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Expression of Aurora kinase A and B in normal and malignant cervical tissue: High Aurora A kinase expression in squamous cervical cancer☆

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ABSTRACT

Objective: Aurora kinases such as Aurora A and B are key regulators of mitosis and tumorigenesis and have been reported to be overexpressed in various malignancies. However, the expression of Aurora kinases in normal and neoplastic cervical tissues remains undetermined.
Study design: Immunohistochemical expression of Aurora A and B kinase was examined in 20 normal cervix, 35 cervical intraepithelial neoplasm 3 (CIN 3) and 95 cervical cancers, including squamous cell carcinoma (SCC) ($n = 76$) and adenocarcinoma (AC) ($n = 19$). Expression of Aurora A and B kinase was confirmed by Western blot. The correlation between Aurora A and B kinases expression and the clinicopathological parameters was analyzed by statistical analysis.
Results: The Aurora A and B expression was significantly increased in carcinoma and CIN 3, compared with normal cervix. However, expression of Aurora A and B showed no significant correlation between CIN 3 and cervical cancer. The nuclear expression of Aurora A showed a significantly positive correlation with the expression of Aurora B ($P = 0.018$). The percentage of Aurora A overexpression between SCCs and ACs showed a significant difference (50% vs. 21.1%, $P = 0.023$). However, there was no correlation of Aurora A and B expression with patient survival.
Conclusion: According to our study, Aurora A and B overexpression is a relatively early phenomenon in the genesis of malignant epithelial neoplasm tumorigenesis. Based on the results of this study, it would be interesting to know whether Aurora kinases play a role in pathogenesis of cervical dysplasia and SCC patients.

1. Introduction

Cancer of the cervix is the second leading cause of cancer deaths in women worldwide and remains a cause of mortality among women of reproductive age in developing countries. Cervical carcinoma progresses through a multistage process of carcinogenesis [1]. For example, cervical intraepithelial neoplasm, a precursor lesion detected in screening programs, can progress to invasive cancer [2].

Mitosis is a highly dynamic phase of the cell cycle and any error can have dramatic consequences, as seen in most tumors, which bear chromosomal anomalies and are often aneuploid. Due to its high complexity, mitosis needs stringent regulators, among which are the Aurora kinases. Aurora proteins belong to a small family of serine/threonin kinases that are key regulators of different steps in mitosis and meiosis [3]. Aurora A localizes to centrosomes during interphase and moves to the spindle poles during early mitosis. In contrast, Aurora B is a chromosomal passenger protein, where it is in a complex with at least three other chromosomal passenger proteins: inner centromere protein, survivin, and the recently described borealin [4]. Several studies on different organisms show a role for Aurora A in centrosome maturation and spindle assembly. On the contrary, Aurora B is part of the spindle checkpoint regulating chromosome cohesion and the bipolar attachment of microtubules. In addition, Aurora B has been proposed to play a role in the control of cytokinesis [5,6]. Since their discovery, Aurora kinases have been implicated in cancer and tumorigenesis [7].

The first data to implicate this family of kinases in tumorigenesis came with the observation that Aurora A and B are

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overexpressed in primary breast and colon tumor samples. Many subsequent studies identified other tumor types, including breast, pancreatic, ovarian and hepatocellular tumors, in which Aurora A was amplified or otherwise overexpressed [8–10] The Aurora kinase family is clearly implicated in tumorigenesis, and its product has been highlighted as a drug target. Several reports have been published describing the first generation of small-molecule inhibitors of Aurora kinase activity.

Recently, Patel et al. showed that human papillomavirus type 16 proteins E6 and E7 cause deregulation of cellular genes such as Aurora A and cdk1, which are known to control the G2–M phase transition and the ordered progression through mitosis [11]. The Aurora kinases expression level in cervical intraepithelial neoplasm (CIN 3) and cervical cancer is still poorly understood. So far, the relationships between Aurora A and B expression and various kinds of cervical cancers, especially adenocarcinomas (ACs) and squamous cell carcinomas (SCCs), have not yet been evaluated.

In this study, we investigated whether high Aurora A and B expression in CIN 3 or cervical cancer is related to the prognostic factors of tumor size, depth of stromal invasion, lymph node metastasis, LVSI, parametrial invasion, stage, and cell type.

2. Materials and methods

2.1. Patients

Cervical cancer samples were collected between January 1996 and December 2000 from patients with different histological types, including SCC ($n = 76$) and AC ($n = 19$), who were treated with radical hysterectomy or radiotherapy. The mean age of the patients was 56.7 ± 12.4 (range, 33–87) years. None of the patients received preoperative adjuvant therapy. We collected tumor specimens 0.5 cm^3 during surgery or biopsy. Samples were stored at $-80\,^{\circ}C$ until analysis. Staging was performed according to the 1988 FIGO classification. The patients' clinico-pathologic characteristics were collected.

Samples were from cervical intraepithelial neoplasm 3 (CIN 3) patients ($n = 35$) who underwent LEEP conization. Histologically normal cervical tissue samples, including from the endocervical and exocervical areas, were obtained from other patients ($n = 20$) treated for a uterine myoma with a total hysterectomy. Cervicitis cases were excluded in the study. Part of the normal cervix, CIN 3 and cervical cancer specimens were fixed in formalin and embedded in paraffin for immunohistochemistry.

The study was approved by the Ethics Committee of the Department of Obstetrics and Gynecology, Taipei Veterans General Hospital; written informed consent was obtained from all patients enrolled in the study.

3. Morphologic criteria and clinical data

After retrieval, two of the pathologists examined the hematoxylin and eosin-stained slides and classified the tumors using commonly accepted criteria [12]. Cellular differentiation, cell type, depth of stromal invasion, tumor size, lymph node metastases, lymph–vascular space invasion, and parametrial invasion were recorded and confirmed by one of the authors (Dr. Lai). Review of the surgical pathology reports yielded information regarding patient age and tumor stage.

4. Immunohistochemistry

Four-micron sections were cut from formalin-fixed tissue embedded in paraffin blocks and mounted onto silane-coated slides. Sections were dewaxed in xylene and rehydrated in a graded alcohol series (100%, 95%, 75%, 60%) and ddH_2O; antigens were retrieved by boiling in buffer (DAKO, USA) for 5 min. Endogenous peroxidase was inhibited by 3% H_2O_2 (R&D Systems, USA). Sections were incubated in primary antibody overnight at 4 °C. The Aurora A and B polyclonal antibody (AB cam) was diluted to 1:150 in antibody diluent (DAKO), with antibodies yielding a high background in the immunohistochemical staining procedures. Following overnight incubation, secondary biotinylated antibodies and streptavidin–HRP-conjugated reagent (R&D Systems) were applied to the sections. Immunoreactive complexes were detected using the peroxidase substrate, AEC (R&D Systems). Slides then were counterstained with aqueous hematoxylin (Shandon, USA) and mounted on a crystal mount (Biomeda, USA). Control sections were incubated with antisera in the presence of 100-fold excesses of the appropriate human Aurora A and B protein, or with isotype-matched immunoglobulin G normal rabbit serum. Control sections were incubated with isotype-matched immunoglobulin G of normal rabbits.

5. Quantification of immunohistochemical staining

Two observers independently examined the immunohistochemistry slides. The specific staining of each antibody was identified in the nucleus and in the cytoplasm. Immunoreactivity in the nucleus was evaluated according to the percentage of positive cells among 500 cells by 2 independent reviewers and calculated as follows: the percentage of cells with intense staining $\times$ 1 + percentage with weak staining $\times$ 0.5. These results were described as a positivity index (PI). When the PI was higher than 20, the case was defined as overexpression [13].

6. Western blot

To confirm the antigenic specificity of the antibodies used for immunostaining, Western blotting was performed as described previously [14]. In brief, 7 fresh tissue specimens (2 from the normal cervix and 5 from cervical cancer SCC and AC) were homogenized and lysed in 0.5 mL NaCl, 0.5% NP-40, 1 mmol/L phenylmethylsulfonylfluoride (Sigma Chemical, St. Louis, MO), 1 μg/mL aprotinin (Boehringer Mannheim, Indianapolis, IN), 1 μg/mL leupeptin (Boehringer Mannheim) and 20 μg/mL TPCK (Boehringer Mannheim). The lysates were centrifuged at 13,000 $\times$ g for 20 min at 4 °C, and the supernatants were stored at $-70\,^{\circ}C$. Extracts equivalent to 50 mg of total protein were separated on sodium dodecyl sulfate-polyacrylamide gels (10% acrylamide). The proteins were then transferred to supported nitrocellulose membranes (Amersham, Buckinghamshire, UK) by applying 100 V for 60 min with a plate electrode apparatus (Semi Dry Blotter II; Ken En Tec, Copenhagen, Denmark). The filters were blocked for 1 h in Tris-buffered saline Tween-20 (TBST) consisting of 0.2 mol/L NaCl, 0.2% Tween-20 and 10 mmol/L Tris (pH 7.4), containing 5% nonfat dry milk and 0.02% NaN_3. Subsequently, the filters were incubated with antibodies against Aurora A (Abcam), Aurora B (Abcam), and β-actin (Biomakor, Rehovot, Israel) (each diluted 1:500) in TBST containing 5% milk and then incubated in antimouse or rabbit IgG (1:1000, Amersham) in TBST containing 2% milk. The filters were washed several times with TBST after each step. The bound antibodies were detected with an enhanced chemiluminescence system (Amersham).

7. Statistical analysis

The relationships between Aurora A and B overexpression and each of the clinico-pathological parameters were analyzed by