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Oncogenes and Tumor Suppressor Genes

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Breast cancer progression involves multiple genetic events, which can activate dominant-acting oncogenes and disrupt the function of specific tumor suppressor genes. This article describes several key oncogene and tumor suppressor signaling networks that have been implicated in breast cancer progression. Among the tumor suppressors, the article emphasizes *BRCA1/2* and *p53* tumor suppressors. In addition to these well characterized tumor suppressors, the article highlights the importance of PTEN tumor suppressor in counteracting PI3K signaling from activated oncogenes such as ErbB2. This article discusses the use of mouse models of human breast that recapitulate the key genetic events involved in the initiation and progression of breast cancer. Finally, the therapeutic potential of targeting these key tumor suppressor and oncogene signaling networks is discussed.

Karyotypic and epidemiological analyses of mammary tumors at various stages suggest that breast carcinomas become increasingly aggressive through the stepwise accumulation of genetic changes. The majority of genetic changes found in human breast cancer fall into two categories: gain-of-function mutations in proto-oncogenes, which stimulate cell growth, division, and survival; and loss-of-function mutations in tumor suppressor genes that normally help prevent unrestrained cellular growth and promote DNA repair and cell cycle checkpoint activation. Epigenetic deregulation also contributes to the abnormal expression of these genes. For example, genes that encode enzymes involved in histone modification are mutated in primary renal cell carcinoma (Dalgliesh et al. 2010; van Haaften et al. 2009). In addition, the involvement of noncoding RNAs in tumorigenesis and tumor metastasis has been recently documented (Croce 2009; Shimono et al. 2009). These can act as oncogenes or tumor suppressor genes, depending on the context. Here, we discuss genes that are frequently altered in breast cancer, focusing on *ErbB2*, *PI3K* (phosphatidylinositol 3 kinase) pathways, *TP53*, *BRCA1/2*, and *PTEN* (phosphatase and tensin homolog deleted on chromosome 10). Genetically engineered mouse models are emphasized because these provide a wealth of biological information. We consider in detail genetic and biochemical studies that have shown that oncogenic proteins and tumor suppressors provide a critical balance in regulation of key pathways that control cell number and cell behavior.

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ONCOGENES AND TUMOR SUPPRESSOR GENES

The identification of oncogenes such as *H-RAS* and tumor suppressor genes such as that encoding retinoblastoma protein (*RB*) involved a combination of functional cloning, linkage analyses, positional cloning, or mutational analyses of genetically predisposed individuals. Comparative genomic hybridization has since revealed various genes that can be amplified or deleted in cancer. The human genome projects further increased the speed of discovery (Bell 2010). Breast cancer genome analyses indicate that there are only a few genes that are frequently mutated but many that are infrequently mutated, providing an explanation for the observed cancer heterogeneity. Complex somatic DNA rearrangements, mostly intrachromosomal tandem duplications, have been found in the breast cancer genomes that have been sequenced. Many of these rearrangements are because of nonhomologous end joining (Stephens et al. 2009). Comparison of genome sequences of primary breast cancer and cancer metastasis show limited de novo mutations in metastasis in addition to significantly shared mutations with the primary tumor (Ding et al. 2010). In a case of metastasis evolved over 9 years, a large number of mutations are novel when compared to the primary tumor (Shah et al. 2009). These studies provide information on tumor evolution and identify pathways critical to breast cancer metastasis.

The *ErbB2*, *PI3KCA*, *MYC*, and *CCND1* (encodes cyclin D1) oncogenes are frequently deregulated in breast cancer. Loss-of-function mutations of *RB* in breast cancer cell lines and primary tumors were reported in 1988 (Lee et al. 1988; T'Ang et al. 1988), but a full appreciation of the tumor suppressor genes affected came much later from studies of hereditary breast cancer. To date, ten tumor suppressor genes, all of which are involved in the regulation of genomic integrity, have been associated with hereditary breast cancer (Walsh and King 2007). *BRCA1* and *BRCA2* mutations are associated with a significantly elevated risk for breast and ovarian cancers. Rare germ-line mutations in *TP53* (the gene encoding p53) and *PTEN* are associated with high risk for various cancers, including breast cancer. Germ-line mutations in *ATM, CHK2, NBS1, RAD50, PALB2*, and *BRIP* all moderately increase breast cancer risk. Among these tumor suppressors, ATM and CHK2 are kinases involved in the DNA damage response. RAD50 is a component of protein complex critical to DNA double-stranded-break end processing. *PALB2* encodes a BRCA2-interacting protein and *BRIP1* encodes a BRCA1-interacting protein with DNA helicase activity. These genes are also mutated in pancreatic, prostate, and other tumors. Tumors develop because of the loss-of-heterozygosity mutations in the remaining normal allele plus other somatic mutations. For example, *BRCA1*-driven breast cancers frequently harbor somatic mutations in *TP53* and *PTEN* (Holstege et al. 2009; Saal et al. 2008).

Several pathways are frequently deregulated in breast cancer as a consequence of mutations in these genes. These include pathways involving key lipid and protein kinases that function in cell growth and survival, the cell cycle machinery, DNA damage response pathways, and apoptosis.

THE PHOSPHATIDYLINOSITOL 3 KINASE (PI3K) PATHWAY

PI3K signaling influences cell growth, survival, metabolism, and metastasis. PI3K is activated by growth factors and signals from the extracellular matrix (Dillon et al. 2007b). Alterations in upstream components of the PI3K pathway, such as receptor tyrosine kinases, and downstream components such as AKT are frequent in breast cancers. The PTEN tumor suppressor antagonizes the PI3K pathway. Loss of PTEN function, and activating mutations in or amplification of the gene that encodes the PIK3 catalytic subunit (*PIK3CA*) are both common.

The Akt (PKB) serine kinase family—AKT1, AKT2, and AKT3—are downstream molecules in the PI3K pathway. These have been implicated in a number of cellular processes, including control of cell proliferation, cell survival, and metabolism (Dillon et al. 2007b). Direct

evidence supporting a role for AKT1 in mammary tumor progression came from studies using transgenic mice that expressed different activated forms of AKT1. Expression of these in the mammary epithelium, although incapable of inducing mammary tumors, resulted in a profound involution defect (Ackler et al. 2002; Dillon et al. 2009; Hutchinson et al. 2004). However, coexpression of an activated AKT1 mutant (AKT1-DD) with an activated ErbB2mutant (NDL) or a PI3K defective middle T oncogene resulted in a decrease in tumor latency in these tumor models (Dillon et al. 2009; Hutchinson et al. 2004). AKT1 coexpression decreased lung metastases, however, in tumor-bearing animals in the MMTV ErbB2 model (Dillon et al. 2009; Hutchinson et al. 2004). Conversely, germ-line deletion of AKT1 profoundly reduced mammary tumor formation in this ErbB2 mouse model of human breast cancer (Ju et al. 2007; Maroulakou et al. 2007). Germ-line deletion of AKT2 accelerated mammary tumor induction in this ErbB2 tumor models (Maroulakou et al. 2007), whereas expression of activated AKT2 in mammary epithelium had little impact on tumor induction but dramatically increased tumor metastasis (Dillon et al. 2009). The enhanced metastatic phenotypes that have been observed in various AKT models have recently been linked to AKT-mediated suppression of the miR 200 microRNA, which suppresses the epithelial–mesenchymal transition in epithelial cells (Iliopoulos et al. 2009). Knocking out another kinase in this pathway—the Snf1-related serine kinase Hunk—abrogates the metastatic phenotype in an ErbB2 mouse tumor model (Wertheim et al. 2009). Oncogenic activation of the different serine kinases may thus have distinct effects on the induction and metastatic phases of mammary tumor development.

Recent studies have also examined the impact of mammary-specific disruption of PTEN. Conditional ablation of PTEN in the mammary epithelium of this MMTV/activated ErbB2 or ErbB2^{KI} strains dramatically accelerated mammary tumor progression (Dourdin et al. 2008; Schade et al. 2009). Gene expression profiling of PTEN-deficient ErbB2 tumors further showed they had acquired many of the salient transcriptional features of the basal breast cancer subtype (Dourdin et al. 2008). Inactivation of a key tumor suppressor pathway thus appears to impact on the breast cancer subtype.

TRANSGENIC MODELS OF ErbB2-INDUCED TUMOR PROGRESSION

ErbB2 is a member of the EGFR family of receptor tyrosine kinases (RTKs). This family comprises four closely related members: EGFR, ErbB2 (Neu, HER-2), ErbB3 (HER-3), and ErbB4 (HER-4) (Hynes and Stern 1994). The importance of ErbB2 in primary human breast cancer is highlighted by the fact that 20%–30% of human breast cancers show elevated levels of ErbB2 because of genomic amplification of the *ErbB2* proto-oncogene (Slamon 1987, 1989). The overexpression of ErbB2 strongly correlates with a negative clinical prognosis in both lymph-node-positive (Antoniotti et al. 1994; Mansour et al. 1994; Ravdin and Chamness 1995) and node-negative (Andrulis et al. 1998) breast cancer patients. Further evidence that overexpression of ErbB2 results in an aggressive tumor type stems from studies showing that elevated ErbB2 expression occurs in many in situ and invasive human ductal carcinomas but is rarely observed in benign breast disorders, such as hyperplasias and dysplasias (Allred et al. 1992; Mansour et al. 1994). Significantly, ErbB-2 may be useful not only as a prognostic marker but also as a predictive marker, given that elevated expression of HER2 predicts tamoxifen resistance of the primary tumor (Pegram et al. 1998) and the response to anti-HER2 targeted therapy such as Herceptin.

Direct evidence supporting a role for the various EGFR family members and their ligands in mammary tumorigenesis comes from studies of transgenic mice. Expression of EGFR or its ligand TGFα in mammary epithelium in transgenic mice resulted in the frequent induction of mammary adenocarcinomas (Brandt et al. 2000; Matsui et al. 1990; Sandgren et al. 1990, 1995). Mammary-epithelium-specific expression of activated ErbB2 similarly rapidly

induced metastatic multifocal mammary tumors (Bouchard et al. 1989; Guy et al. 1996; Lucchini et al. 1992; Muller et al. 1988; Siegel et al. 1999). Whereas expression of the activated *ErbB2* oncogene in murine mammary epithelium is capable of efficiently inducing multifocal mammary tumors, no comparable activating mutations have been detected in human ErbB2 (Lemoine et al. 1990). Thus, the primary mechanism by which ErbB2 induces mammary tumorigenesis in human breast cancer must be through overexpression of the wild-type receptor. To assess whether elevated expression of the wild-type ErbB2 product can indeed induce mammary tumors, Guy et al. generated transgenic mice carrying the *ErbB2* proto-oncogene under the transcriptional control of the MMTV promoter/enhancer (Guy et al. 1992). In contrast to the rapid tumor progression observed in the activated ErbB2-bearing strains, focal mammary tumors arose in these strains after a long latency period (Guy et al. 1992). Tumor progression in these strains is associated with the activation of the ErbB2 tyrosine kinase by somatic mutations in the transgene in at least 70% of the mammary tumors analyzed (Siegel et al. 1994). Significantly, these mutations are confined to a cysteine-rich region of the receptor in the juxtatransmembrane domain and comprise either deletion or insertion of single cysteine residues (Siegel et al. 1994). Further genetic and biochemical analyses have revealed that these cysteine alterations promote the formation intermolecular cysteine bridges between ErbB2 monomers, which results in constitutive receptor dimerization and activation (Siegel and Muller 1996).

Genetic and biochemical analyses of these various transgenic mouse models have also revealed potential roles for other members of the EGFR family. For example, coexpression of the EGFR ligand TGFα and the *ErbB2* proto-oncogene resulted in a dramatic acceleration of tumor progression that correlated with the tyrosine phosphorylation of EGFR and ErbB2 (Muller et al. 1996). In contrast to the parental MMTV/*ErbB2* strains, sporadic mutations were not detected in the *ErbB2* transgene in these mice, presumably because activated EGFR can transphosphorylate ErbB2. Conversely, inhibition of EGFR function can dramatically impair mammary tumor formation in transgenic animals expressing activated ErbB2 in the mammary epithelium (Gillgrass et al. 2003; Lenferink et al. 2000). Another EGFR family member that has been implicated in ErbB2-induced tumor progression is ErbB3. Immunoblot analyses revealed that ErbB3 levels were dramatically elevated during tumor progression in ErbB2 transgenic strains. In fact, quantitative measurement of ErbB3 levels revealed a 10- to 20-fold increase in the levels of ErbB3 protein in tumors compared with adjacent normal tissue (Siegel et al. 1999). Although a dramatic increase in the amount of ErbB3 protein was observed in these strains, equivalent levels of *ErbB3* transcript were detected in normal and tumor tissues (Siegel et al. 1999). Thus, the increased levels of ErbB3 observed in the tumors are the result of an increase in either the stability or the translation of ErbB3.

Tumor progression in transgenic mice expressing the *ErbB2* proto-oncogene is associated with mutations in the cysteine-rich juxtatransmembrane region (Siegel and Muller 1996; Siegel et al. 1994, 1999). Given the large proportion of the ErbB2-induced mammary tumors that possess these activating mutations, there appears to be strong biological selection for them. Although comparable somatic mutations in *ERBB2* have not been detected in human breast cancer, several studies have reported the expression of an alternatively spliced ErbB2 isoform that carries a 16-residue in-frame deletion in the juxtatransmembrane domain (herein referred to ErbB2ΔEx16) that closely resembles the protein generated by the sporadic mutations in the *ErbB2* transgenic mice (Kwong and Hung 1998; Siegel et al. 1999). This splice variant is constitutively active because of its capacity to form disulfide-bonded dimers (Kwong and Hung 1998; Siegel et al. 1999). Because the *ErbB2* cDNA used in the transgenic mice generates an RNA that is incapable of undergoing alternative splicing, the selection for sporadic mutations in these mice may reflect a need to mimic this alternatively

spliced form. Indeed, recent crystal studies with the ErbB2 extracellular domain suggest that ErbB2 does not readily form homodimers owing to electrostatic repulsion (Garrett et al. 2003). Thus, this splice isoform may be required for the production of ErbB2 homodimers through formation of covalent bonds between cysteine residues. Although these genetic studies suggest that the ErbB2ΔEx16 splice variant plays a critical role in ErbB2-driven tumor induction, the biological significance remains to be established.

One limitation of the transgenic mouse models is that expression of ErbB*2* is driven by a strong viral promoter. In an attempt to more closely mimic the events involved in ErbB2-induced mammary tumor progression, we have recently derived transgenic mice that carry a Cre-inducible activated ErbB2 under the transcriptional control of the endogenous *ErbB2* promoter (herein referred as the ErbB2^{KI} model) (Andrechek et al. 2000). In contrast to the rapid tumor progression observed in the MMTV-activated ErbB2 strains, focal mammary tumors arose only after an extended latency period. Tumor progression in these strains is associated with a dramatic elevation in the levels of both ErbB2 mRNA and protein. Remarkably, the elevated expression of ErbB2 also correlates with selective genomic amplification of the activated *ErbB2* allele (Andrechek et al. 2000; Montagna et al. 2002; Hodgson et al. 2005). Thus, as in human breast cancers, amplification of *ErbB2* appears to be a critical event in mammary tumor progression in this mouse model. Its similarity to ERBB2-initiated human breast cancer has been further highlighted by detailed comparative genome hybridization (CGH) analyses of the *ErbB2* amplicon. The results of these studies revealed that both human and mouse *ErbB2* amplicons contain the same core 10 genes closely linked to the *ErbB2* locus (Hodgson et al. 2005).

Tumorigenesis in the ErbB2^{KI} model is also associated with a number of other alterations, including centrosome abnormalities and recurrent deletions of chromosome 4 (Montagna et al. 2002). Further refined mapping of chromosome 4 region has revealed that these tumors have frequently loss of expression of the tumor suppressor 14-3-3σ (Hodgson et al. 2005). Interestingly, loss of 14-3-3σ expression has been noted in many primary human breast cancers (Ferguson et al. 2000; Urano et al. 2002; Vercoutter-Edouart et al. 2001). One potential explanation for selection for loss of 14-3-3σ expression in ErbB2-induced tumors is that 14-3-3σ is involved in cytoplasmic sequestration of the EGR2 transcription factor, which plays a critical role in up-regulating ErbB2 expression (Dillon et al. 2007a). Future studies involving the targeted disruption of 14-3-3σ in mammary epithelium should establish the relative contribution loss that 14-3-3σ plays in ErbB2-driven mammary tumor progression.

Another important issue influencing breast cancer morbidity is recurrence of the cancer after a period of remission. It has been hypothesized that recurrence of a cancer is a consequence of emergence of tumor cells from dormancy (White et al. 2006), and this phenomenon has recently been experimentally validated in several studies. For example, although down-regulation of ErbB2 in an inducible model of mammary tumorigenesis initially results in the regression of mammary tumors, these eventually re-emerge—presumably from dormant tumor cells—after a long latency period (Moody et al. 2002). This re-emergence correlates with up-regulation of the Snail transcription factor in these cells (Moody et al. 2005). Interestingly, Snail controls normal development, epithelial-mesenchymal transitions, and cancer stem cell properties (reviewed in Cobaleda et al. 2007).

IDENTIFICATION OF SIGNALING NETWORKS CRITICAL FOR ONCOGENE-INDUCED MAMMARY TUMOR PROGRESSION

The in vivo importance of oncogene-coupled signaling in mammary tumor progression has recently been shown by experiments using transgenic mice that express phosphorylation mutants of the ErbB2 or PyV mT oncogenes that decouple these oncogenes from particular signaling pathways in the mammary epithelium

(Dankort et al. 2001; Schade et al. 2007). Although mammary epithelial expression of ErbB2 mutants coupled specifically to either the adaptor protein ShcA or Grb2 were capable of efficiently inducing mammary tumors (Dankort et al. 2001), they had dramatically different metastatic outcomes that correlated with distinct transcriptional profiles (Dankort et al. 2001; Schade et al. 2007).

Direct evidence for the importance of ShcA in mammary tumor progression has recently been obtained from studies interbreeding the MMTV/ErbB2 IRES Cre strain (NIC) and a separate strain of mice harboring a conditional knockout of ShcA (Ursini-Siegel et al. 2008). Remarkably, mammary-specific disruption of ShcA in MMTV/NIC mice completely blocked mammary tumor progression in virgin females, suggesting that ShcA signaling is critical for mammary tumor induction. However, the precise role of the ShcA-coupled signaling pathways in ErbB2-induced mammary tumor progression remains to be addressed.

Another important signaling pathway that plays a critical role in ErbB2-induced mammary tumor progression is the c-Src tyrosine kinase. We have previously shown that Src family kinases are activated in mammary tumors derived from MMTV-activated *ErbB-2* transgenic strains (Guy et al. 1994; Muthuswamy and Muller 1995a,b; Muthuswamy et al. 1994). In the case of ErbB-2, activation of c-Src occurred through the direct interaction of the catalytic domain of c-Src with the catalytic domain of ErbB-2 (Kim et al. 2005; Marcotte et al. 2009). Recruitment of c-Src to chimeric EGFR/ErbB-2 receptors possessing the ErbB-2 catalytic domain resulted in disruption of epithelial polarity (Kim et al. 2005; Marcotte et al. 2009).

ONCOGENE-INTEGRIN CROSS TALK IN MAMMARY TUMOR PROGRESSION

An increasing body of evidence suggests oncogenes can activate other classes of transmembrane receptors. For instance, genetic and biochemical analyses have revealed that the α6β1 integrin receptor can associate with activated members of the EGFR family (Weaver et al. 1997). Stimulation of β1 integrin function can thereby result in the enhanced phosphorylation of EGFR family members (Adelsman et al. 1999; Mariotti et al. 2001; Moro et al. 1998). Moreover, activation of TGF-β signaling can induce coclustering of β1-integrin and ErbB2, and thereby promote metastatic invasion (Wang et al. 2009a). Indeed, coexpression of activated TGF-β receptor or its ligand and ErbB2 in the mammary epithelium of transgenic mice dramatically accelerates metastatic progression (Muraoka-Cook et al. 2004; Muraoka et al. 2003; Siegel et al. 2003). It has also been shown that antibodies directed against β1 integrin down-regulate EGFR phosphorylation and can interfere with the morphological transformation of breast tumor cells (Wang et al. 2002; Weaver et al. 1997).

Direct evidence for a role for integrin in mammary tumor progression comes from a recent study involving the conditional ablation of β1 integrin in the PyV mT model. Lack of β1 integrin causes PyV-mT-induced mammary tumors to disappear (Andrechek et al. 2005). Interestingly, this study also showed that tyrosine phosphorylation and subcellular localization of the integrin-associated kinase FAK was compromised in the β1-integrin-null tumor cells (White et al. 2004). This block in tumor progression was associated with a complete block in cell cycle progression without any overt evidence of apoptotic cell death (Andrechek et al. 2005). It was not because of an indirect effect on normal mammary development, because the initial stages of mammary epithelial ductal outgrowth were not perturbed (White et al. 2004). The β1-integrin-deficient PyV mT tumor cells were not able to contribute to tumor development and showed many of the hallmarks of tumor dormancy (White et al. 2004). The mammary tumors that eventually arose were identified as "escapee" populations of epithelial cells: These cells failed to express Cre recombinase owing to the stochastic nature of its expression in MMTV/Cre transgenic strains and thus retained β1-integrin function (White et al. 2004). Conditional disruption of the integrin-coupled FAK pathway also resulted in a profound block in tumor progression

(Lahlou et al. 2007; Luo et al. 2009; Provenzano et al. 2008; Pylayeva et al. 2009). Further evidence for importance of integrin signaling stems from a collaborative study of mice carrying a mutant β4 integrin lacking the carboxy-terminal signaling domain as well as the MMTV/activated *ErbB2* transgene (Guo et al. 2006). The mutant β4 integrin retains the capacity to form hemidesmisomes but cannot signal, owing to truncation of the carboxy-terminal domain (Guo et al. 2006). These mice had a moderate impairment of ErbB2-induced tumor progression, delaying tumor onset 5 weeks (Guo et al. 2006).

ErbB2 SIGNALING IMPACTS ON CELL CYCLE PROGRESSION AND TRANSCRIPTION FACTOR NETWORKS

Ultimately, signaling from activated ErbB2 affects transcription factors and the machinery that controls the cell cycle. Germ-line loss of cyclin D1 in the MMTV/ErbB2 transgenic strains results in a complete block in tumor progression (Yu et al. 2001). Functional Cdk4, a known cyclin D1 partner, is also required for ErbB2-induced tumor progression (Landis et al. 2006; Yu et al. 2006). Conversely, modulation of the levels of the p16 and p27 inhibitors of Cdk function can also have profound effect on ErbB2-driven mammary tumor progression (Bulavin et al. 2004; Muraoka et al. 2002). Key elements of the cell cycle machinery can thus be modulated by oncogenic ErbB2 signaling.

ErbB2-mediated activation of key transcription factors can also influence tumor progression. For example, disruption of Stat3 in mammary epithelium can have a profound impact on ErbB2-driven tumor progression. Although ErbB2 can induce tumors in the absence of Stat3, these tumors fail to metastasize (Ranger et al. 2009). Examination of the gene expression profile of Stat3-deficient tumors revealed that they failed to induce the expression of a number of Stat3 target genes involved in inflammation (Ranger et al. 2009). Disruption of certain key signaling networks thus appears to have selective effects on the metastatic phase of ErbB2-driven tumor progression.

RB AND CELL CYCLE REGULATION

RB regulates the G1-S phase transition by repressing E2F transcription factors that stimulate the expression of genes required for cell cycle progression (reviewed in Burkhart and Sage 2008). Cyclin D1-CDK4 complex phosphorylates RB, leading to its dissociation from the RB-E2F complex, which frees E2F for activation of cyclin D1 and S-phase genes transcription. E2F targets include cell cycle regulatory genes such as cyclin D1 and S-phase genes. The positive feedback loop among RB, E2F, and cyclin D1 allows for cell cycle progression through G1/S and S phases. Deregulation of RB pathways occurs in most cancer and is mediated either by loss-of-function mutation of negative players including RB and CDK inhibitors (CKIs, p15, p16, p21, etc.) or by amplification or overexpression of cyclin D1. Many of these regulators have additional functions; for example, RB plays a role in adipocyte differentiation (Chen et al. 1996) and cyclin D1 has a critical role in development (Bienvenu et al. 2010).

THE p53 PATHWAY

Rare germ-line mutation of *TP53* leads to the Li-Fraumeni familial cancer syndrome. On the other hand, somatic mutations of *TP53* occur in the majority of sporadic cancers. p53 responds to various cellular stress signals and is hailed as the "guardian of genome" (Lane 1992). p53 alteration is common in spontaneously immortalized murine embryonic fibroblasts (Harvey and Levine 1991).

In normal cells, p53 is kept at very low level by p53-interacting protein, MDM2. MDM2 is an E3 ubiquitin ligase that promotes ubiquitination and subsequent proteasomal degradation of p53. Cellular stress induces post-translational modification of p53 and MDM2, leading to stabilization and activation of p53 (reviewed in Levine and Oren 2009; Brown et al. 2009). Mdm2 appears to be intrinsically

active and its deletion results in embryonic lethality (reviewed in Donehower and Lozano 2009; Kruse and Gu 2009). Deletion of *TP53* rescues embryonic lethality in *Mdm2*- and *Mdm4*-deficient mice (Jones et al. 1995; Montes de Oca Luna et al. 1995; Parant et al. 2001). Furthermore, mice carrying a knockin *TP53* allele that shows defective transactivation and Mdm2 binding die during embryogenesis (Johnson et al. 2005). Thus, unrepressed Mdm2 activity is a likely cause of embryonic lethality. Notably, a single nucleotide polymorphism within the first intron of *Mdm2* has been shown to affect promoter strength and affect the level and activities of p53 subsequently (Bond et al. 2004). These studies provide genetic and biochemical evidence of regulation of p53 by MDM2 and MDM4.

p53 binds to DNA in a sequence-specific manner to induce cell cycle checkpoint activation, cellular senescence, apoptosis, or autophagy (reviewed in Menendez et al. 2009; Zilfou and Lowe 2009). Expression of hundreds of genes is directly regulated by p53. For examples, CKI *p21* and Polo-like kinase 1 are important targets of p53 for G1/S and G2-M cell cycle checkpoint activation, respectively (Sur et al. 2009). Centrosome amplification occurs frequently in cells harboring *TP*53 mutations, partly through overactivation of CDKs by cyclin E and cyclin A (reviewed in Fukasawa 2008). This in turn results in chromosome instability, a hallmark of solid tumors.

Loss of p53 allows efficient reprogramming of somatic cells (Kawamura et al. 2009). In mammary gland, loss of p53 result in aberrant asymmetric cell divisions of mammary stem cells (Cicalese et al. 2009). p53 also enhances the maturation of several microRNAs with growth-suppressive functions (Suzuki et al. 2009). These recent results continue to expand our understanding of the tumor suppressor mechanisms of p53.

p53 is inactivated in the cancer stem cells of ErbB2-associated mouse mammary tumors (Cicalese et al. 2009). In this model, cancer stem cells undergo frequent symmetric cell division and expansion. Reactivation of p53 by pharmacological intervention restores asymmetric cell division (Cicalese et al. 2009). In a separate model, expression of the exon-5-6-less *TP53* allele in neural stem cells was followed by the expansion of transient-amplifying progenitor-like cells and subsequent development of malignant astrocytic glioma (Wang et al. 2009b). These studies indicate a role of p53 in the regulation of stem cell division in addition to its well-documented role in transformation.

The majority of cancer-associated mutations in *TP53* are missense mutations of the DNA binding domain. Some mutations induce genetic instability by inactivating ATM (reviewed in Xu 2008). Mouse models of missense mutations corresponding to those in human mutant *TP53* reveal a gain-of-function. These mutant p53 interact with Mre11 and suppress binding of Mre11-Rad50-NBS1 complex to DNA double-stranded breaks (Song et al. 2007). Although mutant p53 levels are increased in most tumors, only low levels of mutant p53 are present in normal mouse tissues initially, which indicates additional events are needed to allow mutant p53 to escape MDM2-dependent degradation (reviewed in Brosh and Rotter 2009).

THE BRCA PATHWAYS

Individuals with mutations in *BRCA1* are predisposed to breast and ovarian cancers. Earlier studies indicated that the majority of *BRCA1*-associated cancers are triple (ERα, PR, and HER-2) negative, basal-type breast cancers (reviewed in Lynch et al. 2008). However, recent reports show that BRCA1-associated breast cancers can be ERα- and PR-positive, especially in aged patients (Atchley et al. 2008; Tung et al. 2010). The *BRCA1* gene encodes a protein of 1863 amino acids with a predicted molecular weight of 220 kDa (Miki et al. 1994). At the amino terminus is a RING domain that mediates the interaction with another RING-domain-containing protein, BARD1. The BRCA1/BARD1 heterodimer shows ubiquitin ligase activity (Chen et al. 2002; Wu-Baer et al. 2003). Exon 11 of *Brca1* encodes approximately 60% of BRCA1. This central region interacts with the DNA repair protein complex Mre11-Rad50-NBS1 and

the transcriptional repressor ZBRK1 (Zhong et al. 1999; Zheng et al. 2000). The Mre11-Rad50-NBS1 complex binds to and processes DNA double stranded breaks. This complex is involved in both nonhomologous end joining and homologous recombinational repair. Mouse embryonic fibroblasts deficient in *Brca1* have impaired homologous recombinational repair (Moynahan et al. 1999). Loss of 53BP1 expression restores homologous recombination in Brca1 mutant cells by promoting ATM-dependent processing of broken DNA ends (Bunting et al. 2010). The carboxyl terminus of BRCA1 contains tandem BRCA1 C-terminal (BRCT) repeats. This region binds to phospho-peptides (Manke et al. 2003; Yu et al. 2003) involved in cell-cycle checkpoints and DNA repair. Several proteins including BACH1, CtIP, Acetyl-CoA carboxylase, Abraxas/CCDC98, and RAP80 interact with the BRCT domain of BRCA1 in a phospho-dependent manner (reviewed in Rodriguez and Songyang 2008). These studies reveal how BRCA1 maintains genomic stability through DNA repair and cell cycle checkpoint activation.

Three BRCA1 protein complexes have been characterized. One complex contains BRCA2 and PALB2, a BRCA2-associated protein (Sy et al. 2009; Zhang et al. 2009). Germ-line mutations of *PALB2* not only associate with hereditary breast cancer, but also is found in Fanconi's anemia and familial pancreatic cancer syndrome. *BRCA2* mutations have been linked to a wide spectrum of cancers. In contrast, *BRCA1* mutation is primarily associated with breast and ovarian cancers. Plausible explanations include the connection between BRCA1, but not BRCA2, and steroid hormone receptors (reviewed in Lee 2008) and the role of BRCA1 in mammary luminal epithelial lineage determination (reviewed in Visvader 2009).

Developmental defects and early embryonic death have been observed in *Brca1* homozygous knockout mice (reviewed in Dasika et al. 1999; Drost and Jonkers 2009). Several further lines of evidence indicate that BRCA1 plays a role in mammary development and differentiation. Mouse mammary tissue harboring a conditional *Brca1* knockout displays abnormal ductal morphogenesis (Xu et al. 1999). Depletion of BRCA1 by RNAi in 3-D cultured MECs impairs acinus formation and up-regulates genes that control proliferation but down-regulates those controlling differentiation (Furuta et al. 2005). Importantly, the epithelial progenitor cell population is expanded in *BRCA1* carriers. This highlights the haplo-insufficiency phenotype of BRCA1 in mammary epithelial differentiation (Lim et al. 2009). The EGFR pathway is activated in the mammary epithelial cells of *BRCA1* carriers (Burga et al. 2009), and may be linked to the expansion of luminal epithelial cells. In contrast, knocking down BRCA1 in primary mammary epithelial cells increases the number of stem/progenitor cells and decreases ERα expression. Loss of heterozygosity of *BRCA1* in the mammary epithelial cells of *BRCA1* carriers leads to the expansion of ALDH1-positive, ER- and PR-negative stem/progenitor cells (Liu et al. 2008). Thus, the effects of BRCA1 on mammary cell differentiation appear to be dosage-dependent (Ginestier et al. 2009).

BRCA2 carriers develop different subtypes of breast cancer. The *BRCA2* gene encodes a protein of 3418 amino acids with a predicted molecular weight of 375 kDa (Wooster et al. 1995). A repeat motif termed the BRC domain that comprises approximately 70 amino acids is present in the middle third of the BRCA2 protein. BRC repeats bind to the Rad51 recombinase (Chen et al. 1998; Wong et al. 1997). BRCA2-deficient cells show defective formation of IR-induced Rad51 foci (Yuan et al. 1999). At the carboxyl terminus of BRCA2 is a region with extensive secondary structure that interacts with the evolutionarily conserved protein DSS1. Based on the 3-D structure, it is predicted that the high-affinity ssDNA-binding and dsDNA-binding domains of BRCA2 play critical roles in homologous recombination (Yang et al. 2002). Indeed, both the formation of Rad51 nuclear filaments and Rad51-mediated strand exchange during homologous recombination are regulated by BRCA2 (reviewed in Thorslund and West 2007).

Brca2 homozygous knockout mice either die during embryogenesis or survive beyond birth, depending on the mutation (Dasika et al.

1999). Mutations in either *p53* or *p21* prolong the survival of *Brca1*$^{-/-}$ and *Brca2*$^{-/-}$ embryos. Several conditional knockout models of these two tumor suppressor genes have been established (see the following).

TRANSGENIC MOUSE MODELS OF BRCA-ASSOCIATED BREAST CANCER

Several different *Brca1* alleles have been generated (Drost and Jonkers 2009). Because of differential splicing, the exon 11-less Brca1 isoform is also present in wild-type mice and the protein product is located in the nucleus—like the full-length Brca1 (Huber et al. 2001). All other alleles are predicted to produce aberrant protein products. Different mutant *TP53* alleles, including null and internally truncated alleles, are generated, which can result in different phenotype. Indeed, mutant but not null mutation promotes expansion of neuronal progenitor cells and subsequent astrocytic glioma formation (Wang et al. 2009b). *Cre* transgenes under the control of various different promoters have been used for the conditional inactivation of *Brca1*. Thus, these models differ in the nature of mutation as well as cell types expressing mutant alleles.

The WAPCre or MMTVCreBrca1$^{\Delta 11}$ Model

Whey acidic protein (WAP) or MMTV promoter driven Cre expression leads to the deletion of exon 11 of the *Brca1* gene in the mammary gland (Xu et al. 1999). Mammary tumors developed in these mice at low frequency after the 10–13 months of latency. Introduction of heterozygous *p53* mutation significantly shortened tumor latency (Brodie et al. 2001). The absence of Brca1 turns the proliferation of ERα-positive cells to an autocrine fashion in contrast to that of the wild-type mice in that estrogen mainly induces proliferation of ERα-negative cells in the mammary gland (Li et al. 2007). This model indicates that exon 11-less Brca1 isoform is deficient in tumor suppression. Within exon 11, a unique ATM phosphorylation site exists. Mice homozygous for the phosphorylation mutant show aging phenotype and have elevated irradiation-induced tumorigenesis (Kim et al. 2009).

The WAPCre^{c}Brca1$^{\Delta 11}$p53$^{\Delta 5\&6}$ Model

Transgenic mice have been generated that carry a constitutively active whey acidic protein promoter-*Cre* transgene (*WAPCre*^{c}) that is expressed in both basal myoepithelial and luminal epithelial cells (Lin et al. 2004). Because expression of this *Cre* transgene does not require pregnancy, the interaction between tumor suppressor genes and ovarian hormones can be addressed. In contrast to wild-type and p53-deficient mammary epithelial cells, proliferation of Brca1- and p53-deficient mammary epithelial cells was uniquely sensitive to progesterone. Furthermore, progesterone receptors were stabilized in these cells. Antiprogesterone treatment in pubertal mice prevented or delayed mammary tumorigenesis (Poole et al. 2006). These mice develop mammary tumors with full penetrancy and a median tumor latency of 6.3 months (Shafee et al. 2008). In line with recent reports (Atchley et al. 2008; Tung et al. 2010), both ERα-positive and ERα-negative tumors were identified and p63 expression was up-regulated. These findings raise the possibility that the cells of origin in *BRCA1*-mediated breast carcinogenesis may be heterogeneous.

The K14-CreBrca1$^{\Delta 5\text{-}13}$p53$^{\Delta 2\text{-}10}$ Model

Keratin 14-*Cre* transgene mediated deletion of *Brca1* and *p53* genes affect epithelial tissues, including mammary myoepithelial cells and the skin epithelium (Liu et al. 2007). A high incidence of poorly differentiated ERα-negative basal tumors developed with a medium tumor latency of 7.0 months, which is consistent with the fact that the keratin 14 promoter drives robust expression in myoepithelial cells.

The β-lactoglobin-CreBrca1$^{\Delta 22\text{-}24}$p53$^{+/-}$ Model

In this model, mammary tumors developed with a median latency of 7.0 months (McCarthy et al. 2007). The *β-lactoglobin* is highly expressed in luminal epithelial cells of the mammary

gland but deletion of the C-terminus of *Brca1* leads to expression of basal-like markers in tumor.

The *Brca1* and *p53* mutations and the cell type(s) targeted differ in each of the above models. Together, the findings from these models and recent studies of *BRCA1*-associated breast cancer indicate that tumor cells can arise from multiple cell types in the mammary gland.

The K14-CreBrca2$^{\Delta 11}$p53$^{\Delta 2\text{-}10}$ Model

The exon 11-less *Brca2* embryos died before E9.5. The K14-*Cre* transgenic approach, however, restricts deletion of *Brca2* exon 11 and *p53* exons 2-10 to the skin, myoepithelial and luminal epithelial cells in the mammary gland, and some other tissues (Jonkers et al. 2001). Mammary and skin tumors developed at high frequencies in these mice (Jonkers et al. 2001). Overall, 5%–30% of adult mammary epithelial cells were targeted in this model; in contrast, 70%–90% were targeted in the *WAPCre*^{c} transgenic model (Lin et al. 2004).

The WAP-CreBrca2$^{\Delta 3\text{-}4/-}$ Model

Knocking a *Cre* gene into the *WAP* locus leads to a 77% incidence of mammary tumors after approximately 1.4-year latency (Ludwig et al. 2001). Surprisingly, invasive adenocarcinomas that are histologically uniform developed in these mice, in contrast to the different breast cancer subtypes seen in *BRCA2* carriers, in which loss of heterozygosity leads to tumorigenesis.

The MMTV-CreBrca2$^{\Delta 9\&10}$ Model

This *Brca2* mutation did not affect pregnancy-induced mammary epithelial expansion and involution (Cheung et al. 2004). Mammary adenocarcinomas developed after approximately 1.6 years latency, and mutations of the *p53* gene were identified in the tumors. Introduction of a *Brca2* mutant allele into *p53*$^{+/-}$ mice significantly skewed the tumor spectrum toward mammary adenocarcinoma.

Despite the fact that both BRCA1 and BRCA2 have a role in DNA double strand break repair, studies of genetically engineered *Brca1* and *Brca2* transgenic mice clearly reveal unique biological functions of these two proteins.

TARGETING ABERRANT PATHWAYS IN BREAST CANCERS

BRCA-deficient mammary tumors are sensitive to cisplatin but not doxorubicin treatment, which is consistent with the observation that BRCA-deficient cells have double strand break repair defects (Shafee et al. 2008). In a small clinical trial, cisplatin treatment for *BRCA1*-associated breast cancers appears to be promising (Byrski et al. 2009). Similarly, *BRCA1*- and *BRCA2*-associated ovarian cancers were responsive to cisplatin or carboplantin treatment; however, resistance developed (Cass et al. 2003). Secondary mutations that restore the open reading frame of *BRCA2* and its DNA repair function were identified in carboplatin-resistant tumors (Edwards et al. 2008; Sakai et al. 2008). Using a synthetic lethality approach, several labs have concluded that *BRCA* deficiency sensitizes cells to poly ADP-ribose polymerase (PARP) inhibitors (see Ashworth and Bernards 2008). Indeed, *Brca1*-associated mammary tumors have been shown to be highly sensitive to PARP inhibitors (Rottenberg et al. 2008).

Breast cancer stem cells became enriched on chemotherapy (Creighton et al. 2009). The CD29^{hi}CD24^{med} cancer stem cells in tumors developed in *WAPCre*^{c}*Brca1*$^{\Delta 11}$*p53*$^{\Delta 5\&6}$ mice expanded in cisplatin-refractory tumors (Shafee et al. 2008). In several breast cancer cell lines, expansion of CD44^{hi}CD24^{med} cells was associated with herceptin resistance (Reim et al. 2009). Strategies to target specific pathways required for the maintenance of cancer stem cells are being developed. For example, metformin, a diabetes drug, (Hirsch et al. 2009) and salinomycin have been shown to target breast cancer stem cells selectively (Gupta et al. 2009). However, differentiation of CD29^{hi} CD24^{med} cancer stem cells to CD29^{-} CD24^{+} cells is likely to be subject to feedback regulation similar to that observed in normal stem cells (Lander 2009). Understanding the mechanisms

underlying such feedback control will provide additional insights into how one might eradicate cancer stem cells.

CONCLUSIONS

Breast cancers are known for their heterogeneity, which is also reflected in the expression of cancer stem cell surface markers (Hwang-Verslues et al. 2009). There are several schools of thoughts on the origin of heterogeneity in cancer: stochastic events (Nowell 1976), the cell of origin (Visvader 2009), expansion of cancer stem cells (Pece et al. 2010), and inter tumor genetic diversity (Park et al. 2010). Results from different cancer models indicate that *Brca1* inactivation in different cell populations can lead to different tumor subtypes. As the tumor progresses, its cancer stem cell content may vary.

Several pathways critical to breast tumorigenesis have been identified and effective pharmacological interventions, including the targeting of steroid hormone receptors and ErbB2 pathways, are now available. To prevent cancer recurrence, new strategies such as targeting of DNA repair pathways, reactivating p53 (reviewed in Brown et al. 2009; Shangary and Wang 2009), and inhibiting cancer stem cells, are being developed. Meanwhile, advances in our understanding of breast cancer oncogenes and tumor suppressor genes will continue to provide insights critical to the development of novel anti-cancer approaches.

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