The Role of Ink4a/Arf in ErbB2 Mammary Gland Tumorigenesis


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ABSTRACT

Most human tumors display inactivation of the p53 and the p16INK4a/pRb pathway. The Ink4a/alternative reading frame (ARF) locus encodes the p16INK4a and p14ARF (murine p19ARF) proteins. p16INK4a is deleted in 40–60% of breast cancer cell lines, and p16INK4a inactivation by DNA methylation occurs in ≤30% of human breast cancers. In mice genetically heterozygous for p16INK4a or Ink4a/Arf, predisposition to specific tumor types is enhanced. Ink4a/Arf+/− mice have increased EpD-Myc-induced lymphomagenesis and epidermal growth factor receptor-induced gliomagenesis. ErbB2 (epidermal growth factor receptor-related protein B2) is frequently overexpressed in human breast cancer and is sufficient for mammary tumorigenesis in vivo. We determined the role of heterozygosity at the Ink4a/Arf locus in ErbB2-induced mammary tumorigenesis. Compared with mouse mammary tumor virus-ErbB2 Ink4a/Arf+/− mice, mouse mammary tumor virus-ErbB2 Ink4a/Arf+/−× Ink4a/Arf−/− mammary tumors showed increased p16INK4a−/−, reduced Ki-67 expression, and reduced cyclin D1 protein but increased mammary tumor apoptosis with no significant change in the risk of developing mammary tumors. These studies demonstrate the contribution of Ink4a/Arf heterozygosity to tumor progression is tissue specific in vivo. In view of the important role of Ink4a/Arf in response to chemotherapy, these transgenic mice may provide a useful model for testing breast tumor therapies.

INTRODUCTION

The complex interplay between tumor suppressor genes and tumor promoting genes has been simplified through the observation of the almost invariant loss of the p53 and p16INK4a/pRb pathway in human tumors (1). Disruption of the Rb pathway in human cancers occurs as a result of inactivation of Rb through mutation or deletion or inactivation of Rb function through viral sequestration, phosphorylation, or dysregulation of components controlling Rb phosphorylation (2). Upregulation of D-type cyclins, mutations of G1-specific, cdk catalytic units, and/or elimination of INK4s (inhibitors of cdk4) contribute to the phosphorylation status and inactivation of Rb (1). Importantly, the components of the Rb pathway behave as a common mutagenic target such that inactivation of one component in general excludes the other.

Inactivation of the Ink4a/ARF locus on human chromosome 9p21 occurs frequently in human cancer. This locus encodes p16INK4a and a second protein translated as an ARF, p19ARF (p14ARF in humans). Inactivation of Ink4a/ARF occurs through mutation, promoter silencing through methylation, and deletion (3). Homozygous deletion is the most common structural lesion resulting in the inactivation of the entire Ink4a/ARF locus, and inactivating point mutations of exon 1a are well described. Intragenic mutations can also affect the second exon common to p16INK4a and p19ARF, although point mutations affecting p16INK4a alone have been described (4). p16INK4a was identified as a protein binding to cdk4 that acts upstream of Rb to cause G1 arrest (5). p19ARF is also capable of inducing cell cycle arrest (6). In contrast with p16INK4a, p19ARF functions in a genetic pathway that involves p53 (7). The MDM2-induced degradation of p53 through the ubiquitin-proteosome pathway is antagonized by p19ARF (8–10).

Murine models of tumorigenesis are of considerable use in assessing the function of candidate tumor suppressors in vivo and developing models for assessing sensitivity to specific chemotherapeutic regimens. In vivo murine models with genetically engineered alterations of the Ink4a/Arf locus have recapitulated oncogenic cooperation observed in cell culture. Ink4a/Arf−/− mice have increased susceptibility to oncogenic effects of Ras, Myc, and an activated EGFR (11–13). Mice heterozygous for Ink4a have enhanced propensity to tumor development, compared with littermate controls (14, 15). Genetic heterozygosity for Ink4a/Arf also has strong and cell type-specific effects (12, 16). Both Ink4a/Arf−/− and Ink4a/Arf+/− melanocytes show defective senescence responses and altered cellular differentiation compared with wild-type melanocytes (17). As the induction of a cellular senescence program may contribute an important safeguard to tumorigenesis, Ink4a/Arf genetic heterozygosity may compromise this function in melanocytes. Lymphomas induced by Myc and gliomas induced by activated EGFR were produced as efficiently in Ink4a/Arf−/− or Ink4a/Arf−/−× Ink4a/Arf−/− mice (12, 13, 16, 18), suggesting Ink4a/Arf haploinsufficiency may have strong effects on tumorigenesis.

Breast carcinogenesis involves a complex interplay between oncogenes, growth factors, steroids, and inactivation of tumor suppressor genes (19, 20). ErbB2 is a member of the EGFR tyrosine kinase family and is overexpressed in 20–30% of human breast cancers (21). Overexpression of ErbB2 through amplification contributes to poor prognosis and may contribute to an aggressive phenotype (reviewed in Ref. 22). Mammary epithelial cell-targeted expression of either a constitutively active ErbB2 or a similarly altered human ErbB2 induces mammary adenocarcinoma with histological features resembling human breast carcinoma (23, 24). Inactivation of tumor suppressor genes in transgenic models of breast cancer has demonstrated oncogene-specific tumor suppressor interactions. Thus, p27Kip1 haploinsufficiency enhanced the rate for ErbB2-induced mammary tumorigenesis (25), and the loss of p21Cip1/INK4A accelerated Ras- but not Myc-induced mammary tumorigenesis (26). The role of Ink4a/Arf in...
mammary tumor suppression was unknown. Because of the important role of INK4a/ARF genetic heterozygosity in several tumor models and death of ErbB2/Ink4a−/− mice from lymphomas, the current studies assessed the role of Ink4a/Arf heterozygosity in ErbB2-induced mammary tumorogenesis in vivo.

MATERIALS AND METHODS

Antibodies, Reagents, and Western Blot Analysis. MCF7 cells were transfected with empty vector or expression plasmids encoding p16INK4a or p19ARF along with pMACS4.1. Transfected MCF7 cells were sorted using an autoMACS cell sorter (Miltenyi Biotec, Auburn, CA). Tissues isolated from MMTV-ErbB2 transgenic mice and cells were lysed in buffer containing 50 mM HEPES (pH 7.2), 150 mM NaCl, 1 mM EGTA, 1 mM EDTA, 1 mM DTT, 0.1% Tween 20, 0.1 mM phenylmethylsulfonyl fluoride, 2.5 mM/ml leupeptin, and 0.1 mM Na3VO4. Total protein was determined using the Bradford Method (Bio-Rad, Hercules, CA). Lysates (100 μg) were separated on 12% SDS-PAGE and blotted onto nitrocellulose. Membranes were blocked with 5% milk in PBS-T for 1 h at room temperature before exposure to primary antibodies to cyclin D1 (AB-3; NeoMarkers, Fremont, CA), p16INK4a, p19ARF, p53 (Santa Cruz Biotechnology, Santa Cruz, CA), or guanine dissociation inhibitor (GDI) (a gift of Dr. P. Bickel). Membranes were washed extensively in PBS-T and incubated with appropriate secondary antibodies conjugated to horseradish peroxidase (Santa Cruz Biotechnology). Membranes were washed extensively with PBS-T, and proteins were visualized using chemiluminescence. Fold increase in protein levels was plotted against a standard of 293T cells transfected with the expression vector for the relevant protein.

Plasmids and Reporter Gene Assays. The luciferase reporter gene constructions for human cyclin D1 (27) and expression plasmids for NeuT (28), pCMV-p16INK4a, pCMV-p16INK4a-p141, (29), and pDNA-p19ARF were described previously.

Cell culture, DNA transfection, and luciferase assays were performed as described previously (27). The MCF7 cells were maintained in DMEM with 10% FCS, 2 mM L-glutamine, 1% nonessential amino acids, 50 U/ml penicillin, and 50 μg/ml streptomycin. pCMV-p16INK4a or pCMV-p16INK4a-p141 were transfected into MCF7 cells using the calcium phosphate method (30). After 24 h, the cells were harvested and assayed for luciferase activity.

Cell Cycle and Ploidy Analysis. cessation of DNA synthesis was determined by BrdUrd incorporation (31). The MCF7 cells were transfected with p16INK4a or p19ARF, and the mean number of foci was determined (mean ± SE, n = 6).

Ink4a/Arf status of offspring. PCR reactions using three primers allowed for simultaneous detection of both the normal and mutant p16INK4a allele in a single reaction. These primers consisted of a common 5′ sense primer, p16-1F (5′-TCCCTCTACATTTCGCG-3′), and two 3′ antisense primers, p16-1R (5′-GGGACGCACATGCTGC-3′) and p16-Nul (5′-CTAGTG-GAGCAGCTGACTT-3′). Reactions were run for 30 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min.

Immunohistochemistry. Immunostaining of the mammary tissue from seven transgenic animals was performed as described previously (32). In each tumor, 500 cells were scored for nuclear cyclin D1 staining. Tissues were fixed in 4% paraformaldehyde, blocked in paraaffin, sectioned at 5 μm, and stained with H&E or used for immunohistochemistry. Sections were deparaffinized in Histo-Clear, rehydrated through a graded series of ethanol, and washed in PBS. After the antigen retrieval by microwave irradiation in 0.01 M trisodium citrate buffer (pH 6.0), the sections were preblocked with 1% horse serum for 1 h, washed, and incubated with a given primary antibody at room temperature for 1 h (cyclin D1 and p16INK4a) or at 4°C overnight (Ki-67). An FITC-conjugated or lissamine-rhodamine-conjugated secondary antibody was added to the sections after washing. Nuclei were counterstained with 4, 6-diamino-2-phenylindole hydrochloride. Samples were imaged with an Olympus IX 70 inverted microscope. In each tumor, 500 cells were scored for nuclear cyclin D1, Ki-67, and p16INK4a staining. Cyclin D1 was detected using the rabbit pAB antibody Ab-3 (NeoMarkers) at 1:200 dilution. Anti-Ki-67 IgG (rabbit pAb antibody) was purchased from Novocasta, Ltd. (Newcastle Upon Tyne, United Kingdom) and used at a dilution of 1:500. Anti-p16INK4a IgG (mouse monoclonal antibody) was obtained from Santa Cruz Biotechnology and used at 1:100 dilution.

Cell Cycle and Ploidy Analysis. Tumor cell cycle parameters were determined using laser scanning cytometry. Tumor samples fixed in formalin and embedded in paraffin were sectioned (5 μm) and placed on slides. Samples were analyzed for DNA content using Laser Scanning Cytometry (CompuCyte, Cambridge, MA). Flow Cytometric Analysis of S phase, tumor samples were processed by standard methods using propidium iodide staining...
of tumor cell DNA (26). Each sample was analyzed by flow cytometry with a 
FACScan Flow Cytometer (Becton-Dickinson Biosciences, Mansfield, MA) 
using a 488-nm laser. Histograms were analyzed for cell cycle compartments 
using ModFit version 2.0 (Verity Software House, Topsham, ME). A mini-
mum of 20,000 events was collected to maximize statistical validity of the 
compartamental analysis.

Tumor Onset, Growth Measurements, and Statistical Analysis. 
Animals were examined visually for the presence of tumors twice weekly. Tumor 
incidence in the MMTV-ErbB2/Ink4a/Arf−/− versus MMTV-ErbB2/Ink4a/ 
Arf−/− mice was compared. Differences in the age at tumor onset between 
mice of the same genotypic groups were compared using Cox proportional 
hazards analysis (34). Once a tumor was detected and had reached 0.5 cm in 
size, its growth was monitored for an additional 5 weeks, after which the 
animal was sacrificed. Tumor measurements were taken approximately every 
7 days using hand calipers, and tumor volume was calculated according to the 
formula: tumor volume (mm³) = (W² × L)/2, where W is width (mm) and L 
is length (mm). Tumor growth curves were generated by plotting tumor 
volume measurements against time. Only female animals were included in this 
study. Kaplan-Meier curves were used to compare death rates and difference 
in survival time assess using the Log-rank test (35).

RESULTS

p16INK4a and p19ARF Inhibit Focus Formation and Cyclin D1 
Abundance in MCF7 Breast Cancer Epithelial Cells. p19ARF in-
hibits Ras-dependent transformation in NIH3T3 cells (6) but requires 
p53 for cell cycle arrest and apoptosis (36). In the human MCF7 breast 
cancer cell line, which expresses ErbB2 and p53, both p16INK4a and 
p19ARF inhibited the formation of foci in soft agar (Fig. 1 , A and B). 
In fibroblasts, p16INK4a inhibits S phase entry, functioning in early but 
not late G1 (29, 37). MCF7 cells were transfected with expression 

vectors for either p16INK4a or p19ARF and subjected to MACS sorting 
with subsequent FACS analysis and Western blotting. The abundance 

Fig. 2. ErbB2-induced activity of the cyclin D1 promoter is repressed by p16INK4a. In 
A, luciferase activity of the cyclin D1 promoter (−1745 CD1LUC) was determined 
In MCF7 cells transfected with expression vectors for oncogenic ErbB2 (A and B) either 
in the presence or absence of cotransfected expression vectors for p16INK4a. The data are 
shown as mean ± SE for n = 8 separate experiments.

Fig. 3. Increased cyclin D1 abundance in Ink4a/Arf−/− mammary tumors and tissues. ErbB2 mammary tumors from 
MMTV-ErbB2 Ink4a/Arf+ or MMTV-ErbB2 Ink4a/Arf−/− mice were analyzed for cyclin D1 abundance by Western 
blotting, and mean abundance is shown (n = 7). The data are 
normalized for the loading control guanine dissociation inhibitor as described in “Materials and Methods.”

of cyclin D1 is rate limiting in the entry of cells into the DNA 
synthetic (S phase) in several cell types (38), including MCF7 cells 
(39), and was therefore assessed in p16INK4a- or p19ARF-transfected 
cells. Expression of p16INK4a and p19ARF was detected by Western 
blotting in cells transfected with the corresponding expression vectors 
for these proteins. Normalized for the loading control guanine disso-
ciation inhibitor, cyclin D1 protein levels were reduced 42 ± 2% by 
p16INK4a. p19ARF-transfected MCF7 cells showed a 68 ± 4.5% re-
duction in cyclin D1 abundance, and the p19ARF target p53 was induced 4-fold (Fig. 1C).

**p16^INK4a and p19ARF Inhibit ErbB2-induced Cyclin D1 Promoter Activity.** To consider the possibility that p16^INK4a may inhibit cyclin D1 abundance through inhibition of the cyclin D1 promoter, the full-length cyclin D1 promoter linked to a luciferase reporter was assessed for activity in MCF7 cells. Previous studies had shown a 40% reduction in cyclin D1 abundance by antisense, reduced ErbB2-induced mammary tumor growth in nude mice (28), and cyclin D1-deficient mice are relatively resistant to ErbB2- and Ras-induced mammary tumorigenesis. Cyclin D1 abundance and promoter activity was induced by coexpression of ErbB2 (Fig. 2A), as shown previously (28), and p16^INK4a expression inhibited ErbB2 induction of the cyclin D1 promoter (Fig. 2A). p16^INK4a also inhibited basal activity of the cyclin D1 promoter (Fig. 2B) but did not inhibit the activity of either the c-fos promoter or the pα1LUC vector (data not shown).

To determine whether reduced p16^INK4a levels were associated with increased abundance of cyclin D1 in vivo, we examined the tissues of Ink4a/Arf^+/− mice (31). We examined the mammary tumor epithelium derived from either Ink4a/Arf^+/− or litter mate Ink4a/Arf^−/− mice in which animals expressed the MMTV-ErbB2 transgene. Mammary tumors from nine separate animals of each genotype were assessed. Cyclin D1 protein levels were increased a mean of 3-fold (2.7-fold, p<0.01, n=9) in the ErbB2 expressing MMTV-ErbB2 tumors (mean S phase, 27% ± 7%, n=9; Fig. 3). A significant increase in the DNA synthetic phase was observed in the MMTV-ErbB2 Ink4a/Arf^+/− mammary tumors (mean S phase, 27% ± 7%, n=9; Fig. 3, B and C). Previous studies of MMTV-Ras mammary tumors had demonstrated an increase in the proportion of mammmary tumor cells in S phase on loss of p16^INK4a (26). The increase in S phase of the tumors on reduction in abundance of Ink4a/Arf is consistent with the G1-S cell cycle checkpoint function of p16^INK4a.

In view of the altered rate of DNA synthesis, we determined the impact of Ink4a/Arf^+/− on the ErbB2 tumor DNA content (Fig. 5D). Approximately 45% of the MMTV-ErbB2 tumors were diploid, with a similar frequency in the MMTV-ErbB2 Ink4a/Arf^+/− tumors (Fig. 5E). These findings are consistent with previous studies of Eμ-Myc
lymphomas in which Ink4a/Arf status did not affect tumor DNA content (13).

Additional analysis of p53 function was performed in the mammary tumors. p19ARF acts as an essential intermediate in oncogene signaling to p53 (9, 36, 40, 41) as part of a p53-dependent failsafe mechanism to counter mitogenic signaling. Oncogenes fail to activate p53 in ARF-null cells and promote proliferation without apoptosis (36, 41). As p53 transcriptionally activates p21Cip1/WAF1, we assessed p53 and p21Cip1/WAF1 abundance in the mammary tumors. Immunoreactive p53 was detectable and correlated with p21Cip1/WAF1 abundance in MMTV-ErbB2 Ink4a/Arf+/− and Ink4a/Arf+/− mammary tumors (Fig. 6, A and B). Although the relative abundance of p53 and p21Cip1/WAF1 was on average higher in tumors with later onset, the correlation was not statistically significant, nor were there significant differences between the Ink4a/Arf+/− and Ink4a/Arf+/− group. These findings are consistent with the similar tumor DNA content of the MMTV-ErbB2 Ink4a/Arf+/− and Ink4a/Arf+/− mammary tumors.

As revealed by histological staining and terminal deoxynucleotidyl transferase-mediated nick end labeling, MMTV-ErbB2 tumors had clearly detectable apoptosis, which was significantly increased in the MMTV-ErbB2 Ink4a/Arf+/− tumors (Fig. 7, A and B). The murine mammary adenocarcinomas strongly resemble the histopathology of human breast comedo carcinoma. Histological analysis was performed using the Annapolis classification of murine mammary tumors. All tumors were of intermediate or intermediate/small cell type with no significant difference between MMTV-ErbB2 Ink4a/Arf+/− and MMTV-ErbB2 Ink4a/Arf+/− tumors (Fig. 7C). In view of the increased DNA synthesis and apoptosis in the MMTV-ErbB2 Ink4a/Arf+/− tumors, we assessed tumor growth rates using serial caliper measurements. Tumor growth rates were identical between MMTV-ErbB2 Ink4a/Arf+/− and MMTV-ErbB2 Ink4a/Arf+/− tumors (Fig. 7D).

Kaplan-Meier curves were constructed to compare death rates from all causes (Fig. 8A), all tumors (Fig. 8B), and breast tumor (Fig. 8C). The MMTV-ErbB2 Ink4a/Arf+/− animals died of lymphomas at T50 = 200 days, consistent with previous studies of Ink4a/Arf+/− mice (31). Although the T50 for the onset of mammary tumorigenesis by Ink4a/Arf genotype suggested Ink4a/Arf+/− (228 days, n = 45), developed tumors earlier than Ink4a/Arf+/− (263 days, n = 30), these differences did not reach significance. None of the MMTV-ErbB2 Ink4a/Arf+/− nor MMTV-ErbB2 Ink4a/Arf+/− animals developed lymphomas. The median survival time (Ink4a/Arf+/−) 240 days, n = 45, Ink4a/Arf+/− 259 days, n = 30) was not significantly different by Log-rank test (Ref. 35; χ2 with 1 degree of freedom = 0.6015, P = 0.438).

Using the Cox proportional hazards model (34), the hazard ratio associated with Ink4a/Arf+/− was 1.22 with 95% confidence interval (0.73, 2.02). Assumption of proportionality was assessed, and a rel-
Ink4a/Arf significant difference in the role of developing tumors compared with The ability to determine whether loss of both infected mammary tumor onset was obscured by the onset of lymphoma (14, 15). Genetic heterozygosity for Ink4a/Arf contributed to enhanced gliomagenesis induced by an activated EGFR (12) and increased lymphomagenesis induced by Eμ-Myc (12, 13, 16, 18). In recent studies, Ink4a/Arf genetic heterozygosity contributed to defective senescence and altered the differentiation program in melanocytes (17). In view of the current findings, together, these studies suggest genetic heterozygosity for Ink4a/Arf has cell type-specific effects.

In the current studies in MCF7 cells, p16INK4a inhibited contact-independent growth, DNA synthesis, and expression of cyclin D1. Cyclin D1 abundance plays a key role in MCF7 cell growth. Both p16INK4a and p19ARF inhibited cyclin D1 abundance in MCF7 cells. The 40% reduction in cyclin D1 protein, in p19ARF-transfected MCF7 cells, may have been mediated through p53, which has been reported to repress cyclin D1 expression (47). Cyclin D1 abundance was increased in Ink4a/Arf–/+ tissues and mammary tumors, associated with reduced p16INK4a. Reciprocal expression of cyclin D1 and p16INK4a has also been reported in human breast cancer (44). ErbB2-induced activity of the cyclin D1 promoter was inhibited by p16INK4a. Repression of the cyclin D1 promoter was specific for p16INK4a, which failed to repress the c-fos promoter or a synthetic serum response element as shown previously (37). Although the current studies suggest p16INK4a can repress cyclin D1 promoter activity and expression of cyclin D1, whether this is dependent on cdk or pRb remains to be determined.

DNA synthesis by FACS and Ki-67 staining was enhanced in the ErbB2 Ink4a/Arf–/+ mammary tumors compared with ErbB2 Ink4a/Arf–/– tumors, suggesting Ink4a/Arf functions as an inhibitor of oncogene-induced mitogenesis in vivo. These in vivo findings may appear paradoxical compared with studies in cultured cells in which oncogenic signaling pathways induce components of the Ink4a/Arf locus and cell cycle arrest (40, 48) but are consistent with other recent in vivo studies. K-Ras, for example, induced proliferation of type II pneumocytes and lung adenocarcinoma in transgenic mice (49, 50). By contrast, in cultured cells, p16INK4a is induced by oncogenic Ras, v-abl (51), E1A (36), and activated MAP kinase (52). Ras or constitutively active MAP/extracellular signal-regulated kinase kinase-induced senescence and arrest in primary murine fibroblasts requires p16INK4a (48). Although Ras induced a growth arrest in cultured cells, accompanied by increased levels of p16INK4a, p19ARF, or p53, this growth arrest was abrogated in the absence of either the Ink4a/Arf or p53 locus (31). Thus, the increased mitogenesis observed in our studies is consistent with findings that cells lacking function of either p53 or p16INK4a undergo uncontrolled mitogenesis and transformation in response to activated Ras-MAP/extracellular signal-regulated kinase kinase signaling (48).

DNA synthesis and cyclin D1 abundance were increased in Ink4a/Arf–/+ mammary tumors; however, increased apoptosis accompanied these tumors, and tumor growth rates were unaltered. Tumor growth rates may be affected by local epithelial events or the heterotypic signals from vascular and inflammatory cells (53, 54). Although no role for Ink4a/Arf in tumor macrophage function has been identified to date, the role of heterotypic signaling in these tumors remains to be determined. In the current studies, tumor ploidy was not significantly altered by heterozygosity for Ink4a/Arf. Loss of p53 function, and hyperproduction of Myc or Cdk4 protein, have been reported to induce hyperploidy (55). Consistent with a role for p53 in genomic stability, tumors arising in ErbB2/p53–/–/2H transgenic mice showed increased aneuploidy compared with ErbB2-induced mammary tumor (56). p19ARF does not appear to control the p53 functions involved in maintaining chromosome stability (13). Thus, although mutations in p53 lead to aneuploidy in culture, a mutation that eliminates p19ARF results in pseudodiploid immortalized cells (7). Eμ-Myc transgene-induced lymphomas remained diploid in the Ink4a/Arf–/+ background but aneuploid in p53–/– (13). The findings
of the current study are consistent with previous studies suggesting p19ARF does not control p53 functions involved in maintaining chromosomal stability.

Reintroduction of p16INK4a into breast cancer epithelial cells induces a cell cycle arrest (57, 58). Breast cancer cell lines are deleted of p16INK4a in ~30% of cases (42), and DNA methylation, an alternate mechanism of p16INK4a inactivation, occurs in ~30% of human breast cancers (43, 44). Primary human breast tumors revealed loss of heterozygosity or allelic imbalance at the Ink4a/Arf locus in 58% of cases. Mutations were not detected in the remaining allele, suggesting Ink4a/Arf may be haploinsufficient for breast tumorigenesis (59). In contrast, however, p16INK4a is overexpressed in primary breast cancer, correlating with poor prognosis (44–46). Because Ink4a/Arf heterozygosity did not affect the rate of ErbB2-induced mammary tumorigenesis, the current studies provide support for an emerging model in which tumor suppressors function in a cell type- and oncogene-specific manner. p27kip1 haploinsufficiency enhanced the rate for ErbB2-induced mammary tumorigenesis (25). p21WAF1/CIP1 deficiency had opposite effects in tumors arising from MMTV-Ras and MMTV-Myc transgenic mice (26). The loss of p21WAF1/CIP1 accelerated Ras-induced mammary tumorigenesis (26). Genetic p21WAF1/CIP1 deficiency, however, did not accelerate Myc-induced mammary tumorigenesis and decreased DNA synthesis in mammary tumors in vivo (26). Many of the genes that control tumor apoptosis in turn influence treatment sensitivity. Chemotherapy and radiation-induced damage can initiate a series of responses, including apoptosis, cell cycle checkpoints, cellular senescence, and mitotic catastrophe. The integrity of these damage response pathways also influences treatment sensitivity. The genetically engineered mice as described herein may therefore recapitulate the complex genetic changes that occur during tumorigenesis and, as such, may be of value in testing chemotherapeutic responses.

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