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Roles and Mechanisms of Estrogen and Estrogen Receptors in Breast Cancer Resistant to Chemotherapy

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1. Introduction

Breast cancer is the most common malignancy of women in many countries including United States and many European countries. Chemotherapy plays a major role in the treatment of advanced breast cancer, either as an adjuvant to primary therapy or as palliation therapy to improve symptoms and prolong survival. The previous 50 years have seen numerous advances in the properties of chemotherapeutic agents. However, a significant proportion of cancers are inherently unaffected by the administration of anticancer drugs. Furthermore, another considerable proportion of patients undergoing chemotherapy display an initial reduction in tumor size and then relapse with a marked insensitivity to a variety of drugs. Both phenomena are brought about by a resistant phenotype, which presents perhaps the single greatest barrier to successful chemotherapy.

Biological mechanisms contributing to drug resistance may be present *de novo* or arise after exposure to anticancer drugs. At present, drug resistance is considered as a multifactorial phenomenon involving several major mechanisms (1, 2). In general, two main groups of factors contribute to the development of drug resistance. The first group includes pharmacological and physiological factors such as drug metabolism and excretion, inadequate access of the drug to the tumor, inadequate infusion rate and inadequate route of delivery. The second group includes cell- or tissue-specific factors. For example, increased repair of DNA damage, reduced apoptotic cell death, altered metabolism of drugs, increased energy-dependent efflux (*e.g.* ATP-binding cassette transporters) of chemotherapeutic drugs and microRNAs are known factors correlated with the development of anticancer drug resistance (1). In recent years, both clinical observations and experimental studies suggested that steroid hormones and their receptors might also affect the therapeutic efficacy of antineoplastic drugs (3-8).

Traditionally, steroid hormones can be grouped into five groups by the receptors to which they bind: glucocorticoids, mineralocorticoids, androgens, estrogens and progestagens (9-11). Previous studies from our laboratory showed that glucocorticoids, such as dexamethasone, could significantly interfere with the antitumor activities of paclitaxel *in vitro* and *in vivo* (3, 4, 12). Further studies suggest that paclitaxel may induce apoptotic cell death through activation of the NF-κB/IκB signaling pathway, whereas glucocorticoids inhibit paclitaxel-induced apoptosis through induction of IκBα synthesis, which antagonizes paclitaxel-mediated activation of NF-κB and subsequently results in inhibition of paclitaxel-induced apoptosis (4, 13, 14). Considering that cancer patients are routinely pretreated with glucocorticoids (such as dexamethasone) before receiving taxanes (*e.g.* paclitaxel, docetaxel) to prevent taxane-related hypersensitivity reactions or other adverse effects, the finding of glucocorticoid-mediated inhibition of paclitaxel-induced apoptosis raises a clinically relevant question as to whether pretreatment of glucocorticoids might actually interfere with the therapeutic efficacy of paclitaxel. We have recently reviewed the influence and impact of glucocorticoids on drug-induced apoptosis (4). The cuurent article is largely focused on the role of estrogen and estrogen receptors on the resistance to chemotherapy and the potential strategies to reverse the resistance or sensitize ER+ breast tumors to chemotherapy.

2. Estrogen and estrogen receptors in the development and treatment of breast cancer

Estrogens, such as 17-β estradiol (E2) in human, are steroidal sex hormones that are synthesized from cholesterol and primarily secreted by the ovaries. They play a major role in the development and maintenance of the reproductive tract as well as in the development of the mammary glands. Estrogens also maintain bone density and reduce cardiovascular system by regulating cholesterol levels and influence some brain structures (15, 16). However, besides their physiological functions, estrogens are also involved in the development and progression of breast and the uterus cancers and can maintain tumor cell proliferation (15, 16).

Estrogen action is primarily mediated by two types of estrogen receptors (ERs), *i.e.* ERα and ERβ. ERs are members of the superfamily of nuclear receptors (17, 18). ERs in the cell nucleus mediate the effects of the ligand E2 by functioning as transcriptional regulators that access various target gene promoters either by directly binding to specific estrogen response elements (EREs) within the promoter or indirectly by interacting with other transcriptional regulators bound to the promoter. Further, several cases of ligand-independent activation of ERα mediated by its phosphorylation by various signaling pathways have been reported (19). In addition, ERα localized in the extra-nuclear compartment (such as the plasma membrane or cytoplasm ER) of target cells, can also mediate several nongenomic effects of estrogen. These non-genomic actions are associated with the activation of a kinase cascade, such as growth factor receptor kinases (*e.g.* epidermal growth factor receptor). By these means, E2 and ERα facilitate pathways involved in the promotion of cell proliferation, inhibition of apoptosis, stimulation of metastasis, and angiogenesis. Although there is growing evidence that the ERβ may inhibit the action of ERα by heterodimerizing with it, the overall role of ERβ in breast cancer remains to be better clarified. A number of reviews have recently been published on the biological roles of estrogens and molecular activities of ERs (15-20). Unless otherwise specified, ER refers to ERα within this review.

Cumulative analysis of tumor biopsies has shown that ERs present in ~65% of human breast tumors (21, 22). This is consistent with the crucial role of the ERα subtype in breast cancer etiology and progression, and with the role played by estrogens as tumor promoters. It has long been known that breast tumors that express the ERα protein (ER+) behave in a fundamentally different fashion than ER-negative (ER-) tumors with regard to their response to hormonal therapies, given that outcomes are often favorable in ER-positive breast tumors treated by adjuvant endocrine therapy alone (23, 24). Neoadjuvant chemotherapy has a well-established role in the management of early-stage, operable breast cancer, and remains the gold standard downstaging systemic therapy in many centers, regardless of ER status. However, the data from other clinical trials or retrospective analyses suggest that ER status might also affect the efficacy of chemotherapy (5-8). Specifically, it has been observed that some chemotherapeutic agents may be less effective in patients with ER+ tumors than those with ER- tumors.

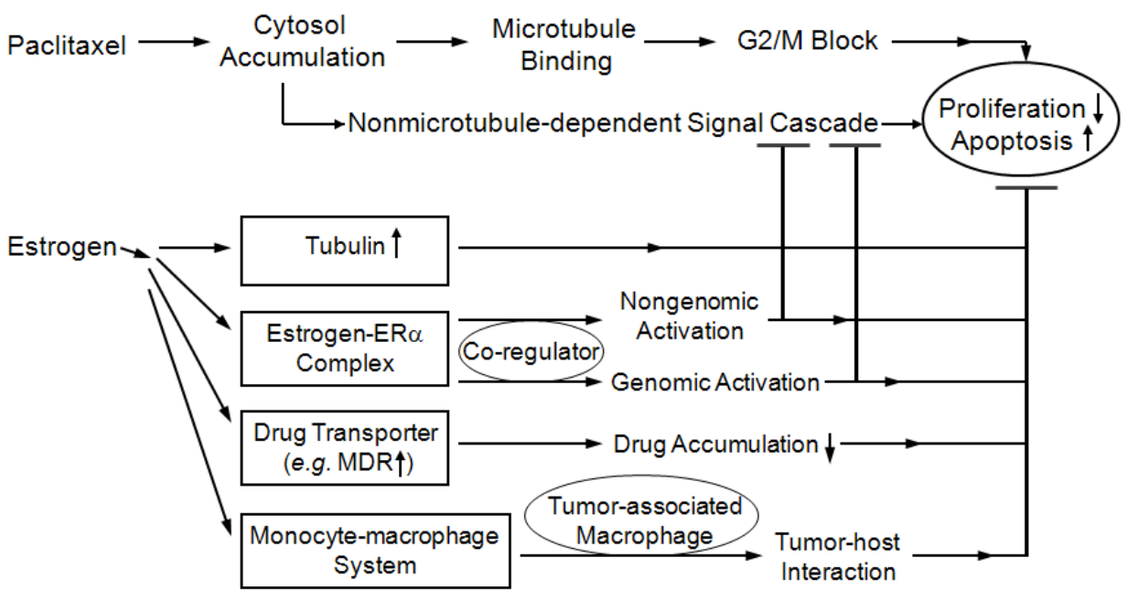

Fig. 1. Hypothesized pathways of estrogen/ER-mediated chemoresistance using paclitaxel as an example. ⊤ and ⊤⋮ represent inhibitory or antagonistic action. MDR, multidrug resistance. There are possible cross-talks between indicated pathways.

3. Current understnding of ER-mediated chemoresistance

More than one decade ago, Lippman ME *et al* first determined the relation between ER and the response rate to cytotoxic chemotherapy in 70 breast cancer patients (6). They found that 34 of 45 patients with low or absent ER values (<10 fmol/mg of cytoplasmic protein) had objective responses to chemotherapy, whereas only 3 of 25 patients with higher ER values (>10 fmol/mg of cytoplasmic protein) responded (p<0.0001). There were no statistically significant differences between the two groups in age, menopausal status, disease-free interval, Karnofsky index or prior therapy. Moreover, differences in sites of involvement or type of combination chemotherapy did not account for the increased response rate in ER- patients. This is the first report suggesting that ER status might be an important predictor of

response to cytotoxic chemotherapy in breast tumors. Since then, evidence from clinical trials or retrospective analyses is accumulating that improvements in chemotherapy disproportionately benefit breast cancer patients with ER- tumors, in which multiple chemotherapeutic regimens have been tested in these studies (6, 25-33), such as taxanes-, anthracycline- and navelbine-containing regimens. More recently, in a retrospective clinical study conducted by us and our collaborators, we found that primary breast cancer patienhts with ER+ tumors achieved significant lower pathologic response than those with ER- breast tumors when treated with preoperative chemotherapeutic regimens including DEC (docetaxel+epirubicin+cyclophosphamide), VFC (vinorelbine/vincristine+5-fluorouracil+ cyclophosphamide) and EFC (epirubicin+5-fluorouracil+cyclophosphamide) (34).

The involvement of ER in chemoresistance has also been confirmed in a number of *in vitro* studies (5, 7, 8, 35-40). For example, ER- breast cancer tissue was found chemosensitive *in vitro* compared with ER+ tissue against six antitumor drugs including carboquone, adriamycin, mitomycin C, aclacinomycin A, cisplatin and 5-fluorouracil (5). When subjected ER+ human breast cancer MCF-7 and ZR-75–1 cells to paclitaxel or to UV irradiation, marked increases in cell apoptosis were induced. However, these responses were significantly reversed by incubation with E2, which was probably mediated through the plasma membrane estrogen receptor (40). Recently, we established several isogenic ER+ cell lines by stable transfection of ERα expression vectors into ER- breast cancer BCap37 cells to investigate the possible influence of ER on the therapeutic efficacy of paclitaxel and *vinca* alkaloids (7, 8). We found that 17-β estradiol significantly reduced the overall cytotoxicity of these antimicrotubule drugs in ERα-expressing BCap37 but had no influence on the ER- parental cells or ER- MDA-MB-468 cells. Further analyses indicate that expression of ERα in BCap37 cells mainly interferes with the apoptotic cell death but not mitotic arrest induced by these drugs. Moreover, we found that the addition of ICI 182,780 (fulvestrant), a selective ER down-regulator, could completely reverse the above resistance observed in ER+ BCap37 cells, and sensitize MCF-7 and T47D cell lines to the treatment of the above drugs (see Fig. 2). These findings further confirmed the correlation between ERα and drug resistance in ER+ tumor cells.

4. Possible mechanisms of ER-mediaed chemoresistance

Estrogens and ERs are well-known for their critical roles in the development and progression of breast tumors, through genomic or non-genomic pathways as described above. Plentiful data also indicate that estrogens and ER are involved in or interact with a number of apoptosis- or proliferation-related signal pathways existed in tumor cells. Therefore, it is believed that through interaction with and/or regulation on specific or various co-regulators or downstream molecules, estrogen/ER induce chemoresistance in tumor cells by promoting tumor growth and/or inhibiting the antitumor effect of chemotherapeutic drugs. Several mechanisms that may contribute to ER-mediated drug resistance are discussed below. It appears that the underlying mechanisms of ER-mediated chemoresistance are quite complicated and specifically related with the tumor models and chemotherapeutic drugs studied.

4.1 Role of apoptosis-related molecules in ER-mediated chemoresistance

Reduced apoptotic cell death or enhanced tumor cell proliferation are major factors involved in drug resistance. Whereas it is not completely understood how estrogen and ER regulate

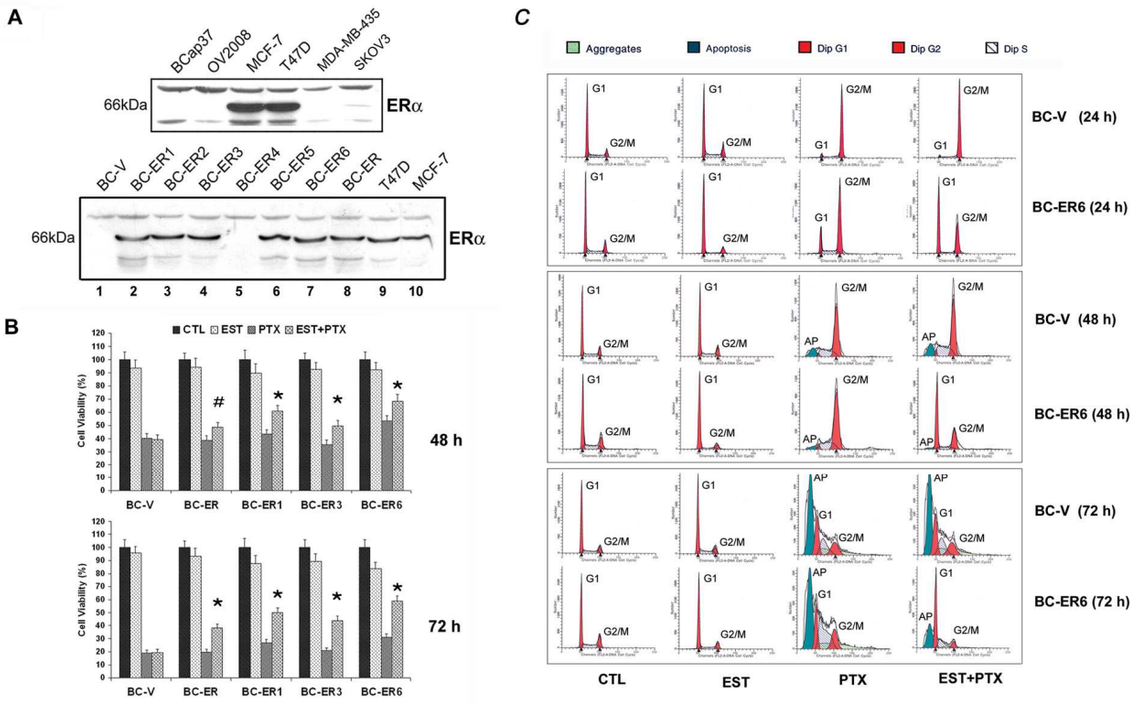

Fig. 2. ERα expression attenuates the anticancer activity of paclitaxel (7). A, protein extracts of BCap37 cells transfected with empty vector (BC-V) or ERα were analyzed by Western blot. T47D and MCF-7 cells were used as positive controls of ERα expression. *B*, cells were treated with 1 nmol/L 17-β estradiol, 50 nmol/L paclitaxel, or the combination treatment in which cells were preincubated with 17- β estradiol for 12 h before paclitaxel treatment. Cell viability was evaluated by MTT assays after both 48 and 72 h of paclitaxel treatment. BC-ER, pooled transfectants of BCap37 transfected with ERα, BC-ER1–7, single clones 1 to 7 of BCap37 transfected with ERα; CTL, control; EST, 17-β estradiol; PTX, paclitaxel. #, $P < 0.05$, when compared with the group treated with paclitaxel alone in the same cell line; *, $P < 0.001$, when compared with the group treated with paclitaxel alone in the same cell line. *C*, cells treated with 1 nmol/L 17-β estradiol, 50 nmol/L paclitaxel, or their combination for indicated time points were harvested, and DNA content stained with propidium iodide for flow cytometric analysis. Peaks corresponding to G1, G2-M, and S phases of the cell cycle and apoptotic cells (AP).

the growth of tumor cells, it is known that hormonal induction of growth factors/receptors such as transforming growth factor α, epidermal growth factor, Her-2 contributes to the proliferative actions of E2 (41-44). Recent studies indicate that several apoptosis-related moleculars or signal pathways, such as bcl-2 and p53, might be involved in E2/ER-mediated resistance to chemotherapy.

Expression of the bcl-2 protein prevents apoptotic cell death induced by a variety of stimuli including most chemotherapeutic agents (45-47). Teixeira C *et al* demonstrated that depletion of estrogen from the medium results in loss of expression of the bcl-2 in MCF-7 cells, whereas reexposure to estrogen markedly induces the bcl-2 expression (48). Moreover, estrogen depletion, the simultaneous treatment of ICI 164,384, or the transfection of bcl-2 antisense significantly sensitized MCF-7 cells to adriamycin, consistent with a decrease in

the bcl-2 levels. Their data suggest that estrogen can promote resistance of ER+ breast cancer cells to chemotherapeutic drugs through a mechanism that involves regulation of the bcl-2, which supports the recent report that bcl-2 expression usually occurs in ER+ breast tumors, whereas ER- breast cancer biopsies tend to lack this protein (48). Another study conducted by Razandi M *et al* showed that in ER+ human breast cancer cells, the apoptosis, activation of c-JNK, phosphorylation of Bcl-2 and Bcl-xl, activation of caspase induced by paclitaxel or UV radiation were significantly reversed by incubation with E2. E2 also independently activated extracellular signal-regulated protein kinase activity, which contributed to the antiapoptotic effects. In addition, our recent studies also demonstrated that E2 significantly inhibited paclitaxel or *vinca a*lkaloids-induced phosphorylations of bcl-2 and c-raf-1, as well as the degradation of IκBα in BCap37 cells transfected with ERα, which was accompanied with decreased sensitivity of BC-ER cells to the above anticancer drugs (7, 8).

In response to various extracellular and intracellular signals, p53 mediates cellular processes, such as apoptosis, cell cycle arrest, and senescence, depending on the signal and the cellular context (49-51). A body of accumulating evidence suggests the possibility of a cross-talk between pathways mediated by ERα and p53. Das GM *et al* demonstrated the direct binding of ERα to p53 both *in vitro* and *in vivo* to endogenous p53 target gene promoters, which subsequently resulted in inhibition of transcriptional activation by p53 (52). They further showed that ERα bound to p53 on endogenous *Survivin* and *MDR1* gene promoters, leading to inhibition of p53-mediated transcriptional repression of these genes. Further, alleviating p53-mediated transcriptional repression of *Survivin* contributes to the ability of ERα to inhibit apoptosis in human breast cancer cells. RNA interference-mediated knockdown of ER resulted in reduced survivin expression and enhanced the propensity of MCF-7 cells to undergo apoptosis in response to staurosporine treatment. These data indicate that countering p53-mediated transcriptional repression of *Survivin* is at least one of the important mechanisms underlying the antiapoptotic function of ERα (53).

4.2 Involvement of tumor growth rate in ER-mediated chemoresistance

Evidence has shown that ER- tumors have a higher growth rate as indicated by a higher labeling index and mitotic index (6). Since many agents used in chemotherapy for breast cancer have some degree of cell-cycle specificity, there might be a correlation between higher growth rate and chemotherapy response (6). Dougherty MK *et al* used three *in vitro* models (MCF-7, T47D and ZR-75) to examine and compare growth rates as well as paclitaxel-induced apoptosis in ER+ and ER- clones with the same originate (54). They found that in T47D and ZR-75 cell lines, loss of ER was associated with a decrease in doubling time and an increase in paclitaxel sensitivity. However, when cell culture conditions were altered to achieve equivalent cell proliferation rates, no difference in paclitaxel sensitivity was observed. Similarly, an ER- clone of MCF-7 cells that did not exhibit an enhanced growth rate compared to its ER+ counterpart also did not show increased paclitaxel sensitivity. In these *in vitro* models, the decreased sensitivity to paclitaxel appears to be correlated closely with the decreased growth rate observed in ER+ breast tumors (54).

4.3 ABC transportors and ER-mediated chemoresistance

The most widely studied phenomenon of drug resistance is multidrug resistance (MDR) that has been linked to overexpression of a membrane associated P-glycoprotein (1, 2), a member of ATP-binding cassette (ABC) transporter family that functions as an efflux pump for

various structurally unrelated anticancer agents, such as the *vinca* alkaloids, anthracyclines and taxanes (1, 2). Several studies have suggested that ABC transporters might be involved in E2/ER-induced drug resistance. For example, E2 increased the cytoplasmic concentration of P-gp in ER+ breast cancer cells that were resistant to doxorubicin treatment (55). In addition, ABCC11 (MRP8) expression is high in high-expressing ER breast cancers, supporting the notion that expression of ABCC11 in ER+ breast cancers may contribute to decreased sensitivity to chemotherapy combinations (56). Interestingly, Sugimoto Y *et al* recently reported that both estrogens (57) and antiestrogens (58) inhibit breast cancer resistance protein (BCRP)-mediated drug resistance. They also found that the physiological levels of E2 down-regulate both endogenous and exogenous BCRP expression in ER+ cells by post-transcriptional mechanisms (59). Moreover, they showed that estrogen decreases P-gp expression in MDR1-transduced, ER+ human breast cancer cells, and this E2-mediated P-gp down-regulation sensitizes tumor cells to vincristine. However, it is possible that the effects of estrogen on P-gp expression may differ in ER+ human breast cancer cells expressing endogenous and exogenous P-gp, which needs to be further assessed in appropriate models (60).

4.4 Enhanced β3-tubulin expression by E2/ER

It has been suggested that certain changes in cytoskeletons, such as tubulin mutations and isoforms, alterations in microtubule-bindings proteins (*e.g.* stathmin, tau), as well as enhanced β3-tubulin expression might be correlated with reduced response to antimicrotubule agent-based chemotherapy or worse outcome in a variety of tumor settings. In *in vitro* studies or in clinical investigations, enhanced expression of β3-tubulin has shown to play a crucial role in the development of chemoresistance to antimicrotubule agents in a variety of tumors such as lung, breast, prostate or orarian cancers (61-66), and has be considered as a predictive marker of paclitaxel resistance (25, 67-70). Nevertheless, the mechanism underlying β3-tubulin expression still remains unclear. In *Drosophila*, β3-tubulin expression is enhanced by an exposure to ecdysone, a steroid hormone, through a transcriptional mechanism (71). Recently, Saussede-Aim J *et al* found that exposure of ER+ MCF-7 cells to estradiol induced β3-tubulin expression in both mRNA and protein levels, while estradiol had no effect on the expression of β3-tubulin in ER- MDA-MB-231 cells (72). They further showed that co-administration of antiestrogens including tamoxifen or fulvestrant, completely abolished the increase of β3-tubulin mRNA levels due to estradiol in MCF-7 cells, implying that estradiol regulates β3-tubulin expression, and thereby induces resistance of ER+ breast tumors to antimicrotubule drugs through an ER-dependent pathway.

4.5 Tumor-host interaction in ER-mediated chemoresistance

Estrogen regulates differentiation, maturation and function of many cell types in monocyte–macrophage system directly or indirectly *via* other cells by autocrine/paracrine mechanisms (73). Estrogen effects on this system are primarily repressive, and mainly mediated by repression of expression of genes for cytokines or modulation of other inflammatory mediators by the ER-dependent or nongenomic pathways. The ER-dependent mechanisms mostly involve modulation of the NF-kappaB pathway for transcriptional regulation of cytokine or other mediator genes. In the context of hormone-regulated cancer, estrogen can influence production of cytokines or other inflammatory mediators by both tumor cells and tumor-invading macrophages (73). The interactions of breast cancer cells with tumor-

associated macrophages, regulation of the monocyte–macrophage system by estrogen and cross-talk between the ER and cytokine-mediated pathways, may play an important role in tumor progression as well as the development of resistance to anticancer treatment (73-75).

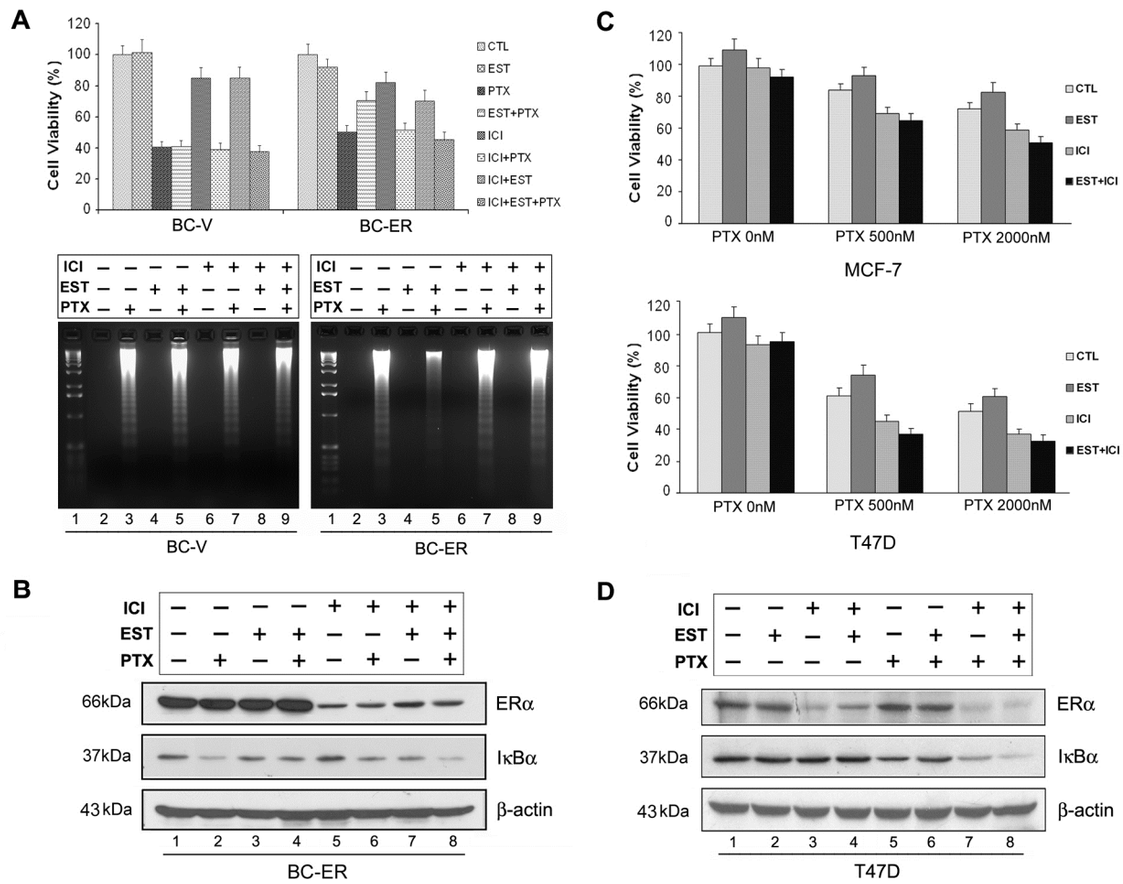

Fig. 3. ICI 182, 780 abrogates the resistance of ERα positive breast tumor cells to paclitaxel (7). BCap37 cells were treated with 1 nmol/L 17-β estradiol, 50 nmol/L paclitaxel, 100 nmol/L ICI 182, 780 or their various combinations. MCF-7 and T47D cells were treated with 100 nmol/L 17-β estradiol, 500 or 2000 nmol/L paclitaxel, 1 μmol/L ICI 182, 780 or their various combinations. *A*, determination of cell viability by MTT assays and apoptosis by DNA fragmentation assays after BC-V and BC-ER cells were exposed to paclitaxel for 48 h. *B*, effect of 17-β estradiol, paclitaxel, ICI 182, 780 and their combinations on the expression of ERα and IκBα in BC-ER cells. *C*, cell viability of MCF-7 and T47D cells after 72 h of paclitaxel treatment with MTT assays. *D*, effect of 17-β estradiol, paclitaxel, ICI 182, 780 and their combinations on the expression of ERα and IκBα in T47D cells. Proteins were extracted from cells after 24 h of paclitaxel treatment.

5. Stratagies to sensitize ER+ breast tumors to chemotherapy

Considering that ERs are expressed in ~65% of human breast cancer, the ER-mediated resistance to chemotherapy has become a big challenge for clinical treatment of breast tumors. Unfortunately, despite the fact that the involvement of ER in drug resistance to

chemotherapy has been observed for more than a decade, very few studies have investigated the potential strategies to reverse the ER-mediated chemoresistance or sensitize ER+ breast tumors to chemotherapy. Because the resistance of ER+ breast tumors to chemotherapy is mainly mediated by activation of estrogen/ER signal pathway, it is logical that agents targeting or inhibiting the ER signal pathway may have the potential to reverse the ER-mediated chemoresistance. Indeed, as described below, a number of studies have shown that antiestrogenic agents in combination with chemotherapeutic drugs are of significant therapeutic benefit in ER+ breast cancer over chemotherapy alone. Moreover, recent investigations indicate that the ER-derived peptide, MicroRNAs specifically targeting ER, as well as agents targeting estrogen-related receptors (ERRs) may hold great promise to sensitize ER+ breast tumors to chemotherapy.

5.1 Sensitization of ER+ breast tumors to chemotherapy by SERMs

Selective estrogen receptor modulators (or SERMs) bind ERs but have a mixed agonist/antagonist profile. Tamoxifen and raloxifene are well-known first and second generations of SERMs, respectively (76-78). New SERMs in clinical development include idoxifene, droloxifene, arzoxifene, acolbifene/EM-800, lasofoxifene, TAT-59, ERA-923, toremifene, GW5638/GW7604, *etc* (76-78). Kurebayashi J *et al* found that concurrent treatment of 5-FU and 4-hydroxytamoxifen (4OHT) additively inhibited the growth of ER+ ML-20 and KPL-1 breast cancer cells but not ER- MDA-MB-231 cells (79). They further demonstrated that 4OHT significantly decreased thymidilate synthase activity, which might increase the antitumor activity of 5-FU (79). However, conflicting observations on the interaction between tamoxifen and chemotherapeutic agents including 5-FU and doxorubicin in terms of antitumor activity have been reported by different laboratories (79-83). In addition, Wu L *et al* showed that arzoxifene and 4OHT can inhibit specifically the repopulation of ER+ MCF-7 and T47D breast cancer cells between courses of weekly treatment with 5-FU or methotrexate (84). Most recently, they further confirmed that combined treatment with arzoxifene given between cycles of 5-FU or paclitaxel can inhibit repopulation of MCF-7 breast cancer xenografts (85). They proposed that scheduling of short-acting antiestrogenic agents between courses of adjuvant chemotherapy for human breast cancer has potential to improve the outcome of treatment. Additionally, the increased etoposide cytotoxicity by tamoxifen as compared to cells treated with either drug alone was observed in brain tumor HTB-14 cells expressing ER, which was accompanied with enhanced inhibition of protein kinase C (PKC) and insulin-like growth factor II (IGF-II) (86).

5.2 Sensitization of ER+ breast tumors to chemotherapy by aromatase inhibitors

One strategy to inhibit the activation of estrogen/ER pathway is to block the conversion of estrogen precursors into estrogen by aromatase inhibitors (AIs) (87). Currently, third-generation aromatase inhibitors, such as the non-steroidal agents anastrozole, letrozole and the steroidal agent exemestane, have been introduced into the market as endocrine therapy in postmenopausal patients, either alone or as part of multiple hormonal therapies (88). In addition to the above AIs, cyclooxygenase (COX) inhibitors also decrease aromatase mRNA expression and enzymatic activity (89). A recent study by Chen D *et al* showed that the combination of paclitaxel with exemestane produced additive antitumor effect in cultured human breast cancer cell lines. Interestingly, this additive effect was independent of ERα expression, but dependent on the presence of androstenedione (90). The effects of AIs on

sensitivity of ER+ breast tumors to chemotherapy remains unclear and need to be further investigated.

5.3 Sensitization of ER+ breast tumors to chemotherapy by SERDs

The pure antiestrogens, also called selective estrogen receptor downregulators (or SERDs), including fulvestrant (ICI 182, 780), ZK-703, ZK-253, RU58668 and TAS-108, act by decreasing the level of ERs through their ubiquitinylation and subsequent targeting to the proteasome (87). Unlike tamoxifen, fulvestrant is a pure antagonist of estrogen-regulated gene expression that could down-regulat ER expression without any concomitant rise in other growth signal pathways, *e.g.*, EGFR or TGF-α (8, 87). Recently, our laboratory demonstrated that pretreatment with fulvestrant significantly prevented E2-induced resistance to paclitaxel and *vinca* alkaloids in human breast cancer BCap37 cells transfected with ER-expressing vector (BC-ER) while down-regulates the protein levels of ERα in BC-ER cells (7, 8). Similar sensitizing effect of fulvestrant was observed in MCF-7 and T47D breast cancer cells expressing endogenous ERα (7, 8). These results provided additional evidence for the correlation between ERα and the resistance of breast tumors to chemotherapeutic drugs such as paclitaxel and *vinca* alkaloids. More recently, through implanted ER- and ER+ BCap37 cells into athymic nude mice, we established isogenic ER- and ER+ xenograft breast tumor models. Subsequently, we demonstrated that co-treatment of fulvestrant could significantly sensitize ER+ breast tumors to paclitaxel (unpublished data). Because fulvestrant has been successfully used in the treatment of ER+ advanced breast tumors, our experimental results may also suggest the clinical strategy to combine fulvestrant with certain chemotherapeutic drugs for the treatment of ER+ breast tumors.

5.4 Other strategies potentially useful for sensitizing ER+ breast tumors to chemotherapy

In addition to the well-known antiestrogens including the SERMs, AIs and SEDMs, studies have been conducted to explore new agents that may interfere the biological responses medicated by E2/ER. One example is the synthesis of ER-derived peptide. Two ER-derived peptides specifically targeting estradiol/ER action, pY-peptide (Ac-Leu-pTyr-Asp-Leu-Leu-Leu-NH2) and Tat-peptide (Ac-EFVCLKSIILLNS-AAA-RKKRRQRRR-NH2) have shown activity to inhibit the growth of ER+ breast tumors *in vitro* and *in vivo* (91, 92). Moreover, accumulating evidence is revealing an important role of MicroRNAs in anticancer drug resistance (93). Adams *et al* reported that MicroRNA (miR)-206 could decrease endogenous ERα in MCF-7 cells *via* two specific target sites within the 3′-untranslated region of the human ER transcript (22, 94). They further found that miR-206 expression was markedly decreased in ER+ human breast cancer tissues, and that the introduction of miR-206 into estrogen-dependent MCF-7 cells led to the suppression of ERα expression and growth inhibition. These data suggest that miR-206 is a key factor for the regulation of ERα expression in breast cancer, which could be a novel candidate for targeting ER (22, 94).

Nuclear receptor estrogen-related receptor (ERR) family, comprising ERRα, ERRβ and ERRγ, are the closest relatives to ERα after ERβ (95) . The ERRs share several biochemical activities with ERs, bind and regulate transcription *via* estrogen response elements (EREs) and extended ERE half-sites termed ERR response elements (ERREs), but do not bind endogenous estrogens. The ERRs act in an analogous fashion as ERα, but the effect of ERRα binding to an ERE or ERRE can be either negative or positive. ERRα likely plays a role in

modulating estrogen responsiveness both by modulating levels of estrogens themselves and expression of estrogen-regulated genes in estrogen target-tissues such as breast cancer. The search for ligands of the ERRs is an active area of research. Targeting ERRs holds great promise and may open new opportunities for the management of breast cancers (95).

As described above, the mechanisms underlying ER-mediated chemoresistance involve ER-coregulatory proteins and cross-talk between plasma membrane-localized ER, nuclear-localized ER and other growth-factor signaling networks, such as EGFR, IGFR, VEGFR and HER2. As a consequence, targeting the ER-coregulators or "cross-talk" pathways may provide opportunities to overcome the ER-mediated chemoresistance, either alone or in combination with agents inhibiting E2/ER activation. However, the mechanisms of ER-mediated chemoresistance need to be further clarified so that effective strategies could be developed to sensitize ER+ breast tumors to chemotherapy.

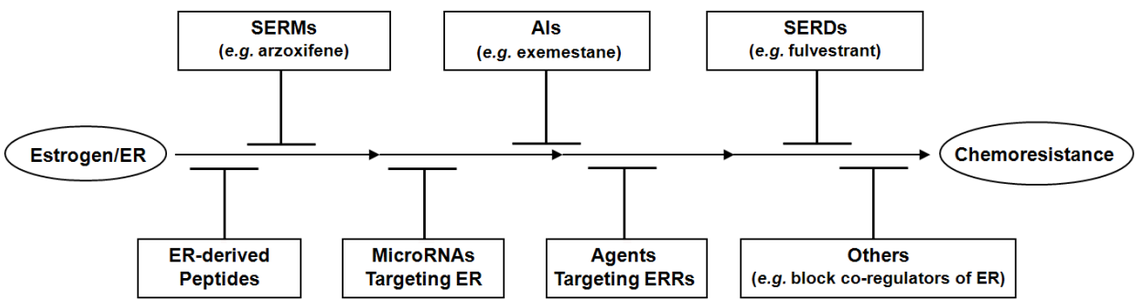

Fig. 4. Possible strategies to reverse ER-mediated chemoresistance or sensitize ER positive tumors to chemotherapy. SERMs, selective estrogen receptor modulators; AIs, aromatase inhibitors; SERDs, selective estrogen receptor down-regulators; ERRs, estrogen-related receptors.

6. Future perspectives

Cumulative data from *in vitro* experiments and clinical investigations have demonstrated the association between ERα expression and the resistance to chemotherapy in breast tumors. However, most of *in vitro* data were based on comparative studies between the tumor cell lines derived from different individuals. Although some paired cell lines were derived under the selective pressure of a low/no estrogen environments, these tumor cells are still not likely to be isogenic because many features, including their proliferative capacity, might have changed due to genetic alterations (54). Thus, it is difficult to elucidate the cellular and molecular mechanisms. The pairs of isogenic breast cell lines generated by stable transfection of ERα or empty vector in our laboratory have provided a valuable model system to investigate the mechanism underlying ERα-mediated breast tumor cell resistance to chemotherapeutic agents. Interestingly, we found that estrogen had marginal effect on microtubule dynamics in breast tumor cells expressing ER (BC-ER) treated with paclitaxel and *vinca* alkaloids, but may decrease the G2-M population through the increase of cells at the G1 phase. This phenomenon is similar to the finding previously reported by Zajchowski *et al*. (96, 97). However, the question still remains whether G1 arrest and decreased G2-M population by estrogen may affect the above drug-induced apoptosis. Further studies are required to elucidate this issue, and it is important to integrate data obtained from breast tumors expressing endogenous ER with those expressing exogenous ER.

Some previous reports about the association between ER status and response to chemotherapy can be confused by the use of chemo-endocrine therapy, where the ER+ population may have responded to the hormonal part of the treatment (98, 99). Moreover, there are heterogeneous in design, in determination of marker and response evaluation, which could be partly responsible for conflicting results about the predictive and prognostic value of these markers (98-103). Therefore, some cautions are required when interpret these results, considering that there are many factors need to be taken into account, such as differences in patient selection, whether the patients were previously untreated with chemotherapy or endocrine therapy, type of chemotherapy, size of the study, follow-up time, different evaluation methods, different cut-off value of ER or other related markers, interactions between combined chemotherapeutic drugs, *etc.*

Compared to the available *in vitro* and clinical reports, very few animal studies have been conducted to determine the role and underlying mechanisms of estrogen and ER in development of chemoresistance, as well as to explorer the potential strategies to reverse the ER-mediated drug resistance. However, appropriate animal models may provide us with easily controlled ways to further evaluate various signal pathways/molecules *in vivo*, to determine the differences between *in vitro* and *in vivo* models, to test chemotherapeutic drugs that we have interests, to investigate agents that may hold promise to sensitize ER+ breast tumors to chemotherapy, either alone or in desirable combinations/sequences. There are less variables need to be taken into account when interpret or analyze the data obtained in animal models compared to clinical patients. The hope to overcome the ER-mediated chemoresistance relies on further clarification of specific pathways or molecules contributing most significantly to the resistance. More exhaustive and systematic attempts to provide this information are essential to reach deeper understandings on ER-mediated chemoresistance in breast tumors. Moreover, it is known that breast cancer patients show a wide range of ER expression levels, and the levels of ER expression in individual patients change during disease progression and/or in response to systemic therapies. Thus, the treatment plan for breast cancer patients might need to be optimized based on the most up-to-date molecular characteristics and responses to therapy in individuals.

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8. References

[1] Raguz, S.;Yagüe, E. Resistance to chemotherapy: new treatments and novel insights into an old problem. *Br. J. Cancer*, 2008, 99(3), 387-391.

[2] Luqmani, Y.A. Mechanisms of drug resistance in cancer chemotherapy. *Med. Princ. Pract.*, 2005, 14 Suppl 1, 35-48

[3] Sui, M.; Chen, F.; Chen, Z.; Fan, W. Glucocorticoids interfere with therapeutic efficacy of paclitaxel against human breast and ovarian xenograft tumors. *Int. J. Cancer.*, 2006, 119, 712–717.

[4] Fan, W.; Sui, M.; Huang, Y. Glucocorticoids selectively inhibit paclitaxel-induced apoptosis: mechanisms and its clinical impact. *Curr. Med. Chem.*, 2004, 403–411.

[5] Maehara, Y.; Emi ,Y.; Sakaguchi, Y.; Kusumoto ,T.; Kakeji, Y.; Kohnoe, S.; Sugimachi, K. Estrogen receptor-negative breast cancer tissue is chemosensitive in vitro compared with estrogen-receptor-positive tissue. *Eur. Surg. Res.*, 1990, 50–55.
[6] Lippman, M.E.; Allegra, J.C.; Thompson, E.B.; Simon, R.; Barlock, A.; Green, L.; Huff, K.K.; Do, H.M.; Aitken, S.C.; Warren, R. The relation between estrogen receptors and response rate to cytotoxic chemotherapy in metastatic breast cancer. *N. Engl. J .Med.*, 1978, 298, 1223–1228.
[7] Sui, M.; Huang, Y.; Park, B.H.; Davidson, N.E.; Fan,W. Estrogen receptor alpha mediates breast cancer cell resistance to paclitaxel through inhibition of apoptotic cell death. *Cancer Res.*, 2007, 67(11), 5337-5344.
[8] Sui, M.; Jiang, D.; Hinsch, C.; Fan, W. Fulvestrant (ICI 182,780) sensitizes breast cancer cells expressing estrogen receptor alpha to vinblastine and vinorelbine. *Breast Cancer Res. Treat.*, 2010, 121(2), 335-345.
[9] McEwen, B.S. Steroid hormones: effect on brain development and function. *Horm. Res.*, 1992, 37, 1-10.
[10] Simons, S.S. What goes on behind closed doors: physiological versus pharmacological steroid hormone actions. *Bioessays*, 2008, 30(8), 744-756.
[11] Dowsett, M.; Folkerd, E.; Doody, D.; Haynes, B. The biology of steroid hormones and endocrine treatment of breast cancer. *Breast*, 2005, 452-457.
[12] Fan, W.; Cheng, L.; Norris, J.S.; Willingham, M.C. Glucocorticoids selectively inhibit taxol-induced apoptosis in a human breast cancer cell line. *Cell Pharmacol.*, 1996, 3, 435–440.
[13] Huang, Y.; Fang, Y.; Wu, J.; Dziadyk, J.M.; Zhu, X.; Sui, M.; Fan,W. Regulation of vinca alkaloid-induced apoptosis by NF-κB/IκB pathway in human tumor cells. *Mol. Cancer Ther.*, 2004, 3, 271–277.
[14] Huang,Y.; Johnson, K.R.; Norris, J.S.; Fan, W. Nuclear factorkappaB/IkappaB signaling pathway may contribute to the mediation of paclitaxel-induced apoptosis in solid tumor cells. *Cancer Res.*, 2000, 60, 4426–4432.
[15] O'Lone, R.; Frith, M.C.; Karlsson, E.K.; Hansen, U. Genomic targets of nuclear estrogen receptors. *Mol. Endocrinol.*, 2004, 18(8), 1859-1875.
[16] Simpson, E.R.; Misso, M.; Hewitt, K.N.; Hill, R.A.; Boon, W.C.; Jones, M.E.; Kovacic, A.; Zhou, J.; Clyne, C.D. Estrogen-the good, the bad, and the unexpected. *Endocr. Rev.*, 2005, 26(3), 322-330.
[17] Cordera, F.; Jordan, V.C. Steroid receptors and their role in the biology and control of breast cancer growth. *Semin. Oncol.*, 2006, 33, 631–641.
[18] Conzen, S.D. Nuclear receptors and breast cancer. *Mol. Endocrinol.*, 2008, 22(10), 2215-2228.
[19] Weigel, N.L.; Zhang, Y. Ligand-independent activation of steroid hormone receptors. *J. Mol. Med.*, 1998, 76, 469–479.
[20] Hanstein, B.; Djahansouzi, S.; Dall, P.; Beckmann, M.W.; Bender, H.G. Insights into the molecular biology of the estrogen receptor define novel therapeutic targets for breast cancer. *Eur. J. Endocrinol.*, 2004, 150(3), 243-255.
[21] Dickson, R.B.; Lippman, M.E. Control of human breast cancer by estrogen, growth factors, and oncogenes. *Cancer Treat. Res.*, 1988, 40, 119-165.
[22] Yamashita, H. Current research topics in endocrine therapy for breast cancer. *Int. J. Clin. Oncol.*, 2008, 13(5), 380-383.
[23] Murphy, L.; Cherlet, T.; Lewis, A.; Banu, Y.; Watson, P. New insights into estrogen receptor function in human breast cancer. *Ann. Med.*, 2003, 35, 614–631.

[24] Ariazi, E.A.; Ariazi, J.L.; Cordera, F.; Jordan, V.C. Estrogen receptors as therapeutic targets in breast cancer. *Curr. Top. Med. Chem.*, 2006, 6(3), 181-202.

[25] Pusztai, L. Markers predicting clinical benefit in breast cancer from microtubule-targeting agents. *Ann. Oncol.*, 2007, 18 suppl 12: xii15-20.

[26] Cocquyt, V.F.; Schelfhout, V.R.; Blondeel, P.N.; Depypere, H.T.; Daems, K.K.; Serreyn, R.F.; Praet, M.M.; Van, Belle, S.J. The role of biological markers as predictors of response to preoperative chemotherapy in large primary breast cancer. *Med. Oncol.*, 2003, 20(3), 221-231.

[27] Faneyte, I.F.; Schrama, J.G.; Peterse, J.L.; Remijnse, P.L.; Rodenhuis, S.; van de Vijver ,M.J. Breast cancer response to neoadjuvant chemotherapy: predictive markers and relation with outcome. *Br. J. Cancer*, 2003, 88(3), 406-412.

[28] International Breast Cancer Study Group (IBCSG). Endocrine responsiveness and tailoring adjuvant therapy for postmenopausal lymph node-negative breast cancer: a randomized trial. *J. Natl. Cancer. Inst.*, 2002, 94(14), 1054-1065.

[29] Berry, D.A.; Cirrincione, C.; Henderson, I.C.; Citron, M.L.; Budman, D.R.; Goldstein, L.J.; Martino, S.; Perez, E.A.; Muss, H.B.; Norton, L.; Hudis, C.; Winer, E.P. Estrogen-receptor status and outcomes of modern chemotherapy for patients with node-positive breast cancer. *JAMA,* 2006, 295(14), 1658-1667.

[30] Gianni, L.; Zambetti, M.; Clark, K.; Baker, J. Cronin, M.; Wu, J.; Mariani, G.; Rodriguez, J.; Carcangiu, M.; Watson, D.; Valagussa, P.; Rouzier, R.; Symmans, W.F.; Ross, J.S.; Hortobagyi, G.N.; Pusztai, L.; Shak, S. Gene expression profiles in paraffin-embedded core biopsy tissue predict response to chemotherapy in women with locally advanced breast cancer. *J. Clin. Oncol.*, 2005, 23(29), 7265-7277.

[31] Colleoni, M.; Minchella, I.; Mazzarol, G.; Nolè, F.; Peruzzotti, G.; Rocca, A.; Viale, G.; Orlando, L.; Ferretti, G.; Curigliano, G.; Veronesi, P.; Intra, M.; Goldhirsch, A. Response to primary chemotherapy in breast cancer patients with tumors not expressing estrogen and progesterone receptors. *Ann. Oncol.*, 2000, 11, 1057-1059.

[32] MacGrogan, G.; Mauriac, L.; Durand, M.; Bonichon, F.;Trojani, M.; de Mascarel, I.; Coindre, J.M. Primary chemotherapy in breast invasive carcinoma: predictive value of the immunohistochemical detection of hormonal receptors, p53, c-erbB-2, MiB1, pS2 and GST pi. *Br. J .Cancer.*, 1996, 74, 1458-1465.

[33] Stearns, V.; Singh, B.; Tsangaris, T.; Crawford, J.G.; Novielli, A.; Ellis, M.J.; Isaacs, C.; Pennanen, M.; Tibery, C.; Farhad, A.; Slack, R.; Hayes, D.F. A prospective randomized pilot study to evaluate predictors of response in serial core biopsies to single agent neoadjuvant doxorubicin or paclitaxel for patients with locally advanced breast cancer. *Clin. Cancer Res.*, 2003, 9(1), 124-133.

[34] Wang, L.; Jiang, Z.; Sui, M.; Shen, J.; Xu, C.; Fan, W. The potential biomarkers in predicting pathologic response of breast cancer to three different chemotherapy regimens: a case control study. *BMC Cancer*, 2009, 9, 226.

[35] Dittrich, C.; Jakesz, R.; Wrba, F.; Havelec, L.; Haas, O.; Spona, J.; Holzner, H.; Kolb, R.; Moser, K. The human tumour cloning assay in the management of breast cancer patients. *Br. J. Cancer*, 1985, 52(2), 197-203.

[36] Aho, A.J.; Mäenpää, J.U.; Kangas, L.; Söderström, K.O.; Auranen, A.A.; Linna, M. Subrenal capsule assay in human breast cancer. Response to cytostatic drug combinations and correlation with receptor status. *Eur. J. Cancer Clin. Oncol.*, 1985, 21(10), 1133-1140.

[37] Kaufmann, M.; Klinga, K.; Runnebaum, B.; Kubli, F. Hormone receptors in breast cancer and response to chemotherapy. *N. Engl. J .Med*, 1979, 300(18), 1052.

[38] Maehara, Y.; Anai, H.; Kusumoto, H.; Sugimachi, K. Poorly differentiated human gastric carcinoma is more sensitive to antitumor drugs than is well differentiated carcinoma. *Eur. J. Surg. Oncol.*, 1987, 13(3), 203-206.
[39] Allegra, J.C.; Lippman, M.E.; Thompson, E.B.; Simon, R. An association between steroid hormone receptors and response to cytotoxic chemotherapy in patients with metastatic breast cancer. *Cancer Res.*, 1978, 38(11 Pt 2), 4299-4304.
[40] Razandi, M.; Pedram, A.; Levin, E.R. Plasma membrane estrogen receptors signal to antiapoptosis in breast cancer. *Mol. Endocrinol.*, 2000, 14(9), 1434-1447.
[41] Lee, A.V.; Cui, X.; Oesterreich, S. Cross-talk among estrogen receptor, epidermal growth factor, and insulin-like growth factor signaling in breast cancer. *Clin. Cancer. Res.*, 2001, 7(12 Suppl), 4429s-4435s discussion 4411s-4412s.
[42] Chalbos, D.; Philips, A.; Rochefort, H. Genomic cross-talk between the estrogen receptor and growth factor regulatory pathways in estrogen target tissues. *Semin. Cancer. Biol*, 1994, 5(5), 361-368.
[43] Kato, S. Estrogen receptor-mediated cross-talk with growth factor signaling pathways. *Breast Cancer*, 2001, 8(1), 3-9.
[44] Levin, E.R. Bidirectional signaling between the estrogen receptor and the epidermal growth factor receptor. *Mol. Endocrinol.*, 2003, 17(3), 309-317.
[45] Levine, B.; Sinha, S.; Kroemer, G. Bcl-2 family members: dual regulators of apoptosis and autophagy. *Autophagy*, 2008, 4(5), 600-606.
[46] Adams, J.M.; Cory, S. Bcl-2-regulated apoptosis: mechanism and therapeutic potential. *Curr. Opin. Immunol.*, 2007, 19(5), 488-496.
[47] van Delft, M.F.; Huang, D.C. How the Bcl-2 family of proteins interact to regulate apoptosis. *Cell Res.*, 2006, 16(2), 203-213.
[48] Teixeira, C.; Reed, J.C.; Pratt, M.A. Estrogen promotes chemotherapeutic drug resistance by a mechanism involving Bcl-2 proto-oncogene expression in human breast cancer cells. *Cancer Res.*, 1995, 55(17), 3902-3907.
[49] Vousden, K.H.; Lu, X. Live or let die: the cell's response to p53. *Nat. Rev. Cancer*, 2002, 2, 594–604.
[50] Vogelstein, B.; Lane, D.; Levine, A.J. Surfing the p53 network. *Nature*, 2000, 408, 307–310.
[51] Hofseth, L.J.; Hussain, S.P.; Harris, C.C. p53: 25 years after its discovery. *Trends Pharmacol. Sci.*, 2004, 25, 177–181.
[52] Liu, W.; Konduri, S.D.; Bansal, S.; Nayak, B.K.; Rajasekaran, S.A.; Karuppayil, S.M.; Rajasekaran, A.K.; Das, G.M. Estrogen receptor α binds p53 tumor suppressor protein directly and represses its function. *J. Biol. Chem.*, 2006, 281, 9837–9840.
[53] Sayeed, A.; Konduri, S.D.; Liu, W.; Bansal, S.; Li, F.; Das, G.M. Estrogen receptor alpha inhibits p53-mediated transcriptional repression: implications for the regulation of apoptosis. *Cancer Res.*, 2007, 67(16), 7746-7755.
[54] Dougherty, M.K.; Schumaker, L.M.; Jordan; V.C.; Welshons W.V.; Curran, E.M.; Ellis, M.J.; El-Ashry, D. Estrogen receptor expression and sensitivity to paclitaxel in breast cancer. *Cancer Biol. Ther.*, 2004, 3(5), 460-467.
[55] Zampieri, L.; Bianchi, P.; Ruff ,P.; Arbuthnot, P. Differential modulation by estradiol of P-glycoprotein drug resistance protein expression in cultured MCF7 and T47D breast cancer cells. *Anticancer Res.*, 2002, 22(4), 2253-2259.
[56] Honorat, M.; Mesnier, A.; Vendrell, J.; Guitton, J.; Bieche, I.; Lidereau, R.; Kruh ,G.D.; Dumontet, C.; Cohen, P.; Payen, L. ABCC11 expression is regulated by estrogen in MCF7 cells, correlated with estrogen receptor alpha expression in postmenopausal breast tumors and overexpressed in tamoxifen-resistant breast cancer cells. *Endocr. Relat. Cancer.*, 2008, 15(1), 125-138.

[57] Imai, Y.; Tsukahara, S.; Ishikawa, E.; Tsuruo, T.; Sugimoto, Y. Estrone and 17-estradiol reverse breast cancer resistance protein-mediated multidrug resistance. *Jpn. J. Cancer Res.*, 2002, 93, 231–235.

[58] Sugimoto, Y.; Tsukahara, S.; Imai, Y.; Sugimoto, Y.; Ueda, K.;Tsuruo, T. Reversal of breast cancer resistance protein-mediated drug resistance by estrogen antagonists and agonists. *Mol. Cancer Ther.*, 2003, 2, 105–112.

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

[60] utoh, K.; Tsukahara, S.; Mitsuhashi, J.; Katayama, K.; Sugimoto, Y. Estrogen-mediated post transcriptional down-regulation of P-glycoprotein in MDR1-transduced human breast cancer cells. *Cancer Sci.*, 2006, 97(11), 1198-1204.

[61] Rosell, R.; Scagliotti, G.; Danenberg, K.D.; Lord, R.V.; Bepler, G.; Novello, S.; Cooc, J.; Crino, L.; Sanchez, J.J.; Taron, M.; Boni, C.; Da Marinis, F.; Tonato, M.; Marangolo, M.; Gozzelino, F.; Di Constanzo, F.; Rinaldi, M.; Solanga, D.; Stephens, C. Transcripts in pretreatment biopsies from a three-arm randomized trial in metastatic nonsmall-cell lung cancer. *Oncogene*, 22(23), 3548–3553.

[62] Seve, P.; Mackey, J.; Isaac, S.; Tredan, O.; Souquet, P.J.; Perol M, Lai, R.; Voloch, A.; Dumontet, C. Class III beta-tubulin expression in tumor cells predicts response and outcome in patients with non-small cell lung cancer receiving paclitaxel. *Mol. Cancer Ther.*, 2005b, 4(12), 2001–2007.

[63] Shalli, K.; Brown, I.; Heys, S.D.; Schofield, A.C. Alterations of beta-tubulin isotypes in breast cancer cells resistant to docetaxel. *FASEB J.*, 2005, 19(10), 1299–1301.

[64] Ranganathan, S.; Benetatos, C.A.; Colarusso, P.J.; Dexter, D.W.; Hudes, G.R. Altered beta-tubulin isotype expression in paclitaxel-resistant human prostate carcinoma cells. *Br. J. Cancer.*, 1998, 77(4), 562–566.

[65] Kavallaris, M.; Kuo, D.Y.; Burkhart, C.A.; Regl, D.L.; Norris, M.D.; Haber, M.; Horwitz, S.B. Taxol-resistant epithelial ovarian tumors are associated with altered expression of specific beta-tubulin isotypes. *J. Clin. Invest.*, 1997, 100(5), 1282–1293.

[66] Gan, P.P.; Pasquier, E.;Kavallaris, M. Class III beta-tubulin mediates sensitivity to chemotherapeutic drugs in non small cell lung cancer. *Cancer Res.*, 2007, 67(19), 9356–9363.

[67] Tommasi, S.; Mangia, A.; Lacalamita, R.; Bellizzi ,A.; Fedele, V.; Chiriatti, A.; Thomssen, C.; Kendzierski, N.; Latorre, A.; Lorusso, V.; Schittulli, F.; Zito, F.;Kavallaris ,M.; Paradiso, A. Cytoskeleton and paclitaxel sensitivity in breast cancer: the role of beta-tubulins. *Int. J. Cancer*, 2007, 120(10), 2078–2085.

[68] Seve, P.; Dumontet, C. Is class III beta-tubulin a predictive factor in patients receiving tubulin-binding agents? *Lancet Oncol.*, 2008, 9(2), 168–175.

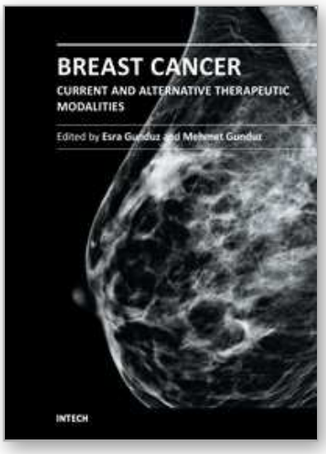
[69] Ferrandina, G.; Zannoni ,G.F.; Martinelli ,E.; Paglia, A.; Gallotta, V.; Mozzetti,S.; Scambia, G.; Ferlini, C. Class III beta-tubulin overexpression is a marker of poor clinical outcome in advanced ovarian cancer patients. *Clin. Cancer Res.*, 2006, 12(9), 2774–2779.

[70] Sève, P., Isaac, S., Trédan, O., Souquet, P.J., Pachéco, Y., Pérol, M., Lafanéchère, L., Penet, A., Peiller, E.L., Dumontet, C. Expression of class III {beta}-tubulin is predictive of patient outcome in patients with non-small cell lung cancer receiving vinorelbine-based chemotherapy. *Clin. Cancer Res.*, 2005, 11(15):5481-5486.

[71] Bruhat, A.; Dreau, D.; Drake, M.E.; Tourmente, S.; Chapel, S.; Couderc, J.L.; Dastugue, B. Intronic and 50 flanking sequences of the Drosophila beta 3 tubulin gene are essential to confer ecdysone responsiveness. *Mol. Cell. Endocrinol.*, 1993, 94(1), 61–71.

[72] Saussede-Aim, J.; Matera, E.L.; Ferlini, C.; Dumontet, C. Beta3-tubulin is induced by estradiol in human breast carcinoma cells through an estrogen-receptor dependent pathway. *Cell Motil. Cytoskeleton*, 2009, 66(7), 378-388.
[73] Härkönen, P.L.; Väänänen, H.K. Monocyte-macrophage system as a target for estrogen and selective estrogen receptor modulators. *Ann. N. Y. Acad. Sci.*, 2006, 1089, 218-227.
[74] Seeger, H., Wallwiener, D., Mueck, A.O. Effects of estradiol and progestogens on tumor-necrosis factor-alpha-induced changes of biochemical markers for breast cancer growth and metastasis. *Gynecol. Endocrinol.*, 2008, 24(10), 576-579.
[75] Kramer, P.R., Kramer, S.F., Guan, G. 17 beta-estradiol regulates cytokine release through modulation of CD16 expression in monocytes and monocyte-derived macrophages. *Arthritis Rheum.*, 2004, 50(6), 1967-1975.
[76] Johnston, S.R. Endocrinology and hormone therapy in breast cancer: selective oestrogen receptor modulators and downregulators for breast cancer - have they lost their way? *Breast Cancer Res.*, 2005, 7(3), 119-130.
[77] Osborne, C.K., Zhao, H., Fuqua, S.A. Selective estrogen receptor modulators: structure, function, and clinical use. *J. Clin. Oncol.*, 2000, 18(17), 72-16.
[78] Howell, S.J., Johnston, S.R., Howell, A. The use of selective estrogen receptor modulators and selective estrogen receptor down-regulators in breast cancer. *Best Pract. Res. Clin. Endocrinol. Metab.*, 2004, 18(1), 47-66.
[79] Kurebayashi, J.; Nukatsuka, M.; Nagase, H.; Nomura, T.; Hirono, M.; Yamamoto, Y,; Sugimoto, Y.; Oka, T.; Sonoo, H. Additive antitumor effect of concurrent treatment of 4-hydroxy tamoxifen with 5-fluorouracil but not with doxorubicin in estrogen receptor-positive breast cancer cells. *Cancer Chemother. Pharmacol.*, 2007, 59(4), 515-525.
[80] Benz, C.; Cadman, E.; Gwin, J.; Wu, T.; Amara, J.; Eisenfeld, A.; Dannies, P. Tamoxifen and 5-fluorouracil in breast cancer: cytotoxic synergism in vitro. *Cancer Res.*, 1983, 43, 5298–5303.
[81] Leonessa, F.; Jacobson, M.; Boyle, B.; Lippman, J.; McGarvey, M.; Clarke, R. Effect of tamoxifen on the multidrug-resistant phenotype in human breast cancer cells: isobologram, drug accumulation, and M(r) 170,000 glycoprotein (gp170) binding studies. *Cancer Res.*, 1994, 54, 441–447.
[82] Osborne, C.K.; Kitten, L.; Arteaga, C.L. Antagonism of chemotherapy-induced cytotoxicity for human breast cancer cells by antiestrogens. *J. Clin. Oncol.*, 1989, 7, 710–717.
[83] Woods, K.E.; Randolph, J.K.; Gewirtz, D.A. Antagonism between tamoxifen and doxorubicin in the MCF-7 human breast tumor cell line. *Biochem. Pharmacol.*, 1994, 47, 1449–1452.
[84] Licun, W.; Tannock, I.F. Selective estrogen receptor modulators as inhibitors of repopulation of human breast cancer cell lines after chemotherapy. *Clin. Cancer Res.*, 2003, 9(12), 4614-4618.
[85] Wu, L.; Tannock, I.F. Effect of the selective estrogen receptor modulator arzoxifene on repopulation of hormone-responsive breast cancer xenografts between courses of chemotherapy. *Clin. Cancer Res.*, 2005, 11(22), 8195-8200.
[86] Ramachandran, C.; Khatib, Z.; Petkarou, A.; Fort ,J.; Fonseca, H.B.; Melnick, S.J.; Escalon, E. Tamoxifen modulation of etoposide cytotoxicity involves inhibition of protein kinase C activity and insulin-like growth factor II expression in brain tumor cells. *J. Neurooncol.*, 2004, 67(1-2), 19-28.

[87] Sola, B.; Renoir, J.M. Antiestrogenic therapies in solid cancers and multiple myeloma. *Curr. Mol. Med.*, 2006, 6(4), 359-368.
[88] Gao, X.P.; Liu, F. New agents in development for breast cancer. *Curr. Opin. Obstet. Gynecol.*, 2007, 19(1), 68-74.
[89] Díaz-Cruz, E.S., Shapiro, C.L., Brueggemeier, R.W. Cyclooxygenase inhibitors suppress aromatase expression and activity in breast cancer cells. *J. Clin. Endocrinol. Metab.*, 2005, 90(5), 2563-2570.
[90] Chen, D.; Hackl ,W.; Ortmann, O.; Treeck, O. Effects of a combination of exemestane and paclitaxel on human tumor cells in vitro. *Anticancer Drugs*, 2004, 15(1), 55-61.
[91] Varricchio, L., Migliaccio, A., Castoria, G., Yamaguchi, H., de Falco, A., Di Domenico, M., Giovannelli, P., Farrar, W., Appella, E., Auricchio, F. Inhibition of estradiol receptor/Src association and cell growth by an estradiol receptor alpha tyrosine-phosphorylated peptide. Mol. Cancer Res., 2007, 5(11):1213-1221.
[92] Castoria, G.; Migliaccio, A.; Giovannelli, P.; Auricchio, F. Cell proliferation regulated by estradiol receptor: therapeutic implications. *Steroids*, 2010, 75(8-9):524-527.
[93] Zheng, T.; Wang, J.; Chen, X.; Liu, L. Role of microRNA in anticancer drug resistance. *Int. J. Cancer*, 2010, 126(1), 2-10.
[94] Adams, B.D.; Furneaux, H.; White, B.A. The micro-ribonucleic acid (miRNA) miR-206 targets the human estrogen receptor-alpha (ERalpha) and represses ERalpha messenger RNA and protein expression in breast cancer cell lines. *Mol. Endocrinol.*, 2007, 21, 1132–1147.
[95] Ariazi, E.A.; Jordan, V.C. Estrogen-related receptors as emerging targets in cancer and metabolic disorders. *Curr. Top. Med. Chem.*, 2006, 6(3), 203-215.
[96] Zajchowski , D.A.; Sager, R.; Webster, L. Estrogen inhibits the growth of estrogen receptor-negative, but not estrogen receptor-positive, human mammary epithelial cells expressing a recombinant estrogen receptor. *Cancer Res.*, 1993, 53, 5004–5011.
[97] Jiang, S.Y.; Jordan, V.C. Growth regulation of estrogen receptor-negative breast cancer cells transfected with complementary DNAs for estrogen receptor. *J. Natl. Cancer Inst.*, 1992, 84(8), 580-591.
[98] DiMarco, B.; Aguggini, S.; Bolsi, G.; Cirillo, F.; Filippini, L.; Betri, E.; Bertoli, G.; Alquati, P.; Dogliotti, L. p53 but not bcl-2 immunostaining is predictive of poor clinical complete response to primary chemotherapy in breast cancer patients. *Clin. Cancer Res.*, 2000, 6, 2751-2758.
[99] Makris, A.; Powles, T.J.; Dowsett ,M.; Osborne, C.K.; Trott, P.A.; Fernando, I.N.; Ashley, S.E.; Ormerod, M.G.; Titley, J.C.; Gregory, R.K.; Allred, D.C. Prediction of response to neoadjuvant chemoendocrine therapy in primary breast carcinomas. *Clin. Cancer Res.*, 1997, 3, 593-600.
[100] Gapinski, P.V.; Donegan, W.L. Estrogen receptors and breast cancer: prognostic and therapeutic implications. *Surgery*, 1980, 88, 386-393.
[101] Hilf, R.; Feldstein, M.L.; Gibson, S.L.; Savlov, E.D. The relative importance of estrogen receptor analysis as a prognostic factor for recurrence or response to chemotherapy in women with breast cancer. *Cancer*, 1980, 45, 1993-2000.
[102] Hilf, R.; Feldstein, M.L.; Savlov, E.D.; Gibson, S.L.; Seneca, B. The lack of relationship between estrogen receptor status and response to chemotherapy. *Cancer*, 1980, 46(12 Suppl), 2797-2800.
[103] Minisini, A.M.; Di Loreto, C.; Mansutti, M.; Artico, D.; Pizzolitto, S.; Piga, A.; Puglisi, F. Topoisomerase IIalpha and APE/ref-1 are associated with pathologic response to primary anthracycline-based chemotherapy for breast cancer. *Cancer Lett.,* 2005, 224(1), 133-139.



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Cancer is the leading cause of death in most countries and its consequences result in huge economic, social and psychological burden. Breast cancer is the most frequently diagnosed cancer type and the leading cause of cancer death among females. In this book, we discussed various therapeutic modalities from signaling pathways through various anti-tumor compounds as well as herbal medicine for this deadly cancer. We hope that this book will contribute to the development of novel diagnostic as well as therapeutic approaches.

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