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Oncogenic and tumor-suppressive mouse models for breast cancer engaging HER2/neu

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Abstract

The human *c-ErbB2* (*HER2*) gene is amplified in ~20 % of human breast cancers (BCs), but the protein is overexpressed in ~30% of the cases indicating that multiple different mechanisms contribute to HER2 overexpression in tumors. It has long been used as a molecular marker of BC for sub-categorization for the prediction of prognosis, and determination of therapeutic strategies. In comparison to ER(+) BCs, HER2(+) BCs are more invasive, but the patients respond to monoclonal antibody therapy with trastuzumab or tyrosine kinase inhibitors at least at early stages. To understand the pathophysiology of HER2-driven carcinogenesis and test HER2-targeting therapeutic agents *in vivo*, numerous mouse models have been created that faithfully reproduce HER2(+) BCs in mice. They include *MMTV-neu* (active mutant or wild type, rat *neu* or *HER2*) models, *neu* promoter-driven *neuNT*-transgenic mice, *neuNT*-knock-in mice at the *neu* locus, and doxycycline-inducible *neuNT*-transgenic models. HER2/neu activates the Phosphatidylinositol-3 kinase-AKT-NF-κB pathway to stimulate the mitogenic cyclin D1/Cdk4-Rb-E2F pathway. Of note, overexpression of HER2 also stimulates the cell autonomous Dmp1-Arf-p53 tumor suppressor pathway to quench oncogenic signals to prevent the emergence of cancer cells. Hence tumor development by *MMTV-neu* mice was dramatically accelerated in mice that lack *Dmp1*, *Arf* or *p53* with invasion and metastasis. Expressions of *neuNT* under the endogenous promoter underwent gene amplification, closely recapitulating human HER2(+) BCs. *MMTV-HER2* models have been shown to be useful to test humanized monoclonal antibodies to HER2. These mouse models will be useful for the screening of novel therapeutic agents against BCs with HER2 overexpression.

Keywords

HER2/neu; Dmp1; Dmtf1; p53; PI3K; breast cancer; transgenic mice; disease model; target therapy; prevention

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Conflicts of Interest

The authors declare no conflicts of interest.

Introduction

Breast cancer (BC) is the second most frequent cause of death from cancer and thus is one of the largest public health issues in the United States and the industrialized nations [1, 2]. It is a heterogeneous disorder that can be categorized into 5 different groups - luminal A, luminal B, HER2, basal, and normal types dependent on the expression of markers for hormone-receptors (estrogen receptor [ER] and progesterone receptor [PR]), human ErbB2 [HER2], and Ki67 [3–7]. In a separate thought, intrinsic subtypes of BCs were determined by GeneChip Microarrays of 1800+ genes for categorization [8, 9]. These subtype classifications of BCs have been used to predict patient outcomes and responsiveness to treatments. BCs that are positive for the ER (luminal A/B) are usually responsive to adjuvant hormonal therapy with anti-estrogens and/or aromatase inhibitors, and thus have a favorable prognosis [2]. On the other hand, ER-negative BCs (HER2 and basal-types) are often associated with aggressive disease, including amplification of *HER2* or c-*Myc* oncogenes and mutation of the *p53* gene with shorter survival [4–6]. Comprehensive molecular portraits of invasive lobular BC showed that mutations targeting *PTEN*, *TBX3*, and *FOXA1* were invasive lobular carcinoma-enriched features [10].

The genomic locus for *HER2* is amplified in 20 % [11], but the protein is overexpressed in 20–30 % of BCs (21% 3+ and 14% 2+ in our specimens [6]), suggesting additional mechanisms for high HER2 expression exist than gene amplification. HER2/neu overexpression is found in metastatic lesions, and thus is associated with poor prognoses [2, 3, 5, 11]. The use of monoclonal antibody to HER2 (trastuzumab, Herceptin®) has been deployed to treat HER2-positive BC, but the prognosis of such patients is poor since >60% of them experience relapse during the first year due to HER2 modifications, defects in the antibody dependent cellular cytotoxicity or in cell arrest and apoptosis or alterations in HER2 signaling components [12, 13]. The basal-type BC constitutes about 20 % of total BC cases and overlaps with the group of triple-negative BC (TNBC) with worst prognosis since anti-hormonal or monoclonal antibody cannot be performed in these patients [14, 15].

The HER/HER family (HER1-HER4) is made up of four structurally related receptor tyrosine kinases (RTKs) with the EGFR as the founding member of the family [16–18]. Activation of the HER family receptors other than HER2 requires binding of a soluble, growth factor ligand located on the receptor that triggers receptor dimerization, phosphorylation, and activation of downstream pathways to elicit response inside the cell. EGFR (HER1) is activated by growth factor-ligands such as epidermal growth factor (EGF), heparin-binding EGF, amphiregulin, or TGF-α [16, 17]. The product of the human *c-ErbB-2* gene (*HER2*) is a 185-kilodalton glycoprotein with protein-tyrosine kinase activity [19, 20], which is a human version of the rat *neu* proto-oncogene [21]. However, it is still an orphan receptor to which no specific ligand has been identified [22]. Conversely, HER3 and HER4 are activated by the heregulin or neuregulin family of growth factors (Fig. 1). Each HER molecule, upon activation by growth factor binding, initiates hetero- or homo-dimerization of receptors. This stimulates auto-phosphorylation of the molecule, followed by trans-phosphorylation of the heterodimerization partner. These phosphorylated tyrosine residues recruit adaptor proteins such as Grb2 and the p85 subunit of the PI3K complex, which initiate the activation of several downstream pathways such as protein kinase B (PKB/Akt)

and the mitogen activated protein kinase (MAPK) pathways (Fig. 1; refs. 23, 24). Activation of the PI3K and Akt by HER2/neu activates mammalian Target of Rapamycin (mTOR), comprised of two complexes, mTORC1 and mTORC2, which, in turn induce protein synthesis that stimulates cell proliferation, migration, and metabolism [25].

The PI3K-Akt signaling has also been linked to the induction of NF-κB (Fig. 1; refs. 23, 26). Two serine threonine IκB kinases (IKK1/2) are responsible for phosphorylating and targeting IκB molecules for degradation. Importantly, both human BC cell lines and primary specimens often show constitutive activation of NF-κB [27], which results in decreased apoptosis and induction of cyclin D1 (Fig. 1), suggesting oncogenic roles of NF-κB in BC development [28].

The transforming potential of HER2/neu has been demonstrated in a number of ways. Both the transforming *neu* oncogene (*neuNT*) and the proto-oncogene *neu* were isolated in the Weinberg's lab through NIH 3T3 transformation assays [21]. The level of expression was shown to be critical for transformation by HER2 [29]. Long terminal repeat - based expression vectors with *neu* were proven to achieve transformation in NIH 3T3 cells [30]. The effect of overexpression in murine mammary epithelium was first analyzed through the creation of *MMTV-neuNT* transgenic mice [FVB/N-Tg(MMTVneu)202Mul/J; ref. 31]. Then a different model was created to overexpress the proto oncogenic form of *neu* under the control of the *MMTV* promoter [32]. Subsequently a mouse model for BC induced by amplification and overexpression of the activated *neu* gene driven by the *neu* promoter was generated [33]. A knock-in model for *neuNT* was also created at the mouse *neu* locus [34]. More recently, a doxycycline (dox)-inducible model for *neuNT* overexpression has been established [35]. Finally, *MMTV*-driven overexpression of human *ErbB2 (HER2)* was generated to test the therapeutic effects of monoclonal antibodies to HER2 since the rat neu protein is not recognized by trastuzumab [36]. The details for these different *HER2/neu*-transgenic models are discussed in this review. These mouse models will be useful to screen drugs that could be effective for novel therapy for human cancers overexpressing HER2, and thus are extremely important tools for future target therapies.

Dmp1, a cyclin D-binding myb-like protein 1, directly binds and activates the *Arf* promoter, thereby stimulates p53 to quench oncogenic Ras signaling [37–48; see 49–51 for Arf reviews; 52–54 for Dmp1 reviews]. To demonstrate the tumor suppressive role for Dmp1 in oncogenic HER2/neu signaling, we analyzed the responsiveness of *Dmp1* and *Arf* promoters to HER2/neu [37]. We also crossed *MMTV-neuNT* mice with *Dmp1*-knockout mice in FVB/N to demonstrate the role of Dmp1 in HER2/neu-driven carcinogenesis [37]. Then we established *MMTV-FlagDmp1α* mice and crossed them with *MMTV-neuNT* to directly demonstrate the tumor suppressive potential of Dmp1α *in vivo* [55]. The results from these studies are also reviewed here.

Oncogenic mouse models for *HER2/neu*

MMTV-mutant neu (neuNT) and MMTV-neu (wild type) models

The cDNA clones for the *neu* oncogene, which is frequently activated in neuro- and glioblastomas of BDIX rats, possessed a valine to glutamic acid substitution in its

transmembrane domain of the epidermal growth factor receptor EGFR [56, 57]. This mutation resulted in the constitutive activation of the receptor in the absence of ligand [58, 59]. c-ErbB2 plays critical roles in ductal morphogenesis of the mammary glands as demonstrated by the results of gene-knockout studies [60, 61]. Although the transmembrane point mutation has not been detected in primary human BCs overexpressing HER2, recent studies have detected an aberrant alternative splice isoform in human BCs lacking 16 amino acids at exon 20 [62, 63; reviewed in 18]. The first transgenic mouse expressing oncogenic rat *neu* (*neuNT*) was reported [31]. Unlike the stochastic occurrence of solitary mammary tumors in transgenic mice bearing the *MMTV-c-myc* or *v-Ha-ras* oncogenes, transgenic mice uniformly expressing the *MMTV-neuNT* gene developed multi-focal mammary tumors at the median age of 197 days in FVB strain (non-parous) with frequent potential to metastasize to the lung [37]. Because these tumors arise synchronously and are polyclonal in origin, expression of the activated *c-neu* oncogene appears to be sufficient to induce malignant transformation in this tissue in a single step (Table 1).

Mice strains bearing the wild type *ErbB2* allele under the control of the *MMTV* promoter have also been established (*MMTV-neu* in Table 1) [32; reviewed in 64]. In contrast to the rapid tumor progression observed in several transgenic strains carrying the activated *neu* transgene, expression of unactivated *neu* in the mammary epithelium resulted in the development of focal mammary tumors with longer latency than activated *neu* (7–12 months dependent on the strain, Table 1). Overexpression of neu in these mammary tumors was associated with elevated intrinsic tyrosine kinase activity of neu and the *de novo* tyrosine phosphorylation of several cellular proteins. Interestingly, many of the tumor-bearing transgenic mice developed secondary metastatic lesions in the lung. These observations suggest that overexpression of the unactivated neu protein induces metastatic disease after long latency [32]. The same group later reported that that activation of *neu* in these transgenic mice occurred through somatic mutations located within the transgene itself [65]. Sequence analyses of these mutations revealed that the tumors contain in-frame deletions of 7 to 12 amino acids in the extracellular region proximal to the transmembrane domain [65]. Since transmembrane deletion of *HER2* is frequently found in human BCs [18, 66], these mice are naturally-occurring models for human BC with HER2 overexpression.

Wild type *neu* and *neuNT* expression under the control of the *neu* promoter - a more physiological model

Although most human BCs are not associated with pregnancy, the promoters that have been used in mouse models of mammary tumors (*MMTV* and whey acidic protein [*WAP*] promoters) require the induction of pregnancy to reach high levels of transcriptional activity [64]. Of note, HER2/neu is expressed in the virgin, pregnant, lactating and regressing mammary gland of the mouse [33], making it difficult for the transgenic promoters, such as those of *MMTV* or *WAP*, to accurately model the consequence of amplification of the *HER2* gene in human BC throughout the course of murine development. To overcome this problem, transgenic mice containing the 1.2 kb upstream sequence of the mouse *neu* gene fused to either the normal or transforming (Val664Glu) rat *neu* gene has been established [33]. With only moderate overexpression of wild type *neu*, the virgin mammary tree underwent lobulo-alveolar hyperplasia throughout the gland [33]. *NeuNT*-transgenic mice

developed solid nests of anaplastic cells, micropapillary adenocarcinoma, or hyperplastic mammary glands producing lactoferrin and lipid droplets (Table 1). The proliferative phenotype was enhanced once the mouse had undergone multiple rounds of pregnancy (mean tumor latency of 14 months in MPs; Table 1) and regression. Rather than reverting to a near virgin state, the gland continued to produce milk proteins and lipids long after weaning. This may be due to the fact that *neu*, under the direction of the transgenic *neu* promoter, is expressed evenly throughout the gland, as can be seen by its proliferative effects in a mammary gland whole mount [33]. In summary, this is an improved mouse model for human BC where *HER2* is amplified and overexpressed since the *neu* gene is driven by the *neu* promoter.

The *neuNT* knock-in mouse model

To test whether expression of activated *neu* under the control of the endogenous promoter in the mammary gland contributes to tumor development, knock-in mice for activated *neu* were developed [34]. They showed accelerated lobulo-alveolar development and formation of focal mammary tumors after a long latency period (mean latency, 8–17 months, Table 1). Thus, the expression of *neuNT* under the endogenous *neu* promoter was not sufficient for to overt mammary carcinogenesis. In comparison to mammary tumors from *MMTV-neuNT* mice, the *neuNT* knock-in mice showed a significantly low metastasis rate [31, 34]. Of note, all tumors isolated from these mice had amplified copies (2–22 copies) of the *neuNT* allele and expressed highly elevated levels of the neu protein. In summary, like human HER2-positive BCs, mammary tumorigenesis in this mouse model requires gene amplification and overexpression of neu [34, 67, 68].

Doxycycline (dox)-inducible mouse mammary tumor models for *neuNT*

To determine the impact of tumor progression on the reversibility of *neu*-induced tumorigenesis, a tetracycline-regulatory system that conditionally expresses activated neu in the mammary epithelium of transgenic mice has been developed (Table 1; ref. 35). When induced with dox, bitransgenic *MMTV-rtTA;TetO-neuNT* mice developed multiple invasive mammary carcinomas, which regressed to a clinically undetectable state following transgene de-induction, indicating that that *neuNT*-initiated tumorigenesis was reversible. Strikingly, extensive lung metastases arising from *neuNT*-induced mammary tumors also rapidly and fully regressed following the disappearance of neu expression. However, despite the near universal dependence of both primary and metastatic tumors on *neuNT*-transgene expression, most animals bearing fully regressed tumors ultimately experienced recurrent disease that progressed to a *neu*-independent state [35]. These findings highlight the importance of elucidation of the mechanisms by which tumor cells escape from their dependence on the initial oncogenic pathways for the treatment of relapsed cancer.

The AMP-activated protein kinase-related molecule Hunk is overexpressed in aggressive subsets of human breast, ovarian, and colon cancers, and is required for metastasis of c-Myc-induced mammary tumors [69]. By crossing $Hunk^{-/-}$ mice with *MMTV-rtTA;TetO-neuNT* mice, Hunk was shown to be essential for tumor development driven by HER2/neu [69]. Most recently, the same group demonstrated the role of Notch signaling in tumor recurrence from dormant residual cells, which are ideal drug targets in BC [70]. Thus this

dox-inducible/de-inducible *neuNT*-transgenic model will be very useful in the analysis of oncogenic signaling cascades as well as for the screening of drugs targeting HER2/neu.

MMTV-driven transgenic mouse models employing human *ErbB2* (*HER2*)

Transgenic mouse models offer the advantage of having immune system components that may be important in the action of antibodies, but are lacking in immunodeficient hosts. Although numerous transgenic mice have been developed using rat *neu*, they cannot be used to test the efficacy of Herceptin® (trastuzumab), a monoclonal antibody to HER2 since the rat neu protein is not recognized by the antibody [36]. To overcome this problem, three different groups made *MMTV*-driven transgenic mice using the human *ErbB2* (*HER2*) gene [36, 66, 71]. In a *MMTV-human ErbB2* model, 76 % of female transgenics developed asynchronous mammary tumors with an average latency of 28 weeks (Table 1; ref. 71). At 24 weeks of age, the founder mouse had a primary mammary tumor of 1 mm^3 that ultimately grew to span two adjacent mammary glands. Later primary mammary tumors (rapidly growing adenocarcinomas) developed in a stochastic manner in the transgenic progeny. Pulmonary metastases were found in 23 % of the female transgenics with high HER2 expression. Sequencing of *HER2* in mammary tumors revealed an in-frame 15-bp deletion in the *HER2* juxtamembranous region [71]. This deletion resulted in the repositioning of two cysteines (634 and 641) to within two amino acids of each other, potentially affecting cysteine-mediated dimerization. These findings are consistent with the published data from the *MMTV-neu*-transgenic model (wild type; ref. 32) that showed the presence of in-frame deletions confined to the juxta-membranous region of the *neu* extracellular domain in mammary tumors via activation of intrinsic tyrosine kinase activity by the *neu* protein [65].

A splice isoform of the HER2 receptor that lacks exon 20 (Δ16HER2) is expressed in many HER2-positive BCs [18], but the impact of Δ16HER2 on tumor pathobiology and its therapeutic response remains uncertain. Therefore *MMTV-ErBB2 (Δ16HER2)* mice have been created to provide genetic evidence that expression of Δ16HER2 was sufficient to accelerate mammary tumorigenesis [66]. A comparative analysis of spontaneous mammary tumor development assay showed that the tumor-free survival (TFS) was shortened from 45 weeks (wild type HER2) to 17 weeks (Δ16HER2). Clinically, HER2-positive BCs from patients who received trastuzumab exhibited a positive correlation in Δ16HER2 and pSRC abundance, consistent with the mouse genetic results [66]. Moreover, patients expressing high pSRC or an activated Δ16HER2 metagene were found to receive the greatest benefit from trastuzumab treatment suggesting that the Δ16HER2 signaling axis is a signature for decreased risk of relapse after trastuzumab treatment [66].

Compound *HER2/neu*-transgenic mice with inactivation of the Dmp1-Arf-p53 tumor suppressor pathway

MMTV-neu;WAP-p53-172H bitransgenic mice

As many as 37 % of tumors arising in *MMTV-neu* (wild type) mice have missense mutations in *p53* [72]. To directly test the cooperativity between wild type *neu* and mutant *p53* in mammary tumorigenesis, they crossed *MMTV-neu* (line N#202; ref. 30) and *WAP-*

p53-172H [70]. The tumor latency was shortened from 240 days (*MMTV-neu*) to 154 days in bitransgenic mice, indicating strong cooperativity (Table 1) [72]. Tumors arising in *WAP-p53-172H; MMTV-neu* bitransgenic mice were anaplastic, aneuploid and exhibited increased apoptosis, in contrast to tumors arising in *p53*-null mice. They recapitulated the two common genetic lesions that occur in human BC, and have shown that *p53* mutation is an important cooperating event in *HER2/neu*-mediated oncogenesis [72].

MMTV-neu; Ink4a/Arf$^{+/-}$ mice

p16^{INK4a} is deleted in 40–60 % of human BC cell lines, and *p16^{INK4a}* inactivation by DNA methylation occurs in 20–30 % of human BCs [73, 74]. To test the effects of *Ink4a/Arf* inactivation in ErbB2-induced breast carcinogenesis, *MMTV-neu* (wild type) mice [32] were crossed *Ink4a/Arf*-null mice [75]. Compared with *MMTV-neu; Ink4a/Arf$^{+/-}$* mice, *MMTV-neu; Ink4a/Arf^{wt}* mammary tumors showed increased p16^{Ink4a}, reduced Ki67 expression, reduced cyclin D1 protein, and decreased apoptosis with no significant change in the risk of developing mammary tumors. Their study demonstrated the contribution of *Ink4a/Arf*-heterozygosity in mammary tumorigenesis [76].

MMTV-neuNT; Dmp1 knockout mice

HER2 overexpression stimulates cell growth in *p53*-mutated cells while it inhibits cell proliferation in those with wild type *p53*, but the molecular mechanism remained poorly understood. We conducted a study to demonstrate the roles of Dmp1 and Arf in HER2/neu signaling and breast carcinogenesis [37]. The *Dmp1* promoter was activated by HER2/neu through the PI3K-Akt-NF-κB pathway, which in turn stimulated *Arf* transcription. Both Dmp1 and p53 were induced in pre-malignant lesions from *MMTV-neu* mice, suggesting that the Dmp1-p53 tumor suppressor pathway plays a crucial role in quenching aberrant HER2 signaling *in vivo*. To study the cooperation between *Dmp1*-loss and HER2/neu overexpression, we crossed *MMTV-neuNT* mice [31] with *Dmp1*-deficient mice [40] in FVB/N (Table 1; ref. 37). HER2/neu-induced mammary tumor development was significantly accelerated from 197 days to 162 days in *Dmp1$^{+/-}$* and 154 days in *Dmp1$^{-/-}$* mice ($p < 0.0001$), with no statistically significant differences between *Dmp1$^{+/-}$* and *Dmp1$^{-/-}$* ($p = 0.23$; Fig. 2A; ref. 37). The tumors from *Dmp1$^{+/-}$* mice expressed the Dmp1 protein, showing haploid insufficiency (37, 77). Molecular analyses of tumors revealed that overexpression of the *Ink4a/Arf* repressor Tbx2/Pokemon [52] was found in >50 % of wild type *MMTV-neuNT* mammary tumors while the deficiency of *Arf* or overexpression of Mdm2 was rare. Tumors from *Dmp1$^{+/-}$* or *Dmp1$^{-/-}$*; *neuNT* mice showed significant downregulation of *p53* and *p21$^{Cip1/WAF1}$*, showing p53 inactivity, with more aggressive phenotypes (i.e. increased invasion and metastasis) than those without *Dmp1* deletion. Notably, endogenous h*DMP1* mRNA decreased when *HER2* was depleted in human BC cells, suggesting the existence of HER2-DMP1 axis in human cells as well [37]. Interestingly, the mouse *Dmp1* gene was hemizygously deleted in ~50 % of *neu* mammary tumors from *Dmp1$^{+/+}$;neuNT* mice with significant downregulation of the Dmp1 protein [37]. *neuNT*-induced mammary tumors are different from human BCs in that the *Ink4a/Arf, Mdm2*, or *p53* locus is not frequently involved. Conversely, we found frequent overexpression of *Ink4a/Arf* repressors in *neuNT* tumors [37]. Further studies will be

required to reveal how these *Ink4a/Arf* repressors collaborate with *Dmp1*-loss in breast (or mammary) carcinoma development.

Tumor prevention model with *MMTV-FLAG Dmp1α*

The study shown above suggests a pivotal role of Dmp1α in quenching hyperproliferative signals from HER2 to the Arf-p53 pathway as a safety mechanism to prevent breast carcinogenesis [37]. To directly demonstrate the role of Dmp1α in preventing HER2/neu-driven mammary tumorigenesis, we established *Flag-Dmp1α* transgenic mice under the control of the *MMTV* promoter (*MMTV-FlagDmp1α* transgenic: *MDTG*) [55]. All mice were viable, but exhibited poorly developed mammary glands with markedly reduced milk production; hence > 50% of the parous females were unable to support the lives of new-born pups. The mammary glands of the *MDTG* mice had very low Ki67 expression, but high levels of Arf, Ink4a, p53, and $p21^{Cip1}$, markers of senescence [55]. None of the transgenic females, regardless of their parous status, exhibited any malignant transformation within two-year observation period (Table 1). In all three strains of *MDTG;neuNT* mice, TFS was significantly prolonged (from 197 to 221 days by median) with decreased weight of mammary tumors [55]. The extent of TFS elongation was dependent on the copy number of the *Flag-Dmp1α* transgene - i.e. the higher, the more protection of tumors [55].

Notwithstanding the tumor-suppressive properties of Dmp1α, mammary tumors arose later in *MDTG;neuNT* mice, irrespective of their transgenic strain. Immunohistochemical studies showed that Flag-Dmp1α and Ki67 protein expressions were mutually exclusive [55] indicating that Flag-Dmp1α-positive areas were not proliferating, and thereby preventing tumor development *in vivo*. Moreover, we saw significant overlap between Flag-Dmp1α-positive areas and cleaved caspase 3 expressing areas in *MDTG;neuNT* tumors [55] indicating that Flag-Dmp1α induces apoptotic cell death as well. In summary, our research provides direct evidence that Dmp1α has a tumor suppressive activity *in vivo* [55]. Future studies should focus on the molecular mechanisms of how the Dmp1α protein is downregulated in the mammary glands of *MDTG;neuNT* mice. Molecules that specifically activate the *Dmp1* promoter or stabilize the Dmp1α protein may thus be effective to induce regression of tumor growth *in vivo*.

Discussion and conclusive remarks

We have reviewed different mouse models of human BC overexpressing wild type or mutant *HER2/neu* driven by *MMTV*, *neu*, or dox-inducible promoters. These mice have been used to analyze the biological behavior of mammary tumors overexpressing HER2/neu (tumor growth, angiogenesis/metastasis), oncogenic signaling pathways, oncogene - tumor suppressor gene interactions [78, 79], and to test therapeutic efficacies of HER(2) tyrosine kinase inhibitors to treat the disease. Accumulating data suggests that the mutant *neu* is more oncogenic than the wild type, and usage of the *neu* promoter for *neu* expression provides more physiological models than *MMTV*-driven ones. *MMTV-HER2* (wild type, Δ16) models have been created since trastuzumab does not recognize the rat neu protein. These mouse models will be useful for the screening of novel humanized monoclonal antibodies that specifically target HER2.

Since human BCs have been categorized into 5 different groups to predict the prognosis and determine therapeutic modalities, it is reasonable to compare gene expression in human BCs and that in gene-engineered mouse models (GEMs). Surprisingly, it has been reported that the *MMTV-neu* model represents the luminal subtypes more than HER2-enriched tumors [80]. Conserved with humans, murine Neu^{Ex} tumors highly expressed several tyrosine kinase pathway-related gene-signatures, namely EGFR and HER2, which would be expected from the nature of the *neu/ERBB2* transgene [81, 82]. Hence *MMTV-neu* tumors regressed with the dual tyrosine kinase inhibitor lapatinib [83]. It has been reported that mice with conditional expression of *neuNT* under the endogenous promoter undergo gene amplification [34], closely recapitulating human HER2+BC [67]. Consistent with the non-invasive nature of mammary tumors induced by expression of *neuNT* under the endogenous promoter, these tumors expressed markers of a highly differentiated state, which are closely linked to HER2 and often co-amplified in non-invasive ductal BCs [68, 84], indicating that it is a faithful model of human BC with *HER2* amplification.

In addition to GEMs employing HER2/neu, patient-derived xenografts (PDXs) generated from fresh specimens recapitulate the diversity of BC and reflect the histopathology, the tumor behavior, and metastatic properties of the original tumor [85, 86]. There are several advantages of PDX models in examination of therapeutic responses to drugs. They are i) one can use human tumor tissue retaining the complexity of genetic/epigenetic abnormalities, which can be applied in the development of individualized molecular therapeutics and ii) stroma from the human tumor microenvironment can be included in the xenograft to mimic the tumor microenvironment. The disadvantages are i) PDX tumor models are expensive and technically challenging, and ii) it is necessary to use immune-deficient mice in which the lymphocyte-mediated response to the tumor is lost; thus we cannot precisely evaluate the tumor growth in immune-competent individuals.

Conversely, the advantages of the GEMs are i) specific genetic abnormalities present in human tumors can be reproduced in an inducible/de-inducible manner in specific tissue, ii) the mice are immune-competent, such that the tumor microenvironment can be reproduced, and iii) multiple genetic interactions can be tested just by crossing GEMs with different genetic backgrounds. The major disadvantage of GEM is that the tumors are mouse tumors, but not human versions where more than four genetic alterations are present; hence it requires multiple crossbreedings to reproduce human conditions. Since both mouse models have advantages and disadvantages, they will complement each other to predict BC patients' responses to novel therapies.

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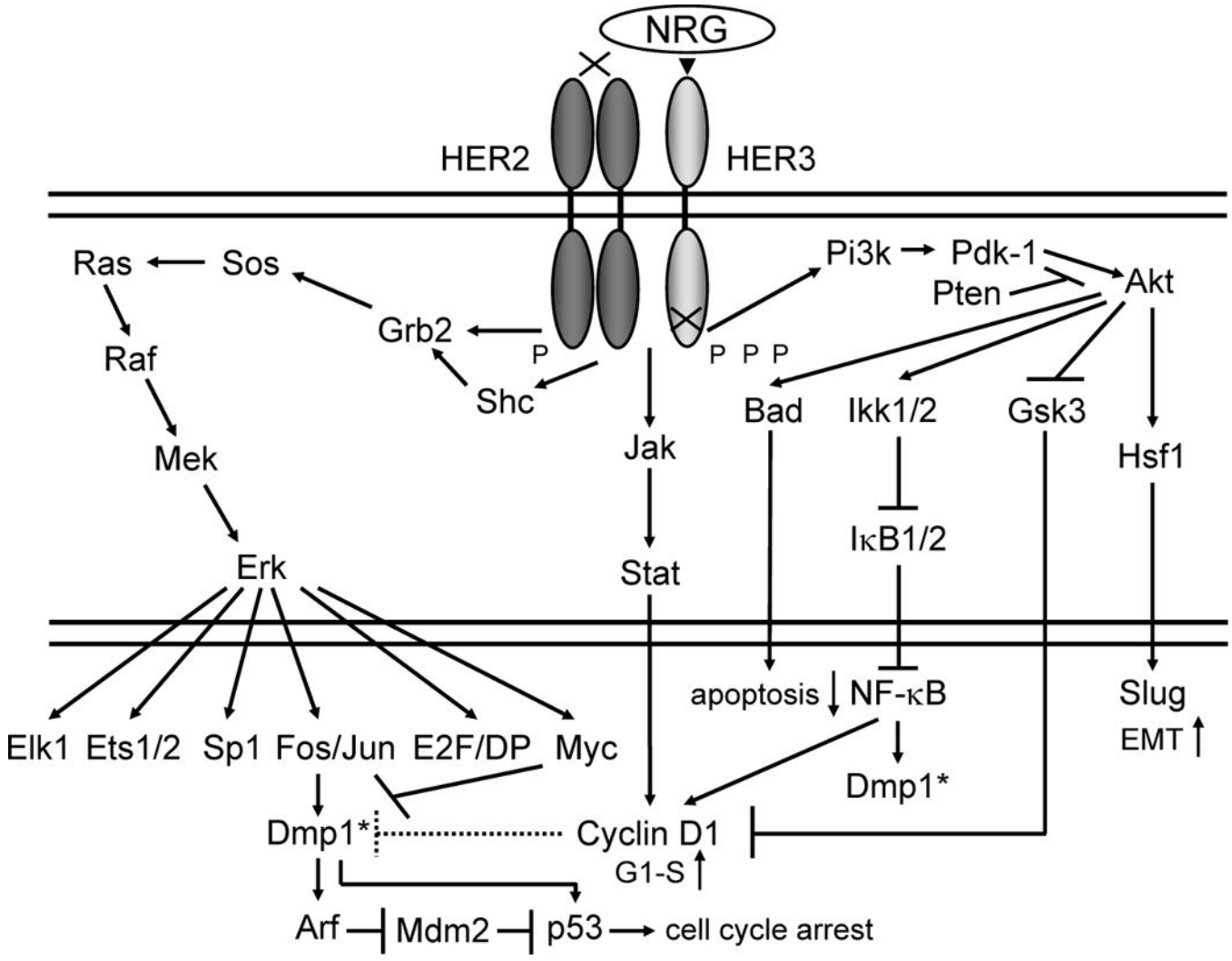

Figure 1. General features of HER2:HER3 signaling involving Ras, Pten, Pi3k, Akt, and NF-κB

The genomic locus for *HER2* is amplified in ~20 % of human BCs, and is associated with aggressive disease with shorter overall and disease-free survival [11]. The protein is overexpressed in ~30 % of BCs in our samples [6] suggesting additional mechanisms for HER2 overexpression exist. The signaling pathways stimulated by the ligand NRG activates ErbB2:ErbB3 (HER2:HER3) heterodimers [17, 23]. Following ligand engagement, HER3 engages and allosterically activates its kinase partner, in this case HER2. Although HER2 molecules make both homo-and hetero-dimers, HER3 do not form homodimers, and does not have protein-tyrosine kinase activity. Phosphorylation of its C-terminal tail leads to recruitment of adapter proteins leading to activation of Pi3k and Ras pathways [23]. Activation of Pi3k leads to phosphorylation of membrane phosphoinositides producing Pip3, which in turn docks the PH domain-containing proteins Pdk1 and Akt [87]. Membrane - bound Akt is phosphorylated and activated by Pdk1. Activated Akt proceeds to phosphorylate a plethora of cellular substrates involved in diverse biological processes. These processes include accelerated G1-S progression as demonstrated by increased cyclin D1 and decreased $p27^{Kip1}$ levels, and enhanced cell survival through increased

phosphorylation of Bad and increased NF-κB levels [17, 23]. Indeed aberrant overexpression of cyclin D1 is frequently observed in human cancers, caused by different mechanisms [88, 89]. The Jak-Stat pathway activation by HER2:HER3 also leads to cell proliferation through cyclin D1 induction. The signaling cascades also stimulate epithelial-mesenchymal transition through stimulation of Hsf1-Slug signaling to cause invasion/metastasis [87]. Thus deregulation of HER2/HER3 can lead to tumorigenesis. Aberrant overexpression of HER2 activates the *Dmp1* promoter to stimulate the Arf-Mdm2-p53 self-autonomous tumor surveillance pathway through Pi3k-Akt-NF-κB and Ras-Raf-Mek-Erk-Jun cascades to eliminate incipient cancer cells by cell cycle arrest or apoptosis [37, 42]. D-type cyclins inhibit the transcriptional activity of Dmp1α in a Cdk-independent fashion on promoters without E2F sites [38, 89], but cyclin D1 collaborates with Dmp1α in *Ink4a* and *Arf* transactivation [42, 48]. Tumor surveillance by the DMP1-p53 pathway is mediated by DMP1α, but not DMP1β, since the latter is an oncogenic splice variant that blocks the tumor-suppressive activity of DMP1α [90]; it also has p53-independent functions for cell proliferation, and is a novel marker for BC [91, 92]. Dmp1*: these are identical molecules.

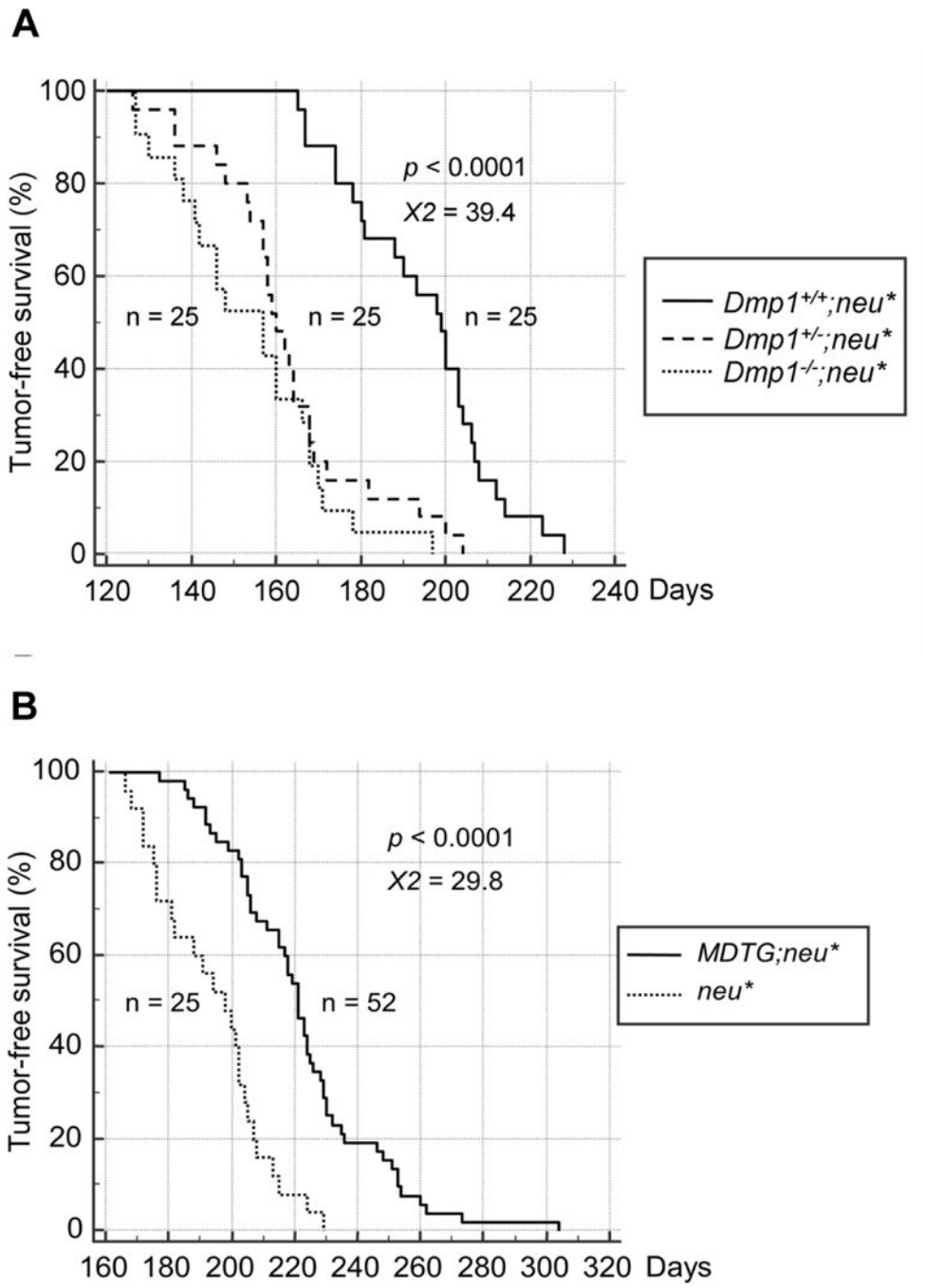

Figure 2. Tumor development in *MMTV-neuNT* mice

A. TFS curves for nulliparous *Dmp1*$^{+/+}$ (straight line), *Dmp1*$^{+/-}$ (discontinuous line), and *Dmp1*$^{-/-}$ (dotted line); *MMTV-neuNT* compound transgenic mice are shown [37]. The median TFS was significantly shortened from 197 days in *Dmp1*$^{+/+}$ mice to 154 and 162 days in *Dmp1*$^{-/-}$ and *Dmp1*$^{+/-}$ mice, respectively (both $p < 0.0001$), with little difference between the two cohorts ($p = 0.23$).

B. TFS curves of *MMTV-FLAG-Dmp1α (MDTG);MMTV-neuNT* and single *MMTV-neuNT* mice [55]. TFS curves for nulliparous *MDTG;neuNT* (straight line, n = 52) and

MMTV-neuNT (dotted line, n = 25) females are shown. The median TFS was significantly prolonged ($p < 0.0001$) from 197 to 221 days with decreased burden of mammary tumors in *MDTG;neuNT* mice in comparison to *neuNT* mice.

Table 1

Mouse models for human breast cancer overexpressing *neu, neuNT, HER2* or *Dmp1α*

The name of the transgene in each mouse model, mean latency of mammary tumor development, pathological findings, potential clinical uses, and references are shown.

Transgene	Mouse model	Mean latency of tumor development	Pathology/genetics	Use	References
neu/ErbB2	*MMTV-neuNT (mutant)*	*Dmp1+/+*: 197 days (NP), 100%	Invasive adca	S, PP, TKI	31
	MMTV-neuNT; Dmp1KO	*Dmp1+/−*: 162 days (NP)	Aggressive adca, multi-focal, larger tumor, highly invasive/ metastatic		37
		Dmp1−/−: 154 days (NP)	Aggressive adca, multi-focal, larger tumor, highly invasive/ metastatic		
	MMTV-neu (wild type)	205 (#202) – 367 (#510) days (MP)	Focal Adca, lung metastasis after long latency (#1)	S, PP, TKI	32, 65
	MMTV-neu; Ink4a/Arf+/−	240 days in *neu* TG, 259 days in *neu; Ink4a/Arf+/−* (nm)	Increased S Phase, higher cyclin D1 and Ki67; increased apoptosis		76
	MMTV-neu; WAP-p53-172H	240 days in *neu* TG, 154 days in *neu;p53-172H* (nm)	Higher grade adca, anaplastic and aneuploid; increased apoptosis		72
	neu promoter - neuNT	14.5 months (MP)	Solid nests of anaplastic cells, micropapillary adca Hyperplastic mammary glands producing lactoferrin and lipid droplets	S, PP, TKI	33
	neuNT knock-in at the neu locus	8 (TM1) - 17 (TM7/8) months (nm)	Accelerated lobuloalveolar development and formation of focal mammary tumors after a long latency (#2)	S, PP, TKI	34, 67, 68
	TetO- neuNT (dox-inducible)	7 weeks with doxycycline (nm)	Multiple invasive mammary adca with metastasis that regresses to a clinically undetectable state following transgene de-induction	S, PP, TKI	35
	MMTV-HER2 (wild type)*	28 weeks (nm)	Adca with areas of solid, tubular, and papillary growth cellular polymorphism with mitosis and metastasis (#3)	S, PP, TKI, MoAb	71
	*MMTV-HER2Δ16**	17 weeks (nm)	Tubular adca consisting of three zones: an outer zone (pale cells), an intermediate zone (darker fusiform cells), and an inner zone (pinkish cytoplasm); ER(−) PR(−) PCNA(+) E-CAD(+)	S, PP, TKI, MoAb	66
Dmp1α	*MMTV-FLAG Dmp1α*	No tumor development in 24 months (NP)	No tumor	S, PP	55
	MMTV-FLAG Dmp1α;neuNT	Delayed mammary tumor development (NP)	Adca; smaller tumors; Flag Dmp1α(+) cells undergo apoptosis, Ki67(−)		
		neuNT: mutant rat neu HER2*: human ErbB2 NP: Nulliparous MP: Multiparous nm: parous status, not mentioned	Adca: Adenocarcinoma #1: in-frame deletions in the extracellular region of neu close to the transmembrane domain #2: gene amplification and protein overexpression of neu #3: in-frame deletion in the juxtamembrane region of HER2	S: signaling pathways PP: pathophysiology TKI: tyrosine kinase inhibitor screening MoAb: monoclonal antibody screening	64 for a review of *MMTV* models