The Landscape of Somatic Chromosomal Copy Number Aberrations in GEM Models of Prostate Carcinoma
Daniella Bianchi-Frias, Susana A. Hernandez, Roger Coleman, Hong Wu, and Peter S. Nelson

Abstract

Human prostate cancer is known to harbor recurrent genomic aberrations consisting of chromosomal losses, gains, rearrangements, and mutations that involve oncogenes and tumor suppressors. Genetically engineered mouse (GEM) models have been constructed to assess the causal role of these putative oncogenic events and provide molecular insight into disease pathogenesis. While GEM models generally initiate neoplasia by manipulating a single gene, expression profiles of GEM tumors typically comprise hundreds of transcript alterations. It is unclear whether these transcriptional changes represent the pleiotropic effects of single oncogenes, and/or cooperating genomic or epigenomic events. Therefore, it was determined whether structural chromosomal alterations occur in GEM models of prostate cancer and whether the changes are concordant with human carcinomas. Whole genome array-based comparative genomic hybridization (CGH) was used to identify somatic chromosomal copy number aberrations (SCNA) in the widely used TRAMP, Hi-Myc, Pten-null, and LADY GEM models. Interestingly, very few SCNAs were identified and the genomic architecture of Hi-Myc, Pten-null, and LADY tumors were essentially identical to the germline. TRAMP neuroendocrine carcinomas contained SCNAs, which comprised three recurrent aberrations including a single copy loss of chromosome 19 (encoding Pten). In contrast, cell lines derived from the TRAMP, Hi-Myc, and Pten-null tumors were notable for numerous SCNAs that included copy gains of chromosome 15 (encoding Myc) and losses of chromosome 11 (encoding p53).

Implications: Chromosomal alterations are not a prerequisite for tumor formation in GEM prostate cancer models and cooperating events do not naturally occur by mechanisms that recapitulate changes in genomic integrity as observed in human prostate cancer. Mol Cancer Res; 13(2); 339–47. ©2014 AACR.

Introduction

To aid in evaluating genetic and environmental factors that influence the development and progression of prostate cancer, murine models have been developed that employ gene targeting technology to alter key signaling programs that regulate aspects of cellular proliferation and survival. The mouse does not naturally develop prostate cancer, and although the rodent prostate differs from the human prostate anatomically, several genetically engineered (GEM) models exhibit attributes also observed in human prostate cancer. Notably, histologic features of prostatic intraepithelial neoplasia (PIN), locally invasive adenocarcinoma, and neuroendocrine carcinoma are recapitulated in GEM prostate cancer models (reviewed in ref. 1, 2). Several models also regress following systemic androgen suppression or pharmacologic inhibition of androgen receptor (AR) signaling, a hallmark of clinical responses observed in human prostate cancer.

The transgenic adenocarcinoma of the mouse prostate (TRAMP) was the first prostate cancer GEM model developed. This model expresses SV40 T-antigen (Tag) under a minimal probasin promoter which specifically targets transgene expression to the prostatic epithelium (3, 4). The full SV40 Tag abrogates the function of the retinoblastoma (RB1), TRP53, and PP2A tumor suppressor proteins (5), each of which are altered in a subset of primary human prostate cancers (6). A second T antigen GEM model, designated LADY, expresses the large T-antigen, lacks small t-antigen, and consequently inactivates only RB1 and TRP53 function. LADY transgenic mice develop murine prostate intraepithelial neoplasia (mPIN) locally invasive adenocarcinoma, and neuroendocrine carcinoma are recapitulated in GEM prostate cancer models (reviewed in ref. 1, 2). Several models also regress following systemic androgen suppression or pharmacologic inhibition of androgen receptor (AR) signaling, a hallmark of clinical responses observed in human prostate cancer.

The transgenic adenocarcinoma of the mouse prostate (TRAMP) was the first prostate cancer GEM model developed. This model expresses SV40 T-antigen (Tag) under a minimal probasin promoter which specifically targets transgene expression to the prostatic epithelium (3, 4). The full SV40 Tag abrogates the function of the retinoblastoma (RB1), TRP53, and PP2A tumor suppressor proteins (5), each of which are altered in a subset of primary human prostate cancers (6). A second T antigen GEM model, designated LADY, expresses the large T-antigen, lacks small t-antigen, and consequently inactivates only RB1 and TRP53 function. LADY transgenic mice develop murine prostate intraepithelial neoplasia (mPIN) that progresses to adenocarcinoma (7). The tumor suppressor PTEN is lost in approximately 40% of human prostate tumors (6) and GEM models with prostate-specific deletion of Pten develop mPIN and ultimately invasive adenocarcinoma and metastasis at a low frequency (8). Increased copy number of the Myc locus and overexpression of MYC protein are also common in human prostate cancer (6). Mouse strains engineered to overexpress MYC in prostate epithelium develop mPIN that progresses to locally invasive adenocarcinoma by 3 to 6 months.

In addition to the histologic similarities between the neoplasms that develop spontaneously in humans and as a consequence of genetic manipulation in the mouse, cross-species comparisons of gene expression have identified syntenic down-stream molecular alterations (8, 9). While concordant changes in
the specific pathway perturbed in the human cancer and corre-
sponding GEM model are anticipated, profiling studies indicate 
that additional alterations accompany the initiating event, and 
the patterns and networks of gene expression exhibit parallels 
with human cancers. This association has been seen in several 
GEM models for breast, lung, colon, and prostate cancer, among 
others (9–12). These observations suggest that cooperating geno-
mic and/or epigenetic events might be shared between species, 
which could explain in part the recurrent deregulation of a subset 
of key obligate genes promoting the transition of premalignant 
cells to invasive neoplasms.

In human carcinomas, phenotypic changes associated with 
gene expression can often be attributed to underlying alterations 
in the genomic architecture of the cancer cell—regions of DNA 
copy gain, DNA loss, aneuploidy, and nucleotide insertions, 
deletions, and base changes. Studies of primary and metastatic 
human prostate cancers report numerous recurrent genomic 
alterations: at the time of diagnosis, the genome of a typical 
primary prostate cancer harbors between 10 to 100 nonsynon-
yymous nucleotide mutations and multiple chromosomal rear-
rangements and somatic copy number aberrations (SCNAs; 
refs. 6, 13–16). In addition to TRP53, RB, PTEN, and MYC 
described above, examples of recurrent genomic aberrations 
include mutations in SPOP (~13%), MEDI2 (5%), rearrange-
ments of the TMRRSS2-ERG locus (~50%), loss of chromosome 
8p (~30–50%), and gain of chromosome 8q (~20–40%).

The clonal and recurrent nature of genomic aberrations in 
human prostate cancers strongly suggests that the genes and/or 
regulatory elements contained in these loci contribute to neo-
plastic growth. Prostate tumors rarely have a single anomaly, 
but rather commonly harbor multiple recurrent genomic alter-
ations, a finding that strongly suggests a requirement for cooperating 
events to effectively drive malignant phenotypes. To date, there is 
little information concerning whether recurrent genomic aberr-
ations in GEM models of prostate cancer associate with neoplasic 
progression and underlie the extensive gene expression alterations 
observed in these models. As chromosomal structural alterations 
dominate the mutational landscape of human prostate cancers, 
we undertook this study to determine whether recurrent SCNAs 
dominate the mutational landscape of human prostate cancers, 
or whether additional alterations accompany the initiating event, and 
assess whether the genomic changes are concordant with those commonly found in 
human prostate cancers.

Materials and Methods
Genetically engineered mouse models
The TRAMP C57BL/6 FVB F1 mice used in these studies were 
generated as follows: C57BL/6 (B6) TRAMP mice were obtained 
from Dr. Norman Greenberg (Fred Hutchinson Cancer Center, 
Seattle, WA) and were subsequently bred by continued back-
crossing to B6 mice (Jackson Labs). FVB/NTac mice were obtained 
from Taconic. B6 TRAMP females were mated with FVB males to 
generate B6/FVB F1 TRAMP animals. Hic-Myc mice were obtained 
from the Mouse Repository of the National Cancer Institute 
Mouse Models of Human Cancer Consortium. Hemizygous Hic-
Myc mice on FVB background were cross-bred with nontransgenic 
FVB breeders from Taconic. B6/FVB F1 TRAMP mice between 24 
and 29 weeks old and Hi-Myc mice between 56 and 72 weeks old 
were sacrificed by cervical dislocation. Spleens were removed and 
snap-frozen in liquid nitrogen. Prostate glands were dissected and 
cut into two pieces, one was processed for histology and the other 
was snap-frozen in liquid nitrogen and stored at ~80°C until 
DNA/RNA extraction. All animals were maintained pathogen free 
in the Fred Hutchinson Cancer Research center animal facility 
that is fully accredited by the Association for Assessment and 
Accreditation of Laboratory Animal Care. The Pb-Cre−/Pten+/−/
GEM was generated and propagated in a C57BL/6.DBA.129/Balb/c 
background as previously described (8). This strain was main-
tained at the University of California Los Angeles by Dr. Hong Wu. 
Prostate glands from Pb-Cre−/Pten+/−/ at age 24 weeks were 
resected, snap frozen, and processed as previously described (8).

Cell lines derived from GEM models of prostate cancer
TRAMP-C2 cells were a generous gift from Dr. Norman Green-
berg (Fred Hutchinson Cancer Research Center, Seattle, WA) and 
were originally derived from a primary prostate tumor of a 32-
week-old Pb-Tag C57BL/6 (TRAMP) mouse (17). Myc-Cap cells 
were established from a primary prostate carcinoma dissected 
from a 16-month-old Hi-Myc transgenic mouse in the FVB inbred 
strain (18). Myc-Cap and TRAMP-C2 cells were maintained in 
DMEM supplemented with 10% FBS at 37°C. Pten-P8 cells 
were established from a primary prostate tumor dissected from a 10-
month-old Pb-Cre−/Pten+/−/ mouse (19). Pten-P8 were main-
tained in DMEM supplemented with 10% FBS (Omega Scientific), 
25 Ag/ml bovine pituitary extract, 5 Ag/ml bovine insulin, and 6 
ng/ml recombinant human EGF (Sigma-Aldrich). Cell lines 
were authenticated within 3 passages of performing CGH studies 
by comparing expression profiles with previously published 
expression profiles and confirming concordant relationships by 
unsupervised clustering.

RNA and DNA extraction
Approximately 5 mg of frozen prostate tumor or spleen were 
ground in liquid nitrogen containing 350 μL of RLT plus buffer, 
and the resulting powder was placed in Eppendorf tubes and store 
at ~80°C until DNA/RNA extraction. DNA and RNA extraction 
was done using the QiaGen AllPrep DNA/RNA kit (Qiagen) 
according to the manufacturer’s instructions. The reference DNA 
(wild-type male C57BL/6 mouse) was purchased from The Jack-
son Laboratory.

Copy number analysis by array comparative genomic 
hybridization
Paraffin-embedded (TRAMP samples) or optimum cutting 
temperature embedded (Hi-Myc and Pb-Cre−/Pten+/−/) prostate 
tumor sections were stained with hematoxylin and eosin according 
to standard protocols. All tissues were cut into 4 or 5 μm 
sections and stained with hematoxylin and eosin (H&E) for 
histologic examination to confirm the presence of neoplastic cells. 
Scale bars in all H&E images for low (4×) and high power (20×) 
magnification are 500 μm and 100 μm, respectively. DNA from 
3 prostate tumors and germline spleen DNA from 3 different mice, 
for each GEM model, and a common reference male C57BL/6 
DNA were used for CGH array analysis using the Agilent SurePrint 
G3 mouse CGH 4 × 180K microarray platform (Agilent Tech-
nologies). The sample preparation and hybridization protocols 
recommended by Agilent were followed. Briefly, we used 1.5 μg of 
tumor or germline spleen DNA and 1.5 μg of reference DNA for 
each analysis. DNA was digested with Rsa I and Alu I and labeled 
by random priming using either Cy3-dUTP or Cy5-dUTP,
respectively. Unincorporated nucleotides were removed using Amicon Ultra-0.5 mL, Ultracel-30 membrane (Millipore), and tumor (or germline) and reference samples were combined. Probes were denatured and preannealed with 50 μg of mouse Cot-1 DNA (Invitrogen), and 71 μL of hybridization master mix was added before loading into array slides. Hybridization was performed at 65°C for 24 hours at 20 rpm. After hybridization, slides were washed and scanned immediately with a DNA Microarray Scanner (Agilent Technologies). Data were extracted from scanned images using Feature Extraction software (Agilent). The text files were then imported for analysis into Genomic Workbench, standard edition 7.0.4.0 (Agilent). We used the Aberration Detection Method 2 (ADM-2) algorithm to identify DNA copy number aberrations. The statistical score represents the deviation of the average of the log ratios from the expected value of zero, in units of SD. The following parameters were used in this analysis: threshold of ADM-2: 7.0; fuzzy zero: ON; GC correction: ON, diploid peak centralization: ON; (minimum number of probes for amplification ≥ 6 and minimum size (Kb) of region for amplification ≥ 0.0 and minimum average absolute log ratio for amplification ≥ 0.25) or (minimum number of probes for deletion ≥ 6 and minimum size (Kb) of region for deletion ≥ 0.0 and minimum average absolute log ratio for deletion ≥ 0.25). Genomic positions were based on the UCSC July 2007 mouse reference sequence (NCBI37/mm9).

Results

Genomic aberrations in the TRAMP GEM model

In the TRAMP model, the onset of prostate tumor development varies depending on the strain background (3, 20). In this study, we evaluated prostatic neoplasms arising in a mixed C57BL/6 × FVB/N background as the FVB genotype exhibits reduced latency, enhanced primary tumor growth, and high rates of metastasis when compared to tumor development in a pure C57BL/6 background (3, 20). We resected the prostate glands from 24- to 29-week-old TRAMP (C57BL/6 × FVB) F1 male mice and analyzed three prostate neuroendocrine carcinomas (NEC, n = 3), two prostate (from littermate mice) with atypical hyperplasia of Tag (AHTag), and used spleen DNA from 3 independent mice as the reference to control for strain-specific genomic variation. DNA from AHTag and neuroendocrine carcinomas were extracted from a defined tumor mass where at least 70% of the region contained hyperplastic/neuroendocrine cells, as determined by H&E stains (Fig. 1A and B, respectively).

A comparison of array CGH signals across the genomes of TRAMP mice and the C57BL/6 reference DNA clearly identified strain background-associated copy number variants (CNV), indicating FVB-specific CNVs such as a copy number loss in the T-cell receptor V-beta gene segment (chr6:41,018,389-41,112,757), typical of the TRAMP strain consistent with previous reports (refs. 21, 22, Fig. 1C).

In prostate neoplasms, the genomes derived from AHTag lesions did not exhibit any SCNAs (Fig. 1C, cyan blue). However, between 3 and 10 SCNAs were identified in neuroendocrine carcinomas (Fig. 1C and supporting Supplementary Table S1), though most were not present in more than one tumor. Gains of whole chromosomes 10, 12 and distal chromosome 11, focal gains in chromosome 1, 3 and 6, heterozygous loss of whole chromosome 16 and focal losses in chromosomes 1, 14 and Y were notable in individual tumors (Fig. 1C). Only three regions were shared in at least two of the 3 neuroendocrine tumors tested. The first was a localized heterozygous deletion (0.13 Mb segment) on chromosome 6 (chr6:63,863,932-63,996,654), a map location for the Grid2 gene (Fig. 1D). The second was a 0.68 Mb deletion on chromosome 8 (chr8: 50,762,466-51,279,003). The third region was a heterozygous copy number loss of the entire chromosome 19 (the location of murine Pten) observed in each of the neuroendocrine carcinomas analyzed (Fig. 1C and E). Of interest, 24% (9/37 cases) of metastatic human prostate cancers evaluated by array CGH exhibited heterozygous loss of the syntenic human genomic region on chromosome 4 encompassing Grid2, (chr4:9,325,550-9,693,649; Supplementary Fig. S1; ref. 6). However, Grid2 transcript levels were not significantly reduced in human or mouse tumors with heterozygous Grid2 loss (Supplementary Fig. S1 and S2).

To determine whether the loss of one chromosome 19 copy results in an overall downregulation of the genes located on this chromosome, we evaluated the corresponding transcript levels by gene expression arrays from an independent sample group. Overall, transcript levels for genes located on chromosome 19 were lower in TRAMP tumors compared with the normal tissues (P < 0.001; Supplementary Fig. S2). However, transcripts encoding the tumor suppressor gene Pten, located in chromosome 19, were not significantly decreased in TRAMP neuroendocrine tumors. This is consistent with a previous study of TRAMP tumors reporting the loss of one Pten allele in 50% of the mice, but no decrease in Pten protein was observed, nor were inactivating mutations in the other Pten allele detected (23). In contrast, transcripts encoding the Men1 tumor suppressor were diminished in tumors relative to benign prostate (P < 0.05; Supplementary Fig. S2). Of interest, mutations in Men1 confer genetic predisposition to neuroendocrine tumors in humans (24) and a subset of heterozygous Men1+ mice are reported to develop prostate pathologies in advanced age (25).

We also evaluated the genomic integrity of tumors obtained from the LADY GEM model (line LPB-Tag 12T-7f) which exhibits several features also found in the TRAMP GEM model such as the development of neuroendocrine tumors. This strain typically develops hyperplasia and carcinomas by 21 weeks of age. No SCNAs were identified in the tumors resected from 21-week-old LPB-Tag 12T-7f mice (see Supplementary Fig. S3 and Supplementary Table S2).

Genomic aberrations in the Pten-null GEM model

In the Pb-Cre;Pten+/− model, high-grade intraepithelial neoplasia or invasive carcinomas are evident by 24 weeks of age (8). We resected prostate glands and the spleens from 24-week Pb-Cre;Pten+/− mice and extracted DNA from whole Pten-null prostate after confirming that ≥70% of each sample comprised neoplastic cells (see Fig. 2A, A’). At the genomic level, Pten-null prostate tumors did not exhibit any unique somatic copy number changes identifiable by CGH (Fig. 2B). All CNAs were present in both germline and tumor tissue DNA, except one copy number loss (chr5: 105142362-105237345) called by the ADM-2 algorithm that appeared to be unique to one Pten tumor (asterisk in Fig. 2B). However, manual inspection of the intensity levels of the probes for that region revealed similar levels across all tumors and germline samples (see Supplementary Fig. S4; Supplementary Table S3). To call aberrations, we required a minimum number of 6 probes to call an...
Figure 1.
Histology and genomic copy number alterations in TRAMP tumors. A–B’, representative microscopic images from H&E-stained TRAMP sections from the prostate glands from which DNA was extracted for CGH analysis. A–A’, TRAMP atypical hyperplasia of Tag (AHTag) at low (4×) and high-power (20×), respectively. B–B’, TRAMP NEC at low (4×) and high-power (20×), respectively. C, copy number alterations (CNAs) in TRAMP mice by array CGH. Genomic DNA from C57BL/6. FVB TRAMP prostate neuroendocrine carcinomas, AHTag lesions, and spleen germline DNA control were hybridized against sex-matched normal mouse C57BL/6 reference DNA using Agilent CGH slides containing 180K probes. Whole genome view of overlaid moving averages (2 Mb window) for the log2 ratios of fluorescence between a sample/reference DNA probe (y-axis) plotted at its genomic position (x-axis), red, blue, and yellow (neuroendocrine tumors; n = 3), cyan blue shades (AHTag n = 2), and green shades (germline n = 3). Aberrations called in any samples (germline and tumor) by the ADM-2 algorithm are identified by a horizontal bar (red, blue, and yellow: neuroendocrine tumors), cyan (AHTag), and green (germline DNA). Aberrations present in at least two (of 3) neuroendocrine tumors are indicated by arrows; those found in individual mice are indicated by arrowheads. (1) Representative CNVs in common between germline, AHTag and neuroendocrine tumors; (2) aberrations called by the ADM-2 algorithm in a single sample that by manual inspection exhibited similar fluorescence intensities in all samples including germline and tumor (see Supplementary Fig. S2, for representative examples); (3) example of a known strain-specific alteration in the FVB strain (a copy number loss in the T-cell receptor V-beta gene segment (chr6:41,018,389-41,112,757). D, zoom-in view of chromosome 6 showing overlaid data points for log2 ratios of the Grid2 gene region. Green, values below log2 = −0.25; red, values above log2 = 0.25. Top, spleen and AHTag samples. Bottom, neuroendocrine tumors showing a SCNAs in the Grid 2 gene. Aberrations called by the ADM-2 algorithm are identified by a horizontal line (bottom). E, zoom-in on whole chromosome 19 showing overlaid data points for log2 ratios. Green, values below log2 = −0.25; red, values above log2 = 0.25. Top, spleen and AHTag samples. Bottom, neuroendocrine tumors showing a copy number loss of whole chr19. The aberration called by the ADM-2 algorithm of the entire chromosome 19 is identified by a horizontal line (bottom).
amplification or deletion. The CNA identified to be unique to one Pten-null tumor comprised only 6 probes in the region. The other germline and tumor samples exhibited overall similar intensity levels in the region, but did not contain the minimum number of probes to call an aberration. Likewise two additional copy number losses that appeared unique to the germline control samples demonstrated similar intensity levels across tumors and germline DNA. Thus, these unique calls are likely to represent germline copy number variation instead of somatic copy number changes.

Genomic aberrations in the Hi-Myc GEM model

The Hi-Myc GEM model is notable for the consistent development of mPIN at 2 to 12 weeks and carcinoma by 24 weeks. We resected the prostates and spleens from each of 3 ARR2/probasin-Myc transgenic mice aged 56 to 84 weeks and confirmed that the prostate tumor regions contained ≥70% neoplastic cells (Fig. 3A). The Hi-Myc mice developed a distinctive tumor mass that could be readily resected from other areas of normal prostate. Array CGH analysis of three prostate carcinomas demonstrated similar intensity levels across tumors and germline DNA. Thus, these unique calls are likely to represent germline copy number variation instead of somatic copy number changes.

Genomic aberrations in cell lines established from TRAMP, Pten-null, and Hi-Myc GEM prostate cancer models

Immortal cell lines from the TRAMP, Pb-Cre;Pten L/L, and ARR2PB-Myc GEM models designated TRAMP-C2, Pten-P8, and Myc-CaP, respectively, have been established and previously characterized (Table 1). We sought to determine whether genomic aberrations identified in the in situ tumors of the GEM models were also found in the derived cell lines, and whether new aberrations occurred during the selection and adaptation of tumor cells to in vitro growth conditions. CGH assays identified multiple genomic aberrations in the three cell lines (Fig. 4). The Myc-CaP line exhibited the greatest number of SCNAS (total of 82 CNAs, with 29 gains and 53 losses) including complete gains of chromosomes 5, 8, 11, 12, 15, 16, and X; partial gain of chromosomes 3, 6, 9, 17, and 19 and partial loss of chromosome 19, and several focal copy number gains and losses, including high-level amplification of the androgen receptor (Ar) locus (see Fig. 4, fuchsia) as reported previously (18). The TRAMP-C2 line (Fig. 4, blue) and Pten-P8 line (Fig. 4, yellow) had fewer...
The genomes of TRAMP-C2 cells (blue) were notable for gain of whole chromosome 15, and losses of chromosomes 1, 7, 9, 10, 14, and X, as well as partial losses of chromosome 2 and 5 (Fig. 4). We did not observe the loss of chromosome 19 or the localized loss in chromosome 6 in the TRAMP-C2 cell line identified in the tissues samples from TRAMP neuroendocrine carcinomas. The Pten-P8 cell line (Fig. 4, yellow) exhibited gains of chromosomes 10, 14, 15, and 17; whole chromosome losses of chromosomes 4, 9, and 13, as well as partial loss of chromosome 7 (Fig. 4A; Supplementary Table S5 for complete list of CNAs). Overall, the cell line genomes were notable for alterations in regions harboring several well-studied oncogenes and tumor suppressor genes (Fig. 4B). These included amplification of the Myc locus and deletion of Trp53.

**Discussion**

In this study, we examined the genomic integrity of tumors derived from four commonly studied GEM prostate cancer models: TRAMP, Hi-Myc, Pten-null (Pb-Cre\(^+\);Pten\(^{floxed/floxed}\)), and LADY. Very few somatic copy number alterations were apparent across these four GEM models: tumors from Hi-Myc, Pb-Cre\(^+\);Pten\(^{floxed/floxed}\), and LADY GEMs demonstrated identical genomic architecture when compared with corresponding germline DNA, with the exception of one Hi-Myc tumor with a somatic gain of chromosome 16.

**Table 1.** Genetically engineered mouse models of prostate cancer (and derived cell lines) assessed in this study

<table>
<thead>
<tr>
<th>GEM prostate cancer models</th>
<th>Reported age at tumor onset</th>
<th>Age (sample collection)</th>
<th>(Reference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRAMP</td>
<td>16 weeks hyperplasia/carcinoma</td>
<td>24–29 weeks</td>
<td>(3, 20)</td>
</tr>
<tr>
<td>Pten</td>
<td>9–29 weeks invasive carcinoma</td>
<td>24 weeks</td>
<td>(8)</td>
</tr>
<tr>
<td>Hi-Myc</td>
<td>≥26 weeks invasive carcinoma</td>
<td>≥56 weeks</td>
<td>(9)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>GEM prostate cancer model-derived cell lines</th>
<th>Mouse source genotype/strain background</th>
<th>Mouse source (age)</th>
<th>Notes</th>
<th>(Reference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRAMP-C2</td>
<td>rPB-SV40Tag [C57BL/6x.FVB] F1</td>
<td>32 weeks</td>
<td>Lost SV40</td>
<td>(17)</td>
</tr>
<tr>
<td>Pten-P8</td>
<td>ARR2Pb-Cre/Pten(^{floxed/floxed}) [C57BL/6;DBA.129/Balb/c]</td>
<td>43 weeks</td>
<td>heterozygous for Pten</td>
<td>(19)</td>
</tr>
<tr>
<td>Hi-Myc</td>
<td>ARR2Pb-Myc-PAI FVB</td>
<td>70 weeks</td>
<td>AR amplification</td>
<td>(18)</td>
</tr>
</tbody>
</table>

*Age at which mice were sacrificed for tissue collection.*
Using a lower resolution array, a previous study reported essentially no genomic aberrations in mPIN lesions and carcinomas from the Hi-Myc model, consistent with our observations using a higher resolution platform (9). The TRAMP neuroendocrine carcinoma was the only tumor type that contained SCNs but these were few and only three recurrent aberrations were identified. Kwabi-Addo and colleagues have previously demonstrated loss of heterozygosity (LOH) near the *Pten* locus in almost 50% of TRAMP (C57BL/6) tumors, but found no evidence that the retained *Pten* allele was inactivated (23). The reported LOH near the *Pten* locus in TRAMP tumors may reflect loss of the entire chromosome 19 as observed in our study. Of interest, a recent report characterizing genomic events in murine models of small-cell lung carcinoma initiated by loss of Trp53 and Rb1 also found high rates of Chr19 loss and *Pten* mutations (26), implicating *Pten* as a common factor in the development of small-cell carcinomas. In contrast to our assessments showing no SCNs in Lady 12T-7f GEM tumors, an allograft model of prostate cancer that originated from a Lady 12T-10 transgenic line (NE-10) showed copy number alterations in metastatic and nonmetastatic grafts (27). The NE-10 line was established from a prostate tumor of a 12T-10 transgenic mouse implanted subcutaneously and serially passed in male athymic nude mice. Several of the genomic aberrations reported in the NE-10 allograft tumors were also observed in our analyses of the TRAMP-C2, Myc-CaP, and *Pten*-P8 cell lines.

There are several reports describing genomic analyses of GEM models of other malignancies. Most studies, including those of breast, lung, liver, melanoma, neuroblastoma, pancreas, and colon cancer models describe relatively few structural and copy number aberrations (28–36). Interestingly, the greatest frequency of genomic alterations have been identified in short-term cultures of primary tumors, mice with telomere dysfunction, or compound models (29, 35, 37–45). The discrepancy between highly unstable human cancer genomes compared with the almost intact genomes observed in the GEM prostate cancer models is striking. Somatic copy number alterations are a common, if not universal, feature of human cancers (46) and the vast majority of prostate cancers harbor multiple recurrent genomic aberrations. A key difference that appears to underlie this cross-species difference in cancer genotypes centers on chromosome biology and structure, particularly telomeres (47). When the mouse genome is engineered to experience telomere dysfunction leading to shortened telomere length, genome instability is evident and tumors acquired a more human cancer-like genome with complex rearrangement and alterations, a subset of which are syntenic to loci altered in human cancers (32, 39, 48). Bojovic and colleagues demonstrated that short telomeres in a breast cancer transgenic model (MMTV-Neu) dramatically increased lung metastasis which correlated with specific alterations in DNA copy number and gene expression (32). Using a murine prostate cancer model driven by *Pten* loss and p53 deletions, Ding and colleagues demonstrated that telomerase dysfunction early in disease onset creates genomic aberrations crucial for telomerase-driven prostate cancer metastasis. Importantly, recurrent structural chromosomal alterations were observed that corresponded to alterations identified in human prostate cancer (39).

In striking contrast to the *in vivo* situation, we found that cell lines derived from tumors with stable genomes exhibited substantial genomic structural changes when propagated *in vitro*. The
chromosomal changes observed in these cell lines are consistent with previous reports. For instance, Pten-P8 has been shown to contain near 6N chromosomes with 113 to 125 chromosomes identified by SKY and G-banding tests (19). AR amplification has been observed in the Myc-CaP cell line (18). Surprisingly, a number of the chromosomal changes observed in these murine prostate cancer cell lines such as loss of chromosomes 4 and 9 and gain of chromosomes 11 and 15 are found in immortalized murine cell lines derived from normal kidney or bladder tissue (49). Clearly, the selective pressures are different for cells growing in laboratory culture than for cells dividing in the context of a tissue with physiologic tumor microenvironment.

Cross-species transcriptome analyses have shown that GEM cancer models and human tumors exhibit significant similarities in gene expression. For example, concordant with changes found in human prostate cancers, Pten-null murine prostate cancers exhibit alterations in the expression of more than 1,000 transcripts including higher levels of clusterin, prostate stem cell antigen, and osteopontin with concomitant downregulation of the Nkx3.1 tumor suppressor (8). Similarly, signatures of transcript profiles generated from the GEM model of Myc-driven prostate cancer classifies subsets of human prostate cancers into Myc-like and non-Myc-like tumors comprised of genes such as Pim-1 with known roles in cooperating with Myc to drive tumorigenesis (9). The lack of structural genomic alterations in these and other GEM tumors indicates that complex pathway interactions are activated and potentially maintained by a single, but robust oncogenic event. The mechanisms underlying the complex gene regulatory alterations that occur in the context of minimal genomic perturbations is unclear. Epigenetic changes could contribute to this complexity. However, it has been recently shown that in contrast to their human counterparts, tumors from GEM models of medulloblastoma, Burkitt lymphoma, and breast cancer harbor very few loci with DNA hypermethylation (50).

This study clearly demonstrates that gross structural chromosomal alterations are not a prerequisite for tumor formation in GEM models of prostate cancer and that secondary cooperating events do not appear to be activated by the mechanisms observed in human prostate cancer, at least during the lifespan of a typical mouse. We cannot rule out that somatic single-nucleotide mutations or small insertions or deletions may occur as secondary events do not appear to be activated by the mechanisms observed in human prostate cancer. Newer models engineering additional alterations into the backgrounds of Pten and p53 loss may provide insights into how these events cooperate, though the fact that these and other changes do not occur de novo suggests fundamental species differences in the genomes and the micro- and macroenvironments that influence tumorogenesis. Furthermore, the stable genomes of mouse models may influence the high response rates of tumors arising in GEM cancer models to cancer-directed therapeutics, and suggests that efforts designed to engineer mouse genomes with a greater degree of instability may result in models that better reflect the biology and treatment responses found in human prostate cancer.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
Conception and design: D. Bianchi-Frias, P.S. Nelson
Development of methodology: D. Bianchi-Frias, S.A. Hernandez, R. Coleman
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): D. Bianchi-Frias, R. Coleman, H. Wu, P.S. Nelson
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): D. Bianchi-Frias, P.S. Nelson
Writing, review, and/or revision of the manuscript: D. Bianchi-Frias, H. Wu, P.S. Nelson
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): D. Bianchi-Frias, P.S. Nelson
Study supervision: D. Bianchi-Frias, P.S. Nelson

Grant Support
D. Bianchi-Frias was supported by a post-doctoral training award from the Department of Defense (PC110970). This work was supported by awards from the Prostate Cancer Foundation (to H. Wu and P.S. Nelson), UO1 CA164188 (to H. Wu and P.S. Nelson), R01 CA165573, and the Pacific Northwest Prostate Cancer SPORE, P50CA97186.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received May 12, 2014; revised August 11, 2014; accepted September 12, 2014; published OnlineFirst October 8, 2014.

References
Genomic Aberrations in Murine Prostate Cancer Models


