doi: http://dx.doi.org/10.5016/1806-8774.2009v11pT102

ARBS Annual Review of Biomedical Sciences

Theme Topic on "Cell Receptors and Signaling"

pdf freely available at http://arbs.biblioteca.unesp.br

2009;11:T102-T113

Crosstalk between the Epidermal Growth Factor Receptor and Androgen Receptor Signaling Pathways in Prostate Cancer

Christopher W Gregory*

Clinsys Clinical Research and Department of Pathology and Laboratory Medicine at the University of North Carolina, Chapel Hill, USA

Received: 15 November 2009; accepted 22 December 2009
Online on 21 February 2010

Abstract

Gregory CW. Crosstalk between the Epidermal Growth Factor Receptor and Androgen Receptor Signaling Pathways in Prostate Cancer. Annu Rev Biomed Sci 2009;11:T102-T113. Malignant cells are characterized by complex and often overlapping signaling pathways that modulate uncontrolled proliferation, leading to tumor growth and cancer progression. Prostate cancer is characterized by a dependence on androgens for growth initiation, and androgen deprivation is the standard treatment for advanced disease. Invariably, progression to castration-resistant prostate cancer (CRPC) occurs. Effective treatment options are currently limited to cytotoxic chemotherapy. In addition to androgen-mediated androgen receptor (AR) signaling in CRPC, multiple growth factor pathways are active, including the epidermal growth factor (EGF) receptor family of tyrosine kinases. Crosstalk between these two pathways has been studied in prostate cancer cell lines, xenograft tumor models, and in prostate cancer patients. This manuscript will focus on EGFR signaling, AR signaling, EGFR-AR crosstalk, and therapies targeted at these receptor-mediated regulatory pathways in CRPC.

Keywords: epidermal growth factor receptor, androgen receptor, prostate cancer, kinase signaling, targeted therapy.

Table of Contents

* **Correspondence**
Clinsys Clinical Research, Inc., 8540 Colonnade Center Drive, Suite 501, Raleigh NC 27615 and Department of Pathology and Laboratory Medicine, University of North Carolina at Chapel Hill, CB# 7525, Chapel Hill NC 27599, USA. E-mail: cgregory@clinsys.com

Acknowledgement: Thank you to Daniel N Wood for critical review of the manuscript and assistance with the illustration.

1. Introduction: Prostate Cancer

Prostate cancer continues to be a significant medical problem in developed countries around the world and is the most frequently diagnosed cancer in males in the United States (American Cancer Society, 2009). Treatment of patients with early-stage, localized disease is effective and the long-term survival outlook is excellent. For patients with locally-advanced or metastatic prostate cancer, androgen deprivation therapy typically induces remission. Unfortunately, development of castration-resistant prostate cancer (CRPC) is common, with median survival of 18-24 months. Treatment with docetaxel (Taxotere®), a cytotoxic agent, is the current standard of care for patients with CRPC. Significant drug development efforts are underway, focused on developing targeted therapies for CRPC, based on the characterization of signaling pathways that exist within malignant cells. In this manuscript, two distinct, but overlapping, signaling pathways (epidermal growth factor receptor [EGFR] and androgen receptor [AR]) will be reviewed and the relevance of the biological crosstalk between these pathways will be explored in the context of targeted therapies for CRPC.

2. Epidermal Growth Factor (EGF) Receptor Signaling

Growth factor-mediated signal transduction networks within living cells enable essential communication with other cells and the extracellular environment to support normal cellular growth, differentiation, adhesion, migration, and apoptosis. Dysregulation of these growth factor pathways has the potential to drive malignant transformation of cells by modulating normal cellular processes, leading to cancer pathogenesis. The epidermal growth factor (EGF) family of receptor tyrosine kinases (RTKs), also called ErbB or HER receptors, represents a set of well-studied biochemical pathways that has been targeted therapeutically to treat cancer and other diseases.

EGF receptors include EGFR (ErbB1, HER1), ErbB2 (HER2, neu in rodents), ErbB3 (HER3), and ErbB4 (HER4). Structurally, EGF receptors (EGFRs) consist of an extracellular N-terminal ligand-binding domain, short transmembrane and juxtamembrane domains, an intracellular tyrosine kinase domain, and an intracellular C-terminal tail (Wieduwilt & Moasser, 2008). A conformational change in response to ligand binding to the ectodomains of the receptors mediates dimer formation and the kinase domain of one monomer phosphorylates tyrosine residues in the C-terminal domain of the other receptor and vice versa, thereby activating the receptor (Weinberg, 2007). A series of downstream kinase signaling pathways (including PLCγ/PKC, PI(3)kinase/AKT, Ras/Raf/MAPK, and Jak/Stat) are activated directly or through adapter proteins resulting in transmission of signals to the nucleus, where transcription factors modulate a biological response.

Multiple growth factor ligands increase the complexity and diversity of signaling controlled by the EGF receptor family (reviewed in Yarden & Sliwkowski, 2001). EGF, transforming growth factor-α (TGF-α), and amphiregulin specifically bind EGFR. HER2 binds no ligand with high affinity, while HER3 binds neuregulin-1 (heregulin), neuregulin-2, and neuroglycan C, even though it has no intrinsic kinase activity. HER4 also binds neuregulin-1 and -2, as well as neuregulin-3 and -4. EGFR and HER4 bind heparin-binding EGF (HB-EGF), epiregulin, and β-cellulin. Ligand binding induces preferential homo- or heterodimer formation between the receptor subtypes. EGF binding to EGFR induces EGFR homodimers or EGFR/HER2 heterodimers, while heregulin binding to HER3 or HER4 leads to HER2/HER3 or HER2/HER4 heterodimers. Although HER2 binds no known ligand, it is the preferred dimerization partner for other EGF receptors.

This diverse family of receptors and ligands regulates a similarly diverse range of normal physiological functions, including mitogenesis, differentiation, and cell survival. Multiple "knockout" studies performed in mice have demonstrated the critical roles of EGFRs. EGFR knockout mice are characterized by failure of multiple organs due to poor development of the epithelium in the skin, lungs, and gastrointestinal tract (Miettinen *et al.*, 1995). Deletion of HER2 or HER3 in mice resulted in early embryonic lethality, supporting a role for these receptors in key developmental events (Lee *et al.*, 1995; Riethmacher *et al.*, 1997). HER3 was also shown to be required for normal development of the cerebellum and the heart. Elimination of HER4 expression has detrimental effects on the embryonic development of neural and cardiac precursors in mice (Gassman *et al.*, 1995).

Due to the central role of the EGFR family in normal physiology and the activation of multiple signaling pathways in parallel, overexpression of the receptors and ligands have been implicated in the formation of premalignant lesions, including those found in the oral cavity, lungs, cervix, and prostate (reviewed in Grandis & Sok, 2004). EGF receptors are also commonly overexpressed in epithelial malignancies, including cancers of the head and neck, breast, lung, ovary, bladder and prostate. Due to the redundancy of the kinase signaling pathways activated by the EGF receptor family from the cell membrane, and the ability of EGF receptors to be translocated into the nucleus, these proteins are considered transcription factors, regulating key pathways within the cell. Crosstalk with nuclear receptor signaling modules has been described in multiple hormone-dependent tumor types, including prostate cancer.

3. Androgen Receptor (AR) Function

Circulating androgens regulate the development and maturation of the male reproductive organs, including the prostate. Since the discovery that androgen deprivation was effective for treating advanced prostate cancer, significant efforts have been made to characterize regulatory pathways that drive the development of this malignancy. Under normal physiological conditions, luteinizing hormone (LH) is produced by the pituitary gland in response to gonadotropin-releasing hormone released by the hypothalamus. LH stimulates testicular Leydig cells to produce testosterone (T), which circulates predominantly bound to sex-hormone binding globulin and albumin. Inside the prostate cell, T is converted to dihydrotestosterone (DHT) by 5α-reductase enzymes, and both steroids bind to the androgen receptor (AR), a ligand-dependent transcription factor that belongs to the nuclear hormone receptor super family. The AR is complexed with chaperone proteins (*e.g.*, HSP90) in the cytoplasm and upon steroid binding, AR monomers bind in an anti-parallel manner to form mature AR homodimers that bind to hormone response elements on androgen-regulated genes as part of a transcription complex with coregulators.

Prostate cancer cells are initially dependent on androgens for growth and tumors regress upon androgen deprivation (surgical castration) or treatment with anti-androgens (chemical castration). Unfortunately, prostate tumors commonly recur after a period of remission and present as a CRPC, characterized by active AR signaling despite the absence of circulating testicular androgens. Multiple mechanisms for the reactivation of AR in CRPC have been proposed, including: amplification of the AR gene; functional mutations that broaden AR ligand specificity beyond T/DHT; increased expression of AR coactivators; changes in steroid metabolism in prostate cancer cells; and crosstalk with growth factor-regulated kinase signaling pathways.

Multiple studies have demonstrated AR gene amplification in approximately 30% of prostate cancer specimens that is associated with an increase in AR protein expression (Visakorpi *et al.*, 1995; Bubendorf *et al.*, 1999; Linja *et al.*, 2001; Ford *et al.*, 2003). Higher AR expression has been shown to increase the sensitivity of prostate cancer cells to low levels of androgen (Gregory *et al.*, 2001) and to maintain or increase expression of androgen-regulated genes (van der Kwast *et al.*, 1991; Chodak *et al.*, 1992; de Vere White *et al.*, 1997; Gregory *et al.*, 1998). AR overexpression was also demonstrated to change the effect of bicalutamide (Casodex®) from an AR antagonist to an AR agonist (Chen *et al.*, 2004).

Mutation of the AR coding sequence is another mechanism for increased AR responsiveness in CRPC. Several of the reported AR mutations allow steroids other than T and DHT to activate the receptor, while still maintaining responsiveness to the cognate ligands (Culig *et al.*, 1993; Peterziel *et al.*, 1995; Tan *et al.*, 1997; Chang *et al.*, 2001; Shi *et al.*, 2002; Taplin, *et al.*, 2003). AR antagonists such as hydroxyflutamide may gain agonist activity with certain AR mutations. While the frequency of AR mutations in androgen-dependent prostate cancer is relatively low, it has been difficult to determine the frequency in CRPC, specifically in bone metastases. Nonetheless, AR mutations may play a role in the development of recurrent disease.

Binding of AR to androgen response elements in androgen regulated genes is characterized by the formation of a multi-unit transcription complex consisting of receptor together with nuclear receptor coactivators. The p160 coactivator family includes steroid receptor coactivator-1 (SRC1) (Onate *et al.*,

1995), transcriptional intermediary factor 2 (TIF2/SRC2) (Voegel *et al.*, 1998) or the mouse homologue glucocorticoid receptor interacting protein (GRIP1) (Hong *et al.*, 1997), and the SRC3 coactivators TRAM1, AIB1, RAC3, ACTR, and p/CIP (McKenna *et al.*, 1999). Overexpression of these coactivators in transient transfection assays increases AR transactivation in response to physiological concentrations of T, DHT and adrenal androgens including androstenedione (ASD) and dihydroepiandrosterone (DHEA) (Tan *et al.*, 2000; Gregory *et al.*, 2001; Heinlein & Chang, 2002). TIF2 and SRC1 were shown to be expressed at higher levels in the nuclei of recurrent prostate cancer specimens compared to benign prostate (Gregory *et al.*, 2001). Similarly, elevated coactivator expression has been demonstrated in breast cancer (Murphy *et al.*, 2000; Graham *et al.*, 2000), ovarian cancer (Anzick *et al.*, 1997), and polycystic ovarian syndrome (Gregory *et al.*, 2002).

CRPC develops in the setting of suppressed systemic androgens. Tumoral steroidogenesis has been proposed as a compensatory mechanism to maintain androgen levels sufficient to activate AR. Locally recurrent CRPC tumors were shown to contain T at levels similar to benign prostate tissue, with lower levels of DHT, DHEA, and ASD (Mohler *et al.*, 2004). T levels were shown to be significantly higher in metastases from surgically castrated prostate cancer patients compared to primary prostate cancers from untreated patients (Montgomery *et al.*, 2008). Additionally, this study demonstrated increased expression of genes for steroidogenic enzymes in CRPC metastases, possibly reflecting an elevated capacity for steroid production from precursors. Down-regulation of steroid 5α-reductase isozymes I and II, which are responsible for converting T to DHT, has been reported in CRPC compared to benign prostate or primary androgen dependent tumors (Titus *et al.*, 2005; Xu *et al.*, 2006; Montgomery *et al.*, 2008). Despite this reduction in steroid 5α-reductase, tumor levels of T and/or DHT are sufficient to activate the AR and drive growth of tumor cells (Gregory *et al.*, 2001).

Activation of growth factor-mediated kinase pathways in CRPC has been proposed as another mechanism for activation of AR signaling in CRPC. Insulin-like growth factor-1 (IGF-1), keratinocyte growth factor (KGF), and EGF have been reported to increase AR transactivation in the absence of androgen (Culig *et al.*, 1996; reviewed in Feldman & Feldman, 2001). Recently, interleukin-6 (IL-6) was demonstrated to induce growth of AR-positive prostate cancer cells and xenograft tumors (Malinowska *et al.*, 2009). Over-expression of HER2 in the LAPC-4 prostate cancer cell line was shown to cause androgen-independent growth and to activate the AR signaling pathway in the absence of androgen (Craft *et al.*, 1999). Likewise, forced over-expression of HER2 in LNCaP cells activated MAPK and prostate-specific antigen (PSA) expression, mediated by AR and possible interaction with the AR coactivator ARA-55 (Yeh *et al.*, 1999). Multiple studies exploring the crosstalk between EGFR and AR have been published and will be detailed below.

4. Crosstalk between EGFR and AR

EGFR expression levels were compared in primary localized prostate cancer tumors and CRPC tumors using immunohistochemical technique and tissue microarrays (Shah *et al.*, 2006). Results demonstrated that EGFR was associated with hormone-refractory status, but likely due to the heterogeneous nature of CRPC, not all tumors showed increased EGFR expression. Another study demonstrated that EGFR was expressed in 69% of CRPC tumors compared to 23% of primary androgen dependent tumors (Hernes *et al.*, 2004). Controversy also exists regarding the elevated expression of HER2 in CRPC, with some studies demonstrating elevated HER2 expression and other studies finding no apparent differences in HER2 between androgen dependent and CRPC tumors (Signoretti *et al.*, 2000; Osman *et al.*, 2001; Calvo *et al.*, 2003). HER3 and its cognate ligand heregulin were reported to be overexpressed in primary androgen dependent prostate cancer compared to benign prostate tissue and there was an association with less favorable prognoses (Leung *et al.*, 1997). HER4 was shown to be over-expressed but heterogeneous (nuclear *vs*. cytoplasmic) in prostate cancer specimens, with no obvious correlation with disease outcome (Ben-Yosef *et al.*, 2007).

Crosstalk between EGFR and AR occurs at multiple levels (see Fig. 1). PC3 prostate cancer cells transfected with AR were used to explore the effects of EGF on invasion and adhesion of cultured cells (Bonaccorsi *et al.*, 2004). EGF-induced EGFR autophosphorylation, PI3-kinase activity, and EGFR internalization were reduced in AR-expressing PC3 cells and AR and EGFR were shown to co-

immunoprecipitate, supporting a direct interaction of the receptors to modulate prostate cancer cell function. Stable expression of a constitutively active mitogen-activated protein kinase kinase kinase-1 (MEKK1) caused apoptosis in AR-positive LNCaP cells but not in AR-negative PC3 or DU145 cells, supporting a link between EGF-mediated MAP kinase signaling and AR (Abreu-Martin *et al.*, 1999). To further establish the link between MAP kinase pathways and AR signaling, a study was conducted using LNCaP cells that showed DHT treatment alone was insufficient to induce ERK1/2 phosphorylation and EGF alone did not induce AR activation, but when DHT and EGF were used together, there was a synergistic effect on AR activation (Mukherjee & Mayer, 2008). The MEK inhibitor U0126 inhibited DHT- and DHT/EGF-mediated AR induction, linking ERK1/2 signaling to AR activation. In addition, DHT treatment of LNCaP cells resulted in down-regulation of EGFR protein, demonstrating the potential for sustained EGFR signaling in the setting of androgen deprivation. In contrast, DHT treatment of LNCaP cells was shown to stimulate EGFR gene expression and this was associated with recruitment of RNA polymerase II to the promoter (Pignon *et al.*, 2009). These discrepant findings may suggest different modes of androgen regulation of EGFR at the transcriptional and post-translational levels. A recent study found that treatment of cultured prostate cancer cells (LNCaP, LAPC4 and C4-2) with EGF or heregulin-β1 caused a rapid decrease in expression of AR protein. Furthermore, AR mRNA levels were decreased in growth factor-treated LNCaP cells and in LNCaP cells adapted to growth in androgen-depleted medium, although the levels of AR protein were not affected, due to increased AR protein stability (Cai *et al.*, 2009).

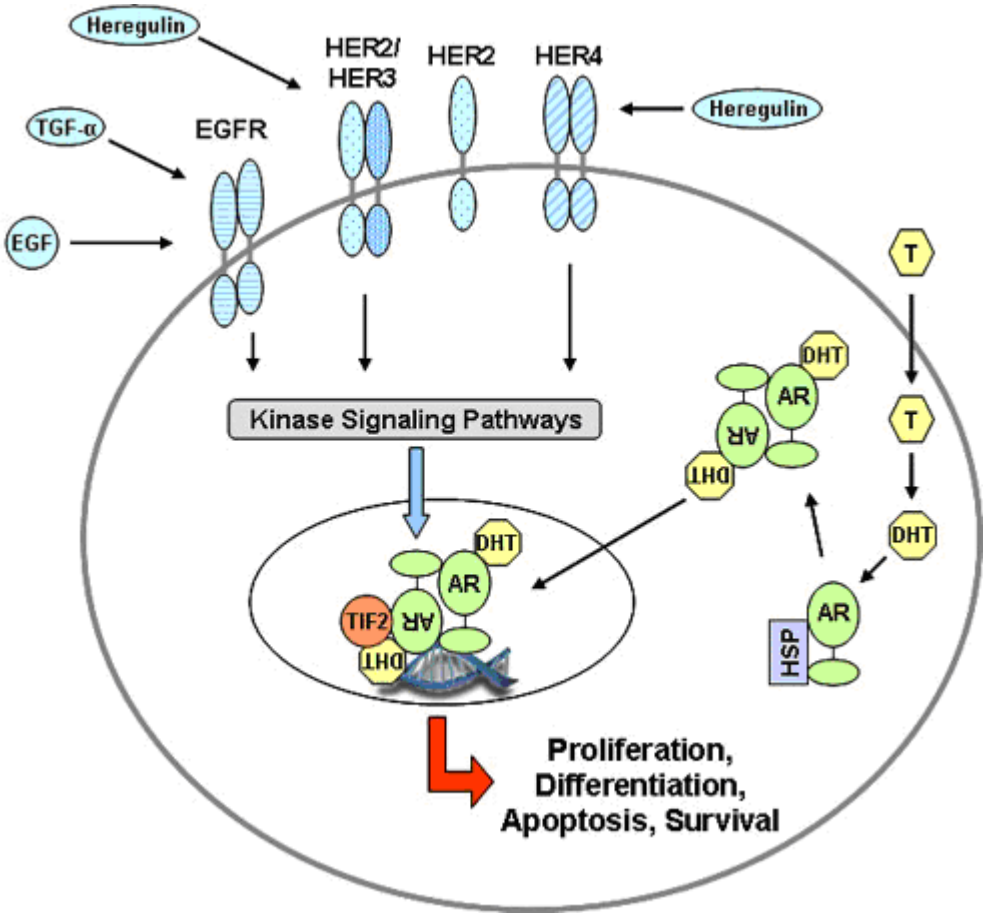

Figure 1. EGFR and AR crosstalk in prostate cancer. Upon binding androgen (DHT), AR dissociates from chaperone proteins, forms an antiparallel homodimer and is imported into the nucleus where, together with steroid receptor coactivators (e.g., TIF2), binds to hormone response elements in genes as part of a transcription complex. EGF receptor family kinases (EGFR, HER2, HER3, and HER4) form homo- or heterodimers upon growth factor binding and activate downstream signaling pathways that can phosphorylate AR, AR coactivators, or other regulatory factors, resulting in growth promoting effects and tumor growth.

To demonstrate a definitive mechanistic link between EGF and AR, the expression of AR was inhibited with small interfering RNA (siRNA) in the CWR-R1 cell line, which decreased EGF-stimulated cell growth (Ponguta *et al.*, 2008). EGF treatment increased AR transcriptional activity that was dependent on phosphorylation of a MAPK consensus site (Ser-515) in the AR N-terminal region and a protein kinase C consensus site (Ser-578) in the AR DNA binding domain. Mutation of Ser-578 to alanine abolished EGF-mediated phosphorylation, eliminated the AR transcriptional response to EGF, and mediated nuclear retention of the receptor in concert with hyperphosphorylation of Ser-515. EGF regulation of AR can also occur through phosphorylation of nuclear receptor coactivators. An EGF-induced increase in TIF2/GRIP1 phosphorylation was shown to be mediated through the MAPK signaling pathway (Gregory *et al.*, 2004). EGF increased androgen-dependent AR transactivation in the CWR-R1 cell line in parallel with increased TIF2/GRIP1. EGF also increased expression of TIF2/GRIP1 in CWR22 xenograft tumors growing in the absence of circulating androgen. Inhibition of TIF2/GRIP1 expression with siRNA caused a decrease in AR transcriptional responses to DHT and EGF, providing a plausible direct link between EGF signaling, increased p160 coactivator levels, and AR activity in CRPC.

Crosstalk between HER2 and AR has also been reported in multiple studies, demonstrating the redundant nature of EGFR family signaling in CRPC. In the 22Rv1 cell line, derived from the CWR22R tumor xenograft, heregulin stimulation resulted in increased HER2 phosphorylation and cell proliferation that could be inhibited by treatment with the anti-HER2 antibody rhuMAb 2C4. This study also demonstrated that growth of CWR22R tumors could be inhibited by treatment of xenograft-bearing mice with the anti-HER2 antibody (Mendoza *et al.*, 2002). Another study found that treatment with rhuMAb 2C4 inhibited the growth of androgen-dependent CWR22 xenograft tumors but also caused increased secretion of PSA in an androgen-independent manner, supporting the possibility of crosstalk between the HER2 and AR signaling pathways (Agus *et al*., 1999). Further characterization of the HER2 kinase signaling pathway in prostate cancer was performed by using the HER2 small molecule inhibitor PKI-166 (Mellinghoff *et al.*, 2004). This study demonstrated that kinase signaling induced by HER2/HER3 receptor dimerization stabilized AR protein and enhanced AR binding to PSA promoter/enhancer DNA sequences. Stabilization of AR was proposed to be a result of HER2-induced protection of AR from ubiquitin-mediated degradation.

Treatment of the CWR-R1 recurrent prostate cancer cell line with heregulin activated HER2 and HER3 and increased androgen-dependent AR transactivation of reporter genes, although endogenous levels of AR protein were unchanged. Lapatinib ditosylate (Tykerb®), a dual tyrosine kinase inhibitor of EGFR and HER2, was a potent inhibitor of HER2 and HER3 tyrosine phosphorylation, AR transactivation, and CWR-R1 cell proliferation induced by heregulin, when compared to gefitinib (Iressa®), an EGFR-specific inhibitor, which was less effective (Gregory *et al.*, 2005). Similarly, in studies performed in LNCaP cells, inhibition of HER2 expression with either an intracellular single-chain antibody or lapatinib resulted in decreased androgen-stimulated cell growth and expression of endogenous PSA mRNA and protein (Liu *et al.*, 2005). Lapatinib was a more effective inhibitor of AR recruitment to, and acetylation of, the PSA enhancer, as compared to gefitinib, and this is consistent with an important regulatory role for HER2 in the modulation of AR transcriptional activity. In a recent study, EGF or heregulin treatment of LNCaP, LAPC4, or C4-2 prostate cancer cells caused a decrease in AR expression that did not occur in the 22Rv1 cell line (Cai *et al.*, 2009). Decreased AR protein was shown to be a result of increased degradation of AR mRNA as regulated by growth factor treatment. In androgen-deprived cells, AR mRNA was rapidly degraded but AR protein levels did not decline due to increased AR stability. Together, these findings support a role for HER2/HER3 regulation of AR function in CRPC, a transformed state in which EGF, heregulin, and other growth factors can activate kinase pathways that influence AR stability, DNA binding, and ultimately, growth promotion.

5. Therapeutic Challenges in Castration-resistant Prostate Cancer (CRPC)

The discovery and description of autocrine/paracrine growth factor signaling pathways in CRPC has generated a great deal of interest in the design and testing of new therapies. Preclinical studies in

prostate cancer xenograft models demonstrated that gefitinib inhibition of EGFR signaling could inhibit tumor growth. Gefitinib was used at a maximum tolerated dose of 150 mg/kg in nude mice bearing androgen-dependent CWR22 tumors or androgen-independent CWR22LD1 or CWR22RV1 tumors. Growth of CWR22 was inhibited by 54% and growth of the androgen-independent variants was inhibited by 76%, over a 2 week treatment cycle (Sirotnak *et al.*, 2002). When combined with the antiandrogen bicalutamide, gefitinib was a more potent inhibitor of tumor growth in the CWR22LD1 model. Coadministration of gefitinib with carboplatin or paclitaxel increased the therapeutic inhibition of tumor growth and tumor-free mice were seen with the gefitinib/paclitaxel combination. A subsequent study was conducted to determine the role of gefitinib in the modulation of metastasis in the PC3 cell line and its highly metastatic PCb2 sub-line (Angelucci *et al.*, 2006). Subcutaneous xenograft growth of both cell lines was inhibited by 50%, whereas formation of bony metastases in nude mice was inhibited by 81% in the PCb2 cell line and 47% in PC3 cells. Gefitinib treatment also reduced the *in vitro* invasive ability of PCb2 cells in response to EGF or bone stromal cell conditioned medium, supporting a possible role for EGFR in CRPC metastasis.

Multiple clinical trials have been conducted to determine the safety and efficacy of gefitinib or erlotinib (Tarceva®) treatment of patients with CRPC (Canil *et al.*, 2005; Curigliano *et al.*, 2007; Gross *et al.*, 2007; Small *et al.*, 2007; Gravis *et al.*, 2008; Nabhan *et al.*, 2009). In all trials, the drugs were generally well-tolerated at daily doses ranging from 150 mg to 500 mg. Over 200 patients were treated across the six studies but efficacy was disappointing, and in most cases there was no clinical response to treatment. One study reported five of 35 patients had stable PSA and 5 patients had a best response of stable disease (Canil *et al.*, 2005). A phase II trial of docetaxel and erlotinib as first-line therapy for metastatic CRPC patients showed minimal efficacy that was comparable to docetaxel alone (Gross *et al.*, 2007). In a phase II erlotinib alone trial of 29 chemotherapy-naïve CRPC patients, two patients achieved a partial response and five demonstrated stable disease (Nabhan *et al.*, 2009). Trastuzumab (Herceptin®) was administered to 18 CRPC patients as a single agent, but demonstrated minimal efficacy. Two patients experienced stable disease based on a PSA decrease to less than 50% of baseline levels, but no patient showed a regression of bone or soft tissue metastases (Ziada *et al.*, 2004). In a recent phase II study of 29 chemotherapy-naïve CRPC patients with rising PSA while receiving androgen deprivation therapy, the dual EGFR/HER2 inhibitor lapatinib was administered at 1500 mg/daily. One of 21 evaluable patients had >50% reduction in PSA and 2 patients achieved stable disease for >5 and >15 months (Whang *et al.*, 2008). Alone or in combination with standard chemotherapeutic agents, EGFR and EGFR/HER2 tyrosine kinase inhibitors have not shown the anticipated efficacy in CRPC that was predicted by preclinical studies.

Several reasons for the lack of effectiveness of these agents have been put forward. First, the possibility exists that kinase signaling is not completely suppressed at the dose levels that have been studied. Administering higher concentrations of the drugs may inhibit more EGF receptors but may also lead to more intolerable toxicities. Second, heterogeneity of CRPC tumors (inter- and intra-patient variability) presents a challenge to therapeutic improvements. Third, receptor-independent kinase signaling downstream likely negates the inhibitory effects of receptor-targeted agents. Fourth, redundancy of growth factor signaling pathways may allow CRPC tumor cells to evade inhibition of EGF receptor family signaling. Fifth, gefitinib and erlotinib are more effective inhibitors of mutant EGF receptors, as found in lung cancers (Pao *et al.*, 2004).

Given the limited effectiveness of agents targeting EGFR family receptor tyrosine kinases in CRPC, unique combination therapies should be explored to overcome these therapeutic limitations. A recent study demonstrated that the Hedgehog signaling pathway may be a viable target in CRPC (Shaw *et al.*, 2008). Patched is the receptor for Hedgehog ligands and inhibits the G-protein coupled receptor Smoothened in the absence of Hedgehog. Hedgehog binding to the Patched receptor leads to disinhibition of Smoothened, activating downstream transcription events. Apoptosis is induced in prostate cancer cells when Hedgehog is inhibited. Hedgehog and EGFR signaling pathways have been shown to be present in circulating tumors cells from CRPC patients. The Hedgehog-specific inhibitor cyclopamine inhibited growth of CRPC cells and synergistic inhibitory effects were noted when used together with gefitinib and lapatinib. Kinase signaling pathways downstream of EGF receptors that have been

implicated in CRPC include the AKT/mammalian target of rapamycin (AKT/mTOR) and MAP kinase pathways. Treatment with rapamycin, an inhibitor of mTOR and PD0325901, an inhibitor of MAP kinase 1 (MEK), caused growth inhibition of cultured prostate cancer cells and prostate tumors in a transgenic mouse model (Kinkade *et al.*, 2008). Growth inhibition was coupled with up-regulation of an apoptotic regulator protein. The combinatorial targeted therapy approach has been employed in multiple clinical trials with temsirolimus and everolimus (mTOR inhibitors) alone or in combination with antiandrogens, gonadotropin analogues, or gefitinib but results from these studies have not been published to date.

6. Summary

Given the dismal clinical outlook for patients with metastatic CRPC, there exists a great need for improved therapies. While docetaxel remains the standard of care for CRPC patients, there is only a modest benefit. Our understanding of EGFR and AR signaling pathways has expanded in recent years and targeted drugs have been designed and tested in clinical trials. Unfortunately, significant therapeutic gains have not been realized to date, likely due to the redundant, parallel, growth-promoting pathways resident in malignant prostate cells. Ongoing drug development programs for patients with CRPC should focus on combinatorial regimens targeting multiple signaling pathways, including those for EGFR and AR.

7. References

Abreu-Martin MT, Chari A, Palladino AA, Craft NA, Sawyers CL. Mitogen-activated protein kinase kinase kinase 1 activates androgen receptor-dependent transcription and apoptosis in prostate cancer. Mol Cell Biol 1999;19:5143-54.

Agus DB, Scher HI, Higgins B, Fox WD, Heller G, Fazzari M, Cordon-Cardo C, Golde DW. Response of prostate cancer to anti-Her-2/neu antibody in androgen-dependent and –independent human xenograft models. Cancer Res 1999;59:4761-4.

American Cancer Society. Cancer Facts & Figures 2009. Atlanta: American Cancer Society, 2009.

Angelucci A, Gravina GL, Rucci N, Millimaggi D, Festuccia C, Muzi P, Teti A, Vincentini C, Bologna M. Suppression of EGF-R signaling reduces the incidence of prostate cancer metastasis in nude mice. Endocr-Related Cancer 2006;13:197-210.

Anzick SL, Kononen J, Walker RL, Azorsa DO, Tanner MM, Guan XY, Sauter G, Kallioniemi OP, Trent JM, Meltzer PS. AIB1, a steroid receptor coactivator amplified in breast and ovarian cancer. Science 1997;277:965-8.

Ben-Yosef R, Sarid D, Vexler A, Lidawi G, Inbar M, Marmor S, Starr A, Yaal Hahoshen N. Nuclear and cytoplasmic expression of ErbB-4 in prostate cancer. Int J Biol Markers 2007;22:181-5.

Bonaccorsi L, Carloni V, Muratori M, Formigli L, Zecchi S, Forti G, Baldi E. EGF receptor (EGFR) signaling promoting invasion is disrupted in androgen-sensitive prostate cancer cells by an interaction between EGFR and androgen receptor (AR). Int J Cancer 2004;112:78-86.

Bubendorf L, Konen J, Koivisto P, Schraml P, Moch H, Gasser TC, Willi N, Mihatsch MJ, Sauter G, Kallioniemi OP. Survey of gene amplications during prostate cancer progression by high-throughput fluorescence in situ hybridization on tissue microarrays. Cancer Res 1999;59:803-6.

Cai C, Portnoy DC, Wang H, Jiang X, Chen S, Balk SP. Androgen receptor expression in prostate cancer cells is suppressed by activation of epidermal growth factor receptor and ErbB2. Cancer Res 2009;69:5202-9.

Calvo BF, Levine AM, Marcos M, Collins QF, Iacocca MV, Caskey LS, Gregory CW, Lin Y, Whang YE, Earp HS, Mohler JL. Human epidermal receptor-2 expression in prostate cancer. Clin Cancer Res 2003;9:1087-97.

Canil CM, *et al.* Randomized phase II study of two doses of gefitinib in hormone-refractory prostate cancer: a trial of the National Cancer Institute of Canada-Clinical Trials Group. J Clin Oncol 2005;23:455-60

Chang CY, Walther PH, McDonnell DP. Glucocorticoids manifest androgenic activity in a cell line derived from a metastatic prostate cancer. Cancer Res 2001;61:8712-7.

Chen CD, Welsbie DS, Tran C, Baek SH, Chen R, Vessella R, Rosenfeld MG, Sawyers CL. Molecular determinants of resistance to antiandrogen therapy. Nature Med 2004;10:33-9.

Chodak GW, Kranc DM, Puy LA, Takeda H, Johnson K, Chang C. Nuclear localization of androgen receptor in heterogeneous samples of normal, hyperplastic, and neoplastic human prostate. J Urol 1992;147:798-803.

Craft N, Shostak Y, Carey M, Sawyers CL. A mechanism for hormone-independent prostate cancer through modulation of androgen receptor signaling by the HER-2/neu tyrosine kinase. Nature Med 1999;5:280-5.

Culig Z, Hobisch A, Cronauer MV, Cato AC, Hittmair A, Radmayr C, Eberle J, Bartsch G, Klocker H. Mutant androgen receptor detected in an advanced-stage prostatic carcinoma is activated by adrenal androgens and progesterone. Mol Endocrinol 1993;7:1541-50.

Culig Z, Hobisch A, Cronauer MV, Radmayr C, Hittmair A, Zhang J, Thurnher M, Bartsch G, Klocker H. Regulation of prostatic growth and function by peptide growth factors. Prostate 1996;28:392-405.

Culig Z, Hobisch A, Cronauer MV, Radmayr C, Trapman J, Hittmair A, Bartsch G, Klocker H. Androgen receptor activation in prostate tumor cell lines by insulin-like growth factor-1, keratinocyte growth factor, and epidermal growth factor. Cancer Res 1994;54:5474-8.

Curigliano G, *et al.* Gefitinib combined with endocrine manipulation in patients with hormone-refractory prostate cancer: quality of life and surrogate markers of activity. Anti-Cancer Drugs 2007;18:949-54.

De Vere White R, Meyers F, Chi SG, Chamberlain S, Siders D, Lee F, Stewart S, Gumerlock PH. Human androgen receptor expression in prostate cancer following androgen ablation. Eur Urol 1997;31:1-6.

Feldman BJ, Feldman D. The development of androgen-independent prostate cancer. Nature Rev Cancer 2001;1:34-45.

Ford OH III, Gregory CW, Kim D, Smitherman AB, Mohler JL. Androgen receptor gene amplification and protein expression in recurrent prostate cancer. J Urol 2003;170:1817-21.

Gassmann M, Casagranda F, Orioli D, Simon H, Lai C, Klein R, Lemke G. Aberrant neural and cardiac development in mice lacking the ErbB4 neuregulin receptor. Nature 1995;378:390-4.

Graham JD, Bain DL, Richer JK, Jackson TA, Tung L, Horwitz KB. Thoughts on tamoxifen-resistant breast cancer. Are coregulators the answer or just a red herring? J Steroid Biochem Mol Biol 2000;74:255-9.

Grandis JR, Sok JC. Signaling through the epidermal growth factor receptor during the development of malignancy. Pharmacol Therapeutics 2004;102:37-46.

Gravis G, Bladou F, Salem N, Goncalves A, Esterni B, Walz J, Bagattini S, Marcy M, Brunelle S, Viens P. Results from a monocentric phase II trial of erlotinib in patients with metastatic prostate cancer. Ann Oncol 2008;19:1624-8.

Gregory CW, Hamil KG, Kim D, Hall SH, Pretlow TG, Mohler JL, French FS. Androgen receptor expression in androgen-independent prostate cancer is associated with increased expression of androgen-regulated genes. Cancer Res 1998;58:5718-24.

Gregory CW, Johnson RT, Mohler JL, French FS, Wilson EM. Androgen receptor stabilization in recurrent prostate cancer is associated with hypersensitivity to low androgen. Cancer Res 2001;61:2892-8.

Gregory CW, He B, Johnson RT, Ford OH, Mohler JL, French FS, Wilson EM. A mechanism for androgen receptor-mediated prostate cancer recurrence after androgen deprivation therapy. Cancer Res 2001;61:4315-9.

Gregory CW, Wilson EM, Apparao KBC, Lininger RA, Meyer WR, Kowalik A, Fritz MA, Lessey BA. Steroid receptor coactivator expression throughout the menstrual cycle in normal and abnormal endometrium. J Clin Endocrinol Metab 2002;87:2960-6.

Gregory CW, Fei X, Ponguta LA, He B, Bill HM, French FS, Wilson EM. Epidermal growth factor increases coactivation of the androgen receptor in recurrent prostate cancer. J Biol Chem 2004;279:7119-30.

Gregory CW, Whang YE, McCall W, Fei X, Liu Y, Ponguta LA, French FS, Wilson EM, Earp HS III. Heregulin-induced activation of HER2 and HER3 increases androgen receptor transactivation and CWR-R1 human recurrent prostate cancer cell growth. Clin Cancer Res 2005;11:1704-12.

Gross M, Higano C, Pantuck A, Castellanos O, Green E, Nguyen K, Agus DB. A phase II trial of docetaxel and erlotinib as first-line therapy for elderly patients with androgen-independent prostate cancer. BMC Cancer 2007;7:142.

Heinlein CA, Chang C. Androgen receptor (AR) coregulators: an overview. Endocr Rev 2002;23:175-200.

Hernes E, Fossa SD, Berner A, Otnes B, Nesland JM. Expression of the epidermal growth factor receptor family in prostate carcinoma before and during androgen-independence. Br J Cancer 2004;90:449-54.

Hong H, Kohli K, Garabedian MJ, Stallcup MR. GRIP1, a transcriptional coactivator for the AF-2 transactivation domain of steroid, thyroid, retinoid, and vitamin D receptors. Mol Cell Biol 1997;17:2735-44.

Kinkade CW, Castillo-Martin M, Puzio-Kuter A, Yan J, Foster TH, Gao H, Sun Y, Ouyand X, Gerald WL, Cordon-Cardo C, Abate-Shen C. Targeting AKT/mTOR and ERK MAPK signaling inhibits hormone-refractory prostate cancer in a preclinical mouse model. J Clin Invest 2008;118:3051-64.

Lee SJ, Lee SD, Park JG, Kim CM, Ryu SH, Suh PG. Overexpression of phospholipase C-gamma 1 in colorectal carcinomas is associated with overexpression of factors that bind its promoter. J Biol Chem 1995;270:16378-16384.

Leung HY, Weston J, Gullick WJ, Williams G. A potential autocrine loop between heregulin-alpha and ErbB3 receptor in human prostatic adenocarcinoma. Br J Urol 1997;79:212-6.

Linja MJ, Savinainen KJ, Saramaki OR, Tammela TLJ, Vassella RL, Visakorpi T. Amplification and overexpression of androgen receptor gene in hormone-refractory prostate cancer. Cancer Res 2001;61:3550-5.

Liu Y, Majumder S, McCall W, Sartor CI, Mohler JL, Gregory CW, Earp HS III, Whang YE. Inhibition of HER-2/neu kinase impairs androgen receptor recruitment to the androgen responsive enhancer. Cancer Res 2005;65:3404-9.

Malinowska K, Neuwirt H, Cavarretta I, Bektic J, Steiner H, Dietrich H, Moser PL, Fuchs D, Hobisch A, Culig Z. Interleukin-6 stimulation of growth of prostate cancer in vitro and in vivo through activation of the androgen receptor. Endocr Rel Cancer 2009;16:155-69.

McKenna NJ, Lanz RB, O'Malley BW. Nuclear receptor coregulators: cellular and molecular biology. Endocr Rev 1999;20:321-44.

Mellinghoff IK, Vivanco I, Kwon A, Tran C, Wongvipat J, Sawyers CL. HER2/neu kinase-dependent modulation of androgen receptor function through effects on DNA binding and stability. Cancer Cell 2004;6:517-27.

Mendoza N, Phillips GL, Silva J, Schwall R, Wickramasinghe D. Inhibition of ligand-mediated HER2 activation in androgen-independent prostate cancer. Cancer Res 2002;62:5485-8.

Miettinen PJ, Berger JE, Meneses J, Phung Y, Pedersen RA, Werb Z, Derynck R. Epithelial immaturity and multiorgan failure in mice lacking epidermal growth factor receptor. Nature 1995;376:337-41.

Mohler JL, Gregory CW, Ford OH III, Kim D, Weaver CM, Petrusz P, Wilson EM, French FS. The androgen axis in recurrent prostate cancer. Clin Cancer Res 2004;10:440-8.

Montgomery RB, Mostaghel EA, Vessella R, Hess DL, Kalhorn TF, Higano CS, True LD, Nelson PS. Maintenance of intratumoral androgens in metastatic prostate cancer: a mechanism for castration-resistant tumor growth. Cancer Res 2008;68:4447-54.

Mukherjee B, Mayer D. Dihydrotestosterone interacts with EGFR/MAPK signaling and modulates EGFR levels in androgen receptor-positive LNCaP prostate cancer cells. Int J Oncol 2008;33:623-9.

Murphy LC, Simon SL, Parkes A, Leygue E, Dotzlaw H, Snell L, Troup S, Adeyinka A, Watson PH. Altered expression of estrogen receptor coregulators during human breast tumorigenesis. Cancer Res 2000;60:6266-71.

Nabhan C, Lestingi TM, Galvez A, Tolzien K, Kelby SK, Tsarwhas D, Newman S, Bitran JD. Erlotinib has moderate single-agent activity in chemotherapy-naïve castration-resistant prostate cancer: final results of a phase II trial. Urol 2009;74:665-71.

Onate SA, Tsai SY, Tsai MJ, O'Malley BW. Sequence and characterization of a coactivator for the steroid hormone receptor superfamily. Science 1995;270:1354-7.

Osman I, Scher HI, Drobnak M, Verbel D, Morris M, Agus D, Ross JS, Cordon-Cardo C. HER-2/neu (p185neu) protein expression in the natural or treated history of prostate cancer. Clin Cancer Res 2001;7:2643-7.

Pao W *et al.* EGF receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib. Proc Natl Acad Sci USA 2004;101:13306-11.

Peterziel H, Culig Z, Stober J, Hobisch A, Radmayr C, Bartsch G, Klocker H, Cato AC. Mutant androgen receptors in prostatic tumors distinguish between amino-acid sequence requirements for transactivation and ligand binding. Int J Cancer 1995;15:544-50.

Pignon J-C, Koopmacsch B, Nolens G, Delacroix L, Waltregny D, Winkler R. Androgen receptor controls EGFR and ERBB2 gene expression at different levels in prostate cancer cell lines. Cancer Res 2009;69:2941-9.

Ponguta LA, Gregory CW, French FS, Wilson EM. Site-specific androgen receptor serine phosphorylation linked to epidermal growth factor-dependent growth of castration-recurrent prostate cancer. J Biol Chem 2008;283:20989-21001.

Riethmacher D, Sonnenberg-Riethmacher E, Brinkmann V, Yamaai T, Lewin GR, Birchmeier C. Severe neuropathies in mice with targeted mutations in the ErbB3 receptor. Nature 1997;389:725-30.

Shah RB, Ghosh D, Elder JT. Epidermal growth factor receptor (ErbB1) expression in prostate cancer progression: correlation with androgen independence. Prostate 2006;66:1437-44.

Shaw G and Prowse DM. Inhibition of androgen-independent prostate cancer cell growth is enhanced by combination therapy targeting Hedgehog and ErbB signaling. Cancer Cell Int 2008;8:3.

Shi XB, Ma AH, Xia L, Kung HJ, de Vere White RW. Functional analysis of 44 mutant androgen receptors from human prostate cancer. Cancer Res 2002;62:1496-1502.

Signoretti S, Montironi R, Manola J, Altimari A, Tam C, Bubley G, Balk S, Thomas G, Kaplan I, Hlatky L, Hahnfeldt P, Kantoff P, Loda M. Her-2-neu expression and progression toward androgen independence in human prostate cancer. J Natl Cancer Inst 2000;92:1918-25.

Sirotnak FM, She Y, Lee F, Chen J, Scher HI. Studies with CWR22 xenografts in nude mice suggest that ZD1839 may have a role in the treatment of both androgen-dependent and androgen-independent human prostate cancer. Clin Cancer Res 2002;8:3870-6.

Small EJ, Fontana J, Tannir N, DiPaola RS, Wilding G, Rubin M, Iacona RB, Kabbinavar FF. A phase II trial of gefitinib in patients with non-metastatic hormone-refractory prostate cancer. BJU Int 2007;100:765-9.

Tan J-A *et al.* Dehydroepiandrosterone activates mutant androgen receptors expressed in the androgen-dependent human prostate cancer xenograft CWR22 and LNCaP cells. Mol Endocrinol 1997;11:450-9.

Tan J-A, Hall SH, Petrusz P, French FS. Thyroid receptor activator molecule, TRAM-1, is an androgen receptor coactivator. Endocrinol 2000;141:3440-50.

Taplin ME, *et al.* Androgen receptor mutations in androgen-independent prostate cancer: Cancer and Leukemia Group B Study 9663. J Clin Oncol 2003;21:2673-8.

Titus MA, Gregory CW, Ford OH III, Schell MJ, Maygarden SJ, Mohler JL. Steroid 5(α)-reductase isozymes I and II in recurrent prostate cancer. Clin Cancer Res 2005;11:4365-71.

Van der Kwast TH, Schalken J, Ruizeveld de Winter JA, van Vroonhover CC, Mulder E, Boersma W, Trapman J. Androgen receptors in endocrine-therapy-resistant prostate cancer. Int J Cancer 1991;48:189-93.

Visakorpi T, Hyytinen E, Koivisto P, Tanner M, Keinanan R, Palmberg C, Palotie A, Tammela T, Isola J, Kallioniemi OP. In vivo amplification of the androgen receptor gene and progression of human prostate cancer. Nature Genet 1995;9:401-6.

Voegel JJ, Heine MJ, Tini M, Vivat V, Chambon P, Gronemeyer H. The coactivator TIF2 contains three nuclear receptor-binding motifs and mediates transactivation through CBP binding dependent and –independent pathways. EMBO J 1998;17:507-19.

Weinberg RA. Growth factors, receptors, and cancer. In: The Biology of Cancer. New York: Garland Science, 2007.

Whang YE, Moore CN, Armstrong AJ, Rathmell W, Godley PA, Crane JM, Grigson G, Morris K, Watkins CP, George DJ. Phase II trial of lapatinib in hormone refractory prostate cancer. ASCO 2008, 156.

Wieduwilt MJ, Moasser MM. The epidermal growth factor receptor family: Biology driving targeted therapeutics. Cell Mol Life Sci 2008;1566-84.

Xu Y, Dalyrmple SL. Becker RE, Denmeade SR, Isaacs JT. Pharmacologic basis for the enhanced efficacy of dutasteride against prostate cancers. Clin Cancer Res 2006;12:4072-9.

Yarden Y, Sliwkowski MX. Untangling the ErbB signaling network. Nature Rev 2001;127-37.

Yeh SY, Lin HK, Kang HY, Thin TH, Lin MF, Chang C. From HER2/neu signal cascade to androgen receptor and its coactivators: a novel pathway by induction of androgen target genes through MAP kinase in prostate cancer cells. Proc Natl Acad Sci USA 1999;96:5458-63.

Ziada A, Barqawi A, Glode LM, Varella-Garcia M, Crighton F, Majeski S, Rosenblum M, Kane M, Chen L, Crawford ED. The use of trastuzumab in the treatment of hormone refractory prostate cancer: phase II trial. Prostate 2004;60:332-7.