Breast Cancer
Vol. 10 No. 2 April 2003

Review Article

Endocrine-Resistant Breast Cancer: Underlying Mechanisms and Strategies for Overcoming Resistance

Junichi Kurebayashi
Department of Breast and Thyroid Surgery, Kawasaki Medical School, Japan.

Estrogen plays important roles in the development and progression of breast cancer. However, one-third of breast cancers fail to respond to endocrine therapy and most endocrine-responsive breast cancers subsequently become resistant to endocrine therapy. A tremendous effort has been made to elucidate the mechanisms responsible for the development of endocrine-resistance in breast cancer. Since the main target molecule of estrogen in breast cancer is estrogen receptor (ER)-α, most studies have focused on investigating quantitative and qualitative changes in ER-α in endocrine-resistant breast cancer. Breast cancers expressing no ER-α fail to respond to endocrine therapy. Some breast cancers expressing ER-α also fail to respond to endocrine therapy and most breast cancers with acquired endocrine resistance retain ER-α expression, which suggests that the disappearance of ER-α in breast cancer cells is not a common cause of resistance to endocrine therapy. Recent molecular biological studies have shown evidence that qualitative and functional changes, such as gene mutations and phosphorylation of ER-α, cause endocrine resistance in breast cancer. In addition, it has been suggested that endocrine resistance could be induced by epigenetic changes, such as hypoxia, in breast cancer tissues. Understanding the precise mechanisms that underlie endocrine resistance may enable clinicians to develop new strategies for retarding or overcoming endocrine resistance in breast cancer.

Breast Cancer 10:112-119, 2003.

Key words: Endocrine resistance, Breast cancer, Growth factor, Signaling pathway, Hypoxia

Accumulated knowledge provided from the results of clinical studies has suggested that endocrine therapy is useful for chemoprevention and treatment for patients with clinical breast cancer in either adjuvant or metastatic settings. However, the clinical benefit provided by endocrine therapy is not available to patients with estrogen receptor (ER)-negative and progesterone receptor (PgR)-negative breast cancer. In addition, one-third of ER-positive recurrent breast cancer cases fail to respond to endocrine therapy. Furthermore, even if recurrent breast tumors respond to first- and/or second-line endocrine therapies, they frequently develop resistance to these therapies after varying durations of response. Most endocrine-responsive breast tumors ultimately become endocrine-resistant.

Breast cancer with acquired resistance to first-line endocrine therapies, such as the antiestrogen tamoxifen frequently responds to second-line endocrine therapies, such as aromatase inhibitors or progestins. In addition, third-line endocrine therapy sometimes provides clinical benefit to patients that suffer from endocrine-refractory breast cancer. These findings support the clinical strategy of treating ER- and/or PgR-positive breast cancer with as many endocrine therapies as possible, and subsequently applying cytotoxic chemotherapy. These findings also suggest that there are several steps or complicated mechanisms causing endocrine resistance.

It should be noted that endocrine resistance is a term sometimes used to designate resistance to a certain endocrine therapeutic agent, such as tamoxifen. Tamoxifen resistance is a common problem in clinics and the underlying mecha-

Reprint requests to Junichi Kurebayashi, Department of Breast and Thyroid Surgery, Kawasaki Medical School, 577 Matsushima, Kurashiki, Okayama 701-0192, Japan.

Abbreviations:
ER, Estrogen receptor; PgR, Progesterone receptor; LH-RH, Luteinizing hormone-releasing hormone; AP-1, Activating protein-1; EGF-R, Epidermal growth factor receptor; PI3K, Phosphatidylinositol-3-OH-kinase; HAT, Histone acetyltransferase; ERBF1, Estrogen receptor promoter B-associated factor 1

Received May 23, 2002; accepted August 26, 2002

nisms have been extensively investigated for the last two decades. Endocrine resistance in breast cancer was divided into four categories by possible mechanisms in the present review as follows: 1) specific resistance to antiestrogens, 2) ligand-independent ER activation, 3) modified ER-interacting proteins, and 4) loss of ER expression. In addition, possible clinical approaches to retard or overcome endocrine resistance are discussed.

Possible Mechanisms of Action Underlying Endocrine Resistance (Table 1)

1) Specific Resistance to Antiestrogens

Tamoxifen has been used as a chemoprevention agent (unfortunately, not in Japan) as well as a therapeutic agent for patients with breast cancer in the preoperative, postoperative and metastatic settings. Other antiestrogens, such as toremifene, have also been used as alternatives to tamoxifen. Therefore, antiestrogen resistance is very common in clinics. In addition, tamoxifen-withdrawal response and tamoxifen-stimulated tumor growth have been observed in clinics and experimental studies[1-3]. Tamoxifen might be deleterious in some antiestrogen-resistant tumors. Several hypothetical mechanisms responsible for antiestrogen resistance have been advocated.

1) Modified tamoxifen metabolism is suspected to cause tamoxifen resistance. Approximately one decade ago, tamoxifen metabolites, a less potent antiestrogenic cis-isomer of 4-hydroxytamoxifen and an estrogenic metabolite, metabolite E, were detected in both animal models and tamoxifen-resistant breast tumors[4]. However, the causal relation between the increased levels of these metabolites and tamoxifen resistance remains to be clarified.

2) Tamoxifen has been known to stimulate ovarian estrogen production and an elevated level of serum estrogen may interfere with the antiestrogenic activity of tamoxifen[5]. Actually, combined tamoxifen and luteinizing hormone-releasing hormone (LH-RH) agonists has been suggested to be superior to either agent alone in clinics[6].

3) Enhancement of estrogenic activity of antiestrogens by a certain mutational change in the ER-α gene or the prominent activating protein (AP)-1-mediated transactivation might induce antiestrogen resistance as well as antiestrogen-stimulated growth[7,8]. However, no common mutation in the ER-α gene has been detected in antiestrogen-resistant breast tumors. A series of experimental and clinical results suggests a positive correlation between up-regulation of AP-1 activity and acquired tamoxifen resistance[9,10]. It has been suggested that cellular oxidative stress induced by tamoxifen might activate signaling pathways leading to the activation of AP-1[8].

Table 1. Action Mechanisms Underlying Endocrine-Resistance in Breast Cancer

Specific Resistance to Antiestrogens
Modified metabolism of antiestrogens
Stimulated estrogen synthesis in ovaries
Enhanced agonistic activity by ER mutations or AP-1 activation
Ligand-independent ER Activation
Phosphorylation of ER by growth factor signaling pathway
ER mutations or ER mRNA variants
Modified ER-interacting Proteins
Quantitative or qualitative changes in cofactors
Interaction with ER-β and its isoforms
Loss of ER Expression
Hypermethylation in the ER promoter
Loss of trans factors, such as ERBF-1
Epigenetic changes, such as hypoxia

2) Ligand-Independent ER Activation

It has been classically presumed that transactivation of target genes by ER requires a ligand, such as estrogen, binding to ER. Recently, experimental studies have revealed that several growth factors and their intracellular signaling pathways can stimulate ER activity in the absence of a ligand. In addition, it has been suggested that this ligand-independent ER activation is caused by the phosphorylation of different sites in the ER protein. These mechanisms may explain the development of endocrine-resistant breast cancer.

1) Overexpression of HER1 (also known as epidermal growth factor receptor, EGF-R) and/or HER2 (also known as ErbB-2) has been detected in 20-30% of primary breast cancers. Experimental and clinical studies have suggested that overexpression of HER1 and/or HER2 causes tamoxifen-resistance[11,12]. Recent fundamental studies also revealed that the intracellular signaling pathway activated by HER1 or HER2, RAS/RAF/MEK/ERK1/2, phosphorylates the serine 118 residue located in ER and results in ligand-independent transactivation of target genes of ER[13-15]. It was also reported that increased ERK1/2 activity correlates with endocrine resistance and shorter survival in patients with breast cancer[16]. Moreover, experimental studies have suggested that the

blockade of these signaling pathways by EGF-R tyrosine kinase inhibitors, MEK inhibitors and the anti-HER2 monoclonal antibody trastuzmab may restore the anti-tumor effect of antiestrogens[17-19].

2) Another intracellular signaling pathway, which is also stimulated by receptor tyrosine kinases, such as HER1 and HER2, is phosphatidylinositol -3-OH-kinase (PI3K)/AKT (also known protein kinase B) pathway. It was reported that phosphorylation of the serine 167 residue located in ER by AKT results in ligand-independent activation of ER[20, 21]. In addition, a negative regulator of AKT, PTEN, which acts as a tumor suppressor, is frequently inactivated in breast cancer[22].

3) Other intracellular signaling pathways, such as protein kinase A induced by G-protein-coupled receptors and the 90-kDa ribosomal S6 kinase RSK, are also known to be involved in ER phosphorylation[23, 24]. However, the roles of these pathways in the development of endocrine resistance remain to be elucidated.

There are other mechanisms that result in ligand-independent ER activation. Certain mutations or mRNA variants of the ER gene may produce a constitutively active form of ER protein.

1) A missense mutation of the tyrosine 537 residue in the ligand binding region of ER was detected in metastatic breast cancer tumors. This mutant ER was reported to be equally active in the absence of ligand or in the presence of estradiol or tamoxifen. Substitution of this residue by different amino acids has been shown to result in the ligand-independent activation of ER together with co-activator recruitment[25, 26]. However, other studies suggest that only 1% of primary breast cancers carry gene mutations of ER in the coding region, most of which did not result in alteration at the protein level[27]. Therefore, the clinical significance of the mutation in endocrine resistance is questionable.

2) Alternatively spliced mRNA lacking specific exons, which encode truncated and potentially constitutively active forms of ER, has been discovered[28]. However, analysis of breast tumors failed to confirm a significant role of the variants in the development of endocrine-resistance. The pathological significance of the ER mRNA variants remains to be determined[29-31].

3) Modified ER-Interacting Proteins

A series of recent studies have revealed that ER-interacting proteins, called cofactors, which include co-activators and co-repressors, have important roles in mediating transcriptional activation of target genes by the ER. These findings suggest that quantitative or qualitative changes of the ER-related cofactors could contribute to the development of endocrine-resistant breast cancer. However, no significant relation has been confirmed between alterations of co-activators or co-repressors and endocrine resistance in breast tumors[32]. However, some experimental findings support these attractive mechanisms.

1) Overexpression of nuclear receptor co-activator protein (NCOA)-1 was reported to increase the agonist activity of tamoxifen in experimental cell systems[33]. This suggests that co-activator overexpression could contribute to endocrine-resistance. In addition, it might be possible that increased histone acetyltransferase (HAT) activity caused by the co-activator up-regulation is also involved in the development of endocrine resistance. Therefore, newly developed HAT inhibitors might be useful in the treatment of endocrine-resistant breast tumors[34].

2) In a mouse model system, it was reported that expression of one of the co-repressors, nuclear co-repressor protein (NCOR)-1, was decreased in tamoxifen-resistant tumors[35]. However, no other studies support this interesting finding in human endocrine-resistant tumors.

3) A novel co-activator, L7/SPA, was discovered and the complex of this co-activator and tamoxifen was reported to lead to increase transcription activation in target genes[36]. Overexpression of this kind of co-activator may contribute to endocrine resistance. However, no study has so far clarified this hypothetical mechanism.

4) A recently discovered ER subtype, ER-β, may play a role in the development of endocrine resistance. A series of studies suggested that the expression of wild-type ER-β in breast cancer cells predicts a favorable prognosis, but an isoform of ER-β gives a poorer prognosis[37-39]. Actually, experimental studies suggested that this isoform reduces ER-α-mediated transactivation in a dominant negative fashion[40]. Further studies are needed to clarify the role of ER-β in the development of endocrine resistance.

4) Loss of ER Expression

Endocrine-resistant breast tumors do not always lose ER-α expression. Therefore, loss of ER-α expression is only one of the possible mechanisms of endocrine-resistance in breast cancer. However, this mechanism of action has long been suggested

Table 2. Clinical Strategies for Overcoming Endocrine-Resistance in Breast Cancer

Inhibition of Growth Factor Signaling Pathway
Purpose: Blocking ligand-independent ER activation
Candidates: trastuzumab, ZD1839 and other tyrosine kinase inhibitors
Retardation of the Development of Resistance
Intermittent endocrine therapy
Alternative endocrine therapy
Combined endocrine therapy
Hypoxic cytotoxins

in Japan as follows: 1) ER-positive breast cancer could consist of so-called mosaic cell mixtures with ER-positive and negative tumor cells. 2) When endocrine therapy effectively kills ER-positive tumor cells, ER-negative tumor cells could selectively grow. 3) Finally, this breast cancer loses ER expression and acquires total endocrine resistance[41].

Another possible mechanism leading to loss of ER expression is that some genetic or epigenetic changes in breast tumors gradually cause a reduction in ER expression. For example, hypermethylation of the promoter region of the ER gene causes transcriptional down-regulation and decreases the expression of ER protein[42]. Another cause of transcriptional down-regulation is decreased expression of transcription-promoting trans elements for ER, such as estrogen receptor promoter B-associated factor (ERBF) 1[43]. However, it is not yet known whether these changes directly cause loss of ER or a loss of ER caused by other mechanisms subsequently induces these changes.

Recently, the inverse relation between the expression of ER and hypoxia-inducible factors such as hypoxia-inducible factor-1α and carbonic anhydrase IX, was reported in breast cancer tissues[44, 45]. In addition, our previous experimental study suggested that hypoxic conditions lead to the post-transcriptional down-regulation of ER-α and its function[44]. These findings suggest that hypoxic microenvironments, which are frequently observed in breast tumors, cause a decrease in ER expression. It has been suggested that hypoxia drives the malignant progression of many types of human cancers[46]. Therefore, it might be possible that hypoxic microenvironments caused by an increase in tumor size or induced by hormonal agents provide a selective pressure for the outgrowth of ER-poor and more aggressive clones and finally cause a loss of ER expression in breast tumors.

New Strategies against Endocrine-Resistant Breast Cancer (Table 2)

As described above, there are many hypothetical action mechanisms underlying endocrine resistance in breast cancer. Some appear to be irreversible and impossible to overcome. However, others appear to be reversible and might be overcome by new strategies. In the last part of this review, new clinically testable strategies are discussed.

1) Inhibition of Growth Factor Signaling Pathway

It was suggested that the up-regulation of growth factor-mediated signaling pathways causes ER phosphorylation, induces ligand-independent ER activation and leads to endocrine-independent and -resistant growth of breast cancer[11-15, 20, 21]. Some recent studies also provided clinical evidence suggesting that breast cancer overexpressing HER1 and/or HER2 together with ER expression is likely to be resistant to endocrine therapy[47, 48]. These findings suggest the following: 1) Inhibitors of the growth factor signaling pathway, such as tyrosine kinase inhibitors, may decrease ER phosphorylation and ligand-independent ER activation. 2) This may induce the estrogen-dependent growth pathway. 3) Then, endocrine therapeutic agents effectively inhibit the growth of breast cancer.

The humanized anti-HER2 monoclonal antibody, trastuzumab, is the only agent that inhibits the growth factor-mediated signaling pathway and has been introduced into clinics[49]. Our previous experimental study suggested that combined treatment with antiestrogen and this antibody effectively inhibits the growth of breast cancer cells expressing both ER and HER2[19]. Several clinical studies launched worldwide tested the clinical usefulness of this combination therapy[50]. Howev-

er, a recent clinical study, in which the anti-tumor effects of the antiestrogen tamoxifen and the aromatase inhibitor letrozole were compared in the neoadjuvant setting, suggested that tamoxifen was significantly less effective than letrozole in ER-positive breast cancer overexpressing HER1 and/or HER2[51]. It was reported that tamoxifen might be deleterious or ineffective in the adjuvant setting for patients with breast cancer overexpressing HER2[52]. These findings suggest that combined treatment with an aromatase inhibitor and this antibody is more suitable for clinical studies recruiting patients with breast cancer overexpresing HER2.

Very recently, an EGF-R-specific tyrosine kinase inhibitor, ZD1839, was introduced into clinics for the treatment of non-small cell lung cancer[53]. This agent effectively inhibits the signaling pathway mediated by EGF-R[54]. It was reported that approximately 20% of breast cancers overexpress EGF-R, and breast cancer overexpressing EGF-R is more aggressive and resistant to endocrine therapy[55]. Our preliminary study also suggested that ER-positive breast cancer overexpressing EGF-R more rapidly acquires endocrine-resistance (unpublished data). Moreover, experimental studies have suggested that ZD1839 is not only effective in breast cancer cells overexpressing EGF-R but is also effective in breast cancer overexpressing HER2[54, 56]. These findings suggest that ZD1839 is also a promising agent for combined use with endocrine therapeutic agents.

2) Retardation of the Development of Endocrine-Resistance

Even if it is difficult to overcome endocrine resistance, it may be possible to prolong the anti-tumor effect of endocrine therapy or retard the occurrence of endocrine resistance. Two hypothetical strategies are introduced and discussed below.

1) Acquired endocrine-resistance has been recognized as an adaptive response of breast cancer cells to endocrine therapy. If so, it can be suggested that intermittent treatment with a single endocrine therapeutic agent, combined treatment with two different agents or alternating use of two different agents may interfere with the adaptation process and retard the occurrence of endocrine-resistance. Prostate-specific antigen is a very sensitive and specific tumor marker, and a new strategy, in which intermittent treatment with an LH-RH agonist is given to patients with advanced prostate cancer, has been tested in clinical trials[57]. In addition, a recent clinical study suggests that alternating treatment with tamoxifen and the aromatase inhibitor aminoglutethimide was more effective than tamoxifen alone in the adjuvant setting for patients with breast cancer[58]. These findings prompted us to start large-scaled prospective clinical trials to test these new treatment modalities.

2) Our recent experimental study suggested that an epigenetic change caused by a hypoxic microenvironment may play an important role in the development of endocrine resistance in breast cancer[44]. If so, hypoxic cytotoxins, such as tirapazamine[59], which specifically kill cancer cells under hypoxic conditions, may be useful for retarding the occurrence of endocrine resistance. Our preliminary findings suggest that the administration of hypoxic toxins restores ER expression in human breast cancer xenografts in athymic nude mice (unpublished data). Further experimental studies are needed to clarify this novel treatment strategy.

Conclusions

Although there are many attractive mechanisms of action possibly responsible for the development of endocrine resistance, a common cascade has not yet been discovered. In other words, many mechanisms may be involved. From a clinical point of view, endocrine therapy appears to be less toxic and provides better quality of life for patients with breast cancer compared with cytotoxic chemotherapy. Therefore, clinicians and researchers have to make a serious effort to develop new strategies for enhancing or prolonging the efficacy of endocrine therapy. With regard to clinical implications, strategies for retarding endocrine resistance, such as the intermittent, alternating or combined endocrine therapy, are testable in clinical trials. In addition, the administration of inhibitors for the growth factor signaling pathway may not only prolong the efficacy of endocrine therapy but also overcome a certain type of endocrine resistance in the near future. Well-designed clinical trials supported by scientific evidence are essential to clarify new promising strategies.

Acknowledgements

This work was supported by Research Project Grants (12-510 and 13-501) from Kawasaki Medical School and

by grants (12671187 and 14571166) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

References

1) Legault-Poisson S, Jolivet J, Poisson R, Poisson R, Beretta-Piccoli M, Band PR: Tamoxifen-induced tumor stimulation and withdrawal response. *Cancer Treat Rep* 63:1839-1841, 1979.
2) Howell A, Dodwell DJ, Anderson H, Redford J: Response after withdrawal of tamoxifen and progestogens in advanced breast cancer. *Ann Oncol* 3:611-617, 1992.
3) Gottardis MM, Jordan VC: Development of tamoxifen-stimulated growth of MCF-7 tumors in athymic mice after long-term antiestrogen administration. *Cancer Res* 48:5183-5187, 1988.
4) Osborne CK: Mechanisms for tamoxifen resistance in breast cancer: possible role of tamoxifen metabolism. *J Steroid Biochem Mol Biol* 47:83-89, 1993.
5) Ravdin PM, Fritz NF, Tormey DC, Jordan VC: Endocrine status of premenopausal node-positive breast cancer patients following adjuvant chemotherapy and long-term tamoxifen. *Cancer Res* 48:1026-1029, 1988.
6) Klijn JG, Beex LV, Mauriac L, van Zijl JA, Veyret C, Wildiers J, Jassem J, Piccart M, Burghouts J, Becquart D, Seynaeve C, Mignolet F, Duchateau L: Combined treatment with buserelin and tamoxifen in premenopausal metastatic breast cancer: a randomized study. *J Natl Cancer Inst* 92:903-911, 2000.
7) Jiang SY, Langan-Fahey SM, Stella AL, McCague R, Jordan VC: Point mutation of estrogen receptor (ER) in the ligand-binding domain changes the pharmacology of antiestrogens in ER-negative breast cancer cells stably expressing complementary DNAs for ER. *Mol Endocrinol* 6:2167-2174, 1992.
8) Schiff R, Reddy P, Ahotupa M, Coronado-Heinsohn E, Grim M, Hilsenbeck SG, Lawrence R, Deneke S, Herrera R, Chamness GC, Fuqua SA, Brown PH, Osborne CK: Oxidative stress and AP-1 activity in tamoxifen-resistant breast tumors in vivo. *J Natl Cancer Inst* 92:1926-1934, 2000.
9) Dumont JA, Bitonti AJ, Wallace CD, Baumann RJ, Cashman EA, Cross-Doersen DE: Progression of MCF-7 breast cancer cells to antiestrogen-resistant phenotype is accompanied by elevated levels of AP-1 DNA-binding activity. *Cell Growth Differ* 7:351-359, 1996.
10) Johnston SR, Lu B, Scott GK, Kushner PJ, Smith IE, Dowsett M, Benz CC: Increased activator protein-1 DNA binding and c-Jun NH2-terminal kinase activity in human breast tumors with acquired tamoxifen resistance. *Clin Cancer Res* 5:251-256, 1999.
11) van Agthoven T, van Agthoven TL, Portengen H, Foekens JA, Dorssers LC: Ectopic expression of epidermal growth factor receptors induces hormone independence in ZR-75-1 human breast cancer cells. *Cancer Res* 52:5082-5088, 1992.
12) Pietras RJ, Arboleda J, Reese DM, Wongvipat N, Pegram MD, Ramos L, Gorman CM, Parker MG, Sliwkowski MX, Slamon DJ: HER-2 tyrosine kinase pathway targets estrogen receptor and promotes hormone-independent growth in human breast cancer cells. *Oncogene* 10:2435-2446, 1995.
13) Kato S, Endoh H, Masuhiro Y, Kitamoto T, Uchiyama S, Sasaki H, Masushige S, Gotoh Y, Nishida E, Kawashima H: Activation of the estrogen receptor through phosphorylation by mitogen-activated protein kinase. *Science* 270:1491-1494, 1995.
14) Bunone G, Briand PA, Miksicek RJ, Picard D: Activation of the unliganded estrogen receptor by EGF involves the MAP kinase pathway and direct phosphorylation. *EMBO J* 15:2174-2183, 1996.
15) Chen D, Riedl T, Washbrook E, Pace PE, Coombes RC, Egly JM, Ali S: Activation of estrogen receptor alpha by S118 phosphorylation involves a ligand-dependent interaction with TFIIH and participation of CDK7. *Mol Cell* 6:127-137, 2000.
16) Gee JM, Robertson JF, Ellis IO, Nicholson RI: Phosphorylation of ERK1/2 mitogen-activated protein kinase is associated with poor response to anti-hormonal therapy and decreased patient survival in clinical breast cancer. *Int J Cancer* 95:247-254, 2001.
17) Kurokawa H, Lenferink AE, Simpson JF, Pisacane PI, Sliwkowski MX, Forbes JT, Arteaga CL: Inhibition of HER2/neu (erbB-2) and mitogen-activated protein kinases enhances tamoxifen action against HER2-overexpressing, tamoxifen-resistant breast cancer cells. *Cancer Res* 60:5887-5894, 2000.
18) Kurokawa H, Arteaga CL: Inhibition of erbB receptor (HER) tyrosine kinases as a strategy to abrogate antiestrogen resistance in human breast cancer. *Clin Cancer Res* 7(12 Suppl):4436s-4442s, 2001.
19) Kunisue H, Kurebayashi J, Otsuki T, Tang CK, Kurosumi M, Yamamoto S, Tanaka K, Doihara H, Shimizu N, Sonoo H: Anti-HER2 antibody enhances the growth inhibitory effect of anti-oestrogen on breast cancer cells expressing both oestrogen receptors and HER2. *Br J Cancer* 82:46-51, 2000.
20) Martin MB, Franke TF, Stoica GE, Chambon P, Katzenellenbogen BS, Stoica BA, McLemore MS, Olivo SE, Stoica A: A role for Akt in mediating the estrogenic functions of epidermal growth factor and insulin-like growth factor I. *Endocrinology* 141:4503-4511, 2000.
21) Campbell RA, Bhat-Nakshatri P, Patel NM, Constantinidou D, Ali S, Nakshatri H: Phosphatidylinositol 3-kinase/AKT-mediated activation of estrogen receptor alpha: a new model for anti-estrogen resistance. *J Biol Chem* 276:9817-9824, 2001.
22) Li J, Yen C, Liaw D, Podsypanina K, Bose S, Wang SI, Puc J, Miliaresis C, Rodgers L, McCombie R, Bigner SH, Giovanella BC, Ittmann M, Tycko B, Hibshoosh H, Wigler MH, Parsons R: PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. *Science* 275:1943-1947, 1997.
23) Chen D, Pace PE, Coombes RC, Ali S: Phosphorylation of human estrogen receptor alpha by protein kinase A regulates dimerization. *Mol Cell Biol* 19:1002-1015, 1999.
24) Joel PB, Smith J, Sturgill TW, Fisher TL, Blenis J, Lannigan DA: pp90rsk1 regulates estrogen receptor-mediated transcription through phosphorylation of Ser-167. *Mol Cell Biol* 18:1978-1984, 1998.
25) Weis KE, Ekena K, Thomas JA, Lazennec G, Katzenellenbogen BS: Constitutively active human estrogen receptors containing amino acid substitutions for tyrosine 537 in the receptor protein. *Mol Endocrinol* 10:1388-1398, 1996.

26) White R, Sjoberg M, Kalkhoven E, Parker MG: Ligand-independent activation of the oestrogen receptor by mutation of a conserved tyrosine. *EMBO J* 16:1427-1435, 1997.
27) Roodi N, Bailey LR, Kao WY, Verrier CS, Yee CJ, Dupont WD, Parl FF: Estrogen receptor gene analysis in estrogen receptor-positive and receptor-negative primary breast cancer. *J Natl Cancer Inst* 87:446-451, 1995.
28) Daffada AA, Johnston SR, Smith IE, Smith IE, Detre S, King N, Dowsett M: Exon 5 deletion variant estrogen receptor messenger RNA expression in relation to tamoxifen resistance and progesterone receptor/pS2 status in human breast cancer. *Cancer Res* 55:288-293, 1995.
29) Leygue E, Huang A, Murphy LC, Watson PH: Prevalence of estrogen receptor variant messenger RNAs in human breast cancer. *Cancer Res* 56:4324-4327, 1996.
30) van Dijk MA, Hart AA, van't Veer LJ: Differences in estrogen receptor alpha variant messenger RNAs between normal human breast tissue and primary breast carcinomas. *Cancer Res* 60:530-533, 2000.
31) Poola I, Speirs V: Expression of alternatively spliced estrogen receptor alpha mRNAs is increased in breast cancer tissues. *J Steroid Biochem Mol Biol* 78:459-469, 2001.
32) Graham JD, Bain DL, Richer JK, Jackson TA, Tung L, Horwitz KB: Nuclear receptor conformation, coregulators, and tamoxifen-resistant breast cancer. *Steroids* 65:579-584, 2000.
33) Webb P, Nguyen P, Shinsako J, Anderson C, Feng W, Nguyen MP, Chen D, Huang SM, Subramanian S, McKinerney E, Katzenellenbogen BS, Stallcup MR, Kushner PJ: Estrogen receptor activation function 1 works by binding p160 coactivator proteins. *Mol Endocrinol* 12:1605-1618, 1998.
34) Lau OD, Kundu TK, Soccio RE, Ait-Si-Ali S, Khalil EM, Vassilev A, Wolffe AP, Nakatani Y, Roeder RG, Cole PA: HATs off: selective synthetic inhibitors of the histone acetyltransferases p300 and PCAF. *Mol Cell* 5:589-595, 2000.
35) Lavinsky RM, Jepsen K, Heinzel T, Torchia J, Mullen TM, Schiff R, Del-Rio AL, Ricote M, Ngo S, Gemsch J, Hilsenbeck SG, Osborne CK, Glass CK, Rosenfeld MG, Rose DW: Diverse signaling pathways modulate nuclear receptor recruitment of N-CoR and SMRT complexes. *Proc Natl Acad Sci USA* 95:2920-2925, 1998.
36) Jackson TA, Richer JK, Bain DL, Takimoto GS, Tung L, Horwitz KB: The partial agonist activity of antagonist-occupied steroid receptors is controlled by a novel hinge domain-binding coactivator L7/SPA and the corepressors N-CoR or SMRT. *Mol Endocrinol* 11:693-705, 1997.
37) Mann S, Laucirica R, Carlson N, Younes PS, Ali N, Younes A, Li Y, Younes M: Estrogen receptor beta expression in invasive breast cancer. *Hum Pathol* 32:113-118, 2001.
38) Jensen EV, Cheng G, Palmieri C, Saji S, Makela S, Van Noorden S, Wahlstrom T, Warner M, Coombes RC, Gustafsson JA: Estrogen receptors and proliferation markers in primary and recurrent breast cancer. *Proc Natl Acad Sci USA* 98:15197-15202, 2001.
39) Omoto Y, Kobayashi S, Inoue S, Ogawa S, Toyama T, Yamashita H, Muramatsu M, Gustafsson JA, Iwase H: Evaluation of oestrogen receptor beta wild-type and variant protein expression, and relationship with clinicopathological factors in breast cancers. *Eur J Cancer* 38:380-386, 2002.
40) Maruyama K, Endoh H, Sasaki-Iwaoka H, Kanou H, Shimaya E, Hashimoto S, Kato S, Kawashima H: A novel isoform of rat estrogen receptor beta with 18 amino acid insertion in the ligand binding domain as a putative dominant negative regular of estrogen action. *Biochem Biophys Res Commun* 246:142-147, 1998.
41) Sonoo H, Kurebayashi J, Iino Y, Inaji H, Watanabe T, Toi M, Kobayashi S, Sato B, Yoshimoto M: Current status and controversial issues concerning endocrine therapy for patients with recurrent breast cancer in Japan. *Breast Cancer* 6:344-350, 1999.
42) Lapidus RG, Nass SJ, Butash KA, Parl FF, Weitzman SA, Graff JG, Herman JG, Davidson NE: Mapping of ER gene CpG island methylation-specific polymerase chain reaction. *Cancer Res* 58:2515-2519, 1998.
43) Yoshida T, Eguchi H, Nakachi K, Tanimoto K, Higashi Y, Suemasu K, Iino Y, Morishita Y, Hayashi S: Distinct mechanisms of loss of estrogen receptor alpha gene expression in human breast cancer: methylation of the gene and alteration of trans-acting factors. *Carcinogenesis* 21:2193-2201, 2000.
44) Kurebayashi J, Otsuki T, Moriya T, Sonoo H: Hypoxia reduces hormone responsiveness of human breast cancer cells. *Jpn J Cancer Res* 92:1093-1101, 2001.
45) Chia SK, Wykoff CC, Watson PH, Han C, Leek RD, Pastorek J, Gatter KC, Ratcliffe P, Harris AL: Prognostic significance of a novel hypoxia-regulated marker, carbonic anhydrase IX, in invasive breast carcinoma. *J Clin Oncol* 19:3660-3668, 2001.
46) Hockel M, Vaupel P: Tumor hypoxia: definitions and current clinical, biologic, and molecular aspects. *J Natl Cancer Inst* 93:266-276, 2001.
47) Nicholson RI, McClelland RA, Gee JM, Manning DL, Cannon P, Robertson JF, Ellis IO, Blamey RW: Epidermal growth factor receptor expression in breast cancer: association with response to endocrine therapy. *Breast Cancer Res Treat* 29:117-125, 1994.
48) Houston SJ, Plunkett TA, Barnes DM, Smith P, Rubens RD, Miles DW: Overexpression of c-erbB2 is an independent marker of resistance to endocrine therapy in advanced breast cancer. *Br J Cancer* 79:1220-1226, 1999.
49) Leyland-Jones B: Trastuzumab: hopes and realities. *Lancet Oncol* 3:137-144, 2002.
50) Slamon D, Pegram M: Rationale for trastuzumab (Herceptin) in adjuvant breast cancer trials. *Semin Oncol* 28 (1 Suppl 3):13-19, 2001.
51) Ellis MJ, Coop A, Singh B, Mauriac L, Llombert-Cussac A, Janicke F, Miller WR, Evans DB, Dugan M, Brady C, Quebe-Fehling E, Borgs M: Letrozole is more effective neoadjuvant endocrine therapy than tamoxifen for ErbB-1- and/or ErbB-2-positive, estrogen receptor-positive primary breast cancer: evidence from a phase III randomized trial. *J Clin Oncol* 19:3808-3816, 2001.
52) Stal O, Borg A, Ferno M, Kallstrom AC, Malmstrom P, Nordenskjold B; South Sweden Breast Cancer Group. Southeast Sweden Breast Cancer Group: ErbB2 status and the benefit from two or five years of adjuvant tamoxifen in postmenopausal early stage breast cancer. *Ann Oncol* 11:1545-1550, 2000.

53) Raben D, Helfrich BA, Chan D, Johnson G, Bunn PA Jr: ZD1839, a selective epidermal growth factor receptor tyrosine kinase inhibitor, alone and in combination with radiation and chemotherapy as a new therapeutic strategy in non-small cell lung cancer. *Semin Oncol* 29 (1 Suppl 4):37-46, 2002.
54) Moulder SL, Yakes FM, Muthuswamy SK, Bianco R, Simpson JF, Arteaga CL: Epidermal growth factor receptor (HER1) tyrosine kinase inhibitor ZD1839 (Iressa) inhibits HER2/neu (erbB2)-overexpressing breast cancer cells in vitro and in vivo. *Cancer Res* 61:8887-8895, 2001.
55) Sainsbury JR, Farndon JR, Needham GK, Malcolm AJ, Harris AL: Epidermal-growth-factor receptor status as predictor of early recurrence of and death from breast cancer. *Lancet* 1 (8547):1398-1402, 1987.
56) Moasser MM, Basso A, Averbuch SD, Rosen N: The tyrosine kinase inhibitor ZD1839 ("Iressa") inhibits HER2-driven signaling and suppresses the growth of HER2-overexpressing tumor cells. *Cancer Res* 61:7184-7188, 2001.
57) Horwich A, Huddart RA, Gadd J, Boyd PJ, Hetherington JW, Whelan P, Dearnaley DP: A pilot study of intermittent androgen deprivation in advanced prostate cancer. *Br J Urol* 81:96-99, 1998.
58) Boccardo F, Rubagotti A, Amoroso D, Mesiti M, Romeo D, Caroti C, Farris A, Cruciani G, Villa E, Schieppati G, Mustacchi G; Italian Breast Cancer Cooperative Group: Sequential tamoxifen and aminoglutethimide versus tamoxifen alone in the adjuvant treatment of postmenopausal breast cancer patients: results of an Italian cooperative study. *J Clin Oncol* 19:4209-4215, 2001.
59) Gandara DR, Lara PN Jr, Goldberg Z, Le QT, Mack PC, Lau DH, Gumerlock PH: Tirapazamine: prototype for a novel class of therapeutic agents targeting tumor hypoxia. *Semin Oncol* 29 (1 Suppl 4):102-109, 2002.