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Review

Neurotrophic factors in prostate and prostatic cancer

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Recent progress in growth factor research has led to a reexamination of the involvement of neurotrophic factors outside their classical domain of the nervous system. These last few years have seen a substantial accumulation of data concerning Nerve Growth Factor (NGF)'s prevalence within the prostate. NGF and its receptors were reported from the normal prostatic tissue, benign hyperplasia and prostatic cancer.

Divergent ideas about the biological role of this factor, its specific distribution pattern within the tissue and its implication in the progression of carcinogenesis have been proposed. Especially the role of NGF in the metastatic process bears direct clinical relevance for research in this area. Many questions remain to be solved like the one on the prevalence of other neurotrophic factors. It is now increasingly becoming clear that neurotrophic factors do play a role in normal physiology and pathology of prostatic cells, opening up new prospects for diagnosis and treatment.

Keywords: neurotrophic factors; nerve growth factor; NGF; CNTF; GDNF

Introduction

In spite of their name, Neurotrophic Factors are increasingly reported to exert a biological function outside their classical domain of the nervous system maintenance and differentiation. The suggestion of an involvement in a plethora of physiological functions in health and disease warrants an in depth investigation of the exact cellular role of this group of growth factors. Recently, there has been an accumulation of data concerning the presence of neurotrophic factors and their receptors in prostate and prostate cancer, suggesting a potential role in the cell biology of these tissues. Moreover, the potential for the use of some of these markers in diagnosis and possibly in therapy, warrants a closer examination.

Neurotrophic factor families

Ever since the early work by Levi-Montalcini and Cohen in the 1950s,[1,2] neurotrophic factors have been known to represent a group of growth factors which are involved in the survival and differentiation of neuronal cells during embryonic and subsequent stages of development.[3] This group of growth factors comprises a number of families: Neurotrophins, Neuropoietic Cytokines and the Heparin-Binding Growth Factors. The Neurotrophin family contains Nerve Growth Factor (NGF), Brain-Derived Neurotrophic Factor (BDNF), Neurotrophin-3 (NT-3), Neurotrophin-4 and -5 (NT-4/5), Neurotrophin-6 (NT-6).

The Neuropoietic Cytokine family is composed of Ciliary Neurotrophic Factor (CNTF) and Interleukin-6 (IL-6) and the family of Heparin-Binding Growth and Differentiation Factors includes acidic and basic Fibroblast Growth Factors (a&bFGF), Midkine (MK) and Pleiotrophin (PTN). The TGF-β-related Glial Cell-Line-Derived Neurotrophic Factor (GDNF) was originally discovered as a neurotrophic factor for midbrain dopaminergic neurons.[4]

All these respective neurotrophic factors mediate their effects through different receptor systems. The neurotrophins bind to high affinity Tyrosine Kinase Receptors (Trk), which can be modulated by the non-related low affinity p75 NGF receptor. CNTF initiates signal transduction via a three-part CNTF-receptor-complex (CNTFRα, LIFRβ and IL-6R/gp130). FGF receptors include a heparan-related low affinity receptor and a tyrosine kinase-containing high affinity receptor.

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Function of the neurotrophic factors

The classical concept of neurotrophic factors holds that innervated tissues supply a signal to the innervating neurons to selectively limit developmental neuronal cell death.[5,6] The first discovered neurotrophic factor, NGF, was shown to fit into this model by regulating cell survival, neurite growth and neurotransmitter production of the sympathetic neurons of the peripheral nervous system.[7] NGF after being produced by the target cell is specifically bound, internalized and carried to the cell soma of the sympathetic neuron by retrograde axonal transport. The accumulation of data, however, has led to a reexamination of this concept and to its adaptation to a more complex model.[8] Neurons derive trophic support also from afferent neurons (anterograde influence), from the sheath of glial cells surrounding them and from themselves (autocrine effects). Considerable heterogeneity is present at all levels of neurotrophic interaction and specificity can be reached by detailed regulation of spatial and temporal expression patterns for all modifications of neurotrophic factors, receptors and intracellular signalling components.

More recently, proof is accumulating that neurotrophic factors also play important roles in regulating neural connectivity in the adult system and possibly in immune function regulation as well.[9,10]

The employment of newly gained insight into these interactions opens strategies to interact therapeutically with a multitude of pathologies.[11–13] Manipulation of neurotrophic interaction is now being tested in many disorders like Amyotrophic Lateral Sclerosis, Motor Neuron Atrophy, Parkinson's disease, Alzheimer's disease and peripheral nerve damage due to physical causes, chemotherapy or diabetes.

Neurotrophic factors in cancer

NGF-receptors have been described in a number of tumors arising from neural crest-derived cells, like neuroblastoma,[14,15] gliomas,[16] medulloblastoma,[17] adrenal tumor,[18] prolactinomas,[19] astrocytoma,[20] melanoma[21] and insulinoma.[22]

Most of these studies describe an implication of NGF in the differentiation processes; in some, the expression of the NGF-receptor has been reported to be associated with improved prognosis.[14] Next to a link with differentiation processes, however, a relation of NGF with the process of invasion is most remarkable. The invasive phenotype of melanoma was reported to be correlated with NGF-receptor presence.[23,24] In human glioma cells NGF could stimulate cell migration in 1 out of 5 glioblastomas studied.[25] The probable mechanism is the NGF induced expression of degradative enzymes like endo-β-glucuronidase heparanase.[26]

Neurotrophic factors in prostate and prostate cancer

NGF has been reported previously to be present in the normal prostate of the guinea pig[27,28] as well as in human prostate cancer tissue.[29] These last few years the presence of NGF in the prostate has been confirmed and insights into the biological meaning are now evolving. Concomitantly, a possible role of NGF in the spread of prostate carcinoma is being unraveled.

In a series of studies Djakiew and coworkers[30–32] demonstrated that a prostatic epithelial cell line and prostatic stromal cells contain and secrete NGF-like protein(s) *in vitro*. It was shown that both cell lines could stimulate each others proliferation rate by the release of these proteins and that anti-NGF antibody could block this paracrine interaction. The NGF-like protein has been described as being localized mainly in stromal cells of the human prostate, benign prostatic hyperplasia (BPH) and adenocarcinoma of the prostate, while the NGF-receptor was found to be localized mainly in epithelial prostatic cells.[33]

In contrast, another research group demonstrated immunohistochemically NGF to be present mainly in the epithelial basal cells of normal human prostate and BPH, and—only to a lesser degree—within stroma.[34] In addition, Paul *et al*[35] showed intense NGF-β immunoreactivity exclusively within the epithelium of both BPH and prostatic adenocarcinoma. The concentration of NGF did not significantly differ between malignant and benign prostate tissues as a whole but morphometry showed that malignant glandular tissue contains less NGF compared to benign glandular tissue. The epithelial localization of NGF is in accordance with observations from other groups.[29,36–38]

A cell population type within the prostate which was specifically thought to be implicated in regulation of growth through paracrine interactions is formed by the neuroendocrine cells.[39] These cells, in which many hormones and other bioactive peptides have been demonstrated, could promote androgen-independent prostate tumor growth after androgen depletion therapy.[40] However, no correlation could be detected in an immunohistochemical study between these neuroendocrine cells and NGF-β.[35]

NGF-receptor expression in prostate cancer

The low-affinity NGF receptor was observed to be present within the normal prostatic epithelium.[33,38] Interestingly, in prostate cancer tissues the low affinity (p75) NGF receptor (LNGFR) was suggested to lose its expression progressively during neoplastic progression of prostatic cells.[41] While normal prostatic tissue expresses the p75 NGF-receptor, the expression was observed to be partly lost in benign and malignant tissue and completely lost in four metastatic tumor cell lines.

Recently, Perez *et al*[42] confirmed this suggestion by showing that the LNGFR, while being present on 100% of normal epithelia and in 100% of prostatic intraepithelial neoplasia (PIN), was lost with malignant transformation. LNGFR content decreased from 37% in well differentiated carcinoma to 36% in moderately differentiated carcinoma, to only 16% in poorly differentiated carcinoma. This way, the presence of the low affinity NGF receptor may form a diagnostic tool in addition to PSA serum levels, which

tend to be still relatively low in well differentiated cancer cases.[42] In addition to the LNGFR, also a functional Trk high-affinity NGF receptor has been described in human prostate epithelium.[43] This receptor being present could explain the responsiveness of prostate tumor cells to NGF in the absence of the low affinity p75 NGF receptor.

A role for NGF in prostate and prostate cancer

The above observations are supportive of the concept of NGF-mediated regulation of cell proliferation in the glandular epithelium of the prostate. This regulation could be either a paracrine action of NGF produced by prostatic stromal cells acting on the epithelium or an autocrine loop by NGF produced by the epithelium itself.

The observed early loss of the low affinity NGF receptor could be the reflection of a potential apoptotic pathway.[44] Such a loss may be instrumental in malignant transformation and immortalization of prostatic epithelial cells.[45] In this way the balance between loss of LNGFR and the persistence of the Trk high affinity receptor could contribute to prostatic carcinogenesis.

In addition to modulation of cell proliferation other biological effects of NGF have been described in prostate cancer cell lines. NGF addition to cultures of the LNCaP prostate tumor cell line stimulates anchorage independent growth of prostate tumor cells, a condition closely parallel to tumor formation *in vivo*.[46] Furthermore, secreted proteins from human prostate stromal cells induced chemotaxis and chemokinesis of prostate tumor cell lines *in vitro*, which could be prevented by anti-NGF antibody.[47] This strongly suggests the implication of NGF in specific episodes of the metastatic cascade.

We have investigated the effects of NGF on *in vitro* invasive capacity of human prostate cancer cells *in vitro*.[48] Addition of NGF to DU-145 prostatic tumor cells could significantly increase the invasion by these cells into basement membrane (Matrigel). Moreover, the secretion of NGF by these cells in culture medium was demonstrated, illustrating an autocrine loop. Contrary to above observations, no effect on the cellular proliferation could be detected.

While these data suggest functional implications of the presence of NGF within prostatic tissue, the exact role of NGF in prostate biology and the significance of NGF-receptors in prostatic carcinoma is far from clear. It seems clear that the overall regulation of prostatic epithelial growth and function is complex and multifactorial, involving many factors and the interaction of different regulators.[49] Figure 1 attempts to summarize NGF's cellular physiology and the hypothetical functional implications of changes observed during malignant transformation.

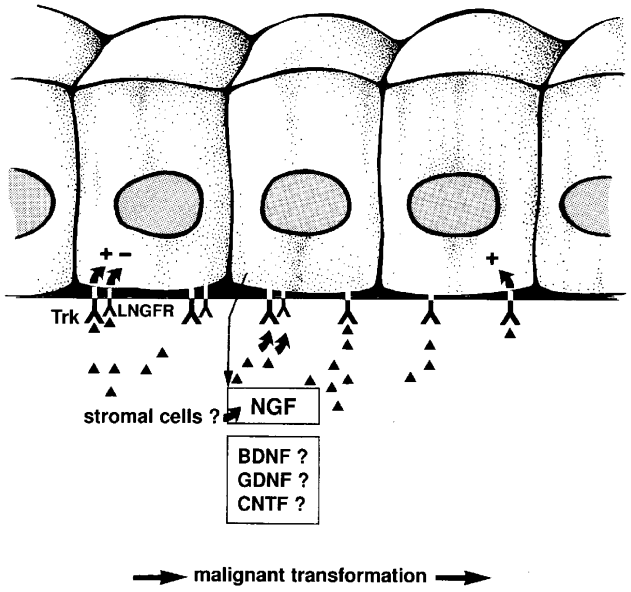

Figure 1 *Effect of NGF (and other neurotrophic factors?) on prostatic epithelial cells through interaction with high-affinity (Trk) and low-affinity (LNGFR) NGF-receptors in different stages of malignant transformation. The loss of LNGFR-expression during malignant transformation of the prostatic epithelium is thought to be responsible for lack of inhibitory signal on cell proliferation.*

Epithelial–neural interactions in prostate cancer

The presence of NGF within the prostate has been determined and compared with neuro-endocrine differentiation.[35] β-NGF levels proved to be of prostatic origin and not to correlate with neuro-endocrine differentiation in malignant tissues. The NGF produced within the prostatic epithelium is thought to recruit sympathetic nerves and hence smooth muscle. Conversely, the growth of the prostate gland has been demonstrated to be facilitated by the autonomic nervous system, possibly through growth factors.[50] In this respect, also the effects of neurotransmitters like norepinephrine have been investigated.[51] While not being neurotrophic factors, strictly speaking, the in depth discussion of these factors is somewhat out of the scope of this review.

A striking spatial relationship of metastatic prostate cancer cells with the neural system is formed by the perineural spread of this tumor. Some authors have suggested that penetration of the periprostatic capsule is almost entirely dependent on the ability of prostate cancer to invade and travel along perineural spaces.[52] Patient screening for perineural spread has been advocated before nerve-sparing prostatectomy is being planned.[53] Especially since the perineural space has been shown not to be an existing lymphatic channel[54] perineural spread cannot be explained as a passive transport of tumor cells within the lympathic flow but must involve an active invasive behavior of the spreading tumor cells. Strikingly, areas of perineural spread have been shown to stain positively for NGF.[29] Similarly, a role for NGF and NGF-receptor has been postulated in perineural invasion in ductal pancreatic tumor.[55] In addition, in experimental prostate cancer metastasis to the spine, the close association of proliferating secondary tumor masses with the sciatic nerve roots at the lumbar spinal cord is prominent.[56] The above observed chemotactic effects of NGF on

invasive prostate tumor cells[48] may explain the close association observed for prostate cancer metastasis with neural tissue.

Questions

A number of tempting questions remain to be answered on the exact cell biology of NGF in prostate cancer and for that matter of other neurotrophic factors as well. Mechanistically, a number of observations still await clarification such as:

(1) The androgen dependency of NGF action. It has been known that levels of NGF-β in some brain areas are regulated by androgens.[57] Castration of male mice could reduce the amount of NGF-β in those areas. Conversely, NGF-β has been shown to influence androgen-binding protein synthesis by Sertoli cells and hence to regulate spermatogenesis.[58] It is not known whether NGF production, binding to receptors or subsequent action within the prostate is influenced by the presence of androgens.

(2) NGF-gamma and prostate specific antigen (PSA) have both been shown to be proteases for Insulin-like Growth Factor-Binding Protein (IGFBP).[59,60] Thus, both could enhance the mitogenic action of IGF in prostatic tissue by reducing the amount of IGFBP. It is not known whether NGF-gamma is present within prostate tissue nor to what level the other NGF subtypes (including NGF-β) exert similar specific proteolytic activity.

(3) A nerve growth factor-inducible A gene (NGFI-A) has been described as an early response transcription factor in the prostate.[61] This gene may be implicated in castration-induced apoptosis in hormone sensitive prostate cancer cells.[62] The biological significance of the presence of NGF in this system remains to be elucidated.

(4) Do other neurotrophic factors play a role in the prostate? Of the many neurotrophic factors known, until recently only NGF had been investigated for the presence in and the effect on prostatic tissue. In a recent study, Dalal and Djakiew[63] detected BDNF and NT-3/4 in addition to NGF in an androgen refractory prostate tumor cell line. An androgen responsive cell line, on the other hand, did not express any of these neurotrophins. We have screened for the presence of various neurotrophic factors in prostatic tumor cell lines and tissue biopsy samples from patients with proven carcinoma (unpublished observation). The preliminary results show that GDNF and CNTF may be expressed on a wide scale in human benign and malignant prostatic tissue. More extensive studies should definitively show the prevalence and significance of these neurotrophic factors in the human prostate and in prostatic tumors.

Future prospects

No doubt, the increasing knowledge of the biological role of growth factors within the prostate will lead to new ways to interact with the regulatory control of vital cellular functions like proliferation, apoptosis, differentiation and invasive behavior. Manipulation of growth factor synthesis, receptor binding or receptor function interaction could provide useful ways to obtain a significant clinical treatment response in prostate cancer. In models for senile dementia of Alzheimer type, such efficacy has been described for a stimulator of NGF synthesis in brain tissue.[64] Recently, a kinase inhibitor (K252a) has been investigated which selectively inhibits activity of the Trk receptors, most probably through inhibition of receptor-Trk phosphorylation.[65] It was shown that K252a could inhibit proliferation of three androgen-independent human prostate carcinoma cell lines. Furthermore, in one prostate tumor cell line this kinase-inhibitor could inhibit Trk receptor phosphorylation, consistent with a role for this receptor in growth regulation in prostate tumor cells. This mechanism may be useful in the design of potential therapies for prostate cancer treatment.

Conclusions

A body of evidence, described above, indicates that neurotrophic factors as well as neurotrophin receptors are present within the normal human prostate, in benign hyperplasia of the prostate and in prostatic cancer. While NGF and its receptors have been demonstrated beyond doubt in prostatic tissue, the first clues are currently arising that additional neurotrophic factors (like BDNF, GDNF, CNTF and NT-3/NT-4) may be present also.

A biological role for these factors in the local physiology has been surmised. Especially, the loss of the low-affinity NGF-receptor appears to be correlated with malignant transformation. Mechanistically, definitive cause–effect relationships have to be established by future research. However, it can be expected that with increasing insight into these mechanisms, new strategies will be developed for diagnosis and therapy of pathological prostatic growth.

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