REVIEW

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Novel roles of prolactin and estrogens in breast cancer: resistance to chemotherapy

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Abstract

Resistance to chemotherapy is a major complication in the treatment of advanced breast cancer. Estrogens and prolactin (PRL) are implicated in the pathogenesis of breast cancer but their roles in chemoresistance have been overlooked. A common feature to the two hormones is activation of their receptors by diverse compounds, which mimic or antagonize their actions. The PRL receptor is activated by lactogens (PRL, GH, or placental lactogen) originating from the pituitary, breast, adipose tissue, or the placenta. Estrogen receptors exist in multiple membrane-associated and cytoplasmic forms that can be activated by endogenous estrogens, man-made chemicals, and phytoestrogens. Here, we review evidence that low doses of PRL, estradiol (E_2), and bisphenol A (BPA) antagonize multiple anticancer drugs that induce cell death by different mechanisms. Focusing on cisplatin, a DNA-damaging drug which is effective in the treatment of many cancer types but not breast cancer, we compare the abilities of PRL, E_2, and BPA to antagonize its cytotoxicity. Whereas PRL acts by activating the glutathione-*S*-transferase detoxification enzyme, E_2 and BPA act by inducing the antiapoptotic protein Bcl-2. The implications of these findings to patients undergoing chemotherapy are discussed.

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Introduction

Each year, over a million women worldwide are diagnosed with breast cancer, accounting for 25% of all female cancers. Treatments include surgery, radiation therapy, chemotherapy, or their combinations. Neoadjuvant chemotherapy is used to reduce tumor size before surgery, while adjuvant chemotherapy is used after tumor excision. Chemotherapy is the mainstay treatment for patients with triple negative tumors (estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (Her-2)) who are resistant to hormone or targeted therapy, and for those with advanced metastatic disease (Coley 2008). Dozens of anticancer drugs have been developed, with treatment options taking into account tumor grade and histology and whether the desired outcome is curative or palliative. Most regimens combine drugs that act by different mechanisms aimed at improving the odds of suppressing tumor growth (Ocana & Pandiella 2008).

While both the selection and success of chemotherapeutic agents have increased, tumor resistance remains a major obstacle, which results in treatment failure. Some tumors are intrinsically resistant to certain drugs, while others acquire resistance following treatment. Resistance can result from drug efflux by transporters, inactivation by detoxification enzymes, altered expression of pro/antiapoptotic proteins, changes in tumor suppressor genes, and increased DNA repair mechanisms (Coley 2008). Whereas hormones such as prolactin (PRL) and estradiol (E_2) have long been implicated in the pathogenesis of breast cancer, their involvement in chemoresistance has been overlooked. The objective of this review is to evaluate emerging evidence that these hormones confer resistance against a variety of chemotherapeutic agents that kill breast cancer cells by different mechanisms and discuss the clinical implications.

PRL and estrogens are dissimilar in chemical structure, receptor characteristics, and signaling mechanisms. Whereas estrogens can bind to several classical (ERα and ERβ) and nonclassical (G protein-coupled receptor 30, GPR30) receptors (Manavathi & Kumar 2006), there is only one receptor (PRLR) for PRL, albeit it exists in several isoforms which couple to different signaling pathways (Swaminathan *et al.* 2008). Yet, there is crosstalk between the two hormones, with PRL

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increasing the expression and phosphorylation of ERα (Carver *et al*. 2009), and E_2 inducing the transcription of both PRL (Duan *et al*. 2008) and the PRLR (Swaminathan *et al*. 2008). Such interactions can result in augmentation, or synergism, between the two hormones.

Several features that are common to PRL and E_2 confound the understanding of their roles in breast cancer. One is the multiple sites of origin, with both hormones reaching the breast from the systemic circulation as well as from local sources (Ben-Jonathan *et al*. 2002, Foster 2008). Thus, blood levels of PRL or E_2 do not reveal the full extent of breast exposure to these hormones. Another is a variable expression of their receptors in tumors, which often depends upon the luminal or basal origin of the tumor. Thus, neither hormone affects tumors that do not express its receptor. Importantly, 80–90% of breast carcinomas express the PRLR (Touraine *et al*. 1998), while ∼75% contain ERα (Karayiannakis *et al*. 1996). This indicates that most breast tumors express both receptors, reinforcing their importance as therapeutic targets. Finally is the issue of receptor promiscuity, with ERs capable of binding steroidal and nonsteroidal compounds (Bai & Gust 2009), and the PRLR capable of binding other lactogens (Goffin *et al*. 2005). Thus, xenoestrogens can mimic or antagonize endogenous estrogens, while GH and placental lactogen (PL) can augment or interfere with PRL actions. Figure 1 illustrates the various compounds that affect breast cancer via their interactions with either the PRLR or ERs.

Properties of selected chemotherapeutic drugs

Over the past 20–30 years, treatment of metastatic breast cancer has evolved from the anthracyclines to taxanes to hormonal and targeted therapy and their combinations (Coley 2008). Here, we focus on those drugs that are affected by PRL and/or estrogens.

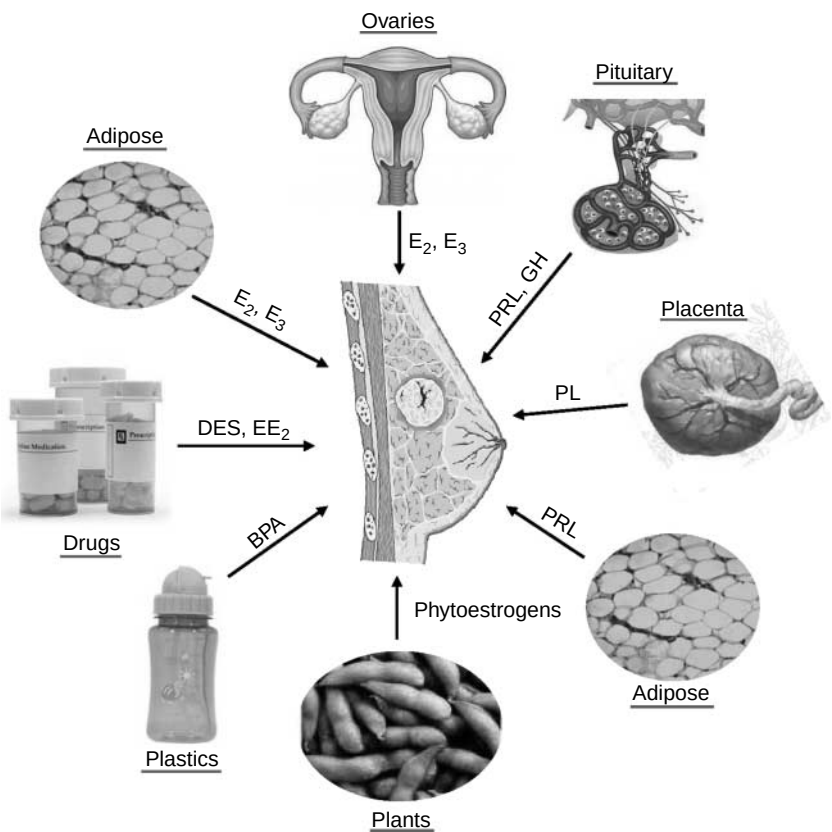

Figure 1 Diagram of the different compounds and their sites of origin which affect breast cancer by activating either the prolactin (PRL) receptor (PRLR) or estrogen receptors (ERs). The right side of the illustration depicts the three structurally related lactogens, prolactin (PRL), GH, and placental lactogen (PL), which bind to the PRLR. The left side shows the variety of compounds which activate either classical or nonclassical ERs. These include natural estrogens: estradiol (E_2) and estriol (E_3); man-made chemicals, diethylstilbestrol (DES), ethinylestradiol (EE_2), and bisphenol A (BPA); and phytoestrogens. Adipose tissue represents the stromal compartment within the breast as well as abdominal and subcutaneous depots. Both PRL and estrogens (likely via aromatization) are also produced within tumor cells themselves, where they act as autocrine/paracrine agents.

Cisplatin is a platinum-based drug that is highly effective against testicular, ovarian, and lung cancers but has limited efficacy as monotherapy in breast cancer patients (Decatris *et al*. 2004). Following uptake into the nucleus, cisplatin interacts with DNA and forms adducts via intrastrand cross-links that induce cell cycle arrest. The DNA can either be repaired by the nucleotide excision pathway, or the cell is destined to die (Kelland 2007). The caspase 3-deficient MCF7 cells are not killed by cisplatin (Blanc *et al*. 2000), although cisplatin can induce apoptosis via a caspase 3-independent mechanism in ovarian cancer cells (Henkels & Turchi 1999).

Doxorubicin (adriamycin) is an anthracycline antibiotics used in multiple cancers, and is considered the standard treatment in breast cancer. Upon entering the nucleus, doxorubicin inhibits topoisomerase II and helicase activities, and interferes with DNA double helix religation. This stops DNA replication and induces apoptosis (Rabbani *et al*. 2005). Apoptosis may occur via activation of pro-apoptotic proteins, since antisense against Bcl-2 and Bcl-xL sensitizes breast cancer cells to this drug (Simoes-Wust *et al*. 2002). Doxorubicin can also cause replicative senescence, as evident by micronuclei formation and senescence-associated β-galactosidase staining (Chang *et al*. 2002).

Taxol (paclitaxel) has been highly successful in treating breast and ovarian cancer, especially in combination with anthracyclines. Taxol targets the microtubules, which mediate alignment of the chromosomes along the equatorial plane prior to segregation to daughter cells. It binds to polymerized tubulin and inhibits microtubule disassembly, thereby suppressing both microtubule treadmilling and dynamic instability (Zhou & Giannakakou 2005). Taxol-induced apoptosis often correlates with phosphorylation of Bcl-2 (Ferlini *et al*. 2003). Apoptosis can occur via caspase 3-dependent or -independent mechanisms (Friedrich *et al*. 2001).

Vinblastine, a vinca alkaloid, is another microtubule-altering drug. Unlike taxol, it binds to monomeric tubulin and prevents its polymerization. Mitosis is blocked at the metaphase/anaphase transition, and the prolonged arrest leads to cell death (Zhou & Giannakakou 2005). Vinblastine also interferes with amino acid, cAMP, and glutathione metabolism, and can induce apoptosis through the nuclear factor κB/inhibitor of κB (NF-κB/IκB) pathway (Huang *et al*. 2004). Apparently, c-Jun protects T47D cells from vinblastine-induced cell death, although it does not prevent the mitotic block (Duan *et al*. 2007). At nontoxic doses, vinblastine inhibits chemotaxis and endothelial cell proliferation, highlighting its antiangiogenic properties (Vacca *et al*. 1999).

Apoptotic signals are also mediated via the tumor necrosis factor (TNF) family of death receptors. The TNF-related apoptosis-inducing ligand (TRAIL) induces oligomerization of the intracellular domains (ICDs) of the death receptors and causes apoptosis in many cancer cell types without killing normal cells (Wang & El Deiry 2003). In recent clinical trials, TRAIL agonists showed no major toxicity, but therapy is limited to patient with TRAIL-sensitive tumors (Bellail *et al*. 2009).

The dependence of tumor expansion and metastasis on angiogenesis has lead to the development of angiogenesis blockers, which inhibit the release of pro-angiogenic proteins such as VEGF, block mitogenic/survival pathways of endothelial cells, or prevent extracellular matrix breakdown (Sessa *et al*. 2008). Over 300 angiogenic inhibitors have been developed, and dozens are in various phases of clinical trials. Avastin, an anti-VEGF monoclonal antibody, was the first antiangiogenic drug shown to prolong patient survival.

Mechanisms of chemoresistance

Resistance to chemotherapy results from many causes, including drug extrusion by transporters, drug metabolism, increased antiapoptotic proteins, decreased pro-apoptotic proteins, and enhanced DNA damage repair. A major problem is that tumors often exhibit resistance to a diversity of chemotherapeutic agents, which act by different mechanisms.

Multidrug resistance transporters, which target structurally diverse drugs, are major contributors to drug resistance. The best characterized are members of the ATP-binding cassette transporters, which include P-glycoprotein, multidrug resistance protein 1 (MRP1), and breast cancer resistance protein (BCRP; Higgins 2007). P-glycoprotein confers resistance against taxol, vinblastine, doxorubicin, and etoposide, with over 40% of breast tumors expressing this transporter (Trock *et al*. 1997). MRP1 confers resistance against anthracyclines, antifolates, and vinca alkaloids, but not against taxanes or cisplatin (Trock *et al*. 1997). Although originally isolated from a drug-resistant MCF7 subline, the BCRP protein is rarely found in E_2-responsive cells, because of its downregulation by estrogens (Imai *et al*. 2005).

The glutathione metabolic pathway confers resistance against environmental insults and drugs. Glutathione-*S*-transferases (GSTs) are phase II detoxification enzymes that catalyze the conjugation of

glutathione to electrophilic compounds, resulting in easily extruded products (Townsend & Tew 2003, McIlwain *et al*. 2006). They inactivate platinum drugs, doxorubicin, cyclophosphamide, and etoposide, but not antimicrotubule drugs. Some GST isozymes inhibit JNK1 via protein:protein interactions and inactivate drugs that act via the mitogen activated protein (MAP) kinase pathway even when they are not subject to conjugation with glutathione. GSTs are inducible enzymes classified by substrate specificity and intracellular distribution into several families, e.g. α, μ, π and θ subtypes. Overexpression of GST π is associated with drug resistance and poor patient survival, while mutations in GST μ and GST π predispose the affected individuals to environmental carcinogens (Shiga *et al*. 1999).

Tumors can acquire drug resistance by overexpressing antiapoptotic proteins (e.g. Bcl-2 and Bcl-xL) or downregulating pro-apoptotic proteins (e.g. Bax). MCF7 cells, which express high levels of Bcl-2, are less responsive to cisplatin, but become sensitized to the drug upon Bcl-2 downregulation by RNA interference (Yde & Issinger 2006). Breast cancer cells that overexpress Bcl-xL are less sensitive to paclitaxel, which correlates with failure of the drug to activate caspase 9 (Wang *et al*. 2005). In addition, Bax overexpression in MCF7 cells restores their sensitivity to various apoptotic agents. Higher Bax expression levels are detected in normal breast epithelium than in adjacent tumors (Bargou *et al*. 1996).

Mutations in tumor suppressors strongly influence cancer cells sensitivity to anticancer drugs. Alterations in p53 are the most common genetic changes in breast cancer, with specific mutations associated with resistance to doxorubicin (Aas *et al*. 1996). The tumor suppressor gene BRCA1 is also frequently mutated in breast cancer. It responds to DNA damage by affecting DNA damage repair. Loss of BRCA1 confers sensitivity to many DNA-damaging agents (Kennedy *et al*. 2004), and its knockdown results in a twofold increase in cell sensitivity to irofulven (Wiltshire *et al*. 2007).

Characteristics of PRL and PRLR

PRL is a 23-kDa hormone of pituitary origin whose main target is the breast, where it stimulates proliferation, differentiation, survival, and secretory activity (Ben-Jonathan *et al*. 2008). Depending upon the cell context and physiological conditions, PRL can exert opposite actions such as proliferation versus differentiation in both malignant and nonmalignant cells. PRL belongs to a family of proteins, named lactogens, which share structural homology and some overlapping functions. The most prominent members are PRL, GH, and PL, which are made of a single polypeptide chain with 2–3 intramolecular disulfide bridges. Lactogens have a high homology in their primary amino acids and a similar tertiary structure, composed of four antiparallel, up–up, down–down helical bundle (Teilum *et al*. 2005).

Unique to humans, PRL is also produced in multiple nonpituitary sites, including the decidua, myometrium, breast, and prostate (Ben-Jonathan *et al*. 1996). Whereas pituitary PRL is controlled by a proximal promoter which requires the pituitary-specific Pit-1 transcription factor for transactivation, expression of extrapituitary PRL is driven by a superdistal promoter (Gerlo *et al*. 2006). The insensitivity of the superdistal promoter to dopamine explains the failure of dopamine agonists, such as bromocriptine, to suppress breast PRL and affect PRL-dependent tumors in patients (Ben-Jonathan *et al*. 2008). Within the normal breast, PRL is produced at much larger quantities by stromal adipocytes than by the epithelium (Zinger *et al*. 2003) and is up-regulated in carcinomas as compared with benign breast epithelium (McHale *et al*. 2008).

The PRLR is a member of the cytokine receptor superfamily, which is nontyrosine kinase, single-pass membrane receptor. It has a three-domain organization: an extracellular ligand-binding domain, which confers specificity, a short transmembrane domain, and an ICD (Swaminathan *et al*. 2008, Clevenger *et al*. 2009). In addition to the most abundant 80 kDa long isoform, shorter variants that couple to different signaling pathways are detectable in breast cancer. Among cell lines, T47D cells express the highest PRLR levels, followed by MCF7, BT483, MDA-MB-468, and BT474 (Peirce *et al*. 2001). The human PRLR is indiscriminate in its binding preferences, with GH binding not only to its receptor (GHR) but also to the PRLR. In contrast, nonprimate GH binds only to the GHR, while PRL binds only to the PRLR but not to GHR in any species (Ben-Jonathan *et al*. 2008). PL does not have a receptor of its own and binds only to the PRLR (see Fig. 1).

Two binding sites on the ligand are required for PRLR activation. One receptor binds to a high affinity site 1, while a second receptor binds to a lower affinity site. This forms an active ternary complex composed of one hormone molecule and receptor homodimer (Teilum *et al*. 2005). The existence of preformed, inactive dimers without a ligand suggests that receptor dimerization is necessary but insufficient for its activation (Clevenger *et al*. 2009). Ligand binding induces relative rotations of the two units, resulting in allosteric reorganization of the ICD. This brings the

ICD and Jak2 kinase into close proximity, enabling their phosphorylation. The lactogens, which differ in critical interacting residues, do not induce identical conformational changes in the receptor (Gertler *et al*. 1996). Instead, each imposes a different stability on the active complex, thereby affecting its dynamics and binding parameters of the associated partners.

Several PRLR antagonists, made by modifications of the PRL molecule, have been generated, which block PRL actions *in vitro* and in experimental animals (Goffin *et al*. 2005). However, their use as an effective treatment in breast cancer patients is uncertain because of their short half-life and the necessity for their administration by injection. Efforts are underway to find small molecules that selectively block the PRLR and can be delivered orally.

Binding of PRL to its receptor activates several signaling pathways, of which the Jak2–Stat5 pathway is the best understood. Jak2 is rapidly activated by PRL and phosphorylates Stat proteins. These dimerize and translocate to the nucleus, where they bind to GAS elements within the promoters of target genes. Stat5a/b mediate many of the PRL actions in normal and malignant breast cells (Clevenger *et al*. 2009). PRL-responsive genes that are involved, directly or indirectly, in cell cycle regulation include cyclin D1, AP-1, c-Myc, and heat shock protein α (Brockman *et al*. 2002, Acosta *et al*. 2003, Gutzman *et al*. 2005, Perotti *et al*. 2008).

Although activation of the Jak2–Stat pathway is critical for lobuloalveolar development and lactation in the normal breast, other PRL-induced pathways are important in breast cancer. One is the Ras–Raf–MAPK pathway, with ERK1/2 and *c-jun* N-terminal kinase being its primary mediators. PRL induces phosphorylation of ERK1/2 in both T47D and MCF7 cells, and can synergize with epidermal growth factor (EGF) to induce ERK (Acosta *et al*. 2003). Activation of the phosphoinositide-3-kinase (PI3K)–Akt survival pathway by PRL has been implicated in cell migration (Maus *et al*. 1999). Crosstalk between PRLR and ERα occurs at several levels. For example, PRL and E_2 cooperatively enhance AP-1 activity (Gutzman *et al*. 2005), and E_2 rapidly phosphorylates Stat5 (Fox *et al*. 2009), while PRL activates the unliganded ER (Gonzalez *et al*. 2009).

Role of PRL in carcinogenesis

The role of PRL in mammary tumorigenesis in rodents has long been recognized, while its involvement in breast cancer only recently became accepted. Prospective studies found a modest association between higher serum PRL levels and cancer risk in both premenopausal and postmenopausal women, primarily those with ER+ tumors (Tworoger & Hankinson 2008). However, shortcomings of epidemiological studies include single blood sample determination and assay standardization. Most importantly, they do not take into account the local production of PRL by the breast or the status of PRLR expression in the tumors.

PRL exerts multiple actions in breast cancer cells, including increased proliferation, enhanced motility, and prolonged survival. Suppression of T47D cell proliferation by PRL antisense oligos, anti-PRL antibodies, and PRLR antagonists served as the evidence for the mitogenic activity of autocrine PRL (Chen *et al*. 1999, Vonderhaar 1999, Llovera *et al*. 2000). The role of autocrine/paracrine PRL is supported by studies with nude mice, where growth of tumors derived from T47D cells is inhibited by treatment with the hPRL antagonist G129R (Chen *et al*. 2002), while PRL overexpressing MDA-MB-435 cells form faster growing tumors (Liby *et al*. 2003).

PRL also affects cytoskeleton modulation, as reveals by its enhancement of breast cancer cell migration and induction of PI3K-dependent membrane ruffling and stress fibers (Maus *et al*. 1999). PRL and its cleaved fragment 16K PRL can stimulate and inhibit angiogenesis respectively, suggesting an indirect role for PRL in carcinogenesis via alterations in tumor blood supply (Clapp *et al*. 2008). Of great importance is a recent report that mouse PRL does not activate the human PRLR (Utama *et al*. 2006), raising issues of interpretation of drug responsiveness of human xenografts in mice, which are unaffected by circulating PRL.

Unlike estrogen, PRL is only a modest mitogen in breast cancer. In fact, Stat5 activation by PRL may be linked to induction of differentiation and suppression of invasion rather than to proliferation (Sultan *et al*. 2005). This is supported by a lower expression of activated Stat5 in node-positive breast cancer than in normal breast or less advanced tumors (Nevalainen *et al*. 2004). Yet, the argument that PRL acts solely as an antimetastatic factor is overreaching, since signaling pathways other than Stat5 are activated by PRL. PRL could serve as a suppressor of metastasis in advanced tumors but as a promoter of cell growth in early tumors. A switch between tumor promotion to suppression is exemplified by transforming growth factor-β (TGFβ), which inhibits the growth of normal epithelial cells but accelerates the malignant process of late-stage tumors (Bachman & Park 2005). Estrogen represents another case of contrasting actions, since in addition to its mitogenic actions it can induce apoptosis under some conditions (Lewis-Wambi & Jordan 2009).

PRL and chemoresistance

Accumulating evidence suggests that PRL opposes cytotoxicity by a wide variety of anticancer drugs. In PC3 prostate cancer cells, TRAIL-induced apoptosis is partially inhibited by PRL, which by itself has no effect on cell proliferation (Ruffion *et al*. 2003). Another group reported that pretreatment of ovarian carcinoma cells with PRL inhibits cisplatin-induced cell death (Asai-Sato *et al*. 2005). PRL also antagonizes apoptosis caused by methotrexate, an antifolate agent, in human promyelocytic leukemia HL-60 cells (Hsu *et al*. 2006).

Epidemiological data suggest that women with elevated blood PRL levels have increased treatment failure and worse survival (Tworoger & Hankinson 2008). Indeed, hyperprolactinemic patients with metastatic breast cancer are less responsive to taxol than those with normal serum PRL levels (Lissoni *et al*. 2001). A small clinical trial revealed better responsiveness in patients treated with a combination of taxol and bromocriptine, compared to those receiving only taxol (Lissoni *et al*. 2002). These data should be replicated in larger trials with PRL inhibitors (i.e. bromocriptine and cabergoline) together with various anticancer drugs. However, an effective blockade of the PRLR would likely be more effective in sensitizing tumors to anticancer drugs than the suppression of pituitary PRL release.

Several studies have focused on PRL as an anticytotoxic factor in breast cancer cells. Ramamoorthy *et al*. (2001) found that induction of apoptosis by cisplatin in T47D cells is enhanced by co-treatment with the hPRL antagonist G129R, suggesting that endogenous PRL is protective. Another antagonist, Δ1-9-G129R-hPRL, potentiates the effects of paclitaxel and doxorubicin in breast cancer cells (Howell *et al*. 2008). In addition, cells that produce PRL, e.g. T47D and MCF7, are more resistant to ceramide-induced apoptosis than those with low or no PRL (Perks *et al*. 2004). PRL can also overcome growth arrest caused by γ-irradiation (Chakravarti *et al*. 2005). None of the above studies, however, have resolved the mechanisms underlying the protective effects of PRL.

Our exploration of the mechanism by which PRL antagonizes anticancer drugs was inspired by the finding that PRL overexpression in MDA-MB-435 cells enhanced tumor growth and up-regulated Bcl-2 (Liby *et al*. 2003). Pretreatment of breast cancer cells with low doses of PRL antagonizes cytotoxicity by taxol, vinblastine, doxorubicin, and cisplatin, albeit at different efficacies (LaPensee *et al*. 2009*b*). We were especially interested in the mechanism by which PRL opposed cisplatin, which has shown only little effectiveness in breast cancer patients. Unlike its strong apoptotic effects in MDA-MB-468 cells, cisplatin is only moderately effective in T47D cells. Reasoning that the resistance of T47D cells may be due to their high endogenous PRL levels, the mechanistic studies were conducted with MDA-MB-468 cells.

Measurement of platinum in nuclear extract by mass spectroscopy reveals that PRL reduces the amount of cisplatin bound to DNA. Lower entry of cisplatin into the nucleus could be due to transporters such as MRP that extrude the drug, or to detoxification enzymes such as GST that inactivate cisplatin (Siddik 2003). Previous work showed that PRL increases hepatic GST activity (Luquita *et al*. 1999). Using inhibitors of the two potential targets, we discovered that GST, but not MRP, accounts for the suppression of cisplatin entry to the nucleus by PRL. This action is mediated by the Jak–Stat and MAPK pathways, but not by PI3K pathway. Subsequent studies show that PRL induces the expression of the GST μ isoform and increases GST enzyme activity in MDA-MB-468 cells (LaPensee *et al*. 2009*b*). The GST μ- and θ-null genotypes are associated with increased survival in women with advanced breast cancer that were treated with chemotherapy (Ambrosone *et al*. 2001). Future studies should determine whether knockdown of specific GST isozymes abrogates the protective effects of PRL.

A model which conceptualizes the mechanism by which PRL confers resistance against cisplatin is presented in Fig. 2. After diffusing into the cell, cisplatin enters the nucleus and binds to DNA, with the ensuing cell cycle arrests leading to apoptosis. Binding of PRL to its receptor induces the activation of Jak–Stat and MAPK pathways, which separately or in concert increase the expression and activity of GST. GST conjugates cisplatin to glutathione, leading to its extrusion from the cell. Consequently, less cisplatin is available for entering the nucleus and inflicting DNA damage. The overall effect of PRL is a marked reduction in cisplatin-induced cell death. In addition to cisplatin, GST confers resistance to doxorubicin but not to the microtubule-altering drugs (L'Ecuyer *et al*. 2004). Thus, the mechanism by which PRL antagonizes drugs which are not substrates for GST may involve alterations in Bcl-2 family proteins.

Estrogens: multiple ligands and diverse receptors

E_2, estriol, and estrone are naturally occurring estrogens, which differ in affinity for the various ERs. They have dissimilar bioactivities, with E_2 being the most potent. From menarche to menopause, the ovaries

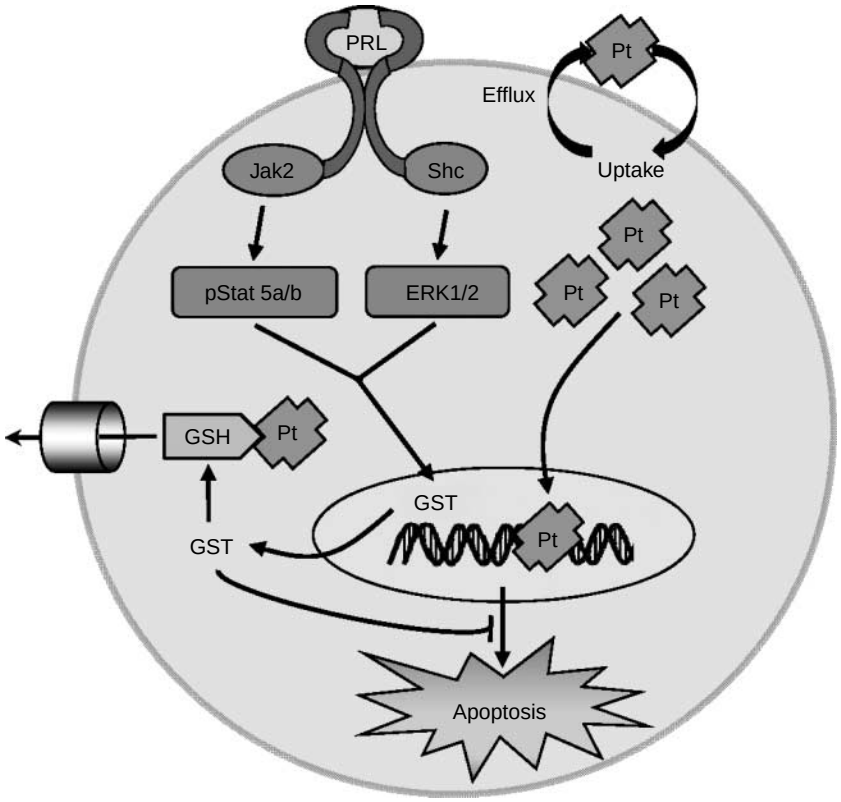

Figure 2 Proposed mechanism by which prolactin (PRL) antagonizes cisplatin-induced apoptosis in breast cancer cells. Cisplatin (Pt) diffuses into the cell and enters the nucleus, where it binds to DNA, causes cell cycle arrest, and induces apoptosis. Binding of PRL to the receptor enables its association with Jak2 and Shc, and the subsequent signaling via Stat5a/b and ERK1/2 pathways. It is through either, or both, of these pathways that PRL increases transcription of the detoxification enzyme glutathione-*S*-transferase (GST). Increased GST activity promotes conjugation of glutathione (GSH) to cisplatin, followed by extrusion of the conjugate from the cell via transporters. Consequently, less cisplatin is available for entry into the nucleus, resulting in decreased apoptosis.

are the primary source of estrogens. After menopause, estrogens can be generated through the conversion of androgens secreted by the adrenals and the ovaries. This process is carried out in sites such as the skin and adipose tissue by the aromatase enzyme complex (Jongen *et al*. 2006; see Fig. 1).

Breast cancer expresses several sex steroid-producing enzymes, including aromatase, 17β-hydroxysteroid dehydrogenase, which catalyzes interconversion among estrogens, and steroid sulfatase, which hydrolyzes sulfated steroids to their bioactive forms (Suzuki *et al*. 2005). Although serum E_2 levels in postmenopausal women are only 5–10% of those before menopause, their tumors are exposed to comparable levels of active estrogens. Indeed, the tumor/plasma ratio of E_2 is >20 in breast carcinomas from postmenopausal women but only 5 in those from premenopausal women (Pasqualini *et al*. 1996). Aromatase inhibitors are effective in blocking the growth of early ER+ tumors (Nabholtz *et al*. 2009). Similar to the higher production of PRL by breast stroma (Zinger *et al*. 2003), estrogen synthesis is higher in the stroma than in epithelial cells due to higher expression of aromatase (Santen *et al*. 1997).

As schematically illustrated in Fig. 1, endocrine disruptors that mimic or antagonize endogenous estrogens are relevant to breast cancer. Estrogen-like compounds include pesticides, industrial chemicals, pharmaceuticals, and plant-derived compounds, all of which can expose humans through food or water supply (Gray *et al*. 2009). Many are lipophilic and can be stored in adipose tissue. The most widely studied are components of plastics, e.g. bisphenol A (BPA; discussed in the next chapter), and detergents such as octyl and nonyl phenols. Chlorinated insecticides, e.g. kepone, dichloro diphenyl trichloroethane (DDT), dieldrin, and methoxychlor, also possess estrogen-like properties. Two unresolved issues are whether early exposure to endocrine disruptors increases the risk of developing breast cancer, and what is the effect of interactions between chemicals in mixtures (Birnbaum & Fenton 2003, Gray *et al*. 2009).

Diethylstilbestrol (DES) and ethinylestradiol are potent pharmaceuticals used to treat symptoms of menopause, as contraceptives and as palliative therapy in advanced prostatic cancer. Given the millions of users, home toilets are the major source of these compounds in wastewater (Falconer 2006). Although excreted into urine as inactive glucoronides or sulfates, some can be degraded in sewage treatment plants and release the active compounds.

Resveratrol, daidzein, quercetin, and genistein represent the most commonly ingested and intensely studied plant-derived phytoestrogens (Martin *et al*. 2007, Mense *et al*. 2008). They show differential binding to ERα and ERβ, exert nongenomic actions, and also affect estrogen biosynthesis and metabolism. Although the general belief is that long-term consumption of phytoestrogens (i.e. soy products) helps in reducing a woman's risk of breast cancer, this notion is controversial (Martin *et al*. 2007, Gray *et al*. 2009).

Estrogens bind to multiple receptors of diverse structure that can be localized in the membrane, cytoplasm, and nucleus. ERα and ERβ differ in their ligand-binding domain, underlying the dissimilar binding affinities of the various estrogenic ligands to the two receptors. ERα is expressed at low levels in the normal breast epithelium (Ricketts *et al*. 1991), but increases in *in situ* carcinomas (Karayiannakis *et al*. 1996). The expression pattern of ERβ is opposite that of ERα, suggesting that loss of ERβ expression indicates breast cancer development and/or progression (Shaaban *et al*. 2003). Following ligand binding, classical ERs dimerize and bind to estrogen response elements in the promoters of target genes. Recruitment of co-regulators results in the formation of complexes that mediate transcription (Nilsson *et al*. 2001). The plethora of cell-specific co-activators and co-repressors account, in part, for the partial agonist versus antagonist activities of tamoxifen in the uterus, breast, bone, and cardiovascular system.

Similar to the differential binding dynamics of the three lactogens to the PRLR (Gertler *et al*. 1996), the various estrogenic ligands can induce distinct changes in ER conformation, thereby altering co-factor recruitment and receptor stability (Bai & Gust 2009). This is exemplified by an induction of rapid ERα degradation by the pure ER antagonist ICI 182 780, but not by E_2 or tamoxifen (Van Den Bemd *et al*. 1999). The ERs also regulate transcription via protein–protein interactions with transcription factors such as the Fos–Jun complex (Normanno *et al*. 2005).

Estrogens can rapidly activate the MAPK and PI3K/Akt signaling pathways, traditionally associated with membrane receptors (Bjornstrom & Sjoberg 2005), but the nature of the receptor(s) involved is controversial. In neurons, pituitary and endothelial cells, G-proteins, ion channels, cytoplasmic protein kinases, and adaptor proteins have been implicated (Manavathi & Kumar 2006, Fox *et al*. 2009). In breast cancer cells, one model stipulates that a subpopulation of ERs is localized to the cell membrane. Steroid receptors do not have transmembrane or kinase domains and thus are unlikely to be incorporated to the cell membrane as integral proteins. Instead, they may interact via palmitoylation of membrane-associated proteins such as caveolin, striatin, and Sch (Song *et al*. 2006). Both IGF1 and EGF receptors are involved in tethering ERα to the membrane and in initiating MAPK and PI3K activation. Although the above model provides an plausible explanation for nongenomic actions of E_2, it does not explain the rapid actions of some xenoestrogens, which have much lower affinities to ERα and ERβ, and yet are active at subnanomolar doses (Watson *et al*. 2007).

GPR30, a 7-transmembrane domain receptor that signals through trimeric G-proteins, represents a different model by virtue of its direct binding to estrogens (Filardo & Thomas 2005, Prossnitz *et al*. 2008). Its actions have mostly been studied in SKBr3 breast cancer cells, which express GPR30 but not classical ERs (Filardo *et al*. 2000). Estrogen signaling can be restored in the ER-negative MDA-MB-231 cells by transfection with GPR30. Binding of E_2 to GPR30 stimulates the cAMP pathways through Gαs, and Src through Gβγ. Subsequently, heparan-bound EGF is released, activates the EGF receptor and its downstream signaling that include MAPK, PI3K, and phospholipase C (PLC) (Filardo & Thomas 2005). Both tamoxifen and ICI act as agonists, rather than antagonists, of GPR30. Expression of GPR30 is higher in invasive carcinoma and is associated with larger tumor size, suggesting that it may be a predictor of aggressive disease (Filardo *et al*. 2006). The relatively high binding affinity of GPR30 to E_2 (K_d of 3 nM) makes this receptor a likely mediator of estrogen actions in ER-negative breast cancer cells (Thomas *et al*. 2005), but its relative role in cells that also express ERα and ERβ is unclear.

Chemoresistance by estrogens

In spite of the abundance of man-made or plant-derived estrogen mimetics which can impact on breast cancer, little is known about their potential interactions with anticancer drugs. In addition, only few studies have examined the role of endogenous estrogens in chemoresistance. This oversight is enigmatic because

stimulation of tumor growth by estrogens involves not only increased cell proliferation but also reduced cell death. This is exemplified by the activation of both the PI3K/Akt survival pathway and Bcl-2 antiapoptotic proteins in breast cancer by estrogens (Huang *et al*. 1997, Rodrik *et al*. 2005). Perhaps research on anticytotoxic effects of estrogens has been hampered by an adherence to the classification of breast cancer cells into those that express classical ERs (estrogen-responsive) and those that do not (estrogen-unresponsive), leading many researchers to ignore cells that do not express ERα or ERβ.

As a follow-up in our studies on antagonism of cisplatin by PRL (LaPensee *et al*. 2009*b*), we ask whether E_2 acts similarly and if so, by what mechanism. Low doses of E_2 (0.01–10 nM) abrogate cisplatin toxicity in T47D and MDA-MBA-468 cells by increasing cell proliferation and decreasing apoptosis (LaPensee *et al*. 2009*a*). Protection by estrogen occurs in the presence of ERα and ERβ antagonists, in ERα-negative MDA-MB-468 cells, and in T47D cells with ERβ knockdown, indicating independence of classical ERs. Since both cell types express GPR30 (LaPensee *et al*. 2009*c*), this receptor is a plausible candidate for transducing survival signals by E_2. Future studies should determine whether GPR30 knockdown abrogates the protective effect of E_2.

Unlike PRL, E_2 does not alter entry of cisplatin into the nucleus, suggesting that its protective effects occur downstream of DNA damage (LaPensee *et al*. 2009*a*). Because previous reports implicated Bcl-2 in estrogen-induced chemoresistance (Teixeira *et al*. 1995, Huang *et al*. 1997), we focused on this antiapoptotic protein. Indeed, E_2 increases Bcl-2 expression in T47D cells, both in the presence and absence of cisplatin, but does not alter Bcl-xL or Bax. A Bcl-2 inhibitor partially abrogates the protection by E_2, indicating that alterations in Bcl-2 may be only part of its mechanism of actions (LaPensee *et al*. 2009*a*).

Other data support the concept that estrogens confer chemoresistance. For example, MCF7 cells depleted of estrogen are twice as sensitive to doxorubicin than estrogen-treated cells (Teixeira *et al*. 1995). Estrogen depletion is accompanied by decreased Bcl-2 expression, and Bcl-2 reconstitution restores resistance to doxorubicin. Others reported that modulation of Bcl-2 levels affects cell sensitivity to taxol (Huang *et al*. 1997). Also, E_2 reduced taxol cytotoxicity in cells overexpressing ERα, with the cells sensitized to taxol by treatment with the ERα antagonist ICI (Sui *et al*. 2007). Estrogen also antagonizes taxol- and radiation-induced apoptosis by altering JNK activity (Razandi *et al*. 2000). A combination of tamoxifen and TRAIL is more effective than each alone in inducing apoptosis in the ERα-negative MDA-MB-231 cells, and in arresting tumor growth in xenografts (Lagadec *et al*. 2008). This sensitization was associated with decreased Bcl-2 and increased Bax levels. Another mechanism by which estrogens can increase chemoresistance is by affecting drug transporters. This was revealed by estrogen-induced increase in cytoplasmic p-glycoprotein in MCF7 cells, which are resistant to doxorubicin cytotoxicity, but not in T47D cells, which are sensitive to the drug (Zampieri *et al*. 2002).

BPA, breast cancer, and chemoresistance

BPA is used in the manufacture of polycarbonate plastics and is the constituent of a wide array of consumer products, including plastic food containers, baby bottles, and the lining of metal food cans (Welshons *et al*. 2006). Migration of BPA into food or water from plastic containers is influenced by the manufacturing process, storage conditions, and heating by users (Kang *et al*. 2006, Le *et al*. 2008). Human exposure to BPA is well documented, with BPA detectable at 0.2–10 ng/ml in serum from most individuals tested (Welshons *et al*. 2006). Being lipophilic, BPA can accumulate in breast adipose tissue (Fernandez *et al*. 2007).

The mechanism by which BPA exerts its actions is enigmatic, since its binding affinity for ERα or ERβ is 10 000- and 1000-fold lower than that of E_2 respectively (Kuiper *et al*. 1998). Yet, BPA at low nanomolar or subnanomolar doses often elicits activities that are similar to those of E_2 (Watson *et al*. 2005, Hugo *et al*. 2008). It has been suggested that BPA binds differentially within the ligand-binding domain of the ERs or recruits a different set of co-activators (Safe *et al*. 2002). In addition, estrogen-related receptors (ERRs) may serve as alternative receptors for transmitting BPA signals (Ariazi & Jordan 2006). Although ERRs do not bind estrogen, ERRγ binds BPA with high affinity (K_d of 5.5 nM; Okada *et al*. 2008). ERRγ is overexpressed in 75% of breast tumors compared to the normal epithelium (Ariazi *et al*. 2002). Phytoestrogens have also been identified as ERRs ligands (Ariazi & Jordan 2006).

BPA rapidly activates nongenomic signaling in many cell types. In MCF7 cells, MAPK and Akt are phosphorylated within 10 min of BPA exposure, similar to that seen with E_2 and other xenoestrogens (Li *et al*. 2006). BPA at low doses induces rapid influx of calcium in breast cancer cells (Walsh *et al*. 2005) and rat hippocampal neurons (Tanabe *et al*. 2006). In cerebellar neurons, Belcher *et al*. (2005) observed a

rapid BPA-induced activation of ERK1/2, PKA, and Src family kinases but not PI3K/Akt pathways. BPA at very low doses (0.01–1 nM) rapidly activates cAMP- and cGMP-dependent protein kinase and triggers rapid phosphorylation of CREB in human testicular seminoma cells (Bouskine *et al*. 2009). These BPA actions are neither reversed by ICI 182 780 nor reproduced by E_2 or DES, leading the authors to conclude that classical ERs are not involved.

The estrogenic activity of BPA was discovered upon noticing that BPA leaching from autoclaved plastic containers increases growth of MCF7 cells (Krishnan *et al*. 1993). In spite of its striking structural resemblance to DES, BPA exhibits a much lower binding affinity to either ERα or ERβ (Ben-Jonathan & Steinmetz 1998). The low binding affinity of BPA to classical ERs explains its inability to exert a strong mitogenic activity in MCF7 cells. One study found increased cell proliferation in response to BPA only at 60 000 times higher concentrations than E_2 (Olsen *et al*. 2003). Another group confirmed that BPA at a relatively high dose (1 μM) showed a modest stimulation of proliferation of MCF7 cells cultured in estrogen-depleted medium, while E_2 was effective at subnanomolar concentrations (Hess-Wilson *et al*. 2006).

Differential gene profiling in response to BPA has also been reported. In one study, BPA induces a different set of genes than E_2 in MCF7 cells, and about 15 genes in ER-null MCF7 cells, leading the authors to conclude that at least some of its actions are independent of ERα (Singleton *et al*. 2004). Another group added BPA to cultures of breast tissue aspirates (Dairkee *et al*. 2008). Expression profiling revealed that BPA is associated with high grade tumors and decreased patient survival. These data suggest that exposure to BPA may contribute to the establishment and/or maintenance of breast tumors. In both studies, however, the BPA doses were at the upper nanomolar to micromolar levels. A major criticism of studies using BPA at very high doses is that they do not reflect human exposure levels to this compound. Since BPA exhibits a 'U'-shaped dose-dependent curve in MCF7 cells (Samuelsen *et al*. 2001), extrapolation from its action, or lack of action, at high doses to its presumed activity at low doses can be misleading.

Our study was the first to report that BPA at environmentally relevant concentrations confers chemoresistance (LaPensee *et al*. 2009*c*). Similar to the actions of PRL and E_2, BPA antagonizes multiple anticancer drugs, showing equimolar potency with E_2 in opposing cisplatin toxicity. These BPA actions do not appear to be mediated via ERα and ERβ, but the receptor involved was not identified (LaPensee *et al*. 2009*c*). In addition to GPR30, we found that both MDA-MB-468 and T47D cells express ERRγ. Given its high binding affinity for ERRγ, BPA may exert its chemoprotective effects via this receptor. ERRγ has been implicated in tamoxifen resistance in a cell line derived from invasive breast carcinoma (Riggins *et al*. 2008). Studies are underway with siRNA directed against ERRγ to examine its involvement in BPA-induced chemoresistance. However, we cannot rule out involvement of as yet unidentified receptor. We are also examining if BPA actions are mediated via genomic versus nongenomic mechanisms.

BPA alone, or in combination with doxorubicin (LaPensee *et al*. 2009*c*) or cisplatin (LaPensee *et al*. 2009*a*) increases Bcl-2 expression. Treatment with a Bcl-2 inhibitor completely blocks the BPA-induced antagonism of cisplatin, whereas it only partially abrogates protection by E_2. This suggests that BPA and estrogen may exert protection against cytotoxicity by somewhat different mechanisms, i.e. antiapoptosis versus mitogenesis. This notion is supported by flow cytometry and BrdU incorporation showing that BPA alone increases cell survival, while estrogen alone increased cell proliferation. Figure 3 schematically illustrates the role of Bcl-2 antiapoptotic protein in mediating chemoresistance by BPA. Note that the mechanism by which BPA exerts chemoresistance against cisplatin differs from that caused by PRL (see Fig. 2).

Summary and perspectives

Hormones have long been implicated in the pathogenesis of breast cancer, but only a few studies have addressed their role in chemoresistance. Mounting evidence indicates that low doses of PRL, E_2, and BPA antagonize multiple anticancer drugs that induce cell death by different mechanisms. PRL opposes cisplatin by increasing GST activity, while E_2 and BPA act by increasing Bcl-2 expression. This serves as an excellent example of why targeting one mechanism of resistance may not be sufficient for slowing down tumor growth or eliminating metastases. Future studies should examine in more detail the potential crosstalk between PRL and E_2 in conferring resistance, and expand *in vitro* studies to pre-clinical models. FDA-approved inhibitors of PRL or E_2, e.g. bromocriptine and tamoxifen, should provide for an easy transition from animal models to clinical trials. As for PRL, blockade of the receptor should be more effective than attempting to reduce the hormone itself.

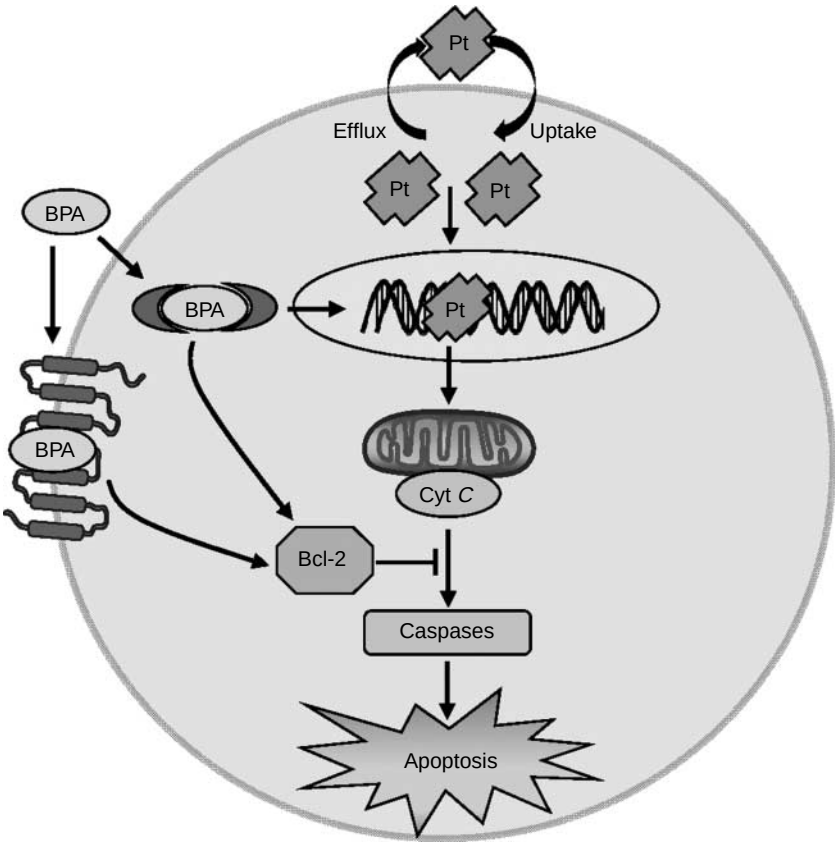

Figure 3 Proposed mechanism by which bisphenol A (BPA) antagonizes cisplatin-induced apoptosis in breast cancer cells. Cisplatin (Pt) diffuses into the cell and enters the nucleus, where it binds to DNA. The ensuing cell cycle arrest leads to the release of mitochondrial cytochrome *C* (Cyt *C*), activation of caspases and apoptosis. BPA binds either to a membrane receptor or diffuses into the cell and binds to a cytoplasmic/nuclear receptor. This results in increased expression of the antiapoptotic protein Bcl-2, which blocks Cyt *C* release and apoptosis. Estradiol (E_2) also antagonizes cisplatin-induced apoptosis by activating similar or different mechanisms. Neither the identity of the receptor(s), which mediate the anticytotoxic actions of BPA/E_2, nor the pathway(s) underlying Bcl-2 activation is known.

A reduction in the ability of PRL and estrogens to confer chemoresistance should have several benefits to breast cancer patients, including an increase in the number as well as efficacy of valuable drugs. For example, drugs such as cisplatin, which has shown success in treating many other types of cancers, could be introduced into breast cancer regimens, while the efficacy of already successful anticancer drugs such as taxol could increase. Many treatment regimens use drugs that act by different mechanisms to improve the chances of suppressing tumor growth. Hence, having more options for combination therapy should especially benefit those patients who undergo second- or third-line anticancer treatment. Furthermore, increased efficacy should enable the use of lower drug doses, thereby reducing the toxicity and side effects associated with high dose therapy and improving the quality of life. Finally, because the actions of E_2, BPA, and possibly other endocrine disruptors may be independent of ERα and ERβ, patients with ER-negative tumors could benefit from the blockade of E_2 and estrogen-like compounds.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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References

Aas T, Borresen AL, Geisler S, Smith-Sorensen B, Johnsen H, Varhaug JE, Akslen LA & Lønning PE 1996 Specific P53 mutations are associated with *de novo* resistance to doxorubicin in breast cancer patients. *Nature Medicine* **2** 811–814.

Acosta JJ, Munoz RM, Gonzalez L, Subtil-Rodriguez A, Dominguez-Caceres MA, Garcia-Martinez JM, Calcabrini A, Lazaro-Trueba I & Martin-Perez J 2003 Src mediates prolactin-dependent proliferation of T47D and MCF7 cells via the activation of focal adhesion kinase/Erk1/2 and phosphatidylinositol 3-kinase pathways. *Molecular Endocrinology* **17** 2268–2282.

Ambrosone CB, Sweeney C, Coles BF, Thompson PA, McClure GY, Korourian S, Fares MY, Stone A, Kadlubar FF & Hutchins LF 2001 Polymorphisms in glutathione-*S*-transferases (GSTM1 and GSTT1) and survival after treatment for breast cancer. *Cancer Research* **61** 7130–7135.

Ariazi EA & Jordan VC 2006 Estrogen-related receptors as emerging targets in cancer and metabolic disorders. *Current Topics in Medicinal Chemistry* **6** 203–215.

Ariazi EA, Clark GM & Mertz JE 2002 Estrogen-related receptor alpha and estrogen-related receptor gamma associate with unfavorable and favorable biomarkers, respectively, in human breast cancer. *Cancer Research* **62** 6510–6518.

Asai-Sato M, Nagashima Y, Miyagi E, Sato K, Ohta I, Vonderhaar BK & Hirahara F 2005 Prolactin inhibits apoptosis of ovarian carcinoma cells induced by serum starvation or cisplatin treatment. *International Journal of Cancer* **115** 539–544.

Bachman KE & Park BH 2005 Duel nature of TGF-beta signaling: tumor suppressor vs. tumor promoter. *Current Opinion in Oncology* **17** 49–54.

Bai Z & Gust R 2009 Breast cancer, estrogen receptor and ligands. *Archiv der Pharmazie* **342** 133–149.

Bargou RC, Wagener C, Bommert K, Mapara MY, Daniel PT, Arnold W, Dietel M, Guski H, Feller A, Royer HD *et al.* 1996 Overexpression of the death-promoting gene bax-alpha which is downregulated in breast cancer restores sensitivity to different apoptotic stimuli and reduces tumor growth in SCID mice. *Journal of Clinical Investigation* **97** 2651–2659.

Belcher SM, Le HH, Spurling L & Wong JK 2005 Rapid estrogenic regulation of extracellular signal- regulated kinase 1/2 signaling in cerebellar granule cells involves a G protein- and protein kinase A-dependent mechanism and intracellular activation of protein phosphatase 2A. *Endocrinology* **146** 5397–5406.

Bellail AC, Qi L, Mulligan P, Chhabra V & Hao C 2009 TRAIL agonists on clinical trials for cancer therapy: the promises and the challenges. *Reviews on Recent Clinical Trials* **4** 34–41.

Ben-Jonathan N & Steinmetz R 1998 Xenoestrogens: the emerging story of bisphenol A. *Trends in Endocrinology and Metabolism* **9** 124–128.

Ben-Jonathan N, Mershon JL, Allen DL & Steinmetz RW 1996 Extrapituitary prolactin: distribution, regulation, functions, and clinical aspects. *Endocrine Reviews* **17** 639–669.

Ben-Jonathan N, Liby K, McFarland M & Zinger M 2002 Prolactin as an autocrine/paracrine growth factor in human cancer. *Trends in Endocrinology and Metabolism* **13** 245–250.

Ben-Jonathan N, LaPensee CR & LaPensee EW 2008 What can we learn from rodents about prolactin in humans? *Endocrine Reviews* **29** 1–41.

Birnbaum LS & Fenton SE 2003 Cancer and developmental exposure to endocrine disruptors. *Environmental Health Perspectives* **111** 389–394.

Bjornstrom L & Sjoberg M 2005 Mechanisms of estrogen receptor signaling: convergence of genomic and nongenomic actions on target genes. *Molecular Endocrinology* **19** 833–842.

Blanc C, Deveraux QL, Krajewski S, Janicke RU, Porter AG, Reed JC, Jaggi R & Marti A 2000 Caspase-3 is essential for procaspase-9 processing and cisplatin-induced apoptosis of MCF-7 breast cancer cells. *Cancer Research* **60** 4386–4390.

Bouskine A, Nebout M, Brucker-Davis F, Benahmed M & Fenichel P 2009 Low doses of bisphenol A promote human seminoma cell proliferation by activating PKA and PKG via a membrane G-protein-coupled estrogen receptor. *Environmental Health Perspectives* **117** 1053–1058.

Brockman JL, Schroeder MD & Schuler LA 2002 PRL activates the cyclin D1 promoter via the Jak2/Stat pathway. *Molecular Endocrinology* **16** 774–784.

Carver KC, Arendt LM & Schuler LA 2009 Complex prolactin crosstalk in breast cancer: new therapeutic implications. *Molecular and Cellular Endocrinology* **307** 1–7.

Chakravarti P, Henry MK & Quelle FW 2005 Prolactin and heregulin override DNA damage-induced growth arrest and promote phosphatidylinositol-3 kinase-dependent proliferation in breast cancer cells. *International Journal of Oncology* **26** 509–514.

Chang BD, Swift ME, Shen M, Fang J, Broude EV & Roninson IB 2002 Molecular determinants of terminal growth arrest induced in tumor cells by a chemotherapeutic agent. *PNAS* **99** 389–394.

Chen WY, Ramamoorthy P, Chen N, Sticca R & Wagner TE 1999 A human prolactin antagonist, hPRL-G129R, inhibits breast cancer cell proliferation through induction of apoptosis. *Clinical Cancer Research* **5** 3583–3593.

Chen NY, Holle L, Li W, Peirce SK, Beck MT & Chen WY 2002 *In vivo* studies of the anti-tumor effects of a human prolactin antagonist, hPRL-G129R. *International Journal of Oncology* **20** 813–818.

Clapp C, Thebault S & Martinez de la EG 2008 Role of prolactin and vasoinhibins in the regulation of vascular function in mammary gland. *Journal of Mammary Gland Biology and Neoplasia* **13** 55–67.

Clevenger CV, Gadd SL & Zheng J 2009 New mechanisms for PRLr action in breast cancer. *Trends in Endocrinology and Metabolism* **20** 223–229.

Coley HM 2008 Mechanisms and strategies to overcome chemotherapy resistance in metastatic breast cancer. *Cancer Treatment Reviews* **34** 378–390.

Dairkee SH, Seok J, Champion S, Sayeed A, Mindrinos M, Xiao W, Davis RW & Goodson WH 2008 Bisphenol A induces a profile of tumor aggressiveness in high-risk cells from breast cancer patients. *Cancer Research* **68** 2076–2080.

Decatris MP, Sundar S & O'byrne KJ 2004 Platinum-based chemotherapy in metastatic breast cancer: current status. *Cancer Treatment Reviews* **30** 53–81.

Duan L, Sterba K, Kolomeichuk S, Kim H, Brown PH & Chambers TC 2007 Inducible overexpression of c-Jun in MCF7 cells causes resistance to vinblastine via inhibition of drug-induced apoptosis and senescence at a step subsequent to mitotic arrest. *Biochemical Pharmacology* **73** 481–490.

Duan R, Ginsburg E & Vonderhaar BK 2008 Estrogen stimulates transcription from the human prolactin distal promoter through AP1 and estrogen responsive elements in T47D human breast cancer cells. *Molecular and Cellular Endocrinology* **281** 9–18.

Falconer IR 2006 Are endocrine disrupting compounds a health risk in drinking water? *International Journal of Environmental Research and Public Health* **3** 180–184.

Ferlini C, Raspaglio G, Mozzetti S, Distefano M, Filippetti F, Martinelli E, Ferrandina G, Gallo D, Ranelletti FO & Scambia G 2003 Bcl-2 down-regulation is a novel mechanism of paclitaxel resistance. *Molecular Pharmacology* **64** 51–58.

Fernandez MF, Arrebola JP, Taoufiki J, Navalon A, Ballesteros O, Pulgar R, Vilchezb JL & Oleaa N 2007 Bisphenol-A and chlorinated derivatives in adipose tissue of women. *Reproductive Toxicology* **24** 259–264.

Filardo EJ & Thomas P 2005 GPR30: a seven-trans-membrane-spanning estrogen receptor that triggers EGF release. *Trends in Endocrinology and Metabolism* **16** 362–367.

Filardo EJ, Quinn JA, Bland KI & Frackelton AR Jr 2000 Estrogen-induced activation of Erk-1 and Erk-2 requires the G protein-coupled receptor homolog, GPR30, and occurs via trans-activation of the epidermal growth factor receptor through release of HB-EGF. *Molecular Endocrinology* **14** 1649–1660.

Filardo EJ, Graeber CT, Quinn JA, Resnick MB, Giri D, DeLellis RA, Steinhoff MM & Sabo E 2006 Distribution of GPR30, a seven membrane-spanning estrogen receptor, in primary breast cancer and its association with clinicopathologic determinants of tumor progression. *Clinical Cancer Research* **12** 6359–6366.

Foster PA 2008 Steroid metabolism in breast cancer. *Minerva Endocrinologica* **33** 27–37.

Fox EM, Andrade J & Shupnik MA 2009 Novel actions of estrogen to promote proliferation: integration of cyto-plasmic and nuclear pathways. *Steroids* **74** 622–627.

Friedrich K, Wieder T, Von Haefen C, Radetzki S, Janicke R, Schulze-Osthoff K, Dörken B & Daniel PT 2001 Overexpression of caspase-3 restores sensitivity for drug-induced apoptosis in breast cancer cell lines with acquired drug resistance. *Oncogene* **20** 2749–2760.

Gerlo S, Davis JR, Mager DL & Kooijman R 2006 Prolactin in man: a tale of two promoters. *BioEssays* **28** 1051–1055.

Gertler A, Grosclaude J, Strasburger CJ, Nir S & Djiane J 1996 Real-time kinetic measurements of the interactions between lactogenic hormones and prolactin-receptor extracellular domains from several species support the model of hormone-induced transient receptor dimeri-zation. *Journal of Biological Chemistry* **271** 24482–24491.

Goffin V, Bernichtein S, Touraine P & Kelly PA 2005 Development and potential clinical uses of human prolactin receptor antagonists. *Endocrine Reviews* **26** 400–422.

Gonzalez L, Zambrano A, Lazaro-Trueba I, Lopez E, Gonzalez JJ, Martin-Perez J & Aranda A 2009 Activation of the unliganded estrogen receptor by prolactin in breast cancer cells. *Oncogene* **28** 1298–1308.

Gray J, Evans N, Taylor B, Rizzo J & Walker M 2009 State of the evidence: the connection between breast cancer and the environment. *International Journal of Occupational and Environmental Health* **15** 43–78.

Gutzman JH, Nikolai SE, Rugowski DE, Watters JJ & Schuler LA 2005 Prolactin and estrogen enhance the activity of activating protein 1 in breast cancer cells: role of extracellularly regulated kinase 1/2-mediated signals to c-fos. *Molecular Endocrinology* **19** 1765–1778.

Henkels KM & Turchi JJ 1999 Cisplatin-induced apoptosis proceeds by caspase-3-dependent and -independent pathways in cisplatin-resistant and -sensitive human ovarian cancer cell lines. *Cancer Research* **59** 3077–3083.

Hess-Wilson JK, Boldison J, Weaver KE & Knudsen KE 2006 Xenoestrogen action in breast cancer: impact on ER-dependent transcription and mitogenesis. *Breast Cancer Research and Treatment* **96** 279–292.

Higgins CF 2007 Multiple molecular mechanisms for multidrug resistance transporters. *Nature* **446** 749–757.

Howell SJ, Anderson E, Hunter T, Farnie G & Clarke RB 2008 Prolactin receptor antagonism reduces the clonogenic capacity of breast cancer cells and potentiates doxorubicin and paclitaxel cytotoxicity. *Breast Cancer Research* **10** R68.

Hsu PC, Hour TC, Liao YF, Hung YC, Liu CC, Chang WH, Kao MC, Tsay G, Hung HC & Liu GY 2006 Increasing ornithine decarboxylase activity is another way of prolactin preventing methotrexate-induced apoptosis: crosstalk between ODC and BCL-2. *Apoptosis* **11** 389–399.

Huang Y, Ray S, Reed JC, Ibrado AM, Tang C, Nawabi A & Bhalla K 1997 Estrogen increases intracellular p26Bcl-2 to p21Bax ratios and inhibits taxol-induced apoptosis of human breast cancer MCF-7 cells. *Breast Cancer Research and Treatment* **42** 73–81.

Huang Y, Fang Y, Wu J, Dziadyk JM, Zhu X, Sui M & Fan W 2004 Regulation of Vinca alkaloid-induced apoptosis by NF-kappaB/IkappaB pathway in human tumor cells. *Molecular Cancer Therapeutics* **3** 271–277.

Hugo ER, Brandebourg TD, Woo JG, Loftus J, Alexander JW & Ben-Jonathan N 2008 Bisphenol A at environmentally relevant doses inhibits adiponectin release from human adipose tissue explants and adipocytes. *Environmental Health Perspectives* **116** 1642–1647.

Imai Y, Ishikawa E, Asada S & Sugimoto Y 2005 Estrogen-mediated post transcriptional down-regulation of breast cancer resistance protein/ABCG2. *Cancer Research* **65** 596–604.

Jongen VH, Hollema H, Van Der Zee AG & Heineman MJ 2006 Aromatase in the context of breast and endometrial cancer – a review. *Minerva Endocrinologica* **31** 47–60.

Kang JH, Kondo F & Katayama Y 2006 Human exposure to bisphenol A. *Toxicology* **226** 79–89.

Karayiannakis AJ, Bastounis EA, Chatzigianni EB, Makri GG, Alexiou D & Karamanakos P 1996 Immunohistochemical detection of oestrogen receptors in ductal carcinoma *in situ* of the breast. *European Journal of Surgical Oncology* **22** 578–582.

Kelland L 2007 The resurgence of platinum-based cancer chemotherapy. *Nature Reviews. Cancer* **7** 573–584.

Kennedy RD, Quinn JE, Mullan PB, Johnston PG & Harkin DP 2004 The role of BRCA1 in the cellular response to chemotherapy. *Journal of the National Cancer Institute* **96** 1659–1668.

Krishnan AV, Stathis P, Permuth SF, Tokes L & Feldman D 1993 Bisphenol-A: an estrogenic substance is released from polycarbonate flasks during autoclaving. *Endocrinology* **132** 2279–2286.

Kuiper GG, Lemmen JG, Carlsson B, Corton JC, Safe SH, van der Saag PT, van der Burg B & Gustafsson JA 1998 Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. *Endocrinology* **139** 4252–4263.

Lagadec C, Adriaenssens E, Toillon RA, Chopin V, Romon R, Van CF, Hondermarck H & Le Bourhis X 2008 Tamoxifen and TRAIL synergistically induce apoptosis in breast cancer cells. *Oncogene* **27** 1472–1477.

LaPensee EW, Fox S, Schwemberger SJ, Afton S & Ben-Jonathan N 2009*a* Bisphenol A and estradiol are equipotent in antagonizing cisplatin cytotoxicity in breast cancer cells. *Cancer Letters* [in press].

LaPensee EW, Schwemberger SJ, LaPensee CR, Bahassi EM, Afton SE & Ben-Jonathan N 2009*b* Prolactin confers resistance against cisplatin in breast cancer cells by activating glutathione-*S*-transferase. *Carcinogenesis* **30** 1298–1304.

LaPensee EW, Tuttle TR, Fox SR & Ben-Jonathan N 2009*c* Bisphenol A at low nanomolar doses confers chemoresistance in estrogen receptor-alpha-positive and -negative breast cancer cells. *Environmental Health Perspectives* **117** 175–180.

Le HH, Carlson EM, Chua JP & Belcher SM 2008 Bisphenol A is released from polycarbonate drinking bottles and mimics the neurotoxic actions of estrogen in developing cerebellar neurons. *Toxicology Letters* **176** 149–156.

L'Ecuyer T, Allebban Z, Thomas R & Vander HR 2004 Glutathione-*S*-transferase overexpression protects against anthracycline-induced H9C2 cell death. *American Journal of Physiology. Heart and Circulatory Physiology* **286** H2057–H2064.

Lewis-Wambi JS & Jordan VC 2009 Estrogen regulation of apoptosis: how can one hormone stimulate and inhibit? *Breast Cancer Research* **11** 206.

Li X, Zhang S & Safe S 2006 Activation of kinase pathways in MCF-7 cells by 17beta-estradiol and structurally diverse estrogenic compounds. *Journal of Steroid Biochemistry and Molecular Biology* **98** 122–132.

Liby K, Neltner B, Mohamet L, Menchen L & Ben-Jonathan N 2003 Prolactin overexpression by MDA-MB-435 human breast cancer cells accelerates tumor growth. *Breast Cancer Research and Treatment* **79** 241–252.

Lissoni P, Vaghi M, Ardizzoia A, Fumagalli E, Tancini G, Gardani G, Conti A & Maestroni GJ 2001 Efficacy of monochemotherapy with docetaxel (taxotere) in relation to prolactin secretion in heavily pretreated metastatic breast cancer. *Neuroendocrinology Letters* **22** 27–29.

Lissoni P, Bucovec R, Malugani F, Ardizzoia A, Villa S, Gardani GS, Vaghi M & Tancini G 2002 A clinical study of taxotere versus taxotere plus the antiprolactinemic agent bromocriptine in metastatic breast cancer pretreated with anthracyclines. *Anticancer Research* **22** 1131–1134.

Llovera M, Pichard C, Bernichtein S, Jeay S, Touraine P, Kelly PA & Goffin V 2000 Human prolactin (hPRL) antagonists inhibit hPRL-activated signaling pathways involved in breast cancer cell proliferation. *Oncogene* **19** 4695–4705.

Luquita MG, Catania VA, Sanchez-Pozzi EJ, Vore M & Mottino AD 1999 Prolactin increases the hepatic content of mu-class subunits of glutathione-*S*-transferase in the rat. *Drug Metabolism and Disposition* **27** 122–124.

Manavathi B & Kumar R 2006 Steering estrogen signals from the plasma membrane to the nucleus: two sides of the coin. *Journal of Cellular Physiology* **207** 594–604.

Martin JH, Crotty S & Nelson PN 2007 Phytoestrogens: perpetrators or protectors? *Future Oncology* **3** 307–318.

Maus MV, Reilly SC & Clevenger CV 1999 Prolactin as a chemoattractant for human breast carcinoma. *Endocrinology* **140** 5447–5450.

McHale K, Tomaszewski JE, Puthiyaveettil R, Livolsi VA & Clevenger CV 2008 Altered expression of prolactin receptor-associated signaling proteins in human breast carcinoma. *Modern Pathology* **21** 565–571.

McIlwain CC, Townsend DM & Tew KD 2006 Glutathione-*S*-transferase polymorphisms: cancer incidence and therapy. *Oncogene* **25** 1639–1648.

Mense SM, Hei TK, Ganju RK & Bhat HK 2008 Phytoestrogens and breast cancer prevention: possible mechanisms of action. *Environmental Health Perspectives* **116** 426–433.

Nabholtz JM, Mouret-Reynier MA, Durando X, Van IP, Al-Sukhun S, Ferriere JP & Chollet P 2009 Comparative review of anastrozole, letrozole and exemestane in the management of early breast cancer. *Expert Opinion on Pharmacotherapy* **10** 1435–1447.

Nevalainen MT, Xie J, Torhorst J, Bubendorf L, Haas P, Kononen J, Sauter G & Rui H 2004 Signal transducer and activator of transcription-5 activation and breast cancer prognosis. *Journal of Clinical Oncology* **22** 2053–2060.

Nilsson S, Makela S, Treuter E, Tujague M, Thomsen J, Andersson G, Enmark E, Pettersson K, Warner M & Gustafsson JA 2001 Mechanisms of estrogen action. *Physiological Reviews* **81** 1535–1565.

Normanno N, Di Maio M, De Maio E, De Luca A, de Matteis A, Giordano A, Perrone F & NCI-Naple Breast Cancer Group 2005 Mechanisms of endocrine resistance and novel therapeutic strategies in breast cancer. *Endocrine-Related Cancer* **12** 721–747.

Ocana A & Pandiella A 2008 Identifying breast cancer druggable oncogenic alterations: lessons learned and future targeted options. *Clinical Cancer Research* **14** 961–970.

Okada H, Tokunaga T, Liu X, Takayanagi S, Matsushima A & Shimohigashi Y 2008 Direct evidence revealing structural elements essential for the high binding ability of bisphenol A to human estrogen-related receptor-gamma. *Environmental Health Perspectives* **116** 32–38.

Olsen CM, Meussen-Elholm ET, Samuelsen M, Holme JA & Hongslo JK 2003 Effects of the environmental oestrogens bisphenol A, tetrachlorobisphenol A, tetrabromobisphenol A, 4-hydroxybiphenyl and 4,4′-dihydroxybiphenyl on oestrogen receptor binding, cell proliferation and regulation of oestrogen sensitive proteins in the human breast cancer cell line MCF-7. *Pharmacology and Toxicology* **92** 180–188.

Pasqualini JR, Chetrite G, Blacker C, Feinstein MC, Delalonde L, Talbi M & Maloche C 1996 Concentrations of estrone, estradiol, and estrone sulfate and evaluation of sulfatase and aromatase activities in pre- and postmenopausal breast cancer patients. *Journal of Clinical Endocrinology and Metabolism* **81** 1460–1464.

Peirce SK, Chen WY & Chen WY 2001 Quantification of prolactin receptor mRNA in multiple human tissues and cancer cell lines by real time RT-PCR. *Journal of Endocrinology* **171** R1–R4.

Perks CM, Keith AJ, Goodhew KL, Savage PB, Winters ZE & Holly JM 2004 Prolactin acts as a potent survival factor for human breast cancer cell lines. *British Journal of Cancer* **91** 305–311.

Perotti C, Liu R, Parusel CT, Bocher N, Schultz J, Bork P, Pfitzner E, Groner B & Shemanko CS 2008 Heat shock protein-90-alpha, a prolactin-STAT5 target gene identified in breast cancer cells, is involved in apoptosis regulation. *Breast Cancer Research* **10** R94.

Prossnitz ER, Arterburn JB, Smith HO, Oprea TI, Sklar LA & Hathaway HJ 2008 Estrogen signaling through the transmembrane G protein-coupled receptor GPR30. *Annual Review of Physiology* **70** 165–190.

Rabbani A, Finn RM & Ausio J 2005 The anthracycline antibiotics: antitumor drugs that alter chromatin structure. *BioEssays* **27** 50–56.

Ramamoorthy P, Sticca R, Wagner TE & Chen WY 2001 *In vitro* studies of a prolactin antagonist, hPRL-G129R in human breast cancer cells. *International Journal of Oncology* **18** 25–32.

Razandi M, Pedram A & Levin ER 2000 Plasma membrane estrogen receptors signal to antiapoptosis in breast cancer. *Molecular Endocrinology* **14** 1434–1447.

Ricketts D, Turnbull L, Ryall G, Bakhshi R, Rawson NS, Gazet JC, Nolan C & Coombes RC 1991 Estrogen and progesterone receptors in the normal female breast. *Cancer Research* **51** 1817–1822.

Riggins RB, Lan JP, Zhu Y, Klimach U, Zwart A, Cavalli LR, Haddad BR, Chen L, Gong T, Xuan J *et al.* 2008 ERRgamma mediates tamoxifen resistance in novel models of invasive lobular breast cancer. *Cancer Research* **68** 8908–8917.

Rodrik V, Zheng Y, Harrow F, Chen Y & Foster DA 2005 Survival signals generated by estrogen and phospholipase D in MCF-7 breast cancer cells are dependent on Myc. *Molecular and Cellular Biology* **25** 7917–7925.

Ruffion A, Al Sakkaf KA, Brown BL, Eaton CL, Hamdy FC & Dobson PR 2003 The survival effect of prolactin on PC3 prostate cancer cells. *European Urology* **43** 301–308.

Safe SH, Pallaroni L, Yoon K, Gaido K, Ross S & McDonnell D 2002 Problems for risk assessment of endocrine-active estrogenic compounds. *Environmental Health Perspectives* **110** (Suppl 6) 925–929.

Samuelsen M, Olsen C, Holme JA, Meussen-Elholm E, Bergmann A & Hongslo JK 2001 Estrogen-like properties of brominated analogs of bisphenol A in the MCF-7 human breast cancer cell line. *Cell Biology and Toxicology* **17** 139–151.

Santen RJ, Santner SJ, Pauley RJ, Tait L, Kaseta J, Demers LM, Hamilton C, Yue W & Wang JP 1997 Estrogen production via the aromatase enzyme in breast carcinoma: which cell type is responsible? *Journal of Steroid Biochemistry and Molecular Biology* **61** 267–271.

Sessa C, Guibal A, Del CG & Ruegg C 2008 Biomarkers of angiogenesis for the development of antiangiogenic therapies in oncology: tools or decorations? *Nature Clinical Practice and Oncology* **5** 378–391.

Shaaban AM, O'Neill PA, Davies MP, Sibson R, West CR, Smith PH & Foster CS 2003 Declining estrogen receptor-beta expression defines malignant progression of human breast neoplasia. *American Journal of Surgical Pathology* **27** 1502–1512.

Shiga H, Heath EI, Rasmussen AA, Trock B, Johnston PG, Forastiere AA, Langmacher M, Baylor A, Lee M & Cullen KJ 1999 Prognostic value of p53, glutathione-*S*-transferase pi, and thymidylate synthase for neoadjuvant cisplatin-based chemotherapy in head and neck cancer. *Clinical Cancer Research* **5** 4097–4104.

Siddik ZH 2003 Cisplatin: mode of cytotoxic action and molecular basis of resistance. *Oncogene* **22** 7265–7279.

Simoes-Wust AP, Schurpf T, Hall J, Stahel RA & Zangemeister-Wittke U 2002 Bcl-2/bcl-xL bispecific antisense treatment sensitizes breast carcinoma cells to doxorubicin, paclitaxel and cyclophosphamide. *Breast Cancer Research and Treatment* **76** 157–166.

Singleton DW, Feng Y, Chen Y, Busch SJ, Lee AV, Puga A & Khan SA 2004 Bisphenol-A and estradiol exert novel gene regulation in human MCF-7 derived breast cancer cells. *Molecular and Cellular Endocrinology* **221** 47–55.

Song RX, Fan P, Yue W, Chen Y & Santen RJ 2006 Role of receptor complexes in the extranuclear actions of estrogen receptor alpha in breast cancer. *Endocrine-Related Cancer* **13** (Suppl 1) S3–S13.

Sui M, Huang Y, Park BH, Davidson NE & Fan W 2007 Estrogen receptor alpha mediates breast cancer cell resistance to paclitaxel through inhibition of apoptotic cell death. *Cancer Research* **67** 5337–5344.

Sultan AS, Xie J, LeBaron MJ, Ealley EL, Nevalainen MT & Rui H 2005 Stat5 promotes homotypic adhesion and inhibits invasive characteristics of human breast cancer cells. *Oncogene* **24** 746–760.

Suzuki T, Miki Y, Nakamura Y, Moriya T, Ito K, Ohuchi N & Sasano H 2005 Sex steroid-producing enzymes in human breast cancer. *Endocrine-Related Cancer* **12** 701–720.

Swaminathan G, Varghese B & Fuchs SY 2008 Regulation of prolactin receptor levels and activity in breast cancer. *Journal of Mammary Gland Biology and Neoplasia* **13** 81–91.

Tanabe N, Kimoto T & Kawato S 2006 Rapid Ca(2+) signaling induced by bisphenol A in cultured rat hippocampal neurons. *Neuroendocrinology Letters* **27** 97–104.

Teilum K, Hoch JC, Goffin V, Kinet S, Martial JA & Kragelund BB 2005 Solution structure of human prolactin. *Journal of Molecular Biology* **351** 810–823.

Teixeira C, Reed JC & Pratt MA 1995 Estrogen promotes chemotherapeutic drug resistance by a mechanism involving Bcl-2 proto-oncogene expression in human breast cancer cells. *Cancer Research* **55** 3902–3907.

Thomas P, Pang Y, Filardo EJ & Dong J 2005 Identity of an estrogen membrane receptor coupled to a G protein in human breast cancer cells. *Endocrinology* **146** 624–632.

Touraine P, Martini JF, Zafrani B, Durand JC, Labaille F, Malet C, Nicolas A, Trivin C, Postel-Vinay MC, Kuttenn F *et al.* 1998 Increased expression of prolactin receptor gene assessed by quantitative polymerase chain reaction in human breast tumors versus normal breast tissues. *Journal of Clinical Endocrinology and Metabolism* **83** 667–674.

Townsend DM & Tew KD 2003 The role of glutathione-*S*-transferase in anti-cancer drug resistance. *Oncogene* **22** 7369–7375.

Trock BJ, Leonessa F & Clarke R 1997 Multidrug resistance in breast cancer: a meta-analysis of MDR1/gp170 expression and its possible functional significance. *Journal of the National Cancer Institute* **89** 917–931.

Tworoger SS & Hankinson SE 2008 Prolactin and breast cancer etiology: an epidemiologic perspective. *Journal of Mammary Gland Biology and Neoplasia* **13** 41–53.

Utama FE, LeBaron MJ, Neilson LM, Sultan AS, Parlow AF, Wagner KU & Rui H 2006 Human prolactin receptors are insensitive to mouse prolactin: implications for xenotransplant modeling of human breast cancer in mice. *Journal of Endocrinology* **188** 589–601.

Vacca A, Iurlaro M, Ribatti D, Minischetti M, Nico B, Ria R, Pellegrino A & Dammacco F 1999 Antiangiogenesis is produced by nontoxic doses of vinblastine. *Blood* **94** 4143–4155.

Van Den Bemd GJ, Kuiper GG, Pols HA & Van Leeuwen JP 1999 Distinct effects on the conformation of estrogen receptor alpha and beta by both the antiestrogens ICI 164,384 and ICI 182,780 leading to opposite effects on receptor stability. *Biochemical and Biophysical Research Communications* **261** 1–5.

Vonderhaar BK 1999 Prolactin involvement in breast cancer. *Endocrine-Related Cancer* **6** 389–404.

Walsh DE, Dockery P & Doolan CM 2005 Estrogen receptor independent rapid non-genomic effects of environmental estrogens on $[Ca^{2+}]$i in human breast cancer cells. *Molecular and Cellular Endocrinology* **230** 23–30.

Wang S & El Deiry WS 2003 TRAIL and apoptosis induction by TNF-family death receptors. *Oncogene* **22** 8628–8633.

Wang Z, Goulet R III, Stanton KJ, Sadaria M & Nakshatri H 2005 Differential effect of anti-apoptotic genes Bcl-xL and c-FLIP on sensitivity of MCF-7 breast cancer cells to paclitaxel and docetaxel. *Anticancer Research* **25** 2367–2379.

Watson CS, Bulayeva NN, Wozniak AL & Finnerty CC 2005 Signaling from the membrane via membrane estrogen receptor-alpha: estrogens, xenoestrogens, and phytoestrogens. *Steroids* **70** 364–371.

Watson CS, Bulayeva NN, Wozniak AL & Alyea RA 2007 Xenoestrogens are potent activators of nongenomic estrogenic responses. *Steroids* **72** 124–134.

Welshons WV, Nagel SC & vom Saal FS 2006 Large effects from small exposures. III. Endocrine mechanisms mediating effects of bisphenol A at levels of human exposure. *Endocrinology* **147** S56–S69.

Wiltshire T, Senft J, Wang Y, Konat GW, Wenger SL, Reed E & Wang W 2007 BRCA1 contributes to cell cycle arrest and chemoresistance in response to the anticancer agent irofulven. *Molecular Pharmacology* **71** 1051–1060.

Yde CW & Issinger OG 2006 Enhancing cisplatin sensitivity in MCF-7 human breast cancer cells by down-regulation of Bcl-2 and cyclin D1. *International Journal of Oncology* **29** 1397–1404.

Zampieri L, Bianchi P, Ruff P & Arbuthnot P 2002 Differential modulation by estradiol of P-glycoprotein drug resistance protein expression in cultured MCF7 and T47D breast cancer cells. *Anticancer Research* **22** 2253–2259.

Zhou J & Giannakakou P 2005 Targeting microtubules for cancer chemotherapy. *Current Medicinal Chemistry: Anti-Cancer Agents* **5** 65–71.

Zinger M, McFarland M & Ben-Jonathan N 2003 Prolactin expression and secretion by human breast glandular and adipose tissue explants. *Journal of Clinical Endocrinology and Metabolism* **88** 689–696.