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SIGNAL TRANSDUCTION PATHWAYS MODULATE AR TRANSCRIPTIONAL ACTIVITY

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INTRODUCTION

The development and maintenance of the prostate is dependent on the proper functioning of the androgen receptor (AR) in response to the androgenic steroids testosterone (T) and dihydrotestosterone (DHT). In addition to androgens, paracrine and autocrine regulation of cell growth by peptide growth factors and cytokines contribute to prostate homeostasis. Unlike steroid hormones, growth factors regulate cellular responses through binding to membrane tyrosine kinase receptors. Growth factor or cytokine binding initiates a phosphorylation cascade that ultimately results in phosphorylation of transcription factors or transcription factor interacting proteins. In the prostate, AR is among the transcription factors whose activity is influenced by signal transduction cascades and disruption of the normal interaction between signal transduction and AR transactivation may contribute to the progression of prostate cancer.

Prostate cancer progression is often associated with alteration of growth factor expression or the receptors for growth factors in the tumor (Djakiew, 2000; Russell *et al.*, 1998). However, many of these growth factors may influence prostate cancer through mechanisms that do not involve AR. For example, basic fibroblast growth factor (bFGF or FGF-2) is elevated in the tumors and serum of some prostate cancer patients (Cronauer *et al.*, 1997). However, AR transcription is not stimulated by bFGF in prostate cancer cell lines (Culig *et al*., 1994). Both the AR positive LNCaP and AR negative DU145 prostate cancer cell lines proliferate in response to bFGF, suggesting that the effect of bFGF is independent of AR (Nakamoto *et al*., 1992). In addition to functioning as a mitogenic factor, bFGF is angiogenic and its production by prostate cancer cells may contribute to tumor vascularization (Russell *et al*., 1998). Similarly, some growth factor stimulated pathways have not yet been shown to effect AR transactivation. The nuclear kinase casein kinase 2 (CK2) is stimulated in prostate cancer cells by epidermal growth factor and FGF-7 through an undefined pathway (Guo *et al*., 1999) and CK2 activity is elevated in prostate cancer samples (Yenice *et al*., 1994). Although AR contains a putative CK2 phosphorylation site at serine 118, mutation of this site does not influence AR transcription or nuclear localization in response to androgen (Jenster *et al*., 1994). However, alteration in signaling by the epidermal growth factor family, transforming growth factor beta (TGFβ), interleukin 6 (IL6), and proline rich kinase 2 (Pyk2) have all been implicated in prostate cancer progression and in the modulation of AR transcriptional activity and will therefore be the focus of this review.

A number of reports have suggested that alteration in the phosphorylation status of steroid receptors, including AR, permits "ligand-independent" transcriptional activation in that no exogenous addition of agonistic ligands is necessary for receptor transactivation to be detected. Dephosphorylation of AR mediated by PKA stimulation (Blok *et al*., 1998; Nazareth and Weigel, 1996) or mitogen activated protein (MAP) kinase phosphorylation of ER (Bunone *et al*., 1996; Tremblay *et al*., 1999) have both been proposed to result in ligand independent activation. These studies were typically performed in charcoal stripped serum, which reduces the steroid hormone content of serum by 90-95%, but does not completely remove steroids (Kirkland *et al*., 1976). Therefore, such phosphorylative regulation may be more accurately considered to allow transcription in the presence of low concentrations of ligand. Physiologically, a particular steroid hormone is seldom completely absent, but may be present at dramatically reduced concentrations, as with prostate cancer patients who have undergone chemical or surgical castration as part of androgen ablation therapy. The ability of AR to become transcriptionally active at castration levels of androgens due to alteration in growth factor signaling may contribute to the development of hormone refractory prostate cancer.

The direct phosphorylation of steroid receptors is known to have both a positive and negative influence on transcriptional activity (Shao and Lazar, 1999). One mechanism through which phosphorylation can alter steroid receptor transcription is by influencing the interaction between the receptor and coactivators. For example, overexpression of the receptor tyrosine kinase Her2 enhances the interaction between AR and the AR coactivator ARA70 (Yeh *et al.*, 1999). Similarly, MAP kinase phosphorylation of ERβ (Tremblay *et al.*, 1999) or the orphan receptor SF-1 (Hammer *et al.*, 1999) stimulates coactivator recruitment by these receptors. In contrast, Akt phosphorylates AR at serines 210 and 790 (Lin *et al.*, 2001; Wen *et al.*, 2000) resulting in a decrease in AR transcriptional activity and resistance to androgen induced apoptosis (Lin *et al.*, 2001). A constitutively active allele of Akt reduces the interaction between AR and the AR coactivator ARA70 (Lin *et al.*, 2001). Similarly, inhibition of PI3 kinase, an upstream activator of Akt, increases the ability of AR coactivators to enhance AR transcription (Lin *et al.*, 2001). Therefore, the Akt phosphorylation of AR may reduce AR transcription by interfering with the ability of AR to recruit coactivators. In the case of the glucocorticoid receptor (GR), phosphorylation by MAP kinase or glycogen synthase kinase-3 (GSK-3) reduces ligand dependent transcription (Krstic *et al.*, 1997; Rogatsky *et al.*, 1998). It is possible that GR phosphorylation by these kinases also reduces the interaction between the receptor and its coregulators.

Steroid receptor transcriptional activity can also be modulated by alteration in the phosphorylation status of steroid receptor interacting proteins in addition to the receptor itself. PKA stimulation enhances transcription by the progesterone receptor (PR), although the phosphorylation status of PR is not altered (Beck *et al.*, 1992; Wagner *et al.*, 1998). Instead, PKA activation may enhance PR transcription by reducing the interaction between PR and the corepressors NCoR and SMRT (Wagner *et al.*, 1998). In contrast, phosphorylation of the SMRT by casein kinase 2 stabilizes its interaction with the thyroid hormone receptor (TR), resulting in reduced TR transcriptional activity in the presence of thyroid hormone (Zhou *et* al., 2001). Stimulation of MAP kinase by epidermal growth factor results in the phosphorylation of the SRC family of coactivators (SRC-1, TIF2, and SRC-3), increasing their ability to enhance nuclear receptor transactivation (Font de Mora and Brown, 2000; Lopez *et al.*, 2001; Rowan *et al.*, 2000; Rowan *et al.*, 2000). MAP kinase mediated phosphorylation of SRC-1 and SRC-3 stimulates recruitment of CBP/p300 (Font de Mora and Brown, 2000; Rowan *et al.*, 2000). MAP kinase therefore not only stimulates the direct phosphorylation of a number of steroid receptors, including ER (Bunone *et al.*, 1996; Tremblay *et al.*, 1999), it may also facilitate the recruitment of a coregulator complex through the additional phosphorylation of coactivators. AR transcriptional activation has been found to be enhanced by a member of the MAP kinase cascade, mitogen activated protein kinase kinase kinase 1 (MEKK1) (Abreu-Martin *et al.*, 1999). It is not yet known whether this kinase directly phosphorylates AR or activates other kinases capable of AR phosphorylation. However, because kinases downstream of MEKK1 can phosphorylate the SRC coactivators, it is possible that MEKK1 mediated enhancement of AR transcriptional activity occurs through the recruitment of a phospho-SRC/CBP coactivator complex in a manner similar to ER, as depicted in Figure 1. In addition to coregulators, some growth factors directly mediate the phosphorylation and activation of non-nuclear receptor transcription factors. For example, TGFβ stimulation results in the phosphorylation and nuclear translocation of the Smad transcription factors (Massague and Wotton, 2000) and IL-6 can modulate transcription through the STAT proteins (Akira, 1997; Smith *et al*., 2001). Members of the Smad and STAT transcription factors are able to interact with AR to influence AR transcriptional activity. The interaction between AR and these proteins may be an important mechanism contributing to the effect of these growth factors on the progression of prostate cancer.

EPIDERMAL GROWTH FACTOR FAMILY

The Her or ErbB family of receptor tyrosine kinases mediate diverse cellular processes, including proliferation and differentiation (Olayioye *et al*., 2000). Overexpression of the Her tyrosine kinases has been associated with multiple types of cancer, including those of the breast and prostate (Agus *et al*., 2000; Menard *et al*., 2000; Saloman *et al*., 1995; Signoretti *et al*., 2000). Four structurally related members of this family have been characterized: the prototypic epidermal growth factor receptor (EGFR, also called Her1 or ErbB1), Her2 (ErbB2/neu), Her3 (ErbB3), and Her4 (ErbB4). Each of these receptors carries an extracellular domain containing two cysteine rich regions separated by the peptide ligand binding domain, a transmembrane domain, and a large cytoplasmic domain that contains a tyrosine kinase and multiple tyrosine autophosphorylation sites (Riese and Stern, 1998; Saloman *et al*., 1995). The ligands of the Her receptors, the EGF-related peptide growth factors, are bivalent in nature and show differential receptor affinity (Riese and Stern, 1998; Tzahar *et al*., 1997). EGF, TNFα and amphiregulin bind specifically to EGFR while betacellulin, epiregulin, and heparin-binding EGF bind to both EGFR and Her4 (Riese and Stern, 1998). Neuregulin (NRG)-1 and NRG-2 are able to bind Her3 and Her4. However, NRG-3 and NRG-4 are only able to bind Her4 (Carraway *et al*., 1997; Olayioye *et al*., 2000; Riese *et al*., 1995). No ligand has been identified for Her2 and it is currently thought that Her2 functions primarily as a heterodimeric partner for the other Her family members. The N-terminal of the EGF-related growth factors binds to their primary receptor at high affinity. The C-terminal of the ligands has low affinity and broad specificity for the receptors which enables the formation of homo- and heterodimers between the Her receptors (Tzahar *et al*., 1997). The EGF-related growth factors exhibit a differential binding affinity and pH to their primary receptor, a characteristic that influences the signal strength and duration (Olayioye *et al*., 2000; Waterman *et al*., 1998). Differences in signal duration can result in profoundly different cellular effects. For example, in PC12 cells the sustained activation of the MAP kinase pathway results in differentiation while transient MAP kinase activation leads to proliferation (Marshall, 1995). The preferred heterodimerization partner of the Her receptors in Her2 (Graus-Porta *et al*., 1997; Tzahar *et al*., 1996) and the formation of Her2 heterodimers slows ligand dissociation from Her3, Her4, and to a lesser degree EGFR (Jones *et al*., 1999; Karunagaran *et al*., 1996; Tzahar *et al*., 1996). In addition, Her2 prevents lysosomal degradation of EGFR and allows receptor recycling to the cell membrane allowing more prolonged signaling (Lenferink *et al*., 1998). These properties may contribute to the oncogenicity of Her2 when it is overexpressed in tumor cells.

Ligand induced dimerization of the Her receptors stimulates the receptor tyrosine kinase resulting in phosphorylation of specific residues in the cytoplasmic C-terminal which in turn provides binding sites for mediators of cellular signal transduction pathways. The tyrosine kinase of Her3, however, is impaired and phosphorylation of the Her3 C-terminal occurs through transphosphorylation by heterodimeric partners (Guy *et al*., 1994; Pinkas-Kramarski *et al*., 1996). C-terminal receptor phosphorylation provides binding sites for signal transduction adapter proteins, such as Shc and Grb2, and kinases, including Src and the p85 subunit of PI3 kinase (Olayioye *et al*., 2000; Weiss and Schlessinger, 1998). Each of the receptors contain distinct but overlapping signal molecule binding sites that are subject to tyrosine phosphorylation (Olayioye *et al*., 2000). The specific target sites that are phosphorylated depend on the receptor dimerization partner. For example, EGF induced EGFR homodimers are able to recruit both Shc and Grb2. However, EGFR heterodimerized with Her4 shows a different C-terminal phosphorylation profile compared to EGF induced homodimers, and the EGFR-Her4 heterodimer is unable to recruit Grb2 (Olayioye *et al*., 1998). All Her family members are able to activate the mitogen-activated protein kinase (MAP kinase) pathway through Shc and/or Grb2. However, PI3 kinase is preferentially activated by Her3 containing dimers due to the multiplicity of p85 binding sites. EGFR is able to activate signaling through phospholipase C-γ (PLCγ) in addition to MAP kinase due to the ability of PLCγ to bind directly to the phosphorylated receptor (Olayioye *et al*., 2000). The capacity for heterodimerization therefore allows for signal diversification in response to ligand binding by permitting the stimulation of multiple signal transduction pathways.

The amplification and/or overexpression of the Her family members, particularly EGFR and Her2, have been observed clinically in a number of cancer types, including malignancies of the brain, urinary tract, and male and female reproductive systems (Saloman *et al*., 1995). Overexpression of Her2 is found in 10-40% of breast tumors and is associated with poor prognosis in patients with nodal metastases (Menard *et al*., 2000; Slamon *et al*., 1987). However, whether Her2 is overexpressed in prostate cancer is more controversial, possibly due to methodological differences in tissue preparation and the antibodies used. Although initial studies were unable to detect Her2 protein or mRNA in prostate cancer samples (Klotz *et al*., 1990; McCann *et al*., 1990), more recent studies have found Her2 protein to be elevated (Kuhn *et al*., 1993; Moriote *et al*., 1999; Sadasivan *et al*., 1993; Signoretti *et al*., 2000). It is possible that increased expression of Her 2 in prostate carcinomas is related to the development of androgen independence. Signoretti and colleagues (Signoretti *et al*., 2000) have observed an increase in the proportion of Her2 positive prostate tumors in patients receiving total androgen ablation (TAA) therapy prior to surgery compared to patients treated by surgery alone. A further increase in Her2 positive cases was seen in patients who had developed androgen insensitive (AI) metastases (Signoretti et al., 2000). This correlation with treatment and androgen sensitivity was independent of tumor stage and grade. These observations are consistent with previous studies that found elevated Her2 in tumor cells compared to normal adjacent epithelia, but that the level of Her2 expression or percentage of Her2 positive tumors did not correlate with tumor stage (Kuhn *et al*., 1993; Myers *et al*., 1994). The Her2 positive tumors from AI patients and those receiving TAA also expressed AR and PSA (Signoretti *et al*., 2000). This final observation provides clinical support for the previous suggestion that overexpression of Her2 may contribute to androgen independence in prostate cancer. Overexpression of Her2 in the normally androgen sensitive LNCaP cells allows androgen independent cell proliferation and decreases the tumor latency of xenografts in castrated mice (Craft *et al*., 1999; Yeh *et al*., 1999). Her2 overexpression was also found to induce androgen independent expression from the PSA promoter that could not be completely blocked by antiandrogens (Craft *et al*., 1999; Yeh *et al*., 1999). It has therefore been suggested that elevated Her2 may protect prostate cancer cells from TAA, at least in part, by allowing transcriptional activation of AR in the absence of ligand or under conditions of extremely low circulating androgen. These cells, possibly in combination with other genetic events, may give rise to androgen independent tumors (Signoretti *et al*., 2000).

Because of the ability of Her2 to heterodimerize with other Her family members, it is possible that it stimulates AR transcriptional activity through any of the signal transduction pathways linked to the Her receptors. However, to date Her2 stimulation of AR has been reported through MAP kinase and PI3 kinase. As shown in Figure 1, the MAP kinase and PI3 kinase pathways are not completely distinct and activation of one pathway may either stimulate (Campbell *et al*., 1998; Downward, 1998; Yart *et al*., 2001) or inhibit (Rommel *et al*., 1999; Zimmerman and Moelling, 1999) the other, possibly in a cell type or signal specific manner. Overexpression of Her2 in prostate cancer cells has been demonstrated to enhance AR transcription in the presence of DHT (Yeh *et al*., 1999), raising the possibility that elevated Her2 may permit the proliferation of malignant prostate cells prior to therapeutic intervention. Overexpression of Her2 increased proliferation of xenografts in intact mice, consistent with this hypothesis (Craft *et al*., 1999). Yeh, et al have shown that the effect of Her2 on AR transcriptional activity in the presence of androgen occurs at least partly though the MAP kinase pathway (Yeh *et al*., 1999). Mutation of a conserved MAP kinase consensus site at S514 in the AR N-terminal or treatment with the MKK-1 inhibitor PB98059 partially reduces Her2 stimulated enhancement of AR transcription in DU145 cells (Yeh *et al*., 1999). Her2 stimulation of AR transactivation enhances the interaction between AR and the AR coregulator ARA70 (Yeh *et al*., 1999), although it has not yet been determined whether this increase is due solely to MAP kinase phosphorylation of AR or if phosphorylation of ARA70 also contributes to this effect. Phosphorylation of ERβ and SF-1 by MAP kinase has been found to enhance the ability of those receptors to recruit coactivators (Hammer *et al*., 1999; Tremblay *et al*., 1999), suggesting this represents a general regulatory mechanism of nuclear receptors. MAP kinase also phosphorylates SRC family coactivators enhancing their ability to form a coactivator complex to facilitate transcription (Font de Mora and Brown, 2000; Lopez *et al*., 2001; Rowan *et al*., 2000; Rowan *et* al., 2000). Therefore it is possible that both mechanisms contribute to the enhanced interaction between AR and ARA70 found with Her2 overexpression. It is unclear if Her2 activates MAP kinase through homodimerization induced by a high concentration of Her2 at the cell surface or through heterodimerization with other Her receptors conferring sensitization to endogenous EGF-related ligands secreted by the cells (Connolly and Rose, 1990; Grasso *et al*., 1997). Clinically, elevated EGF and TNFα have been detected in prostate cancer cells compared to benign tissue, although alteration of EGFR in prostate cancer specimens is contradictory between different studies (Russell *et al*., 1998). EGF has been found to enhance AR transcription in the presence of androgen (Gupta, 1999; Reinkainen *et al*., 1996). However, EGF has been found to suppress AR transcription in LNCaP cells (Henttu and Vihko, 1993; Langeler *et al*., 1993). It therefore remains unclear to what extent the effect of Her2 on AR transcription in prostate cancer is mediated by Her2 alone or through heterodimerization with other EGF receptor family members.

In LNCaP cells, expression of constitutively active Her2 (caHer2) enhances AR transcription at the very low level of androgen found in charcoal stripped serum present in the media. Addition of androgen further increases AR transcription in the presence of caHer2 (Wen *et al*., 2000). The Her2 mediated AR transactivation is reduced by transfection of a dominant negative mutant of Akt, a PI3 kinase target (Wen *et al*., 2000). Akt is able to bind directly to AR and phosphorylates AR at S213 in the N-terminal and at S791 in the AR ligand binding domain (Wen *et al*., 2000). These observations suggest that Akt phosphorylation of AR can enhance AR transcription at a low level of androgen. Akt activity is increased in androgen independent xenograft tumors (Graff *et al*., 2000). Therefore, stimuli that increase Akt activity, including Her2, may contribute to the progression of prostate cancer. The activity of PI3 kinase, and thus Akt, is regulated by the phosphatase PTEN (Di Cristofano and Pandolfi, 2000; Simpson and Parsons, 2001; Stambolic *et al*., 1998). PTEN functions as a tumor suppressor and loss of PTEN function is observed in a number of human cancers, including prostate cancer (Cairns *et al*., 1997; Di Christofano *et al*., 1998; Li *et al*., 1997). LNCaP and PC-3 cells lack endogenous PTEN (Ramaswamy *et al*., 1999). Exogenous PTEN expression in these prostate cancer lines results in growth inhibition and repression of AR transcription (Davies *et al*., 1999; Li *et al*., 2001; Lin and Chang, manuscript in preparation), consistent with a stimulatory effect of Akt on AR transcription. However, PTEN is also able to inhibit AR transcription directly. PTEN interacts with androgen bound AR and decreases AR protein stability (Lin and Chang, manuscript in preparation). Although Her2 apparently enhances AR activity through PI3 kinase and Akt (Wen *et al*., 2000), the direct binding of PI3 kinase to Her2 has not been reported (Olayioye *et al*., 2000) but LNCaP cells have been characterized as expressing a high level of endogenous Her3, known to bind PI3 kinase (Grasso *et al*., 1997). The AR activation with Her2 overexpression in LNCaP cells was observed in the absence of exogenous Her3 ligands (Wen *et al*., 2000), possibly indicating that elevated Her2 sensitizes Her2-Her3 heterodimers to low levels of Her3 ligands present in cell culture serum or that Her2 homodimers have a previously unreported ability to activate PI3 kinase/Akt signaling. However, elevated expression of Her3 and NRG have been detected in some human prostate cancers (Leung *et al*., 1997) suggesting that autocrine stimulation of Her2-Her3 heterodimers can occur and allow AR transcription in patients treated with TAA therapy. Another possibility is that PI3 kinase is activated by Her2 through crosstalk between the MAP kinase and PI3 kinase pathways (Figure 1). PI3 kinase dependent activation of Ras (Yart *et al.*, 2001) and Ras dependent activation of PI3 kinase (Campbell *et al.*, 1998; Downward, 1998; Okano *et al.*, 2000) have both been reported in response to EGF stimulation. It is possible that Her2 enables communication between these two pathways in LNCaP cells.

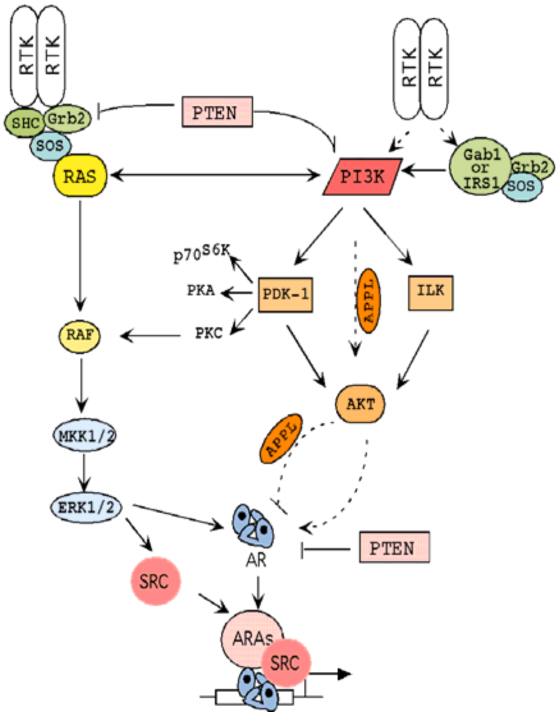

Figure 1. Crosstalk between the MAP kinase and PI3 kinase pathways activated by receptor tyrosine kinase (RTK) stimulation. As discussed in the text, both MAP kinase and PI3 kinase/Akt kinase can influence the phosphorylation of AR and AR coactivators (ARAs), including members of the SRC family. The inhibition of AR by Akt and APPL is discussed below in the IL-6 section. The MAP kinase pathway signals to JNK and p38 kinases, in addition to ERK 1/2 as shown here (Lewis et al., 1998). The relative importance of the different MAP kinases in AR or AR coregulator phosphorylation in prostate cancer cells has yet to be determined.

TGFβ And The Smad Proteins

TGFβ is the prototypic member of a family of polypeptide growth factors that also includes bone morphogenic protein and Mullerian inhibiting substance. In the normal prostate, TGFβ functions as a growth inhibitor of prostatic epithelia and possibly functions as a differentiation factor for prostatic stroma (Lee *et al*., 1999). TGFβ signaling occurs through the type 1 and type 2 TGFβ receptors (TβR1 and TβR2, respectively), both of which are serine/threonine kinases. TGFβ binds to TβR2 which then recruits and phosphorylates TβR1. Loss of either receptor results in insensitivity to TGFβ. As shown in Figure 2, the direct effectors of TGFβ signaling are the Smad proteins that function as phosphorylation regulated transcription factors. Phosphorylation of TβR1 by TβR2 activates the TβR1 kinase that in turn phosphorylates Smad2 and Smad3. Phosphorylation of Smad2 and Smad3 by TβR1 is required for their accumulation in the nucleus. Prior to nuclear entry, Smad2 and Smad3 associate with the co-Smad, Smad4. Co-Smads are not directly regulated by the TGFβ receptors but their association with Smads are necessary for transcriptional regulation of many Smad induced genes (Massague and Chen, 2000). In addition to activation of the Smad transcription factors, TGFβ also activates Ras and the MAP kinase signaling pathway (Kretzschmar *et al*., 1999; Mulder, 2000; Oft *et al*., 1996; Yan *et al*., 1994). The Erk2 MAP kinase can phosphorylate Smad2 and Smad3, preventing nuclear translocation and gene activation (Kretzschmar et al., 1999). In normal cells, this negative regulatory loop is thought to moderate Smad2/Smad3 activation (Kretzschmar *et al*., 1999).

In the normal prostate, TGFβ is predominantly produced by prostatic stromal cells (Nemeth *et al*., 1997; Wong *et al*., 2000) while epithelial cells demonstrate a greater expression of TβR1 and TβR2 (Kim *et al.,* 1996; Wong *et al*., 2000). TGFβ functions as a paracrine inhibitor of normal prostate epithelial cell proliferation (Lee *et al*., 1999; Martikainen *et al*., 1990; Sutkowski *et al*., 1992) and is thought to be a mediator of castration induced epithelial apoptosis (Kyprianou and Isaacs, 1989; Lee *et al*., 1999; Martikainen *et al*., 1990). *In vitro*, TGFβ inhibits the growth of primary human prostate epithelial cells (Sutkowski *et al*., 1992) and of the human prostate cell lines PC-3 and DU145 (Wilding, 1991). The prostate cancer cell line TSU-Pr1 has alternately been reported to proliferate (Lamm *et al*., 1998) or be growth inhibited (Guo and Kyprianou, 1998) in response to TGFβ treatment. These divergent results may be due to differences in cell culture conditions, which are known to influence the TGFβ responsiveness of some cell lines (Barrack, 1997). Several observations suggest that a decreased sensitivity to the inhibitory effect of TGFβ contributes to prostate cancer cell proliferation and cancer progression. Clinically, reduced expression of TβR1 and TβR2 in prostate tumors is correlated with increasing tumor grade (Kim *et al*., 1998; Kim *et al.,* 1996) and loss of TβR1

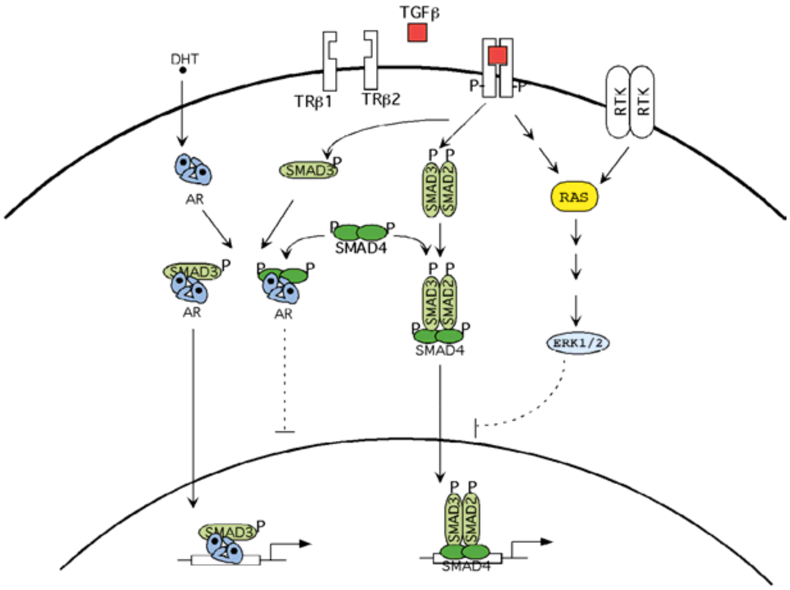

Figure 2. Mechanism of signal transduction by TGFβ. Binding of TGFβ to TRβ1 and TRβ2 stimulates phosphorylation of the Smad transcription factors and activation of the MAP kinase pathway. Phosphorylated Smad3 and Smad4 have been shown to interact with AR to regulate AR transcriptional activity. As discussed in the text, the disruption of this pathway in prostate cancer may contribute to aberrant AR activation.

is associated with serological recurrence (as measured by an increase in PSA after hormonal therapy) and decreased survival (Kim *et al*., 1998). The androgen sensitive prostate cancer cell line LNCaP does not respond to TGFβ due to a decrease or absence of TGFβ receptors (Guo and Kyprianou, 1998; Kim *et al*., 1996). Stable transfection of TβR2 in LNCaP cells (LNCaP-RII) restores the ability of TGFβ to inhibit cell growth and significantly reduces tumor formation in SCID mice (Guo and Kyprianou, 1998; Guo and Kyprianou, 1999). The suppression of tumorigenicity in LNCaP-RII cells is associated with an increase in cellular apoptosis (Guo and Kyprianou, 1999). Resistance to growth inhibition by TGFβ may also be due to over-stimulation of Ras. Transfection of oncogenic Ras mutants into epithelial cells, including DU145 (Park *et al*., 2000), has been shown to prevent growth inhibition by TGFβ (Houck *et al*., 1989; Longstreet *et al*., 1992; Schwartz *et al*., 1988). Stimulation of the MAP kinase pathway by constitutively active Ras or MEK1 mutants, or by EGF treatment, reduces Smad2 and Smad3 nuclear accumulation through phosphorylation of the linker region (Kretzschmar *et al*., 1999). These phosphorylation sites are distinct from those recognized by the TGFβ receptors (Kretzschmar *et al*., 1999).

In addition to a reduced sensitivity to TGFβ, prostate cancers may over-express TGFβ (Barrack, 1997; Eastham *et al*., 1995; Truong *et al*., 1993). Elevated serum TGFβ is associated with poor prognosis in prostate cancer patients (Shariat *et al*., 2001; Stravodimos *et a*l., 2000; Wikstrom *et al*., 1998). In addition, elevated TFGβ is correlated with increased primary tumor vascularization, tumor metastasis, serological recurrence (Shariat *et al*., 2001; Stravodimos *et al*., 2000; Wikstrom *et al*., 1998). TGFβ, in addition to acting as a growth inhibitor of prostate epithelial cells, can stimulate angiogenesis and cell motility (Yang and Moses, 1990). Sublines of the prostate cancer MLL cells that are growth-inhibited by TGFβ show a TGFβ-induced increase in motility, which may contribute to metastasis (Barrack, 1997). Therefore it is possible that a combination of reduced TGFβ sensitivity to growth inhibition and secretion of TGFβ resulting in vascularization or altered epithelia-stroma interaction contributes to a more aggressive prostate tumor phenotype.

The clinical observations that an elevation of serum TGFβ is associated with elevated serum PSA (Stravodimos *et al*., 2000), and that loss of TβR1 is a prognostic indicator for a reduced time to serological recurrence as determined by increased serum PSA (Kim *et al.,* 1998), suggest that interaction between the TGFβ pathway and AR transcriptional activity may exist. AR has been reported to interact with Smad3 (Hayes *et al*., 2001; Kang *et al*., 2001). Transfection of AR into the AR negative prostate cells DU145 and PC3 and co-treatment with DHT and TGFβ results in an increase in AR transcription. Co-transfection of Smad3 results in a further increase in AR transactivation (Kang *et al*., 2001). However, the ability of Smad3 to enhance AR transactivation is reversed by overexpression of the co-Smad, Smad4. Smad4 is also able to directly interact with AR and this interaction decreases the interaction between AR and Smad3 (Kang and Chang, unpublished observations) (Figure 2). In the normal prostate, Smad4 may function to moderate AR transcription and prostate epithelial cell proliferation. It has not yet been determined whether Smad4 levels are altered in prostate cancer but it is possible that a decrease in Smad4 enables Smad3 to enhance AR transcription and facilitate prostate cancer progression. This mechanism may be important in a subset of prostate cancer that retain the TGFβ receptors (Kim *et al.*, 1998; Kim *et* al., 1996). In these cases, autocrine production of TGFβ may phosphorylate Smad3, which enhances AR transcription in the presence of low levels of Smad4. Smad4 is not directly responsive to TGFβ (Massague and Chen, 2000), but it is possible that the AR response to TGFβ is modulated by Smad4 levels.

Alternatively, Hayes and colleagues observed that AR transcriptional activity was reduced by exogenous transfected Smad3 in cells treated with TGFβ and DHT (Hayes *et al.*, 2001). This is consistent with a model in which TGFβ plays a modulatory role for the proliferative effect of DHT-bound AR in normal prostate epithelial cells. In prostate cancer cells, the decreased sensitivity to TGFβ due to reduced TGFβ receptor levels results in decreased phosphorylated Smad3. In prostate cancer cells with this phenotype, the decreased phosphorylated Smad3 removes an inhibitory mechanism for AR transcription, allowing cellular proliferation and PSA expression even at the low levels of androgen present after androgen ablation therapy. It is therefore possible that two distinct mechanisms of TGFβ-responsiveness operate in prostate cancer cells depending on the expression level of different proteins in the TGFβ signaling pathway.

IL-6 AND THE JAK-STAT PATHWAY

The cytokine IL-6 functions in multiple physiological systems including immunology, bone metabolism, reproduction, and neural development (Keller *et al.*, 1996). IL-6 functions to regulate cellular differentiation, proliferation, or growth inhibition in a cell type specific manner (Keller *et al.*, 1996). In the context of neoplasia, IL-6 has been found to induce proliferation of cells derived from renal cell carcinomas, mammary carcinomas, Kaposi's sarcoma, lymphomas, and myelomas (Chiu et al., 1996; Klein *et al.*, 1989; Miki *et al.*, 1989; Miles *et al.*, 1990). In contrast, estrogen receptor positive breast cancer cell lines and cells derived from benign melanomas have been found to be growth inhibited by IL-6 (Chiu *et al.*, 1996; Lu and Kerbel, 1993). Elevated serum levels of IL-6 have been found in patients with metastatic prostate cancer (Adler *et al.,* 1999; Hoosein *et al.*, 1995; Twillie *et al.*, 1995), particularly those with hormone refractory disease (Drachenberg *et al.*, 1999; Hoosein *et al.*, 1995), suggesting that IL-6 may play a role in the progression of prostate cancer.

The receptor for IL-6 is composed of an IL-6 specific subunit (IL-6R) and a signal transducing subunit, gp130. IL-6 binding to IL-6R induces the formation of a multimeric complex containing two IL-6R and two gp130 molecules (Murakami *et al.*, 1993; Ward *et al.*, 1994), as shown in Figure 3. The formation of this complex results in autophosphorylation of the Janus tyrosine kinases (JAK1, JAK2, and TYK2) that in turn phosphorylate gp130 (Akira, 1997; Stahl *et al*., 1994). Phosphorylated gp130 is able to recruit the transcription factors STAT1 and STAT3 to the complex, resulting in their phosphorylation. The phosphorylated STAT proteins form homo- or heterodimers and translocate to the nucleus where they function as transcriptional regulators (Darnell *et al*., 1994; Wen *et al*., 1995). In addition to activation of the JAK-STAT pathway, IL-6 also induces the MAP kinase pathway through two distinct mechanisms. IL-6 mediated activated JAK is able to phosphorylate Shc, the upstream activator of Ras (Akira, 1997; Cutler *et al*., 1993). Alternatively, IL-6 has been shown to induce gp130 and Her2 association and phosphorylation resulting in MAP kinase activation in LNCaP cells (Chen *et al*., 1999; Qiu *et al*., 1998). STAT1 and STAT3 are phosphorylated at serine residues by members of the MAP kinase signal cascade (Chung *et al.,* 1997; Gollob *et al*., 1999; Lim and Cao, 2001; Turkson *et al*., 1999; Wen *et al*., 1995). The MAP kinase mediated phosphorylation of STAT3 influences the tyrosine phosphorylation status and contributes to maximal transcriptional activation (Lim and Cao, 2001; Turkson *et al*., 1999; Wen *et al*., 1995). The PI3 kinase pathway is also stimulated in LNCaP and PC-3 cells by IL-6 (Chung *et al*., 2000; Qiu *et al*., 1998). In these cell lines, IL-6 increases the interaction between the p85 subunit of PI3 kinase and gp130 and enhances p85 phosphorylation (Chung *et al*., 2000). Inhibition of IL-6 induced PI3 kinase activity by wortmannin causes apoptosis in LNCaP cells (Chung *et al*., 2000), suggesting that this pathway may contribute to prostate cancer cell survival.

Studies of the effect of IL-6 on prostate cancer cell growth and transcriptional activation of AR have yielded conflicting results. One possible reason for the divergent observations is the number of different pathways induced by IL-6 that can influence AR transcription, as shown in Figure 3. Several investigators have found PC-3 and DU145 cells are unaffected or show slight growth inhibition in response to IL-6 treatment (Degeorges *et al*., 1996; Spiotto and Chung, 2000). IL-6 has also been reported to result in growth inhibition and neuroendocrine differentiation of LNCaP cells (Qiu *et al*., 1998; Spiotto and Chung, 2000; Spiotto and Chung, 2000). In DU145 cells, the transcriptional activity of transfected AR is not influenced by IL-6 treatment. However, inhibition of PI3 kinase activity using LY294002 results in IL-6 enhancement of AR transcription in the presence of androgen (Lin and Chang, unpublished observations) (Figure 3B). AR can be phosphorylated by Akt, a downstream target of PI3 kinase (Lin *et al*., 2001; Wen *et al*., 2000). Lin *et al.* found that DHT induced transcription by AR was inhibited by constitutively active Akt in DU145 cells (Lin *et al*., 2001) (Figure 1). Similarly, inhibition of PI3 kinase enhanced AR transcription (Lin *et al*., 2001).

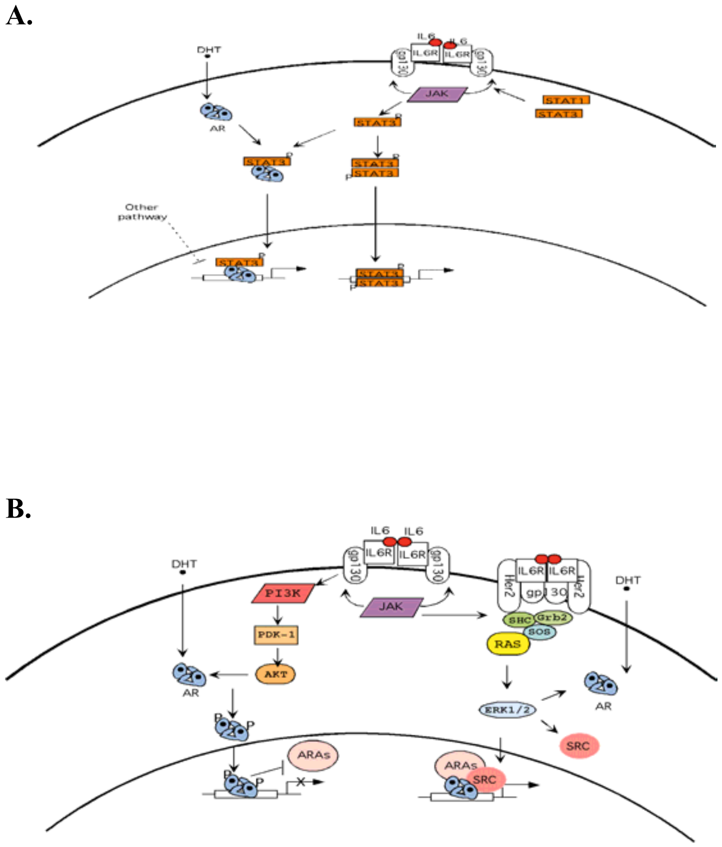

Figure 3. Signal transduction by IL-6. A. The direct effectors of the IL-6 signal are the STAT transcription factors. The STAT factors can function as homo- or heterodimers, although only the STAT3 homodimer is shown here for simplicity. As discussed in the text, STAT3 interacts with AR to enhance AR transcriptional activity. Stimulation of other pathways may inhibit AR transcription. B. One pathway activated by IL-6 that inhibits AR transcriptional activity is the PI3 kinase pathway. It has not yet been determined whether inhibition of AR by Akt alters the AR interaction with STAT3. IL-6 may also activate the MAP kinase pathway either through the JAK kinases or through dimerization between the IL-6 receptor and Her2 (here shown as RTK). As discussed in the text, AR transcriptional activity in some model systems is enhanced by IL-6 in a MAP kinase dependent manner.

This effect is due at least in part to a decrease in the interaction between AR and AR coactivators, including ARA70 (Lin *et al.*, 2001). The effect of Akt may also be due to association between AR and the Akt bridging protein APPL (Mitsuuchi *et al.*, 1999) (Figure 1). APPL binds to AR to inhibit DHT induced transcription through a mechanism dependent on Akt function (Lin and Chang, unpublished observations). Akt mediated inhibition of AR is in contrast to the observation showing Her2 can stimulate AR transcription through PI3 kinase and Akt (Wen *et al.*, 2000). One explanation for these divergent observations is that the relative strength of the PI3 kinase induction is different in the two experimental designs and contributes to a different AR transcriptional endpoint. This is similar to the observed differences in cellular response depending on the relative strength and duration of MAP kinase stimulation (Marshall, 1995). Extracellular signals that result in a prolonged or strong activation of the PI3 kinase pathway would therefore be expected to inhibit AR transcriptional activity. IL-6 treatment may represent one such condition. Expression of a constitutively active Her2 would be expected to result in a strong stimulation of MAP kinase but possibly a weaker activation of PI3 kinase than would be provided by transfection of a constitutively active Akt. While the effect of Akt on AR phosphorylation is the same in the two experimental systems, the strength of the PI3 kinase signal, or the balance between MAP kinase and PI3 kinase stimulation, may influence AR interacting proteins that contribute to the AR transcriptional response.

In contrast to the negative effect of IL-6 on AR transcription and LNCaP cell growth, other investigators have observed stimulation of cell growth in DU145, PC-3, and LNCaP cells with IL-6 treatment (Lou *et al.*, 2000; Okamoto *et al.*, 1997). AR transcriptional activity can be enhanced by IL-6 in LNCaP cells and in DU145 cells transfected with AR (Chen *et al.*, 2000; Hobisch *et al.*, 1998; Matsuda *et al.*, 2001). The observed increase in AR transactivation with IL-6 treatment is blocked by the MAP kinase inhibitor PD98059 suggesting that the IL-6-MAP kinase pathway is required for enhancement of AR transcriptional activity (Chen *et al.*, 2000; Hobisch *et al.*, 1998) (Figure 3B). AR transcription may also be enhanced through interaction with phosphorylated STAT3, as shown in Figure 3A (Chen *et* al., 2000; Matsuda *et al.*, 2001). STAT3 has been found to coimmunoprecipitate with AR in cells treated with IL-6 or overexpressing JAK1 (Chen *et al.*, 2000; Matsuda *et al.*, 2001). Transfection of a dominant negative mutant of STAT3 into LNCaP cells abrogates IL-6 enhancement of AR activation (Chen *et al.*, 2000; Matsuda *et al.*, 2001).

The reasons for these divergent results are not completely understood. It has been suggested that altered levels of intracellular kinases exist in the prostate cancer cell lines used by different laboratories (Hobisch *et al*., 2000). However, in the studies that found IL-6 induced prostate cell growth inhibition, with one exception (Qiu *et al*., 1998) IL-6 was added to media containing 3-10% fetal calf serum (Chung *et al*., 1999; Degeorges *et al*., 1996; Spiotto and Chung, 2000). In the studies in which IL-6 was found to stimulate prostate cancer cell growth or enhance AR transcriptional activity, IL-6 and androgen were added to serum free or defined media (Chen *et al*., 2000; Hobisch *et al.,* 1998; Lou *et al*., 2000; Matsuda *et al*., 2001; Okamoto *et al*., 1997). This suggests that a factor may be present in fetal calf serum that reverses the growth stimulatory effect of IL-6 on prostate cancer cells. Because the proliferative effect of IL-6 is mediated via the MAP kinase pathway and the inhibitory effect by PI3 kinase, it is possible that fetal calf serum factors contribute to a stronger induction of PI3 kinase with IL-6 treatment. The identification of such a putative factor could be of potential therapeutic benefit in the treatment of prostate cancer.

In light of the conflicting cell culture models, the mechanism resulting in the association between elevated serum IL-6 and hormone refractory metastatic prostate cancer remains unclear. It is possible that in advanced prostate cancer, secondary mutational events result in loss of growth inhibition by IL-6 (Chung *et al*., 1999; Spiotto and Chung, 2000). Alternatively, elevated exposure of the tumor to IL-6 may result in constitutive activation of STAT3 and other IL-6 mediated signaling pathways. IL-6 can function as an anti-apoptotic factor in hepatocytes (Kovalovich *et al*., 2001) but it is not known if it serves a similar function in prostate cells. Constitutive STAT3 activation could contribute to cancer progression by enhancing AR transcriptional activity in conditions of low circulating androgens as found in patients treated with androgen ablation therapy (Chen *et al*., 2000; Hobisch *et al*., 1998). Constitutive activation of STAT3 has been observed in a number of tumor types, including multiple myelomas, squamous cell carcinomas, and mammary carcinomas (Turkson and Jove, 2000). Inhibition of STAT3 in multiple myeloma derived cells and in a head and neck squamous cell carcinoma xenograft enhanced apoptosis, suggesting that STAT3 may be an important mediator of cell survival in some cancer types (Grandis *et al*., 2000; Turkson and Jove, 2000) and raising the possibility that the AR-STAT3 interaction could promote survival of prostate cancer cells.

PYK2 AND ARA55

Proline rich kinase 2 (PYK2/CAKβ/RAFTK/FAK2) is a cytoplasmic protein tyrosine kinase that is highly homologous to focal adhesion kinase (FAK) (Avraham *et al*., 1995; Sasaki *et al*., 1995). FAK is associated with cellular focal adhesions and becomes autophosphorylated upon stimulation of the integrin cell adhesion receptors, resulting in regulation of cytoskeletal architecture and cell motility (Alpin *et al*., 1998). In contrast, PYK2 is predominantly perinuclear and a proportion translocates to focal adhesions upon integrin stimulation by extracellular matrix proteins or activation of G-protein receptors (Litvak *et al*., 2000; Matsuya *et al*., 1998). Phosphorylation and activation of PYK2 occurs in response to integrin stimulation, however this response may be cell type specific (Astier *et al*., 1997; Sasaki *et al*., 1995; Schlaepfer and Hunter, 1998). In B cells, β1 integrin stimulation increases tyrosine phosphorylation of PYK2 (Astier *et al*., 1997). However, fibronectin stimulation of rat fibroblasts does not alter the phosphorylation status of PYK2 (Sasaki *et al*., 1995). It is possible that differential integrin expression contributes to the cell type specific phosphorylation of PYK2. PYK2 phosphorylation can also be induced by an increase in intracellular Ca^{+2}, PI3 kinase, or treatment growth factors and cytokines, including IL-6 and TNFα (Avraham *et al*., 2000; Koziak *et al*., 2001; Liu *et al*., 1997). The mechanism through which PYK2 is phosphorylated by these stimuli has not yet been determined. The phosphorylation and activation of PYK2 allows the recruitment of the adapter proteins Grb2 and Shc, resulting in activation of the MAP kinase pathway (Dikic *et al*., 1996). In addition, a direct phosphorylation target of PYK2 is ARA55/Hic-5 (Fujimoto *et al*., 1999; Ishino *et al.,* 2000; Shibanuma *et al*., 1994), a protein known to function as an AR coregulator in prostate cancer cells (Fujimoto *et al*., 1999). Although ARA55 has been shown to interact with FAK (Fujita *et al*., 1998; Osada *et al.,* 2001), ARA55 is not a phosphorylation target of FAK (Fujita *et al*., 1998). ARA55 is located in the nucleus and in focal adhesions (Matsuya *et al*., 1998; Thomas *et al.,* 1999; Yang *et al*., 2000), and in some cell types may translocate to focal adhesions with PYK2 (Osada *et al*., 2001).

The phosphorylation of ARA55 by PYK2 may contribute to the regulation of prostate epithelial cell growth and AR transcriptional activity. The normal prostate expresses ARA55 mRNA (Fujimoto *et al*., 1999) and phosphorylated PYK2 is found in normal prostatic epithelial cells (Stanzione *et al*., 2001). ARA55 was initially characterized as a coactivator of AR (Fujimoto *et al*., 1999). However, PYK2 mediated phosphorylation of ARA55 blocks the interaction between AR and ARA55, reducing AR transcription in prostate cancer cell lines (Wang and Chang, manuscript in preparation). Clinically, a progressive reduction in PYK2 expression is observed with increasing tumor grade in prostate cancer samples (Stanzione *et al*., 2001). Significantly, 19/19 prostate tumors with Gleason scores of 7 to 9 showed a complete loss of PYK2 immunoreactivity (Stanzione *et al.,* 2001). A decrease in activated PYK2 would be expected to result in a decrease in ARA55 phosphorylation, allowing ARA55 to enhance AR

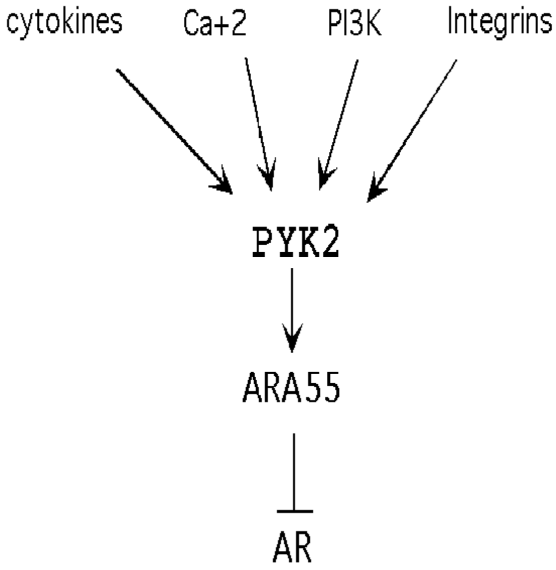

Figure 4. Phosphorylation of the coactivator ARA55 by PYK2 decreases the interaction between ARA55 and AR, leading to a decrease in AR transcription.

transcription and contribute to prostate cell proliferation. However, PYK2 and ARA55 may also regulate prostate cancer cell growth independent of the androgen receptor. Overexpression of PYK2 induces apoptosis in several epithelial, fibroblastic, and multiple myeloma cells lines in the absence of exogenous androgen (Chauhan *et al.*, 1999; Ueda *et al.*, 2000; Xiong and Parsons, 1997). ARA55 overexpression in the AR negative cell line NIH3T3 reduces cell spreading on fibronectin (Nishiya *et al.*, 2001) and an increase in ARA55 expression is associated with senescence in fibroblasts and TGFβ induced senescence in osteoblastic cells (Shibanuma *et al.*, 1994; Shibanuma *et al.*, 1997). These observations suggest that PYK2 and ARA55 may regulate cell motility and cell death through multiple mechanisms.

GSK-3, β-Catenin And AR

Glycogen synthase kinase 3 (GSK-3) was initially characterized as a regulator of glucose metabolism. Insulin activates PI3 kinase and Akt, which in turn phosphorylates and inactivates GSK-3. Inhibition of GSK-3 allows activation of glycogen synthase and thus the metabolism of glucose (Sakanaka *et al.*, 2000). GSK-3 is also involved in the regulation of β-catenin in response the Wnt family of growth and differentiation factors (Gumbiner, 1995; Willert and Nusse, 1998) and potentially the phosphorylative regulation of AR (Wang and Chang, unpublished observations). When GSK-3 is active, it is able to interact with AR and reduce AR transcription of PSA gene in LNCaP cells (Figure 5A). The inhibition of AR transcriptional activation requires the function of the GSK-3 kinase domain. Mutation of the kinase domain of GSK-3 or inhibition of GSK-3 activity by LiCl relieves the inhibition of AR activity (Wang and Chang, unpublished observations). Active GSK-3 also phosphorylates β-catenin resulting in the association of β-catenin with adherens junctions (Willert and Nusse, 1998) (Figure 5A). β-catenin functions both as a cytoskeletal protein (Gumbiner, 1995; Willert and Nusse, 1998), and as a transcriptional coregulator to AR (Trucia *et al.,* 2000) and the T cell factor (TCF) family of transcritpion factors (Huber *et al*., 1996). β-catenin forms a bridge linking the actin cytoskeleton to adherens junctions formed by cadherin and α-catenin (Gumbiner, 1995; Willert and Nusse, 1998). Wnt binding to its membrane receptor results in the phosphorylation and inactivation of GSK-3. In the absence of GSK-3 kinase activity, β-catenin is not phosphorylated and accumulates in the cytoplasm due to increased protein stability (Willert and Nusse, 1998) (Figure 5B). The increase in available β-catenin enables it to complex with TCF and AR in the nucleus and enhance transcriptional activation (Brannon *et al*., 1997; Trucia *et al*., 2000). The activities of β-catenin in cell adhesion and as a coactivator are separable. β-catenin mutants that are unable to interact with α-catenin are able to transduce the Wnt signal (Orsulic and Peifer, 1996).

Mutations of β-catenin have been detected in prostate cancer and occur focally within tumors, suggesting that β-catenin mutation is a late event in cancer progression (Voeller *et al*., 1998). One prostate cancer associated β-catenin mutant, β-catenin S33F, enhances AR transcription in response to the normally weak adrenal androgens androstenedione and DHEA (Trucia *et al*., 2000). This and similar mutations could contribute to prostate cancer progression by enabling cancer cells to survive androgen ablation therapy in conditions of low testicular androgens. In addition to mutation, the coactivator function of β-catenin may be increased in prostate cancer due to an increase in Wnt signaling. Wnt-5A is not expressed in the normal prostate but is found in prostate carcinoma samples (Iozzo *et al*., 1995). It is possible that aberrant expression of Wnt-5A by prostate cancer cells leads to autocrine inhibition of GSK-3 and increases the available β-catenin leading to enhancement of AR transcription. Elevated Wnt-5A may also enhance AR transcription by preventing GSK-3 from phosphorylating and inhibiting AR. However, inhibition of GSK-3 can occur through other pathways than Wnt, including the Akt and hepatocyte growth factor (HGF)

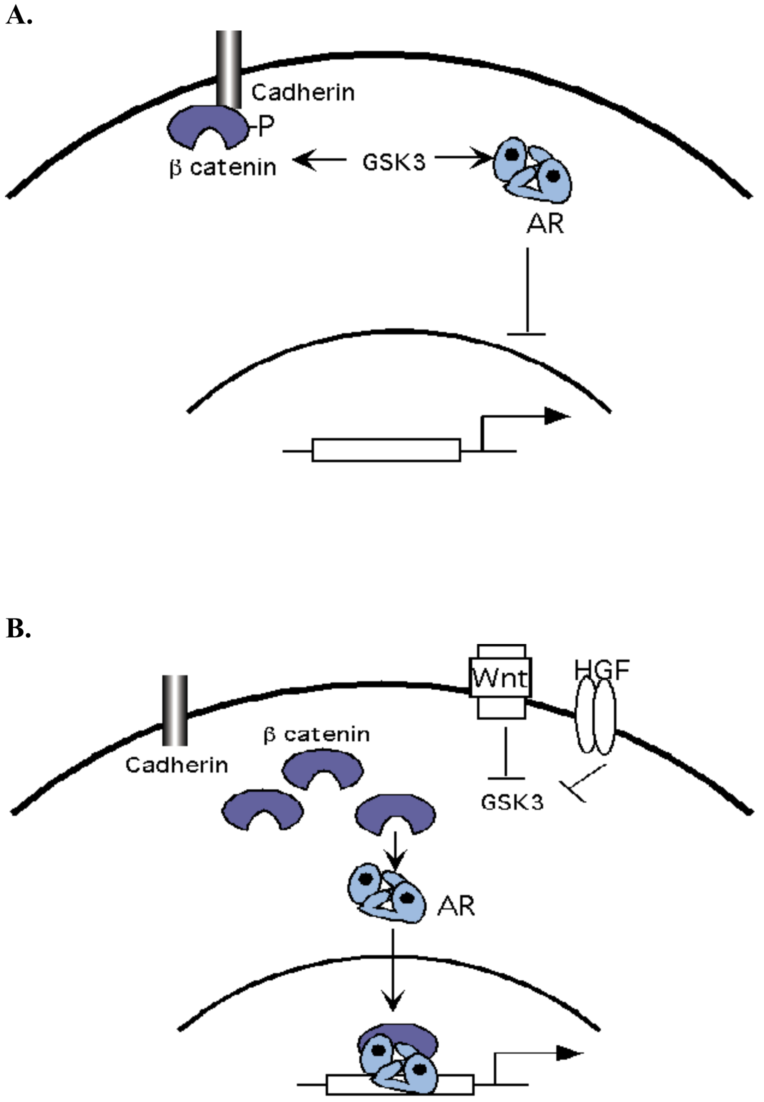

Figure 5. GSK-3 and AR transcription. ***A.*** *GSK-3 phosphorylates the coactivator β-catenin, limiting its availability and mediating its association with cadherin and adherens junctions. GSK-3 also interacts with AR to inhibit AR transcription.* ***B.*** *Phosphorylation of GSK-3 represses its ability to phosphorylate β-catenin and allows AR transcription. Unphosphorylated β-catenin is available to function as a coactivator of TEF (not shown) and AR.*

(Delcommenne *et al*., 1998; Hemmings, 1997; Morin, 1999; Papkoff and Aikawa, 1998). Because AR is a direct target for Akt (Lin *et al*., 2001), it is possible that the suppressive effect of Akt phosphorylation of AR is dominant over stimulation of β-catenin. The cytokine HGF binds to the proto-oncogene c-met to stimulate prostate cancer cell proliferation and elevated c-met expression is observed in prostate carcinomas (Djakiew, 2000; Humphrey *et al.,* 1995; Pisters *et al*., 1995). Although an effect of HGF on AR transcriptional activation has not yet been demonstrated, it is possible that HGF contributes to prostate cancer metastases partly through increasing the availability of β-catenin to AR.

CONCLUDING REMARKS

The involvement of multiple growth factor mechanisms in the progression of prostate cancer makes them an attractive pharmacological target to supplement androgen ablation therapy. Recently, antibodies directed against Her2 have been shown to be effective in the treatment of breast cancer in combination with other chemotherapeutic agents (Pegram *et al*., 1999; Pegram and Slamon, 1999). Because Her2 expression is elevated in hormone refractory prostate cancer (Kuhn *et al*., 1993; Moriote *et al.,* 1999; Sadasivan *et al*., 1993; Signoretti *et al*., 2000), Her2 antibody treatment may also be therapeutically beneficial. The EGF receptor has also been targeted either by antibodies directed towards extracellular domains or through chemical inhibitors of receptor tyrosine kinase activity (Mendelson and Baselga, 2000). Although EGF receptor directed therapies have been primarily used for head and neck carcinomas, it is possible that these therapies may also be useful in the subset of EGF receptor positive prostate cancers (Mendelson and Baselga, 2000; Russell *et al*., 1998). However, different strategies may be needed to address the prostate cancers that progress due to inactivation of signal transduction pathways that normally function to inhibit epithelial proliferation, as is the case with PYK2 and possibly TGFβ (Kang *et al*., 2001; Kim *et al.,* 1998; Stanzione *et al*., 2001). Reconstitution or reinduction of these pathways may prove difficult without the use of gene therapy. It is possible that compounds can be isolated that pharmacologically mimic the effect of inhibitory signaling pathways. Because many growth factors implicated in prostate cancer are able to promote tumorigenic processes such as angiogenesis in addition to influencing AR transcriptional activation, growth factor directed therapy combined with androgen ablation potentially enables more than one aspect of tumor growth to be simultaneously targeted.

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