Clinical Research Paper

Expression and clinical significance of DNA-PKcs in nasopharyngeal carcinoma

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Key words: nasopharyngeal carcinoma, DNA-PKcs, DNA double-strand break (DSB), immunohistochemistry

Background and Objective: DNA double-strand break (DSB) is the main mechanism of tumor cell death after irradiation. Homologous recombination (HR) and DNA nonhomologous end-joining (NHEJ) are two important ways to repair DSB. The catalytic subunit of DNA-dependent protein kinase (DNA-PKcs), an essential protein of NHEJ, plays a major role during DSB. This study was to investigate the expression of DNA-PKcs in nasopharyngeal carcinoma (NPC), and analyze its correlation to clinicopathologic features and prognosis of NPC. Methods: The expression of DNA-PKcs protein in 223 specimens of NPC tissues was detected by immunohistochemistry. The correlation of DNA-PKcs expression to clinicopathologic features and prognosis of NPC were analyzed. Results: The overexpression rate of DNA-PKcs in 223 NPC specimens was 36.8%. The expression of DNA-PKcs had no significant correlations to gender, age, pathological type and N staging of NPC (p > 0.05), but had remarkable correlations to TNM, T and M staging (p < 0.05). The five-year overall survival rate was significantly lower in patients with overexpression of DNA-PKcs than in those with low expression of DNA-PKcs (54.6% vs. 79.4%, p < 0.05). T, N, M staging and the expression of DNA-PKcs were independent predictors for the overall survival of NPC (p < 0.05). Conclusions: DNA-PKcs is positively expressed in the majority of NPC tissues. The expression level of DNA-PKcs is an important factor affecting the prognosis of NPC, which could be used as a prognostic predictor for NPC.

Radiotherapy is the main treatment for nasopharyngeal carcinoma (NPC). The therapeutic effect of NPC differs among patients, which might be owing to their different radiation damage repair capacity. The ionizing radiation kills tumor cells mainly through the mechanism of DNA double-strand break (DSB). There are two repairing ways: DNA nonhomologous end-joining (NHEJ) and homologous recombination (HR). Activation of DNA-dependent protein kinase (DNA-PK), including Ku70, Ku80 proteins and DNA-PK catalytic subunit (DNA-PKcs) is critical for NHEJ; while taxia telangiectasia mutated (ATM) plays an important role in HR. DNA-PKcs, the main repair protein for DNA double-strand break (DSB), has become a key research point in recent years. Scientists have been investigating its role in tumor pathogenesis, development, diagnosis and therapy. Shintani et al. discovered that the expression level of DNA-PKcs was significantly elevated after radiation in oral squamous carcinoma. Our previous studies also revealed that the expression and activity of DNA-PKcs in NPC cells were significantly increased after radiation; while the employment of DNA-PKcs antisense reversed the resistance of NPC cells to radiation and increased the radiation sensitivity. These results indicate a close correlation between DNA-PKcs and radiation sensitivity of tumors.

The correlation between DNA-PKcs and radiation sensitivity of tumors has been proved by studies in vitro. However, the expression of DNA-PKcs, the correlation of DNA-PKcs to prognosis, and the possibility of DNA-PKcs guided clinical diagnosis and therapy in human tumors remain unclear. The aim of this study was to examine the expression of DNA-PKcs in 223 NPC tissues, thus to investigate its correlation to clinicopathologic features and prognosis of NPC.

Data and Methods

Sample resources and patient data. Two hundred and twenty-three well-preserved NPC paraffin-embedded specimens were all collected from pathologically confirmed NPC patients diagnosed and treated in Sun Yat-sen University Cancer Center from July 1994 to December 1999. The patients were 13–68 years old, with a median age of 48 years. Detailed clinical data of the patients are shown in Table 1. All patients underwent radical radiation therapy (RT). The primary tumor was irradiated to a dose of 66 to 70 Gy. Additional (boost) RT of 8 to 12 Gy was delivered for residual tumor and destructed skull base after a standard dose of 66 to 70 Gy. The neck received 50 to 70 Gy depending on the lymph node involvement, 50 Gy for node-negative necks and 60 to 70 Gy for node-positive necks. A daily fraction of 2.0 Gy, and five fractions per week were delivered using cobalt-60 or a linear accelerator. The standard RT technique consisted of opposing lateral faciocervical fields to cover...
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Reagents. DNA-PKcs rat anti-human monoclonal antibody was purchased from Santa Cruz Company, USA. The SP-9000 immunohistochemistry kit was bought from Beijing Zhongshan Company.

Methods. Continuous sections of 4 µm thick were obtained from each paraffin embedded specimen. The expression of DNA-PKcs in NPC tissues was detected using immunohistochemistry (IHC). Sections were first deparaffinaged twice by dimethyl benzene; then dehydrated by alcohol of different concentrations. H2O2 (3%) was added to eliminate endogenous peroxydase for 15 min, followed by conventional repair using EDTA (pH8.0). Subsequently, sections were microwave heated in an oven for 10 min, cooled for 2 min, and heated at a mid-high temperature for 10 min to fully expose the antigen site. Sections were blocked with normal goat serum (Solution A in the SP-9000 kit) for 15 min at room temperature, followed by three times of rinse with 1 × PBST (1‰ Tween). Then sections were incubated with peroxidaze-conjugated streptavidin (Solution C in the SP-9000 kit) for 15 min at room temperature, rinsed with 1 × PBST (1‰ Tween) for three times. DAB substrate kit was applied for colorization under room temperature away from light. For a negative control, the primary antibody was replaced by PBS.

Determination of results. DNA-PKcs staining was mainly dark yellow or brown granules located in cellular nucleus, while a few brown yellow granules were located in the endochylema. Determination of IHC results were based on scoring criteria proposed by Xu et al. The classification of staining intensity was as follows: 0 point, no coloration; 1 point, light yellow; 2 points, dark yellow and 3 points, brown. The percentage of positive cells was scored as: 1 point, ≤ 10%; 2 points, 10 – 50%; 3 points, 50 – 75%; 4 points, > 75%. Samples were categorized into overexpression (> 4) or low expression (≤ 4) group based on the multiplication of the score of staining intensity and the score of the percentage of positive cells. Negative expression is included into low expression group. All results were independently determined by at least two pathologists.

Follow-ups. Patients were followed up via telephone interviews or re-examinations at out-patient clinic (OPC) after discharge until July 27, 2007. The longest period of follow up was 143 months, with an average period of 70 months.

Statistical analysis. All data were analyzed using SPSS 13.0 software. The Pearson chi-square test was adopted to analyze the correlation of DNA-PKcs expression to clinicopathologic features of NPC. The Kaplan-Meier survival curve was employed for univariate analysis and the log-rank test was used to analyze the difference in survival curves. The Cox model was used for multivariate analysis. A p value of less than 0.05 was regarded as significantly different.

Results

Expression of DNA-PKcs in NPC tissues. The expression of DNA-PKcs in NPC tissues is shown in Figure 1. Positive staining of DNA-PKcs was mainly located in the nuclei of cancer cells. Among

Table 1 Clinicopathologic features of 223 patients with nasopharyngeal carcinoma

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Cases</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>168</td>
<td>75</td>
</tr>
<tr>
<td>Female</td>
<td>55</td>
<td>25</td>
</tr>
<tr>
<td>Pathologic type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PDSC</td>
<td>213</td>
<td>95</td>
</tr>
<tr>
<td>VNCC</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>TNM stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>27</td>
<td>12</td>
</tr>
<tr>
<td>II</td>
<td>75</td>
<td>34</td>
</tr>
<tr>
<td>III</td>
<td>63</td>
<td>28</td>
</tr>
<tr>
<td>IV</td>
<td>58</td>
<td>26</td>
</tr>
<tr>
<td>T stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>102</td>
<td>46</td>
</tr>
<tr>
<td>T2</td>
<td>43</td>
<td>19</td>
</tr>
<tr>
<td>T3</td>
<td>42</td>
<td>19</td>
</tr>
<tr>
<td>T4</td>
<td>36</td>
<td>16</td>
</tr>
<tr>
<td>N stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N0</td>
<td>136</td>
<td>61</td>
</tr>
<tr>
<td>N1</td>
<td>37</td>
<td>17</td>
</tr>
<tr>
<td>N2</td>
<td>31</td>
<td>14</td>
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<tr>
<td>N3</td>
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<td>8</td>
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<tr>
<td>M stage</td>
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<td></td>
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<td>M0</td>
<td>208</td>
<td>93</td>
</tr>
<tr>
<td>M1</td>
<td>15</td>
<td>7</td>
</tr>
</tbody>
</table>

PDSC: poorly differentiated squamous carcinoma; VNCC: vesicular nucleus cell carcinoma.

the nasopharynx and upper cervical lymphatic drainage region, with one lower anterior cervical field to cover the lower cervical region. After 36 to 40 Gy, opposing lateral preauricular fields were used for the primary region, and anterior split neck fields were used for the cervical region. When the nasal cavity or post-styloid area had been invaded, an anterior facial field or postauricular field was added as a supplementary field.

Figure 1. The positive expression of DNA-PKcs in human nasopharyngeal carcinoma cells (SP×400)
Expression and clinical significance of DNA-PKcs in nasopharyngeal carcinoma

223 specimens of NPC tissues, overexpression of DNA-PKcs was detected in 82 cases (36.8%).

Correlations between overexpression of DNA-PKcs and clinicopathologic features of NPC. The correlation between overexpression of DNA-PKcs and clinicopathologic features of NPC is shown in Table 2. The expression of DNA-PKcs had no significant correlations to gender, age, pathological type and N staging (p > 0.05), while had remarkable correlations to TNM, T and M staging (p < 0.05).

Correlations of DNA-PKcs expression to the prognosis of NPC.

Results of univariate analysis. Kaplan-Meier overall survival curves of NPC patients with different expression levels of DNA-PKcs are shown in Figure 2. Differences in various indexes were examined by the log-rank test. The overall survival rate was significantly lower in the DNA-PKcs overexpression group than in the low expression group (X^2 = 19.191, p < 0.05).

Results of multivariate analysis. The Cox regression model was adopted to analyze the correlations between clinicopathologic features, including gender, age, pathological type, T, N, M staging and the expression level of DNA-PKcs to the prognosis of NPC. T, N, M staging and the expression level of DNA-PKcs were independent predictors for the overall survival rate of NPC (p < 0.05, Table 3).

Discussion

DNA is the main targeting molecule of radiation-induced cell death. Ionizing radiation can induce DNA single strand break (SSB), double strand break (DSB), and DNA-protein cross link. DSB is the most important DNA damage pattern for radiation-induced cell death, and the DSB repairing capability is the main factor affecting cell survival and death. There are two major pathways of DSB repair, HR and NHEJ. Ku80 and DNA-PKcs are the two major components of DNA-PK. When DSB is induced by rays or cytotoxic drugs, the subunits of Ku70 and Ku80 immediately form a heterodimer, which combines with the damaged DNA via end-joining, and activates DNA-PKcs via the NHEJ pathway to participate in repair of a DNA double strand break gap and maintain genome integrity.6 Besides directly participating in the repair of DNA double strand break via the NHEJ pathway, as a serine/threonine kinase, DNA-PK can also promote the expression and phosphorylation of p53 protein,7,8 and activate another anti-oncogene, WAF1/CIP1, to produce p21 protein. p21 can inhibit proliferating cell nuclear antigen (PCNA) and various cyclins, thus to block damaged cells at G1 phase for DNA repair or to activate apoptosis if the damage is too severe to be repaired.9,10

Many studies have revealed DNA-PKcs expression in tumor tissues and its correlation to the prognosis. Shintani et al.2 adopted IHC to analyze DNA-PKcs expression in an oral squamous carcinoma cell line and oral squamous carcinoma tissues after pre-operative radiotherapy. They found an increase of DNA-PKcs expression after radiotherapy, and a positive correlation between DNA-PKcs and tumor resistance towards ionizing radiation, which suggest the potential of using DNA-PKcs as a new target for radiosensitization therapy. Hosoi et al.1 studied 12 cases of colon carcinoma and found significantly higher levels of DNA-PKcs protein and mRNA in tumor tissues than in adjacent normal tissues. Considering the high consistency between DNA-PKcs and the recognition factor of the promoter region of transcription factor

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**Table 2**

<table>
<thead>
<tr>
<th>Item</th>
<th>Cases</th>
<th>Overexpression</th>
<th>Low expression</th>
<th>p value</th>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>168</td>
<td>104(62)</td>
<td>64(38)</td>
<td>0.474</td>
</tr>
<tr>
<td>Female</td>
<td>55</td>
<td>37(67)</td>
<td>18(33)</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 50</td>
<td>129</td>
<td>80(62)</td>
<td>49(38)</td>
<td>0.660</td>
</tr>
<tr>
<td>≥ 50</td>
<td>94</td>
<td>61(65)</td>
<td>33(35)</td>
<td></td>
</tr>
<tr>
<td>Pathological type</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PDSC</td>
<td>213</td>
<td>134(63)</td>
<td>79(37)</td>
<td>0.905</td>
</tr>
<tr>
<td>VNCC</td>
<td>10</td>
<td>7(70)</td>
<td>3(30)</td>
<td></td>
</tr>
<tr>
<td>TNM stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I/II</td>
<td>102</td>
<td>73(72)</td>
<td>29(28)</td>
<td>0.018</td>
</tr>
<tr>
<td>II/IV</td>
<td>121</td>
<td>68(56)</td>
<td>53(44)</td>
<td></td>
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<tr>
<td>T stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1/T2</td>
<td>145</td>
<td>100(69)</td>
<td>45(31)</td>
<td>0.015</td>
</tr>
<tr>
<td>T3/T4</td>
<td>78</td>
<td>41(53)</td>
<td>37(47)</td>
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<tr>
<td>N stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N0/N1</td>
<td>173</td>
<td>112(65)</td>
<td>61(35)</td>
<td>0.384</td>
</tr>
<tr>
<td>N2/N3</td>
<td>50</td>
<td>29(58)</td>
<td>21(42)</td>
<td></td>
</tr>
<tr>
<td>M stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M0</td>
<td>208</td>
<td>137(66)</td>
<td>71(34)</td>
<td>0.002</td>
</tr>
<tr>
<td>M1</td>
<td>15</td>
<td>4(27)</td>
<td>11(73)</td>
<td></td>
</tr>
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**Table 3**

<table>
<thead>
<tr>
<th>Variable</th>
<th>B</th>
<th>SE</th>
<th>Wald</th>
<th>p</th>
<th>Exp(B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA-PKcs</td>
<td>0.723</td>
<td>0.244</td>
<td>8.808</td>
<td>0.003</td>
<td>2.060</td>
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<tr>
<td>T stage</td>
<td>0.461</td>
<td>0.133</td>
<td>11.963</td>
<td>0.001</td>
<td>1.586</td>
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<tr>
<td>N stage</td>
<td>0.499</td>
<td>0.157</td>
<td>10.134</td>
<td>0.001</td>
<td>1.647</td>
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<tr>
<td>M stage</td>
<td>1.150</td>
<td>0.429</td>
<td>7.185</td>
<td>0.007</td>
<td>3.158</td>
</tr>
</tbody>
</table>

PDSC: poorly differentiated squamous carcinoma; VNCC: vesicular nucleus cell carcinoma.

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![Figure 2. Kaplan-Meier overall survival curves of the nasopharyngeal carcinoma patients with overexpression or low expression of DNA-PKcs](image-url)
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SP1, it is suggested that the increase of DNA-PKcs is correlated to the expression of SP1 protein.

Studies on DNA-PKcs expression in NPC tissues and its correlation to the prognosis of NPC are relatively limited. In 2005, Lee et al.12 conducted a study on 66 cases of NPC patients using IHC. The results exhibited that the higher the expression level of DNA-PKcs, the poorer was the therapeutic effect after radiation therapy. However the expression of DNA-PKcs imposed no significant influence on the local control rate, disease-free survival, and metastases-free survival. Results of the present study showed that the overall survival was significantly lower in DNA-PKcs overexpression group than in low expression group; and DNA-PKcs was an independent predictor for the overall survival of NPC. Compared with the results obtained by Lee et al.,12 we further confirmed the influence of DNA-PKcs on the prognosis of NPC using a greater number of samples, resulting in higher objectivity. Moreover, we observed the correlation between DNA-PKcs and T, M staging. The higher the T and M stage, the higher was the expression of DNA-PKcs. With the increase of the T stage, the possibility of recurrence increased and the prognosis was poorer,13 which suggest that DNA-PKcs might be associated with the recurrence of NPC. Newton et al.14 and Weinert et al.15 decoded the amino acid sequence of DNA-PK and ATM protein kinase domain, revealing their correlation to PI3K. In recent years, studies on PI3K-AKT signaling pathway have exhibited that PI3K/AKT is closely correlated to tumor genesis and development, and plays an important role in tumor metastasis. Therefore it is deduced that DNA-PKcs might play a crucial role in NPC metastasis. Overexpression of DNA-PKcs could lead to a poor prognosis.

In summary, DNA-PKcs is an important predictor for the prognosis of NPC patients. It is likely to play a key role in the recurrence and metastasis of NPC, and thus provide some prognostic guidance for patients.

Acknowledgements

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References