The breast cancer susceptibility gene BRCA1 encodes an 1863-amino acid protein that acts as a tumor suppressor. The biochemical function of BRCA1 is unknown, and there are conflicting results describing its subcellular location. We have identified a 220-kDa protein, which is reactive with three antibodies raised against the amino- and carboxyl-terminal regions of BRCA1. Immunofluorescence staining with an antibody to the carboxyl terminus of BRCA1 localized the protein to the nucleus of breast, ovarian, and cervical carcinoma-derived cell lines. A similar result was observed by biochemical subcellular fractionation that indicated that the 220-kDa protein was localized primarily to the nucleus of cell lines established from breast carcinomas. In addition to the 220-kDa protein, one antibody, C-20, also recognized a 180-kDa protein in MDA-MB-468 total cell lysates that was not detected by the other two antibodies. Several observations suggest the 180-kDa protein is the epidermal growth factor (EGF) receptor: (i) C-20 reacted avidly with a 180-kDa protein immunoprecipitated by an antibody to the EGF receptor; (ii) an EGF receptor antibody detected a 180-kDa protein immunoprecipitated by C-20; (iii) the affinity purified EGF receptor was both immunoprecipitated and detected on immunoblots by the C-20 antibody but not another BRCA1 antibody; (iv) similar phosphopeptide maps were generated from the EGF receptor and the 180-kDa BRCA1 antibody; (v) the C-20 immunizing peptide bears sequence identity map was distinct from the 220-kDa phosphoprotein; and (v) the C-20 immunizing peptide bears sequence identity to the EGF receptor. These results indicate that BRCA1 is a 220-kDa nuclear protein and that the 180-kDa protein reported previously may be unrelated to BRCA1.

Nearly 50% of hereditary breast and ovarian cancers occur as a result of mutations in the breast and ovarian cancer susceptibility gene, BRCA1 (1). BRCA1 encodes a novel 1863-amino acid protein with a predicted molecular weight of approximately 207,000 (2). Although the cellular function of BRCA1 is unknown, epidemiological studies suggested that it acts as a tumor suppressor (3–5). This conclusion was supported by data that showed germ line mutations in BRCA1 in familial breast and ovarian cancers (6, 7). In support of its role as a tumor suppressor, recent studies indicated that expression of wild type BRCA1 in breast or ovarian cancer cell lines reverts the transformed phenotype and represses tumor growth of breast cancer-derived cells in mice (8).

A role for BRCA1 in cell proliferation is also apparent from recent studies on its expression during mouse development and in brca1-deficient mice. Targeted disruption of the gene at exons 5–6 results in an embryonic lethal and mice that are unable to develop past day 7.5. This is attributed to a failure of the proliferative burst necessary at this stage of gestation (9). However, mice deficient in brca1 due to disruption at exon 11 develop until days 10–13 of gestation and suffer from neuroepithelial abnormalities probably due to problems with cell growth (10). Additional data have shown that in the developing mouse, brca1 is expressed during cell differentiation and functional differentiation of the mammary gland (11, 12), suggesting that it may be involved in modulating cell growth. In spite of the evidence that indicates that BRCA1 is involved in cell growth control, its biochemical function is not known.

Initial studies on BRCA1 identified proteins differing in size and subcellular location. Chen et al. (13) showed that BRCA1 was 220 kDa and was aberrantly localized to the cytoplasm of cells or tumors derived from breast or ovarian cancers, whereas in normal breast or ovarian cells or tissues BRCA1 was found in the nucleus. Recently, these results have been challenged by Scully et al. (14), who found BRCA1 localized primarily to the nucleus in tumor-derived cell lines. Additionally, the 220-kDa BRCA1 protein is apparently up-regulated when quiescent cells are stimulated to divide by the addition of serum (15), and expression of BRCA1 RNA seems to be increased prior to DNA synthesis (15, 16). In contrast, a 180–190-kDa form of BRCA1 has been observed in Western immunoblots (16–18). Jensen et al. (17) report that the 180–190-kDa BRCA1 is localized to secretory vesicles where it exhibits granin-like properties and is secreted by the cell in a hormone-dependent manner. More recent results have identified five different isoforms of BRCA1 ranging in size from 85 to 210 kDa (16). Differences between the observed size and location of BRCA1 could be due to alternative RNA splice variants or to techniques and reagents, although all of these reports utilized antibodies directed against the carboxyl-terminal region of the protein (13–18). With the function of BRCA1 unknown, it is possible that several isoforms of the protein may exist, and further analysis is necessary to sort through the conflicting data.

Using polyclonal antibodies generated against large fragments of the amino- and carboxyl-terminal regions of BRCA1, we have examined the discrepancy in location and size of BRCA1 in cell lines derived from breast adenocarcinomas as well as ovarian and cervical carcinomas. Our results demonstrate that BRCA1 is primarily localized to the nucleus with an apparent molecular weight of 220,000.

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Antibodies against Different Regions of BRCA1 Recognize 180- and 220-kDa Proteins—To identify BRCA1 in cells, we raised polyclonal antibodies to amino acids 8–475 (BPA-1) and 1283–1863 (BPA-2) of the BRCA1 protein and compared them to each other.

**RESULTS**

**Analysis of BRCA1 Localization**

**Antibodies and Materials**

Antibodies C-20 and D-20 were raised against amino acids 1843–1862 and 2–21, respectively, of BRCA1 and were obtained from Santa Cruz Biotechnology Inc. (Santa Cruz, CA). Antibodies BPA-1 and BPA-2 were raised against the affinity purified BRCA1 fragments 8–475 and 1283–1863, respectively, produced in the baculovirus/Sf9 cell system. The antibodies were affinity purified on columns containing the appropriate BRCA1 protein fragment immobilized on CNBr-activated Sepharose (Pharmacia Biotech Inc.). Anti-EGF receptor antibody, Ab4, was obtained from Oncogene Science (Uniondale, NY), and horseradish peroxidase-conjugated antibodies were purchased from Bio-Rad. Human recombinant EGF and LA22, anti-EGF receptor antibody, were obtained from Upstate Biotechnology Inc. (Lake Placid, NY). Affinity purified EGF receptor was purchased from Sigma. Cell culture reagents, media, and fetal bovine serum were obtained from Life Technologies, Inc. 

**Methods**

**Cell Culture and Lysis—**MCF7 (HTB 22 and SKBR3 (HTB 30) cells were maintained in high glucose Dulbecco's modified Eagle's medium (DMEM) supplemented with penicillin and streptomycin, t-glutamine, and 10% fetal bovine serum (FBS). For MCFT 1 × nonessential amino acids were also added. OVCAR3 (HTB 161) cells were grown in RPMI 1640 medium supplemented with 10 μg/ml insulin and 20% FBS. SW480 (CCL 228) cells were maintained in Leibovitz L15 supplemented with penicillin and streptomycin, t-glutamine, and 10% FBS. MDA-MB-468 (HTB 132) and MDA-MB-453 (HTB 131) cells were initially grown in Leibovitz L15 supplemented with 10% FBS and then transferred into high glucose DMEM supplemented with t-glutamine and 10% heat-inactivated FBS and grown in 5% CO2, C33A (HTB 31) cells were grown in Eagle's minimum essential medium with nonessential amino acids, 1 mM sodium pyruvate, 10% FBS, and Earle's balanced salts.

Cells were rinsed twice with ice-cold PBS and lysed in Triton lysis buffer (20 mM Tris-HCl, pH 8.0, 137 mM NaCl, 1 mM EGTA, 1% Triton X-100, 10% glycerol, 1.5 mM MgCl2) in the presence of protease and phosphatase inhibitors (200 mM vanadate, 50 mM NaF, 1 mM Pefabloc, 1 mM diithiothreitol, 10 μg/ml leupeptin, 10 mg/ml pepstatin, 10 mg/ml aprotinin). Proteins were normalized and BRCA1 protein immunoprecipitated with either BPA-1 (1 μg), BPA-2 (1 μg), C-20 (2.5 μg), or D-20 (2.5 μg). Proteins were size separated through a 6% acrylamide gel and transferred to Immobilon-P.

**Antibodies against Different Regions of BRCA1 Recognize 180- and 220-kDa Proteins—**TRC1 and TRC2 (13 g) were then incubated with 10% FBS and then transferred into Immobilon-P and hybridized with C-20, BPA-1, or LA22.

**Biochemical Subcellular Localization of BRCA1—**Cells were seeded at subconfluency in 6-well plates containing coverslips, and after 2 days the coverslips were removed and fixed in methanol at −20°C for 20 min and then washed in PBS followed by PBS/0.05% saponin. The fixed cells were incubated at 37°C in PBS containing 10% milk, 0.05% saponin, and the indicated BRCA1 antibody at a final concentration of 2 μg/ml. The cells were washed again in PBS/0.05% saponin and then incubated with fluorescein isothiocyanate-conjugated goat anti-rabbit IgG (Sigma) at a final dilution of 1:32 and DAPI at 0.1 μg/ml. Stained cells were visualized by fluorescence using a Zeiss axiovert 100 TV microscope at 100×.

**Immunocytochemical Subcellular Localization of BRCA1—**Cells were seeded at subconfluency in 6-well plates containing coverslips, and after 2 days the coverslips were removed and fixed in methanol at −20°C for 20 min and then washed in PBS followed by PBS/0.05% saponin. The fixed cells were incubated at 37°C in PBS containing 10% milk, 0.05% saponin, and the indicated BRCA1 antibody at a final concentration of 2 μg/ml. The cells were washed again in PBS/0.05% saponin and then incubated with fluorescein isothiocyanate-conjugated goat anti-rabbit IgG (Sigma) at a final dilution of 1:32 and DAPI at 0.1 μg/ml. Stained cells were visualized by fluorescence using a Zeiss axiovert 100 TV microscope at 100×.

**Biochemical Subcellular Fractionation—**Cells were lysed in Triton lysis buffer (20 mM Tris-HCl, pH 7.4, 10 mM NaCl, 3 mM MgCl2, 1 mM EGTA, 1 mM EDTA) plus inhibitors (1 mM vanadate, 10 μg/ml phenylmethylsulfonyl fluoride, 0.1 mM leupeptin, 200 kalirikine units/ml aprotinin) and allowed to swell for 10 min prior to Bounce homogenization (40 strokes) with a tight fitting pestle (type B). Low speed centrifugation (575 × g) for 5 min resulted in a nuclear pellet and postnuclear (supernatant) fraction. The postnuclear fraction was further clarified by centrifugation (150,000 × g, 30 min) to separate membrane (pellet) and cytosolic (supernatant) fractions. The membrane fraction was washed with HBL, whereas the cytosolic (supernatant) was centrifuged again (150,000 × g, 30 min) to remove any membrane contaminants. To remove membrane and cytosolic contaminants, nuclear fractions were washed five times in HBL with 0.1% Nonidet P-40. All fractions were adjusted to 50 mM Tris-HCl, pH 7.4, 100 mM NaCl, 3 mM MgCl2, 1 mM EDTA, 1 mM EGTA, 0.5% deoxycholate, 0.1% SDS, 0.2% Nonidet P-40 plus inhibitors, centrifuged at 12,000 × g for 15 min to remove insoluble material, and the supernatants were removed to a fresh tube. BRCA1 was immunoprecipitated with BPA-2 as described above. The immunoprecipitated proteins were size separated through an SDS gel, transferred to Immobilon-P, and immunoblotted with C-20. Remaining portions of the fractions were digested in 4 × SDS sample buffer, boiled, and electrophoresed through SDS-Tris-glycine gels. Following transfer to Immobilon-P, the membranes were probed with antibodies against α-tubulin or p380.

1 The abbreviations used are: EGF, epidermal growth factor; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; PBS, phosphate-buffered saline; BSA, bovine serum albumin; Tricine, N-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine; DAPI, 4',6-diamidino-2-phenylindole.
BPA-1 and BPA-2, and to a lesser extent, by C-20 (Fig. 1, lane 4) prepared from established breast cancer cell lines were analyzed by immunoblotting using antibodies BPA-1, BPA-2, and C-20 as denoted below the panels at left. The arrows indicate 220-kDa protein present in lysates and co-migrating with 10 ng of recombinant purified BRCA1 (BV Brca1). Lysates from the indicated cells were also subjected to immunoprecipitation with antibodies BPA-1, BPA-2, C-20, and D-20 and the precipitates analyzed by immunoblotting with BPA-2 (panels on the right). The positions and molecular weights (×10⁻³) of the pre-stained standards are indicated between the panels.

An additional polypeptide with a molecular mass of approximately 160 kDa was also detected by BPA-1 and BPA-2. The C-20 antibody recognized numerous polypeptides, most notably a prominent 180-kDa protein present in the MDA-MB-468 and SKBR3 cells. To determine if the 220-kDa protein detected in cell lysates was BRCA1, we performed immunoprecipitations from the same cell lysates as well as from SW480 colon cancer cell lysates and analyzed the precipitates by immunoblotting with BPA-2 (Fig. 1, right panel). Antibodies BPA-1, BPA-2, and C-20 but not D-20 all efficiently immunoprecipitated the 220-kDa protein recognized by BPA-2, whereas most of the proteins observed in crude lysate samples were absent. This 220-kDa protein co-migrated with BV BRCA1, the full-length BRCA1 produced in the baculovirus/Sf9 cell system. Immunoblotting with antibodies BPA-1 and C-20 also recognized the 220-kDa protein in these immunoprecipitations (not shown). The 180-kDa protein detected by C-20 in the MDA-MB-468 and SKBR3 but found only at low levels in MCF7 lysates was not immunoprecipitated at all by BPA-1 or BPA-2. It is likely that both the 180- and 220-kDa proteins react directly with the C-20 antibody because their detection was significantly diminished by preincubation of the antibody with excess immunizing peptide (not shown). These results suggest that the 220-kDa protein is BRCA1 because it is recognized by antibodies made to both the amino and carboxyl terminus.

The C-20 Antibody Recognizes the EGF Receptor—Failure to detect the 180-kDa protein with either BPA-1 or BPA-2 prompted us to further characterize this protein. Because MDA-MB-468 cells express high levels of the EGF receptor (21), we tested the reactivity of the C-20 antibody against immunoprecipitates of the EGF receptor. The C-20 antibody detected a 180-kDa protein immunoprecipitated from cell lysates by LA22, an antibody directed against the EGF receptor (Fig. 2A, lane 1). Additionally, a 180-kDa protein immunoprecipitated by C-20 was recognized in Western blots by Ab4, another antibody directed against the EGF receptor (Fig. 2A, lane 4). The C-20 immunoprecipitated protein co-migrated with the EGF receptor immunoprecipitated by LA22 and blotted with Ab4 (Fig. 2A, lane 3). Both C-20 and LA22 immunoprecipitated a 180-kDa phosphoprotein from MDA-MB-468 cells metabolically labeled with [³²P]orthophosphate (Fig. 2B, lower arrow). In both C-20 and LA22 immunoprecipitates, phosphorylation of the 180-kDa protein was enhanced when the cells were stimulated with EGF (Fig. 2B, compare lanes 1 and 2 with lanes 3 and 4). Furthermore, the phosphopeptide map of the 180-kDa phosphoproteins immunoprecipitated by LA22 or C-20 and digested with V8 protease were similar (Fig. 2C). The only difference in the phosphopeptide map was a doublet at 51 kDa (Fig. 2C, lower arrow) apparent in the C-20 180-kDa protein peptide digest but not in the peptide digest of the immunoprecipitated EGF receptor (Fig. 2C, lanes 1 and 2). This difference is probably partially phosphorylated...
EGF receptor because the lower band co-migrates with a phosphopeptide in the unstimulated cells (Fig. 2, lane 1), and the upper band co-migrates with a phosphopeptide in the EGF-stimulated cells (Fig. 2C, lane 2). However, the doublet may be due to a receptor associated phosphoprotein or another protein immunoprecipitated by C-20.

To further assess the C-20 reactivity with the EGF receptor, affinity purified EGF receptor from A431 cells was subjected to an in vitro kinase reaction in the presence of [γ-32P]ATP. Equivalent fractions of the phosphorylation reaction were immunoprecipitated with C-20, BPA-1, or LA22. Both C-20 and LA22 immunoprecipitated the in vitro phosphorylated EGF receptor with similar efficiency (Fig. 3A, lanes 2 and 3). Under these conditions, the phosphorylated EGF receptor was not immunoprecipitated by BPA-1 (Fig. 3A, lane 4). After an overnight exposure at −70 °C, a weak signal is apparent in Fig. 3A both lanes 4 and 5, indicating that the purified receptor had a weak affinity to protein A-Sepharose (data not shown). Furthermore, the purified EGF receptor was recognized in Western blot analysis with either LA22 or C-20 but not with BPA-1 (Fig. 3B, lanes 1–3, respectively). Preincubation of C-20 with the immunizing peptide significantly reduced hybridization to the receptor (data not shown). These results suggested that C-20 cross-reacts with the EGF receptor.

The 220-kDa Protein Recognized by C-20 Is Distinct from the 180-kDa Protein—C-20 also immunoprecipitated a 220-kDa phosphoprotein (Fig. 2B, upper arrow), which co-migrated with in vitro phosphorylated BV BRCA1 (not shown). Analysis of the 220-kDa protein from stimulated and unstimulated MDA-MB-468 cells labeled with [32P]orthophosphate revealed similar phosphopeptide profiles (Fig. 2C, lanes 5 and 6) and a distinctly different profile from the 180-kDa proteins (Fig. 2C, compare lanes 1–4 with lanes 5 and 6). The similarity between the phosphopeptide profile of the 220-kDa protein from the unstimulated and EGF-stimulated cells suggested that it was not phosphorylated in an EGF-dependent manner. Furthermore, the 220-kDa phosphoprotein immunoprecipitated from [32P]orthophosphate-labeled EGF-stimulated cells showed no apparent increase in tyrosine phosphorylation as assessed by alkali treatment of the gel, nor was there an increase in the amount of the 220-kDa protein immunoprecipitated with antiphosphotyrosine antibodies and immunoblotted with C-20 (data not shown).

Because C-20 and BPA-1 both recognize a 220-kDa protein (Fig. 1), we wanted to determine whether these proteins were similar. MCF7 cells were labeled with [32P]orthophosphate and a 220-kDa protein was immunoprecipitated from cell lysates with either BPA-1 or C-20 (Fig. 4, top panel). The V8 proteolytic phosphopeptide profile of the 220-kDa protein immunoprecipitated by C-20 was similar to the BPA-1 immunoprecipitated protein (Fig. 4, bottom panel). Phosphopeptides marked by an asterisk in Fig. 4 are also apparent in Fig. 2C (lanes 5 and 6). These results indicate that the 220-kDa proteins recognized by antibodies developed against different regions of BRCA1 are similar.

BRCA1 Is Localized to the Nucleus of Transformed Cell Lines—Because the subcellular location of BRCA1 may provide important clues as to its function, immunohistochemical staining to localize BRCA1 was employed utilizing BPA-2. A punctate nuclear staining pattern was observed in breast cancer-derived cell lines, MCF7, SKBR3, MDA-MB-468, MDA-MB-453, as well as from cell lines derived from ovarian or cervical carcinomas, OVCAR3 and C33A, respectively (Fig. 5). This pattern was not observed in control panels, nor was there any florescence observed in the red channel, indicating that the staining pattern shown in Fig. 5 was not the result of nonspecific immunofluorescence (data not shown). This type of staining suggests a nuclear localization in either breast or nonbreast cancer-derived cells similar to that reported by Scully et al. (14) using monoclonal antibodies raised against BRCA1 protein. These results suggest that BRCA1 is principally found in the nucleus of the breast cancer, ovarian, and cervical carcinoma-derived cell lines examined here, although some cytoplasmic
staining was also apparent.

To further assess the localization of BRCA1, biochemical fractionation analysis of the breast cancer-derived cell lines, MDA-MB-468 and MCF7, was performed. Using the method of Fazioli et al. (20), equal numbers of cells were fractionated into three distinct subcellular compartments: cytoplasm, membrane, and nuclear. To remove contaminating membrane or cytoplasmic proteins, the nuclear fraction was washed extensively with Nonidet P-40 buffer. The volume of lysate for each fraction was normalized, and BRCA1 was immunoprecipitated from equal portions of the cytosolic, membrane, and nuclear fractions using BPA-2. As shown in Fig. 6, BRCA1 appears to be localized mainly to the nuclear fraction in both cell lines, although there does appear to be some BRCA1 in the membrane and cytosolic fractions, particularly in the MCF7 cells. The level of BRCA1 in the cytosolic and membrane portions may be somewhat exaggerated because these fractions were normalized by volume of lysate from the same number of cells rather than protein concentrations. Protein analysis indicated that there was approximately 2–4-fold more protein per immunoprecipitation for the cytosolic and membrane fractions than for the nuclear fraction (data not shown). There appeared to be minimal contamination between the purified subcellular compartments (Fig. 6) as shown by blotting equal portions of the fractions with antibodies against α-tubulin and p300, which define the cytosolic and nuclear fractions, respectively (14, 22). These results clearly demonstrate that BRCA1 is localized predominantly to the nuclear fraction of these cell lines.

**Fig. 6. Biochemical fractionation of MCF7 and MDA-MB-468 cells.** Cytosolic (C), membrane (M), or nuclear (N) fractions were prepared as described under “Experimental Procedures,” and BRCA1 was immunoprecipitated with BPA-2, subjected to electrophoresis, and then immunoblotted with C-20. As a control, cytosolic lysates were incubated with protein A-Sepharose (PAS). Equivalent volumes of cytoplasmic, membrane, and nuclear fractions were subjected to electrophoresis and then immunoblotted with either p300 or α-tubulin antibodies to distinguish nuclear and cytosolic fractions, respectively.

**DISCUSSION**

The results presented here demonstrate that at least one isoform of BRCA1 has a molecular weight of approximately 220,000, and in the cell lines examined, it was localized primarily to the nucleus. We believe that this 220-kDa protein is BRCA1, because it was recognized by antibodies raised against disparate regions of BRCA1 and co-migrated with the BRCA1 protein purified from baculovirus-infected Sf9 cells. Furthermore, it is unlikely that the antibodies are recognizing different proteins because the 220-kDa protein immunoprecipitated by two different BRCA1 antibodies had a similar phosphopeptide profile when analyzed with V8 protease. These results suggest that the protein we have immunoprecipitated is likely to be at least one isoform of BRCA1. Our results showing a 220-kDa size for BRCA1 are in agreement with those shown recently by several labs using C-20 or other antibodies made against peptide regions of BRCA1 (13, 14). Using established cell lines, we have been unable to detect a 180-kDa BRCA1 protein with any of the three antibodies shown here. If there was a 180-kDa BRCA1, we might have expected the V8 digest of the 32P-labeled 180-kDa protein immunoprecipitated from MDA-MB-468 cells to be distinct from the EGF receptor immunoprecipitated from the same cells. Because no such difference was observed, we can only conclude that if a 180-kDa BRCA1 exists, it is either not a phosphoprotein or the V8 phosphopeptide profile of this BRCA1 isoform and the EGF receptor are indistinguishable. As demonstrated by V8 proteolytic analysis, EGF did not stimulate a dramatic change in phosphorylation on the 220-kDa BRCA1 protein. This result is limited because subtle changes in BRCA1 phosphorylation may not be detected with this type of analysis.

Clues to the function of BRCA1 may come from determining its location in the cell. Previously, Chen et al. (13) reported that BRCA1 was not translocated to the nucleus in breast and ovarian cancer-derived cell lines and pleural effusions as is thought to occur in normal mammary or ovarian tissues. By both immunostaining and biochemical subcellular fractionation, we found 220-kDa BRCA1 to be located primarily in the nucleus of the cancer cells examined. We have confirmed this result in MCF7 cells by using other biochemical fractionation methods (data not shown). Although some BRCA1 was found in the cytoplasmic and membrane fractions of MDA-MB-468 and MCF7 cell lines, there was significantly less than that found in the nucleus of the same cells. BRCA1 was immunoprecipitated from equal volumes of the cytoplasmic, nuclear, or membrane
lysates, and the protein levels were higher in the cytoplasmic and membrane fractions than nuclear fraction (data not shown). As a result, the amount of BRCA1 in the membrane or cytoplasmic fractions was a smaller proportion of the total protein in those fractions relative to the amount of BRCA1 observed in the nucleus. The low level of BRCA1 in the cytoplasm and membrane fractions shown here does not support the hypothesis that BRCA1 is inappropriately localized to the cytoplasm in cell lines derived from tumors. Furthermore, we found that BRCA1 was localized to the nucleus of two non-breast cancer-derived cell lines, OVCAR3 and C33A. Our results may represent differences in reagents or techniques; however, the nuclear localization for BRCA1 is in agreement with recently reported results (14).

The 220-kDa BRCA1 protein identified by BPA-1 and BPA-2 is also recognized by C-20; however, this antibody also immunoprecipitated and reacted in immunoblots with a 180-kDa protein (Fig. 1). Several pieces of data presented here suggest that C-20 recognizes the 180-kDa EGF receptor: (i) C-20 will immunoprecipitate the affinity purified EGF receptor and recognizes the purified receptor in Western blots; (ii) EGF receptor immunoprecipitated from cell lysates was recognized by C-20, and reciprocally, a 180-kDa protein immunoprecipitated from cell lysates by C-20 was recognized by the EGF receptor antibody Ab4 and co-migrated with the EGF receptor; and (iii) a phosphoprotein immunoprecipitated by C-20 has a phosphopeptide profile similar to the EGF receptor. A computer-generated BestFit analysis (23) indicated 64% similarity between a portion of the C-20 immunizing peptide (amino acids 1843–1856 of BRCA1) and amino acids 1007–1020 of the human EGF receptor (Ref. 24; Genbank® Sequence). Six of seven amino acids between 1850–1856 of BRCA1 were identical to six amino acids of the EGF receptor between 1014 and 1020. These similarities may explain the cross reactivity between C-20 and the EGF receptor.

Previous reports using C-20 identified BRCA1 as a 180–190-kDa protein (16–18). While this manuscript was in revision, a 220-kDa BRCA1 protein identified by BPA-1 and BPA-2 reportedly was detected by other antibodies (13, 14).

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