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Cross-talk between ER and HER2 in breast carcinoma

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ABSTRACT

In tumors in which estrogen receptor (ER) and growth factor signaling pathways are simultaneously active, there is a bidirectional cross-talk that results in a positive feedback cycle of cell survival and proliferation stimuli. Beside the postulated inverse correlation between ER and HER2 (human epidermal growth factor receptor 2) as a consequence of repressive feedback signaling loop, there are also other mechanisms regarding ER-HER2 interactions. It seems that MAPK (mitogen-activated protein kinase) pathway has a central role in synergistic action between ER and HER2 in normal mammary gland development, as well in the breast cancer. MAPK pathway is hyperstimulated in cells that overexpress HER2 as a consequence of HER2 gene amplification. In ER+ tumors, MAPK phosphorylates and activates either ER itself or ER coregulators, enhancing the transcriptional activation potential of ER. ER and HER2 signaling could interact on multiple levels (genomic or non-genomic) and therefore might induce reduced ER expression or might increase ER function. Based on our own research, dominant effect of postulated cross-talk was not related to HER2-induced reduced expression of ER (no difference in quantitative levels of ER in ER+ tumors regarding their HER2 status and no difference in progression-free time between ER+HER2- and ER+HER2+ patients) as presented. The importance of understanding ER-HER2 cross-talk is not only because of its significance in breast cancer progression, but because it seems to be fundamental factor in endocrine resistance that can improve treatment strategies, especially targeting MAPK pathway.

KEY WORDS: *Breast Neoplasms; Receptors, Estrogen; Receptor, Epidermal Growth Factor; Receptor, erbB-2; Receptor Cross-Talk*

INTRODUCTION

Breast cancer development and progression involves complex interaction between hormonal receptors and growth factor signaling pathways. Although ER status and transcriptional profile of ER+ and ER- tumors is the main discriminative factor for breast cancer phenotype, breast cancer remains more heterogeneous disease with many different phenotypes. That is the result of various different growth regulatory pathways that could be activated and the possibility that virtually all signaling pathways can cross-talk, underlying the complexity of disease progression. Tumor cells are able to produce growth factors by autocrine means and frequently express several classes of growth factor receptors and their downstream signaling elements. Responses to growth factors include enhancement of proliferation, cell survival, motility, invasiveness and angiogenesis. Thus, it is not surprising that inappropriate activation of growth factor signaling cascades, either through the enhanced supply of growth factor ligands or via increased activation of their receptors or signaling elements can associate with aggressive tumor biology and poor patient prognosis. Moreover, there is considerable evidence that increased signaling through such pathways promotes in vitro and in vivo resistance to various treatment strategies in breast cancer cells. Increase in growth factor signaling in breast cancer could be considered as adaptive event that should facilitate cell survival in the presence of therapy and ultimately development of resistance.

It has been found that synergism and coordination exist between ER and growth factor signaling that can even substitute for estrogen in supporting the growth and the survival of breast cancer cells. Molecular identification and validation of candidate ER cross-talk pathways will likely lead to clinically important prognostic markers and targets for the application of novel therapeutics in combination with standard endocrine agents. Assuming that ER and HER2 are currently the two most clinically relevant biomarkers in breast cancer it is logical to suggest that substantial cross-talk exists between these two signaling pathways in breast cancer. The complex and pleiotropic action of ER could be explained by the intense, bidirectional cross-talk with growth factor signaling cascade, occurring at multiple levels. The importance of understanding this cross-talk is not only because of its significance in breast cancer progression, rather because it seems to be fundamental factor in endocrine resistance.

Gene microarray and other studies indicate that ER-positive breast cancers can be divided into clinical subsets with extremely different outcomes that range from tumors with good prognosis and endocrine responsiveness to others with *de novo* or acquired endocrine resistance and risk of early relapse (1,2). The clinical responsiveness of ER-positive breast cancers to the antiestrogen tamoxifen correlates positively with the absolute expression level (fmol/mg protein) of tumor ER (3,4). Additionally, there are preclinical and clinical reports that link antiestrogen resistance with tumor overexpression of one or more members of the HER family of receptor tyrosine kinases (5). In particular, up to 15% of newly arising breast cancers are not only ER-positive but also overexpress the HER2 as a result of oncogene amplification. Several clinical studies have shown that these ER+HER2+ breast cancers have significantly lower ER and PR content than ER+HER- breast cancers (6) Supporting these clinical observations, ER+ breast cancer cell lines engineered to overexpress HER2 retain their ER positivity but show marked reductions in their ER content (7). While this downregulation of ER expression as a consequence of ER-HER2 cross-talk,

may partially explain the reduced antitumor activity of antiestrogens against ER+HER2+ breast cancers relative to ER+HER2- cancers, how HER2 activation downregulates ER expression remains unknown. However decreased expression of ER by HER2 is not the only consequence of ER-HER2 cross-talk and there are many other possible interactions that we tried to summarize in this review.

ER – BASIC BIOLOGY AND MECHANISM OF ACTION

ER is a member of the steroid nuclear receptor superfamily of ligand-regulated transcription factors (8). ER has highly conserved functional and structural features, such as DNA binding domain and the ligand-binding domain. Transcription activation has been attributed to two regions: the N terminal activation function (AF-1) and ligand-dependent AF-2 in C terminal ligand binding domain. AF-1 (ligand independent) and AF-2 (ligand-dependent) can function independently and synergistically to activate transcription and promote signaling depending on the cell and promoter context.

Mechanisms underlying activation of ER involve phosphorilation (9). There are still several controversial issues such as whether both tyrosine and serine are phosphorylated and which phosphorilation are ligand dependent. However, in the absence of estrogen (ligand-independent activation of ER), other signaling pathways can modulate ER through phosphorylation. In these cross-couplings between growth factors and ER, the mediators are the guanine nucleotide binding protein $p21^{ras}$ and the mitogen-activated protein kinase (MAPK) (10). $P21^{ras}$ functions as an intermediate between the membrane associated growth factor receptor-tyrosine kinase and MAPK phosphorylation cascades. The various MAPK family members play a complex role in the determination of cell growth, differentiation and apoptosis and this is thought to involve a balance between competing MAPK pathways. Estrogen binding to ER induces phosphorylation of the Ser-118 residue at the ligand-independent AF-1 domain and this site correspond to the consensus phosphorylation site for MAPK (11) Phosphorilation of AF-1 at Ser-118 result in activation of ER in ligand-independent manner, thereby perhaps contributing to endocrine resistance. Surprisingly, research of Sarwar et al. shows that Ser-118 phosphorylation is higher in more differentiated tumors, suggesting that phosphorylation at this site is associated with a good prognosis in patients not previously treated with endocrine agents (12). In addition, Ser-118 phosphorylation was elevated in tumor biopsies taken from patients who had relapsed following tamoxifen treatment. These data are consistent with the view that Ser-118 phosphorylation is a feature of normal ER function and that increases in levels of phosphorylation at this site may play a key role in the emergence of endocrine resistance in breast cancer. MAPK mediated phosphorylation of the Ser-118 potentiates the function of ligand-independent AF-1 domain. MAPK are activated through several distinct signaling pathways, especially tyrosine kinase membrane receptors (such as HER2) activated by growth factors. Such cross-talk is basis for synergistic action between estrogen and growth factor action in normal mammary gland development, as well in the breast cancer.

ER plays a key role in normal breast development and breast cancer progression. ER represents the important target for breast cancer treatment, because of the ability of ER to respond to multiple inputs and to control expression of multiple downstream genes. To understand the consequences of cross-talk between ER and growth factors and their receptors it is important to know basic modes of ER action, that could be genomic and non-genomic (13). Genomic mode of action (nuclear initiated steroid signaling) could be classical (transcriptional regulation of the estrogen-regulated genes containing an estrogen response element (ERE) in their promoters) or non-classical (interaction with other transcription factors and regulation of gene expression at alternative regulative DNA sequences i.e. non-ERE sites). Non-genomic effects (membrane-initiated steroid signaling) refers to ability of ER to interact with and activate growth factor receptors (EGFR, HER-2) cellular tyrosine kinases, mitogen activated protein kinases (MAPK), phosphatidylinositol 3 kinase and Akt (protein kinase B). The genomic and non-genomic mechanisms of actions are not mutually exclusive and many interactions between these pathways exist.

HER2 – BASIC BIOLOGY AND MECHANISM OF ACTION

HER2 oncogene encodes a transmembrane tyrosine kinase growth factor receptor. It is amplified and overexpressed in 20% to 25% of human breast cancers and is frequently related to aggressive tumor growth and metastatic activity, leading to poorer clinical course of disease (shorter disease-free interval and overall survival) (14). Similar to ER status and HER2 status affects expression of significant number of genes representing multiple biochemical pathways. HER2 + or HER2- breast cancer could be distinguished by their gene expression profiles (15) that results in differences in biological effects and consequently clinical implications.

HER2 may be considered as a master regulator of the HER network and plays a crucial role in the network of cell-signaling processes controlling normal growth and development (16). HER2 is a preferred dimerization partner for inter-receptor interactions within HER family and co-receptor for many different ligands. Moreover, HER2 has a potent tyrosine kinase domain, which shows activity even in the absence of heterodimer formation (the ligand-independent tyrosine kinase activity (17). This could be especially important when HER2 is overexpressed. Signaling by HER2–containing receptor combinations is relatively prolonged and results in enhanced activation of signaling pathways such as the MAPK route (18). Overexpression of HER2 promotes formation of more HER2 heterodimers and the result of their action is potent signaling, enhanced responsiveness to growth factors, selective growth advantage and malignant growth. Oncogenic action of HER2 is a result of hyperactivated HER2 signaling network that results in deregulation of the cell cycle and a key mediator of cell proliferation is MAPK pathway.

ER AND HER2 INTERACTIONS

Breast cancer growth is regulated by coordinated actions of the ER and various growth factor receptor signaling (e.g., HER2 amplification). In tumors in which ER and HER2 pathways are simultaneously active there is a bidirectional cross-talk that results in a positive feedback cycle of cell survival and proliferation stimuli. Enhanced cross-talk between ER and HER2 may be involved in the development of a hormone-independent phenotype in breast cancer cells and the resistance to hormonal therapy. Activation of different growth factor-driven signaling pathways accompanies each step of development of resistance to estrogen-antiestrogen manipulation and may promote tumor cell growth in a different ways: by suppression of ER expression and function (promote evolution of ER- phenotype) and by increasing of ER function. Alterations in ER cross-talk pathways clinically linked with resistance but not related to ER-HER2 interactions have also been described and include: enhanced activation of the gene-regulating transcription factor complex, AP-1 (19), deregulated PI3/Akt (20), protein kinase Cα (21), and the insulin-like growth factor I (22) signaling pathways.

The most popular model of cross-talk is one in which elevated HER2 signaling causes ER to exhibit diminished transcriptional activity through either the transcriptional down-regulation of the ER gene (23) posttranslational modification of ER (phosphorilation) (24) or the induction of ER–binding corepressors (25). It has been suggested that in ER+ breast tumors, particularly those with highly active HER2, a cross-talk is established in a way that estrogen activates growth factor signaling and the growth factor signaling pathway further activates ER (26). ER and HER2 signaling could interact on a genomic or non-genomic level and therefore might induce reduced ER expression or, on the other hand, might increase ER function, but in all this cases the net result could be altered responsiveness to endocrine manipulation.

Altered ER expression

Lack of ER expression is clearly the main mechanism of de novo resistance to hormonal therapy. Since chronic ER activation by estrogen can be associated with ER downregulation, it is certainly feasible that constitutive / chronic activation of ER by growth factor signaling could similar result in decline in ER (27). Numerous studies indicated this

mutually repressive feedback signaling loop between ER and HER2, resulting in inverse correlation and probably reflecting the interrelationship of endocrine and paracrine signals important in normal mammary gland development as well as in cancer. Transfection of constitutive active HER2 results in significant reduction in the expression of ER mRNA and protein and in marked reduction of estrogen-regulated genes, leading to the development of estrogen-independent phenotype (28). On the other hand, administration of estrogen to breast cancer cell lines results in transcriptional repression of HER2. HER2 promoter could be suppressed by estrogen-induced downregulation (29). According to Konecny et al. who analyzed relationship between HER2 and ER levels as continuous variables, ER+HER2+ patients had statistically significant lower quantitative levels of ER than ER+HER2– tumors (30). There is inverse relationship between ER positivity and HER2 positivity, such that only about 50% of HER2-positive tumors are ER-positive in contrast to around 75% of the whole population (31). Dowsett et al. showed also that, beside inverse correlation between HER2 and ER status (quantitative values), ER+HER2+ primary breast carcinomas show an impeded antiproliferative response to endocrine therapy (32). It could be suggested that dominant effect of ER-HER2 cross-talk is down-regulation of ER gene transcription. However, existence of ER+HER2+ or ER-HER2- phenotypes indicates that postulated inverse correlation is not absolute.

However, loss of ER expression has been demonstrated only in 17% to 28% of patients with acquired resistance to tamoxifen (33). Also, if the effects of HER2 were mediated primarily through effects on ER transcriptional activity (genomic mechanism of cross-talk), it would be expected that a substantial number of the genes in HER2+ER+ phenotype should be ER-induced genes. However, this is not the case. Newest hypotheses propose that, in addition to current models where HER2 acts primarily by disrupting the transcriptional activity of ER, a significant fraction of effects of HER2 on ER+ breast cancer may involve ER-independent mechanisms of gene activation, contributing to clinically aggressive phenotype (34). Based on our research (unpublished data), that included 100 metastatic breast cancer patients (treated with different kinds of therapy, alone or in combination, in adjuvant and metastatic setting), there is only a trend toward to a weak inverse correlation between ER and HER2. Chromogenic in situ hybridization (CISH) for detection of HER2 gene amplification was performed on paraffin-embedded tissue sections and quantitative levels of steroid receptor contest were determined using radio-ligand binding assay. Our findings indicate that there is no difference in quantitative levels of ER in ER+ tumors regarding their HER2 status (Figure 1).

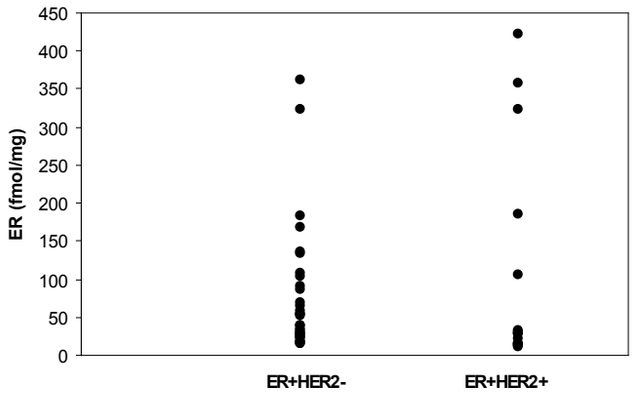

Figure 1. The distribution of ER quantitative values (fmol/mg) in ER+HER2- and ER+HER2+ breast cancer phenotypes; Mann-Whitney test (z=0.913, p >0.05)

Moreover, follow-up of these patients during the course of metastatic disease, showed that there was no difference in progression-free time between ER+HER2- and ER+HER2+ patients (Figure 2), implying that in our case, dominant effect of postulated cross-talk was not related to HER2-induced reduced expression of ER. As a variety of studies tend to show that HER2 + tumors are less likely to respond to endocrine therapy, there are studies that do not support this notion. For example, the study of Arpino et al. neither supports the importance of cross-talk between HER2 and ER for tamoxifen resistance nor that the amplification of HER2 can be independent predictor for tamoxifen resistance (35).

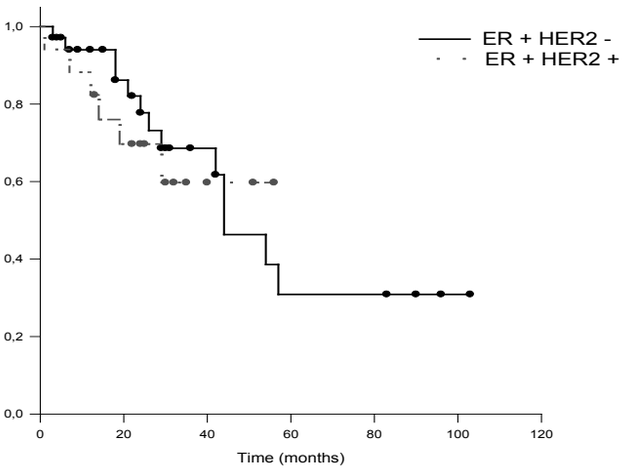

Figure 2. Survival curves for progression-free time during follow up of ER+HER2- and ER+HER2+ breast cancer patients (log-rank test, p>0.05).

Role of MAPK

Activation of the EGFR-HER2 signaling pathway initiates a kinase signaling cascade that has a variety of effects on the tumor cells, including inhibition of apoptosis, stimulation of cell proliferation, enhanced invasion and cell motility, and induction of angiogenesis stimuli. Cell survival and proliferation are mediated predominantly through the phosphatidylinositol 3-kinase (P13K)/Akt and the Erk1/2 MAPK pathway. HER2 upregulation (amplification) results in hyperstimulation of the mitogen-activated protein kinase extracellular signal-regulated kinase Erk1/2. Erk1/2 pathway is frequently upregulated in breast cancer and the expression of this pathway regulates the expression of genes with roles in the invasiveness of breast cancer cells. Hyperstimulation of Erk1/2 kinase, that is a downstream signaling effector of several receptor tyrosine kinases, is a common event associated with the progression of breast tumors and tumor cells to more invasive phenotype (36). HER2 overexpressing cells have elevated levels of activated Erk1/2. These kinases are also important for ER activity in ER+ tumors because they phosphorylate and activate either ER itself or ER coregulators. This phosphorilation augments the transcriptional activation potential of ER and enhances its effects on cell proliferation and survival. Oh et al. showed that constitutive activation of stably transfected HER2 leads to a MAPK/Erk induced down-regulation of ER that is reversible via abrogation of MAPK activity. These data suggests that up-regulated growth factor signaling via MAPK is directly linked to loss of ER expression and generation of the ER- phenotype (37). In tumors expressing both ER and abundant HER2, these two pathways provide a strong stimulus for tumor growth and may contribute to hormonal resistance. There are many findings that support involvement of MAPK in ER and HER2 cross-talk. Increased activity of MAPK signal cascade is associated with decreased survival time in ER+ breast cancer patients and antiestrogen resistance. Blockade of MAPK pathway using the MAPK – inhibitor (UO126) has been found to restore the inhibitory effect of tamoxifen on ER-mediated transcription and cell proliferation in MCF-7 cells transfected with HER2 (38).

Growth factors are known to stimulate the ligand-independent activity of ER through the activation of MAPK and the direct phosphorylation of ER. Current models of ER action suggest that it modulates the rate of transcription through interactions with basal transcription effectors via the recruitment of a variety of cofactors. ER interacts with coactivators and

corepressors that enhance or inhibit its activity on target genes. Coactivators such as A1B1 recruit acetyltransferases to the promoter site, which help to unwind the DNA, allowing gene transcription to occur (39). Reducing the levels of A1B1 significantly impedes ER mediated effects, not only on gene transcription, but also on tumor growth in experimental models (40). A1B1 is overexpressed in 65% of breast cancer suggesting important role in breast cancer development and progression (41). Osborne et al. demonstrated poor disease-free survival for patients receiving adjuvant tamoxifen, whose tumors express high levels of HER2 and the ER coactivator A1B1 (42). A1B1 is phosphorylated by kinases in the HER2 pathway such as MAPK. MAPK activation significantly increase recruitment of A1B1 and TIF-1 and enhances interactions between ER and these coactivators, but mechanism is not fully understood. Recent studies suggest that these cofactors can be phosphorylated by MAPK in addition to ER and it is well known that phosphorylation enhance trancriptional activity (43). One of the reasons for enhanced ER signaling and tumor cell growth may be ability of MAPK (beside direct phosphorilation of ER) to phosphorylate ER coactivators, suggesting a novel mechanism by which the MAPK signaling pathway is coupled to the regulation of gene transcription by modulation of A1B1 transactivation capacity.

Importance of non-genomic ER-HER2 signaling mechanism

Beside genomic so called classical way of ER action as a transcriptional regulator in the nucleus (nuclear – initiated steroid signaling), there is recently identified ER functions that can occur very rapidly in the cell before gene transcription tales place. This ER action may occur outside the nucleus or even in the cell membrane (non-genomic or membrane-initiated steroid signaling) (44). This membrane ER can modulate activities through several signaling pathways normally thought to be regulated by growth factors. Membrane ER can associate with and activate a variety of growth factor signaling molecules such as insulin-like growth factor receptor (IGF1-R), P13 K, Shc and Src (45). Activation of Src leads to activation of matrix metalloproteinase, which cleave EGF from the cell membrane, freeing it to bind to and activate the growth factor receptors on the cell surface. In this way, ER can rapidly activate the kinase cascade leading downstream to activation of Erk1/2 MAPK and Akt thereby providing strong survival and proliferative signals to the breast cancer cell. In addition these kinases can phosphorylate ER and its coregulators to augment nuclear ER signaling. In breast cancer cells with low levels of EGFR or HER2, these membrane functions of ER may be modest, but in tumor cells with abundant EGFR or HER2 (for which has been shown to potentiate membrane ER signaling in response to both ER and tamoxifen and can sequester ER outside the nucleus), this membrane-initiated steroid signaling may contribute more substantially to tumor growth. The recent study (46) showed for the first time that ER redistribution to the cytoplasm and its interaction with HER2 is important downstream effect of HER2 overexpression, HER2 overexpression did indeed promote ER colocalization with HER2 in the cytoplasm. HER2 deregulation was accompanied by the presence of ER in the cytoplasmic compartment with a concurrent reduction in the level of nuclear ER indicating the possibility for ER and HER2 cross-talk in cytoplasmic compartment. The observed hyperstimulation of Erk1/2 may be important for localization of ER.

These results provide a new explanation for the aggressiveness of HER2-overexpressing, ER+ breast cancer cells. Phosphorylation of ER and its coregulatory proteins can augment nuclear ER function resulting in a tumor that is highly dependent on estrogen for growth, but also a tumor that might be resistant to tamoxifen because of activation of membrane ER by tamoxifen (47). In general, enhanced growth factor signaling especially MAPK hyperactivation, such as in the context of HER2 amplified tumors, could lead to increased non-genomic actions of ER.

CONCLUSION

In summary, it seems that both non-genomic (membrane) and genomic (nuclear) ER signaling influence and are influenced by growth factor signaling pathways resulting in endocrine resistant cells. In such cells, membrane ER rapidly activates cell surface tyrosine kinase receptors such as HER2, leading to signaling through MAPK and other pathways. These protein kinases are able to phosphorylate nuclear ER in its AF-1 domain and their coactivators. This results in re-activation of ER-mediated transcription (even in the presence of antiestrogen), increase in growth factor expression, and reinforce the signaling loop. HER2 overexpression could thus serve to augment this signaling loop, markedly increasing MAPK activation and its target coactivator A1B1 to subsequently enhance nuclear ER signaling.

Although there are many studies dedicated to this issue, the importance of cross-talk between ER and HER2 in breast cancer is not established yet. Preclinical and clinical studies suggest that HER2 positive status confers a relative resistance to endocrine treatment, with moderate significance. At present, HER2 status is not used for selection or prediction of endocrine treatment in primary or in metastatic breast cancer, because the level of available evidence does not support it and data are still conflicting (48). However, no matter what way of cross-talk is dominant in ER-HER2 interaction, central role belongs to MAPK. The most important clinical implication of such findings is the need for increased use of growth factor pathway inhibitors (gefitinib, trastuzumab) or other treatments that inhibit these kinases (or even downstream intermediates in Erk1/2 MAPK pathway) in combination with tamoxifen, since monotherapy is not likely to be optimal in ER+HER2+ tumors.

Note

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