Distinct Luminal-Type Mammary Carcinomas Arise from Orthotopic Trp53-Null Mammary Transplantation of Juvenile versus Adult Mice

David H. Nguyen¹, Haoxu Ouyang¹, Jian-Hua Mao², Lynn Hlatky³, and Mary Helen Barcellos-Hoff¹

Abstract

Age and physiologic status, such as menopause, are risk factors for breast cancer. Less clear is what factors influence the diversity of breast cancer. In this study, we investigated the effect of host age on the distribution of tumor subtypes in mouse mammary chimera consisting of wild-type hosts and Trp53 nullizygous epithelium, which undergoes a high rate of neoplastic transformation. Wild-type mammary glands cleared of endogenous epithelium at 3 weeks of age were subsequently transplanted during puberty (5 weeks) or at maturation (10 weeks) with syngeneic Trp53-null mammary tissue fragments and monitored for one year. Tumors arose sooner from adult hosts (AH) compared with juvenile hosts (JH). However, compared with AH tumors, JH tumors grew several times faster, were more perfused, exhibited a two-fold higher mitotic index, and were more highly positive for insulin-like growth factor receptor phosphorylation. Most tumors in each setting were estrogen receptor (ER)-positive (80% JH vs. 70% AH), but JH tumors were significantly more ER-immunoreactive (P = 0.0001) than AH tumors. A differential expression signature (JvA) of juvenile versus adult tumors revealed a luminal transcriptional program. Centroids of the human homologs of JvA genes showed that JH tumors were more like luminal A tumors and AH tumors were more like luminal B tumors. Hierarchical clustering with the JvA human ortholog gene list segregated luminal A and luminal B breast cancers across datasets. These data support the notion that age-associated host physiology greatly influences the intrinsic subtype of breast cancer. Cancer Res; 74(23); 7149–58. ©2014 AACR.

Introduction

Breast cancer is a heterogeneous disease, or rather, a collection of neoplastic diseases. As tumor characteristics are highly correlated with prognosis, there has been considerable investment in defining classes and standardizing the characterization of breast tumors based on traditional pathology, marker analysis, imaging features, and molecular analysis. Human epidermal growth factor receptor 2 (HER2) amplification distinguishes a specific breast cancer subtype that provides a robust molecular target in therapy (1). Estrogen receptor (ER) status is important not only because ER-positive (ER⁺) tumors are managed with antiestrogen treatments but also because ER status is associated with different age groups and with different outcomes. ER-negative (ER⁻) tumors are more frequent in young women and have a worse prognosis (2). Among ER⁻ breast cancers there are also several diseases. ER⁻ and progesterone receptor (PR)⁻ cancers tend to be more poorly differentiated and aggressive. Triple-negative breast cancers that lack both hormone receptors and HER2 amplification are usually invasive ductal carcinomas that have a high rate of relapse (3).

Molecular profiling discriminates between five and 20 tumor types depending on the algorithms and datasets used (reviewed in ref. 4). As defined by Perou and colleagues (5–7), transcriptomic analysis of human breast cancer comprises at least six different intrinsic molecular subtypes: luminal A, luminal B, HER2, basal-like, claudin-low, and normal-like. Molecular classification is not synonymous with clinical marker status using ER, PR, and HER2. For example, although ER⁺ tumors predominate in postmenopausal women and have a relatively low relapse rate, molecular characterization revealed that ER⁺ breast cancers can be divided into the so-called luminal A cancers, which are associated with a longer disease-free survival than that of luminal B cancers. The luminal A and B subtypes are known to maintain the expression of ESR1 transcript and protein, along with the canonical mammary luminal fate regulators GATA3 and FOXA1 (8, 9), but the luminal B subtype has a strong signature of proliferation.

Although tumor characteristics are clearly linked with prognosis, it is less clear how these distinct features arise.

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One source of biologic distinction between breast tumors is thought to be the cell of origin at transformation (10). A "cells of origin" autonomous view of breast cancer is bolstered by several studies in which disruption of the progenitor fate regulator, BRCA1, via promoters specific to the basal mammary gland compartment, produced tumors that resembled human basal-like breast cancers (11–13). Alternatively, deregulation of key transcriptional regulator, whether by mutation or epigenetic alteration, may divert cells to a particular state. For example, GATA3, a necessary transcriptional factor for differentiation of luminal cells, is associated with the luminal subtype of breast cancers (14–16). External factors are suggested by epidemiologic studies of girls exposed to ionizing radiation, who develop triple-negative breast cancer at an early age (17, 18), and the association of specific tumor types with obesity (19). Several recent studies have turned attention on the stroma to derive prognostic value by using expression profiling of stromal and extratumoral tissues (20). Using microdissected stroma from breast cancer, Finak and colleagues (21) found a stroma-derived prognostic predictor that stratifies disease outcome based on a signature of immune mediators, hypoxia, and angiogenesis. Analysis of the expression profiles from invasive breast cancer and ductal carcinoma in situ provides evidence that stromal biology is a key determinant of progression (22). Consistent with the influence of distinct microenvironment subtypes, an active versus inactive cancer-adjacent microenvironment is associated with aggressiveness and outcome of ER+ human breast cancers (20).

The stromal factors that contribute to breast cancer subtype distribution are not mutually exclusive and may be further influenced by more systemic processes. We used a mouse model to show that mice exposed to ionizing radiation and subsequently transplanted with uniradiated donor tissue preferentially develop ER+ breast cancers (23). These tumors exhibit a gene expression program characterized by signatures of inflammation and stem cell biology. Somewhat surprisingly, the murine host radiation profile also segregates sporadic human breast cancers according to intrinsic subtype, i.e., basal from luminal, and within basal cancers, the irradiated murine host profile mediated by TGFβ associated with claudin-low cancers (24). This bioinformatic analysis suggests that common processes may underlie the etiology of both radiation-preceded and sporadic basal-type cancers. These data led to the hypothesis that host biology profoundly influences the etiology of breast cancer intrinsic subtypes (25).

As puberty is a period of significant physiologic changes that alter the hormonal milieu, which in turn directs mammary morphogenesis, in this study, we used a mammary chimera model to compare the development of Trp53-null cancer as a function of host age. More tumors arose from Trp53-null outgrowths in adult mice compared with those transplanted during puberty, but the influence of puberty was manifested in development of rapidly growing, strongly ER+ luminal tumors. We found that the transcriptional profile of the features that differ between tumors arising from juvenile versus adult transplantation segregates human luminal intrinsic breast cancer subtypes.

Materials and Methods

Animals

All animal experiments were performed at Lawrence Berkeley National Laboratory with institutional review and approval. BALB/c mice were purchased from The Jackson Laboratory and housed 4 per cage, fed with Lab Diet 5008 chow and water ad libitum. Trp53-null BALB/c mice were bred in-house under similar conditions. For transplantation experiments, the epithelial rudiments in inguinal glands of 3-week-old mice were surgically removed. At 5 or 10 weeks of age, both cleared mammary glands of host mice were transplanted with an approximately 1 mm3 fragment of previously frozen Trp53-null BALB/c mammary gland harvested and pooled from three or more inguinal glands of 8- to 10-week-old donor mice. Mice were monitored by palpation two to three times per week for 13 months. Once a palpable tumor was detected, tumors were measured with calipers twice weekly until reaching 1 cm3, at which point the first tumor was resected using survival surgery. Tumors were divided and frozen in liquid nitrogen for RNA extraction, or formalin-fixed followed by paraffin embedding. The mouse was further observed until the ipsilateral recurrence or a second tumor developed in the contralateral fatpad, which was monitored as above. If no tumors developed by experiment termination, which was 13 months after transplantation, then a wholemount was prepared to confirm transplantation efficiency. A gross necropsy was performed upon termination. An informative transplant was defined as that which had an epithelial outgrowth evident by tumor development or in the wholemount at sacrifice.

Immunohistochemistry

Sections were deparaffinized and rehydrated before antigen unmasking according to the manufacturer’s instructions (Vector Labs; #H-3300), washed once with phosphate-buffered saline (PBS), and blocked with 0.5% casein and 0.1% Tween20/PBS for 1 hour at room temperature. Primary antibodies to ER (C1355; Millipore/Upstate; #06-935), PR (Fisher Scientific; RM-9102-50), Foxa1 (Abcam; ab23738), Gata3 (Santa Cruz Biotechnology; SC-268), or phospho-IGF1 receptor (IGFR; Abcam; Ab39398) were diluted in Superblock Blocking Buffer (Pierce; #37515) and incubation was done at 4°C, overnight. The slides were then washed, followed by incubation with peroxide-conjugated secondary antibody, washed, and counterstained with hematoxylin. Histopathologic characteristics of the tumors were reviewed by two observers blinded to the experimental details of the mouse models. ER status was scored using the Allred method (26).

Expression profiling

Total RNA quality and quantity were determined using Agilent 2100 Bioanalyzer and NanoDrop ND-1000, Illumina-Mouse WG-6 v2.0 Expression Beadchips were used according to the manufacturer’s protocol. The raw values (non-normalized and non-background-corrected) were exported from Beadstudio and read into R/Bioconductor using the Lumi package. Intensity values were then log transformed and quantile normalized. Background subtraction was not
performed. The main effect of no background correction for Illumina data is that the fold-change estimation for low expression probes tends to be lower, but low expression yet high fold-change probes are more likely to be false positives. Gene expression data are archived on Gene Expression Omnibus (GEO) under accession number GSE56726.

Unsupervised hierarchical clustering (UHC) was done using Gene Cluster v3.0 software and heatmaps were visualized using Java TreeView v1.1.4r3 software. Expression data were mean centered. Gene clustering was done by an uncentered-correlation was done by Spearman rank correlation. Significance of Analysis of Microarray (SAM) analysis was done in a tandem-bootstrapping scheme as previously described (23). This approach derived a profile of 243 genes modulated by at least 1.5-fold in juvenile host (JH) tumors in 100% (13 of 13) of the secondary bootstraps. Enriched pathways within the 243 genes were identified with Ingenuity Pathway Analysis (IPA).

Statistical analysis
Statistical analysis was performed using Prism (GraphPad). Time to 2 × 2 mm² tumor occurrence per informative fatpad was plotted using the Kaplan–Meier method with significance determined by the log-rank test. Tumor growth curves in a treatment group were fitted to an exponential curve and then averaged into one curve for each treatment group, as previously reported (23). Differences between treatment groups were determined using the χ² test, Mann–Whitney test, or two-tailed Student t test, which were considered statistically significant at P < 0.05. The K-S test was used to determine the difference in distributions of human breast cancers that were classified by the centroids and then stratified by plotting in two dimensions.

Results
Age at transplantation affects Trp53-null mammary carcinogenesis
The mouse mammary gland develops after birth, starting at 4 weeks of age with the onset of the ovarian hormones of puberty. The inguinal mammary gland can be dissected of its endogenous epithelia at 3 weeks of age (27). Afterward, a mammary fragment from a donor mouse can be transplanted into the empty fatpad and it will grow out to form a ductal tree by 6 to 8 weeks. Here, we use the mammary chimera model to test the effect of age by characterizing the occurrence and type of tumors arising from Trp53-null outgrowths initiated in a host undergoing puberty compared with those that arise from transplantation into an adult. Trp53-null mammary fragments from 8- to 10-week-old syngeneic donors were transplanted into 5-week-old (juvenile) or 10-week-old (adult) BALB/c hosts; tumor occurrence was monitored for 13 months after transplantation (Fig. 1A). Significantly more palpable tumors were obtained in mice that were adults at transplantation (Fig. 1B). The extent of morphogenesis and successful transplantation frequency were comparable regardless of age at transplantation. The average size of sub-clinical neoplastic lesions (i.e., those that did not form frank, palpable tumors) evident in

Image: Figure 1. Trp53-null transplantation into JH delays tumorigenesis compared with AH. A, cartoon of experimental scheme in which the epithelial rudiments of host mice were surgically removed from the inguinal mammary gland. A mammary fragment from a syngeneic Trp53-null BALB/c mouse was transplanted into the empty fatpad at either 5 weeks of age (middle of puberty) or 10 weeks of age (young adult). Tumor development was tracked for 13 months. B, Kaplan–Meier analysis tracking the time to tumor. Transplants into AH (red) had a median tumor latency of 347 days, with 55% (n = 89) of successful outgrowths forming tumors. Transplants into JH fatpads (pink; n = 55) formed tumors at a frequency of 27% after 13 months (log-rank test, P = 0.003).

carmine-stained outgrowth wholemounts was similar for transplantation at each host age (data not shown). Age at transplantation did not affect the distribution of tumor histopathology (summarized in Supplementary Table S1), which included adenocarcinomas, spindle cell carcinomas, and squamous carcinomas as previously reported for this Trp53-null model (23). Adult host (AH) tumors (n = 89) had a median latency of 347 days, and 55% of informative fatpads were tumor bearing by experiment termination. In contrast, the median latency of JH tumors (n = 55) could not be calculated because only 27% of informative fatpads developed palpable tumors.

Transplantation during puberty results in more aggressive tumors
Although fewer tumors arose from transplantation during puberty, once the tumor was detected, JH tumors grew significantly faster compared with AH (Fig. 2A). The tumor growth rate of tumors arising from transplantation to adults was comparable with that observed in previous experiments (23). Moreover, the difference of tumor growth rate was replicated in cohort of mice transplanted as adults versus juveniles in an independent study (28). Mitotic figures that included the spectrum of condensed chromosome states (Fig. 2B) were quantified in high-powered fields of view (n = 10) per hematoxylin and eosin (H&E)–stained tumor. Consistent with increased growth rate, mitotic figures were increased 2-fold in JH tumors compared with AH tumors (Fig. 2C). We next examined the degree of vascular perfusion. Red blood cells
are auto-fluorescent under the UV filter in H&E-stained tissue. We quantified the average area of red blood cell cross-sections encapsulated within a membrane lining to exclude areas of hemorrhage to measure the size of perfused vessels (Fig. 2D). The size of perfused vessels in JH tumors was twice that of AH tumors (Fig. 3E). Thus, the age of the host, juvenile, or adult at the time of \textit{Trp53}-null mammary transplantation affects the features of tumors that arise during the following year.

Transplantation during puberty results in a luminal breast cancer subtype

The BALB/c \textit{Trp53}-null mammary transplant model recapitulates the histologic (29, 30) and molecular diversity of human breast cancer (24, 31). Eighty percent of the tumors arising from JH were ER\textsuperscript{+} by the Allred score (26), compared with less than 40\% of AH tumors (4 of 11). Similarly, PR immunoreactivity in PR\textsuperscript{+} tumors from JH tended to be higher (JH: mean, 11\%–30\%; AH: mean, 2\%–10\%). Eighty percent of JH tumors (8 of 10) were also positive for Foxa1. The intensity and frequency of Foxa1 positivity were comparable between JH and AH tumors. We also examined Gata3 by immunostaining; however, the results were not informative, with only one positive nuclear staining out of 20 tumors (10 JH and 10 AH). Overall, JH tumors exhibit features of a luminal cancer subtype.

We next conducted genome-wide microarray analysis using total RNA from JH tumors (\textit{n} = 15) and AH (\textit{n} = 12) tumors using Illumina Mouse WG-6 V2.0 Beadchips. UHC of all tumors did not segregate JH from AH tumors (data not shown). SAM analysis was done in a tandem-bootstrapping scheme, as previously reported (23), to derive a profile of genes modulated by at least 1.5-fold in 100\% (13 of 13) of the secondary
Adult expression program induced by the JH: detected in these tumors, there was a clear luminal gene score (38). Consistent with the high ER protein expression, proliferation, cancer, and tumor morphology (Fig. 4B, IPA score 43), cellular growth and included embryonic development, organ development, and revealed that the global processes occurring in these 243 genes most JH tumors apart from AH tumors (data not shown). IPA of the 27 tumors by these 243 genes resulted in segregation of compared with AH tumors (Supplementary Table S1). UHC Table S2) that represented the biology of JH tumors were more immunoreactive for ER than tumors from AH (JH: mean, 10) from transplants into AH as classi

Figure 3. Tumors from transplantation during puberty are more highly immunoreactive for ER. A, 80% of tumors (n = 21) from transplants into JH were ER-immunoreactive compared with 70% of tumors (n = 10) from transplants into AH as classified according to the Allred score. B, representative images of ER immunohistochemistry in tumors from juvenile or AH (bar, 60 μm), C, comparison of the frequency component of the Allred score revealed that tumors from JH were more immunoreactive for ER than tumors from AH (JH: mean, 11%–33%, max, >66%; adult: mean, 1%–10%, max, 11%–33%; ***; P = 0.0004, Mann–Whitney test).

boosts in JH tumors. This yielded 243 genes (Supplementary Table S2) that represented the biology of JH tumors compared with AH tumors (Supplementary Table S1). UHC of the 27 tumors by these 243 genes resulted in segregation of most JH tumors apart from AH tumors (data not shown). IPA revealed that the global processes occurring in these 243 genes included embryonic development, organ development, and organ morphology (Fig. 4A, IPA score 43), cellular growth and proliferation, cancer, and tumor morphology (Fig. 4B, IPA score 38). Consistent with the high ER protein expression detected in these tumors, there was a clear luminal gene expression program induced by the JH: Foxa1, Areg, and Krt18 (Fig. 4C). In addition, Igf2 and Ccnld1 (cyclin D1) transcripts were also increased, consistent with the increased mitotic index detected in JH tumors (32, 33). Beyond growth induction, the Areg gene product has been reported to have proangiogenic properties, which would be consistent with the increased perfusion observed in JH tumors (34).

Enhanced IGF1 signaling associates with the luminal subtype of breast cancer

Studies in human and mouse have shown that enhanced insulin-like growth factor (IGF) signaling is associated with breast cancer phenotype (reviewed in ref. 35), including poor prognosis (36), and IGF signaling regulates proliferation in the normal mammary gland (37, 38). Because JH tumors were highly proliferative and exhibited an increase in Igf2 transcript, we examined phosphorylated IGF receptor (IGFR) levels in JH tumors. Both Igf1 and Igf2 bind to IGFR and ligand binding of the IGFR leads to its phosphorylation. Phosphorylated IGFR was detected with an antibody targeting the Y1161 residue, which represents the activated state of the receptor. Positive cells were located near the tumor border were characterized by membrane staining (Fig. 4D and E). Phosphorylated IGFR staining was semiquantitative assessed as the percentage of a tumor that exhibited positive staining. Phosphorylated IGFR was increased 25-fold in JH tumors (P = 0.026) compared with AH tumors (Fig. 4F).

Human orthologs of the JvA243 profile segregates luminal breast cancers

The high ER protein expression and luminal transcriptional program in the JH tumors clearly suggested that these tumors were of a luminal subtype. We then determined the utility of 243-gene JH profile in discerning differences between human breast cancers using the Fredlund dataset, which represents 1,608 breast cancers compiled from 10 independent datasets on the Affymetrix U133A platform. Human orthologs of 161 genes, represented by 314 probes, of the 243 mouse genes were present on the Affymetrix Human U133A platform. To further explore this association, we developed a JvA centroid table, which maintains the relative direction of the genes that are differentially expressed in tumors arising in juvenile versus AH (Supplementary Table S3). Genes in the JvA centroid table were the human homologs mouse JvA genes, which are referred to as the JHb and AHb centroids, respectively. Human breast cancers (n = 1,608) from Fredlund were classified according to the Allred frequency score as the percentage of a tumor that exhibited positive staining. Phosphorylated IGFR was increased 25-fold in JH tumors (P = 0.026) compared with AH tumors (Fig. 4F).
LumB cancers are much more proliferative than LumA cancers (reviewed in ref. 8), we determined how the proliferation program in the 243 genes affected segregation. Removal of 31 proliferation-related genes in the 161 human orthologs left 130 human genes represented by 240 probes (Supplementary Table S2). The proliferation-divested profile showed even stronger segregation of LumB cancer in the Ivshina dataset (16% in sub-tree 1, 63% in sub-tree 2; $\chi^2, P < 0.0001$; Fig. 6B). The same effect was observed on 110 grade-1 LumA or LumB cancers ($\chi^2, P < 0.001$; Fig. 6C). Effective segregation of LumA from LumB cancers without the proliferation-related genes suggests that the JvA 243 profile captures a biologic distinction between LumA and LumB that is more than just increased proliferation. Taken together, the bioinformatic analyses suggest that the JvA gene list identifies a distinct luminal breast cancer subtype.

**Discussion**

To date, studies using the *Trp53*-null mammary chimera at different ages, genotypes, or physiologic states suggest that it is a robust model for studying breast cancer diversity (23, 31, 42–44). Our studies using this model suggest that the host environment profoundly influences the type of tumor that eventually develops long after mammary outgrowth is complete. Here, we show that the age at transplantation elicits phenotypically and genomically distinct tumors that have features strongly reminiscent of the human luminal intrinsic subtypes. Although transplantation of AH generated more tumors...
Human breast cancer. Centroids defined by the human orthologs of the JvA were informative in regards to intrinsic tumor types in the JvA. Moreover, ER and phosphorylated IGFR staining was perfused than those arising from outgrowths initiated in adult mice. Notably, proliferation mediated by IGFR is important in cancer overall, is strongly associated with poor prognosis in breast tumors (36), and increases susceptibility in familial breast cancer (46).

Given the diversity of Tp53 tumor phenotypes, these specimens provide a meaningful model to further examine specific genomic alterations associated with particular tumor types. One means by which host physiology may influence tumor type is by causing epigenetic changes. The Cancer Genome Atlas project integrated copy number, RNA, methylation status, and miRNA data from approximately 300 to 460 human breast cancer genomes for each breast cancer subtype (9). Breast cancers positive for ER have a distinct methylene profile compared with the more aggressive ER cancer (47). Methylation is a key component of differentiation-associated patterns of transcription in normal development of the mammary gland (48). Both hypo- and hypermethylation changes are associated with disease. DNA methylation can confer long-term memory of early life events (49). Consistent with this, high estrogen exposure during neonatal development induces epigenetic imprinting in the form of gene methylation that is reflected in uterine and prostate cancers arising later on in adulthood (50, 51). Indeed, breast cancer molecular subtypes defined by transcriptomic profiling can also be characterized by methylene profiling (52), suggesting that the two may go hand-in-hand. Identical hypermethylated genes occur in histologically normal ducts that are adjacent to preinvasive and invasive lesions, suggesting a common loco-regional etiology mediated by methylation (9, 53, 54).

We speculate that the distinct tumor subtypes that arise as a function of host biology in the mammary chimera model could be rooted in factors that influence cell intrinsic processes of either initiation or early progression during specific physiologic states, which in this case is puberty. The relative short age of the JvA profile stratified luminal from basal-like human breast cancers and luminal A from luminal B breast cancers. In support of this, JvA clustered luminal A and B intrinsic subtypes across public datasets.

An intriguing question is how the physiology and microenvironment during puberty promotes the development of highly ER mammary cancers. The mammary gland in mice and humans develops into maturity after birth. The onset of puberty initiates the estrus cycle that releases cyclical surges of estrogen and progesterone, two of the main hormones that induce ductal morphogenesis. Concomitantly, growth hormone and IGF stimulate longitudinal growth of the entire organism, while also being essential regulators of mammary gland development (37, 38). Phosphorylated IGFR staining was strikingly elevated in the highly ER JH tumors compared with AH tumors. Involvement of IGF signaling is consistent with the highly ER luminal phenotype. It has been shown that in mice, serum IGF1 level was significantly higher during the late puberty (5–8 weeks of age) compared with the level during the early puberty (3–5 weeks of age; ref. 45). It is possible that this growth factor surge promotes the carcinogenesis of JH tumor while for AH tumor, such growth-stimulation is lacking. This potential mechanism will be investigated in the further studies where different hormones are evaluated at different ages. Notably, proliferation mediated by IGFR1 is important in cancer overall, is strongly associated with poor prognosis in breast tumors (36), and increases susceptibility in familial breast cancer (46).

Figure 5. Luminal A human breast cancers are more similar to JH tumors. A, centroids (JHh and AHh) representing the human orthologs of the JvA 243-gene profile were used to stratify 1,063 luminal (blue) or basal-like (red) human breast cancers (x-axis, AH; y-axis, JH). JHh vs. AHh centroid stratification distinguished luminal cancers from basal-like cancers, with the distribution of basal-like cancers shifting toward the positive AHh axis (K-S test, \( P = 1.11E^{-33} \)). This suggests that basal-like cancers are more similar to tumors arising from transplantation to AH. B and C, the centroid stratification of luminal A and luminal B cancers shows that luminal A cancers shift toward the positive JHh axis and away from the positive AHh axis, while luminal B cancers are shifted toward the positive AHh axis and away from the positive JHh axis (K-S test, \( P = 3.17E^{-16} \)).
window of 5 weeks between transplantation resulting in JH and AH tumors suggest that events occurring in roughly the first month of transplant outgrowth affects the specific tumor type. The transplanted tissue fragments contain about 2,000 epithelial cells, of which 1 to 4 are stem cells based on functional repopulation assays (28).

Trp53-null tissue gives rise to morphologically normal ductal outgrowths by 3 months posttransplantation; over the course of the next 6 to 9 months, morphologically aberrant lesions become evident. This effect of transplantation at different ages is consistent with our studies in irradiated hosts in which tumor type was affected even though only the host was irradiated months before tumor development. A strong radiation signature in the mammary gland expression profiles is present at 1 week after exposure, is much less robust at 4 weeks, and is no longer discernable 12 weeks after irradiation, yet is evident in tumors arising months later (23, 28).

In multistage carcinogenesis models, cancer is initiated by genomically aberrant cells whose proliferation results in acquisition of additional mutations to establish the malignant phenotype. Concomitant subversion of tissue suppressive function, evasion of the immune system, and recruitment of a permissive stroma involves a dynamic interplay between malignant and nonmalignant cells that is equally important (25). Our data from the Trp53 mammary chimera model show that tumor latency and type is highly influenced by differences in host biology at the time of transplantation due to exogenous conditions (e.g., ionizing radiation exposure, diet) or endogenous factors (e.g., host genotype, age). The idea that tumor type is determined by biology outside the epithelial cell genome is provocative in that it suggests that tumor type is not the sum of random genomic alterations and that, once understood, such host biology might be a target for chemoprevention. Extrinsic carcinogenesis processes can be viewed in terms of environmental imprinting or ecologic selection, although it is unclear which is more likely without more detailed studies or modeling. The mammary chimera model provides the means to somewhat uncouple cellular (i.e., initiation) and systemic responses to physiologic or environmental (e.g., life style) factors during carcinogenesis.

Conclusions
Age is clearly a primary factor associated with the risk of detecting clinically evident cancer, but it has been less clear how age intersects with the carcinogenic process. Here,
mammary Tp53-null carcinogenesis advanced more rapidly from transplantation to adult compared with pubertal hosts, but tumors arising from transplantation during puberty had distinct hallmarks (e.g., faster growth rate, more mitoses, and greater perfusion) than those tumors arising from transplantation to an adult mouse. Preliminary data from middle-aged mice (i.e., transplanted at 10 months of age) indicate that tumorigenesis is reduced but the frequency of ER- tumors substantially increased (unpublished data). The mammary chimera model thus provides novel evidence that both the latency and subtype of cancer is strongly influenced by factors beyond genomic alterations within the tumor cells.

The distinct biology of murine tumors arising from tissue undergoing morphogenesis in the context of puberty suggests that neoplastic transformation at a young age promotes a luminal tumor phenotype. These data support the notion that tumor intrinsic subtype is influenced by physiologic status at the time of putative initiation. Future studies will determine how systemic factors particular to puberty act to promote luminal-type tumors. Potential mechanisms include those affecting initiation, supporting survival of cells with particular mutations, or generating a stroma permissive for particular initiation events.

Disclosure of Potential Conflicts of Interest
D.H. Nguyen is a consultant at Creative Bioinformatics Consulting and is Editor-in-Chief and President of Cancer InCytes Magazine. No potential conflicts of interest were disclosed by the other authors.

References


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