Virchows Archiv A Pathol Anat (1993) 422:185–192

Virchows Archiv A
Pathological Anatomy and Histopathology

Prostatic intra-epithelial neoplasia: expression and location of proliferating cell nuclear antigen in epithelial, endothelial and stromal nuclei*

R. Montironi, C. Magi Galluzzi, L. Diamanti, I. Giannulis, E. Pisani, M. Scarpelli

Institute of Morbid Anatomy and Histopathology, University of Ancona, Nuovo Ospedale Regionale, Via Conca, I-60020 Torrette di Ancona, Italy

Received August 11, 1992 / Received after revision November 6, 1992 / Accepted November 10, 1992

Abstract. The expression and location of proliferating cell nuclear antigen (PCNA) immunostaining in epithelial, endothelial and stromal nuclei were assessed in prostatic intra-epithelial neoplasia (PIN). It was then compared with patterns in benign lesions and in invasive adenocarcinomas of the prostate. The PCNA-positive nuclei showed homogeneous or granular types of staining, or a mixture of both, and a gradation in the intensity of staining. Nuclei with granular and mixed patterns appeared lighter brown than those with a homogeneous pattern, which were darker and more often noted in PIN and invasive adenocarcinomas than in benign lesions. For epithelial PCNA-stained nuclei, the proportions in the two grades of PIN were greater than in benign prostatic hyperplasia (mean 3.16%, SE 0.31%) and prostatic atrophic ducts and acini (mean 0.56%, SE 0.09%), the values decreasing from the nuclei in the basal position towards those in the luminal layer. In grade 1, the category mean values were 9.51% (SE 1.14%) in the basal, 7.02% (SE 1.27%) in the intermediate and 6.02% (SE 0.90%) in the luminal position. In grade 2, the category mean values were 13.81% (SE 1.42%) in the basal position, 10.99% (SE 1.17%) in the intermediate and 7.91% (SE 1.43%) in the luminal position. In small and large acinar adenocarcinomas, the proportions of positive nuclei were 8.66% (SE 0.30%) and 9.06% (SE 0.30%), respectively. The category mean values in the cribriform adenocarcinomas were 14.40% (SE 0.61%) in the basal position, 11.84% (SE 1.30%) in the intermediate and 9.26% (SE 0.66%) in the luminal position. As in PIN, the proportions of immunostained nuclei in the adenocarcinoma with cribriform pattern decreased from the basal towards the luminal layer. In the solid/trabecular adenocarcinomas, the category mean value in the cell layer adjacent to the stroma was 17.60% (SE 2.92%), whereas in the other cell layers it was lower than that in the cells adjacent the stroma (mean 13.88%, SE 1.71%). For capillary endothelial and stromal cells, the percentages of PCNA-stained nuclei were much lower than those in the epithelial component. The lowest mean values were obtained in benign lesions, whereas the highest were in invasive adenocarcinomas, the percentages in PIN being intermediate.

Key words: Prostate – Proliferating cell nuclear antigen – Prostatic atrophy – Benign prostatic hyperplasia – Prostatic intra-epithelial neoplasia

Introduction

Prostatic intra-epithelial neoplasia, or intraductal dysplasia of the prostate, is considered to be a premalignant lesion which can progress to invasive adenocarcinoma (Bostwick and Brawer 1987; Kastendieck and Helpap 1989; McNeal and Bostwick 1986). PIN affects ducts and acini and is basically defined as proliferation and anaplasia of luminal (or secretory) cells (Bostwick and Brawer 1987). The changes are not abrupt but increase progressively and are based on the subjective evaluation of architectural features (epithelial crowding, stratification and spacing), cytological features (nuclear enlargement and size variability, chromatin pattern, and nucleolar frequency and prominence), together with intactness or disruption of the basal cell layer and basement membrane (associated features) (McNeal and Bostwick 1986). These groups of features have been evaluated quantitatively, allowing us to acquire an objective knowledge of PIN and of its progression towards the invasive phase of the adenocarcinoma (Deschenes and Weidner 1990; Hansen and Ostergard 1990; Helpap 1988; Montironi et al. 1990a, b, 1991, 1992b; Patein et al. 1991).

Neither qualitative nor quantitative data on the location of proliferating nuclei have been published and scant information on the state of proliferation of PIN

* This paper was read in part at the Workshop on Chemoprevention of Premalignant and Early Malignant Lesions of the Prostate, Scottsdale, Arizona, USA, 27–29 March, 1992

Correspondence to: R. Montironi

is available in the literature (Deschenes and Weidner 1990; Hansen and Ostergard 1990; Patein et al. 1991). This prompted us to evaluate the expression and location of the proliferating cell nuclear antigen (PCNA) in epithelial, endothelial and stromal nuclei in PIN in comparison with benign lesions, such as prostatic atrophic ducts and acini (PADA) and benign prostatic hyperplasia (BPH), and with prostatic invasive adenocarcinoma (PAC).

Materials and methods

The present study was performed on histological sections of 80 prostatectomy/prostatic adenectomy cases retrieved from the slide files of the Institute of Morbid Anatomy and Histopathology at the University of Ancona. The ages of the patients ranged from 60 to 70 years. No patient had received chemotherapy, hormonal therapy or radiation therapy before surgery.

The archive material had been fixed for 24–48 h in neutral buffered formalin (4%), dehydrated in alcohols (50%, 70%, 90%, 95% twice, 100% twice), cleared in xylene (twice) and embedded in paraffin. Using haematoxylin- and eosin-stained sections, one member of our team (M.S.) reviewed all the histological slides, checked the quality of the material and selected the slides for quantitative evaluations. Because prostate lesions were being sought, the slides selected mainly covered the peripheral zone of the prostate. The following morphological patterns of prostatic disease were investigated: BPH (20 cases), PADA (20 cases), PIN of low grade (PIN1; 10 cases) and of high grade (PIN2; 10 cases), and PAC (5 cases with cribriform, 5 with solid/trabecular, 5 with small acinar and 5 with large acinar pattern) (Mostofi et al. 1980). All the invasive adenocarcinomas were stage B, according to Jewett (1975). The part of the section to be evaluated was marked on the slide with a pen. Areas with acute and chronic inflammation were carefully avoided. PIN was selected in slides where PAC was not present, so as to avoid the risk of measuring the intraductal spreading of adenocarcinomatous cells; in those cases where the PIN grade was not homogeneous, the highest was always selected. Since the prostatic epithelium adjacent to PIN and PAC usually shows a proliferative pattern (Bostwick and Brawer 1987), BPH was selected in case where PIN and PAC were not present.

Conventional histological serial sections (5 μm thick) were cut from formalin-fixed, paraffin-embedded material. Sections were mounted on poly-L-lysine-coated glass slides and air dried overnight at room temperature. Sections were dewaxed in xylene, rehydrated through graded alcohols and then immersed in 3% hydrogen peroxide in methanol for 10 min to block endogenous peroxidase activity. Sections were then washed in phosphate buffered saline (PBS), and normal goat serum was applied for 10 min to reduce non-specific antibody binding. The monoclonal anti-PCNA antibody (PC10, Dako) was used at a concentration of 0.5 μg/ml for 10 min at room temperature. Biotinylated goat anti-mouse antibody was used as the linker molecule and was applied at a dilution of 1 in 100 for 10 min. After further washing, sections were incubated in streptavidin-horseradish peroxidase complex (Cambridge Biosciences) at a 1 in 100 dilution for 10 min. Diamino-benzidine-hydrogen peroxide (Sigma) was used as chromogen and Mayer's haematoxylin counterstain was applied. Sections were dehydrated in alcohol, cleared in xylene, and mounted in DPX. To ensure consistency of PCNA staining between batches, a known positive control PAC was included in each round. The experiment was repeated if there was any measurable fluctuation in the PCNA count of this control. Negative controls were included by performing duplicate assays, on one of which the primary antibody was replaced with PBS.

For the analyses of PCNA expression and location, the sections were evaluated by one of our team (L.D.) using a Leitz Orthoplan microscope equipped with a ×63 objective and with an eyepiece graticule (Montironi et al. 1992a). All identifiable staining was regarded as positive. The percentage of PCNA-stained nuclei from a minimum of 2000 nuclei per case (1000 epithelial, 500 endothelial and 500 stromal cells) was then evaluated. In the epithelial component, in particular, the percentage of stained nuclei was separately calculated in each cell layer (see below). The time needed to analyse each case was approximately 90 min.

The data were stored in an Apple Macintosh II computer. StatView II software was used for the calculation of the category mean and standard error (SE) as well as for statistical analyses (Kruskal-Wallis and Mann-Whitney tests; Spearman's rank correlation).

Reproducibility was tested by duplicate evaluations of the PCNA-related feature in six cases (two of BPH, two of PIN and two of PAC). No statistically significant differences were found.

Results

PCNA immunostaining was confined to the nucleus with the exception of cells in mitosis, which showed faint cytoplasmic staining. Nuclei with pyknotic chromatin were not stained. The nucleoli did not show a consistent pattern of staining and many remained unstained. In particular, the positive nuclei showed homogeneous or granular types of staining, or a mixture of both, and a gradation in the intensity of staining. Nuclei with homogeneous patterns appeared darker brown than the lighter granular and mixed patterns. Darker nuclei, generally not seen in the PADA but present to some extent in BPH, were quite frequently noted in PAC, mainly among the epithelial cells adjacent to the stroma. In PIN1 and PIN2, darker nuclei were seen almost as often as in invasive adenocarcinomas.

In BPH, the ducts and acini appeared lined by a two-cell type epithelium, i.e. the basal cell layer and the luminal (secretory) cell layer. PCNA immunostaining was almost exclusively seen in the basal cell layer (Fig. 1), whose category mean value was 3.16% (SE 0.31%). As for the luminal cells, PCNA positive nuclei were exceptionally rare (three positive nuclei in all the secretory cells evaluated). In PADA basal cells were not identifiable by light microscopy. The ducts and acini appeared lined by cells with scanty cytoplasm. The proportion of PCNA-immunostained nuclei was 0.56% (SE 0.09%), i.e. much lower than in BPH (Mann-Whitney: $P < 0.0005$).

PIN was characterized by the presence of cells that appeared stratified and crowded. PIN1 included cases that showed morphological features of mild and/or moderate dysplasia, PIN2 showing those of severe dysplasia and/or carcinoma in situ (Montironi et al. 1990a). In both grades the percentage of positive nuclei was counted separately for the cells adjacent to the basement membrane, for the cells bordering the lumen and for the cells in the position intermediate between the basal and the luminal layers. In both, the proportions of immunostained nuclei decreased from the basal position towards the lumen (Fig. 2). For PIN1, the category mean values were 9.51% (SE 1.14%) in the basal, 7.02% (SE 1.27%) in the intermediate and 6.02% (SE 0.90%) in the luminal position (Kruskal-Wallis $P < 0.1$; Mann-Whitney: basal vs intermediate $P < 0.05$, basal vs luminal $P < 0.05$, intermediate vs luminal $P < 0.8$). For PIN2,

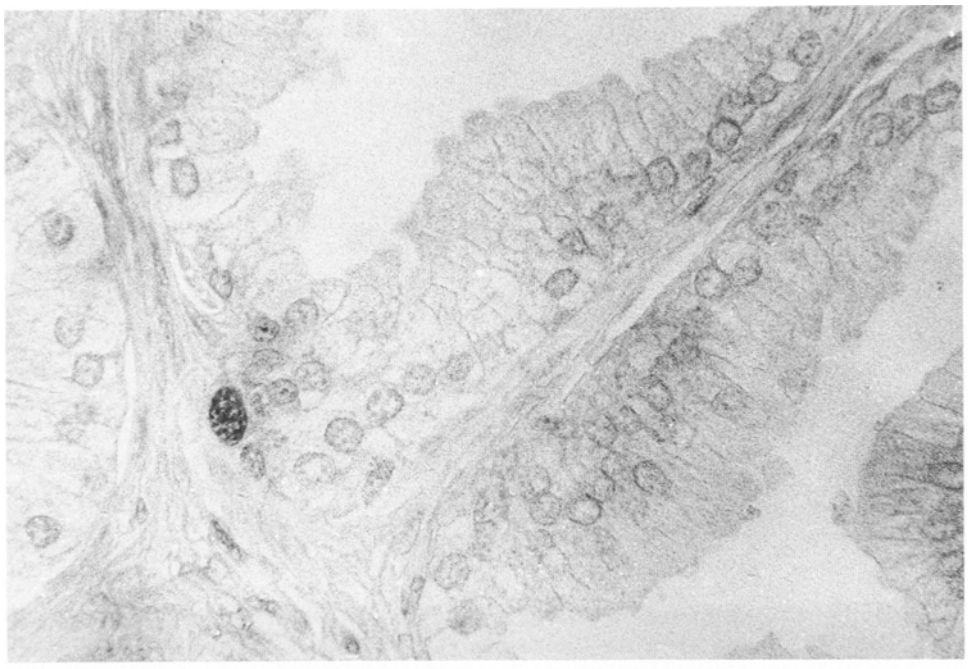

Fig. 1. Anti-proliferating cell nuclear antigen (PCNA) immunocytochemistry in benign prostatic hyperplasia (BPH). Note staining of a nucleus in the basal cell layer. Immunoperoxidase with haematoxylin counterstain, ×400

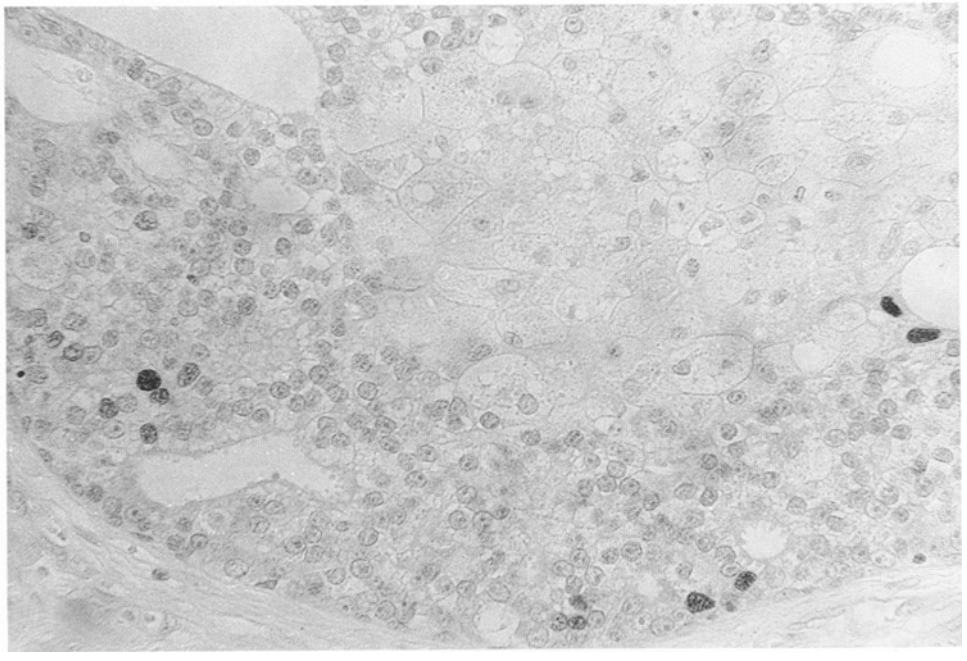

Fig. 2. Prostatic intra-epithelial neoplasia (PIN) labelled with anti-PCNA shows positivity of nuclei in all the cells layers. Immunoperoxidase with haematoxylin counterstain, ×250

the category mean values were 13.81% (SE 1.42%) in the basal position, 10.99% (SE 1.17%) in the intermediate and 7.91% (SE 1.43%) in the luminal position (Kruskal-Wallis $P<0.01$; Mann-Whitney: basal vs intermediate $P<0.2$, basal vs luminal $P<0.01$, intermediate vs luminal $P<0.05$). In PIN1 the percentages were slightly lower than in PIN2 (Mann-Whitney: basal PIN1 vs basal PIN2 $P<0.05$; intermediate PIN1 vs intermediate PIN2 $P<0.01$; luminal PIN1 vs luminal PIN2 $P<0.4$) and higher than in BPH (Mann-Whitney: basal PIN1 vs BPH $P<0.0005$).

For adenocarcinomas with cribriform pattern (large acinar structures filled with epithelial cells forming multiple gland-like lumens), the proportions of immunostained nuclei, slightly greater than in PIN2, decreased from the basal position, or adjacent to the stroma, towards the lumen (Fig. 3). In fact, the category mean values were 14.40% (SE 0.61%) in the basal position, 11.84% (SE 1.30%) in the intermediate and 9.26% (SE 0.66%) in the luminal position (Kruskal-Wallis $P<0.05$; Mann-Whitney: basal vs intermediate $P<0.2$, basal vs luminal $P<0.01$, intermediate vs luminal $P<0.2$; basal cribriform vs basal PIN2 $P<0.8$). In the solid/trabecular pattern, the percentage of positive nuclei was counted separately for the cells adjacent to the stroma and for the cells in the other cell layers. The category mean value

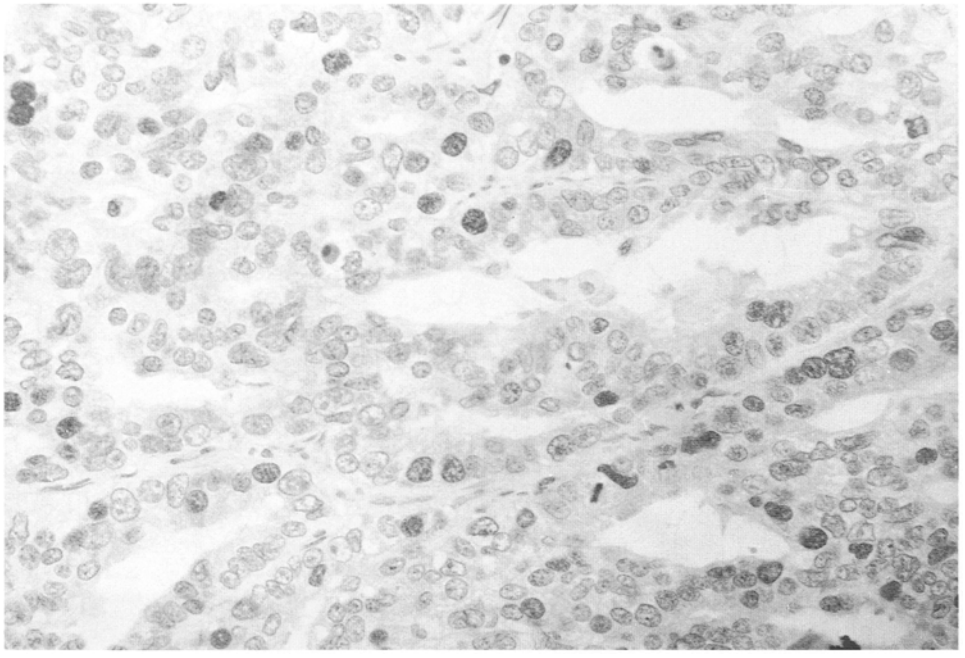

Fig. 3. Adenocarcinomas with cribriform pattern immunostained with anti-PCNA. Immunoreactivity is clearly seen in all the nuclear layers. Immunoperoxidase with haematoxylin counterstain, ×250

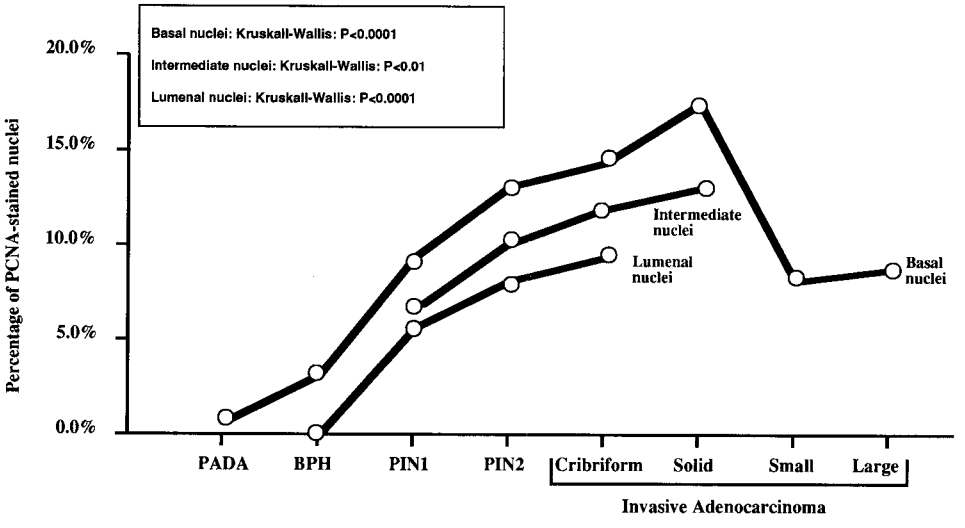

Fig. 4. Line chart of PCNA staining of the basal, intermediate and luminal nuclei in the epithelial component. The proportions in prostatic intra-epithelial neoplasia (*PIN1* and *PIN2*) are greater than in prostatic atrophic ducts and acini (*PADA*) and benign prostatic hyperplasia (*BPH*), the values decreasing from the basal position towards the luminal layer. For PIN1, the value is slightly lower than in PIN2. The percentages in adenocarcinomas with cribriform and solid/trabecular patterns are higher than in other categories; as with PIN, the values in the cribriform decrease from the basal towards the luminal layer

in the cell layer adjacent to the stroma was 17.60% (SE 2.92%), whereas in the other cell layers it was lower than that in the cells adjacent the stroma (mean 13.88%; SE 1.71%) (Mann-Whitney: $P<0.2$). The proportions of immunostained nuclei were highest in comparison with other adenocarcinoma categories. The percentage of immunostained nuclei in the small acinar pattern was somewhat similar to those of PIN1 and close to that in the large acinar pattern, i.e. 8.66% (SE 0.30%) and 9.06% (SE 0.30%), respectively (Mann-Whitney: small acinar vs basal PIN1 and large acinar vs basal PIN1 $P<0.8$; small acinar vs large acinar $P<0.6$) (Fig. 4).

Morphologically, capillaries in the lesions appeared as short or elongated vessels with clearly identifiable endothelial nuclei. In the BPH, PADA and PIN categories, the capillaries were located in close contact with or in proximity to the basement membrane of ducts and acini. In contrast, in the PAC category, the capillaries in general appeared within the tumour stroma and septa (Montironi et al. 1992b). The percentages of PCNA-stained nuclei were much lower than those in the epithelial component. The lowest mean values were seen in PADA and BPH: 0.21% (SE 0.04%) and 0.24% (SE 0.05%), respectively. The highest mean values were seen in PAC: cribriform 1.04% (SE 0.19%), solid/trabecular 1.12% (SE 0.22%), small acinar 0.92% (SE 0.20%) and large acinar 0.94% (SE 0.23%), respectively. The category mean value in PIN1 was 0.80% (SE 0.26%), i.e. between the percentages seen in benign lesions (BPH and PADA) and those in the adenocarcinomas. The category mean value in PIN2 was 0.94% (SE 0.15%), similar to that obtained in small and large acinar adenocarcinomas (Fig. 5).

The stroma surrounding the ducts and acini with BPH, PADA and PIN lesions and within the PAC septa was of the fibromuscular type. The percentages of

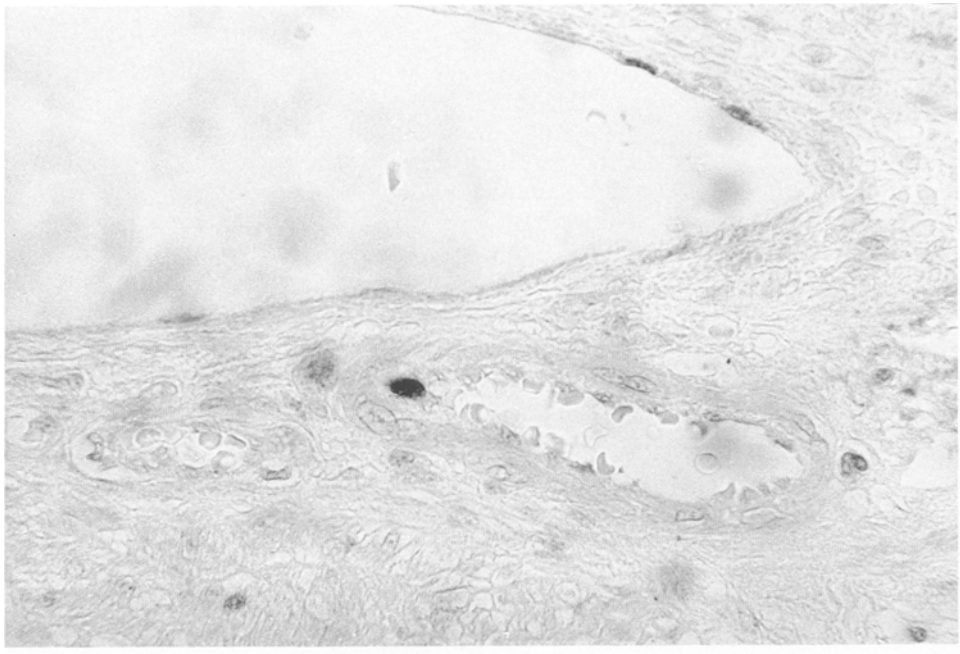

Fig. 5. PCNA immunostaining of an endothelial nucleus. Immunoperoxidase with haematoxylin counterstain, ×250

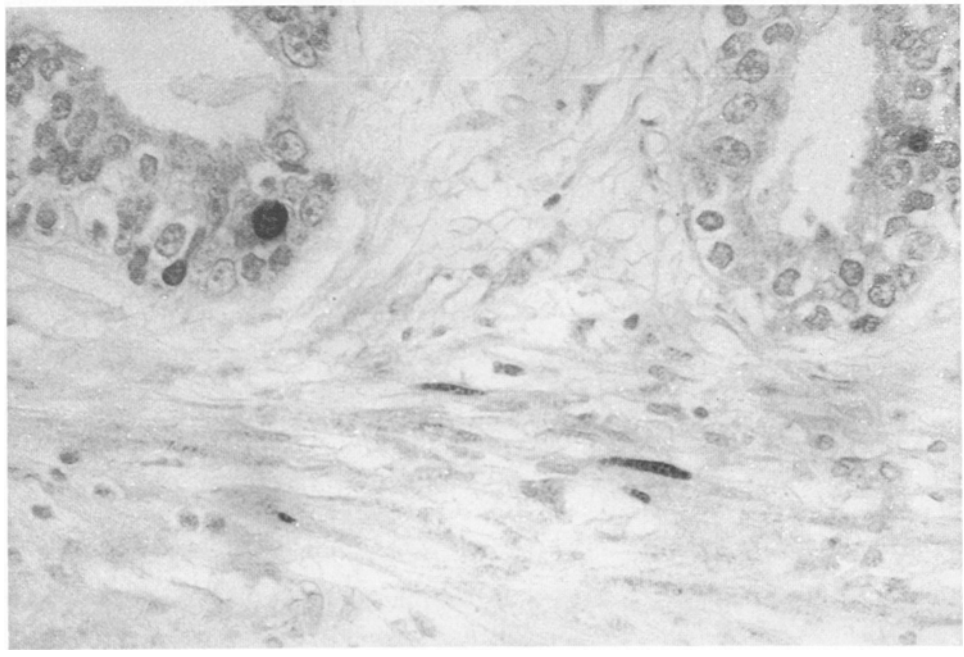

Fig. 6. PCNA immunostaining of stromal nuclei. Immunoperoxidase with haematoxylin counterstain, ×250

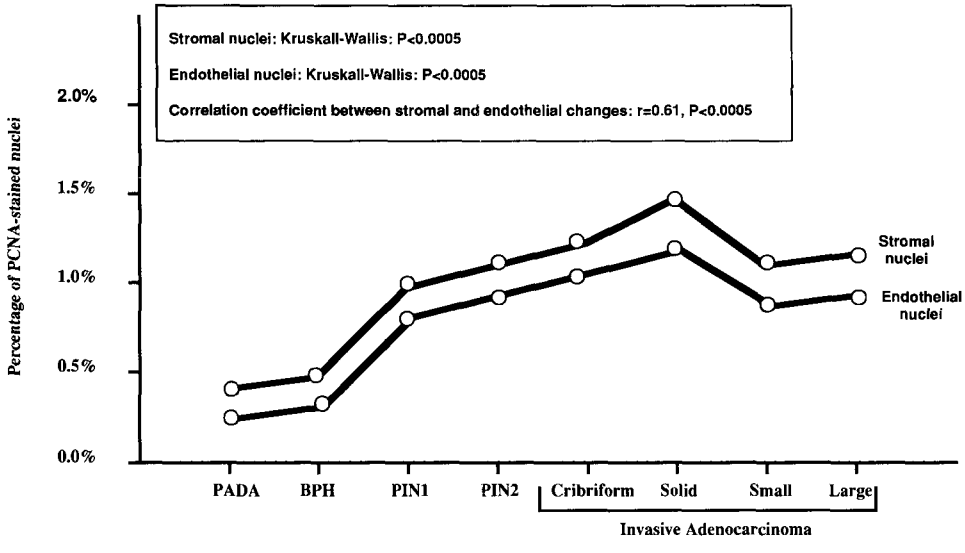

Fig. 7. Line chart of PCNA staining in the capillaries and stroma. There is a significant correlation in the nuclear PCNA expression between the percentages of endothelial nuclei and of stromal nuclei

PCNA-stained nuclei were much lower than those in the epithelial component and slightly higher than in the endothelium (Figs. 6, 7). As in the capillaries, the lowest mean values were seen in PADA (mean 0.32%, SE 0.05%) and BPH (mean 0.40%, SE 0.08%), the highest being in PAC (cribriform: mean 1.16%, SE 0.32%; solid/trabecular: mean 1.40%, SE 0.2%; small acinar: mean 1.05%, SE 0.22; large acinar: mean 1.08%, SE 0.22%). The category mean value in PIN1 was 0.96% (SE 0.22%), i.e. between the percentages seen in benign lesions and those in adenocarcinomas. The category mean value in PIN2 was 1.04% (SE 0.12%), i.e. similar to that obtained in small and large acinar adenocarcinomas. The significance tests for capillary endothelial and for stromal nuclei gave the same results, i.e. Kruskall-Wallis test: $P<0.0005$; Mann-Whitney test: PADA vs BPH $P<0.8$; PADA vs PIN, PADA vs PAC, BPH vs PIN, BPH vs PAC, PIN vs PAC $P<0.0001$. There was a significant correlation between epithelial and endothelial changes ($r_s=0.71$, $P<0.0005$), between epithelial and stromal changes ($r_s=0.67$, $P<0.0005$), and between endothelial and stromal changes ($r_s=0.61$, $P<0.0005$).

Discussion

We noted that the positive nuclei showed homogeneous or granular types of staining, or a mixture of both, and that the nuclei with homogeneous patterns appeared darker brown than those with lighter granular and mixed patterns. Takahashi et al. (1991) also mentioned that PCNA-positive tumour cells increased in staining intensity from common melanocytic naevi through dysplastic naevi to malignant melanoma. It is well known that PCNA is present in the highest concentrations during the S phase (DNA synthesis phase); the level decreases during G2, is lowest during mitosis, and increases during G1 (Celis and Celis 1985; Coltrera and Gown 1991; Garcia et al. 1989; Kamel et al. 1991; Linden et al. 1992; Morris and Mathews 1989). This was also observed in breast carcinomas, where the proportions of PCNA-positive nuclei were compared with the cell cycle fractions determined with static and flow cytometric techniques (Montironi et al. 1992a). In our opinion, these results and observations suggest that DNA synthesis is practically absent in PADA, is present only to some extent in BPH, is highest in PAC and is either intermediate or close to PAC in PIN.

We do not know why nuclei with pyknotic chromatin were not stained by the anti-PCNA antibody. We assume that these nuclei belong to the group of apoptotic cells, which are not cycling and therefore not expressing the PCNA antigen.

Our work showed that the values of the epithelial PCNA-stained nuclei in PIN lesions are higher than in benign lesions and close to the values in PAC indicating that the epithelial proliferating fractions are progressively greater from BPH through PIN to PAC. The data we obtained in BPH and PAC are similar to those determined by Ki-67 immunostaining and published by Raymond et al. (1988). According to these authors, BPH showed an average Ki-67 score of 4%, significantly less than the 16.3% mean growth fraction found in PAC. In our study, the mean value in BPH was 3.16%, whereas the percentages in PAC ranged from 8.66% (small acinar pattern) to 17.60% (solid/trabecular pattern). Little information on the proliferation state is available in the PIN literature. It has been investigated to a small extent by DNA static cytometry and by analyses of silver-stained nucleolar organizer regions (AgNORs) (Deschenes and Weidner 1990; Hansen and Ostergard 1990; Patein et al. 1991). For the nuclear DNA content, Patein et al. (1991) found that the DNA proliferation index, derived from DNA histogram analysis, showed values which increased with the severity of PIN. Quantitative analyses of AgNORs, a marker related to cell proliferation (Derenzini et al. 1990), showed that AgNOR counts and area increase from BPH to PIN and to PAC (Deschenes and Weidner 1990; Hansen and Ostergard 1990). Our PCNA-related study confirms the information obtained with these types of evaluation and agrees with earlier studies performed by Helpap (1980), who investigated atypical hyperplasia of the prostate using the thymidine labelling index. According to this author, the mean labelling index of atypical hyperplasia, a diagnostic category in which he probably included PIN along with other proliferative lesions, was similar to that of poorly differentiated adenocarcinomas and cribriform carcinoma. Our results agree with this observation. In fact, the proportions of PCNA-stained nuclei in the adenocarcinomas with cribriform and solid/trabecular patterns are slightly greater than in PIN2, whereas in the small and large acinar patterns the proportions are similar to those in PIN1. This also indicates that the degree of cell proliferation in the PAC varies according to the morphology and agrees with the concept that there is correlation of prognosis with the morphological patterns and, therefore, with the degree of cell proliferation (Raymond et al. 1988).

PCNA immunostaining also allowed the identification of the precise location of the proliferating cells in relation to the epithelial architecture. In BPH, only the nuclei in the basal position express PCNA. Cell kinetic organization within normal cell population has already been investigated in some epithelia, for instance in the intestinal crypts and in the squamous cell epithelium (Wright 1984), mostly with techniques other than PCNA immunostaining. In the intestinal crypts, it has been observed that mitotic activity is confined to the lower two-thirds of the crypt. No cell division occurs in the upper crypt, which is regarded as a transit compartment. From here cells move towards the villus to differentiate. In the squamous cell epithelium, the basal layer represents the proliferative compartment, from which the cells migrate to the surface in order to become mature or differentiated. This type of information on spatial distribution of proliferating cells was recently confirmed by Garcia et al. (1989), Hall et al. (1990) and Wilson et al. (1990) using anti-PCNA antibodies. The organization of cell proliferation and differentiation in the normal prostate epithelium could be considered to resemble the ordered structure of the squamous cell epithelium more closely

than the intestinal crypt. In particular, the basal cell layer of prostate ducts and acini, which contains cells either able to divide or dividing, is the proliferative compartment. The luminal layer, which is unable to proliferate because it contains post-mitotic maturing and mature cells, is the differentiated compartment (McNeal 1988).

PIN lesions show positive nuclei in all cell layers, even though the proportion of positive nuclei decreases from the basal position, through the intermediate layers, to the luminal cells. A similar pattern was seen in invasive cribriform adenocarcinomas. This indicates firstly that the main location of the epithelial cell proliferation in benign, preneoplastic and neoplastic lesions is the cell layer adjacent to the stroma; secondly, that cells able to proliferate appear in all layers of PIN and PAC, thus resulting in the expansion of the proliferative compartment; and, thirdly, that even though proliferating cells are present in all cell layers, their proportion decreases towards the lumen because the cells acquire some degree of differentiation. The type of expansion of the proliferative compartment seen in the ducts and acini with PIN changes has been investigated in the squamous cell epithelium of the uterine cervix using techniques other than PCNA immunostaining (Mariuzzi et al. 1989). In the cervix it has already been shown that the width of the proliferative compartment is related the degree of dysplasia, occupying the entire epithelial thickness in carcinoma in situ. In these lesions, the differentiating and differentiated compartments decrease in width and/or disappear. For the intestine, Wilson et al. (1990) observed a significant expansion of the proliferative compartment in the colonic crypts of subjects with polyps. What has not been observed previously in preneoplastic lesions is that the proportion of proliferating cells decreases from the basal layer towards the lumen.

The PCNA-related data obtained in the capillary endothelial cells confirm the results of a previous paper (Montironi et al. 1992b) in which progressive changes in the capillary pattern were associated with an increasing number of endothelial nuclei. A similar result was obtained by Chodak et al. (1980). They investigated "angiogenic activity" as a marker of the development of preneoplastic and neoplastic lesions of the human bladder in an experimental study in which human bladder biopsies were implanted into a rabbit iris. The numbers of endothelial cells per capillary lumen and endothelial cell mitoses were found to be greater in invasive carcinoma and in association with biopsies containing carcinoma in situ and atypical epithelium than normal. It has been proved that the increased number of endothelial cells is an expression of the proliferation of the endothelial cells themselves (Brem et al. 1977; Cavallo et al. 1972). Hobson and Denekamp (1984) investigated the proliferation of vascular endothelium using tritiated thymidine. They found large differences in labelling indices between neoplastic and normal tissue.

The stromal cells surrounding the ducts and acini with PIN changes and the tumour nodules show that the nuclear expression of PCNA increases from BPH and PADA through PIN to PAC and parallels that in the epithelium and in the endothelium. These findings support the concept that the progression of PIN to PAC represents a complex process which simultaneously involves the epithelium and the capillaries as well as the stroma (Montironi et al. 1992c). Even though, to the best of our knowledge, there are no previous studies on the degree of stromal nuclear proliferation in prostate lesions, there are papers which suggest the existence of mesenchymal-epithelial interactions in prostatic development and carcinogenesis. For instance, McNeal (1983) proposed that the aetiology of BPH may be based upon reactivation of embryonic-like inductive activity within the stroma cells of BPH nodules. The possible role of stromal-epithelial interactions in prostatic carcinogenesis was emphasized by Cunha and Donjacour (1987).

In conclusion, analysis of PCNA-stained nuclei allowed precise evaluation of the expression of proliferating cells and the exact identification of their location in PIN. The main advantage of using PCNA immunostaining was that this technique could be applied in routinely processed histological sections in which the tissue architecture and cell-to-cell relationships were preserved and appeared to be of fundamental help in the identification of nuclei expressing PCNA. In this study, cells were read as either positive or negative, even though some qualitative description of PCNA patterns and intensity was given. Further analyses are currently underway in this institute to quantify variations in nuclear immunostaining patterns and intensity. Additional cell-cycle-related information may be forthcoming.

References

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

Brem SS, Gullino PM, Medina D (1977) Angiogenesis: a marker for neoplastic transformation of mammary papillary hyperplasia. Science 195:880–882

Cavallo T, Sade R, Folkman J, Cotran RS (1972) Tumor angiogenesis: rapid induction of endothelial mitoses demonstrated by autoradiography. J Cell Biol 54:408–411

Celis JE, Celis A (1985) Cell cycle-dependent variations in the distribution of the nuclear protein cyclin proliferating cell nuclear antigen in cultured cells: subdivision of S phase. Proc Natl Acad Sci USA 82:3262–3266

Chodak GW, Haudenschild C, Gittes RF, Folkman J (1980) Angiogenic activity as a marker of neoplastic and preneoplastic lesions of the human bladder. Ann Surg 192:762–771

Coltrera MD, Gown AM (1991) PCNA/Cyclin expression and BrdU uptake define different subpopulations in different cell lines. J Histochem Cytochem 39:23–30

Cunha GR, Donjacour AA (1987) Stromal-epithelial interactions in normal and abnormal prostatic development. In: Coffey DS, Bruchovsky N, Gardner WA Jr, Resnick MI, Karr JP (eds) Current concepts and approaches to the study of prostate cancer. Liss, New York, pp 251–272

Derenzini M, Pession A, Trerè D (1990) The quantity of nucleolar silver-stained proteins is related to proliferating activity in cancer cells. Lab Invest 63:137–140

Deschenes J, Weidner N (1990) Nucleolar organizer regions (NOR) in hyperplastic and neoplastic prostate disease. Am J Surg Pathol 14:1148–1155

Garcia RL, Coltrera MD, Gown AM (1989) Analysis of proliferative grade using anti PCNA/Cyclin monoclonal antibodies in fixed, embedded tissues. Am J Pathol 134:733–739

Hall PA, Levison D, Woods AL, Yu CC-W, Kellock DB, Watkins JA, Barnes DM, Gillett CE, Camplejohn R, Dover R, Waseem NH, Lane DP (1990) Proliferating cell nuclear antigen (PCNA) immunolocalization in paraffin sections: an index of cell proliferation with evidence of deregulated expression in some neoplasms. J Pathol 162:285–294

Hansen AB, Ostergard B (1990) Nucleolar organizer regions in hyperplastic and neoplastic prostatic tissue. Virchows Arch [A] 417:9–13

Helpap B (1980) The biological importance of atypical hyperplasia of the prostate. Virchows Arch [A] 387:307–317

Helpap B (1988) Observations on the number, size and localisation of nucleoli in hyperplastic and neoplastic prostatic disease. Histopathology 13:203–211

Hobson B, Denekamp J (1984) Endothelial proliferation in tumours and normal tissues: continuous labelling studies. Br J Cancer 49:405–413

Kamel OW, LeBrun DP, Davis RE, Berry GJ, Warnke RA (1991) Growth fraction estimation of malignant lymphomas in formalin-fixed paraffin-embedded tissue using anti-PCNA/Cyclin 19A2. Am J Pathol 138:1471–1477

Kastendieck H, Helpap B (1989) Prostatic "dysplasia/atypical hyperplasia". Urology 34 [Suppl]:28–42

Jewett HJ (1975) The present status of radical prostatectomy for stage A and B prostatic cancer. Urol Clin North Am 2:105–123

Linden MD, Torres FX, Kubus Y, Zarbo RJ (1992) Clinical application of morphologic and immunocytochemical assessments of cell proliferation. Am J Clin Pathol 97 [Suppl 1]:S21–S29

Mariuzzi GM, Montironi R, Di Loreto C, Sisti S (1989) Multiparametric quantitation of the progression of uterine cervix preneoplasia towards neoplasia. Pathol Res Pract 185:606–611

McNeal JE (1983) The prostate gland: morphology and pathobiology. Monogr Urol 4:3–37

McNeal JE (1988) Normal histology of the prostate. Am J Surg Pathol 12:619–633

McNeal JE, Bostwick DG (1986) Intraductal dysplasia: a premalignant lesion of the prostate. Hum Pathol 17:64–71

Montironi R, Scarpelli M, Sisti S, Braccischi A, Gusella P, Pisani E, Alberti R, Mariuzzi GM (1991a) Quantitative analysis of prostatic intraepithelial neoplasia on tissue sections. Anal Quant Cytol Histol 12:366–372

Montironi R, Scarpelli M, Sisti S, Braccischi A, Mariuzzi GM (1990b) Morphologic and quantitative analyses of intraductal dysplasia of the prostate. Anal Cell Pathol 2:277–285

Montironi R, Braccischi A, Matera G, Scarpelli M, Pisani E (1991) Quantitation of the prostatic intra-epithelial neoplasia. Analysis of the nucleolar size, number and location. Pathol Res Pract 187:307–314

Montironi R, Diamanti L, Magi Galluzzi C, Mangili F, Cantaboni A (1992a) S-phase fraction analysis in DNA static cytometry in breast cancer. Comparison with proliferating cell nuclear antigen (PCNA) immunostaining. Acta Stereol 11:99–108

Montironi R, Magi Galluzzi C, Diamanti L, Taborro R, Scarpelli M, Pisani E (1992b) Prostatic intra-epithelial neoplasia. Qualitative and quantitative analyses of the blood capillary architecture on thin tissue sections. Pathol Res Pract (in press)

Montironi R, Scarpelli M, Magi Galluzzi C, Diamanti L (1992c) Aneuploidy and nuclear features of prostatic intraepithelial neoplasia (PIN). J Cell Biochem 164:47–53

Morris GF, Mathews MB (1989) Regulation of proliferating cell nuclear antigen during the cell cycle. J Biol Chem 264:13856–13864

Mostofi FK, Sesterhenn I, Sobin LH (1980) Histologic typing of prostate tumours. WHO, Geneva, pp 17–20

Patein M, Michel P, Van Velthoven R, Pasteels J-L, Brawer MK, Davis JR, Nagle RB, Kiss R (1991) Morphonuclear relationship between prostatic intraepithelial neoplasia and cancer as assessed by digital cell image analysis. Am J Clin Pathol 96:628–634

Raymond WE, Leong A S-Y, Bolt JW, Milios Y, Jose JS (1988) Growth fractions in human prostatic carcinoma determined by Ki-67 immunostaining. J Pathol 156:161–167

Takahashi H, Strutton GM, Parsons PG (1991) Determination of proliferating fractions in malignant melanomas by anti-PCNA/cyclin monoclonal antibody. Histopathology 18:221–227

Wilson RG, Smith AN, Bird CC (1990) Immunohistochemical detection of abnormal cell proliferation in colonic mucosa of subjects with polyps. J Clin Pathol 43:744–747

Wright NA (1984) Cell proliferation in health and disease. In: Anthony PP, MacSween RNM (eds) Recent advances in histopathology 12. Churchill Livingstone, Edinburgh, pp 17–33