Immunohistochemical Evaluation of PCNA, p53, HSP60, HSP10 and MUC-2 Presence and Expression in Prostate Carcinogenesis

FRANCESCO CAPPELLO1,2, FRANCESCA RAPPA2, SABRINA DAVID1, RITA ANZALONE1 and GIOVANNI ZUMMO1

1Human Anatomy Section, Department of Experimental Medicine and 2Institute of Pathological Anatomy, University of Palermo, Italy

Abstract. Background: The study of the expression of different biological markers in non-neoplastic, pre-neoplastic and neoplastic lesions of prostate could help to better understand their role in carcinogenesis and to find new diagnostic and prognostic tools. Materials and Methods: In the present work we evaluated, by immunohistochemistry, the presence and the expression of PCNA, p53, HSP60, HSP10 and MUC-2 in a series of nodular hyperplasia, low- and high-grade prostatic intraepithelial lesions and adenocarcinomas. Results: Our data confirmed that: 1) PCNA expression could be related to the grade of progression of cancer; and that 2) p53 mutation could be a late event in prostate carcinogenesis. Moreover, we reported that: 1) HPS60 and HPS10 were overexpressed early in prostate carcinogenesis; and that 2) MUC-2 is absent in both tumoral and non-tumoral prostatic tissue. Conclusion: We suggest the further examination, by molecular and genetic studies, of the role of HPS60 and HSP10 during carcinogenesis of the prostate as well as of other organs.

Cancer is a multi-step process. Prostatic intraepithelial neoplasia (PIN) is a fundamental step during carcinogenesis of the prostate (1). These pre-malignant lesions were classified in three categories, depending on the level of dysplasia: mild (PIN-1), moderate (PIN-2) and severe (PIN-3). More recently, they were divided into only two groups, low-and high-grade PIN (L-PIN and H-PIN), the former including PIN-2 and PIN-3 (2).

A number of studies suggest that the tumoral progression from PIN to invasive cancer follows a predictable natural course, (3, 4), although the cellular mechanisms responsible for this progression remain unclear (5-7). However, numerous works have focused their attention on the expression of different biological markers during prostatic carcinogenesis, to better understand their role in carcinogenesis and to test them as diagnostic and prognostic tools (8-11).

In a recent work on a series of 63 prostate adenocarcinomas of moderate Gleason's grade, we supported the hypothesis that p53 mutation is a late event in prostate carcinogenesis (12). Moreover, in two more of our recent studies, we found an overexpression of the heat-shock protein (HSP) 60, a mitochondrial protein, during cervical (13) and colorectal (14) carcinogenesis. HSP60 is a molecular chaperon involved in protein folding. In addition, more recently we found an analogous overexpression of HSP10 in the same carcinogenetic models (manuscript in preparation). HSP10 is another mitochondrial chaperon functionally related to HSP10. Both proteins are also involved in apoptosis activation (15). Finally, the presence of MUC-2, a highly glycosylated protein, in tumoral and non-tumoral prostates is as yet poorly understood (16).

In this work we performed an immunohistochemical evaluation of PCNA, p53, HSP10, HSP60 and MUC-2 in non-neoplastic, pre-neoplastic and neoplastic prostatic tissues, to evaluate and to compare their presence and expression.

Materials and Methods

The files of the Institute of Pathological Anatomy of the University of Palermo, Italy, were searched to select a number of cases of modular hyperplasias (NH), L-PIN, H-PIN and prostate carcinomas with an intermediate grade of differentiation (PC). All specimens were formalin-fixed and paraffin-embedded. We selected 10 cases of each of them and we performed an immunohistochemical analysis, with avidin-biotin complex (LSAB2, DAKO, Cat. No K677), using the primary antibodies shown in Table 1. Non-immune sera were substituted for negative controls and appropriate positive controls were run concurrently. 3-3-Diaminobenzidine (DAB chromogen solution, DAKO, Cat. No. K3467) was used as developing chromogen. A nuclear counterstaining with hematoxylin (DAKO, Cat. No. S2029) was finally performed.

The immunostainings were evaluated by two independent observers who counted the percentage of positive tumoral cells in 10 HFP for each case. The results were semiquantitated on a scale of 0-3 as follows: mild positivity (0-35% of positive epithelial cells); ++; moderate positivity (35-66%); +++; strong positivity (67-100%); +++++. Moreover, a one-way analysis of variance was used to determine the presence of significant differences within the averages of the groups (NH, L-PIN, H-PIN and PC), using the Student's t-test. Differences between the means of the values were regarded as significant when p < 0.05 was obtained.

Correspondence to: Dr. Francesco Cappello, Via alla Falconara 120, 90136 Palermo, Italy. e-mail: franeapp@hotmail.com

Key Words: Carcinogenesis, prostate, heat-shock proteins.

0250-7005/2003 $2.00 +.40
Table I. The primary antibodies used in this study.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>PCNA</th>
<th>P53</th>
<th>HSP60</th>
<th>HSP10</th>
<th>MUC2</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAKO</td>
<td></td>
<td></td>
<td>SIGMA</td>
<td>STRESSGEN</td>
<td>NOVOCASTRA</td>
</tr>
<tr>
<td>monoclonal</td>
<td></td>
<td></td>
<td>monoclonal</td>
<td>polyclonal</td>
<td>monoclonal</td>
</tr>
<tr>
<td>M0879</td>
<td>M7001</td>
<td>H4149</td>
<td>SPA-110</td>
<td>NCL-MUC2</td>
<td></td>
</tr>
</tbody>
</table>

Table II. The immunohistochemical results.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>PCNA</th>
<th>p53</th>
<th>MUC-2</th>
<th>HSP60</th>
<th>HSP10</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-PIN</td>
<td>+</td>
<td>-</td>
<td></td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>H-PIN</td>
<td>+</td>
<td>-</td>
<td></td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>PC</td>
<td>+++</td>
<td>++</td>
<td></td>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>

Results

The immunostaining results are summarized in Table II. NH resulted negative for all tested antibodies in both glandular and stromal component (Figure 1). L-PIN and H-PIN indicated a similar positivity for PCNA, HSP10 and HSP60, while they were negative to p53 and MUC-2. In particular, they indicated a mild positivity for PCNA and a strong positivity for HSP60 and HSP10, above all in the cytoplasm of the epithelial cells (Figure 2). Finally, PC showed a strong positivity for PCNA, HSP60 and HSP10, and a moderate positivity for p53. MUC-2 was always negative (Figure 3).

Statistical analysis indicated that there were no significant differences in epithelial cell immunopositivity of PCNA, HSP60 and HPS10 between L-PIN and H-PIN (p > 0.05) (Figure 4). By contrast, the immunoreactivity of PCNA in PC increased significantly (p < 0.0001) (Figure 4a). Finally, HSP60 and HPS10 did not show any significant difference between PIN and PC (p > 0.05) (Figure 4b, c).

Discussion

PIN is the most widely recognized precursor lesion to adenocarcinoma of the prostate. PIN is encountered with increasing frequency on needle biopsy specimens in routine uropathology. The distinction between L-PIN and H-PIN is critical. Although several authors combine PIN-1 and PIN-2 as L-PIN (17-19), the majority of authors agree that PIN-2 and PIN-3 should be considered H-PIN (2, 20). In the current study, we did not find any discrepancy in the expression of the studied antibodies between L-PIN and H-PIN. These data could indicate that these antibodies are not useful to discriminate these entities. PCNA is a sensitive indicator of cellular growth fraction (21). It is detectable throughout most of the cell cycle, not just in cells in active DNA synthesis or division (22). Many studies have indicated correlation between tumor grade and proliferative activity on needle biopsy (22-24). In our study, PCNA positivity in L-PIN and H-PIN was mild. In contrast, PC showed a strong positivity.

These data could confirm that PCNA could be a useful prognostic marker for correlating proliferative activity with tumor progression.

Many studies have suggested a role of p53 mutation in prostate carcinogenesis (25-28). Other studies indicated that p53 reactivity was strongly related to progression of prostate carcinoma (29, 30). We recently supported the hypothesis that p53 mutation is a late event in prostate carcinogenesis in a study on 63 Gleason 6 (3+3) prostate adenocarcinomas (12). In the present study we found that p53 was negative in L-PIN and H-PIN, as analogously described by Yaman (31), while we found its accumulation only in carcinomas. Our results are in agreement with other data presented in the literature and they could confirm that p53 mutation could be a late event in prostate carcinogenesis.

HSPs are a family of molecular chaperons involved above all in protein folding in mitochondria (32-34). We recently described the cytoplasmic over-expression of HSP60 in excocervical (13) and colorectal carcinogenesis (14). In particular, we found a moderate-strong positivity for HSP60 in cervical dysplasias and colorectal tubular adenomas, suggesting that HPS60 overexpression was a early event in these carcinogenic models. Moreover, our recent experiments have analogously discovered an early overexpression of HSP10 in both excocervical and colorectal carcinogenesis (manuscript in preparation). The results of the present study suggested that HSP60 and HSP10 are also overexpressed early during prostate carcinogenesis. This should justify molecular and genetic studies to clarify if HSPs are functional in the cytoplasm during carcinogenesis and what their role could be away from mitochondrial chaperons, i.e. in controlling apoptotic pathways.

MUC-2 is a highly-glycosylated protein present in a number of glandular tissues, such as small and large intestinal mucosa. It is a well-demonstrated diagnostic and prognostic tool in intestinal and colorectal cancer (35, 36). Its presence and expression in prostate cancer progression is not well understood (16). In our study, this marker resulted negative in all studied tissues, and thus we could consider it of poor value.
In conclusion, in this paper we have supported the hypotheses that: 1) PCNA expression could be related with the grade of progression of cancer, and 2) the mutation of p53 could be a late event in prostate carcinogenesis. Moreover, this is the first work that describes an early HPS60 and HPS10 overexpression in prostate carcinogenesis; their simultaneous cytoplasmic presence could indicate that they are functional and it could address further studies to discover their role outside the mitochondria during carcinogenesis. Finally, the absence of MUC-2 in both tumoral and non-tumoral prostatic tissue confirmed its scarce role as a diagnostic tool in prostatic carcinoma.

Acknowledgements

We are grateful to Dr. M. Salvato M. Campione and Mr. S. Gentile for their technical support. This work was in part supported by funds "Progetto Giovani Ricercatori, anno 1999", Italy.
Figure 2. PIN (upper left, H&E 10X) lightly-positive for PCNA (middle left) and strongly-positive for HSP60 (middle right) and HSP10 (lower right) (10X). p53 (lower left) and MUC-2 (upper right) were negative (10X). Insets: higher magnification (40X).

References

8 Sakr WA, Ward C, Grignon DJ and Haas GP: Epidemiology and
Figure 4. Statistical evaluation of the presence of significant differences between the media of the immunohistochemical results. $p = 0.05$. 
chaperonin (hsp60) is over-expressed during colorectal carcinoma. Eur J Histochem. in press, 2003.

Received October 21, 2002
Accepted January 13, 2003