Molecular Cancer Research

DNA Damage and Cellular Stress Responses

KIAA0101 Interacts with BRCA1 and Regulates Centrosome Number

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Abstract

To find genes and proteins that collaborate with BRCA1 or BRCA2 in the pathogenesis of breast cancer, we used an informatics approach and found a candidate BRCA interactor, KIAA0101, to function like BRCA1 in exerting a powerful control over centrosome number. The effect of KIAA0101 on centrosomes is likely direct, as its depletion does not affect the cell cycle, KIAA0101 localizes to regions coincident with the centrosomes, and KIAA0101 binds to BRCA1. We analyzed whether KIAA0101 protein is overexpressed in breast cancer tumor samples in tissue microarrays, and we found that overexpression of KIAA0101 correlated with positive Ki67 staining, a biomarker associated with increased disease severity. Furthermore, overexpression of the *KIAA0101* gene in breast tumors was found to be associated with significantly decreased survival time. This study identifies KIAA0101 as a protein important for breast tumorigenesis, and as this factor has been reported as a UV repair factor, it may link the UV damage response to centrosome control. *Mol Cancer Res; 9(8); 1091–9. ©2011 AACR.*

Introduction

Breast cancer, one of the most common malignancies affecting women worldwide, is attributable in about 40% of familial breast cancer cases to mutations in either one of the known breast cancer associated genes, *BRCA1* or *BRCA2* (1, 2). The search for other "BRCA" genes has not identified any new candidate gene, though there are families with breast cancer predisposition and no known mutation of either *BRCA1* or *BRCA2* (3, 4). It is possible that the remaining familial cases of breast cancer are due to gene mutations that have low penetrance for the breast cancer phenotype, and this low penetrance would complicate their discovery. We hypothesize that potential protein–protein interactions inferred from gene expression data can reveal genes/proteins that interact with either BRCA1 or BRCA2 in their biological functions, and these may be important markers for breast cancer.

Previous work to identify BRCA1-interacting proteins from gene expression data has utilized a network modeling strategy to identify genes that are potentially associated with breast cancer (5). In that study, microarray results from a single large microarray data set were used to find genes that had mRNA levels that correlated with *BRCA1*, *BRCA2*, *ATM*, and *CHK2* in all of the samples. Results identified 164 genes that were candidate BRCA1- and BRCA2-interacting proteins. To focus on specific candidates from among these 164 genes, omic data sets were used to rank individual genes/proteins in the BRCA-centered network. One gene/protein identified in the generated network was *HMMR*, and experimental results revealed functional associations with BRCA1 that were previously unknown. Specific single-nucleotide polymorphisms (SNP) in the *HMMR* locus were shown to be associated with an increased risk for breast cancer in specific populations of humans. Thus, the network modeling strategy was effective and showed that it can be used in discovering new cancer-associated genes and generating functional interactions between its components (5).

Depletion of BRCA1 in mammary-derived cells in tissue culture results in centrosome amplification (6), a phenotype that is commonly seen in early-stage human tumors including breast tumors (7, 8). Centrosomes are nonmembranous organelles that are essential in establishing bipolar spindles in mitotic cells and thus are important for the control of proper chromosome segregation into daughter cells (9). Normally, centrosome duplication happens only once during the cell cycle in coordination with the replicating DNA. Having exactly 2 centrosomes in dividing cells is crucial for the formation of bipolar spindles and thus for the appropriate segregation of chromosomes into progeny cells. BRCA1 regulates centrosome duplication through its E3 ubiquitin ligase

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Note: Supplementary data for this article are available at Molecular Cancer Research Online (http://mcr.aacrjournals.org/).

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doi: 10.1158/1541-7786.MCR-10-0503

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activity where it ubiquitinates γ-tubulin (a centrosomal protein) and thereby prevents centrosome reduplication within the same cell cycle (6, 10, 11). HMMR was identified to be functionally and physically associated with BRCA1. HMMR depletion resulted in centrosome amplification, the same phenotype that was seen with the depletion of BRCA1 (5). Finding new genes that collaborate with BRCA1 in this phenotype is thus important because it will eventually lead to the identification of genes that might contribute to the pathogenesis of breast cancer.

In this study, we utilize a similar informatics strategy, using multiple publicly available microarray data sets, to find genes/proteins that have high correlation with the mRNA levels of *BRCA1*, *BRCA2*, and *HMMR*. We further screen the candidate interactor for relevance to breast cancer using an online resource (Oncomine) to identify changes in gene expression in tumor samples (12). For the proteins that pass these 2 filters, we test whether they interact with BRCA1-regulated biological processes using tissue culture–based functional assays. In this coexpression analysis, *KIAA0101* was one of the genes that had consistently high coexpression levels with the reference genes, and Oncomine analysis revealed its association with increased metastasis and higher cancer grade. Analysis of the KIAA0101 protein in cells revealed that its concentration must be precisely controlled for the regulation of centrosomes, as either depletion or overexpression of the protein results in the disruption of centrosome duplication control. Our results indicate that the concentration of the KIAA0101 protein must be finely modulated, and in many breast tumors with aggressive phenotype, we detected that this protein is overexpressed. In addition, KIAA0101 overexpression correlated with lower breast cancer patient survival rates. Controlling centrosome number is a major regulatory step in the prevention of genomic instability, and by being correlated with increased tumor aggressiveness and poor patient survival rates, KIAA0101 stands out as a promising biomarker for breast cancer.

Materials and Methods

Cell lines and cell culture

Hs578T (ATCC cell line HTB-1216) and HeLa S3 cells (ATCC cell line CCL-2.2) were cultured according to the American Type Culture Collection (ATCC) recommendations. HeLa cells were double blocked in S-phase by treatment with 2 mmol/L thymidine (Sigma) for 18 hours, followed by growth in thymidine-free medium for 9 hours and reblocking in 2 mmol/L thymidine for another 18 hours. Cells were blocked in M phase by growth in 2 mmol/L thymidine for 18 hours, followed by growth in thymidine-free medium for 3 hours, and 100 ng/mL of nocodazole (Sigma) for 12 hours.

Whole-cell extracts were made from monolayers of cells by incubating the cells in 50 mmol/L Tris, pH 7.9, 300 mmol/L NaCl, 0.5% NP40, 1 mmol/L EDTA, and 5% glycerol, for 30 minutes at 0°C, followed by centrifugation to remove cell debris.

Centrosome duplication assay

The assay was done in HeLa and Hs578T cells. siRNA and green fluorescent protein (GFP)-centrin plasmid (13) transfection was done using Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol, and cells were fixed 48 hours posttransfection. GFP-centrin-2 marks centrioles, and these were counted by fluorescence microscopy using a Zeiss Axiovert 200 M microscope. Specific siRNA constructs used were as follows. The control oligonucleotide targets luciferase mRNA (5′ to 3′, UCG AAG UAU UCC GCG UAC GTT), 3 different siRNAs were specific for the *KIAA0101* mRNA (5′ to 3′, GGA AUU GGA GAA UUC UUU AGG UUG U, ACA ACC UAA AGA AUU CUC CAA UUC C, and AUG AAA CUG AUG UCG AAU UAG UGG C).

Homologous recombination assay

HeLa-DR cells (14) are a derivative of HeLa cells that contain a recombination substrate (15) in its genome. Following transfection of a plasmid for the expression of the I-*Sce*I endonuclease, homologous recombination repair results in gene conversion changing an inactive GFP allele into an active allele. The effect of KIAA0101 depletion on homologous recombination was tested using published methods (14).

Construction of KIAA0101 expression vectors and antibody production

The full-length open reading frame of *KIAA0101* was cloned by PCR using MGC full-length cDNA clones (Open Biosystems). The full-length *KIAA0101* gene was subcloned into a pcDNA3 vector for expression in mammalian cells and into pET21a for expression of the hexahistidine-tagged protein from bacteria. Details of the subcloning strategy are available from the authors. The expression and purification of bacterially expressed KIAA0101 protein was by standard methods, including Ni-NTA metal ion affinity chromatography followed by *S*-methyl sulfonate ion exchange column. Immunization was conducted by a commercial vendor (Cocalico Biologicals) and then the generated antibody was antigen affinity purified using recombinant KIAA0101 protein bound to Affi-Gel 10 (Bio-Rad).

Antibodies

Antibodies specific to α-tubulin (Sigma) was used at a dilution of 1:20,000. Cyclin A and cyclin B antibodies (Santa Cruz) were used at a dilution of 1:1,000. KIAA0101 antibody was used at a dilution of 1:250 in Western blots and 1:100 in immunofluorescence. The antiserum was affinity purified using the full-length recombinant KIAA0101 protein using standard procedures and was used at a dilution of 1:75 for immunohistochemistry. The immunohistochemistry was conducted by the OSU pathology core resource.

Immunofluorescence microscopy

Cells were fixed in methanol and then blocked with 5% bovine serum albumin (BSA/PBST) for 1 hour at room temperature. The cells were then incubated with the

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affinity-purified primary antibody at a 1:100 dilution in 5% BSA/PBST overnight. Cells were then washed in PBST and then incubated in anti-rabbit conjugated with either Texas red or fluorescein isothiocyanate (FITC) for 1 hour. Sections were then washed in PBST and then incubated with 4′,6-diamidino-2-phenylindole (DAPI; Invitrogen) for 5 minutes. Cells were washed and mounted in ProLong Gold antifade reagent (Invitrogen).

For the centrosome assay, cells were fixed with methanol, stained in DAPI, and then incubated with wheat germ agglutinin conjugated with Texas red (Invitrogen) to stain the cell membrane. Cells were then washed and mounted in ProLong Gold antifade.

Coimmunoprecipitation

The BRCA1 antibody (16) was prebound to the protein A agarose beads for 2 hours at 4°C. Then the whole-cell extract was added to the beads and rotated end over end overnight at 4°C in 50 mmol/L Tris, 300 mmol/L NaCl, 0.5% NP40, 1 mmol/L EDTA, and 5% glycerol. The beads were then washed 5 times in cold PBS. 1.5× sample buffer was then added to the moist beads and the beads were then boiled for 10 minutes.

Calculation of PCC values

In each data set, we identify the probe set with the maximum mean expression value for each reference gene. The Pearson's correlation coefficient (PCC) values for all the probe sets in that data set with respect to the selected anchor gene probe set are computed and the probe sets are sorted on the basis of the PCC values in a descending order. Probe sets with high PCC values (larger than the threshold) are selected and voted over multiple reference genes. Both processes were implemented using MATLAB scripts and software is available on request.

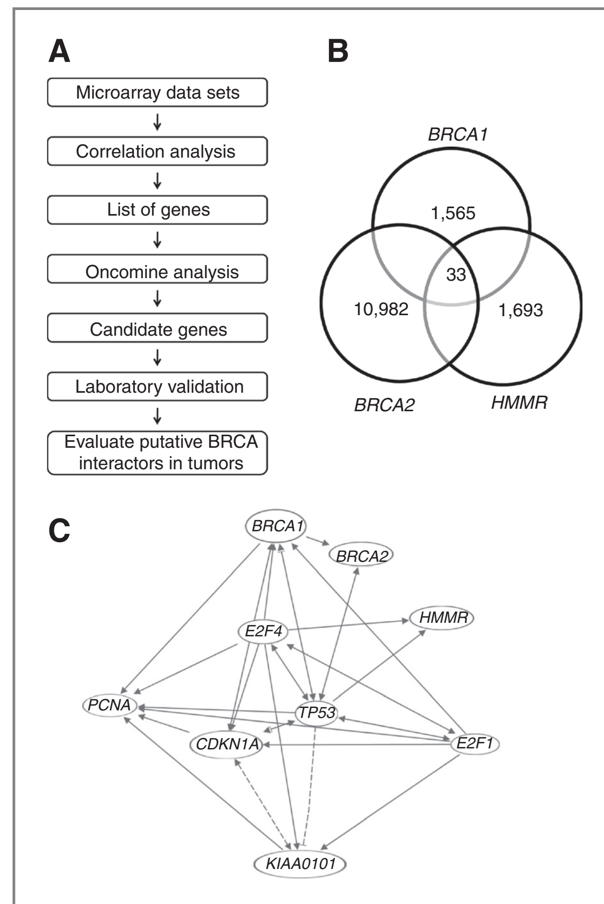

Figure 1. Summary of the workflow to discover candidate BRCA interactors. A, the summary of the workflow used for the discovery of BRCA interactors. B, an example taken from GEO data set GDS2367 with 33 genes/proteins that correlated with the 3 reference genes with a PCC value > 0.6. C, Ingenuity Pathway Analysis revealing links from *BRCA1*, *BRCA2*, and *HMMR* to *KIAA0101*.

Results

We followed a strategy based on the observations that genes that function together tend to have similar expression patterns (17–19) revealing functional modules (20). We applied coexpression analysis to data sets deposited in the Gene Expression Omnibus database (21) to initiate our search for genes that are likely to be coexpressed with the reference genes: *BRCA1*, *BRCA2*, and *HMMR*. BRCA1 and BRCA2 are established as highly penetrant tumor suppressors for breast cancer, and HMMR was previously identified as associated with BRCA1 in the control of centrosome function (5). We hypothesized that by using this approach (Fig. 1A), we could discover new genes and fill the gaps in the BRCA pathway. In this coexpression analysis, the gene *KIAA0101* consistently revealed high levels of correlation with *BRCA1*, *BRCA2*, and *HMMR* in multiple data sets and was thus identified as a candidate BRCA interactor (Fig. 1B). Oncomine analysis showed that its expression level correlates with severity of the breast tumor (Supplementary Fig. S1). Though published data on *KIAA0101* was low, a regulatory circuit was identified that links *BRCA1*, *BRCA2*, and *HMMR* to *KIAA0101* (Fig. 1C). At the time we began the work on this study, there were very few published studies on *KIAA0101*. In 2 publications, *KIAA0101* was observed among a number of genes whose expression was elevated in cancer (22, 23). In addition, one study renamed this protein p15(PAF), as it associated with the DNA repair factor, proliferating cell nuclear antigen (PCNA; ref. 24). A recent finding revealed that KIAA0101/p15(PAF) protein is important in the response to DNA damage caused by UV irradiation (25). Given that the analysis revealed this protein to have its mRNA expression highly correlated with the BRCAs, we evaluated this protein in the context of BRCA1-dependent processes in control of homologous recombination and in centrosome duplication.

KIAA0101 regulates centrosome number

BRCA1 has been shown to regulate the pathways that control centrosome number and homologous recombination in cultured cells. We first examined the effect of KIAA0101 depletion on centrosome number by transfecting

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into cells siRNAs specific for *KIAA0101*. The cell lines tested included a breast tumor–derived cell line (Hs578T) and the cervical epithelial cell line, HeLa. Because the siRNAs were cotransfected with a plasmid that expresses GFP-centrin-2, which localizes to the centrioles (13), centrosome amplification was then determined using fluorescence microscopy. KIAA0101 depletion resulted in centrosome amplification. In HeLa cells, 10% of the cells depleted of KIAA0101 had supernumerary centrosomes, whereas control siRNA depletion resulted in 2% of the cells with extra centrosomes. Similarly, in the breast cancer tissue culture cell line, Hs578T, KIAA0101 depletion resulted in 23% of the cells having supernumerary centrosomes, whereas 3% of control cells had amplified centrosomes (Fig. 2A-B). Similar results of centrosome amplification were observed when transfecting 2 other siRNA oligonucleotides specifying *KIAA0101* (Supplementary Fig. S2A), indicating that these results from the depletion of KIAA0101 are not due to off-target effects of the siRNA. These results reveal that depletion of KIAA0101 results in the same phenotype generated upon depletion of BRCA1, supernumerary centrosomes. Interestingly, overexpression of KIAA0101 also resulted in centrosome amplification in both cell lines, a phenotype that was not observed with the overexpression of BRCA1 (Supplementary Fig. S2B) and thus was unique to KIAA0101. In the Hs578T cells, 21% of cells overexpressing KIAA0101 had supernumerary centrosomes as compared with 5% in cells transfected with the empty vector. In HeLa cells, 14% of the cells overexpressing KIAA0101 had supernumerary centrosomes, compared with 5% of vector control transfected cells (Fig. 2C-D). We also tested simultaneous depletion of BRCA1 and KIAA0101 but no additive phenotype was detected (Supplementary Fig. S2C). A modest additive effect was observed when BRCA1 was depleted and KIAA0101 was simultaneously overexpressed (Supplementary Fig. S2C). Taken together, our results clearly show the major role of KIAA0101 in the regulation of centrosome number in the cell. The concentration of KIAA0101 must be precisely regulated: If KIAA0101 abundance is either too high or too low, it results in centrosome number defects.

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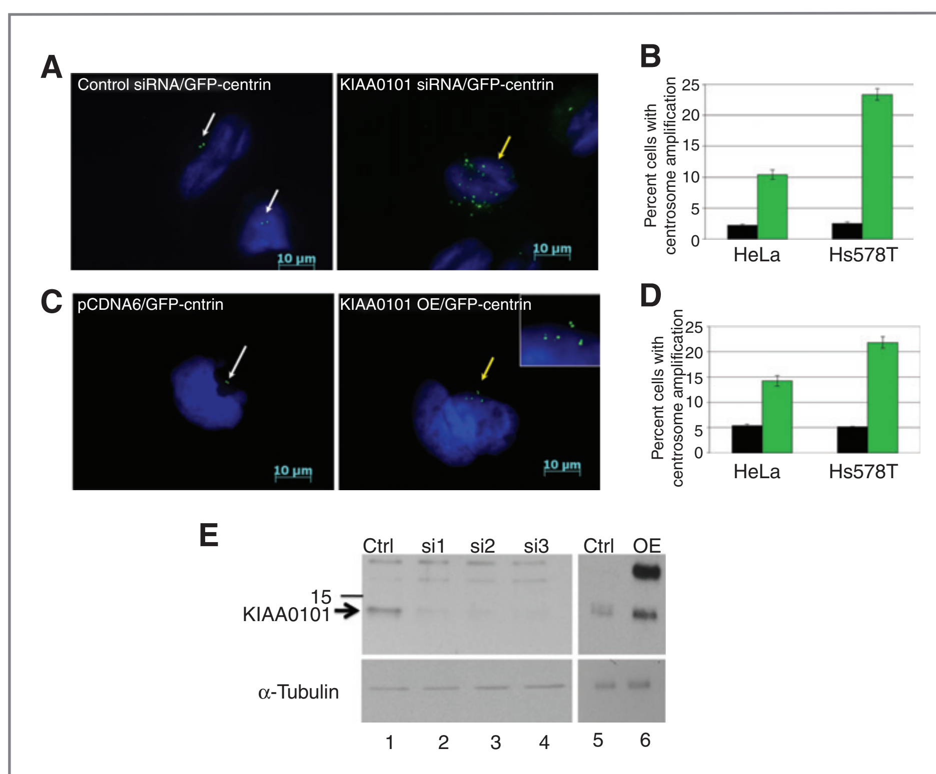

Figure 2. KIAA0101 controls centrosome number. A, KIAA0101 depletion results in centrosome amplification. Fluorescence microscopy results for GFP-centrin are shown in Hs578T cells transfected with control (left) or KIAA0101 (right) siRNA. White arrows indicate normal centrosomes; yellow arrows indicate centrosome amplification. (Bar: 10 μm) B, the percentages of cells with centrosome amplification in HeLa and Hs578T cells upon depletion of KIAA0101 are shown (green bar). The percentages of cells transfected with a control siRNA with aberrant centrosomes are indicated with black bars. C, fluorescence microscopy results for GFP-centrin are shown in Hs578T cells overexpressing KIAA0101 by transfection of an expression plasmid. The white arrow indicates normal centrosomes; the yellow arrow indicates centrosome amplification. (Bar: 10 μm). D, the percentages of cells with centrosome amplification in HeLa and Hs578T cells upon overexpression of KIAA0101 (green bar) or vector (black bar). E, an immunoblot for KIAA0101 protein is shown. Lysate from HeLa cells transfected with the control siRNA (lane 1) was compared with 3 different siRNAs specific for KIAA0101 (si; lanes 2–4). Lysate from empty vector-transfected HeLa cells (Ctrl, lane 5) was compared with the lysate from cells transfected with the KIAA0101 expression plasmid (OE; lane 6). The KIAA0101-specific antibody used for immunoblots was crude antiserum. Reprobing the same membrane for α-tubulin provides the loading control (bottom).

Because *KIAA0101* was identified because of correlated mRNA levels with 2 homology-directed recombination (HDR) repair factors, BRCA1 and BRCA2, we tested whether KIAA0101 depletion would block HDR using a cell line that scores gene conversion events secondary to homologous recombination (14, 15). KIAA0101 depletion resulted in no detectable reduction in GFP-positive cells, indicating that the KIAA0101 protein does not share with BRCA1 and BRCA2 the functional role in homologous recombination (Supplementary Fig. S3).

KIAA0101 protein levels vary according to the cell cycle with peak concentrations during S and G_2 phases

We tested the stage of the cell cycle at which KIAA0101 protein concentration is highest. HeLa cells were double blocked in thymidine to obtain cells synchronized in early S-phase or blocked in thymidine and subsequently in nocodazole to obtain cells in early mitosis. In each case, the cell cycle was released by changing the medium and harvesting cell lysates at 0, 3, 6, and 9 hours postrelease. This protocol results in a set of lysates distributed throughout the cell cycle. The expression level of KIAA0101 protein at different positions in the cell cycle was determined using Western blot analysis comparing KIAA0101 to cyclin A and cyclin B. KIAA0101 expression paralleled to a certain extent the expression of cyclin A, suggesting that KIAA0101 levels increase in S and G_2 and decrease after the initiation of mitosis (Fig. 3A). Because centrosomes duplicate early in S-phase (26, 27), this temporal distribution of KIAA0101 protein could be consistent with inhibiting the overduplication of the centrosome.

We next tested whether KIAA0101 protein was required for passage through the normal cell cycle. KIAA0101 protein was depleted by RNA interference and whether the population of cells was blocked at any particular stage of the cell cycle was determined by flow cytometry at time points 48, 72, and 96 hours postdepletion. We found that KIAA0101 depletion had no effect on the cell-cycle progression (Fig. 3B).

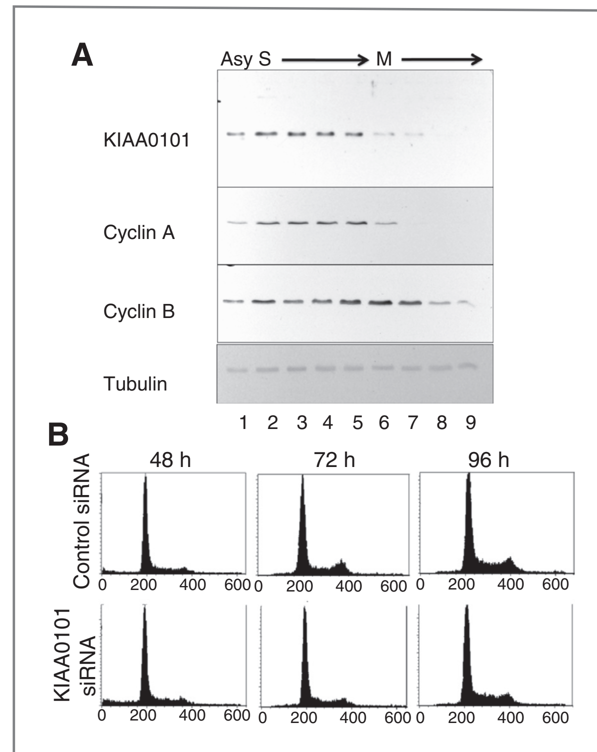

Figure 3. KIAA0101 abundance is cell cycle controlled. A, lysates of HeLa cells were double blocked with thymidine and released for 0 (lane 2), 3 (lane 3), 6 (lane 4), and 9 (lane 5) hours. Lysates of HeLa cells treated with a thymidine block followed by nocodazole block and release were harvested at 0 (lane 6), 3 (lane 7), 6 (lane 8), and 9 (lane 9) hours. Lysates from asynchronous cells (Asy) were analyzed in lane 1. Immunoblots were probed for KIAA0101, cyclin A, cyclin B, and α-tubulin. The membranes were cut at 37 kD to blot them with KIAA0101 antibody and cyclin antibodies at the same time. B, cell-cycle analysis by flow cytometry 48, 72, and 96 hours postdepletion of KIAA0101. Top, DNA content of HeLa cells transfected with control siRNA, and bottom, DNA content of HeLa cells transfected with KIAA0101 siRNA.

KIAA0101 protein localizes to the nucleus and a perinuclear space coincident with the centrosome

We next investigated the subcellular localization of endogenous KIAA0101 protein by immunofluorescence microscopy. In HeLa cells, KIAA0101 was detected colocalized with nuclei and with an asymmetrical perinuclear staining pattern (Fig. 4A). This pattern of KIAA0101 had been previously observed and characterized as mitochondrial (24). We observed in addition a bright focus of KIAA0101 stain at a single site adjacent to the nuclear membrane that could be consistent with localization to the centrosome. This was tested directly by expressing GFP-tagged centrin-2 protein, which localizes to the centrosomes, and in 100% of the HeLa and Hs578T cells, the bright focus of KIAA0101 stain correlated with the centrin-2 stain indicating its localization to regions that are coincident with both the mother and the daughter centrioles (Fig. 4B top; Supplementary Fig. S4, left). In fact, in the case of the Hs578T breast cancer cell line, there was very little nuclear stain for KIAA0101, and nearly all of the KIAA0101 protein was detected in this perinuclear position that included the centrosomes (Fig. 4B, top). Depletion of KIAA0101 (Fig. 4B, bottom) resulted in an overall decrease in the intensity of the immunofluorescence signal, the focus coincident with the centrosome region was lost, and supernumerary centrosomes were apparent. Overexpression of the protein along with extra centrosomes was also shown using immunofluorescence (Supplementary Fig. S4, right). These results suggest that the effect of KIAA0101 protein on centrosomes may be direct. Similar to BRCA1 (11), KIAA0101 was present at the centrosomes at all the different stages of the cell cycle; however, its levels increased during S–G_2 (Supplementary Fig. S5).

To test whether the KIAA0101 protein directly interacts with BRCA1, we conducted a coimmunoprecipitation experiment of the 2 endogenous proteins (KIAA0101 and BRCA1). KIAA0101 and BRCA1 were found to associate in endogenous protein complexes in HeLa cells

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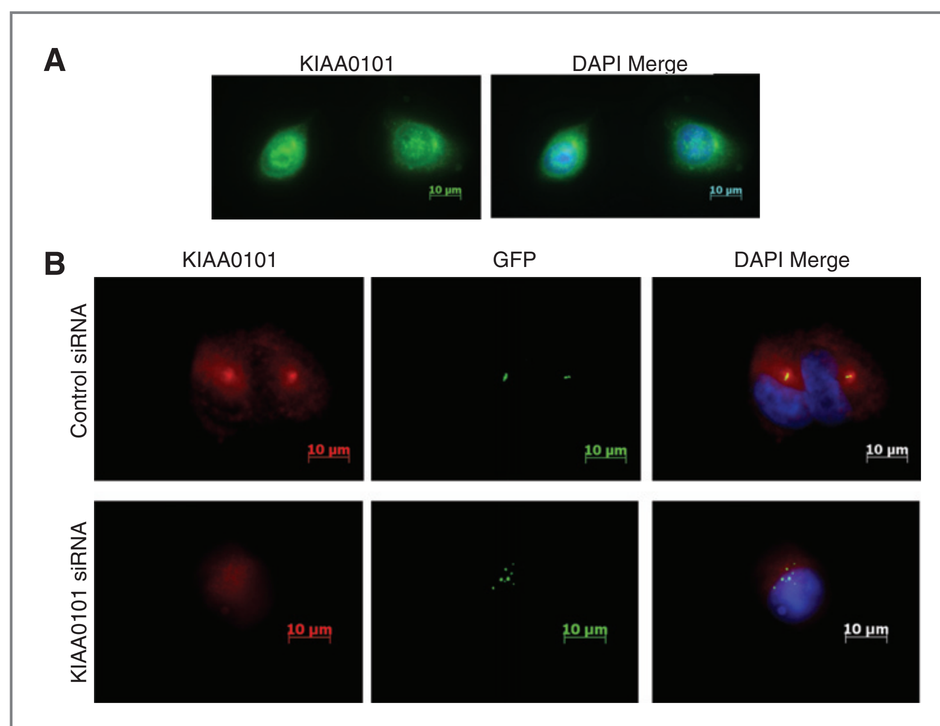

Figure 4. Colocalization of KIAA0101 with the centrosome. A, immunofluorescence microscopy of KIAA0101 (green) and the DAPI counterstain (blue and merged, right only) showing nuclear and asymmetric perinuclear localization of KIAA0101 in HeLa cells. (Bar: 10 μm). B, Hs578T breast cancer cells were transfected with control (top) or KIAA0101-specific (bottom) siRNAs for 48 hours and stained as indicated. KIAA0101 (left), GFP-centrin (middle), and the merged image containing also DAPI counterstaining (right) show colocalization of KIAA0101 to foci and to cytoplasmic regions coincident with the centrosomes in Hs578T cells that had been transfected with the control siRNA. Depletion of KIAA0101 resulted in diminished stain for KIAA0101 and extra centrosomes. (Bar, 10 μm).

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(Fig. 5), suggesting that KIAA0101 may regulate centrosome duplication by direct physical interaction with BRCA1.

Overexpression of KIAA0101 protein in breast tumor samples correlates with a biomarker for high proliferation and aggressive tumors

Because depletion of either KIAA0101 or BRCA1 resulted in the same phenotype in the centrosome assay, we tested for changes in the expression of KIAA0101 protein in clinical samples using antibody-based stain of breast tumors in tissue microarrays (TMA). Western blot analysis confirmed the high specificity of the affinity-purified antibody that we have generated against KIAA0101, as the only band detected by this antibody preparation migrated at a position consistent with a 12-kD polypeptide (Supplementary Fig. S6) and which was depleted by siRNAs specific for *KIAA0101* (Fig. 2). Previous studies have indicated overexpression of *KIAA0101* mRNA by microarray analyses in a number of tumors including breast, pancreatic, and liver tumors (23, 28, 29), but such results on mRNA abundance may not reflect protein content. Here, we find that KIAA0101 protein, as detected by immunohistochemistry, was overexpressed in the breast cancer tissue. In one example, a breast tumor sample was compared with adjacent normal breast tissue (Fig. 6). In the normal tissue, the epithelial

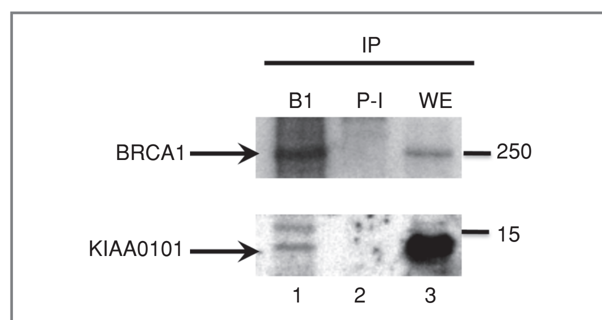

Figure 5. BRCA1 and KIAA0101 proteins are in a complex. HeLa whole-cell extract (WE) was immunoprecipitated (IP) with BRCA1-specific antiserum (B1; lane 1) or matched control preimmune serum (P-I; lane 2) and analyzed by immunoblotting for BRCA1 (top) and for KIAA0101 (bottom). Five percent of the WE was analyzed in lane 3. Full-length blots are presented in Supplementary Figure S7.

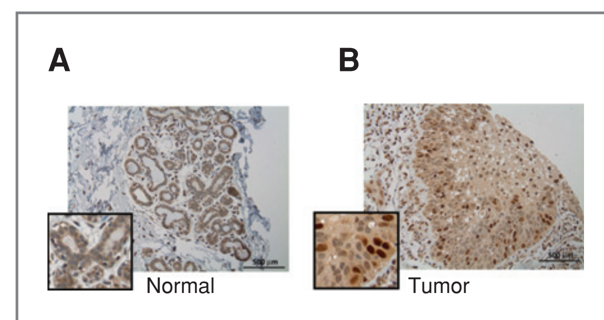

Figure 6. Overexpression of KIAA0101 in breast cancer tumors. A, normal breast tissue with background KIAA0101 staining observed as a pale brown cytoplasmic background stain. B, an invasive ductal carcinoma from the same patient as the normal sample, has increased expression of KIAA0101 seen as dark brown nuclei. Insets show higher magnification views of representative sections of the same image. (Bar, 500 μm).

Table 1. Comparison of the expression level of KIAA0101 to different biomarkers of breast cancer in 2 independent TMA studies

	Total tumor samples	% KIAA0101 positive	Biomarkers	% KIAA0101 positive
TMA1	80	29/80 (36.3)	Ki67 negative	0/6 (0)
			Ki67 intermediate	11/41 (26.8)
			Ki67 positive	18/33 (54.5)[a]
	81	30/81 (37.04)	ER negative	19/43 (44.2)
			TNBC	14/26 (53.8)[b]
TMA2	256	138/256 (53.9)	ER negative	44/72 (61.1)
			TNBC	28/46 (60.9)

[a]Fisher test $P = 0.006$.
[b]Fisher test $P = 0.048$.

cells stained pale brown in the cytoplasm with blue counterstained nuclei apparent (Fig. 6A). In the tumor sample from the same patient, many cells were apparent with intensely brown-stained nuclei indicating overexpression of KIAA0101 (Fig. 6B).

We next analyzed and compared the expression of KIAA0101 to the expression of known breast cancer biomarkers such as Ki67, estrogen receptor (ER), progesterone receptor (PR), and Her2, using TMA analysis. We used 2 different tumor sets: the first containing 81 breast tumor samples that included all 4 biomarkers and the second containing 256 samples stained for ER, PR, and Her2. We applied a grading system for immunohistochemical results with KIAA0101 that is similar to that applied to analyzing the ER biomarker in which the percentage of cells with positive nuclei and the intensity of stain are both scored. If 10% of the epithelial cell nuclei were positive for the KIAA0101 antigen, then the sample was considered to be positive. Overall, in the 2 TMAs, KIAA0101 was overexpressed in 37% and 54% of the tumors. This was compared with 25% of normal breast samples (5 of 20 samples). The number of normal samples analyzed was too small to give confidence to the increase in KIAA0101 detected in the tumor samples.

Next, we compared the expression of KIAA0101 to the expression of Ki67 in 80 tumor samples taken from the first TMA set. Ki67 is a cellular marker associated with proliferation and when positive is correlated with a high risk of relapse and a worse survival in patients with early breast cancer (30). Overexpression of KIAA0101 was significantly correlated with high positive staining of Ki67 ($P = 0.006$) indicating a strong correlation between overexpression of KIAA0101 and the aggressiveness of the tumor (Table 1). In both TMA sets, a trend was observed with KIAA0101 overexpression in more than 50% of the triple-negative breast cancer (TNBC) tumors (ER^-, PR^-, and $Her2^-$; Table 1). In one of the TMAs, the correlation of KIAA0101 overexpression with TNBC was statistically significant, but in the second TMA, this observed trend did not exceed the 95% confidence level.

We queried a data set containing mRNA abundance in breast tumors and patient survival (31). The data were divided into 2 groups high *KIAA0101* expressers and low expressers. We found that those patients who overexpressed *KIAA0101* had significantly shorter survival time. As a measure of this, the 75% survival rate of each group was about 5 years for the high expressers and about 14 years for the lower expressers (Fig. 7).

We interpret the results from the KIAA0101 protein stain in breast tumors and from the survival curve for high *KIAA0101* expressing breast tumors that KIAA0101 overexpression is strongly correlated with aggressive, proliferative breast cancer cases. Because many proliferative breast tumors are ER^- or TNBCs, there is a trend correlating KIAA0101 overexpression with these tumor types. If

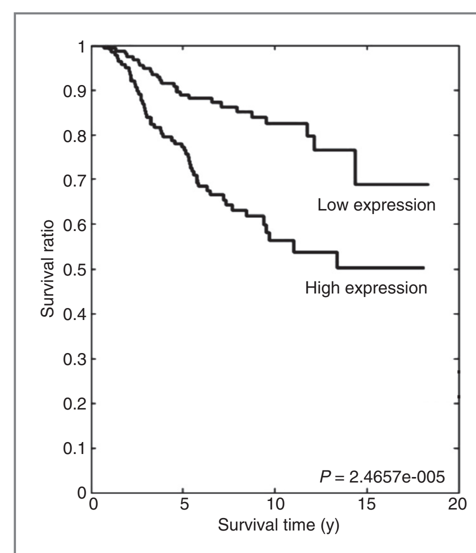

Figure 7. Overexpression of KIAA0101 correlates with worse survival rates. Data taken from the van't Veer microarray data set (31, 39) showing significant decrease in the average survival rate of breast cancer patients with tumor samples with high KIAA0101 expression (bottom) versus low KIAA0101 expression (top; $P = 2.5$ e-005). Log-rank test was used to determine the statistical significance.

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KIAA0101 is to be a useful biomarker, more tumors will need to be analyzed that also include outcome information to determine whether KIAA0101 overexpression can be used to stratify breast tumors in a novel way.

Discussion

In this study, we discovered that (i) KIAA0101 is a potential BRCA1 interactor; (ii) KIAA0101 protein concentrations are critical to appropriate control of centrosome duplication; (iii) KIAA0101 protein is a cell-cycle–regulated protein most abundant during S and G_2 phases; (iv) depletion of KIAA0101 does not affect passage through the cell cycle; (v) KIAA0101 localizes in the cell to the centrosome and to a perinuclear position that contains the centrosomes; (vi) KIAA0101 is in complex with BRCA1 protein; (vii) KIAA0101 is overexpressed in Ki67-positive breast cancer tumors and its overexpression is loosely correlated with TNBCs; and (viii) KIAA0101 overexpression in breast tumors results in lower survival rates than the low-expressing tumors.

Depletion of KIAA0101 resulted in significant centrosome amplification, a phenotype associated with BRCA1 depletion in breast cancer cell lines. Interestingly, KIAA0101 overexpression also resulted in the same phenotype, suggesting that the concentration of KIAA0101 must be precisely balanced for the regulation of centrosome duplication. Because both depletion and overexpression of KIAA0101 resulted in centrosome amplification, it is possible that KIAA0101 participates in 2 mechanisms that control centrosome number. One mechanism would be sensitive to KIAA0101 depletion, and another sensitive to KIAA0101 overexpression. Further work is targeted at identifying these mechanistic causes of centrosome amplification secondary to changes in KIAA0101 protein levels. In 2 separate articles on hepatocellular carcinoma, upregulation (23) or downregulation (32) of *KIAA0101* correlated with the development of the disease. Thus, our observation of precise KIAA0101 concentration control being critical for regulation of centrosomes is consistent with observations that either high or low concentrations of this protein are associated with hepatic cancers. KIAA0101 levels increased in S and G2 phases of the cell cycle, a time when KIAA0101 could, like BRCA1 (10, 11), block overduplication of centrosomes. Because KIAA0101 depletion did not affect cell-cycle progression and because KIAA0101 localized to centrosomes, our results are consistent with a direct role of KIAA0101 protein in regulating this organelle.

KIAA0101 protein had previously been identified as a factor associated with PCNA and important in the repair of UV-damaged DNA (25). The association with PCNA led to the anticipation that KIAA0101 would stimulate homologous recombination, and it was thus surprising that KIAA0101 depletion had no effect on this DNA repair process. Many DNA repair proteins also affect centrosome biology (33–38) and clearly KIAA0101 protein levels are critical for controlling centrosome number. However, like BRCA1, most of the DNA repair proteins that are involved in the regulation of centrosome number are known to be involved in the repair of DNA double-strand breaks, but KIAA0101 is different in that it is a UV-specific repair factor that connects this repair pathway to the centrosome.

Is KIAA0101 a tumor suppressor? Because KIAA0101 depletion causes centrosome amplification (this study) and because KIAA0101 is important in the UV damage response (25), it is possible that KIAA0101 is a tumor suppressor. However, it was its overexpression that was observed in a significant number of breast tumors, suggesting that it could be an oncogene. Further work is needed to determine the effects on cell growth and transformation of KIAA0101 depletion or overexpression.

Analysis of breast cancer tumors in TMAs showed KIAA0101 to be overexpressed in about 45% of breast cancer tumors, and it revealed a correlation between KIAA0101 and Ki67, a proliferation marker, suggesting an association between overexpression of KIAA0101 and increased chances of relapse and worse survival rate. These results of overexpression of KIAA0101 in Ki67-positive tumors are consistent with the Oncomine analysis that linked KIAA0101 mRNA levels with high-grade tumors. The utility of KIAA0101 as a protein biomarker for breast cancer is, however, currently unclear. KIAA0101 overexpression tended to be more common in ER-negative and triple-negative tumors, but the trend had borderline significance. Rather, KIAA0101 overexpression appears to be an independent marker from the current breast cancer classifiers. Thus, KIAA0101-positive immunohistochemistry may prove to be an important tool for the prediction of prognosis in early breast cancers that might eventually help guide the therapy for patients suffering from the invasive and aggressive form of the disease. Current work is aimed at analyzing more TMAs with matched patient outcome data to find how KIAA0101 immunohistochemistry can contribute to prognostication or to prediction of outcome for breast cancer patients.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

The authors thank S. Jones and J. Bacher of the OSUMC Pathology core for preparation of slides and conducting immunohistochemical analyses and the members of the Parvin Laboratory for helpful advice during the course of this study.

Grant Support

This work was supported by the NIH grants CA141090 and CA111480 to J.D. Parvin.

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Received November 8, 2010; revised June 2, 2011; accepted June 7, 2011; published OnlineFirst June 14, 2011.

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