Prostate Epithelium-derived Ets Transcription Factor mRNA Is Overexpressed in Human Breast Tumors and Is A Candidate Breast Tumor Marker and A Breast Tumor Antigen

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ABSTRACT

Purpose: There is a need to identify novel breast tumor-associated molecules with a potential as diagnostic/prognostic markers of breast cancer as well as targets of vaccine and drug discovery against this cancer.

Experimental Design: We used a combination of digital differential display and reverse transcription-PCR (RT-PCR) methods to identify breast tumor-associated cDNAs.

Results: It was found that prostate epithelium-derived Ets transcription factor (PDEF) and five other cDNAs occur at high frequency in the cDNA libraries from normal human breast tissue and human breast tumors. In contrast, these cDNAs are either undetectable or present at low frequencies in the cDNA libraries from other normal human tissues. RT-PCR expression analysis of PDEF showed it to be overexpressed in 14 of 20 primary human breast tumors and in one metastases tested. Also, consistent with the digital differential display data, RT-PCR analysis of PDEF expression showed highly restricted expression in normal human tissues. Furthermore, we show that PDEF transcript levels are 192-fold higher in the peripheral blood of a breast cancer patient in comparison with two normal individuals and another breast cancer patient. In contrast to PDEF, RT-PCR analysis of the expression of the other three cDNAs, including MYL5, Hs.44017, and Hs.215937, showed that these cDNAs are expressed in several normal human tissues.

Conclusions: These results suggest that PDEF is a breast tumor-associated cDNA and should be further evaluated for its potential as a breast tumor marker and a breast tumor antigen.

INTRODUCTION

We have been interested in the identification of breast tumor-associated cDNAs that are overexpressed in breast tumors in comparison with normal breast tissue and that show restricted or low level expression in other normal human tissues. Such cDNAs can provide a starting point for a number of studies if their expression is frequently induced in tumor cells. Thus, frequent overexpression in the tumor tissue suggests a role in the initiation and/or progression of breast cancer, and such cDNAs should be evaluated as diagnostic/prognostic markers as well as targets of drug discovery. In addition, frequent overexpression of cDNA in tumors when accompanied by restricted/low level expression in normal tissues provides a basis for its evaluation as a tumor antigen for developing vaccines against breast cancer.

Over the years, various approaches have been used for the identification of tumor-associated molecules. Thus, monoclonal antibodies were used to define a large number of normal tissue antigens as well as tumor-associated antigens (1). In parallel, various molecular biology techniques were developed to identify differences between normal and tumor tissues, e.g., differential display and screening of enriched cDNA libraries after cDNA subtraction (2, 3). Similarly, tumor-specific CTLs and serum from cancer patients are being used to identify tumor-associated antigens (4–6).

We evaluated previously (7) a modified differential display method for identification of breast tumor-associated cDNAs with limited success. In this report, we show that comparison of cDNA libraries from different normal and tumor tissues by the DDD method (8) is a useful approach for identification of tumor-associated cDNAs. Using this method, we found that PDEF and five other cDNAs are frequently present in the cDNA libraries from normal human breast tissue and human breast tumors and they are either undetectable or present at low frequency in the cDNA libraries from other normal human tissues. RT-PCR analysis of PDEF expression showed results similar to those obtained by the DDD method providing independent confirmation of the DDD-derived data. These and other data presented in this report show that PDEF is a breast tissue/tumor-associated cDNA and is a candidate breast tumor marker and breast tumor antigen.

MATERIALS AND METHODS

DDD Method. The DDD method used in this study was accessed from the Cancer Genome Anatomy Project site (8).

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3 The abbreviations used are: DDD, digital differential display; PDEF, prostate epithelium-derived Ets transcription factor; RT-PCR, reverse transcription-PCR.

Real-time PCR. For real-time PCR, 2 μg of total RNA from each tissue was first reverse transcribed. Briefly, RNA was mixed with 1 μl (100 pmol) of oligo(dT) and incubated at 70°C for 10 min. The reaction mixture was chilled on ice and centrifuged, and then 200 μM each deoxynucleotide triphosphate, 2 units/μl RNase inhibitor, and 2 units/μl reverse transcriptase (Promega) in a total volume of 10 μl was added. Reverse transcription was performed in a PTC-100 and included one cycle of reverse transcription at 42°C for 1 h and enzyme inactivation at 95°C for 10 min. After reverse transcription, the reaction mixture was 5-fold diluted with diethyl pyrocarbonate-treated water. Ten μl of this diluted reaction mixture of synthesized cDNA was used as a template in real-time PCR.

Real-time PCR amplification was performed on an ABI Prism 7700 sequence detector system (PE Applied Biosystems) using the TaqMan universal PCR mix according to the directions of the supplier. The amplification was performed in Micro Aamp optical tubes (PE Biosystems, Foster City, CA) using primers and probes designed for specific detection of PDEF. PDEF-specific sense primer 5′-GTGAAGAGAGCCTGGACCAG-3′, antisense primer 5′-TTGAGGACTGCCAAGGTG-3′, and a TaqMan-labeled probe 5′-CGAGGTGACTCTCATGCTCGG-3′ were used. A 79-bp PDEF-specific sequence was amplified by these primers that flanked the probe sequence. The reaction mixture contained cDNA synthesized from 500 ng of total RNA as template, 5 pmol each primers, 7.5 pmol TaqMan fluorescent label probe, and 1× TaqMan universal PCR master mix (PE Applied Biosystems) in a total volume of 25 μl.

In the experiment for detection of PDEF transcripts in peripheral blood, a combination of conventional RT-PCR and real-time PCR were used sequentially.

RESULTS

Search for Breast Tumor-associated cDNAs Using DDD. To compare the specific cDNA frequencies in the cDNA libraries from human normal and tumor tissues, we used the online DDD method from the Cancer Genome Anatomy Project site (8). Table 1 shows the identities of various tissues and the cDNA libraries used for this comparison. The cDNA libraries from prostate tumors were included in this comparison to serve as internal controls because this should identify prostate-associated cDNAs, some of which are described previously (10).

Two-hundred and seventeen cDNAs showed differential representation in the cDNA libraries from specific tissues in comparison with one or more of the other tissues (data not shown). From this collection of cDNAs, we specifically selected those that showed high frequency representation in normal breast tissue and breast tumors and undetectable or low representation in other normal tissues. As shown in Table 2, six cDNAs including PDEF, UBp62, CB561, MLY 5, EST Hs.44017, and EST Hs.215937 were found with these characteristics, and among these, PDEF expression characteristics were most remarkable. Thus, PDEF was detected at about a 20-fold higher frequency in the cDNA libraries from breast tumors (relative frequency, 0.04212) in comparison with those from normal breast tissue (relative frequency, 0.00267). Moreover, it occurs at much lower relative frequencies in the cDNA

libraries from prostate tumors and is virtually undetectable in those from other normal human tissues including the normal prostate.

Also of interest (in Table 2) is the representation of the three prostate-specific cDNAs including ACPP, MSMB, and KLK3. These cDNAs are represented restrictively in normal prostate and in prostate tumors. Among these, KLK3 cDNA is already known to encode the prostate-specific antigen. Another noteworthy point is that the cDNA specific for glyceraldehyde-3-phosphate dehydrogenase is represented well in all of the normal tissues, so it was not examined further in this study.

Because breast tumors arise from epithelial cells, we also compared the cDNA representation by DDD method in the cDNA libraries from microdissected epithelial cells from normal and tumor breasts. The results were very similar to those shown in Table 2 except that PDEF representation was about 11-fold higher in the cDNA libraries from breast tumors in comparison with that from normal breast tissues (data not shown). To obtain independent evidence in support of these observations, expression of PDEF was analyzed by RT-PCR.

**Analysis of PDEF mRNA Expression in Normal Tissues**

Expression of UBp62, CB561, MYL5, Hs.44017, and Hs.215937 in Normal Human Tissues. A previously published study (11) showed that UBp62 RNA is expressed in all of the normal tissues, so it was not examined further in this study. Similarly, CB561 was known previously (12) to be expressed in many neuroendocrine tissues including neuronal cell bodies and/or fibers in the gut, blood vessels, retina, and posterior pituitary and in the endocrine cells of the gut, anterior and intermediate lobes of the pituitary, heart muscle, and adrenal medullary chromaffin cells. For this reason, CB561 was also not examined further in this study. RT-PCR analysis of the expression of the remaining three cDNAs including MYL5, Hs.44017,
Fig. 1  RT-PCR analysis of PDEF and β-actin RNA expression. Top panel, results of expression analysis for PDEF; lower panel, expression analysis for β-actin. Lane 1, various size markers with marker lengths shown only in the top panel. Lanes 2–13 (top panel), RT-PCR results of PDEF expression in various normal human tissues followed by lanes 14–19 showing expression data for PDEF in five primary human breast carcinomas and one metastasis. The bottom panel for analysis of β-actin expression has identical distribution of normal and tumor tissues in various lanes. Normal tissues in various lanes are: Lane 2, breast; Lane 3, brain; Lane 4, heart; Lane 5, kidney; Lane 6, liver; Lane 7, lung; Lane 8, prostate; Lane 9, skeletal muscle; Lane 10, spleen; Lane 11, testis; Lane 12, trachea; and Lane 13, uterus. The lanes for tumor tissues are: Lane 14, primary breast tumor 1; Lane 15, metastasis from primary tumor 1; Lane 16, primary breast tumor 2; Lane 17, primary breast tumor 3; Lane 18, primary breast tumor 4; and Lane 19, primary breast tumor 5. Amplification was for 25 cycles, and the PCR-amplified PDEF cDNA in the top panel (indicated by an arrow) was 452 nucleotides long.

Fig. 2 A and B, RT-PCR analysis of Hs.44017 (A, top panel) and Hs.215937 (B, lower panel) mRNA expression. Specific sense 5’-CTCGCCCGCCAGCTAGCGAAGA-3’ and antisense primer 5’-CGGGCGCGCGACGCTCCGAG-3’ were used to amplify 355-bp Hs.44017-specific cDNA in the top panel. Similarly, specific sense 5’-TAGACATAATGTTCAGCC-3’ and antisense primer 5’-CCTTCTTCTCCTTGTGCACAGAAT-3’ were used to amplify Hs.215937-specific 400-bp fragment. In top panel, tissue designation for various lanes are: Lane 1, size markers; Lane 2, breast; Lane 3, brain; Lane 4, heart; Lane 5, kidney; Lane 6, liver; Lane 7, lung; Lane 8, skeletal muscle; Lane 9, skeletal muscle (from the Tissue Procurement Facility of the Roswell Park Cancer Institute); Lane 10, prostate; Lane 11, testis; Lane 12, trachea; and Lane 13, uterus. In the lower panel, tissue designation for various lanes are: Lane 1, size markers; Lane 2, colon; Lane 3, heart; Lane 4, intestine; Lane 5, liver; Lane 6, lung; Lane 7, skeletal muscle; Lane 8, prostate; Lane 9, spleen; Lane 10, testis; Lane 11, trachea; and Lane 12, breast.

and Hs.215937 cDNAs was carried out using specific primers derived from their known sequences. The data showed that, unlike PDEF, each of these cDNAs was significantly expressed in several normal human tissues. Specifically, as shown in Fig. 2A, Hs.44017 was significantly expressed in most normal human tissues. In contrast, MYL5 and Hs.215937 showed somewhat restricted expression in several normal human tissues. RT-PCR expression data for Hs.215937 in various normal human tissues is shown in Fig. 2B. On the basis of these observations, Hs.44017, Hs.215937, and MYL5 (data not shown) cDNAs were not evaluated further for their expression characteristics in breast tumors.

Analysis of PDEF Expression in Paired Normal and Tumor Tissues from Breast Cancer Patients. To get an idea about the range of variation in the expression of PDEF in normal and tumor tissues from breast cancer patients, we performed PDEF expression analysis in such tissues from five breast cancer patients. Expression was analyzed by the conventional semi-quantitative one-step RT-PCR (Fig. 3A), as used previously in Fig. 1, and by real-time quantitative PCR (Fig. 3B). As shown in these figures, PDEF expression varied both in the normal tissues from different patients (Fig. 3A) as well as in the tumors from these patients (Fig. 3, A and B). Quantitative comparison by real-time PCR of the matching normal and tumor tissues from individual patients showed that overexpression of PDEF in tumors varied over a wide range, i.e., from about 5-fold in patient 4 to about 90-fold in patient 2 (Fig. 3B). However, because of significant variation in the expression of PDEF in normal breast tissues from the five patients (Fig. 3A), we used a normal breast RNA sample from Clontech Labs as a control in subsequent comparison with PDEF expression in breast tumors.

Range of Variation of PDEF Expression in 20 Breast Tumors. We have now analyzed PDEF expression in 20 primary breast tumors by real-time PCR. Of those, 14 showed overexpression that varied over a wide range. Using Clontech breast RNA as a control, five tumors expressed PDEF at more than 20-fold higher levels, another three between 10–20-fold
higher levels, and the remaining six expressed PDEF at a few fold to 10-fold higher levels than those in the normal breast. The data are shown in Table 3 and are representative of two separate experiments with <20% inter-experiment variability.

The data shown in Table 3 also validates the use of control breast RNA from Clontech in these experiments. Because Clontech control was generated from eight women, all of whom were under 45 years of age, there remained a question of whether this control is representative of expression levels of normal breast tissue from patients, most of whom are significantly older. Table 3 shows fold overexpression for 11 of 20 tumor samples for which both "normal" breast tissue from the same patient as well as the control from Clontech were used for assessing overexpression in tumors. These patients included those with tumor sample numbers 1, 2, 6, 7, 8, 9, 10, 12, 13, 15, and 16. In four of these cases with tumor numbers 1, 2, 6, and 13, overexpression of PDEF in tumors was similar whether the patient’s normal breast or Clontech RNA was used as control, and this reflects similar expression levels of PDEF in the normal breast of these patients as in the Clontech sample. Of the remaining seven cases, three patients with tumor numbers 7, 10, and 15 showed apparent lower fold overexpression of PDEF in tumors when using the patient’s normal breast as control versus the Clontech control. This is because of the relatively higher level of PDEF expression in the normal breast tissues from these patients in comparison with the Clontech control. In contrast, in the other four of the seven patients including those with tumor numbers 8, 9, 12, and 16, fold PDEF overexpression in tumors was higher when the normal breast tissue from those patients was used as control versus the Clontech control. Again, this apparent discrepancy results from relatively lower level expression of PDEF in the normal breast tissue of these four patients in comparison with the normal Clontech control. The combined data from these 11 patients showed that the level of PDEF expression in the Clontech control is representative of normal breast tissue from patients because roughly similar numbers of patients showed PDEF expression levels below or above the Clontech control, as well as similar to this control. Moreover, the range of overexpression in tumors was similar with both controls. Thus, when using patient’s “normal” tissue as control, the range of overexpression in tumors varied from 4.38-fold for tumor number 10 to 91.7-fold for tumor number 1. Similarly, when using Clontech RNA as control, the range of overexpression varied from 5.57-fold for tumor 8 to 127.11-fold for tumor number 1. These data show that there does not seem to be a significant bias introduced in this study as a result of using Clontech normal breast RNA as control.

PDEF-specific Transcript Levels in the Peripheral Blood of Breast Cancer Patients and Normal Individuals.

To determine the potential of PDEF as a marker of breast cancer, we analyzed peripheral blood samples from two breast

### Table 3: Fold overexpression of PDEF in individual tumors

<table>
<thead>
<tr>
<th>Primary tumor number</th>
<th>Fold overexpression in comparison with patient’s normal breast sample</th>
<th>Fold overexpression in comparison with normal breast sample from Clontech</th>
</tr>
</thead>
<tbody>
<tr>
<td>1(^a)</td>
<td>91.7</td>
<td>127.11</td>
</tr>
<tr>
<td>2(^b)</td>
<td>23.05</td>
<td>25.81</td>
</tr>
<tr>
<td>3(^a)</td>
<td>ND(^b)</td>
<td>Not overexpressed</td>
</tr>
<tr>
<td>4(^a)</td>
<td>ND</td>
<td>12.22</td>
</tr>
<tr>
<td>5(^a)</td>
<td>ND</td>
<td>21.11</td>
</tr>
<tr>
<td>6</td>
<td>Not overexpressed</td>
<td>Not overexpressed</td>
</tr>
<tr>
<td>7(^b)</td>
<td>38.05</td>
<td>116.16</td>
</tr>
<tr>
<td>8(^b)</td>
<td>88.03</td>
<td>5.57</td>
</tr>
<tr>
<td>9(^b)</td>
<td>20.25</td>
<td>Not overexpressed</td>
</tr>
<tr>
<td>10(^b)</td>
<td>4.38</td>
<td>15.86</td>
</tr>
<tr>
<td>11</td>
<td>ND</td>
<td>3.29</td>
</tr>
<tr>
<td>12</td>
<td>23.58</td>
<td>6.73</td>
</tr>
<tr>
<td>13</td>
<td>Not overexpressed</td>
<td>Not overexpressed</td>
</tr>
<tr>
<td>14</td>
<td>ND</td>
<td>9.71</td>
</tr>
<tr>
<td>15</td>
<td>5.60</td>
<td>22.92</td>
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<td>16</td>
<td>5.24</td>
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</tr>
<tr>
<td>17</td>
<td>ND</td>
<td>12.99</td>
</tr>
<tr>
<td>18</td>
<td>ND</td>
<td>Not overexpressed</td>
</tr>
<tr>
<td>19</td>
<td>ND</td>
<td>6.96</td>
</tr>
<tr>
<td>20</td>
<td>ND</td>
<td>2.98</td>
</tr>
</tbody>
</table>

\(^a\) Sample used in the experiments shown in Figure 1.

\(^b\) Sample used in the experiments shown in Figure 3.

ND, not determined.

Fig. 3  A and B, semiquantitative and quantitative expression of PDEF in matching normal and tumor tissues from five breast cancer patients. In this analysis, samples were selected that represented variation in the range of overexpression seen by nonsaturating PCR as shown in Fig. 1. Fig. 3A shows such a nonsaturating semiquantitative PCR for 25 cycles for five representative matching normal and tumor samples. B shows fold overexpression of the PDEF RNA in the tumor sample in comparison with the matching normal breast sample derived from the quantitative real-time PCR assay on the same samples. The respective samples (left to right) in A and B are from patients identified as tumor numbers 7, 8, 9, 10, and 2; four of which were different from those used in Fig. 1.
cancer patients and two normal individuals for the presence of PDEF-specific mRNA. In this experiment, first, a conventional RT-PCR was performed for 40 cycles using the PDEF-specific primer pair, previously used in the experiments described in Figs. 1 and 3. Then, equal aliquots from the conventional PCR were subjected to real-time PCR using a separate set of nested PCR primers and a probe as described in "Materials and Methods." As shown in Fig. 4, a peripheral blood RNA sample from one of the patients (PPB1) showed a PDEF-specific amplification profile with a ΔC_T value substantially lower than that seen in the samples from the two normal individuals (NPB1 and NPB2) and the second breast cancer patient (PPB2). In addition, this Figure shows the amplification profiles of 10-fold serial dilutions (TS1 to TS5) of RNA from the primary tumor of the patient PPB1. A standard curve was then generated from the ΔC_T values from the serial dilutions. Plotting of the ΔC_T values from patients and normal samples on this standard curve showed that the patient PPB1 had PDEF-specific transcripts at a 192-fold higher level in her peripheral blood than the two normal individuals and the second breast cancer patient. These results suggest that the patient PPB1 has circulating breast tumor cells in her peripheral blood. A pathology report of this patient supports our conclusion because all of the 35 resected lymph nodes from this patient were positive for invading breast tumor cells. In contrast, 2 of the 13 resected lymph nodes from the second breast cancer patient were positive for breast tumor cells.

DISCUSSION

By using two independent methods including DDD and RT-PCR in this work, we demonstrate that PDEF is overexpressed in a large fraction (70%) of human breast tumors and is barely expressed in the normal human tissues except prostate and trachea. PDEF is a recently described (9) new member of the Ets transcription factor family. Members of this family are implicated in the regulation of proliferation, differentiation, and oncogenic development (13) and are the downstream effectors of the Ras/mitogen-activated protein kinase cascade. Their expression can be regulated in a tissue-specific manner, and their binding to DNA may be facilitated by other proteins, e.g., serum response factors as well as transcription factors of other families (13). On the basis of these observations, we believe that PDEF may have a role in the neoplastic progression of the mammary tissue.

The DDD method is useful for initial identification of cDNAs that are expressed preferentially in particular tissues or frequently overexpressed in tumors. Because this method uses pools of cDNAs from groups of cDNA libraries from specific tissues, the results obtained can be influenced by various parameters. In particular, cDNA libraries from microdissected versus bulk tissues would yield quite different relative frequencies of specific cDNAs. In addition, the relative frequencies of specific cDNA in different pools are influenced by how fre-
quenty a particular cDNA is differentially expressed in the individual libraries of that pool. Thus, Her2/neu that is overexpressed in about 20 to 30% of breast tumors was not identified as a breast tumor-associated cDNA in our comparison. Conversely, unusually high representation of a cDNA in one of the libraries of a pool of cDNA libraries can also yield incorrect impression of differential expression of that cDNA in most libraries of that pool. Moreover, the number of cDNAs sequenced in each cDNA library used in the DDD analysis can affect the accuracy of the data obtained by this method. We believe that this may be a significant cause of the incongruent results obtained by the DDD and the RT-PCR methods in this study. Thus, in most cDNA libraries used in this analysis, only a few hundred to several hundred cDNAs were sequenced. This limits the DDD comparison to relatively abundant cDNAs. Consequently, many of the relatively low abundant cDNAs are incorrectly scored as absent from the cDNA libraries from various normal human tissues by the DDD method. This creates the misleading impression of restricted expression of such cDNAs in normal tissues. At the same time, some of these cDNAs appear to be differentially expressed between the tumor and the corresponding normal tissue because of overexpression in tumors. Thus, the usefulness of the DDD method is dependent on the quality of the input data. To enhance the accuracy of the DDD method, a large number (a few thousand to several thousand) of cDNAs should be sequenced from each cDNA library that is used in the DDD comparison.

For the present, the DDD method seems most useful for initial identification of candidate cDNAs with a potential for tumor/tissue specificity, and it is imperative that the results of DDD analysis be verified by independent methods. The RT-PCR analysis that we used in the present study showed that of the six cDNAs initially identified by DDD, only PDEF has the expression characteristics of a breast tumor-associated cDNA. Nonetheless, given the difficulty of finding novel breast tumor-associated cDNAs, the DDD method remains a useful bioinformatics tool for this purpose. We are aware that PDEF and other cDNAs were reported previously (14) to be associated with breast tumors solely on the basis of DDD analysis. Our identification of these cDNAs was independent and was also reported previously (15).

It has been shown by Northern blot hybridization that PDEF mRNA is expressed at particularly high levels in the prostate gland and at low levels in the ovary, breast, and salivary glands. All tissues regulated by steroids (9). PDEF transcripts were shown not to be present in other tissues or organs. This tissue distribution conforms to our results derived from RT-PCR analysis. Our new findings are that PDEF is frequently overexpressed in human breast tumors and also shows significant expression in normal tissue. At present, the expression of PDEF at the protein level is not known. However, if a prostate-specific transcription factor reported recently (16) as human prostate-specific Ets is identical to PDEF, then PDEF protein is indeed expressed in the prostate gland, more precisely in the nucleus of prostate epithelial cells. In addition, functional expression of PDEF protein has been demonstrated in LNCaP and CV-1 tumor cell lines (9). Further systematic expression of PDEF at the protein level in human breast tumors remains to be undertaken.

Breast tumor recurrences and metastases are currently diagnosed by clinical symptoms and imaging procedures and by the use of serum markers. These methods require a threshold tumor volume that usually occurs at late stages of tumor spread. More recently, RT-PCR methods are being evaluated to detect rare tumor cells in the peripheral blood or in the bone marrow aspirates from breast cancer patients (17–19). In some studies (20–21), the presence of tumor cells in these tissues correlated with poor prognosis. Our demonstration of elevated levels of PDEF mRNA in the peripheral blood lymphocyte of a breast cancer patient (Fig. 4) suggests the presence of breast tumor cells in the circulation of this patient. The pathology report of this patient supports this conclusion, because all of the lymph nodes from this patient were infiltrated with tumor cells. In addition, PDEF expression in the primary tumor of this patient was 23-fold higher than that in the normal breast. The lack of elevated levels of PDEF in the peripheral blood of the second breast cancer patient is also interesting, both with respect to the relatively fewer lymph nodes involved (2 of 13) and the lack of overexpression of PDEF in the primary tumor of this patient. These results suggest that PDEF overexpression may be linked to breast cancer progression. However, we have failed to find a correlation between the level of PDEF overexpression in tumors with other known pathological indicators including tumor size or grade. This may be because of the relatively small sample size in this study. Alternatively, PDEF expression may affect other nonconventional parameters, e.g., the immunogenicity of tumors. Thus, aberrant PDEF expression in breast tumors may enhance their immunogenicity and subsequently increase the time interval to tumor recurrence and/or the overall survival of the patients. Because the tumor samples used in this study are fresh surgery specimens from recently diagnosed breast cancer patients, this information is not available. In the future, we plan to study PDEF protein expression in archival breast tumors so that a correlation between the level of PDEF protein expression and the disease stage, clinical aggressiveness, and/or overall survival of the patients can be assessed.

It is becoming increasingly apparent that immunotherapy of cancer is a viable alternative to other nonimmunological strategies for systemic treatment of cancer (22–24). However, evidence (25–30) indicates that tumor cells frequently undergo loss of expression of tumor antigens and/or MHC class I antigens, and this may lead to tumor escape from adoptive immunotherapy or vaccine-induced immunity. To minimize tumor escape, induction of immunity against multiple tumor antigens is most desirable. However, for most human cancers including breast cancer, the repertoire of known tumor antigens is relatively small. The expression characteristics of PDEF described in this report qualify PDEF as a candidate breast tumor antigen. In particular, the frequent overexpression restricted to breast tumors suggests that once its expression is confirmed at the protein level in breast tumors, PDEF may be used for development of multivalent breast cancer vaccines. We recognize that clinical relevance of PDEF as a breast tumor antigen and breast tumor marker remains speculative at this point. We are presently working to generate antibodies against PDEF to study PDEF protein expression in tissue sections from large panels of archival primary breast tumors and breast tumor metastases.
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