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REVIEW ARTICLE

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# Morphogenetic concepts of normal and abnormal growth in the human prostate

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**Abstract** Benign prostatic hyperplasia (BPH) and prostate cancer are multifactorial disease processes, involving a growing number of biochemical, genetic and epigenetic factors. Their pathogenesis, however, remains poorly understood. The present review examines current morphogenetic concepts of normal and abnormal growth in the human prostate. This includes the role of basal cells in organogenesis and cancerogenesis, the impact of cell–matrix interactions, and the importance of cellular heterogeneity in tumour progression and hormone-insensitive growth. Knowledge of morphogenesis and morphology is required in any scientific approach to BPH and prostate cancer.

**Key words** Morphogenesis · Benign prostatic hyperplasia · Prostate cancer

## Introduction

Proliferative disorders of the human prostate have become a growing medical problem, because of their major impact in terms of morbidity, mortality and health care costs. Benign prostate hyperplasia (BPH) occurs with such a high frequency in men over 50 years of age that it is often considered a age-related, physiological process. Prostate cancer is the most commonly diagnosed male malignancy in Western countries, and represents the second leading cause of cancer-related death in the United States [64]. Despite its clinical magnitude, there are important biological issues with regard to the development of benign and malignant prostatic growth.

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Many questions on the molecular and cellular basis of proliferative disease processes in the human prostate remain unanswered. This reflects the complex composition of the gland into three distinct anatomical zones (transition, central and peripheral zones) and the cellular diversity of the prostatic epithelial cell system [1, 46]. The basic cell types making up the prostatic epithelium (secretory luminal cells, basal cells and endocrine cells) clearly differ in their hormonal regulation and marker expression. Secretory luminal cells require continuous support by circulating androgens. Like common prostatic adenocarcinoma, this phenotype expresses the nuclear androgen receptor (AR), prostate-specific antigen (PSA), and cytokeratins 8 and 18 [1, 5, 50, 52, 57, 62]. The current concept of prostatic cancerogenesis therefore postulates a secretory luminal origin of common adenocarcinoma [26, 50, 52, 57, 60, 62]. Basal cells, the second most important phenotype of benign glands, are androgen independent, proliferate under oestrogen stimulation, and may express nuclear oestrogen and progesterone receptors [1, 26, 39, 60, 63]. The marker profile of basal cells differs significantly from those of secretory luminal cells and adenocarcinoma. Basal cells lack PSA and express high-molecular weight cytokeratins that are specific for this phenotype [26, 50, 52, 57, 62]. Finally, endocrine-paracrine cells constitute the third phenotype that produces a number of regulatory peptides, including serotonin, calcitonin, and parathyroid hormone-related peptides [27, 28].

The cellular diversity of the prostatic epithelium suggests the existence of pluripotent stem cells, but the phenotype and localization of putative stem cells are ill defined in the human prostate [1, 39]. In particular, the significant differences between the three basic cell types are difficult to reconcile with a unitarian concept relating the various epithelial cell lineages in a hierarchical pathway of differentiation. Earlier morphological studies have suggested that basal cells function as a generative cell population that can give rise to all differentiated progeny [20, 23, 25, 31, 36, 40, 47]. However, the putative stem cell role of basal cells has not been universally accepted

[1, 26, 29, 30, 50, 52, 62]. The present review examines selected areas of contemporary prostate research related to morphogenesis of BPH and prostate cancer.

## The role of basal cells in benign prostatic growth

In recent years it has become increasingly evident that the basal cell layer houses the prostatic stem cell population [2, 6]. This concept is based on the occurrence of intermediate differentiation between the three basic cell types encountered in the prostatic epithelium. Immunohistochemical studies have shown that basal cells may express basal cell-specific cytokeratins and PSA or the endocrine marker chromogranin A simultaneously [13]. This observation clearly indicates that basal cells have the differentiation potency to give rise to secretory luminal cells and endocrine cells via intermediate phenotypes [13, 66]. Cell kinetic studies indicate that the proliferation compartment of the normal and hyperplastic epithelium is located in the basal cell layer. In fact, 70% of proliferating epithelial cells express basal cell-specific cytokeratins, while the remaining 30% of cycling cells are found in the secretory epithelium [12]. Conversely, endocrine-paracrine cells lack proliferative activity, and represent a post-mitotic, terminal differentiated cell population [7, 14]. The putative stem cell function of basal cells is further supported by the differential expression of the mitochondrial oncoprotein Bcl-2 in the prostatic epithelial cell system [16, 22]. *Bcl-2* is a potent suppressor of programmed cell death and is expressed preferentially in stem cell and proliferation compartments of typical self-renewing tissues (e.g. gastrointestinal tract). In the transition and peripheral prostate zone, *Bcl-2* is restricted to the proliferation compartment (basal cells) and is undetectable in secretory luminal cell types [16]. Downregulation of *Bcl-2* in the secretory epithelium suggests that these cells undergo apoptosis after terminal differentiation and are replaced by generative basal cells.

Regulation of basal cells involves a network of hormonal control, growth factors and adhesive interactions with epithelial basement membranes and other extracellular matrix molecules. It is well established that basal cells are androgen-independent in androgen-cycling experiments and proliferate under oestrogen stimulation [1, 29, 30, 39]. Although androgen independent, basal cells are androgen responsive: they express both the nuclear AR and the 5α-reductase isoenzyme 2, which is crucial for the dihydrotestosterone (DHT)-forming process [5, 15]. In addition to androgens and oestrogens, other nonsteroidal growth factors are implicated in the control of basal cells [49, 60]. In human prostate tissue, basal cells express a number of growth factor receptors, including epidermal growth factor receptor (EGF-R), $p185^{erbB-2}$, $p180^{erb\ B-3}$, c-met, and tumour suppressor genes (nm23-H1) [49, 60]. It is likely that these regulatory peptides are involved in maintaining the proliferative function of basal cells. Another important aspect of basal cell control refers to adhesive interactions with epithelial basement membranes (BM). Experimental studies have shown convincingly that prostatic epithelial cells require BM components for in vitro growth and differentiation [32]. In human prostate tissue, basal cells exhibit polarised distribution of integrin receptors (α6β1, α2β1, α6β4) and hemidesmosome-associated proteins (BP180, BP220, HD1) [10, 41, 51]. It seems likely that formation of stable hemidesmosomes and adhesive interactions with BM contribute significantly to the integrity and biological function of basal cells [3, 51].

## Morphogenesis of glandular hyperplasia

The cumulative data outlined above indicate that the prostatic epithelium is maintained by two functional compartments [2, 6] (Fig. 1). The proliferation compartment is androgen independent and consists of basal cells [12]. The secretory epithelium constitutes the differentiation compartment, which is androgen dependent but has a limited proliferative capacity [12]. The growth rate within the proliferative compartment is regulated by a network of growth factor receptors (e.g. EGF-R, $p185^{erbB-2}$, $p180^{erb\ B-3}$) and *Bcl-2*, preventing basal cells from the androgen-regulated apoptotic cell death [16, 49, 60]. The proliferation compartment most probably houses the prostatic stem cell population that gives rise to all epithelial cell lineages via intermediate cell types [2, 6, 13] (Fig. 1). Differentiation processes within this cell system depend on a hormonal balance between circulating androgens and oestrogens. Oestrogen stimulation induces basal cell hyperplasia by preventing the differentiation process from basal cells to secretory luminal cells [2, 6]. The oestrogen effect is balanced by androgens, which induce the maturation process from basal cells to secretory luminal cells [2, 6]. This indicates that the turnover of the secretory epithelium depends largely on the number of androgen-responsive target cells in the proliferation compartment (basal cells) [2, 5, 6]. Accordingly, an increase in the total number of androgen-responsive basal cells accelerates the differentiation process towards secretory luminal cells and thus leads to glandular hyperplasia (Fig. 2). How can this concept be reconciled with our current understanding of BPH development? The biochemical approach of BPH suggests an age-related increase in the oestrogen/androgen ratio in prostate tissue, resulting from increased oestrogen levels in the stromal compartment and also decreased DHT levels and reduced 5α-reductase activity in epithelial compartments [43, 58]. The differential tissue distribution of nuclear AR and ER (oestrogen receptors) indicates that both stromal and basal cells are targets of the hormonal imbalance between androgens and oestrogens [5, 63]. It is conceivable that the age-related decrease in the DHT-forming process in the epithelial compartment increases AR gene expression in the basal cell layer, making basal cells more sensitive to the low levels of bioavailable androgens [5]. This hormonal imbalance may result in glandular hyperplasia by accelerating the differentiation

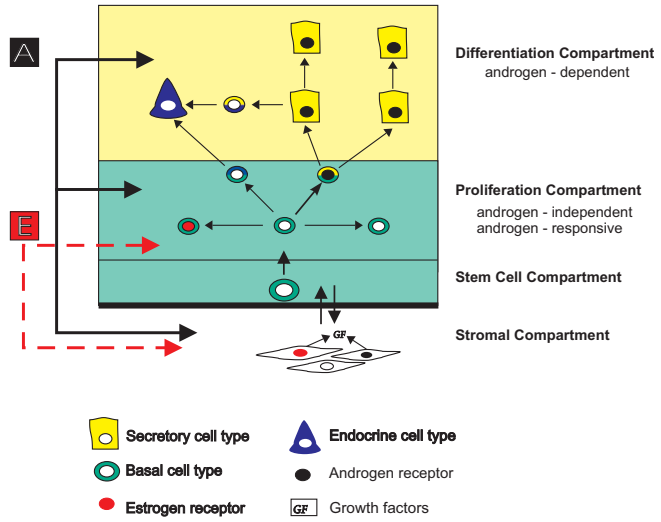


**Fig. 1** Stem cell concept for the organization of the prostatic epithelium. The model illustrates basic differentiation and proliferation processes encountered within the epithelial cell system of the human prostate. From [6], with permission

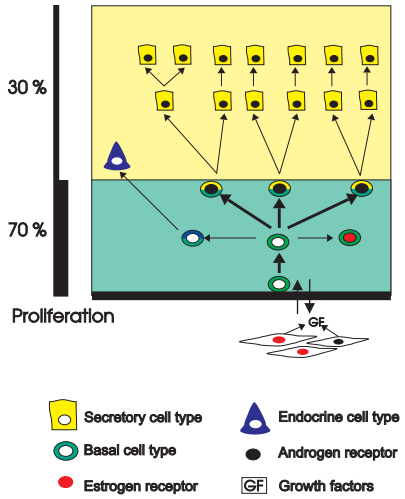


**Fig. 2** Morphogenesis of glandular hyperplasia. The age-related decrease of the dihydrotestosterone (DHT)-forming process increases androgen receptor gene expression in basal cells. The resulting increase of androgen-responsive basal cells in the proliferation compartment results in glandular hyperplasia by accelerating the differentiation process from basal cells to secretory luminal cell types. From [6], with permission

process from basal cells to secretory luminal cells (Fig. 2). Alternatively, stromal derived growth factors may also influence the androgen sensitivity of basal cells in the proliferation compartment. In summary, the proposed stem cell concept suggests that hypersensitization of basal cells to circulating androgens is crucial for the development of glandular hyperplasia.

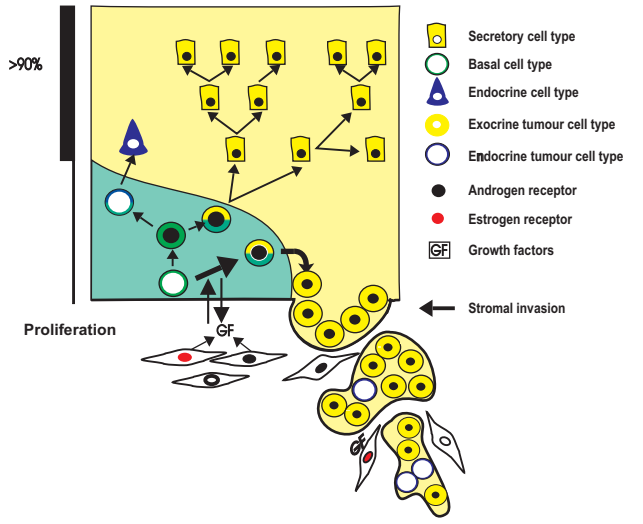


**Fig. 3** Morphogenesis of prostatic adenocarcinoma. The malignant transformation of the prostatic epithelium results from severe differentiation and proliferation disorders involving abnormal expression of growth factor receptors and oncogene products. Transformed stem cells located in the basal cell layer lose critical adhesive elements and acquire exocrine features. Invasive tumour cells produce basement membranes, which provide a supporting scaffold for penetration through the extracellular matrix during the process of stromal invasion and metastasis. Exocrine tumour cells, the major phenotype of prostatic adenocarcinoma, are androgen responsive and retain the DHT-forming process even in hormone-refractory stages of the disease. The progressive emergence of endocrine differentiation in prostatic adenocarcinoma reflects the differentiation potency of its stem cells and has prognostic implications. Endocrine tumour cells devoid of the nuclear androgen receptor and proliferative activity are likely to escape androgen deprivation and radiation therapy. Neurosecretory peptides produced by these cells can exercise growth-promoting effects on adjacent exocrine tumour cells and thus maintain tumour growth in an androgen-deprived milieu. From [6], with permission

## Differentiation and proliferation abnormalities in early phases of prostatic cancerogenesis

High-grade prostatic intraepithelial neoplasia (HGPIN) is considered to be the most likely precursor of peripheral zone cancer [17, 18, 48]. This lesion most frequently develops within pre-existing duct-acinar units of the peripheral zone and shows nuclear atypias similar to those found in poorly differentiated carcinomas. Tumours deriving from HGPIN tend to be poorly differentiated and clinically significant. HGPIN has a high predictive value as a marker for adenocarcinoma, and its identification in biopsy specimens warrants a further search for concurrent invasive cancer [17, 18, 48].

These premalignant lesions result from abnormal differentiation and proliferation processes within the prostatic epithelial cell system. Cell kinetic studies have shown that the basal cell layer loses its proliferative function, while secretory luminal cells acquire enhanced proliferative capacity [12]. In HGPIN, less than 10% of cycling cells are detected in the former proliferation compartment. The proliferative zone shifts to secretory luminal cells in the differentiation compartment [12].

Extension of the proliferative zone to the differentiation compartment is a typical premalignant proliferation disorder. Similar changes have been described in colorectal adenomas and other premalignant lesions. The proliferative abnormalities encountered in HGPIN are most probably related to an aberrant expression of growth factor receptors and tumour suppressor genes.

A number of biomarkers, including p185 $^{erbB-2}$, p180 $^{erbB-3}$, c-met and nm23-H1 restricted to basal cells in normal conditions, are overexpressed in the secretory epithelium of HGPIN [49, 60]. In addition, severe regulatory disorders of programmed cell death occur during the early phases of prostatic cancerogenesis. Approximately 20% of HGPIN overexpresses the apoptosis suppressor *Bcl-2* in the secretory epithelium, which has potential implications [16]. Recent immunohistochemical studies using double-label techniques have shown that the *Bcl-2*-mediated process does not depend on the proliferative capacity, but on the androgen sensitivity of the dysplastic epithelium [16]. In fact, HGPIN overexpressing *Bcl-2* tend to down-regulate the nuclear AR, as documented by markedly reduced levels of nuclear AR [16]. This indicates that HGPIN lesions with abnormal *Bcl-2* expression are probably resistant to androgen-mediated programmed cell death. Clinico-pathological studies have shown that subsets of HGPIN persist after neoadjuvant total androgen blockade, documenting their androgen insensitivity [17, 18]. The abnormal expression of *Bcl-2* in HGPIN also interferes with normal differentiation processes by protecting the differentiation compartment from apoptotic cell death. The resulting increased life span of the transformed cells, together with the high proliferation rate, provides an environment in which genetic instability can occur. The most common numerical alterations detected in HGPIN so far include gains of chromosomes 8, 7, 10, indicating that genes on these chromosomes may play a role in early phases of prostatic cancerogenesis [18, 56]. The overall frequency of numeric chromosomal anomalies reported is remarkably similar in HGPIN and in invasive cancer, suggesting that they have a similar pathogenesis [18, 56]. In summary, virtually all phenotype and genotype data amassed in recent years clearly support the concept that HGPIN is the precursor of most prostatic adenocarcinomas, particularly those arising in the peripheral zone [18, 19].

Another potential precursor of prostatic adenocarcinoma is atypical adenomatous hyperplasia (AAH). This lesion arises predominantly in the transition zone and is usually associated with hyperplastic nodules [18, 33, 48]. AAH refers to an architecturally atypical, small acinar lesion that mimics low-grade (Gleason patterns 1 and 2) adenocarcinoma arising in the transition zone [18, 33, 48]. There is some evidence to suggest that AAH is a precursor of transition zone cancer, including morphological similarities between AAH and small acinar adenocarcinoma [18]; increased incidence with autopsy cancer (31% vs 15% without cancer at autopsy) [18]: topographical relationship with transitional zone cancer [18]; proliferation rates and AgNOR counts intermediate between hyperplasia and low-grade adenocarcinoma [33, 37]; and evidence of genetic instability. Allelic imbalance was found in 7 of 15 cases of AAH, indicating a genetic link with adenocarcinoma [21].

However, AAH does not reveal typical premalignant differentiation and proliferation abnormalities as found in HGPIN. Although the proliferative activity of AAH is increased, the proliferation zone and *Bcl-2* expression are restricted to basal cells as described in benign glands [6, 12]. In conclusion, the biological and clinical significance of AAH is uncertain. AAH may be a precursor of low-grade adenocarcinoma, particularly of the transition zone, although its predictive value for adenocarcinoma is currently unknown [18, 33, 48]. At present, the morphogenesis of low-grade, small acinar adenocarcinoma remains controversial [48]. Many of these lesions obviously derive from inconspicuous duct-acinar units without any morphological intermediate between nondysplastic epithelium and small acinar adenocarcinoma. Novel phenotype and genotype markers are required to define malignancy-associated changes that are currently undetectable at the histology level in the human prostate.

## Adhesive interactions in preinvasive lesions and invasive prostate cancer

Adhesive interactions in premalignant lesions do not differ significantly from those encountered in benign prostate glands. HGPIN retains BM and expresses the same BM proteins and integrin receptors as are described in normal or hyperplastic conditions [10, 35, 41, 51]. This obviously reflects the presence of basal cells in HGPIN. Nevertheless, more subtle alterations are detectable at the mRNA level. In situ hybridization (ISH) studies have shown that HGPIN reveals increasing transcriptional activity of BM coding genes in secretory luminal cell types, reaching steady state levels of adjacent cancer cells [54]. The abnormal gene expression may modify the biochemical composition of the underlying BM, which may in turn interfere with critical adhesive interactions [54]. Dramatic changes occur during early stromal invasion, when the transformed epithelium loses basal cell differentiation. During this process, HGPIN loses a number of hemidesmosome-forming proteins and associated adhesive molecules, including BP180, BP230, HD1, collagen VII, β3- and γ2-subchains of laminin 5, and α6β4-integrins [35, 41, 51]. There is little doubt that normal acini and HGPIN require these adhesive elements to maintain the basal cell integrity. Accordingly, the inability of transformed cells to synthesize hemidesmosome-forming proteins is obviously a key step in the progression of HGPIN to early invasive cancer.

Both experimental and morphological data suggest that prostate cancer cells require BM to invade the stroma [3, 8–10]. Immunohistochemical studies have shown that invasive tumour cells are separated from the host tissue by BM and express integrin receptors that mediate

attachment to these BM-like matrices [8–10]. This particular tumour/stroma relation encountered in prostatic adenocarcinoma is maintained through the various stages of the disease, including high-grade lesions, therapy-induced changes, and metastasis [8–10]. The functional significance of BM has also been demonstrated in vitro, showing that prostate cancer cell lines generally require reconstituted BM (Matrigel) to be tumorigenic in athymic mice [53, 55]. Recent ISH analysis in human tissue indicates that BM components are produced predominantly by tumour cells and not by the host tissue [54]. High steady state levels of BM-coding genes were reported in high-grade carcinomas and lymph node metastasis, suggesting that the BM-forming process increases with tumour progression and metastasis [54]. It is quite clear that the functional role and biochemical nature of BM differ in normal and neoplastic conditions. Immunohistochemical studies have shown that the immunoreactivity of normal and neoplastic BM components differs in their differential susceptibility to pepsin treatment, suggesting conformational differences in the location of epitopes on the molecule [8]. As mentioned above, neoplastic BM lack hemidesmosome-associated laminin 5, collagen VII, and type IV collagen $\alpha 5$ and $\alpha 6$ chains [24, 35, 41, 51].

In summary, existing data indicate that formation of de novo synthesized BM and adhesion via specific receptors are crucial for the process of stromal invasion and metastasis in prostate cancer [3].

## Implications of phenotype heterogeneity in prostate cancer

Common prostatic adenocarcinoma is composed predominantly of exocrine tumour cells sharing phenotype similarity with secretory luminal cells (for example PSA; cytokeratins 8, 18) [50, 64]. The second most important phenotype reveals neuroendocrine (NE) differentiation. Immunohistochemical studies using the panendocrine marker chromogranin A (ChrA) have shown that NE differentiation occurs in virtually all common adenocarcinomas [27, 28]. Extensive and multifocal NE features are found in approximately 10% of all prostatic malignancies [27, 28]. Recent clinico-pathological studies suggest that NE differentiation predicts tumour progression after radical prostatectomy and radiation therapy in clinically advanced disease [34, 61]. The potential prognostic implications of NE differentiation have attracted increasing attention in contemporary prostate cancer research [27, 28]. Recent progress in this field indicates that the proliferation compartment of prostatic adenocarcinoma consists of exocrine cell types, while NE tumour cells remain in a quiescent state within the cell cycle [7, 14]. Accordingly, the prognostic significance of NE differentiation cannot be explained by the proliferative capacity of the NE phenotype. Other regulatory mechanisms are likely to be involved in this process. NE tumour cells secrete a number of regulatory products with growth-promoting activity, including serotonin, bombesin, and parathyroid hormone-related peptides [27, 28]. Immunohistochemical studies using double-label techniques have shown that NE tumour cells are associated with increased proliferative activity of adjacent exocrine cells, which probably reflects a paracrine influence on cell proliferation [7].

Concerning the morphogenesis of NE differentiation in prostatic adenocarcinoma, it is most unlikely that NE tumour cell type derives from nonproliferating, terminal differentiated NE cells of normal acini or HGPIN [2, 4, 6, 13, 65]. The frequent occurrence of amphicrine (PSA- and ChrA-positive) tumour cells supports the concept that NE tumour cells derive from exocrine (PSA-positive) cell populations that acquire endocrine features during tumour progression [4, 13].

Another important aspect of NE differentiation refers to its potential implication in the development of androgen insensitivity [4]. Although androgen dependent in early stages, locally advanced adenocarcinomas generally recur and progress during androgen deprivation therapy. The molecular mechanisms responsible for development of hormone-refractory disease are complex, and involve AR gene mutations, AR gene amplifications, and downstream signalling pathways [42]. Immunohistochemical studies have shown that hormone-refractory and metastatic adenocarcinomas continue to express the nuclear AR and $5\alpha$-reductase isoenzymes 1 and 2 at high levels [11, 15, 38, 45]. Continuous expression of nuclear AR in an androgen-deprived milieu probably involves a high level of AR gene amplification, which is a frequent event in recurrent tumours but has not yet been detected in primary tumours [42, 59]. An alternative pathway by which prostate cancer cells escape hormonal control is provided by the process of NE differentiation [4]. In fact, NE tumour cells detected by ChrA consistently lack the nuclear AR in primary carcinoma and recurrent disease [11, 44]. This clearly indicates that NE phenotypes belong to those cell populations in prostatic adenocarcinoma that are initially androgen insensitive and refractory to hormonal therapy.

## Morphogenesis of prostate cancer

In the early phase of prostatic cancerogenesis, the prostatic epithelium accumulates phenotype and genotype changes, resulting in severe differentiation and proliferation disorders (Fig. 3). The proliferation abnormalities encountered in HGPIN (i.e. extension of the proliferation zone) are associated with aberrant expression of growth factor receptors ($p185^{erbB\text{-}2}$, $p180^{erb\ B\text{-}3}$, c-met) and tumour suppressor genes (nm23-H1) [12, 49, 60]. Overexpression of the *Bcl-2* gene product in subsets of HGPIN confers resistance to the androgen-mediated apoptotic cell death and may increase genetic instability [16, 22]. Transformed stem cells lose their basal cell phenotype and acquire exocrine features (Fig. 3). The definite loss of basal cell differentiation is a critical

point in the progression to invasive cancer, and is most probably related to the inability of transformed cells to produce hemidesmosome-forming proteins [3, 41, 51]. Invasive tumour cells produce BM-like matrices, which are crucial for anchorage and penetration through the host tissue during stromal invasion and metastasis [8–10, 54]. Common prostatic adenocarcinoma is composed mainly of exocrine cell types, which remain androgen responsive through the various stages of the disease [38, 45]. These cells express the nuclear AR and 5α-reductase 1 and 2 even in metastatic, recurrent and hormone-refractory disease [15, 38, 45] (Fig. 3). Amplification of the AR gene is an attractive hypothesis to explain the continuous expression of nuclear AR at high levels in an androgen-deprived milieu [42, 59]. The presence of nuclear AR, however, does not imply androgen dependence. AR gene mutations can lead to an altered AR protein responding to oestrogen, progesterone and other steroids [42].

The second most important phenotype of common adenocarcinoma shows NE differentiation, which has potential prognostic implications [4, 27, 28]. NE tumour cells probably derive from exocrine cell types during tumour progression, which reflects the differentiation repertoire of prostatic stem cells [13]. NE differentiation occurs exclusively in the G0 phase of the cell cycle, indicating that these cells are more resistant to radiation therapy and cytotoxic agents than proliferating (exocrine) tumour cells [14]. Conversely, the various neurosecretory products secreted by NE tumour cells may induce proliferation of adjacent exocrine cells via paracrine mechanisms [4, 7]. The lack of detectable nuclear AR clearly indicates that NE tumour cells are androgen insensitive [11, 44]. These data suggest that NE differentiation can affect the natural history and prognosis of prostate cancer by different pathogenetic pathways.

## Conclusion

BPH and prostate cancer are complex disease processes involving phenotype, epigenetic and genetic factors. In each methodical approach, knowledge of prostatic cellular heterogeneity and morphology is essential. This highlights the role of pathologists in contemporary prostate disease research.

The proliferative and putative stem cell function of basal cells illustrates the paramount importance of this particular phenotype in normal prostatic growth and in the development of BPH and prostate cancer. The molecular basis of basal cell control remains largely unknown, however. Both experimental and morphological studies suggest that basement membranes and their adhesive interactions with transformed cells have important implications for the multistep process of stromal invasion. NE differentiation has received little attention in molecular studies, despite existing clinical and morphological data supporting its role in tumour progression and androgen-insensitive growth. The morphogenetic data reviewed in this article may provide a conceptual framework for studying the impact of biochemical and genetic factors on prostate disease processes.

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