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Interaction of IGF Signaling and the Androgen Receptor in Prostate Cancer Progression

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Abstract The insulin-like growth factor type I receptor (IGF-IR) has been suggested to play an important role in prostate cancer progression and possibly in the progression to androgen-independent (AI) disease. The term AI may not be entirely correct, in that recent data suggest that expression of androgen receptor (AR) and androgen-regulated genes is the primary association with prostate cancer progression after hormone ablation. Therefore, signaling through other growth factors has been thought to play a role in AR-mediated prostate cancer progression to AI disease in the absence of androgen ligand. However, existing data on how IGF-IR signaling interacts with AR activation in prostate cancer are conflicting. In this Prospect article, we review some of the published data on the mechanisms of IGF-IR/AR interaction and present new evidence that IGF-IR signaling may modulate AR compartmentation and thus alter AR activity in prostate cancer cells. Inhibition of IGF-IR signaling can result in cytoplasmic AR retention and a significant change in androgen-regulated gene expression. Translocation of AR from the cytoplasm to the nucleus may be associated with IGF-induced dephosphorylation. Since fully humanized antibodies targeting the IGF-IR are now in clinical trials, the current review is intended to reveal the mechanisms of potential therapeutic effects of these antibodies on AI prostate cancers. J. Cell. Biochem. 99: 392–401, 2006.

Key words: insulin-like growth factor type I receptor (IGF-IR); androgen receptor (AR); androgen-independent (AI) prostate cancer; AR co-regulators

In the presence or possibly absence of androgen ligand, the androgen receptor (AR) translocates from the cytosol to the nucleus and functions as a transcriptional factor, which may be necessary or even crucial for the progression of prostate cancer [Scher and Sawyers, 2005]. Classically, in the absence of androgen ligand, AR remains in the cytosol and is not active. Thus, it is of particular interest that malignant prostate cancer progression occurs frequently in men who have been surgically or chemically castrated. The progression of prostate cancer after castration has been termed androgen-independent (AI) prostate cancer. More interestingly, animal studies showed that when the expression of AR was disrupted, prostate cancer ceased to progress [Taplin and Balk, 2004]. All these together posed a conundrum if the AR, rather than the androgen ligand, is a driving force in prostate cancer progression. If so, it would suggest that the AR is functioning in a non-classical manner in the absence of steroid ligand. Although non-genomic mechanisms for AR function have been proposed through an interaction with SRC–Raf–Ras–Map kinase in the cytosol rather than the nucleus, this "traditional" non-genomic mechanism also requires the presence of androgen ligand and would not explain progression of disease in a ligand-independent manner [Kousteni et al., 2001; Pandini et al., 2005].

The concept of AR functioning in AI progression was first proposed by Mohler and colleages

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[Gregory et al., 1998; Mohler et al., 2004]. In relevant studies, tumor biopsies were taken from prostate cancer patients who had been androgen ablated and presented with progression of the cancer [Gregory et al., 1998; Mohler et al., 2004] In these samples, the AR primarily resided in a nuclear location, contrary to what had been expected in a castrated environment. This may in part due to residual levels of androgen in the prostate tissue. When tissue levels of androgen, testosterone, and dihydrotestosterone (DHT), were measured, although lower than in non-castrated men, they were still detected in the nanomolar range in many of the castrated men [Titus et al., 2005a]. This subtle level of tissue androgen may account for the nuclear localization of the AR and signal to activate an AR transcriptional program. The failure of castration to completely abolish intraprostatic androgens has also been evidenced in the study where normal men were placed on a GnRH antagonist for 4 weeks and in whom serum levels of testosterone (T) and DHT were clearly in the castrate range (Page and Bremner, personal communication). The source of the androgens in these castrate men has yet to be determined; however, the most likely source would be conversion from adrenal androgens. The prostate has active 5α-reductase systems for both isoforms I and II ensuring that circulating T can be readily converted to DHT in the prostate [Titus et al., 2005b]. In addition, recent microarray data has shown that the prostate contains mRNAs for the enzymes necessary for the conversion of cholesterol precursor into DHT; however this conversion has not been demonstrated in the prostate. Anti-androgen drugs, such as biclutamide, have not been shown to alter the translocation of the AR to the nucleus in prostate specimens from men treated with combined androgen blockade [Mohler et al., 2004]. Therefore, it is not clear whether it is the low levels of androgens driving prostate cancer progression in castrated men. Until a total androgen ablation mechanism in men is developed, the importance of residual androgens in tumor progression cannot be determined.

Castration studies on prostate cancer xenograft and transgenic mouse models support the speculation that residual androgen production following castration is only the partial driving force for tumor progression. Since mice do not produce adrenal androgens to any significant degree, castration in a mouse results in "complete androgen ablation" [Van Weerden et al., 1992]. In these models, tumors progress from androgen-dependent (AD) to AI following castration in spite of the fact that prostate specific androgen levels decrease to nearly undetectable levels, suggesting that residual androgens are unlikely to play a part in post-castration tumor progression [Thalmann et al., 2000; Corey et al., 2003]. We and others have shown that, in these models, the majority of tumor nuclei still contain AR after castration although some of the AR moves from the nucleus to the cytoplasm (Fig. 1). Furthermore, androgen-regulated genes continue to be expressed in "AI" disease [Corey et al., 2003]. Together, these data suggest that other mechanisms beyond the traditional ligand-receptor interaction of AR signaling are responsible for AD to AI prostate cancer progression.

Alterations in co-regulators of the AR, which may enhance ligand-independent AR translocation to the nucleus and binding to DNA, have been suggested as one of the mechanisms for ligand-independent AR signaling [Gregory et al., 1998; Fujimoto et al., 1999; Kang et al., 1999; Sadar, 1999; Sadar and Gleave, 2000; Mohler et al., 2004]. It has been suggested that some peptide growth factors can act directly at the androgen-binding domain of the AR or indirectly through modifying the phosphoryla-

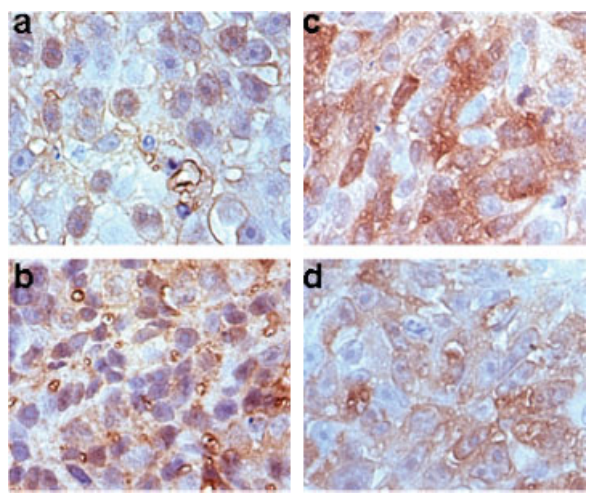

Fig. 1. IGF-IR signaling-induced translocation of AR into the nucleus in xenograft human prostate tumors. **a**: AR compartmentalization in the nucleus in intact animals. **b**: Blocking IGF-IR signaling with antibody A12 caused cytoplasmic retention of AR in intact animals. **c**: AR in the nucleus in castrated animals. **d**: A12 induced marked AR retention in the plasma in castrated animals.

tion status of the AR or its co-regulators to initiate AR signaling [Culig et al., 1994, 1995; Sadar, 1999; Sadar and Gleave, 2000; Lin et al., 2001]. In this "Prospectus" we examined the interactions between AR function and the activation of type 1 insulin-like growth factor receptor (IGF-IR). Among peptide growth factor-induced cell signaling, IGF activated IGF-IR signaling is a potential driving force for the growth of AI prostate cancer for several reasons as listed in Table I. In the following sections, we will examine the evidence for each of these components of potential interaction between the IGF-IR and AR.

IGF-IR IS NECESSARY FOR CELL TRANSFORMATION

Fibroblasts from IGF-IR knock out mice R^- do not transform spontaneously when compared to R^{wt} control cells. When the IGF-IR is re-expressed in these fibroblasts, transformation takes place. In SV40T immortalized prostate epithelial cells, inhibition of IGF-IR expression with an antisense construct significantly decreases colony formation in soft agar, a marker of transformation. In studies when growth hormone and IGF deficient LID mice were crossed with the transgenic prostate cancer (TRAMP) mouse, tumor development was significantly delayed Majeed et al., 2005). All these studies suggest an essential role of IGF-IR in cellular transformation. Hongo et al. [1998] have identified specific tyrosine residues on the β-subunit of the IGF-IR that are crucial for the transforming actions of the IGF-IR [O'connor et al., 1997; Liu et al., 1998].

TABLE I. Evidence for Interaction of the IGF-IR and AR in Prostate Cancer

1.	The IGF-IR is necessary for cell transformation
2.	Clinical data, although somewhat controversial suggests that higher levels of IGF-I in the serum of men predicts men at risk for developing clinical prostate cancer
3.	Androgens increase IGF-IR levels in prostate epithelial cells
4.	IGF-IR signaling alters AR phosphorylation
5.	IGF-IR signaling alters the AR transcriptional profile
6.	IGF-IR signaling effects translocation of the AR to the nucleus
7.	IGF-IR ligands increase in the progression of prostate cancer and are particularly abundant in bone where prostate cancer metastases are most abundant
8.	Xenograft models of prostate cancer respond differently to IGF-IR inhibition depending on the presence or absence of androgens
9.	IGF binding proteins (IGFBP) that enhance signaling of IGF ligands through the IGF-IR are increased in the period immediately after castration
10.	Inhibition of the IGF-IR in conjunction with castration
11.	Transcription factors that stimulate the IGF-IR promoter are also regulated by androgens

Since prostate cancer rarely develops in the absence of androgens, it is suspected that androgens are at least permissive in the transformation process of prostate epithelial cells. However, it should be noted that expression of the AR is necessary for normal luminal prostate epithelium to develop. It is suggested that maintaining certain levels of IGF-IR expression in the prostate may be necessary in normal prostate differentiation, increased levels of IGF-IR expression may be required for the prostate epithelia transformation process, and decreased IGF-IR expression may be required for prostate cancer malignant progression. This is consistent with the clinical findings that the levels of IGF-IR decrease following the initial transformation of the epithelium [Tennant et al., 1996]. This concept has been corroborated by the decrease in tumor metastases and increase in apoptosis associated with the re-expression IGF-IR in prostate cancer xenograft cell lines [Plymate et al., 1997a,b].

It should be pointed out that not all studies have shown an increase in IGF-IR expression during early prostate epithelia transformation or a decrease in IGF-IR expression in the progression to malignant prostate epithelia [Hellawell et al., 2002]. This may due to discrepancies in the choice of antibodies or technique in immunohistochemistry studies. The IGF-IR is a tyrosine kinase receptor that is only activated when located on the cell surface; although rapidly internalized upon activation, it is also rapidly processed through the golgi to be re-expressed on the cell surface.

CLINICAL DATA SUGGESTS THAT MEN IN THE HIGHER QUARTILES OF SERUM IGF-I LEVELS ARE AT A GREATER RISK FOR DEVELOPING PROSTATE CANCER

Large scale epidemiologic studies, such as the Physician's Health Study, have suggested that men with higher serum levels of IGF-1 as well as androgens may be at increased risk of developing prostate cancer in the following 6–9 years [Chan et al., 1998; Pollak, 2000; Pollak et al., 2004]. Also, in these studies serum levels of IGFBP-3 were inversely correlated with the risk of developing prostate cancer [Chan et al., 1998]. Of further note, the risk of cancer developing was more attributable to serum

IGF-I or IGFBP-3 than to serum testosterone. However, other studies have not shown an association of risk for prostate cancer with serum levels of IGF-I [Harman et al., 2000; Chen et al., 2005]. One should be aware that the risk of developing prostate cancer was not the primary end point of any of these studies nor did the results of the epidemiologic studies indicate a direct link between the IGF system and the risk of cancer.

ANDROGENS INCREASE IGF-IR EXPRESSION IN PROSTATE EPITHELIAL CELLS

We had initially detected an increase in IGF-IR expression at protein and mRNA levels in androgen-responsive prostate epithelial cell lines [Plymate et al., 2004]. This observation was subsequently confirmed by other investigators [Pandini et al., 2005]. The mechanism by which androgens increase the IGF-IR expression has been a topic of controversy. Pandini et al. [2005] have shown in their models that the increase in IGF-IR protein induced by androgens does not require nuclear translocation of the AR and is only partially blocked by bicluta-mide. On the other hand, this effect of AR on IGF-IR expression was completely inhibited by the ERK1/2 inhibitor PD980259 [Pandini et al., 2005]. These data suggested a "non-genomic" effect of androgen. This group further confirmed their findings using a mutated AR that will not translocate to the nucleus and demonstrated that the mutanted AR can activate the cytoplasmic Src–Raf–Ras–Map kinase pathway and enhance the transcriptional activity of IGF-IR promoter [Pandini et al., 2005]. Other investigators have not found that activation of this pathway is necessary for androgen-induced increases in IGF-IR expression [Plymate et al., 2004]. Other mechanisms including an increase in KFL6 (Kruppel factor like 6) in response to androgens have been suggested from the study in LnCaP lines (Levine-personal communication). We have shown that KFL6 increases IGF-IR expression by binding to the IGF-IR promoter [Rubinstein et al., 2004]. We have also shown in prostate cell lines that androgens can increase IGF-IR protein expression without an increase in its mRNA expression level, suggesting a post-transcriptional modification of IGF-IR expression, such as mRNA stability [Plymate et al., 2004]. Despite the existing controversials on the mechanisms, all the studies have consistently showed that androgens signaling through the AR result in increased IGF-IR protein expression in prostate epithelium, which is associated with increased phosphorylation of IGF-IR and increased cell proliferation in response to IGF ligands. However, it is not understood whether the induction of increased IGF-IR is part of the differentiation process of prostate epithelium or part of the mechanism for tumor progression. Since both IGF and androgens are necessary for epithelial differentiation, induction of increase in IGF-IR expression as part of the differentiating function of androgens may appear reasonable. On the other hand, increasing IGF-IR expression would be a mechanism by which androgens could enhance transformation and progression of prostate cancer.

IGF-IR ACTIVATION ALTERS AR PHOSPHORYLATION

One mechanism by which IGF-IR signaling could directly affect the function of the AR would be to alter AR phosphorylation. Studies by Lin et al. [2001] first suggested a role of IGF signaling in AR function. They observed that androgen induced apoptosis in AR transiently transfected DU-145 cells and treatment with IGF-1 decreased the transcriptional activity of the AR and inhibited apoptosis. We subsequently found that the effects on IGF-IR signaling on AR activity depended on whether the cells were from an orthotopic or a metastatic lesion [Plymate et al., 2004]. If the tumor was in the orthotopic site, IGF-IR activation inhibited AR transcription under a probasin promoter (AAR3) [Plymate et al., 2004]. In contrast, when the tumor was in the metastatic site, IGF-IR activation enhanced AR transcriptional activity on the AAR3 promoter. Interestingly, the effect of IGF-IR activation on the AR transcriptional activity in both primary and metastatic tumors appears to be mediated through the PI3K/AKT pathway [Plymate et al., 2004]. Lin et al. subsequently demonstrated that the effects of IGF on AR activity occurred in a biphasic manner in LnCaP cells: suppressing AR transcriptional activity at low passage numbers but enhancing AR transcriptional activity at high passage numbers [Lin et al., 2001]. Whether the effect is due to IGF-initiated phosphorylation of AR is rather controversial. Lin et al described that IGF-I phosphorylates AR at serines 210

and 790 [Lin et al., 2001], whereas Gioeli et al. [2002] failed to find any sites on the AR that were phosphorylated by IGF through a peptide terminal degeneration technique. We examined the effect of IGF-IR activation on AR phosphorylation in AR-transfected M12 (M12AR) cells. We showed that AR phosphorylation was decreased in the presence of IGF-I and that this effect was blocked by an inhibitory IGF-IR antibody A12 (Fig. 2a). Our newest study indicated that serine 16 on the AR is a potential site of dephosphorylation whereas serine 81 on the AR is a potential site of phosphorylation by IGF (Fig. 2b). The reasons for discrepancies between studies are not entirely clear. One possible reason for differences in phosphorylation would be differential expression of PP2A in different cell types.

IGF-IR SIGNALING EFFECTS TRANSLOCATION OF THE AR TO THE NUCLEUS

Phosphorylation of the AR may result in several changes that could alter the AR transcriptional functions. One of these effects could be translocation of the AR to the nucleus. Whereas AR phosphorylation was thought to be necessary for nuclear translocation, recent data has shown that phosphorylation of AR at serine 650, which takes place after the AR is in the nucleus and bound to DNA, results in the export of AR from the nucleus [Gioeli et al., 2006]. Thus, the process of dephosphorylation of specific serines on the AR may account for retention of AR in the nucleus and accentuated signaling. As we have shown in Figure 2, IGF decreases phosphorylation of the AR in our M12AR cells. We also have evidence that IGF can enhance AR nuclear translocation in the absence of androgens and that this effect can be inhibited by an IGF-IR inhibitory antibody (Fig. 3a). We have also demonstrated the changes in AR compartmentalization in nuclear and cytoplasmic fractions in response to IGF using Western blot analyses (Fig. 3b). Using the AAR3 probasin reporter assay, we show a significant transactivation of the AR in the absence of androgen and enhanced AR activation in the presence of androgen by IGF-I in M12AR cells. The AR transactivation response to IGF can be blocked by the IGF-IR antibody A12. These data indicate that even in the absence of androgen, IGF can induce transactivation of the AR. Whether this is attributed to changes in phosphorylation of the AR as we have discussed or to the recruitment of AR co-

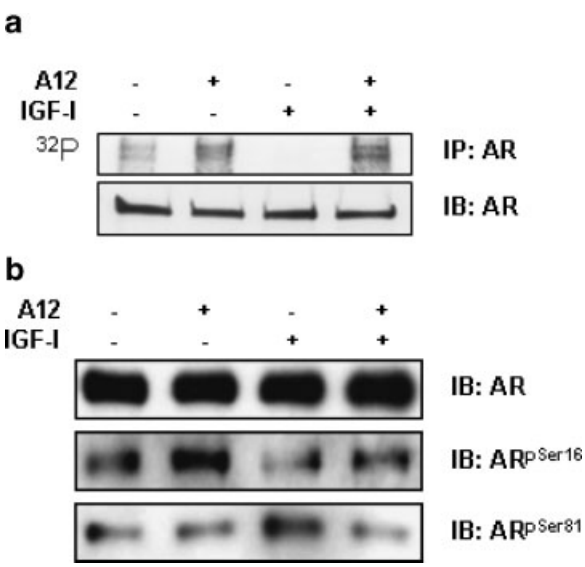

Fig. 2. IGF-I induces AR dephosphorylation. **a**: M12AR cells were labeled with ortho-^{32}P. Cell lysates were immunoprecipitated (IP) with AR-specific antibody. IB, western blotting. **b**: M12AR cells were IB with serine-specific anti-AR antibody.

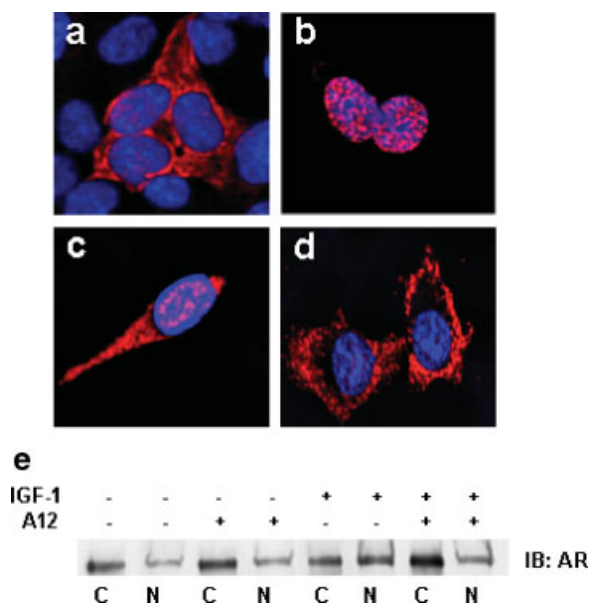

Fig. 3. Confocal image and cell fractionation showing IGF-I-induced AR translocation into the nucleus in M12AR cell lines. **a**: M12AR cells in IGF-I, DHT free medium. **b**: M12AR cells in medium containing 10^{-8}M of DHT. **c**: M12AR cells in medium containing 10 ng/ml of IGF-I. **d**: Medium containing 10 ng/ml of IGF-I and 10 μg/ml of anti-IGF-IR antibody A12. **e**: AR in cytosol and nuclear fractions of M12AR cells under various culture conditions. Red fluorescence. AR, androgen receptor. IGF-I, insulin-like growth factor I. DHT, dihydrotestosterone.

factors, or to both has yet to be determined. Regardless, these studies suggest that, in castrated patients, the increase in AR expression coupled with intact IGF-IR signaling can lead to AR-mediated AI prostate cancer progression. This marks the IGF-IR a potential therapeutic target in post-castrated prostate cancer.

XENOGRAFT MODELS OF PROSTATE CANCER RESPOND DIFFERENTLY TO IGF-IR INHIBITION DEPENDING ON THE PRESENCE OR ABSENCE OF ANDROGENS

We have reported in prostate cancer human xenograft models that inhibition of the IGF-IR with A12 results in a decreased rate of tumor growth in AD and AI tumors [Wu et al., 2005]. However, when we examined the mechanisms by which A12 caused decrease in growth rate, we noted marked differences depending on whether the tumors were AD or AI. In the AD tumors we found that A12 treatment resulted in a combination of apoptosis and G1 cell cycle arrest, whereas in the AI tumors we found that tumor cells arrested in G2 with no occurrence of apoptosis [Wu et al., 2005]. The question arose as to whether these differences in responses were due to a change in the character of the tumor or the absence of androgen. In order to address this issue, we implanted the AI tumor into intact animals. As predicted, tumor growth was inhibited in the A12 treated animals compared to vehicle treated controls. Interestingly, a majority of these tumors displayed an apoptotic response and G1 cell cycle arrest as opposed to the lack of apoptosis when implanted in the castrated animals. To determine potential mechanisms for this effect of androgen on the tumors, we performed cDNA microarray analyses of A12-treated AI tumors from castrated and intact animals and found marked differences in the gene expression profiles (Fig. 4). Some genes such as PP2A and TSC-22 were regulated in opposite direction with A12 treatment, depending on the presence or absence of androgens. It is of interest that TSC-22 has been shown to be androgen-regulated and its expression decreases from benign prostate luminal epithelium to cancer. Another interesting gene differentiated expressed is IGFBP-5, which has been demonstrated to increase post-castration and is associated with recovery from castration-induced apoptosis [Miyake et al., 2000a].

IGF BINDING PROTEINS (IGFBP) THAT ENHANCE SIGNALING OF IGF LIGANDS THROUGH THE IGF-IR ARE INCREASED IN THE PERIOD IMMEDIATELY AFTER CASTRATION

Following castration, IGFBP-2 and IGFBP-5 have been shown to increase significantly in both human prostate and mouse models of prostate cancer. Both of these IGFBPs can increase IGF-ligand signaling through the IGF-IR and enhance recovery from castration induced apoptosis and cell cycle arrest. These two IGFBPs accomplish this task by binding to extracelluar matrix and maintaining a higher concentration of IGF ligand in the proximity of the IGF-IR [Jones et al., 1995; Russo et al., 1997; Kiyama et al., 2003]. The functional importance of these changes has been demonstrated by the studies of Miyake et al. [2000b] in which over-expression of these IGFBPs in LnCaP cells markedly enhances cell growth following androgen withdraw. Using antisense oligonucleotides to IGFBP-2 or IGFBP-5, this group was able to demonstrate the stimulatory effects of the IGFBPs on tumor growth [Kiyama et al., 2003].

INHIBITION OF THE IGF-IR IN CONJUNCTION WITH CASTRATION THERAPY FOR PROSTATE CANCER

These studies suggest that blocking IGF-IR signaling at the time of castration would enhance the effects of androgen withdraw. Preliminary studies in our laboratory using mouse xenograft models have shown a marked enhancement of the castration effect on prostate tumor growth with the inhibitory IGF-IR antibody A12. Potential mechanisms of the augmented effect of A12 on androgen withdraw may include suppression of Survivin, a member of the Inhibitor of Apoptosis (IAP) family of proteins that has been shown to play a role in the recovery process of anti-androgen therapy [Zhang et al., 2005].

IGF-IR ACTIVATION CAN STIMULATE AR CO-FACTORS THAT ENHANCE AR SIGNALING

Insulin-like growth factor may also influence AR signaling by increasing the expression of AR

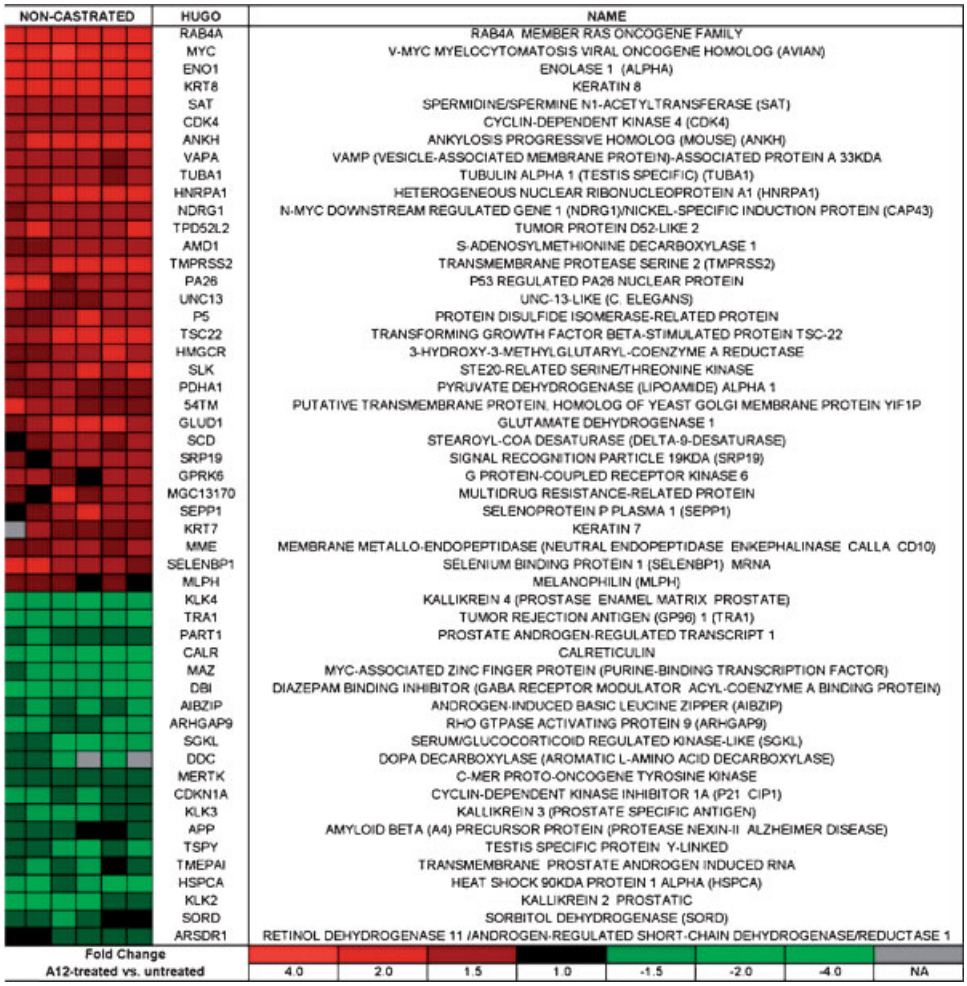

Fig. 4. cDNA microarray expression values of androgen-regulated genes differentially expressed in LuCaP 35V tumors from A12-treated relative to untreated non-castrated mice. There were 82 unique genes known to be androgen-regulated which had significantly consistent gene expression across all samples as compared to no change by a one-sample *t*-test in SAM (<1% FDR significance cut-off used). The scale represents fold-change in A12-treated relative to untreated tumors.

co-stimulatory factors. Given the known 100 or more AR co-regulatory factors, it is not surprising that IGF-IR activation would enhance the expression or activation of one or more co-regulators of the AR. Amongst them, TIF-2 (GRIP-1) and insulin degrading enzyme (IDE) are of particular interest. Studies in a series of human prostate specimens from men with prostate cancer, Mohler and Wilson have demonstrated an increased expression of TIF-2 in most of the recurrent AI prostate cancers that also have a high levels of AR in the nucleus [Gregory et al., 2001]. The same group has also shown the coincidence of increased TIF-2 expression with the recurrence of AI human prostate cancer in xenograft models. Mohler has also demonstrated that overexpression of TIF-2 in vitro can increase AR transcriptional activity in the presence of the physiological concentrations of adrenal androgen. Studies have shown that IDE is a potent co-stimulator of AR transcriptional activity and the ability of IDE to bind to the AR can be regulated by insulin and IGF ligands [Kupfer et al., 1994]. In addition, as the name implies, IDE can degrade insulin, IGF-I and IGF-II [Udrisar et al., 2005].

CONCLUSION

In this review, we have summarized our current understandings of the interactions between the IGF system and the AR (Fig. 5).

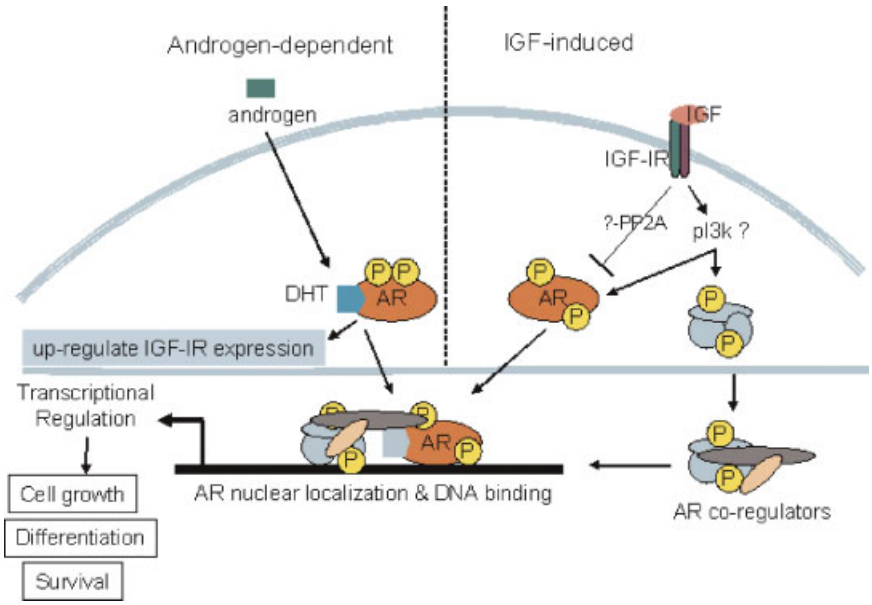

Fig. 5. Interactions of the IGF system with AR signaling. pI3k, phosphoinositide 3-kinase. PP2A, protein phosphotase 2A. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

The ability of IGF signaling to potentiate the transcriptional activity of the AR in the face of low to no androgen makes the IGF system, especially the IGF-IR, a strong candidate that leads progression of AI prostate cancer through AR signaling.

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