Summary

Purpose: Aurora kinase family plays an important role in mitosis and cell cycle organization. Aurora-A is an important member of the aurora kinase family and its expression increases the genomic instability and contributes to carcinogenesis. In this study, the prognostic role of Aurora-A expression in colorectal cancer (CRC) was assessed.

Methods: Metastatic CRC patients, whose diagnoses were histopathologically confirmed and who were followed up at the Antalya Education and Research Hospital between 2008 and 2010, were included in the study. Aurora-A expression was assessed with immunohistochemistry.

Results: A total of 40 patients were included in the study. Aurora-A expression was determined as positive in 33 (82.5%) patients and as negative in 7 (17.5%). No significant correlation was determined between Aurora-A expression and tumor location, metastatic location and histological subtype (p=0.549, 0.511, and 0.709, respectively). Also, no significant correlation was determined between Aurora-A expression and overall survival (p=0.202). Median survival was 8.7 months (95% confidence interval/CI 6.9-10.4) in patients with negative Aurora-A expression, whereas it was 22.6 months (95% CI 12-33.3) in patients with positive Aurora-A expression (p=0.202).

Conclusion: Despite the lack of statistical significance, we speculate that Aurora-A overexpression may have a positive effect on the survival of patients. With this regard, there is a need for further comprehensive studies examining the relation and effect of Aurora-A expression on survival and response to treatment.

Key words: Aurora-A, colorectal cancer, metastasis, prognosis

Introduction

CRC is the most common malignancy of the gastrointestinal system and an important cause of morbidity and mortality. It is the third most common malignancy in males and the second in females [1]. In our country it is the fourth most common cancer in males, following lung, prostate and bladder cancers and the third most common cancer in females, following breast and thyroid cancers [2].

In the 7th edition of the American Joint Committee on Cancer (AJCC), in addition to preoperative high levels of CEA (carcinoembryonic antigen), satellite tumor deposits that do not represent residual nodal disease and discontinuous extramural tumor deposits, tumor regression after neoadjuvant chemotherapy, circumferential surgical margin, microsatellite instability (MSI), perineural invasion (PNI), lymphovascular invasion (LVI) and KRAS mutation status are suggested as prognostic factors [3].

The process from normal colonic epithelium to invasive carcinoma takes 7-12 years [4]. During this process, involved are many genetic and epigenetic factors. Activation of oncogenes plays an important role. Aurora kinase family has important effects in mitosis and the organization of the cell cycle. Aurora-A is an important member of the Aurora kinase family. It has been demonstrated that Aurora-A expression increases the genomic instability and contributes to carcino-
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genesis [5]. These changes in DNA content can develop as a result of duplication or separation of centrosomes, chromosome separation and defects in division of the cytoplasm, thus, Aurora kinases are considered as oncogenes. It has been demonstrated that Aurora-A is overexpressed in breast, bladder, colon, ovary, pancreas, head and neck, and lung cancers [6-12].

The purpose of this study was to assess the expression status of Aurora-A and its possible relationship with prognosis in a cohort of patients with metastatic CRC.

Methods

Patient selection

Forty histopathologically diagnosed metastatic CRC patients, who were treated and followed up at our center at Antalya Education and Research Hospital Medical Oncology Clinic between 2008 and 2010, were included in the study. Patients, whose imaging and clinical staging studies were completed, were evaluated again according to the AJCC 7 staging system. Age, gender, grade of differentiation, stage of disease and treatment information were retrieved from the patient records.

Immunohistochemistry

Tumor specimens obtained after surgery or endoscopy, were fixed in 10% formaldehyde. After fixation, tumor specimens were embedded in paraffin and paraffin blocks were cut in slices of 4 µm thickness and initially they were stained with hematoxylin and eosin.

Tissue sections were de-paraffinized in incubator at 60 °C for 1 h. Then, they were kept in xylene for 10 min and in 100% alcohol for 5 min, and finally washed in distilled water. Slides were kept in a solution buffered with 10% citrate in a microwave oven at maximum power (800 watts) for 15 min. Afterwards, the power was decreased by half for the slides to be kept in the microwave oven for an additional 20 min. Slides brought out of the microwave oven were kept at room temperature for 20 min. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide for 20 min. Slides washed with distilled water were treated with phosphate buffered saline (PBS) for 5 min x 3 times with protein blocking agent (Novocastra Protein Block, Newcastle, UK). After being kept in primary antibody (mouse monoclonal antibody AURKA (ab13824), 1/100 dilution; Abcam Inc, Cambridge, MA) for 30 min, they were taken into PBS to be washed for 5 min. Afterwards, slides were treated with biotinylated secondary antibody (Vector Laboratories, Burlingham, CA) for 20 min and washed with PBS for 5 min and were kept with peroxidase conjugated antibody for 20 min. Following this, the slides were washed in PBS for 5 min and kept in chromogenic 3,3'-diaminobenzidine (DAB) for 5 min. Slides were then washed with tap water, counterstained with hematoxylin-eosin and were dehydrated, dried and covered with Entellan. After staining, the specimens were examined under Nikon Eclipse 80i microscope.

Immunohistochemical scoring

If the specimens showed high level of expression, they were examined under low magnification. Poor expression or negative results were examined under high magnification. Expression rates for the positive tumor cells were evaluated by two pathologists who were unaware of the patients’ clinical features. With nuclear Aurora-A expression in <10% of the tumor cells this was accepted as negative staining and positivity was defined with nuclear expression in >10% of the tumor cells. Staining in inflammatory cells was used as internal control (Figures 1,2).

Figure 1. Aurora-A negativity in a poorly differentiated adenocarcinoma case (immunoperoxidase, Aurora-A x100)

Figure 2. Generalized nuclear Aurora-A positivity (immunoperoxidase, Aurora-A x100) accompanied with cytoplasmic staining in a well differentiated adenocarcinoma case.
Statistics

Statistical analyses were performed by using SPSS software for Windows 15.0 (SPSS Inc, Chicago, Ill). Compliance of variants to normal distribution was assessed using visual (histograms and probability graphs) and analytical methods (Kolmogorov-Smirnov/ Shapiro-Wilk tests). In Kolmogorov-Smirnov test, p-values>0.05 were considered as normal distribution. Differences between groups were estimated using x² test and Man Whitney U test. The relationship of immunohistochemical results with survival was assessed with Kaplan-Meier method and statistical differences were estimated with Log-rank test. Factors identified to show statistical significance for predicting survival in univariate analysis were further analysed by multivariate Cox regression analysis. A p-value<0.05 was considered as statistically significant.

Results

A total of 40 patients, 15 (37.5%) of whom were female and 25 (62.5%) male, was included in the study. The mean patient age was 58.7±13.5 (range 31-82) (Table 1). Thirty four (85%) patients had co-morbidities, the most common of which was Type 2 diabetes mellitus in 21 (52.5%) patients. Other co-morbid diseases were hypertension in 7 (17.5%) patients, cerebrovascular disease in 2 (5%) and atherosclerotic heart disease in 1 (2.5%); more than one co-morbid disease was found in 3 (7.5%) patients. Twelve patients (29.3%) had history of smoking.

Tumor localization was in the colon in 28 patients (70%) and in the rectum in 12 (30%). Of the colon tumors 15 (37.5%) were in the right colon and 13 (32.5%) in the left colon. Histology showed that 38 (95%) patients had adenocarcinoma. Signet-ring cell adenocarcinoma was found in 2 (5%) patients. Concerning tumor grade of 21 patients the results revealed grade 1 in 1 patient (2.5%) grade 2 in 10 (25%) and grade 3 in 10 (25%) patients. Most common metastatic locations were the lung in 6 (15%) patients, peritoneum in 5 (12.5%), and bone in 3 (7.5%) patients; multiple metastases were found in 2 (5%) patients. Aurora-A expression was positive in 33 (82.5%) patients, and negative in 7 (17.5%). There wasn’t any relation determined between Aurora-A expression and gender, age and smoking (p=0.742, p=0.967, and p=0.420). There wasn’t any significant relation between Aurora-A expression and tumor localization, metastatic location, and histological subtype (p=0.549, 0.511, and 0.709, respectively) (Table 2). A significant relation was found between Aurora-A expression and tumor histological grade (p=0.025; Table 2).

Mean follow up time was 19.2±12.8 months (range 0.95-51.2). Median patient overall survival was 22.6 months (95% CI 12-33.3) (Figure 3). Two-year overall survival was 48.8%.

Univariate analysis showed no relation between the presence of co-morbid diseases, age, gender, smoking history, primary tumor localization, metastasis location and survival (p=0.827, 0.364, 0.298, 0.682, respectively).

There wasn’t any significant correlation determined between Aurora-A expression and overall survival (p=0.202) (Figure 4). Median survival was determined as 8.7 months (95% CI 6.9-10.4) in those with negative Aurora-A expression and as 22.6 months (95% CI 12-33.3) in those with positive Aurora-A expression (p=0.202).

Table 1. Results of basic blood and serum biochemistry examinations

<table>
<thead>
<tr>
<th>Normal range</th>
<th>Mean±standard deviation</th>
<th>Median</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>58.7±13.5</td>
<td>60</td>
</tr>
<tr>
<td>BUN (mg/dL)</td>
<td>8-23</td>
<td>14</td>
</tr>
<tr>
<td>CRE (mg/dL)</td>
<td>0.5-1.3</td>
<td>1.05±1.42</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>0-33</td>
<td>31.3±35.4</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>0-32</td>
<td>27.9±23.5</td>
</tr>
<tr>
<td>LDH (U/L)</td>
<td>120-300</td>
<td>408.1±440.8</td>
</tr>
<tr>
<td>Total bilirubin (mg/dL)</td>
<td>0-1.2</td>
<td>0.87±1.15</td>
</tr>
<tr>
<td>Direct bilirubin (mg/dL)</td>
<td>0-0.2</td>
<td>0.31±0.34</td>
</tr>
<tr>
<td>WBC (10³/mm³)</td>
<td>5.98-10.04</td>
<td>8.29±3.28</td>
</tr>
<tr>
<td>PLT (10³/mm³)</td>
<td>182-369</td>
<td>344.3±122.4</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>11.2-15.7</td>
<td>11.6±1.22</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>3.5-5.2</td>
<td>3.68±0.61</td>
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Although the mortality of CRC has decreased in the last 20 years due to diagnostic improvements and therapeutic progression it still ranks second among cancer-related deaths [13]. Identification of genes correlated with carcinogenesis and research of ways to silence them can contribute to improved patient survival.

The roles of Aurora kinases in mitosis and cell cycle are known. It has been demonstrated that their overexpression increases genomic instability (aneuploidy) and contribute to carcinogenesis. These changes in DNA content can
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Develop as a result of duplication or separation of centrosomes, chromosomes and defects in division of the cytoplasm. With the demonstration of their increased expression in different cancers and cancer cell lines, Aurora kinases are defined as oncogenes [14]. Lassman et al. demonstrated that Aurora-A expression gradually increases in the normal mucosa-adenoma-carcinoma development process [15].

In our study we found Aurora-A overexpression in 82.5% of metastatic CRC patients using immunohistochemistry. Baba et al. found Aurora-A overexpression in 18% (98/517) in non-metastatic patients and in 21% (15/71) in metastatic patients cases (p>0.05) [16]. Different from our study, in the study of Baba et al. Aurora was considered positive in case of mild or moderate and strong staining in 50% or more of the tumor cells. Thus, Aurora-A expression rate might be found lower than the expression rate found in our study.

Dotan et al. assessed the Aurora-A gene expression in CRC patients with polymerase chain reaction (PCR) and determined high expression in 69% (41/59) of the cases. In the same study, Aurora-A overexpression was determined as positive with immunohistochemistry in 33% (9/20) of the patients with high gene expression as determined with PCR [17]. In that study, similar to ours, > 10% staining was accepted as positive.

This difference in Aurora-A overexpression rates in these studies may be due to the different number of patients, stage of disease, immunohistochemical method and technical differences.

We didn’t notice any relation between the Aurora-A positive expression and gender, age, tumor localization, smoking, metastatic location, and tumor histological characteristics. Similar to our study, Baba et al. observed no relation between Aurora-A expression and age, tumor localization and stage in all CRC cases; they only found a relation with chromosomal instability [16].

The results of studies examining the relation between Aurora-A expression and survival are conflicting. Pohl et al. didn’t detect any significant relation between Aurora-A expression and survival in their study in which they evaluated Aurora-A and -B gene expression and single nucleotide polymorphism in 41 metastatic CRC patients, similar to our study [18]. Dotan et al., demonstrated that the survival of the group with high Aurora-A gene expression was better compared with the survival of the group with poor expression [17]. Although no statistical significance was determined in our study, median overall survival was 22.6 months in Aurora-A positive patients and 8.7 months in Aurora-A negative patients. This could be possibly attributed to the low number of patients in our study.

Aurora-A is overexpressed in CRC and many other cancers acting as oncogene. Treatments targeting Aurora kinases before or after cytotoxic treatments have been intensely researched preclinically and clinically. It is considered that Aurora-A overexpression causes drug resistance in cancer cells, and treatments targeting Aurora kinases can prevent this resistance and activate the apoptotic pathways in cancer cells [19,20].

Our study was not without some limitations. Among them we include its retrospective nature, the low number of patients, the fact that many patients were diagnosed with biopsy of the primary lesion, some patients were in early-stage disease at the time of diagnosis and in metastatic stage during follow up. On the other hand, despite the lack of statistical significance, we believe that Aurora-A overexpression may have an impact on the survival of the patients. In this regard, prospective studies with larger numbers of patients, examining the relation and effect of Aurora-A expression on survival and the response to treatments are needed.

References


