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Spontaneous immunogenicity of ribosomal P0 protein in patients with benign and malignant breast lesions and delay of mammary tumor growth in P0-vaccinated mice

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A common carboxyl-terminal epitope (C-22 P0) of the ribosomal P proteins (P0, P1 and P2) was shown to elicit autoantibodies in systemic lupus erythematosus (SLE) and in head and neck cancer patients. In this report we provide evidence for the *in vivo* immunogenicity of the P0 protein in breast cancer patients. Using recombinant P proteins, we demonstrated that sera from breast carcinoma patients (8/75) displayed significant reactivity to P0 protein when compared with healthy donor sera (0/45). Four out of the eight sera showed simultaneous reactivity to all P proteins. Breast benign tumor (3/17) and mammary hyperplasia (3/17) patient sera also showed significant reactivity to P proteins, thus suggesting that the occurrence of P protein autoantibodies might reveal mammary cell cycle dysregulation. Patient sera reacting with all P proteins recognized C-22 P0. Anti-P0 autoantibodies did not correlate with prognostic parameters of breast carcinomas. High level expression of C-22 P0 was found in mammary carcinomas compared with normal adjacent epithelium and benign lesions. To determine the antitumor activity of P0 as an immunogen, BALB-*neu*T transgenic mice displaying age-related breast cancer progression were vaccinated using xenogeneic P0 at the stage of mammary atypical hyperplasia. P0 vaccination significantly delayed the onset of mouse mammary tumors that overexpressed C-22 P0. Sera from P0 vaccinated mice recognized C-22 P0. Evidence for immunity to the P0 protein, its overexpression in carcinomas and its peculiar surface localization on cancer cells, along with its antitumor activity as an immunogen might be relevant for the use of P0 protein in monitoring cancer progression and in planning immunotherapeutic strategies. (*Cancer Sci* 2011; 102: 509–515)

Breast cancer is one of the most frequent and deadly cancers.[1–3] Although the survival of patients has increased in recent decades, many patients still die from metastatic relapse.[1–3] Accumulation of molecular alterations induces breast cancer cell genetic instability, which leads to an invasive and resistant phenotype.[4,5] The characterization of novel molecular targets might allow the definition of reliable diagnostic/prognostic factors and the development of targeted therapies. Many autoantibodies have been proposed as diagnostic or prognostic markers in breast cancer patients.[6–9] Most of them are directed against self-antigens that are overexpressed in tumors.[10–12] The repertoire of autoantibodies overlaps with that typical of patients with autoimmune diseases.[13] Autoantibodies are useful serological markers in the diagnosis of systemic autoimmune diseases including systemic lupus erythematosus (SLE).[14–17]

The immune recognition of self-antigens in breast cancer has important implications that go beyond the discovery of novel biomarkers, since autoantibodies detected in cancer patients have been shown to target several key molecules involved in the carcinogenesis process.[6] Recent reports suggest that the machinery involved in protein synthesis might furnish novel anti-cancer drugs or immunological targets.[6] Indeed, increased cell proliferation, a characteristic of aggressive cancer, entails an increase in protein synthesis.[6] The ribosomal P proteins (P0, 38 kDa; P1, 19 kDa; and P2, 17 kDa) form the ribosomal stalk of the 60 S ribosomal subunit in eukaryotic cells.[18,19] P proteins take part in the regulation of protein synthesis.[20–22] A few reports describe overexpression of ribosomal P proteins in human cancer.[23–25] Recently it was demonstrated that ectopic overexpression of P0 enhanced cell proliferation in breast and liver carcinoma cells and affected tumorigenesis.[26]

P0 exists as a free protein in the cytoplasm and on the surface of cancer cells.[25,27,28] Autoantibodies to the P proteins were reproducibly found in SLE.[29–31] An anti-P immunodominant epitope (C-22 P0) was found to be located within the 22 amino acid C-terminal peptide shared by P proteins.[32,33]

Recently we demonstrated immunogenicity of P0 protein in head and neck cancer patients and overexpression of C-22 P0 in invasive carcinomas.[25]

In the present study, we investigate the humoral immune response to P proteins in breast cancer and benign lesion patients along with C-22 P0 expression in matched mammary tissue specimens. We also explored the efficiency of vaccination with P0 protein in counteracting the growth of mammary tumors developed in BALB-c mice transgenic for the neu oncogene (BALB-*neu*T).

Materials and Methods

Cell lines, monoclonal antibody (MAb) 2B2 and recombinant P0, P1 and P2 ribosomal proteins. Neu overexpressing BALB-*neu*T mammary cancer cells (H-2d) (TUBO) were kindly provided by Professor G. Forni (University of Torino).[34] MAb 2B2, which recognizes the C-22 P0 epitope and prokaryotic recombinant proteins were previously described.[25] Protein concentration was determined by Bradford protein assay (Bio-Rad, Hercules, CA, USA).[35] Goat anti-human and anti-mouse IgG peroxidase-conjugated antibodies were purchased from Sigma (Milan, Italy).

Tissues and sera. Tissue and sera of patients were obtained according to the ethical guidelines of the Policlinico of Tor

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Vergata ''PTV'', Rome. Sera from 75 breast carcinoma, 17 mammary hyperplastic lesion and 17 benign tumor (papilloma, $n = 2$; fibroadenoma, $n = 14$; tubular adenoma, $n = 1$) patients were collected prior to therapy and compared with 45 healthy donor sera. Sera were obtained after informed consent. The clinical stage of the carcinoma patients included stage 0 ($n = 11$), stage I ($n = 21$), stage IIa ($n = 13$), stage IIb ($n = 2$), stage IIIa ($n = 8$), stage IIIb ($n = 7$), stage IIIc ($n = 3$) and stage IV ($n = 10$). Mammary tissue specimens from these patients could be obtained for 30 invasive and 13 *in situ* carcinomas. Benign lesions including hyperplastic lesions and benign tumors were informative in 34 cases. Adjacent normal epithelium was informative in 28 specimens.

Western blotting. Purified ribosomal P-GST and GST proteins (0.5–2.5 μg/lane) as well as mouse mammary carcinoma (TUBO) cell extracts (80 μg/lane) were separated by SDS-PAGE.[25,36] Membranes were incubated with MAb 2B2, human or mouse sera. The 1:100 human serum dilution was used. Sera from BALB-*neu*T mice were collected prior to immunization and 7 days after the final boost. Membranes were incubated with mouse sera at different dilutions (1:250, 1:1000 and 1:4000). The immunocomplexes were visualized as previously described.[25] The intensity of the specific bands was expressed in densitometric units (DU) and obtained using the NIH ImageJ software after blot scanning.[37] Titer was defined as the dilution factor required to reach a DU of 100 000.

Enzyme-linked immunosorbent assay (ELISA) for determination of antibodies to the C-22 P0 peptide. ELISA was performed as previously described.[25] The carboxyl-terminal 22 amino acid peptide (C-22 P0) (NH2-AKVEAKEESEESDEDMGFGLFD-COOH) was synthesized by Primm (Milan, Italy).[25,32,33] The ErbB4 283 peptide (Santa Cruz Biotech, Santa Cruz, CA, USA) was used as the control peptide. ELISA plates (Pro-bind, Falcon, NJ, USA) were coated overnight with 1–5 μg/well of the peptides. Plates, after blocking, were incubated overnight at 4°C with human (1:100) and mouse (1:200) sera. The immunocomplexes were visualized as previously described.[25] For human sera, any serum with an optical density (OD) exceeding the mean of healthy donor sera OD values plus 3 standard deviations (SD) was considered positive for the presence of autoantibodies to C-22 P0.

Immunohistochemical analysis. Expression of C-22 P0 on tissues was determined by immunoperoxidase staining after incubation with MAb 2B2 or MOPC-21 (1 μg/mL) as previously described.[25,38] No reactivity was observed using MOPC-21 (data not shown). Paraffin sections were also processed for immunohistochemistry using a Ventana XT automated (Ventana Medical System Inc., Roche, Tuscon, AZ, USA) slide stainer to test the following prognostic markers: estrogen receptor (ER) using Ventana CONFIRM anti-ER (SP1) rabbit monoclonal antibody; progesterone receptor (PR) using Ventana CONFIRM anti-PR (1E2) rabbit monoclonal antibody; Ki-67 using Ventana CONFIRM anti-Ki-67 (30-9) rabbit monoclonal antibody; HER2 using Ventana PATHWAY anti-HER-2/neu (4B5) rabbit monoclonal antibody. HER-2 gene FISH analysis was performed using HER2 FISH (fluorescence *in situ* hybridization) pharmDx kit (Dako, Glostrup, Denmark A/S).

Semiquantitative C-22 P0 expression in human tissues was estimated at ×200 magnification in at least 10 fields by two investigators in a blind fashion, who used a previously described score system with minor modifications.[39] C-22 P0 expression level (negative, 0; weakly positive, 1; moderately positive, 2; and strongly positive, 3) and percentage of positive cells (negative, 0; 1, ≤25%; 2, >25–≤50%; 3, >50–≤75%; and 4, >75%) were scored and the results reported as mean ± standard deviation. The interobserver reproducibility was >95%.

A tumor was considered negative for ER and PR expression if <5% of tumor cell nuclei were stained. The HER2 expression level was scored based on membrane staining intensity: 0 (negative) and 1 + (discontinuous and weak) staining were considered negative, 2 + (continuous and weak to moderate) staining was considered borderline, and 3 + (continuous and strong) was considered positive. Borderline cases have been solved by FISH analysis. Accordingly, the presence of less than two copies of the HER2 gene was scored as negative, while that of two or more copies was scored as positive. Only distinct nuclear staining was used for scoring Ki-67 expression, which was scored as low (15% or less immunoreactive cells) or high (>15% immunoreactive cells). Nuclear grading was assessed by evaluating nuclear size, monomorphism or pleomorphism, dispersion of chromatin, number of nucleoli and mitosis, and scored as low (G1 and G2) and high (G3).

Transgenic BALB-*neu*T mouse colony. Transgenic BALB-*neu*T male mice[34] were routinely mated with BALB/c females (H-2d; Charles River, Calco, Italy) in the animal facilities of Tor Vergata's University. Progenies were confirmed for the presence of the transgene by PCR.[40,41] Individually tagged virgin females were used. Mice were handled in compliance with European Union and institutional standards for animal research. Original male BALB-*neu*T mice were kindly provided by Professor G. Forni (University of Torino).

Vaccination protocol. BALB-*neu*T female mice were intraperitoneally immunized with 25 μg of human GST-P0 (six mice) or GST (eight mice) proteins in 50 μL of Titermax Gold Adjuvant (Sigma–Aldrich, St. Louis, MO, USA), starting at 6 weeks of age. Mice were then boosted three times at 4-week intervals (complete immunization schedule: 6, 10, 14, 18 weeks). Tumor growth was monitored until all mammary glands displayed a palpable tumor or masses exceeding 15 mm diameter.[40] At this point the mice were killed.

Immunofluorescence staining and fluorescence-activated cell sorting (FACS) analysis of C-22 P0 on TUBO cells. Indirect immunofluorescence and FACS analysis were carried out on native cells at 4°C, using MAb 2B2, MOPC-21 (1 μg/mL) or sera from GST-P0 or GST vaccinated mice (1:500) as previously described.[25,38] To stain native cells, TUBO were grown for 48 h with or without 10% serum.

Statistical analysis. Statistical significance of prolonged median tumor-free survival was determined by log-rank survival analysis using Prism4 GraphPad software.[42] Differences in the presence of autoantibodies and their association with prognostic parameters as well as variation in C-22 P0 expression were evaluated by Fisher's exact test and Student's *t*-test, respectively. Statistical associations were considered significant at a *P*-value ≤0.05.

Results

Immunoreactivity of breast cancer patient sera with recombinant ribosomal P proteins. Using the GST-P proteins and the GST as a negative control, serum from patients with breast carcinomas ($n = 75$), benign tumors ($n = 17$), mammary gland hyperplasia ($n = 17$) or from healthy donors ($n = 45$) was subjected to qualitative western blotting analysis. Representative experiments are shown in Figure 1A. None of the sera from healthy donors displayed reactivity with P proteins (Table 1). Conversely, 8 out of 75 sera from breast cancer patients reacted with the P0 protein. The prevalence of anti-P0 autoantibodies in breast cancer patients was significant compared with that of healthy donors ($P = 0.02$) (Table 1). Four out of the eight positive sera from breast cancer patients showed simultaneous reactivity to all P proteins (Table 1). Autoantibodies to P proteins were also found in sera from patients with mammary benign lesions (6/34) (Table 1). The incidence of anti-P0 autoantibodies in these patients (4/34) was significant compared with that of healthy donors ($P = 0.03$). Simultaneous reactivity to all P

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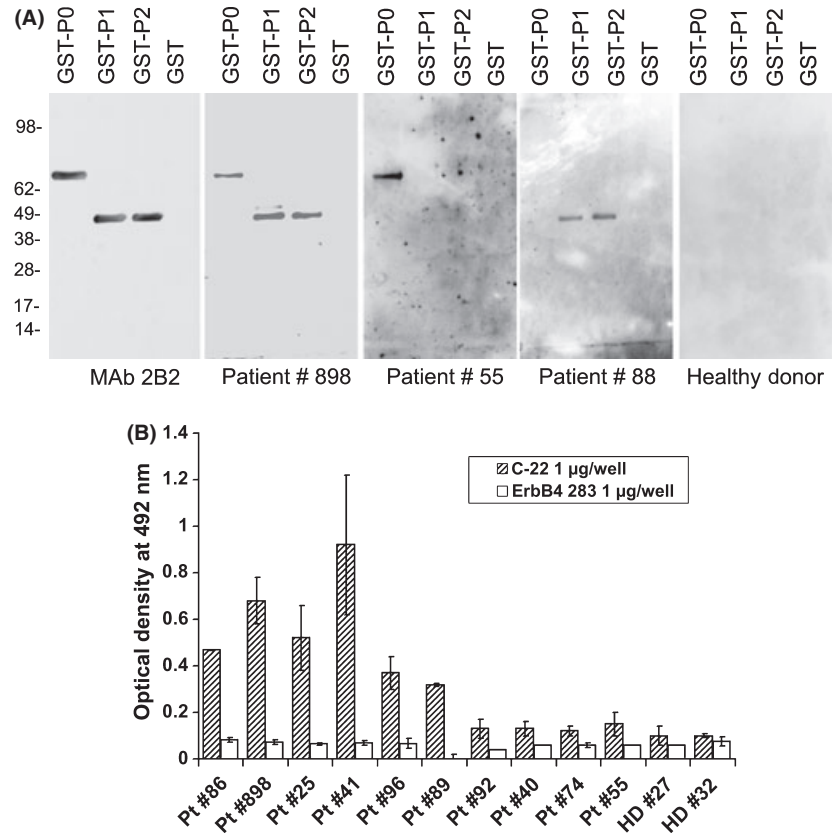

Fig. 1. Detection of anti-P ribosomal antibodies in breast cancer patients. (A) Western blot analysis of GST-tag P proteins and GST using serum from breast cancer patients (#898, #55), a breast papilloma patient (#88) and a healthy donor. MAb 2B2 was used as a positive control. (B) ELISA showing immunoreactivity of patients (Pt) or healthy donor (HD) sera with the C-22 P0 peptide. The ErbB4 283 peptide was used as the control peptide. Experiments were repeated twice in duplicate. Column optical density values represent the mean ± standard deviation.

proteins suggested reactivity to a single immunogenic epitope shared by different P proteins or to multiple epitopes specific for different P proteins. However, sera from patients with breast cancer (#86, #898, #25 and #41) or benign lesions (#96 and #89) that recognized all P proteins were able to bind C-22 P0 (Fig. 1B). Accordingly, sera from carcinoma patients recognizing only the P0 protein (#92, #40, #74 and #55) did not react with C-22 P0, thus indicating recognition of epitope(s) different from C-22 P0. No reactivity with the C-22 P0 peptide was found in healthy donor sera (Fig. 1B).

Anti-P0 autoantibodies are not associated with hormone receptor status, HER2 overexpression, Ki-67 expression and nuclear grading (Table 2). Immunity to P0 protein did not correlate with either lymph node status or clinical stage. P0 autoantibodies were observed in patients with clinical stage 0 (n = 1), I (n = 1), IIa (n = 4) and IIb (n = 1). Only one patient simultaneously reacting with P0, P1 and P2 exhibited a clinical stage IIIa. However, tumors of patients with autoantibodies to P proteins were positive for ER and PR, showed a low expression level of HER2 and Ki-67 (except one patient) and displayed a low nuclear grading (Table 2). In addition, the significant occurrence of P0 autoantibodies in patients with mammary benign lesions suggests that the anti-P0 humoral response might reveal mammary cell cycle dysregulation.

Expression of C-22 P0 in human normal and pathological mammary tissues. To assess the state of C-22 P0 expression in tumors of patients with or without anti-P0 serum reactivity, immunohistochemical analysis was performed using MAb 2B2. MAb 2B2 staining in pathological lesions was compared to that of adjacent normal breast epithelium. The latter showed a C-22 P0 weak expression confined to the basal cell layer (Fig. 2A). Conversely, C-22 P0 high-level expression throughout all cell layers was restricted to invasive carcinomas (Fig. 2A). It is worth noting that fibroblasts and endothelial cells of inflammatory tissue adjacent to the tumors also expressed C-22 P0, whereas mesenchymal cells in benign lesions did not. Benign hyperplastic epithelium showed higher intensity and homogeneity of epithelial cell staining than normal epithelium but less than carcinomas (Fig. 2A).

Table 1. Immunoreactivity of breast cancer patient sera with ribosomal P proteins

Serum origin	Serum reactivity with			
	P0	P1 + P2	P0 + P1 + P2	Sum P0
Healthy donors (n = 45)	0	0	0	0
Carcinomas (n = 75)	4	0	4	8 (P = 0.02)†
Benign lesions (n = 34)	2	2	2	4 (P = 0.03)‡

†Carcinomas and ‡benign lesions *versus* healthy donors.

Overall, C-22 P0 was weakly expressed in normal epithelium (Fig. 2B). By comparison, C-22 P0 showed significantly higher staining intensity and number of positive cells in carcinomas than normal epithelium or benign lesions (Fig. 2B). However, overexpression of C-22 P0 was not associated with hormone receptor status, HER2 overexpression, Ki-67 expression and nuclear grading of invasive carcinomas.

***In vivo* effect of vaccination with xenogeneic P0 protein on neu oncogene-mediated mammary carcinogenesis.** BALB-*neu*T mice transgenic for the neu oncogene exhibit reproducible transition from normal epithelium to multifocal breast carcinoma in a precise temporal progression.[34,40,41] C-22 P0 expression was assessed by immunohistochemistry in invasive carcinomas of BALB-*neu*T mice. High expression levels of C-22 P0 were found in the cytoplasm and cell membrane of breast carcinomas cells (Fig. 3A).

BALB-*neu*T mice were immunized with the xenogeneic human GST-P0 or with GST protein as a control. Vaccination was initiated at 6 weeks of age when mammary glands

Table 2. Relationship between anti-P0 autoantibodies and prognostic parameters

Prognostic parameters	Anti-P0 antibody-positive patients 8 cases (10.66)	Anti-P0 antibody-negative patients 67 cases (89.36)
ER+	8 (100)	56 (83.6)
ER−	0	11 (16.4)
PR+	8 (100)	56 (83.6)
PR−	0	11 (16.4)
HER2+	0	6 (8.95)
HER2−	8 (100)	61 (91.05)
Ki-67 ≤ 15%	7 (87.5)	36 (53.73)
Ki-67 > 15%	1 (12.5)	31 (46.27)
LNG	8 (100)	54 (80.6)
HNG	0	13 (19.4)
Lymph node status		
N0	4 (50)	32 (47.76)
N+	4 (50)	35 (52.24)

Values given in parentheses are expressed as percentage. ER, estrogen receptor; HNG, high nuclear grading; LNG, low nuclear grading; PR, progesterone receptor.

displayed atypical hyperplasia. By week 17, tumors had developed in all control mice immunized with GST protein. By contrast, none of the mice immunized with the GST-P0 fusion protein exhibited signs of tumor growth at this stage (Fig. 3B). Indeed, in GST-P0 vaccinated mice, tumors began to form at week 19, and at week 24 had affected all immunized mice. Accordingly, vaccination with the GST-P0 fusion protein produced a significant delay in the onset of mammary tumors ($P = 0.012$) (Fig. 3B).

Serum antibodies of GST-P0-BALB-*neu*T vaccinated mice target C-22 P0. The antibody response to the P0 protein elicited in GST-P0-BALB-*neu*T vaccinated mice was investigated by western blotting analysis using the BALB-*neu*T mammary tumor cells (TUBO) as the antigen source. MAb 2B2 detected on TUBO cells a strong immunoreactive protein of 38 kDa, consistent with the molecular weight of P0 (Fig. 4A). Analysis of one representative serum from BALB-*neu*T GST-P0 vaccinated mice and one representative of the GST vaccinated mice is depicted in Figure 4A. Criteria of serum positivity comprised the appearance of an immunoreactive band in TUBO cells co-migrating with the one visualized by MAb 2B2. Antibodies to murine P0 were detected in all six GST-P0 vaccinated mice and not in those vaccinated with GST. Mice vaccinated with human GST-P0 developed a high antibody titer (1:2075, $P < 0.001$ *versus* GST vaccinated mice) to murine P0. In addition, sera from GST-P0 ($n = 5$) but not from GST ($n = 5$) vaccinated mice were able to specifically recognize the C-22 P0 by ELISA (Fig. 4B). This result indicates that the C-22 P0 represents a B cell immunodominant epitope in mouse as well.

Sera from P0-vaccinated mice bind the membrane of mammary cancer cells. To determine whether MAb 2B2 and anti-P0 serum antibodies targeted the C-22 P0 exposed on the membrane of TUBO cells, indirect immunofluorescence was carried out. Our results demonstrated the expression of C-22 P0 on the membrane of TUBO cells grown in the presence of serum (Fig. 5A). However, serum deprivation resulted in enhanced C-22 P0 membrane expression (Fig. 5A). In addition, sera from GST-P0 vaccinated mice bound the membrane of TUBO cells similarly to MAb 2B2. A representative reactivity of one GST-P0 and GST-vaccinated mouse serum is reported (Fig. 5A). The increased plasma membrane expression of the C-22 P0 epitope upon serum deprivation was confirmed by FACS analysis. The percentage of MAb 2B2-stained TUBO cells increased from 48% to 75% in serum-deprived cells. Similarly, the mean fluorescence intensity (MFI) increased from 13 to 24 (Fig. 5B). An enhanced number of C-22 P0-stained cells following serum

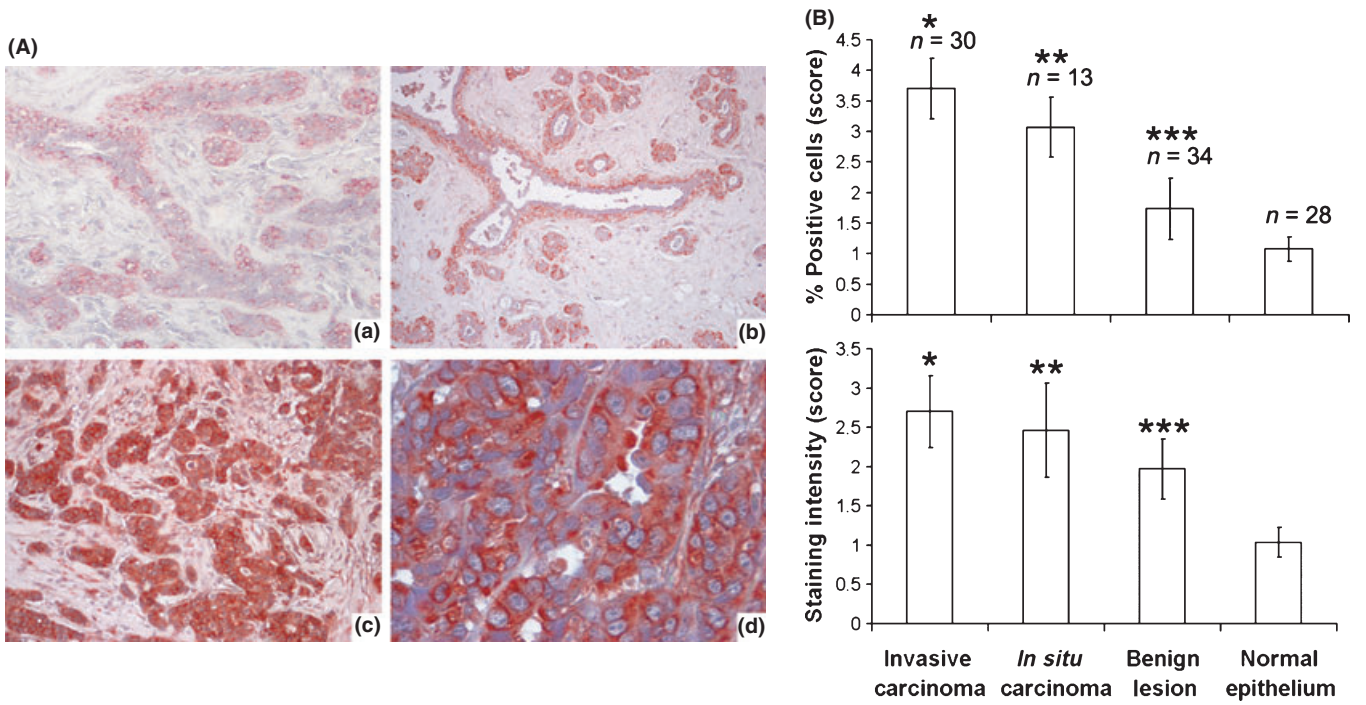

Fig. 2. Expression of C-22 P0 in normal and pathological human mammary tissues. (A) Immunohistochemical detection of C-22 P0 by MAb 2B2 in human mammary tissue. (a) normal mammary epithelium (×100); (b) fibroadenoma (×100); (c) invasive carcinoma (×100); and (d) invasive carcinoma (×400). (B) Bar graph showing a semiquantitative evaluation of C-22 P0 expression. *$P \leq 0.0001$, invasive carcinoma *versus* adjacent normal epithelium and *versus* a benign lesion. **$P \leq 0.0001$, *in situ* carcinoma *versus* normal epithelium. ***$P \leq 0.0001$, benign lesions *versus* normal epithelium.

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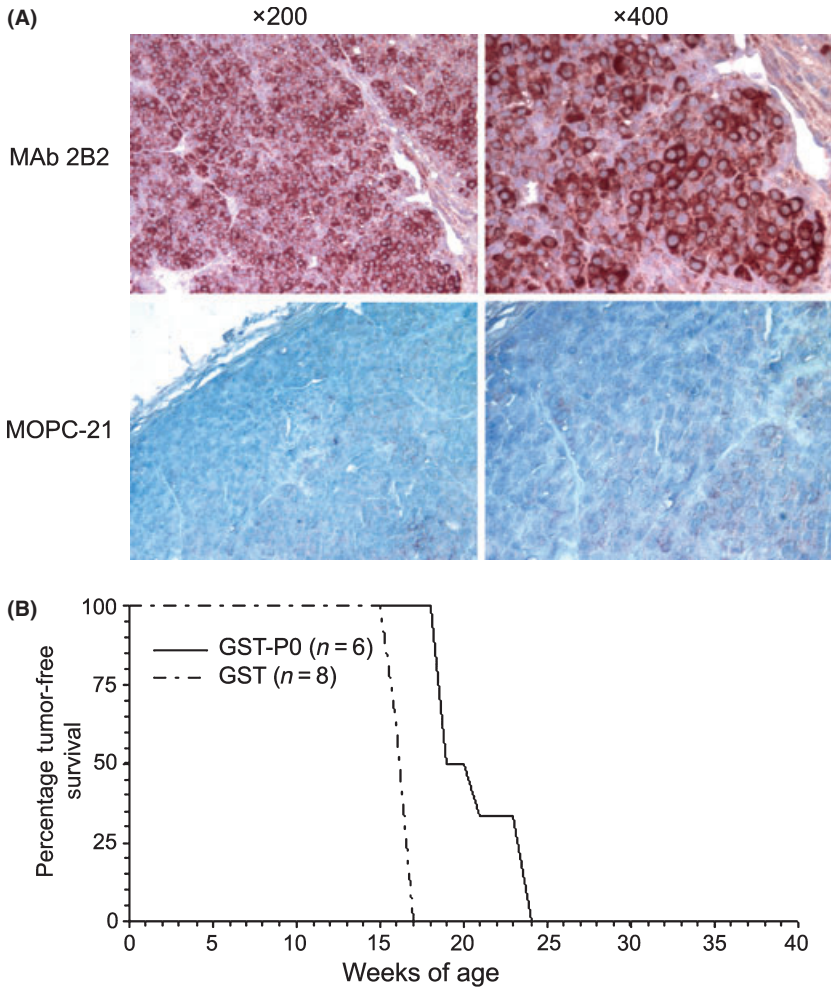

Fig. 3. C-22 P0 expression in invasive carcinoma of BALB-*neu*T mice and delay of neu-mediated mammary carcinoma growth by vaccination of BALB-*neu*T mice with xenogeneic human P0. (A) Immunohistochemical detection of C-22 P0 using MAb 2B2 in mammary carcinomas of BALB-*neu*T mice. Original magnification, ×200 and ×400. (B) Tumor-free survival in BALB-*neu*T mice vaccinated at 6 weeks of age. Six and eight mice were immunized with GST-P0 and GST, respectively ($P = 0.012$).

withdrawal was also revealed by serum from GST-P0-vaccinated mouse (36% *vs* 80%) (Fig. 5B). Collectively, these results show that C-22 P0 might represent an *in vivo* target for anti-C-22 P0 antibodies.

Discussion

It was recently demonstrated that ectopic overexpression of the ribosomal acidic P0 protein enhanced cell proliferation of breast and liver carcinoma cells.[26] In addition, increased expression of P0 was found in hepatocellular and colorectal cancer.[23,24] We recently demonstrated that the carboxyl-terminal epitope of P0 (C-22 P0) was overexpressed in head and neck squamous cell carcinomas.[25] Autoantibodies to P0 and to the C-22 P0 epitope are considered a hallmark of SLE.[30–33] We earlier reported a significant humoral immune response to P0 and to the C-22 P0 in head and neck cancer patients.[25] Overall, the immune recognition of self-antigens is emerging as an important topic in human cancer.[6–13] Autoantibodies in breast cancer sera are proposed as reporters of breast tumorigenesis.[6]

In the present study, we demonstrate the presence of a significant spontaneous humoral response to P0 protein in breast cancer patients. Autoantibodies to P0 were detected in the sera of 8 out of 75 patients with malignant breast cancer as well as four out of 34 patients with benign lesions. Although C-22 P0 appears to be immunodominant in four carcinoma patients, immunity to P0 was not restricted to this epitope at least in the other four carcinoma patients (Table 1). Conversely, autoantibodies restricted to P1 and P2 proteins were observed in the sera of patients with mammary benign lesions only (2/34). The presence of P protein autoantibodies might reveal mammary cell cycle dysregulation. In light of this evidence, further studies in a larger cohort of patients will be necessary to determine whether antibodies to P0 or P1 and P2 could be considered as a potential biomarker of cancer progression. However, anti-P0 autoantibodies were not associated with hormone receptor status, HER2 overexpression, Ki-67 expression and nuclear grading of breast carcinomas. Immunity to P0 protein did not correlate with either lymph nodes status or clinical stage. We earlier demonstrated that cellular stress leads to an increase of C-22 P0 on the pharynx cancer cell surface.[25] It should also be taken into account that within large tumors a proportion of cancer cells are exposed to stress conditions and, accordingly, are prone to necrotic and apoptotic cell death, both of which can favour the induction of autoreactive immune responses.[13,43,44]

The antibodies' response to P0 protein in breast cancer and benign lesion patients was not associated with P0 protein overexpression. In fact, all invasive carcinomas compared with normal epithelium showed a significant overexpression of C-22 P0, which was strongly and homogeneously expressed throughout all epithelium. Overexpression of C-22 P0 was not associated with hormone receptor status, HER2 overexpression, Ki-67 expression and nuclear grading of malignant tumors.

Nevertheless, given that superior cell proliferation, which is a characteristic of aggressive carcinomas, needs enhanced ribosomal synthesis, it may be thought that molecules involved

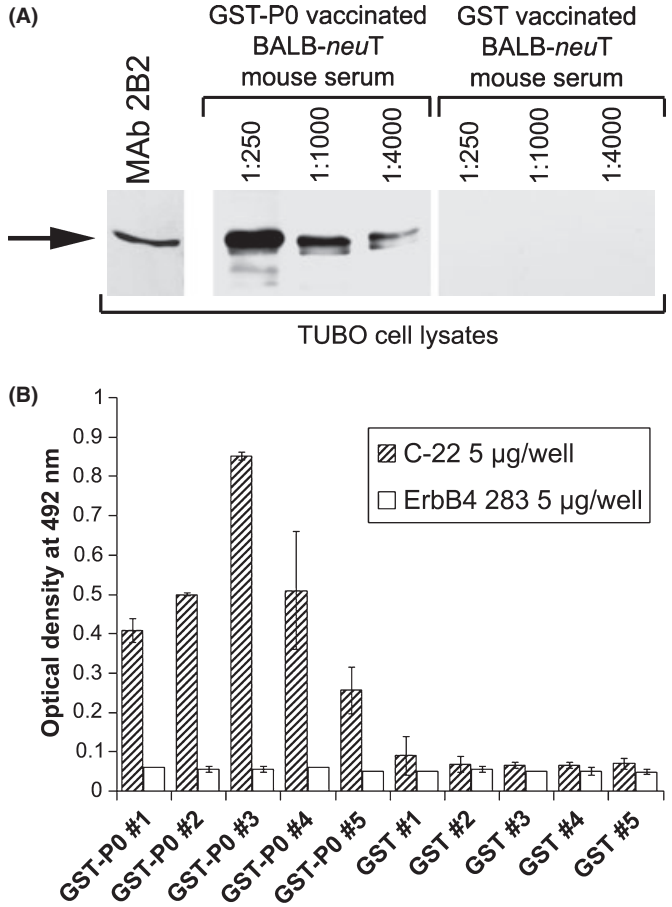

Fig. 4. Antibody response to murine P0 in mice vaccinated with human GST-P0 protein. (A) Mouse sera were assayed at different dilutions. MAb 2B2 served as a positive control. GST-P0 #1 and GST #1 vaccinated mouse sera reactivity is shown. (B) ELISA showing immunoreactivity of vaccinated mouse GST-P0 (GST-P0 #) or GST (GST #) sera with the C-22 P0 peptide. The ErbB4 283 peptide was used as the control peptide. Experiments were repeated twice in duplicate. Column optical density values represent the mean ± standard deviation.

in protein synthesis (e.g. P0 protein) might offer novel immunological targets. To this end, we determined the effect of P0 vaccination on neu-mediated tumorigenesis by using female BALB-*neu*T mice immunized with the xenogeneic human P0. Here, we provide evidence for a significant delay of neu-mediated mammary carcinoma growth by vaccination of BALB-*neu*T mice with human P0 protein. The extent of tumor growth interference *in vivo* was associated with high serum levels of antibodies, which were able to recognize the murine P0 protein expressed on mouse mammary cancer cells as well as the C-22 P0. Accordingly, C-22 P0 might represent an *in vivo* target for anti-C-22 P0 antibodies. It should be highlighted that monoclonal antibodies against P0 were able to penetrate into living cells and cause apoptosis of a human acute T cell leukemia in culture.[45] In addition, anti-P0 antibodies were shown to penetrate into live hepatoma cells and cause cellular dysfunction in culture.[28,46] Accordingly, anti-P0 antibodies elicited by vaccination, apart from activating immunocompetent cells, might directly hamper the growth of mammary cancer cells in BALB-*neu*T mice.[13] It is also worthy to point out that anti-P ribosomal protein autoantibodies were found in some patients with SLE and in some cases associated with psychiatric manifestations, nephritis or hepatitis.[47] Thus, it should be taken into account that anti-P0 antibodies could induce adverse reactions in cancer patients, which can also be dependent on the titer of antibodies. On the other hand, the prevalence of anti-ribosomal P antibodies in SLE patients was shown to be diverse in different ethnic groups and some reports failed to find any relationship of anti-P antibodies and neuropsychiatric SLE or liver and renal diseases in SLE patients.[47,48]

The molecular characterization of self-antigens targeted by autoantibodies in cancer patients, the knowledge of the mechanisms triggering and sustaining autoantibody production and the definition of autoantibodies' biological effects might all help in designing anti-cancer diagnostic and immunotherapeutic strategies. Evidence for immunity to the ribosomal P0 protein, its high-level expression in carcinomas and its surface localization on cancer cells, along with P0's ability to delay growth of neu-mediated mammary carcinomas in BALB-*neu*T mice when used as immunogen, might be relevant for the use of P0 protein in monitoring cancer progression and in planning immunotherapeutic strategies.

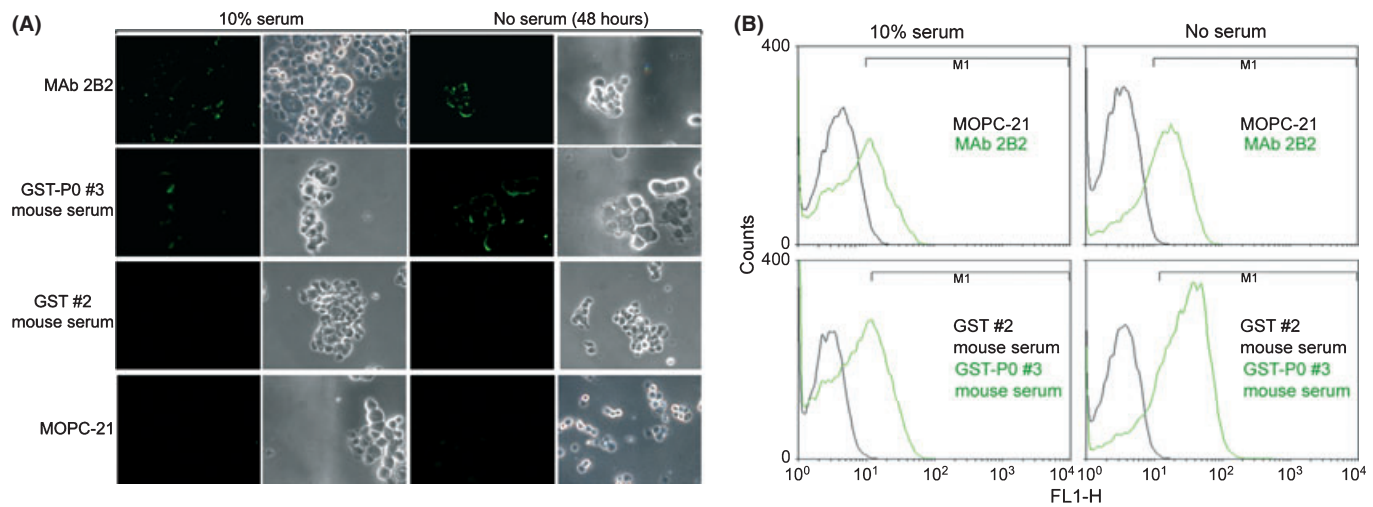

Fig. 5. Mouse vaccinated P0 serum targets C-22 P0 on the membrane of mammary cancer cells (TUBO). (A) Indirect immunofluorescence using MAb 2B2 and P0-vaccinated mouse serum (GST-P0 #3) indicated C-22 P0 surface expression on native TUBO cells. MOPC-21 and GST-vaccinated mouse serum (GST #2) did not react with TUBO cells. Cell morphology was determined by phase-contrast microscopy (IAS version 007 000). (B) FACS analysis: percentage of positive TUBO cells and MFI using MAb 2B2 and P0-vaccinated mouse serum (GST-P0 #3) were determined on the M1 cell-labelled population.

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Disclosure Statement

The authors have no conflict of interest.

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