Chemotherapy often results in the generation of cancers that are resistant not only to the specific drugs used in the regimen but also to a variety of structurally and functionally unrelated anticancer drugs, and this phenomenon is known as multidrug resistance (MDR). The MDR phenomenon can easily be reproduced in the laboratory by growing the drug-sensitive cultured cancer cells in the presence of anticancer drugs. This ability has facilitated in identifying several overexpressed MDR-related proteins as the mediators of the MDR development in cancers (1). One of these proteins is P-glycoprotein (Pgp), a 1,280–amino acid polypeptide (2, 3), which is well established in cancers (1). The MDR phenomenon can easily be reproduced in the laboratory by growing the drug-sensitive cultured cancer cells in the presence of anticancer drugs. This ability has facilitated in identifying several overexpressed MDR-related proteins as the mediators of the MDR development in cancers (1). One of these proteins is P-glycoprotein (Pgp), a 1,280–amino acid polypeptide (2, 3), which is well established in cancers (1). As the NH$_2$11 antibody detects Pgp present in cells and tissues, we conclude that the amino acid sequence to which this antibody was raised is highly antigenic and the antibody is useful in the detection of Pgp by a variety of immunologic methods.

Abstract

**Purpose:** Cancers exposed to chemotherapy develop multidrug resistance, a major cause for chemotherapy failure. One mechanism of multidrug resistance development is due to overexpression of P-glycoprotein (Pgp) in these cancer cells. Thus, a prechemotherapy evaluation of Pgp in cancer cells aids in the design of alternative regimens that can circumvent such failure. As few Pgp-specific antibodies are available in detecting low levels of Pgp, there is a need for preparing an antibody that allows the detection of Pgp by various immunologic methods.

**Experimental Design:** We selected the amino acid stretch 11 to 34 in the cytoplasmically located NH$_2$ terminus of Pgp as antigen, which was chemically synthesized and used to raise an antibody in a rabbit, termed NH$_2$11 antibody. We compared the properties of NH$_2$11 antibody with that of the well-characterized Pgp-specific antibody, C219, by Western blotting, immunoprecipitation, immunocytochemistry, and immunohistochemistry.

**Results:** Immunoblotting analysis suggested that NH$_2$11 antibody efficiently interacts with both recombinant and constitutively expressed Pgp in cancerous and noncancerous human cells. Immunoprecipitation reactions indicated that the NH$_2$11 antibody selectively immunoprecipitates Pgp. Immunocytochemical analyses indicated that the NH$_2$11 antibody detects Pgp in drug-resistant breast cancer cells as well as in human prostate and breast adenocarcinoma tissue sections.

**Conclusion:** As the NH$_2$11 antibody detects Pgp present in cells and tissues, we conclude that the amino acid sequence to which this antibody was raised is highly antigenic and the antibody is useful in the detection of Pgp by a variety of immunologic methods.

Chemotherapy often results in the generation of cancers that are resistant not only to the specific drugs used in the regimen but also to a variety of structurally and functionally unrelated anticancer drugs, and this phenomenon is known as multidrug resistance (MDR). The MDR phenomenon can easily be reproduced in the laboratory by growing the drug-sensitive cultured cancer cells in the presence of anticancer drugs. This ability has facilitated in identifying several overexpressed MDR-related proteins as the mediators of the MDR development in cancers (1). One of these proteins is P-glycoprotein (Pgp), a 1,280–amino acid polypeptide (2, 3), which is well established in cancers (1). The MDR phenomenon can easily be reproduced in the laboratory by growing the drug-sensitive cultured cancer cells in the presence of anticancer drugs. This ability has facilitated in identifying several overexpressed MDR-related proteins as the mediators of the MDR development in cancers (1). One of these proteins is P-glycoprotein (Pgp), a 1,280–amino acid polypeptide (2, 3), which is well established in cancers (1). As the NH$_2$11 antibody detects Pgp present in cells and tissues, we conclude that the amino acid sequence to which this antibody was raised is highly antigenic and the antibody is useful in the detection of Pgp by a variety of immunologic methods.

Although most studies correlate the expression of Pgp with the development of MDR in many cancers, detection of Pgp has been imperfect due to the low specificity of many commonly used antibodies of Pgp and due to the variations in the methodologies used to detect Pgp (1). The currently available antibodies for the detection of Pgp can generally be classified based on the location of their interacting epitopic region in the Pgp molecule as those recognizing intracellular regions and those recognizing extracellular regions. The C219 antibody recognizes two defined epitopes, 568-VQVALD-573 and 1213-VQVELD-1218, and the C494 antibody recognizes the epitope 1028-PNTLEGN-1034 in the Pgp molecule (8), all three of which are predicted to be intracellular in location. Although the JSB-1 antibody is shown to recognize the intracellular region of Pgp, the exact location of its epitope is unclear (9). The antibodies UIC2 and 4E3, which recognize extracellular regions of Pgp, seem to be conformation dependent (10–12). Although all of these antibodies recognize Pgp, in general, their use in the analysis of Pgp structure or detection by immunohistochemistry is limited due to their varied degrees of immunoreactivity and specificity (1).

The long-term goal of our laboratory is to understand the Pgp-mediated MDR in cancers. To accomplish this goal, we raised an antibody in rabbit against a synthetic peptide epitope comprising amino acids 11-AKKNFFKLNKSEKDKKEKKPTV-34 in the Pgp molecule. The results of characterization presented here show that this antibody, in addition to being...
able to detect Pgp by a variety of immunologic methods, it is also capable of detecting Pgp in frozen tissue sections. Thus, we expect that this antibody will serve as a valuable diagnostic tool to detect Pgp in tumor biopsy samples as well as in normal tissues that express low amounts of this drug transporter.

**Materials and Methods**

**Cell lines and cell culture conditions.** The Adriamycin-resistant breast cancer cell line (MCF-7/Adr), was kindly provided by Dr. Kenneth Cowan, Eppley Institute, UNMC, Omaha, NE (13). The cells (MCF-7/Adr, HEK-293, and prostate PC3) were grown in DMEM containing high glucose (4.5g/L), antibiotics (100 units/mL penicillin and 100 µg/mL streptomycin), and 10% (v/v) fetal bovine serum in a 37°C humidified incubator with 5% CO2. Chinese hamster ovary cells were grown and maintained in F-12 medium containing 10% fetal bovine serum.

**NH211 antibody.** To raise antibody against the NH2-half of Pgp, the amino acid sequence 11-AKKKNFFKLNNKSEKDKKEKKPTV-34 in the Pgp molecule was chosen as an epitope based on its overall hydrophilicity. Synthesis of this immunizing peptide and coupling to carrier protein, keyhole limpet hemocyanin, and generation of antibodies in rabbit were carried out at AnimalPharm Services (Heidelberg, CA). Western blotting was used to screen for the presence of antibodies in the test bleed sera. Serum collected from rabbit 3030 exhibited strong and specific binding to Pgp, which was termed NH211 antibody.

**Reverse transcription-PCR.** Standard molecular biology methods were followed in these studies. Detection of MDR1 transcripts in cells was carried out by the reverse transcription-PCR (RT-PCR). Briefly, cells were grown in 35 mm dishes to near confluency. Total RNA was extracted using the RNeasy Mini kit (Qiagen, Valencia, CA) according to the manufacturer’s instructions. First strand was synthesized using 10 µg total RNA in a reverse transcription reaction using the Prostar First Strand RT-PCR kit (Stratagene, Cedar Creek, TX). PCR was carried out in a total volume of 50 µL containing 600 ng first strand mixture, Deep Vent DNA polymerase (New England Biolabs, Beverly, MA), and the following primer pair: forward primer 5’-GTCTACATGGATGA-GATTTGTCGCAGACAAACGCT-3’ and reverse primer 5’-CTTGCCGGCCGTTCACTCCTCGTGGC-3’. A total amount of 570-bp DNA fragment from bases 1,482 to 2,052 of the MDR1 cDNA. PCR products were separated on a 1% agarose gel containing ethidium bromide. The DNA fragments were visualized by the Bio-Rad Gel Doc 2000 system (Hercules, CA).

**Immunocytochemistry.** MCF-7/Adr cells grown on coverslips were fixed with 2% paraformaldehyde for 15 minutes and incubated in PBS containing 1% goat serum and 0.25% NP40 for 1 hour. The cells were fixed with 2% paraformaldehyde for 15 minutes and incubated in PBS containing 1% goat serum and 0.25% NP40, 0.5% sodium deoxycholate, 0.1% SDS, and 10% (v/v) fetal bovine serum in a 37°C humidified incubator with 5% CO2. Chinese hamster ovary cells were grown and maintained in F-12 medium containing 10% fetal bovine serum.

**Immunoprecipitation.** The S9 insect cells infected with the MDR1 cDNA carrying baculovirus as described previously (15) were lysed 3 days after infection in radioimmunoprecipitation assay buffer [PBS containing 1% NP40, 0.5% sodium deoxycholate, 0.1% SDS, and protease inhibitors (Roche Diagnostics, Mannheim, Germany)]. Cells were also lysed in buffer [20 mmol/L MOPS, 2 mmol/L EGTA, 2 mmol/L EDTA, 2 mmol/L orthovanadate, 30 mmol/L NaCl (pH 7.0)] containing either 1% NP40, 1% Triton X-100, or 1% octylglucoside. The cells were lysed in the above buffers for 10 minutes at 4°C, and the insoluble material was removed by centrifugation at 14,000 rpm for 15 minutes. The supernatants were incubated with 1 µL NH211 antibody [1:30,000 (v/v)], preimmune serum [1:150 (v/v)], or C219 [1:1,000 (v/v)] and 25 µL protein A/G agarose suspension (Santa Cruz Biotechnology) overnight at 4°C. The beads were washed thrice with the respective lys buffers (1 mL each) and then resuspended in 50 µL Laemmli dis aggregating buffer. Equal volumes (10 µL) of immunoprecipitates were analyzed by Western blotting as described previously (15).

**SDS-PAGE and Western blotting.** The cells growing in the tissue culture dishes were scraped into the growth medium and centrifuged at 2,000 rpm for 5 minutes in a microcentrifuge. The pellet cells were washed once with ice-cold PBS, and the cellular proteins were precipitated with 6% (w/v) trichloracetic acid. The precipitated proteins in the cell lysates were dissolved in Laemmli dis aggregating buffer and separated on 7.5% SDS-PAGE gels and transferred electrophoretically onto polyvinylidine difluoride membranes (16, 17). The immunoblots were developed using the either NH211 or C219 antibody in conjunction with horseradish peroxidase–coupled secondary antibody and the enhanced chemiluminescence assay kit (Amersham, Piscataway, NJ) as described previously (18).

**Results**

**Western blotting analysis of NH211.** To facilitate the identification of NH2-half of Pgp, we searched the cytoplasmically located NH2 terminus for a highly hydrophilic stretch of >20 amino acids lacking cysteine residues as an antigen for raising an antibody. This search resulted in the identification of 11-AKKKNFFKLNNKSEKDKKEKKPTV-34 in the Pgp molecule as an epitope. This peptide was synthesized chemically and injected i.p. in a rabbit, whose preimmune serum did not interact with any proteins in the S9 insect cells or MCF-7 cell lysates (data not shown). Serum collected after three boosters was termed NH211 antibody, as it was directed against an amino acid sequence at the NH2 terminus beginning at residue 11. The NH211 antibody was tested by Western blotting for its

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3We are aware that the identity of the National Cancer Institute–supplied Adriamycin-resistant breast cancer cell line, MCF-7/Adr, is being identified as NCI/Adr recently. Because this cell line was characterized by Dr. Cowan and his coworkers previously (13), we preferred the name MCF-7/Adr to describe the cell line used in this article.

**Immunohistochemistry.** Paraffin-embedded human prostatesctomy specimens with adenocarcinoma (Gleason grade 7) and the human breast adenocarcinoma specimens were deparaffinized in neat xylene for 20 minutes. The slides were then treated for 3 minutes each with 100%, 95%, 70%, 30%, and 0% ethanol in water to bring about gradual dehydration of tissue sections. Slides were washed with PBS followed by incubation with 10% normal goat serum dissolved in PBS for 60 minutes at room temperature. The tissue sections were then incubated overnight at 4°C with preimmune serum obtained from rabbit 3030 [1:30,000 (v/v) dilution], NH211 antibody [1:30,000 (v/v) dilution], or C219 antibody [1:3,000 (v/v) dilution] diluted in PBS containing 1% (w/v) bovine serum albumin. After washing thrice in PBS (10 minutes each), slides were incubated with the corresponding Alexa Fluor 488–conjugated secondary antibody (Molecular Probes, Eugene, OR) at 1:1,000 (v/v) dilution in PBS for 60 minutes at room temperature. The slides were washed with PBS as described above and mounted in Antifade solution (Molecular Probes). The Alexa Fluor 488 fluorescence in the tissue sections was analyzed by Leica (Leica Microsystems, Wetzlar, Germany) DMIRE fluorescence microscope (14).

**Immunoprecipitation.** The S9 insect cells infected with the MDR1 cDNA carrying baculovirus as described previously (15) were lysed 3 days after infection in radioimmunoprecipitation assay buffer [PBS containing 1% NP40, 0.5% sodium deoxycholate, 0.1% SDS, and protease inhibitors (Roche Diagnostics, Mannheim, Germany)]. Cells were also lysed in buffer [20 mmol/L MOPS, 2 mmol/L EGTA, 2 mmol/L EDTA, 2 mmol/L orthovanadate, 30 mmol/L NaCl (pH 7.0)] containing either 1% NP40, 1% Triton X-100, or 1% octylglucoside. The cells were lysed in the above buffers for 10 minutes at 4°C, and the insoluble material was removed by centrifugation at 14,000 rpm for 15 minutes. The supernatants were incubated with 1 µL NH211 antibody [1:30,000 (v/v)], preimmune serum [1:150 (v/v)], or C219 [1:1,000 (v/v)] and 25 µL protein A/G agarose suspension (Santa Cruz Biotechnology) overnight at 4°C. The beads were washed thrice with the respective lys buffers (1 mL each) and then resuspended in 50 µL Laemmli dis aggregating buffer. Equal volumes (10 µL) of immunoprecipitates were analyzed by Western blotting as described previously (15).

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ability to recognize Pgp. Pgp expressed in Sf9 insect cells via infection with a MDR1 cDNA-harboring baculovirus was used as a source of Pgp (15). The initial experiments indicated that the serum could be used at a dilution of 1:30,000 (v/v) in the Western blotting analysis without diminishing the intensity of Pgp in the test samples. Figure 1A shows a typical Western blotting analysis of Pgp expressed in Sf9 insect cells using NH211 antibody. A single ~140-kDa protein is reactive with the serum, which was absent in Sf9 insect cells infected with a sham baculovirus. To corroborate that it is Pgp that was recognized by the test serum, a duplicate blot was probed with C219 antibody at a dilution of 1:3,000 (v/v). Figure 1B shows that C219 antibody also recognized an identical ~140-kDa band in the Pgp-containing membranes. Although not shown, the 140-kDa Pgp in the blots developed with NH211 could be superimposed after reprobing with C219 antibody and vice versa (see Fig. 2). To further show that the antibody interacted with the above epitope in Pgp, a Western blot was developed with the NH211 antibody that was mixed with or without the immunizing peptide at a protein ratio of 1:1 (w/w). Figure 1C shows that the NH211 antibody recognizes the 140-kDa Pgp (left). However, the NH211 antibody did not recognize Pgp in the presence of immunizing peptide (right). These results suggested that NH211 interacts with the above amino acid sequence in the Pgp molecule.

**Immunoprecipitation of P-glycoprotein with NH211 antibody.**

To determine if the NH211 antibody could immunoprecipitate Pgp, Pgp expressed in Sf9 insect cells was solubilized in buffers containing NP40, Triton X-100, or octylglucoside as well as in

**Fig. 1.** Western blotting analysis of Pgp by NH211 and C219 antibodies. Membrane fraction (10 μg protein) obtained from the Sf9 insect cells expressing the human Pgp (15) was separated by SDS-PAGE followed by immunoblotting. The blots were probed with NH211 (A) or C219 (B) antibody. As control, membrane fraction (10 μg protein) obtained from Sf9 insect cells infected with a sham baculovirus was used. The ~140-kDa protein band (arrow) is the nonglycosylated form of Pgp (15). Pgp samples were loaded in six lanes and the membrane blot was cut into halves for immunoblotting analysis. C, immunoblots developed with NH211 antibody mixed without (left) or with (right) immunizing peptide as mentioned in Materials and Methods.

radioimmunoprecipitation assay buffer. The solubilized Pgp was then immunoprecipitated as described in Materials and Methods. We have employed different detergents in these experiments as membrane proteins needed to be solubilized from the lipid bilayer and to determine a suitable detergent that does not disrupt the antigen-antibody interactions. Figure 2 shows that the 140-kDa Pgp was solubilized in all of the above buffers containing different detergents (lanes L1-L4), which was detected by both NH211 (Fig. 2A) and C219 (Fig. 2B) antibodies. In these analyses, antibody-reactive ~80-kDa proteins are also present, which are proteolytic products of Pgp. Pgp thus solubilized in different detergents was immunoprecipitated using either NH211 or C219 as described in Materials and Methods. The immunoprecipitates obtained using NH211 and C219 antibodies were analyzed by Western blotting by probing with C219 and NH211 antibodies, respectively. Figure 2A (lanes IP1-IP4) shows that the NH211 antibody immunoprecipitated the 140-kDa protein, which is reactive with C219 antibody, indicating that this 140-kDa protein is Pgp. Similarly, the C219 antibody immunoprecipitated the 140-kDa Pgp, which is reactive with NH211 antibody (Fig. 2B, lanes IP1-IP4). Pgp was not immunoprecipitated when preimmune serum was used (data not shown). Thus, immunoreactivity of 140-kDa protein with both NH211 and C219 antibodies together suggest that the immunoprecipitated protein is Pgp.

**Detection of P-glycoprotein in cultured cell lines.** To determine if the NH211 antibody also recognizes Pgp expressed constitutively in mammalian cell lines, Western blotting analysis was carried out on cell lysates obtained from the drug-sensitive breast cancer MCF-7, Adriamycin-resistant MCF-7/Adr<sup>B</sup>, prostate cancer PC3, human kidney HEK-293 cells, and the Chinese hamster ovary cells. Figure 3A shows that the NH211 antibody did not react with any protein in the drug-sensitive MCF-7 cells. However, a single ~170-kDa protein was
reactive with the NH211 antibody in MCF-7/AdrR and HEK-293 cells. Interestingly, the prostate cancer cells (PC3 cells) also contained significant amount of a similar ~170-kDa protein. No protein was detectable in the Chinese hamster ovary cells, which could be either due to the absence of Pgp in these cells or due to the lack of sequence homology in the epitope region of the human and hamster Pgp forms. To confirm that the ~170 kDa detected by the NH211 antibody is Pgp, the drug-sensitive MCF-7 and Adriamycin-resistant MCF-7/AdrR cell lysates were analyzed by Western blotting using C219 antibody. Figure 3B shows that the C219 antibody also recognizes the ~170-kDa protein present in the MCF-7/AdrR cells, suggesting that NH211 antibody recognizes Pgp expressed in the mammalian cells.

Reverse transcription-PCR analysis of the MDR1 transcript. To further determine that NH211 antibody recognized protein in the above cell lines was Pgp, the presence of MDR1 mRNA was determined by RT-PCR using primers specific for the human MDR1 gene as described in Materials and Methods, and the results are shown in Fig. 4. As expected from the use of the designed primers, the RT-PCR amplified a 573-bp DNA fragment in all of the human cell lines, suggesting the presence of MDR1 mRNA in these cell lines. Interestingly, the staining intensity of this DNA fragment in MCF-7/AdrR, HEK-293, and PC3 cells correlates well with the intensity of Pgp protein in these cells (Fig. 3A). However, the 573-bp DNA fragment was also present in the drug-sensitive MCF-7 cells, although these cells do not contain Pgp protein (Fig. 3A). Although not shown, we have sequenced cDNA fragment amplified from the MCF-7 cells, which clearly indicated that it is the MDR1 cDNA. Presence of MDR1 mRNA and absence of Pgp in drug-sensitive MCF-7 cells were also noticed by other investigators (19).

Detection of P-glycoprotein expressed in the presence of anticancer drugs. Pgp expression in drug-resistant cells is generally known to increase with exposure to anticancer drugs. Thus, the MCF-7/AdrR cells were grown overnight in the presence of different anticancer drugs and the levels of Pgp were determined by Western blotting using the NH211 antibody. Figure 5 shows the expression of Pgp increases significantly when cells were grown in the presence of etoposide, mitoxantrone, and doxorubicin, all of which are known Pgp transport substrates. However, the amount of Pgp expressed in the

**Fig. 3.** Western blotting analysis of Pgp expressed in mammalian cells. Lysates from MCF-7, MCF-7/AdrR, Chinese hamster ovary (CHO), HEK-293, and prostate cancer PC3 cells (20 μg protein) were separated on SDS-PAGE followed by immunoblotting. A, blot developed with NH211 antibody. B, blot developed with C219 antibody. Arrow, ~170 kDa Pgp.

presence of methotrexate, a non-Pgp substrate, remained nearly unchanged. Incubation of drug-sensitive MCF-7 cells in these anticancer drugs resulted in cell death without the expression of Pgp (data not shown). These results are expected as Pgp induction is well established to be a gradual process, requiring exposure to a gradual increase in the concentration of anticancer drugs to these cells. Taken together, these results suggest that anticancer drugs, which are Pgp transport substrates, increase the Pgp levels, and these can be detected by Western blotting using NH211 antibody.

Detection of P-glycoprotein in drug-resistant MCF-7/AdrR cells by immunocytochemistry. To determine if the NH211 antibody, which was raised against a Pgp peptide, is useful in the detection of Pgp by immunocytochemical methods, the MCF-7/AdrR cells were grown on coverslips and immunostained with the NH211 antibody. Pgp staining was not observed when the immunizing peptide at a ratio of 1:1 (w/w; data not shown). Although the C219 antibody stained the plasma membranes of these cells similarly, the staining intensity was weak (data not shown). These data suggested that the NH211 antibody interacts with Pgp in cells and thus is useful in detecting Pgp.

Detection of P-glycoprotein in the human prostate and breast adenocarcinoma tissue sections. To determine if NH211 antibody is useful in detecting Pgp in tissues, the human prostatic adenocarcinoma and breast adenocarcinoma tissue sections were analyzed by immunohistochemistry as described in Materials and Methods. Figure 7 shows the immunostaining of the prostate adenocarcinoma sections with C219 (Fig. 7A) and NH211 (Fig. 7B) antibodies, which indicated that mainly the basal cells lining the epithelial compartment of the prostate gland were immunoreactive with these antibodies. The
preimmune serum did not immunoreact with any of these regions (data not shown). These results are consistent with the observations of Kawai et al. (20), suggesting that Pgp expression is predominantly restricted to the basal cells of the prostate glands and can be detected by the NH211 antibody.

Immunostaining of consecutive microsections of breast adenocarcinoma tissues with C219 (Fig. 7C) and NH211 (Fig. 7D) showed prominent staining of the luminal side of the ductal epithelial cells and slight to moderate staining of cells within the stromal compartment. The preimmune serum did not immunostain with any of the regions in the sections (data not shown). Although not shown, the immunostaining carried out with NH211 antibody mixed with immunizing peptide completely abolished this Pgp staining. These observations together suggested that the NH211 antibody is useful in detecting Pgp in tissue samples by immunohistochemistry analysis as well.

**Discussion**

Overexpression of Pgp in cancers is a major factor contributing to chemotherapy failure. In spite of this importance (see Introduction), few antibodies are currently available for the accurate detection of Pgp in the clinical test samples. The major limitations of these commercially available antibodies are their limited usefulness in one or the other cell biological and biochemical methods and prohibitive costs combined with a minimal storage life in laboratory conditions, as we have found loss of function of these antibodies in antibody-based detection assays. To circumvent these limitations, to determine Pgp levels in tumor specimens obtained from tissue procurement facilities, and to use in the biochemical analysis of Pgp, we selected a cytoplasmically located hydrophilic region in the Pgp molecule as an epitope to which polyclonal NH211 antibody was raised. The results presented here indicate that this antibody interacts with Pgp with high affinity, which is comparable with the affinity and usefulness of the well-characterized C219 Pgp antibody in biochemical assays. In addition, we have shown that the NH211 antibody is also useful in immunocytochemistry and immunohistochemical analysis of Pgp.

In addition to characterizing the usefulness of this antibody, this study corroborates that drug resistance results from the overexpression of Pgp. For instance, results presented in Fig. 3 shows that Pgp expression is absent in the drug-sensitive MCF-7 cells, whereas it is high in the drug-resistant MCF-7/AdrR cells. Interestingly, both of these MCF-7 cells contain the MDR1 transcript, suggesting that the MDR1 translation is negatively regulated in drug-sensitive cells and such regulation is altered during the development into MDR. Furthermore, our results of Fig. 6 show that Pgp is localized in the plasma membrane of these drug-resistant cells. Immunohistochemistry analysis on the breast adenocarcinomas suggested that the Pgp expression is high in cells lining the ductules (Fig. 7). It is interesting to note that the C219 antibody also recognized precisely the similar regions in the tissue sections as the NH211 antibody, but with slightly lower staining intensity under identical fluorescence gain. As both of the NH211 and C219 antibodies immunostained identically, and the immunizing peptide inhibited the immunostaining of NH211 antibody effectively (data not shown), these results suggest that the NH211 antibody is specific and valuable for identifying Pgp expression in cancer tissues.

Significant amount of Pgp is also detected in the human kidney HEK-293 cells, which may play a role in filtering out the toxic byproducts of metabolism and/or xenobiotics in the blood. Although prostate cancers are known to develop MDR, few reports indicate that such MDR is mediated by Pgp expression (20, 21). Data presented here indicate that the prostate cancer PC3 cells express small amounts of Pgp (Fig. 3), which is further supported by the presence of MDR1 transcript in these cells (Fig. 4). In addition, our immunohistochemical analysis of prostate adenocarcinoma suggested that Pgp is predominantly expressed in basal cells (Fig. 7). These observations together
suggest that a small amount of contamination of basal cell population, which express high levels of Pgp (Fig. 7), in the cultured laboratory prostate cancer cell lines would be sufficient to result in the variation in the detection of Pgp. It is also likely that the use of Pgp antibodies with variable sensitivity could also account for this discrepancy.

Together, the data presented here indicate that the epitope located at the NH₂ terminus of Pgp serves as an excellent antigen. The NH₂11 antibody is highly specific in detecting Pgp by the Western blotting, immunocytochemistry, and immunohistochemistry methods. The NH₂11 antibody is also useful in purifying Pgp from cell membrane by immunoprecipitation and in the analysis of Pgp structure and function (18, 22).

Acknowledgments

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Fig. 7. Immunohistochemistry of Pgp. The paraffin-embedded human prostate adenocarcinoma sections (A and B) and breast adenocarcinoma sections (C and D) were immunostained using C219 (A and C) or NH₂11 (B and D) antibody in conjunction with the Alexa Fluor 488–coupled appropriate secondary antibody. The Alexa Fluor 488 fluorescence was visualized in a Leica fluorescence microscope and the digital images were acquired. Arrows, cells with bright fluorescence due to binding of antibody to Pgp.

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Characterization of a New Antibody Raised against the NH₂ Terminus of P-Glycoprotein


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