The mechanisms and managements of hormone-therapy resistance in breast and prostate cancers

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Abstract

Breast and prostate cancer are the most well-characterized cancers of the type that have their development and growth controlled by the endocrine system. These cancers are the leading causes of cancer death in women and men, respectively, in the United States. Being hormone-dependent tumors, antihormone therapies usually are effective in prevention and treatment. However, the emergence of resistance is common, especially for locally advanced tumors and metastatic tumors, in which case resistance is predictable. The phenotypes of these resistant tumors include receptor-positive, ligand-dependent; receptor-positive, ligand-independent; and receptor-negative, ligand-independent. The underlying mechanisms of these phenotypes are complicated, involving not only sex hormones and sex hormone receptors, but also several growth factors and growth factor receptors, with different signaling pathways existing alone or together, and with each pathway possibly linking to one another. In this review, we will discuss the potential mechanisms of antihormone-therapy resistance in breast and prostate cancers, especially focusing on the similarities and differences of these two cancers. We will also discuss novel agents that have been applied in clinical practice or with clinical potential in the future.

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Introduction

The endocrine system is critical for growth, maturation, and coordination of the human body. However, malignancies can also arise from the organs influenced by endocrine-system-secreted hormones. Among these malignancies, the most widely studied are breast cancer and prostate cancer. Apart from skin cancers, breast cancer and prostate cancer are the primary diagnosed cancer and secondary cause of cancer death in women and men, respectively, in the United States (Jemal *et al.* 2004). These two types of cancer are not only similar in their epidemiological patterns but also possess similar pathological entities. Both of them are hormone-related cancers. They depend on specific steroid hormone receptors, such as estrogen receptor (ER),

progesterone receptor (PR), and androgen receptor (AR), to mediate hormone effects on the initiation and progression of diseases. With the improvement of diagnostic methodologies, the incidence of both cancer types have increased, which is most likely attributed to early diagnosis, but the cancer death rate of breast and prostate cancers has continued to decline since 1992 and 1995, respectively (Jemal *et al.* 2003). However, to date curative treatments for both advanced cancers have not yet been established. Understanding the underlying pathogenesis of disease progression is the pivotal prerequisite for developing effective therapeutic and preventive strategies of these two cancers.

Since ER and AR mediate the hormone effect on tumor initiation and progression in breast and prostate cancer, several selective ER modulators (SERMs;

Katzenellenbogen and Katzenellenbogen 2002, Jordan 2003*a*, 2003*b*) and selective AR modulators (SARMs) that specifically inhibit receptor function by means of hormone deprivation or hormone blockade have been applied in clinics as both therapeutic and preventive strategies. Tamoxifen (TAM), the most commonly used SERM, competes with estrogen having a higher binding affinity to ER. Although TAM-binding ER can also translocate into the nucleus, the TAM–ER transcription complex is incomplete and insufficient to initiate downstream target gene transcription which is required for estrogen-dependent tumor growth (Piccart *et al.* 2003). Flutamide (FLU) and bicalutamide are both pure antiandrogen agents; they inhibit androgen from binding to AR and its subsequent translocation (Brogden & Chrisp 1991). In order to get maximal androgen blockade, antiandrogen agents usually combine gonadotropin-releasing hormone (GnRH) agonist to suppress the compensatory-elevated luteinizing hormone (LH) and follicular-stimulating hormone (FSH; Labrie *et al.* 1993).

About 75% of breast cancer patients are ER- or PR-positive. TAM is the most frequently used drug as an adjuvant therapy for ER- and/or PR-positive breast cancer patients (Kiang & Kennedy 1977, Early Breast Cancer Trialists' Collaborative Group 1998, Fisher *et al.* 1998, Schiff *et al.* 2000).

After a 5-year administration of adjuvant TAM, the proportional recurrence reduction was 47%, and the corresponding mortality reduction was 26% (Early Breast Cancer Trialists' Collaborative Group 1998). TAM can also be used as a first-line therapy for metastatic breast cancer patients if their tumors are hormone receptor-positive and can be used as a chemoprevention agent for high-risk patients (Fisher *et al.* 1998). Although TAM is effective as an adjuvant and chemopreventive agent, there is still a significant proportion of patients who develop breast cancer or relapse breast cancer even after taking TAM, and the emergence of resistance in metastatic breast cancers is usually not preventable (Muss 1992). In prostate cancer, the majority of cases are AR-positive and androgen-ablation therapy by surgery, medicine, or combination is mandatory to those patients with locally advanced prostate cancer or metastatic disease. However, the response to hormone therapy is temporary and relapse is eventually inevitable. Chemoprevention is considered to be particularly important to reduce incidence of prostate cancer because of its diagnosis in elderly men, and even a modest delay in the neoplastic development could result in a substantial reduction in the incidence of this clinically detectable disease. Unlike breast cancer, chemoprevention for prostate cancer has just

emerged and under evaluation. A recent prostate cancer prevention trial has been done to show that finasteride, a 5α-reducatase inhibitor that inhibits the conversion of testosterone to a more potent androgen, dihydrotestosterone, has a chemopreventive effect for prostate cancer development (Thompson *et al.* 2003). However, in this study they point out that finasteride also increases the risk of high-grade prostate cancer. This result implies that hormone deprivation for prostate cancer may promote the phenotypic progression of those tumor cells that are able to survive the acute period of the therapy. The selection pressure driven by hormone therapy seems play the same roles in both breast and prostate cancer.

The phenotypes of these resistant/relapsed tumors can be roughly categorized into: (1) receptor-positive, hormone-dependent, but resistant to or even stimulated by the first-line antihormone agents; (2) receptor-positive, hormone-independent; and (3) receptor-negative, hormone-independent. Several mechanisms have been proposed for these phenotypes. However, a common scenario is that once the tumor develops resistance to antihormone therapy it will become more aggressive and correlate with poor prognosis. Therefore understanding the mechanism of resistance emergence and the methods to overcome the resistance is critical for the treatment of hormone-therapy-resistant tumors. In this review, we will discuss the similarities and differences between breast cancer and prostate cancer in the development of hormone resistance and the therapeutic options (Table 1).

Sex hormone — sex hormone receptor-modulating mechanisms

In human beings, secretion of sex hormones is controlled by the hypothalamus-pituitary-sex gland axis. For premenopausal women, ovaries produce almost all of the estrogen, and the primary estrogen produced is 17β-estradiol; for postmenopausal women, peripheral tissues, such as the adrenal gland, peripheral adipose tissue, and even the breast itself are the main organs producing estrogen. In men, the testes produce the majority of circulating testosterone, which is converted into the more powerful dihydrotestosterone (DHT) by 5α-reductase in target organs. A small portion of testosterone is produced by adrenal glands. Although the secretion of testosterone will decrease during aging, testes are still the major source of androgen in the elderly (Table 2).

Both ER and AR belong to the steroid nuclear receptor superfamily. The basic domain structures of

Table 1 Resistance mechanism and corresponding therapeutic countermeasures

Receptor	Ligand dependence	Resistance mechanism	Therapeutic countermeasures
ER-/AR-positive	Positive	Receptor amplification Increased circulating hormone Increased endogenous hormone Receptor hypersensitivity Changing ratio of coregulators	Total hormone ablation by antihormone agent and GnRH agonist Aromatase inhibitor (BC) Fulvestrant (BC) MAPK-pathway inhibitors Chemotherapy SERM SARM
ER-/AR-positive	Negative	Receptor mutation Crosstalk with other growth factors and receptors and receptors Bypass receptors	Hsp90 inhibitor Anti-EGFR antibodies (IMC-C225, ABX-EGF) Anti-Her-2 antibodies (trastuzumab, 2C4) Tyrosine kinase inhibitors (gefitinib, OSI-774, CI-1033, PKI166, GW572016) Emodin E1A Bcl-2 antisense Chemotherapy
ER-/AR-negative	Negative	Loss of receptors	Chemotherapy

BC, breast cancer; EFGR, epidermal growth factor receptor; Hsp, heat-shock protein; MAPK, mitogen-activated protein kinase.

Table 2 The major hormone source and antihormone therapy agents of breast cancer and prostate cancer

	Breast cancer	Prostate cancer
Major sex hormone	17β-Estradiol	DHT
Major source of sex hormone	Premenopause: ovary Postmenopause: peripheral adipose tissue, adrenal gland, breast	Majority: testis Minority: adrenal gland
First-line antihormone agents	TAM Aromatase inhibitor	FLU GnRH agonist
Chemoprevention agents	TAM Aromatase inhibitor	Finasteride

the nuclear receptor superfamily include an N-terminal region with the activation function 1 (AF-1) domain, a DNA-binding domain (DBD) with two zinc-finger structures, and a hinge region and ligand-binding domain (LBD) at the C-terminus (Fu *et al.* 2003). The LBD includes the activation function 2 (AF-2) domain and 12 α-helices that project away from the hormone-binding groove in the absence of ligand. Without ligand binding, ER/AR associates with chaperone proteins, heat-shock proteins (Hsps), and the immunophilin complex. After ligand binding, receptors dissociate from chaperone proteins, and form homodimers or heterodimers that can bind to the estrogen- and androgen-response elements in the promoter regions of target genes. This will release corepressors and recruit coactivators, thereby forming a transcriptional complex and initiating target gene transcription (Feldman & Feldman 2001, Osborne *et al.* 2001, Isaacs *et al.* 2003)

There are two ER genes on different chromosomes, ER-α on chromosome 6q25.1, and ER-β on chromosome 14q22-25. ER-α is usually the dominant isoform and correlates with most of the prognostic factors in breast cancers (Fuqua *et al.* 2003). Both proteins have compatible binding affinities for estradiol and similar binding domains for ligand and DNA; they also have two transcriptional domains, AF-1 and AF-2. The transcriptional function of AF-1 and AF-2 is tissue- and promoter-specific. The transcription function of AF-1 is ligand-independent, and it is closely related to the phosphorylation status of ER, and can be induced by mitogen-activated protein kinases (MAPKs), growth factors and oncogenes (Fu *et al.* 2003), while

AF-2 is completely ligand-dependent (Kato *et al.* 2000). AF-1 and AF-2 of ER can activate transcription independently or synergistically. ER-α and ER-β have opposite effects on transcription initiated by AP-1, SP-1, or cAMP-response elements (CREs; Paech *et al.* 1997, Castro-Rivera *et al.* 2001, Liu *et al.* 2002). Especially in a low-estrogen environment such as in cases of aromatase-inhibitor treatment, the inhibitory effect of ER-β could become more prominent as an antitumor mechanism (Jordan 2003*a*). Thus compounds that are antagonists for ER-α may be agonists for ER-β at these sites. However, the function and prognostic significance of ER-β is still not clear (Hall & McDonnell 1999, Palmieri *et al.* 2004, Speirs *et al.* 2004). For those breast cancer cells with ER expression, the ratio between ER-α and ER-β may change during carcinogenesis, it may also serve as an important marker in predicting the response to certain kinds of specific selective estrogen-modulator therapy (Dotzlaw *et al.* 1999). In contrast to ER, no AR isoform has been found to date. Without ligand binding, ER mainly stays in nucleus but the majority of AR stays in cytoplasm. Upon ligand binding, the AR homo-dimer will translocate into the nucleus. In addition, androgens can stabilize AR protein level 6-fold compared to the level without ligand binding. However, estrogen binding will accelerate ER degradation. These biological differences between ER and AR could be used as different targeting strategies (Table 3).

Mechanisms of resistance

There are several mechanisms that have been proposed to be responsible for the emergence of hormone resistance in breast cancers and prostate cancers. These resistance mechanisms can occur at the pre-receptor level, such as a change in the hormone level, at the receptor level, or at the post-receptor level. Based on the three major phenotypes mentioned above, we summarize several possible mechanisms for the development of resistance (Figure 1). These events can occur alone or together, dependent on the individual case,

and therefore the next step of therapy after the emergence of resistance should depend on the underlying mechanism for the resistance.

Receptor-positive, ligand-dependent

Enhancing receptor expression

Long-term hormone deprivation may select for cancer cell clones that enhance the expression of receptors to compensate for the low-level ligand environment. In long-term estrogen deprivation of MCF-7 cells, ER-α was found to be upregulated 4–10-fold (Santen *et al.* 2003). The same situation was reported for ER-β from clinical samples (Speirs *et al.* 1999). In prostate cancer AR gene amplification is rarely found in primary cancer; after androgen-ablation therapy, approximately 30% of tumors become androgen independent due to an increase of AR expression (Feldman & Feldman 2001). Additionally, the gain of AR gene copies in primary prostate cancer due to X-chromosome polysomy was observed (Ropke *et al.* 2004). This may due to the selective effect that comes from the low level of androgen after androgen ablation, which favors clonal expansion of cancer cells expressing a higher level of AR. These tumors are still hormone-dependent, and they may respond to second-line therapy with total hormone ablation.

Increased circulating hormone

TAM binds to ER, and FLU binds to AR to antagonize sex hormones in the tumors; however, they also bind to the receptors in pituitary gland and hypothalamus, which may interrupt the negative-feedback pathways of sex hormones. As a result GnRH is secreted continuously, ultimately producing a hyperstimulatory effect on the ovaries or testes resulting in oversecretion of estrogen or androgen. This may explain the elevated estradiol levels that were noted in some premenopausal breast cancer patients after TAM administration (Ravdin *et al.* 1988). To overcome this problem, GnRH analogues can be combined with antihormone agents to reach total estrogen or androgen ablation (Robertson & Blamey 2003).

Table 3 Comparison of ER and AR

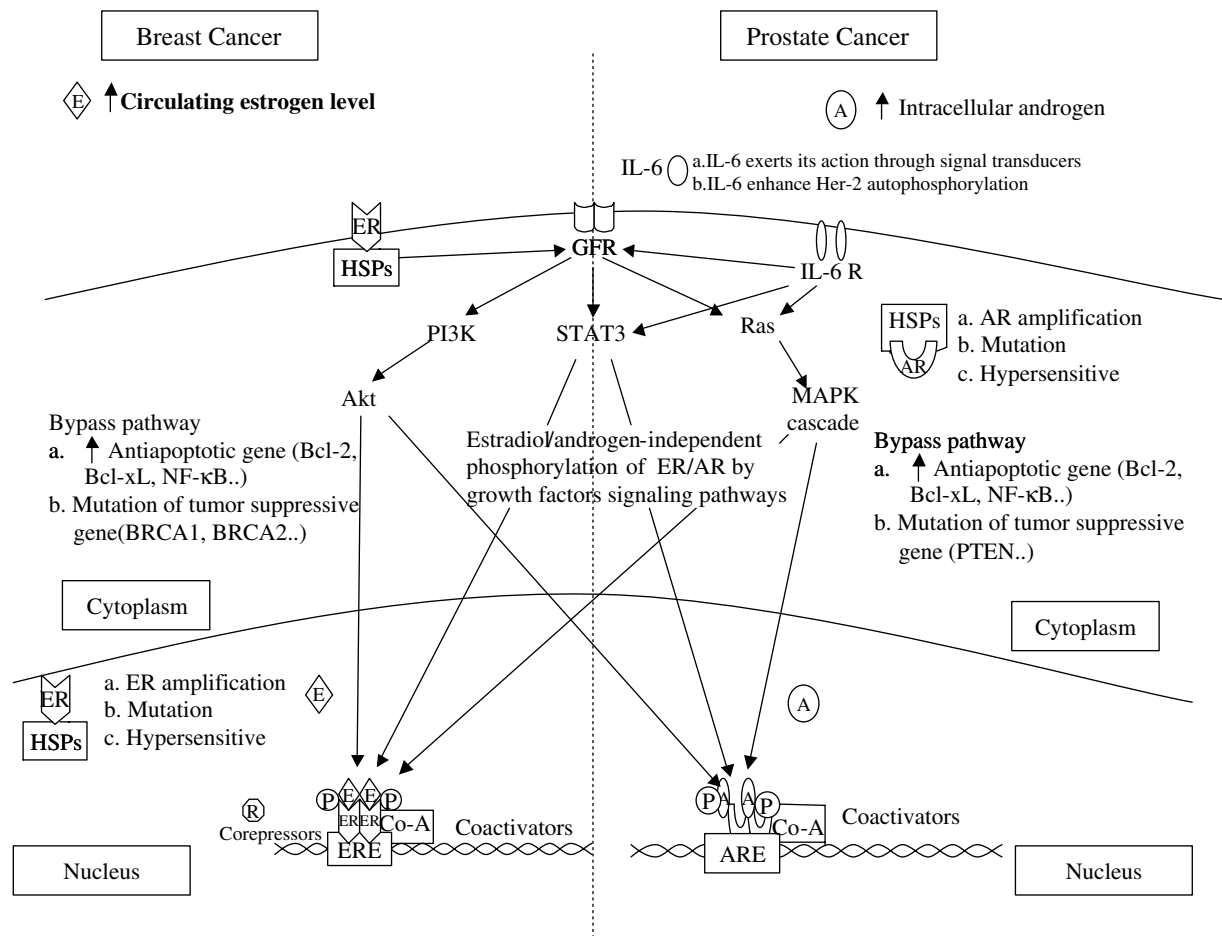|	ER	AR
Expression before treatment	75%	>90%
Mutation as the cause of resistance	<10%	30%
Location before ligand binding	Nucleus	Cytoplasm
Isoform	ER-α/ER-β	No isoform
Dimer formation after ligand binding	Homo-/heterodimer	Homodimer only
Stability after ligand binding	Accelerated degradation	More stable

Figure 1 Summary of several mechanisms that have been proposed to be responsible for the emergence of hormone resistance in breast cancers and prostate cancers. These mechanisms make up the three phenotypes. A androgen, AR androgen receptor, ARE androgen responsive element, Co-A coactivator, E estrogen, ER estrogen receptor, ERE estrogen responsive element, GFR growth factor receptor, IL-6 interleukin 6, IL-6R interleukin 6 receptor, PI-3K phosphatidylinositol 3-kinase, R corepressor, STAT3 signal transducer and activator of transcription 3.

Increased endogenous hormone

In females, estrogen production can be converted from androgen by aromatase in the breast. The transcriptional control of aromatase is different between normal tissue and breast cancer tissue (Harada *et al*. 1993). Increased aromatase expression and activity have been noted in breast tumors compared to peritumor tissues (Goss & Strasser 2002). This increased production of aromatase comes from the interaction between breast cancer cells and associated fibroblasts and inflammatory cells in the adjacent stroma. Breast cancer cells synthesize prostaglandin E_2, and inflammatory cells produce not only prostaglandin E_2 but also interleukin 6 (IL-6), IL-11, and tumor necrosis factor α (TNFα), all of which stimulate fibroblasts to produce aromatase. This mechanism is related closely to cyclooxygenase 2 (COX-2; Johnston & Dowsett 2003). Also in long-term

estrogen-deprived breast cancer cells, aromatase activity increases adaptively (Yue *et al*. 1999). Both of these cases lead to higher local estrogen concentration and can overcome the effect of anti-estrogen agents. This may explain in part why aromatase inhibitors elicit a better clinical response than TAM in clinical studies (Wong & Ellis 2004).

In males, long-term androgen-ablation therapy may select prostate cancer cells with higher 5α-reductase activity, which can produce more DHT from adrenal androgen, thus providing a higher intracellular DHT level to compensate for the low level of circulating testosterone (Navarro *et al*. 2002). Men of African descent, who have the highest incidence of a polymorphism in the gene of 5α-reductase in which a valine residue at codon 89 is substituted by a leucine, have been reported to have higher 5α-reductase activity and

have a particularly high incidence of prostate cancer with poor prognosis (Ruijter *et al.* 1999).

Receptor hypersensitivity

Receptors may have congenital or acquired mutations that change their sensitivity to ligands. A study of a typical breast hyperplasia found that a mutation of ER-α (Ala-908→Gly) affects the border of the hinge and hormone-binding domains of ER-α and shows increased sensitivity to estrogen. This mutation may promote or accelerate the development of cancer from premalignant breast lesions (Fuqua *et al.* 2000). Long-term deprivation of estrogen, for instance with TAM treatment, can induce hypersensitivity of breast cancer cells to estradiol (Berstein *et al.* 2004). Such adaptive hypersensitivity may go through a rapid, nongenomic plasma membrane receptor-mediated pathway: estradiol binds to ER-α, then phosphorylates Shc, Shc then binds to Grb-2 and SoS, resulting in the rapid activation of MAPK through Ras, Raf, and MAPK/extracellular-signal-regulated kinase (ERK) kinase (MEK), and then the phosphorylation of AF-1 on ER-α (Santen *et al.* 2003). Several agents can block this pathway, including: aromatase inhibitors, which block the estrogen production from the peripheral tissue; the pure anti-estrogen fulvestrant (Howell 2001); farnesyl-thiosalicylic acid, an inhibitor of GTP-Ras binding to its membrane acceptor site (Berstein *et al.* 2003); and the MEK inhibitor U0126 (Martin *et al.* 2003).

Upregulation of AR sensitivity to low-level androgen was also found in a prostate cancer animal model (Gregory *et al.* 2001*b*). Under androgen-ablation conditions, AR from the recurrent prostate cancer was highly expressed, with increased stability and nuclear localization, making the tumor cells more sensitive to the growth-promoting effect of DHT. The concentration of DHT needed for growth stimulation was four orders of magnitude lower in androgen-independent prostate cancer (AIPC) cells than in androgen-dependent LNCaP cells. Also chronic activation of Ras/MAPK signaling could cause or contribute to the development of AIPC cells (Bakin *et al.* 2003).

Coregulator regulation in breast and prostate cancers

In recent years a large number of nuclear and steroid receptor coregulators, including coactivators and corepressors, have been cloned and characterized to regulate receptor-mediated transactivation. After ligands bind their receptors, these coregulators are recruited to the promoters of target genes through protein — protein interaction, enhancing or reducing the nuclear receptor-mediated transcription of responsive genes (Klinge 2000). Coactivators are protein complexes with intrinsic histone acetyltransferase activity that affect transcription by modifying the chromatin structure in a ligand-dependent manner; corepressors are proteins associated with unligated nuclear receptors that recruit histone deacetylase complexes and inhibit transcription. The extent and direction of transcription of responsive genes are influenced not only by the types of ligands, but also by specific coregulators. The ratio of coactivators to corepressors may also determine the direction of gene transcription. As shown in Table 4, many coactivators have been identified as enhancing the ligand-induced transcriptional activity for both AR and ER. The most well-characterized is the steroid receptor coactivator (SRC) family, which contains SRC-1, transcriptional intermediary factor 2 (TIF2), and Amplified in Breast Cancer (AIB1)/SRC-3. Members of the SRC family of coactivators typically interact with the LBD of nuclear receptors through LXXLL motifs (where L is leucine and X is any amino acid) that form α-helices. The LXXLL domains of the coactivators interact with the nuclear receptor partly through the hydrophobic surface of the receptor AF-2 domain. The ER dimer binds to SRC-1 through an interaction between the ER LBD and the LXXLL motifs of SRC-1. SRC-1 and TIF-2 interact primarily with the AR N-terminus and possibly the DBD and this interaction, in contrast to several other nuclear receptors, does not require the coactivators to contact intact LXXLL motifs (Spencer *et al.* 1997, Bevan *et al.* 1999, He *et al.* 1999). Although the crystal structure of AR suggests that ligand-binding induced LBD conformations similar to ER and potentially generates a similar coregulator interaction surface, functional analyses of the full-length receptors suggest that distinct differences exist between the coregulator interaction domains of AR and ER. This may be because the interaction between the AR N-terminus and the LBD generates a potential coregulator in interaction structure that differs from that of ER. Since different target cells express different levels of coactivators and corepressors, which accounts for cell-specific regulation of responsive gene expression, increased activity of coactivators can lead to the emergence of resistance. Additionally, coactivators can interact with other transcriptional factors (Lee *et al.* 1998), and as a result they also enhance transcription without ligand binding. Several coactivators are important both in breast cancer and prostate cancer for the development of hormone independence. Disruption of SRC-1 results in partial hormone resistance, particularly to thyroid hormone (Xu *et al.* 1998). In

Table 4 The common AR and ER coregulators

Transcriptional coregulator	Other nuclear receptor targets	Functions	Selected references
SRC-1 (NcoA-1)	GR, PR, TR, RXR	Unlike ER and other receptors, which interact with SRC-1 through their LBD, AR interacts through its N-terminal and DBDs. Interacts with CBP/p300 and possesses weak acetyltransferase activity.	Bevan *et al.* 1999, He *et al.* 1999, Spencer *et al.* 1997
AIB1/SRC-3 (Rac3, ACTR, TRAM1, p/CIP)	PR, TR, RAR	Interact with CBP/p300 and possess acetyltransferase activity.	Anzick *et al.* (1997), Chen *et al.* (1997), Yeh *et al* (1998).
RIP140	PPARγ, PPARα	Influences the transcriptional activity of PPARγ and PPARα. May recruit histone deacetyltransferases.	Bevan *et al.* (1999), Treuter *et al.* (1998)
SRA	PR, GR	Enhances transactivation through AF-1 domain of GR and PR. Functions as an RNA transcript and associated with an SRC-1-containing coregulator complex.	Lanz *et al.* (1999)
p300/CBP, pCAF	TR, RAR, RXR	General coregulators with intrinsic histone acetyltransferase activity. Facilitates the AR N/C interaction.	Aarnisalo *et al.* (1998), Chakravarti *et al.* (1996), Ikonen *et al.* (1997)
BRCA1	PR	Breast cancer susceptibility gene. Interacts with CBP. Enhances AR transactivation activity but reduces ER transactivation activity.	Fan *et al.* (2001), Miki *et al.* (1994), Yeh *et al.* (2000)
Src	ERβ	Src tyrosine kinase activity stimulated by E2 and DHT for non-genomic steroid action.	Kousteni *et al.* (2001), Migliaccio *et al.* (2002)
TIF2 (GRIP1, NcoA2)	TR,VDR, RAR, GR, PR	Interacts with CBP and functions as a general coregulator for nuclear receptors.	Berrevoets *et al.* (1998), He *et al.* (1999),
Tip60	PR	Interacts with AR through Hing LBD domain. A member of the MTST/SAS family of histone acetyltransferases. Also functions as coactivator for PR.	Hong *et al.* (1997), Brady *et al.* (1999)
Zac-1	TR, GR	Interacts with AR through LBD domain. In Hela cells, coactivation is synergistic with TIF2.	Huang & Stallcup (2000)
ARA267 (NSD1)	TR, RAR, RXR	Interacts with AR through both N- and C-terminal domains. Contains SET and PHD domains.	Huang *et al.* (1998), Wang *et al.* (2001)
ARA70 (RFG, ELE1)	PPARγ, GR	Enhances the transactivation of both wild-type AR and AR (T877A) in response to DHT and E2. Functions as a bridge factor for TFIIB and p/CAF.	Heinlein *et al.* (1999), Yeh & Chang (1996), Yeh *et al.* (1998)

PPAR, peroxisome proliferator-activated receptor; RAR, retenoic acid receptor; RXR, retenoid X receptor; GR glucocorticoid receptor; TR thyroid receptor; VDR Vitamin D receptor; CBP, cAMP-response-element-binding protein; E2: 17 beta-estradiol; MYST/SAS: (*M*02, *Ybf2/Sas3, Sas2,* and *TIP* 60)/(something about silencing) domain; SET (Su(var)3-9, Enhancer-of-zeste, trithorax) methyl transferase domain; PAD: plant homeodomain.

addition, results from different groups indicated that SRC-1 is involved in the progression of prostate cancers. Using reverse transcriptase PCR, Fujimoto and colleagues (2001) found that the expression levels of SRC-1 were higher in higher-grade prostate cancers or cancers with a poor response to endocrine therapy. At the same time, it has been reported that SRC-1 expression was elevated, together with the expression of AR, in recurrent prostate cancers (Gregory *et al.* 2001*a*). Previous studies found that SRC-2 is also overexpressed in recurrent prostate cancers. Over-expression of SRC-1 and SRC-2 confers on AR an increased sensitivity to the growth-stimulating effects of low-androgen concentrations. This change may contribute to prostate cancer recurrence after androgen-deprivation therapy. High levels of SRC-1 in uterus and breast are known to enhance the agonistic effect of TAM (Katzenellenbogen & Katzenellenbogen 2002); SRC-1 can also interact synergistically with CRE-binding protein to activate ER-mediated transcription in a ligand-independent manner (Jackson *et al.* 1997), and this activity can be inhibited by corepressor SMRT (Smith *et al.* 1997).

In TAM-treated breast cancer patients, high AIB1/SRC-3 expression was associated with worse disease-free survival, which is indicative of TAM resistance (Osborne *et al.* 2003). Compared with the widespread expression of SRC-1 and SRC-2, expression of AIB1/SRC-3 is restricted to few tissues, including the uterus, the mammary gland and the testis (Suen *et al.* 1998). Disruption of AIB1/SRC-3 gene in mice causes severe growth and reproductive defects, such as the retardation of mammary gland development (Xu *et al.* 2000). Amplification and overexpression of AIB1/SRC-3 in human breast and ovarian cancers have been observed (Anzick *et al.* 1997, Chen *et al.* 1997, Yeh *et al.* 1998). It has been reported that the AIB1/SRC-3 amplification/overexpression was correlated with ER and PR positivity (Bautista *et al.* 1998).

Breast cancer (BRCA1) is a breast cancer-susceptibility gene, and its mutations are correlated with an increased risk of breast and ovarian cancers (Martin *et al.* 2001). Interestingly, BRCA1 was shown to function as a ligand-independent corepressor for ER and PR and enhance the ligand-dependent AR trans-activation in the presence of exogenous SRC family members (Miki *et al.* 1994, Yeh *et al.* 2000, Fan *et al.* 2001). Thus, it is reasonable to speculate that the normal expression of BRCA1 probably protects the breast from tumorigenesis by suppressing the ER and PR signaling pathway and promoting AR activity in prostate cancer development. The ARAs, AR-associated proteins, is a group of factors that can bind to AR and modulate its transcriptional activity. Based on their molecular masses, these factors were named ARA70, ARA160, ARA54, ARA55, ARA267 and ARA24 (Kang *et al.* 1999, Yeh *et al.* 1999). Studies on the expression patterns of ARA70 in different cell lines and human cancer samples showed that the expression of ARA70 was decreased in prostate cancer (Yeh *et al.* 1999) and breast cancer (Kollara *et al.* 2001). In breast, loss of ARA70 protein expression was found in 60% of Human Epidermal growth factor receptor (HER)2-positive breast cancers, whereas only 33% of HER2-negative breast cancer samples lost the expression (Kollara *et al.* 2001). Since androgen plays an inhibitory role for breast cancer cell growth, and HER2 stimulates the growth of breast cancers, loss of the expression of AR and/or ARA70 in breast might confer a growth advantage to these cells. In prostate, ARA70 mRNA is highly expressed in the normal epithelial cells, while benign prostatic hyperplasic and cancer cell lines express either lower or no ARA70 (Kollara *et al.* 2001).

Receptor-positive, ligand-independent

Receptor mutations

Mutations of receptors can occur during the initiation of tumor formation, or can develop after therapy begins. Mutations in the ligand-binding domains of receptors may change not only their binding affinity and sensitivity to circulating ligands, but also the specificity of the receptors to their ligands. In this case receptors are able to bind substitutes such as other circulating steroid hormones, or even antihormone agents such as TAM or FLU to stimulate cell growth.

In breast cancer, ER mutation is not a major cause of hormone resistance since it is seen in fewer than 10% of TAM-resistant breast cancer patients (Achuthan *et al.* 2001). However, in prostate cancer, AR mutations are found in about 30% of the metastases that are resistant to hormone therapy (Navarro *et al.* 2002). In addition, the mutation rate is significantly increased in metastatic sites compared to primary tumors (Marcelli *et al.* 2000), especially after androgen-ablation therapy (Taplin *et al.* 1995). Many of the mutations are gain of function and are located in the ligand-binding domain, which results in inappropriate activation of AR by steroid hormones other than androgen and AR antagonists (Buchanan *et al.* 2001). For instance, several mutant ARs such as Thr-877→Ala, Leu-701→His, and Leu-701→His/Thr-877→Ala have a broadened spectrum of ligand responsiveness, and bicalutamide works as an agonist for these mutants (Hara *et al.* 2003). These mutations are considered to be responsible for the phenomenon

of 'antiandrogen withdrawal syndrome' (Nelson *et al.* 2003).

ER/AR crosstalk with other growth factors

During the progression from hormone-dependent to independent cancers, the crosstalk between ER/AR and other growth factor pathways is a complicated issue. There are several growth factors and their receptors involved in this process. The crosstalk between growth factor pathways and ER/AR occurs at multiple levels and is bidirectional with different importance in breast and prostate cancers. Novel therapies against these growth factors are emerging as alternative choices for the prevention and treatment of hormone-resistant breast cancer or prostate cancer.

Many of the estrogen-responsive genes code for peptide growth factors, membrane-bound tyrosine kinase receptors (TKRs), and several cellular signaling molecules, and usually their transcription is inversely correlated with ER expression (Schiff *et al.* 2004). On the contrary, the genotropic activity of ER is enhanced by several growth factor signaling pathways, including epidermal growth factor (EGF), insulin-like growth factor type I (IGF-1), and transforming growth factor-α (TGF-α), which enhance the phosphorylation of AF-1 on Ser-118 of ER by MAPK. This phosphorylation enhances the nuclear localization of coactivators and their interaction with nuclear ER, producing ligand-independent transcriptional activity of AF-1, even in the presence of TAM, and is related to antihormone resistance (Kato *et al.* 1995, Schiff *et al.* 2003). Thus, increasing the expression of growth factors or upregulation of their receptors or the downstream signaling elements can promote antihormone-therapy resistance.

Membrane ERs can also function like growth factor receptors by binding to p85, a regulatory subunit of phosphoinositide 3-kinase (PI3K) at the cell membrane, leading to activation of the protein kinase Akt and the subsequent downstream signaling (Migliaccio *et al.* 2002). Therefore, membrane ER enhances the transcriptional activity of nuclear ER through a nongenotropic pathway. Both genotropic and non-genotropic actions seem to be complementary, even synergistic, not only for cellular growth but also for the emergence of hormone resistance in a low-estrogen environment (Schiff *et al.* 2003). In summary, ER may suppress the expression of other growth factor receptors and long-term estrogen suppression can reactivate the expression of membrane TKRs. This results in increased growth factor signaling, and ERK1/2 MAPK, and PI3K/Akt activities, alteration of ER subcellular localization and enhancement of

the nongenotropic action, stimulating the malignant phenotype (Kumar *et al.* 2002, Schiff *et al.* 2004). More and more evidence suggests that the crosstalk between ER and these signaling pathways is upregulated or activated in endocrine-resistant breast cancers, and may be the major cause of endocrine resistance (Johnston *et al.* 2003). In an *in vitro* study, dual inhibition of MAPK with U-0126 and PI3K with Ly294002 have been found to decrease the sensitivity of ER to estradiol (Yue *et al.* 2003).

Among all the growth factors and growth factor receptors, the EGF receptor (EGFR) family seems to play a major role in promoting hormone refractory transition, in particular HER-2/*neu* is well known to associate with poorer prognostic phenotypes including high-grade histology, high proliferation rate, and ER negativity. There is also a tendency for HER-2/*neu*-overexpressed breast cancer to be less responsive to anti-estrogen therapies (Revillion *et al.* 1998). Introducing HER-2/*neu* cDNA into breast cancer cells promotes ligand-independent downregulation of ER, and converts cancer cells from estrogen-dependent to estrogen-independent (Pietras *et al.* 1995). Its signaling pathway can also disrupt the TAM-induced interaction of ER with the transcriptional corepressor N-CoR (Kurokawa *et al.* 2000, Kurokawa & Arteaga 2001). High HER-2/*neu* expression constitutively activates PI3K/Akt. Active Akt renders MCF-7 cells from estrogen-dependent to -independent, and treating these cells with TAM actually stimulates instead of inhibiting their growth (Faridi *et al.* 2003). Since HER-2/*neu* overexpression is closely related with MAPK hyper-activity and TAM resistance, inhibiting MAPK can reverse TAM resistance in HER-2/*neu*-overexpressed breast cancer cells (Kurokawa *et al.* 2000).

The crosstalk between AR and growth factor signaling pathways of prostate cancer is very similar to that of breast cancer (Nelson *et al.* 2003). EGF, IGF-I, and keratinocyte growth factor all activate AR, especially IGF-I, and the AR antagonist casodex blocks this activation completely (Culig *et al.* 1994). This indicates that the activation is AR-dependent. Membrane-bound TKRs, especially HER-2/*neu*, were also observed to be involved in the progression to AIPC, as overexpression of HER-2/*neu* increases MAPK and Akt activities, phosphorylates AR, and then turns on downstream target genes in a ligand-independent manner (Lin *et al.* 2001).

The cytokine IL-6 is also related to the growth of breast and prostate cancers and the emergence of hormone resistance through its interaction with TKRs and intracellular signaling pathways. In both breast cancer and prostate cancer, elevated circulating levels

of IL-6 are associated with worse prognosis, especially in AIPC (Drachenberg *et al.* 1999, Salgado *et al.* 2003). Serum IL-6 levels are significantly elevated in hormone-refractory prostate cancer patients as compared with earlier stages of the disease or with benign prostate hyperplasia (Drachenberg *et al.* 1999). In breast cancer, IL-6 increases intracellular aromatase activity (Honma *et al.* 2002). IL-6 also facilitates the formation of bone metastasis of both breast cancer and prostate cancer by stimulating osteoclasts (Roodman 2001). In prostate cancer, IL-6 is the most potent nonsteroidal regulator of AR activity. It alone causes the activation of AR to approximately 50% of the maximal activity induced by androgen (Culig 2003). IL-6 regulation of AR activity and prostate cancer growth occurs through MAPK and signal transducer and activator of transcription 3 (STAT3; Chen *et al.* 2000, Culig *et al.* 2002). It can also stimulate the autophosphorylation of HER-2/*neu* in prostate cancer cells and subsequently activate the downstream kinase signaling pathways (Qiu *et al.* 1998). Thus, IL-6 is important in mediating and enhancing the transition from hormone-dependent to hormone-independent of breast and prostate cancer cells.

Since long-term estrogen deprivation induces the expression of growth factors and growth factor receptors, and overexpressed growth factors and growth factor receptors facilitate the emergence of antihormone resistance, the question arises as to whether it is possible to not only treat antihormone-resistant cancer, but also to prevent the development of resistance by early treatment with anti-growth factor agents, either alone or in combination with anti-estrogen agents. Several experiments using such combinations have been reported, and have shown promising results (Schiff *et al.* 2004).

Receptor-positive, receptor/ligand-independent (bypass pathway)

The mechanisms involved in hormone-refractory transition are complicated. Several complementary or alternative pathways may occur simultaneously, some of which are capable of bypassing receptors completely. Inhibition of genotropic or nongenotropic pathways can lead to apoptosis in breast and prostate cancer cells. Blocking the apoptotic cascades by enhancing antiapoptotic genes, decreasing proapoptotic genes or through mutations in oncogenes or tumor-suppressor genes all are possible mechanisms for the bypass pathway.

The antiapoptotic gene Bcl-2 is overexpressed in more than half of all human cancers. Overexpression of Bcl-2 occurs in 40–80% of human breast tumors (Nahta & Esteva 2003) and also occurs frequently in prostate cancers. Overexpression of Bcl-2 is associated with the resistance to hormonal therapy and chemotherapy in both breast cancers and prostate cancers. This phenotype may come from the selection effect of antihormone agents. Treatment with Bcl-2 antisense in a prostate cancer animal model delays the emergence of androgen independence (Gleave *et al.* 1999). Transcriptional factor nuclear factor κB (NF-κB) modulates the expression of genes involved in cell proliferation, differentiation, apoptosis, and metastasis. Constitutive activation of NF-κB is noted in ER-negative breast cancer cells, in TAM-resistant MCF-7 cells (Gu *et al.* 2002), and during breast cancer progression to hormone-independent growth (Nakshatri *et al.* 1997).

Mutation or decreased expression of tumor-suppressor genes is also an alternative pathway. Tumor-suppressor genes are involved in the DNA-repair process to maintain genomic integrity, cell-cycle control, and induction of apoptosis in damaged cells and regulation of transcription. Failure of tumor-suppressor genes increases the probability of accumulation of replication errors and genomic instability, making cells less responsive to apoptotic signals, and contributing to an increase in hormone-independence and tumor aggressiveness. The most common mutated tumor-suppressor gene is p53 (Levine *et al.* 1991). In hormone-refractory prostate cancers p53 was positively increased during hormonal therapy from 17% of untreated primary tumors to 40% of hormone-refractory recurrences. The percentage of mutations was significantly higher in cases of AR gene amplification (Koivisto & Rantala 1999). PTEN (phosphatase and tensin homologue deleted on chromosome 10) is another tumor-suppressor gene that has been noted to be mutated in both breast cancer and prostate cancer (Li *et al.* 1997). In breast cancer, BRCA1 and BRCA2 mutations are well known for hereditary and sporadic breast cancers (Wooster & Weber 2003). Recently these two genes were found to be related to prostate cancer development (Rosen *et al.* 2001).

Receptor-negative, hormone-independent

Since ER/AR are responsible for the ligand-induced signal transduction, ER/AR are also the main targets of antihormone agents. With decreased expression of ER/AR these therapeutic agents lose their targets, which may be a cause of resistance. In fact, absence of ER expression is the most common mechanism of *de novo* resistance in breast cancers, whereas a complete loss of

ER expression is not common in acquired resistance (Clarke *et al.* 2003). Decreased expression of receptors usually comes from hypermethylation of the responsible genes. DNA hypermethylation of the AR promoter region leading to AR downregulation has been identified in 30% of hormone-refractory prostate cancers, compared with only 10% in untreated primary tumors (Suzuki *et al.* 2003). Reverse DNA hypermethylation by cytosine DNA methyltransferase inhibitors restores androgen responsiveness in androgen-refractory prostate cancer cells, making them sensitive to growth inhibition by antiandrogen agents (Izbicka *et al.* 1999). Hypermethylation of a CpG island in the 5′ region of the ER gene is seen in ER-negative breast cancer cells; demethylation of this site reactivates ER gene expression (Ferguson *et al.* 1995). The same condition has also been reported in AR-negative prostate cancer cells (Jarrard *et al.* 1998).

Novel agents targeting sex hormone receptors in hormone-resistant breast and prostate cancers

In recent years, there has been growing interest in the development of nonsteroidal modulators for steroid hormone receptors as therapeutic agents. SERMs, SARMs, and nonsteroidal modulators for progesterone receptor have been successfully developed (Zhi *et al.* 1998, 2000, Hamann *et al.* 1999, Mitlak & Cohen 1999, Weryha *et al.* 1999). These modulators are well characterized for their better receptor specificity and selectivity than steroidal ligands, and are more flexible in structural modification for pharmacologic properties. More importantly, with these nonsteroidal chemicals, it may achieve tissue-selective actions and thus generate compounds with diverse purpose suitable for specific therapeutic needs.

SERMs

Estrogens are widely used clinically to control reproduction and for hormone therapy and the treatment of menopausal symptoms in women. Although beneficial in these contexts, estrogen use has also been implicated as a risk factor in breast and uterine cancer, particularly since the first published report from the Women's Health Initiative (Rossouw *et al.* 2002), suggesting that a greater measure of flexibility to control unwanted side effects would be desirable. Consequently, the recognition of SERMs as agents able to elicit estrogenic effects in a tissue-specific manner has expanded the potential population that could benefit from ER ligand therapies. The prototypic

SERM is the *trans* isomer of TAM (Gottardis & Jordan 1987, Gottardis *et al.* 1988). Although it was first proposed to use for regulating fertility, it has been applied primarily as a drug to treat breast cancer (Harper & Walpole 1967, Williamson & Ellis 1973). The ability of TAM to inhibit ER action has long been considered integral to its utility in the breast cancer arena, and this is consistent with numerous studies and clinical trials demonstrating an effect of TAM in ER-positive cells or breast tumors and an absence of any significant activity in those lacking ER expression (Early Breast Cancer Trialists' Collaborative Group 1998). The subsequent observation of the estrogen-like effects of TAM in the human skeleton (Love *et al.* 1992) was important to the conceptualization of SERMs as potential drugs for indications other than breast cancer. The success of TAM as a SERM has been a driving force in the search for new SERMs as well as selective modulators for other nuclear/steroid receptors. Raloxifene, like TAM, exhibits anti-estrogen activity in the breast and estrogen activity in the skeleton. However, raloxifene lacks the significant uterotropic activity associated with TAM and therefore represents an improved agonist/antagonist profile (Delmas *et al.* 1997, Ettinger *et al.* 1999). In addition, a number of other compounds, including lasofoxifene, arzoxifene, and bazedoxifene, are under development, which may one day be of clinical use for chemoprevention of breast cancer or treatment and prevention of osteoporosis (Baracat *et al.* 1999, Suh *et al.* 2001). Consequently, the recognition of SERMs as agents able to elicit estrogenic effects in a tissue-specific manner has expanded the potential population that could benefit from ER ligand therapies.

SARMs

Chemicals that regulate the transcriptional activity of AR can be further categorized into structural (steroidal and nonsteroidal) and functional (androgenic and antiandrogenic) classes. Steroidal androgens, mainly testosterone and its derivatives, have been used clinically as replacement therapies for androgen deficiency (Bagatell & Bremner 1996). Antiandrogens are used to counteract the undesirable actions of excessive androgens (e.g. to treat acne, hirsutism, male-pattern baldness, and androgen-dependent prostate cancer; Neumann 1982, McLeod *et al.* 1993). Nonsteroidal antiandrogens, such as FLU (Eulexin), nilutamide (Anandron), and bicalutamide (Casodex), bind to the AR LBD and, therefore, are devoid of antigonadotropic, anti-estrogenic, and progestational effects. These agents are advantageous over

steroidal antiandrogens (e.g. megestrol acetate, cyproterone acetate) in terms of specificity and selectivity (Cockshott *et al.* 1990, Teutsch *et al.* 1994). Whereas steroidal antiandrogens have been used clinically for a long period of time, nonsteroidal androgens were not conceptualized until very recently. Although androgen therapies are currently available, they are based primarily on delivery of testosterone or its derivatives by injections or skin patches (Negro-Vilar 1999). Neither approach is optimal because injections result in undesirable fluctuations in serum testosterone levels, and skin patches are associated with irritation and rashes. Oral preparations of currently available androgens are not recommended because of their relatively low efficacy, fluid retention, liver toxicity, prostatic hypertrophy, and gynecomastia. Therefore, the goal of preservation of positive androgen effects in some tissues, while minimizing negative side effects in other tissues, has stimulated a search for SARMs. Recently, a group of nonsteroidal androgens that are electrophilic derivatives of bicalutamide and hydroxyflutamide was discovered (Dalton *et al.* 1998). Also, several analogs of quinoline-based AR antagonists, notably tricyclic pyridinodihydroquinoline derivatives, showed promising anabolic effects without any significant action on the prostate and seminal vesicles (Edwards *et al.* 1999, Hamann *et al.* 1999, Higuchi *et al.* 1999, Zhi *et al.* 1999). The selective action of these compounds on muscle and bone tissues implies important clinical applications for these androgen analogs in the treatment of elderly men and patients with wasting diseases. These studies marked the emergence of a novel category of pharmacological agents with potential applications in androgen therapy. Assessments of the *in vivo* SARM activity of these compounds are underway in animals as well as in humans, and they show a promising tissue-selective activity profile. Animal experiments with one such SARM, LGD2226, revealed that it prevented loss of bone mineral density associated with orchidectomy in rats; in contrast, LGD2226 did not stimulate prostate weights above those observed for intact rats (Negro-Vilar 1999). The discovery of nonsteroidal androgens provides an opportunity to identify agents with superior pharmacokinetic profiles to steroidal androgens and implicates the possibility to obtain tissue-selective AR modulators.

Fulvestrant

To overcome crosstalk between ER and other growth factors, several novel agents have been evaluated, some of which are currently in clinical practice. The most straightforward method is to diminish functional ER. Fulvestrant (Faslodex; ICI 182,780), is a pure anti-ER agent which competes with estrogen for binding to ER, with much higher affinity (89 versus 2.5% binding affinity of estrodiol; Wakeling *et al.* 1991, Morris & Wakeling 2002). Fulvestrant reduces the rate of ER dimerization, increases ER degradation (Fawell *et al.* 1990), and reduces ER shuttling from the membrane to the nucleus by blocking its nuclear uptake (Dauvois *et al.* 1993). The loss of ER not only abrogates the transcriptional effect of estrogen, but also blocks the activation of ER by other growth factors. More importantly fulvestrant blocks the agonistic effects of both estrogen and TAM without a demonstrable estrogen-agonistic effect (Wakeling & Bowler 1988, Howell 2001). A single dose of fulvestrant has been shown to decrease ER, PR, and Ki-67 levels significantly in the primary breast cancers of postmenopausal women compared to TAM (Robertson *et al.* 2001). For the treatment of postmenopausal women with receptor-positive breast cancers, fulvestrant was at least as effective as aromatase inhibitors and TAM (Howell *et al.* 2002, 2004, Osborne *et al.* 2002). The long-term efficacy and side effects of fulvestrant still need to be followed.

Hsp90 inhibitors

As a chaperone protein, Hsp90 binds to over 100 kinds of proteins that are involved in multiple signaling pathways utilized by cancer cells for growth and survival. These proteins include steroid hormone receptors (ER, AR, PR), growth factor receptors (EGFR, HER-2), several kinases (Akt, c-RAF-1), transcriptional factors, and mutated or chimeric signaling proteins (mutated p53, p210[Bcr-Abl]; Isaacs *et al.* 2003). Hsp90 serves as an important regulator to control the folding, intracellular disposition, and proteolytic turnover of many key regulatory proteins of cell growth, differentiation, and survival. It is constitutively expressed at 2–10-fold higher levels in tumor cells compared to normal cells (Isaacs *et al.* 2003), its protective effect allows tumor cells to tolerate the mutation of multiple critical signaling molecules that would otherwise be lethal. Therefore it may be important for cancer cell development and survival (Bagatell & Whitesell 2004). Hsp90 inhibitors including geldanamycin, the derivative product of geldanamycin –17-allylaminogeldanamycin (17-AAG), help to degrade several oncogenic proteins, and block several oncogenic pathways (Workman 2004). More surprisingly, the Hsp90 that is derived from tumor cells has a 100-fold-higher binding affinity for 17-AAG than

the Hsp90 from normal cells (Kamal *et al.* 2003). This information suggests that Hsp90 is a good target of 17-AAG for treating cancers. In breast cancer and prostate cancer, Hsp90 inhibitors can effectively downregulate ER and AR protein levels (Segnitz & Gehring 1997, Bagatell *et al.* 2001). Hsp90 inhibitors downregulate HER-2/*neu*, inhibit Akt activation, and enhance paclitaxel-induced apoptosis in HER-2/*neu*-overexpressed breast cancer cells (Basso *et al.* 2002, Solit *et al.* 2003). Thus Hsp90 inhibitors can be used to target hormone-dependent and -independent breast and prostate cancers with high activities of growth factor signaling pathways. A clinical trial combining 17-AAG and paclitaxel together against HER-2/*neu*-overexpressed breast cancer and prostate cancer is currently ongoing.

Novel agents targeting tyrosine kinase receptor in hormone-resistant breast and prostate cancers

Since crosstalks with other growth factors and their receptors — especially tyrosine kinase receptors — are important mechanisms for the emergence of antihormone resistance, an alternative method to block or reverse this resistance is targeting the growth factors and receptors themselves. Tyrosine kinases are tightly regulated enzymes that play an important role in the control of most fundamental cellular processes, including cell proliferation, differentiation, metabolism, migration, and survival. There are several potentially effective interventions of the signaling pathways of tyrosine kinase receptors, including agents targeting growth factors; anti-growth factor receptor antibodies such as trastuzumab (Herceptin), which target HER-2/*neu*-overexpressed breast cancer, and pertuzumab (2C4), which binds to a different epitope of HER-2/*neu* ectodomain than trastuzumab; monoclonal antibodies against EGFR such as IMC-C225 and a fully humanized anti-EGFR monoclonal antibody ABX-EGF; low-molecular-mass EGFR-specific tyrosine kinase inhibitors, such as gefitinib (ZD1839 or Iressa), which works by competing at the ATP-binding site on the tyrosine kinase domain of EGFR; OSI-774 (Tarceva), which specifically blocks the kinase activity of EGFR and EGFR autophosphorylation; CI-1033, which acts as a pan-EGFR tyrosine kinase inhibitor, and PKI-166, which is a reversible tyrosine kinase inhibitor and can block the enzymatic activity of HER-2/*neu*. All of these agents are presently in clinical trials or clinical practices and have been reviewed extensively (Johnston *et al.* 2003, Normanno *et al.* 2003, Madhusudan & Ganesan 2004). Thus, we will not discuss these agents here. Instead, we will describe a couple of novel agents that may have potential to interfere tyrosine kinase receptors.

Emodin

Emodin (3 methyl-1,6,8-trihydroxyanthraquinone), isolated from *Polygonum uspidatum*, is an inhibitor of protein tyrosine kinases (Jayasuriya *et al.* 1992). Emodin also initiates apoptotic pathways in cancer cells, such as hepatoma (Jing *et al.* 2002), cervical cancer (Srinivas *et al.* 2003), and leukemia cells (Chen *et al.* 2002). Emodin suppresses the autophosphorylation and transphosphorylation activities of HER-2/*neu* tyrosine kinase, resulting in tyrosine hypophosphorylation of p185neu in HER-2/*neu*-overexpressing breast cancer cells and non-small cell lung cancer (Zhang *et al.* 1995, Zhang & Hung 1996), suppressing their growth and sensitizing these tumors to several chemotherapeutic agents including paclitaxel, doxorubicin, etoposide, and cisplatin. *In vivo* Emodin also represses the growth of HER-2/*neu*-overexpressed breast cancer cells and sensitizes these cells to paclitaxel (Zhang *et al.* 1999). Since emodin inhibits tyrosine kinase activity, suppresses HER-2/*neu* phosphorylation, and enhances chemosensitivity of breast cancers, it has the potential to be an alternative therapy for HER-2/*neu*-overexpressed, hormone-resistant breast cancers.

Type 5 adenovirus early region 1A (E1A) protein

The E1A of human adenovirus type 5 encodes the proteins that activate viral transcription, thus permitting viral replication in infected cells (Flint & Shenk 1989). E1A was reported to have an antitumor growth effect and anti-metastasis effect (Pozzatti *et al.* 1988*a, b*, Frisch 1991, Chinnadurai 1992, Frisch & Mymryk 2002). E1A can downregulate HER-2/*neu* expression in human tumor cells, and inhibits the growth of HER-2/*neu*-overexpressed cancer cells *in vitro* and *in vivo* (Yu *et al.* 1990, 1991, 1993). There are several molecular mechanisms that may contribute to this anti-cancer effect of E1A: E1A can suppress HER-2/*neu* gene expression; E1A inhibits activation of NF-κB through suppression of IκB kinase (IKK) activity and IκB phosphorylation, rendering cells to be more sensitive to environmental stress (Shao *et al.* 1997, 1999, 2001); E1A also negatively regulates the expression of Axl, which is a transforming receptor tyrosine kinase essential for tumor cell growth (Lee *et al.* 1999). When combined with chemotherapeutic agents, E1A can sensitize cells to the cytotoxic effect of drugs (Lowe *et al.* 1993, Ueno *et al.* 1997). This sensitizing effect,

which can be observed in a preclinical gene therapy setting using an orthotopic breast cancer animal model, may, through E1A, activate p38 and inactivate Akt (Liao & Hung 2003, Liao *et al.* 2004).

The first clinical trial of E1A gene therapy was initiated in 1996, which was the first gene-therapy trial focused on breast cancers and ovarian cancers (Hortobagyi *et al.* 2001). In this trial, E1A was delivered by a specific liposome into the thoracic cavity of breast cancer patients with pleural effusion, or into the peritoneal cavity of ovarian cancer patients with ascites. After E1A treatment, HER-2/*neu* expression in cancer cells derived from patients with HER-2/*neu*-overexpressing cancers decreased significantly. In addition, the local concentration of TNFα increased. E1A was known to sensitize TNFα-induced apoptosis; thus the enhanced TNFα may help E1A-mediated anti-cancer activity (Wold 1993, Shao *et al.* 1999). There are other clinical trials focused on breast, ovarian, and head and neck cancer that have been reported indicating the feasibility of the E1A gene therapy (Yoo *et al.* 2001, Villaret *et al.* 2002, Madhusudan *et al.* 2004).

In summary, E1A as a therapeutic gene can be used in several kinds of cancer. Its function is through not only downregulating HER-2/*neu* expression, but also interfering with mitogenic signaling pathways. These effects may work on hormone-dependent or -independent breast cancers, and may reverse hormone resistance.

Conclusion

The endocrine system controls the long-term coordination of whole body organs. A dysregulated response to hormones, in some cases, leads organs to receive the hormone as a growth stimulant and results in malignancy. Breast cancer and prostate cancer are the most well-known and well-studied examples of this. Although in most cases the cancers are initially sex-hormone-positive and responsive to antihormone therapy, the complicated interactions in these cells eventually will make the resistance inevitable. From previous reviews we know the emergence of resistance is composed of several mechanisms, which can work alone or in cooperation with each other, making treatment more complicated and difficult. However, the more we know about the mechanisms, the better we can predict the prognosis of patients and, more importantly, prevent the emergence of resistance and treat patients with different phenotypes.

One such example is the aromatase inhibitors. In postmenopausal women, aromatase can inhibit the production of estrogen from different sources, without the stimulatory effect from ligand, and aromatase inhibitors can prevent the emergence of resistance more effectively than TAM, as shown in several clinical trials including primary tumors, metastatic tumors, and chemoprevention trials (Fricker 2004). Following aromatase inhibitors, the pure anti-estrogen agent, fulvestrant, was shown to be more effective than aromatase inhibitors in postmenopausal women (Coleman 2003).

Cancer cells are heterogenous. There are differences not only between different individuals but also between different cells in the same individual. Currently there are emerging new tests to examine the phenotypes of cancers before we start to treat them or before we change the treatment protocols. Additionally, the therapeutic methods can be single or combination methodologies. In treating hormone-dependent cancers, there are also several preclinical and clinical trials combining antihormone agents with anti-growth factor agents to block crosstalk, therefore preventing and overcoming antihormone resistance. It is optimistic that combination therapies may soon provide effective strategies for the treatment of breast cancers and prostate cancers, and prevent the emergence of resistance.

Acknowledgements

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