Monoclonal Antibody PR92 with Restricted Specificity for Tumor-associated Antigen of Prostate and Breast Carcinoma

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ABSTRACT

A unique mouse hybridoma, PR92, was obtained using human prostate adenocarcinoma cell line DU145. The monoclonal antibody produced by the PR92 clone was reactive with DU145, MCF-7 (breast adenocarcinoma), and CHAGO (lung carcinoma), but not with normal cell lines and 16 other cell lines of cancerous origin. A homologous solid-phase sandwich radioimmunoassay (PR92-RIA) was developed utilizing PR92 monoclonal antibody. The PR92-RIA recognized unique antigen present in DU145 cell extract but did not detect 16 other known tumor-associated markers. In preliminary studies, the PR92-RIA measured greater than 5 units/ml level of PR92 monoclonal antibody-binding antigen in 23 of 31 (74%) serum specimens of active prostate cancer and 27 of 31 (87%) active breast carcinoma patients. Only 1 of 79 (1%) sex-matched normal donors and 1 of 57 (2%) benign disease control patients showed the serum antigen level greater than 5 units/ml. A high degree of correlation was observed between the PR92 antigen activity and the clinical status of four prostate and four breast cancer patients during therapeutic treatment. Thus, the PR92-RIA detects new tumor-associated antigen which may be useful in detection and monitoring of prostate and breast carcinoma patients.

INTRODUCTION

Hybridoma technologies have provided means of identifying many new tumor-associated antigens. These antigens were characterized by their immunoreactivity toward specific monoclonal antibodies and were proposed for use as diagnostic and prognostic aids in the clinical management of tumor disease states. For example, the hybridoma clone developed with human colorectal carcinoma cell line SW1116 and epithelial ovarian carcinoma cell line OVCA433 led to the discoveries of tumor-associated antigens CA 19-9 (1) and CA 125 (2), respectively.

Prostate carcinoma ranks third in cancer incidence in men in the United States, following skin cancer and lung carcinoma (3). PAP and PA have been widely used as prostate tumor-associated markers and found to be elevated in sera of prostastic carcinoma patients, especially those in an advanced disease state (4-6). However, neither marker is ideal as a serological marker owing to lack of adequate sensitivity or specificity (5, 7).

Previous investigators prepared monoclonal antibodies against human prostate cancer cell line DU145 or PC3 (8-12). Hybridoma clones were commonly selected based on the observation that MAbS recognize the immunized tumor cells and cross-react less with normal cells or other benign or malignant cell lines of different origin. The selected MAbS generally did not recognize PAP nor PA and exhibited unique staining patterns with pathological prostatic tissues in immunohistochemical studies.

We describe in this paper development of a new hybridoma cell line PR92 which produced MAb with restricted specificity in recognizing tumor cell lines and, in addition, a high degree of specificity and sensitivity in a serological radioimmunoassay, in identifying prostate and breast carcinomas.

MATERIALS AND METHODS

Cell Lines. The following cell lines were obtained from American Type Culture Collection (Rockville, MD) and grown in the media as recommended by the supplier: DU145; PC3; T24; J82; HT1197; HT1376; RT4; M1A; DLD-1; HT29; MCF-7; MDA-MB; BT-20; C33A; SKUT-1B; and ACHN. DU145 cell line was isolated originally by Stone et al. (13) from a lesion in the brain of a patient with metastatic carcinoma of the prostate and a 3-yr history of lymphocytic leukemia. In addition, the cell line 647V from Dr. B. Liu (University of California, Los Angeles, CA) and CHAGO from Dr. J. Braatz (National Cancer Institute, Frederick, MD) were used in this study. Normal human cells of blood type ABH and lymphocytes were prepared from the blood of healthy normal donors. Primary human embryonic kidney cells, cultured and supplied by Dr. Tribby (Abbott Labs, North Chicago, IL), were also used.

Serum Specimens. Clinical serum specimens were provided by Dr. H. Fritsche at M. D. Anderson Hospital (Houston, TX), Dr. A. Malkin at Sunnybrook Medical Center (Toronto, Canada), and Dr. R. Vessella at the V. A. Hospital (Minneapolis, MN). The patient history and the clinical data at the time of sampling were provided by the attending physicians at the institutions. Serum specimens of normal healthy donors were obtained from laboratory workers at Abbott Labs and also at South Chicago Community Hospital (Chicago, IL). Informed consent was obtained from each specimen donor at the respective institutions. All specimens were aliquoted and stored at — 20°C until tested.

Production of Hybridomas. Female BALB/c mice were given injections of DU145 cells following the procedure described by Starling et al. (10). The spleens of the mice were removed for subsequent hybridization. The serum samples from each mouse were assayed for antibodies to DU145 cell using a microtiter plate that had been previously coated with DU145 cell fragments (see the following section). The mouse spleen cells were fused with cells from mouse myeloma cell line SP2/0-Ag14 according to the procedure of De St. Groth and Scheidegger (14). Hybridoma clones which produced antibodies exhibiting strong specificity to DU145 cell line were selected for further propagation to obtain pure clones for amplification. Following the subcloning process, supernatant solutions were screened against DU145 cell fragments, Hek extract, purified CEA, and PAP (Abbott Labs.). Sixteen hybridomas showing strong binding with DU145 cell line but essentially no binding with Hek extract, CEA, and PAP were selected. Pristane-primed female BALB/c mice were given injections of 45 × 10⁶ hybridoma cells. The ascitic fluid suspensions were passed in vivo every 7 to 10 days. Antibodies produced by each of the 16 clones were further tested for binding specificity using the plates that had been coated with cell fragments of normal cells or various tumor cell lines.

Preparation of Cell-coated Plate. Viable cells of 10 to 20 × 10⁶ cells/ml in Hanks' balanced salt solution (Gibco, Grand Island, NY) containing 2 mm phenylmethylsulfonyl fluoride and 20 μl/ml of trasyol were sonicated for 2½ min to disrupt the cells into fragments. The fragments, equivalent to 5 × 10⁶ cells/70 μl, were added to each well of a microtiter plate (Dynatech, Alexandria, VA), and the plate was centrifuged for 3 min at 1400 × g on a Beckman J-6 centrifuge.
isolated mucin extract were gifts of Dr. G. Hass and J. Slota (Abbott Labs.).

To assess immunological specificity of PR92-RIA, some of the recognized tumor-associated markers were tested for a dose-response phenomenon. The standard reagents included in the commercially available immunoassay kits were used as a source of PAP, CEA, AFP, and ferritin (Abbott Labs.); PA (Yang Labs., Bellevue, WA); and CA 19-9, CA 125, and CA 15-3 (Centocor, Malvern, PA), respectively. Human actin, fibrinogen, and β₂-microglobulin were obtained from Calbiochem (La Jolla, CA). Human blood group substances and human colon mucin extract were gifts of Dr. G. Hass and J. Slota (Abbott Labs.).

Molecular Weight Determination. Molecular weight of PR92 MAb-binding protein was estimated by high-performance liquid chromatography (HPLC unit; Perkin-Elmer, Norwalk, CT) with a Bio-Sil TSK 250 gel filtration column (Bio-Rad, Richmond, CA), using Bio-Rad gel filtration standard (Bio-Rad) as the molecular weight marker. The column eluent was monitored by PR92-RIA to measure the PR92 MAb binding-protein level in each fraction.
ease patient populations. This serum panel was used to compare the different RIA systems in relation to each other. The overall results (not shown) indicated that the MAb of H92C149 in combination with itself (homologous) has superior sensitivity and specificity to other combinations of MAb in RIA systems in identifying the serum specimens from prostate and breast cancer patients. Thus, we designated this monoclonal antibody, PR92 MAb, and investigated its molecular characteristics and explored potential application in developing a clinical test.

Production and Purification of PR92 MAb. In vitro culture of H92C149 clone to produce PR92 MAb has not been successful. Therefore, H92C149 hybridoma cells were passed in vivo using primed mice, and PR92 MAb was isolated from mouse ascitic fluid. In addition to PR92 MAb of the IgG1 subtype, a small but significant amount of nonspecific IgG2a and IgG3 was present in the ascitic fluids. Thus, PR92 MAb was isolated from the other nonspecific immunoglobulins, in Protein A column procedure, by elution at pH 5.5.

Evaluation of PR92-RIA. A typical standard curve of PR92-RIA is shown in Fig. 1. One batch of soluble DU145 cell extract was assigned as the primary standard reagent containing an arbitrary 100 units/ml of the PR92 MAb-binding antigen, as assayed by the PR92-RIA. To determine the immunological specificity, the PR92-RIA was tested with a number of recognized tumor-associated antigens, including PAP, PA, CEA, and AFP. The results presented in Table 2 indicate that the PR92-RIA specifically detects a unique antigen present in the DU145 cell extract.

Serum specimens from the patients diagnosed to have cancer of the kidney, ovary, bladder, liver, thyroid, colorectum, esophagus, stomach, brain, head, neck and cervix, as well as the sera from patients with melanoma and leukemia, were subjected to the PR92-RIA testing. The clinical status of a cancer patient was determined by the attending physician as progressive (advance of disease), remission (diminution of disease symptoms), response (improvement resulting from therapy), stable (no change in status), or NED. For the purpose of analysis, the serum specimens of cancer patients were grouped, in this study, as progressive status or nonprogressive (remission, stable, quiescent, NED) status. The results are shown in Fig. 2.

Serum specimens from 58 normal males over 50 yr old, 29 specimens from patients with benign prostate disease, and 30 from prostate cancer patients at clinically progressive status were assayed by the PR92-RIA. The results are shown in Fig. 3. One hundred ten serum specimens, obtained from normal female subjects and patients with benign or malignant breast disease, were also assayed by the PR92-RIA, and the results are shown in Fig. 4. Specimens listed in Figs. 2 to 4, with values greater than 50, 11, and 30 units/ml, respectively, could not be reassayed due to limited specimen volume.

Serum specimens of four prostate cancer patients at Stage D were collected over a period ranging from 108 to 719 days, stored at −20°C, and analyzed by the PR92-RIA. The results were correlated to clinical evaluations of the disease state of the patients at the time of sampling. The resulting data are set forth in Fig. 5. In a similar manner, serum specimens from four patients with breast cancer at Stage IV, taken at clinically relevant times, were assayed by the PR92-RIA, and the results are presented in Fig. 6.

The molecular weight distribution of the PR92 MAb-binding protein (PR92 antigen) was investigated by subjecting DU145 cell extract to high-performance liquid chromatography. Analysis of the column eluent by PR92-RIA revealed that PR92 antigen eluted as a single peak of which the molecular weight corresponded to about 470,000.

DISCUSSION

Many investigators have generated hybridomas using established tumor cell lines (8, 9, 10, 12, 17, 18) and tested the antibodies produced by the hybridomas against a variety of normal, benign, or malignant cells. The hybridomas whose MAb displayed specific affinity to certain types of cancer cells were selected for detailed investigation, such as immunohistochemistry. The objective of the present study was to search for hybridomas, producing monoclonal antibodies that can specifically recognize prostate tumor-associated antigen(s) that may
be present in serum or urine specimens of the prostate cancer patients. It was hoped that the MAb could provide a base to develop an immunodiagnostic test for detection and/or prognosis of a prostate neoplasm.

PR92 MAb is distinct from the monoclonal antibodies D83.21, α-Pro3, and KR-P8, which were raised against prostate carcinoma cell lines DU145 and PC3 (8–10). The other monoclonal antibodies were reported to bind both DU145 and PC3 cells as well as some bladder cancer cell lines. The PR92 MAb bound to DU145 cells, but did not bind PC3 nor any of the six bladder carcinoma cell lines tested. Raynor observed that KR-P8 MAb strongly interacted with some antigens present in human seminal plasma (9). The PR92-RIA, however, failed to detect any seminal antigen.3

3 Unpublished observation.
The PR92-RIA demonstrated immunological specificity by displaying a positive dose-response phenomenon with the soluble antigens in DU145 cell extract (Fig. 1), while showing negative responses with 16 other tumor-associated proteins (Table 2). This observation indicates that the antigenic determinant(s) of the molecules detected by PR92 MAb is distinct from any of the known tumor-associated proteins. It is noteworthy that the PR92-RIA did not recognize mucin molecules related to CA 19-9, CA 125, CA 15-3, and a human colonic mucin preparation. This, however, does not rule out the possibility that PR92 antigen may be a glycoprotein derived from some type of mucins.

Serum specimens of patients with benign or malignant diseases were screened by the PR92-RIA. For the purpose of comparing the serum PR92 antigen level in different groups, 5 units/ml of PR92 antigen were assumed to be the highest value of normal specimens. The results presented in Fig. 2 revealed that 1 of 10 specimens of benign disease cases and, among cancer cases, 6 of 16 ovarian, 1 of 16 bladder, 1 of 6 liver (or thyroid), 1 of 10 esophageal (or stomach) carcinomas, and 1 of 10 melanoma (or leukemia) cases showed greater than 5 units/ml of PR92 antigen level. In contrast, the serum specimens from patients with progressive prostate or breast carcinoma showed generally higher PR92 antigen activity compared to the specimens of normal and benign disease controls and nonprogressive prostate or breast carcinoma cases. This observation motivated us to investigate the potential utility of the PR92-RIA in detecting progressive prostate or breast carcinoma cases.

Of the specimens from 58 normal males and 29 patients with benign prostate and urogenital diseases, only one showed PR92 antigen activity greater than 1 unit/ml. In contrast, 23 of 31 specimens from progressive prostate cancer patients displayed reactivity above 5 units/ml. It should be noted that 21 of 23 specimens from cancer patients at Stage C/D showed an elevated PR92 antigen level, while 5 of 7 specimens of Stage A/B patients yielded the antigen level below 5 units/ml. A similar trend was observed in the study of breast carcinoma cases. Of 21 serum specimens from normal female volunteers and 28 from patients with benign breast disease, 1 displayed reactivity above 5 units/ml. Among 61 diagnosed breast cancer patients, 30 were at a clinically nonprogressive state, while 31 were judged to have progressive cancer. Compared to 27 of 31 specimens from progressive breast cancer patients, only 3 of 30 from nonprogressive cancer patients displayed a PR92 antigen level greater than 5 units/ml. Considering that the majority of the progressive breast cancer cases examined here were at Stage C/D, these results suggest a significant clinical correlation between the elevation of serum PR92 antigen activity and the advance of tumor stage in prostate or breast cancer patients.

The potential use of the PR92-RIA as an indicator of clinical status of a patient was studied with a series of serum specimens. The results depicted in Figs. 5 and 6 indicate a general increase in PR92 antigen activity above the normal level with progression of malignant disease and a decrease when a patient responds to clinical treatment. There appears to be a high degree of correlation between the PR92 antigen level and the clinical status of the prostate and breast cancer patients. These findings indicate that the sensitivity of the PR92-RIA is such that it may be useful as an indicator of a prostate or breast cancer disease state.

We presented in this paper some evidence that PR92 MAb specifically recognizes some cancer cell lines. The amount of the PR92 antigen in serum specimens of prostate and breast cancer patients appeared to depend upon the tumor stage, but
more extensive study, including a large number of documented serum specimens, is necessary to establish this relationship. Comparison of the PR92-RIA with other recognized prostate or breast tumor marker-assays may reveal the merit of this new serum test in detection or monitoring of cancer. Preliminary data in our laboratory suggest that the PR92 antigen is a glycoprotein with an approximate molecular weight of 470,000 and may be secreted into body fluids during the development of malignant disease. An investigation is currently in progress to elucidate molecular characteristics and to understand any physiological function of the PR92 antigen.

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REFERENCES

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