



Published in final edited form as:

*Anticancer Res.* 2006 ; 26(0): 4235–4243.

## HER-2 and NF- $\kappa$ B as the Targets for Therapy-resistant Breast Cancer

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### Abstract

HER-2 (also called ErbB2 or Neu) tyrosine kinase, one of the four members of ErbB receptor family (ErbB1, i.e., EGFR, ErbB2, ErbB3 and ErbB4), plays a critical role in the control of diverse cellular functions involved in differentiation, proliferation, migration and cell survival via multiple signal transduction pathways. Overexpression of HER-2, observed in HER-2-positive breast cancer patients, is believed to cause the tumor resistance to an array of anti-cancer agents and poor prognosis. Although HER-2 antibodies have shown growth inhibitory effects, more efficient molecular targets against HER-2-mediated tumor resistance need to be developed. The molecular mechanisms underlying HER-2-mediated tumor resistance, especially the connections between HER-2 and therapy-resistant signaling networks, need to be further investigated. NF- $\kappa$ B, a key stress transcription factor that can initiate a pro-survival network, was found to be activated in many cancer cells overexpressing HER-2 and to be responsible for the radiation resistance in HER-2 transfected breast cancer cells. Recent findings in literature and data from this laboratory suggest a possible co-operation between HER-2 and NF- $\kappa$ B in signaling tumor resistance to radiotherapy. This review will discuss the mechanisms of HER-2 mediated NF- $\kappa$ B signaling pathway and potential target for therapeutic intervention.

### Keywords

HER-2; NF- $\kappa$ B; herceptin; drug resistance; radiotherapy resistance; review

### Introduction

HER-2 is a proto-oncogene which encodes a 185 kDa (1255 amino acids) transmembrane receptor tyrosine kinase (RTK) in various tissues of epithelial, mesenchymal and neuronal origin (1, 2). It maps to human chromosome 17q21, and is closely related in structure to the epidermal growth factor receptor (EGFR) (3, 4). EGFR proteins exist as monomers in the cell membrane. Upon ligand binding (except HER-2), these receptors form homo- or heterodimers to regulate diverse biological processes, such as proliferation, differentiation, cell mobility and apoptosis, *via* different signal transduction pathways (5, 6). Although there is no specific ligand for HER-2, it appears that it acts as a preferred co-receptor to form heterodimers with other EGFR members for the initiation of signal transduction (7, 8). In cells overexpressing HER-2, including those of the breast, spontaneously activated homodimers can occur in the absence of a ligand and constitutive receptor activation (9). Following dimerization, HER-2 undergoes autophosphorylation on specific tyrosine residues within the regulatory domain (10, 11). HER-2 is localized to the cell membrane with two

cysteine-rich extracellular dimerization domains, a transmembrane domain and an intracellular tyrosine kinase domain (3, 12, 13). Although HER-2 is a membrane-bound protein, it was found to enter the nucleus by endocytosis (14) and function as a transcriptional regulator (15).

HER-2 overexpression or amplification, found frequently in many types of human cancers, including breast, ovarian, lung, gastric and oral cancers (2, 16–22), increases cell proliferation and survival (23), and induces tumor resistance to anticancer therapies (16, 24). Breast cancer is the most common cancer and the second leading cause of cancer related death in women in the United States (25). Although a normal level of HER-2 is required for the regulation of normal breast growth and development (26), amplification and overexpression of HER-2 causes the disruption of normal cellular control and the formation of aggressive breast tumor cells (18, 27). HER-2 level is considered as the predictive marker for the diagnosis of metastatic breast cancer and it is an important factor for treatment plan design (28, 29). Many breast cancer patients benefit from radiotherapy combined with chemotherapeutic agents. These combined modalities improve the local control of tumor growth and increase survival rates. However, accumulating reports suggest that chemoresistance can be induced following radiation (radio-chemoresistance), which challenges the overall effectiveness of the combined modality therapy. Most importantly, therapy-resistance is strikingly increased when tumor cells are HER-2 positive. For instance, overexpression of HER-2 has been related to an increased risk of local relapse in breast cancer patients who received conservative surgery and radiation therapy (29). These results suggest that HER-2-mediated therapy-resistance involves the anti-radiation signaling network.

## HER-2 and Breast Cancer

Breast cancer cells expressing high levels of EGF receptors are associated with an aggressive clinical behavior (30). Approximately 30% of breast cancer patients showed genetic alterations in the HER-2 gene causing an increased amount of the growth factor receptor protein on the tumor cell surface. Patients with HER-2 positive cancers show a more aggressive disease, greater likelihood of recurrence, poorer prognosis, and decreased survival compared to patients with HER-2-negative breast cancer. A causal link between HER-2 overexpression and tumor progression was further evidenced by experimental results that HER-2 transfected cells showed increased metastasis *in vivo*, and if HER-2 is inhibited by monoclonal antibody, antisense constructs or adenovirus 5 ELA gene products, the malignant phenotype was reversed (31). Therefore, targeting HER-2 has the potential to be an efficient approach in anticancer treatments. However, HER-2 monoclonal antibody "trastuzumab" (marketed as "herceptin"; a recombinant humanized monoclonal antibody against the extracellular domain of HER-2) produces remission in 11-15% of patients with metastatic breast cancer. This result indicates that the majority of the patients become resistant to herceptin, although they initially respond to this monotherapy. Another monoclonal antibody pertuzumab, which blocks dimerization of HER-2, alone or together with herceptin could not significantly reduce the viability of herceptin-resistant breast cancer cells (32, 33). Also, a combined therapy of herceptin and chemotherapeutic agents (*e.g.*, paclitaxel and docetaxel) resulted in little increase in patient survival (34). These clinical data strongly suggest that targeting HER-2 alone is insufficient for breast cancer patients and the signaling network causing HER-2-mediated tumor resistance needs to be elucidated.

Radiotherapy is an important therapeutic intervention following breast conservative surgery (35, 36). Richard *et al.* reported that HER-2 monoclonal antibody (rhuMAbs) modulates repair of IR-induced DNA damage and enhances radiosensitivity of HER-2 positive breast cancer cells (37). The potential role of HER-2 in the modulation of sensitivity to radiation is

assumed to be due to the signal generated by antibody binding to HER-2 receptor that blocks DNA repair in HER-2-overexpressing cells (38). This concept is supported by clinical studies that demonstrate earlier local relapse in HER-2-positive breast cancer patients following conservative surgery accompanied by radiotherapy. Although it is well documented that genetic factors contribute to the outcome of radiotherapy (39), redox imbalance, repair of DNA damage, cell cycle adjustment, by-stander effects, genomic instability, as well as adaptive tumor resistance may contribute to a complex network that determines the efficacy of anticancer radiotherapy. The exact molecular mechanisms regulating tumor response to radiation are largely unknown, although a group of stress responsive effector genes were found to be responsible for increased resistance to radiotherapy (40, 41). Recently, data reported by our group and others strongly suggest a pro-survival signaling network involving NF- $\kappa$ B and HER-2 (40, 42, 43). HER-2 was found to regulate the constitutive activation of NF- $\kappa$ B in HER-2-overexpressing breast cancer cells (40, 44). Therefore, targeting both HER-2 and NF- $\kappa$ B could be an efficient strategy in the management of HER-2 positive breast cancer patients.

## HER-2-mediated Chemoresistance

Tumor resistance to multiple chemotherapeutic agents, a major cause of failure of anti-cancer therapy, is believed to be related to intrinsic, as well as acquired during treatment (45, 46). The most commonly used anti-neoplastic agents in the treatment of disseminated breast cancer are adriamycin, methotrexate and cyclophosphamide. Cell lines selected for resistance to adriamycin often develop cross-resistance to structurally dissimilar anti-neoplastic drugs with different mechanisms of cytotoxic action, a phenomenon called pleiotropic or multidrug resistance (MDR) (45). One of the mechanisms that may contribute to chemoresistance is the activation of oncogenes, including HER-2, Bcl-2, Bcl-XL, Ras, c-Jun, c-Fos, or mutant p53 (46). HER-2 transfected human lung cancer cells showed enhanced resistance to chemotherapies (47). The level of HER-2 is the only independent predictor for chemoresistance to doxorubicin, etoposide, and probably cisplatin. Although intrinsic chemoresistance almost certainly is a multifactorial process, overexpression of HER-2 may be an important factor for the chemoresistance of non-small cancer lung cells (48). A clinical study conducted by Gusterson *et al.* demonstrates that HER-2-overexpressing breast cancer cells are less responsive to adjuvant chemotherapy regimens consisting of cyclophosphamide, Methotrexate and 5-fluorouracil (CMF) than tumors that have normal expression of HER-2 (49). In another clinical study, patients with metastatic breast cancers showed that elevated HER-2 serum protein levels are associated with a lower rate of response to chemotherapy compared to those with normal HER-2 levels (29% *versus* 59%) (50).

Herceptin has been shown to induce therapeutic responses in patients with primary operable breast cancer through antibody-dependent cellular cytotoxicity (ADCC) (51). It activates the PTEN phosphatase, which results in rapid dephosphorylation of Akt and inhibits cell proliferation (52). Although clinical studies established that herceptin is active against HER-2-overexpressing breast cancer cells (16, 17), the time to disease progression is short (median duration is 9 months) (17). Therefore, herceptin monotherapy, approved in 1998 by the US Food and Drug Administration, is not an efficient treatment for many cancer patients. Recent studies using herceptin combined with chemotherapeutic drugs, including paclitaxel and docetaxel, increases the time to disease progression and the survival of breast cancer patients. However, in most patients disease progression begins again within a year (34). These results indicate that targeting other key signaling elements as well as HER-2 is critical to improve the survival of HER-2-overexpressing breast cancer patients.

## HER-2-mediated Radioresistance

Radiotherapy is widely used in the treatment of breast cancer and reduces the risk of loco-regional recurrence (53). Patients treated with breast conservation surgery routinely receive radiotherapy as an additional treatment. However, breast cancer cells have been shown to be relatively refractory to IR-induced DNA damage and apoptosis (54), although the molecular mechanism underlying the radioresistant phenotype is largely unknown. Several clinical studies showed that the recurrence risk after treatment with surgery and radiation is higher among HER-2-positive breast cancer patients. Stimulated with epidermal growth factors, MCF-7 human breast cancer cells showed more radioresistance than the untreated cells (55). Compared to parental cell line, HER-2 transfected MCF-7 cells showed enhanced resistance to IR-induced apoptosis and increased post-radiation clonogenic survival (37, 56). Previous studies indicated that ionizing radiation itself can mimic the function of growth factors to activate EGFR, which then activates the downstream mitogen-activated protein kinase (MAPK) pathway (57, 58). Other radiation-induced factors, such as p53 status of the cell, induction of Bax and Bcl-2 families of proteins, NF- $\kappa$ B activation, relative levels of insulin like growth factor and insulin-like growth factor binding proteins, and PI3K/Akt pathway activation, may modulate the apoptotic response to DNA-damaging agents (54).

EGFRs, especially HER-2, have become the target to treat cancers that are resistant to radiotherapy (59). Anti-HER-2 monoclonal antibody, rhumAbHER2 (trastuzumab/herceptin), enhances radiation-induced growth inhibition in human head and neck carcinomas (60). Rao *et al.* found that a potent EGFR tyrosine kinase inhibitor CI-1033 that inhibits the proliferation of HER-2-overexpressing breast cancer cells, reduced clonogenic survival by 65-fold with radiation compared to cells that received irradiation alone (59). The study conducted by Pietras *et al.* indicated that herceptin is able to radiosensitize HER-2-overexpressing MCF-7 cells. They found that the combination of herceptin and radiation can synergistically reduce tumor formation in nude mice (37). Another study showed that breast cancer cells treated with herceptin significantly sensitized the apoptosis induced by radiation in cell lines with high levels of HER-2 (such as BT474, SKBR3, and MDA453), but not in cell lines with low levels of HER-2 (such as MCF7, ZR75B and MDA468) (61). Herceptin is hypothesized to sensitize HER-2-positive tumors to irradiation by blocking the growth-promoting signals required to induce cell proliferation and survival through HER-2, although the signaling pathways have not been clearly identified. Further research indicated that MAPK and PI3K/Akt pathways are involved in HER-2-mediated resistance to radiation-induced apoptosis in breast cancer cells (53, 56). Wortmannin, a PI3K inhibitor, induces radiosensitization in MCF-7 and other cell lines (62), and inhibition of MAPK decreases radioresistance in A431 human squamous carcinoma cells (63).

## Mechanisms of NF- $\kappa$ B Activation

NF- $\kappa$ B is a dimeric transcription factor, which acts as a master regulator of stress and immune responses in many cell types, since it constitutes a primary means of relaying an extracellular, immunologically relevant signal into nucleus to initiate a genetic program. NF- $\kappa$ B was originally identified as a protein bound to a sequence in the immunoglobulin kappa light chain enhancer in B cells (64). It tightly controls genes for stress responses, inflammation and apoptosis. NF- $\kappa$ B family consists of five members of the Rel family: RelA (also called p65), RelB, c-Rel, p50/p105 (also called NF- $\kappa$ B1) and p52/p100 (also called NF- $\kappa$ B2). Although the heterodimer of p50 and p65 is shown to be the most abundant form of NF- $\kappa$ B (65), different combinations of homo- or hetero-dimers can be formed to regulate the intrinsic NF- $\kappa$ B specificity (66–69). NF- $\kappa$ B DNA binding sites that are present in the promoter region of many genes are capable of binding to p50 homodimers, p50/p65 or

p50/c-Rel heterodimers, suggesting that NF- $\kappa$ B can regulate the expression of different effector genes (70).

Under resting conditions, the NF- $\kappa$ B complex is formed by a p50 homodimer or a p50/p65 heterodimer bound to a member of the I $\kappa$ B family (70, 71). In the cytosol of unstimulated cells, the nuclear localization signal of NF- $\kappa$ B is effectively hidden through the non-covalent binding with I $\kappa$ B. Members of the mammalian I $\kappa$ B family include I $\kappa$ B $\alpha$ , I $\kappa$ B $\beta$ , I $\kappa$ B $\gamma$ , I $\kappa$ B $\epsilon$ , Bcl3, p105 and p100, of which the most studied is I $\kappa$ B $\alpha$ . Following stimulation, DNA-binding subunits p50 and p52 that carry a Rel homology domain (RHD) are proteolytically released from p105 and p100, respectively. The RHD contains a nuclear localization sequence (NLS) and is involved in dimerization, sequence-specific DNA binding and interaction with the inhibitory I $\kappa$ B proteins. Bcl3 functions as a transcriptional activator with p50 or p52 homodimers, rather than an inhibitor of NF- $\kappa$ B. Using various stimuli, including TNF- $\alpha$ , PMA, LPS, interleukins and UV or IR, it has been well established in many cell lines that signal-induced activation of NF- $\kappa$ B typically occurs through phosphorylation of I $\kappa$ B proteins at Ser-32 and Ser-36 in I $\kappa$ B $\alpha$ , and Ser-19 and Ser-23 in I $\kappa$ B $\beta$  *via* ubiquitin-dependent protein kinase, followed by ubiquitination at nearby lysine residues and degradation by the 26S proteasome (72–74). Upon degradation of I $\kappa$ B protein, NF- $\kappa$ B translocates to the nucleus where it either binds to a specific 10-base-pair consensus site GGGPuNNPyPyCC (Pu = purine, Py = pyrimidine and N = any base) or interacts with other transcription factors thereby regulating gene transcription. Although it has been suggested that the degraded I $\kappa$ B may still be associated with NF- $\kappa$ B in mammalian cells, activated NF- $\kappa$ B typically exists as a dimeric protein, and this transcriptionally active form possesses both DNA-binding and transactivation domains. NF- $\kappa$ B activates transcription from a wide variety of promoters, including that of its own inhibitor I $\kappa$ B $\alpha$ . The newly synthesized I $\kappa$ B $\alpha$ , enters the nucleus and removes NF- $\kappa$ B from its DNA-binding sites and transports it back to the cytoplasm, thereby terminating NF- $\kappa$ B-dependent transcription (75, 76).

Acetylation and deacetylation events are post-translational control mechanisms that play critical roles in the activation of NF- $\kappa$ B. Evidence suggests that NF- $\kappa$ B-dependent transcription requires multiple co-activators *i.e.*, p300/CBP, P/CAF and SRC-1/NcoA-1 that possess histone acetyltransferase (HAT) activity (77–79). The interactions between NF- $\kappa$ B and these HATs suggest a link between acetylation events and NF- $\kappa$ B-mediated transactivation. A role for acetylation in the regulation of NF- $\kappa$ B-mediated transactivation has emerged with the finding that histone deacetylase inhibitors (HDACi) (such as trichostatin A or sodium butyrate) enhance NF- $\kappa$ B-dependent gene expression in the presence of TNF- $\alpha$  (80–82). In addition, NF- $\kappa$ B heterodimer p50/p65 can be acetylated at multiple lysine residues (83), which is believed to regulate the function of transcriptional activation, DNA-binding affinity and I $\kappa$ B $\alpha$  affinity. Interestingly, the p50 subunit, that does not possess a transactivation domain, is acetylated *in vitro* by p300/CBP (84). The enhanced p50 acetylation *in vivo* is found to be correlated with increased p50 binding to the cyclooxygenase-2 (COX-2), an important NF- $\kappa$ B-regulated effector (85). Acetylation of p65 is also detected *in vivo* (86) with stimulation of TNF- $\alpha$  and PMA (87). In both cases, p65 is acetylated following overexpression of p300/CBP and p65 is deacetylated through a specific interaction with histone deacetylase-3 (HDAC-3) (82, 87, 88). These results strongly suggest that acetylation of p65 increases its ability to bind to DNA. The exact role of the association of NF- $\kappa$ B with HATs and HDACs remains to be elucidated.

## NF- $\kappa$ B Activation in Human Breast Cancers

Aberrant activation of NF- $\kappa$ B is associated with various human cancers (89–91), which indicates that this transcription factor may play crucial roles in neoplastic cell growth. The



constitutive NF- $\kappa$ B activation in some cancers is believed to be caused by genetic alterations in genes encoding NF- $\kappa$ B components, I $\kappa$ B $\alpha$ , or upstream regulators of NF- $\kappa$ B, *e.g.*, deregulated expression or activation of upstream kinases. Activated NF- $\kappa$ B was detected in cultured cells from ER-negative breast cancer cells (92). Also, Debajit *et al.* found activated NF- $\kappa$ B predominantly in the ER-negative and ER-negative/HER-2 positive compared to ER-positive breast cancers (93), suggesting the importance of NF- $\kappa$ B signaling in specific classes of breast cancers. Selective inhibition of NF- $\kappa$ B in an ER-negative and HER-2 positive human breast tumor cell line (SKBr3) blocked heregulin-induced proliferation and resulted in apoptosis (93). Lack of NF- $\kappa$ B activation in ER-positive breast cancer cells is consistent with the observation that stimulation of the ER-positive pathway blocks NF- $\kappa$ B activation (94). A recent publication by Akane *et al.* showed that a novel IKK $\beta$  inhibitor IMD-0354 prevented the proliferation of ER-positive (MCF-7) and -negative (MDA-MB231 and HMC1-8) breast cancer cells (95). This result of NF- $\kappa$ B activation in ER-positive MCF-7 cells is contrasted with that reported by Debajit *et al.* Overall, the constitutive activation of NF- $\kappa$ B in breast tumor cells indicates an active role of this transcription factor in signaling the response to anticancer therapy. The relationship between NF- $\kappa$ B and HER-2-mediated breast cancer resistance is an important issue to be investigated.

## HER-2 and NF- $\kappa$ B Connection in Signaling Tumor Resistance

It has been well-documented that overexpression of HER-2 increases cell proliferation and survival (23) and causes NF- $\kappa$ B activation (42). The PI3K/Akt pathway is involved in HER-2 mediated NF- $\kappa$ B activation (44). Both IKK-dependent and independent pathways contribute to the deregulation of NF- $\kappa$ B in breast cancers. The IKK-independent pathway involves calpain-mediated I $\kappa$ B $\alpha$  degradation (44). This pathway also requires PI3K and its downstream kinase Akt, which is subject to inhibition by the tumor suppressor phosphatase PTEN. In another study, Akt-mediated NF- $\kappa$ B activation blocked apoptosis in HER-2-expressing cells (96). It is therefore highly possible that PI3K/Akt pathway mediated by HER-2 expression is involved in radio- or chemo-induced NF- $\kappa$ B activation that regulates downstream effector genes required for HER-2-mediated tumor resistance against therapeutic regimens. To support this notion, the following section describes a tight correlation between Akt and HER-2-mediated NF- $\kappa$ B activation in radiation-treated HER-2 overexpressing cells (40, 44).

Although HER-2 has been shown to initiate signaling responsible for tumor response to chemo- and radiotherapy (97), the key elements causing HER-2-mediated radio- or chemo-resistance have not been well established. We found that NF- $\kappa$ B and its radioresistant effector genes are activated with HER-2 overexpression (40). Overexpression of HER-2 also enhanced NF- $\kappa$ B activation and stable transfection of mutant I $\kappa$ B (MCF-7/Her-2/mI $\kappa$ B) or treatment with herceptin, inhibited NF- $\kappa$ B activation and radiosensitized MCF-7/HER-2 cells (40). Basal and IR-induced Akt was found to be activated in MCF-7/HER-2 cells and inhibited by herceptin. To determine the downstream signaling effectors, cyclin B1, cyclin D1, Bcl-2 and Bcl-XL, but not the pro-apoptotic Bad and Bax, were found to be up-regulated in MCF-7/HER-2 cells with a striking enhancement in expression of Bcl-2 and Bcl-XL. IR further induced the expression of cyclin B1 and cyclin D1 that can be reduced by herceptin treatment. These results thus suggest that overexpression of HER-2 is able to enhance NF- $\kappa$ B in response to IR, and a specific pro-survival network downstream of NF- $\kappa$ B is required for HER-2-mediated radioresistance (40). However, although our data showed an agreement between the decreased apoptotic cells and increased clonogenic survival, we cannot exclude the possibility that apoptosis-independent cell death (*e.g.*, necrosis and mitosis-linked cell death) may also contribute to the radioresistant phenotype observed in MCF-7/Her-2 cells. Clinically, expression of cyclin B1 has been identified as an important factor when evaluating radioresistance of patients with squamous cell carcinoma

(98, 99) and those with regional recurrence of head and neck tumors treated by radiotherapy (100). Together with HER-2-induced activation of Bcl-2 and Bcl-XL, our results demonstrate a tight regulation of cyclin B1 with the prosurvival network regulated by NF- $\kappa$ B in HER-2-overexpressing cells. Using IR treated human breast carcinoma MCF-7 cells (41) and human keratinocytes (101), we have reported a temporary radioresistance phenotype associated with activation of both NF- $\kappa$ B and mitochondrial antioxidant MnSOD. Overexpression of MnSOD has been shown to protect cells from mitochondria-initiated apoptosis (102, 103). Since NF- $\kappa$ B is able to induce MnSOD expression by TNF- $\alpha$  and IL- $\beta$  (104) and NF- $\kappa$ B binding sites have been located in the regulatory regions of the SOD2 gene that encodes MnSOD (104–106), NF- $\kappa$ B-mediated MnSOD activation might function as a major downstream effector of the HER-2 pathway. Alteration of mitochondrial apoptosis in HER-2-overexpressing cells is worth being investigated.

Although overexpression of HER-2 is clearly linked with NF- $\kappa$ B activation, as described above, recently, we observed that NF- $\kappa$ B may also activate HER-2 expression. The 10-base-pair NF- $\kappa$ B DNA binding sequence, *i.e.*, consensus site (gggacgacc; located between -364 and -355) is located in the promoter region of HER-2. Using a luciferase reporter assay, we observed that 5 Gy IR induced HER-2 activation in breast cancer MCF-7 and MDA-MB-231 cells (unpublished data). The increased reporter gene transcription was reduced to the control (sham-irradiated cells) levels if the NF- $\kappa$ B binding sequence was deleted. These results strongly suggest that NF- $\kappa$ B induces HER-2 gene transcription in HER-2-negative cancer cells. This is an important finding that should be further investigated. Overall, published results and our own data indicate that HER-2 and NF- $\kappa$ B may function in a mutually dependent pattern to enhance cell survival. Figure 1 shows that overexpression of HER-2 first activates the PI3K/Akt pathway (one of the most important pathways for cell growth, proliferation, and survival) for NF- $\kappa$ B activation that, in turn, up-regulates a group of pro-survival genes including mitochondrial antioxidant enzyme MnSOD (involved in mitochondrial function), cyclin B1 (involved in cell cycle regulation and apoptosis) as well as HER-2 itself, leading to the radio- and/or chemo-resistant phenotype in HER-2 positive cancer cells.

## Conclusion

Clinically, humanized HER-2 antibody herceptin has been used over the past 7 years alone or together with chemotherapeutic drugs as an important anti-cancer agent for the treatment of HER-2-positive breast cancer patients. However, the majority of patients who initially respond to herceptin generally acquire resistance within 1 year. Therefore, further elucidation of HER-2-mediated therapy-resistance will allow for the design of more efficient targets to enhance herceptin anticancer efficiency. Data presented in this review strongly suggest that HER-2-activated NF- $\kappa$ B plays a critical role in HER-2-mediated tumor resistance. Importantly, NF- $\kappa$ B may, in turn, activate HER-2 gene expression causing radio-and/or chemo-resistance in HER-2-negative cancer cells. Therefore, NF- $\kappa$ B and HER-2 appear to be mutually dependent in signaling pro-survival in breast cancer cells. Thus, combination of NF- $\kappa$ B inhibitor and herceptin may promise a novel therapeutic strategy for breast cancer patients.

## Acknowledgments

We thank our collaborators, postdoctoral fellows and graduate students at the School of Health Sciences, Purdue University for their perceptive discussion and support. This work was partially supported by the National Institutes of Health (RO1 CA101990) and by the Office of Science (BER), DOE low-dose radiation research program (Grant No. DE-FG02-05ER63945) to JJ Li.

## Abbreviations

<b>ER</b>	estrogen receptor
<b>EGFR</b>	epidermal growth factor receptor
<b>HAT</b>	histone acetyltransferase
<b>HDAC</b>	histone deacetylase
<b>HER-2</b>	human epidermal growth factor receptor-2
<b>I<math>\kappa</math>B</b>	inhibitor kappa B
<b>IKK</b>	inhibitor kappa B kinase
<b>IR</b>	ionizing radiation
<b>MAPK</b>	mitogen-activated protein kinase
<b>MnSOD</b>	manganese-containing superoxide dismutase
<b>NF-<math>\kappa</math>B</b>	nuclear factor-kappaB
<b>PI3K</b>	phosphatidylinositol 3 kinase
<b>PTEN</b>	phosphatase and tensin homolog deleted on chromosome ten

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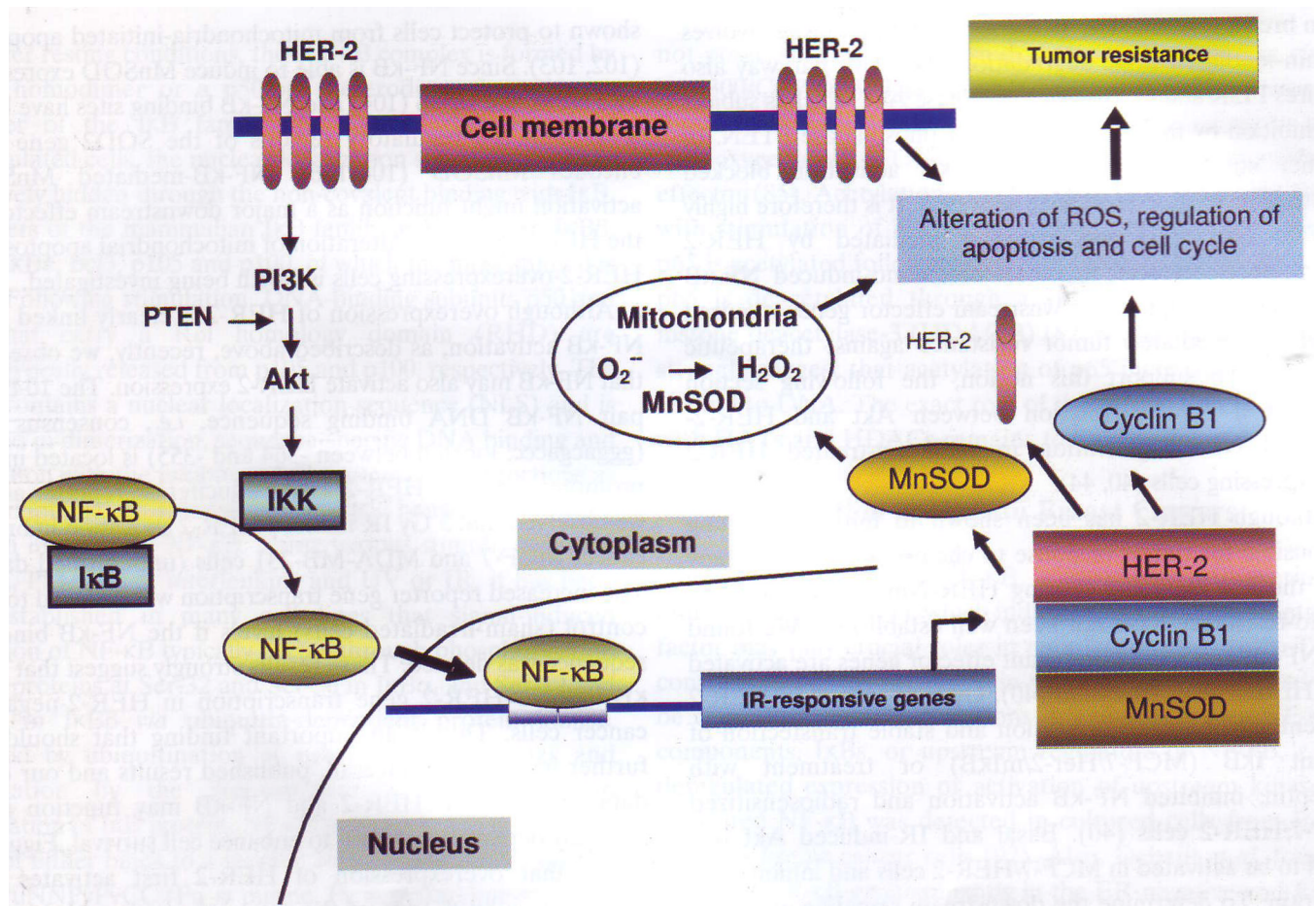
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**Figure 1.** Signaling network of HER-2-mediated NF- $\kappa$ B activation in radio- and/or chemo-resistant breast cancer cells. overexpression of HER-2 firstly activates p13K/Akt pathway that, in turn, activates NF- $\kappa$ B. Activated NF- $\kappa$ B then induces a group of pro-survival genes including cyclin B1, mitochondrial antioxidant MnSOD and importantly, the HER-2 gene itself, leading to the radio-and/or chemo-resistant phenotype in HER-2-positive cancer cells.