

Stem cells in prostatic epithelia

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Summary. The normal prostate is, structurally and functionally, a highly complex glandular tissue in which populations of epithelial and stromal cells interact, one with the other, and are under a constant state of proliferation, differentiation, elimination and selective secondary replenishment so that functional integrity of the tissue is maintained. The ability of normal prostatic tissue to maintain its structure and function is dependent upon retention of cells, generally regarded as 'stem cells', which are able to respond by proliferation and selective differentiation within a wide range of phenotypic alternatives. With respect to cells in the epithelial compartment, replenishment is possible at several levels from within distinct pathways of normal cellular differentiation. It is now appreciated that fully differentiated prostatic epithelial cells retain a far greater degree of phenotypic 'plasticity' than was earlier apparent from morphological examination of the intact tissue. This inherent plasticity, coupled with the ability of the intact tissue to respond to diverse environmental (particularly humoral) stimuli by regenerating a wide and divergent spectrum of functional prostatic epithelial phenotypes is its strength – but also its weakness. Disturbance and distortion of the homeostatic regulatory mechanisms, whether physical or humoral, which control the normal sequence of epithelial proliferation, differentiation and elimination exposes these cells, particularly multipotent 'stem cells', to an increased probability of genetic change, thus resulting in either transient, or permanent, neoplastic transformation.

Keywords: stem cells, prostatic epithelia, stromal/epithelial interaction, multi-lineage differentiation, origins of neoplasia.

Background

It is a biological axiom that to maintain integrity of any tissue, loss of parenchymal cells must be compensated by an equivalent replacement with cells of identical phenotype. Such replacement may occur either by

mitosis within a population of already-differentiated cells or by *de novo* replacement through selective differentiation following mitotic proliferation of a precursor stem cell population. Such stem cells may be either completely undifferentiated, or partially differentiated, with a restricted repertoire of residual potential differentiation. In mature glandular epithelial tissues such as breast, pancreas and prostate, both types of cellular recruitment occur. Given that the composition of normal prostatic epithelium includes the complex interaction of

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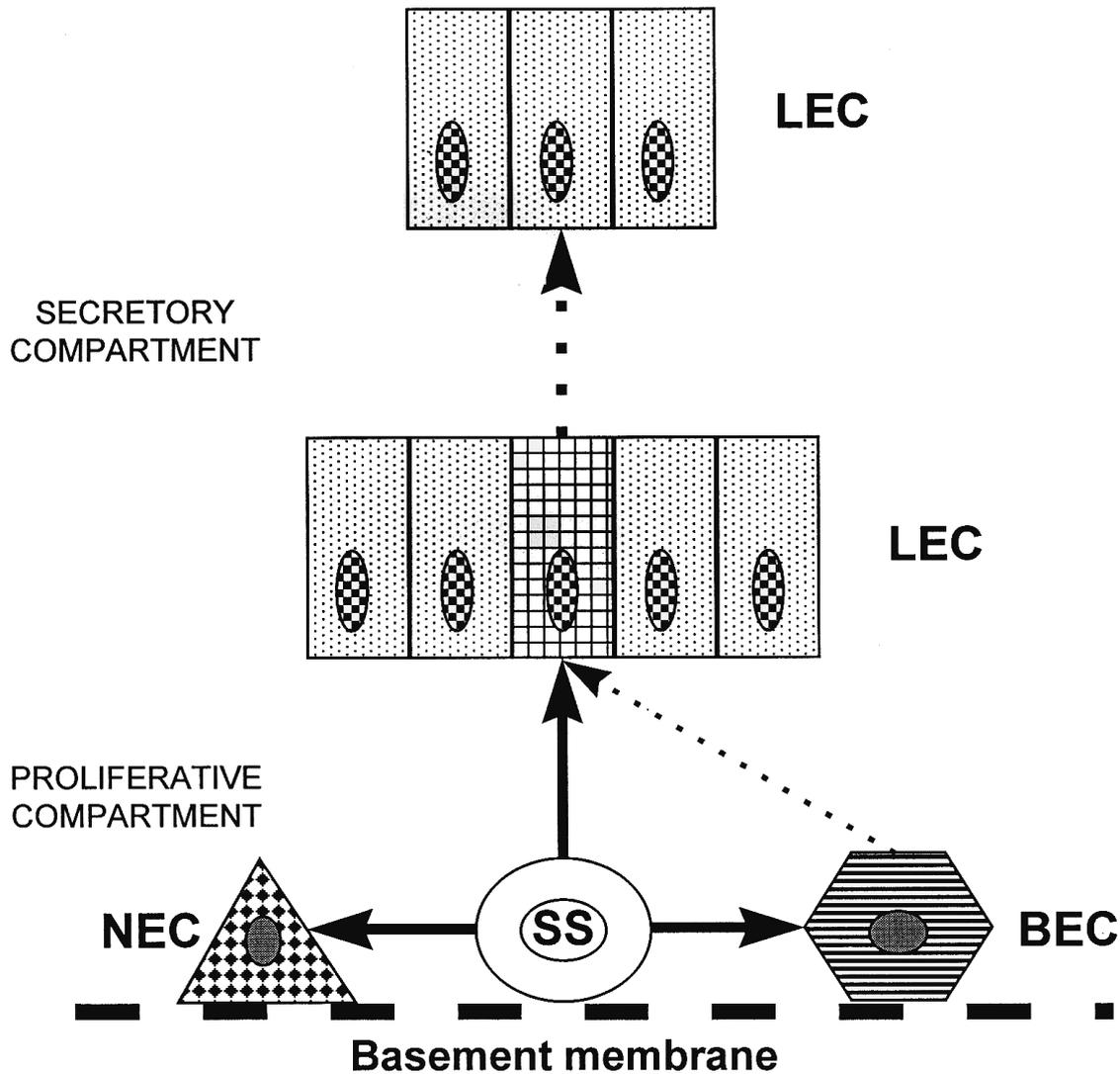


Figure 1. Schematic diagram indicating the inter-relationships of the various prostatic epithelial cell-types in the fully differentiated normal adult gland. Prostatic stem cells (SS) located within the basal layer give rise to basal epithelial cells (BEC) and to basal neuroendocrine cells (NEC) as well as to luminal epithelial cells (LEC). It is most probable that these routes of cellular differentiation are exclusive and unidirectional. However, it is likely that at least some BEC's retain a residual capacity to proliferate, to undergo further vectorial differentiation to LEC's and possibly even to NEC's, as well as to additional BEC's. Reverse differentiation along these routes has not been reported.

the three phenotypically distinct cell types of elongate basal cells, small polygonal neuroendocrine cells and cuboidal/columnar luminal (secretory) cells, it has now become apparent how these three individual cell lineages share a common origin and are related in a precursor-progeny sequence in which basal epithelial cells play a fundamental role in normal prostatic growth as well as in the initiation and progression of at least some forms of prostate cancer.

Strictly, there is no such single entity as 'prostatic stem cells'. The human prostate gland is a complex tissue

which undergoes stromal and epithelial maturation and differentiation in at least four distinct phases during progression from embryogenesis through to adulthood (Aumuller 1983). The first is an early embryonic phase in which endodermally derived epithelial cells from the definitive urogenital sinus infiltrate and proliferate within surrounding mesenchymal tissues. Proliferation of these pre-prostatic epithelial cells does not happen at this time in a common or unified manner throughout the developing gland but may be divided into five subanatomic regions, each of which giving rise to a separate and

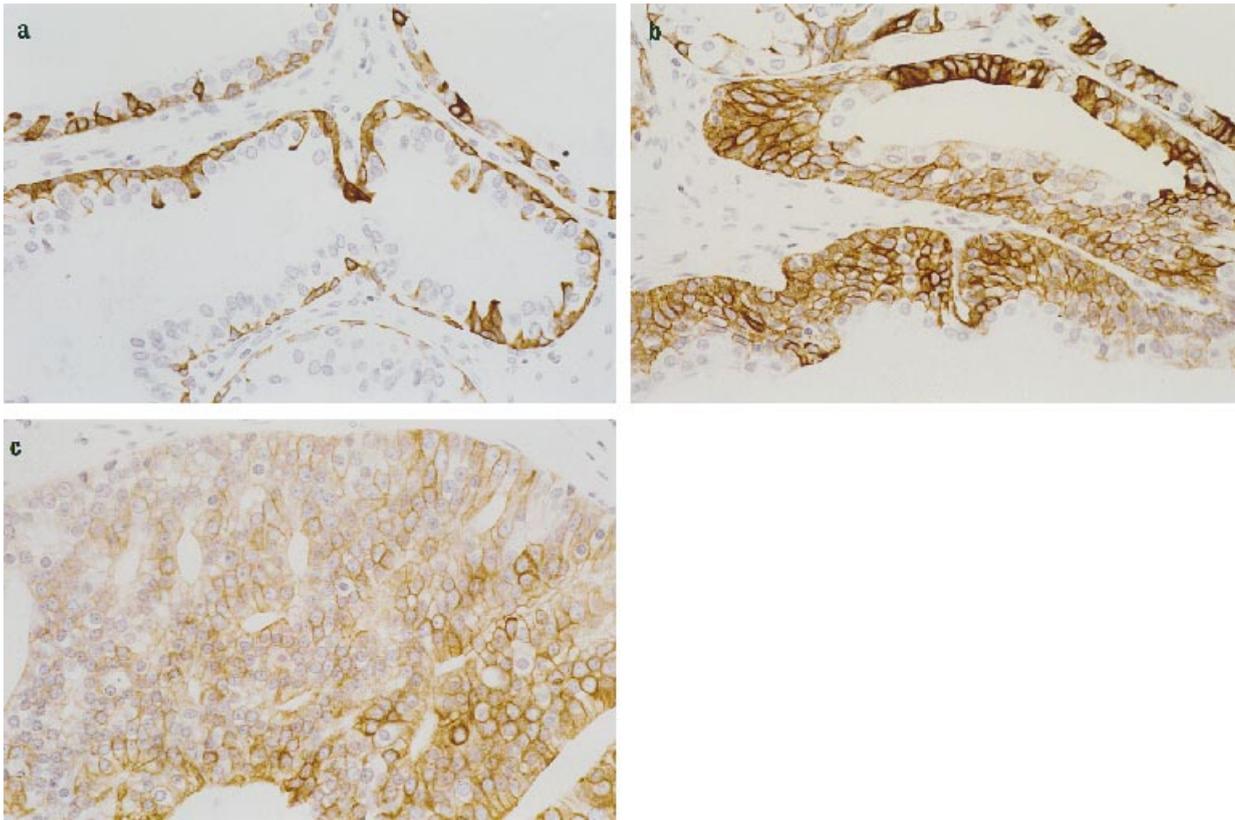


Figure 2. a, Normal, nonhyperplastic and non-neoplastic prostatic epithelium stained with for molecular weight cytokeratins with antibody AE1/AE3 revealing the phenotypically characteristic basal epithelial cells located as a monolayer beneath the glandular luminal epithelial cells. (Magnification $\times 350$). b, Basal cell hyperplasia in which there is proliferation of AE1/AE3-positive basal epithelial cells, but without dysplastic or dyskaryotic appearances. An intact layer of luminal epithelial cells is present lining the surface of the majority of the epithelial compartment. These latter appear unremarkable and indistinguishable from those in (a). (Magnification $\times 350$). c, Hyperplastic and seriously dysplastic basal epithelial compartment in which there is heterogeneous staining by antibody AE1/AE3. There is no single intact basal epithelial layer. There is also no morphologically distinguishable luminal epithelial compartment comparable to that seen in (a) or (b). Instead the proliferating basal compartment contains irregularly arranged epithelial cells. The nuclei contain clumped chromatin as well as prominent nucleoli. (Magnification $\times 350$).

subanatomically defined component of the eventual mature gland. When one considers the differences in propensity and predisposition to neoplasia occurring within different parts of the adult prostate, it is a moot point (but not semantic), as to whether or not each of the five zones thereafter contains epithelial stem cells which are phenotypically distinct from each other. The second phase of prostatic development, also embryonic in origin, occurs between the eighth month of pregnancy through to the second postnatal month when there is a distinct period of morphogenesis and cellular (epithelial and mesenchymal) regression. The third phase is an infantile 'resting' period extending between the second postnatal month through to age 10 or 12 years, depending upon the individual child. Although euphemistically classed as a 'resting' period, it is likely that subcellular, particularly

molecular-biological, events which determine future phenotypes of individual groups of cells within the epithelium and possibly also the stroma, are occurring throughout this time. Thereafter, the fourth phase, pubertal maturation, extends from age 12 through to 18 years in which there is an enhanced phase of cellular proliferation, differentiation and tissue morphogenesis. From the age of 20 years through to old age, prostatic components (epithelial and stromal) are not static since cells in both these populations are involved in a continuous range of activities which include cell proliferation, apoptosis, variable responses to hormonal influences, transdifferentiation, etc. Individual factors controlling many of these events are only just becoming defined so that an understanding of the processes interpreting and co-ordinating them remains extremely scanty at the present time.

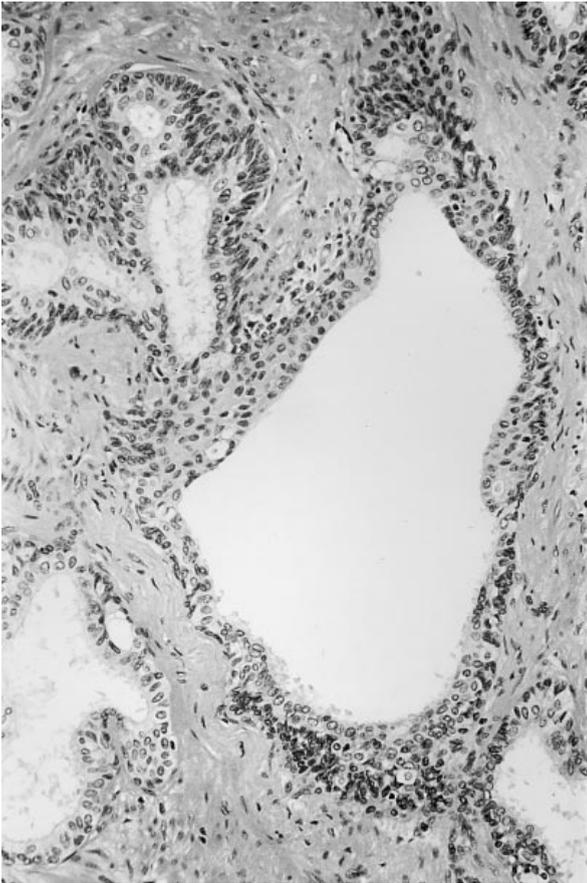


Figure 3. Enlarged terminal ductule at a site adjacent to its associated lobule in which there is a prominent focus of basal cell hyperplasia together with an region of benign squamous metaplasia below which the apparently normal basal layer remains intact. (Magnification $\times 180$).

Studies of adult prostatic tissues performed during the past decade have identified some of the phenotypic characteristics of these putative epithelial stem cells now recognized to be located in the inconspicuous basal cell layer of cells around the periphery of prostatic terminal ducts, ductules and acini. These elongate cells contain scant cytoplasm and separate the underlying basement membrane from overlying luminal epithelial cells (Dermer 1978). Basal cells develop following the androgenic stimulation of immature prostatic epithelium which occurs at puberty (Wernert & Seitz 1987). However, the observation that basal cells develop only during postnatal prostatic parenchymal differentiation suggests that these should be regarded as 'second-order' while their progenitor (embryological) cell of origin (i.e. 'first-order') stem cells continue to exist. This is not a semantic concept without practical application, but an important

problem which is fundamental to understanding the aetiopathogenesis of prostatic epithelial hyperplasia and cancer, particularly since strong evidence now indicates that at least some of the genetic events leading to prostatic neoplasia and to prostate cancer take place within this population of basal cells. It is those genetic events (whether mutational, genetic imprinting or other) which occur within the embryological 'first-order' stem cells and predispose an individual to prostate cancer. Thereafter, such genetic modifications will be inevitably transmitted to all prostatic epithelial progeny throughout the subsequent lifetime of that individual. Conversely, 'second-order' stem cells, in which genetic events of a potentially malignant nature are induced postnatally, may be effaced by obliteration followed by proliferation of nondefective first-order stem cells, and hence their effects eradicated.

Basal cell compartment in normal prostate

There are several lines of evidence to support the notion that the basal cell layer contains the prostatic epithelial stem cell population. Using double-label techniques for phenotypic markers, the three basic epithelial cell types have been clearly demonstrated to be linked in a precursor-progeny relationship (Bonkhoff & Remberger 1996). Among prostatic epithelial cell types, basal cells have the greatest capacity to differentiate into all epithelial cell lineages through intermediate phenotypes (Bonkhoff *et al.* 1994a). The proliferative compartment of both normal and hyperplastic epithelium resides in the basal epithelial cell (BEC) layer (Figure 1). Approximately 70% of proliferating epithelial cells are phenotypically basal (Figure 2). The remaining 30% of proliferating cells belong within the luminal secretory epithelium (LEC), whereas chromogranin A-expressing endocrine-paracrine cells comprise an entirely postmitotic cell population (Bonkhoff *et al.* 1991, 1995) and normally located within the basal epithelial layer.

With respect to the physiological replenishment of cells occurring within normal prostatic epithelium, basal cells are the most capable of dividing and differentiating into other cell types such as secretory cells (Dermer 1978; Cleary *et al.* 1983; Wernert & Seitz 1987). Although both basal cells and secretory cells retain the ability to divide, the usual proliferative compartment is the basal cell layer (Bonkhoff *et al.* 1994b). In normal (non transformed, non-neoplastic) prostatic tissue, transition forms have been reported between basal cells, secretory luminal and neuroendocrine cells, although rarely – and only in tissue culture (Heatfield *et al.* 1980; Bonkhoff *et al.* 1994). Prostate-specific antigen (PSA) and prostatic

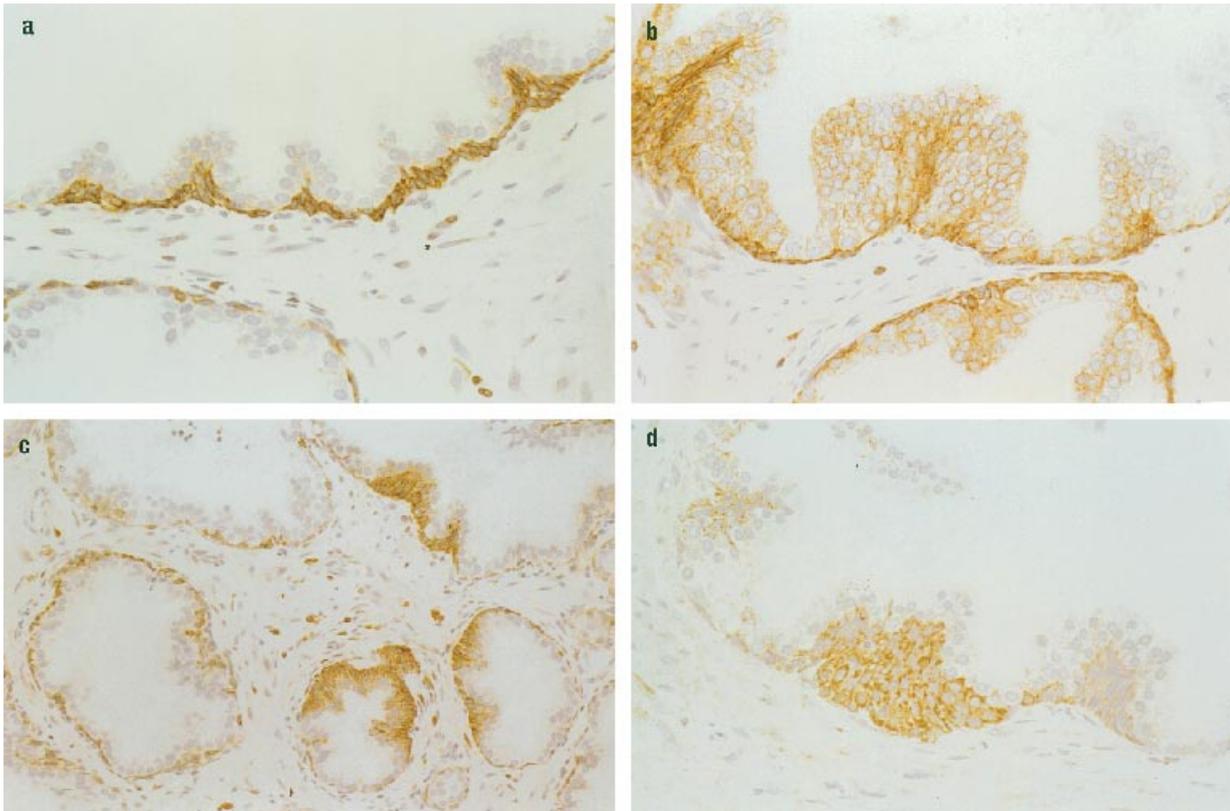


Figure 4. a, Normal prostatic epithelium in which there is characteristic expression of Bcl-2 by cells within the basal epithelial compartment. There is an overlying intact layer of luminal epithelial cells which do not express Bcl-2 protein. (Magnification $\times 350$). b, Atypical epithelial hyperplasia of papillary type in which proliferating cells are morphologically of basal-type. Although expression of Bcl-2 is occurring predominantly within the basal layer, there is significant expression of this protein by the overlying hyperplastic epithelium. (Magnification $\times 350$). c, Focally enhanced expression of Bcl-2 protein within reactive but non-neoplastic prostatic epithelium. Within the five glands illustrated, there is polarization of hyperplasia towards the central region of the stroma. These appearances strongly suggest the presence of a trophic factor arising from the stroma and causing local hyperplasia of the glandular epithelium, with concomitant enhanced expression of Bcl-2 protein. (Magnification $\times 350$). d, Focal basal epithelial cell hyperplasia of nondysplastic and non-neoplastic type in which there is significantly enhanced expression of protein Bcl-x. The overlying luminal epithelium appears intact and unremarkable and does not express this protein. Adjacent nonhyperplastic basal epithelial cells express only very low levels of Bcl-x protein. (Magnification $\times 350$).

acid phosphatase (PAP) immunoreactivity are present in a subset of basal cells (Devaraj & Bostwick 1993), suggesting that basal cells can acquire the immunophenotype of secretory cells. Basal cells also retain the ability to undergo metaplasia, including squamous differentiation, in prostatic infarction and in myoepithelial differentiation in sclerosing adenosis (Figure 3). Other evidence supporting the stem cell origin of basal cells includes presence of apoptosis-suppressing Bcl-2 oncoprotein (Colombel *et al.* 1993), and androgen-independence but androgen-responsiveness of basal cells as documented by the presence of 5- α -reductase isoenzyme-2 and expression of the nuclear androgen receptor (Bonkhoff *et al.* 1996).

Evidence of the stem cell role for basal cells in prostate

epithelia is provided by the selective expression of different members of the Bcl-2/bcl-x family of regulatory proteins which block programmed (apoptotic) cell death in both the stem cell and proliferation compartments of normal tissues (Hockenberry *et al.* 1991; Long-Lu *et al.* 1996). In all normal tissues, Bcl-2 and its homologue Bcl-x (comprising alternately spliced variants Bcl-xL and Bcl-xS) together with proteins Bcl-w and Mcl-1, protect cells from a wide variety of apoptotic stimuli. These proteins, while conserving structural similarities in defined regions, are differentially regulated such that it is the relative *ratios* of these regulators, particularly Bcl-xL and Bcl-xS (Minn *et al.* 1996) which promote cell survival (or allow cell death), rather than the absolute value of any single protein. One mechanism by which

Table 1. Phenotypical characteristics of prostatic differentiated basal and luminal epithelial cells

Cellular feature	Basal	Luminal
HMWCK*	+	-
Proliferative activity	+	-
Secretory activity	-	+
EGF synthesis	-	+
Bcl-2 protein	+	-
Bcl-x protein	+	-
bFGF-synthesis	+	-
PSA synthesis	-/±	+
PAP synthesis	-/±	+
5-(reductase-2	+	+
Nuclear androgen receptor	+	-
Androgen independence	+	-
Androgen responsiveness	+	-
Oestrogen receptor	-/±	+
Progesterone receptor	-/±	+

* HMWCK, High molecular weight cytokeratin (34 β -E12). \pm indicates either transient positivity or very small subsets of these cells may be consistently positive.

Bcl-xL maintains cell survival is through regulating the permeability of the intracellular membranes to which it is distributed. The ion conducting channel(s) formed by Bcl-xL display multiple conductance states that have identical ion selectivity (Minn *et al.* 1997). Although not yet demonstrated, other members of the apoptosis-inhibitors may also from similar ion-selective channels through which homeostasis of the intracellular environment is stabilized and maintained. For their efficacy, Bcl-2 and Bcl-xL are not only mutually interrelated, but they are also dependent upon normal p53 function (Zhan *et al.* 1996) acting through an inducible intermediary protein. Conversely, *bax* and *bak* facilitate cell death by inducing and promoting apoptosis. One mechanism now confirmed is through the heterodimerizing of Bax with Bcl-xL to yield a structure which is not competitively inhibited by Bcl-xS (Minn *et al.* 1996). Irrespective of tissue-type (epithelial, neural or reticuloendothelial) these general principles are uniformly applicable.

In the normal prostate, Bcl-2 protein expression is exclusively restricted to the basal cell layer (Figure 4), whereas the secretory epithelium lacks this oncoprotein in both normal and hyperplastic conditions (Colombel *et al.* 1993; Bonkhoff & Remberger 1996). Down-regulation of Bcl-2 within the differentiation compartment suggests that secretory luminal cells are permitted to undergo programmed cell death after terminal differentiation, thereafter to be replaced by generative basal cells (Bonkhoff & Remberger 1996). In prostatic epithelial hyperplasia, dysplasia and neoplasia, expression of anti-apoptotic proteins is not a characteristic of normal, hyperplastic or mildly dysplastic situations – but is

enhanced in high-grade primary malignancies and in metastatic prostatic disease. However, in accordance with the pattern of expression and the figures reported for p53 (Foster *et al.* 1992), Bcl-2 and Mcl-1 are expressed by only a small proportion of high grade malignancies (Krajewska *et al.* 1996). In contrast, the pro-apoptotic protein Bax is expressed in all cases of dysplasia and malignancy, irrespective of grade with high percentages of immunopositive cells and strong immunointensity typically occurring regardless of tumour grade, and suggesting that other mechanisms promoting prostatic epithelial cell survival are likely to be operating.

The proliferative compartment of the prostatic epithelium is able to accumulate biologically active dihydrotestosterone, which binds with high affinity to the appropriate androgen receptor. Since subsets of basal cells are androgen-responsive, it is likely that the effect of androgen on these particular cells induces differentiation towards secretory luminal cell types (Bonkhoff & Remberger 1996). Immunohistochemically, the elongate basal cells which contain the stem cell population are distinct from the argentaffin neuroendocrine cells, which comprise the third discrete lineage of prostatic epithelial differentiation. These three basic epithelial cell types are clearly differentiated by their marker expression and hormonal regulation. The luminal secretory epithelium requires continuous support by androgens for its maintenance and widely expresses nuclear androgen receptor, prostatic specific antigen (PSA) and cytokeratins 8 and 19, similar to that reported in common prostate cancer (Ware 1994). Basal cells may focally and transiently express nuclear receptors for oestrogens (ER) and progesterone (PR), but consistently lack nuclear androgen receptor. With the exception of the small defined population of cells, already described, basal cells lack PSA but strongly express high molecular weight cytokeratins which may be identified immunohistochemically using antibodies such as 34 β -E12 (Allsbrook & Pfeiffer 1997) or AE1/AE3. The endocrine-paracrine cell, the third epithelial phenotype of prostatic epithelium, is characterized by endocrine markers such as chromogranin A (Figure 5).

In addition to androgens and oestrogens, nonsteroidal growth factors are implicated in the control of basal cells. Epidermal growth factor (EGF) is required by prostatic epithelial cells for *in vitro* proliferation (Ware 1994). In the human prostate, EGF is produced by secretory luminal cells, whereas the basal cell layer (proliferative compartment) strongly expresses the EGF receptor. Insulin-like growth factor-1 (IGF-1), nerve growth factor (NGF) and members of the fibroblast growth factor family, particularly basic FGF, also affect proliferation since the

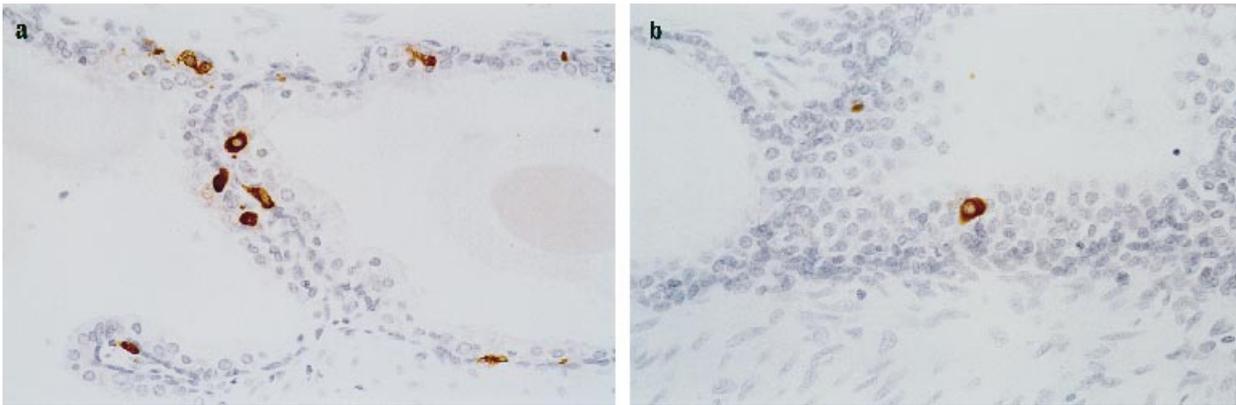


Figure 5. a, Normal prostatic epithelium containing chromogranin-positive neuroendocrine cells, located entirely within the basal epithelial cell layer. (Magnification $\times 350$). b, Basal epithelial hyperplasia of nondysplastic and non-neoplastic type in which there is neo-expression of neuroendocrine cell characteristics, identified by chromogranin A-positive staining, in which the neuroendocrine cell is not located within the basal layer but is present within the upper (sublittoral) luminal epithelial compartment. (Magnification $\times 350$).

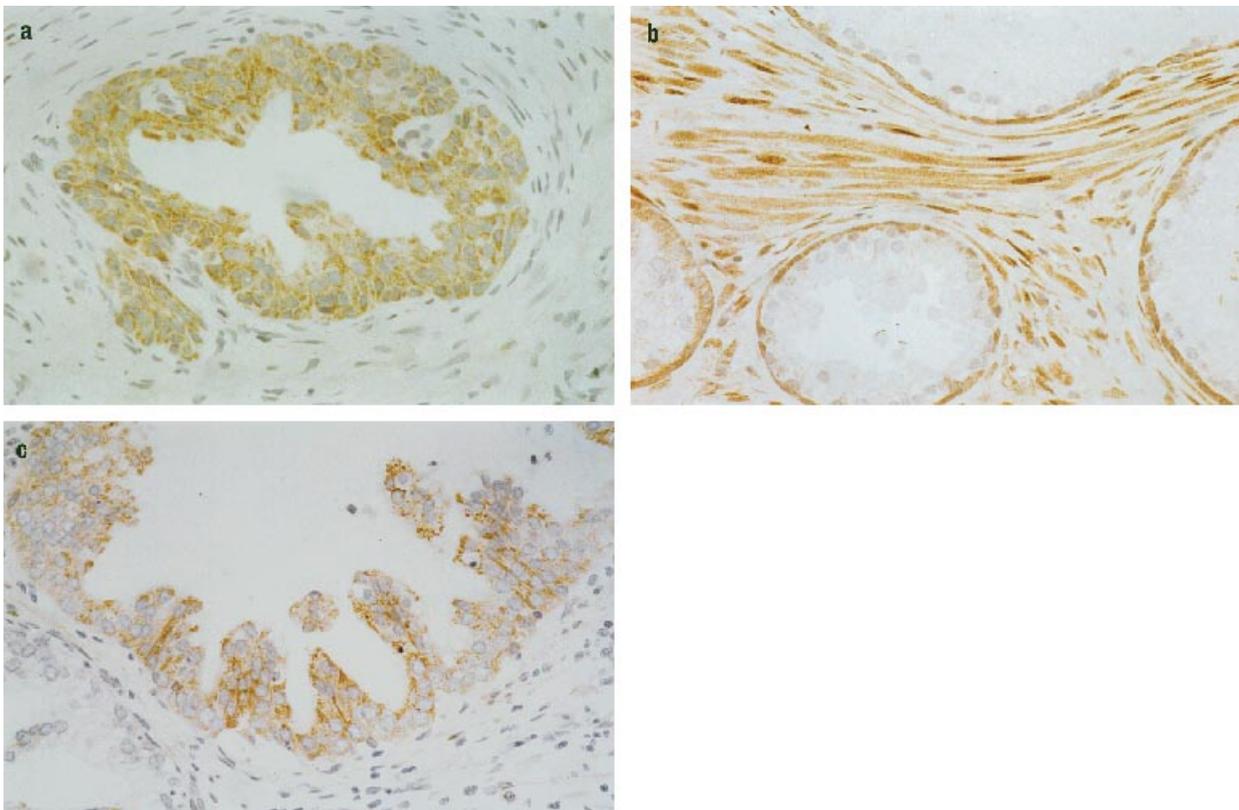


Figure 6. a, Prominent expression of α -FGF by hyperplastic basal epithelial cells. Normal prostatic epithelium, and nondysplastic prostatic epithelium, do not express α -FGF. The appearance of strong α -FGF staining in this situation is an important indicator that the epithelial cell hyperplasia is probably dysplastic, irrespective of the morphological appearance. (Magnification $\times 350$). b, Strong β -FGF expression by basal epithelial cells of prostatic glands together with myoepithelial cells within the stromal compartment. The glandular luminal epithelial cells do not express β -FGF. c, Neo-expression of β -FGF by hyperplastic prostatic epithelium, in which the expression β -FGF extends throughout all layers of prostatic epithelium, with enhanced expression in the luminal rather than the basal layers.

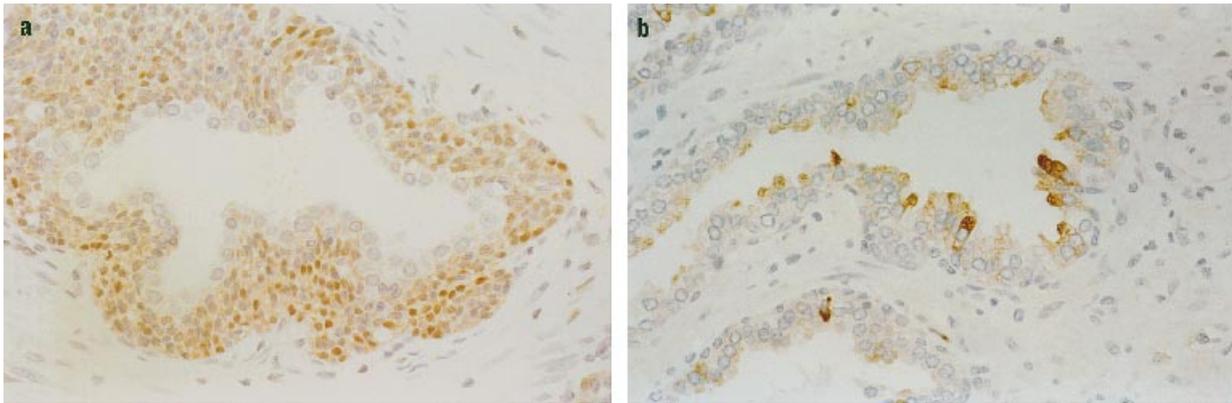


Figure 7. a, Prominent oestrogen receptor expression within nuclei of hyperplastic basal epithelial cells. The overlying layer of morphologically unremarkable luminal epithelial cells do not express oestrogen receptor. (Magnification $\times 350$). b, Focal and heterogeneous expression of oestrogen receptor protein within the cytoplasm of luminal epithelial cells in nonhyperplastic, nondysplastic, prostatic epithelium. This is a characteristic appearance in this situation, there being no functional association between the appearances in basal cell hyperplasia (7a) and in normal, nonhyperplastic, prostatic epithelium. The cytoplasmic appearances of the latter are believed to indicate nonfunctional receptor protein. (Magnification $\times 350$).



Figure 8. a, Isolated prostatic organoid of pure epithelial cells devoid of stromal components cultured *in-vitro* for 7 days within a three-dimensional collagen matrix. Dedifferentiation of cells has occurred throughout the structure with loss of 'basal' and 'luminal' phenotypic characteristics. Cell division is continuing to promote the outgrowth of solid epithelial cords into the surrounding matrix. (Magnification $\times 130$). b, Growing tip of a solid epithelial projection similar to those shown in (a). All cells comprising the tip are phenotypically undifferentiated and undergo repeated cycles of mitosis as the forward progression of the solid structure continues. Stress-lines are clearly visible within the collagen. (Magnification $\times 320$). c, Formalin-fixed and paraffin wax-embedded transverse section of extension shown in (b) which has been stained using monoclonal antibody AE1/AE3 to confirm expression of high molecular weight cytokeratins. Neo-differentiation of the basal phenotype is occurring in only those epithelial cells at the stromal interface. Appropriate staining-patterns for β -FGF but not EGF or PSA are also identified in this location. Centrally, differentiation of a luminal epithelial phenotype may be demonstrated both morphologically and immunohistochemically. (Magnification $\times 420$).

pertinent receptors are expressed by cells within the basal layer (Figure 6). With respect to EGF and its receptor (EGFr), these form a positive-feedback loop in the non-neoplastic prostate, but which becomes disrupted during neoplasia as evidenced by loss of the majority of EGFr from the tumour cells and failure to maintain an appropriate intracellular topographical distribution for the EGFr which is retained. While basic FGF is expressed only by basal epithelial cells in the normal prostate (Deshmukh *et al.* 1997) acidic FGF is not expressed at immuno-histochemically identifiable levels until the epithelial cells have started to develop early morphological features of dysplasia.

Based on the data outlined above, prostatic epithelium is composed of two functional compartments (Table 1). The proliferative compartment is androgen-independent and localized within the basal cell layer. The luminal epithelium represents the secretory compartment which is androgen-dependent but has a limited proliferative potential (Bonkhoff *et al.* 1994). The growth rate within the proliferative compartment is regulated by EGF and other growth factors (IGF, NGF, FGF) affecting proliferation, and by Bcl-2 which blocks programmed cell death. Thus, the proliferative compartment (basal cell layer) contain a small stem cell population which gives rise to all epithelial cell lineages via intermediate phenotypes. These differentiation processes within the prostatic cell system are regulated in a balanced vectorial manner by a combination of circulating steroid hormones together with a local nonsteroidal paracrine effect. Oestrogens cause basal cell hyperplasia *in vivo*, indicating that the differentiation process from basal to secretory luminal cells is also arrested by oestrogens (Figure 7). Conversely, androgens induce differentiation towards a secretory luminal-cell phenotype. Accordingly, turnover of the secretory epithelium largely depends upon the number of androgen-responsive target cells within the proliferative basal cell layer compartment, which is mediated by balanced trophic stimulation, either simultaneously or sequentially, with oestrogen and androgen.

Interaction of basal cells with stroma

The epithelial basement membrane in the prostate, as in other organs, represents a structural and functional interface between the epithelial compartments and the extracellular matrix. Its integrity maintains a structural and functional relationship between the secretory parenchymal epithelium and the stroma. All basement membranes are biochemically characterized by a similar grouping of proteins which include type IV collagen (Bornstein & Sage 1980), laminin (Timpl *et al.* 1979) and heparin

sulphate proteoglycan (Hassell *et al.* 1980; Lozzo 1985). Immunohistochemical studies have shown that basement membrane constituents, such as laminin and type IV collagen, are absent in many invasive tumours when compared with their benign counterparts (Albrechtsen *et al.* 1981; Siegal *et al.* 1981; Barsky *et al.* 1983; Liotta *et al.* 1983; Charpin *et al.* 1986; Willebrand *et al.* 1986; Furness & Lam 1987). Recently, the presence and distribution of laminin has been evaluated immunohistochemically in normal, in hyperplastic and in neoplastic human prostate tissues. These findings showed that laminin expression is directly correlated with prostate epithelial differentiation (Sinha *et al.* 1989). In the foetal prostate, acinar basement membranes were characterized by a thin, regular and continuous band of immunohistochemical staining. In adult normal prostate, and in various non-neoplastic conditions, including hyperplasia, atrophy and prostatitis, acinar basement membranes were locally thickened but also contained regions in which laminin or type IV collagen were focally absent. It is suggested that such loss of basement membrane determinants may reflect a normal physiological process of local repair and remodelling – indicating that the basement membranes and their interaction with the adjacent epithelium are not static but remain in a constantly dynamic relationship.

In contrast with the study by Sinha *et al.* (1989), intracytoplasmic laminin or type IV collagen immunoreactivity in adult normal, in hyperplastic or in neoplastic prostatic epithelium has not been identified. This discrepancy may be due to differences in antibody specificities. In contrast, intracytoplasmic laminin, type IV collagen and heparan sulphate proteoglycan immunoreactivity were identified in foetal glands. The findings of this study suggested that basement membrane components of acinar membranes are synthesized by the secretory epithelium. This is an interesting and important hypothesis which deserves further evaluation since the protein-synthetic capacity of immature prostatic epithelium and of post androgen-stimulated epithelium are probably quite distinct in many respects. In particular, it would be of significance to know whether synthesis of prostatic basement membrane constituents lies outside the regulation of androgenic hormones.

The proteolytic enzyme pepsin, an endopeptidase with highly specific substrate requirements, together with immunohistochemistry, has been employed to dissect the conformational structures of basement membrane components in prostatic epithelial stromal and neoplastic basement membranes. Analysis of the effects of pepsin digestion on the demonstration of basement membrane components in ethanol- and formalin-fixed tissues has

provided strong evidence that laminin, type IV collagen and heparan sulphate proteoglycan immunoreactivity differ in normal acinar and in stromal as well as in neoplastic basement membrane structures. The selective enhancement-effect of pepsin on basement membrane antigens is well recognized (Sinha *et al.* 1989) and may be related to its ability to solubilize portions of the molecule otherwise masking potentially immunoreactive epitopes (Barsky *et al.* 1984). Thus, the observed differential susceptibility by different basement membrane constituents to the intensity of pepsin exposure most likely reflects conformational differences in the expression of epitopes within the particular proteins comprising acinar, stromal and neoplastic basement membranes. No differences between type IV collagen and laminin immunoreactivity were found in stromal, epithelial or neoplastic basement membranes. When compared with laminin and type IV collagen, heparan sulphate proteoglycan reactivity was weak and revealed distinct basement membranes only in foetal prostate and

prostatic adenocarcinoma. These findings appear to indicate that epithelial basement membranes can be modulated in two ways: (i) individual protein components may be synthesized or not; (ii) depending upon the precise type of prostatic tissue (neonatal, adult, neoplastic, inflammatory, etc.) individual components may be synthesized but subject to conformational changes which, in turn, influence their interaction with adjacent cells, including the epithelium.

In keeping with earlier data obtained from the *in vitro* culture of human breast (Foster *et al.* 1983) and other fully differentiated epithelial tissues, prostatic epithelial cells are able to undergo both proliferation and subsequent differentiation when placed in an appropriate physicochemical environment (Hayward *et al.* 1992). Full differentiation of the proliferated cells to yield the complete spectrum of prostatic epithelial phenotypes is dependent upon their maintenance in a three-dimensional supportive collagenous matrix and stimulation with appropriate humoral additives (Foster *et al.* 1996).

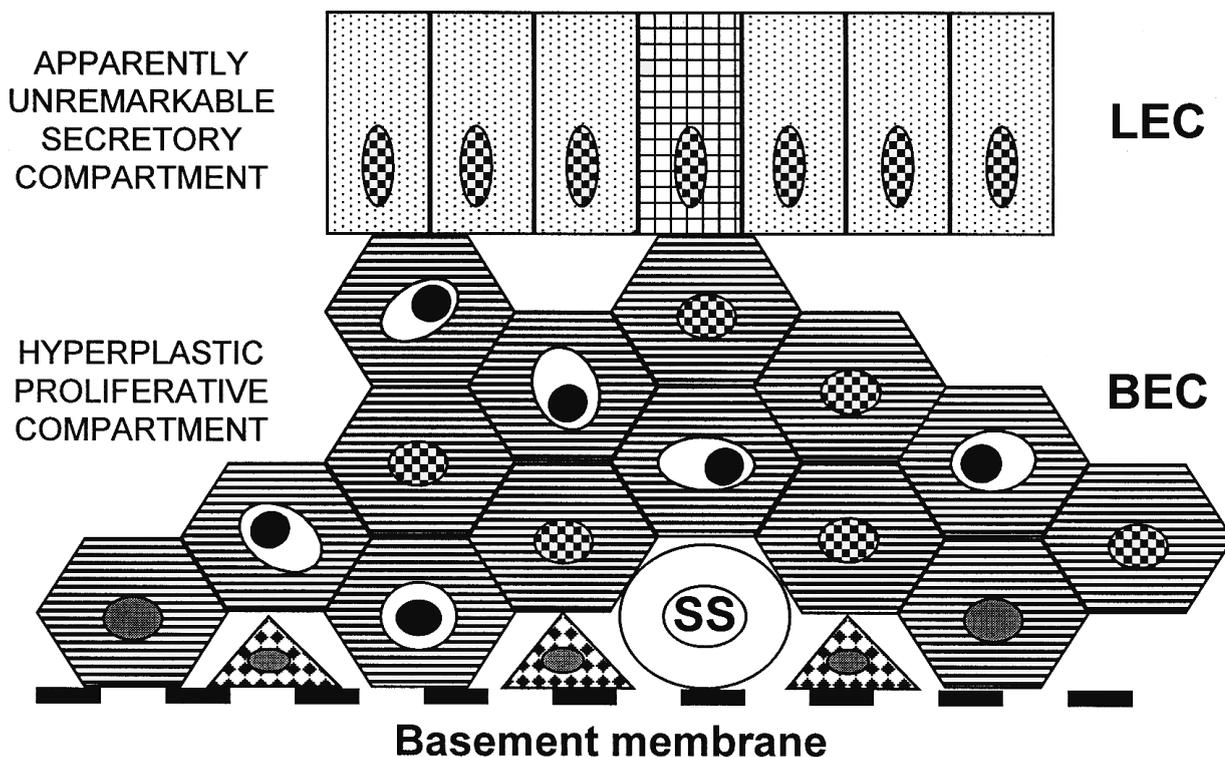


Figure 9. Schematic diagram of basal cell hyperplasia in which it is presumed that the original stem cell compartment remains constant but in which there is hyperplasia of the BEC's. Some proliferating basal cells may contain genetic mutations, as indicated by the open nuclei and prominent nucleoli. It is not yet known whether any cells from this group progress to re-populate the luminal (secretory) epithelial compartment (LEC's), which is a distinct likelihood in at least some examples of prostatic hyperplasia. Nevertheless, in the majority of cases examined, the overlying LEC's are either apparently unremarkable, or have undergone focal metaplasia to a squamous morphology.

However, it is independent of the presence of viable stromal mesenchymal cells. In such experiments, while some attrition of isolated prostatic epithelial cells usually occurs following mechanical or enzymatic injury incurred during the extraction process, mitotic activity may be demonstrated in both the peripheral (basal) and internal (luminal) populations. Nevertheless, the greater proportion of proliferative activity occurs in the majority of peripheral cells, particularly those comprising the newly developing ductular structures (Figure 8). These *in-vitro* tissue-culture data further emphasize the observations made on intact mature prostatic tissues that virtually all basally located prostatic epithelial cells retain the ability to undergo both proliferation and differentiation. While in that location, the cells appear to be protected from terminal commitment to programmed self destruction (apoptosis), possibly by expression of stress-related proteins such as hsp70, as well as through other mechanisms involving *bax* or *bak*, already described.

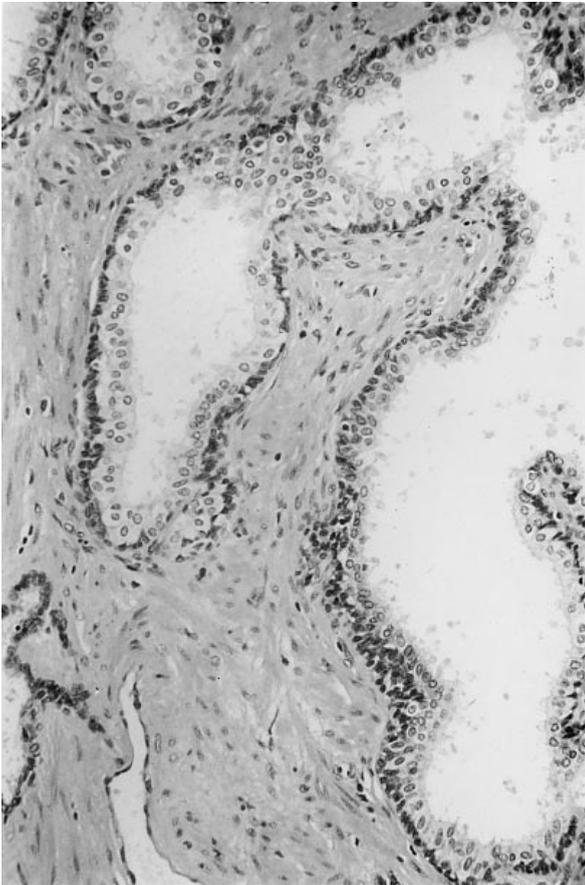


Figure 10. Florid basal cell 'crowding' but without significant multilayering. The overlying luminal epithelium shows the characteristic 'budding' associated with flat PIN. (Magnification $\times 350$).

However, it is not yet known whether activation of some, or any, of these mechanisms in centrally located (luminal) prostatic epithelial cells could permit the reversal of phenotypic differentiation such that any formerly differentiated epithelial cells not already committed to apoptosis might undergo further cell division and subsequent differentiation to yield an identical spectrum of prostatic epithelial phenotypes to that developing from the basal cell population. Such a process would account, at least in part, for some of the anomalous appearances of cell-proliferation and differentiation occurring in pathological, but non-neoplastic, prostatic epithelia.

Basal cells in prostatic epithelial hyperplasia

Basal cell hyperplasia is an entity recognized by pathologists and consists of a thickness of two or more

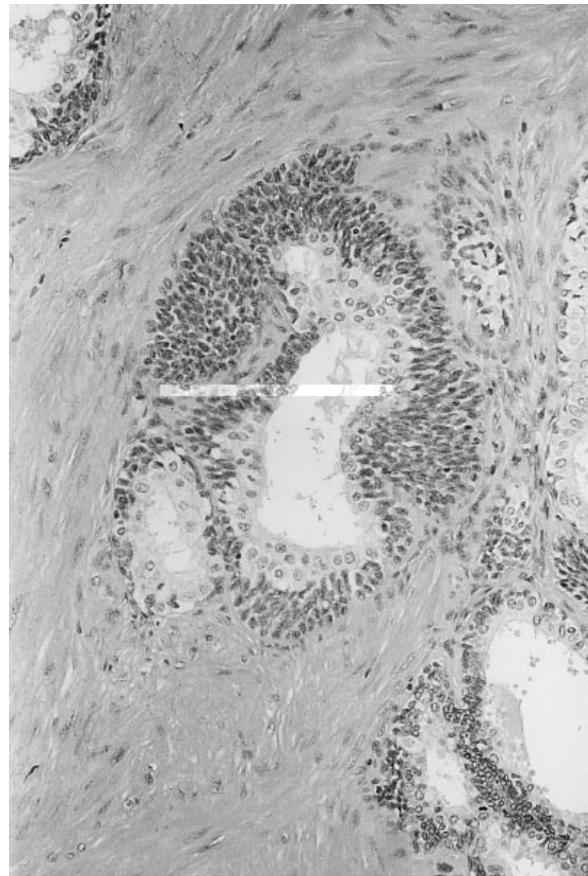


Figure 11. Florid basal cell hyperplasia behaving in a 'Pagetoid' manner undermining and elevating the morphologically unremarkable luminal epithelium. Diagnostically, these appearances, as do those in Figure 2, require accurate differentiation from transitional cell carcinoma of urothelial origin migrating along prostatic ductular and acinar structures. (Magnification $\times 350$).

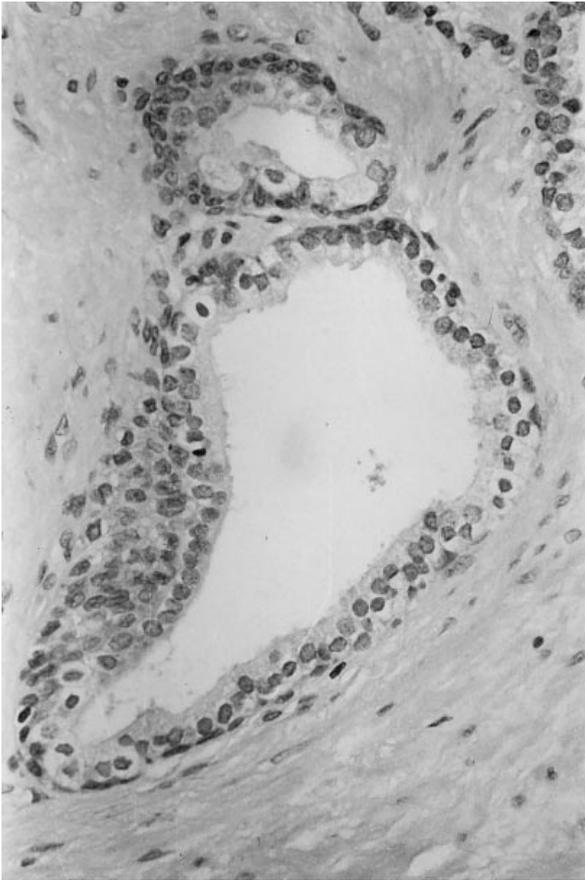


Figure 12. Atypical epithelial hyperplasia in which cells intermediate in morphology between basal and fully differentiated luminal cell-types have accumulated. These cells contain enlarged nuclei, some with prominent nucleoli. Some of the immediately overlying luminal epithelial cells contain similarly anomalous cytological features. (Magnification $\times 350$).

basal-type epithelial cells adjacent to the basement membrane cells at the periphery of prostatic acini (Figure 9). While a minimum thickness of two basal cells is said to be required for diagnosis, some authorities consider this criterion to be arbitrary (Leu *et al.* 1986). This definition also fails to take account of the lateral crowding of basal cells, which is recognized as a focal or general phenomenon in many otherwise benign prostatic specimens (Figure 10). Morphologically, basal cell hyperplasia can appear as small nests of cells surrounded by a few concentric layers of compressed stroma, often associated with chronic inflammation. The nests may be solid or cystically dilated, and occasionally are punctuated by irregular round luminal spaces, creating a cribriform pattern (Devaraj & Bostwick 1993). Basal cell hyperplasia typically involves only part of an acinus and sometimes protrudes into the lumen,

retaining the overlying secretory cell layer; less often symmetric duplication of the basal cell layer is observed at the periphery of the acinus (Figure 11). The cells in basal cell hyperplasia are enlarged, ovoid, or round and plump, with large, pale ovoid nuclei, finely reticular chromatin, and a moderate amount of cytoplasm. Nucleoli are usually inconspicuous (smaller than $1\ \mu\text{m}$ diameter), although they are enlarged in atypical basal cell hyperplasia. Basal cell hyperplasia is rarely associated with atypical adenomatous hyperplasia (Bostwick & Devaraj 1997).

Histologically, basal cell hyperplasia with sclerosis refers to the presence of delicate, lace-like fibrosis or dense irregular sclerotic fibrosis and hyperplastic smooth muscle surrounding and distorting hyperplastic basal cell aggregates. Clear cell change is common in basal cell hyperplasia and other forms of basal cell proliferation, often with a cribriforming pattern. Squamous metaplasia is found infrequently, usually in association with infarction. Chronic inflammation is common but not specific. Nuclear grooves are infrequent. Nuclear 'bubble' artefact, appearing as intranuclear vacuoles, is frequently observed in formalin-fixed specimens, although not in frozen sections, and appears more prominent in basal cells than in secretory luminal cells. Focal calcification is evident in some lesions and may be present within the basal cell nests. Basal cell hyperplasia associated with androgen ablation as with oestrogenic stimulation usually shows prominent squamous metaplasia.

Atypical basal cell hyperplasia

Atypical basal cell hyperplasia is identical to basal cell hyperplasia except for the inclusion of large, prominent nucleoli (Bonkhoff & Remberger 1993; Devaraj & Bostwick 1993) which are round to oval and lightly eosinophilic (Figure 12). In the majority of cases chronic inflammation is present, suggesting that the characteristic nucleomegaly is reactive. A morphological spectrum of nucleolar size is observed in basal cell proliferations and considered atypical only when more than 10% of cells exhibit prominent nucleoli. There is no apparent clinical significance to atypical basal cell hyperplasia, but it is a pitfall to a histopathological diagnosis.

Prostatic intraepithelial neoplasia (PIN)

Prostatic intraepithelial neoplasia (PIN) defines to the appearances occurring in the middle region of the spectrum extending between morphologically normal and frankly malignant prostatic epithelium (Epstein & Armas

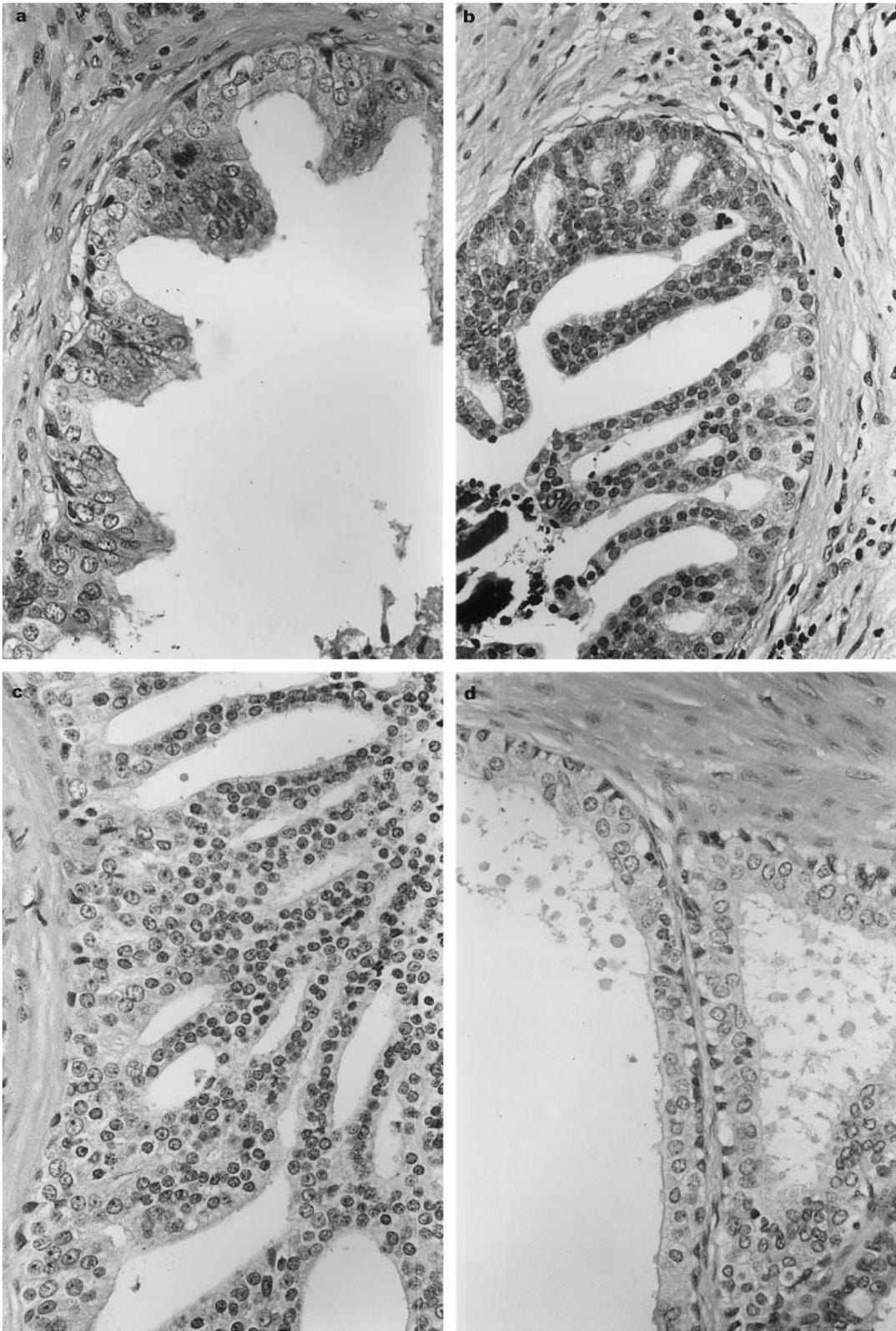


Figure 13. Morphological appearances of high grade PIN. a, tufting; b, micropapillary; c, cribriform; d, flat. Note the distinctive appearance of the characteristic 'budding' from the luminal plasma membrane in d. (Magnification $\times 350$).

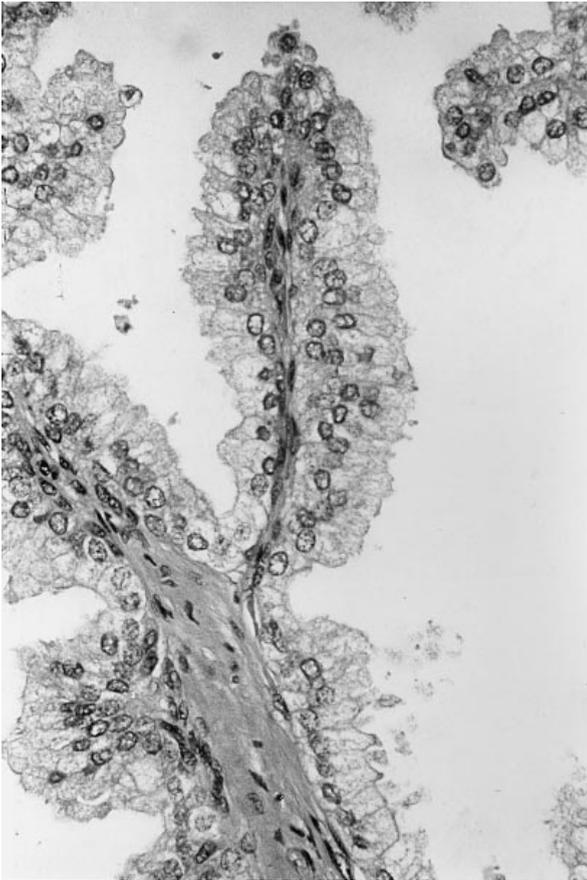


Figure 14. Non-neoplastic papillary hyperplasia in which the crowded luminal-type epithelial cells are located on fine fibrovascular core structures, but without intervening basal cells. Nuclei are finely granular and without nucleoli. There are no features suggesting PIN. (Magnification $\times 350$).

1992; Devaraj & Bostwick 1993;). Originally classified into three groups, the consensus view endorsed by an international conference held at the Mayo Clinic in 1995 was that PIN should be considered only as either 'low' (formerly '1') or 'high' (formerly '2' or '3') grade (Bostwick & Brawer 1987). It is characterized by a range of architectural and cytological factors which include progressive loss of the epithelial two-cell arrangement with eventual disappearance of basal cells (Montironi *et al.* 1996). During this process, the overall proliferative role of the affected epithelium may remain constant, or become increased, as the balance between cellular proliferation and atrophy by apoptosis becomes deranged. While hyperplasia is not *per se* an obligatory characteristic of PIN, it is a frequent accompaniment. However, there is no absolute requirement for the original steady-state within the epithelium to become altered while mutational

events shift the affected populations of cells from normality through dysplasia to absolute neoplasia (carcinoma *in situ*) and, eventually, to invasive malignancy. Thereafter, as apoptotic regulatory mechanisms fail, there is a common tendency for numbers of malignant cells to increase by geometric progression. However, this is uncommon and hyperplasia is recognized as an important component in the three architecture patterns (Figure 13) of tufting, micropapillary and cribriform (Bostwick 1996). The fourth pattern (flat) is explained by a retained balance between hyperplasia and atrophy. Diagnostically, these appearances require differentiation from benign epithelial hyperplasia (Figure 14), which has no future neoplastic implications. Prostatic intraepithelial neoplasia is not detectable by transrectal ultrasonography, MRI scanning or any technique other than microscopic examination (Bostwick *et al.* 1993). With respect to diagnostic criteria, the cytological hallmarks of PIN are nuclear and nucleolar enlargement (Figure 15). In the most severe foci, nuclei are usually uniformly enlarged, although condensed hyperchromatic forms may also be found. Increased variability of nuclear morphology is associated with low-grade intraepithelial neoplasia.

With respect to the clinical management of patients, the importance of diagnosing PIN is its high predictive value as a prognostic marker for the occurrence of adenocarcinoma. Evidence to support this association as a functional relationship has been obtained from several sources. Davidson compared 100 patient needle-biopsies of high-grade PIN with 112 control biopsies matched for clinical stage, patient age and serum PSA (Lee *et al.* 1989). In subsequent biopsies of both cohorts of patients, adenocarcinoma appeared in 35% of those with PIN when compared to 13% of the controls. Of the parameters measured, PIN provided the highest risk-ratio. Only thereafter were patient age and serum PSA concentrations the next most significant predictors of cancer. Conversely, the *amount* of PIN on biopsy, the architectural pattern of PIN, patient ethnic origin, digital rectal examination findings and transrectal ultrasound results were NOT candidate predictors. With respect to an initial diagnosis of PIN, the likelihood of detecting prostate cancer in successive biopsies performed on an individual patient was greater in those undergoing more than one biopsy on any single occasion (44%) than in those in which only one biopsy was performed (32%).

High grade PIN is currently identified in up to 16% of contemporary needle biopsies, separately from that encountered in transurethral resection specimens (Davidson *et al.* 1995), thus further emphasizing the differences between peripheral prostatic tissue sampled by needle biopsy and control tissues taken on TURP. All

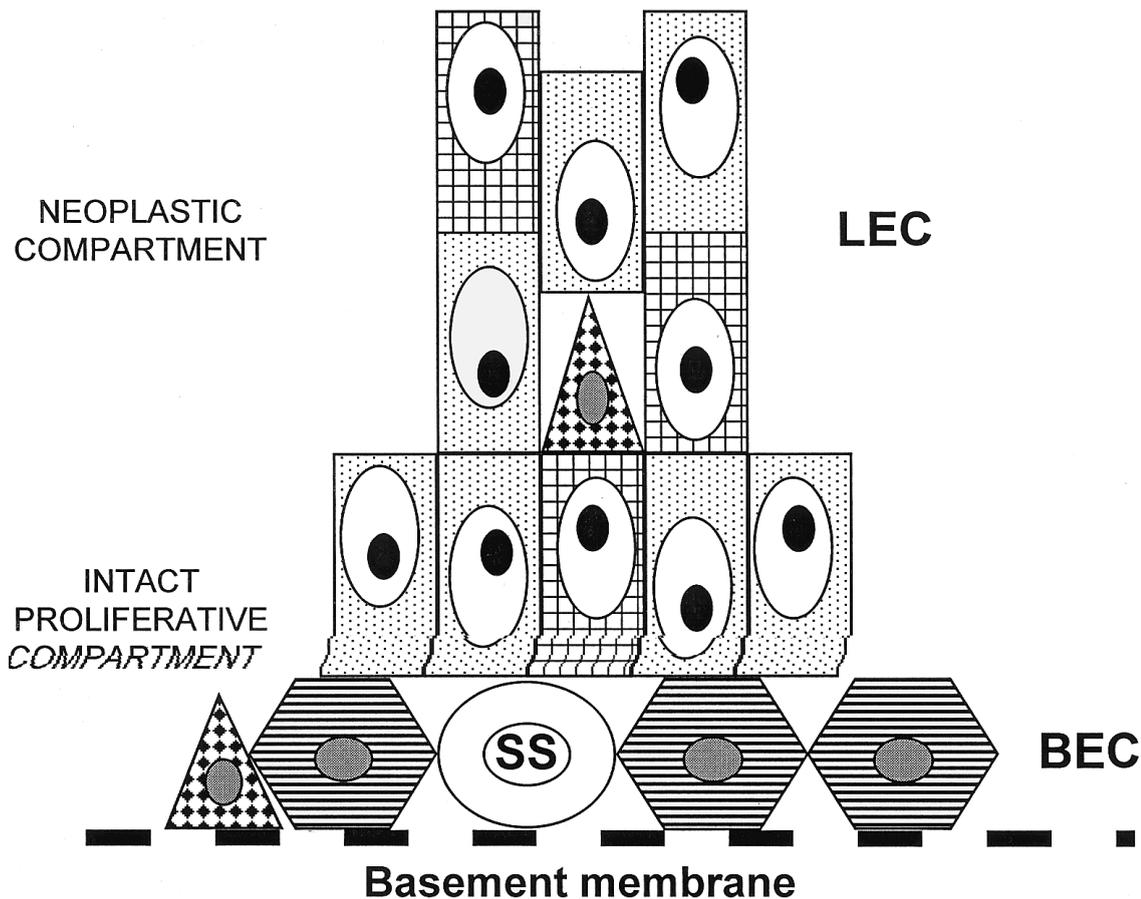


Figure 15. Schematic diagram of prostatic intraepithelial neoplasia (PIN) in which a genetic mutation within the stem cells (SS), basal epithelial cell or luminal (secretory) epithelial compartments give rise to new populations of luminal epithelial cells with the architectural appearances of multilayering (except *flat* PIN) together with the characteristically enlarged nuclei containing prominent nucleoli. Differentiation within this population can also give rise to the appearance of anomalous neuroendocrine cells (triangular) within the luminal compartment (see also Figure 5). In PIN, the layer of basal epithelial cells (BEC's) and the adjacent basement membrane remain intact.

surgical pathologists reporting prostatic biopsies should routinely search for PIN, grade it according to conventional criteria, and report it to the submitting urologists as a benign but potentially premalignant entity in order to ensure close follow-up of affected patients. When PIN is encountered in TURP specimens, all residual chippings should be embedded and examined microscopically. To differentiate high grade PIN from *in-situ* or invasive cancer, the presence of basal cells may be confirmed immuno-histochemically using antibodies such as AE1/AE3 or 34 β -E12 which recognize high molecular weight cytokeratins (Bostwick *et al.* 1995). Where basal cells are preserved as an intact or fragmented basal layer in PIN, absence of basal cells is an important marker raising a high suspicion of prostate cancer. While identification of PIN, whether of low- or high-grade should not dictate or

influence therapeutic decisions (Allsbrook & Pfeiffer 1997) follow-up at between 3- to 6-month intervals for a minimum of two years is recommended and thereafter at 12 month intervals for life (Montironi *et al.* 1996).

Stromal cells

Hitherto, this review has concentrated on an examination of stem cells within the epithelial compartment of the prostate. However, as is clear from the forgoing discussion, these epithelial cells depend for their phenotype and function upon the biochemical composition and structure of the basement membranes with which they are in direct physical contact, and also upon the cellular composition and metabolic activity of surrounding non-epithelial stromal cells. This latter compartment is

presently undergoing intense scrutiny, particularly with respect to the role of its contained cells in benign prostatic hyperplasia and in modulating neoplastic transformation within the epithelium. Work in this laboratory has shown that the stroma is not a phenotypically static collection of cells but is undergoing constant dynamic change. At the earliest embryonic stage, the presence of sympathetic neurones penetrating the substance of the developing prostate during weeks 10–20 of intrauterine life cause neo-expression of smooth muscle-associated proteins within a population of stromal cells previously considered to be undifferentiated mesenchymal (fibroblastic) in type. In later life, after the development of clinically apparent benign prostatic hyperplasia, and irrespective of the presence or absence of neoplastic transformation within the epithelium, a proportion of cells within the stromal compartment remains 'plastic' in that they also respond by modulating expression of smooth muscle contractile proteins in response to altered concentrations of noradrenaline, at least in tissue culture (Smith *et al.* 1997). In common with other glandular tissues in the body there is also a population of circulating mesenchymal cells within the prostate, particularly of reticuloendothelial and lymphoid types. While these cells lie outside the remit of this current review, they do play an integral role in determining response of the individual prostate gland to infection, inflammation and neoplasia, particularly through the secretion and action of highly potent cytokines. Such molecules, which are extremely effective paracrine mediators at very low concentrations, secondarily influence any response by epithelial stromal cells to alterations in cell-regulatory events such as apoptosis and neoplasia. At the present time, details of the complex interaction between these two groups of cells may only be surmised since the individual mechanisms have not yet been elucidated.

Based upon a series of detailed studies performed *in vitro* and *in vivo*, Cunha *et al.* (1996) have proposed a single hypothesis which unifies prostatic developmental biology and carcinogenesis. The principle underlying this hypothesis is the reciprocal interaction occurring between epithelial and mesenchyme during prostatic development and which is followed by a similar reciprocal homeostatic interaction of epithelium and smooth muscle in adulthood. It is suggested that, under constant androgenic drive, prostatic stromal smooth muscle stimulates prostatic epithelium through a combination of physical and humoral stimuli to maintain differentiation and to repress proliferation. Conversely, the same physico/humoral interaction maintains smooth muscle differentiation of prostatic stromal cells. The absolute requirement of androgen to initiate and to maintain this epithelial

differentiation has been confirmed both *in vitro* and *in vivo*. Where luminal epithelium was selectively lost, or did not develop following withdrawal, or in the absence of androgen, differentiation of prostatic luminal and basal epithelial cell-types did not occur (Hayward *et al.* 1996). These experiments not only provide compelling evidence dispelling the notion that adult prostatic tissue is terminally and irrevocably differentiated but demonstrate the immense potential for a wide range of further differentiation retained by a population of apparently fully-differentiated prostatic epithelial cells. Rather than being fixed into a terminal phenotype, a functionally-definable and responsive group of adult prostatic epithelial cells retain a developmental plasticity equivalent to their undifferentiated foetal counterparts and, following application of appropriate physico-chemical stimuli, are capable of being reprogrammed *in situ* to express completely new morphological and functional phenotypes (Lipschutz *et al.* 1996).

Conclusions

The human intact normal prostate is, both structurally and functionally, a highly complex glandular tissue. Cells within the epithelial compartment are subject to continuous proliferation and differentiation to compensate for a wide range of degenerative and destructive processes. In order that this process of replenishment might be maintained, it is probable that there are at least two interrelated proliferation compartments, each containing stem cells for the particular population. Fundamental or 'first-order' prostatic epithelial stem cells reside within the basal layers of terminal ductules and acini within the normal prostate gland. Physically, these cells allow replenishment of the three broad categories of functional cell-types within prostatic epithelium namely basal myo-epithelial, basal neuroendocrine and luminal secretory. Phenotypically, many of the characteristics of basal cell stem cells have been identified and described earlier in this review. It has also been demonstrated, particularly from immunohistochemical observations made on prostatic luminal epithelial cells in the intact tissue, that within this population of differentiated cells there is a local or 'second order' stem cell population able to replenish some luminal epithelial cells, but which do not under *normal* circumstances, generate the alternative differentiated epithelial lineages of basal and neuroendocrine cells.

Control of the individual phenotypes expressed within normal human prostatic epithelium is exercised by both physical (architectural) and humoral constraints. However, epithelial tissue-culture experiments performed on isolated prostatic epithelial cells *in vitro* have clearly

shown that fully differentiated prostatic epithelium retains a far greater degree of phenotypical 'plasticity' than is immediately apparent from examination of the intact tissue. Strong evidence now indicates that when the normal physical and humoral constraints are withdrawn from the dissociated and isolated epithelium, the majority of these cells undergo dedifferentiation to an uncommitted phenotype expressing none of the characteristic ultrastructural or immunohistochemical features of the differentiated cell-types within the normal gland. Subsequently, the processes of cell-division, lineage-differentiation and organ morphogenesis occur throughout the reorganizing epithelial structures and are not limited to only those cells which were, respectively, external (basal) or internal (luminal). Thus, while it is undoubtedly true that prostatic epithelial cells do become irreversibly differentiated and committed to a single unique phenotypic lineage at some point during their lifespan, current evidence suggests that this 'point of no return' is much later than previously anticipated. It is likely, although not yet proven, that the critical point is attained only when ratios of particular intracellular control proteins (e.g. Bcl-xL, Bcl-xS and bax) reach critical values and an individual epithelial cell becomes committed to apoptosis – whenever that final process might occur. Until that specific point is reached, individual prostatic epithelial cells, whatever their differentiation status (with the possible exception of neuroendocrine), retain the ability to undergo cell division and also remain phenotypically 'plastic' such that, under appropriate (but *abnormal*) physical and humoral stimuli, they modulate their phenotypic characteristics.

Prostatic basal epithelial cells are of particular pathological importance since it is within this population that genetic events predispose an individual to prostatic neoplasia – whether this be along the basal hyperplasia route or the luminal PIN route. Just as occurs in other differentiated tissues (the well-recognized paradigm being lymphoid neoplasia), genetic mutational events occurring at different stages in the spectrum which extends from undifferentiated basal stem cells through to fully differentiated basal myoepithelial cells, basal neuroendocrine cells or luminal secretory cells, will determine the type and nature of the resulting neoplasm. Both positive and negative feedback-loops conducting information between environmental structures and the neoplastic epithelia quickly establish a new steady-state in which the developing epithelial cells exhibit new and characteristic phenotypes. Hence, any loss of control proteins such as Bcl-xL or bax, or a mutation in *p53*, will significantly alter the homeostatic mechanisms regulating the sizes of the basal and luminal epithelial compartments. Cells within these compartments now

responding to powerful humoral stimuli which include both oestrogen and testosterone together with any abnormal analogues which might be produced, result in the establishment of new phenotypic populations of epithelial cells in which normal intercellular arrangements become significantly disturbed. Susceptibility to further promotion of neoplastic events now become significantly enhanced within each of these new populations.

In summary, human normal mature prostatic epithelium within the intact organ contains populations of basally located first-order totipotent stem cells able to replenish the entire epithelial compartment by cell-proliferation and differentiation. A second-order stem-cell population is also present within luminal epithelial cells, and which is able to respond to local demands for increased functional/secretory epithelial activity. Nevertheless, these two groups of stem cells may only be defined within intact normal prostatic tissue and do not reflect the residual or reserve potential within the remaining majority of fully differentiated, prostatic epithelial cells for both cell division and alternative differentiation. It is this inherent 'plasticity', coupled with the ability of the intact tissue to respond by regenerating the complete spectrum of functional prostatic epithelial phenotypes, which is both its strength – and also its weakness. Disturbance and distortion of these physical and humoral regulatory mechanisms controlling the normal sequence of epithelial proliferation, differentiation and apoptosis immediately exposes the cells within any of these compartments to the liability of genetic change resulting in transient, or permanent, neoplastic transformation.

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