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Seminar article

Oncogenic activation of androgen receptor

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Abstract

Background: There is considerable evidence implicating the aberrant activation or "reactivation" of androgen receptor in the course of androgen-ablation therapy as a potential cause for the development of castration-resistant prostate cancer. Several non-mutually exclusive mechanisms including the inappropriate activation of androgen receptor (AR) by non-steroids have been postulated. The present work is aimed to understand the role of neuropeptides released by neuroendocrine transdifferentiated prostate cancer cells in the aberrant activation of AR.

Objectives: The study was designed to study how neuropeptides such as gastrin-releasing peptide activate AR and to define the crucial signal pathways involved, in the hope to identify therapeutic targets.

Methods and Materials: Androgen-dependent LNCaP cell line was used to study the effects of bombesin/gastrin-releasing peptide on the growth of the cell line and the transactivation of AR. The neuropeptide was either added to the media or introduced as a transgene in LNCaP cells to study its paracrine or autocrine effect on LNCaP growth under androgen-deprived conditions. The activation of AR was monitored by reporter assay, chromatin immunoprecipitation (ChIP) of AR, translocation into the nucleus and cDNA microarray of the AR response genes.

Results: Bombesin/gastrin releasing peptides induce androgen-independent growth of LNCaP in vitro and in vivo. It does so by activating AR, which is accompanied by the activation of Src tyrosine kinase and its target c-myc oncogene. The bombesin or Src-activated AR induces an overlapping set of AR response genes as androgen, but they also a unique set of genes. Intriguingly, the Src-activated and androgen-bound ARs differ in their binding specificity toward AR response elements, indicating the receptors activated by these 2 mechanisms are not conformationally identical. Finally, Src inhibitor was shown to effectively block the activation of AR and the growth effects induced by bombesin.

Conclusion: The results showed that AR can be activated by neuropeptide, a ligand for G-protein coupled receptor, in the absence of androgen. The activation goes through Src-tyrosine kinase pathway, and tyrosine kinase inhibitor is a potentially useful adjunctive therapy during androgen ablation.

Keywords: Neuroendocrine differentiation; Neuropeptides; Src tyrosine kinase; Androgen receptor activation; Tyrosine kinase inhibitor; Hormone refractory prostate cancers

Introduction

Prostate cancer (PCA) represents the most frequently diagnosed malignancy of men in the United States. PCA is a hormonally regulated malignancy and AR plays an important role in disease progression. One of the most troubling aspects of PCA progression is the conversion from an androgen-dependent to independent (AI) state, which at present defies any effective treatment. In the majority of end-stage, hormone-refractory (HR) tumors, AR continues to be expressed and appears to be activated by castration levels of androgen and adrenal androgens [1–3]. Thus, a required step towards solving the clinical problem of prostate cancer androgen independence, becomes one of understanding how AR is inappropriately activated and how to inhibit such aberrant signals.

AR and androgen-independence

AR plays a vital role in the development of male reproductive organs. Genetic defects in AR results in the failure to develop a prostate gland, a notion corroborated by recent

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AR knockout experiments in mice [4,5]. In normal development, androgen is primarily required for differentiation functions. By contrast, during the development of PCA, androgen becomes a growth and survival factor for tumor cells. At the early stage of localized and metastatic PCA, proliferation depends on androgen, and androgen-ablation therapy is highly effective in controlling the disease. Treatment success however is only short-lived, as AI or HR clones eventually grow out, resulting in clinically unmanageable metastasis and mortality. Analysis of clinical AI PCAs revealed that over 90% express AR and androgen-response genes, indicating that the AR remains active and suggesting that AR is inappropriately activated in the absence of or at castration levels of testicular and adrenal androgens. Four mechanisms have been postulated to account for aberrant AR activation in AI tumors: (1) activation of AR by non-steroids via deregulated signals, (2) genetic mutations of AR, rendering the receptor hyperactive, (3) amplification or overexpression of AR and its coactivators, which sensitizes cells toward a low level of androgen, and (4) the increase of intracrine androgen. These 4 mechanisms are not mutually exclusive, and indeed it is likely that they work in concert to induce HR PCA. The fourth mechanism is a provocative new concept presented in this meeting (see the articles by Dr. P. Nelson and Dr. S. Balk). In this article, we will be elaborating on the first mechanism with recent results from our laboratory.

Androgen-independent AR activation by non-steroids

AR mediates androgen action by being a transcriptional factor that binds specific DNA sequences and recruits RNA polymerase II and a basal transcriptional complex for efficient transcription of cellular genes. The transcriptional activity of AR is mediated by coregulators (coactivators and corepressors) [6,7] which, in response to androgen's binding to AR and nuclear translocation, are assembled in a dynamic way at different response elements along the genome. The best recognized coactivators are the histone acetylases, such as p300/CBP [8,9] and the p160 SRC (steroid receptor coactivator) family [10–15]. These coactivators drive transcription by remodeling chromatin via histone acetylation and by recruiting RNA polymerase to the promoter, as we recently demonstrated [16]. The molecular basis for AR activation by androgen is a conformational change of AR induced by androgen binding, allowing the coactivators to associate. This process, however, is modulated—and in some cases, overridden—by phosphorylation, which has been postulated to be an underlying reason for AI activation of AR by non-steroidal agonists. Examples include interleukin-6 (IL-6) activation of AR via ERK phosphorylation of SRC-1 [17], EGF activation of AR via ERK phosphorylation of SRC-2 [18], IGF-1 activation of AR via AKT phosphorylation of AR [19], and neuropeptide activation of AR via the ERK pathway [20]. Indeed, in vitro phosphorylation and activation of AR by serine/threonine kinases ERK [21,22], AKT [23], PKA [24,25], and PKC [26,27] have been reported. Other studies further implicate casein kinase 2 (CK2) in the androgen response and growth of PCA [28–30]. Additional post-translational modifications of AR such as sumoylation, acetylation, and protease cleavage, which affect AR activity, have also been identified [31–33]. While serine/threonine kinases are direct modulators of AR and its transcriptional machinery, they are not the immediate effectors of growth factors, cytokines, or chemokines. Indeed, we and others showed that IL-6, EGF, and neuropeptide all activate tyrosine kinases in PCAs [20,34,35]. Our studies identified a tyrosine kinase complex involving Src/Etk/FAK to be a common target for all the above described non-steroid ligands, and for the case of neuropeptides, we present evidence that inhibitors of these kinases may have therapeutic value in the treatment of AI tumors.

Neuroendocrine differentiation and the development of hormone-refractory PCA

Our interest in neuropeptide and its possible role in HR PCA stems from the well documented observation that increased neuroendocrine cells accompany the development of HR PCA. We first demonstrated that IL-6, a progression factor of HR PCA, induced neuroendocrine differentiation of LNCaP [35], which is now supported by several studies reported in the literature [36–39]. Others reported that androgen-deprivation and forskolin also induced neuroendocrine differentiation of LNCaP, indicating the propensity of this cell line to undergo neuroendocrine differentiation [40–42]. The connection of neuroendocrine differentiation to androgen withdrawal was subsequently confirmed in the in vivo xenograft systems [43,44], and had strong clinical implications. We found that the neuroendocrine cells have acquired strong apoptosis resistance, but are growth arrested; they themselves are thus not malignant, yet they are endowed with the potential to release cytokines, chemokines, and growth factors, which fuel the surrounding undifferentiated prostate cancer cells to grow, migrate, and survive, especially under the harsh conditions of androgen withdrawal). The "neurokines" released by these cells include gastrin-releasing peptide (GRP, the homolog of bombesin), neurotensin, PTHrP, IL-8, relaxin, VEGF, factors implicated in chemotaxis, survival, angiogenesis, and bone metastasis [45]. Significantly, the work by Deeble et al. [46] and by Jin et al. [47] showed that neuroendocrine cells, when co-injected with CaP xenograft in a paracrine fashion enhance tumorigenesis, in support of the above hypothesis.