CASE REPORT

High grade prostatic intraepithelial neoplasia with squamous differentiation

M Melissari, A Lopez Beltran, R Mazzucchelli, E Froio, D G Bostwick, R Montironi

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An unusual variant of prostatic intraepithelial neoplasia with prominent and extensive squamous differentiation is described. The lesion was identified in the transition zone of a 79 year old man with a three year history of increasing urinary obstructive symptoms and a clinical diagnosis of benign prostatic hyperplasia who underwent simple prostatectomy. Two years after surgery, prostatic biopsies showed atrophy and mild chronic inflammation, with no evidence of malignancy. This unusual intraepithelial lesion seems not to have been described before and may represent a new variant of high grade prostatic intraepithelial neoplasia (HGPIN) with squamous differentiation.

High grade prostatic intraepithelial neoplasia (HGPIN) is the most likely precursor of prostatic adenocarcinoma.^{1,2} The usual cell type comprising HGPIN is a glandular secretory epithelial cell. Multiple variants of HGPIN have been reported, including mucinous, foamy, inverted, signet ring, and small cell neuroendocrine,^{3–5} most of which are extremely rare. Similar features are occasionally seen in prostatic adenocarcinoma, underscoring the close relation between HGPIN and carcinoma. Squamous differentiation has been described in the benign prostate and prostatic carcinoma, but to our knowledge has not previously been reported in HGPIN.

CASE HISTORY

A 79 year old white man presented in late 2002 with a three year history of increasing urinary obstructive symptoms for which he had been treated with tamsulosin, an $\alpha(1A)/\alpha(1D)$ -adrenoceptor antagonist α blocker. There was no history of other drugs or hormonally active supplements, and he described being on a typical Mediterranean diet. Digital rectal examination was normal. Total serum prostate specific antigen (PSA) was 6.3 ng/ml, with a free to total ratio of 0.28. Transrectal ultrasound revealed diffuse enlargement. The clinical diagnosis was benign prostatic hyperplasia, and suprapubic (simple) prostatectomy was undertaken, resulting in successful normal voiding. Two years after surgery, sextant needle biopsies of the peripheral and central zones showed mild chronic inflammation in the periglandular stroma and atrophy, with no evidence of malignancy. PSA decreased after surgery and remained below 2.0 ng/ml.

METHODS

The suprapubic prostatectomy specimen weighted 50 g and measured 5×4×3 cm. The cut surface was white and nodular. Initially, 10 tissue blocks were randomly taken and embedded in paraffin. Subsequently the remainder of the specimen was submitted and histologically examined. Serial sections (4 μ m) were stained with haematoxylin and eosin, periodic acid-Schiff, and Alcian blue at pH 2.5.

Immunoperoxidase studies were performed on formalin fixed, paraffin embedded tissues using the Dako EnvisionTM system. The following antibodies were used: cytokeratin 34betaE12, (DakoCytomation, Denmark; 1:40 dilution); AE1/AE3 (BioGenex, San Ramon, California, USA; prediluted), p63 (DakoCytomation; 1:50 dilution), prostate specific antigen (DakoCytomation; 1:750 dilution), α -methylacyl-CoA racemase or AMACR (P504S; Corixa, Seattle, Washington, USA; 0.5 μ g/ml dilution), α -smooth muscle actin (BioGenex; prediluted), S-100 protein (BioGenex, prediluted), Ki-67 (DakoCytomation; 1:50 dilution), and chromogranin A (DakoCytomation; 1:100 dilution) and p53 (Novocastra, Newcastle upon Tyne, UK; 1:40 dilution). The immunohistochemical staining was recorded as either positive or negative. At least 500 cells were counted in each case.

In situ hybridisation (ISH) was employed to detect the presence of human papilloma virus (HPV), using a wide spectrum HPV biotinylated DNA probe, prediluted for ISH. The tyramide signal amplification system for biotinylated probes was used as detection system. Known positive and negative control tissues were included in the analysis and gave appropriate results.

Karyometry was undertaken by one of us (RMA) on haematoxylin-eosin (H&E) stained histological sections. A Zeiss-Kontron IBAS-AT image analyser (Munich, Germany) combined with a Zeiss light microscope equipped with a 100× oil immersion objective was used. Fifty nuclei were measured for each component (see below) and for the normal prostate (secretory cells). For comparison, 50 control nuclei were measured in typical lesions from other similarly prepared cases, including foci of low grade PIN, high grade PIN, adenocarcinoma (Gleason score 2+2), benign prostate with squamous differentiation, and squamous cell carcinoma in situ of the cervix.

RESULTS

Pathological findings

Typical histological and immunohistochemical findings of benign prostatic hyperplasia (BPH) were identified, with no evidence of malignancy.

Scattered ductules and acini distant from the periurethral region and amid the normal glands within the BPH nodules revealed HGPIN (fig 1, panels A and B).² Architectural growth patterns were micropapillary and tufted. The presence of basal cells was apparent by H&E staining and was confirmed by immunohistochemistry (see below). Luminal mucin (Alcian blue positive) was seen in some ductules and acini. A second component consisted of stratified glycogenated squamous epithelium protruding into and partially filling the lumens. The cells showed moderate nuclear

Abbreviations: BPH, benign prostatic hyperplasia; HGPIN, high grade prostatic intraepithelial neoplasia; ISH, in situ hybridisation; PIN, prostatic intraepithelial neoplasia

Figure 1 (A) Ductules and acini with HGPIN with squamous differentiation. The two components appear side by side, with direct transition from one to another. (B) HGPIN characterised by basophilic cytoplasm and enlarged nuclei with prominent nucleoli. The cells are stratified and crowded. Luminal mucin is seen. (C) Glycogenated squamous differentiation with nuclear atypia in association with typical HGPIN. (D) The secretory cells in the glandular component stain positively for PSA. (E) p63-Immunoreactive nuclei in the basal position are present in the glandular component, whereas positive nuclei are seen in all the cells layers of the squamous component. (F) The squamous component is diffusely keratin AE1/AE3 positive. HGPIN, high grade prostatic intraepithelial neoplasia.

pleomorphism, irregularly distributed chromatin, and nucleolar prominence. Mitotic figures were very rare. The cells located most centrally within the lumens showed shrunken nuclei, well defined cell borders, and moderately clear (empty) cytoplasm rich in glycogen (that is, koilocytosis-like feature) (fig 1C). HGPIN and the squamous component were intimately admixed, with direct transition from one to another. Squamous differentiation was not present in benign acini, and there were no ducts or acini with HGPIN that lacked the squamous component.

Focal and mild chronic inflammation was present in the stroma, away from the ducts and acini, with the features of HGPIN.

Immunohistochemistry and in situ hybridisation

The secretory cells of HGPIN expressed PSA (fig 1D), and were negative for p63 and keratin 34 β E12. Scattered p63 (fig 1E) and keratin 34 β E12 positive cells were seen at the periphery, corresponding to basal cells seen in the H&E stained sections. A few cells stained positively for p53. Very rare cells were positive for chromogranin A. Ki-67 immunostaining revealed several positive nuclei in all cell layers.

The cells in the squamous component were negative for PSA and chromogranin A, and positive for p63 (fig 1E), keratin AE1/AE3 (fig 1F), and keratin 34 β E12. In addition, p53 positive cells and several Ki-67 positive nuclei were present in all cell layers, with the greatest number at the periphery. This is a feature seen in squamous cell carcinoma in situ.⁶

α -Smooth muscle actin, S-100 protein, and α -methylacyl-CoA racemase were negative in HGPIN and in the foci of squamous differentiation. In situ hybridisation did not reveal the presence of HPV DNA.

Karyometry

Morphometric analysis carried out in the benign prostate of the current case and in control cases showed that the nuclear

and nucleolar area (expressed in μm^2) increased from benign prostate to low grade PIN, to high grade PIN, and to adenocarcinoma. In particular, the values in the secretory cells of HGPIN in the present case were similar to those obtained in the HGPIN case used as control (present case: mean (SD) of the nucleolar area = 50.35 (11.58) and of the nucleolar area, 3.41 (0.79); control: mean (SD) of the nucleolar area = 48.60 (9.12) and of the nucleolar area, 3.66 (0.94)).

The values in the squamous component of the present case were intermediate between those obtained in the control benign prostate with squamous differentiation and those obtained in squamous cell carcinoma in situ of the cervix.

DISCUSSION

The HGPIN lesion described here was characterised by the simultaneous presence of a glandular and a squamous cell component. The former was similar in terms of morphology, immunohistochemistry, and karyometry to the usual HGPIN seen in patients with clinically detected cancer. The latter, with the features of the glycogenated type of squamous differentiation or transdifferentiation, was similar to squamous cell carcinoma in situ.⁶ The two components were side by side, never sharply separated or confined to separate glands, and always in touch with the periphery of the glands, thus suggesting that they derived from a common precursor cell showing divergent differentiation. The differential diagnosis is with basal cell hyperplasia, a lesion demonstrating cell stratification and associated with a squamous component. However, in basal cell hyperplasia, p63 positive nuclei are present in all cell layers, whereas PSA is negative.⁷

Our patient's main concern was benign prostatic hyperplasia, for which he had been treated with tamsulosin for three years. There is no evidence to suggest that α blockers like tamsulosin induce cells changes, such as squamous differentiation or metaplasia, so we cannot attribute the findings here to drug treatment. Further, the patient denied

Take home message

An unusual type of prostatic intraepithelial neoplasia appears to represent a new variant: high grade prostatic intraepithelial neoplasia with squamous differentiation

taking drugs other than the one mentioned above, and his diet included only the typical elements of the Mediterranean diet. Finally, the absence of metaplastic changes in benign acini distinct from HGPIN argues strongly against drug induced or diet induced changes, as these would be expected to affect the prostate diffusely.

Squamous differentiation has been described in the benign prostate, including BPH, and in prostatic carcinoma. In the former it has also been called squamous metaplasia. To our knowledge, it has not previously been reported in HGPIN. In normal prostatic epithelium it is secondary to inflammation, alteration in hormonal milieu, or injury. It can also occur as a result of treatment, such as hormonal therapy, radiation therapy, and cryosurgery.⁸ This divergent differentiation in normal prostate is not considered a precursor for prostatic carcinoma and does not constitute a risk factor for detection of carcinoma.

Many carcinomas in the prostate with squamous differentiation, including adenosquamous carcinoma, arise after radiation or hormonal therapy. Adenosquamous carcinoma⁹⁻¹¹ is defined by the presence of both glandular (acinar) and squamous cell carcinoma components. The two components could be distinct or could show direct transition, as in the HGPIN lesion described here. The grade of the adenocarcinoma ranges from moderately to poorly differentiated. The histological grade of the squamous elements is also variable, with reports of well, moderately, and poorly differentiated growths. The glandular tumour component generally expresses PSA and prostate specific acid phosphatase, whereas the squamous component displays high molecular weight cytokeratins.¹⁰

In summary, to the best of our knowledge, this unusual form of HGPIN has not previously been reported and appears to represent a new variant: high grade prostatic intraepithelial neoplasia with squamous differentiation.

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Authors' affiliations

M Melissari, E Froio, Department of Pathology and Medicine of Laboratory, Section of Pathological Anatomy and Histology, University of Parma, Parma, Italy

A L Beltran, Department of Pathology, Reina Sofia University Hospital and Faculty of Medicine, Cordoba, Spain

R Mazzucchelli, R Montironi, Section of Pathological Anatomy and Histopathology, Polytechnic University of the Marche Region, School of Medicine, Ancona, Italy

D G Bostwick, Bostwick Laboratories, Glen Allen, Virginia, USA

Correspondence to: Professor Rodolfo Montironi, Section of Pathological Anatomy and Histopathology, Polytechnic University of the Marche Region (Ancona), School of Medicine, Umberto I Hospital, Via Conca 71, I-60020 Torrette, Ancona, Italy; r.montironi@univpm.it

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