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Expression of genes coding for pS2, c-erbB2, estrogen receptor and the H23 breast tumor-associated antigen

A comparative analysis in breast cancer

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Expression of the gene coding for a new breast tumor-associated antigen, H23, was compared to expression of genes coding for pS2, c-erbB2 and estrogen receptor (ER). Comparison involved mRNA expression in normal and malignant breast tissues as well as in non-breast tumors. Results obtained by RNA dot blot and Northern hybridizations showed that expression of the H23 antigen coding gene is a discriminatory marker in human breast cancer. It is expressed in 92% of breast tumors whereas 69%, 62% and 56% of breast tumors demonstrate significant mRNA levels of c-erbB2, ER and pS2, respectively. Non-malignant or normal breast tissue expresses much lower levels of the H23 antigen mRNA. From the comparative analysis presented here it is concluded that the gene coding for H23 antigen furnishes a most useful marker for human breast cancer.

Gene expression; Breast tumor antigen H23; Breast tumor antigen pS2; Oncogene erbB2; Estrogen receptor; Breast cancer

1. INTRODUCTION

The identification of tumor-specific markers is of great importance in cancer diagnosis. Several breast tumor-associated markers have recently been described [1–6] including mucin-like antigens [2,3], pS2 antigen [5], erbB2 oncogene [4] and estrogen receptor, ER [6].

A gene designated I7.5 [7] coding for the H23 breast tumor-associated antigen [12], was isolated and characterized in Keydar's laboratory [7] and full-length cDNAs coding for various forms of the antigen were recently described by Wreschner et al. [8,9]. An internal fragment of the gene, designated '3b' [10], codes for multiple 20 amino acid repeats, similar to cDNA fragments described by two other groups [3,11]. These multiple repeats appear to be highly immunogenic. One mAB contained (H23) recognizes an epitope contained within the repeat sequence and detects antigen in 91% of breast carcinomas [12].

Although all the markers mentioned above (pS2, erbB2, ER and the H23 antigen) seem to be breast tumor-associated, there are some discrepancies in the literature regarding their specificity for breast cancer.

To evaluate which of these different markers may be most significant, their expression levels were analyzed in both malignant and benign breast tumor tissues as well as in non-breast tumors.

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2. MATERIALS AND METHODS

2.1. *Tissues*

Tumor and 'normal' tissue specimens were obtained from Sheba and Sourasky Medical Centers, Tel Aviv. The malignant breast tumors (BT) were all adenocarcinomas. The corresponding 'N' samples (BN) represent 'normal' tissue adjacent to the tumor. BF designates breast fibroadenomas. Tissue samples (both benign and normal) were also obtained from other organs including thyroid, stomach, colon, bladder and ovary.

2.2. *RNA extraction*

RNA was extracted from frozen tissues by the guanidium thiocyanate/CsCl method [13]. PolyA$^+$ RNA was purified by oligo dT chromatography [14].

2.3. *Blot hybridization analysis*

RNA dot and Northern blots were prepared according to Maniatis [15] using Gelman nylon membranes. Prehybridization and hybridization were performed as described [15]. After washing, blots were exposed to Kodak X-ray film for 1–7 days. Film densitometry was performed with LKB 2222-020 Ultrascan XL 11 laser densitometer. The amounts of mRNA hybridized with each probe were normalized to 18S rRNA present in the same samples as detected by hybridization with ^{32}P-labeled 18S rRNA cDNA.

2.4. *Plasmids*

The plasmid containing the 3b cDNA fragment was isolated by M. Hareuveni [10]. Plasmids containing inserts of ER and pS2 coding sequences were kindly provided by P. Chambon [16,17]. The plasmid recombinant for the erbB2 gene was constructed by T. Yamamoto [18] and kindly provided by Y. Shilo.

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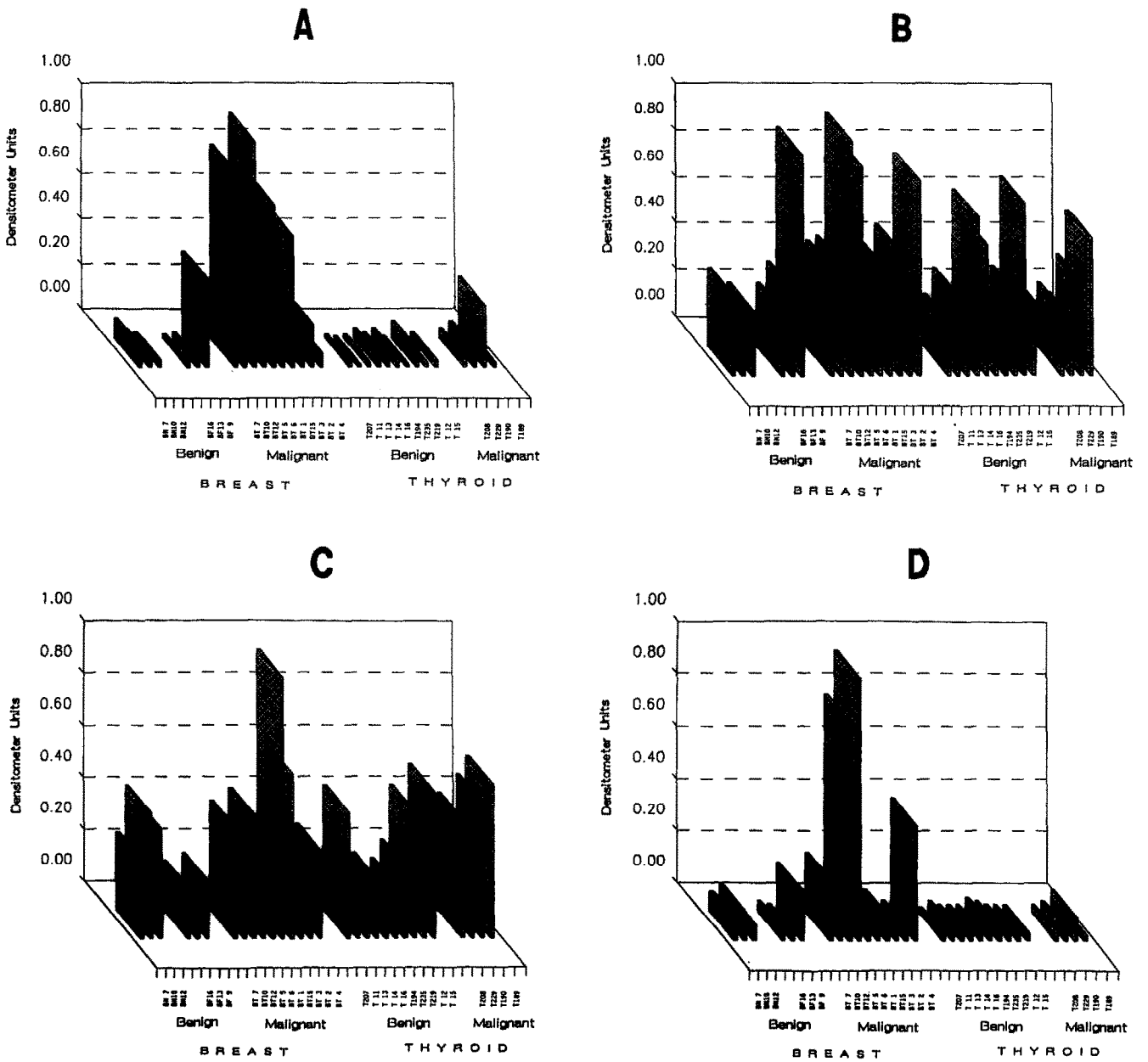

Fig. 1. Analysis (RNA dot blot) of the H23 antigen, pS2, c-erbB2 and ER mRNA expression in breast and thyroid malignant and non-malignant tumors. The dot blot was hybridized with: (A) ^{32}P-labeled 3b cDNA coding for part of the H23 antigen; (B) ^{32}P-labeled pS2 cDNA; (C) ^{32}P-labeled c-erbB2 cDNA; (D) ^{32}P-labeled ER cDNA. The key for samples is as follows: Breast samples: BT, adenocarcinoma; BN, 'normal' tissue adjacent to the tumor; BF, benign fibroadenoma. Thyroid samples: T11, toxic adenoma; T13, T14, T16 and T207, follicular adenomas; T208, T229 and T190, papillary carcinomas; T189, anaplastic carcinoma; T194, thyroiditis; T235, colloidal nodular; T12, T15 and T219, goiter.

3. RESULTS

3.1. *Expression of the H23 antigen mRNA*

Fig. 1A shows that H23 antigen mRNA is highly expressed in most (9/10) breast carcinoma samples as analyzed by RNA dot blotting. The mean level of hybridization in malignant breast tumors is about 7 times higher than in 'normal' breast tissues adjacent to the biopsied malignant samples. In some cases H23 antigen mRNA expression is even higher (see for example BT12 showing 33-fold increased expression).

The fibroadenomas BF13 and BF16 (Fig. 1A) show levels of H23 antigen mRNA expression comparable to those seen in 'normal' breast tissues. One breast fibroadenoma, BF9, contained H23 antigen mRNA at levels about 4-fold higher than that observed in 'normal' tissues. This sample was obtained from a patient who had previously undergone mastectomy of the contralateral breast because of an adenocarcinoma.

In non-breast epithelial glandular tissues such as benign thyroid samples, H23 antigen mRNA is almost undetectable. In contrast, thyroid papillary carcinomas

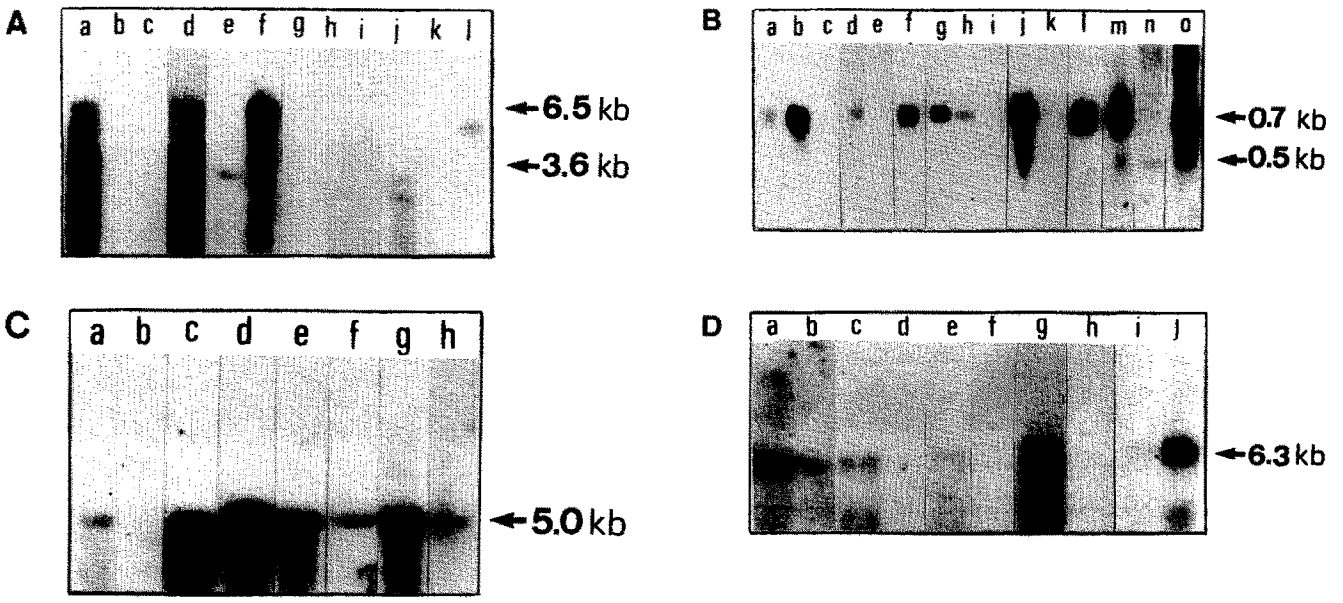

Fig. 2. Northern blot analysis of mRNA expression in breast and non-breast tumors. 30 μg of total RNA or 5 μg of polyA$^+$-selected mRNA from breast and non-breast tumors were electrophoresed on 1.4% agarose gel, transferred to nitrocellulose membrane and hybridized with the following ^{32}P-labeled cDNAs: (A) 3b cDNA coding for part of the H23 antigen. Lanes: a, BT10; b, BN10; c, BF13; d, T47D cell line; e, BT4; f, BT2; g, bladder malignant tumor; h, kidney tumor; i, kidney normal tissue; j, stomach carcinoma; k, thyroid anaplastic carcinoma; l, thyroid papillary carcinoma. (B) pS2 cDNA. Lanes: a, BT6; b, BT4 polyA$^+$; c, BT4 polyA$^-$; d, BT11; e, BT14; f, stomach carcinoma; g, colon tumor; h, colon normal tissue; j, bladder tumor; k, bladder normal tissue; l, ovarian ascitic cells; m, BT12; n, T190; o, BT1. (C) erbB2 cDNA. Lanes: a, BT2 polyA$^+$; b, BT2 polyA$^-$; c, BT4 polyA$^+$; d, BT15; e, T208 polyA$^+$; f, T16; g, kidney tumor; h, ovarian ascitic cells. (D) ER cDNA. Lanes: a, ovarian tumor; b, ovarian normal tissue; c, T190; d, T194; e, BN2; f, BT2; g, BT4; h, BN4; i, BF16; j, BT15.

show low, yet detectable levels of H23 antigen mRNA expression. Varying detectable levels of H23 antigen mRNA are also seen in stomach (Fig. 2A) and colon carcinomas (data not shown); in these cases expression is 3.5–12 times lower than that observed in breast tumors.

Northern blot analysis (Fig. 2) demonstrates significant expression of H23 antigen mRNA in breast tumors. The mRNAs are heterogeneous in size, due to either individual differences in the number of 60 bp repeat units [3,7,8–11], or differential splicing events [9].

3.2. *Expression of the pS2 mRNA*

Probing the same RNA dot blot with pS2 cDNA shows that expression of the pS2 gene is less specific for breast cancer (Fig. 1B). Although pS2 mRNA is mainly detected in H23 antigen-positive malignant breast tumors, hybridization with pS2 cDNA is also observed in benign breast tumors (fibroadenoma), which are H23 antigen mRNA-negative. Similar levels of pS2 mRNA expression are observed both in breast carcinomas and in malignant and benign thyroid tissues (Fig. 1B). High expression of pS2 is also noted in stomach, colon, bladder and ovarian tumors (Fig. 2B).

Northern blot analysis demonstrates 0.7 kb pS2 mRNA in 9 out of 16 breast carcinomas as well as in several non-breast tumors (Fig. 2B).

3.3. *Expression of the erbB2 mRNA*

Dot blot analysis of erbB2 mRNA shows significant levels in breast and thyroid tumors (Fig. 1C). Northern blot hybridization confirms this observation demonstrating the presence of 5 kb erbB2 mRNA in 12 out of 16 breast carcinoma tissues. Several malignant (3/4) and benign (3/10) thyroid tumors (Fig. 1C) as well as kidney and ovarian tumor samples have significant expression (Fig. 2C). Increased amounts of erbB2 mRNA are detected in some samples of stomach, colon and lung carcinomas (data not shown).

3.4. *Expression of the ER mRNA*

Probing the RNA dot blot with ER cDNA shows markedly higher levels of ER mRNA expression in breast cancer samples than in other tissues analyzed (Fig. 1D). In 3 out of 10 breast carcinomas, however, ER mRNA is not detected. Low levels of ER are also seen in a benign breast fibroadenoma.

Northern blot hybridization demonstrates the presence of 6.3 kb ER mRNA in 11 out of 16 breast carcinomas. This mRNA is also seen in ovarian and thyroid papillary tumors (Fig. 2D).

4. DISCUSSION

The comparative analysis of H23 antigen, pS2, erbB2 and ER gene expression revealed that the H23 antigen

may be considered a discriminating marker for breast cancer. Its increased expression was observed in 92% of tested breast carcinomas whereas the erbB2 oncogene was expressed only in 69%, ER gene in 62%, and pS2 gene in 56% of breast tumors. The expression in breast cancer of genes coding for ER, pS2 and erbB2, as presented here, is, therefore, in good agreement with the data obtained by others [4,5,12,19].

Although H23 antigen mRNA is also found in some non-breast epithelial tumors, such as stomach, thyroid and colon, its relative level of expression in these malignancies is significantly lower than that observed in breast tissue.

It is not clear why the gene coding for H23 antigen mRNA is overexpressed in breast cancer. However, as one of the antigen forms is likely a transmembrane receptor-like protein [8,9], it may be an element of a signal transducing system. Its increased expression in breast tumor cells may, therefore, reflect an involvement in cell growth.

H23 antigen mRNA was found to be significantly expressed in one breast fibroadenoma sample (BF9) isolated from a patient with a history of adenocarcinoma in the contralateral breast, indicating that increased H23 antigen expression may precede the transformation of a cell to malignancy.

Overexpression of the pS2 gene is not restricted to breast cancer as compared to expression of the H23 antigen mRNA. Rio et al. [5] consider pS2 a breast cancer-specific marker and found that 49% of breast carcinomas expressed pS2 antigen. In our study a comparable 56% of breast tumors expressed pS2 mRNA. However, we have also observed relatively high levels of pS2 expression in bladder and stomach carcinomas, ovarian ascitic tumor cells and various thyroid tumors. Recently, Rio et al. [20] reported that pS2 antigen is also found in normal stomach mucosa. The combined data thus show that pS2 gene expression is less specific for breast cancer than expression of the H23 antigen gene.

Overexpression of the erbB2 gene in breast cancer and its possible clinical significance has been recently reported [2,4]. We also found that 69% of breast carcinomas overexpressed erbB2 mRNA. This observation is in accordance with others reporting overexpression of 5.0 kb erbB2 mRNA in human mammary tumor cell lines [22] and in human breast tumors [4,23]. However, the specificity of overexpression of erbB2 in human breast tumors has been recently questioned [24]. Results obtained in this study as well as data published by others [4,21,23] show that the c-erbB2 gene is highly expressed not only in breast tumors, but also in tumors of thyroid, ovarian, colon, stomach and lung origin. Since c-erbB2 overexpression is observed in various tumors, it might be more a tumor-associated than an organ (breast)-specific marker.

The level of ER gene expression in malignant breast tissue has clinical significance. Our analysis reveals that 62% of breast carcinomas synthesize ER mRNA. McGuire and De La Gras [19] also showed that about 70% of breast tumor patients have detectable levels of tumor ER. The amounts of ER mRNA in some tumors (BT5, BT12) were up to 15–20-fold higher than those observed in normal tissues. However, since ER mRNA is detected only in 60–70% of breast tumors, its role as a diagnostic tool in breast cancer is limited in comparison to H23 antigen mRNA. The value of ER gene expression in predicting the clinical response to hormone therapy is nevertheless indisputable [25].

In conclusion, a comparative analysis of four breast cancer-related genes revealed that expression of the recently described H23 antigen gene is highly correlated with the malignant phenotype of breast cells and therefore may be of significant diagnostic value.

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