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Review

Molecular aspects of androgenic signaling and possible targets for therapeutic intervention in prostate cancer

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ABSTRACT

The androgen axis is of crucial importance in the development of novel therapeutic approaches for non-organ-confined prostate cancer. Recent studies revealed that tumor cells have the ability to synthesize androgenic hormones in an intracrine manner. This recognition opened the way for the development of a novel drug, abiraterone acetate, which shows benefits in clinical trials. A novel anti-androgen enzalutamide that inhibits androgen receptor (AR) nuclear translocation has also been developed and tested in the clinic. AR coactivators exert specific cellular regulatory functions, however it is difficult to improve the treatment because of a large number of coregulators overexpressed in prostate cancer. AR itself is a target of several miRNAs which may cause its increased degradation, inhibition of proliferation, and increased apoptosis. Truncated AR occur in prostate cancer as a consequence of alternative splicing. They exhibit ligand-independent transcriptional activity. Although there has been an improvement of endocrine therapy in prostate cancer, increased intracrine ligand synthesis and appearance of variant receptors may facilitate the development of resistance.

Contents

1. Introduction

Traditionally, endocrine therapy in prostate cancer has been based on administration of drugs which lower endogenous androgen levels or inhibit binding of dihydrotestosterone to the C-terminal part of the androgen receptor (AR). Non-steroidal anti-androgens hydroxyflutamide and bicalutamide have replaced steroidal drugs such as cyproterone acetate which has a progestagenic structure. An advantage of using hydroxyflutamide and bicalutamide in prostate cancer therapy is the lower risk of side effects which are attributed to steroidal receptor antagonists. At higher concentrations, progestagenic steroids may induce activation of the AR. However, also treatment with non-steroidal anti-androgens fail and several mechanisms relevant to the failure of non-steroidal anti-androgens have been identified. Recently, novel drugs which target the AR axis have been developed and tested in clinical trials. The main purpose of this review is to discuss some of the

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molecular mechanisms associated with AR signaling in prostate cancer cells and how those mechanisms could be exploited for novel therapeutic agents.

Identification of the AR as a therapy target in castration therapy-resistant prostate cancer has resulted from immunohistochemical, *in vitro*, and *in vivo* studies using novel tumor models. In contrast to previous models which mostly utilized rat prostate cancer cells, studies with human material obtained from patients who failed endocrine therapy demonstrated that the AR is expressed in primary cancer and in metastases [1,2]. Enhanced expression of the AR could be explained by several molecular mechanisms such as amplification of the AR gene or increased stability of mRNA or protein which is considered an adaptation mechanism in tumors subjected to androgen withdrawal [3,4]. Although ligand-independent activation of the AR by growth factors, growth factor-related receptors, cytokines, neuropeptides, and compounds that increase protein kinase A levels has been intensively investigated in prostate cancer, its significance in the development of therapy-refractory disease is still a matter of discussion. Another potential mechanism of AR activation is the potentiation of effects of low concentrations of androgens by a non-steroidal compound. Neuroendocrine peptides such as bombesin can enhance AR phosphorylation and activity in the presence of low androgen doses [5]. This mechanism may be in part relevant to neuropeptide-promoted progression of prostate cancer towards castration therapy resistance. The process of neuroendocrine differentiation is suppressed by the AR [6]. AR knock-down increases the levels of neuroendocrine markers such as beta-tubulin or nestin.

Studies on androgen signaling in prostate cancer progression have been facilitated by the introduction of novel model systems. AR and prostate-specific antigen (PSA) expression were observed in the proliferating cells of the recurrent CWR22 xenograft thus indicating the importance of the AR in tumor progression [7]. On the basis of those findings, it is not surprising that inhibition of AR expression by neutralizing antibodies in sublines that represent castration therapy-refractory stage, such as LNCaP C4 and Rf cells, causes inhibition of proliferation [8]. A plausible concept for prostate cancer progression suggests that there is a heterogenous expression of the AR in primary tumor and metastatic tissues. In some prostate cancer cells methylation of the promoter of the AR gene leading to a diminished expression was reported [9]. In conclusion, it should be kept in mind that there may be obvious differences in AR expression between primary tumors at different stages of prostate carcinogenesis and tumor metastases.

One important obstacle in improving therapy of prostate cancer is inability to uncouple proliferative and differentiation responses of androgen signaling. It is worthwhile to mention that AR-positive cell lines are more sensitive to an antiproliferative effect of vitamin D than AR-negative cells [10]. If AR signaling is blocked by an antiandrogen, the growth-inhibitory effect of vitamin D is reduced. In such a case, restoration of androgen responsiveness may be beneficial for a subgroup of prostate cancer patients. To date, it was not possible to selectively enhance pro-differentiation properties of the AR at the same time when a drug of interest could inhibit pro-proliferative responses. Those findings also raise the questions about a possible partial restoration of androgenic response in castration therapy-resistant prostate cancer favoring the proliferative rather than the differentiation response.

All those studies served as a basis for intensive analysis of molecular mechanisms which are relevant to facilitation of tumor progression by the AR. Best clinical evidence of AR activity estimation in castration-resistant prostate cancer is the rise of PSA levels that accompanies the recurrence of the disease. Thus, the AR axis is still a valuable target in castration-resistant prostate cancer. In this review, we focus on several novel aspects of AR action such as intracrine mechanisms of steroids synthesis, complex interactions of AR with coactivators or corepressors, mutual regulation of miRNA and AR, and the role of truncated AR in prostate cancer (Fig. 1).

2. Intracrine androgen synthesis and therapeutical implications

Reactivation of steroidogenic pathways in recurrent prostate cancer was reported in a study by Holzbeierlein and associates who analyzed changes in gene expression during hormonal therapy [11]. One of the rate limiting enzymes in sterol synthesis, squalene monooxygenase was up-regulated in castration therapy-resistant prostate cancer. In the following years, this issue has been extensively investigated by other laboratories. Mostaghel and associates reported that conventional endocrine therapies do not eliminate expression of many androgen target genes [12]. Testosterone and dihydrotestosterone levels were measured in benign and malignant prostates including those obtained after therapies. It was found that intracellular levels of testosterone do not decrease in castration therapy-resistant prostate cancer and are sufficient for AR activation [13,14]. Alterations in expression of intratumoral enzymes implicated in steroidogenesis from progesterone to adrenal androgens and testosterone has been intensively investigated in prostate cancer tissues. It was found that several enzymes, namely FASN, CYP17A1, HSD3B1, HSD17B3, CYP19A1, and UGT2B17 are up-regulated, whereas SRD5A2 was down-regulated [15]. Thus, a ''backdoor pathway'' of androgen synthesis is active in advanced prostate cancer (Fig. 1a). Consequently, these alterations in enzyme expression resulted in an increase in the levels of intracellular testosterone in prostate cancer xenografts from castration therapy-resistant tumors. Locke and associates have demonstrated that intracellular androgen synthesis increases in sublines of LNCaP cells during tumor progression due to up-regulation of expression of steroidogenic enzymes [16]. In the LNCaP progression model, that group showed that there is an increased expression of respective enzymes in androgen-independent cellular passages. The ability of the AR-negative tumor cells PC-3 and DU-145 to synthesize testosterone from cholesterol was documented as well. This indicates that prostate cancer cells which are not dependent on testicular androgens may contribute to tumor progression by increasing intracellular androgen production [17]. Taken together, those studies identified intracrine androgen synthesis as a mechanism that compensates for the effects of androgen ablation. Intratumoral conversion of androstanediol to dihydrotestosterone in a castration-resistant xenograft has resulted in a high intratumoral level of the active androgen [18]. Therefore, it was postulated that the effects of classic androgen ablation may be combined with drugs which target intracrine steroidogenic pathways. On the basis of the findings, therapeutic inhibition of CYP17, a key enzyme involved in the synthesis of adrenal androgens from progesterone, has been proposed in prostate cancer. A novel inhibitor of the enzyme, abiraterone, was synthesized at the Cancer Research UK Center [19]. In comparison to the previously used drug ketoconazole, abiraterone acetate is 20 times more potent. It was recently reported that abiraterone acetate also inhibits 3beta-hydroxysteroid dehydrogenase which is responsible for the conversion of dehydroepiandrosterone to androstenedione and androstenediol to testosterone turnover [20]. Abiraterone has an inhibitory effect on AR mRNA and protein expression [21] and may therefore reverse AR transcriptional activity which is induced by residual ligands [22]. Although this review is largely focused on basic research on androgen signaling in prostate cancer, it should be emphasized that clinical trials with abiraterone indicate an improvement in medical treatment. Patients who previ-