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## Invited Review

# A nuclear function for the tumor suppressor BRCAI

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**Summary.** The breast and ovarian cancer susceptibility gene *BRCA1* has been recently cloned and revealed an open reading frame of 1863 amino acids, but a lack of significant homology to any known protein in the database has led to few clues about its functions. One of the first steps to investigate the function of BRCA1 was to define its subcellular localization. Several reports have led to contradictory findings that include: nuclear localization in normal cells and cytoplasmic in breast and ovarian cancer cells; nuclear in both normal and cancer cells; cytoplasmic and secreted to the extracellular space; present in tube-like invaginations of the nucleus; and colocalizing with the centrosome. As is apparent, the subcellular localization has been the most controversial aspect of BRCAl biology and is a key point to uncover its functions. In this paper we review the published data on subcellular localization of BRCAl with special emphasis on the antibodies and techniques used. We conclude that there is now overwhelming evidence to support a nuclear localization for BRCAl, both in normal and cancer cells. In addition, several BRCAl-interacting proteins have been isolated and they are preferentially located in the nucleus. Evidence supporting a physiological function for BRCAl during DNA repair and transcriptional activation is also discussed.

**Key words:** Breast cancer, Tumor suppressor gene, BRCAI, Antibody specificity

**Abbreviations:** GST: Glutathione-S-transferase; NLS: nuclear localization signal; CEFs: chicken embryo fibroblasts; SDS: sodium dodecyl sulfate; DAPI: 4',6-diamidino-2-phenylindole; FBS: fetal bovine serum; PBS: phosphate buffered saline

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### Introduction

Mutations in BRCAl account for approximately 45% of the families with high incidence of breast cancer and 80% of families with high incidence of both breast and ovarian cancer (Easton et al., 1993). Identification of human BRCAl by positional cloning techniques revealed an open reading frame coding for 1863 amino acids with no statistically significant homology to proteins in the database, with the exception of a zinc-binding RING finger motif (C3HC4) in the N-terminal region (Miki et al., 1994). This motif is found in several proteins that have their functions mediated through DNA binding (Saurin et al., 1996). In addition, the presence of two putative nuclear localization signals (NLSs; aa 500-508 and 609-615) and an excess of negatively charged residues in the C-terminal region of BRCAl suggested a function for BRCAI in transcriptional regulation (Miki et al., 1994). Using a different computational strategy, Koonin et al. partitioned the BRCA1 sequence into putative globular and non-globular domains and used the globular domains to perform iterative searches in the database (Koonin et al., 1996). These studies defined a globular domain repeated in tandem in the C-terminal region of BRCAI, named BRCT (for BRCAl C-terminal domain) also present in 53BP1, a p53 binding protein (Koonin et al., 1996). This study was later extended and defined a superfamily of proteins containing the BRCT domains involved in DNA damage and cell cycle checkpoints (Bork et al., 1997; Callebaut and Mornon, 1997). Collectively, the fact that the RING finger, the NLSs, the excess of negatively charged residues and the BRCT domains are conserved in human, dog, rat and mouse *Brca1*, suggests that these regions are significant for BRCAl function (Abel et al., 1995; Lane et al., 1995; Sharan et al., 1995; Szabo et al., 1996; Bennet et al., 1999).

### Is BRCAl aberrantly localized in breast and ovarian cancer?

One of the first strategies to understand the biochemical function of BRCAl was immunofluorescence

and immunocytochemical analysis. Chen et al. (1995a,b) initially characterized BRCA1 as a 220 kDa nuclear phosphoprotein in normal cells as well as in cells derived from tumors other than breast and ovarian cancer. Interestingly, in the majority of breast and ovarian cancer cell lines and cells obtained from malignant pleural effusions of these tumors, BRCA1 appeared to be mislocalized to the cytoplasm (Chen et al., 1995a,b). Previous experiments raised questions about the role of BRCA1 in sporadic breast and ovarian cancer since no mutations in BRCA1 had been found in sporadic breast cancers and very few in ovarian cancers (Futreal et al., 1994). Mislocalization of BRCA1 to the cytoplasm suggested that a deficient nuclear transport mechanism might disrupt BRCA1 function in sporadic tumors in the absence of loss-of-function mutations. However, these authors only performed biochemical fractionation in HBL100 cells, and there was no fractionation data of the cancer cell lines showing mislocalization (Chen et al., 1995a,b).

In 1996, Scully et al. (1996a) reexamined the question by using an affinity purified polyclonal antibody as well as seven monoclonals (for a comprehensive list of BRCA1 antibodies published in the literature see Table 1; for a comparative study of several antibodies see Wilson et al., 1999) raised against various epitopes and found a consistent "nuclear dot" pattern in cell lines fixed with neutral paraformaldehyde, or methanol, or 70% ethanol. Moreover, biochemical fractionation analysis of three cancer cell lines (SKOV-3, MCF-7 and U20S) confirmed the presence of BRCA1 in the nuclear but not in the cytoplasmic fractions (Scully et al., 1996a). Although some of the antibodies showed weak cytoplasmic staining, confocal microscopy studies could not demonstrate colocalization of the signals derived from different antibodies, strongly arguing for non-specific cross-reactivity in the cytoplasm. Paraffin-embedded sections fixed with alcoholic formalin were shown to generate nuclear, both nuclear and cytoplasmic, as well as cytoplasmic staining. However, when these sections were treated with microwave heating, the staining was predominantly cytoplasmic suggesting that artifacts due to sample preparation may contribute to the confusion in BRCA1 location. In fact, cell lines where BRCA1 had been shown to be nuclear both by subcellular fractionation of unfixed cells and by immunostaining, displayed variable results when subjected to different fixation and heating conditions (Scully et al., 1996a).

To circumvent specificity problems with antisera to native BRCA1 epitopes, Chen et al. (1995b) ectopically overexpressed an N-terminal FLAG-tagged BRCA1 to show the tagged protein to be in the nucleus of normal cells but in the cytoplasm in a series of breast cancer cell lines. A caveat of ectopic overexpression is that high levels of protein can saturate subcellular compartments and result in the presence of the protein where it is not normally found under physiological conditions. Moreover, overexpression of BRCA1 can also cause toxicity and induce changes in cell morphology (Wilson et al., 1997). An important issue that remains is the localization of BRCA1 in rapidly proliferating cells versus contact-inhibited cells. Contact-inhibited cells have very low, in many cases undetectable, BRCA1 levels (Chen et al., 1996a; Jin et al., 1997). This may also be a source of artifactual results because antibodies that show both nuclear and some cytoplasmic staining will show only cytoplasmic staining when cells are contact inhibited due to the absence BRCA1 and presence of cross-reacting species in the cytoplasm. Examining the published results, cells presenting cytoplasmic staining are found in close contact with other neighboring cells. Immunostaining of cells expressing BRCA1 (HBL100) and chicken embryo fibroblasts (CEFs) that lack BRCA1 judged by low stringency southern blots (Miki et al., 1994), is a particularly revealing example of cytoplasmic cross-reactivity (Fig. 1A). Cells expressing BRCA1 (HBL100), show both a nuclear dot pattern and a diffuse cytoplasmic staining, whereas cells lacking BRCA1 (CEFs) show only a diffuse cytoplasmic staining. Moreover, to confirm the absence of BRCA1 in CEFs, immunoprecipitations were performed with cellular extracts obtained sequentially with (i) a mild Triton-containing buffer (HNTG) that extracts cytosolic proteins and (ii) subsequently with a harsher SDS-containing buffer (RIPA) (Fig. 1B). Typically, these studies confirm that BRCA1 is present only in the nuclear fraction and not in the cytoplasm of HBL100 and MCF-7 cells. In addition, immunoreactivity against BRCA1 is not observed CEFs, confirming the absence of BRCA1 in these cells.

### Is BRCA1 a granin?

The discovery of a granin sequence in BRCA1 suggested that BRCA1 and BRCA2 might be secretory proteins (Jensen et al., 1996a). Granins are a family of highly variable proteins that share a 10 amino acid motif and participate in secretory pathways (Ozawa and Takata, 1995). Interestingly, using polyclonal antibodies detected a 190 kDa in cell lysates and a 180 kDa protein from baculovirus lysates expressing the recombinant protein rather than the typical 220 kDa protein (Table 1). Furthermore, the majority of the reacting species localized to the membrane fraction and a small amount to the cytoplasm. The staining of primary human mammary epithelial cells revealed a granular pattern in both nucleus and Golgi complex (Jensen et al., 1996a). Later, using confocal microscopy Coene et al. suggested that the nuclear dot pattern represents cross-sections of cytoplasmic invaginations and that BRCA1 was mostly perinuclear (Coene et al., 1997). Although antibodies cross-reactivity may explain Golgi staining, additional evidence for BRCA1 being secreted is still lacking.

Several observations, following this initial publication, rebut the idea that BRCA1 is a granin. That includes the fact that the antibodies (Table 1) used in the

**Table 1.** α-BRCA1 antibodies.

| ANTIBODY | BRCA1 EPITOPE[a] | USES | LOCALIZATION (REFERENCE)[b] | PROTEIN SIZE[c] AND OTHER COMMENTS |
|---|---|---|---|---|
| α-BRCA1 | (G) 762-1315 | IP[d], IB, IF | Nuclear in normal cells and cells from tumors other than breast and ovary and cytoplasmic in breast and ovarian cancer cells (Chen et al., 1995a,b). | 220 kDa (Chen et al., 1995a,b). Mouse polyclonal |
| α-BRCA1 | 860-881 | IF, IB | Cytoplasmic tube-like invaginations of the cytoplasm in both normal and breast cancer cells (Coene et al., 1997). | 190, 220 and 240 kDa (Coene et al., 1997). Rabbit polyclonal. |
| α-BRCA1 | 1848-1863 | IF, IB | Cytoplasmic tube-like invaginations of the cytoplasm in both normal and breast cancer cells (Coene et al., 1997). | 190, 220 and 240 kDa (Coene et al., 1997). Rabbit polyclonal. |
| α-BRCA1 BgI | (G) 341-748 | IP | n.s.[e] | 220 kDa (Chen et al., 1995). Mouse polyclonal. |
| α-BRCA1N | (G) 1-302 | IP | | 220 kDa (Chen et al., 1996). Mouse polyclonal. |
| A | 2-20 | IB, IP | Nuclear and some cytoplasmic in fractionation experiments (Ruffner and Verma, 1997). | 220 kDa (Ruffner and Verma, 1997; Wilson et al., 1999). Rabbit polyclonal. |
| A19 | 1847-1863 | IP, IB | Nuclear dot pattern both in normal and cancer cell lines (Scully et al., 1996). | 220 kDa (Scully et al., 1996). Rabbit polyclonal. |
| AP11 | (G) 1313-1863 | IP, IB, IF | n.s. | n.s. Mouse monoclonal. |
| AP12 | (G) 1313-1863 | IP, IB, IF | n.s. | 220 kDa (Wilson et al., 1999). Mouse monoclonal. |
| AP16 | (G) 1313-1863 | IP, IB, IF | nuclear dot pattern both in normal and cancer cell lines (Scully et al., 1996; Jensen et al., 1996b). Variable in paraffin-embedded cell pellets depending on conditions used (Jensen et al., 1996b). | 220 kDa (Scully et al., 1996; Wilson et al. 1999). Mouse monoclonal. |
| B | 70-89 | | | 220 kDa (Ruffner and Verma, 1997). Rabbit polyclonal. |
| B112 | (G) 2-355 | IF, IB, IP | Diffuse nuclear staining and absent from nucleoli (Wilson et al., 1997). | 230 kDa (Wilson et al., 1997); 220 kDa (Wilson et al., 1999). Rabbit polyclonal. |
| BPA-1 | 8-475 | IB | n.s. | 220 kDa (Thomas et al., 1996). Rabbit polyclonal. |
| BPA-2 | 1293-1863 | IB, IF | Nuclear dot pattern both in normal and cancer cell lines (Thomas et al., 1996). | 220 kDa (Thomas et al., 1996). Rabbit polyclonal |
| BR64 | | IF | Nuclear dot pattern (Jensen et al., 1998). | 220 kDa (Jensen et al., 1998). Rabbit monoclonal, available through Upstate Biotechnology. |
| C | 768-793 | IB, IP, IF | n.s. | 220 kDa (Ruffner and Verma, 1997; Wilson et al., 1999). Rabbit polyclonal. |
| C-19 | 1844-1863 | IP | n.s. | 180-190 kDa (Jensen et al., 1996a)[f]. Rabbit polyclonal. |
| C-20 | 1843-1862 | IP, IF, IB, | Predominantly granular cytoplasmic, with nuclear and Golgi staining (Jensen et al., 1996a). Nuclear and associated with the centrosome during mitosis (Hsu and White, 1998). Nuclear (Thakur et al., 1997; Wilson et al., 1999). | 220 kDa (Chen et al., 1995; Thomas et al., 1996; Thakur et al., 1997; Wilson et al., 1999); 180-190 kDa (Jensen et al., 1996a,b)[f]; 230 kDa (Wilson et al., 1996); 185 kDa (Gudas et al., 1995). Rabbit polyclonal, available through Santa Cruz Biotechnology. Cross-reacts with EGFR and HER2 (Wilson et al., 1996). |
| D | 1847-1863 | IB, IP | Nuclear and some cytoplasmic in fractionation experiments (Ruffner and Verma, 1997). | 220 kDa (Ruffner and Verma, 1997). Rabbit polyclonal |
| D-20 | 1-20 | IB, IF | n.s. | 190 kDa (Jensen et al., 1996a); 220 kDa (Wilson et al., 1999). Rabbit polyclonal, available through Santa Cruz Biotechnology. Does not cross-react with EGFR and HER2 (Wilson et al., 1996). |

continued

| | | | | |
|---|---|---|---|---|
| GLK2 | 1839-1863 | IF | Cytoplasmic in cell lines. Cytoplasm or no signal in paraffin-embedded cell pellets depending on conditions used (Jensen et al., 1996b). | Mouse monoclonal. |
| I-20 | 1823-1842 | IB | n.d.[g] | 230 kDa (Wilson et al., 1996). Rabbit polyclonal, available through Santa Cruz Biotechnology. Does not cross-react with EGFR and HER2 (Wilson et al., 1996). |
| Ki-8 | 903-919 | IP, IB | Nuclear in fractionation experiments (Zhang et al., 1997). | 215 kDa (Zhang et al., 1997). Mouse monoclonal. |
| M-20 | mouse BRCA1 C-terminus | IB, IF | n.d. | 215 kDa (Zhang et al., 1997). Goat polyclonal, available through Santa Cruz Biotechnology. |
| MS110 | (G) 1-304 | IP, IB, IF | Nuclear dot pattern both in normal and cancer cell lines (Scully et al., 1996, 1997b; Jensen et al., 1996b; Hsu and White, 1998; Wilson et al., 1999). Variable in paraffin-embedded cell pellets depending on conditions[h] used (Jensen et al., 1996b). | 220 kDa (Scully et al., 1996a; Hsu and White, 1998; Scully et al., 1997b; Wilson et al., 1999). Mouse monoclonal, available as Ab-1 from Oncogene Research Products. |
| MS13 | (G) 1-304 | IF, IP, IB | Nuclear dot pattern both in normal and cancer cell lines. Variable in paraffin-embedded cell pellets depending on conditions used (Scully et al., 1996, 1997b; Jensen et al., 1996b) | 220 kDa (Scully et al., 1996, 1997b; Wilson et al., 1999). Mouse monoclonal, available as Ab-2 from Oncogene Research Products. |
| N25 | 1-25 | IF | Cytoplasmic in cell lines. Cytoplasm or no signal in paraffin-embedded cell pellets depending on conditions used (Jensen et al., 1996b). | Mouse monoclonal. |
| SD112 | (G) 758-1313 | IP, IB | n.s. | 220 kDa (Wilson et al., 1999). Mouse monoclonal. |
| SD118 | (G) 758-1313 | IP, IB, IF | n.s. | 220 kDa (Wilson et al., 1999). Mouse monoclonal. |
| SD123 | (G) 758-1313 | IP, IB, IF | n.s. | 220 kDa (Chen et al., 1998; Wilson et al., 1999). Mouse monoclonal. |
| SG11 | (G) 1847-1863 | IF, IP | Nuclear dot pattern both in normal and cancer cell lines. Variable in paraffin-embedded cell pellets depending on conditions used (Scully et al., 1996; Jensen et al., 1996b). | Mouse monoclonal, available as Ab-3 from Oncogene Research Products. |
| ZB1 | (G) 13-75 | IB, IP | Nuclear with some amount cytoplasmic in fractionation experiments (Aprelikova et al., 1996). | 220 kDa (Aprelikova et al., 1996). Rabbit polyclonal. |
| 6B4 | (G) 341-748 | IP, IB | | 220 kDa (Chen et al., 1996a; Aprelikova et al., 1996). Mouse monoclonal. |
| 17F8 | (G) 762-1315 | IP, IB, IF | Nuclear at lower (< 3 $\mu$g/ml) concentrations of antibody (Wilson et al., 1999). | 220 kDa (Wilson et al., 1999). Mouse monoclonal, available through GeneTex. |
| 24G11 | | IP, IB | n.d. | 220 kDa (Chen et al., 1995b). Mouse monoclonal. |
| 113 | 673-1365 | IP, IB, IF | Nuclear (Wilson et al., 1999). | 220 kDa (Wilson et al., 1999). Rabbit polyclonal. |
| 115 | 673-1365 | IP, IB, IF | | 220 kDa (Wilson et al., 1999). Rabbit polyclonal. |
| 579 | 903-919 | IP, IB | n.d. | 215 kDa (Zhang et al., 1997). Rabbit polyclonal. |

The references in this table are not extensive. Due to space limitations we have focused on the initial papers. A direct comparison of several monoclonal and polyclonal antibodies has been recently published (Wilson et al., 1999). [a]: all the antibodies have been raised against human BRCA1 epitopes unless otherwise stated. (G): antibody was raised against a GST-fusion protein. The remaining antibodies were raised using synthetic or recombinant peptides. [b]: In this table we have chosen to be conservative and not to consider experiments reported but not shown in the original papers. [c]: Although many antibodies described here showed several reactive bands, the size shown is that of the reactive species considered by the authors to represent the full length BRCA1. [d]: The uses described here were the ones reported in the original. We have not considered experiments reported but not shown. IP: immunoprecipitation; IF: immunofluorescence; IB, immunoblots. [e]: Not shown. [f]: 190 kDa species was detected from cell lysates and the 180 kDa species was detected from expressing the recombinant protein in baculovirus. [g]: Not done. [h]: Conditions vary in fixatives (neutral or alcoholic formalin) and in heat-induced epitope retrieval (no treatment; microwave or pressure cooker treatment).

majority of the experiments cross-reacts with human EGF Receptor and HER2, tyrosine kinase receptors that are frequently amplified in human breast and ovarian cancers (Wilson et al., 1996). Secondly, the statistical significance of the presence of the granin sequence in BRCA1 has been challenged, particularly allowing for a substitution in one of the motif's invariant position found in rat, dog and mouse Brca1 (Bradley and Sharan,

1996; Koonin et al., 1996). In addition, an alternative splice form of BRCA2 was found lacking the granin sequence, and shown by linkage analysis not to predispose to disease but to be a benign polymorphism (Mazoyer et al., 1996). Thirdly, the concept of BRCA1 being a tumor suppressor acting via the extracellular space has been challenged on the basis that it would be unlikely that a loss of heterozygosity in BRCA1 locus would cause a cell autonomous defect (Bradley and Sharan, 1996). In the scenario suggested by Jensen et al. (1996a), cells in breast and ovary tissue carrying no functional BRCA1 (after loss of hetero-zygosity) would still be bathed by the extracellular milieu containing BRCA1 secreted by the neighboring cells, making it unlikely to explain tumor initiation. To date, there has been no independent corroboration to the notion that BRCA1 is secreted.

### The controversy resolved

The characterization of the putative nuclear localization signals (NLS) found in BRCA1 has been a key point to establish it as a nuclear protein (Miki et al., 1994). Two groups have identified slightly different NLSs [$^{503}$KRKRRP$^{508}$, $^{606}$PKKNRLRRKS$^{615}$ and $^{651}$KKKKYN$^{656}$ (Chen et al., 1996b); $^{501}$KLKRK RR$^{507}$ and $^{607}$KKNRLRRK$^{614}$ (Thakur et al., 1997)]. Chen et al. (1996b) reported that NLSs 503-508 and NLS 606-615, but not NLS 651-656, are crucial for nuclear localization as site directed mutagenesis of these sites result in cytoplasmic localization of BRCA1. Further studies have found that *BRCA1* interacts with the Importin-α subunit of the nuclear transport signal receptor. Thakur et al. (1997) also found that NLS 501-507 is critical for nuclear localization whereas deletion mutants lacking NLS 607-614 are nuclear. The discrepancy may be explained by the different mutations employed (site-directed mutagenesis versus deletion mutants). In any event, the characterization of the NLSs supports the idea that BRCA1 is a nuclear protein.

Thomas et al. (1996) have developed different antibodies (Table 1) and confirmed the nuclear localization of the 220 kDa *BRCA1* in both normal and cancer cell lines through immunofluorescence and biochemical fractionation. Moreover, Wilson et al. (1997) confirmed the nuclear staining using a panel of overexpressed epitope-tagged BRCA1 and biochemical fractionation of cells overexpressing BRCA1. Interestingly, they describe the major alternative splice variant *BRCA1* Δ11b (~ 110 kDa), that lacks the NLSs and localizes preferentially to the cytoplasm. This variant might be responsible for cytoplasmic staining in immunofluorescence since it conserves the N- and C-terminal epitopes against which the majority of the antibodies have been raised against. Even in the case of BRCA1 Δ11b variant, no evidence was found of staining in Golgi or endoplasmic reticulum (Wilson et al., 1997). Other groups raised additional antibodies (Table 1) used

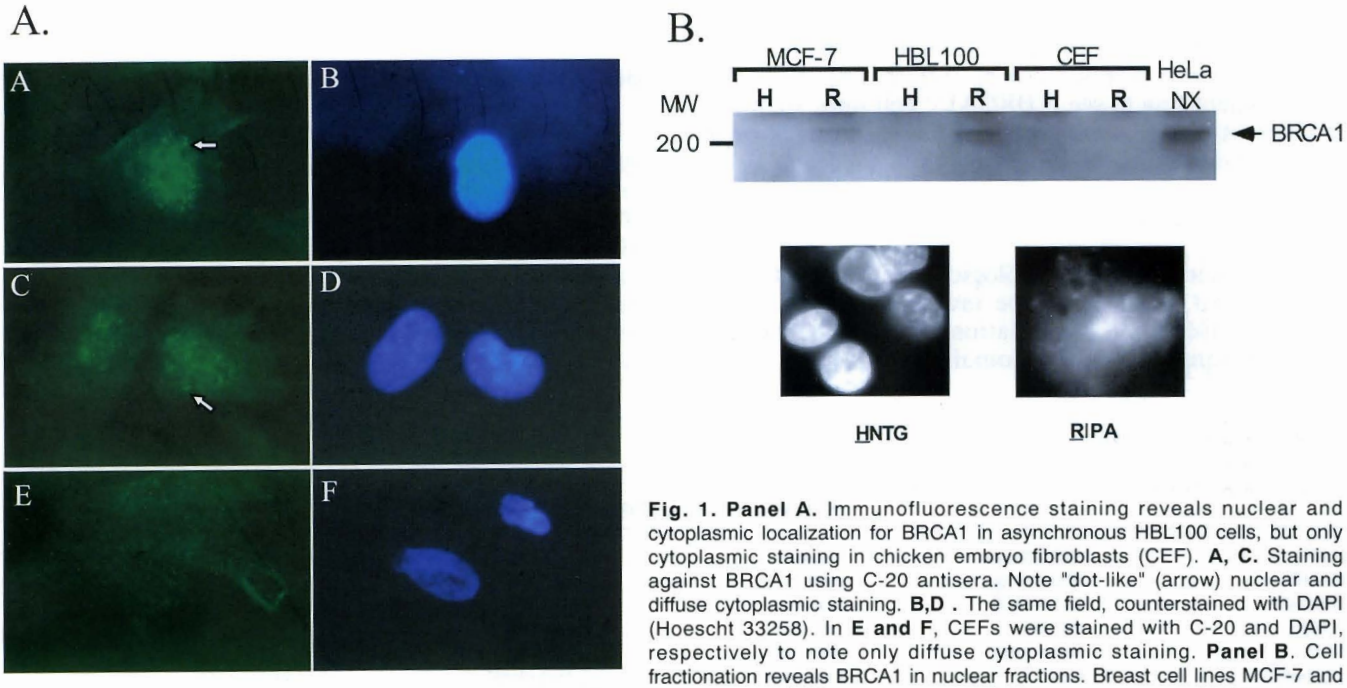


**Fig. 1. Panel A.** Immunofluorescence staining reveals nuclear and cytoplasmic localization for BRCA1 in asynchronous HBL100 cells, but only cytoplasmic staining in chicken embryo fibroblasts (CEF). **A, C.** Staining against BRCA1 using C-20 antisera. Note "dot-like" (arrow) nuclear and diffuse cytoplasmic staining. **B,D .** The same field, counterstained with DAPI (Hoescht 33258). In **E and F**, CEFs were stained with C-20 and DAPI, respectively to note only diffuse cytoplasmic staining. **Panel B**. Cell fractionation reveals BRCA1 in nuclear fractions. Breast cell lines MCF-7 and HBL100, and CEFs were lysed in 1% Triton-containing HNTG buffer, which is insufficient to lyse nuclear membranes or SDS-containing RIPA buffer, which readily lyses nuclear membranes (see lower DAPI panels). Detergent extracts from HNTG, RIPA, or high salt nuclear extracts (NX) were subsequently immunoprecipitated with C-20 antiserum and further immonoblotted with the same antiserum. BRCA1 was detected with HRP-conjugated secondary antibody.

for biochemical fractionation (Aprelikova et al., 1996; Thomas et al., 1996; Ruffner and Verma, 1997; Zhang et al., 1997) and immunoperoxidase staining (Rao et al., 1996) and have come to the conclusion that *BRCA1* is a nuclear protein with a molecular mass of 215-240 kDa.

Recently, Wilson et al. (1999) have undertaken an important task of comparing several different antibodies derived both from different laboratories and commercial sources in a variety of situations and consistently showed that BRCA1 is nuclear and attribute some early findings of cytoplasmic mislocalization to high concentrations of antibody used (see also Fig. 1). This study is the more comprehensive panel of antibodies tested so far and presents a compelling argument for a nuclear localization of BRCA1. More importantly, they present a series of biochemical fractionations using different antibodies to demonstrate nuclear localization. Table 1 in Wilson et al. (1999) summarizes the panel of antibodies, techniques and results obtained in parallel experiments. This table, used in conjunction with the Table 1 shown here, will be of special interest to the pathologist since it deals in detail with antibodies and conditions for immunohistochemistry in tissue blocks.

## A function for BRCA1 in spindle checkpoints?

Hsu and White (1998) have presented interesting data suggesting the association of BRCA1 with the centrosome, more specifically with γ-tubulin during mitosis. Considering that the huge amounts of tubulin present in the cells are a potential cause of artifacts in immunoprecipitations, confirmation using other methods (in vitro binding assays or GST-fusion pull down) and other antibodies (besides MS110 and the problematic C-20) is needed before we can be certain. Importantly, it will be interesting to see if $BRCA1^{-/-}$ cell lines are prone to aberrant chromosome segregation caused by failure in spindle checkpoints.

## A nuclear function?

Both genetics and cell biological data have led to the proposal that BRCA1 may be involved in DNA repair and in transcriptional regulation. BRCA1 contains a transcriptional activation domain localized to its C-terminus (Chapman and Verma, 1996; Monteiro et al., 1996). Interestingly, introduction of disease-predisposing mutations disrupted transcriptional activation (Chapman and Verma, 1996; Monteiro et al., 1996) while benign polymorphisms did not (Monteiro et al., 1997). In support of the proposed role in transcriptional regulation, BRCA1 has been found to be associated with the RNA polymerase II holoenzyme, through RNA helicase A (Scully et al., 1997a; Anderson et al., 1998) and to act as a coactivator for p53-mediated gene expression (Somasundaram et al., 1997; Ouchi et al., 1998; Zhang et al., 1998).

Co-localization studies by Scully et al. (1997b) have also uncovered an interaction of BRCA1 with Rad51, the homolog of bacterial RecA, suggesting a role for BRCA1 in DNA repair. In addition, DNA damage induces changes in BRCA1 subnuclear location and phosphorylation strengthening the idea that *BRCA1* is involved in DNA repair (Scully et al., 1997c; Thomas et al., 1997). Additional findings support the notion of BRCA1 may act in DNA repair, including the fact that blastocysts from $Brca1^{-/-}$ mice are more sensitive to DNA damage and tend to accumulate chromosomal abnormalities (Shen et al., 1998). Secondly, *BRCA1* has been found to be in a complex with BRCA2 (Chen et al., 1998), the product of the other major breast and ovarian cancer susceptibility gene that has also been shown to be involved in DNA repair (Connor et al., 1997; Sharan et al., 1997; Patel et al., 1998). Interestingly, a recent finding that BRCA1 is required for transcription-coupled repair in murine cells indicates that the above hypotheses for BRCA1 function are not mutually exclusive (Gowen et al., 1998).

## BRCA1-Interacting proteins are nuclear

Screening methods, such as the yeast two-hybrid system, which are unbiased for proteins in any particular cellular compartment, have revealed the interaction of BRCA1 with two previously characterized proteins, c-Myc (Wang et al., 1998), a proto-oncogene that functions as a transcription factor and, CtIP a protein implicated in the CtBP pathway of transcriptional repression that was independently cloned by two laboratories (Wong et al., 1998; Yu et al., 1998). Both

**Table 2.** BRCA1-interacting proteins.

| INTERACTING PROTEIN | BRCA1 BINDING SITE | LOCATION OF INTERACTING PROTEIN | FUNCTION OF INTERACTING PROTEIN | REFERENCE |
|---|---|---|---|---|
| BAP1 | RING finger | nuclear | ubiquitin hydrolase | Jensen et al., 1998 |
| BARD1 | RING finger | nuclear | unknown, repair (?) | Wu et al., 1996 |
| BRCA2 | 1314-1863 | nuclear | transcription (?), repair | Chen at al., 1998 |
| c-Myc | 433-511 | nuclear | transcription | Wang et al., 1998 |
| CtiP | 1651-1863 | nuclear | transcription | Wong et al., 1998; Yu et al., 1998 |
| γ-tubulin | n.d. | cytoplasm | cytoskeletal | Hsu et al., 1998 |
| Importin-α | NLSs | nuclear/cytoplasm | nuclear import | Chen et al., 1996b |
| p53 | 224-500 | nuclear | transcription | Ouchi et al., 1998; Zhang et al., 1998 |
| RAD51 | 758-1064 | nuclear | repair | Scully et al., 1997b |
| RNA helicase A | 1650-1800 | nuclear | transcriptional | Anderson et al., 1998 |

proteins are nuclear and their interaction with BRCA1 is consistent with the proposed role of BRCA1 in transcription.

Two previously unknown proteins were also found, BARD1, a protein of unknown function containing a RING finger and two BRCT domains (Wu et al., 1996) and BAP-1 a protein with ubiquitin hydrolase activity (Jensen et al., 1998). With exception of γ-tubulin, all the proteins found to interact in vivo with *BRCA1* are nuclear or participate in nuclear import (Table 2).

## Conclusion

The controversy about BRCA1 is a cautionary tale for cell biologists as well as pathologists and illustrates the difficulty to unambiguously assign a location to a protein of unknown function. There is an overwhelming body of direct evidence that points to a nuclear localization and function for BRCA1 in normal and cancer cells. Similarly, there is an increasing amount of indirect evidence, both from interacting proteins and from functional studies of BRCA1, that support the data for the nuclear localization for BRCA1. It is too soon to evaluate the functional significance and relevance of the interacting proteins identified in several screening approaches. One way to assess the significance of the interaction is to show that interaction is disrupted by disease-causing mutations, which has been shown in some cases. In any case, results from interaction experiments seem to support the notion that BRCA1 is involved in repair and in transcriptional regulation.

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