EVALUATION OF THE INFLUENCE OF PROTEIN P53 IN PENILE CARCINOMA

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ABSTRACT

Introduction: The expression of protein p53 and its influence in the biological behavior of penile epidermoid carcinoma (PEC) were studied according to its effect on the following parameters: histologic staging, clinical and pathologic staging, and prognostic factors, such as survival curve and risk of death due to the tumor.

Material and Methods: We studied 55 patients with PEC who underwent surgical treatment from 1979 to 1995. Protein p53 was studied by immunohistochemical analysis (clone DO-7-DAKO A/S, Denmark) in the most representative surgical specimens of the primary tumor and their metastasis. The intensity of p53 expression was determined by the number of tumor cell nuclei stained in the tumor and was classified into four groups: group 1 - up to 25%; group 2 - 26 to 50%; group 3 - 51 to 75% and group 4 - more than 75% stained nuclei. The relationship between the p53 expression in the tumor cells and the studied parameters was analyzed.

Results: Rising rates of p53 expression correlate with low degrees of cell differentiation (p = 0.053). Twelve patients died due to the tumor during this study. Our data have shown that higher p53 expression in the tumor correlates with worse prognosis (p = 0.025). There was no significant correlation between the presence of p53 and the clinical or pathological stage of the tumor.

Conclusion: Our data show that there is significant p53 expression in the PEC. The higher p53 expression, the worse the patient prognosis and the greater is the tumor biological aggressiveness. The tumor becomes more aggressive in accordance with the increase in protein intensity in the tumor cell nuclei.

Key words: penis; carcinoma; tumor; protein p53; prognosis

INTRODUCTION

Nowadays, it is believed that some genes, with or without mutations, can be related to the natural history of neoplasias. The suppressive gene of tumors p53 is the most expressive example, because it presents mutations in more than 50% of the studied cases of neoplasias (1). Nigro et al. (1989) have studied 20 mutations in the gene p53 in many types of tumors: colon, brain, lung and breast. They have discovered that these mutations occur, more frequently, in four specific regions of the gene. In 1979, protein p53, product of this gene, was identified as a nuclear 53-kilodalton (kd) phosphoprotein, linked to the large antigen T of virus SV 40, being initially considered as a protein produced by an oncogene (3).

P53 is involved in the cellular cycle control in its natural state. Whenever there is cellular aggression with DNA modification, this protein causes a halt in the cellular cycle. During this period, the cell has time to repair the damage or, if it is too severe, a process of programmed cellular death is started (4).
Protein p53 detection can be made through immunohistochemistry, using antibodies which allow its identification in the cell nuclei, also detecting both the natural protein and its mutations (5).

The action of protein p53 can be affected by mutations or interactions with other cellular or viral proteins (6). The large antigen T of virus SV 40 and protein E1B from Adenovirus 5 can link themselves to the protein p53, resulting in a complex in which the p53 is more stable, but non-functional (7). The protein codified by the ORF E6 of human papillomavirus (HPV) can link itself to the p53, making it inactive and accelerating its destruction by the ubiquitin system (8). P53 expression is altered in many tumors and this process can be related to the prognosis of some, such as hepatocellular, colorectal and esophageal carcinoma (9-11).

Penile carcinoma is a disease which tends to present regional growth and mainly lymphatic dissemination, first to inguinal lymphnodus, and then to other organs. The stage of the disease is related to the prognosis and to patients survival (12).

The objective of this study is to define the importance of p53 expression in the biological behavior of penile epidermoid carcinoma as well as its influence in variables such as clinical and pathologic staging, histological grading, prognostic factors and survival curve.

MATERIAL AND METHODS

Fifty-five patients diagnosed with primary penile epidermoid carcinoma, surgically treated from January 1979 to December 1995, were retrospectively studied. In all of the cases there were pathologic specimens and medical records available for evaluation.

Patients’ ages varied from 23 to 80 years (mean: 57.2) and the size of the penile lesions varied from 1 to 8 cm in the longest axis (mean: 3.4 cm).

From the 55 patients, 48 had postoperative follow-up which varied from 5 to 120 months, with mean of 31.6 months.

With an established histopathologic diagnosis of penile epidermoid carcinoma, all patients were submitted to clinical staging of the tumor which included, besides physical examination, an abdominal ultrasonography, a chest plain film, and, in some cases, an abdomen computed tomography.

We have used the criteria proposed by Jackson to analyze the clinical stage as follows (13): Stage I – 17 cases (30.9%), Stage II – 22 cases (40.0%), Stage III – 14 cases (25.5%), Stage IV – 2 cases (3.6%). This classification was used because the TNM classification was not universally used in the beginning of this study and, according to some reports, there is a strong correlation between both classification systems in penile epidermoid carcinoma (12).

Penile amputation was used to treat all primary lesions being partial in 44 cases, total in 8, and in three cases emasculations with partial resection of the scrotum were performed. In the cases of total amputation or emasculation a perineal uretrostomy was made. Thirty-nine patients underwent bilateral inguinal lymphadenectomy at least one month after treating the primary carcinoma, and 2 underwent iliac lymphadenectomy.

The histopathologic specimens were revised by a single pathologist, using Broders classification (14).

The other factors, such as histologic staging, clinical and pathologic staging, and death due to the tumor, were analyzed taking into account the presence of protein p53.

The streptavidin-biotin method was used to detect protein p53. This method uses three reagents, based on the streptavidin ability to connect itself to the biotin. The first reagent, or primary antibody, is specific to the antigen to be localized; in other words, protein p53. The second agent, or secondary antibody, is connected to the biotin and is able to link itself to the primary antibody. The third reagent is a complex peroxidase combined with biotin and streptavidin, in which free sites are linked to the secondary antibody. The visualization of the reaction is made by using cromogenic substract and hematoxilin staining.

Microscopic slides were analyzed by counting the positive nuclei using the light microscope with a 400X magnification, considering 500 cells of 10 sites in the most representative areas of the tumor. Incision of large intestine adenocarcinoma significantly positive to the monoclonal antibody p53 was used as positive control. Protein p53 expression was
classified into 4 groups: group 1)- up to 25% of stained nuclei; group 2)- 26 to 50% of stained nuclei; group 3)- 51 to 75% of stained nuclei; group 4)- more than 75% of stained nuclei to protein p53.

The same incision of large intestine adenocarcinoma was used as negative control; however, the specific anti-serum of protein p53 was replaced by a non-related anti-serum.

The statistical analysis emphasized the detection of factors associated with the clinical presentation of these tumors as well as prognostic factors. The \( \chi^2 \) test (chi-square) or Fisher’s exact test were used. Kappa’s reproducibility rate was used to compare the behavior of the 2 methods.

Survival expectancy was analyzed through Kaplan-Meier’s survival curves in relation to the presence of protein p53. Logistic regression was used in order to obtain the predictive factors of death due to the tumor. The results of risk of death analysis were obtained according to the Cox’s regression technique.

5% significance level was used in all tests.

RESULTS

Three recidivations of partial penectomies (5.4%) and four of lymphadenectomies were observed during the study period. From the 41 cases of lymphadenectomies, 19 presented metastasis to lymph nodes (46.3%), in which 11 were unilateral and 7 bilateral to inguinal lymph nodes and 1 to iliac lymph nodes. The patient with iliac lymphnodus metastasis had previously had inguinal metastasis.

Out of the 48 patients who completed follow-up, there were 14 deaths (30.4%), being 12 (27.3%) related to the tumor.

Most of the tumors had low or intermediate histologic grades: 18 cases (32.7%) were grade I, 26 cases (47.3%) were grade II, and 11 cases (20.0%) were grade III.

Protein p53 tested positive in 30 cases (54.5%) and negative in 25 (45.5%).

The tumors tested more highly positive to protein p53 presented higher histologic grade (less differentiated). Four out of five patients with G-III and positive expression to protein p53 had more than 50% of the nuclei stained to protein p53. This

![Figure 1 - Positive reaction to protein p53 with less than 25% of the tumor cell nuclei stained to the protein (immunostaining for protein p53, X400).](image1)

![Figure 2 - Positive reaction to protein p53 with more than 75% of the tumor cell nuclei stained to the protein (immunostaining for p53, X400).](image2)
difference is close to reach statistically significant values, when compared to well and moderately differentiated tumors (Table-1).

Clinical and pathologic staging were not related to protein p53. Table-2 shows these results in relation to the pathologic staging.

When the cases of death due to the tumor were analyzed, a statistically significant difference was observed. In protein p53 positive patients, the higher the expression of p53, the worse was the prognosis (Table-3). The occurrence of death in patients with 50 to 75% of tumor cellular nuclei stained to protein p53 was 9.95 times higher than for the patients who had lower protein expression. When patients with more than 75% of stained nuclei were analyzed, the risk of death was 15.28 times higher, with confidence interval of 95%.

Our data show that the higher if p53 expression in the tumor, the less is the probability of life expectancy (Figure-3).

### Table 1 - Comparison of protein p53 presence in well (grade I) and moderately (grade II) differentiated tumors versus undifferentiated ones (grade III).

<table>
<thead>
<tr>
<th>Grade</th>
<th>p53</th>
<th>1+</th>
<th>%</th>
<th>2+</th>
<th>%</th>
<th>3+</th>
<th>%</th>
<th>4+</th>
<th>%</th>
<th>Total</th>
</tr>
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<tbody>
<tr>
<td>1 + 2</td>
<td></td>
<td>10</td>
<td>22.73</td>
<td>7</td>
<td>15.91</td>
<td>7</td>
<td>15.91</td>
<td>1</td>
<td>22.27</td>
<td>19</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>1</td>
<td>9.09</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>9.09</td>
<td>3</td>
<td>27.27</td>
<td>6</td>
</tr>
<tr>
<td>n</td>
<td></td>
<td>11</td>
<td>7</td>
<td>8</td>
<td>4</td>
<td>25</td>
<td>55</td>
<td>20</td>
<td>25</td>
<td>11</td>
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Fisher p = 0.053

### Table 2 - Relation between pathologic staging and protein p53 expression (No. = 41).

<table>
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<tr>
<th>PS</th>
<th>I</th>
<th>%</th>
<th>II</th>
<th>%</th>
<th>III</th>
<th>%</th>
<th>IV</th>
<th>%</th>
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<tr>
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<td>0</td>
<td>8</td>
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<td>40</td>
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<td>2+</td>
<td>2</td>
<td>28.57</td>
<td>4</td>
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<td>0</td>
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</tr>
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<td>1</td>
<td>16.67</td>
<td>1</td>
<td>16.67</td>
<td>3</td>
<td>50</td>
<td>1</td>
<td>16.67</td>
<td>6</td>
</tr>
<tr>
<td>4+</td>
<td>0</td>
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<td>33.33</td>
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<td>0</td>
<td>2</td>
<td>66.67</td>
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<td></td>
</tr>
<tr>
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<td>6</td>
<td>30</td>
<td>7</td>
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<td>4</td>
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<td>3</td>
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<td>8</td>
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<td>8</td>
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<td>8</td>
<td>11</td>
<td>41</td>
</tr>
</tbody>
</table>

1+ = up to 25% stained nuclei to protein p53; 2+ = 26 to 50% stained nuclei to protein p53; 3+ = 51 to 75% stained nuclei to protein p53; 4+ = more than 75% stained nuclei to protein p53; PS = pathologic staging

### DISCUSSION

Protein p53 is a proteinaceous product of the p53 gene. In its natural state, it is linked to the cellular cycle control. If there is harm to the DNA, protein p53 is activated, causing an interruption in the cellular cycle. During this period, the cell can repair the damage or, if it is too severe, a process of programmed cellular death can be initiated. Therefore, the gene works as the guardian of the genome (4). On the other hand, when there are alterations in the gene due to genetic mutations or interaction with cellular or viral proteins, its action affected, which facilitates the perpetuation of cellular cycle errors. Gene p53 is altered in a significant number of tumors; therefore, this gene has been widely studied in the literature (15).

Protein p53 works as a transcription inhibitor due to its interaction with other genes. It has a very short life span, of approximately 20 minutes, in its...
natural and active forms. However, its concentration is always increased if there is cellular aggression (16).

Immunocytochemistry can detect natural and mutant forms of protein p53; however, due to the short life span of the former, only mutant forms are often detected. In some cases, the wild form is detected, in vitro, in recently divided normal keratinocytes and also in lymphocytes, in response to the mitogenic stimulation (17).

There are few studies focusing on the amount of tumor cell nuclei stained to the protein and its influence on cell differentiation and prognosis. The amount of stained cells to protein p53 may be important, as it has been suggested in the literature that p53 expression in a few cells would be the normal response to alterations in the tumor cells genome (18).

It was observed that the mutation of protein p53 is related to a more reserved prognosis in rare cases of uterine cervix carcinoma not associated with HPV (19).

The expression of protein p53 is correlated with grade, staging of the tumor, progression probability and, also, with a worse survival rate in cases of bladder cancer (20).

In this study, it was observed that 54.5% of the cases (30 patients) tested positive to protein p53 and, from these patients, 20% had less than 25% of the nuclei stained and 7.3% had more than 75% of the nuclei stained.

### Table 3 - Relation between number of deaths due to the tumor and protein p53 presence (No.= 46).

<table>
<thead>
<tr>
<th>p53</th>
<th>1+</th>
<th>%</th>
<th>2+</th>
<th>%</th>
<th>3+</th>
<th>%</th>
<th>4+</th>
<th>%</th>
<th>Neg</th>
<th>%</th>
<th>Total</th>
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<tbody>
<tr>
<td>Death</td>
<td>(n)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>7</td>
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<td>6</td>
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<td>2</td>
<td>5.88</td>
<td>1</td>
<td>2.4</td>
<td>18</td>
<td>52.94</td>
<td>34</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>25</td>
<td>3</td>
<td>25</td>
<td>6</td>
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<td>25</td>
<td>6</td>
<td>25</td>
<td>18</td>
<td>52.94</td>
<td>46</td>
</tr>
</tbody>
</table>

1+ = up to 25% stained nuclei to protein p53; 2+ = 26 to 50% stained nuclei to protein p53; 3+ = 51 to 75% stained nuclei to protein p53; 4+ = more than 75% stained nuclei to protein p53.

### Figure 3 - Survival curve in relation to protein p53 expression in the tumors.
We observed a worse degree of cellular differentiation in neoplasias with a higher percentage of stained nuclei to protein p53. This result has almost reached statistical significance, when well and moderately differentiated tumors were compared to the undifferentiated ones.

After studying 42 patients with penile epidermoid carcinoma, Lam et al. obtained positive results to protein p53 in 40% of the cases. These authors have found higher percentages of protein p53 presence in more differentiated tumors, which contradicts our findings (21).

Considering the pathologic staging, when table-2 is observed, it can be noticed that the frequency of patients with metastatic neoplasia increases, in relation to the increase in tumor cell nuclei stained to protein p53. However, these findings did not show statistical significance.

Protein p53 had a severe impact on patients prognosis, as the higher the number of stained nuclei to the protein, the higher the occurrence of death. Most of the patients who presented more than 75% of the nuclei stained died during the period of analysis (Table-3).

The survival curve also showed that the higher the percentage of stained cells, the fewer the chances of survival (Figure-3).

The probability coefficient (odds ratio) for patients death related to protein p53 expression was significant, as patients with 50 to 75% of stained nuclei in the tumor were 9.95 times more likely to die due to neoplasia. When these percentages are higher, these rates reach 15.28 times.

CONCLUSIONS

The incidence of protein p53 in penile epidermoid carcinoma is high, and the tumors with unfavorable histology have greater amounts of the protein in the cell nuclei.

Risk of death due to the tumor is significantly higher in patients with high p53 expression. The abnormal expression of protein p53 is significantly related to the biological behavior of penile epidermoid carcinoma.

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