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

Katalin Hegyi, Kristóf Egervári, Zsuzsa Sándor, Gábor Méhes

Department of Pathology, University of Debrecen Medical and Health Science Center, Debrecen, Hungary

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 ± 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. 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.

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 consequent 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 9.0 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 1:1000) 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 I (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 observed in 36 (72%) and 27 (54%) cases, respectively. ER2 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 ± 8.8) with a subset of breast carcinomas showing unexpectedly high Aurora B expression. The cell
proliferation capacity expressed by the MIB-1 index was in the range of 1–95 (mean 19.2, SD 8.20.7) in the same tumors. A strong correlation was found between Aurora B and MIB-1 expression (r = 0.77, fig. 1) suggesting a relation due to the increase of the G2/M phase of the cell cycle.

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 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 ± 0.06; AURKB copy number mean = 0.64/chromosome 17, SD ± 0.03 in codeleted cases.

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

<table>
<thead>
<tr>
<th>Variable</th>
<th>Number of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histotype</td>
<td></td>
</tr>
<tr>
<td>Ductal</td>
<td>45</td>
</tr>
<tr>
<td>Lobular</td>
<td>5</td>
</tr>
<tr>
<td>ER status</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>14</td>
</tr>
<tr>
<td>Positive</td>
<td>36</td>
</tr>
<tr>
<td>PR status</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>23</td>
</tr>
<tr>
<td>Positive</td>
<td>27</td>
</tr>
<tr>
<td>c-erbB2 score</td>
<td></td>
</tr>
<tr>
<td>–</td>
<td>16</td>
</tr>
<tr>
<td>+</td>
<td>11</td>
</tr>
<tr>
<td>++</td>
<td>14</td>
</tr>
<tr>
<td>+++</td>
<td>9</td>
</tr>
<tr>
<td>HER2 amplification</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>37</td>
</tr>
<tr>
<td>Positive</td>
<td>13</td>
</tr>
</tbody>
</table>

ER = Estrogen receptor; PR = progesterone receptor.
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 codelleted cases the copy number of chromosome 17 was found to be between 2.44 and 3.9 (mean $\pm$ SD $3.18 \pm 0.18$) while in cases with normal gene copy numbers it was between 1.6 and 3.19 ($2.1 \pm 0.05$, $p < 0.0001$, 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 ($0.9 \pm 0.18$), and between 0.48 and 1.6 ($1.01 \pm 0.2$), 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-
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 ± 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 AMI ≤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. 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 ×40.
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 genetic 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-

![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.](image)

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

<table>
<thead>
<tr>
<th></th>
<th>Polysomic cells (n = 19)</th>
<th>Disomic cells (n = 31)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>range</td>
<td>mean ± SD</td>
<td>range</td>
</tr>
<tr>
<td>p53-expressing cell fraction</td>
<td>0–100</td>
<td>21.16 ± 35.57</td>
<td>0–90</td>
</tr>
<tr>
<td>Aurora B-expressing cell fraction</td>
<td>0–35</td>
<td>7.42 ± 8.67</td>
<td>0–35</td>
</tr>
<tr>
<td>Mitosis index</td>
<td>5–54</td>
<td>21.26 ± 10.25</td>
<td>2–68</td>
</tr>
<tr>
<td>MIB-1-positive cell fraction</td>
<td>5–70</td>
<td>21.37 ± 15.48</td>
<td>1–95</td>
</tr>
<tr>
<td>Aurora B/MIB-1 index</td>
<td>0–1</td>
<td>0.31 ± 0.26</td>
<td>0–2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>range</th>
<th>mean ± SD</th>
<th>range</th>
<th>mean ± SD</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP53/chromosome 17</td>
<td>0.49–1.11</td>
<td>0.81 ± 0.2</td>
<td>0.74–1.44</td>
<td>0.99 ± 0.13</td>
<td>0.0006</td>
</tr>
<tr>
<td>AURKB/chromosome 17</td>
<td>0.48–1.13</td>
<td>0.91 ± 0.2</td>
<td>0.82–1.59</td>
<td>1.08 ± 0.18</td>
<td>0.0036</td>
</tr>
</tbody>
</table>

Loss of TP53 and AURKB could be stated in cases with chromosome 17 aneusomy which was statistically significant for both loci.
1.7p13 loss was strongly associated with the aneusomy of chromosome 17 (p < 0.0001).

Fig. 6.

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. 7.

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

Fig. 8.

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.

Fig. 9.
nopositive cells is mainly determined by the proportion-
al increase of the G2/M phase where the kinase is ex-
pressed 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 ex-
pression 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 rela-
tive 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 fol-
licular germinal centers of reactive lymph nodes rises up
to 0.6, suggesting that approximately 60% of the prolif-
erative compartment is in the G2/M phase of the cell cy-
cle. 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 poten-
tially relies on parallel stimulatory actions. Aggressive
tumors excluded by Her2 positivity/steroid receptor neg-
ativity showed a significantly higher Aurora B protein ex-
pression, but following the relativation to the proliferat-
ing cell fraction (AMI) they were not significantly differ-
ent. 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 vari-
ables 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 ana-
yzed. 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 cor-
relation between AURKB and TP53 copy alterations; de-
letions 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. Nu-
mericalterations 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 genera-
tion and survival of malignant cell clones with 17p13 lo-
cus 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 deficien-
cies in the chromosome passenger complex leading to mi-
totic errors while p53 deficiency helps the cell to survive
due to insufficient activation of the intrinsic apoptotic
pathways. These parallel effects finally increase the com-
plexity of mitotic abnormalities and generate aneuploid
cell populations.

In summary, Aurora B expression determined by IHC
could not be established as an independent biological fac-
tor as it seems to be strongly proliferation-dependent. Ge-
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 re-
gion 17p13 with allelic loss of TP53 and AURKB delin-
eates a breast cancer group with high cell proliferation
capacity and chromosomal instability, the clinical signif-
cicance of which should be further evaluated.

References

1 Mendelin J, Grayson M, Wallis T, Visscher
DW: Analysis of chromosome aneuploidy in
breast carcinoma progression by using fluo-
rescence in situ hybridization. Lab Invest

2 Hanahan D, Weinberg RA: The hallmarks of

3 Nigg EA: Mitotic kinases as regulators of cell
division and its checkpoints. Nat Rev Mol

4 Keen N, Taylor S: Aurora-kinase inhibitors

5 Carmina M, Earnshaw WC: The cellular ge-
ography of aurora kinases. Nat Rev Mol Cell

6 Lengauer C, Kinzler KW, Vogelstein B: Ge-
etic instabilities in human cancers. Nature

7 Lukasiewicz KB, Lingle WL: Aurora A, cen-
trosome structure, and the centrosome cy-
619.

8 Marumoto T, Zhang D, Saya H: Aurora-A – a
guardian of poles. Nat Rev Cancer 2005;5:
42–50.

9 Bolanos-Garcia VM: Aurora kinases. Int J

10 Katayama H, Brinkley WR, Sen S: The Au-
rora kinases: role in cell transformation and

11 Vader G, Medema RH, Lens SM: The chro-
omosomal passenger complex: guiding Auro-
ra-B through mitosis. J Cell Biol 2006;173:
833–837.


