Minireview

# Epidermal growth factor, neurotrophins and the metastatic cascade in prostate cancer

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**Abstract Although cancer of the prostate (CaP) is the most commonly occurring cancer in males, there are major limitations in its diagnosis and long-term cure. Consequently, understanding the molecular mechanisms involved in the progression of CaP is of particular importance for production of pharmacological and biological agents to manage the disease. The development of the normal prostate is regulated by stromal–epithelial interactions via endocrine and paracrine factors, such as androgens and growth factors, which act as precise homeostatic regulators of cellular proliferation. Importantly, after a period of hormonal therapy, CaP shifts from an androgen-dependent to an androgen-independent state with a concomitant switch from paracrine to autocrine growth factor stimulation and subsequent upregulation of growth factor expression. Thus, growth factors and their receptors have a pivotal role in CaP. This is emphasized by current evidence obtained from clinical specimens as well as several in vitro and in vivo models strongly suggesting that epidermal growth factor and the neurotrophins (nerve growth factor, brain derived neurotrophin factor, neurotrophin-3 and neurotrophin-4/5) together with their tyrosine kinase receptors could play a very significant role in CaP progression.**


*Keywords:* Prostate cancer; Metastatis; Neurotrophins; trk; Epidermal growth factor receptor; Epidermal growth factor

## 1. Introduction

Cancer of the prostate (CaP) is the most commonly diagnosed cancer in males, afflicting one man in nine over the age of 65 [1]. In order to understand the initiation and progression of CaP, it is necessary first to elucidate the molecular mechanisms involved in the development of the normal prostate gland. It is well established that stromal–epithelial interactions, through endocrine and paracrine factors, extracellular matrix components, cell–cell contact together with locally produced androgens and growth factors, are involved in normal prostate development and function [2–4]. There is also evidence that regulation of prostatic growth is maintained by androgen dependent secretion of paracrine growth factors which, during normal development, behave as mitogens of prostatic epithelial cells [3,4]. Importantly, this balance is destabilized in CaP and, in particular, during therapeutic CaP treatment where epigenetic deregulation of cell growth control can occur due to acquisition of androgen-independent autocrine growth factor stimulation [3–5]. Such stimulation has been implicated in tumour growth, angiogenesis and metastasis which, in turn, involve cellular proliferation, adhesion, secretion and motility [3–5]. Therefore, analysis of the deregulation of the functional relationship between growth factors and their receptors is central to determining the pathogenesis of CaP.

Current knowledge regarding the roles of epidermal growth factor (EGF) and the neurotrophins – nerve growth factor (NGF), brain derived nerve growth factor (BDNF), neurotrophin-3 (NT-3) and neurotrophin 4/5 (NT-4/5), as well as their tyrosine kinase receptors – epidermal growth factor receptor (EGFR) and members of the trk family (trk A, trk b and trk c) – suggest strongly that these systems have a fundamental role in stromal–epithelial interactions during the initiation and progression of CaP.

## 2. Basic cellular architecture of the human prostatic duct

The basic functional units of the normal adult prostate are ducts composed of epithelial cells associated with stromal cells. There are at least three different epithelial cell types which have been characterized by their morphology, functional properties and possible role in carcinogenesis (Fig. 1). The prevailing epithelial cell type is the secretory luminal cell [6,7]. These are differentiated and androgen-dependent cells able to secrete proteins such as growth factors. As well as androgen receptors, these cells express cytokeratins 8 and 18 and the cell surface marker CD57 [6–8]. The second epithelial cell type is the basal cell. These cells are located primarily in the underlying basement membrane and form a uniform continuous layer. Some basal cells may also be present among the luminal cells [6,7,9]. Basal cells, which appear not to be secretory, are characterized by the expression of CD44, cytokeratins 5, 14 and possible low levels of androgen receptor [6,7,9]. Interestingly, analysis of the cytokeratin expression patterns suggested also the presence of transient populations of cells with basal/luminal properties that may be stem cells [10,11]. The third type of epithelial cell is the neuroendocrine cell [12]. These cells are a minor population scattered throughout the basal layer and provide paracrine signals able to support the growth of luminal cells [12,13]. Neuroendocrine cells are androgen-independent but express chromogranin A and serotonin [12,13]. The stroma is mainly composed of smooth muscle type cells, associated with a

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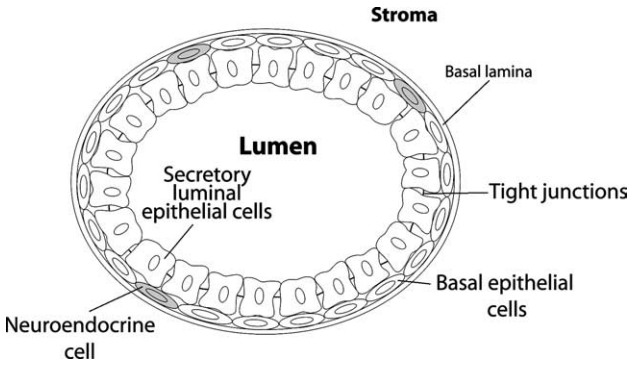


Fig. 1. Schematic representation showing a typical normal human prostate duct. Within the adult duct, the positions of luminal, basal and neuroendocrine epithelial cells as well as the stroma can be observed. It should be noted that there is no morphological distinction between epithelial neuroendocrine and basal cells.

matrix composed of fibrillar proteins (collagens and fibronectins), fibroblasts, adipocytes, lymphocytes, endothelial cells, pericytes and neuromuscular tissue [14–16]. The smooth muscle cells produce growth factors such as EGF and neurotrophins such as NGF [15] and appear to play a significant role in prostate development and maintenance of homeostasis [15]. The current view is that the stroma is characterized by a constant modulation of different and individual cell phenotypes, which oscillate between those of fibroblastic origin to those of differentiated smooth muscle type and most likely others that still need to be identified [17].

## 3. Prostate cancer and its progression

Fig. 2 illustrates the main stages in the progression of CaP. Prostatic intraepithelial neoplasia (PIN) has been suggested to be the precursor of CaP [18,19]. PIN is a continuum from low- to high-grade lesions confined to the prostatic capsule/duct [18,19]. PIN can have allelic imbalance as well as architectural and cytological properties resembling invasive carcinoma, such as disruption of the basal layer and low levels of expression of markers (such as matrix metalloproteinase-2 and kallikreins) associated with early invasive carcinoma [18]. PIN can be followed by invasive carcinoma, characterized by loss of the basal lamina, over proliferation of basal and luminal cells and full expression of matrix metalloproteinase-2 and kallikreins [20]. Subsequently, invasive carcinoma may progress to a more aggressive form capable of local invasion of the seminal vesicles [20,21]. Finally, metastasis may occur primarily to bone and then to lung, and can be lethal. Importantly, in patients undergoing androgen ablation treatment, the transition from invasive carcinoma to metastasis is characterized by a shift from an androgen-dependent to an androgen-independent state [20,21]. The initial pathology of malignant cell migration within the prostate is predominantly by direct extension around prostatic nerves. This invasion, via the micro-lymphatic system, follows the course of nerve branches to the superior pedicle and inferior pedicle where capsule penetration is most common [20].

It is important to note that CaP rarely arises spontaneously in animals (with the exception of dog), thus, to investigate the mechanisms involved in the progression of this type of neoplasia, emphasis has been placed on the production of human

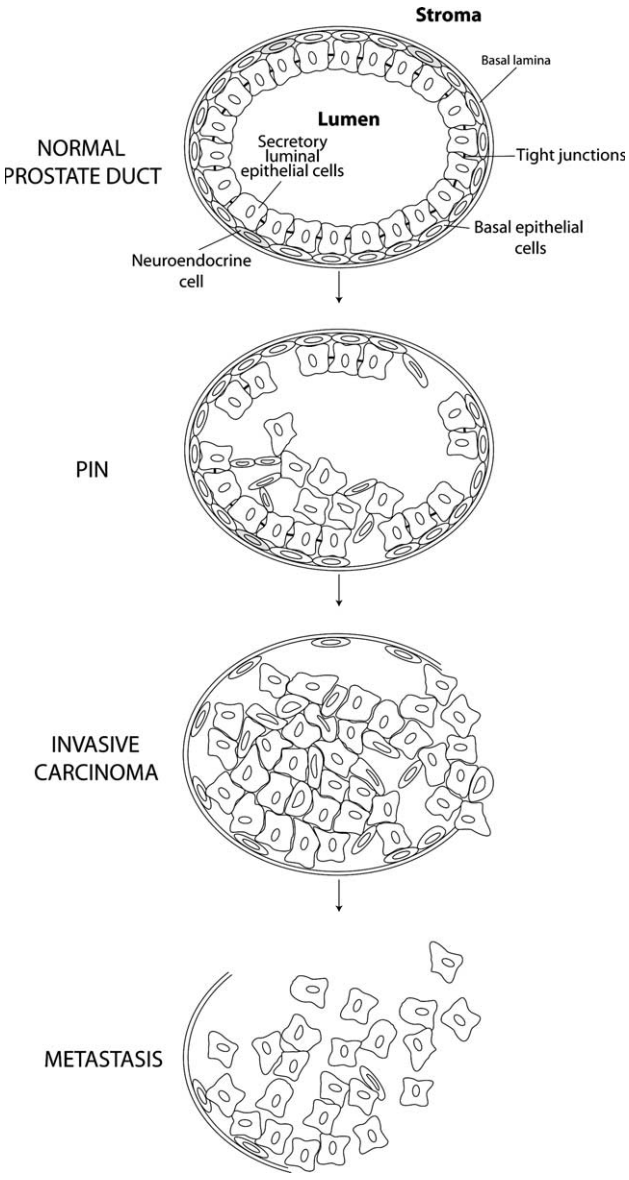


Fig. 2. Schematic representation of human prostate cancer progression. The integrity of normal adult duct is maintained by its androgen dependence. Subsequently, with the onset of prostatic intraepithelial neoplasia (PIN), there is disorganization of luminal and basal epithelial cells. PIN leads to invasive carcinoma and the full disarray of both luminal and basal cells with concomitant loss of the basal lamina. Finally, during metastasis there is migration to other body sites of malignant epithelial cells which, when patients undergo androgen withdrawal treatment, become androgen independent.

xenografts, transgenic and knockout mice as well as model cell lines. We should strongly emphasize, therefore, that although it is possible that observations made on given cellular models may not individually accurately represent human CaP, results obtained from different systems taken together could throw light on the various clinical aspects of CaP progression.

## 4. Tyrosine kinase receptors and their ligands in CaP

This section describes what is currently known about the involvement of EGF, neurotrophins and their receptors in the

progression of metastatic CaP (the role of TGFα will be mentioned only within the context of the role of EGF). Fundamentals of the structure and function mechanisms of these growth factors and their receptors have been reviewed previously [22–25].

### 4.1. EGF

In the normal adult prostate, EGF can be detected as a secreted protein [5]. ELISA has shown EGF to be present in the prostatic fluid of adult prostate [26]. Also, immunohistochemical and ELISA analyses of EGF expression in normal prostate epithelial cells showed that it is produced by luminal cells, specifically in the luminal/apical region of these cells, and released into the lumen of the prostatic duct [27,28]. Interestingly, immunocytochemical and ELISA studies of TGFα have shown it to be expressed and secreted by smooth muscle cells of the stroma in normal adult prostate [28–30].

Further analysis of EGF and TGFα expression demonstrated that normal and low grade PIN as well as localized carcinomas lack or have very low EGF and TGFα expression [28–30]. However, poorly differentiated tumours and highly metastatic CaP cell lines such as PC-3 and DU-145 were shown to express detectable levels of EGF and TGFα [30]. These results taken together give an indication that high amounts of EGF and TGFα are preferentially expressed in poorly differentiated as well as metastatic tumours but not in localized and differentiated carcinomas or normal prostate.

### 4.2. EGFR

Comparative immunohistochemical analysis of EGFR expression in biopsies of normal prostate, PIN, together with carcinomas of the prostate with or without severe intraductal dysplasia showed strong EGFR immunostaining in basal but not luminal cells of normal prostate, very diffuse/weak cytoplasmic staining in poorly differentiated carcinomas and metastatic samples as well as a discontinuous basal pattern of staining in PIN [31–33]. A thorough analysis of PIN demonstrated that low-grade PIN had strong positive staining whereas in high-grade PIN levels of staining had only moderate intensity [33]. Furthermore, analysis of EGFR mRNA expression showed that localized or more differentiated samples appeared to express significantly more mRNA than normal prostate epithelium [33]. However, comparable studies of EGFR mRNA expression of CaP specimens did not find a significant difference in levels or any correlation across various types or grades; similar results were obtained by immunohistochemical staining of EGFR protein in sections of the same samples [34,35].

Because of the ambiguity in the results obtained by several laboratories, a comparative analysis of EGFR was carried out in foetal, neonatal, pre-pubertal and young adult glands with PIN and carcinoma [36]. This investigation showed that EGFR protein is strongly and exclusively expressed in basal cells of foetal samples [36]. In neonatal and prepubertal glands, EGFR continued to be present only in basal cells [36]. In normal adult prostate, detectable EGFR levels were strictly localized in basal cells and in the lateral plasma membranes of luminal cells. Irrespective of grade, PIN showed moderate-diffuse staining of EGFR in the majority of luminal cells. Moderate-diffuse staining was also observed in carcinomas [36].

A possible model based on the evidence presented by this investigation together with what is known, to date, about the expression of EGF and TGFα is illustrated in Fig. 3. In adult prostate, this model accounts for the response to stromally produced TGFα, by EGFR present on the basolateral membrane, below the tight junctions of the luminal cells (Fig. 3(a)). In this situation, EGFR cannot be accessed by the high levels of EGF produced by the apical region of the luminal cells but only the low concentrations of the stromally produced TGFα. Thus, TGFα acts as a paracrine factor mediating proliferation by activating receptors located in the luminal cells [34,36]. During tumorigenesis, however, when the epithelial cells become disorganized and the tight junctions are destabilized, EGF can access the baso-lateral surface and activate EGFR.

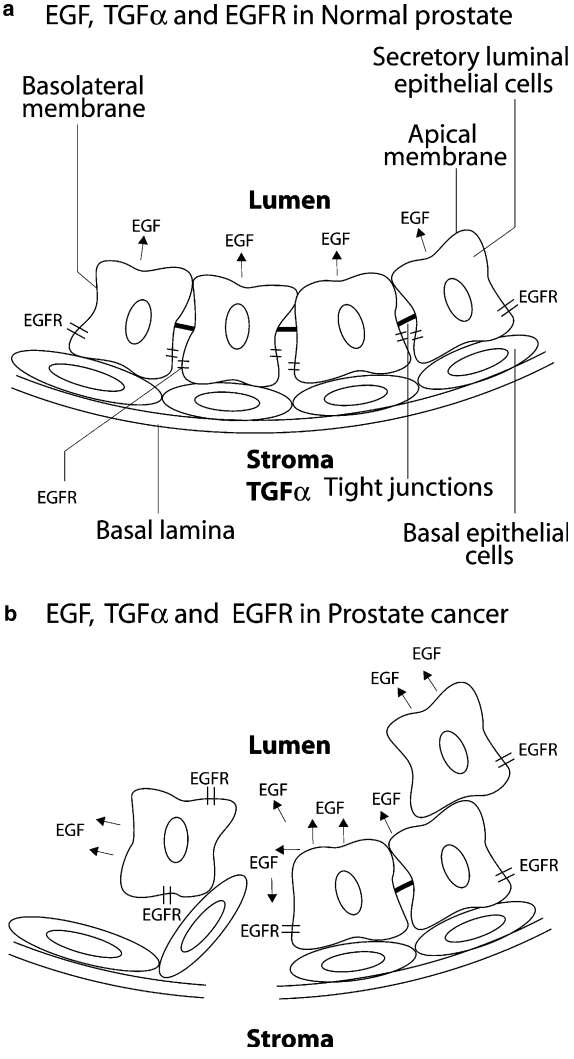


Fig. 3. (a) Diagram showing expression of EGF, TGFα and EGFR in normal and malignant prostate accini. In normal adult prostate, the secretory luminal epithelial cells are well organized and only separated by the tight junctions. This allows secretion of EGF into the lumen and avoids autocrine stimulation of EGF receptors located in the basolateral membranes, below the tight junctions. Thus, EGFR molecules in this location are only stimulated by TGFα produced by the stroma. (b) In the neoplastic accini and as the basal and luminal cells become disorganized, EGFR molecules, located in the basolateral membranes, become exposed to EGF secreted from the apical membrane, thus resulting in an autocrine stimulating proliferation loop.

This autocrine stimulation overrides the paracrine loop and enhances the autonomous progression of cancerous growth of the prostate [34,36]. Importantly, autocrine stimulation can lead to gradual androgen-independence and partial independence from stromally produced growth factors, which in turn, could promote a multilayered epithelium with the ability to metastasize [37,38] (Fig. 3(b)). Since TGFα is present in CaP tissues, it is possible that TGFα is also an autocrine growth stimulator of CaP.

Interestingly in foetal tissue, EGFR and TGFα were shown to be co-expressed in proliferating epithelia at a time when androgen receptors were absent, suggesting the possibility that an androgen-independent autocrine intra-epithelial mechanism may modulate growth and differentiation of prostatic epithelium also during development [36]. Thus, it appears that an intra-epithelial autocrine signalling pathway similar to that found in the developing prostate is recapitulated in neoplasia. Indeed, a number of developmental genes have been shown to be re-expressed in cancer and may be termed ''onco-foetal genes'' [39].

In summary, the EGF/TGFα/EGFR autocrine loop is involved in early development of the prostate. With further maturation, the loop increasingly assumes a paracrine character enabling interactions between smooth muscle of the stroma and epithelial cells. This is lost during progression to neoplasia. However, whilst there is considerable support for the model shown in Fig. 3, further work is required for full verification.

Finally, there is some evidence that in androgen responsive LNCaP cells, EGF can also activate the androgen receptor to initiate a signalling cascade [40]. Similarly, recent data demonstrate that EGFR levels can be increased by the addition of androgens to LNCaP cells [41]. These results suggest that other possible autocrine loops could also be involved in CaP progression.

### 4.3. EGFRvIII

Mutations of EGFR are very common in several human tumours including CaP [42]. The most common is the type III mutation (EGFRvIII) which has a deletion in the extracellular ligand binding domain [43]. EGFRvIII lacks amino acid residues 6–273 in the extracellular domain, reducing its size from 170 to 145 kD. This deletion promotes a conformational change similar to the one induced by ligand binding to wild type EGFR and thus gives rise to a constitutively active receptor [43,44]. Accordingly, EGFRvIII undergoes self-dimerization which is dependent on core glycosylation [45]. Expression of EGFRvIII appears to increase as CaP progresses [42]. EGFRvIII mRNA and protein can be detected in PIN as well as invasive and metastatic CaP but not in normal prostate; higher levels of expression have been seen in malignant tissue. Interestingly, EGFRvIII appears to be localized in the cytoplasm and perinuclear areas of epithelial cells [42]. However, the functional consequence(s) and detailed localization pattern are not known and need to be investigated further.

### 4.4. Neurotrophins

Prostate is the most abundant source of NGF outside the nervous system [46]. NGF and NGF- immunoreactive proteins secreted by the prostate are able to stimulate prostatic epithelial growth [46,47]. Because of their mitogenic effects on these cells, the expression and role of neurotrophins have been analysed extensively in normal and neoplastic prostatic tissue. Immunocytochemistry of normal prostate and primary carcinoma samples showed NGF to be localized in the stroma of normal prostate [47]. Furthermore, a comparative analysis of mRNA as well as DNA from stromal smooth muscle cells of normal prostate and non-metastatic LNCaP cells (known to be growth stimulated by NGF) showed that only smooth muscle cells expressed mRNA for NGF and BDNF [48] (Fig. 4(a)). Further analysis showed that NT-3 was not detected in either smooth muscle or normal epithelial cells, indicating that there might be other potential sources of NT-3 expression in normal prostate. Similarly, NT-4/5 did not appear to be expressed in smooth muscle cells [48].

An investigation to determine whether neurotrophins were produced by CaP cell lines showed that in the non-metastatic LNCaP cells, neurotrophin expression was not detected [49]. In contrast, metastatic DU145 and PC-3 cells secreted mea-

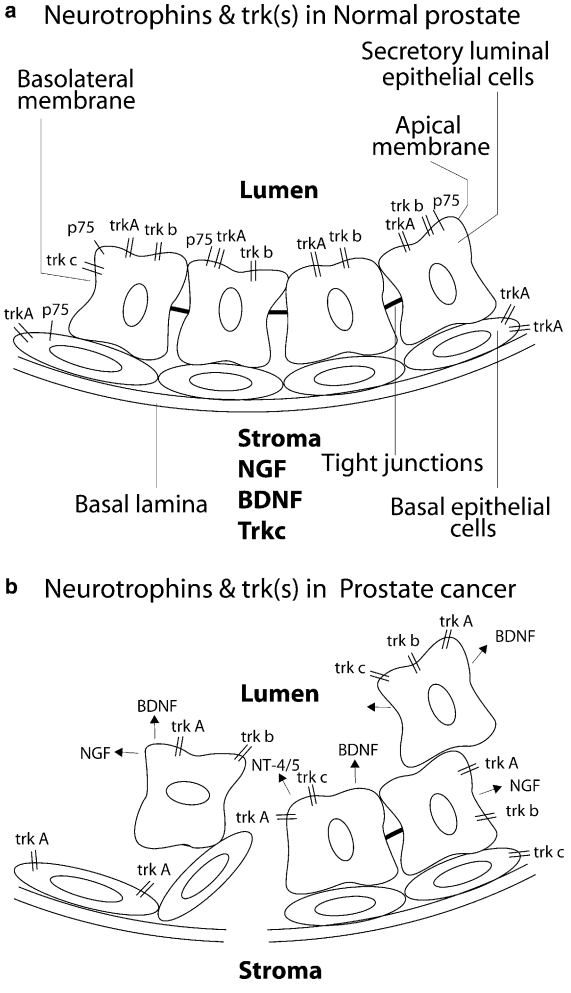


Fig. 4. (a) Diagram showing the expression of neurotrophins and their receptors in normal and malignant prostate accini. In normal adult prostate, the secretory luminal epithelial cells are well organized and only separated by the tight junctions. These cells preferentially express trk A, trk b and p75 and can only be stimulated with stromally produced NGF and BDNF in a paracrine fashion. (b) In the neoplastic accini, the luminal cells become disorganized, lose p75 expression and produce NGF, BDNF and NT-4/5, which are able to stimulate trkA, trk b and trk c in an autocrine fashion.

surable amounts of NGF, as determined by ELISA, and secreted NGF was able to induce differentiation of rat pheochromocytoma PC12 cells [50,51]. PC-3 cells were also similarly shown to secrete detectable BDNF [50]. These results suggested that malignancy could involve a switch from paracrine to autocrine control of neurotrophin activity (Fig. 4(b)). However, a more thorough investigation using other CaP models is needed to define this possibility.

### 4.5. trk(s)

Immunocytochemical analysis of normal prostate and CaP tissue biopsies showed the expression of members of the trk family (trk(s)) in epithelial cells of all tissues studied [47]. trk(s) were found to be localized in the epithelial component of the ducts, specifically, in both basal and luminal epithelial cells [47] (Fig. 4(a)). Further immunocytochemical analysis of normal and malignant prostate tissues showed that the majority of normal ductal basal cells expressed trk A and sporadically the androgen receptor, but not trk b or trk c [52] (Fig. 4(a)).

In order to determine the role of trk(s) in CaP, comparative mRNA and DNA analyses of stromal smooth muscle cells of normal prostate and LNCaP cells were performed. These experiments showed that smooth muscle cells expressed mRNA only for trk c, whereas LNCaP cells expressed all trk(s) [47] (Fig. 4(b)). A more detailed investigation of the levels of mRNA transcripts for each individual trk member in LNCaP cells found that trk c was highly expressed when compared to trk A and trk b [52]. Thus, it is possible, as in the case of EGF, TGFα and EGFR, that trk(s) and neurotrophin expression in normal prostate is involved in paracrine mechanisms in such a way that NGF and BDNF produced by the stromal smooth muscle cells are able to stimulate trk A and probably trk b when expressed by the epithelial cells (Fig. 4(a)). As CaP progresses, however, NGF, BDNF and NT-4/5 produced by epithelial cells are able to stimulate trk family members, also expressed by epithelial cells, in an autocrine fashion (Fig. 4(b)).

A recent immunohistochemical analysis of specimens obtained from hormone-treated and non-treated patients showed that both normal and malignant prostatic epithelial cells were able to express all neurotrophins and their receptors. In normal tissue, neurotrophins were observed in luminal secretory cells, whereas trk(s) were present in basal cells [53]. In this investigation, NGF was not detected in stromal cells, suggesting that the stroma might not necessarily be a source of neurotrophins and that instead, neurotrophin paracrine stimulation was provided by luminal cells [53]. These results can only be treated as preliminary, since they involved only immunocytochemical detection and although they are indicative of an alternative model of paracrine stimulation, further experiments are needed to fully verify their validity.

The combined role of neurotrophins and trk(s) in CaP has been assessed by a comparative in vitro analysis of the invasive capacity of LNCaP, PC-3 and DU145 cell lines; results showed that NGF enhanced the invasiveness of these cell lines in Matrigel [49,50]. The effects of NGF as well as NT-4/5 involved time- and dose-dependent expression of heparanase (a heparan sulfate-specific endo β-D-glucuronidase), which is an important molecular determinant of metastasis [54]. Also, exposure of LNCaP cells to NGF increased their proliferation and the mitogenic action of NGF appeared to be mediated by trk A. Overall, these data suggested that activation of trk(s) by their ligands could lead to proliferation and metastatic progression of CaP.

In summary, autocrine expression and activity of neurotrophins in prostate may enhance extracapsular invasion and migration of metastatic cells along the microlymphatic system. Once outside the prostate, autocrine neurotrophin expression may provide a sufficient source of these factors to maintain the viability and proliferative capacity of the trk(s)-expressing tumour cells during metastasis to distant sites.

### 4.6. p75

The role of the low-affinity NGF receptor p75 (a member of the tumour necrosis family [24,25]) has also been assessed in normal and neoplastic prostate. p75 has been detected only in normal tissue and found to be localized unevenly in the epithelial component of the ducts [47]. Immunocytochemistry of normal and primary carcinoma samples also showed that the majority of normal ductal basal cells expressed p75 [55]. Interestingly, luminal cells of normal prostate expressed trk A and p75; however, when compared to the expression in basal cells, trk A was detected at higher levels than p75 [55] (Fig. 4(a)). In all cases, p75 was not detected in primary carcinomas [47,52]. In order to determine the stage at which the expression of p75 is lost during CaP progression, the presence of p75 was evaluated in PIN and malignant tissues categorized into well, moderately and poorly differentiated primary carcinomas [55]. It was found that p75 was present in PIN and loss of p75 was directly related to the grade of malignancy with poorly differentiated carcinomas having undetectable p75 [54]. This change was also seen in vitro, analysis of normal prostate epithelia showed expression of trk(s) and p75 [56], whereas p75 was not detected in DU-145, PC-3 and LNCaP cell lines [48,57].

To elucidate the function of p75, LNCaP cells, which do not express p75, were stably transfected with this receptor. Expression of p75 decreased the dose dependent stimulation of proliferation by NGF [58]. NGF deprivation and treatment with anti-NGF antibodies significantly increased the proportion of epithelial cells undergoing apoptosis, whereas addition of NGF rescued cells from programmed cell death [58]. These results would suggest that p75 is a negative modulator of epithelial cell growth by inducing apoptosis. Hence, loss of p75 appears to contribute to malignancy by promoting proliferation and suppression of growth inhibitory pathway(s) [48,59].

The role of p75 in relation to trk(s) has also been assessed. Analysis of trk(s) stimulation in different cell lines showed that BDNF was not directly involved in receptor activation [47]. However, since binding of neurotrophins to p75 would inhibit cell death, it is very likely that BDNF binding to p75 could induce survival of epithelial cells and NGF binding to trk A induced proliferation, resulting in the overall stimulation of growth [47]. These findings have been supported by mRNA analysis of DU145 and PC-3 cells showing high levels of NGF and BDNF transcripts [48]. In conclusion, in normal prostate and CaP, autocrine NGF may only interact with trk A, autocrine NT-4/5 with both trk b and trk A, while BDNF may only stimulate p75.

In order to ascertain whether the absence of p75 expression in CaP is due to full or partial deletion of the *p75* gene, DNA from DU145, PC-3 and LNCaP cells was analysed and it was found that lack of p75 expression was not due to deletion [60]. Furthermore, reverse transcription polymerase chain reaction,

RNAse protection and chromatin immunoprecipitation assays showed detectable transcription of p75 [60]. Transient transfection with the 3′ end untranslated region of p75 (a region involved in mRNA stabilization by cytosolic factors able to interact with elements in this region) did not promote the expression of p75 protein, thereby suggesting that specific cytosolic factors could be lost during CaP progression thus leading to mRNA instability and loss of p75 expression [60].

### *4.7. Neurotrophin precursors*

There is evidence that neurotrophins are synthesized as precursors that are proteolytically cleaved to biologically active neurotrophins [46,48,61]. The NGF precursor is proteolytically cleaved at amino acids −71 to −43 and −40 to −3. Analyses of these products have been carried out using specific antibodies, which revealed that mature NGF was not expressed by stromal cells of normal prostate; instead, two precursors (35 and 27 kD) together with a partially processed 22 kD form were detected [48]. To determine whether mature or cleaved NGF products were able to activate trk A in malignant cells, the effects of the precursors together with mature NGF were tested on B5 cells (a derivative cell line from DU145 cells) and were shown to stimulate receptor phosphorylation/activation [61]. Importantly, a more recent study showed that NGF precursors can activate p75 and induce apoptosis without stimulating trk A [62]. These results raise the possibility that the role of NGF precursors is to activate p75 and to maintain optimal cell numbers during normal prostate development.

## 5. Pharmacological and clinical aspects

Because of the potential role of growth factors in CaP, several types of pharmacological and biological agents have been produced to target EGFR and members of the trk family. The most promising class of compounds that block EGFR are small-molecule inhibitors of its kinase activity [63,64]. These belong to the aniliquinazolines and act by competitively binding and inhibiting the ATP binding domain [63,65]. Clinically, the most advanced analogue is ZD-1839, which has several effects on EGFR-expressing cells such as induction of cell arrest, increasing apoptosis and reduction of proliferation [63,65]. Animal models treated orally with ZD-1839 showed significant anti-tumour effects, which could be additive or supra-additive when administered in combination with cytotoxic compounds such as topoisomerase II inhibitors and anti-metabolites [63,65].

Another approach used to block the activity of EGFR is use of monoclonal antibodies [66,67]. The antibodies compete for the ligand binding domain and thus inhibit receptor activity. Clinically, the most advanced antibody is IMC-225 [67]. This is a chimera generated by attaching the Fv (variable) regions of the original anti-EGFR murine monoclonal antibody to a human $IgG_1$ constant region. This antibody induces apoptosis and inhibits proliferation, angiogenesis, invasion/metastasis and cell proliferation after chemotherapy or radiation of tumour cells [67]. Clinical trials of breast carcinoma using IMC-255 in combination with cytotoxic drugs such as cysplatin have provided patients with a stable non-progressive disease [66,67]. Combination trials for CaP have not yet been reported.

Ribozymes, which are RNA molecules capable of site specific cleavage of transcripts with the overall effect of reducing or blocking protein translation, have been tested as possible specific agents against EGFRvIII [68]. EGFRvIII-specific ribozymes have been found to decrease tumour growth by reducing proliferation of EGFRvIII-expressing cells as well as decreasing colony formation in soft agar assays [68]. Ribozymes have not yet been tested clinically against CaP.

As in the case of EGF/EGFR, it has become clear that there is a need to expand the pharmacology that could ablate neurotrophin stimulation of members of the trk family to suppress CaP progression. The indolocarbazole K252a is a well known inhibitor of trk kinase [64,69–71] and its analogue CEP-751 has potent anti-tumourigenic activity [71]. CEP-751 selectively inhibits CaP growth in tumour assays when using human and rat prostate carcinoma cell lines injected into Copenhagen rats [69,70]. The anti-tumour effects were independent of androgen sensitivity, tumour growth rate or metastatic ability [69,70]. Further analysis of the effects of CEP-751 by itself or when combined with androgen ablation produced transient tumour regression, independent of the effects on the cell cycle, in the Dunning R-3327 H rat CaP model [71]. Castration alone induced tumour regression, which was followed by re-growth after 4–6 weeks, whereas castration together with intermittent CEP-751 treatment resulted in prolonged tumour regression. Similar results were obtained when CEP-701 (an analogue of CEP-751) was administered orally together with a gonadotrophin-releasing hormone agonist, Leuprolide, to induce androgen ablation [71]. These results indicated that treatment with CEP-751 or CEP-701 combined with either surgical or chemical androgen ablation could reduce tumour burden more efficiently than either surgical removal or chemical ablation alone, and further suggested that these combined therapies could induce CaP cell death [69,70]. Importantly, effective doses of these pan-trk inhibitors did not appear to block trk signalling in epithelial cells or to induce death or proliferation of normal basal and luminal cells [71].

## 6. A possible association of EGF and NGF with voltage-gated $Na^+$ channel expression in CaP

A novel approach to understanding the pathophysiology of CaP suggested that upregulation of voltage-gated $Na^+$ channels (VGSCs) could be an accelerating factor in CaP metastasis [72]. This notion emerged from two sets of findings. First, whole-cell patch clamp recordings showed consistently that VGSCs occur only in strongly metastatic CaP cells of both rat and human [73,74]. Second, blockage of VGSC activity using the highly specific neurotoxin, tetrodotoxin, suppressed Matrigel invasion by some 50% [73,74]. VGSC activity also potentiated CaP cells endocytic membrane activity, consistent with a role in control of secretion [75]. Furthermore, a positive correlation was found between invasiveness and VGSC expression in numerous rat and human CaP cell lines [76]. The concept to emerge from this work is that functional VGSC expression potentiates a number of cellular behaviours integral to the metastatic cascade and thus accelerates metastatic disease. If so, VGSC expression could be a significant factor in differentiating 'slow' from 'fast' progressing cancers of the prostate and could enable potentially metastatic CaP to be detected whilst still confined to the gland.

Although the mechanism(s) responsible for the VGSC upregulation is not known at present, both NGF and EGF are candidates. Accordingly, application of NGF to PC12 cells significantly increased the expression of the Nav 1.7 (PN1) subtype of VGSC [77]. Interestingly, this is the predominant VGSC subtype found in metastatic CaP cell lines of both rat and human [78]. Also, BDNF has most recently been shown to be involved in ligand mediated Nav 1.9 activation via trk b [79]. Importantly, EGF has also been shown to increase VGSC activity in PC12 cells [77], GH3 cells [80] and rat CaP MAT-LyLu cells (Ding et al., manuscript in preparation). An intriguing possibility, therefore, is whether a positive feedback between growth factor (GF) release and VGSC upregulation could occur in CaP progression, as follows:

VGSC expression → VGSC upregulation → GF release
→ VGSC upregulation → CaP progression

Such a scheme, operating in either paracrine or autocrine mode, could be a significant 'self-potentiating', thus accelerating factor in CaP. Further work is required to test this scheme and elucidate its potential relevance to metastasis.

## 7. Conclusion

In conclusion, EGF and neurotrophins have potent effects on CaP progression, especially in conditions when it becomes androgen refractory. Thus, EGF, neurotrophins, and their receptors are viable new targets for treating metastatic CaP. However, more work remains to be done in order to fully elucidate their mode of action and mechanisms of involvement in CaP as well as to exploit their clinical potential.

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