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Androgen-Independent Prostate Cancer: Potential Role of Androgen and ErbB Receptor Signal Transduction Crosstalk

Soha Salama El Sheikh, Jan Domin†, Paul Abel‡, Gordon Stamp* and El-Nasir Lalani**

Departments of *Histopathology, †Renal Medicine, ‡Surgery, Imperial College, Hammersmith Hospital Campus, London, UK

Abstract

In prostate cancer (PC), increasing evidence suggests that androgen receptor (AR) signalling is functional under conditions of maximal androgen blockade. PC cells survive and proliferate in the altered hormonal environment possibly by interactions between growth factor–activated pathways and AR signalling. The present review article summarizes the current evidence of this crosstalk and focuses on the interactions among the ErbB receptor network, its downstream pathways, and the AR. The potential role of this crosstalk in the development of androgen independence and in relation to antiandrogen therapy is discussed. Such interactions provide insight into possible complementary or additional strategies in the management of PC.

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Introduction

Prostate cancer (PC) is the commonest cancer affecting men in the western world. The number of new cases of PC registered in England and Wales (age-standardized) increased by 104% from 1971 and 1993, whereas the number of deaths increased by 38% between 1971 and 1998 [1]. In the year 2000, there were an estimated 180,400 new cases and 31,900 deaths in the United States [2]. Conventional therapies involve surgical or pharmacological castration [clinically referred to as maximum androgen blockade (MAB) when combined with antiandrogen therapy] with the intent of maximally diminishing the availability and action of androgen on the androgen receptor (AR). Over time, the PC cells overcome the need for androgen as a survival, growth, and differentiating factor and become androgen-independent (AI) [3,4]. Evidence from xenograft models and clinical material indicates that this change to AI growth could result from either the outgrowth of a preexisting (pretreatment) AI clone or adaptive responses to androgen deprivation, which allow the evolution of AI clones [5,6]. Under conditions of MAB, autocrine growth factor loops could enhance growth and mitogenic signalling through the activation of tyrosine kinase–coupled receptors and subsequently second messengers such as mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K), and other effector pathways.

In the last decade, it has become clear that signalling pathways are not simply linear sequences of interactions downstream of an activated receptor. Growth factors appear not to act independently of the AR in the prostate. Crosstalk between these signal transduction pathways has been implicated in maintaining PC survival in an androgen-poor environment [7]. In this review, evidence demonstrating the potentially important crosstalk mechanisms that bypass classical androgen dependence is presented.

AR

The AR is a member of the nuclear receptor superfamily of transcription factors [8]. It can be localized to the cytoplasm or nucleus and its topographic localization is a reflection of its functional state (active/inactive). In its inactive state, AR associates with heat shock proteins (hsp70 and hsp90) [9,10]. In the absence of ligand, cytoplasmic AR is degraded. In the presence of the ligand, testosterone, and its more potent derivative dihydrotestosterone (DHT), the AR–hsp complex is disrupted and the AR undergoes conformational change that allows phosphorylation, dimerization, and translocation of the more stable ligand–receptor complex to the nucleus [11,12]. In the nucleus, the dimerized AR–ligand complex interacts with coactivator molecules (e.g., ARA54, ARA70) [13] and initiates gene transcription of androgen-regulated genes by binding to specific androgen response elements (AREs). (The different states of the AR and its localization are illustrated in Figure 1, *A* and *B*.) The gene activation pathways initiated by androgens impact upon the processes of prostatic cellular proliferation, survival, and differentiation [14].

AI growth may be attributed to secondary genetic mutations, which allow the AI PC cells to survive and proliferate despite the paucity/absence of androgen and imply that signalling through the AR is no longer active

Abbreviations: AI, androgen independence; AR, androgen receptor; MAB, maximal androgen blockade; PIP_3, 3′-phosphoinositides; PC, prostate cancer

Address all correspondence to: Prof. E.-N. Lalani, Department of Histopathology, Division of Investigative Sciences, Faculty of Medicine, Imperial College, Hammersmith Hospital Campus, Du Cane Road, London W12 0NN, UK. E-mail: e.lalani@ic.ac.uk

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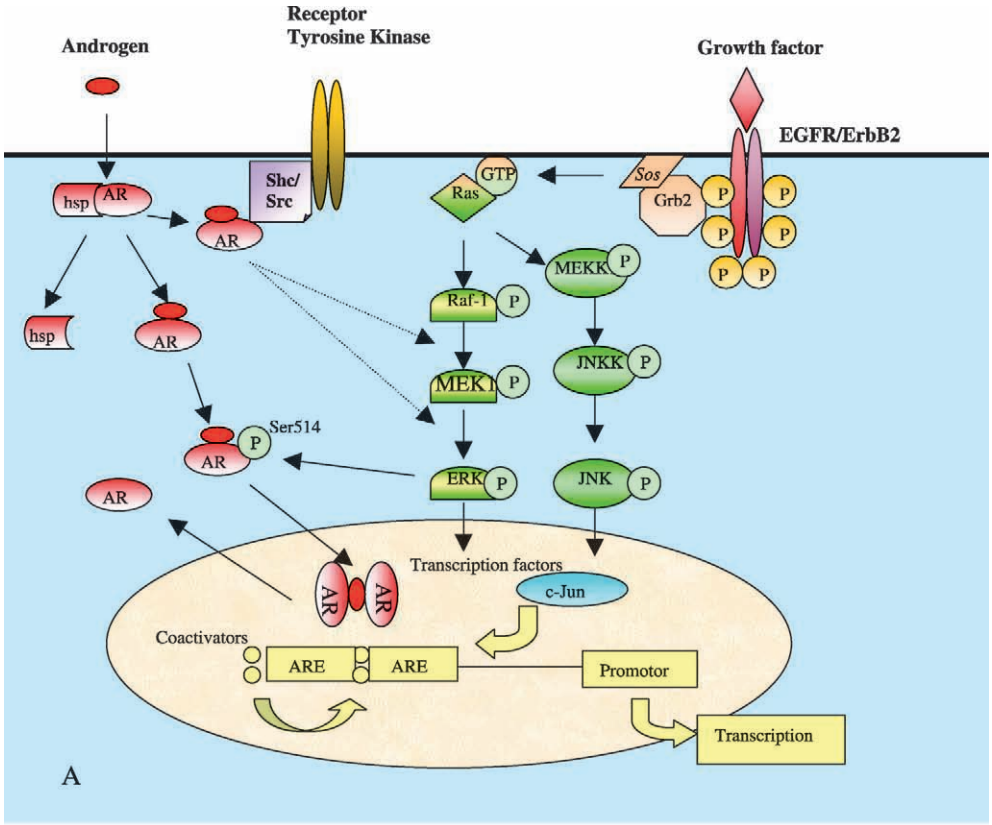
[15–17]. However, in nearly all cases of AI PC, the persistent expression of AR and of androgen-regulated genes such as PSA indicates that AR signalling is not always bypassed but is indeed functional and active [7].



Augmentation of AR-mediated signal at lower androgen levels can occur by amplification of the AR gene itself. AR gene amplification was observed in 30% of recurrent PC tumor specimen after androgen deprivation therapy [18–23]. AR hypersensitivity and increased AR protein stability have also been implicated in AI PC progression [24]. Several *in vitro* studies on human PC cell lines have shown that mutations in the AR can make the receptor "promiscuous"— whereby the AR can be activated by a number of different ligands such as testosterone, DHT, estrogen, progesterones, and the adrenal androgen dihydroepiandrosterone (DHEA) [25]. To complicate matters further, antiandrogens such as flutamide, hydroxyflutamide, and bicalutamide can also activate mutant AR [26]. These studies suggest that AR mutations in PC may provide a survival advantage for AR-mutated clones in response to treatment with antiandrogens as flutamide [27,28]. In clinical PC, AR mutations have been observed [29,30] and are thought to be the most likely explanation for the "antiandrogen withdrawal syndrome." This syndrome is a well-established phenomenon in PC where a subset of patients will benefit from withdrawal of antiandrogen hormonal therapy and exhibit decreasing PSA values and clinical improvement [31]. Other researchers have reported that antiandrogens, hydroxyflutamide, bicalutamide, cyproterone acetate, RU58841, and other compounds such as genistein and RU486, can promote the interaction between AR and its coactivator, ARA70, in a dose-dependent manner [32]. Increased expression or mutations in AR coactivators can activate the AR in the absence of DHT [e.g., cofactor ARA70 specifically conferred the androgenic effect from 17 β-estradiol (E2) and hydroxyflutamide to AR] [33,34].

An alternative model of AR activation in the setting of MAB involves nonsteroid receptor signal transduction pathways [35]. AR can be activated in a ligand-independent manner by a number of growth factors including insulin-like growth factor-1 (IGF-1), epidermal growth factor (EGF), keratinocyte growth factor (KGF), and interleukin-6 (IL-6). Each of these has been shown to transcriptionally activate an androgen-responsive reporter construct in the absence of ligand or synergistically in conjunction with androgens. The mechanistic details are, however, unknown [36–38]. The effect exerted by growth factors on the AR suggests that these signalling pathways are not mutually exclusive. They point towards the existence of crosscommunication between ligands, at the level of their cognate receptors or through their intracellular downstream kinase cascades, and the AR. This allows the emergence of AI cells, which can survive and proliferate under androgen ablation, leading to recurrence and metastasis [7,37].

Data on the importance of the interaction between polypeptide growth factors like EGF and the ErbB network of receptors (or their secondary mediators) with the AR in favor of PC survival are now rapidly emerging. This article reviews these interactions and focuses on their potential role in promoting AI growth.

AR and the ErbB Receptors

ErbB receptors are typical receptor tyrosine kinases activated downstream of EGF and EGF-like ligands. They include four members: EGF receptor EGFR/ErbB1, ErbB2 (or HER2/Neu), HER3/ErbB3, and HER4/ErbB4. All EGFR family members are characterized by a modular structure consisting of an extracellular ligand binding domain, a transmembrane region, and an intracellular part harboring the highly conserved tyrosine kinase domain. Ligand binding induces the formation of homodimers or heterodimers and the phosphorylation of tyrosine residues, which serve as docking sites for a variety of signal transducers (Figure 1, *A* and *B*) [39,40]. This family of receptors plays a critical role in the proliferation, migration, survival, and differentiation of target cells. Dysregulation of signalling by the ErbBs has been implicated in the pathogenesis and progression of human cancers [39,40].

ErbB1/EGFR

EGF is a mitogen required for normal prostatic epithelial cell growth and is present in large amounts in human prostatic fluid [41,42]. mRNA and protein expression of its receptor, EGFR, have been demonstrated in human PC, benign prostatic hyperplasia (BPH), and normal prostate

Figure 1. *(A) The interaction between the AR and the ErbB/MAPK pathway. Parallel pathways activated by androgen and the ErbB receptor intersect at several levels. Following hormone binding in the cytoplasm, the AR–hsp–containing complex is dissociated and the steroid receptor is rapidly translocated to the nucleus. In the nucleus, AR dimerizes and binds to the DNA double helix at specific sequences called AREs. The DNA-bound AR dimer recruits a multiprotein complex containing members of the basal transcription machinery and other coactivators or corepressors (not shown), which control the transcriptional AR response. AR is then dissociated from the DNA and shuttled back to the cytoplasm where it can reassociate with hsp or ligand. Growth factors initiate signalling by binding to and sequentially activating the ErbB receptors, adaptor molecules like Grb2 and Sos, and the GTP exchange factor Ras. This in turn activates the three-tiered MAPK cascade. Crosstalk may occur in the cytoplasm, for example, when AR complexes with Shc/Src (two adaptor molecules recruited by activated receptor tyrosine kinases) to activate the MAPK pathway, leading to phosphorylation of ERK. Alternatively, ErbB2 activates the AR through ERK phosphorylation at Ser514, thereby suggesting an influence of these two pathways on each other. c-Jun is activated downstream of MEKK, which enhances the homodimerization of AR with DNA and consequently the AR response to androgen. The requirement for AR in MEKK1-induced apoptosis is not shown in this figure. (B) The interaction between AR and ErbB/PI3K/Akt. Similar to (A), ligand binding of the AR initiates dissociation from hsp, phosphorylation, and dimerization. Growth factors activate ErbB2/ErbB3 complex and recruit PI3K, which produces 3′-phosphoinositides (PIP_3) and recruits PDK1 and Akt, resulting in their activation. Akt then inactivates a variety of proapoptotic molecules including Bad, caspase 3, GSK-3β, and the forkhead transcription factors (FKHR). In addition, Akt targets the AR and phosphorylates it at Ser210 and Ser790. This phosphorylation promotes PC cell survival by controversial mechanisms (marked by the question marks). Some workers hypothesize that this is through activation of AR transcription. Alternatively, it may protect the cells from androgen-induced apoptosis through ubiquitylation and degradation of the AR within an Akt/Mdm2/AR complex. Another mechanism of AR activation by Akt is through GSK-3β inactivation and nuclear accumulation of β-catenin. In the cytoplasm, AR rapidly activates PI3K and increases intracellular PIP_3 by an as yet unknown mechanism. PTEN, the negative regulator of this pathway, is not shown in this figure.*

[43,44]. Transforming growth factor-α (TGF-α) is another ligand for EGFR. The existence of a stimulatory autocrine loop involving EGF, TGF-α, and EGFR has been suggested from *in vivo* observations in high-grade prostatic intraepithelial neoplasia (PIN) and PC. These lesions showed a higher expression of membranous EGFR, ErbB2, and cytoplasmic TGF-α than low-grade PIN or BPH [45]. This stimulatory loop appeared to be important in the growth of three human PC cell lines: PC-3, LNCaP, and DU145. The DU145 cell line was apparently dependent on this autocrine loop for cell proliferation [46]. The importance of the EGFR in promoting the survival of PC cells was demonstrated in the CWR22 xenograft model of PC, in which castration was associated with an initial reduction in EGFR expression. EGFR returned to comparable or higher levels following the administration of androgen or in relapsed AI tumors (CWR22R) [47].

DHT and EGF are thought to play complementary roles in regulating the proliferation of prostate cells. The combination of DHT and EGF has been shown to enhance the proliferation of the MDA PC2a and MDA PC2b human PC cell lines through a convergent stimulatory mechanism. This promoted progression through the cell cycle by increasing cyclin-dependent kinase-2 (CDK2) activity and accelerating the downregulation of the CDK inhibitor $p27^{kip1}$ compared to either ligand alone [48]. In addition, an interactive stimulation of EGFR synthesis by DHT [47–51] and of AR synthesis by EGF [48] has been described in several androgen-sensitive PC cell lines. DHT increased both EGFR expression and receptor–ligand affinity, resulting in increased EGF binding and an enhanced mitogenic response to EGF [47–51]. This effect of DHT can be blocked by the AR antagonist bicalutamide, which indicates that these effects require the AR [47].

ErbB2 (HER2/Neu)

ErbB2 is one of the best-studied genes involved in human malignancy. In PC, ErbB2 is considered a potential surrogate biomarker for screening chemopreventive agents in short-term Phase II trials [52,53]. Unlike other members of the ErbB family, ErbB2 has no known ligand and is the preferred heterodimerization partner within the EGFR family. Heterodimers containing ErbB2 induce signals with the strongest biological activity [39]. EGFR and ErbB2 serve as receptors for EGF [40] and so they are likely to be involved in an interaction between the EGF and AR signalling pathways.

Studies of the role of ErbB2 in PC remain inconclusive. Previous work has shown widely divergent levels of ErbB2 expression in primary PC, probably owing to methodological differences in the studies. Some authors have demonstrated ErbB2 protein overexpression and/or gene amplification in a subset of PC patients and in premalignant lesions [54–58]. ErbB2 protein was expressed at statistically significant higher levels in PC treated by androgen deprivation therapy compared to untreated cancer [57,59]. On the other hand, some investigators have not found overexpression of ErbB2 in PCs [60] and most have not shown amplification of the ErbB2 gene [59,61,62]. Several authors have suggested that elevated serum levels of the extracellular portion of ErbB2 in patients with metastatic PC provide further supporting evidence of its potential role [63,64]. Using the LAPC-4 mouse xenograft model, Craft et al. [65] showed that AI sublines of human PC xenografts expressed higher levels of ErbB2 than androgen-dependent sublines. They also showed that overexpression of ErbB2 confers AI growth to the androgen-dependent LNCaP cell line. In the absence of androgen, overexpression of ErbB2 activated the transcription of prostate specific antigen PSA (a process that requires a functional AR but is not inhibited by the antiandrogen bicalutamide) [65]. The failure of bicalutamide to block PSA induction by ErbB2 is consistent with clinical AI PC and indicates that ErbB2 interacts with the AR pathway distal to the interaction between androgens and AR.

The biochemical mechanisms of this crosstalk are unclear, but the failure of ErbB2 to activate a single high-affinity AR binding site points either to the involvement of an intermediate protein, or that ErbB2 may optimize AR function by activating Ras and other signalling pathways (Figure 1*A*). Ras proteins are molecular switches with the ability to interact and activate several effector molecules. Among those, Raf-1 kinase, PI3K, and Ral-GDS are the best characterized. Raf activates the mitogenic MEK/ERK kinases pathway, whereas PI3K regulates the PKB/Akt cascade, involved in the control of proliferation, metabolism, and apoptotic responses (both pathways are discussed in detail below and outlined in Figure 1, *A* and *B*, respectively). Yeh et al. [66] demonstrated ErbB2 activation of the AR through activating the MAPK pathway as well as promoting the interaction of AR and ARA70 coactivator. ErbB2 overexpression thereby favors AR activation at very low levels of androgen.

ErbB2 has also been involved in signalling downstream of other ligands such as IL-6. As previously mentioned, IL-6 is a nonsteroidal activator of the AR whose level is frequently elevated in sera of patients with metastatic PC [67]. Through a novel mechanism that involves the heterodimerization of the IL-6 receptor and ErbB2, IL-6 activated both ErbB3 and MAPK in LNCaP cells [68].

ErbB3/HER3 and ErbB4/HER4

Neu differentiation factor/heregulin (HRG) belongs to a family of polypeptide growth factors that bind to receptor tyrosine kinases ErbB3 and ErbB4. HRG binding induces ErbB3 and ErbB4 heterodimerization, activating downstream signal transduction. ErbB3 differs from other ErbB family members in that it possesses diminished kinase activity and is largely dependent upon other ErbB kinases, in particular ErbB2 [39,69].

HRG is present in normal human adult prostate and BPH, where it may function as a paracrine physiological differentiation factor [70,71]. Analysis of clinical PC specimens indicates that overexpression of ErbB3 has been linked to a less favorable prognosis [72]. In PC cell lines, HRG inhibited the growth of AR-positive but not AR-deficient cells [71]. The exact sites of crosstalk between HRG and AR signalling pathways remain to be elucidated. However, the fact that

only AR-positive PC cells appear to be influenced by HRG supports the concept that the physiological function of HRG on prostatic cells is optimal in the presence of AR.

Ebp1 is a recently identified ErbB-3 binding protein that possesses an important motif, which mediates interactions with nuclear hormone receptors and is thought to provide a link between the ErbBs and AR. Ebp1 has been shown to bind to AR *in vitro* and *in vivo*. It inhibited ligand-mediated transcriptional activation of AR-regulated genes such as the PSA growth of AR-positive LNCaP cells [73].

Reports on the ErbB4 in the prostate are few. ErbB4 protein is strongly expressed by normal prostate luminal cells. Only 23% of PC specimens and none of the PC cell lines examined so far expressed ErbB4 [71]. More investigations into the role of ErbB4 in the prostate are required.

Interactions between the AR and the ErbB receptors are summarized in Table 1.

AR and Signal Transduction Pathways

Steroid receptors are phosphoproteins. Phosphorylation of human steroid hormone receptors is generally believed to positively or negatively modify their transcriptional activity rather than act as an on–off switch. Phosphorylation–dephosphorylation events are influenced by ligand binding, which may affect both ligand-dependent and ligand-independent receptor functions [74]. Such events may predominantly serve to fine-tune aspects of receptor regulation, perhaps by influencing the integration of signals from other pathways or by modulating the subcellular localization, trafficking, and degradation of receptor proteins [74–76]. Analogous to other members of the steroid receptor superfamily, AR is highly phosphorylated [77] and therefore sensitive to growth factor–initiated signalling pathways. Site-directed mutagenesis has confirmed three phosphorylation sites on the AR: two in the N-terminal domain (Ser81, Ser94) and one in the hinge region (Ser650) [78]. Identification of these AR phosphorylation sites allows the study of protein kinase(s), which may be involved in the phosphorylation of AR under conditions of androgen ablation.

MAPKs

MAPKs are mediators of cellular responses to many extracellular stimuli [79]. At least three subgroups have been identified: extracellular signal–regulated kinase (ERK), c-Jun N-terminal protein kinase (JNK), also known as stress-activated protein kinase (SAPK), and p38/HOG (reactivating protein kinase). These three MAPK subfamilies represent similar, yet distinct protein kinase cascades. Each consists of a module of three cytoplasmic kinases: a MAPK kinase kinase (MAPKKK), a MAPK kinase (MAPKK), and the MAPK itself [80–82].

The best understood MAPK signal transduction pathway in mammalian cells is that formed by the Raf/MEK/ERK. Proliferative signals provided by growth factors activate the MAPK pathway through their cognate receptors. Receptor tyrosine kinase activation and phosphorylation recruit adaptor proteins Grb2 and Sos. Sos is a GTP exchange factor, which activates the small G-protein Ras. This in turn activates the MEK kinase (MEKK) Raf, the MAPK/ERK (MEK) kinase, and subsequently MAPK/ERK [79,82]. ERK

Table 1. A Summary of the Major Interactions between the AR and ErbB-Mediated Signalling.

	Interaction with the AR	Effect on cell
EGF/EGFR	EGF augments DHT and AR signalling, and stimulates AR synthesis [48]	Enhanced proliferation and cell cycle progression
	DHT stimulates EGFR synthesis [47–51]	Enhanced mitogenic response to EGF
ErbB2	Transcriptional activation of PSA [65], enhancement of AR coactivator binding [66], activation of MAPK signalling downstream of IL-6 [68]	Ligand-independent activation of AR, which promotes survival and proliferation of PC in the androgen-depleted environment
HRG/ErbB3	HRG acts on AR positive cells only [71] possibly through ErbB-3 binding protein Ebp1, which binds to AR *in vitro* and *in vivo* [73]	Inhibition of ligand-mediated transcriptional activation of AR and reduced growth of AR-positive cells
MAPK/ERK2	Activation of AR transcription downstream of ErbB2 and IL-6 [66], phosphorylation of the AR at Ser514 [66] and SRC-1 [93]	Optimal ligand-dependent and ligand-independent activation of the AR
	AR activates MAPK in a complex with Src/Shc [90,91].	A rapid nongenomic effect that cannot be inhibited by antiandrogens [89]
MEKK1	Transcriptional activation of AR [94] possibly through its downstream target c-Jun, which interacts with the DNA binding domain/hinge region of AR [96,97]	Potentiation of the functional interaction between N-terminus and C-terminus of AR to enhance DNA binding and gene transcription
	Activates apoptotic pathways only in AR-expressing cells [94]	Apoptosis of PC cells
Akt	Phosphorylates AR at Ser210 and Ser790, which either activates transcription of AR-regulated genes in the absence of androgens [109,114]; alternatively, phosphorylation and inactivation of GSK-3β increased nuclear levels of β-catenin, which elevates AR activity [118]	Increased survival of PC cells in the absence of androgen
	Repression of AR transcriptional activity [119] by increasing AR degradation in a complex that includes Akt, Mdm2, and the AR [122]	Increased survival of PC when androgen favors PC apoptosis

activity is increased in many human tumors including PC. Both ERK expression and activation are postulated to mediate important roles in the initiation and progression of PC [83,84]. In the AI PC DU145 cell line, the constitutive phosphorylation of ERK2, a hallmark of MAPK activation has been reported and can be blocked by several EGFR inhibitors [85].

The MAPK pathway is also a target of steroid receptors (Figure 1). In addition to being transcription factors, both estrogen receptor (ER) and AR activate MAPK [86–90]. In a complex with Src/Shc, AR activates ERK-1 and ERK-2 in PC cells to stimulate their proliferation [90]. This rapid effect of the AR (within 2 minutes) [89,91] is distinct from its genotropic effects. It may be effected by less fully developed or broader receptor conformations initiated by brief association with the ligand [91]. In nonprostatic cells, the ligand binding domains of ER and AR were sufficient to stimulate an anti–apoptotic response through Src/Shc/ERK, a response which was eliminated by nuclear targeting of the receptor [91]. The activation of ERK by AR is specific for this class of kinases and cannot be inhibited by antiandrogens [89]. These findings imply that the current therapeutically available antiandrogens may not be totally effective in abrogating all androgenic activities in target cells.

Conversely, MAPK pathways were shown to activate the AR and thus mimic androgen signalling in the androgen-depleted environment [92]. MAPK was required for both ligand-dependent and ligand-independent activation of the AR downstream of ErbB2 and IL-6 [65,66,93]. ErbB2 was shown to induce androgen-dependent gene activation in the absence of ligand through the phosphorylation of AR in the N-terminal segment at amino acids 511–515 by MAPK (ERK2) [66]. The phosphorylation of steroid receptor coactivator-1 (SRC-1) by MAPK was also required for optimal ligand-independent activation of the AR by IL-6 [93].

In contrast to the Raf/MEK/ERK pathway, MEKKs comprise a family of related serine–threonine protein kinases that regulate MAPK signalling pathways, leading to c-Jun N terminal kinase and p38. These MAPK pathways are induced by cellular stress, inflammatory cytokines, and G-protein–coupled protein agonists. They have been implicated in apoptosis, oncogenic transformation, and inflammatory responses in various cell types. MEKK1 mediates Ras-dependent activation of JNK cascades by growth factor receptors through an association with Grb2 [82] (Figure 1). The link between MEKK1 and AR signalling is contradictory and puzzling. The MEKK1 pathway may contribute to the progression of PC to AI by modulating the activation of AR and its transcriptional response to ligands [94]. A constitutively active MEKK1 stimulated natural and artificial androgen response promoter templates in an AR-dependent manner and induced transcriptional activation of AR-regulated genes in the absence of androgens. Furthermore, a dominant negative mutant of MEKK1 impaired the activation of the AR by androgen [94]. The molecular basis of this crosstalk is unclear. MEKK1 possibly activates signalling cascades (such as c-Jun) that indirectly lead to posttranslational modifications of the AR and affect its function. Homodimerization of the AR and other steroid receptors is required for these transcription factors to bind DNA. This homodimerization is thought to result from an intramolecular or intermolecular interaction between the amino and carboxyl termini of the receptor [95]. The downstream target of MEKK1/JNK, c-Jun, interacts with the DNA binding domain/hinge region of AR to potentiate the functional interaction between N-terminal and C-terminal and enhance AR DNA binding and gene transcription [96,97].

MEKK1 induced apoptosis in diverse non–AR-expressing cells in response to stress or DNA-damaging agents [82]. Interestingly in PC, MEKK1 induces an apoptotic effect only when the AR signalling pathway is intact [94]. The requirement for AR in mediating the apoptotic effect of MEKK1 apparently contrasted with the role of AR signalling as a survival and/or proliferative factor in prostate secretory epithelial cells. However, AR signalling has been detrimental to PC growth and survival in certain settings (e.g., the effects of high concentrations of androgen on the AR-positive LNCaP cells or transfection of AR into AR-negative PC3 and DU145 cells) [98,99].

PI3K

Phosphorylation of phosphatidylinositol (PtdIn) at the D3 position by extracellular stimuli plays a major role in cell survival. Through activation of protein kinases such as the phosphoinositide-dependent protein kinases (PDK1 and PDK2) and Akt/protein kinase B (Akt/PKB), PtdIn 3,4,5-trisphosphate (PIP_3) inhibits apoptosis and influences other intracellular metabolic functions. Targets phosphorylated by Akt include the proapoptotic proteins (e.g., BAD), caspase 9, and the forkhead transcription factors as well as enzymes involved in intracellular metabolism such as glycogen synthase kinase-3 (GSK-3β) [100–102] (Figure 1*B*). PTEN (phosphatase and tensin homologue deleted on chromosome 10) is the negative downregulator of the PI3K pathway acting as a PtdIn phosphatase. Loss of PTEN function results in the constitutive activation of Akt. Tumor cells affected in this manner may escape dependence on extracellular survival factors and may become more resistant to agents that induce apoptosis [103]. The increased frequency of PTEN dysfunction and/or mutations in prostate and other cancers underscores the potential importance of signals provided by the PI3K/Akt pathway in the development and proliferation of cancer cells [104–106].

The PI3K/Akt signalling axis has been described recently as a dominant growth factor survival pathway in PC, which at best can only be partially compensated by a MAPK-sensitive step [107,108]. In the LNCaP PC model of AI, PI3K signalling was activated at the onset of androgen deprivation and progressively increased thereafter [107,109]. Androgen ablation increased PI3K/Akt activation, possibly by promoting the abnormal establishment of autocrine growth factor loops and/or enhancing the engagement of already-present growth factor signalling pathways [6,110] (Figure 2). PI3K

promoted survival of the acute effects of androgen deprivation and promoted proliferation of AI PC cells by diminishing the expression of the cyclin-dependent inhibitor $p27^{kip1}$ [111] and/or increasing its degradation [107]. ErbB2-induced AR transactivation is mediated only partially through the MAPK pathway and requires a functional PI3K/Akt pathway [109]. Inhibition of PI3K signalling by dominant negative Akt or the PI3K inhibitor LY294002 completely abolished AR activation by ErbB2, whereas PD98059, an MAPK inhibitor, inhibited it only partially [66,109]. Attenuation of the PI3K/Akt pathway either by pharmacological inhibitors or PTEN triggered a rapid and extensive apoptosis in LNCaP cells [107,108,112,113]. This response could not achieved by MAPK inhibition and was prevented by pretreatment with androgens or growth factors [107,108,112,114]. The mechanism by which androgens can overcome the apoptotic response to PI3K inhibition is yet unknown. It may result from PI3K activation and/or PTEN inhibition and it clearly involves the AR [108,114]. Some researchers demonstrated that in AR-positive cells, DHT rapidly activated PI3K and increased intracellular PIP_3 in an *in vitro* kinase assay (Figure 1*B*) [89,115], whereas others failed to observe any effect of DHT on PI3K or PTEN [114,116]. Alternatively, androgens may protect from PTEN-induced apoptosis either by activating a survival pathway that is independent of Akt, or by activating the same survival pathway at steps downstream of Akt [112,116]. PI3K/Akt has been shown to be a key regulator of AR expression in mouse vas deferens epithelial cells and in LNCaP cells. Inhibition of the PI3K pathway strongly decreased both basal and DHT-induced levels of AR [117]. Exposure of LNCaP to LY294002 pretreatment inhibited the increased expression of PSA mRNA normally induced by DHT [117,118].

Protein Kinase B/Akt

Transfection of a constitutively active Akt maintains PC survival in an androgen-poor or growth factor–poor environment [107,111]. In addition to its negative effects on the proapoptotic machinery, some studies have demonstrated that activated Akt specifically interacted with and phosphorylated both ERα (at Ser167) and AR (two sites): N-terminal Ser210 and C-terminal Ser790 [109–121]. AR phosphorylation by Akt was shown to promote PC survival, but whether it activated or inhibited AR gene transcription was unclear. Several groups have reported the activation of the AR transcription by Akt in the absence of androgen [109,114]. In support of the above findings, PTEN, the negative regulator of the PI3K pathway, antagonized AR signalling and androgen-induced cell proliferation and repressed the transcriptional activity of the AR as well as PSA production [114]. In contrast, Lin et al. [119] reported that Akt inhibited androgen/AR–induced apoptosis and blocked AR target genes such as *p21*, thereby promoting PC survival. The constitutively active forms of PI3K or Akt repressed the transactivation of AR and the interaction between AR and its coactivators. Additionally, the phosphorylation of AR by Akt appears to be critical for AR ubiquitylation and subsequent degradation by the proteasome [122]. This effect of Akt on AR was markedly reduced if two serine residues (Ser210 and Ser790) were replaced with alanine, preventing Akt induced AR phosphorylation, or in a Mdm2-null cell line compared with the wild-type cell line [122]. Other researchers have been unable to demonstrate a physical protein–protein interaction between Akt and AR or phosphorylation of AR by Akt *in vitro* [114,118]. They proposed that β-catenin acted as a mediator in the crosstalk between PI3K and androgen signalling [118]. PI3K/Akt induced the phosphorylation and inactivation of GSK-3β,

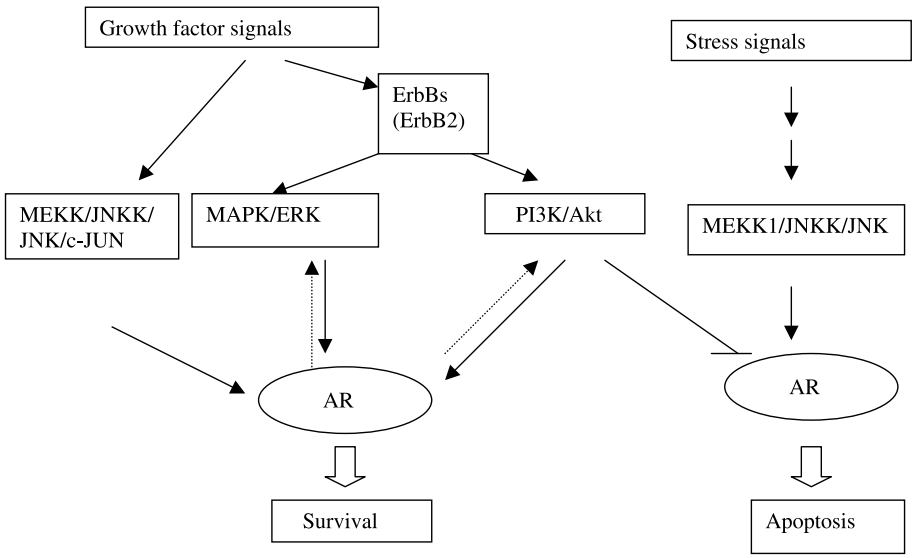


Figure 2. *A synopsis of the crosstalk between AR and the ErbB cascade. This figure provides a brief outline of the interplay of AR and ErbB cascade in promoting PC survival. Growth factor signals augment AR survival signals and enhance AR transactivation through the MAPK/ERK, MEKK/JNKK/c-Jun, and the PI3K/Akt pathways. This enhancement is shown in vitro to occur both in the presence and absence of ligand. Apoptotic responses initiated by stress signals acting through MEKK/JNKK/JNK and the AR can be subverted by activation of the PI3K/Akt pathway downstream of growth factors, thereby allowing PC cell survival.*

which in turn increased nuclear levels of β-catenin. Increased β-catenin elevates AR activity, stimulating PC growth and survival [118].

The interaction between the AR and the PI3K/Akt pathway is summarized in Figure 1*B* and in Table 1.

Conclusion

The development of AI PC is one of the major problems in its treatment. A shift in growth support from androgens to growth factors may enhance the ability of PC cells to survive the acute effects of androgen withdrawal and to proliferate in androgen-depleted conditions. The continued expression of AR and AR-regulated genes in AI PC suggests that alternative signalling pathways are utilized to activate the AR. Elements of the AR and ErbB pathways interact, cross over, and converge on targets downstream of each other's signalling cascades to promote AI PC cell survival (summarized in Table 1 and Figure 2). It is noteworthy to mention that studies revealing AI mechanisms of AR activation have been performed *in vitro*. Additionally and more importantly, the ligand-free environment that is achievable in the laboratory may not be reflected in the clinical situation because very low levels of androgen remain in patients' serum (castrate testosterone is defined as less than 50 ng/dl) despite surgical or pharmacologic castration.

Although manipulative *in vitro* data or descriptive *in vivo* data have not yet resolved the mechanism of AI growth, identification of these interactions and their physiological relevance enhances understanding of the possible means by which PC cells escape their requirement for androgen. This understanding suggests that inhibition of these signalling interactions could convert AI PC back to a hormone-sensitive state and offers possible additional strategies in the management of PC.

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