

Androgen-Receptor Coregulators Mediate the Suppressive Effect of Androgen Signals on Vitamin D Receptor Activity

Huei-Ju Ting,^{1,*} Bo-Ying Bao,^{2,*} Cheng-Lung Hsu,¹ and Yi-Fen Lee¹

Departments of ¹Urology and ²Chemical Engineering, University of Rochester, 601 Elmwood Ave., Rochester, NY 14642

Overexpression of androgen receptors (AR) in PC-3 cell, and treatment of 5 α -dihydrotestosterone in LNCaP cells lead to the suppression of VDR transactivation. Competition for shared coregulators between AR and VDR is one possible mechanism to explain the suppressive effect of androgen-AR signals on VDR activity. Among the AR coregulators we tested, ARA54, ARA70, supervillin, and gelsolin were found to enhance VDR transactivation. Further characterization of the interaction between ARA54 or ARA70 and VDR demonstrated a direct interaction between VDR and ARA70, but no association between ARA54 and VDR. The LXXLL motif of ARA70 is essential for interaction with VDR and partially responsible for its function as a coactivator of VDR. The suppression of VDR transactivation by AR signal was restored by overexpression of ARA70, but not ARA54. Together, ARA70 and ARA54 modulate VDR transactivation, and the competition for ARA70 mediates the suppressive effect of androgen-AR on VDR transactivation.

Key Words: 1 α ,25-Dihydroxyvitamin D₃; vitamin D₃ receptor; androgen receptor; coregulators.

Introduction

Treatment with 1 α ,25-dihydroxyvitamin D₃ (1,25-VD) inhibits proliferation and promotes differentiation in several types of cancers (1). The implications for clinical usefulness has triggered a number of studies regarding the use of 1,25-VD to treat prostate cancer (PCa) (2). Among several established human PCa cell lines, including LNCaP, DU145, PC-3, ALVA-3, and MDA PCa, treatment with 1,25-VD led to antiproliferation effects, but in varying degrees (3,4). For example, DU145, PC-3, ALVA-3, and MDA PCa

2a display less growth inhibition with 1,25-VD (<20% inhibition) than LNCaP and MDA PCa 2b (approx 50%). These varied degrees of 1,25-VD growth inhibition indicate cells develop resistance to 1,25-VD treatment and hence become less sensitive to 1,25-VD. The receptor for 1,25-VD (VDR), which transmits the ligand signal to the nucleus and regulates the transcription of target genes, is universally expressed in the PCa cells studied (3,4). The antiproliferation effect of 1,25-VD is suggested to be partly correlated with VDR expression level and transactivity (3). Signals affecting VDR activity are potentially involved in the development of 1,25-VD resistance. The fact that 1,25-VD-resistant cells are also androgen-independent implies that the defects in 1,25-VD signaling may be a result of the altered molecular context derived in such forms of PCa.

Androgens are important for the growth of PCa, and anti-androgens have been used therapeutically for decades. Pharmacological or surgical androgen ablation therapy is commonly used to treat PCa patients. Although tumors shrink significantly after therapy, the majority of patients develop hormone-refractory PCa. Numerous mechanisms revealed to date demonstrate that diverse signaling pathways are involved in developing resistance to androgen ablation (5). First, mutant ARs with broad ligand sensitivity have been found to utilize hormones other than androgen to transmit growth-promoting signals (5). Second, amplification of growth hormone signals, such as HER2/neu, in PCa can stimulate growth and eventually bypass AR (5). Third, overexpression of coregulators, such as SRC-1, TIF-2, and gelsolin, in androgen-independent PCa has been shown, that may magnify AR activity in the presence of trace amounts of androgen (6,7). A more recent finding also demonstrated that hydroxyflutamide, an antiandrogen, could activate the MAPK pathway, an effect that might contribute to the development of PCa resistance to androgen ablation (8). Either one of mechanisms or a combination of several mechanisms described above can result in the development of androgen-independent cell growth, and therefore play roles in 1,25-VD resistance.

Most coregulators associate with and modulate more than one steroid receptor. AR coregulators, originally identified as AR-associated proteins, also modulate many other steroid receptors. As previously reported, ARA54 enhances AR and progesterone receptor (9); ARA70 enhances peroxisome

*These two authors contributed equally to this paper.

Received November 8, 2004; Revised December 15, 2004; Accepted December 16, 2004.

Author to whom all correspondence and reprint requests should be addressed: Yi-Fen Lee, PhD, Departments of Urology and Chemical Engineering, University of Rochester, 601 Elmwood Ave., Rochester, NY 14642. E-mail: YiFen_Lee@urmc.rochester.edu