

CYCLING ACTIVITY OF BENIGN PROSTATIC EPITHELIAL CELLS DURING LONG-TERM ANDROGEN BLOCKADE: EVIDENCE FOR SELF-RENEWAL OF LUMINAL CELLS

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SUMMARY

Combined androgen blockade (CAB) therapy (LHRH agonist and flutamide) for 6 months leads to marked regressive changes of the prostate gland. This is associated with a reduction in the ratio of luminal to basal cells in the peripheral zone (PZ) in hyperplastic glands of the transitional zone (TZ). To examine the cycling activity of luminal and basal cells, double immunostaining was performed. For precise definition of basal cells, the basal cell-specific antibody 34 β E12 was used, while cycling cells were identified by the MIB-1 antibody. In 6-month-treated specimens, the fraction of cycling luminal cells increased from 0.3 to 2.5 per cent in the PZ and from 0.2 to 3.9 per cent in the TZ. This was associated with an inversion of the ratio of MIB-1-labelled luminal to basal cells, with values of 5.7:1 (PZ) and 3.9:1 (TZ), compared with 1:4 (PZ) and 1:5 (TZ) in untreated specimens. The predominance continued for at least 11 years of CAB. The findings strongly suggest that luminal cells are capable of self-renewal under conditions of low androgen levels. The substantial decrease of prostatic volume on long-term CAB implies that cell loss outweighs cell proliferation. © 1998 John Wiley & Sons, Ltd.

KEY WORDS—prostate; proliferation; regeneration; stem cells; immunohistochemistry; androgen antagonists; LHRH agonist

INTRODUCTION

Human prostate glands are composed of a predominant population of luminal cells with secretory activity, along with basal cells and a small number of dispersed neuroendocrine cells. Basal cells selectively express high molecular weight cytokeratins (HMWK) detectable by the monoclonal antibody 34 β E12,¹ while luminal cells express the androgen receptor (AR), a molecule that is lacking in most basal cells as well as in the chromogranin A-defined neuroendocrine cells.^{2,3} Androgen deprivation has been shown to result in characteristic changes: namely a substantial decrease of gland size caused by considerable cell loss, associated with vacuolation of the residual luminal cells and prominence of the basal cell layer.^{4,5} Experimental studies on the rat prostate have revealed a rapid loss of luminal cells after castration.^{6,7}

Two opposing models have been put forward to explain the development and regeneration of the prostate gland. According to the first model, the basal cell layer represents the regenerative compartment, which gives rise to both basal and luminal cells during developmental growth or during regeneration after castration-induced loss of luminal cells. Proliferation of basal cells and subsequent (androgen-dependent) differentiation into luminal cells would be required for

complete regeneration of the gland.^{8,9} In this model, basal cells would include a small, slowly cycling fraction of stem cells with an unlimited capacity for androgen-independent proliferation and a larger fraction of amplifying cells, rapidly proliferating after cell loss. Another model, largely based on cell-kinetic studies of the developing rat prostate, proposes that luminal cells are fully capable of self-renewal, without a contributory role of basal cells.¹⁰

We anticipated that a study of the cycling activity of human prostatic basal and luminal cells under conditions of severe and chronic cell loss due to prolonged CAB might help to resolve this issue.

MATERIALS AND METHODS

Tissue specimens

Prostatectomy specimens were obtained from 14 patients with clinically localized prostate cancer, of whom seven received neoadjuvant CAB⁴ for 6 months until surgery. Sections of four cystoprostatectomy specimens additionally provided untreated benign prostatic glandular tissues. Specimens fixed in buffered formalin (pH 7.4) were processed routinely for paraffin embedding. From each treated case, a section was selected that did not contain residual cancer or prostatic intra-epithelial neoplasia. From eight additional patients, prostatic needle biopsies were available that were taken after 5–11 years of CAB. Although this therapy was discontinued 1–6 months before the biopsies were

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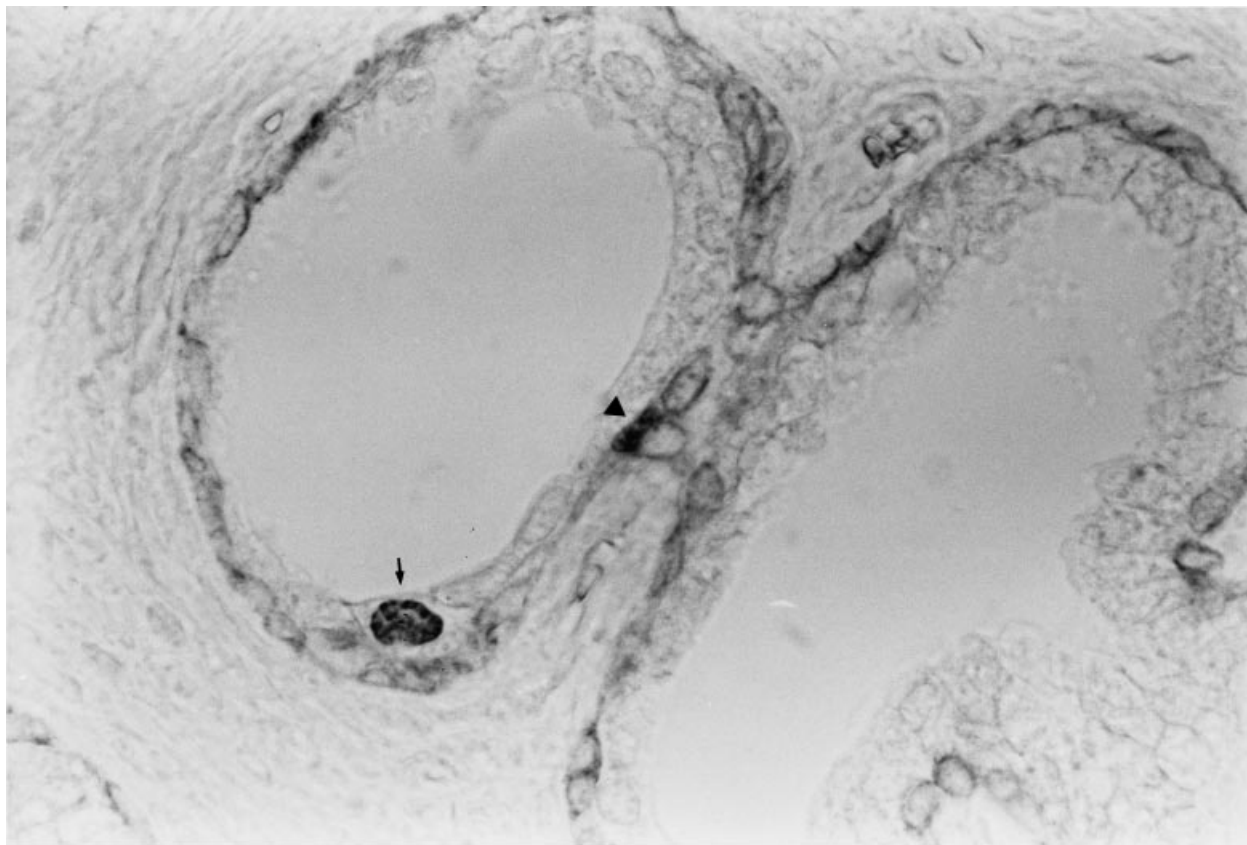


Fig. 1—Transitional zone prostatic gland after 6 months of CAB. Using immunohistochemical double labelling, basal cells are selectively stained with anti-HMWK antibody. Note the MIB-1-labelled luminal (arrow) and basal cell (arrow-head) ($\times 40$)

taken, serum androgen levels had not returned above castration levels at that time (data not shown). Transrectal ultrasonography revealed that in these patients, the prostate was substantially reduced in size.

Immunohistochemistry

To allow the unequivocal distinction of MIB-1-labelled luminal and basal cells (Fig. 1), sequential double immunostaining was performed with the mouse monoclonal antibody MIB-1 (Immunotech, France), followed by staining with 34 β E12 (DAKO, U.S.A.) against HMWK. After antigen retrieval of deparaffinized rehydrated sections,³ immunoreactivity for MIB-1 was visualized using avidin–biotin–peroxidase complexes (DAKO, Denmark) and for anti-34 β E12 with an alkaline phosphatase-labelled avidin (Lab Vision, U.S.A.) method. In addition, double staining was performed for AR and HMWK, by replacing the MIB-1 monoclonal antibody by an overnight incubation with the monoclonal antibody F39.4.³ Immunostaining for PSA with the monoclonal antibody ER-PR8 was performed using the avidin–biotin–peroxidase method.

Quantitation

Sections double-labelled for MIB-1 and HMWK were examined at $\times 400$ magnification and the relative number of HMWK-positive (basal) cells and unstained

(luminal) cells was determined by counting about 500 cells per section. The MIB-1 labelling index of basal and of luminal cells was determined by counting 500 nuclei of HMWK-positive and of HMWK-negative cells per section and expressing MIB-1 reactivity as a percentage of basal cells and of luminal cells, respectively. This number proved sufficient to detect the considerable differences among the groups under study. Areas with signs of prostatitis were avoided. The ratio of MIB-1-labelled luminal and basal cells in 6-month-treated specimens was also assessed separately by determination of the distribution of 50 MIB-1-labelled nuclei per section over the HMWK-positive and -negative cell compartment. In untreated specimens, lower numbers were assessed owing to the very low frequency of MIB-1-positive glandular cells. Because of the small numbers of glandular cells in the prostatic needle biopsies, the distribution of all MIB-1-labelled cells over the basal and luminal compartment was assessed.

RESULTS

Histological examination of 6-month-treated prostatectomy specimens revealed regressive changes of the glandular structures, including vacuolation of luminal cells, and prominence of the basal cell layer. Quantitative data obtained by analysis of untreated and treated prostatectomy specimens are listed in Table I. The

Table I—Cycling activity of glandular cells in untreated and treated prostate glands

CAB treatment	Peripheral zone		Transitional zone	
	Untreated (n=7)	6 months (n=7)	Untreated (n=4)	6 months (n=6)
<i>l/b</i> ratio*	1.9 (1.4–2.4)	1.0 (0.7–1.1)	3.4 (2.0–4.7)	0.8 (0.6–1.0)
MIB-1 % luminal cells	0.3 (0.0–1.0)	2.5 (0.8–6.4)	0.5 (0.0–1.2)	3.4 (2.0–5.4)
MIB-1 % basal cells	0.9 (0.2–2.2)	0.5 (0.0–1.8)	3.1 (2.2–3.8)	1.0 (0.4–2.0)
MIB-1 ratio <i>l/b</i>	0.25†	5.6 (3.2–7.3)‡	0.2 (0.1–0.3)‡	3.9 (3.2–4.6)‡

**l/b* ratio is the number of luminal cells/number of basal cells in a given number of glands.

†Based on the distribution of a total of 193 MIB-1-labelled cells of six cases.

‡Based on the distribution of 50 MIB-1-positive nuclei per case.

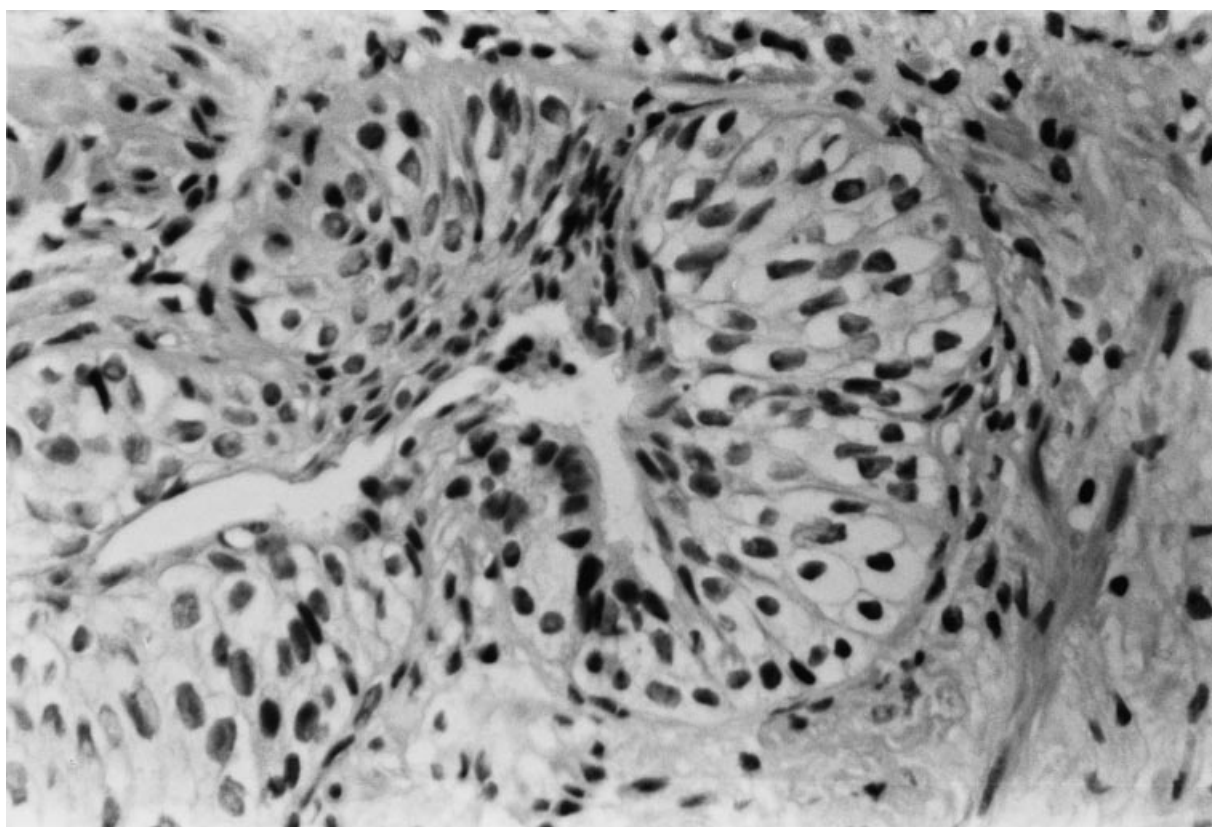


Fig. 2—Prostatic needle biopsy obtained after 5 years of CAB, showing residual glandular structures ($\times 40$). Luminal cells can be identified by their columnar morphology and their lining of the small lumen

figures concerning untreated specimens conform to those reported previously.^{8,11} Exposure to 6 months of CAB resulted in a decreased ratio of luminal to basal cells and an increased MIB-1 score of the remaining luminal cells in the peripheral zone (PZ) and nodular glandular areas of the transitional zone (TZ). Strikingly, the ratio of MIB-1-labelled luminal to basal cells was reversed in both PZ and TZ glands. Luminal cells of treated glands expressed a low level of nuclear AR and PSA. Sporadic mitotic figures were noted in the luminal cell compartment.

The histology of needle biopsies of men given continuous CAB for more than 5 years revealed residual glandular structures, occasionally with a distinctive morphology (Fig. 2) characterized by an accumulation of

HMWK-positive cells with extensive cytoplasmic vacuolation. Some residual luminal cells continued to display faint nuclear AR staining, but PSA expression was largely absent. In several needle biopsies, large areas of AR-positive fibromuscular tissue were present, entirely lacking glandular structures. Double labelling for MIB-1 and HMWK revealed a MIB-1 ratio of HMWK-negative (luminal) to HMWK-positive (basal) cells of 4.3:1 on a total number of 131 MIB-1-positive cells counted in eight cases.

DISCUSSION

Two models for prostatic glandular regeneration have been put forward: a 'classical' stem cell model

attributing stem cells with multilineage potential, as well as their direct progeny, the amplifying cells, to the basal cell layer; and a 'two-lineage' model that considers both basal and luminal cells each separately capable of self-renewal. The validity of the former model was supported by the observation in the human prostate that under normal conditions, the cycling fraction of basal cells far exceeded that of the luminal cells.^{8,12} From the perspective of the two-lineage model, this observation could also be explained by a more rapid turnover of basal cells than luminal cells. Indeed, in the untreated human prostate, apoptotic bodies are more frequently found in the basal cell layer than in the luminal cell layer.¹² Co-localization of basal cell-specific cytokeratins and luminal cell-specific markers after androgen-stimulated regeneration of 7-day-castrated rat prostates gave additional support to the 'classical' stem cell model.⁹ On the other hand, it was reported by Evans and Chandler that during development of the rat prostate, the luminal cell compartment contains the largest fraction of proliferating cells.¹⁰ Similarly, it was shown in the rat that after castration-induced cell loss, androgen-induced regeneration led to an enhanced proliferative response particularly of luminal cells.^{7,13}

Following castration, the rapid loss of luminal cells in the rat prostate is not compensated during the first week by entrance of basal or luminal cells into the cell cycle.^{6,7} Westin *et al.* also did not observe Ki-67-positive cells in normal human prostatic glandular tissue at 7 days after androgen ablation.¹⁴ Montironi *et al.* reported a slight increase in luminal cells labelled with proliferating cell nuclear antigen, following 3 months of CAB.¹² Strikingly, our data demonstrate that CAB for 6 months and longer was accompanied by strongly enhanced cycling activity, as defined by MIB-1 labelling in the luminal cells. It is possible that a lag period exists for the development of cycling activity after androgen deprivation, or that the rate of progression through the cell cycle is markedly reduced under androgen deprivation.

Our data strongly suggest that human prostatic luminal cells are capable of self-renewal after cell loss in an environment largely depleted of androgens. The accompanying shift of the cycling fraction from the basal cell layer to the luminal cell layer, persisting even after 11 years of CAB, is opposed to the 'classical' stem cell model, which would predict vigorous basal cell proliferation in an attempt to restore the severe and chronic loss of luminal cells. On the other hand, the large gland-free prostatic stromal areas and the considerably reduced size of the prostate imply that on very-long-term androgen deprivation, luminal and basal cell proliferation cannot compensate adequately for chronic cell loss.

The persistence of a low level of AR expression in some residual luminal cells after several years of CAB suggests that these cells remain androgen-responsive. Indeed, in one case exposed to 11 years of CAB, which was discontinued 21 months before biopsy, full restoration of intensely PSA-positive normal glandular structures was seen.

The two stem cell models for prostatic glandular regeneration may be reconciled by assuming that in the presence of androgens a certain number of AR-positive basal cells¹⁵ may differentiate into luminal cells, to compensate for luminal cell loss after androgen deprivation. In our opinion, the luminal cell compartment must contain at least a population of amplifying cells capable of persistent proliferation as a reaction to chronic luminal cell loss. Following the current paradigm that stem cells and their direct progeny are the targets for oncogenic events,¹⁶ our data imply that luminal cells represent a likely target population.

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