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## Mammary Tumorigenesis following Transgenic Expression of a Dominant Negative CHK2 Mutant

Eunice L. Kwak<sup>1</sup>, Sang Kim<sup>1</sup>, Jianmin Zhang<sup>1</sup>, Robert D. Cardiff<sup>2</sup>, Emmett V. Schmidt<sup>1</sup>, and Daniel A. Haber<sup>1</sup>

<sup>1</sup> Massachusetts General Hospital Cancer Center and Harvard Medical School, Charlestown, Massachusetts

<sup>2</sup> Center for Comparative Medicine, University of California-Davis, Davis, California

### Abstract

A truncating allele of the cell cycle checkpoint kinase *CHK2* is present in 1% of the population, conferring a moderate increase in breast cancer risk, and inactivation of *chk2* enhances mammary tumorigenesis in mice with targeted inactivation of *brca1*. We used the mouse mammary tumor virus (MMTV) promoter to target expression of a kinase-dead *CHK2* allele (D347A). Mammary tumors, of predominantly micropapillary histology, developed in 40% of *MMTV-CHK2-D347A* transgenic mice with an average latency of 20 months. Tumors metastasized to lung and spleen; tumor-derived cell lines were frequently aneuploid and showed suppression of irradiation-induced p53 function. Primary hematopoietic malignancies were also observed in the spleen, another site of MMTV expression. The increased rate of tumor formation in *MMTV-CHK2-D347A* mice, compared with the relatively low incidence in *chk2*-null mice, provides a model to study modifiers of *CHK2*-dependent transformation.

### Introduction

*CHK2* (also known as *CHEK2*) encodes a serine/threonine kinase that activates cell cycle checkpoints in response to DNA damage, thereby contributing to the maintenance of genomic integrity (1–3). In yeast (*S. pombe*), the Chk2 orthologue Cds1 mediates the G<sub>2</sub>-M checkpoint, phosphorylating Cdc25C in response to activation by the ATM/ATR orthologue Rad-3. In mammalian cells, *CHK2* is activated by ATM primarily in response to double-stranded DNA damage although the downstream events triggered by *CHK2* activation seem to be more complex.

Genetic evidence of *CHK2* inactivation in human cancer syndromes supports its relevance in DNA damage response pathways. We initially discovered germ line *CHK2* mutations as part of a screen of checkpoint regulators in families with variants of the multi-cancer Li-Fraumeni syndrome that lack characteristic mutations in *p53* (4). Subsequent studies by an international *CHEK2* consortium showed that a specific truncating mutation, 1100delC, is present in 1% of the population and in 5% of cases with familial breast cancer, suggesting that it confers a low to moderate increase in cancer risk (5). Follow-up studies involving >10,000 breast cancer cases have provided further support for the 1100delC *CHK2* mutation as a “modifier” of cancer risk, even in women unselected for family history (6). In aggregate, epidemiologic studies have

thus identified *CHK2* as a low-penetrance gene, conferring predisposition to breast cancer and potentially to other tumors, including prostate cancer (7–9).

The cellular mechanisms underlying *CHK2* function have been defined in cultured cell lines in which activation of *CHK2* by double-stranded DNA damage results in the phosphorylation of a variety of targets, including *CDC25A* (serine-123), *CDC25C* (serine-216), *p53* (serine-20), and *BRCA1* (serine-988), among others. For example, in U2OS osteosarcoma cells, ionizing radiation–induced phosphorylation of *p53* on serine-20 by *CHK2* seems to mediate *p53* stabilization and *G*<sub>1</sub> cell cycle arrest (10). In U2OS and other cancer cell lines, *CDC25A* degradation is triggered by its phosphorylation by *CHK2* in response to DNA damage, thereby preventing activation of cyclin E–associated *CDK2* and activating the S-phase checkpoint (11). In embryonic stem cells, maintenance of *G*<sub>2</sub> cell cycle arrest after irradiation is mediated by phosphorylation and inactivation of *cdc25c* by *chk2* (12). A link to *BRCA1* is also supported by radiation-induced phosphorylation of serine-988 by *CHK2*, leading to the dispersal of *BRCA1* nuclear dots (13). Nonetheless, the physiologic targets of *CHK2*-mediated phosphorylation that are responsible for its suppression of tumorigenesis *in vivo* remain to be defined.

Two mouse models of *chk2* inactivation have been reported: the first targeting the COOH-terminal kinase domain (14) and the second resulting in an NH<sub>2</sub>-terminal truncation (exons 2–5; ref. 15). *Chk2*-null thymocytes derived from both have abnormalities in ionizing radiation–induced *p53*-mediated apoptosis. A modest increase in tumor susceptibility was reported in one strain following dimethylbenzanthracene-induced skin carcinogenesis (15) whereas the other developed rare lymphomas and a single case of mammary carcinoma (14). More recently, inactivation of *chk2* was found to enhance mammary tumorigenesis induced by mammary-specific targeting of *brca1* (16). This observation contrasts with human epidemiologic studies in which increased prevalence of *CHK2* 1100delC in familial breast cancer cohorts, but not in *BRCA1*-linked pedigrees, was proposed as evidence of functional redundancy between the *BRCA1* and *CHK2* pathways (5,6). Interestingly, Kim et al. (17) have recently created mice lacking the murine equivalent of *brca1* serine-988, the phosphorylation target of activated *chk2*. These mice were predisposed to the development of late-onset tumors, thus supporting a direct role of *chk2* on *brca1* function.

Here, we tested the role of *chk2* in mammary tumorigenesis by targeted overexpression of a kinase-dead D347A mutant, which functions as a dominant-negative protein (10,11,13). Following serial pregnancies to drive expression of the mouse mammary tumor virus (MMTV) promoter, mice harboring the transgene reproducibly developed multiple mammary carcinomas, a subset of which showed metastases to lung and spleen. Immortalized cell lines generated from *CHK2*-D347A–derived mammary tumors showed attenuation of the *p53*-dependent response to DNA damage. These results support a role for *CHK2* inactivation in breast tumorigenesis.

## Materials and Methods

### DNA constructs and transgenic mice

The transgenic expression construct was derived from human *CHK2* cDNA encoding the D347A mutation, driven by the *MMTV-LTR*, with 650 bp of *β-globin* intron sequence and 580 bp of *hGH* poly(A) sequence. The linearized *MMTV-CHK2-D347A* construct was injected into fertilized FVB/N pronuclei (Beth Israel Deaconess Hospital Transgenic Facility) resulting in seven transgenic mice that contained the transgene as assessed by Southern blot of tail DNA. Immunoblot analyses of mammary and splenic tissue taken from progeny postpartum females confirmed the expression of the *CHK2* transgene (human-specific *CHK2* antibody from Santa Cruz Biotechnology, Santa Cruz, CA).

All mice were provided routine care in accordance with institutional guidelines and genotyped using standard procedures. Colonies of FVB/N-Tg(MMTV-CHK2-D347A) mice were repeatedly bred to wild-type FVB mice (Taconic Farms, Germantown, NY) and monitored for the development of mammary tumors. Mice were sacrificed on tumor development or at 2 years of age. FVB/N-Tg(MMTVneu) 202 Mu1 mice were obtained from The Jackson Laboratory (Bar Harbor, ME) and mated to Tg(MMTV-CHK2-D347A) mice to yield doubly heterozygous Tg(MMTV-CHK2-D347A/c-neu) or heterozygous c-neu transgenic lines. These mice were not mated during the period of tumor development.

### Cell culture and cytogenetic studies

After dissection, tissues from mammary tumors, mammary glands, spleens, lungs, liver, and brain were immediately placed in freshly prepared 4% paraformaldehyde for subsequent histologic analyses or were frozen on dry ice and placed at  $-80^{\circ}\text{C}$  for biochemical studies. To establish mammary tumor cell lines, tumor fragments were minced in MAM media (DMEM/F12, Life Technologies, Inc., Gaithersburg, MD; epidermal growth factor, 5 ng/mL, Sigma; insulin 1  $\mu\text{g}/\text{mL}$ , Sigma; fetal bovine serum, 2.1%; penicillin/streptomycin, 1%) and treated with collagenase or trypsin-EDTA (Life Technologies). Attached cells were maintained in MAM media. For karyotype analysis, proliferating cultures of CHK2-D347A, c-myc, and c-neu tumor cells were submitted to the Dana-Farber/Harvard Cancer Center Cytogenetics Core facility for Giemsa banding. For BRCA1 staining, cultured tumor cells were irradiated and stained with antibody to BRCA1 phosphoserine-988 (Santa Cruz Biotechnology) followed by immunofluorescence microscopy.

### CHK2 expression and p53 analysis

To show the dominant-negative properties of CHK2-D347A *in vitro*, primary human foreskin fibroblasts were retrovirally infected with the *MSCV-SVG* vector or *MSCV-SVG* containing *CHK2-D347A*, and immunoblotting of lysates was done using antibody recognizing human p53 (Santa Cruz Biotechnology). Following tumor development, frozen mouse tissue was pulverized with a chilled mortar and pestle, lysed in SDS sample buffer, followed by SDS-PAGE and probing of immunoblots with human-specific anti-CHK2 antibody (Santa Cruz Biotechnology). Cultured c-myc, c-neu, or CHK2-D347A derived mammary tumor cells were lysed in SDS sample buffer and immunoblotted with antibodies to CHK2, CDC25A (Santa Cruz Biotechnology), p53 (Novocastra), or tubulin (Upstate, Charlottesville, VA). For Northern blots, total RNA was isolated from cultured tumor cells (RNeasy kit, Qiagen, Valencia, CA) and analyzed by standard methods. All experiments involving  $\gamma$  irradiation were done using a cesium irradiator. Big Dye (ABI) sequencing reactions were done on genomic DNA using primers spanning each exon of *p53*.

## Results and Discussion

Substitution of alanine for aspartic acid at codon 347 within the kinase domain of CHK2 inactivates its enzymatic activity and has been shown to function as a dominant negative in cultured cell lines (10,11,13). Accordingly, we generated a CHK2-D347A construct (Fig. 1A) and confirmed its effect following retroviral infection into primary human foreskin fibroblasts. Ionizing radiation-induced stabilization of p53 (Fig. 1B) and induction of the target genes *MDM2* and *p21* (not shown) were suppressed in cells expressing CHK2-D347A compared with vector-infected cells. An MMTV-driven CHK2-D347A construct was used to generate seven founder transgenic mice, of which two with the highest expression of the dominant-negative construct within MMTV-LTR target tissues (including mammary gland and spleen in both lines) were selected for further study (Fig. 1C).

Female transgenic mice expressing MMTV-CHK2-D347A were monitored for the development of mammary tumors (Fig. 1D and E). Of 70 females bred repeatedly to maximize expression of CHK2-D347A, 27 (39%) developed at least one histologically confirmed mammary carcinoma. The majority of these mice developed multiple tumors with an average latency of 20 months (range 15–26 months; Fig. 1D, transgenic line 1). Of six mice that were sacrificed at 24 months without gross evidence of mammary tumors, three (50%) had mammary hyperplasia on histologic analyses. Thirty-three females from CHK2-D347A line 2 were also studied, of which 10 (30%) developed mammary carcinomas with an average latency of 21.5 months (range, 18.5–26 months; Fig. 1D). Of the 37 mice from both lines that developed mammary tumors, metastatic lesions to the lung were detected in five cases and a metastatic lesion to the spleen was detected in one case (Table 1).

All tumors tested expressed the *CHK2-D347A* transgene, which was readily distinguished from endogenous mouse *chk2* using a human-specific antibody. The predominating histology of mammary carcinomas was micropapillary in at least 9 of 47 cases analyzed from both transgenic lines and papillary in an additional 3 cases (Table 1; Fig. 2). Micropapillary tumors are characterized by epithelial cells with short papillae lacking a distinct fibrovascular stalk, an unusual histology that has been previously described in STAT 5 and CK2 $\alpha$ -driven mammary tumors (18,19) and is clearly distinguishable from that of the well-studied *erbB2*-driven or *c-myc*-driven mammary tumor models (20,21). A subset of *CHK2-D347A*-driven tumors had other histologies, including epithelioid, spindle cell, intermediate cell, glandular, and adenosquamous. Mammary hyperplasia was common in mice without tumors as well as in the unaffected glands of those that developed carcinomas. Interestingly, mammary tumors from conditional *brca1*-null mice with and without p53 inactivation also showed a variety of histologic phenotypes, ranging from hyperplasia to high-grade invasive adenocarcinomas with various morphologic features (22).

The FVB strain is commonly used in the creation of transgenic mice because of its reproductive fitness and large zygotic pronuclei (23). Serial mating and advancing age in FVB mice have been linked recently to the development of pituitary adenomas, which secrete prolactin and contribute to mammary hyperplasia and, rarely, formation of noninvasive tumors (24). As expected, pituitary abnormalities were observed in a subset of *CHK2-D347A* mice; of 45 pituitaries analyzed, 28 had a pituitary adenoma and 6 showed hyperplasia. However, these pituitary abnormalities were not required for mammary tumor formation: of 11 *CHK2-D347A* mice with histologically normal pituitary glands, 5 developed mammary tumors and 2 had mammary hyperplasia. Furthermore, the characteristics of spontaneous mammary tumors in FVB mice differ from those observed in MMTV-*CHK2-D347A* strains. Whereas mammary hyperplasia is common under the influence of prolactin, mammary tumors are infrequent, occur singly, have a predominantly adenosquamous histology, and do not metastasize (24,25). The frequent occurrence of multiple tumors in MMTV-*CHK2-D347A* mice, their metastatic potential, and distinct histology all point to a *CHK2*-driven effect. Nonetheless, it is possible that the combination of mammary gland proliferation associated with serial breeding, together with expression of *CHK2-D347A*, may underlie the striking mammary carcinogenesis observed here, which was not evident in *chk2*-null mice (see below).

Whereas this study was designed to investigate the contribution of *chk2* function in mammary tumorigenesis, mice from the two transgenic lines also developed primary abnormalities of the spleen, an organ that is also targeted by MMTV and which showed high levels of *CHK2-D347A* expression in both lines. Spleens from 9 of 31 mice analyzed had hematopoietic abnormalities ranging from myeloid hyperplasia to frank leukemia (Table 1). Such MMTV-driven abnormalities in the spleen have also been reported with *c-myc* transgenic mice (21). Of note, *chk-2* null mice were reported to develop spontaneous lymphomas (15). Together with the

hematopoietic abnormalities in CHK2-D347A mice, these observations support a role for CHK2 in tumorigenesis beyond that observed in mammary tissue.

The role of CHK2 as a guardian of genomic stability and the epidemiologic data indicating that CHK2 inactivation may function as a genetic modifier suggested that its loss may facilitate tumor formation driven by other stimuli. Therefore, we tested for potential interactions between MMTV-CHK2-D347A and a well-characterized mouse model of mammary tumorigenesis, MMTV-c-neu overexpression (26). Sixty-four virgin CHK2-D347A/c-neu double heterozygous mice and 59 c-neu heterozygous mice were monitored for tumor development in the absence of serial mating. The average tumor-free survival was 44.2 weeks in the CHK2-D347A/c-neu mice as compared with 49.5 weeks in the c-neu mice ( $P = 0.0093$ ; Fig. 1E), indicating a potential collaborative effect between *chk2* inactivation and c-neu overexpression in determining mammary tumor predisposition. The spectrum of histology observed in tumors from double heterozygous mice reflected that of c-neu rather than CHK2-D347A.

To study the characteristics of CHK2-D347A-driven tumor cells, we established cell lines from 20 of 24 (83%) primary mammary tumors. Remarkably, such cell lines were readily established from CHK2-D347A tumors, which is unusual for most mouse mammary tumor models. In contrast, the single cell line that could be generated from a mammary tumor arising spontaneously in a wild-type FVB mouse with a pituitary adenoma underwent senescence within three passages. As previously noted, immortalized tumor cell lines were infrequently generated from MMTV-c-neu tumors (10%) although establishment of MMTV-c-myc cell lines was generally successful (90%). CHK2-D347A-derived cell lines showed persistent expression of the transgene by both immunoblotting and reverse transcription-PCR analyses (data not shown).

Given the role of CHK2 in the maintenance of genomic stability, we first evaluated the chromosomal integrity of cells from CHK2-D347A tumors. Karyotypes from early-passage cells derived from these tumors showed varying degrees of abnormality, ranging from gain or loss of single chromosomes to gross aneuploidy (Fig. 3A). By comparison, metaphase chromosomes from c-neu-driven tumor cells were predominantly euploid whereas chromosomes from c-myc-driven tumor cells were more frequently aneuploid, consistent with previously published karyotypic analyses (27,28). No chromosomal abnormalities characteristic of BRCA1 defects, such as quadriradial chromosomes (29), were detected in CHK2-D347A tumor cell lines.

The development of a mouse model for mammary tumorigenesis also made it possible to test the integrity of specific cellular pathways in epithelial tumor cells driven at least in part by *chk2* inactivation. Previous analyses of downstream pathways in cells with inactivated *chk2* have primarily been carried out in normal fibroblasts (14,15). We first tested the integrity of the p53 pathway using nine cell lines derived from CHK2-D347A mouse tumors. None of these lines had acquired a mutation in p53 either *in vivo* or during culture *in vitro* as determined by nucleotide sequencing (data not shown). Gamma irradiation of these cells resulted in reduced stabilization of p53 protein levels as compared with cell lines derived from either MMTV-c-myc or MMTV-c-neu tumors (Fig. 3B). Irradiation of CHK2-D347A tumor cells also resulted in reduced induction of the p53-target gene *mdm2* (Fig. 3C), consistent with the partial compromise of p53-dependent ionizing radiation-induced DNA damage signals reported in *chk2*-null thymocytes (14,15). Interestingly, levels of p53 protein and *mdm2* mRNA were generally elevated in the absence of ionizing radiation in CHK2-D347A tumor-derived cells, possibly reflecting baseline genomic instability in these cells. Whereas the function of p53 as a regulator of *mdm2* was compromised in CHK2-D347A tumor cells, we could not detect a reproducible functional alteration of p53-dependent cell cycle checkpoints in CHK2-D347A tumor cells as compared with cells from c-neu and c-myc tumors (data not shown).



In contrast to the p53 pathway, we did not detect consistent abnormalities in either *cdc25A* or *brca1* pathways in CHK2-D347A tumor cells. In U2OS cells, phosphorylation of CDC25A by CHK2 contributes to its degradation following irradiation (11) but CHK2-D347A mammary tumor cells generally showed preservation of irradiation-induced *cdc25A* degradation, as did *c-myc* and *c-neu* tumor-derived cells (data not shown). In addition, formation of *brca1* nuclear dots following ionizing radiation was not altered in CHK2-D347A tumors cells as shown by immunofluorescence staining using antibody to phosphoserine-988 (not shown). Thus, despite their similar delayed tumorigenesis phenotype, the molecular events driving tumorigenesis in our dominant negative CHK2 model may differ from the targeted disruption of BRCA1 serine-988 recently reported by Kim et al. (17). Thus, the quantitative defect in p53-mediated DNA-damage response evident in CHK2-D347A mammary tumor-derived cells may contribute to the tumorigenic effect of CHK2 inactivation *in vivo*.

## Concluding Remarks

Modifier or low-penetrance genes are now thought to account for most cases of breast cancer predisposition within the general population (30). Given their prevalence in the population, *CHK2* mutations seem to constitute the first such low-penetrance allele identified in breast cancer, with an associated cancer risk confirmed by multiple epidemiologic analyses. Because few high-prevalence/low-penetrance cancer genes have yet been identified, the functional properties of CHK2 may be particularly informative in searching for other genes that also contribute to cancer predisposition in the general population. CHK2 is noteworthy in that it functions at a critical crossroad in multiple pathways that mediate the response to DNA damage, including p53 itself, but functional redundancy with CHK1 is likely to explain the mild phenotype of CHK2 inactivation in cultured cells, in mouse models, and potentially in human tumorigenesis. Population-based analyses of other genetic variants in checkpoint regulators will be required to determine whether they may also function as modifiers in human breast cancer.

The mouse model described here differs from the phenotype of *chk2*-null mice, which showed only modest tumor susceptibility. This might result from differences in genetic background associated with the FVB/N strain (CHK2-D347A) and C57BL/6 mice (*chk2*-null). Alternatively, tumor development in CHK2-D347A mice may reflect additional properties attributable to overexpression of a dominant-negative protein, such as sequestration of components of the DNA-damage response. Finally, it is also possible that the repeated breeding protocol used to induce MMTV-LTR expression provides a hormonally driven proliferative stimulus that cooperates with inactivation of *chk2* in mediating tumorigenesis. Such potential interactions between genetic and hormonal factors are particularly relevant, given the known contribution of estrogen exposure to human breast cancer.

Whereas the precise mechanism of tumorigenesis initiated by the dominant-negative *CHK2* transgene awaits further experiments, the fact that these mice develop tumors provides the first opportunity to study *chk2*-mediated signaling in primary malignant epithelial cells driven by *chk2* inactivation. This mouse model may prove particularly valuable in studying the interaction between genetic and hormonal risk factors in mammary carcinogenesis.

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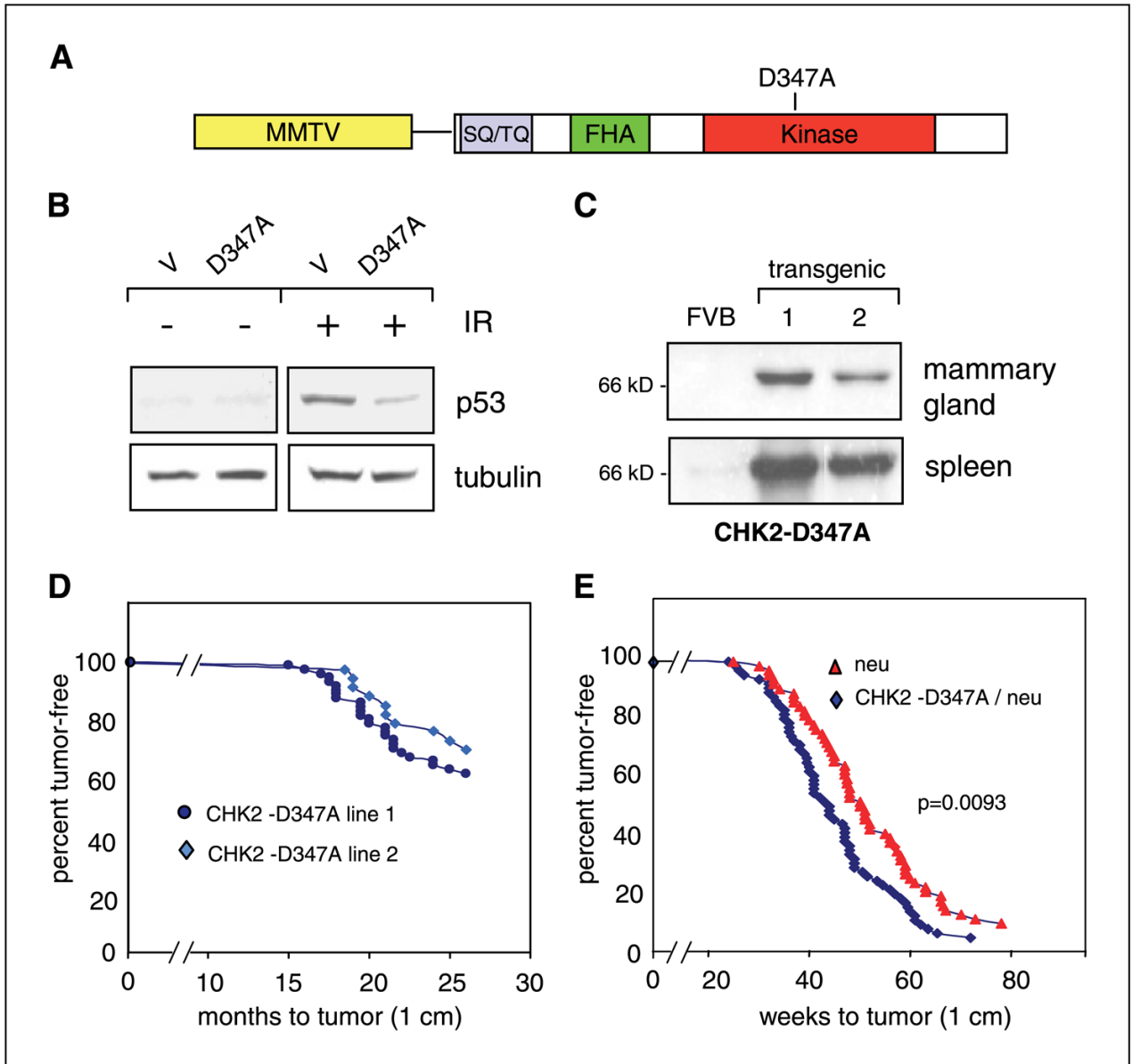
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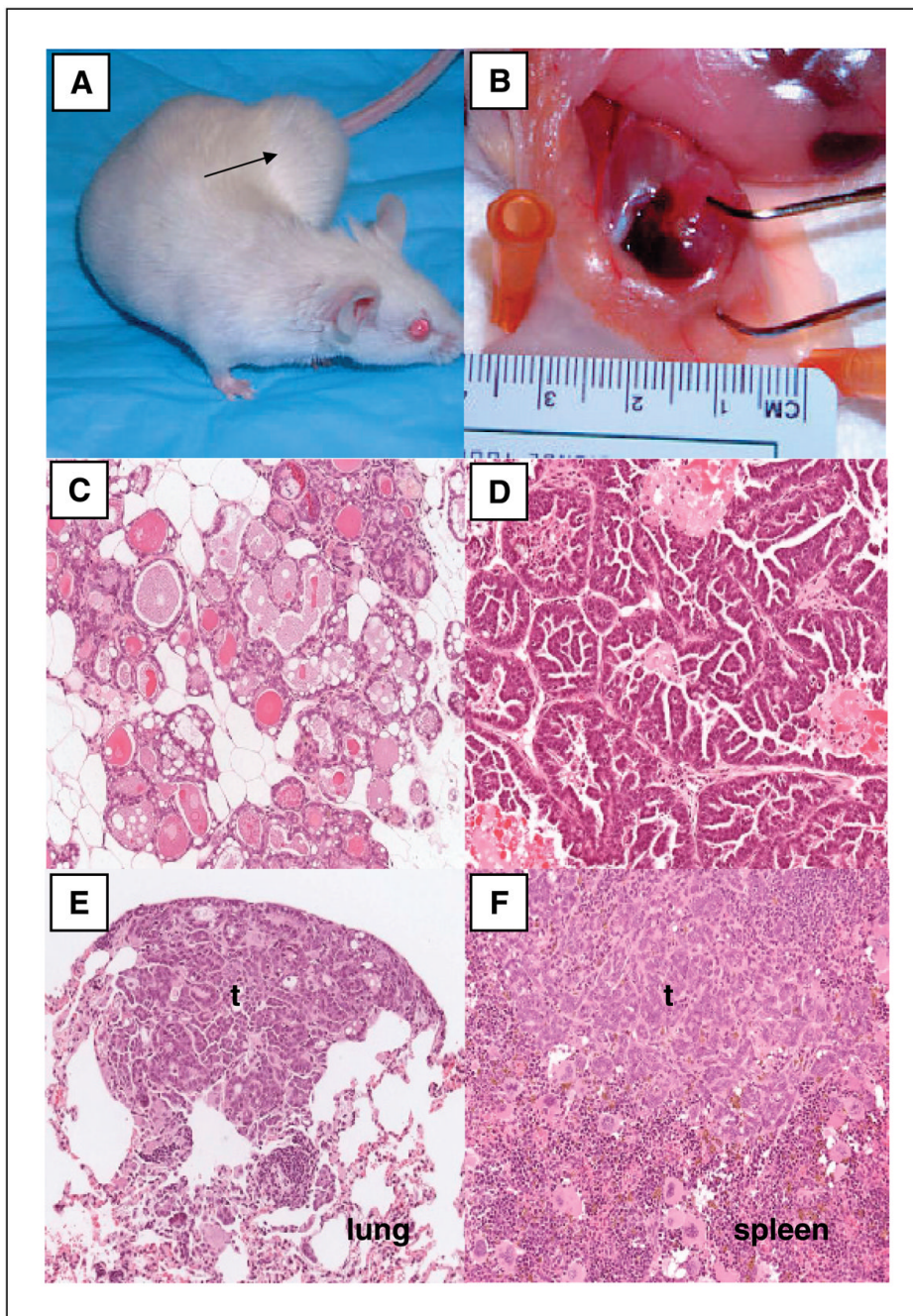
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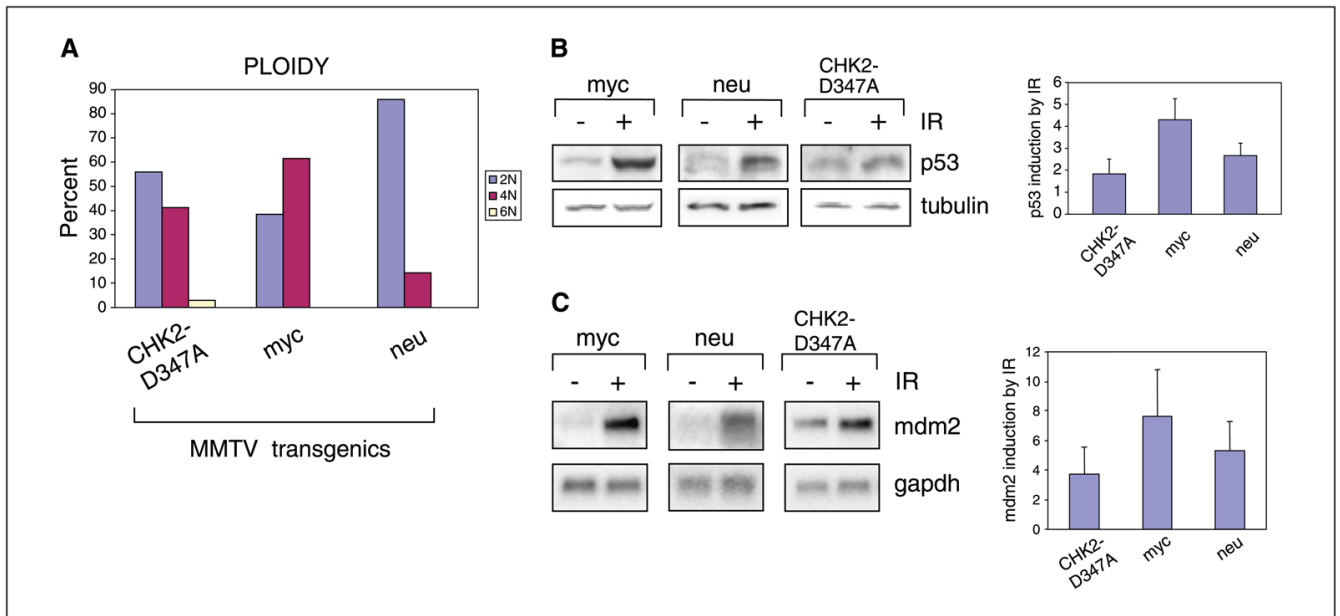
**Figure 1.**

Induction of mammary tumors by *MMTV-CHK2-D347A*. **A**, schematic diagram of the *CHK2-D347A* transgene containing the MMTV promoter driving expression of full-length cDNA encoding human CHK2, including the serine/threonine/glutamine-rich motif (*SQ/TQ*), the forkhead-associated homology domain (*FHA*), and the kinase domain in which aspartic acid is replaced by alanine at amino acid position 347. **B**, Western blot of protein lysates from primary human foreskin fibroblasts infected with either MSCV-SVG retroviral vector (*V*) or MSCV-SVG-CHK2-D347A (*D347A*). Lysates from infected cells were harvested 90 minutes after 10 Gy of ionizing radiation (*IR*). Equal microgram quantities of lysates were analyzed using antibody to human p53 or tubulin (control). **C**, mammary gland and spleen from wild-type FVB and two *MMTV-CHK2-D347A* lines. Equal quantities of lysates were immunoblotted and probed with antibody to human CHK2. **D**, Kaplan-Meier curve of tumor-free survival in two lines of *MMTV-CHK2-D347A* transgenic mice. In line 1 (dark blue), 27

of 70 mice (39%) developed palpable mammary tumors (1 cm) with an average latency of 20 months. In line 2 (*light blue*), 10 of 33 mice (30%) developed tumors with an average latency of 21.5 months. *E*, Kaplan-Meier curve of tumor-free survival in MMTV-*c-neu* mice (*red*) and MMTV-CHK2-D347A/*c-neu* double heterozygotes (*blue*). Fifty-nine *c-neu* mice developed mammary tumors with an average latency of 49.5 weeks and 64 CHK2-D347A mice developed mammary tumors with an average latency of 44.2 weeks ( $P = 0.0093$ ).



**Figure 2.** Histopathology of MMTV-CHK2-D347A–driven tumors. *A*, MMTV-CHK2-D347A mouse with mammary tumor (*black arrow*). *B*, dissected mammary gland from a CHK2-D347A mouse. Upper arm of tweezer marks the tumor; lower tweezer arm marks unaffected mammary tissue. *C* to *F*, representative tissue sections stained with H&E showing mammary hyperplasia (*C*), mammary tumor with micropapillary histology (*D*), metastatic tumor (*t*) to the lung (*E*), and metastatic tumor to the spleen (*F*). Magnification,  $\times 20$ .

**Figure 3.**

Functional abnormalities in MMTV-CHK2-D347A-derived tumor cells. **A**, ploidy of mitoses from seven independent CHK2-D347A tumor-derived cell lines (56% of mitoses were 2N, 41% were 4N, 3% were 6N), two c-myc cell lines (38% of mitoses were 2N, 62% were 4N), and two c-neu cell lines (86% of mitoses were 2N, 14% were 4N). Cells were karyotyped at passages 2 to 5. Virtually all CHK2-D347A cells with 2N ploidy exhibited gains or losses of individual chromosomes. **B**, quantitation of p53 protein levels following ionizing radiation. Immunoblot analysis of lysates from c-myc, c-neu, and CHK2-D347A mammary tumor-derived cells following 10-Gy ionizing radiation. Equal quantities of protein lysates (tubulin control) were analyzed 1 hour after ionizing radiation and representative samples are shown. Densitometric analysis of multiple immunoblots showing p53 expression following ionizing radiation for three cell lines derived from CHK2-D347A, c-myc, and c-neu tumors. *Columns*, mean fold induction (standardized to baseline); *bars*, SD. **C**, Northern blot analysis of mdm2 expression in c-myc, c-neu, and CHK2-D347A mammary tumor cells 2 hours after ionizing radiation (10 Gy). Representative samples are shown with glyceraldehyde-3-phosphate dehydrogenase (*gapdh*) control. Densitometric quantitation of multiple experiments showing mdm2 induction by ionizing radiation for CHK2-D347A tumors (nine cell lines), c-myc tumors (five cell lines), and c-neu tumors (three cell lines). *Columns*, mean fold induction (standardized to baseline); *bars*, SD.

**Table 1**

Histologies of tumors from MMTV-CHK2-D347A mice

Mammary tumor histology	<i>n</i> = 47
Micropapillary/papillary	12 (26%)
Adenocarcinoma	27 (57%)
Adenosquamous/squamous	8 (17%)
Spleen histology	<i>n</i> = 31
Myeloid hyperplasia	3 (10%)
Myeloid dysplasia	2 (6%)
Increased myeloid immaturity	2 (6%)
Myeloid leukemia	1 (3%)
Lymphoid leukemia	1 (3%)
Site of metastatic disease	<i>n</i> = 32
Lung	5 (15%)
Spleen	1 (3%)

NOTE: *N* refers to number of cases examined from mice representing both transgenic lines with gross evidence of mammary abnormality. Mice without gross evidence of tumor were not routinely submitted for histologic examination. Three mice developed clinical mammary tumors but were not available for histologic analyses. The majority of mice developed multiple tumors, sometimes with several histologies occurring in the same mouse.