Social Isolation Reduces Mammary Development, Tumor Incidence, and Expression of Epigenetic Regulators in Wild-type and p53-Heterozygotic Mice

Nina S. Hasen1,2,3, Kathleen A. O’Leary2, Anthony P. Auger1,3, and Linda A. Schuler1,2

Abstract

Chronic stress is associated with more rapid tumor progression, and recent evidence suggests that stress may contribute to social and ethnic disparities in the incidence and mortality of breast cancer. We evaluated the p53+/− FVB/N mouse as a model to investigate effects of chronic social stress on mammary gland development, gene expression, and tumorigenesis. We individually housed (IH) wild-type and p53+/− female FVB/N mice, starting at weaning. At 14 weeks of age, both wild-type and p53+/− IH mice showed strikingly reduced mammary development compared with group-housed (GH) controls, with IH mice having significantly fewer preterminal end buds. This morphologic difference was not reflected in levels of mammary transcripts for estrogen receptor-α or progestin receptor. However, IH increased levels of mRNA for the kisspeptin receptor in the medial preoptic area of the hypothalamus, associated with reduced duration of estrous cycles. Furthermore, IH altered mammary transcripts of genes associated with DNA methylation; transcripts for methyl-binding protein 2 and DNA methyltransferase 3b (DNMT3b), but not DNMT1 and DNMT3a, were reduced in IH compared with GH females. Interestingly, the glands of p53+/− females showed reduced expression of all these mediators compared with wild-type females. However, contrary to our initial hypothesis, IH did not increase mammary tumorigenesis. Rather, p53+/− GH females developed significantly more mammary tumors than IH mice. Together, these data suggest that social isolation initiated at puberty might confound studies of tumorigenesis by altering mammary development in mouse models. Cancer Prev Res; 3(5); 620–9. ©2010 AACR.

Introduction

Breast cancer is a complex disease in which multiple endocrine, paracrine, and intracellular systems interact in both the development and the progression of tumors. Because these systems are sensitive to social experience, particularly social stress, it has been suggested that one component of the observed social and ethnic disparities in breast cancer incidence and mortality might be higher levels of social stress in vulnerable populations. Several epidemiologic studies have associated life stress with breast cancer incidence, although a 2003 meta-analysis showed that only the death of a spouse significantly increases risk (1). In contrast, progression of breast cancer has been more definitively linked with stress (for reviews, see refs. 2, 3). Depression has been correlated with reduced cell-mediated immunity, and higher risk for metastasis (4). Biomarkers that are sensitive to social stress were predictive of breast cancer recurrence in a large, multi-ethnic cohort of women (5). African American women have a higher incidence of early onset breast cancer and are more likely to die from the disease (6). African American women also report more stressful life events, higher levels of perceived stress, and lower social support than Caucasian women (7). A retrospective analysis of data from the Black Women’s Health Study documented a positive correlation between experience of racial bias and risk for breast cancer in this population (8). Although it seems likely that stressful experiences may play a role in the increased risk and mortality of African American women, the underlying mechanisms are poorly understood. Many factors impede the study of social stress and physiology in human populations, including long life spans, inaccuracy of self-reporting, ethical considerations, and high levels of genetic variation.

Animal models circumvent many of these complications, and provide more tractable systems for studying the interplay of social factors and breast cancer. Studies in rats have shown a relationship between social isolation and mammary tumors (9, 10), and social isolation of murine recipients of ovarian tumor xenografts increases tumor progression (11). Genetically defined mouse...
models play critical roles in the investigation of tumorigenesis (12), as well as studies of behavior and stress (13). They allow us to interrogate the net outcome of complex interactions in response to specific perturbations, including not only physiologic stimuli, but also environmental factors, such as social experiences. This capacity to investigate the effect of experience on physiology is especially important in the context of addressing health disparities, where it is likely that interactions between genes and the environment contribute to differences in disease incidence and progression (14). Mice, like humans, are exquisitely sensitive to changes in their environment, and therefore are excellent models for investigating the underlying mechanisms of these interactions.

In this study, we evaluated a mouse model to study the effect of social stress on mammary development and tumorigenesis. To model social stress, we chose individual housing, a well-validated paradigm for modeling the effects of chronic stress in female mice (15). To examine interactions between chronic stress and tumorigenesis, we compared wild-type (WT) FVB/N females to those heterozygous for the p53 gene, modeling the Li-Fraumeni syndrome in women (16, 17). Mutations of this gene predispose to tumorigenesis in multiple organs, including the breast. p53 is commonly mutated in “triple negative” breast tumors, the aggressive tumor subtype that predominates in young African American women. We examined the interplay between social isolation and p53 heterozygosity on mammary gland morphology and gene expression in young adult mice, and followed a subset of the group-housed (GH) and individually housed (IH) females were sacrificed at 14 weeks of age to examine the effects of social isolation and p53 heterozygosity on physiology prior to the onset of disease. On that morning, these mice were given a brief social behavior test consisting of a 5-minute interaction with a novel WT, GH female mouse in a clean cage in a different room from the colony. This interaction was videotaped and the following behaviors scored offline by an observer blind to the test condition of the mice: latency to first contact with the novel mouse (with contact defined as placement of the focal animal’s nose on the novel animal), number of contacts, total time spent in contact, number of rears (with rearing defined as the animal rising onto its hind legs), total time spent rearing, and number of digs (with digging defined as the animal rearranging bedding material with its front paws). IH mice were alternated with GH mice. Following the 5-minute test, each animal was returned to its home cage and the home cage returned to the colony room.

### Materials and Methods

#### Genotyping and maintaining mice

p53 heterozygous (p53+/−) mice (18) were crossed into the FVB/N background for more than 10 generations, and were maintained in the FVB/N genetic background. Experimental p53+/− females were generated by crossing p53+/− and WT-FVB/N animals from our existing colony. Tail biopsies were collected at weaning and offspring were screened for the p53 mutation, as described previously (ref. 19; primer sequences in Table 1). Mice were housed and handled in accordance with the Guide for Care and Use of Laboratory Animals in facilities accredited by the Association for the Assessment and Accreditation for Laboratory Animal Care. All procedures were approved by the University of Wisconsin-Madison Animal Care and Use Committee.

#### Social isolation and behavioral testing

At 21 days, female mice were weaned and either housed singly or in groups of three to four. Mice remained in their housing conditions until sacrifice or natural death. A subset of the group-housed (GH) and individually housed (IH) females were sacrificed at 14 weeks of age to examine the effects of social isolation and p53 heterozygosity on physiology. Mice were sacrificed by CO2 gas inhalation and handled in accordance with the Guide for Care and Use of Laboratory Animals in facilities accredited by the Association for the Assessment and Accreditation for Laboratory Animal Care. All procedures were approved by the University of Wisconsin-Madison Animal Care and Use Committee.

#### Collection of tissue: 14-week cohort

Two to three hours after the completion of the social behavior test, animals were killed with CO2 gas. The brain

### Table 1. Primer sequences

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<th>Reverse</th>
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<tr>
<td></td>
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<td>(wt) ACA GCG TGG TAC CTT AT</td>
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and one caudal mammary gland were rapidly dissected, frozen, and stored at −80°C. Trunk blood was collected at the same time, centrifuged and the resulting serum stored at −20°C. The contralateral chain of glands was dissected, mounted on a glass slide, and fixed in 10% neutral buffered formalin overnight and stored in 70% ethanol until processed. This "whole-mounted" tissue was stained with carmine alum, dehydrated with graded ethanol, cleared of fat with xylene, and stored in glycerol until analysis. Glands were viewed through a dissecting microscope, and the number of visible hyperplastic lesions counted.

**Serum corticosterone assay**

Plasma levels of corticosterone were measured using an enzyme immunoassay (Cayman) according to the instructions of the manufacturer.

**Cytology**

To assess the effects of housing condition on estrous cycling, we examined vaginal cytology daily from both GH (n = 7) and IH (n = 6) FVB/N WT mice for 21 consecutive days, following established protocols (20).

**Collection of tissue: end stage cohort**

The remainder of the GH and IH mice remained in their housing conditions until they reached end stage, defined as a tumor of 1.5 cm or deteriorating health status. All animals underwent necropsy, and all mammary glands were examined for tumors. One chain of mammary glands, contralateral to a tumor (if present), was whole-mounted, and processed as above for analysis of epithelial morphology. Invasive mammary carcinomas were confirmed by a board-certified pathologist. In addition, the opposite cranial gland was evaluated histologically for microscopic lesions.

**Assessment of mammary gland morphology**

Digital photos were taken of whole-mounted caudal mammary glands of 14-week-old females using a digital camera mounted on a dissecting microscope. An observer blind to the experimental treatment identified a 7 mm × 3 mm rectangle extending caudally from the lymph node, along the gland margin, and counted the number of terminal end buds (defined as club-shaped structures at the end of a duct or branch), preterminal end buds (defined as outgrowths not at the ends of ducts or branches), and established ductal branching points within this rectangle.

**Tissue processing**

Brains were defrosted to −10°C, and sectioned at 300 μm on a cryostat. Micropunches were taken from the appropriate sections for the medial preoptic area (MPOA) at −0.30 mm from the bregma (21), rapidly re-frozen and stored at −80°C (22). RNA was extracted from brain and mammary gland samples using the AllPrep DNA/RNA Mini Kit from Qiagen. Concentrations of RNA in each sample were measured using the Qubit quantitation platform (Invitrogen).

**Quantitative reverse transcription-PCR**

cDNA was generated, and quantitative reverse transcription-PCR was carried out essentially as described (23), using 18S RNA as an internal control. Quantitative reverse transcription-PCR results were calculated using the ΔΔCT method. Estrogen receptor-α (ERα) primers were synthesized by Integrated DNA Technologies. All other primers were synthesized by Invitrogen. Primer accession numbers and sequences are listed in Table 1.

**Statistical analyses**

Behavioral tests and mammary morphologic measurements were analyzed using two-way ANOVAs with genotype and housing condition as factors. Serum corticosterone and vaginal cytology were analyzed using a one-way ANOVA or unpaired Student's t test, unless otherwise noted. These values were analyzed using two-way ANOVAs, unless otherwise noted. Whenever data were square root–transformed to achieve equal variance and/or normal distribution, this is indicated in the figure legend. The Holm-Sidak test was used for all post hoc analyses. Survival was analyzed using the Gehan-Breslow test and curves drawn using the Kaplan-Meier method. Mammary tumor and lesion rates were analyzed using the Fisher exact test. All statistical analyses were done using SigmaStat software (Systat, Inc.).

**Results**

**Behavioral effects of isolation**

Individual housing has been shown to elevate indices of stress and anxiety in female mice (15), including reducing exploratory behavior. After 14 weeks of IH, both WT and p53⁺⁻ female mice in our study showed significantly different behavioral responses from GH mice, indicating altered social function and a reduction in exploratory behavior. In a social interaction test, IH mice reared less often (P < 0.001) and for less time (P < 0.001), made more contacts with a novel intruder (P = 0.01), and spent more time in contact with the intruder (P < 0.001) than GH mice (Fig. 1A and B). These findings replicate those of other studies (15, 24, 25), and show that the IH treatment altered behavioral systems associated with the stress response. Serum, collected from p53⁺⁻ GH and IH females between 14:00 and 16:00 hours during the animals' light cycle, did not contain different levels of corticosterone (P = 0.868; Fig. 1C). This lack of altered corticosterone might be strain-dependent; the FVB/N strain has been shown to have lower levels of corticosterone in response to chronic stress (26).

**Mammary gland morphology and individual housing**

At puberty, activation of epithelial ERα initiates elongation of the ductal rudiment throughout the mammary fat
pad (27). The ductal tree develops increasing complexity throughout subsequent estrous cycles in nonparous females, which may be accompanied by alveolar budding in some mouse strains, including FVB/N (28). To examine the effects of housing conditions on mammary structures, we examined whole-mounted mammary glands of both males, which may be accompanied by alveolar budding throughout subsequent estrous cycles in nonparous females (27). The ductal tree develops increasing complexity in a clean cage and then a novel, female mouse was introduced to the cage for 5 min. A, IH mice of both genotypes spent significantly more time in contact with the novel mouse; B, GH mice spent significantly more time rearing up on their hind legs, a behavior associated with investigation of the environment (n = 5 for each group in the novel conspecific test). C, serum corticosterone collected 2 to 3 h after the novel conspecific test showed no difference between IH and GH animals. Corticosterone from GH and IH p53−/− animals were compared using a standard EIA kit. GH (n = 8), IH (n = 8). Different uppercase letters denote overall statistical differences in housing or genotype (P ≤ 0.05). Different lowercase letters denote statistical differences found in post hoc comparisons between experimental groups (P ≤ 0.05). Columns, mean; bars, SEM.

Fig. 1. GH and IH mice respond differently to the presence of a novel conspecific, but show no difference in serum corticosterone. Mice were placed in a clean cage and then a novel, female mouse was introduced to the cage for 5 min. A, IH mice of both genotypes spent significantly more time in contact with the novel mouse; B, GH mice spent significantly more time rearing up on their hind legs, a behavior associated with investigation of the environment (n = 5 for each group in the novel conspecific test). C, serum corticosterone collected 2 to 3 h after the novel conspecific test showed no difference between IH and GH animals. Corticosterone from GH and IH p53−/− animals were compared using a standard EIA kit. GH (n = 8), IH (n = 8). Different uppercase letters denote overall statistical differences in housing or genotype (P ≤ 0.05). Different lowercase letters denote statistical differences found in post hoc comparisons between experimental groups (P ≤ 0.05). Columns, mean; bars, SEM.

Estrous cycling and individual housing

Reduced preterminal budding is associated with reduced progesterone action (29), and suggests altered estrous cycles. Therefore, we examined the effects of housing on estrous cycling by daily monitoring of vaginal smears. Periodicity was somewhat irregular in both GH and IH mice, with no significant differences in the number of days in estrus (P = 0.271), number of days in diestrus (P = 1.0, Mann-Whitney rank sum), or number of days in proestrus (P = 0.428). However, isolated mice had a significantly shorter average number of days between the onset of estrous cytology than did GH mice (P = 0.002; Fig. 3A). Given the effects of IH on gland morphology and cycling, we compared levels of mammary transcripts for ERα and progesterone receptor (PR) among the four groups. Housing did not significantly affect ERα or total PR mRNA (Fig. 3B and C) nor PRβ transcripts (data not shown). However, p53 heterozygosity significantly reduced transcript levels for both receptors. To determine if social isolation altered the expression of genes governing metabolic processes, as recently reported in female mice similarly isolated (25), we quantitated mRNA for ATP citrate lyase (Acyl). As shown in Fig. 4C, transcripts for this gene were elevated in IH females of both genotypes (P = 0.028).

p53 status, individual housing, and kisspeptin receptor expression

Kisspeptin and its cognate receptor, Kiss1r, have been shown to modulate the hypothalamic-pituitary-gonadal axis (30, 31), and mediate some effects of stress on secretion of luteinizing hormone and follicle-stimulating hormone (32). We therefore wondered if the Kisspeptin system might be altered in response to IH. We focused on the receptor because ligand expression in the brain is sensitive to reproductive state (30), and examined kiss1r transcripts in the brains and mammary glands of IH and GH mice of both genotypes (Fig. 4). Both housing and p53 status affected the levels of kiss1r mRNA in the MPOA of the hypothalamus (Fig. 4A). Overall, IH animals showed higher levels of kiss1r mRNA than GH mice (P = 0.043).

In the mammary gland, kiss1r mRNA was strongly reduced in p53−/− females (P = 0.019; Fig. 4B). This effect was focused in the IH groups; mammary kiss1r transcripts
were twice as high in WT IH mice, compared with p53+/−
IH females (P = 0.03).

Effect of housing and p53+/− status on epigenetic
regulators

We hypothesized that IH might alter neoplastic process-
es in our model by altering the expression of genes implic-
cated in methylation of DNA. As shown in Fig. 5, we
found striking effects of housing and/or p53 status on
mRNA levels of DNA methyltransferases (DNMT1,
DNMT3a, DNMT3b) and methyl-binding protein 2
(Mecp2). The mammary glands of IH females, both WT
and p53+/−, contained significantly reduced Mecp2
transcripts (P < 0.001; Fig. 5D). DNMT3b transcripts
were similarly altered by housing conditions (P = 0.002;
Fig. 5C). There was an IH trend effect on DNMT3b
mRNA in WT females (P = 0.08), which was highly signifi-
cant in p53+/− females (P < 0.001). In contrast, neither
DNMT1 nor DNMT3a transcripts were affected by housing
(Fig. 5A and B).

Notably, p53 heterozygosity reduced transcripts for all
of these epigenetic regulators (P < 0.001; Fig. 5), indica-
Fig. 2. IH mice developed fewer
mammary preterminal end
buds. Photomicrographs of
carmine-stained whole-mounted
caudal mammary glands of WT
group-housed (A), WT individually
housed (B), p53+/− group-housed
(C) and p53+/− individually housed
(D) female mice at 14 wk of age.
E, housing significantly affected
the number of preterminal end
buds (defined as all buds not
located at the tip of a duct). WT
GH (n = 6), IH (n = 5); p53+/− GH
(n = 10), IH (n = 10). F, housing
does not alter transcripts for
cytokeratin 8. WT GH (n = 5), IH
(n = 5); p53+/− GH (n = 6), IH (n = 9).
Different uppercase letters denote
overall statistical differences in
housing or genotype (P ≤ 0.05).
Columns, mean; bars, SEM.

ting a strong effect of this genetic modification on this
system.

Social isolation, survival, and tumorigenesis in
p53+/− mice

Initially, the IH mouse cohort seemed to sicken
or develop tumors more rapidly than the GH controls
(Fig. 6A). Indeed, survival of IH mice at 1 year of age
was significantly reduced (P < 0.021). The earliest deaths
were primarily due to thymic lymphomas in both housing
groups. However, this difference disappeared as the study
progressed, and there was no significant effect of housing
on overall survival (P = 0.96). Likewise, the proportion of
females that had developed tumors at end stage was not
significantly different for IH and GH females (GH: 93%,
n = 15; IH: 82%, n = 16; Fisher exact test, respectively).
However, housing did significantly affect the incidence
of mammary tumors (Fig. 6B): IH mice were less likely
than GH mice to develop mammary tumors (P = 0.012).
Indeed, only a single invasive mammary carcinoma was
found in one end stage IH female, compared with six single
tumors in GH females. Nonetheless, IH p53+/− females did

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develop some mammary histologic abnormalities, and the quantity was not statistically different from GH glands. Housing did not significantly affect the incidence of any other tumor type.

**Discussion**

In this study, we examined the effects of social isolation on development and tumorigenesis of the mammary gland. Using this established model for chronic stress combined with p53 heterozygosity, mimicking the Li-Fraumeni syndrome in women, we showed that social experience alters mammary gene expression, development, and tumorigenesis. Females housed individually beginning at puberty, regardless of genotype, displayed underdeveloped mammary ductal trees, which correlated with reduced estrous cycle length, significantly elevated kiss1r transcripts in the MPOA of the hypothalamus, and reduced mammary transcripts for the epigenetic regulators, Mecp2 and DNMT3b. Despite morphologic similarity to mammary glands of WT female controls, p53 heterozygotes, regardless of housing status, displayed a very different profile of gene expression, characterized by reduced mammary transcripts for ERα, PR, Kiss1r, and all four epigenetic regulators. Contrary to our hypothesis, IH reduced the incidence of end-stage mammary tumors in p53+/− females. Our findings indicate that social isolation initiated at puberty inhibits mammary development and alters its epigenome, which might confound studies of mammary tumorigenesis.

Both WT and p53+/− IH females showed a significant and unpredicted lack of mammary development. Although ductal elongation was complete, glands of IH young adult females of both genotypes exhibited reduced numbers of preterminal end buds. This phenotype suggests altered progesterone responsiveness (29), but we found no significant difference in either ERα or PR mRNA levels in IH animals. However, differences in estrous cycling in IH and GH mice suggest altered function of the hypothalamic-pituitary-gonadal axis, which may lead to these differences in morphology.
p53 is a powerful tumor suppressor, and exerts its actions via multiple pathways, including regulation of the cell cycle and apoptosis (33, 34). Genetic defects in one allele result in the Li-Fraumeni syndrome in humans (16, 17). Afflicted individuals are at high risk for multiple cancers, including a 55% chance of breast cancer in women by age 45 (35). Here, we used a mouse model heterozygous for a well-studied germ line mutation of p53.

Fig. 4. Kiss1r transcript levels in the brain and mammary gland of 14-wk-old females are modulated by housing and genotype. A, Kiss1r transcript levels in the MPOA of the hypothalamus. Levels were higher in IH compared with GH animals. WT GH (n = 2), IH (n = 5), p53<sup>+/−</sup> GH (n = 8), IH (n = 6), B, Kiss1r transcript levels in the mammary glands (MG). p53<sup>+/−</sup> status also drove lower expression of Kiss1r, with WT IH animals showing higher levels of expression than p53<sup>+/−</sup> IH animals. WT GH (n = 5), IH (n = 5), p53<sup>+/−</sup> GH (n = 9), IH (n = 10). Different upper case letters denote overall statistical differences in housing or genotype (P ≤ 0.05). Different lowercase letters denote statistical differences found in post hoc comparisons between experimental groups (P ≤ 0.05). Columns, mean; bars, SEM.

Fig. 5. Housing and p53 status differentially modulate transcripts for DNMT and Mecp2 in mammary glands of 14-wk-old WT and p53<sup>+/−</sup> females. A, both GH and IH p53<sup>−−</sup> mice had lower levels of DNMT1 mRNA, and there was a trend towards an effect of isolation in WT animals; B, loss of a p53<sup>−−</sup> allele led to reduced DNMT3a mRNA; C, both isolation and loss of a p53<sup>−−</sup> allele diminished the expression of DNMT3b mRNA; and D, both isolation and loss of a p53<sup>−−</sup> allele diminished the expression of MeCP2. WT GH (n = 5), WT IH (n = 5), and p53<sup>+/−</sup> IH (n = 10). Different uppercase letters denote overall statistical differences in housing or genotype (P ≤ 0.05). Different lowercase letters denote statistical differences found in post hoc comparisons between experimental groups (P ≤ 0.05). Columns, mean; bars, SEM.
this gene (18). We backcrossed this mutation, originally developed in 129/SV × C57BL/6 mice, into the FVB/N genetic background, to study its behavior in the context of a mouse strain that is extensively used for transgenic models of cancer. This change in strain background increased overall tumor incidence (>90%), and reduced latency, compared with the original report (18). Interestingly, the distribution of tumor types in GH $p53^{+/−}$ FVB/N females also shifted toward a predominance of mammary tumors, more similar to the human syndrome.

Social isolation, conferred by individual housing of normally social species, is a stress paradigm which is relevant to human disease. Indeed, use of this stressor increases the progression of ovarian tumors in mice (11), and two recent studies also linked this stress to progression of mammary tumors in rat (10) and mouse (25) models. In the latter study, Williams and colleagues used a very similar experimental paradigm and the same mouse strain (FVB/N) as we employed in our study. In seeming contrast to our observed reduced incidence of invasive mammary carcinomas in IH $p53^{+/−}$ females, they showed that isolation increased mammary lesions and tumor burden induced by the C3(1)/SV40 T-antigen (Tag) transgene (25).

The C3(1)/SV40 Tag model differs from $p53$ heterozygotes in several important respects. Transformation induced by SV40-Tag also involves loss of $p53$ function, but retinoblastoma protein is also inactivated (36). Moreover, C3(1) targets Tag expression to only epithelial cells, in contrast to the germ line $p53^{+/−}$ model used in our study. The consequences of these differences on tumor incidence and progression in the context of social isolation merit additional investigation. However, the effect of IH on mammary development in both WT and $p53^{+/−}$ females found in our study re-emphasizes the importance of a long-understood consequence of isolation: IH exerts complex effects on estrous cycling (37, 38), and consequently, steroid hormone action. Particularly, when housing treatments are initiated at puberty, this could disrupt normal mammary development, which may impede tumorigenic processes (e.g., ref. 39). Furthermore, mammary epithelial populations and potential tumor precursors expand rapidly at this time (40); manipulations that interfere with these events may also inhibit oncogenesis. In addition, ovarian steroids are potent mitogenic stimuli for hormonally responsive mammary lesions, which include those induced by the absence of $p53$ in other strain backgrounds (41). By reducing mammary development and ovarian steroid action, IH might mask the possible effects of stress on tumor incidence from stimuli, such as $p53$ heterozygosity, which may be relatively weak compared with viral oncogenes. Thus, experimental social isolation, particularly in the vulnerable peripubertal period, may confer additional complications in efforts to tease out any relationship between psychosocial factors and breast tumor incidence, as opposed to cancer progression. Techniques that reliably induce stress without depriving subjects of pheromonal cues that drive estrous cycling might be more appropriate for studies of mammary cancer.

Contrary to our expectations, IH also did not alter the overall survival of $p53^{+/−}$ females in our study, despite behavioral and physiologic evidence of increased stress. Larger studies examining cell-specific loss of $p53$ alleles.
may reveal the effects of isolation on individual tumor types. However, in the initial months after weaning, IH animals died at a more rapid rate. The convergence of the survival rates with time suggests that some, but not all, mice could \textquotedblleft adapt\textquotedblright{} to the higher stress level. This is consistent with a growing body of literature suggesting that individual temperaments play a role in the experience of stress and risk for disease (42, 43).

Kisspeptin was originally identified as an antimitastic agent in breast and other cancers (44). This ligand and its cognate receptor, Kiss1r, also play critical roles in regulating the maturation and function of the hypothalamic-pituitary-gonadal axis in humans, non-human primates, and rodents (31, 45). Kiss1r transcripts in the hypothalami and MPOA of adults have been shown to be modulated by several stressors (32, 46), supporting the hypothesis that kisspeptin mediates the effect of stress on the estrous cycle by modulation of gonadotropin secretion. The altered cycling and higher kiss1r mRNA levels in the MPOA of IH females in our study, regardless of genotype, suggests that IH may be exerting its effects on mammary development in part via the action of kisspeptin on the hypothalamic-pituitary-gonadal axis. A similar mechanism may also mediate the delayed mammary development reported in isolated Sprague-Dawley rats (10).

Studies exploring the effect of maternal care have shown that social experience in infancy could alter the methylation of genes in the brain (47, 48). Similarly, studies in adult animals show that injury to the central nervous system induces changes in the epigenome (49). Here, we showed that postpubertal social isolation could also result in lower expression of genes regulating methylation in the mammary gland. The reduced DMNT3b and Mecp2 mRNA that we observed might reflect the relatively underdeveloped glands; DNA methylation and Mecp2 both play a role in some (50), but not all (51), aspects of mammary development. Alternatively, IH may alter this system regardless of mammary maturation. Examination of the effect of IH on fully mature glands would further illuminate this relationship.

Mammary glands of p53+−/− females exhibited reduced transcripts for all four epigenetic regulators. This is surprising in light of the association of heterozygosity of p53 with regional hypermethylation of CpG islands, repression of DNMT1 transcription by p53, and corepression of some promoters by p53 and DNMTs (52, 53). However, global hypomethylation as well as localized hypemethylation are hallmarks of cancer (54). DNA hypomethylation and reduced DMNT3b, in particular, increase chromosomal instability (54, 55), and global 5-methylcytosine levels are reduced in breast as well as in prostatic disease (56, 57). Hypomethylation might be particularly important for early neoplastic events (54). Together, these observations indicate a need for more studies of the role of epigenetic changes in tumorigenesis secondary to mutations in p53.

Our study shows that social isolation initiated at puberty exerts complex physiologic effects, including dysregulation of mammary development and expression of epigenetic modulators. In the context of p53 heterozygosity, this experimental paradigm unexpectedly reduces the incidence of mammary tumors, highlighting the neglected interplay between social experience, mammary development, and tumor biology. These studies, in the context of the literature, underscore the need for further investigation of the effects of social experience on the risk and progression of mammary cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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