

## Biological aggressiveness evaluation in prostate carcinomas: immunohistochemical analysis of PCNA and p53 in a series of Gleason 6 (3+3) adenocarcinomas

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We selected 63 prostate tumors with Gleason's grade 6 (3+3), commonly showing both tubular and cribrous patterns. We compared in both patterns the expression of two of the most used biologic markers: PCNA and p53, with the aim to verify the validity of the Gleason's grading system to compare the morphologic grade with biologic aggressiveness and prognostic value. We did not find any statistical difference in the protein immunopositivity, indicating that both patterns could have identical biologic behaviour; then we confirmed the validity of Gleason's system for considering both tubular and cribrous patterns as an intermediate grade of tumoral differentiation. Moreover, we found a linear relationship between the increase of PCNA and the accumulation of mutated p53; this datum could confirm the hypothesis that p53 mutation is a late event in prostate carcinogenesis.

Key words: proliferative index, tumoral differentiation, prostate cancerogenesis, tumoral prognosis.

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Prostate adenocarcinoma is one of the most frequent malignancy and the second leading cause of cancer-related deaths in men in Western Countries (Boring et al., 1992). It determined a great focus on its pathogenesis as well as diagnostic and prognostic criteria and therapeutic approach.

Gleason's system is the most widely used grading of prostate carcinoma, based on the tissue aspect at low magnification (Gleason, 1977). The value is obtained identifying the predominant and the second most prevalent architectural pattern and assigning a grade from 1 to 5, with 1 being the most differentiated and 5 undifferentiated (Gleason, 1977).

The main advantages of this system are that i) it is easy to learn and apply and ii) it was well demonstrated its fairly well correlation with the prognosis (Kramer et al., 1980). The most frequent causes of pitfall are i) the multifocality of the prostate cancer with simultaneous variety of differentiation and ii) the tendency to undergrade the bioptic material when a minimal amount of tumor is present. The latter can determine difficulty in valuing the variability of neoplastic gland size and shape, especially important to identify the Gleason's grade 3. Moreover, the combination of tumoral grade with other prognostic variables to determine multiple prognostic indexes allows greater precision in predicting outcome (Kramer et al., 1980).

Gleason 6 (3+3) is one of the most commonly found grade. It is characterised by both tubular and/or cribrous pattern of tumoral glands. Commonly, adenocarcinomas of other sites with a tubular pattern are thought a more differentiated form than the cribrous ones, considering the latter as an expression of an intermediate differentiation. On the contrary, the presence of one (or both) of these patterns in prostate adenocarcinoma, implies an intermediate grade of differentiation. Nevertheless,

to our knowledge, none study looked for the presence of discrepancies between these two pattern in terms of biologic characterisation and behaviour.

Here, we studied two important prognostic indexes in a large series of Gleason 6 (3+3) prostate adenocarcinomas: proliferating cell nuclear antigen (PCNA) and p53. PCNA is retained one of the most important index to estimate the tumoral proliferate activity (proliferating index, PI), while p53 is the widest studied tumor suppressor gene, involved in regulation of cell cycle. The objective was to verify the presence (or absence) of a discrepancy in the expression of these antigens in both tubular and cribrous patterns.

### Materials and Methods

Files of the Institute of Pathology of the University of Palermo were searched for cases of prostate carcinoma, Gleason 6 (3+3), during the period between 1st January 1998 and 31st December 2001. All specimens were been formalin-fixed and paraffin-embedded. We selected 63 cases, in which there were simultaneously present tubular and cribrous areas. Seven micrometers sections were stained with H&E for routinely diagnosis. Moreover, we performed an immunohistochemical analysis.

We immunostained with avidin-biotin complex (LSAB2, DAKO), using primary antibodies against PCNA (Clone PC10, DAKO, Cat. No. M 0879) and p53 (Clone DO-7, Dako, Cat. No. M 7001) respectively diluted at 1:50 and 1:40. Non-immune serum was substituted for negative controls. Appropriate positive controls were run concurrently for both antibodies tested. Aminoethylcarbazol (AEC chromogen, DAKO, Cat. No. K0697) was used as develop chromogen. A light nuclear counterstaining with hematoxylin was finally performed.

To better confirm the data, the immunostainings were repeated three times on two sections for each specimen. Two independent observers carried out the microscopical analysis. They valued: PCNA, counting the percentage of positive tumoral cells in 10 HPF; p53, counting the number of tumoral glands in which at least one cell expressing antigenic positivity was present in 10 HPF.

We using the *One way analysis of variance* (ANOVA) to determine the presence of significant differences within the data. Differences between the means of the values were regarded as significant when a  $p < 0.05$  was obtained. Moreover, we used the "r-Pearson" model to verify the presence of a linear

relationship between PCNA and p53 immunopositivity (t-tab: 2,021).

### Results

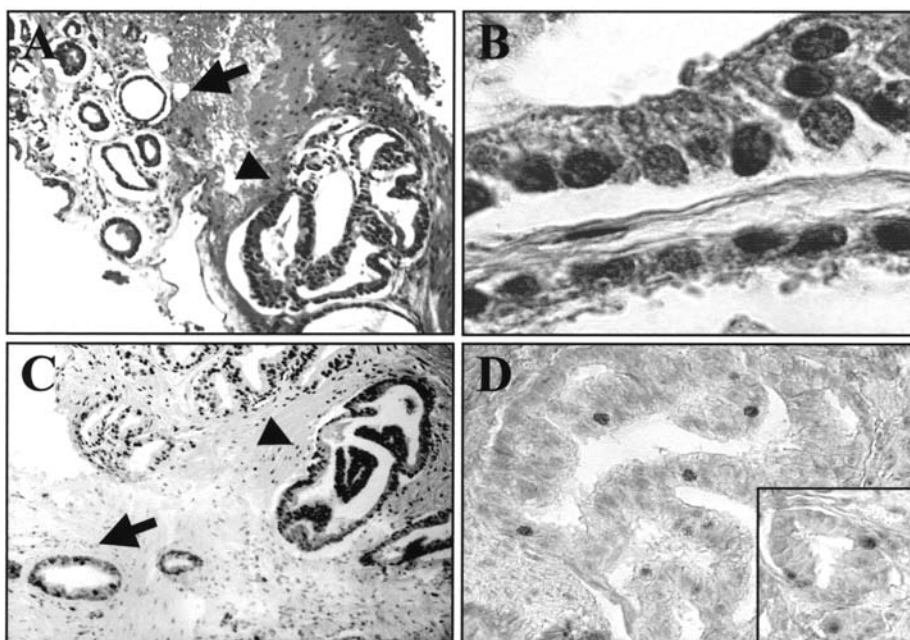
We observed 63 cases of prostate adenocarcinoma with both cribrous and tubular pattern. Tumoral tissue showed closely packed small glands, usually irregularly separated, with poorly defined edge and smoothly circumscribed masses of cribriform epithelium (Figure 1a). Moreover, tumoral cells present in the glands differed from surrounding benign tissue in both cytoplasmic or nuclear features. In particular, prominent nucleoli, nuclear enlargement, hyperchromatic nuclei, amphophilic cytoplasm and mitotic figures were common features (Figure 1b). In addition, basal cells were frequently absent and corpora amillacea were rare. Finally, high-grade PIN (prostatic intraepithelial neoplasia) foci were occasionally found in proximity of tumoral glands.

Immunohistochemistry demonstrated the presence of PCNA in all examined specimens (100%), while p53 was present only in 40 of 63 cases (63,5%). In particular, the positivity of PCNA ranged between 20% and 55% of the tumoral cells (in mean 35%) (Figure 1c), while p53 was expressed in a number of tumoral glands varying between 0% and 45% (in mean 14%) (Figure 1d). In addition, both PCNA and p53 positivity were occasionally found in high-grade PIN foci (0-5%), while normal glands showed rare and negligible positivity only to PCNA (0-1%).

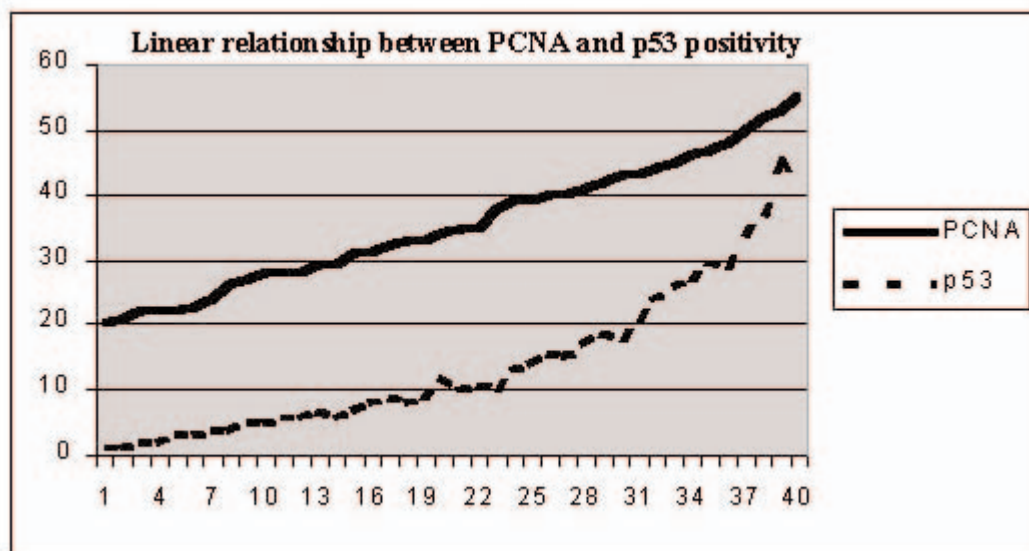
At the statistical analysis, no significative difference resulted in positivity between these two markers in both tubular and cribrous areas ( $p > 0.05$ ). In particular, we found PCNA positivity in mean in 34% of tubular area and 35% of cribrous area cells, while p53 was found in mean in 13% and 15% of tubular and cribrous glands, respectively. Moreover, a linear relationship between PCNA and p53 expression was found (t=2.235; t-tab=2.021;  $t > t$ -tab), examining the 40 cases in which both markers were simultaneously present (Figure 2).

### Discussion

Proliferating cell nuclear antigen (PCNA) is one of the most studied proliferation-associated protein. It was demonstrated to be a strong sensitive indicator of cellular growth fraction (PI), because it is detectable throughout most of the cell cycle, during active DNA synthesis or cell division (Hall et al., 1990). Moreover, Visakorpi reported a strong correlation between high level of PCNA staining and poor



**Figure 1.** Histologic and immunohistochemical aspects of studied tumors. A: H&E stained section showing both patterns: tubular (arrow) and cribriform (arrow-head), 10 $\times$ . B: H&E showing the features of the tumoral cells, 40 $\times$ . C: PCNA positivity in both tubular (arrow) and cribriform (arrow-head) patterns, 10 $\times$ . D: cribriform area showing nuclear positivity to p53. Inset: p53 positivity in tubular area, 25 $\times$ .



**Figure 2.** Graphic showing the linear relationship between PCNA and p53 positivity.

prognosis for patients with prostate carcinoma (Visakorpi, 1992). In addition, Montironi et al. (1993) showed that the number of PCNA-stained nuclei increased from small and large acinar patterns through cribriform to solid or trabecular patterns. Moreover, there was also a tendency for a stronger staining at the invasive edge of the neoplastic area than in the central region.

P53 is a tumor suppressor gene frequently altered in malignant human tumors (Oren, 1992; Cappello et al., 2001). In case of DNA damage, functional

(wild) p53 may block cell-cycle progression in late G1 phase or may trigger an intrinsic mechanism of apoptosis (Levine et al., 1991, Cappello et al., in press). By contrast, the abnormal p53 protein produced by mutant gene is ineffective and more stable than the wild type protein; it becomes less sensitive to proteolysis and it tends to accumulate in the nucleus, so being easily detected by immunohistochemistry (Brunner et al., 1993). We used the monoclonal antibody clone DO-7 that recognised both wild-type and mutant p53; however, because the wild

type protein is rapidly degraded, its physiologic levels remain below the immunohistochemical detectable threshold. In this way, p53 immunoreactivity is likely to reflect p53 gene mutations (Papadopoulos et al., 1996). Numerous studies suggested the role of p53 mutation in the pathogenesis of prostate cancer, even if recent studies reported conflicting results about the incidence of nuclear p53 accumulation (Samaan et al., 1993; Kallakury et al., 1994; Fox et al., 1993; Shurbaji et al., 1995).

In our study, PCNA resulted positive in all cases, with a mean percentage of 35% tumoral cells. By contrast, p53 was present in 40 of 63 cases, with a mean value of 14% of tumoral glands. It should mean that the studied tumors had a moderate proliferation index and an intermediate differentiation. Moreover, we found a linear relationship between PI and p53 positivity. This datum indicated a correlation between the increase of proliferating index and the accumulation of mutated p53. This is in agreement with Papadopoulos et al. (1996) that found a highly significant correlation between p53 overexpression and tumor stage. They suggested the mutation of the p53 gene was not an initial event in prostate carcinogenesis. It implied the existence of a strong correlation between p53 and tumor grade, suggesting also that aberrant p53 expression goes along with a loss of differentiation. Indeed, Papadopoulos et al. (1996) found that tumors with increased p53 positivity also exhibited a significant higher proliferation index, this latter corroborating the former observation. In conclusion, in this work we studied a series of prostate adenocarcinomas with an intermediate grade of differentiation (Gleason 6: 3+3). There were simultaneously present both tubular and cribriform areas. Generally, the presence of a tubular pattern in adenocarcinomas of other sites than prostate addresses the diagnosis towards a well-differentiated tumor, while the cribriform aspect is commonly an index of intermediate differentiation. Notwithstanding this, we did not find any statistical difference between PCNA and p53 expression in both tubular and cribriform patterns of prostatic adenocarcinomas. Therefore, our results confirmed the validity of the Gleason's system to attribute an intermediate grade of differentiation at both tubular and cribriform patterns.

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