Aurora Kinase B Expression in Breast Carcinoma: Cell Kinetic and Genetic Aspects

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Key Words

Aurora kinase B · p53 antigens · Kinase deregulation · Cell proliferation · Aneuploidy

Abstract

Background: Mitotic deregulations may contribute significantly to cell division errors and the development of aggressive tumor cells. The mitotic kinase Aurora B is essential for chromosome segregation. Its gene is located at 17p13 in close proximity to the TP53 gene. Although the frequent alteration of this locus is well known, the information about the AURKB status and protein expression is limited. **Methods:** 50 breast carcinoma cases were evaluated for 17p13 status and chromosome 17 ploidy by FISH and for Aurora B protein by immunohistochemistry. **Results:** Aurora B protein expression showed a strong correlation with cell proliferation (regression coefficient = 0.77). Therefore, the Aurora B/MIB-1 index was used as a measure of expression, which showed a wide range (1–35%, mean 0.32, SD $\pm$ 0.28). A gain in the 17p13 chromosome locus could not be shown while a deletion was stated in 10/50 cases including a subset with TP53 and AURKB codeletion in 6/10 cases. The loss of TP53/AURKB loci strongly correlated with aneusomy ($p < 0.0001$). **Conclusion:** Elevated Aurora B expression frequently occurs due to an increased cell proliferation rate in breast carcinoma.

ma. Codeletion of TP53 and AURKB at 17p13 indicates a concerted mechanism leading to the survival of cell clones with deficient mitotic kinase function which could contribute to the formation of aneuploid cells and an aggressive tumor phenotype.

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Introduction

Deregulation of the mitotic apparatus significantly contributes to genetic instability [1] leading to highly aberrant cell clones with nuclear DNA content abnormalities [2]. According to their central role in the regulation of the mitotic progress mitotic kinases have been linked to tumorigenesis and tumor aggressiveness [3]. The spindle assembly checkpoint in the early phase of cell division is critical to ensure that all chromosomes are attached correctly. In the background of these processes Aurora kinases have been identified as important regulators [4].

The Aurora family of serine/threonine kinases includes 3 members, Aurora kinase A, B and C [5]. Although the catalytic domains are highly conserved, Aurora kinases have diverse functions and also show different cellular localization [4].

Aurora A functions as a regulator of the centrosome cycle and mitotic spindle assembly. Located at 20q13, its gene is frequently deleted in breast cancer [6, 7] but was also found to be amplified in various epithelial tumors [8]. Very little is known about Aurora C, which is expressed in the testis in a tissue-specific manner [5, 9].

During the normal cell cycle Aurora B kinase is expressed in the late G2/M phase and remains active in the process of mitosis [10]. It plays an essential role in chromosome segregation and cytokinesis, chromosome condensation and cohesion [9, 11]. Accordingly, Aurora B kinase and interacting proteins play a key role in the maintenance of normal ploidy during cell division [5]. In reverse, the deregulation of this complex was shown to correlate with aneuploidy [12–14]. The AURKB gene itself is located at 17p13 [15]. The 17p13 locus harbors the TP53 gene, which is also exposed to genetic aberrations in cancer [16] resulting in the loss of p53 activity and the consecutive disruption of apoptosis, thereby accelerating tumorigenesis in transgenic mice [17].

Aneuploidy is a common feature of advanced breast cancer [1]. One of the most frequent chromosomes involved is chromosome 17 [18]. The aim of the present study, therefore, was to investigate the potential associations between Aurora B expression during the cell cycle, AURKB and TP53 gene status as well as chromosome 17 copy number changes as an indicator of mitotic defects.

Materials and Methods

Patients and Samples

Histological samples of 50 patients (aged between 32 and 93 years) with invasive ductal and lobular breast carcinoma were recruited to the study. Clinical data were available for all patients. Specimens consisted of surgically resected carcinomas and tumor biopsies. Samples were fixed in formaldehyde for 24 h and processed routinely into paraffin blocks.

Immunohistochemical Staining

Immunohistochemical staining (IHC) was performed according to our standard routine protocol. p53 and MIB-1 antigens were retrieved in citrate buffer pH 6.0 and in Epitope Retrieval Solution (Novocastra Laboratories, Newcastle upon Tyne, UK) and EDTA at pH 9.0 for Aurora B in a high-pressure cooker. Tissue sections were incubated with monoclonal mouse anti-human p53 (DAKO, Glostrup, Denmark; dilution 1:100), monoclonal mouse anti-human Ki-67 (Clone MIB-1; DAKO; dilution 1:1,000) and polyclonal rabbit anti-human Aurora B (AbCam plc, Cambridge, UK; dilution 1:100) primary antibodies. Horseradish peroxidase-conjugated secondary antibody (Envision System; DAKO) was used as a detection system.

Immunopositive cells were determined semiquantitatively in a percentage of the total tumor cell fraction by two independent

observers. The c-erbB2 immunohistochemistry was scored according to Sapino et al. [19].

FISH Analysis

Chromosome 17 and HER2/neu copy number was determined with a direct labeling method, using Pathvision™ HER-2 DNA probe kit (Vysis/Abbott Laboratories Inc., Downers Grove, Ill., USA). TP53 and AURKB copy number was evaluated using Poseidon™ Repeat Free™ p53 and SE 17 and Poseidon Repeat Free AURKB/SE 17 probes (Kreatech Diagnostics, Amsterdam, The Netherlands).

Deparaffinized tissue sections were pretreated according to the manufacturer's instructions with some modifications. The slides were finally counterstained with DAPI (Vysis/Abbott Laboratories Inc.) and coverslipped.

FISH signals were detected with an Olympus BX51 fluorescence microscope equipped with DAPI, FITC and Texas Red filters and the Isis imaging system (MetaSystems, Altlußheim, Germany) for image acquisition. Chromosome 17, HER2/neu, TP53 and AURKB signals were counted in at least 100 nuclei in each case with a 100x immersion oil objective. According to previous studies the HER2 gene status was considered normal when the HER2/CEP17 ratio was between 1.8 and 2.2, and high-grade HER2 amplification was stated at a ratio above 4.0. The chromosome 17 copy number was considered normal between 1.5 and 2.3, monosomy was stated below 1.5 and polysomy/aneusomy was defined at copy numbers of ≥ 2.3 /cell nucleus. AURKB and TP53 deletion was defined at copy numbers ≤ 0.8 /chromosome 17 based on earlier measurements on normal tissue sections.

Statistical Analysis

XLSTAT statistical software was used for statistical analysis. Mean and standard deviations (SD) were calculated for each group and analyzed with Student's t test. p values < 0.05 were considered statistically significant. Correlations between datasets were obtained using linear regression.

Results

Clinical and Biological Features of Breast Carcinomas Studied

Altogether 50 breast tumor samples were evaluated, 45 diagnosed as invasive ductal carcinoma morphology and the remaining 5 cases as invasive lobular carcinomas. ER and PR positivity was observed in 36 (72%) and 27 (54%) cases, respectively. Her2 positivity/gene amplification was identified in 13/50 cases (26%). Clinicopathological features are shown in table 1.

Aurora B Expression and Cell Proliferation in Breast Carcinoma

The rate of Aurora B-expressing cells in the studied breast carcinoma cases was found in the range of 1–35% (mean 6.15, SD $\pm$ 8.8) with a subset of breast carcinomas showing unexpectedly high Aurora B expression. The cell

Fig. 1. Correlation of Aurora B and MIB-1 protein expression determined by immunohistochemistry in breast carcinoma (n = 50). A strong (linear) correlation was found between Aurora B and MIB-1 expression ($r = 0.77$) suggesting a relation due to the increase of the G2/M phase of the cell cycle.

Table 1. Histopathological characteristics of the breast carcinomas evaluated (n = 50)

Variable	Number of cases
Histotype	
Ductal	45
Lobular	5
ER status	
Negative	14
Positive	36
PR status	
Negative	23
Positive	27
c-erbB2 score	
–	16
+	11
++	14
+++	9
HER2 amplification	
Negative	37
Positive	13

ER = Estrogen receptor; PR = progesterone receptor.

proliferation capacity expressed by the MIB-1 index was in the range of 1–95 (mean 19.2, SD $\pm$ 20.7) in the same tumors. A strong correlation was found between the Aurora B- and MIB-1-positive fractions ($r = 0.77$, fig. 1), indicating that an increased expression of Aurora B is a feature of tumors with a high proliferating fraction. Breast

carcinomas with HER2 amplification presented with an elevated Aurora B fraction ($p = 0.015$) but they also showed a significantly higher MIB-1 proliferation index ($p = 0.018$; fig. 2).

To obtain a more accurate picture on Aurora B expression relative to the proliferating cells the ratio of the Aurora B- and the MIB-1-expressing cell fraction (Aurora B/MIB-1 index; AMI) was calculated which was in the range of 0–1 (mean 0.32, SD $\pm$ 0.28; fig. 3). According to parallel observations on highly proliferating normal cell lines and reactive changes, the group of low and high Aurora B-expressing tumors was determined by using an AMI cutoff set to 0.3. Of the breast carcinomas studied, 20/50 (40%) showed an AMI higher than 0.3 and thus were considered as Aurora B overexpressing (fig. 4). The AMI was found to be statistically independent of the HER2 status.

Copy Number Alterations of the Chromosome Loci AURKB and TP53 and of Chromosome 17

None of the 50 cases evaluated by FISH analysis displayed amplification or gain of 17p13 using either of the probes specific for AURKB or TP53. On the contrary, the loss of 17p13 was occasionally demonstrated due to the FISH signal loss relative to chromosome 17 signals. We observed TP53 signal deletion in 10 cases (20% of all cases), which was associated with the loss of the AURKB locus in 6 cases (12%) (TP53 copy number mean = 0.55/chromosome 17, SD $\pm$ 0.06; AURKB copy number mean = 0.64/chromosome 17, SD $\pm$ 0.03 in codeleted cas-

Fig. 2. Results of the immunohistochemical determination of Aurora B and MIB-1 according to the Her2 status in breast cancer. A significantly higher rate of Aurora B and MIB-1-expressing cell fraction could be measured in HER2-amplified cases.

es). There was a strong correlation between the mean copy numbers of AURKB and TP53 genes ($r = 0.73$; fig. 5).

No direct association between AURKB/TP53 deletion and HER2 gene status could be stated. The loss of one of the AURKB and/or TP53 alleles was obvious in only 4/12 (33.3%) of the HER2 amplified cases.

Chromosome 17 copy number changes, determined by the use of a pericentromeric alpha satellite FISH probe, were seen in 19/50 cases (38 %), and the range was between 2.3 and 3.9 copies/cell nucleus (table 2). In AURKB and TP53 codeleted cases the copy number of chromosome 17 was found to be between 2.44 and 3.9 (mean $\pm$ SD 3.18 ± 0.18) while in cases with normal gene copy numbers it was between 1.6 and 3.19 (2.1 ± 0.05 , $p < 0.0001$, statistically significant; fig. 6).

Using these FISH data, the relative copy numbers of TP53 and AURKB genes/chromosome 17 were also calculated (table 3). The copy number of TP53 and AURKB was between 0.49 and 1.4 (0.9 ± 0.18), and between 0.48 and 1.6 (1.01 ± 0.2), respectively. An imbalance at the loci TP53/AURKB could be detected in cases with chromosome 17 aneusomy compared with cases bearing normal chromosome 17 numbers ($p = 0.001$ and $p = 0.004$, re-

Fig. 3. The AMI according to HER2 status. The ratio was found to be unchanged in HER2-amplified cases. Asterisks represent extreme outliers.

Color version available online

Fig. 4. Microscopic appearance of Ki-67 (MIB-1) (**a, c**) and Aurora B (**b, d**) immunostaining. Tissues were hematoxylin counterstained. **a, b** A case with an AMI value of 0.25. **c, d** Increased fraction of Aurora B-expressing cells (AMI = 0.88). Original magnification $\times 40$.

spectively). Cases showing AURKB and TP53 codeletion all belonged to the chromosome 17 aneusomy group (6/19 cases, 31.6%).

Aurora Kinase B Expression and Chromosome 17 Copy Number Changes

To learn more about the relation of Aurora B expression and numeric chromosome aberrations the protein expression was evaluated in the context of 17p13 and centromeric chromosome 17 copy numbers. Neither p53, MIB-1 or Aurora B evaluated by IHC showed a correlation with chromosome 17 copy number.

In cases with normal AURKB gene copy numbers the Aurora B-expressing cell fraction was in the range of $0-35$ (6.47 ± 9.26) and $0-10$ (3.83 ± 3.44) in cases with gene copy number losses; however, the difference was statistically not significant ($p = 0.5$; fig. 7).

Furthermore, AURKB/TP53 codeleted breast carcinomas showed an AMI between 0 and 0.25 (0.15 ± 0.1), while cases with intact 17p were between 0 and 1 ($0.36 \pm$

0.3) for the same parameter ($p = 0.1$, not significant; fig. 8).

The evaluation of the AURKB copy number relative to the Aurora B expression fraction did also not show a clear correlation. Cases displaying very high AMI values presented slightly higher signal AURKB FISH copy numbers (1.1 ± 0.2) in contrast to tumors with $\text{AMI} \leq 0.3$, the majority of which had copy numbers below 1 (0.9 ± 0.2 , $p = 0.004$; fig. 9).

Discussion

Mitotic kinases such as the Aurora kinase B are potential biomarkers for chromosome instability and mitotic errors leading to aneuploidy/aneusomy and aggressive tumor phenotype. The expression of Aurora B can also be studied in tissue conditions by immunohistochemistry, as described previously [20–22]. The activity of Aurora B in normally growing cell populations is limited to

Fig. 5. Correlation of AURKB and TP53 copy numbers relative to chromosome 17 number in breast cancer ($r = 0.73$) as stated by FISH analysis. Six cases showed copy loss for both FISH probes.

Table 2. Cell kinetic data in relation to chromosome 17 aneusomy as determined by FISH analysis

	Polysomic cells ^a (n = 19)		Disomic cells (n = 31)		p value
	range	mean $\pm$ SD	range	mean $\pm$ SD	
p53-expressing cell fraction ^b , %	0–100	21.16 $\pm$ 35.57	0–90	15.71 $\pm$ 26.87	0.55
Aurora B-expressing cell fraction, %	0–35	7.42 $\pm$ 8.67	0–35	5.37 $\pm$ 8.8	0.43
Mitosis index ^c	5–54	21.26 $\pm$ 10.25	2–68	13.6 $\pm$ 15.04	0.06
MIB-1-positive cell fraction, %	5–70	21.37 $\pm$ 15.48	1–95	17.58 $\pm$ 23.22	0.54
Aurora B/MIB-1 index	0–1	0.31 $\pm$ 0.26	0–2	0.44 $\pm$ 0.5	0.31

^a Polysomy was stated if FISH signal copy exceeded 2.3/cell nucleus.

^b p53, Aurora B and MIB1 were estimated in percentage of tumor cells following IHC.

^c Mitosis index was counted in 10 high-power fields.

Table 3. Relative number of TP53 and AURKB signals in relation to the chromosome 17 copy number

	Chromosome 17 polysomy (n = 19)		Chromosome 17 disomy (n = 31)		p value
	range	mean $\pm$ SD	range	mean $\pm$ SD	
TP53/chromosome 17	0.49–1.11	0.81 $\pm$ 0.2	0.74–1.44	0.99 $\pm$ 0.13	0.0006
AURKB/chromosome 17	0.48–1.13	0.91 $\pm$ 0.2	0.82–1.59	1.08 $\pm$ 0.18	0.0036

Loss of TP53 and AURKB could be stated in cases with chromosome 17 aneusomy which was statistically significant for both loci.

the G2/M phase of the cell cycle. When evaluated independently as a single variable, the proportion of the Aurora B-expressing cells was reported to be increased in malignant tumors, which is often interpreted as overexpression. However, biological reasons such as specific ge-

netic aberrations or gene expression deregulation were not described so far. According to our results the expression of Aurora kinase B is frequently increased in breast carcinoma but strongly dependent on cell proliferation. We assume that the elevated fraction of Aurora B-immu-

Fig. 6. 17p13 loss was strongly associated with the aneusomy of chromosome 17 ($p < 0.0001$).

Fig. 7. Aurora B expression changes considering AURKB copy numbers. Decreased values were shown in cases with AURKB deletion ($p = 0.5$, not significant). Asterisks represent extreme outliers.

Fig. 8. Changes in AMI values considering 17p13 status. A decreasing trend was shown in cases with 17p13 loss ($p = 0.1$, not significant).

Fig. 9. Correlations of relative AURKB gene copy number reflected by the AMI. The copy number of AURKB tends to be increased in cases with AMI > 0.3 .

nopositive cells is mainly determined by the proportional increase of the G2/M phase where the kinase is expressed in a function-specific manner. Therefore, the relative expression of the Aurora B protein is better reflected by the AMI which represents the Aurora B expression in relation to the whole proliferative fraction of the tumor. The data indicate a linear correlation between Aurora B and MIB-1 expression. By the use of this system only a few breast carcinoma cases presented with a relative Aurora B increase equivalent to overexpression (>0.3 AMI). According to our earlier data the AMI detectable in highly proliferative B lymphoid cell populations of follicular germinal centers of reactive lymph nodes rises up to 0.6, suggesting that approximately 60% of the proliferative compartment is in the G2/M phase of the cell cycle. This unexpectedly high value is possibly associated with the enrichment of G2/M phase cells that can rarely be seen in breast carcinoma. Significant upregulation of Aurora B expression in breast cancer seems to be rare and the highly dynamic overproduction of the protein potentially relies on parallel stimulatory actions. Aggressive tumors excluded by Her2 positivity/steroid receptor negativity showed a significantly higher Aurora B protein expression, but following the relativation to the proliferating cell fraction (AMI) they were not significantly different. As a result of detailed evaluation no clear impact of the Aurora B overexpression determined by IHC could be stated following correlation with the most common variables in breast carcinoma.

Unlike the reported changes of the Aurora A kinase gene AURKA, FISH analysis of the AURKB locus did not show any gains or amplifications in the samples analyzed. On the contrary, frequent loss of the AURKB gene located at chromosome locus 17p13 was demonstrated. 17p13 harboring the TP53 gene is commonly deleted in a large set of solid tumors and leukemias [23, 24]. Our mo-

lecular cytogenetic studies also represented a strong correlation between AURKB and TP53 copy alterations; deletions at 17q13 including both TP53 and AURKB were stated in 6/50 cases.

Despite its limitations in histological sections, the copy number of chromosome 17 determined by FISH could be efficiently used as a measure of aneusomy. Numeric alterations of this particular chromosome could be observed with a high frequency in breast carcinoma and were associated with general mechanisms leading to higher grade chromosomal instability and aggressivity [25, 26]. According to our present results, losses at 17p13 and chromosome 17 polysomy/aneusomy determined by FISH analysis correlated significantly. Although the number of TP53/AURKB codeleted cases was low these findings gave rise to further ideas regarding the generation and survival of malignant cell clones with 17p13 locus deficiency. One can speculate on a synergic effect of the two genes involved by the codeletion at the mentioned region: loss of AURKB may serve for regulatory deficiencies in the chromosome passenger complex leading to mitotic errors while p53 deficiency helps the cell to survive due to insufficient activation of the intrinsic apoptotic pathways. These parallel effects finally increase the complexity of mitotic abnormalities and generate aneuploid cell populations.

In summary, Aurora B expression determined by IHC could not be established as an independent biological factor as it seems to be strongly proliferation-dependent. Genetic determination of Aurora B overexpression, e.g. gene amplification, is not a common feature in breast cancer. On the other hand, an imbalance at the chromosomal region 17p13 with allelic loss of TP53 and AURKB delineates a breast cancer group with high cell proliferation capacity and chromosomal instability, the clinical significance of which should be further evaluated.

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