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

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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 HSP60 and HSP10 during carcinogenesis of the prostate as well as of other organs.*

Cancer is a multi-step process. Prostatic intraepithelial neoplasm (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).

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In a recent wotk on a series of 63 prostate adeno-carcinomas 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, HSP10 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 I. 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. S2020) was finally performed.

The immunostainings were evaluated by two independent observers who counted the percentage of positive tumoral cells in 10 HPF for each case. The results were semiquantitated on a scale of 0-3 + as follows: mild positivity (0-33% of positive epithelial cells): +; moderate positivity (34-66%): ++; 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.

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Table I. *The primary antibodies used in this study.*

PCNA	P53	HSP60	HSP10	MUC2
DAKO	DAKO	SIGMA	STRESSGEN	NOVOCASTRA
monoclonal	monoclonal	monoclonal	polyclonal	monoclonal
1:50	1:100	1:500	1:500	1:100
M0879	M7001	H4149	SPA-110	NCL-MUC2

Table II. *The immunohistochemical results.*

	PCNA	p53	MUC-2	HSP60	HSP10
NH	-	-	-	-	-
L-PIN	+	-	-	+++	+++
H-PIN	+	-	-	+++	+++
PC	+++	++	-	+++	+++

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 immunoexpression f 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 exocervical (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 exocervical 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.

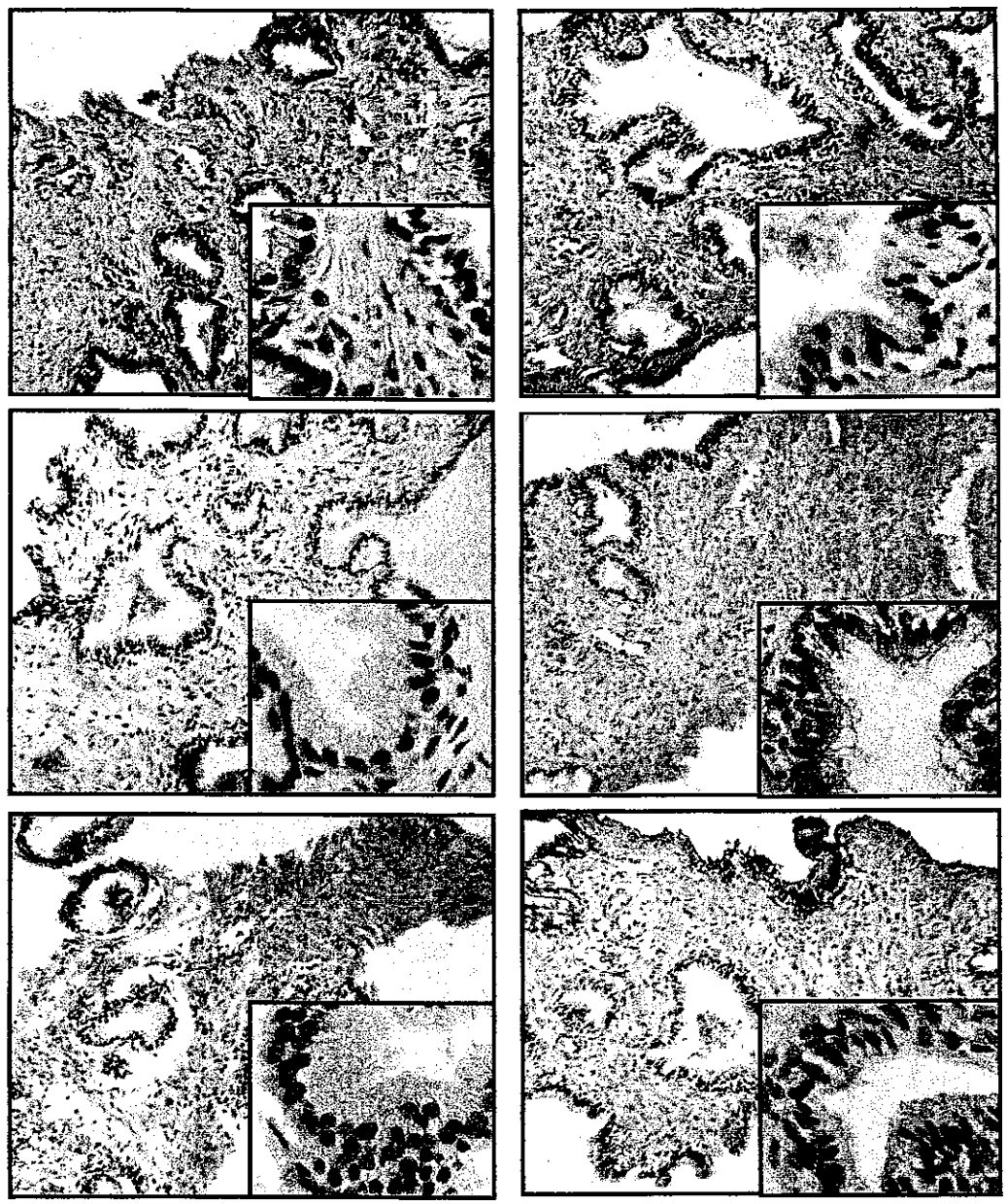

Figure 1. *Nodular hyperplasis (upper left, H&E 10X)-negative at immunohistochemistry for all markers: PCNA (middle left), p53 (lower left), MUC-2 (upper right), HSP60 (middle right) and HSP10 (lower right) (10X). Insets: higher magnification (40X)*

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.

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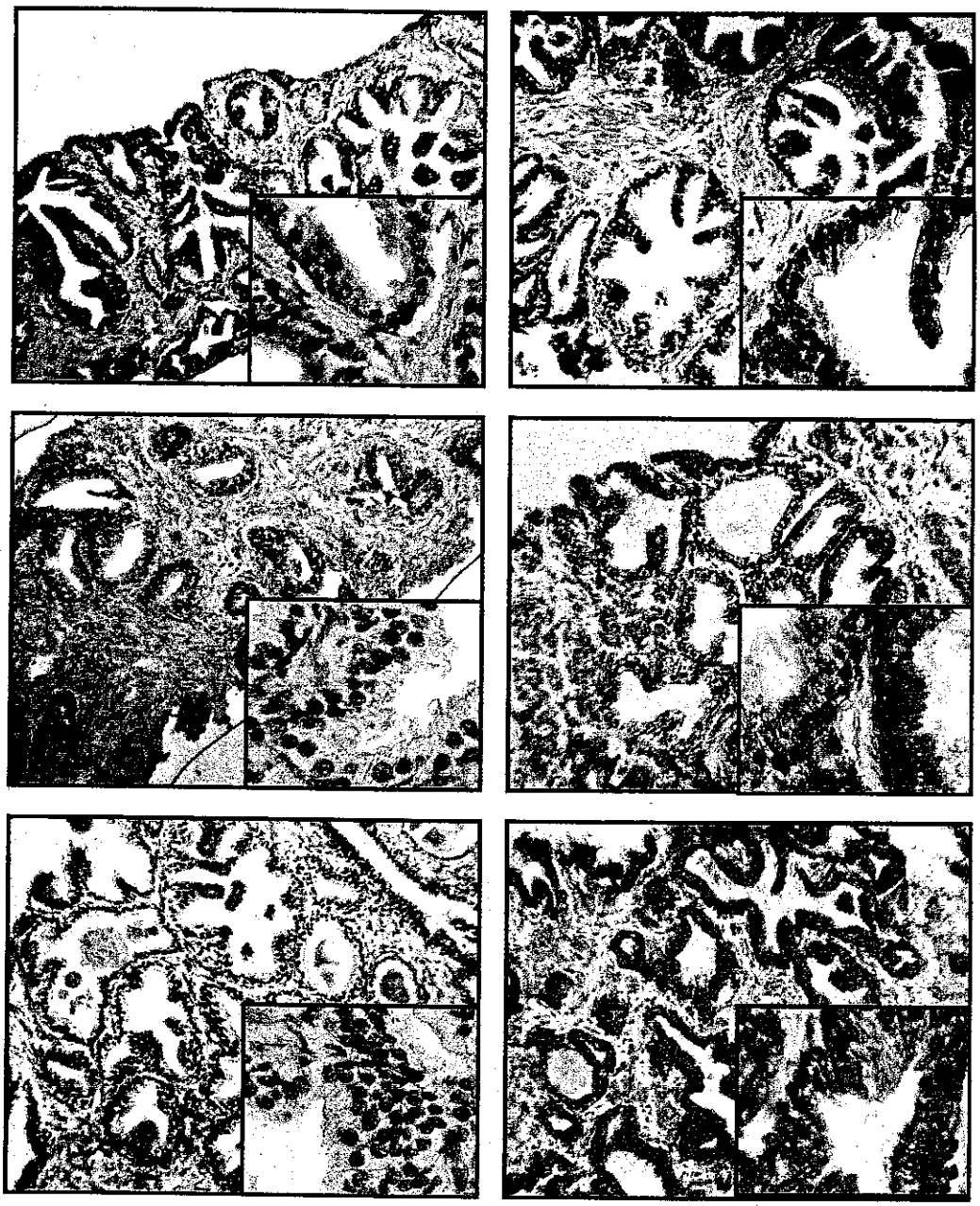

Figure 2. *PIN (upper left, H&E 10X) lightly-positive for PCNA (middle left) and strongly-positive for HSP60 (middle right) and HSP10 (lower right) (10X). p53 (lower left) and MUC-2 (upper right) were negative (10X). Insets: higher magnification (40X).*

References

1 McNeal JE and Bostwick DG: Intraductal dysplasis: a premalignant lesion of the prostate. Hum Pathol *17:* 64-71, 1986.

2 Epstein JI, Grignon DJ, Humphrey PA, McNeal JE, Sesterhenn IA, Troncoso P and Wheeler TM: Interbserver reproducibility in the diagnosis of prostatic intraepithelial neoplasis. Am J Surg Pathol *19:* 873-886, 1995.

3 Bostwick DG and Brawer MK: Prostatic intra-epithelial neoplasia and early invasion in prostate cancer. Cancer *59:* 788-794, 1987.

4 Brawer MK: Prostatic intraepithelial neoplasia: a premalignant lesion. J Cell Biochem *Suppl 16G:* 171-174, 1992.

5 Bostwick DG: Prostatic intraepithelial neoplasia (PIN): current concepts. J Cell Biochem, *Suppl 16H:* 10-189, 1992.

6 Colanzi P, Santinelli A, Mazzucchelli R, Pomante R and Montironi R: Prostatic intraepithelial neoplasia and prostate cancer: analytical evaluation. Adv Clin Path *2:* 271-284, 1998.

7 Montironi R, Mazzucchelli R and Pomante R: Preneoplastic lesions of the prostate. Adv Clin Path *1:* 35-47, 1997.

8 Sakr WA, Ward C, Grignon DJ and Haas GP: Epidemiology and

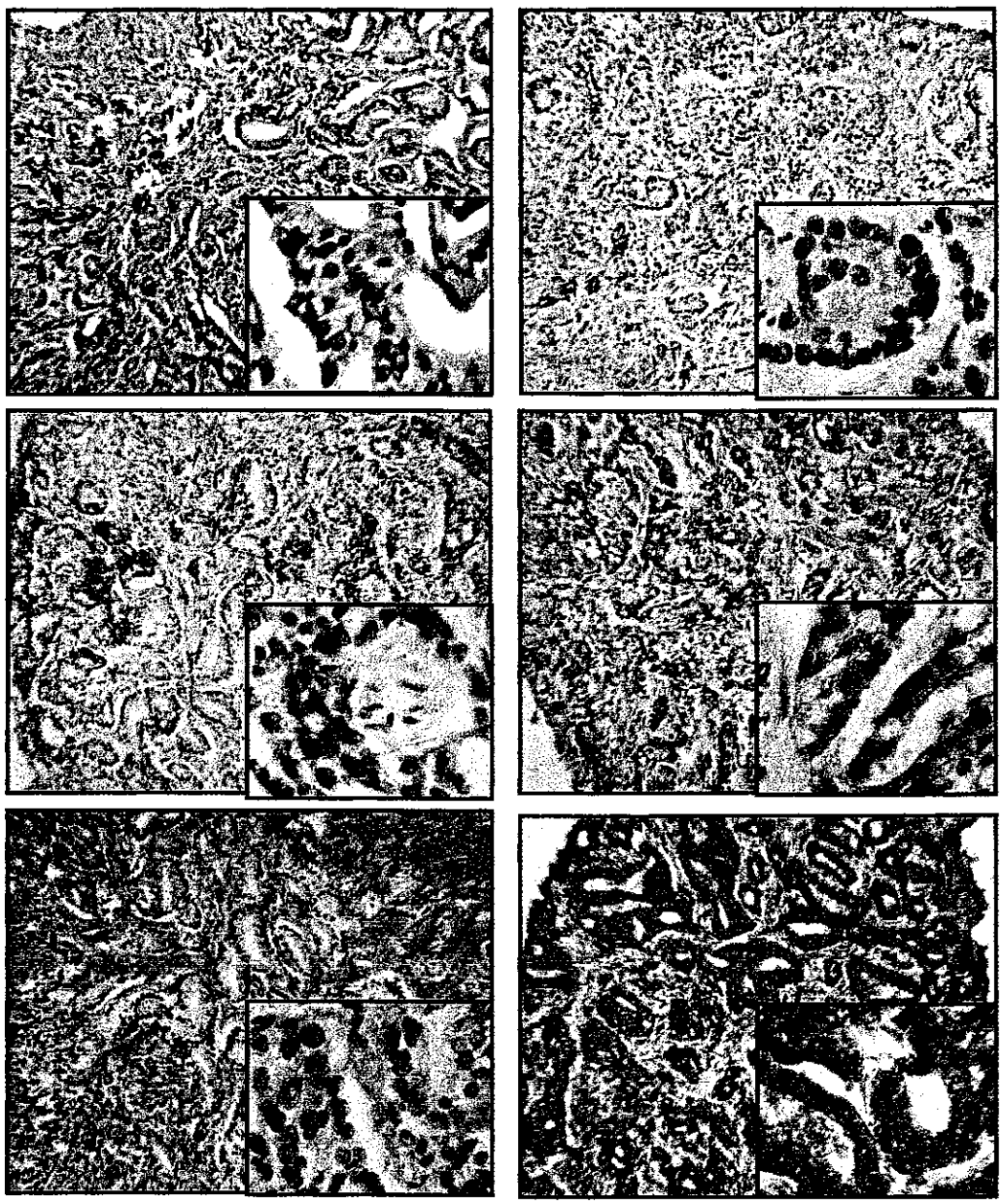

Figure 3. *Prostatic carcinoma strongly-positive for PCNA (middle left), HSP60 (middle right) and HSP10 (lower right) (10X), and moderately-positive for p53 (lower left). MUC-2 resulted negative (upper right) (10X). Insets: higher magnification (40X).*

molecular biology of early prostatic neoplasia. Mol Urol *4:* 109-115, 2000.

9 Sakr WA and Partin AW: Histological margin of risk and the role of high-grade prostatic intraepithelial neoplasia. Urology *57:* 115-120, 2001.

10 Alsikafi NF, Brendler CB, Gerber GS and Yang XJ: High-grade prostatic intraepithelial neoplasia with adjacent atypia is associated with a higher incidence of cancer on subsequent needle biopsy than high-grade prostatic intraepithelial neoplasia alone. Urology *57:* 296-300, 2001.

11 Prange W, Erbersdobler A, Hammerer P, Graefen M, Hautmann SH, Hautmann RE, Huland H and Henke RP: Significance of high-grade prostatic intraepithelial neoplasia in needle biopsy specimens. Urology *57:* 486-490, 2001.

12 Cappello F, Palma A, Martorana A, Rappa F, Cabibi D, Barresi E, Melloni D, Farina F and Aragona F: Comparative PCNA and p53 evaluation in tubular and cribrous patterns of Gleason 6 prostate adenocarc inoma. Eur J Histochem in press, 2003.

13 Cappellδ F, Bellafiore M, Palma A, Marciano V, Martorana G, Belfiore P, Martorana A, Farina F, Zummo G and Bucchieri F: 60kDa Heat Shock Protein (HSP60) expression increases during carcinogenesis of uterine exocervix. Pathobiology, in press, 2003.

14 Cappello F, Bellafiore N, Palma A, David S, Marciano V, Bartolotta T, Sciume C, Modica G, Farina F, Zummo G and Bucchieri F: 60kDa

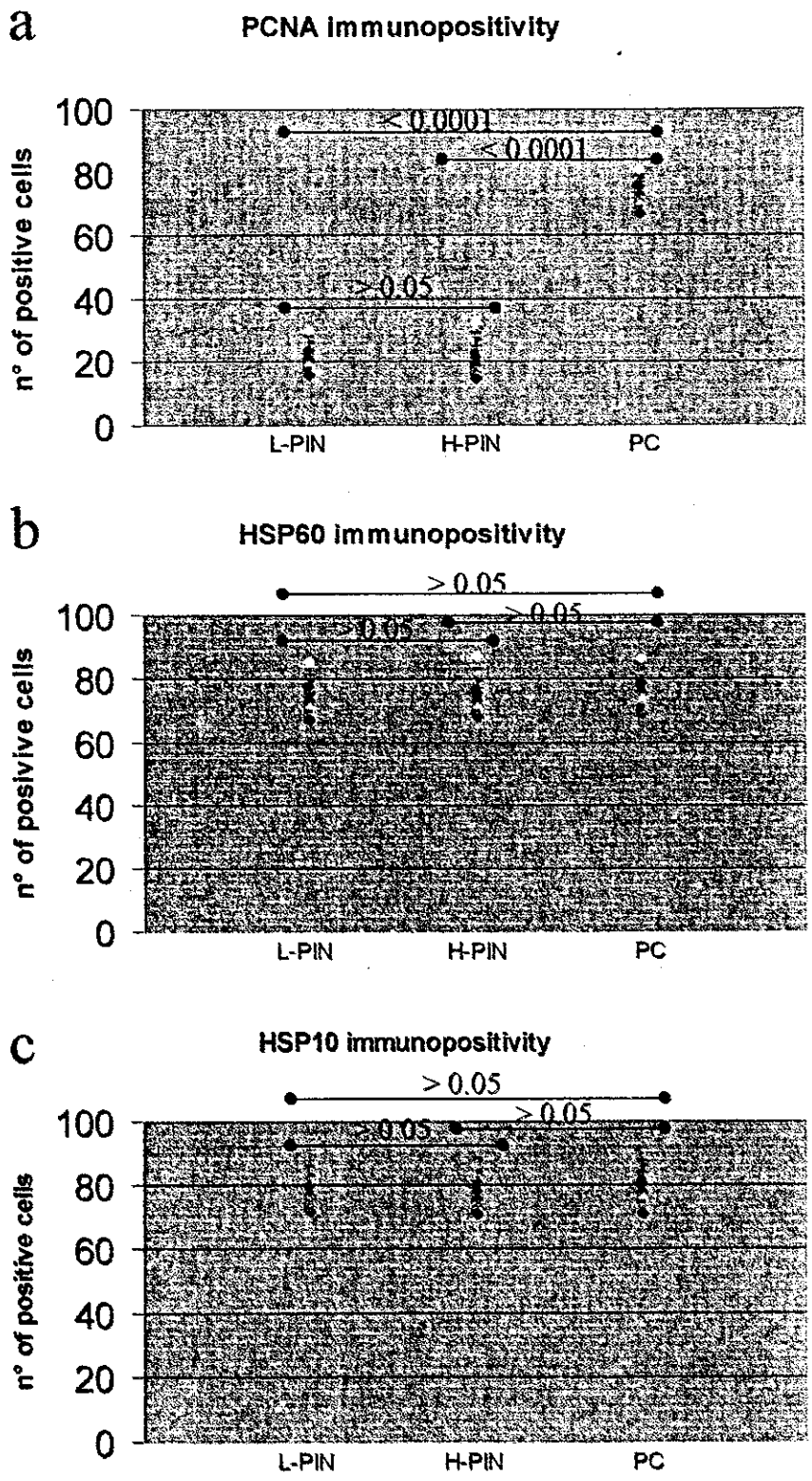

Figure 4. *Statistical evaluation of the presence of significant differences between the media of the immunohistochemical results. p = 0.05.*

chaperonin (hsp60) is over-expressed during colorectal carcinogenesis. Eur J Histochem in press, 2003.
15 Lin KM, Lin B, Lian IY, Mestril R, Sheffler IE and Dillmann WH: Combined and individual mitochondrial HSP60 and HSP10 expression in cardiac myocytes protects mitochondrial function and prevents apoptotic cell deaths induced by simulated ischemia-reoxygenation. Circulation *103:* 1787-1792, 2001.
16 Cohen RJ, McNeal JE and Baillie T: Patterns of differentiation and proliferation in intraductal carcinoma of the prostate: significance for cancer progression. Prostate *43:* 11-19, 2000.
17 Montironi R, Scarpelli M, Sisti S, Braccischi A, Gusella P, Pisani E, Alberti R and Mariuzzi GM: Quantitative analysis of prostatic intraepithelial neoplasia on tissue sections. Anal Quant Cytol Histol *12:* 366-372, 1990.
18 Crissman JD, Sakr WA, Hussein ME and Pontes JE: DNA quantification of intraepithelial neoplasia and invasive carcinoma of the prostate. Prostate *22:* 155-162, 1993.
19 Jones EC and Young RH: The differential diagnosis of prostate carcinoma: its distinction from premalignant and pseudocarcinomatous lesions of the prostate gland. Am J Clin Pathol *101:* 48-64, 1994.
20 Drago JR, Mostofi FK and Lee F: Introductory remarks and workshop summary. Urology *34(Suppl):* 2-3, 1989.
21 Hall PA, Levison DA, Woods AL, Yu CC, Kellock DB, Watkins JA, Barnes DM, Gillett CE, Complehohn R and Dover R: Proliferating cell nuclear antigen (PCNA) immunolocalization in paraffin section: an index of cell proliferation with evidence of deregulated expression in some neoplasms. J Pathol *162:* 285-294, 1990.
22 Spires SE, Jennings CD, Banks ER, Wood DP, Davey DD and Cibull ML: Proliferating cell nuclear antigen in prostatic adenocarcinoma: correlation with established prognostic indicators. Urology *43:* 660-666, 1994.
23 Nemoto R, Kawamura H, Miyakawa I, Uchida K, Hattori K, Kioso K and Harada M: Immunohistochemical detection of proliferating cell nuclear antigen (PCNA)/cyclin in human prostate adenocarcinoma. J Urol *149:* 165-169, 1993.
24 Visakorpi T: Proliferative activity determinated by DNA flow cytometry and proliferating cell nuclear antigen (PCNA) immunohistochemistry as a prognostic factor in prostatic carcinoma. J Pathol *168:* 7-13, 1992.
25 Effert PJ, Neubauer A, Walther PJ and Liu ET: Alteration of the p53 gene are associated with the progression of a human prostate carcinoma. J Urol *147:* 789-793, 1992.
26 Bookstein R, MacGrogan D, Hilsenbeck SG, Shakey F and Allred DC: p53 is mutated in a subset of advanced-stage prostate cancer. Cancer Res *53:* 3369-3373, 1993.
27 Navone NM, Troncoso P, Pisters LL, Goodrow TL, Palmer JL, Nichols WW, von Eschenbach AC and Conti CJ: p53 protein accumulation and gene mutation in the progression of human prostate carcinoma. J Natl Cancer Inst *85:* 1657-1669, 1993.
28 Matsushima H, Sasaki T, Goto T, Hosaka Y, Homma Y, Kitamura Y, Kawabe K, Sakamoto A, Murakami T and Machinami R: Immunohistochemical study of p21waf1 and p53 proteins in prostatic cancer and their prognostic significance. Hum Pathol *29:* 778-783, 1998.
29 Kallakury BV, Figge J, Ross JS, Fisher HA, Figge HL and Jennings TA: Association of p53 immunoreactivity with high Gleason tumor grade in prostatic adenocarcinoma. Hum Pathol *25:* 92-97, 1994.
30 Shurbaji MS, Kalbfleisch JH and Thurmond TS: Immunohistochemical detection of p53 protein as a prognostic indicator in prostate cancer. Hum Pathol *26:* 106-109, 1995.
31 Yaman O, Ozdiler E, Orhan D, Sak SD, Baltaci S, Tulunay O and Gogus O: Immunohistochemical determination of p53 protein in prostatic cancer and prostatic intraepithelial neoplasms. Urol Int *58:* 199-202, 1997.
32 Harti F, Hlodan R and Langer T: Molecular chaperones in protein folding: the art of avoiding sticky situations. Trends Biochem Sci *19:* 20-25, 1994.
33 Weissman JS, Hohl CM, Kovalenko O, Kashi Y, Chen S, Braig K, Saibil HR, Fenton WA and Horwich AL: Mechanism of GroEL action: productive release of polypeptide from a sequestered position under GroES. Cell *83:* 577-587, 1995.
34 Ryan MT, Naylor DJ, Hoi PB, Clark MS and Hoogenraad NJ: The role of molecular chaperones in mitochondrial protein import and folding. Int Rev Cytol *174:* 127-193, 1997.
35 Durrant LG, Jacobs E and Price MR: Production of monoclonal antibodies recognising the peptide core of MUC2 intestinal mucin. Eur J Cancer *30A:* 355-363, 1994.
36 Velcich A, Yang W, Heyer J, Fragale A, Micholas C, Viani S, Kucherlpati R, Lipkin M, Yang K and Augenlicht L: Colorectal cancer in mice genetically dificient in the mucin Muc2. Science *295:* 1726-1729, 2002.

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