Short Communication

Loss of Aurora A/STK15/BTAK Overexpression Correlates with Transition of in Situ to Invasive Ductal Carcinoma of the Breast

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Abstract

The biological mechanisms involved in the progression of ductal carcinoma in situ (DCIS) to invasive breast cancer are not fully understood. We previously have shown that the putative oncogene Aurora-A/STK15/BTAK, encoding a centrosome-associated kinase that regulates centrosomes and chromosome segregation, is amplified in human breast cancer. In this study, archival breast tissue specimens of histologically confirmed DCIS lesions with adjacent invasive carcinoma and morphologically nonmalignant mammary ducts were analyzed immunohistochemically for expression of STK15. Statistically significant differences in overexpression of STK15 was found between invasive cancer and either nonmalignant mammary ducts (P < 0.0001) or DCIS lesions (P < 0.0005). Abnormalities in centrosome size and number was detected in the samples analyzed and 56% (14 of 25) of the cases also showed aneuploidy reflected in >2 signals of chromosome 3 and 17. Our data demonstrate that STK15 overexpression correlates with centrosome anomalies and aneuploidy in DCIS, and loss of STK15 overexpression is associated with progression of in situ to ductal invasive breast carcinoma.

Introduction

Ductal carcinoma in situ (DCIS) accounts for 12–14% of newly diagnosed breast cancer cases in the United States, and ~50% of patients with DCIS may develop invasive breast cancer within 24 years of diagnosis (1–4). The biological mechanisms involved in the progression of DCIS to invasive cancer are not fully understood. We previously have shown that centrosome-associated STK15/BTAK/Aurora-A kinase, a member of a novel serine/threonine kinase family that includes the prototypic yeast IPL1 and Drosophila aurora kinases, involved in regulating centrosomes and chromosomal segregation, is associated with human breast carcinogenesis. Overexpression of this gene was shown to induce abnormal centrosome duplication/distribution, aneuploidy, and transformation in mammalian cell lines (5). In yeast, the temperature sensitive ipl1 gene mutants missegregate chromosomes, resulting in polyploidy (6). Loss of function of aurora kinases in Drosophila inhibits separation of centrosomes and leads to formation of abnormal mitotic spindles (7).

STK15 overexpression in invasive ductal carcinoma of the breast has earlier been reported to occur in 94% (31 of 33) of cases (8), although only 12% of primary breast cancers were reported to have STK15 amplification (5). This observation is similar to what has recently been observed in human bladder cancer, where STK15 overexpression was detected in ~77% of invasive cancer, but the gene was amplified in only ~35% of these cases (9).

Evaluation of STK15 expression patterns in adjacent invasive breast cancer, DCIS, and morphologically nonmalignant mammary ducts in the same slide to detect the extent of correlation between STK15 expression levels and different stages of disease has not been reported thus far. The present study, done along these lines, revealed that loss rather than gain of STK15 overexpression is associated with progression of in situ to invasive ductal carcinoma of the breast. The study further demonstrates that elevated expression of STK15 is an early event in breast carcinogenesis correlating with centrosome anomalies and aneuploidy.

Materials and Methods

Identification of Patient Material. Paraffin-embedded archival breast tissue specimens and surgical pathology reports for 37 nonconsecutively examined DCIS with invasive component were identified from the pathology database of The University of Texas M. D. Anderson Cancer Center during the period of 1986–1999. H&E-stained slides of each DCIS case were reviewed and graded by a pathologist (A. A. S.) using standard grading criteria, as described previously (10). Sociodemographic characteristics and data on reproductive variables were abstracted from patients’ medical records.

 Immunohistochemical Analysis of STK15. Before using STK15 antibody, the pathologist (A. A. S.) examined the H&E-stained specimen to locate the relevant DCIS region of each case and then marked the DCIS region of interest and adjacent normal breast tissue and invasive ductal carcinoma to determine STK15 overexpression. A rabbit polyclonal anti-STK15 antibody developed in our laboratory raised against a COOH-terminal peptide was used for immunohistochemical localization of STK15 protein expression in the tissue as described previously (5). The specificity of the antibody is shown by Western blotting and immunohistochemistry in breast carcinoma cell line BT474 and in a near diploid nontumorigenic breast epithelial cell line MCF10 using the rabbit polyclonal
anti-STK15 antibody (Fig. 1). Semiquantitative evaluation of the staining intensity for immunohistochemical localization of STK15 was performed by independent investigators (A. A. S., A. H. S. S.) in a blinded manner. The intensity of immunohistochemical staining and positive nature of each specimen was scored on a two-point scale as follows: (a) −, for no detectable expression; and (b) +, for moderate to strong expression. The tumors were classified as positive for STK15 overexpression when >10% of the cells showed strong cytoplasmic expression.

Paraffin-embedded sections of human breast carcinoma cells with known overexpression of the STK15 protein identified in our previous studies were used as positive control. The baseline expression level of STK15 protein in normal breast epithelium was tested on paraffin-embedded sections of tissue obtained from individuals with nonmalignant breast disease.

Expression of STK15 was determined simultaneously in DCIS lesions, invasive ductal carcinoma, and adjacent normal breast tissue.

**Fluorescence in Situ Hybridization.** Formalin-fixed, paraffin-embedded tissue slides from 25 DCIS patient tumor samples were deparaffinized and analyzed by dual-color fluorescence in situ hybridization with the Vysis centromeric α-satellite DNA fluorescence in situ hybridization probes CEP 3 (SpectrumGreen) and CEP 17 (SpectrumOrange) according to our previously published protocol (5). Hybridization was carried out for 24–30 h. Nuclei were stained with 4′,6-diamidino-2-phenylindole and visualization of the assay was done using a Nikon Optiphot-2 fluorescence microscope. About 50 clearly resolvable individual nuclei in each specimen were evaluated for aneuploidy by counting the number of cells with two and more than two fluorescent signals within each nucleus. Such computing of only the modal number of two and more than two signals underestimate the true chromosome instability (CIN) value (11) but is used to avoid the compounding effect of nuclear truncation artifact in the tissue sections (12).

**Immunohistochemical Detection of Centrosomes.** To analyze centrosome number and structure, paraffin-embedded tissue sections were immunohistochemically stained with an antibody against pericentrin, a conserved integral centrosome protein according to the published protocol (12). Inclusion of controls in the form of histological sections of nonmalignant mammary gland epithelium and touch preparations of lymphocytes from normal lymph nodes allowed identification of structural and numerical abnormalities in centrosomes in the cancer tissue specimens. Centrosomes were considered structurally abnormal when they were at least twice the size of those seen in control cells, and more than two in number were recorded as representing numerical abnormality in the specimens analyzed.

**Statistical Analysis.** STK15 expression was categorized as overexpressed or not overexpressed. A cutoff point of 10% positive cells was considered overexpressed in nonmalignant mammary ducts, DCIS lesions, and adjacent invasive cancer. The McNemar χ² test was used to compare proportions of STK15 overexpression between adjacent normal mammary ducts and DCIS and invasive ductal carcinoma. All statistical computations were performed using the Statistical Package for the Social Sciences (SPSS, Inc., Chicago, IL) and Statistica computer software programs (StatSoft, Inc., Tulsa, OK). P < 0.05 was considered statistically significant.

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![Image](https://via.placeholder.com/150.png?text=Fig. 1) A. Western blot analyses of STK15 expression in breast carcinoma cell line BT474 and in a near diploid nontumorigenesis breast epithelial cell line MCF10 using the rabbit polyclonal anti-STK15 antibody. The specificity of the antibody is reflected in the detection of a single protein band in the top panel, and the absence of any detectable band in the middle panel in which the antibody was preabsorbed by the peptide antigen against which the antibody was raised. The presence of similar intensity β-actin bands in the lanes in the bottom panel indicates comparable amounts of protein loading from the cell lysates. B, immunohistochemical assay of STK15 expression in the cell lines, using paraffin-embedded sections of cells reflecting high expression in the breast carcinoma cell line BT474 and very low expression in MCF10 cells.

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**Table 1**  Patient characteristics

<table>
<thead>
<tr>
<th>Race</th>
<th></th>
<th>Median age at diagnosis (range)</th>
<th>52 years (33–72)</th>
</tr>
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<tr>
<td>White</td>
<td></td>
<td>White</td>
<td>23</td>
</tr>
<tr>
<td>Black</td>
<td></td>
<td>Black</td>
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<td>Hispanic</td>
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<td>Unknown</td>
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<tr>
<td>Nuclear grade</td>
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<td>3</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Necrosis</td>
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</tr>
<tr>
<td>Focal</td>
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<td>7</td>
<td></td>
</tr>
<tr>
<td>Extensive</td>
<td></td>
<td>14</td>
<td></td>
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Median age at menarche (range) 13 years (9–16)

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**Table 2**  Comparison of STK15 overexpression in invasive ductal carcinoma of the breast with adjacent normal mammary ducts and ductal carcinoma in situ

<table>
<thead>
<tr>
<th>Invasive Breast Cancer</th>
<th>Negative</th>
<th>Positive</th>
<th>P</th>
</tr>
</thead>
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<tr>
<td>Adjacent normal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>8 (22)</td>
<td>0</td>
<td></td>
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<tr>
<td>Positive</td>
<td>17 (46)</td>
<td>12 (32)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Total</td>
<td>25 (68)</td>
<td>12 (32)</td>
<td></td>
</tr>
<tr>
<td>Ductal carcinoma in situ</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>11 (30)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>14 (38)</td>
<td>12 (32)</td>
<td>0.0005</td>
</tr>
<tr>
<td>Total</td>
<td>25 (68)</td>
<td>12 (32)</td>
<td></td>
</tr>
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</table>
Results
The majority of the patients whose specimens were analyzed were white women and their median age at diagnosis was 52 years. The median age at menarche and age at first childbirth was 13 and 24 years, respectively. Of the 37 specimens evaluated, the majority was grade 2 (38%) and grade 3 (54%) DCIS lesions. Focal or extensive necrosis was present in 23 of 37 (57%) DCIS lesions (Table 1). Overexpression of STK15 in nonmalignant mammary ducts, DCIS lesions, and adjacent invasive ductal carcinoma was 78, 70, and 32%, respectively. We observed highly statistically significant differences in overexpression of STK15 between invasive cancer and either nonmalignant mammary ducts ($P < 0.0001$) or DCIS lesions ($P < 0.0005$; Table 2). An example of STK15 expression in normal breast epithelial cells and DCIS is shown in Fig. 2, A and B. Expression level of STK15 was down-regulated in invasive ductal carcinoma compared with adjacent nonmalignant mammary ducts or DCIS (Fig. 3). STK15 overexpression was not significantly different between DCIS and adjacent nonmalignant mammary ducts ($P > 0.45$). STK15 overexpression did not differ significantly between nuclear grades of DCIS.

Twenty-one of the 37 specimens were available for analysis of numerical and structural abnormalities in centrosomes. Two parameters were used to evaluate the abnormal status of centrosomes, larger size and more than two in number (Fig. 2, C and D). The proportion of tumor cells with centrosome abnormalities in each tumor varied from ~30 to ~100%. All of the tumors showed centrosome abnormalities. Abnormal structure was detected in all but one tumor analyzed, although this tumor showed more than the normal number (more than two) of centrosomes. Three of the tumors revealed the expected one or two centrosomes, but the sizes of these centrosomes were found to be grossly larger compared with those seen in normal cells.

![Fig. 2. A and B show immunohistochemical assay of STK15/Aurora-A expression in normal mammary epithelial ducts (A) and in ductal carcinoma in situ (DCIS) lesions (B). C and D show immunostained centrosomes (arrows) detected with an anti-pericentrin antibody in lymphocytes from normal lymph nodes (C) and in the tumor cells from DCIS lesions (D). E and F show fluorescence in situ hybridization analyses of normal diploid mammary epithelial cells (E) and of aneuploid tumor cells (F) from DCIS lesion performed with centromeric probes for chromosome 17 (green signal) and chromosome 3 (red signal). Note high expression of STK15/Aurora-A (B), abnormal centrosomes (D), and aneuploidy (F) in the tumor cells from DCIS lesions.](image-url)
For the two parameters of centrosome defects analyzed, the pattern was somewhat heterogeneous within tumors. Because STK15 overexpression has earlier been reported to induce CIN by causing segregation defects during mitotic proliferation of cells, we investigated if malignant lesions with STK15 overexpression also reveal CIN manifested in the form of aneuploidy. Aberrant modal chromosome number or aneuploidy is a somatic mutation event that is frequently detected in a wide variety of tumors. It has been proposed that the nonmodal distribution of chromosomes in tumor cells reflect a CIN phenotype that results because of defective mitotic checkpoint function allowing missegregation of chromosomes during mitosis. In this study, we analyzed if nonmodal distribution of chromosomes 3 and 17 reflect CIN in the DCIS and invasive carcinomas. Of the 25 specimens analyzed, 14 (56%) showed more than two signals of chromosomes 3 and 17. Eleven of these 14 (79%) cases revealed high expression of STK15. In all of the cases, at least ≥20% cells showed aneuploid chromosomal constitution, and in all of the specimens, aneuploidy for either one of the two chromosomes was detected. Examples of chromosomal instability of chromosome 3 and 17 are shown in Fig. 2F.

Discussion

We found overexpression of STK15 was not present in invasive ductal carcinoma of the breast when compared with adjacent DCIS or adjacent morphologically nonmalignant mammary ducts in the same slide. We did not observe differential overexpression of STK15 in higher grade DCIS compared with lower grade DCIS lesions. Although, the published breast cancer literature has reported elevated STK15 expression in invasive breast cancer (8), we found this not to be true in the cases analyzed in this study. Overexpression of STK15 in DCIS and adjacent nonmalignant mammary ducts suggest that high expression of STK15 may be more relevant in tumor initiation than progression in breast carcinogenesis.

Studies have shown that several forms of genetic instability associated with distinct molecular mechanisms can be found in human tumors (11). One of the most well-characterized forms of instability involves inactivation of DNA mismatch repair genes reflected in the expansion or shortening of microsatellite sequences, but it can be documented in only a minor proportion of tumors, which typically retain a diploid or near-diploid karyotype (13). Inactivation of mitotic spindle checkpoint genes such as BUB and MAD2 has been implicated as responsible for aneuploidy, but again, mutant BUB and decreased expression of MAD2 could be documented in only a small fraction of aneuploid solid tumors (13). We have described STK15 amplification in virtually all of the human bladder tumors analyzed by fluorescence in situ hybridization in a recently published study that also revealed strong correlation of STK15 gene amplification/overexpression with the degree of chromosomal instability detected in this malignancy (9). The results suggested that STK15 plays an important role in bladder
cancer by contributing to the development of aneuploid cell populations with aggressive phenotype. Similar correlation between STK15 overexpression and aneuploidy has been reported earlier in human breast and gastric cancer (14, 15). Molecular mechanisms underlying chromosomal instability leading to aneuploidy is not yet fully understood. In normal cells, the metaphase spindle is a bipolar structure comprised of microtubules emanating from centrosomes at each pole with centrosomes aligned at the midzone. Centrosomes appear to play an important role in the spindle assembly process (16, 17), regulating proper segregation of chromosomes during cell division. Because spindles are organized in part by centrosomes, it is possible that abnormal centrosome function can lead to missegregation of chromosomes resulting in chromosomal instability and consequent aneuploidy. It is relevant in this context to note that multipolar spindles have often been observed in human cancers in situ, and abnormal number of centrosomes have been seen in variety of cancer sites, including breast (12, 14, 18). Our findings that overexpressed STK15 kinase, commonly affected in human cancer, indeed affects centrosome number, and chromosome missegregation in mammalian cells makes it a relevant target of investigation to understand the molecular pathway involved in the induction of chromosomal instability and aneuploidy during malignant transformation process. Relevance of this pathway in the initiation of breast cancer has recently been strongly suggested with an experimental animal model in which overexpression of STK15 and centrosome amplification were found to be early events in rat mammary carcinogenesis (19). Altered expression of STK15 as an early event in human ovarian carcinogenesis has also been reported in a recently published study (20). These authors have reported that activation and overexpression of STK15 kinase is more frequently detected in early-stage, low-grade ovarian tumors.

Although the biological basis of STK15 overexpression in DCIS and adjacent nonmalignant mammary ducts remains unexplained, one may speculate that overexpression of STK15 causes genomic instability as an early event associated with DCIS, which subsequently leads to clonal expansion of one or more aneuploid clones even in absence of STK15 overexpression. Alternatively, it is also possible that a subset of invasive cancer may develop de novo by mechanisms independent of STK15 pathway. The high incidence of STK15 overexpression in DCIS and in the adjacent nonmalignant mammary epithelium in the same tissue, nonetheless, appear extremely significant in view of a recent publication identifying STK15 as a candidate tumor susceptibility gene in mouse and human (21). This study revealed that a genetic variant of STK15, preferentially amplified and overexpressed, is associated with degree of aneuploidy in human colon tumors consistent with a role for this gene in human cancer susceptibility. It would be interesting to investigate, in the future, if a specific allelic variant is also preferentially overexpressed in DCIS, making it possible to develop an allele-specific detection system for breast cancer risk assessment. These findings, taken together, indicate that STK15 has the strong potential to become (a) an early marker of cellular and glandular disorganization, genomic instability, and breast cancer risk and (b) a molecular target for preventive drug development.

References
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