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## Aurora B kinase expression in laryngeal squamous cell carcinoma and its prognostic implications

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Keywords:	Aurora B, Survivin, Aurora A, Larynx; Squamous cell carcinoma, Prognostic factor



# **Aurora B kinase expression in laryngeal squamous cell carcinoma and its prognostic implications**

**Running title: Aurora B expression in LSCC**

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## **Keywords**

Aurora B; Survivin; Aurora A; Larynx; Squamous cell carcinoma; Prognostic factor

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**ABSTRACT**

**Aims:** To investigate the clinical and prognostic significance of Aurora B in laryngeal squamous cell carcinomas (LSCC).

**Methods and results:** Aurora B protein expression was analyzed in a series of 259 LSCC. In addition, the expression of the proliferation index (Ki67) and other proteins involved in cell cycle control, such as Aurora A, survivin and p53 was also determined. Aurora B was highly expressed in 55.4% of LSCC. High Aurora B expression levels were correlated with tumour recurrence ( $P = 0.01$ ), dead of disease ( $P = 0.05$ ), and decreased disease-free survival ( $P = 0.013$ ) and overall survival ( $P = 0.04$ ). Survivin expression was neither associated with clinicopathological characteristics nor with survival. However, survivin expression in the nucleus paralleled Aurora B expression ( $P = 0.014$ ). Aurora A expression was significantly associated with increased tumour grade ( $P = 0.008$ ). Multivariate analysis indicated that Aurora B was an independent predictor for LSCC-specific disease-free survival (hazard ratio [HR], 2.10; 95% confidence interval [95% CI], 1.25-3.52 [ $P = 0.005$ ]) and overall survival (HR, 1.91; 95% CI, 1.01-3.34 [ $P = 0.023$ ]).

**Conclusions:** Aurora B may be a novel prognostic biomarker for LSCC and a potential therapeutic target in this type of tumour.

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INTRODUCTION

Laryngeal squamous cell carcinoma (LSCC) is a common disease of the upper respiratory tract, and approximately 12,000 new cases are estimated to occur yearly in the United States, with nearly 4,000 expected deaths.<sup>1</sup> It is one of the most common malignancies in Europe, with about 45,000 new cases per year and 21,000 deaths.<sup>2</sup> In Spain, about 4,500 cases are diagnosed yearly with 1,500 estimated deaths.<sup>3</sup> The prognosis of LSCC is based on commonly recognized prognostic factors, including site of the primary tumour, histological grade, neck metastasis, and stage. However, patients with LSCC of the same clinical stage often show different clinical course, suggesting that the clinicopathological parameters alone may be insufficient to predict the prognosis. Biological markers that can identify the lesions with more aggressive phenotype and worse prognosis still need to be identified.

The genetic alterations associated with head and neck squamous cell carcinoma are numerous and include a variety of different pathways.<sup>4</sup> The Aurora family of serine/threonine kinases contains three members in mammalian cells, designated Auroras A, B, and C, which share the highest degree of sequence homology in their catalytic domains.<sup>5</sup> However, their expression pattern and cellular localization differ markedly. Auroras A and B are expressed in many different cell types, whereas the expression of Aurora C seems to be restricted to testicular tissue.<sup>6</sup> Aurora A is required for centrosome maturation and division.<sup>7-9</sup> Aurora B is localized to the late-interphase chromosomes proximal to the centromere, from where, during mitosis, it regulates the phosphorylation and thus the mitotic activity of many substrates<sup>10,11</sup> Together with survivin, inner centromere protein, and borealin, Aurora B constitutes a chromosome passenger complex that regulates several aspects of mitosis, such as the spindle

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checkpoint and cytokinesis.<sup>12</sup> Aurora C has also been reported to localize the chromosome passenger complex, but little is known about its function.<sup>13</sup>

Both Auroras A and B are overexpressed in several tumour types.<sup>14,15</sup> Aurora A is located in chromosome 20q13.2, a region commonly amplified in human tumours, including those in colon, breast, pancreas, ovary, and prostate.<sup>14</sup> Although aberrant expression of Aurora A has been found in a variety of human tumours, it does not always correlate with gene amplification. In this sense, we have recently demonstrated amplification of *AURKA* in 27.6% of ovarian carcinomas although 58.8% of tumours overexpressed Aurora A<sup>16</sup>, suggesting that the expression of Aurora A is likely to be regulated not only by gene amplification, but also by other mechanisms that have been previously described such as transcriptional activation and/or suppression of protein degradation.<sup>17</sup> The contribution of Aurora B to carcinogenesis has been less studied than that of Aurora A. Aurora B is located in chromosome 17p13.1, a region not typically amplified in human cancer. However, overexpression of Aurora B has been demonstrated in a variety of human cancers, including astrocytomas,<sup>18</sup> malignant mesotheliomas,<sup>19</sup> seminomas,<sup>20</sup> colorectal,<sup>21</sup> prostate,<sup>22</sup> endometrial,<sup>23</sup> thyroid,<sup>24</sup> non-small cell lung,<sup>25</sup> oral squamous cell,<sup>26</sup> and ovarian<sup>16</sup> cancers, at various pathological stages, with a tendency to group in higher grades of malignancy.

Survivin is a multifunctional protein that inhibits apoptosis, regulates cell division and enhances angiogenesis. Although rarely expressed in terminally differentiated adult tissues, upregulation of survivin is found in many cancers, usually associated with increased aggressiveness and decreased survival.<sup>27</sup>

Aurora B protein expression has not been previously characterized in human LSCC by immunohistochemistry, and its role in LSCC prognosis has not been well documented. In the present study our aim was to investigate Aurora B protein expression in a large

series of LSCC. To further evaluate the effect on patient prognosis and clinical characteristics of other key players of cell cycle regulation, we also performed immunohistochemistry for Aurora A, survivin, p53 and Ki67, and we examined the relationship between clinicopathological findings and the expression of these biomarkers in tumour cells. Moreover, we compared Aurora B, Aurora A, and survivin with other classical prognostic parameters to evaluate the effectiveness of these molecules as prognostic biomarkers in LSCC.

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## MATERIAL AND METHODS

### *Patient selection and tissue samples*

Surgical tissue samples of LSCC from 259 patients, obtained in the period between 1990 and 1996, were retrieved from the archive of the Department of Pathology, University Hospital La Paz, Madrid (Spain). None of the patients had been treated previously. Tissue samples were fixed in 10% formalin and embedded in paraffin. Standard haematoxylin and eosin stains were used to establish the diagnosis and to grade the tumours into well (grade I), moderately (grade II) and poorly (grade III) differentiated, according to the World Health Organization classification. Tumour size and clinicopathological stage of tumours were classified according to the 1988 TNM classification of the American Joint Committee of Cancer (AJCC).<sup>28</sup> Follow-up data from the patients were registered by means of protocols that the otolaryngologists fulfilled throughout the follow-up of the patients. Disease-free survival (DFS) and overall survival (OS) were the main endpoints of this study. DFS was calculated from the day of surgery to the date of recurrence, either locoregional or distant. OS was calculated from the day of surgery to the date of either death or the last follow-up. Approval for the study was obtained from the Local Ethics Committee (Comité Ético de

Investigación Clínica del Hospital Universitario La Paz, ref. PI-349). Detailed patients characteristics and histopathologic data are shown in Table 1.

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### ***Tissue microarray construction***

Representative areas of the tumours were selected on haematoxylin and eosin-stained sections and marked on individual paraffin blocks. Two tissue cores (1 mm in diameter) were obtained from each specimen. The tissue cores were arrayed into a receptor paraffin block using a tissue microarray workstation (Beecher Instruments, Silver Spring, MD), as described previously.<sup>29</sup> A haematoxylin and eosin-stained section of the array was reviewed to confirm the presence of morphologically representative areas of the original lesions.

### ***Immunohistochemistry***

Immunohistochemistry (IHC) was performed on 4-µm sections of formalin-fixed, paraffin-embedded tissues. Briefly, the tissue sections were deparaffinized and rehydrated in water, after which antigen retrieval was carried out by incubation in EDTA solution, pH 8.2 at 50°C for 45 minutes in an autoclave. Endogenous peroxidase and non-specific antibody reactivity was blocked with peroxidase blocking reagent (Dako, Glostrup, Denmark) at room temperature for 15 minutes. The sections were then incubated for 60-90 minutes at 4°C with the following antibodies: Aurora A monoclonal antibody (clone JLM28, Novocastra Laboratories, Newcastle Upon Tyne, UK, dil. 1:50), Aurora B polyclonal antibody (Abcam pcl, Cambridge, UK, dil. 1:50), survivin polyclonal antibody (Abcam pcl, dil. 1:400), p53 monoclonal antibody (clone DO-7, Novocastra Laboratories, dil. 1:100), and Ki67 monoclonal antibody (clone MIB1, Dako, dil. 1:100). Detection was performed with EnvisionPlus Detection System (Dako).

### *Analysis of immunohistochemical stains*

For Aurora B, evaluation of the IHC slides was performed using a semiquantitative scoring system for both, intensity of stain and percentage of positive cells (labelling frequency percentage). For the intensity, the grading scale ranged from non-detectable signal [0] to strong signal [2] seen at low power. A weak to moderate signal seen at low to intermediate power was designated 1. Labelling frequency was scored on a four-tiered system as 1 (0-25%), 2 (26-50%), 3 (51-75%), or 4 (76-100%). The multiplicative expression index (MEI) was obtained by totalling the scores of intensity and percentage, as previously reported.<sup>30</sup> Expression level of Aurora B was defined as low (MEI, 1-4) or high (MEI, >4). For Aurora A, the MEI could not be established, as the intensity ranged only from non-detectable signal (0) to weak signal (1), and the labelling frequency ranged between 0-60%. Therefore, according to the mean value of the registered scores, we established a cut-off rate of 5% to consider the expression of Aurora A as positive, as previously published.<sup>16</sup> Survivin expression was assessed as the percentage of cells with nuclear and/or cytoplasmic immunostaining. Expression of p53 was evaluated as the percentage of tumour cells with nuclear-positive immunostaining. The Ki67 labelling index was expressed as the percentage of positively stained nuclei in the tumour cells.

### *Statistical analysis*

Statistical analysis was carried out using 9.0 SPSS software for Windows (SPSS Inc, Chicago, IL). All tests were 2-sided and used a significance level of 0.05. Qualitative data were registered as absolute frequencies and percentages; quantitative data were expressed as median, range, and/or mean and standard deviation. Frequency tables were tested by Fisher test for comparison of discrete variables. Correlations between the expression of Aurora A and B, survivin, p53 and Ki67 were examined using

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Spearman's rank correlation test. Analysis of disease-free and overall survival data were carried out using Kaplan-Meier method plots and log-rank test was used to compare the survival distributions of two samples. Factors with significant effects on disease-free and overall survival were identified with univariate and multivariate Cox proportional hazards regression model. To accommodate non-proportional hazards interactions were built between covariates and time into the Cox regression model as time-dependent covariates. These interactions with a non significant effect were excluded from the final model.

## RESULTS

### *Aurora B, survivin, and Aurora A protein expression and clinicopathological characteristics*

Mean expression values for Aurora A, Aurora B, survivin, p53 and Ki67 are shown in Table 2. For Aurora B the staining was predominantly nuclear (Figure 1). Aurora B was highly expressed (MEI >4) in 139 (55.4%) of 251 LSCC in our study. Tumours with high expression of Aurora B (MEI >4) showed a higher rate of recurrence and death of disease than those tumours with a low expression of Aurora B protein (MEI ≤4) (37.2% versus 21.3%,  $P = 0.01$ , and 28.7% versus 17.9%,  $P = 0.05$ , respectively) (Table 3). Aurora B protein expression was not related to location of tumour (glottic versus non-glottic), T category (T1-2 versus T3-4), tumour grade, regional lymph node or distant metastasis, and tumour stage (I-II versus III-IV) (Table 3).

Survivin expression was assessable in 234 samples. All cases showed cytoplasmic staining for survivin; however, in some tumours, survivin was detected both in the nucleus and the cytoplasm of tumour cells (106 tumours, 45.23%) (Figure 2). Survivin expression was not associated with any of the clinicopathological variables studied (Table 3). However, survivin expression in the nucleus paralleled Aurora B expression

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( $P = 0.014$ ); thus, mean nuclear survivin expression in tumours with high expression of Aurora B was  $12.19\% \pm 17.88$  (range 0-100%) *versus*  $6.73\% \pm 12.64$  (range 0-60%) in those tumours with low expression of Aurora B.

The immunohistochemical staining pattern for Aurora A was cytoplasmatic (Figure 3). Aurora A was expressed ( $>5\%$ ) in 57 (22.4%) of 255 tumours. Aurora A protein expression was significantly associated with tumour grade; thus, 33.3% of grade 3 tumours expressed Aurora A compared to 17.5% of grade 1 and 2 tumours ( $P = 0.008$ ) (Table 3). The expression of Aurora A was not related to location of tumour (glottic *versus* non-glottic), T category (T1-2 *versus* T3-4), regional lymph node or distant metastasis, tumour stage (I-II *versus* III-IV), tumour recurrence or final status of patient (alive *versus* dead) (Table 3).

Correlation between the expression of Aurora B and Aurora A, survivin, p53 and Ki67 was examined using a Spearman rank correlation test. A positive correlation was obtained between survivin expression both in the nucleus and cytoplasm and the mean expression of Aurora B ( $P = 0.009$ ,  $R = 0.18$ , and  $P = 0.001$ ,  $R = 0.23$ , respectively) and Ki67 ( $P < 0.001$ ,  $R = 0.25$ , and  $P = 0.015$ ,  $R = 0.16$ , respectively). There was also a positive correlation between the expression of Aurora A and p53 ( $P = 0.003$ ,  $R = 0.20$ ), and the expression of Aurora A and B and Ki67 ( $P = 0.011$ ,  $R = 0.17$ , and  $P = 0.003$ ,  $R = 0.2$ , respectively).

**Association between disease-free survival, overall survival, and Aurora B and A protein expression in LSCC**

The mean disease-free survival was  $43.54 \pm 31.01$  and mean overall survival was  $46.56 \pm 30.7$  months. Sixty patients (28.6%) died due to their cancer. In the univariate analysis, Kaplan-Meier method showed that patients with high expression of Aurora B (MEI  $>4$ ) had a decreased disease-free survival compared with patients whose tumours expressed

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Aurora B protein at lower levels ( $\text{MEI} \leq 4$ ) (mean survival of 72 months, 95% confidence interval [CI], 62-82, *versus* 89 months, 95% CI, 81-98;  $P = 0.013$ , respectively) (Figure 4). Regarding overall survival, patients with high expression of Aurora B ( $\text{MEI} > 4$ ) showed a significant decreased survival time compared to those patients with a low expression of Aurora B protein ( $\text{MEI} \leq 4$ ) (mean survival of 84 months, 95% CI, 75-92, *versus* 122 months, 95% CI, 111-133;  $P = 0.04$ , respectively) (Figure 5). There was no correlation between disease-free survival or overall survival and survivin and Aurora A protein expression.

The multivariate analysis using the Cox regression model adjusted to clinicopathological variables (tumour site [glottis *versus* other], T category [T1-2 *versus* T3-4], histological grade (G1-2 *versus* G3), tumour stage (I-II *versus* III-IV) and lymph node metastasis) showed Aurora B protein expression as an independent prognostic factor for both disease-free survival ( $P = 0.005$ , hazard ratio [HR] = 2.10, 95% CI, 1.25-3.52) and overall survival ( $P = 0.023$ , HR = 1.91, 95% CI, 1.01-3.34) (Table 4).

## DISCUSSION

This is, to our knowledge, the first study that investigates Aurora B protein expression in LSCC. Using immunohistochemical analysis of a large series of tumours, we showed frequent high expression of Aurora B in LSCC (55.4%), suggesting that Aurora B plays a role as tumour marker in LSCC. Aurora B is reported to form complexes with inner centromere protein and survivin. These complexes are thought to be involved in the regulation of chromosome alignment, segregation, and cytokinesis.<sup>10,31</sup> In LSCC, Aurora B was localized in the nucleus, in line with previous reports on other tumour types.<sup>16,20-26</sup> Since recent studies have identified the histone H3 protein as an important substrate for Aurora B kinase,<sup>11,32</sup> nuclear localization of Aurora B seems to be functionally important. Moreover, high expression of Aurora B was correlated with

nuclear expression of survivin. This is consistent with the results of previous studies in non-small cell lung carcinoma<sup>25</sup> and *in vitro* studies which show that cells lacking survivin expression upon targeting by antisense oligonucleotide treatment have a lower Aurora B kinase activity.<sup>33</sup>

Overexpression of Aurora B has been related to poor prognosis in recent reports of endometrial carcinoma,<sup>23</sup> non-small cell lung carcinoma<sup>25</sup> and mesothelioma.<sup>19</sup> In our study, tumours with high expression of Aurora B showed a decreased disease-free survival and overall survival, thus suggesting that Aurora B plays a role in LSCC tumour progression. Moreover, the multivariate analysis adjusted to tumour site, T category, histological grade, lymph node metastasis, and tumour stage by Cox proportional hazards regression showed Aurora B expression as an independent prognostic factor for both disease-free survival and overall survival. Aurora B has been proposed to promote tumour progression through mechanisms such as the loss of mitotic control and abnormal proliferation.<sup>11</sup> In this regard, we found a positive correlation between Aurora B levels and the tumour proliferation rate assessed by the immunostaining for the proliferation marker Ki67, similar to the data published for astrocytomas,<sup>18</sup> endometrial,<sup>23</sup> thyroid,<sup>24</sup> and non-small cell lung carcinomas.<sup>25</sup>

In LSCC, survivin expression has been related to poor patient survival and tumour invasion, suggesting that survivin protein frequently exhibits dysregulated expression and may contribute to the malignant transformation of the laryngeal squamous epithelia, and a possible association with the stronger aggressiveness.<sup>34-36</sup> Thus, it is postulated that the state of survivin expression might be an important discriminator in the progression of LSCC. In our study, survivin expression was not correlated with any patient characteristics of adverse disease. On the other hand, it has been previously reported that the expression of survivin in the nucleus may have prognostic

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significance.<sup>37,38</sup> In our cases of LSCC, however, we did not observe any association between localization of survivin (cytoplasmic or nucleocytoplasmic) and survival or other clinicopathological parameters.

We showed that Aurora A expression increases with lower tumour differentiation, consistent with what has been recently reported for the levels of Aurora A mRNA quantified by real-time reverse transcription PCR in head and neck squamous cell carcinomas.<sup>39</sup> These data suggest that expression of Aurora A contributes to poor prognosis in LSCC patients, as described in other tumour types showing a correlation between the up-regulation of Aurora A and tumour progression and clinical aggressiveness.<sup>15</sup> Aurora A has been shown to interact with many important cellular proteins, including p53. Aurora A is a key regulatory component of the p53 pathway, and high expression of Aurora A phosphorylates p53, leads to an increased p53 degradation, thus facilitating oncogenic transformation.<sup>40</sup> In this sense, in this study we found a correlation between expression of Aurora A and p53; nevertheless the interrelationship between these molecules and tumour progression is, however, still unclear.

Targeted therapies alone and in combination with cytotoxic therapy have recently been successfully introduced into the treatment of some solid tumours. In the last years huge progress has been made in both understanding the basic biological role of Aurora A and B kinases and in the development of small molecular inhibitors that seem to have a very interesting preclinical activity.<sup>41,42</sup> These are presently being tested in clinical studies in cancer patients.<sup>43</sup> In this sense, our results show that Aurora B could be a new therapeutic target in LSCC.

In conclusion, we have reported for the first time the presence of Aurora B expression in a large series of LSCC, and its correlation with adverse tumour features. Our results

suggest the involvement of Aurora B in LSCC progression. Studies aimed at further understanding of Aurora B in tumour progression in LSCC are warranted.

For Peer Review

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**FIGURE LEGENDS**

**Figure 1.** Expression of Aurora B protein in the nuclei of the tumour cells.

**Figure 2.** Expression of survivin in the tumour cells. Note the strong immunostaining of the nuclei of the tumour cells (same case as depicted in Figure 1).

**Figure 3.** Expression of Aurora A is observed in the cytoplasm of the tumour cells.

**Figure 4.** Kaplan-Meier curve showing the association between the expression of Aurora B and disease-free survival.

**Figure 5.** Kaplan-Meier curve showing the association between the expression of Aurora B and overall survival.

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For Peer Review

**Table 1.** Clinicopathological characteristics of the patients with LSCC

	<b>n (%)</b>
<b>Total patients</b>	259
<b>Age, median (range), y</b>	60.38 (29-87)
<b>Sex (n=259)</b>	
Male	255 (98.5)
Female	4 (1.5)
<b>Tumour site (n=246)</b>	
Glottic	61 (24.8)
Supraglottic	87 (35.4)
Subglottic	5 (2)
Transglottic	93 (37.8)
<b>T category (n=252)</b>	
T1	63 (25)
T2	46 (18.3)
T3	90 (35.7)
T4	53 (21)
<b>Histological grade (n=253)</b>	
Well differentiated (G1)	61 (24.1)
Moderately differentiated (G2)	112 (44.3)
Poorly differentiated (G3)	80 (31.6)
<b>Lymph node metastasis (n=259)</b>	
N <sub>0</sub>	177 (68.3)
N <sub>+</sub>	82 (31.7)
<b>Distant metastasis (n=252)</b>	
M <sub>0</sub>	231 (91.7)
M <sub>+</sub>	21 (8.3)
<b>Tumour stage (n=252)</b>	
I	56 (22.2)
II	36 (14.3)
III	71 (28.2)
IV	89 (35.3)
<b>Recurrence (n=252)</b>	
Yes	54 (21.4)
No	198 (78.6)
<b>Disease-free survival, median, mo</b>	43.54
<b>Overall survival, median, mo</b>	46.56
<b>Final status</b>	
DOD	60 (28.6)
Alive	150 (71.4)

LSCC, laryngeal squamous cell carcinoma; DOD, dead of disease

**Table 2.** Mean protein expression values for Aurora B, survivin, Aurora A, p53 and Ki67.

	Mean (%)	± SD	Range (%)
<b>Aurora B</b>	80.29	31.72	0-100
<b>Survivin (nuclear)</b>	9.81	15.92	0-100
<b>Survivin (cytoplasm)</b>	93.13	14.91	25-100
<b>Aurora A</b>	4.86	9.92	0-60
<b>p53</b>	30.80	37.77	0-100
<b>Ki67</b>	40.49	25.65	5-100

SD, Standard deviation

**Table 3.** Relationship between Aurora B, survivin and Aurora A protein expression and clinicopathological variables.

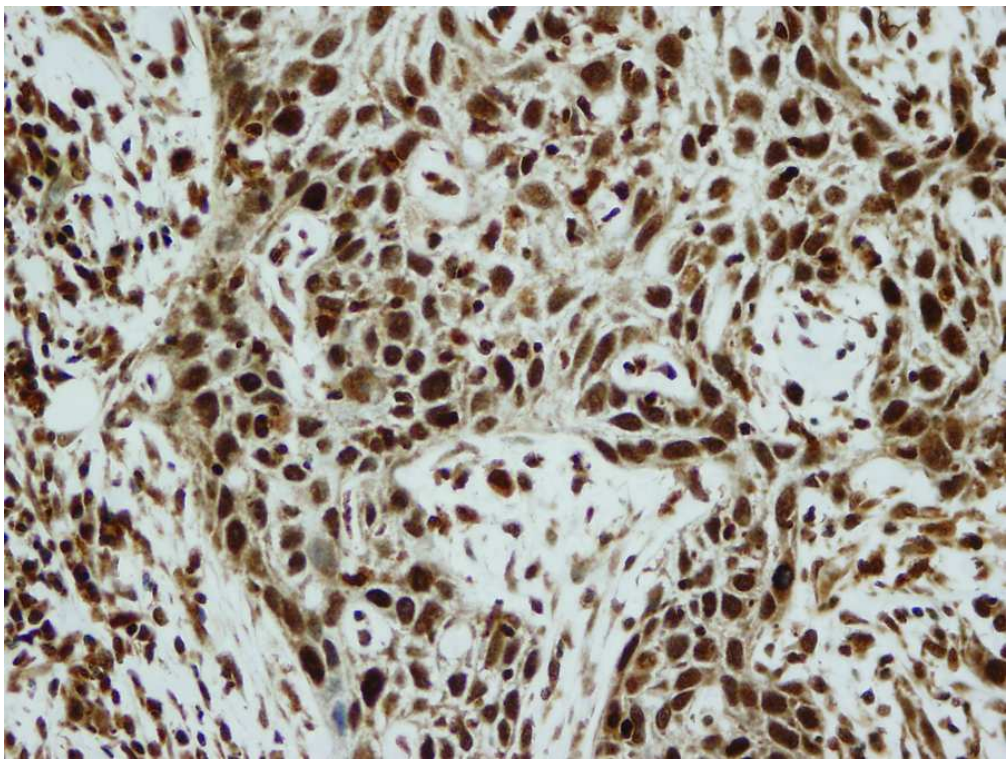
		Aurora B			Survivin			Aurora A		
		MEI ≤4 (n, %)	MEI >4 (n, %)	P	C (n, %)	N/C (n, %)	P	≤5% (n, %)	>5% (n, %)	P
Tumour site	Glottic	27 (47.4)	30 (52.6)	0.54	23 (52.3)	21 (47.7)	0.74	47 (79.7)	12 (20.3)	0.72
	Non-glottic	77 (42.5)	104 (57.5)		93 (55.4)	75 (44.6)		140 (76.5)	43 (23.5)	
T category	T1-T2	45 (43.3)	59 (56.7)	0.80	48 (52.7)	43 (47.3)	0.78	82 (75.9)	26 (24.1)	0.44
	T3-T4	63 (45)	77 (55)		70 (55.1)	57 (44.9)		113 (80.1)	28 (19.9)	
Histological grade	G1-G2	71 (43)	94 (57)	0.41	76 (51.4)	72 (48.6)	0.25	141 (82.5)	30 (17.5)	0.008
	G3	39 (48.8)	41 (51.3)		43 (60.6)	28 (39.4)		52 (66.7)	26 (33.3)	
Lymph node metastasis	N <sub>0</sub>	74 (43.3)	97 (56.7)	0.67	86 (57)	65 (43)	0.24	142 (81.1)	33 (18.9)	0.13
	N <sub>+</sub>	34 (46.6)	39 (53.4)		32 (47.8)	35 (52.2)		53 (71.6)	21 (28.4)	
Distant metastasis	M <sub>0</sub>	101 (44.9)	124 (55.1)	0.63	111 (55)	91 (45)	0.44	180 (78.9)	48 (21.1)	0.41
	M <sub>1</sub>	7 (36.8)	12 (63.2)		7 (43.8)	9 (56.3)		15 (71.4)	6 (28.6)	
Tumour stage	I-II	36 (41.4)	51 (58.6)	0.59	43 (57.3)	32 (42.7)	0.57	72 (79.1)	19 (20.9)	0.87
	III-IV	72 (45.9)	85 (54.1)		75 (52.4)	68 (47.6)		123 (77.8)	35 (22.2)	
Recurrence	No	85 (78.7)	81 (62.8)	0.01	85 (71.4)	68 (71.6)	1.00	134 (71.3)	37 (69.8)	0.86
	Yes	23 (21.3)	48 (37.2)		34 (28.6)	27 (28.4)		54 (28.7)	16 (30.2)	
Final status	Alive	92 (82.1)	97 (71.3)	0.05	91 (75.8)	80 (79.2)	0.63	148 (75.1)	46 (83.6)	0.21
	DOD	20 (17.9)	39 (28.7)		29 (24.2)	21 (20.8)		49 (24.9)	9 (16.4)	

MEI, Multiplication expression index; C, Cytoplasmic staining; N/C, Nuclear and cytoplasmic staining; DOD, Dead of disease

**Table 4.** Prognostic value of Aurora B expression adjusted for clinicopathologic parameters by Cox proportional hazards regression.

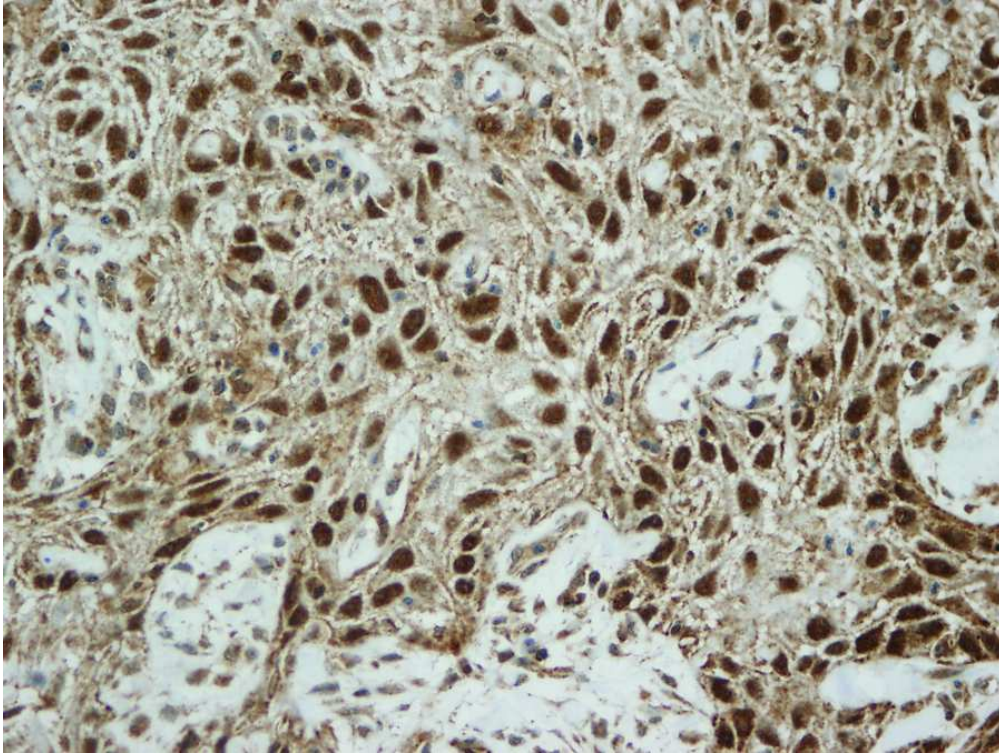
Univariate		DFS		OS		
Prognostic factor	<i>P</i>	HR	95% CI	<i>P</i>	HR	95% CI
Tumour site	0.016	0.47	0.26-0.87	0.001	0.25	0.11-.58
T category	0.001	2.52	1.50-4.25	<0.001	4.93	2.49-9.74
Histologic grade	0.042	1.67	1.02-2.74	0.154	1.48	0.86-2.55
Lymph node metastasis	<0.001	3.34	2.10-5.44	<0.001	3.43	2.05-5.74
Tumour stage	<0.001	3.05	1.72-5.42	<0.001	6.09	2.76-13.43
Aurora B expression	0.019	1.81	1.10-2.90	0.043	1.74	1.02-2.90
Multivariate		DFS		OS		
Prognostic factor	<i>P</i>	HR	95% CI	<i>P</i>	HR	95% CI
Tumour site	0.305	0.69	0.34-1.40	0.067	0.4	0.15-1.07
T category	0.379	1.54	0.59-4.07	0.25	2.03	0.61-6.80
Histologic grade	0.637	0.88	0.50-1.52	0.26	0.72	0.40-1.28
Lymph node metastasis	<0.001	2.70	1.48-4.93	0.007	2.28	1.25-4.15
Tumour stage	0.904	1.07	0.33-3.5	0.535	1.6	0.36-7.10
Aurora B expression	0.005	2.10	1.25-3.52	0.023	1.91	1.01-3.34

DFS, Disease-free survival; OS, Overall survival; HR, Harzard ratio; 95% CI indicates 95% confidence interval.

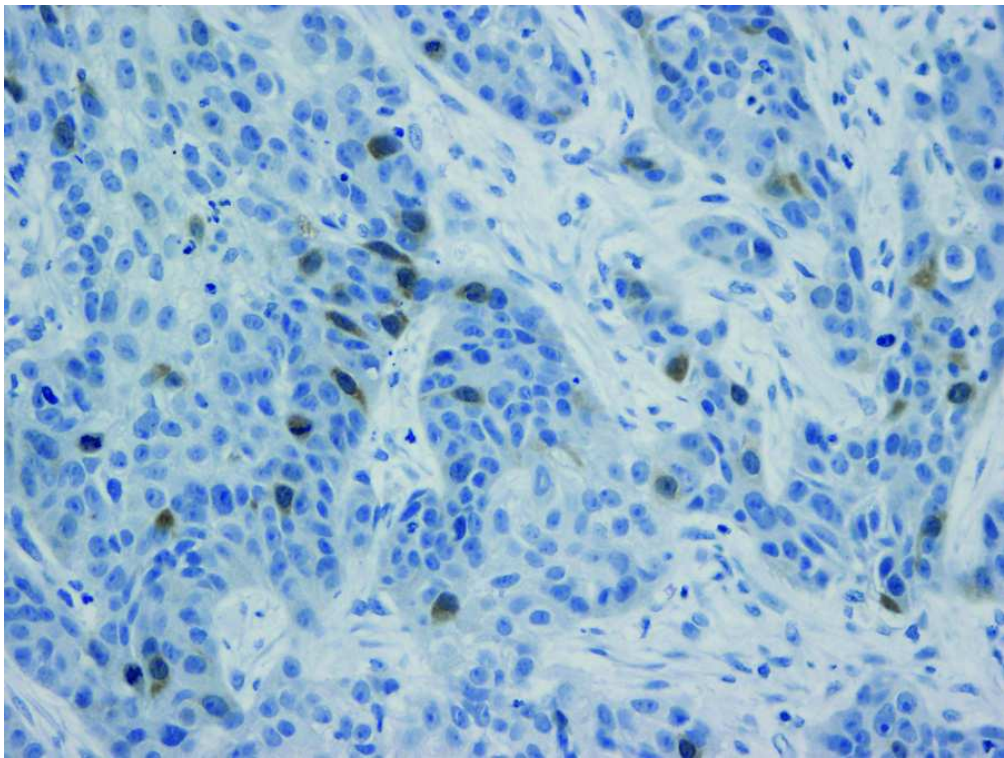


Expression of Aurora B protein in the nuclei of the tumour cells  
76x57mm (300 x 300 DPI)

Review

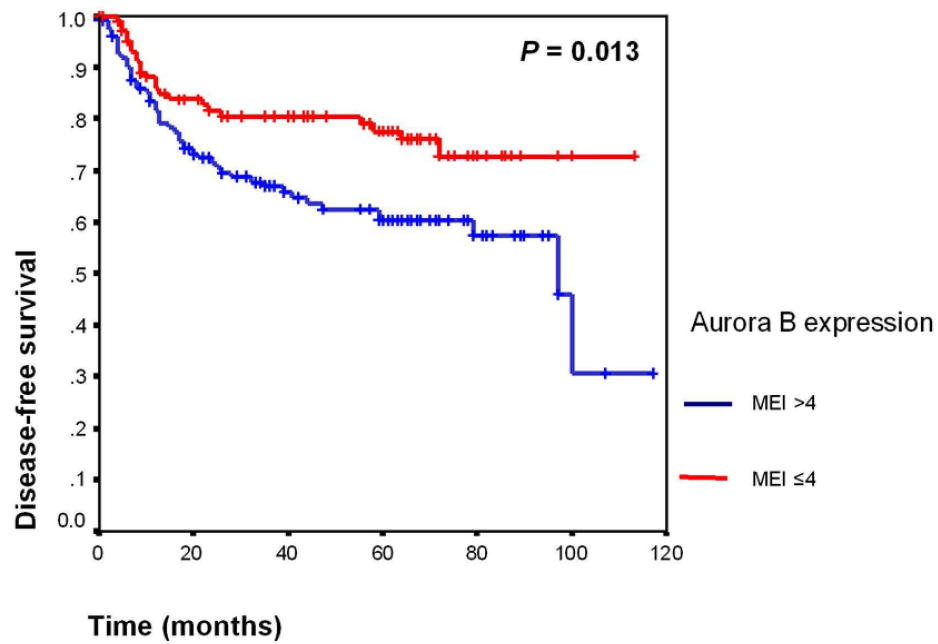


Expression of survivin in the tumour cells. Note the strong immunostaining of the nuclei of the tumour cells (same case as depicted in Figure 1)  
76x57mm (300 x 300 DPI)

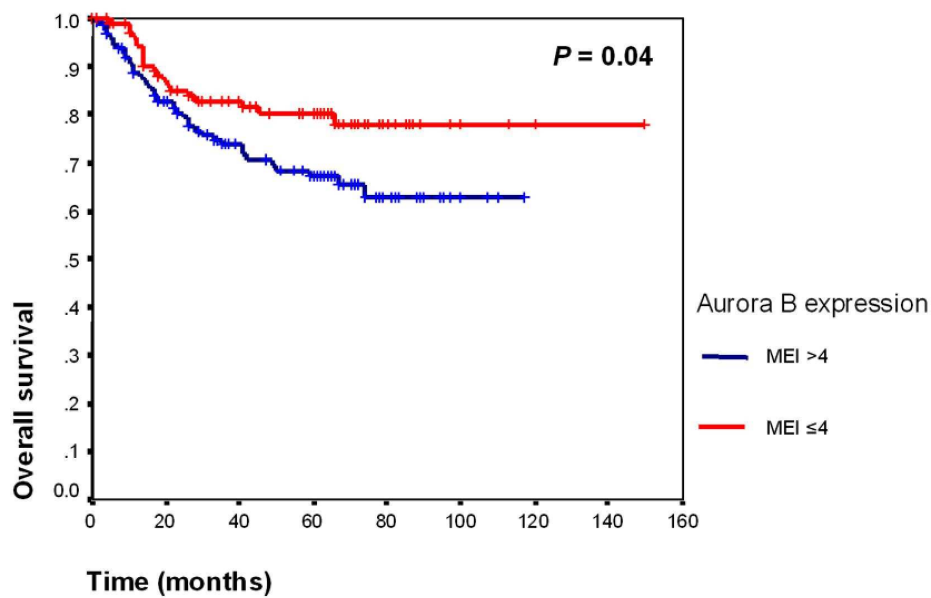


Expression of Aurora A is observed in the cytoplasm of the tumour cells  
76x57mm (300 x 300 DPI)

Review



Kaplan-Meier curve showing the association between the expression of Aurora B and disease-free survival  
76x56mm (600 x 600 DPI)



Kaplan-Meier curve showing the association between the expression of Aurora B and overall survival  
76x54mm (600 x 600 DPI)