor agents) and for tumor detection [6, 7].

In normal breast tissue, secretory component (SC) is present in epithelial cells and during lactation it plays a central role in the transport of IgA into colostrum. Both SC and IgA are present in high concentrations in colostrum and milk where they contribute to the immune protection of the neonate [19]. Breast tumor tissue, which is primarily epithelial cell in origin, reportedly contains SC, as measured by immunofluorescence [8] and immunoperoxidase [9] staining techniques. As measured in the blood of breast cancer patients, SC has been shown to be elevated compared to normal controls [20].

Secretory component is located not only in breast tissue, but also on epithelial cells which line mucosal surfaces such as the lung, gut, mouth, and uterus [12]. At all of these surfaces the function of SC is to transport IgA into external secretions. A number of studies carried out in this laboratory have indicated that SC is under hormonal control in the uterus [24, 28]. In ovariectomized rats, secretion of SC is enhanced by estradiol and diminished when progesterone is administered together with estradiol [25]. These studies have led to the conclusion that the increase in SC levels in uterine secretions of the intact rat is due to the rise in blood estrogen that occurs at the proestrous stage of the estrous cycle [23]. During the human menstrual cycle, SC levels in uterine secretions increase between the time of ovulation and menses [26]. Since both estrogen and progesterone are elevated at this time, the increase may be a net effect of the prevailing hormone balance.

The objectives of this study were: (1) to measure the concentrations of SC in tumors from patients with breast cancer by a quantitative radioimmunoassay (RIA); (2) to examine the conditions for stability of SC in these samples; and (3) to relate the levels of SC in tumors to disease stage and patient age.

Materials and methods

Tumor tissues from biopsy or surgery were immediately frozen on dry ice or in liquid nitrogen. At the time of assay, frozen tissues were weighed and placed in 3–7 volumes of ice cold 10 mM Tris, 1.5 mM EDTA, 10% glycerol buffer containing 0.1% (v/v) monothioglycerol (TED buffer). Samples were disrupted using 3 × 5 bursts of a Brinkman PCU-2 polytron set at speed 5, with 55 s cooling periods between bursts. Homogenates were spun at 100,000 *g* for 30 min to obtain high speed supernatants (cytosols) which were kept frozen at −20 °C until they were assayed. Patient age and tumor type were obtained from information provided by cooperating physicians.

Reagents for the RIA included purified colostral SC and rabbit anti-human SC [26], and goat anti-rabbit IgG (Miles Laboratories Elkhart, IN). The anti-human SC antibody recognizes primarily free SC, which is SC not bound to IgA. Iodination of purified human SC was per-

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formed by the IODO-GEN method [15]. The procedure was as previously described [26] except that reacted material was applied to a 3.5 ml Biogel P60 column equilibrated with 0.1 M $NaPO_4$ containing 1 mg/ml bovine plasma albumin (BPA). The column elution was used to separate ^{125}I-SC from free ^{125}I.

A previously characterized RIA [26] was used to measure SC. Briefly, 20 µl of the unknown sample or standard was placed in 1.5 ml microfuge tube and 30 µl of ^{125}I-SC and 20 µl of rabbit anti-human SC (1/1000) was added. After equilibration for 60 min, 30 µl of goat-anti rabbit IgG (1/60) was added to each tube and equilibration continued for an additional 90 min at room temperature. After incubation, assay tubes were centrifuged (10,000 g × 4 min), the pellets were washed once with 80 µl of the appropriate protein free buffer, and the final pellets were counted in a Packard Multi-Prias 2 gamma counter.

In order to assay tumor cytosols prepared in TED buffer, standards were prepared in TED buffer containing 1 mg/ml BPA. Antibodies and ^{125}I-SC were mixed in 50 mM Tris buffer containing 25 mM KCl, 5 mM $MgCl_2$ and 1 mg/ml BPA. When serum SC was measured, standards were in Dulbecco's phosphate-buffered saline (without Ca^{2+} and Mg^{2+}; [5]) containing 1 mg/ml BPA. Washes were in the same buffer as the antibodies except that no BPA was present in the wash.

Protein assays on all tumor cytosols were done according to the method of Lowry et al. [14]. Data were analyzed by the Student's t-test.

Results

Presence and stability of SC in serum and cytosol

The results shown in Table 1 indicate that SC can be measured in the serum of cancer patients and in high speed cytosols obtained from breast tumor tissue. The serum values of SC were 2.6 times greater in the three cancer patients than in four normal controls. From the values in Table 1, we have estimated the possible influence of serum contamination in the measurement of cytosolic SC. Based on an assumption that no more than 5% (by weight) of the tumor mass consists of blood, and allowing for the volume of buffer that the tumor was diluted in before cytosol preparation, the increase in SC contributed by serum would at most be 5% of the final tumor SC measured.

Also shown in Table 1 is evidence for additivity in the measurement of serum and cytosolic SC. Additivity was checked by adding exogenous SC to samples of serum or cytosol and measuring the subsequent ability to recover SC by RIA. When the SC in the original sample was subtracted from the value obtained in the presence of exogenous SC, the difference equaled the standard amount added to each sample. This indicates that SC in cytosol and serum samples was not masked by specific or nonspecific interference. Serial dilution of cytosol resulted in curves that were parallel to the SC standard curve, indicating that no nonspecific augmentation or diminution of the SC measured in these samples was taking place.

To assess the decay of SC in tumor cytosols stored as frozen samples, cytosols from two different patients were prepared and aliquots were stored at −20 °C. At various times after freezing, aliquots were removed, thawed, and assayed for evidence of SC degredation. As shown in Table 2, no change was seen in the concentration of SC as measured in cytosols frozen for up to 5 months. In addition, when known amounts of SC were added to stored cytosols, no evidence for interference was detected. In another set of samples (data not shown) aliquots of cytosol were repeatedly frozen and thawed on the same day. Repeated freeze-thaw (up to 10×) had no effect on measured SC in these samples.

SC in primary and metastatic tumors

Figure 1 shows the results of the assay of 95 tumors as a function of patient age. Samples from metastatic disease and primary tumors are presented separately. The age of the patients ranged from 28 to 97 years and these were divided into three groups. The first group included premenopausal women of less than or equal to 49 years of age. The other two groups, 50 to 69 years and 70 years or older, were postmenopausal women. In all three groups, SC positive primary tumors had values which ranged widely between the concentrations of 4 ng/mg protein and 600 ng/mg protein. About 84% of tumors for which information was available were infiltrating ductal carcinoma.

The other group of tumors shown in Fig. 1 were metastatic lesions from women between the ages of 31 and 85 years. In the majority of cases, lesions were located either in the chest wall adjacent to the mastectomy scar or found in the adjoining lymph nodes. The SC values from metastatic tumors never exceeded 46 ng/mg protein.

A one-way analysis of variance [4] was used to examine the differences in the average levels of the logarithms of the SC concentrations of the four groups. Logarithms of

Table 1. Measurement of secretory component (SC) in serum and cytosol: Test for additivity

Sample	n	ng/20 µl	Added SC (ng)	Recovered SC (ng)
Normal serum	4	16.8± 4.6	11.4±0.2	11.6±1.4
Patient serum	3	44.0±14.2	11.4±0.2	11.8±2.2
Tumor cytosol	4	3.8± 0.4	4.4±0.4	5.5±0.4

Serum and cytosol samples were analyzed for evidence of interference in the measurement of SC by radioimmunoassay (RIA). Known amounts of SC standard (added SC) were added to tubes containing serum, cytosol or buffer. SC was determined by RIA for SC standard (4 tubes/assay), serum or cytosol (3–4 samples, in duplicate), and sample plus standard (for each sample in duplicate). The difference between sample plus standard ans sample gave "recovered SC." The lack of a significant difference between "Added SC" and "recovered SC" indicates that serum and cytosol did not interfere with the RIA

Table 2. Effect of time frozen on secretory component (SC) content in tumor cytosol samples (ng SC/ng protein)

Weeks:	0[a]	1	2	3	4	5	6	7	8	9	12	18	20
$Sample_1$	11.9[b] ± 2.7				17.9 ± 3.7			10.8 ± 3.2			10.5 ± 0.2		18.2 ± 8.6
$Sample_2$	14.4 ± 2.1	17.7 ± 0.8	16.1 ± 0.8		11.9 ± 0.7			14.6 ± 0.7				11.7 ± 1.4	

Cytosol samples were prepared by polytron disruption and high speed centrifugation. Aliquots (20 µl) were frozen at −20 °C. At various times after freezing samples were thawed and assayed for SC by RIA.

[a] Fresh tumor, nonfrozen cytosol [b] Mean ± SE; 3–4 samples/group

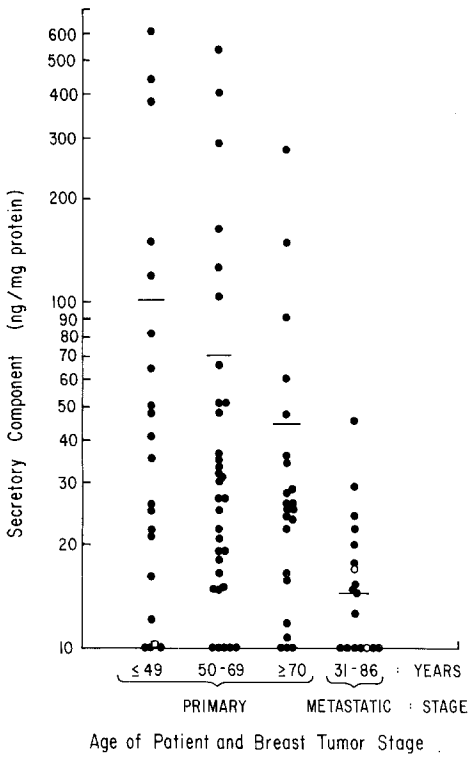

Fig. 1. Secretory Component (SC) in tumor samples from patients with primary or metastatic breast cancer. SC in cytosol prepared from tumor samples was measured by radioimmunoassay (RIA) and normalized to cytosolic protein. Each circle represents the mean of duplicate determinations from an individual patient. The mean of each group is indicated by the horizontal bar. Metastatic tumors contained no more than 46 ng SC/mg cytosolic protein and had a mean SC value which was statistically different from that of the primary tumors. The open square represents a patient under 50 years of age who had had a hysterectomy. The open circles are premenopausal women under 50 with metastatic cancer

the SC values have a distribution that is less skewed and closer to Gaussian than the raw values. The log is a monotonic, one to one transformation and as such, results for the transformed data are applicable to the initial values. There was no significant difference ($P=0.52$) between the average log of the SC concentrations of the primary tumors from the three age groups. However, a significant difference was found between the average log of the SC concentration of the primary tumors from these groups when compared with the average log SC values of the metastatic tumors ($P=0.0003$).

Several exceptions were noted in our tumor population. One was a 36-year-old woman with SC levels of 10.4 fmol/mg protein who had had a complete hysterectomy and therefore did not have regular menstrual cycles and cannot be classed as premenopausal. Other exceptions, found in the 50 to 69 year age group, were four younger women who were still premenopausal or perimenopausal. Included in the group of metastatic lesions is one uterine tissue specimen. The cancer in this tissue was not clearly a metastasis of a primary breast tumor. The patient, however, had been operated on for primary infiltrating ductal carcinoma of the right breast 2 months previously. Uterine SC was 12.5 ng/mg protein in this patient. Unfortunately insufficient breast tumor tissue was available for assay of SC in this individual.

Discussion

In the present study we have evaluated the concentration of human SC in primary and metastatic breast tumors. Through the use of a quantitative sensitive RIA for SC we have shown that SC is a stable protein which resists degradation in cytosol during storage, and demonstrated that the levels of SC vary widely in different tumor samples.

Previous studies demonstrating the presence of SC in mammary tumors have relied on immunocytochemical techniques. Harris et al. [8] analyzed 20 primary tumor samples and found positive anti-SC immunofluorescence to be more intense than that found in normal breast tissue. Hsu et al. [9] reported SC positive cells in medullary carcinoma using immunoperoxidase staining. The present study demonstrates that SC in breast tumors can be measured quantitatively and that SC levels in different tumor samples can be compared.

Studies from this laboratory have shown that the antibody used to measure breast tumor SC detects primarily free SC and not SC bound to IgA [26]. Similar free SC specific antibody was used by Harris et al. [8]. Hsu et al. [9] demonstrated positive staining for SC in samples which also showed a positive reaction for IgA. One function of SC is to transfer IgA into lumenal secretions. Co-secretion of SC and IgA have been demonstrated in uterine secretions [25], gut secretions [1], and tears [22]. Measurement of IgA by RIA in the tumor cytosols needs to be examined to determine whether both IgA and SC are elevated in tumor samples as well.

As a part of this study, we demonstrated that SC was stable in cytosols stored for up to 5 months. In addition, repeated freezing and thawing did not interfere with the

measurement of SC in stored cytosols. Since SC is a small glycoprotein molecule [2], our finding that no degradation occurred under a variety of conditions is consistent with the known stability of glycoproteins in general [27].

The levels of SC in primary tumor samples measured in this study varied widely (Fig. 1). One explanation suggested by the work of Hsu et al. [9] is that variations in SC levels are due to differences in tumor type: medullary vs infiltrating ductal carcinoma. Based on our findings this explanation appears unlikely since the majority of our samples (84%) were infiltrating ductal carcinoma and all showed variation.

Another explanation for the variation in SC levels in breast tumors is that tumor SC may be related to the endocrine balance of the patient and to the steroid-hormone receptor concentration in the tumors. Studies from our laboratory have shown that estrogen elevates SC and IgA in uterine luminal secretions of rats and that progesterone decreases luminal SC and IgA [25]. More recently, we have found that SC levels in uterine secretions also change during the menstrual cycle of women; SC was highest during the secretory phase (postovulatory), lower during the proliferative phase (preovulatory), and lowest during menstruation [26]. Our data in this study, indicate that wide variations in SC in primary tumors were present in both premenopausal and postmenopausal populations. While this finding suggests that tumor SC is independent of menopausal status, it does not exclude the possibility that hormones influence SC concentrations. Wide variations in the levels of receptors for both estradiol and progesterone occur in all age groups [16]. Estrogen and progesterone receptors are presently used as indicators of the responsiveness of cancers to treatment by additive or ablative hormone therapy [18]. Receptor levels are indicative of the long-term prognosis of patients since invasive metastasis is more common in individuals with low receptor levels than in those with high receptor levels [17]. We have recently reported that in evaluations of 76 patients, the levels of sex hormone receptors may be correlated with tumor SC such that tumors which are estrogen or progesterone receptor positive have higher mean SC values than do tumors which are receptor negative [21]. Whether the levels of SC found in primary and metastatic tumors correlate directly with receptor levels and hormone response to endocrine therapy is currently under examination in our laboratory.

An intriguing finding of the present study was that mean SC levels were lower in metastatic tissues than in any group of primary tumors examined. Low levels of tumor SC may reflect either reduced production of SC, greater binding of SC to IgA, or an increased secretion of SC synthesized by the tumors. The latter possibility would result in higher local concentrations of SC surrounding tumors with low cytosolic SC (e.g., those with a potential for metastasis), and is particularly interesting in view of a recently proposed immunosuppressive role for SC. Crago et al. [3] have shown that the SC rich fractions of human colostrum are capable of inhibiting incorporation of ^{3}H-thymidine into pokeweed mitogen stimulated peripheral blood lymphocytes. Whether tumor SC plays an immunoregulatory role which in turn influences tumor growth and the potential for metastasis remains to be established.

In summary, these studies show that SC can be measured quantitatively in both primary and metastatic breast cancer and that under conditions of prolonged storage as well as repeated freezing and thawing the stability of SC is not altered. Furthermore, whereas primary tumors displayed a wide range of SC concentrations, metastatic lesions had SC values which were lower as a group. The results suggest that SC may have importance in providing a connection between endocrine control of tumor growth and immune regulation leading to metastatic disease.

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