

## ORIGINAL ARTICLE

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# Aurora A overexpression in breast cancer patients induces taxane resistance and results in worse prognosis

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## Summary

**Purpose:** The aim of this study was to determine the expression level of Aurora A in human breast cancer tissues and to test whether there is a relationship between its expression levels and clinicopathological parameters including response to taxanes, tumor grade, estrogen receptor (ER) status, human epidermal growth factor receptor 2 (HER2) status, and overall survival (OS).

**Methods:** We retrospectively analyzed paraffin-embedded tissue sections from 49 metastatic breast cancer patients whose clinical outcomes had been tracked after taxane treatment. The expression status of Aurora A was defined by immunohistochemistry (IHC) using the anti-Aurora A antibody.

**Results:** Aurora A was overexpressed in 73% of the examined specimens. There was significant correlation between high Aurora A expression and decreased taxane sensitiv-

ity ( $p=0.02$ ). There was no association between the clinicopathological parameters including histologic grade, ER positivity and triple negative molecular subtype and the level of Aurora A expression. However, HER2 positive tumors showed significantly higher Aurora A expression compared with HER2 negative tumors ( $p=0.02$ ). Kaplan-Meier survival analysis failed to show a significant correlation between expression levels of Aurora A and OS although patients with low Aurora A levels had a marginally longer survival compared to patients with high levels.

**Conclusion:** Our data suggest that Aurora A may be a promising predictive and prognostic marker in patients with breast cancer.

**Key words:** Aurora A, breast cancer, HER2, predictive, prognostic, taxanes

## Introduction

Breast cancer is second leading cause of death from cancer in women [1]. Despite the multimodal treatment with surgery, radiotherapy and chemotherapy, many patients show disease progression and die of their disease. Taxanes and anthracyclines are the best chemotherapeutic agents for breast cancer although a proportion of patients do not benefit from chemotherapy [2]. For this reason, determination of predictive and prognostic factors in breast cancer may help select those pa-

tients more likely to derive a clinical benefit.

Taxanes (paclitaxel and docetaxel) are microtubule-stabilizing agents. They result in defective spindle formation by increasing polymerization and inhibiting depolymerization of microtubules. Defective spindle formation activates the mitotic checkpoint and causes cell cycle arrest, resulting in apoptosis [3]. Spindle assembly checkpoint (SAC) monitors the attachment of spindle microtubules to the kinetochore of each sister chroma-

tid for accurate chromosomal segregation in mitosis. SAC consists of various proteins including budding uninhibited by benzimidazoles proteins (BUB1-3), Bub1-related protein kinase (BubR1), mitotic arrest deficient proteins (MAD 1-3), centromere-associated protein-E (CENP-E), cell-division cycle protein 20 (Cdc20), Aurora A, Aurora B and survivin. Mad2, BubR1, Bub3 and Cdc20 form the mitotic checkpoint complex (MCC), which delays anaphase onset by inhibiting the Anaphase Promoting Complex/cyclosome (APC/C) until all kinetochores are attached to microtubules [4]. This regulatory system prevents aneuploidy by ensuring the segregation of only one copy of each pair of duplicated sister chromatids [3].

Aurora A, a serine/threonine kinase, is essential in correcting the function of SAC and the accurate timing of mitosis. In many different malignant diseases, its abnormal expression has been demonstrated. High expression levels of Aurora A interfere with the Mad2-Cdc20 signal in mitosis, overriding the mitotic checkpoint even in the presence of defective spindle formation [5]. Moreover, Aurora A overexpression prolongs mitosis and results in decreased post-mitotic G1 arrest, due to inactivation of the p53 checkpoint by phosphorylation of serine residues resulting in a loss of p53 G1-checkpoint control [6]. Considering that anti-mitotic drugs target microtubules, correct functioning of the Aurora A would seem crucial for an appropriate drug response.

Previous *in vitro* studies performed in many different cancer cell lines have reported that overexpression of Aurora A can alter the sensitivity to microtubulizing drugs and may result in chemotherapy resistance [5,7-11]. Moreover, there have been studies showing that its expression levels may be associated with survival [12-16]. In our study, we retrospectively identified the expression levels of Aurora A protein in paraffin-embedded breast cancer tissue samples obtained from patients with metastatic breast cancer and evaluated the role of Aurora A expressions in predicting treatment response to taxanes. Survival and correlation with clinicopathological parameters including tumor grade, ER positivity, HER2 status, and triple negative molecular subtypes were also analyzed to determine the prognostic value of Aurora A in these patients.

## Methods

### *Patient and tissue samples*

Cases were retrospectively selected from the re-

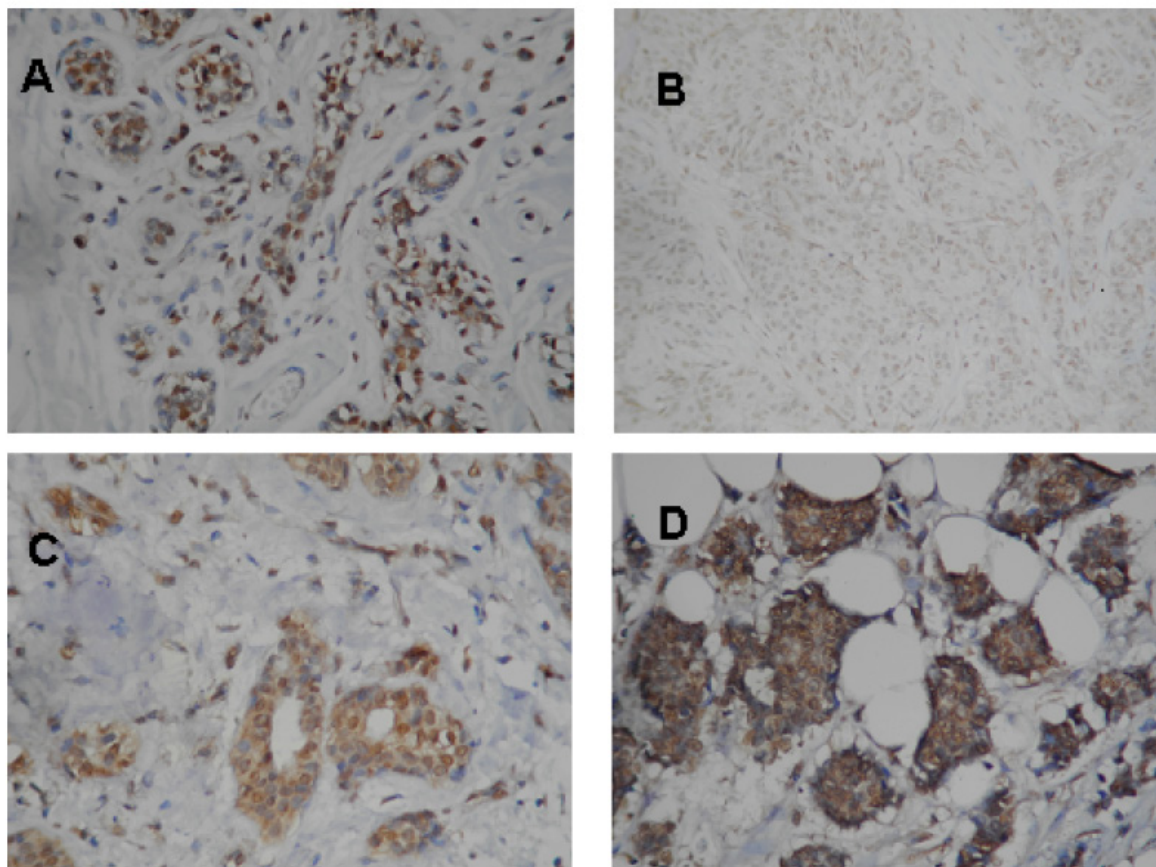
cords of Goztepe Medical Park Oncology Hospital and Bursa Ali Osman Sonmez Oncology Hospital between the years 2010-2015. The study has been approved by the Goztepe Medical Park Hospital Ethics Committee under the title '*Retrospective analysis of tissue samples by immunohistochemistry (IHC)*'.

Eligibility criteria were as follows: (a) Responding and non responding patients who were administered single agent taxane in the metastatic setting (to exclude the effect of other chemotherapeutics on response as a bias source in patient selection in responding patients); (b) Non responding patients who were administered combination of taxanes with any other chemotherapeutic agent in the metastatic setting; (c) Patients who had new biopsy for metastatic disease, if long time had elapsed from initial diagnosis to metastatic disease occurrence.

The taxanes had been administered as either weekly paclitaxel (80 mg/m<sup>2</sup>) or docetaxel every 3 weeks (75 mg/m<sup>2</sup>). A total of 49 patients who met the eligibility criteria were stratified according to treatment response to taxanes into two groups as responders and non-responders. The responders' group (patients with complete response, partial response and stable disease) and non responders' group (progressive disease) were defined according to the Response Evaluation Criteria in Solid Tumors (RECIST). Moreover, patients were stratified as positive, negative and triple negative according to ER, progesterone receptor (PR) and HER2 status respectively. In addition, patients were also stratified according to disease histologic grade as low/intermediate (grade 1-2) and high grade (grade 3).

### *Immunohistochemical evaluation*

Formalin-fixed and paraffin-embedded tissue specimens of primary or metastatic breast cancer collected in the Pathology Department archive at Goztepe Medical Park Hospital and Bursa Ali Osman Sonmez Oncology Hospital were used for IHC staining. These specimens were cut (4-5 µm) and stained with hematoxylin and eosin. A representative slide of each case was selected for IHC studies. Sections 4-5 µm thick were placed on electrostatic-charged slides (X-traTM, Surgipath Medical Industries, Richmond, Illinois, USA) and dried at 60°C for at least two hours and stained with Aurora A antibody (GeneTex Clone C3, CA, USA, 2 hours incubation at a dilution of 1:800). IHC staining was carried out according to standard streptavidin-biotin-peroxidase method. A previously identified strongly staining tumor section was used as a positive control and a section without primary antibody was used as a negative control. Aurora A expression was generally both cytoplasmic and nuclear, although several cytoplasmic-only specimens were also seen. As Aurora A was stained in nearly all of the breast cancer cells as well as in normal breast epithelial cells (weak staining), Aurora A expression on tumor cells was evaluated according to the immunostaining intensity. The staining



**Figure 1.** Immunohistochemistry staining using the primary antibody againsts Aurora A (x 200). **(A)** Aurora-A immunostaining in normal breast epithelium; **(B)**, **(C)** and **(D)**: Aurora-A immunostaining in breast cancer tissue; brown color indicates antibody binding and intensity of staining. **(B)** weak (1+); **(C)** moderate (2+); **(D)** strong (3+) expression.

intensity was scored on the following three-point scale: score 0: no staining; score 1: weak intensity (weaker than that in normal control epithelium or equivalent to normal control epithelium) (Figure 1A and B); score 2: moderate intensity (Figure 1C); score 3: strong intensity (Figure 1D). Scores 0-1 and 2-3 (staining signal stronger than that in normal control epithelium) were defined as low expression and high expression, respectively.

#### Statistics

Each clinicopathological variable was compared between the Aurora A positive and negative expression groups, and evaluated with  $\chi^2$  test or Fisher's exact test. OS time was calculated using the Kaplan-Meier method as the time from the date of diagnosis to the date of death or last follow up. Differences in survival among the groups were compared using the log-rank test.  $P < 0.05$  (two-tailed) was considered statistically significant. All statistical analyses were performed using SPSS, version 15.

## Results

### *Aurora A expression in breast cancer and its relationship to the clinical effectiveness of taxanes*

Thirty-six tumors (73%) showed positive expression for Aurora A. Of all patients, 43% (21/49) were resistant to taxanes. Of Aurora A negative tumors patients 85% (11/13) had taxane-responsive disease, compared with 47% of Aurora A positive tumors (17/36) ( $p=0.02$ ). Moreover, 38% of patients with Aurora A negative tumors (5/13) achieved complete disease response, compared with 8% of Aurora A positive tumors (3/36) ( $p=0.02$ ). The association between the response to taxanes and the expressions of Aurora A is summarized in Table 1.

### *Association of Aurora A expression with clinical parameters*

There was no significant association between

clinical parameters such as tumor grade, ER positivity, molecular subtypes and expression level of Aurora A protein. However, all of HER2 positive tumors (11/11) had high Aurora A expression, compared with 66% of HER2 negative tumors (25/38) ( $p=0.02$ ). There was a significant association between HER2 positive tumors and the level of Aurora A expression (Table 1).

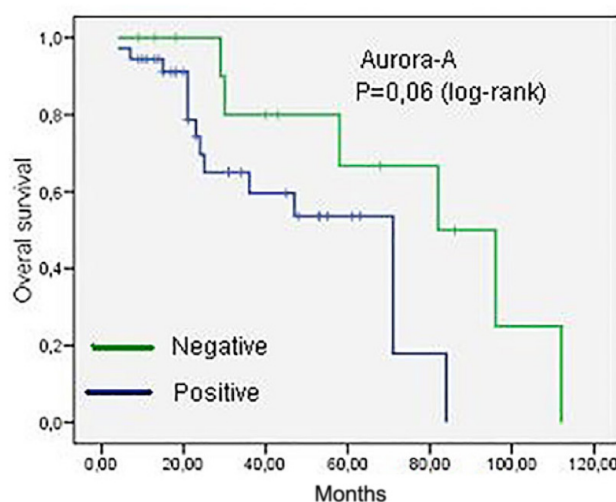
#### Association of Aurora A expression with survival

A total of 49 metastatic breast cancer patients were evaluated for OS. Twenty of 49 patients (41%) died from the date of diagnosis to last follow up. The longest OS was 112 months. The median survival time in patients with positive and negative Aurora A expression was 71 and 82 months, respectively (log-rank test,  $p=0.06$ , Figure 2).

## Discussion

Aurora A is one of the multiple genes identified as candidate oncogenes. It is located on the long arm of chromosome 20 and is involved in centrosome amplification, genomic instability and oncogenic transformation in various human cancers [17-22]. Association of Aurora A with chemotherapy sensitivity and clinical significance of its overexpression have been recently investigated. Overexpression of Aurora A is correlated with tumor progression and clinically aggressive disease in breast cancer [13-16], gastrointestinal cancers [23-26], bladder cancer [27], glial tumors [28] and gynecological cancers [29,30]. In this study, we focused on the prognostic and predictive significance of Aurora A expression in breast cancer.

Previous *in vitro* and *in vivo* studies performed in many different cancer cell lines and xenograft models had reported that overexpression of Aurora A resulted in decreased taxane sensitivity [9,10]. However, in clinical studies performed to confirm these preclinical observations, contradictory results had been reported. Contrary to expectations, Lassmann et al. reported that Aurora A overexpression was associated with improved OS in optimally debulked epithelial ovarian cancer patients receiving taxol/carboplatin therapy [31]. Noguchi reported that high Aurora A mRNA levels were associated with a lower response rate to docetaxel only in ER-negative breast cancers but not in ER-positive breast cancers [32]. In a recent study Tamotsu et al. showed that patients with high Aurora A levels had a higher response rate to chemoradiation, which consisted of 5-Fluorouracil plus cisplatin and radiation with 40 Gy



**Figure 2.** Overall survival in Aurora A positive and negative patients. Despite the lack of significant correlation, patients with negative Aurora A expression had a marginally longer overall survival.

in esophageal squamous cell carcinoma [33]. In our study, 73% of patients with breast cancer had Aurora A positive tumors. This proportion was similar to previous reports, ranging from 62 to 93% in breast cancer [34,35]. In the present study we confirmed the *in vitro* findings in cancer cell lines by showing the strong association between high Aurora A expression and resistance to taxanes ( $p=0.02$ ). In addition, the complete response rate of patients with low Aurora A expression was

**Table 1.** Associations between clinicopathological variables and the expressions of Aurora A

Variables	N	Aurora A expression		p value
		High	Low	
Responders	28	17	11	0.02
Non responders	21	19	2	
Triple negative	10	6	4	0.30
Other subtypes	39	30	9	
ER positive	35	26	9	0.80
ER negative	14	10	4	
HER2 positive	11	11	0	0.02
HER2 negative	38	25	13	
CR	8	3	5	0.02
PR, PD and SD	41	33	8	
Grade 1-2	32	22	10	0.50
Grade 3	17	14	3	

CR: complete response, PR: partial response, SD: stable disease, PD: progressive disease, ER: estrogen receptor

significantly higher than in those with high expression of Aurora A. We did not find any association with tumor grade and ER positivity; however, HER2 positive tumors had significantly higher Aurora A expression than those with HER2 negative tumors.

Currently, inhibitors of Aurora kinases are under preclinical and clinical development [36]. However, whether high Aurora A expression results in worse prognosis is controversial. Despite the lack of statistical significance, Goktas et al. reported that Aurora A overexpression may have a positive effect on the survival of metastatic colorectal patients [37]. In study of Royce et al. Aurora A expression in primary breast tumors was correlated with nuclear grade but not with prognosis [12]. Nadler et al. reported that high Aurora A expression was strongly associated with decreased survival and was associated with high nuclear grade and high HER2 expression [14]. In a current meta-analysis, Weier and Mao reported significant correlation between increased Aurora A expression and distant metastases in ER-positive breast cancers but not in HER2 and basal-like subtype [15]. In a study performed in 766 node-negative breast cancer patients who did not receive chemotherapy, Siggelkov et al. showed that patients with higher Aurora A expression had a shorter metastasis-free survival in ER-positive

breast carcinomas but not in ER-/HER2- nor in HER2+ carcinomas [16]. Moreover, they reported that Aurora A RNA levels correlated with histological grade tumor size and HER2 positivity. In our study we failed to show a significant correlation between the expression level of Aurora A and OS, although patients with low Aurora A level had a marginally longer survival time compared to those with high level. Our results were related with clinical parameters and survival may reflect some limitations due to the small size of the sample, heterogeneity in the distribution of cases for ER, and triple negative cases and to the presence of censored data.

In summary, Aurora A was overexpressed in about 73% of patients with breast cancer, and high Aurora A expression was associated with decreased taxane sensitivity. HER2 positive tumors showed higher Aurora A expression than those with HER2 negative tumors. Despite the lack of statistical significance, patients with high Aurora A expression tended to have shorter OS. In this clinicopathological study, our findings confirmed the preclinical results that high Aurora A expression was associated with decreased taxane sensitivity. Despite the relatively limited number of cases, our data imply that Aurora A may be a promising predictive and prognostic marker in patients with breast cancer.

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