

Report

Prostate specific antigen in breast cancer, benign breast disease and normal breast tissue

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Abstract

Prostate specific antigen (PSA) is a tumor marker used widely for the diagnosis and monitoring of prostatic adenocarcinoma. Recently, we provided evidence that PSA may also be produced by breast tumors. In this report we examined quantitatively the PSA levels in 199 breast tumors, 48 tissues with benign breast disease (BBD, 34 fibroadenomas), and 36 normal breast tissues. Significant amounts of PSA (≥ 0.030 ng of PSA per mg of total protein) were found in 28% of breast tumors, 65% of BBD tissues, and 33% of normal breast tissues. PSA positivity in breast tumors was highest in stage I disease (34%) and decreased with disease stage (24% in stage II and 18% in stage III–IV). Using polymerase chain reaction amplification we have shown PSA mRNA presence in patients with PSA protein-positive tissues (benign and malignant) but not in patients with PSA protein-negative tissues. Our data suggest that PSA is expressed frequently by normal breast tissue, by tissue of benign breast diseases, and by breast cancer tissue. Highest expression is seen in benign breast disease and lowest expression in advanced stage cancerous tissue. As PSA production is mediated by steroid hormones and their receptors, we propose that PSA may be a new marker of steroid hormone action in the normal or diseased female breast. The role of this enzyme in the development of breast diseases including breast cancer is currently unknown.

Introduction

Prostate specific antigen (PSA) was recently found in breast cancer extracts and its presence was closely associated with presence of steroid hormone receptors [1]. Patients with PSA-positive cancer tended to have earlier stage disease [2]. In comparison to

PSA-negative breast cancer, patients with PSA-positive cancer have longer relapse-free survival regardless of other clinical and pathological features, suggesting that PSA is a new favourable prognostic indicator in breast cancer [3].

PSA is a 33 kDa glycoprotein with serine protease activity [4, 5]. The production of PSA in the

prostate is up-regulated by androgen, through the androgen receptor [6]. PSA was initially thought to be exclusively produced by prostatic epithelial cells. However, there is now compelling evidence indicating that PSA is not prostate specific [2, 7–14]. The major difference in PSA production between the prostate and other tissues is that the amount produced by other tissues is comparatively much less. In terms of tissue regulation, some of the PSA-producing non-prostatic tissues, such as breast, endometrium, and ovary, are similar to the prostate since their growth and differentiation are controlled by steroid hormones.

The production of PSA by the female breast has been confirmed by several lines of evidence. Steroid hormone receptor-positive breast cancer cell lines like T-47D and MCF-7, but not steroid hormone receptor-negative cell lines like BT-20, have the ability to produce PSA *in vitro* after stimulation by steroid hormones [15]. Fluids secreted by breast epithelial cells such as milk and breast cyst fluid contain PSA [16, 17]. Normal breast contains PSA, especially in women receiving oral contraceptives [18]. PSA mRNA has been identified in cancerous and normal breast tissue [18, 19]. In this study we have examined and compared the PSA content of 199 breast cancers, 48 breast tissues from patients with benign breast diseases, and 36 normal breast tissues. Our data suggests that PSA is a biomarker secreted by breast tissue in normal states as well as in benign and malignant diseases.

Materials and methods

Breast tissue specimens

Tissue specimens from 48 women with histologically confirmed benign breast disease (BBD) were collected during surgery at the Department of Gynecologic Oncology, University of Turin, between January 1992 and September 1994. The specimens were snap-frozen in liquid nitrogen immediately after surgical resection, and were stored at -80°C until use. The ages of women with BBD were between 12 and 78 years with a median of 38 years. The histological diagnosis included 34 fibroadenomas

(71%), 3 papillomas (6%), 1 cyst (2%), 2 sclerosing adenosis (4%), 7 fibroadenosis (15%), and 1 phyllodes tumor (2%).

Tumor specimens from 199 patients with primary breast cancer were collected at the Department of Gynecologic Oncology, University of Turin, between January 1992 and May 1993. These specimens were snap-frozen in liquid nitrogen immediately after surgical removal and were stored at -80°C until use. The ages of these patients were between 29 and 93 years with a median of 57 years. Breast cancer was confirmed by histopathological examination in all cases.

Breast tissue specimens from 36 women undergoing cosmetic breast reduction surgery at the Toronto Hospital or at the Toronto East York General Hospital between September 1993 and November 1994 were also collected and were assumed to be normal breast tissues. The specimens were frozen on dry ice immediately after surgical resection and were later stored at -70°C until use. Of the 36 women, 30 had breast specimens collected from both breasts. The ages of these women were between 15 and 61 years with a median of 34 years.

Cytosol preparation

Cytosol extracts from these tissue specimens were prepared as follows. Approximately 0.2 g of tissue from each specimen was pulverized manually with a hammer to a fine powder at -80°C . The cells were lysed for 30 minutes on ice with 1 mL of lysis buffer, containing 50 mmol/L Tris, pH 8.0, 150 mmol/L NaCl, 5 mmol/L EDTA, 10 g/L Nonidet NP-40 surfactant, and 1 mmol/L phenylmethanesulfonyl fluoride. The lysates were centrifuged at 15,000 g at 4°C for 30 minutes, and the supernatants were assayed for PSA and total protein.

Measurement of PSA and total protein

A time-resolved immunofluorometric PSA assay was used to measure PSA concentration in the cytosolic extracts. Each extract was measured in duplicate. The PSA assay, described in detail elsewhere

[20], has a detection limit of 0.01 ng/ml. Total protein in each sample was measured in duplicate using a commercial method (Pierce Chemical Co. Rockford, IL 61105). PSA concentration in all cytosols is expressed as ng of PSA per mg of total protein.

RNA extraction, cDNA synthesis, PCR procedure, and Southern blot analysis

RNA from selected tissues was extracted, reverse-transcribed, and amplified with PSA gene-specific primers. The PCR product was run on agarose gels, stained with ethidium bromide and subsequently transferred to nylon membranes and hybridized to a radioactive PSA cDNA probe. These procedures have been described in detail elsewhere [19]. Actin mRNA was amplified as a positive control.

Statistical analysis

PSA concentrations in the breast cytosols were compared between groups of women using the Wilcoxon rank sum test. Women were also categorised

Table 1. PSA concentration in the cytosol of breast cancer, benign breast disease, and normal breast tissues

	Cancer (n = 199)	Benign breast disease (BBD) (n = 48)	Normal (n = 36)
Quartiles	PSA value (ng/mg)		
Lowest	0	0	0
25%	0.012	0.013	0.011
Median	0.020	0.067	0.016
75%	0.034	0.24	0.042
Highest	8.85	2.43	0.84
Wilcoxon rank sum test for: Cancer and BBD, $p < 0.001$; Cancer and Normal, $p = 0.529$; and BBD and Normal, $p = 0.008$.			
PSA status	Number (%)		
< 0.03 ng/mg	143 (71.9)	17 (35.4)	24 (66.7)
≥ 0.03 ng/mg	56 (28.1)	31 (64.6)	12 (33.3)
Chi-square test for: Cancer and BBD, $p < 0.001$; Cancer and Normal, $p = 0.527$; and BBD and Normal, $p = 0.005$.			

dichotomously based on the PSA concentration in the specimens (PSA-positive and PSA-negative) using a cutoff value of 0.03 ng/ml [1]. The number of women with PSA-positive breast specimens were compared between the three patient groups using the contingency table method and the chi-square test (or Fisher's exact test when this was necessary).

Results

PSA in breast cancer

PSA concentrations in the 199 breast cancer patients ranged from 0 to 8.8 ng/mg with a median of 0.020 ng/mg (Table 1). The PSA positivity rate was 28% in the group of all cancer patients (Table 1), 33% in patients under the age of 50, and 26% in patients at age 50 or older (Table 2). PSA-positive tumors were found in 34% of stage I, 24% of stage II, and 18% of stage III or IV disease.

PSA in benign breast disease (BBD)

PSA levels in the cytosol of BBD patients were between 0 and 2.43 ng/mg with a median value of 0.067 ng/mg (Table 1). PSA concentration in BBD was significantly higher than in breast cancer ($p < 0.001$) or in normal breast tissue ($p = 0.008$). Of the 48 BBD patients, 65% had PSA-positive lesions, a positivity rate that was significantly higher than the PSA positivity rate in breast cancer ($p < 0.001$) or in

Table 2. PSA status in the cytosols of breast cancer, benign breast disease, and normal breast tissue by age groups

PSA status	Cancer	Benign breast disease (BBD)	Normal
Age under 50 yr			
Number (%)			
< 0.03 ng/mg	38 (66.7)	14 (38.9)	18 (66.7)
≥ 0.03 ng/mg	19 (33.3)	22 (61.1)	9 (33.3)
Age 50 yr or over			
Number (%)			
< 0.03 ng/mg	105 (73.9)	3 (25.0)	1 (33.3)
≥ 0.03 ng/mg	37 (26.1)	9 (75.0)	2 (66.7)

normal breast tissue ($p = 0.005$). The PSA positivity rate in BBD seemed to have been increased in older patients but the number of patients at age 50 or older was relatively small (Table 2). PSA was found in 65% of 34 patients with fibroadenomas and in 64% of the remaining 14 patients with all other histological types of BBD combined (data not shown).

PSA in normal breast

PSA was also found in normal breast tissue extracts. PSA concentrations in the 38 normal women were between 0 and 0.84 ng/mg with a median of 0.016 ng/mg (Table 1). The levels of PSA in normal breast tissues were significantly lower than in tissues from BBD, but they were not different from those of the cancer tissues ($p = 0.529$). The proportion of PSA-positive samples in normal women was also significantly lower than in women with BBD (33% vs 65%, Table 1), but was not significantly different from that in breast cancer patients although the PSA positivity rate was slightly higher in the normal tissues than in the cancer (33% vs 28%, Table 1). As only 3 women (Table 2) underwent cosmetic breast reduction surgery at age 50 or older, the difference in PSA positivity rates according to patient age could not be examined reliably in this

Table 3. PSA concentration in the cytosol of normal breast tissues

	Right breast (n = 30)	Left breast (n = 30)
Quartiles	PSA value (ng/mg)	
Lowest	0.000	0.002
25%	0.007	0.005
Median	0.012	0.012
75%	0.034	0.016
Highest	0.53	0.84
Wilcoxon rank sum test for right and left breast, $p = 0.446$		
PSA status	Number (%)	
< 0.03 ng/mg	21 (70.0)	27 (90.0)
≥ 0.03 ng/mg	9 (30.0)	3 (10.0)
Chi-square test for right and left breast, $p = 0.053$		

group of normal women. However, breast cancer patients with advanced disease (stage III–IV) were less frequently PSA-positive (18%) than women with normal breasts (33%).

Of the 38 patients undergoing breast reduction surgery, 30 had breast specimens collected from both sides of the breast. The comparison of PSA concentrations between the two sides is presented in Table 3. With regard to the PSA concentration, no significant difference was observed ($p = 0.446$) between the two breast sides. However, when we examined the PSA status (i.e. PSA-positive versus PSA-negative), we found 2 women with PSA-positive specimens from both sides of their breasts, 7 women with PSA-positive specimen only from the right side, and 1 with PSA-positive specimen only from the left side (data not shown). This data suggest that the PSA-positivity rate was slightly higher in the right breast than in the left breast (30% versus 10%, $p = 0.053$).

Molecular characterization of PSA in benign breast disease

We have previously demonstrated that PSA mRNA could be detected by polymerase chain reaction amplification in PSA protein-positive breast tumors [19] and in PSA protein-positive normal breast tissue [18]. The sequence of this mRNA was identical to the sequence of mRNA isolated from prostatic tissue. In this work, we have amplified RNA isolated from four BBD tissues and one PSA-positive breast tumor (positive control) and hybridized the PCR product with a radiolabeled PSA cDNA probe. Two BBD tissues had PSA protein concentration < 0.010 ng/mg (samples A, B, PSA-negative) and two had PSA protein concentration of 1.66 (sample C) and 1.51 (sample D) ng/mg, respectively (PSA-positive). After reverse transcription, PCR amplification, and agarose gel electrophoresis of the PCR products, we detected the expected 569 base pair fragment in sample C only (Figure 1A). However, after hybridization with a radiolabeled probe, sample D was also positive (Figure 1C, D). In Figure 1C and D we demonstrate that along with the expected 569 bp hybridization band,

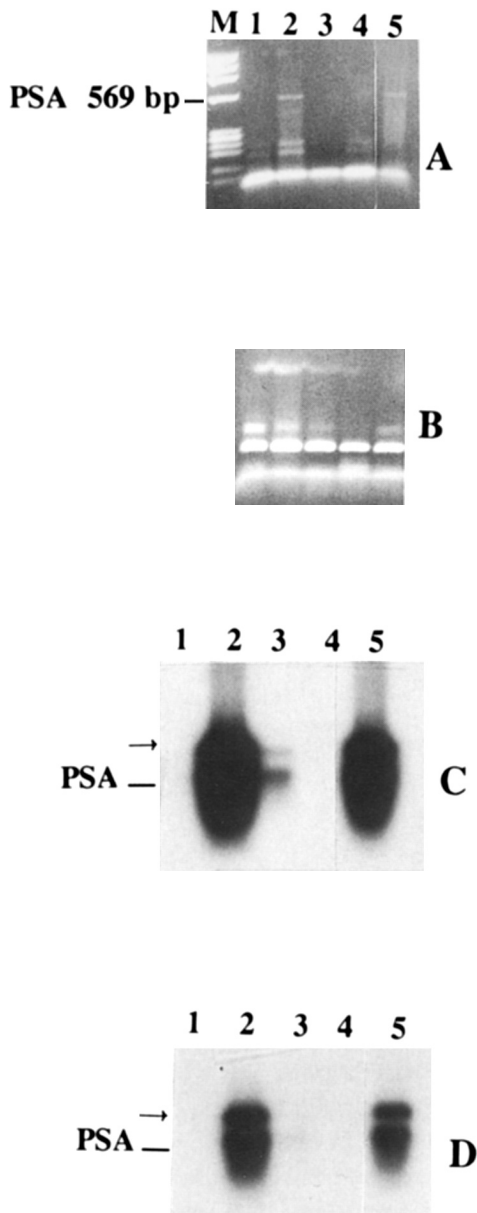


Figure 1. PSA expression in fibrocystic disease. (A) M, molecular weight markers. Lanes 1 and 4 represent PSA protein-negative fibroadenomas and lanes 2 and 3 PSA protein-positive fibroadenomas. Lane 5 represents a PSA protein-positive breast tumor. The ethidium bromide-stained agarose gel of the RT-PCR products shows visible 569 bp bands in lanes 2 and 5. (B) Actin RT-PCR used as an internal control. (C) Southern blot and hybridization of gel shown in (A). Positive bands appear in lanes 2, 3 and 5. Exposure time was 5 h. (D) As in C but with exposure time of 30 min. For more discussion see text. The arrow indicates a PCR product with a different molecular weight.

another hybridization band is also revealed and is present in the specimens from benign breast disease and breast cancer. We speculate that this band represents PSA mRNA generated through alternative splicing as suggested previously by others [21].

Discussion

PSA immunoreactivity in breast cancer cytosols was first detected with highly sensitive immunological techniques [1, 20]. High performance liquid chromatography and Western blot analysis demonstrated that the immunoreactive species has a molecular weight of 33 KDa [2], identical to the molecular weight of seminal plasma PSA and serum free PSA [5]. Positive identification of the immunoreactive species was achieved by molecular analysis. Using reverse transcription-polymerase chain reaction (RT-PCR) amplification we have demonstrated that PSA mRNA was present in PSA protein-positive breast tumors and not in PSA protein-negative tumors [19]. Sequencing of the PCR product confirmed the identity between PSA mRNA from breast and prostatic tissue. The capability of the female breast to produce PSA was further demonstrated by finding relatively large amounts of PSA in milk of lactating women [16] and breast cyst fluid [17]. Furthermore, using breast cancer cell lines in culture, we have shown that these can produce PSA when stimulated by androgens, progestins, and glucocorticoids, but not estrogens [15]. Breast cancer cell lines and tumors produce PSA only when they are steroid hormone receptor-positive, underlining that the regulation of PSA gene expression in breast cancer is under the control of steroid hormones and their receptors. In the prostate, PSA production is induced by androgen through androgen receptors [6].

PSA is a serine protease with trypsin-like and chymotrypsin-like enzymatic activity [4]. PSA in seminal plasma is involved in semen liquefaction by cleaving a seminal vesicle protein [22]. The physiological role of PSA in the prostate and other steroid hormone-regulated tissues is poorly understood at present. However, there is recent evidence that PSA may play an important role in growth regula-

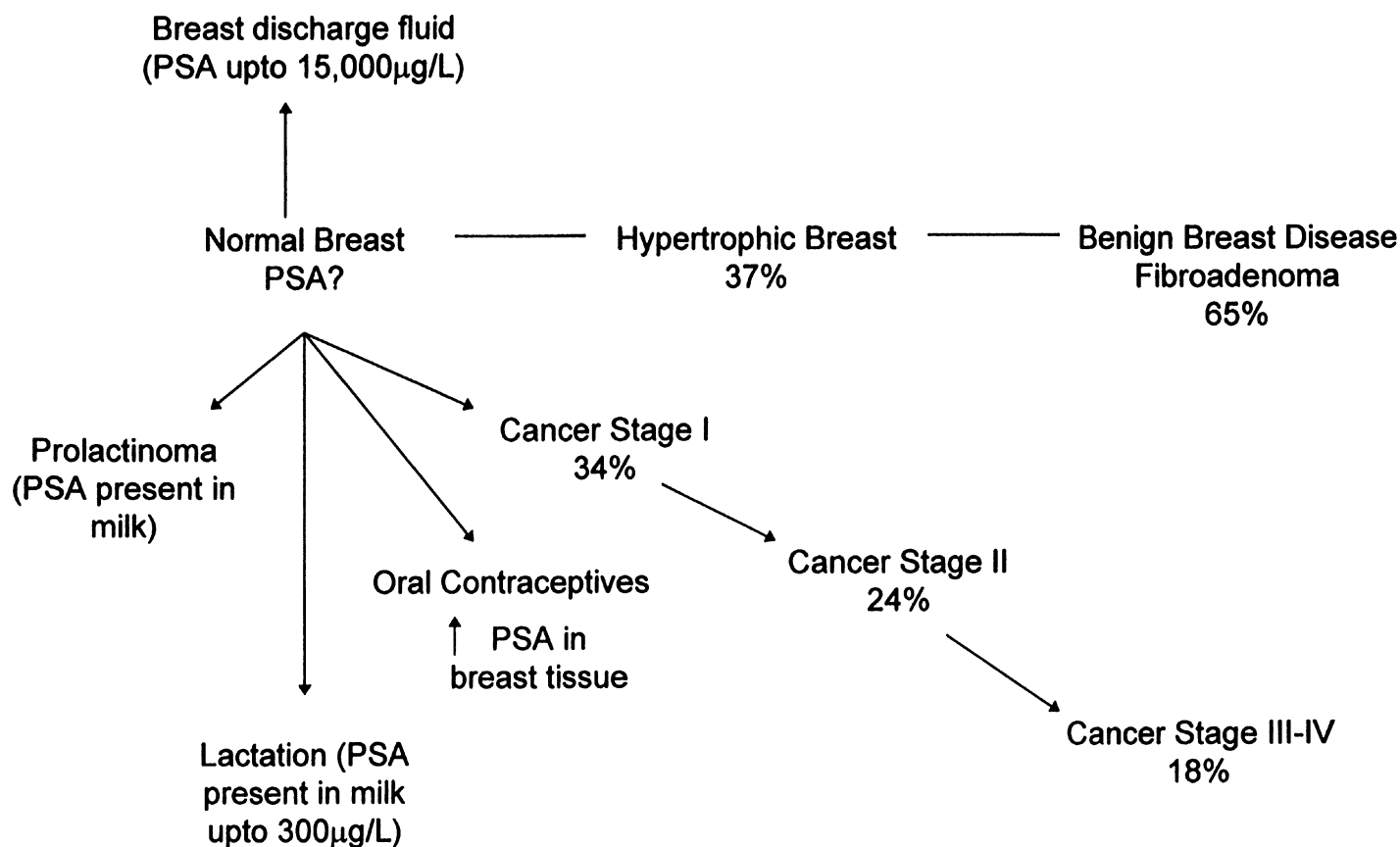


Figure 2. PSA expression and regulation in the breast. Percentages indicates PSA positivity rate based on a cutoff level of 0.03 ng of PSA per mg of total protein. ↑ indicates PSA upregulation. Data on PSA in prolactinoma is based on 1 patient with galactorrhea who had elevated PSA (PSA ~ 20 µg/L) in breast fluid from both breasts (unpublished data). Data of PSA in milk during lactation and PSA upregulation by oral contraceptives have been published elsewhere [16, 17].

tion. PSA was found in amniotic fluid [23] and milk of lactating women [16]. A possible relationship between PSA and human transforming growth factor β (TGF- β) has been suggested by Killian et al. [24].

Associations between PSA concentration and levels of insulin-like growth factor binding protein (IGFBP) 2 and 3, which are regulators of insulin-like growth factor I and II (IGF-I, IGF-II) were observed in the serum of prostate cancer patients [25]. Another study has demonstrated that PSA can proteolytically degrade IGFBP-3 in seminal plasma [26]. A recent report describes steroid hormone-independent activation of androgen receptors in prostate cancer cells by IGF-I and other growth factors [27]. These new data converge to the proposal that there is a relationship and possibly a regulatory loop between PSA and growth factors, growth factor binding proteins, and some cytokines. One possibility is that PSA releases IGF-I by digesting IGFBP-3 and that IGF-I binds to the androgen re-

ceptor to induce PSA production as well as to exert other physiological actions.

In this study we found that PSA was present in normal breast tissue as well as in non-malignant and malignant breast tissue. This phenomenon is also seen in the prostate, where PSA is produced by normal, hyperplastic, and cancerous prostatic tissue. Most benign breast disease tissues were fibroadenomas (71%). Evidence of increased risk of breast cancer in women with fibroadenoma is controversial [28–32]. Some epidemiological studies found a slightly increased risk for breast cancer among women with fibroadenoma while others failed to identify such risk.

In this work we found substantial differences in PSA positivity rates between BBD and cancer but we found no difference between normal breast and breast cancer. These apparently conflicting data can be explained by considering recent findings and new hypotheses of breast cancer initiation and pro-

gression in humans [33]. We have reported that PSA production in the breast is regulated by steroid hormone receptors, mainly androgen and progesterone receptors [1, 15]. It is now known that among normal breast epithelial cells only 4–26% contain steroid hormone receptors, while the rest are steroid hormone receptor-negative [33]. However, more than 60% of the breast tumors are steroid hormone receptor-positive. We would thus propose that the equal positivity rates between normal tissue and cancerous breast tissue implies that many steroid hormone receptors in the breast cancer tissue are not biologically functional since they cannot mediate PSA production. If the defect is in the receptor molecules themselves or in post-receptor abnormalities, it is currently unknown. This notion is also supported by clinical data showing that approximately 30–40% of steroid hormone receptor-positive tumors do not respond to antihormonal treatment. Our data further suggest that in fibroadenomas, as many as 70% of the tissues are receptor positive and that the receptors are functional, mediating PSA production. A hypothetical scheme describing PSA expression in various disease and normal states in the breast is presented in Figure 2.

In a previous report we documented that PSA production in the normal breast is upregulated by oral contraceptives [18]. Unfortunately, information on oral contraceptive use in the 38 women described in this report was not available and the association between PSA expression and oral contraceptives could not be examined.

All normal breast tissues described here were obtained during breast reduction surgery. These patients had enlarged breasts which necessitated reduction, indicating that their breasts were hypertrophic. Large breasts are also associated with unbalanced steroid hormone regulation. Therefore, PSA production by truly normal breast tissue is still undetermined and it will not likely become readily known because of unaccessibility of such tissue.

In summary, we have shown that PSA is present in the cytosols of normal breast tissue, tissue from benign breast diseases, and cancerous breast tissue. PSA positivity rate was highest (65%) in non-malignant breast diseases and similar (30%) in normal breast and cancerous tissue. These findings

suggest that PSA production in these tissues may be regulated by mechanisms which involve derangement of balance between the various steroid hormones and their receptors and also expression of non-functional receptors or deranged post-receptor pathways. Based on the information presented, PSA can now be regarded as a molecule secreted by breast tissue in normal, benign, and malignant diseases. PSA has already been shown to be a favourable prognostic indicator in breast cancer [3]. The physiological role of PSA in normal breast, and its role in the promotion or prevention of benign breast disease and breast cancer, remain to be determined.

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