Regulation of androgen receptor activity by tyrosine phosphorylation

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Summary

The androgen receptor (AR) is essential for the growth of prostate cancer cells. Here, we report that tyrosine phosphorylation of AR is induced by growth factors and elevated in hormone-refractory prostate tumors. Mutation of the major tyrosine phosphorylation site in AR significantly inhibits the growth of prostate cancer cells under androgen-depleted conditions. The Src tyrosine kinase appears to be responsible for phosphorylating AR, and there is a positive correlation of AR tyrosine phosphorylation with Src tyrosine kinase activity in human prostate tumors. Our data collectively suggest that growth factors and their downstream tyrosine kinases, which are elevated during hormone-ablation therapy, can induce tyrosine phosphorylation of AR and such modification may be important for prostate tumor growth under androgen-depleted conditions.

Introduction

Prostate cancer is the second leading cause of cancer death among men in Western countries. Patients with advanced prostate cancer initially benefit from androgen-ablation therapy, which leads to temporary tumor remission due to apoptosis of androgen-sensitive tumor cells. However, recurrence of androgen-independent tumors is inevitable for most patients and renders the conventional hormone therapy ineffective (Denmeade and Isaacs, 2002). It has, therefore, become a focus of intensive study to understand the mechanisms underlying the development of hormone-refractory prostate cancer (Debes and Tindall, 2004; Feldman and Feldman, 2001). The androgen receptor (AR), a member of the steroid hormone receptor family, is primarily responsible for mediating the physiological effects of androgens by binding to specific DNA sequences, known as androgen response elements (AREs), which regulate transcription of androgen-responsive genes (Heinlein and Chang, 2004). Increasingly, clinical findings demonstrate that a majority of androgen-ablation therapy-resistant prostate cancers still express AR and androgen-dependent genes, indicating that the AR-signaling pathway is functional in the absence of androgens or in the presence of low levels of androgens (Culig et al., 2000). Several independent studies also showed that AR is essential for both hormone-sensitive and -refractory prostate cancer (Chen et al., 2004; Zegarra-Moro et al., 2002).

Mutations and amplification of AR, alterations in protein kinases, growth factors, and nuclear receptor coactivators have all been proposed to modulate AR signaling and may, therefore, play key roles in the development of androgen independence of prostate cancer (Feldman and Feldman, 2001; Gelmann, 2002). Mutations in the ligand-binding domain of AR are shown to broaden the ligand-binding profile of the mutant receptor (Veldscholte et al., 1990; Zhao et al., 2000). However, the frequency of AR mutation is generally low and probably only accounts for less than 10% of the cases surveyed (Taplin et al., 2003). Upregulation of the enzymes involved in steroid synthesis in some recurrent prostate tumors and activation of AR via the intracrine mechanism have also been reported (Titus et al., 2005a). However, the tissue androgen levels did not correlate with clinical prognosis (Titus et al., 2005b). Recently, the increased AR expression level was shown to associate with the development

SIGNIFICANCE

Recurrent prostate cancer is resistant to commonly used hormonal therapy and is the major cause of patient death. Study of altered signaling events in hormone-refractory prostate cancer cells will allow us to develop more effective regimens and more reliable prognostic markers. In this study, we show that the androgen receptor can be phosphorylated by tyrosine kinase Src and such modification appears to be important for prostate cancer cell growth under low-androgen conditions. Our results suggest that the level of AR tyrosine phosphorylation may serve as a diagnostic tool to predict patient outcome in response to hormone-ablation therapy and inhibition of tyrosine phosphorylation of AR may be an effective intervention target for hormone-refractory prostate cancer.

of resistance to antiandrogen therapy (Chen et al., 2004). The cross-talk between growth factor and AR-signaling pathways in prostate cancer cells has been well documented. The expression of several peptide growth factors, such as EGF/TGFα, IL-6, and IGF-1, are reported to be elevated during progression to hormone-refractory human prostate cancer (Bartlett et al., 2005; Di Lorenzo et al., 2002; George et al., 2005; Krueckl et al., 2004; Lorenzo et al., 2003). These autocrine/paracrine factors can either induce the androgen-independent activation of AR transcriptional activity or sensitize AR to low concentrations of androgens (Culig et al., 1994; Gregory et al., 2004; Ueda et al., 2002). A substantial body of literature suggests that AR is regulated directly by phosphorylation. Several protein kinases, including the mitogen-activated protein kinase (MAPK), Akt/PKB, cAMP-activated protein kinase A (PKA), and protein kinase C (PKC), have been shown to modulate AR transcriptional activity by phosphorylating serine or threonine residues in AR or its coactivators, such as the transcriptional intermediary factor 2 (TIF2) and the steroid hormone receptor coactivator 1 (SRC1) (Gregory et al., 2004; Lin et al., 2001; Ueda et al., 2002; Yeh et al., 1999). The expression of erbB2/HER-2/neu, a member of the EGF receptor family tyrosine kinases, is increased in a subset of hormone-refractory LAPC4 xenografts (Craft et al., 1999). Forced overexpression of erbB2 in androgen-dependent prostate cancer cells promotes androgen-independent growth (Craft et al., 1999; Yeh et al., 1999). In addition to erbB2, several other non-receptor tyrosine kinases, including Src, FAK, and Etk/BMX, have been implicated in activation of AR transcriptional activity in response to nonsteroid stimuli such as IL-6 and bombesin (Lee et al., 2001, 2004). It is also shown that Src kinase is directly associated with AR upon androgen treatment through a nongenotropic pathway (Kousteni et al., 2001; Migliaccio et al., 2000). However, the mechanisms by which these tyrosine kinases regulate AR transcriptional activity in prostate cancer cells are not well understood yet.

Here, we report that AR is tyrosine phosphorylated in prostate cancer cells in response to growth factors. Our data suggest that growth factors and their downstream tyrosine kinases, which are elevated during hormone-ablation therapy, can induce tyrosine phosphorylation of AR and such modification may contribute to androgen-independent activation of AR or sensitize AR to low levels of hormone. Our findings provide a mechanism by which hormone-refractory prostate cancer cells continue to grow under androgen-depleted conditions.

Results

Elevated tyrosine phosphorylation of AR in hormone-refractory prostate tumor xenografts

We observed that the level of tyrosine phosphorylation is significantly increased in hormone-refractory prostate xenograft tumors compared to their hormone-sensitive counterparts (Figure 1A). Interestingly, the AR protein immunoprecipitated from hormone-refractory tumors could also be recognized by the anti-phosphotyrosine antibody (Figure 1B), suggesting that AR is tyrosine phosphorylated. Moreover, tyrosine phosphorylation of AR was significantly increased in hormone-refractory tumors compared to their hormone-sensitive counterparts (Figure 1B). To find out which tyrosine kinase might be responsible for the increased AR tyrosine phosphorylation, we examined the activity of a panel of tyrosine kinases, including erbB2, FAK, and Src. We only consistently detected the elevated Src kinase activity in all hormone-refractory samples while the protein level of Src remained largely unchanged (Figure 1C).

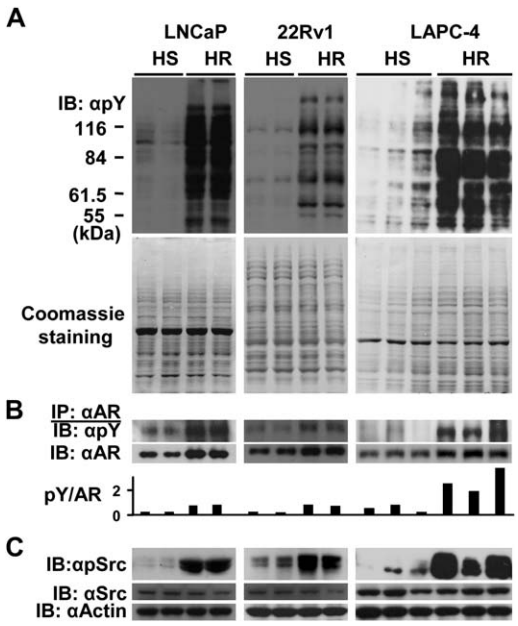

Figure 1. Increased tyrosine phosphorylation in hormone refractory prostate tumor xenografts

A: Hormone-sensitive (HS) and refractory (HR) xenograft tumors were derived as described in Experimental procedures. The tumor lysates were immunoblotted with anti-phosphotyrosine (αpY) antibody. The sample loading was monitored by Coomassie Blue staining (bottom).

B: The immunoprecipitated AR was subjected to immunoblotting with the antibodies indicated. The ratio of pY/AR is shown at the bottom. For each group, HR is higher than HS ($p < 0.05$).

C: Immunoblot with anti-phospho-SrcY416(αpSrc) to determine the Src kinase activity.

Tyrosine phosphorylation of AR in prostate cancer cells

To test whether AR could be tyrosine phosphorylated in prostate cancer cells in response to extracellular stimuli, we treated LNCaP cells with several growth factors and hormones, respectively. As shown in Figure 2A, all tested stimuli, except for DHT, could induce tyrosine phosphorylation of AR in LNCaP cells under our experimental conditions. We also showed that EGF induced a transient tyrosine phosphorylation of AR in all tested AR-positive prostate cancer cell lines with similar kinetics, which peaked at 5 min and tapered by 30 min (Figure 2B). Interestingly, the EGF-induced AR tyrosine phosphorylation was concomitant with the increased phosphorylation of Y416 of Src kinase (Figure 2C), an indicator of Src kinase activity, and was significantly diminished by the selective Src kinase inhibitors PP2 and SU6656 (Figure 2D), as well as the siRNA specific for Src (Figure 2E), suggesting that it is, at least partially, dependent on Src kinase activity. Figure 2F shows that the constitutively active SrcY527F could induce tyrosine phosphorylation of the endogenous AR in LNCaP cells as well as the exogenous AR in COS-1 cells, while the kinase-inactive mutant SrcK295M failed to do so. These data together indicate that EGF-induced AR tyrosine phosphorylation is mediated by Src kinase.