Published in final edited form as:
Mol Cancer Res. 2011 August ; 9(8): 1091–1099. doi:10.1158/1541-7786.MCR-10-0503.

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 since 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. Further, 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 since this factor has been reported as a UV repair factor, it may link the UV damage response to centrosome control.

Keywords

KIAA0101; BRCA1; BRCA2; breast cancer; centrosomes

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

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Conflicts of interest: The authors report no conflicts of interest.

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 in order to identify genes that are potentially associated with breast cancer (5). In that study, microarray results from a single large microarray dataset 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. In order 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 SNPs 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 non-membranous 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 two 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 activity where it ubiquitinates gamma 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 datasets, 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 on-line resource (Oncomine) to identify changes in gene expression in tumor samples (12). For the proteins that pass these two 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 since 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 recommendations. HeLa cells were double blocked in S phase by treatment with 2 mM thymidine (Sigma) for 18h, followed by growth in thymidine-free medium for 9h and re-blocking in 2 mM thymidine for another 18h. Cells were blocked in M phase by growth in 2 mM thymidine for 18h, followed by growth in thymidine-free medium for 3h, and 100 ng/ml of nocodazole (Sigma) for 12h.

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

Centrosome duplication assay

The assay was done in HeLa and Hs578T cells. Small interfering RNA (siRNA) and GFP-centrin plasmid (13) transfection was done using Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol, and cells were fixed 48 hours post-transfection. GFP-centrin2 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), three 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-SceI 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 ORF 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 performed 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 1:1000. 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 immunohistochemstry. The immunohistochemistry was performed by the OSU pathology core resource.

Immunofluorescence microscopy

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

Co-immunoprecipitation

The BRCA1 antibody (16) was prebound to the protein-A-agarose beads for 2 hrs at 4ºC. Then the whole cell extract (WE) was added to the beads and rotated end-over-end overnight at 4ºC in 50 mM Tris, 300 mM NaCl, 0.5% NP40, 1 mM EDTA, and 5% glycerol. The beads were then washed 5 times in cold PBS. 1.5X sample buffer was then added to the moist beads and the beads were then boiled for 10 min.

Calculation of PCC values

In each dataset, we identify the probe set with the maximum mean expression value for each reference gene. The PCC values for all the probe sets in that dataset with respect to the selected anchor gene probe set are computed and the probe sets are sorted based on 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.

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 datasets deposited in the Gene Expression Omnibus database (21) to initiate our search for genes that are likely to be co-expressed 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 (Figure 1A) we could discover new genes and fill the gaps in the BRCA pathway. In this co-expression analysis, the gene *KIAA0101* consistently revealed high levels of correlation with *BRCA1*, *BRCA2*, and *HMMR* in multiple datasets and was thus identified as a candidate BRCA interactor (Figure 1B). Oncomine analysis showed that its expression level correlates with severity of the breast tumor (Supp. Figure S1). Though published data on *KIAA0101* was low, a regulatory circuit was identified that links *BRCA1*, *BRCA2*, and *HMMR* to *KIAA0101* (Figure 1C). At the time we began the work on this study, there were very few published studies on *KIAA0101*. In two 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), since it associated with the DNA repair factor, PCNA (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 into cells short interfering RNAs (siRNA) specific for *KIAA0101*. The cell lines tested included a breast-tumor derived cell line (Hs578T) and the cervical epithelial cell line, HeLa. Since the siRNAs were co-transfected 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 (Figure 2A). Similar results of centrosome amplification were observed when transfecting two other siRNA oligonucleotides specifying *KIAA0101* (Supp. 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, over-expression of KIAA0101 also resulted in centrosome amplification in both cell lines, a phenotype that was not observed with the over-expression of BRCA1 (Supp Fig. S2B) and thus was unique to KIAA0101. In the Hs578T cells, 21% of cells overexpressing KIAA0101 had supernumerary centrosomes as compared to 5% in cells transfected with the empty vector. In HeLa cells, 14% of the cells overexpressing KIAA0101 had supernumerary centrosomes, compared to 5% of vector control transfected cells (Figure 2A). We also tested simultaneous depletion of BRCA1 and KIAA0101 but no additive phenotype was detected (Supp Fig. S2C). A modest additive effect was observed when BRCA1 was depleted and KIAA0101 was simultaneously overexpressed (Supp 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.

Since *KIAA0101* was identified due to correlated mRNA levels with two 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 (Supp. Figure S3).

KIAA0101 protein levels vary according to the cell cycle with peak concentrations during S and G2 phases

We tested the stage of the cell cycle at which KIAA0101 protein concentration is highest. HeLa cells were double-blocked in thymidine in order to obtain cells synchronized in early S phase or blocked in thymidine and subsequently in nocodazole in order 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 post release. 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 G2 and decrease after the initiation of mitosis (Figure 3A). Since centrosomes duplicate early in S phase (26, 27), this temporal distribution of KIAA0101 protein could be consistent with inhibiting the over-duplication of the centrosome.

We next tested whether KIAA0101 protein was required for passage through the normal cell cycle. KIAA0101 protein was depleted by RNAi, 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 post depletion. We found that KIAA0101 depletion had no effect on the cell cycle progression (Figure 3B).

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 (Figure 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 (Figure 4B *top*; Supp. Figure 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 (Figure 4B, *top*). Depletion of KIAA0101 (Figure 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 (Figure 4B, *bottom*). Over-expression of the protein along with extra centrosomes was also shown using immunofluorescence (Supp. Figure S4, *right*). These results suggest that the effect of KIAA0101 protein on centrosomes may be direct. Similar to BRCA1 (Sankaran, et al., 2005), KIAA0101 was present at the centrosomes at all the different stages of the cell cycle, however, its levels increased during S/G2 (Supp. Figure S5).

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

Since 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. Western blot analysis confirmed the high specificity of the affinity-purified antibody that we have generated against KIAA0101 since the only band detected by this antibody preparation migrated at a position consistent with a 12 kD polypeptide (Supp. Figure S6) and which was depleted by siRNAs specific for *KIAA0101* (Figure 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 (IHC), was overexpressed in the breast cancer tissue. In one example a breast tumor sample was compared with adjacent normal breast tissue (Figure 6A). In the normal tissue, the epithelial cells stained pale brown in the cytoplasm with blue counter-stained nuclei apparent (Figure 6Aa). In the tumor sample from the same patient, many cells were apparent with intensely brown stained nuclei indicating overexpression of KIAA0101 (Figure 6Ab).

We next analyzed and compared the expression of KIAA0101 to the expression of known breast cancer biomarkers such as Ki67, ER, PR, and Her2, using tissue microarray (TMA) analysis. We used two different tumor sets: the first containing 81 breast tumor samples that included all four biomarkers and the second containing 256 samples stained for ER, PR, and Her2. We applied a grading system for IHC 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 two TMAs, KIAA0101 was overexpressed in 37% and 54% of the tumors. This was compared to 25% of normal breast samples (5/20 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 (Figure 6B). 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-negative; Figure 6B). 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 dataset containing mRNA abundance in breast tumors and patient survival (31). The data were divided into two 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 of each group was about 5 years for the high expressers, and about 14 years for the lower expressers (Figure 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. Since many proliferative breast tumors are ER-negative or TNBC, then there is a trend correlating KIAA0101 overexpression with these tumor types. If KIAA0101 is to be a useful biomarker, more tumors will need to be analyzed that also include outcome information in order to determine whether KIAA0101 overexpression can be used to stratify breast tumors in a novel way.

Discussion

In this study, we discovered: 1.) KIAA0101 is a potential BRCA1-interactor; 2.) KIAA0101 protein concentrations are critical to appropriate control of centrosome duplication; 3.) KIAA0101 protein is a cell cycle regulated protein most abundant during S and G2 phases; 4.) depletion of KIAA0101 does not affect passage through the cell cycle; 5.) KIAA0101

localizes in the cell to the centrosome and to a perinuclear position that contains the centrosomes; 6.) KIAA0101 is in complex with BRCA1 protein; 7.) KIAA0101 is overexpressed in Ki67-positive breast cancer tumors and its overexpression is loosely correlated with TNBC; and 8.) 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 two mechanisms that control centrosome number. One mechanism would be sensitive to KIAA0101 depletion, and one mechanism sensitive to KIAA0101 overexpression. Further work is targeted at identifying these mechanistic causes of centrosome amplification secondary to changes in KIAA0101 protein levels. In two separate papers 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 over-duplication of centrosomes. Since KIAA0101 depletion did not affect cell cycle progression, and since 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? Since KIAA0101 depletion causes centrosome amplification (this study), and since 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 tissue microarrays 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 IHC may prove to be an important tool for the prediction of prognosis in

early breast cancers that might eventually help guide the therapy of patients suffering from the invasive and aggressive form of the disease. Current work is aimed at analyzing more tissue microarrays with matched patient outcome data in order to find how KIAA0101 IHC can contribute to prognostication or to prediction of outcome for breast cancer patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We are grateful to S. Jones and J. Bacher of the OSUMC Pathology core for preparation of slides and performing of the IHC analyses. We are grateful to the members of the Parvin lab for helpful advice during the course of this study. This work was supported by the National Institute of Health CA141090 and CA111480 to J.D.P.

References

1. Serova OM, Mazoyer S, Puget N, Dubois V, Tonin P, Shugart YY, et al. Mutations in BRCA1 and BRCA2 in breast cancer families: are there more breast cancer-susceptibility genes? Am J Hum Genet. 1997 Mar; 60(3):486–95. [PubMed: 9042907]
2. Thompson D, Szabo CI, Mangion J, Oldenburg RA, Odefrey F, Seal S, et al. Evaluation of linkage of breast cancer to the putative BRCA3 locus on chromosome 13q21 in 128 multiple case families from the Breast Cancer Linkage Consortium. Proc Natl Acad Sci U S A. 2002 Jan 22; 99(2):827–31. [PubMed: 11792833]
3. Mangia A, Chiarappa P, Tommasi S, Chiriatti A, Petroni S, Schittulli F, et al. Genetic heterogeneity by comparative genomic hybridization in BRCAx breast cancers. Cancer Genet Cytogenet. 2008 Apr 15; 182(2):75–83. [PubMed: 18406868]
4. Oldenburg RA, Meijers-Heijboer H, Cornelisse CJ, Devilee P. Genetic susceptibility for breast cancer: how many more genes to be found? Crit Rev Oncol Hematol. 2007 Aug; 63(2):125–49. [PubMed: 17498966]
5. Pujana MA, Han JD, Starita LM, Stevens KN, Tewari M, Ahn JS, et al. Network modeling links breast cancer susceptibility and centrosome dysfunction. Nat Genet. 2007 Nov; 39(11):1338–49. [PubMed: 17922014]
6. Starita LM, Machida Y, Sankaran S, Elias JE, Griffin K, Schlegel BP, et al. BRCA1-dependent ubiquitination of gamma-tubulin regulates centrosome number. Mol Cell Biol. 2004 Oct; 24(19): 8457–66. [PubMed: 15367667]
7. Lingle WL, Lutz WH, Ingle JN, Maihle NJ, Salisbury JL. Centrosome hypertrophy in human breast tumors: implications for genomic stability and cell polarity. Proc Natl Acad Sci U S A. 1998 Mar 17; 95(6):2950–5. [PubMed: 9501196]
8. Pihan GA, Purohit A, Wallace J, Knecht H, Woda B, Quesenberry P, et al. Centrosome defects and genetic instability in malignant tumors. Cancer Res. 1998 Sep 1; 58(17):3974–85. [PubMed: 9731511]
9. Nigg EA. Centrosome aberrations: cause or consequence of cancer progression? Nat Rev Cancer. 2002 Nov; 2(11):815–25. [PubMed: 12415252]
10. Ko MJ, Murata K, Hwang DS, Parvin JD. Inhibition of BRCA1 in breast cell lines causes the centrosome duplication cycle to be disconnected from the cell cycle. Oncogene. 2006 Jan 12; 25(2):298–303. [PubMed: 16170356]
11. Sankaran S, Starita LM, Groen AC, Ko MJ, Parvin JD. Centrosomal microtubule nucleation activity is inhibited by BRCA1-dependent ubiquitination. Mol Cell Biol. 2005 Oct; 25(19):8656–68. [PubMed: 16166645]
12. Rhodes DR, Yu J, Shanker K, Deshpande N, Varambally R, Ghosh D, et al. ONCOMINE: a cancer microarray database and integrated data-mining platform. Neoplasia. 2004 Jan–Feb; 6(1):1–6. [PubMed: 15068665]

13. D'Assoro AB, Stivala F, Barrett S, Ferrigno G, Salisbury JL. GFP-centrin as a marker for centriole dynamics in the human breast cancer cell line MCF-7. Ital J Anat Embryol. 2001; 106(2 Suppl 1): 103–10. [PubMed: 11729945]
14. Ransburgh DJ, Chiba N, Ishioka C, Toland AE, Parvin JD. Identification of breast tumor mutations in BRCA1 that abolish its function in homologous DNA recombination. Cancer Res. 2010 Feb 1; 70(3):988–95. [PubMed: 20103620]
15. Pierce AJ, Hu P, Han M, Ellis N, Jasin M. Ku DNA end-binding protein modulates homologous repair of double-strand breaks in mammalian cells. Genes Dev. 2001 Dec 15; 15(24):3237–42. [PubMed: 11751629]
16. Schlegel BP, Starita LM, Parvin JD. Overexpression of a protein fragment of RNA helicase A causes inhibition of endogenous BRCA1 function and defects in ploidy and cytokinesis in mammary epithelial cells. Oncogene. 2003 Feb 20; 22(7):983–91. [PubMed: 12592385]
17. Agrawal H. Extreme self-organization in networks constructed from gene expression data. Phys Rev Lett. 2002 Dec 23.89(26):268702. [PubMed: 12484863]
18. Jansen R, Greenbaum D, Gerstein M. Relating whole-genome expression data with protein-protein interactions. Genome Res. 2002 Jan; 12(1):37–46. [PubMed: 11779829]
19. Jansen R, Yu H, Greenbaum D, Kluger Y, Krogan NJ, Chung S, et al. A Bayesian networks approach for predicting protein-protein interactions from genomic data. Science. 2003 Oct 17; 302(5644):449–53. [PubMed: 14564010]
20. Tornow S, Mewes HW. Functional modules by relating protein interaction networks and gene expression. Nucleic Acids Res. 2003 Nov 1; 31(21):6283–9. [PubMed: 14576317]
21. Barrett T, Troup DB, Wilhite SE, Ledoux P, Rudnev D, Evangelista C, et al. NCBI GEO: mining tens of millions of expression profiles--database and tools update. Nucleic Acids Res. 2007 Jan; 35(Database issue):D760–5. [PubMed: 17099226]
22. Collado M, Garcia V, Garcia JM, Alonso I, Lombardia L, Diaz-Uriarte R, et al. Genomic profiling of circulating plasma RNA for the analysis of cancer. Clin Chem. 2007 Oct; 53(10):1860–3. [PubMed: 17717129]
23. Yuan RH, Jeng YM, Pan HW, Hu FC, Lai PL, Lee PH, et al. Overexpression of KIAA0101 predicts high stage, early tumor recurrence, and poor prognosis of hepatocellular carcinoma. Clin Cancer Res. 2007 Sep 15; 13(18 Pt 1):5368–76. [PubMed: 17875765]
24. Simpson F, Lammerts van Bueren K, Butterfield N, Bennetts JS, Bowles J, Adolphe C, et al. The PCNA-associated factor KIAA0101/p15(PAF) binds the potential tumor suppressor product p33ING1b. Exp Cell Res. 2006 Jan 1; 312(1):73–85. [PubMed: 16288740]
25. Turchi L, Fareh M, Aberdam E, Kitajima S, Simpson F, Wicking C, et al. ATF3 and p15PAF are novel gatekeepers of genomic integrity upon UV stress. Cell Death Differ. 2009 May; 16(5):728–37. [PubMed: 19219066]
26. Kuriyama R, Borisy GG. Centriole cycle in Chinese hamster ovary cells as determined by whole-mount electron microscopy. J Cell Biol. 1981 Dec; 91(3 Pt 1):814–21. [PubMed: 7328123]
27. Tsou MF, Stearns T. Mechanism limiting centrosome duplication to once per cell cycle. Nature. 2006 Aug 24; 442(7105):947–51. [PubMed: 16862117]
28. Yu P, Huang B, Shen M, Lau C, Chan E, Michel J, et al. p15(PAF), a novel PCNA associated factor with increased expression in tumor tissues. Oncogene. 2001 Jan 25; 20(4):484–9. [PubMed: 11313979]
29. Hosokawa M, Takehara A, Matsuda K, Eguchi H, Ohigashi H, Ishikawa O, et al. Oncogenic role of KIAA0101 interacting with proliferating cell nuclear antigen in pancreatic cancer. Cancer Res. 2007 Mar 15; 67(6):2568–76. [PubMed: 17363575]
30. de Azambuja E, Cardoso F, de Castro G Jr, Colozza M, Mano MS, Durbecq V, et al. Ki-67 as prognostic marker in early breast cancer: a meta-analysis of published studies involving 12,155 patients. Br J Cancer. 2007 May 21; 96(10):1504–13. [PubMed: 17453008]
31. van de Vijver MJ, He YD, van't Veer LJ, Dai H, Hart AA, Voskuil DW, et al. A gene-expression signature as a predictor of survival in breast cancer. N Engl J Med. 2002 Dec 19; 347(25):1999–2009. [PubMed: 12490681]

32. Guo M, Li J, Wan D, Gu J. KIAA0101 (OEACT-1), an expressionally down-regulated and growth-inhibitory gene in human hepatocellular carcinoma. BMC Cancer. 2006; 6:109. [PubMed: 16646990]

33. Fukasawa K, Choi T, Kuriyama R, Rulong S, Vande Woude GF. Abnormal centrosome amplification in the absence of p53. Science. 1996 Mar 22; 271(5256):1744–7. [PubMed: 8596939]

34. Griffin CS, Simpson PJ, Wilson CR, Thacker J. Mammalian recombination-repair genes XRCC2 and XRCC3 promote correct chromosome segregation. Nat Cell Biol. 2000 Oct; 2(10):757–61. [PubMed: 11025669]

35. Kanai M, Tong WM, Sugihara E, Wang ZQ, Fukasawa K, Miwa M. Involvement of poly(ADP-Ribose) polymerase 1 and poly(ADP-Ribosyl)ation in regulation of centrosome function. Mol Cell Biol. 2003 Apr; 23(7):2451–62. [PubMed: 12640128]

36. Kraakman-van der Zwet M, Overkamp WJ, van Lange RE, Essers J, van Duijn-Goedhart A, Wiggers I, et al. Brca2 (XRCC11) deficiency results in radioresistant DNA synthesis and a higher frequency of spontaneous deletions. Mol Cell Biol. 2002 Jan; 22(2):669–79. [PubMed: 11756561]

37. Tutt A, Gabriel A, Bertwistle D, Connor F, Paterson H, Peacock J, et al. Absence of Brca2 causes genome instability by chromosome breakage and loss associated with centrosome amplification. Curr Biol. 1999 Oct 7; 9(19):1107–10. [PubMed: 10531007]

38. Yamaguchi-Iwai Y, Sonoda E, Sasaki MS, Morrison C, Haraguchi T, Hiraoka Y, et al. Mre11 is essential for the maintenance of chromosomal DNA in vertebrate cells. EMBO J. 1999 Dec 1; 18(23):6619–29. [PubMed: 10581236]

39. van‘t Veer LJ, Dai H, van de Vijver MJ, He YD, Hart AA, Mao M, et al. Gene expression profiling predicts clinical outcome of breast cancer. Nature. 2002 Jan 31; 415(6871):530–6. [PubMed: 11823860]

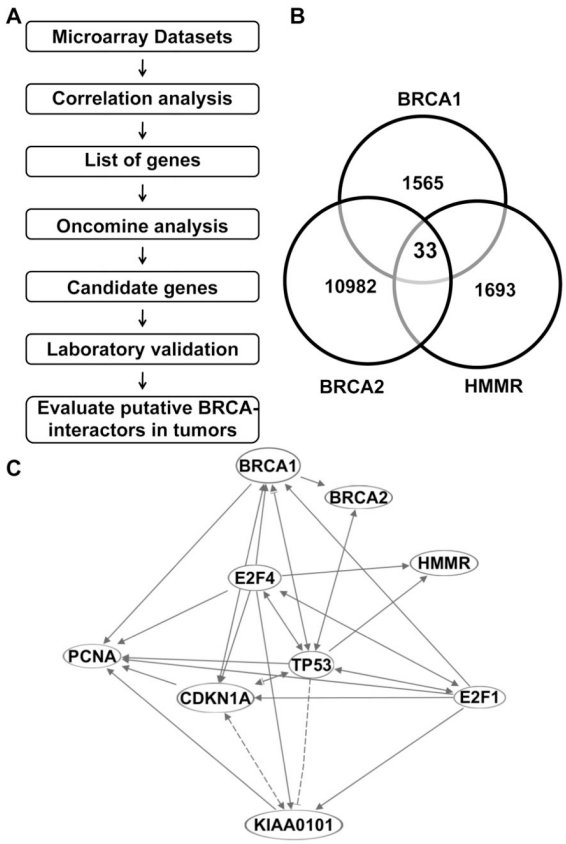


Figure 1. Summary of the workflow to discover candidate BRCA-interactors

A. The summary of the workflow used for the discovery of BRCA-interactors

B. An example taken from GEO dataset GDS2367 with 33 genes/proteins that correlated with the three reference genes with a PCC value > 0.6.

C. Ingenuity Pathway Analysis revealing links from BRCA1, BRCA2, and HMMR to KIAA0101.

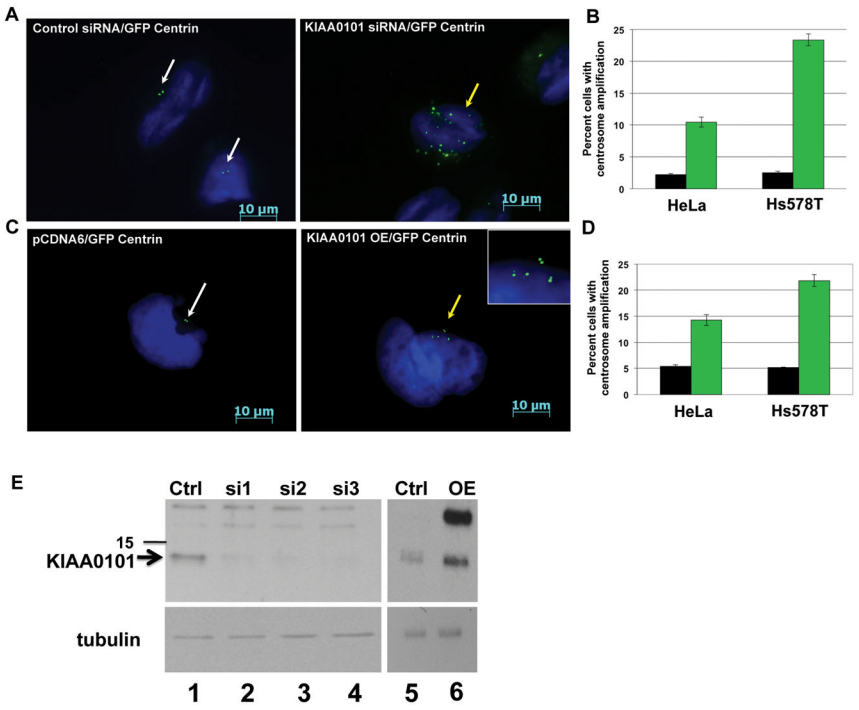


Figure 2. KIAA0101 controls centrosome number

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 10μm)

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 10μm)

D. The percentages of cells with centrosome amplification in HeLa and Hs578T cells upon over-expression 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 three 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. Re-probing the same membrane for alpha-tubulin provides the loading control (*bottom panel*).

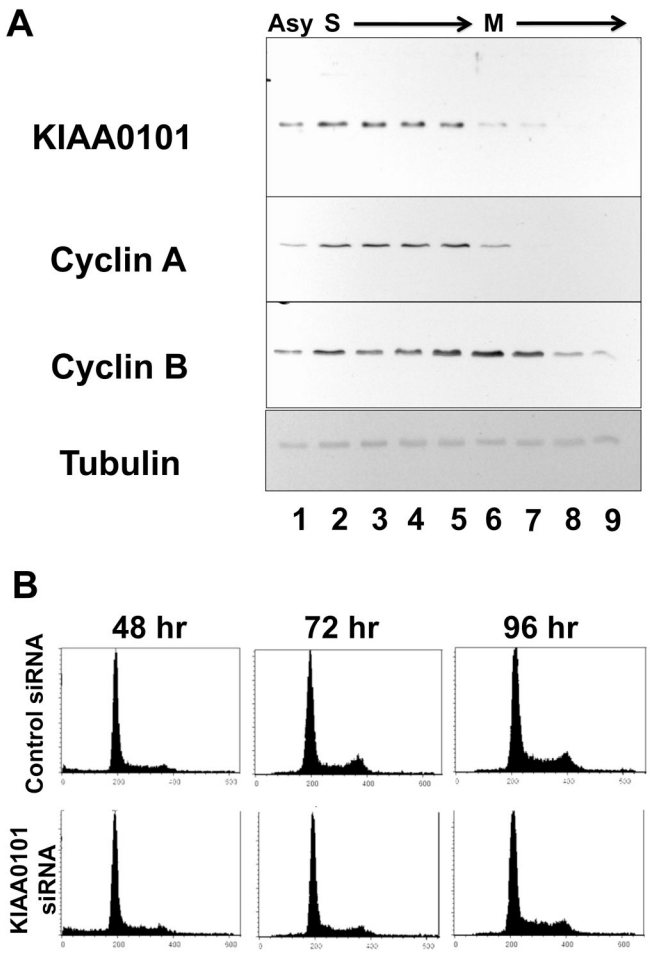


Figure 3. KIAA0101 abundance is cell cycle controlled

A. Lysates of HeLa cells were double-blocked with thymidine and released for 0h (lane 2), 3h (lane 3), 6h (lane 4), and 9h (lane 5). Lysates of HeLa cells treated with a thymidine block followed by nocodazole block and release were harvested at 0h (lane 6), 3h (lane 7), 6h (lane 8), and 9h (lane 9). Lysates from asynchronous cells (Asy) were analyzed in lane 1. Immunoblots were probed for KIAA0101, cyclin A, cyclin B, and alpha-tubulin. (The membranes were cut at 37KD to blot them with KIAA0101 antibody and Cyclin antibodies at the same time.)

B. Cell cycle analysis by flow cytometry 48, 72, and 96 hrs post depletion of KIAA0101. The upper panel shows DNA content of HeLa cells transfected with control siRNA, and the lower panel shows the DNA content of HeLa cells transfected with KIAA0101 siRNA.

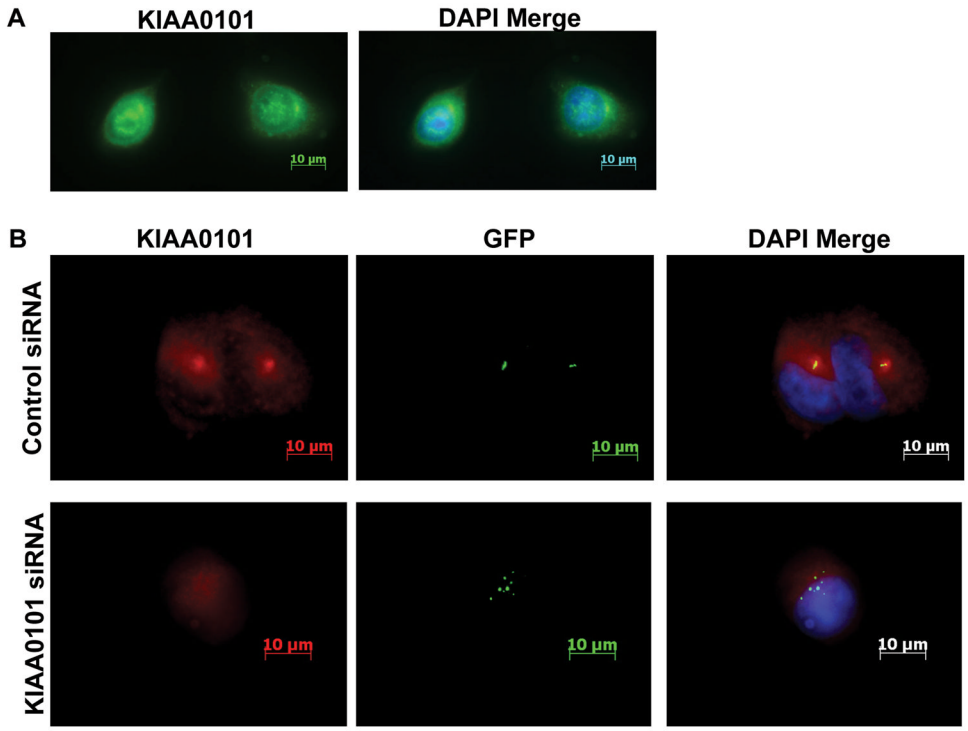


Figure 4. Co-localization of KIAA0101 with the centrosome

A. Immunofluorescence microscopy of KIAA0101 (green) and the DAPI counterstain (blue and merged, *right* panel only) showing nuclear and asymmetric perinuclear localization of KIAA0101 in HeLa cells. (Bar 10µm)

B. Hs578T breast cancer cells were transfected with control (*top*) or KIAA0101-specific (*bottom*) siRNAs for 48h 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 10µm).

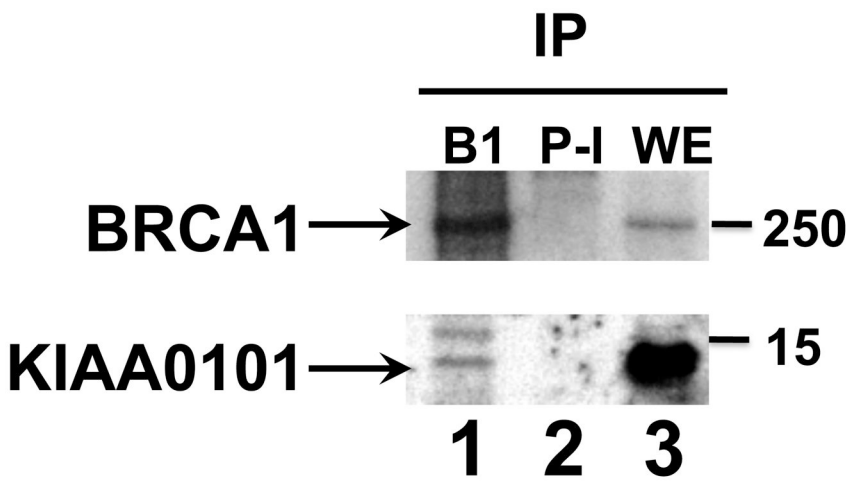
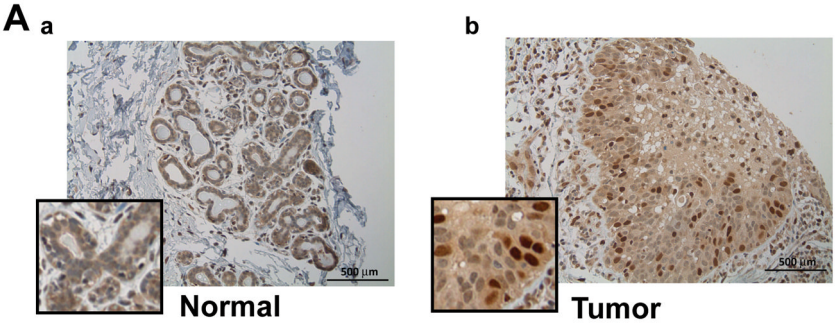


Figure 5. BRCA1 and KIAA0101 proteins are in a complex

HeLa whole cell extract (WE) was immunoprecipitated with BRCA1-specific antiserum (B1; lane 1) or matched control pre-immune serum (P-I; lane 2) and analyzed by immunoblotting for BRCA1 (*top panel*) and for KIAA0101 (*bottom panel*). 5% of the WE was analyzed in lane 3. Full length blots are presented in Supplementary Figure S7.

A



B

	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%)*
	81	30/81 (37.04%)	ER Negative	19/43 (44.2%)
			TNBC	14/26 (53.8%)**
TMA2	256	138/256 (53.9%)	ER Negative	44/72 (61.1%)
			TNBC	28/46 (60.9%)

Figure 6. Overexpression of KIAA0101 in breast cancer tumors

A. (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 500µm)

B. A table comparing the expression level of KIAA0101 to different biomarkers of breast cancer in two independent TMA studies. (*) Fisher test P-value = 0.006. (**) Fisher test P-value = 0.048. TNBC: Triple negative breast cancer.

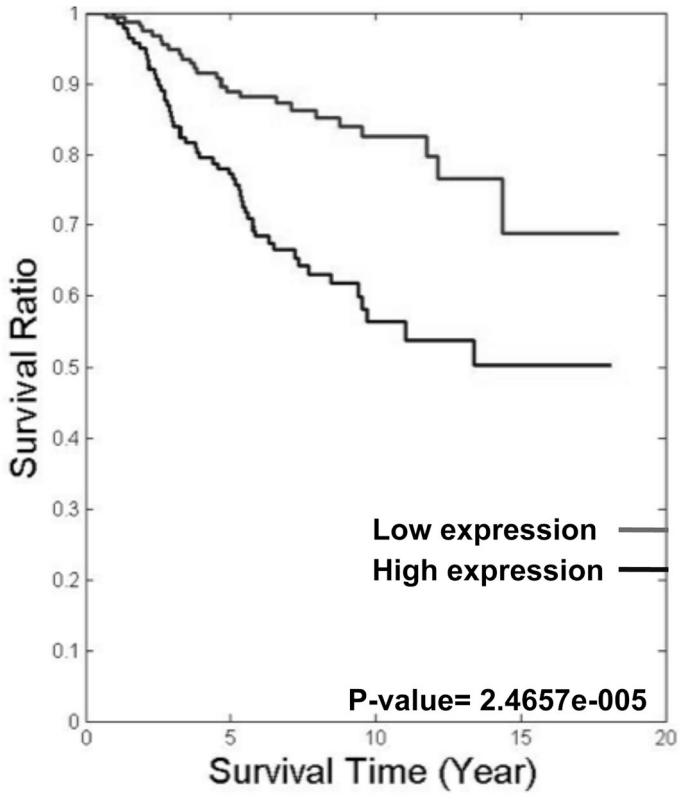


Figure 7. Overexpression of KIAA0101 correlates with worse survival rates

Data taken from the van't Veer microarray dataset (31, 39) showing significant decrease in the average survival rate of breast cancer patients with tumor samples with high KIAA0101 expression (black) versus low KIAA0101 expression (gray) (P-value = 2.5 e-005). Log-rank test was used to determine the statistical significance.