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Proliferating cell nuclear antigen/cyclin in incidental carcinoma of the prostate

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Abstract. Monoclonal antibody to proliferating cell nuclear antigen (PCNA) has been used to identify the growth fraction in ten cases of benign prostatic hyperplasia (BPH), in 20 prostatic microcarcinomas (PMC) and in 30 cases of infiltrating prostatic carcinoma (PC). Ten year follow-up was available on all cases by means of clinical, serological, radiological and echographic examinations. The percentage of PCNA-staining nuclei was independently counted by two observers. Statistical analysis showed significant differences between PCNA/cyclin score of BPH and PMC without recurrences with respect to those of PMC with progression and of PC. PCNA immunostaining may represent a reliable method for assessing cellular proliferative activity. It may be used as a more powerful diagnostic hallmark of PMC than patterns of non-malignant microglandular proliferation and is also a useful additional test for assigning histological grades to PMC and PC. Statistical analysis indicated that PCNA/cyclin index was an independent significant prognostic indicator of predicting malignant progression ($P \leq 0.01$) and survival rates ($P \leq 0.05$) of PC and PMC (>5 mm diameter).

Key words: Proliferating cell nuclear antigen – Prostate cancer – Prostatic microcarcinoma – Prognosis

Introduction

Stage A (Whitmore 1973) prostatic carcinoma (PC) is a small cancer undetectable by means of clinical investigation that has been divided by Jewett (1975) into A1 (or focal) and A2 (or diffuse). Golimbu et al. (1978) and Battaglia (1991) suggested that A2 tumours should be included in the B2 stage because of its poorer prognosis than B1. On this basis, the only incidental PC is represented by a focal spreading tumour of microscopic size (stage A1) or prostatic microcarcinoma (PMC). PMC is characterized by identification of no more than 3 microscopic foci in all sections (pT1a of TNM staging system) altogether <10 mm in total principal diameters (Battaglia et al. 1979, 1982) or by the discovery of no more than 5% of the lesion in the resected tissue (Battaglia 1991).

The prevalence of microtubular configuration with monolayered cells and lack of basal cells, verified by negativity of the basal-cell antibody (keratin 903), abnormal histoarchitecture with irregular distribution of the glands, packed back to back, and infiltration of muscular fibres, have been reported by Battaglia et al. (1979), to be histological criteria that define PMC. Moreover, the presence of clear or dark cells with slight nuclear atypia, without mitotic figures, slightly larger and more hyperchromatic nuclei, but with prominent and enlarged nucleoli (Helpap 1988) are the only relevant cytological features of PMC that are the same as those of well-differentiated PC.

Several investigations, including those of DNA index (Losi et al. 1991; Forsslund et al. 1992), AgNOR counts (Hansen and Østergärd 1990; Botticelli et al. 1991; Mammaeva et al. 1991) and Ki-67 antigen expression (Galle et al. 1989; Oomens et al. 1991) have been proposed as prognostic determinants in PC and in PMC. Currently, a commercial monoclonal antibody to the proliferating cell nuclear antigen (PCNA/cyclin), an auxiliary protein to DNA polymerase delta (Bravo et al. 1987; Prelich et al. 1987), can be used to estimate growth fraction. PCNA/cyclin is a highly conserved acid nuclear protein with an apparent molecular weight of about 36,000 Da, synthesized in late G1 and S phase (Bravo and Celis 1980; Kurki et al. 1986). Immunohistochemical studies have shown a close relationship in topographic nuclear distribution during S phase between PCNA and tritiated thymidine (Kurki et al. 1986; Galand and Degraef 1989), suggesting its association with clusters of initiated DNA replicative units.

The aim of this study was to assess PCNA/cyclin distribution in PMC with different clinical and biological

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behaviour and to compare the PCNA mean value index of PMC with benign lesions and grades 1, 2, 3 of PC. The findings might provide relevant clinical correlations.

Materials and methods

Sixty prostates of patients aged between 61 and 70 years, who underwent subtotal prostatectomy for benign prostatic hyperplasia (BPH; 10 cases), PMC (20 cases) and pT2a (B1) infiltrating (< 50% of a lobe) PC (30 cases) were collected. Ten cases of each prostatic lesion (BPH, PMC without malignant progression, PMC with malignant progression and grades 1, 2, 3 PC of the UICC) were selected. A 10-year follow-up (1983–1992) of all patients was performed (from 6-month to 2-year intervals) by digital rectal examination (DIRE), prostatic specific antigen (PSA) serum levels, radiographic and transrectal ultrasound (TRUS) investigations.

Paraffin histological sections (4 μm thick) were immunostained using the anti-PCNA antibody (PC10, Dako, Denmark) at a final dilution of 1:200, following the streptavidin-biotin immunoperoxidase method and using diaminobenzidine to detect the presence of the peroxidase with haematoxylin as counterstain. PCNA was detected in the nucleus which stained dark brown and exhibited a granular or uniform pattern. The labelling index for PCNA was independently determined by two observers and corresponded to the percentage of positive nuclei among 1000 cells on an optical grid, using a ×40 objective.

The multiple range test of the Student Newman Keuls procedure, Mantel Cox method, Cox proportional hazards model with risk type Loglin and regression stepwise, life table and Student's *t*-test were used to calculate statistical analysis of postoperative progression and survival rates of PMC and PC.

Results

In 10 cases of BPH and in 10 cases of PMC without progression (PMC-NP), PSA serum levels were normal (<4 ng/ml), and no relapses, residual carcinomas, or metastases were observed by means of DIRE, TRUS or radiological examinations. At the last clinical assessment all these patients were alive and in good health.

In 6 cases of PMC with progression (PMC+P) elevated PSA serum levels (>4 ng/ml), and capsular and extracapsular extensions were documented, and in 4 cases (PMC+P) bone metastases were found. Five out of these 10 patients died from neoplastic progression 8 years after surgery, and 1 patient died after 7 years, due to cardiovascular complications.

The patients with grade 1 PC died of neoplastic progression with elevated PSA serum level (>4 ng/ml), and bone and brain metastases after 6 (2 cases), 7 (3 cases), 8 (4 cases) and 9 (1 case) years, whereas those with grade 2 PC died after 4 (2 cases), 5 (2 cases), 6 (4 cases) and 7 (2 cases) years and those with grade 3 PC after 3 (2 cases), 4 (2 cases), 5 (2 cases) and 6 (4 cases) years.

As shown in Fig. 1, the lowest PCNA/cyclin score was found in BPH (range from 1 to 9; mean 5.2 ± 0.6), whereas the highest value was in PC, grade 3 (range from 31 to 59; mean 42.3 ± 1.2). PMC-NP showed <5 mm main diameter, whereas PMC+P had main diameter >5 mm and sometimes much more ominous histological findings such as hyperchromatic cell nuclei or cribriform patterns. In the latter cases, nuclear

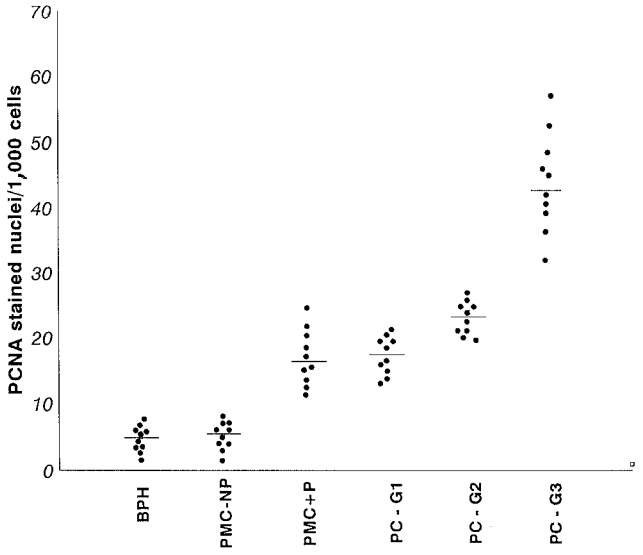

Fig. 1. Distribution of proliferating cell nuclear antigen/cyclin values in different prostatic lesions. *Bars* indicate mean values. BPH, Benign prostatic hyperplasia; PMC-NP, microcarcinoma without progression; PMC+P, microcarcinoma with progression; PC, infiltrating prostatic carcinoma; G1, G2, G3, histological grades of PC

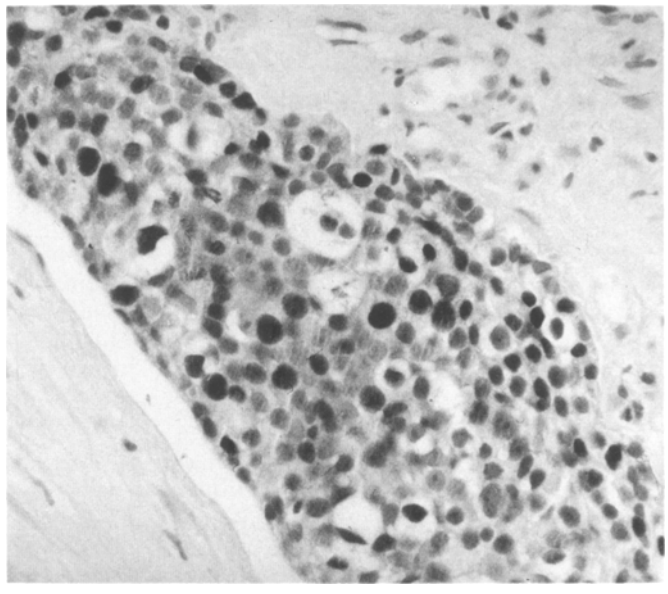

Fig. 2. Proliferating cell nuclear antigen/cyclin immunostaining of sections from prostatic microcarcinoma with progression. (Streptavidin-biotin immunoperoxidase, ×250)

PCNA/cyclin immunostaining (Fig. 2) was quite similar to that of grade 2–3 PC (Fig. 3).

Statistical analysis showed no differences in PCNA/cyclin index between BPH and PMC-NP, whereas PC and PMC+P had a higher mean value of PCNA/cyclin than previous groups. Moreover, the PCNA/cyclin index was higher in poorly differentiated tumours ($P \leq 0.01$ in PC G3), whereas grade 1 PC and PMC+P presented quite similar scores ($16.58 \pm 2.48 / 18.58 \pm 3.67$).

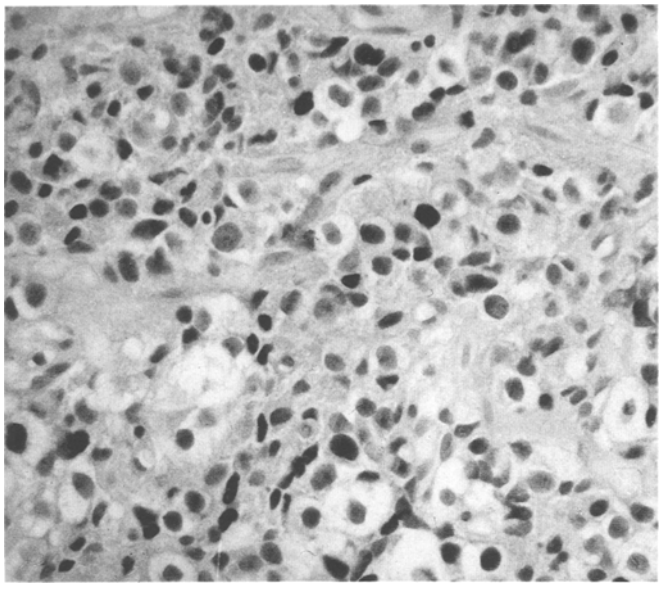

Fig. 3. Proliferating cell nuclear antigen/cyclin-immunostaining of sections from infiltrating prostatic carcinoma. (Streptavidin-biotin immunoperoxidase, ×250)

The 3-, 4- and 5-year survival rates (YSR) for G 3 PC were 80%, 60% and 40%, respectively; the 4-, 5- and 6-YSR for G2 PC 80%, 60% and 20%; the 6-, 7- and 8-YSR for G1 PC 80%, 50% and 10% and the 7- and 8-YSR for PMC+P 90% and 40%. The Cox proportional hazard model with stepwise selection was used to estimate PCNA index in each group of all 60 cases. Analysis demonstrated χ^2 4.18 for PCNA with the P value statistically significant ($P \leq 0.05$).

Discussion

PMC has to be differentiated from various benign conditions and precancerous lesions. In several non-malignant epithelial conditions the presence of basal cells, detected by means of keratin 903 antibody, may be useful in identifying clear cell cribriform hyperplasia without nuclear anaplasia (Mostofi et al. 1992) and tubular or cribriform typical or atypical basal cell hyperplasias (Epstein and Armas 1992). Moreover, in sclerosing adenosis the cells are considered to be of myoepithelial nature, because of the strong positivity for keratin 903 and actin antibodies (Collina et al. 1992). Basal cells may be absent focally in florid glandular, post-atrophic (Mostofi et al. 1993) and post-undeveloped prepubertal prostatic unit (Battaglia 1989) hyperplasia, or completely in microacinar hyperplasia. In all these cases no nuclear nor nucleolar atypia is detected (Mostofi et al. 1992, 1993). However, PMC may be preceded by two different premalignant lesions: prostatic intraepithelial neoplasia (Bostwick and Brawer 1987) or dysplasia (McNeal and Bostwick 1986; Kastendieck and Helpap 1989; McNeal et al. 1991) and atypical adenomatous hyperplasia (AAH; Kastendieck 1980; Kovi and Mostofi 1989; Mostofi et al. 1993). AAH, adenosis (Brawn 1982) and PMC show the same histological features with abnormal architecture and dispersion in the stroma, cytological pattern, biological behaviour, predictive prognosis and malignant progression (about 7% of cases). They may be considered to be the same neoplastic lesion. In these cases additional histological sections are mandatory to ensure that an extensive infiltrating PC is not found.

Studies concerning the prognostic role of PCNA/cyclin index in PC give conflicting results. Harper et al. (1992) and Spires et al. (1992) reported a good correlation between PCNA/cyclin score and histological grading and prognosis, whereas Hanna et al. (1992) and Visakorpi (1992) found no correlation between PCNA/cyclin expression and prognosis. The present study showed a lower PCNA/cyclin score in histological grade 1 than in grades 2 and 3 PC. Moreover, the mean values of PCNA/cyclin-immunostained nuclei of PMC-NP were quite similar to those expressed in BPH and significantly lower than those detected in PMC+P that had quite similar PCNA mean values with respect to grade 1 PC (PMC-NP < PMC+P < G2,G3 PC). Therefore, PCNA/cyclin index in PC indicates a close relationship to histocytological grades and in PMC, together with size (< or > 5 mm main diameter), may represent a potential diagnostic tool for differentiating PMC from foci of extratubulo-alveolar proliferations with or without atypia.

In terms of clinical correlations, the predictive value of the survival rates for PCNA score in PMC+P was statistically significant ($P \leq 0.05$), and they were also indicators of malignant progression of PC and PMC+P compared with PMC-NP ($P \leq 0.001$). In conclusion, the PCNA index seems to be an additional or independent prognostic indicator of tumour growth and of clinical progression ($P \leq 0.001$) and survival rates ($P \leq 0.05$) in PC and PMC.

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