Overexpression of DNA-binding Protein B Gene Product in Breast Cancer as Detected by in Vitro-generated Combinatorial Human Immunoglobulin Libraries

Daniel B. Rubinstein, Alexi Stortchevoi, Michael Boosalis, Raheela Ashfaq, and Thierry Guillaumé

Section of Hematology/Oncology [D. B. R., A. S.] and Cancer Research Center [M. B.], Boston University School of Medicine, Boston, Massachusetts 02118; Department of Surgical Pathology, Division of Cytopathology, University of Texas Southwestern Medical Center, Dallas, Texas 75360 [R. A.]; and Laboratory of Experimental Oncology, University of Louvain, 1200 Brussels, Belgium [T. G.]

ABSTRACT

Molecules differentially expressed or overexpressed by malignant cells can serve in detecting and tracking of tumor. Additionally, they potentially can be applied in histologic-specific anticancer therapy. Few breast cancer-associated candidate molecules have been identified. Here we describe the use of combinatorial immunoglobulin [antigen-binding fragment of immunoglobulin molecule (Fab) fragment] phage libraries generated from patients with breast carcinoma to identify cancer-associated gene expression. The libraries were enriched for tumors-binding Fab by 3 logs and yielded a group of antibodies against DNA-binding protein B (DbpB), a 35-kDa thrombin-inducible nuclear factor and member of the Y-box family of proteins, which are known to act both negatively in selective gene suppression and positively as promoters of gene transcription. Sequencing of the anti-DbpB showed a degree of heterogeneity and substitutions suggesting that the Fabs selected from the combinatorial library represented a variety of anti-DbpB immune response and did not simply arise from in vitro amplification by PCR of a single or limited numbers of immunoglobulin genes. Sequencing of the DbpB molecule expressed in malignant breast cancer showed no evidence of tumor-specific mutations. Evaluation of levels of DbpB gene product expression however showed the molecule to be constitutively expressed in normal nonmalignant breast tissues but to have consistently differentially higher expression in breast cancer. Immunohistological staining revealed DbpB to be present both intracellularly and on the cell surface, which suggests it may be a means whereby malignant cells repair and replicate DNA in a selectively advantageous manner as compared with nonmalignant cells. DbpB expression in breast cancer may advance the basic understanding of the role of Y-box binding proteins as regulatory agents, and in defining malignant cell phenotypes. In addition, DbpB and the antibodies generated against it may have direct application in tumor detection and in molecule-targeted immunotherapy.

INTRODUCTION

In an effort to improve tumor screening for diagnosis, epidemiology, and prevention, extensive efforts have been undertaken during the past 20 years to identify antitumor Abs and their corresponding Ags (1). In the 1980s, studies focused on generating murine monoclonal Abs (2–4), whereas during the past decade, efforts have centered more on developing vaccines and potential candidates for tumor-specific T cell clones. Although proving to be very promising, autologous methods using the unamplified Ab repertoire run the risk that antitumor Abs, even highly tumor-specific ones, may be present in the serum at titers too low to be detected even in patients self-inoculated by tumor. In addition, although identifying potentially significant tumor Ags, serum-based studies do not allow for cloning and characterization of the genes encoding the serum antitumor Abs themselves.

Combinatorial human immunoglobulin libraries provide an in vitro means of generating Abs that is not dependent on direct detection of serum immunoglobulin as in SEREX nor limited by the need for culturing immunoglobulin-producing lymphocytes as required in generating immunoglobulin-producing hybridomas. In generating these libraries, the entire repertoire of genes encoding immunoglobulin V heavy and V light chains are transcribed, amplified in vitro, and expressed as either functional Fab fragments or single-strand constructs (19). By amplification and cloning of expressed human immunoglobulin genes, up to 1011 Ab-binding specificities can be created and simultaneously assayed for tumor-specific Abs (20). As a result of in vitro gene amplification, even rarely expressed antitumor Abs can be detected, including those having titers too low to be detected in studies using unamplified autologous human or murine serum.

Studies in recent years have identified DNA sequences with high affinity to nuclear matrix proteins. Within sections of sequence denominated matrix attachment regions (MARs) there are ATC-rich regions of DNA that can lead to unwinding by contiguous base unrolling when subjected to negative supercoiling (21). Such regions (22) have been shown to be the preferential target for cell-specific protein factors such as sequence-binding protein 1 (SATB1) which is predominantly expressed in T cells (23) and the B-cell factor known as Bright (24) as well as serving as binding sites of other nonchromatin proteins such as nucleolin (25), p53 (26), and p114 (27). Once attached, DNA-binding proteins can mediate levels of specific gene expression.

Here we report the differential expression of DbpB in breast cancer cells. DbpB is a 35-kDa thrombin-inducible nuclear factor and member of the Y-box family of proteins, which act both negatively in gene suppression and positively as promoters of gene transcription (28–4985).
30). Expression of this DNA-associated protein in malignant breast cells was identified by combinatorial immunoglobulin (Fab) libraries generated from individuals with breast cancer. Abs (Fab fragments) specifically binding to DpbB were selectively enriched, cloned, and characterized as were the DpbB molecules themselves. Evaluation of expression levels shows the molecule to be constitutively expressed in normal nonmalignant breast tissues with differentially higher expression in breast cancer. DpbB may find use in advancing the basic understanding of the role of Y-box binding as regulatory agents and in defining breast cancer cell phenotypes. Phenotypic characterization may potentially prove to be highly useful in diagnostic, preventive, and epidemiological studies of early breast cancer.

**MATERIALS AND METHODS**

**Construction of Human Combinatorial Phage-Fab Libraries.** To widen the phenotypic variety of tumor types for generation of the Fab library, peripheral blood mononuclear cells were drawn from a group of 18 patients divided between those newly diagnosed and those with longstanding (greater than 5 years) breast cancer. Total RNA was extracted (Qiagen, Valencia, CA) and reverse-transcribed with polyadenylated nucleotide as primers. The resultant reverse transcription cDNA was used as template for PCR amplification. The primers and the strategy used in amplifying human ant reverse transcription cDNA was used as template for PCR amplification.

**Table 1** Primers used for immunoglobulin chain amplification

| VH genes       | V5' `GGTGGCAATGAGCAGACATGATGTCGAGCAGCTGGTCTGGTGTGCAGCAGTGGTCTGGTGCTCC-3' |
| V4 genes       | V5' `GGTGGCAATGAGCAGACATGATGTCGAGCAGCTGGTCTGGTGTGCAGCAGTGGTCTGGTGCTCC-3' |
| VL5a           | V5' `GGTGGCAATGAGCAGACATGATGTCGAGCAGCTGGTCTGGTGTGCAGCAGTGGTCTGGTGCTCC-3' |
| VL5b           | V5' `GGTGGCAATGAGCAGACATGATGTCGAGCAGCTGGTCTGGTGTGCAGCAGTGGTCTGGTGCTCC-3' |
| CL5b           | V5' `GGTGGCAATGAGCAGACATGATGTCGAGCAGCTGGTCTGGTGTGCAGCAGTGGTCTGGTGCTCC-3' |

**EXPRESSION OF DpbB IN BREAST CANCER**

**Cells.** Breast cancer cells were derived at the time of tumor resection from the fresh primary tumors of two patients with advanced disease. Methods of tumor cell isolation were adapted from procedures described previously (32, 33). After digestion of grossly fatty areas, a section of tumor was cut into 1 mm cubes and digested for 2 h at 37°C in mammary epithelial growth medium (MEGM; Clonetics, Walkersville, MD) containing collagenase and disase (Sigma, St. Louis, MO). Isolated cells were maintained in MEGM. Malignant cells derived from two primary tumors denominated MF and MW were confirmed by fluorescence-activated cell sorting using the anticytokinin Ab CAM 5.2 (Becton-Dickinson, Mountain View, CA). Nonmalignant HMEC's derived from primary tissue were obtained from Clonetics (Clonetics, Rockville, MD).

**Panning of Immunoglobulin Combinatorial Library on Monolayered Cultured Cells.** Selection (panning) of the immunoglobulin combinatorial library was carried out in 25-cm² flasks. To optimize identification of breast cancer-specific Fabs, the library of Fab-bearing phages were repeatedlypreadsorbed on nonmalignant human epithelial cells before exposure to breast cancer cells. This process of phage subtraction, as we and others have described previously (34, 35), is designed to remove the majority of Fabs that bind to epithelial cell-surface Ags other than those present on tumor.

Nonmalignant cells (HMECs) were grown to near confluence on flask surfaces. After two washes with PBS, the flask surfaces and the cell monolayer were blocked with milk/PBS, washed with PBS, and incubated with 5–10 × 10¹³ polyethylene glycol-precipitated Fab-bearing phage particles. Phages that failed to bind to the HMEC monolayer were removed and transferred on to a confluent monolayer of breast cancer cells in a 2.5-cm² well flask for an additional 2-h exposure. After removal of the phage supernatant, the tumor cells were vigorously washed with PBS (8, 12, and 16 washings for the second, third, and fourth rounds of panning, respectively) to remove nonbinding phage. Phase binding to tumor cells were eluted with 0.1 M glycine (pH 2.2), then neutralized with 0.5 M Tris-HCl (pH 9.1). At each round of panning, the tumor-eluted phagemids were reinfected into fresh Escherichia coli XL1-Blue in the presence of helper phage. The degree of enrichment of tumor-adherent Fab at each step was determined by plating an aliquot of the eluted material and counting the colony-forming units/ml.

**Screening of Breast Cancer cDNA Expression Library with Phage-bearing Fab.** A breast cancer cDNA library (MDA-MB-435S) constructed in the λ-ZAP Express vector (Stratagene) was screened with phage-bearing Ab fragments. Plates (5 × 10³) were plated and transferred to nitrocellulose membranes in triplicate. Screening was done on duplicate membranes while an identical third copy of each membrane was simultaneously screened with phage alone (phage displaying no Fab); this third membrane served to distinguish nonspecific binding of phage itself to cDNA clones. After thorough absorption on an E. coli phage lysate to remove cross-reactive Abs, phage-Fab diluted in TBST (Tris-buffered saline with 0.05% Tween 20) were incubated for 1 h with the membrane. After multiple washes with TBST, the nitrocellulose membranes were incubated with the murine monoclonal anti-M13 Ab (Amersham/Pharmacia, Uppsala, Sweden). After additional washes, the membranes were incubated with alkaline phosphatase-conjugated goat anti-mouse Ab, washed four times with TBST and immersed in nitroblue tetrazolium-5-bromo-4-chloro-3-indolyl phosphate (NBT-BCIP) substrate to reveal sites of Ab binding. Plates identified as positive on both copies of the membrane were subcloned. Clones of interest were purified and excised, and the plasmid DNA inserts were sequenced using T3 and T7 primers.

**DNA Fingerprinting of Clones: Determination of Diversity of the Combinatorial Immunoglobulin Library.** To determine the diversity of the selected (enriched) libraries, individual recombinant clones were selected at random. DNA inserts from 20 clones were directly amplified with a pair of phF²-specific primers consisting of a 5' primer (TATGACCATGATTACGCAA) situated upstream from the pelB leader sequence and a 3' primer (CACAGTTTCAAGACTGCTATGC) downstream in gene 3. The PCR products containing the V₅ and V₃ genes were digested with BstNI, run on agarose gels to verify correct size (36), and sequenced.

**Northern Analysis.** Northern blots (Invitrogen, Carlsbad, CA) with mRNA from breast cancer and normal mammary epithelium (equalized for amounts of mRNA/sample lane) were hybridized with a ³²P-labeled EcoRI-Xhol 350-bp cDNA fragment of the DpbB gene prepared by random priming.
using a primer labeling kit (Promega, Madison, WI). The probe was generated by amplification with a pair of oligonucleotide primers derived from the DbpB gene sequence (GenBank) including an upstream primer (AATGGTTCAAT-GTAAGGAAC) and a downstream sequence (GCTGCATAGATAATGCA-GAT), Hybridizations with RNA blots were performed as we described previously (37). The levels of hybridizing mRNA were quantitated by densiometric scanning of the autoradiograms using a LKB Ultrascan Laser densiometer and the densiometric values calculated as differences in DbpB mRNA expression.

**Immunostaining.** Paraffin sections were cut on a rotary microtome, mounted on glass slides (Superfrost; Fisher), and baked overnight. Subsequently, the sections were deparaffinized in xylene and ethanol, placed in Dako Target Retrieval solution (pH 6.0) Dako, Carpinteria, CA] and rinsed thoroughly with deionized water. Immunostaining was performed at room temperature and carried out using a Dako Autostainer. Reagents were used as supplied in the Envision Plus Detection kit (Dako). Sections were incubated with primary Ab (anti-DbpB; gift of Dr. Olga I. Stenina, Cleveland Clinic Foundation, Cleveland, OH) for 30 min, followed by incubation in EnVision peroxidase, a labeled dextran polymer. This was followed by incubation in diaminobenzidine (DAB) and buffer substrate, counterstaining with hematoxylin blue in Richard Allen Bluing Reagent, and dehydrated in a graded series of ethanol and xylene. Known positive sections were included in each run to ensure the presence of proper staining, and rabbit immunoglobulin in PBS was run as negative controls.

**RESULTS**

**Analysis of V_{H} and V_{L} Gene Diversity of the Combinatorial Library.** To demonstrate the degree of diversity of the combinatorial immunoglobulin (Fab) library and to confirm that generation of the library did not merely result in a limited number of V_{H} genes repeatedly amplified in vitro by PCR, 20 clones from the library were randomly selected. The amplified genes from these clones were digested with BstNI, a frequently cutting enzyme, and the resultant fragments analyzed on agarose gels. Results of cutting patterns are shown in Fig. 1A indicating the diverse nonrepetitiveness of randomly selected members of the immunoglobulin library.

**Subtractive Panning: Enrichment of Phage Displaying Fab Adherent to Breast Cancer Cells.** To optimally identify Fabs specifically binding tumor Ags, the Fab-bearing phage library was preadsorbed on monolayers of nonmalignant human epithelial cells before each round of selection. This process of phage subtraction was designed to remove the majority of Fabs that bind to epithelial cell surface Ags other than those present specifically on tumor cells.

Phage-Fab not adherent to the nonmalignant monolayer were exposed (panned) on monolayers of breast cancer cells. Four rounds of selection yielded a 3-log rise in breast cancer-binding phage-Fab from 1.4 x 10^{5} to 1 x 10^{7} pu/ml as determined by reinflection of the binding phage in fresh E. coli XL1-Blue and counting the resulting colonies (plaque-forming units/ml; Fig. 2, ●, top curve). As a control measure for the effectiveness of the preabsorption step, aliquots of the Fab library were similarly preadsorbed on nonmalignant cell monolayers but then panned on nonmalignant cells. Four rounds of panning on nonmalignant cells yielded no enrichment of phage binding (Fig. 2; ■, bottom curve). An identical confirmatory second panning was carried out with the same phage-Fab library and cell monolayers to minimize the possibility that the library enrichment resulted from some artifactual systemic error.

**Screening of the Enriched Phage Library on Breast Cancer cDNA Expression Libraries.** Approximately 5 x 10^{5} phage plaques from breast cancer cDNA expression library were plated, lifted on to nitrocellulose membranes, and screened in duplicate with the enriched Fab library as described in the Methods section. In initial screening, a group of positives plaques were identified. The specificity of Fab binding to the plaques was confirmed on a second set of duplicate nitrocellulose membranes from which 43 clones were identified and isolated (not shown). In addition to the duplicate membranes, triplicate membranes with phage bearing no Fab were screened as negative controls. Five of the candidate 43 clones reacted strongly with phage alone. To exclude the possibility that the murine monoclonal anti-M13 Ab used in detection was not itself reacting with the cDNA breast cancer library, anti-M13 Ab was exposed to membranes bearing the λZAP Express library, which had not been reacted with the phage-Fab library.

The cDNAs from the 38 expression clones, confirmed to bind the breast cancer immunoglobulin library, were recloned, excised, and ligated into the plasmid pBK-CMV. The excision was successful in 32 of 38 candidate clones. DNA from these plasmids was digested with EcoRI and XhoI, electrophoresed in 1% agarose gels to identify DNA restriction profiles. The profiling segregated into seven distinct groups with the largest containing 24 clones with a similar restriction pattern.

**Diversity of the Combinatorial Library Enriched for Breast Cancer Binding.** As initial indication of diversity, the library enriched for phage-Fab binding to breast cancer cells was digested with the common-cutting enzyme BstNI, similarly to the digestion carried out on the unselected combinatorial library. Representative examples of clone diversity are shown in Fig. 1B. In addition, to directly demonstrate the diversity of the enriched library, 18 clones were randomly selected for sequencing (Fig. 3). Because of in vivo higher expression of certain V_{H} genes as compared with others, and because of PCR amplification used in generation of the library, a degree of gene redundancy was to be expected. Of the group of genes selected, 12 clones containing more than 20 amino acid sequence differences were found (Fig. 3). Because the clones were selected at random, the level of gene heterogeneity suggests an overall high degree of diversity among the library enriched for binding to breast cancer cells. Seven of the 12 V_{H} genes are members of the VH1 gene family, whereas only 1 is a member of VH3, the largest of the V_{H} families. Despite this preponderance of the VH1 genes, the number and place-
Fig. 2. Enrichment of Fabs specifically adherent to breast tumor cells. The Fab-displaying phage library was preadsorbed on nonmalignant epithelial cells grown as a monolayer on the surface of 25-cm² flasks. Fabs that did not adhere to the nonmalignant monolayer were collected and placed on monolayers of growing breast cancer cell lines. Four rounds of panning (see Text) yielded a 3-log rise in the titer of phage-Fab binding to breast cancer cells, from 1.4 × 10⁹ to 1 × 10¹² (*, top curve). Similar preadsorption of phage-Fab on non-malignant cells followed by panning on the same non-malignant cells resulted in no enrichment (**, bottom curve).

Levels of mRNA expression were quantitated by densitometric scanning of the autoradiographs using a LKB Ultrascan Laser densitometer (Fig. 4, bottom panel). All of the densitometric values, including those of the mRNA derived from nonmalignant sources (Lanes 4–7) were determined by subtraction of background intensities of the blot in the immediate area of each hybridization lane. The intensities of hybridization of the tumor-derived mRNAs (Fig. 4, Lanes 1–3, 5 and 6) are expressed as a percentage of intensity of the nonmalignant mRNAs. Although consistently elevated in all malignant tissue examined, the degree of increased mRNA expression was heterogeneous.

Immunostaining. To directly confirm expression of the 35-kDa DbpB gene product, anti-DbpB (28) was used for histological staining. Immunostaining was carried out on both malignant tissue (from 10 different tumors) and nonmalignant breast tissues (seven proliferative nonmalignant breast tissues and two atypical breast sections). Histological materials were stained with anti-DbpB Ab and visually developed as described in the “Materials and Methods” section. DbpB expression in breast tumor is both intracellular and on the cell surface (Fig. 5B). Even among different tumors, there is heterogeneity of DbpB expression: In addition to the intense expression of the DbpB gene product seen in eight samples, less intense (2+) staining was seen in two of the breast tumors (denominated D0-7 and DO-38; Table 2). Staining in proliferative and atypical non-malignant tissues was visually graded as 1+ on a scale of 1 to 3 (Fig. 5A). This level of constitutive gene expression is consistent with levels of transcription seen in the Northern analysis (Fig. 5), and inherent sensitivity in a three-step staining process contributes to amplify even small amounts of the DbpB protein in the sections.

Immunostaining for DbpB expression in tissues other than breast were also carried out in material from a variety of tissue types including normal nonmalignant colon (four sections), lung (3 sections), ovary (6 sections) and endometrial (5 sections) tissues. In a degree similar to that seen in normal breast (Table 2), DbpB was detectable in nonmalignant tissues.

DISCUSSION

Studies in recent years have identified DNA sequences with particular high affinity to surrounding proteins in the nucleus referred to as matrix attachment regions (MARs) and a number of proteins have been found to have DNA site-specific binding (21–27). In
addition to sequence-specific targeting in DNA of nonmalignant cell, several proteins have been found to bind specifically to the DNA sequence sites in tumor cells. HMG-I(Y) has been shown to be present in very low levels in normal adult tissues whereas it is elevated in thyroid, prostate, colorectal, and several other tumor types (39). In addition, levels of DNA binding by p114 have been found to correlate with the degree of breast cancer aggressiveness (27), which suggests that gene sequences that are bound may be sites of important regulatory events. In MDA-MB-231 breast cancer cells, the level of HMG-I(Y) can be down-modulated by signals from the extracellular matrix through signaling by heregulin (HRG)-erb B (40). This down-signaling is directly linked to tumor metastatic phenotype leading to a reduction in its invasive capability in vitro (40).

DbpB is a member of Y-box-binding proteins that have been demonstrated to have regulatory function in the expression of a variety of molecules including MHC class-II Ags (29, 41) and granulocyte macrophage colony-stimulating factors (30). Sequencing of the DbpB gene has shown it to be a 35-kDa protein that is not only constitutively expressed but can be induced by thrombin (28). Our finding of low levels of DbpB mRNA in normal nonmalignant breast tissues (Table 2; Fig. 4) is consistent with reports of constitutive DbpB gene transcription (28).

In this study, indications of increased DbpB expression in breast cancer comes from several lines of evidence. DbpB was detected by combinatorially generated Abs that on sequencing were found to be not a single, repeated sequence but a group of Fabs with differing CDR regions (including CDR3), which suggests that gene sequences that are bound may be sites of important regulatory events. In MDA-MB-231 breast cancer cells, the level of HMG-I(Y) can be down-modulated by signals from the extracellular matrix through signaling by heregulin (HRG)-erb B (40). This down-signaling is directly linked to tumor metastatic phenotype leading to a reduction in its invasive capability in vitro (40).

DbpB is a member of Y-box-binding proteins that have been demonstrated to have regulatory function in the expression of a variety of molecules including MHC class-II Ags (29, 41) and granulocyte macrophage colony-stimulating factors (30). Sequencing of the DbpB gene has shown it to be a 35-kDa protein that is not only constitutively expressed but can be induced by thrombin (28). Our finding of low levels of DbpB mRNA in normal nonmalignant breast tissues (Table 2; Fig. 4) is consistent with reports of constitutive DbpB gene transcription (28).

In this study, indications of increased DbpB expression in breast cancer comes from several lines of evidence. DbpB was detected by combinatorially generated Abs that on sequencing were found to be not a single, repeated sequence but a group of Fabs with differing CDR regions (including CDR3), which suggests a diverse immune response to DbpB. In addition, Northern hybridization with probes generated with oligonucleotides derived from known sequences of the DbpB gene showed differential binding of mRNA from tumor cells (Fig. 4), and direct immunostaining with anti-DbpB demonstrated increased expression of the DbpB gene product (Fig. 5). Not surprisingly, phenotypic variability even among tumors of the same histo-

logical type accounts for the varying results in various levels of DbpB expression in the breast cancer tissues examined, both at the transcriptional (Fig. 4) and translational (Fig. 5) levels. At the same time however, the sequences of DbpB in breast cancer cell clones showed no evidence of a tumor-specific gene mutation; all of the cloned DbpB sequences that were examined were identical. The fact that DbpB belongs to Y-box proteins having DNA-binding sequences with transcriptional regulatory functions (29, 41, 30) may account for its heterogeneous expression in different breast cancer cells.

The association of DbpB with breast cancer should not, however, be overinterpreted. Despite the similarity of DbpB with known regulatory Y box-binding proteins, and immunohistochemical staining (Fig. 5) showing tumor association, it remains to be seen whether there is any correlation between DbpB expression and tumor biology, metastatic potential, and hormonal dependence, and whether the level of DbpB gene expression changes in the sometimes long natural history of breast cancer. As in the case of Her2/neu, recognizing a
to identify Ag-specific Fab and single-chain fragments of variable combinatorial immunoglobulin libraries have been successfully used to associate Ags such as DbpB, which may ultimately prove useful in vitro. Rather, the Fabs described here were used to identify a specific tumor correlate, examining its function by ectopic expression, understanding its cellular function, and, finally, using it in therapeutic application are distinct goals (14). These studies are in progress.

The generation of tumor-specific Abs by combinatorial human immunoglobulin libraries has been most successfully reported in melanoma (9), whereas few have been shown to specifically bind epithelial tumors. This is likely attributable to the much stronger antigenicity of melanoma as compared with epithelial tumors (42). In addition, the use of breast cancer cell lines that have undergone phenotypic changes in the course of numerous cell passages may have hampered the identification of antiepithelial tumor Abs. Selection of Ag-specific Abs in phage libraries has been described primarily against known purified Ags (19). Identifying Abs (expressed as either Fab or single-chain fragments of variable regions of immunoglobulin) to molecules present on the surface of whole cells has remained a more difficult challenge because of the complexity of the Ags and the variable orientation of their epitopes on the cell surface. A number of strategies have been tried to identify cell-surface Ags, including cell sorting by flow cytometry, magnetic bead selection, and subtraction (34, 35, 43–48). To best examine unaltered cell surface proteins in the present study, we used only cells from fresh primary breast tumors. In addition, enrichment for antitumor Fabs was carried out directly on growing nontransfected breast cancer cells as antigenic targets in situ growing nontransfected breast cancer cells as antigenic targets with the objective of identifying novel cancer-associated proteins. As a result of targeting whole cells, distinct anti-DbpB Fabs cannot be isolated from the overall in vitro-generated immunoglobulin library. However, now having identified DbpB as an Ag of interest, purified DbpB can, in turn, be used as an antigenic target to isolate the Fabs within the library that bind it, and those Fabs (and the gene encoding them) can be cloned.

The cellular location of DbpB in breast cancer cells is significant. Staining showed the molecule to be present both intracellularly and on the cell surface (Table 2; Fig. 5), which accounts for our ability to detect DbpB using intact cells as Ab targets. Mechanisms of enhanced DNA repair to which DbpB contributes have been associated with tumor propagation and suggest a possible means whereby malignant cells repair and replicate DNA in a selectively advantageous manner, as compared with nonmalignant cells (52, 53). The fact that anti-DbpB Abs were identified using short-term, confluent, in vitro cultured whole tumor cells as the target suggests that the DbpB molecule is present on the intact tumor cell surface. The presence of the molecule on the cell surface is further suggested by histoimmunologic detection with anti-DbpB on intact cells that were not solubilized before fixing on to slide surfaces (Fig. 5). Nevertheless, localization of the DbpB at the cell surface does not exclude concomitant shedding of the molecule caused by accelerated tumor cell turnover. As in all candidate tumor-associated Ags, the extracellular shedding of Ag may have implications in potential use in the diagnosis and tracking of tumor (54).

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REFERENCES

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