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## The oncogene HER2; Its signaling and transforming functions and its role in human cancer pathogenesis

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### Abstract

The year 2007 marks exactly two decades since HER2 was functionally implicated in the pathogenesis of human breast cancer (Slamon et al. 1987). This finding established the HER2 oncogene hypothesis for the development of some human cancers. The subsequent two decades have brought about an explosion of information about the biology of HER2 and the Human Epidermal Growth Factor Receptor (HER) family. An abundance of experimental evidence now solidly supports the HER2 oncogene hypothesis and etiologically links amplification of the HER2 gene locus with human cancer pathogenesis. The molecular mechanisms underlying HER2 tumorigenesis appear to be complex and a unified mechanistic model of HER2 induced transformation has not emerged. Numerous hypotheses implicating diverse transforming pathways have been proposed and are individually supported by experimental models and HER2 may indeed induce cell transformation through multiple mechanisms. Here I review the evidence supporting the oncogenic function of HER2, the mechanisms that are felt to mediate its oncogenic functions, and the evidence that links the experimental evidence with human cancer pathogenesis.

### INTRODUCTION

HER2 and neu are the human and rodent homologues of an oncogenic growth factor receptor that were identified and named independently in the early 1980s from rodent and human models, but soon found to be homologues of each other. The *neu* oncogene was initially described as a transforming oncogene discovered in a carcinogen induced rat brain tumor model (Shih et al. 1981). This gene was found to be homologous to the *v-erbB* (avian erythroblastosis virus) viral oncogene and the cellular epidermal growth factor receptor (EGFR) gene (Schechter et al. 1985; Schechter et al. 1984). In independent studies, an EGFR-related gene was found to be amplified in a human breast cancer cell line and named Human Epidermal Growth Factor Receptor-2 (HER2)(King et al. 1985). The HER2 protein product was related to and had tyrosine kinase activity similar to EGFR (Akiyama et al. 1986). Subsequent cloning of two other related human genes and the post-genome characterization of the human kinome completed the description of this family of four members (Kraus et al. 1989; Plowman et al. 1993; Manning et al. 2002). These four members are commonly referred to as EGFR (HER1, erbB1), HER2 (erbB2, HER2/*neu*), HER3 (erbB3), and HER4 (erbB4).

The analysis of tumors from mouse models and from human cancer cells has led to the description of numerous variants and mutants of HER2 and neu and adherence to appropriate nomenclature is essential in order to avoid confusion. While ErbB2 is used to

refer to the gene across both human and rodent species, HER2 is used in reference to the human gene and gene product and neu is used in reference to its rodent counterparts. Table 1 lists the various HER2 and neu species that have been described in the literature and the nomenclature and aliases that are often used to refer to them. Each of these species are discussed in the sections below and Table 1 is provided for reference and to clarify the distinctions between these variants.

## THE SIGNALING FUNCTIONS OF HER PROTEINS

The HER family proteins are type I transmembrane growth factor receptors that function to activate intracellular signaling pathways in response to extracellular signals. Their structure consists of an extracellular ligand binding domain, a transmembrane domain, and an intracellular tyrosine kinase domain (Figure 1). The function of this family is simplest in *C. Elegans* where signaling is mediated by a single ligand and a single receptor and slightly more complex in *Drosophila* where four ligands signal through a single receptor (Lacener and Sternberg 2000; Moghal and Sternberg 2003). The system is far more complicated in mammals where the functions of this family are performed by at least twelve ligands and four receptors. Readers are referred to several recent excellent reviews of HER family signaling and functions (Yarden and Sliwkowski 2001; Barnes and Kumar 2004; Mendelsohn and Baselga 2000; Prenzel et al. 2001; Olayioye et al. 2000). While the reasons behind such multiplicity in this system are not well understood, much is now known regarding the molecular basis underlying their signaling activities. Upon ligand binding to their extracellular domains, HER proteins undergo dimerization and transphosphorylation of their intracellular domains. These phosphorylated tyrosine residues dock numerous intracellular signaling molecules leading to activation of a plethora of downstream second messenger pathways and crosstalk with other transmembrane signaling pathways leading to diverse biological effects (reviewed in (Barnes and Kumar 2004; Prenzel et al. 2001; Bazley and Gullick 2005; Yarden and Sliwkowski 2001)). The structural bases for receptor dimerization have been coming to light in the past few years by cryotollagraphic data. The extracellular domain of HER proteins can exist in a closed inhibited or an open active conformation. Ligand binding induces a conformational change in their extracellular domain that induces the active conformation and promotes their dimerization and consequent transphosphorylation (Burgess et al. 2003). Partner selection appears to be a key determinant of signaling activity among HER proteins and their signaling functions follow a distinct hierarchical order favoring heterodimers over homodimers. HER2 has the strongest catalytic kinase activity and HER2-containing heterodimers have the strongest signaling functions (Tzahar et al. 1996; Graus-Porta et al. 1997). The expansion of the HER family in mammalian systems has been associated with functional differentiation necessitating interdependence rather than promoting independent or redundant functions. This is exemplified by HER2 and HER3 which are functionally incomplete receptor molecules. Unlike the other members of the family, the extracellular domain of HER2 does not pivot between active and inactive conformations and constitutively exists in an activated conformation (Garrett et al. 2003; Cho et al. 2003). Consistent with its constitutively active conformation, HER2 lacks ligand binding activity and its signaling function is engaged by its ligand-bound heterodimeric partners (Sliwkowski 2003). On the other hand HER3, unlike the other members, lacks ATP binding within its catalytic domain and is catalytically inactive (Sierke et al. 1997). Consistent with this, the signaling functions of HER3 are mediated entirely through the kinase activity of its heterodimeric partners (Kim et al. 1998). Even chimeric kinase-active HER3 constructs fail to signal without hetero-partners suggesting that HER3 even lacks the ability to homodimerize and is an obligate heterodimerization partner (Berger et al. 2004). Although individually they are incomplete signaling molecules, a large body of evidence not only establishes HER2 and HER3 as obligate partners but their complex forms the most active signaling heterodimer of the

family and essential for many biologic and developmental processes (Sliwkowski et al. 1994;Tzahar et al. 1996;Britsch et al. 1998;Vijapurkar et al. 2003;Goodearl et al. 2001;Vartanian et al. 2000;Keely and Barrett 1999;Horan et al. 1995;Wallasch et al. 1995b).

The HER proteins are widely expressed and functionally important in non-hematopoietic tissues (Press et al. 1990; Real et al. 1986). They are each essential in mammalian development and gene disruption models in mice reveal that they are critically involved in the development of multiple organ systems including the brain, skin, lung, and gastrointestinal tract (Miettinen et al. 1995; Sibia and Wagner 1995; Morris et al. 1999; Sibia et al. 1998; Threadgill et al. 1995; Lee et al. 1995; Gassmann et al. 1995; Riethmacher et al. 1997; Britsch et al. 1998; Erickson et al. 1997). Their functional importance in adult mammary gland development escapes analysis in these germline deletion models due to embryonic lethality but is nicely demonstrated by alternative models that inactivate EGFR or HER2 in pubertal mammary tissue (Andrechek et al. 2005; Xie et al. 1997).

## THE TRANSFORMING POTENTIAL OF HER2 AND NEU

### Transformation by neu

The data supporting the transforming potential of human HER2 and rodent *neu* is irrefutable. The rodent *neu* oncogene was initially identified in a screen for oncogenes using a rat carcinogen-induced tumor model and shown to transform NIH3T3 cells (Shih et al. 1981; Schechter et al. 1984). The *neu* oncogene also transforms mammary epithelial cells *in vitro* (Brandt et al. 2001). After cloning of the normal *c-neu* allele, it was determined that transforming function in the *neu* oncogene was conferred by a point mutation within the transmembrane domain resulting in a V664E mutated protein named neuT (Bargmann et al. 1986)(figure 1, region C). The V664E mutation promotes receptor dimerization and enhanced tyrosine kinase activity (Weiner et al. 1989b). Numerous mouse transgenic models have confirmed the role of this oncogene in tumorigenesis. The activated *neu* oncogene (*neuT*) expressed in mice mammary tissue (MMTV-*neuT* mice) induces adenocarcinomas. This appears as a single step whole gland transformation or in a more typical stochastic tumor formation in different backgrounds (Muller et al. 1988; Bouchard et al. 1989). The *c-neu* protooncogene overexpressed in mammary tissue of mice (MMTV-*neu* mice) also induces tumor formation, although in the majority of tumors this occurs after acquisition of deletion mutations within the extracellular juxtamembrane region which promote dimerization and enhanced kinase activity (figure 1, region A)(Guy et al. 1992; Siegel and Muller 1996; Siegel et al. 1994). The development of tumors in MMTV-*neu* transgenic mice likely involves the acquisition of additional genetic abnormalities since these tumors frequently have LOH at specific genomic loci (Cool and Jolicoeur 1999; Ritland et al. 1997). In particular, the inactivation of p53 is descriptively and etiologically linked with *neu* induced mouse mammary tumors (Li et al. 1997). Additional transgenic models demonstrate that overexpression or activation of *neu* is tumorigenic in other tissues as well, including skin, biliary tract, and prostate (Table 2). The tumorigenic function of activated *neu* has also been confirmed in two rat models by luminal retroviral infection (Wang et al. 1991). The transforming functions of *neu* require its tyrosine kinase activity as demonstrated by kinase-inactivating mutational studies (Weiner et al. 1989a). The rodent data amounts to a body of highly consistent and indisputable evidence that activation of *neu* is tumorigenic. The MMTV-*neu* mice have become the workhorse model of HER2 tumorigenesis *in vivo*.

### Transformation by HER2

The data with regards to human HER2 is also compelling however there appears to be significant differences between the rodent and human genes. While rodent *neu* appears to

require mutational activation for tumorigenicity, human HER2 appears to hold tumorigenic potential through overexpression alone. An engineered mutation within the transmembrane region of HER2 (figure 1, region C) does increase its kinase and transforming activities similar to *neu*, however this mutation does not appear to occur spontaneously (Segatto et al. 1988). Overexpression of HER2 in NIH3T3 or NR6 mouse fibroblasts induces cell transformation and tumorigenic growth (Di Fiore et al. 1987; Hudziak et al. 1987; Chazin et al. 1992). Overexpression of HER2 in MCF-7 breast cancer cells increases their invasiveness and tumorigenicity (Benz et al. 1992). Overexpression of HER2 in human mammary epithelial cells induces proliferative advantage, transformed characteristics, tumorigenic growth, and in 3D models induces proliferative and anti-apoptotic changes that mimic early stages of epithelial cell transformation (Muthuswamy et al. 2001; Woods Ignatoski et al. 2003). Transgenic expression of the human HER2 cDNA in mouse mammary breast tissue induces metastatic mammary tumors similar to *neu* (Finkle et al. 2004). However in this cross-species MMTV-HER2 transgenic model, the resulting mouse tumors have frequent deletion mutations within the extracellular domain of the HER2 transgene similar to that seen with MMTV-*neu* mice. However neither mutations within the transmembrane domain (analogous to *neuT*, see figure 1 region C) or the extracellular domain (analogous to *neu8142/neu8342*, figure 1 region A) of HER2 have ever been reported in naturally occurring human cancers and despite these experimentally acquired mutations, human breast cancers appear to be always characterized by overexpression of wildtype HER2. Interesting theories regarding this discrepancy have been proposed and are reviewed below in the section concerning  $\Delta$ HER2.

### HER2 overexpression in human cancer

The relevance of the experimental data to human disease is supported by a substantial body of clinical data. Overexpression of the HER2 protein, either through gene amplification or through transcriptional deregulation is seen in approximately 25-30% of breast and ovarian cancers and confers worse biological behavior (Slamon et al. 1989). Initial conflicting reports regarding the prognostic relevance of HER2 were resolved with improved methodologies and the overwhelming data now confirms this initial landmark genetic-biologic finding (nicely reviewed in (Ross et al. 2003)). Breast cancers can have up to 25-50 copies of the HER2 gene and up to 40-100 fold increase in HER2 protein expression resulting in up to 2 million receptors expressed at the tumor cell surface (Kallioniemi et al. 1992; Lohrisch and Piccart 2001; Venter et al. 1987) (Figure 2). Evidence suggests that HER2 amplification is an early event in human breast tumorigenesis. HER2 amplification is seen in nearly half of all in situ ductal carcinomas without any evidence of invasive disease (Liu et al. 1992; Park et al. 2006). HER2 status is maintained during progression to invasive disease, nodal metastasis and distant metastasis (Park et al. 2006; Latta et al. 2002; Carlsson et al. 2004; Tsuda et al. 2001). Therefore although HER2 amplified breast cancers appear to have a higher propensity for progression and worse prognosis compared to non-amplified breast cancers, the amplification is an early event and defines a subtype of breast cancer not a later stage of it. This is further underscored by gene expression profiling studies that show that HER2 amplified breast cancers comprise a specific disease subset with a unique molecular portrait and this portrait is maintained during progression to metastatic disease (Perou et al. 2000; Weigelt et al. 2005). HER2 amplified breast cancers have biologic characteristics that distinguish them from other types of breast cancers. These include increased sensitivity to certain cytotoxic chemotherapeutic agents, resistance to certain hormonal agents, and increased propensity to metastasize to the brain (Ross et al. 2003; Gabos et al. 2006). HER2 overexpression and amplification is also seen in subsets of gastric, esophageal, and endometrial cancers, also associated with worse disease (Morrison et al. 2006; Yano et al. 2006; Mimura et al. 2005) and also seen rarely in cancers of the oropharynx, lung, and bladder (Khan et al. 2002; Hirsch et al. 2002; Latif et al. 2003). The

etiologic role of HER2 overexpression in diseases other than breast cancer remain to be defined.

### HER2 kinase domain mutations in human tumors

Large scale resequencing efforts have only begun to screen human tumors for somatic mutations. Somatic mutations within the HER2 kinase domain have been described in a small subset of lung cancers, predominantly in Asian populations, and very rarely in gastric, colorectal, and breast cancers (figure 1 region D)(Stephens et al. 2004;Shigematsu et al. 2005;Lee et al. 2006). Much less is currently known about the biology of these rare HER2 mutations. At least one of these mutations has been shown to enhance HER2 kinase activity and increase its transforming efficiency (Wang et al. 2006). Although numerous mouse models have established the tumorigenic potential of *neu* through overexpression, HER2 mutation in lung cancers is not known to be associated with overexpression or gene amplification. As such, a tumorigenic function of this mutated HER2 cannot be presumed and awaits the appropriate transgenic mouse models for analysis. In breast tissue, activated *neu* is not by itself transforming and requires amplification and overexpression to induce tumorigenesis (Andrechek et al. 2000). Much of the data discussed in this review pertains to the overexpression model of HER2 transformation. Our understanding of the biology of kinase-domain mutated HER2 is still in its infancy and ongoing studies in this area will undoubtedly shed further light on the biological characteristics of this HER2 mutant, its role in tumorigenesis, and its similarities or differences with overexpressed HER2.

### HER2 polymorphism in humans

Although somatic mutations in the transmembrane domain of HER2 analogous to the *neu* oncogene have not been seen in human tumors, polymorphism in the transmembrane domain of HER2 (figure 1 region C) has been described. In particular, the I665V variant of HER2 has increased potential for dimerization and signaling (Fleishman et al. 2002). This has led some investigators to propose that the I665V allele of HER2 may confer increased susceptibility to breast cancer. Although an initial large study indeed found such an association with breast cancer risk, several subsequent studies have shown conflicting results with the majority of them showing no risk associated with the I665 genotype (Xie et al. 2000;Benusiglio et al. 2005;Montgomery et al. 2003). The preponderance of evidence at this time does not appear to support an association between HER2 polymorphism and breast cancer risk, although this continues to be debated.

## MECHANISMS OF HER2-MEDIATED TUMORIGENESIS

The evidence that increased expression and activity of *neu* or HER2 induces cell transformation and tumorigenesis is overwhelming and was reviewed above. The data regarding the pathways that mediate, that are required for, and that cooperate with HER2 induced transformation are much more diverse and most likely HER2 induces transformation through a number of signaling pathways. The relative contribution of these pathways to tumorigenesis are difficult to know. The mechanisms and pathways for which there is significant evidence are reviewed below and are schematically depicted in Figure 3.

### Overactivity vs overexpression

In the simplest mechanistic model, HER2 induces transformation through increased kinase activity and overphosphorylation of itself and cellular substrates. In this model, the sole consequence of overexpression is to increased cellular HER2 activity. But a limitation of this model has been that it fails to explain why in human cancers HER2 activity is always elevated through overexpression and mutational activation of HER2, analogous to rodent *neuT*, is never seen in human breast cancers. One explanation that has been offered is that



neu can be activated through a single base pair mutation whereas the analogous activation of HER2 requires a two base pair mutation, making this much less likely to occur spontaneously in the human gene. However a more plausible explanation has been that increased expression of HER2 is an essential aspect of its transforming function. This presents a more complex model of transformation, but there is substantial evidence to support it. While high level expression of neu or neuT is tumorigenic in transgenic models, converting the endogenous neu to neuT by knock-in methodology leads to architectural distortion but is insufficient to induce tumorigenesis (Andrechek et al. 2000). These mice do eventually develop tumors with long latency, however these tumors are characterized by amplification and overexpression of the mutant neu allele supporting the hypothesis that overexpression is mechanistically required for transformation by HER2. A number of mechanistic models are able to propose how overexpression of HER2 can disrupt signaling and promote tumorigenic functions. Overexpression of HER2 can change the composition of HER family dimers, significantly increasing HER2-containing heterodimers and HER2 homodimers. Evidence discussed below suggests these increases can deregulate cell polarity and cell adhesion. In addition, evidence discussed below also shows that HER2-containing dimers have prolonged signaling activity and evade signal attenuation increasing signaling potency. Increased total expression of HER2 carries with it an increase in expression of subsets of HER2 including nuclear HER2 and the rare  $\Delta$ HER2 isoform, and the increase in each of these may be functionally relevant to cell transformation. These are also discussed individually below.

The recent discovery that rare types of lung cancers have HER2 kinase domain mutations that confer increased kinase activity without overexpression may be inconsistent with the overexpression model. But it is too soon to challenge this model since very little is yet known about the biology of the kinase domain mutated HER2. Studies forthcoming in the coming years will determine whether this HER2 mutant is transforming without overexpression and establish its role in the evolution of lung cancer. A unified model of HER2 transformation that also explains why HER2 is deregulated through overexpression in breast and ovarian cancers but through mutation in lung cancers is impossible to propose at this time and must await a much better understanding of its biology in lung cancers.

### **Deregulation of HER family signaling dynamics**

At the lateral level HER2 overexpression causes increased HER2 heterodimerization with EGFR and HER3 (Hendriks et al. 2003a; Karunagaran et al. 1996)(figure 3). This interferes with the endocytic regulation of EGFR. EGFR is unique among the HER family in that it undergoes endocytic degradation after ligand mediated activation and homodimerization, in contrast to the other members of the family which undergo endocytic recycling (Baulida et al. 1996). EGFR-HER2 heterodimers similarly evade endocytic degradation in favor of the recycling pathway and have increased signaling duration and potency (Lenferink et al. 1998; Waterman et al. 1998). Therefore HER2 overexpression results in increased EGFR membrane expression and activity (Huang et al. 1999; Wang et al. 1999; Hendriks et al. 2003b). But it seems unlikely that HER2 requires EGFR for its transforming function since HER2 also transforms NR6 fibroblasts which lack EGFR expression (Chazin et al. 1992). The analogous dispensibility of EGFR in epithelial models of HER2 transformation has not been specifically demonstrated. Overall, compared with other members of the family, HER2 is least subject to inactivating mechanisms and its recruitment into heterodimeric signaling complexes leads to prolonged signaling. As such, HER2 overexpressing cells have significantly prolonged activation of downstream MAPK and c-jun following stimulation with EGFR or HER3 ligands compared with low HER2 cells (Karunagaran et al. 1996).

## Signaling through HER3 and downstream PI3K/Akt

The functional role of HER3 is much better established. HER3 is widely expressed in breast cancers (Lemoine et al. 1992). Heregulin regulates HER2 through coexpressed HER3 (Wallasch et al. 1995b). HER2 and HER3 cooperatively induce transformation and in fact HER3 is an obligate partner in HER2 induced transformation (Holbro et al. 2003; Alimandi et al. 1995). Tumors from MMTV-*neu* mice have increased HER3 expression and phosphorylation and elevated HER3 expression is similarly seen in HER2 overexpressing human tumors (Siegel et al. 1999). The most important oncogenic signaling function of the HER2-HER3 complex appears to be activation of the PI3K/Akt pathway (figure 3). HER2 lacks binding sites for the p85 subunit of PI3K whereas HER3 has seven p85 binding phosphotyrosine containing motifs (Prigent and Gullick 1994; Soltoff et al. 1994; Schulze et al. 2005). Growth factor mediated activation of PI3K and Akt by HER2 is mediated specifically through phosphorylation of HER3 (Soltoff et al. 1994; Hellyer et al. 2001; Fedi et al. 1994). Cell transformation by overexpressed HER2 is also associated with increased HER3 phosphorylation and activation of PI3K/Akt signaling (Holbro et al. 2003; Alimandi et al. 1995; Ram and Ethier 1996; Tokunaga et al. 2006; Wallasch et al. 1995a). Tumors from MMTV-*neu* mice also have activation of PI3K signaling (Amundadottir and Leder 1998). This is corroborated by clinical studies which show the frequent activation of Akt in HER2 overexpressing tumors (Tokunaga et al. 2006; Zhou et al. 2004). The existing data strongly suggests that the transactivation of HER3 and downstream PI3K/Akt seems to be a critical tumorigenic function of overexpressed HER2. This is consistent with numerous other lines of evidence that highlight a central role for Akt in tumorigenesis. Akt functions in the crossroads of multiple signal transduction pathways that regulate numerous cellular functions critically important for cancer cells including cell proliferation and survival, cell size and response to nutrient availability, glucose metabolism, epithelial-mesenchymal transition and cell invasiveness, genome stability, and angiogenesis. The critical function of Akt in cancer is now widely recognized but will not be discussed in this review. Readers are referred to a number of excellent recent reviews of this topic ((Vivanco and Sawyers 2002; Luo et al. 2003; Paez and Sellers 2003; Testa and Bellacosa 2001) and the entire *Oncogene* vol 24; issue 50 (11/14/2005)). The abundance of data reviewed above suggests that HER2 induced tumors activate Akt through HER3 and PI3K.

## Alternative HER2 transcript

The fact that deletion mutations in the extracellular region of the receptor seen in *neu* overexpressing mouse mammary tumors are not seen in human tumors has produced a dilemma and an interesting hypothesis to resolve it. A naturally occurring but rare human HER2 RNA transcript has been cloned that is alternatively spliced and lacks a single 48bp coding exon resulting in the in-frame deletion of 16 amino acids from the juxtamembrane region overlapping with the area of deletion mutations seen in *neu* induced tumors. This alternative HER2 protein, named  $\Delta$ HER2, has increased ligand-independent signaling activity and increased transforming potency similar to the mutated *neu* gene (Kwong and Hung 1998; Siegel et al. 1999). This deletion removes cysteine residues in *neu* and in HER2, disrupting the disulfide bond structure of the proteins and leaving unpaired cysteine residues available for intermolecular bonding. Experimental models have confirmed that  $\Delta$ HER2 has increased transforming activity compared with wtHER2 in several models, including *in vitro* and transgenic models, and  $\Delta$ HER2 has significantly higher dimerization promoted by intermolecular disulfide-bond stabilization, and significantly increased tyrosine phosphorylation compared with wtHER2 (Kwong and Hung 1998; Siegel et al. 1999; Castiglioni et al. 2006). Although the activated  $\Delta$ HER2 transcript is a normal byproduct of HER2 transcription, its significant increase in HER2 amplified tumors has been proposed as a mechanism underlying HER2 tumorigenesis.  $\Delta$ HER2 transcripts have been detected in a majority of breast tumors and normal breast tissue and cell lines and reported by two groups

to comprise 4-9% of total HER2 transcripts (Siegel et al. 1999; Castiglioni et al. 2006). This hypothesis of HER2 induced tumorigenesis implies that the presence of the naturally occurring  $\Delta$ HER2 obviates the need for human HER2 to undergo mutational activation. The human MMTV-human HER2 mouse transgenic model is seemingly consistent with this. In this model, mouse mammary tumors are induced by overexpression of the human HER2 cDNA, which does not have a  $\Delta$ HER2 transcriptional by product associated with it (Finkle et al. 2004). Interestingly, the majority of tumors in this model have acquired deletion mutations of the juxtamembrane region of the human HER2 transgene similar to the *neu* transgenic model. This is consistent with the hypothesis that there is a selection pressure for this mechanism of activation, whether it is through gene mutation or whether it is through increased expression of  $\Delta$ HER2. More definitive proof of the causative role of  $\Delta$ HER2 awaits the development of experimental reagents that selectively target and inactivate the  $\Delta$ HER2 isoform.

### Role of Src kinases

Since HER family proteins signal predominantly through the recruitment of proteins to their tyrosine phosphorylated residues, their transforming functions could be in large part mediated through these interacting proteins, in particular SH2 domain containing proteins. Evidence suggests that src kinase are important second messengers of HER2. Src kinases are potently transforming and tumorigenic when constitutively activated, and their activation is seen in many human tumor types, including breast cancers with or without HER2 overexpression (Reissig et al. 2001; Ottenhoff-Kalff et al. 1992; Belsches-Jablonski et al. 2001). An association between src activation and HER2 overexpression has been reported in pre-invasive carcinomas of the breast (Wilson et al. 2006). Mammary tumors that arise in MMTV-*neu* transgenic mice have activation of c-src and c-yes (Muthuswamy et al. 1994; Muthuswamy and Muller 1995a). *In vitro* models corroborate a direct link between src and HER2. Transformation of mammary epithelial cells by HER2, but not by H-Ras, results in the activation of c-src (Sheffield 1998). Therefore it seems plausible that src functions downstream of oncogenic HER2. But a clear mechanistic model of how HER2 activates src and proof that this activation is essential for tumorigenesis has not yet emerged. Src directly interacts with HER2 within the HER2 catalytic domain (Muthuswamy and Muller 1995b; Belsches-Jablonski et al. 2001; Kim et al. 2005). It has been suggested that HER2 activates src through increasing its expression and stability, or by directly phosphorylating src on tyr215 in its SH2 domain (Tan et al. 2005a; Vadlamudi et al. 2003). Inhibition of src kinases in HER2 overexpressing tumors cells using src-selective tyrosine kinase inhibitors has produced different phenotypes including the selective inhibition of invasive and prometastatic characteristics in one report and total growth inhibition and apoptosis in another report (Tan et al. 2005b; Belsches-Jablonski et al. 2001). A specific function in promoting migration, invasion, and metastasis would be highly consistent with the known functions of src in regulating focal adhesions and integrin signaling, and regulation of the actin cytoskeleton. Consistent with this, HER2 mediated activation of c-src in epithelial cells results in loss of polarity, disruption of cell-cell adhesions, and anchorage independent growth (Kim et al. 2005; Sheffield 1998). In contrast to a downstream function of c-src, recent reports show c-src can also function upstream of HER2. C-src enhances HER2-HER3 dimerization and increases their auto- and transphosphorylations and signaling activities (Ishizawar et al. 2006). C-src also phosphorylates HER2 at tyr877 within the activation loop of the kinase domain and increases the kinase activity of HER2 (Xu et al. 2007). These data imply that, in addition to functioning downstream, src kinases may also function upstream or midstream of the transforming functions of HER2 (figure 3). A multi-level involvement of src kinases would make them critical enhancers of HER2 driven tumorigenesis. Genetic crosses to prove an essential role of src in *neu* induced mammary tumorigenesis have been difficult to do due to the poor health and survival of src null mice.



## Disruption of cell adhesion and cell polarity

A hallmark of many epithelial cancers is the loss of cell polarity and cell adhesion. Recent evidence demonstrates that deregulated HER2 signaling can disrupt cell polarity and cell adhesion. HER2, by virtue of its interaction with ERBIN (ErbB2 interacting protein), normally localizes to the basolateral surface of epithelial cells where it likely mediates crosstalk with ligand secreting stromal cells (Borg et al. 2000; Shelly et al. 2003; De Potter et al. 1989). The experimental homodimerization and activation of HER2 leads to disruption of tight junctions, loss of cell polarity, and proliferative disarray in breast epithelial cell acinar structures, although the basal membrane is preserved and invasive features are not induced (Muthuswamy et al. 2001). Activated HER2 disrupts apical-basal polarity through its interaction with components of the Par polarity complex including PAR6 (partition protein 6) and aPKC (atypical protein kinase C) (Aranda et al. 2006). On the other hand, the experimental heterodimerization of HER2 with EGFR promotes the invasive phenotype mediated through pathways including PI3K, Ras, and PLC $\gamma$  (phospholipase C $\gamma$ ) (Zhan et al. 2006). These studies were done using engineered synthetic ligand-activated constructs that can discriminate between the functions of homodimers and heterodimers of HER2 in cells grown in 3D models (Muthuswamy et al. 1999). It is inferred from these studies that overexpressed HER2 in cancers, through increased HER2 homodimers and HER2-EGFR heterodimers mediates the loss of polarity and deregulated cell adhesion typical of epithelial cancers (figure 3).

## Promoting the invasive phenotype

Multiple downstream signals may be mediating the invasive phenotype associated with HER2 overexpression in tumor cells. Downstream signals that have been implicated in the invasive phenotype include activation of PI3K (Ignatoski et al. 2000), PKC- $\alpha$  and src (830), FAK (Benlimame et al. 2005; Ignatoski et al. 2000), downregulation of  $\alpha$ 4 integrin (Woods Ignatoski et al. 2003), induction of  $\beta$ 4 integrin (Gambaletta et al. 2000), and TGF- $\beta$  (Seton-Rogers et al. 2004). The functional significance of  $\beta$ 4 integrin in particular has recently been established in genetic models. HER2 physically interacts with  $\beta$ 4 integrin (Falcioni et al. 1997) and tumors arising in MMTV-*neu* mice have delayed onset and reduced invasion and metastases if  $\beta$ 4 integrin signaling is disrupted genetically (Guo et al. 2006). Furthermore, *ex-vivo* studies demonstrated that the proliferative phenotype of *neu* is mediated through c-jun and the disruption of cell adhesions is mediated through STAT3.

## Cell cycle deregulation

Deregulation of cell cycle control, in particular G1/S checkpoint control, leads to uncontrolled proliferation and is a hallmark of many cancers. The two cell cycle regulators that have emerged as downstream targets of oncogenic HER2 are cyclin D1 and p27 (figure 3).

HER2 overexpression in breast epithelial cells deregulates G1/S control through upregulation of cyclin D1, E and cdk6, and degradation of p27 (Timms et al. 2002). Human tumor studies have only compared cyclin D1 expression among different breast cancers and have found no significant increase in cyclin D1 expression in HER2 overexpressing tumors compared to their non-overexpressing counterparts (Ahnstrom et al. 2005; Yang et al. 2004; Loden et al. 2003). It remains possible that cyclin D1 is widely overexpressed in breast cancers and it occurs through other pathways in tumors without HER2 overexpression. Consistent with this, a necessary but not rate-limiting role of cyclin D1 for HER2-induced tumorigenesis has been shown in transgenic models. MMTV-*neu* mice have almost complete protection from tumor formation in a cyclin D1-null background (Lee et al. 2000; Yu et al. 2001). With prolonged intervals hyperplastic lesions eventually develop due to cyclin E compensation (Bowe et al. 2002). Consistent with the requisite role of cyclin D1,

its catalytic partner cdk4 is also required for tumorigenesis in MMTV-*neu* mice (Reddy et al. 2005). Other lines of evidence also corroborate the hypothesis that HER2 induces transformation through increased cyclin D1/cdk4 activity. Expression of the cyclin D1/cdk4 inhibitor p16 in breast tissue also protects against tumors in MMTV-*neu* mice (Yang et al. 2004).

HER2 regulates p27 through multiple mechanisms that regulate its localization and its proteolysis. Akt is activated in HER2 amplified breast cancers (reviewed above) and activated Akt phosphorylates p27 inhibiting its function by excluding it from the nucleus (Viglietto et al. 2002; Liang et al. 2002; Shin et al. 2002). Several clinical studies have also established that HER2 overexpressing breast cancers have reduced p27 expression compared to other types of breast cancers (Loden et al. 2003; Spataro et al. 2003; Newman et al. 2001). HER2 mediates p27 degradation likely through mechanisms that involve MAPK (Lenferink et al. 2001; Yang et al. 2000; Donovan et al. 2001). Consistent with a functional role for p27, HER2 induced cell transformation can be inhibited by forced expression of p27 (Yang et al. 2001). The cell cycle function of oncogenic HER2 is likely mediated specifically through its regulation of cyclin D1 and p27 since other G1/S defects such as loss of p16 or Rb are not seen in HER2 amplified breast cancers, whereas they are common in other types of breast cancer (Yang et al. 2004).

### The role of transmembrane mucins

While in the simplest scenario, HER family receptor interactions are driven by their affinities for each other, there is evidence that other transmembrane proteins can facilitate their interactions. HER2 stably interacts with the membrane mucin Muc4. The interaction is mediated through one of two EGF-like domains in ASPG-1, the membrane associated subunit of Muc4 and may play a role in regulating the polar localization of HER2 (Carraway, III et al. 1999; Ramsauer et al. 2003). The experimental induction of Muc4 expression in cells lacking Muc4 leads to increase cell surface retention of HER2 and HER3 and increased HER2-HER3 signaling activity (Funes et al. 2006)(figure 3). The evidence that Muc4 potentiates HER2 signaling has led to the hypothesis that it may promote HER2 tumorigenic signaling even in tumors that do not overexpress HER2. Consistent with this hypothesis, increased expression of Muc4 is associated with worse prognosis in several types of cancer (Tsutsumida et al. 2006; Tamada et al. 2006; Saitou et al. 2005; Shibahara et al. 2004). Further analysis of this hypothesis awaits models that more definitively demonstrate the role of Muc4 in promoting tumorigenic HER2 function.

### The role of HER2 neighbors

While all the experimental preclinical models are generated by specific overexpression of the singular HER2 gene, the overexpression of HER2 in naturally occurring breast cancers is almost always due to amplification of a segment of chromosome 17 at 17q12-q21 that contains many genes in addition to HER2. Therefore the human disease involves a more complex genetic basis than the experimental models provide. The effort to identify the genes co-amplified with HER2 in human breast cancer have been greatly facilitated by modern techniques and access to the human genome database. The structure of the HER2 amplicon has now been characterized using a variety of techniques including southern blotting, FISH, and CGH, as well as looking for highly expressed neighboring genes by RT-PCR and microarray expression analyses ((Luoh 2002; Kauraniemi et al. 2001) and reviewed in (Kauraniemi and Kallioniemi 2006)). The HER2 amplicon is relatively small and fairly constant among many breast tumors and spans a minimal region of approximately 280kb centered around the HER2 locus (Kauraniemi et al. 2003)(figure 4). This region contains 10 transcribed genes, of which six are overexpressed in tumors as a result of amplification of the region, suggesting that they may be the biologically relevant genes. These include

HER2, GRB7, MLN64, PNMT, MGC9753, and MGC14832. GRB7 is an SH2-containing adaptor protein and is functionally linked with HER2 as it is known to bind phosphorylated HER2 and mediate aspects of cell migration (Janes et al. 1997). MLN64 is a late endosomal membrane protein that binds sterols and is felt to be involved in cholesterol transport (Alpy and Tomasetto 2006). PNMT (phenylethanolamine N-methyltransferase) is a catecholamine biosynthetic enzyme and is unlikely to be important for HER2 tumorigenesis. The functions of the hypothetical proteins MGC9753 and MGC14832 are currently unknown. In the mouse knock-in model of HER2 tumorigenesis which occurs through spontaneous gene amplification, the mouse tumor HER2 amplicon is highly similar to the human breast cancer amplicon and contains the same genes, consistent with the hypothesis that genes in addition to HER2 are selected for in the amplification event (Hodgson et al. 2005). Studies to determine the functional relevance of these genes in HER2 driven tumorigenesis are ongoing.

### HER2 linked genomic abnormalities

In addition to the genes directly located within the 17q12 HER2 amplicon, HER2 amplified breast cancers often have numerous other genomic alterations that are characteristic of this tumor type and much less common in breast tumors without HER2 amplification. These include amplifications in 17q22-24, just distal to the HER2 amplicon as well as amplifications in 20q and deletions in 18q (Isola et al. 1999). These areas may also contain genes that through gain or loss of function could cooperate to establish the biology of HER2 amplified tumors. The topoisomerase IIa (TOP2A) gene on 17q21 is frequently co-amplified with HER2 and may explain why HER2 overexpressing breast cancers are particularly sensitive to treatment with topoisomerase inhibitors (Mano et al. 2006).

Of particular interest, the PTPN1 gene encoding protein tyrosine phosphatase 1B (PTP1B) on 20q13 is often amplified in HER2 amplified breast cancers (Tanner et al. 1996). Although tyrosine phosphatases frequently play negative feedback roles in growth factor receptor signaling, the frequent amplification and overexpression of PTPN1 in HER2 amplified tumors has led to the hypothesis that it may have a tumor promoting function in these tumors. Consistent with this hypothesis, tumor formation in MMTV-*neu* mice is significantly delayed or prevented in the PTPN1-null background (Tires-Alj and Neel 2007). The molecular mechanisms mediating the supportive tumorigenic role of PTP1B remain to be worked out.

### TRANSCRIPTIONAL TARGETS OF HER2

While the function of HER proteins as transmembrane growth factor receptors is well understood and innumerable lines of evidence are consistent with its growth factor signaling function, a nuclear function as a transcription factor has also been proposed. In early two-hybrid screens for Neu interacting proteins, the cytoplasmic domain of Neu used as a bait was unexpectedly found to have transcriptional transactivating activity (Xie and Hung 1994). Consistent with a transcriptional function, a small percentage of cellular HER2 is seen in nuclei and DNA binding and transcriptional activation of at least one promoter has been reported (Wang et al. 2004). A transcriptional function inherent in HER2 significantly widens the realm of mechanisms by which overexpressed HER2 can mediate tumorigenic functions. The transcriptional function of HER2 is clearly not the principal mechanism underlying its transforming functions, since kinase function is essential for *neu* induced transformation (Weiner et al. 1989a). But transcriptional targets may be enhancing its transforming functions. So far the COX-2 gene has been reported as a direct target of HER2. However the upregulation (and downregulation) of numerous other genes has been described in HER2 amplified tumors and in fact these tumors have a gene expression profile unique to HER2 amplified breast cancers (Perou et al. 2000). Many of these genes are likely

consequences of HER2 overexpression, although some may be directly driven by HER2 transcriptional function. The pathways which current evidence suggests are functionally relevant are reviewed below. The role of many other genes remains to be defined.

### Induction of COX-2

The inducible prostaglandin synthase cyclooxygenase-2 (COX-2) is overexpressed in HER2 amplified breast cancers (Subbaramaiah et al. 2002). HER2 directly regulates COX-2 expression through transcriptional induction (Vadlamudi et al. 1999; Wang et al. 2004). COX-2 knockout mice are impaired in their ability to support MMTV-*neu* induced mammary tumorigenesis (Howe et al. 2005). Interestingly, this phenotype seems to be related to impaired angiogenic signaling. Whether this is due to defective tumor cell angiogenic signaling or host angiogenic signaling is not currently defined. As such, the role of tumor COX-2 in HER2-induced transformation remains to be defined.

### Induction of CXCR4

HER2 induces the expression of the chemokine receptor CXCR4 in transfection models and indeed increased expression of CXCR4 is seen in HER2 overexpressing breast cancers (Li et al. 2004). Chemokine receptors have been functionally linked with the metastatic properties of breast cancers (Muller et al. 2001) leading to the hypothesis that the prometastatic properties of HER2 overexpressing tumors may be mediated through the increased expression of relevant chemokine receptors. Consistent with this hypothesis, the increased migratory activity induced by the experimental overexpression of HER2 can be suppressed by anti-CXCR4 antibodies (Li et al. 2004).

### Induction of ETS

ETS (E26 transformation specific) transcription factors are almost universally overexpressed in HER2 amplified breast cancers and they are also overexpressed and are essential in MMTV-*neu* induced mammary tumorigenesis (Shepherd et al. 2001; Neve et al. 2002). HER2 overexpression specifically leads to the bimodal activation of the ETS transcription factor ER81 (Goel and Janknecht 2003) which mediates the induction of expression of the catalytic subunit of telomerase (Goueli and Janknecht 2004).

### Other downstream or cooperating pathways

HER2 overexpression leads to increased expression of hypoxia inducible factor 1 $\alpha$  (HIF-1 $\alpha$ ) through Akt leading to increased expression of VEGF and increased surface expression of fibronectin receptors (Laughner et al. 2001; Li et al. 2005; Spangenberg et al. 2006). VEGF expression is also induced by HER2 overexpression through a HIF-1 $\alpha$  independent mechanism (Loureiro et al. 2005).

Numerous other pathways have been implicated in HER2-mediated transformation, although they are much less well characterized. HER2 overexpressing breast cancers are characterized by increased expression of MMP-2 (matrix metalloproteinase -2) and MMP-9 (matrix metalloproteinase 9) (Pellikainen et al. 2004). HER2 overactivity also promotes the activation of the nuclear factor kappa-B (NF- $\kappa$ B) anti-apoptotic pathway (Makino et al. 2004). Involvement of myc downstream of HER2 has been proposed although it remains to be defined and may be complex (Hynes and Lane 2001).

## TUMOR DEPENDENCE ON HER2

While the tumorigenic potential of HER2 overexpression has been clearly demonstrated in numerous model systems (see above), its suitability as a drug target depends on whether clinically advanced tumors continue to be dependent on HER2 for survival and progression.

This dependency, recently described as oncogene-addiction, implies that such cancers can be effectively treated and possibly cured with drugs that inactivate the oncogene product (Weinstein 2002). Alternatively, genomic instability can lead to the mutational activation of additional pathways that could compensate for the pharmacologic inactivation of the tumor-initiating oncogene making such an oncogene less effective as a drug target.

### HER2 knockdown models

Since the identification of HER2 as an oncogene in human tumors, numerous approaches have been undertaken to demonstrate the dependence of HER2 overexpressing tumors on HER2. A number of studies using antisense, ribozyme, or siRNA methodologies to suppress HER2 expression in human cancer cell lines consistently show that HER2 overexpressing tumor cells are dependent on HER2 and undergo growth inhibition and apoptosis in cell culture, or tumor regression *in vivo*, in the absence of HER2 expression, while tumor types that do not overexpress HER2 are not sensitive to HER2 knockdown (Colomer et al. 1994; Juhl et al. 1997; Roh et al. 2000; Faltus et al. 2004; Choudhury et al. 2004). A kinase-dead mutant of activated HER2 competes with and reverses the transformed phenotype induced by activated HER2 (Messerle et al. 1994). Intracellularly expressed single chain antibodies that target and inactivate HER2 revert HER2 induced transformation or induce apoptotic cell death in HER2 overexpressing tumor cells (Beerli et al. 1994; Deshane et al. 1996).

### HER2 withdrawal models

Tetracycline inducible systems offer even more elegant models for analysis of oncogene addiction. NIH3T3 cells transformed by tetracycline regulated overexpression of HER2 revert from the transformed phenotype and their mouse implanted tumors regress when HER2 expression is withdrawn (Baasner et al. 1996; Schiffer et al. 2003). Tetracycline induced expression of activated HER2 in squamous epithelia of mice results in severe hyperplastic abnormalities of squamous epithelial tissues, which reverse upon withdrawal of the HER2 transgene expression (Xie et al. 1999). Tumors in MMTV-*neuT* mice are also dependent on continued oncogene expression. In the MMTV-rtTA/TetO-NeuNT bitransgenic variant of this model regulated by doxycycline, when expression of the *neuT* oncogene is induced in the mammary tissue of adult mice, this leads to the formation of multiple mammary tumors and lung metastases, and the entire primary tumor and metastatic disease fully regresses when *neuT* expression is withdrawn (Moody et al. 2002).

Each of these models is subject to specific criticisms. For example the antisense or siRNA approaches have non-specific effects, the NIH3T3 fibroblast models are not representative of what is principally an epithelial oncogene in humans, and the transgenic models may be too simplistic and understate the genetic complexity of the human disease. But taken in aggregate, the existing data from all the different models and approaches is highly consistent and collectively makes a highly compelling case that HER2 induced tumors are addicted to HER2. This has made HER2 one of the most sought after targets in cancer drug development.

## CONCLUSION

Numerous experimental models now solidly support the hypothesis that HER2 overexpression promotes tumorigenesis and the finding of HER2 amplification and overexpression in many human breast cancers and its link with the biology of this disease now clearly implicate this oncogene in the pathogenesis of this type of human cancer. Numerous well supported experimental models implicate diverse mechanisms involved in HER2 mediated tumorigenesis and a unified mechanistic model cannot currently be proposed. It is possible that the position of HER2 in the signaling web places it in control of



numerous pathways that can contribute to malignant progression such that the amplification of HER2 as a single event can substitute for several events in the multi-step model of carcinogenesis. Indeed HER2 amplified breast cancers occur at an earlier age than other types of breast cancers consistent with a fast-track route to oncogenesis in these cancers (Crowe et al. 2006; Swede et al. 2001). The highly oncogene-addicted nature of these cancers also attests to a HER2 function that is not easily supplanted, possibly due to its multiple oncogenic roles. The critical driving role of HER2 in human cancers, and the large number of patients affected by this type of cancer has made HER2 an ideal target for the development of rationally designed anti-cancer drugs.

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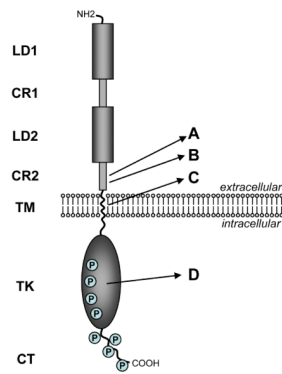


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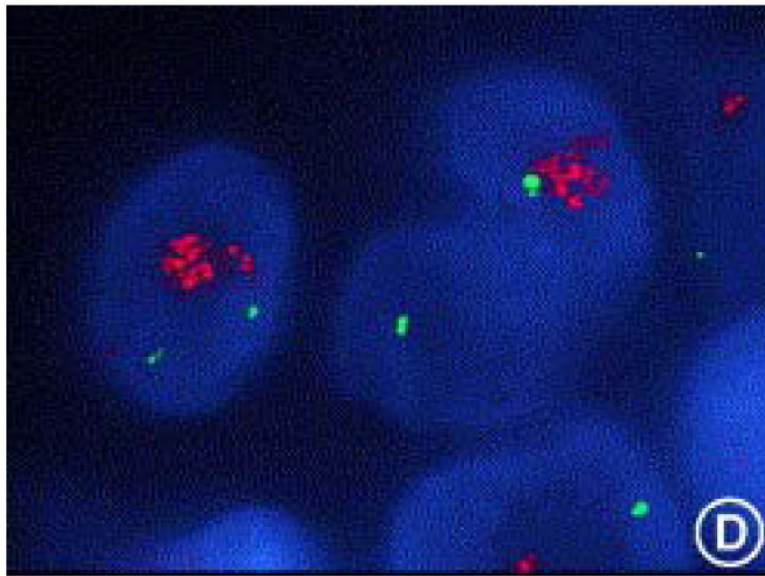
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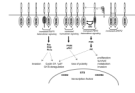
**Figure 1.**

Structure of the HER2 and Neu proteins. The domain structure is shown on the left consisting of two ligand binding regions (LD1 & LD2), two cysteine-rich regions (CR1 & CR2), a short transmembrane domain (TM), a catalytic tyrosine kinase domain (TK), and a carboxy terminal tail (CT). Numerous sites of tyrosine phosphorylation within the TK and CT domains are indicated by circled P. The letters on the right point to specific areas that are altered or mutated in certain naturally occurring or experimentally induced cancers discussed in the text. A) site of somatic mutations found in tumors arising in MMTV-*neu* mice. B) site of the 48bp deletion in the naturally occurring human  $\Delta$ HER2 isoform. C) site of the mutation in the neuT oncogene initially discovered in a rat carcinogen induced tumor model and subsequently used in numerous *in vitro* and transgenic experimental models. D) site of mutations found in rare cases of human lung cancers.



**Figure 2.** FISH analysis of HER2 amplification. A human breast cancer specimen hybridized with a HER2 gene probe (in green) and a chromosome 17 centromeric probe (in red) showing significantly increased HER2 gene copy number compared with the chromosome 17 control. (Hicks and Tubbs 2005) Reprinted from Human Pathology 36, p256, Copyright 2005, with permission from Elsevier.



**Figure 3.**

Schematic of the signaling abnormalities resulting from HER2 overexpression that are felt to contribute to tumorigenesis. HER2 overexpression results in increased HER2 containing dimers of all kinds. Increased HER2-EGFR dimers drive proliferative and invasive functions. Increased HER2 homodimers disrupt cell polarity. Increased HER2-HER3 dimers drive proliferative, survival, invasive, and metabolic functions. Increased HER2 expression results in an increase in the rare  $\Delta$ HER2 isoform with more potent signaling characteristics. Several transcription factors are induced in HER2 overexpressing cells resulting in a plethora of gene expression changes.



**TABLE 1**

Common nomenclature of HER2 rodent and human variants

Name, aliases	Description	Site mutated (figure 1)	Where found
neu, c-neu, wtneu, neu proto-oncogene	rodent cellular homolog of HER2	-	in rat and mouse cells
neuT, neuNT, neu oncogene, neu <sup>V664E</sup>	rat neu containing a transmembrane domain mutation, has potent transforming activity	C	discovered in a rat carcinogenesis model, used as a potent experimental oncogene
neu8142, neu8342	rat neu containing deletion mutations in the extracellular domain	A	found in tumors from MMTV-c-neu transgenic mice
HER2, ErbB2, c-ErbB2	human cellular HER2	-	in human cells
HER2 <sup>V659E</sup>	engineered transforming mutant of human HER2, analogous to neuT	C	never found in nature, engineered for experiments
$\Delta$ HER2	isoform of human HER2 missing one exon	B	normally occurring minor isoform found wherever HER2 is expressed
HER2 <sup>YVMA</sup>	HER2 with a kinase domain mutation and increased kinase activity	D	found in some lung cancers

TABLE 2

Reported mouse transgenic models of HER2/neu

Reference	Promoter	Gene	Phenotype
(Muller et al. 1988)	MMTV	neuT transgene	early, multiple mammary tumors by 3 months, short survival
(Bouchard et al. 1989)	MMTV	neuT transgene	mammary tumors stochastically, 5-10 months
(Guy et al. 1992)	MMTV	c-neu transgene	mammary tumors stochastically, 4-12 months, frequent lung metastases
(Andrechek et al. 2000)	endogenous	neuT knock-in	late mammary tumors, 12-17 months, with transgene amplification
(Weinstein et al. 2000)	murine neu	c-neu transgene	abnormal involution, focal mammary abnormalities in multiparous females, 1-2 years
(Weinstein et al. 2000)	murine neu	neuT transgene	abnormal involution, some late mammary tumors in multiparous females, 14 months
(Finkle et al. 2004)	MMTV	human HER2 transgene	multiple mammary tumors, 7 months, some lung mets
(Moody et al. 2002)	MMTV-rTA / TetO	neuT transgene	rapid mammary tumors within 6 weeks and frequent lung mets, regress without dox
(Xie et al. 1998)	human Keratin 14	neuT transgene	severe hyperplastic lesions of skin, hair follicles, esophagus, perinatal lethal
(Bol et al. 1998)	bovine keratin 5	neuT transgene	severe hyperplastic lesions of skin, esophagus, papillomas, early death
(Kiguchi et al. 2000; Kiguchi et al. 2001)	bovine keratin 5	c-neu transgene	hyperplasia, alopecia, papillomas, skin carcinomas, biliary carcinomas, survive 6-12 months
(Xie et al. 1999)	K14-rTA-TetRE	neuT transgene	rapid dox induction of hyperplastic lesions in skin, hair follicles, esophagus, regress without dox
(Li et al. 2006)	probasin	neuT transgene	prostatic intraepithelial neoplasia and invasive prostate cancer, 1-2 years