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Simultaneous haploinsufficiency of *Pten* and *Trp53* tumor suppressor genes accelerates tumorigenesis in a mouse model of prostate cancer

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Abstract

Tumor suppressor gene *PTEN* is important in the initiation and progression of human prostate carcinoma, whereas the role of *TP53* remains controversial. Since *Pten/Trp53* double conditional knockout mice show earlier onset and fast progression of prostate cancer when compared to *Pten* knockout mice, we asked whether heterozygosity of these two tumor suppressor genes was sufficient to accelerate prostatic tumorigenesis. To answer this question we examined prostatic lesion progression of *Pten/Trp53* double heterozygous mice and a series of controls such as *Pten* heterozygous, *Pten* conditional knockout, *Trp53* heterozygous and *Trp53* knockout mice. Tissue recombination of adult prostatic epithelium coupled with embryonic rat seminal vesicle mesenchyme was used as a tool to stimulate prostatic epithelial proliferation. In our study, high-grade prostatic intraepithelial neoplasia (PIN) was found with high frequency at 8 weeks post-tissue recombination transplantation. PIN lesions in *Pten/Trp53* double heterozygous mice were more severe than those seen in *Pten* heterozygous alone. Furthermore, morphologic features attributable to *Pten* or *Trp53* loss appeared to be enhanced in double heterozygous tissues. LOH analysis of *Pten* and *Trp53* in genomic DNA collected from high-grade PIN lesions in *Pten* heterozygous and *Pten/Trp53* double heterozygous mice showed an intact wild-type allele for both genes in all samples examined. In conclusion, simultaneous heterozygosity of *Pten* and *Trp53* accelerates prostatic tumorigenesis in this mouse model of prostate cancer independently of loss of heterozygosity of either gene.

Keywords

Prostate cancer; Tumor suppressor genes; *Pten*; *Trp53*; Mouse models; Pathology; AKT; Tissue recombinants; Embryonic mesenchyme

1. Introduction

Tumor suppressor genes are extremely important in the initiation and progression of prostate cancer (Bookstein, 1994). Among them, *PTEN* and *TP53* are of specific interest due to the growing evidence of their role, individually and in combination, in the pathogenesis of prostate carcinoma.

PTEN encodes a dual specific phosphatase that counteracts many important cellular growth and survival pathways. One of the main substrates of this membrane phosphatase is the enzyme phosphatidyl-inositol kinase 3 (PIP3), which activates the AKT pathway and results in increased cell survival. *PTEN* also directly and indirectly regulates cell motility, migration and differentiation, as well as the cell cycle via interactions with focal adhesion kinase (FAK), mitogen activated protein kinases (MAPK) and cyclin D, respectively (Di Cristofano et al., 1998; Li and Sun, 1998; Mamillapalli et al., 2001; Radu et al., 2003; Sun et al., 1999; Yuan et al., 2000; Zheng et al., 2003).

In humans, loss of *PTEN* was identified in many primary and metastatic prostate carcinomas, as well as in prostate cancer cell lines (Cairns et al., 1997; Li et al., 1997; Suzuki et al., 1998; Wang et al., 1998). Recapitulating the human disease, *Pten* heterozygous mice as well as prostate conditional *Pten* knockout mice develop hyperplasia, PIN and invasive carcinomas with potential to metastasize (Di Cristofano et al., 1998, 2001; Kwabi-Addo et al., 2001; Podsypanina et al., 1999; Stambolic et al., 2000; Trotman et al., 2003; Wang et al., 2003).

The important tumor suppressor gene *TP53* induces apoptosis in the face of cellular damage posed by intrinsic or extrinsic stressors, such as hypoxia and DNA damage. Contradictions as to the direct role of *TP53* in prostate cancer abound in the literature, but *TP53* mutations appear to be more common in high-grade, metastatic and androgen-independent human prostate cancer (Bookstein et al., 1993; Chi et al., 1994; Costa-Pereira and Cotter, 1999; Gumerlock et al., 1997; Meyers et al., 1998).

Aside from the tumor suppressor functions of each individual gene, numerous interactions between the gene products *in vivo* have been reported: *PTEN* regulates the transcriptional activity, DNA-binding avidity and MDM-2-mediated degradation of *TP53* (Di Cristofano et al., 2001; Freeman et al., 2003; Mayo et al., 2002; Sheng et al., 2002; Stambolic et al., 2001). *PTEN* also mediates the inhibition of AP-1 and NF-kappa B by the tumor suppressor protein *TP53* (Wang et al., 2005). In addition, *TP53* regulates mTOR (target of rapamycin), an important downstream kinase in the PIP3/AKT pathway, inhibited by *PTEN* (Feng et al., 2005).

In vivo, lesion progression in *Pten/Trp53* double prostate conditional knockout mice indicates significant cooperation between *Pten* and *Trp53* (Chen et al., 2005). Double conditional knockout mice develop prostate cancer by 2 weeks post-puberty, with disease progression and death by 7 months of age. This is in contrast to the longer latency of prostate cancer development in *Pten* conditional knockout mice, and to the fact that the conditional *Trp53* knockout mouse fails to develop prostatic lesions.

In light of these data, the hypothesis was that simultaneous haploinsufficiency of both *PTEN* and *TP53* was enough to accelerate prostatic tumorigenesis. Because the latency for onset of

prostate lesions in *Pten/Trp53* double heterozygous mice is prohibitively long, this question was addressed by comparing prostate lesion progression in tissue recombinants made with rat seminal vesicle mesenchyme (rSVM) and mouse prostatic epithelium (mPRE) of different genetic make-ups. Our results show that simultaneous heterozygosity of *Pten* and *Trp53* accelerates prostate tumorigenesis. In this model, the combined effect of these two genes in early lesion progression cannot be explained by LOH events since all PIN lesions examined retained their wild-type alleles for both *Pten* and *Trp53*. Finally, we discuss morphologic hallmarks of the dysfunction of each of the two genes, providing a basis for interpretation of prostatic lesions in mouse models with alterations in *Pten*, *Trp53* or both.

2. Material and methods

2.1. Animal tissues

All prostate glands were obtained from post-pubertal mice (around 11 weeks of age) of the following genotypes: *Pten*^{+/-} on a C57BL/6J background (Banach-Petrosky et al., 2006; Gao et al., 2006; Podsypanina et al., 1999), *Pten* prostate conditional knockout mice on a C57BL/6xDBA2-129/Balb/c background (Wang et al., 2003) and *Trp53*^{+/-} and *Trp53*^{-/-} on a 129 SvJ background (Mao et al., 2003). Prostatic epithelium from wild-type (WT) post-pubertal male mice was used in control tissue recombinants. Pilot studies revealed that tissue recombinants made of rSVM and *Trp53*^{+/-} mouse prostatic epithelium (mPRE) were indistinguishable from WT tissue recombinants (SSC, personal communication). Thus, this group was removed from subsequent experiments.

2.2. Tissue dissections

Mice were sacrificed by CO₂ asphyxiation followed by cervical dislocation. Prostate glands were removed and placed in Dulbecco's modified Eagle's medium (DMEM). Ducts of the anterior prostate were microdissected for the grafting experiments as described previously (Sugimura et al., 1986). Paired samples of adjacent pre-graft tissue were examined histologically and verified to be normal.

2.3. Grafting and tissue recombination

Early lesions in each genetic background were examined in tissue recombinants composed of rat seminal vesicle mesenchyme (rSVM) plus ductal segments from *Pten*^{+/-}, *Pten*^{-/-}, *Trp53*^{-/-} and *Pten/Trp53* double heterozygous prostates. To obtain rSVM from newborn Sprague-Dawley rat pups (Simonsen Laboratories, Inc., Gilroy, CA), seminal vesicle epithelium was removed with fine forceps from dissected seminal vesicle rudiments after a brief trypsin digestion. The tissue recombinants were prepared by combining rSVM with a 300–500 μm segment of mouse prostate duct (mouse prostate epithelium (mPRE)) to yield rSVM+mPRE recombinants, cultured on nutrient agar medium overnight and then grafted under the kidney capsule of 45–60-day-old, intact male nude mice (Charles River, Wilmington, MA) as described previously (Cunha and Donjacour, 1987; Donjacour and Cunha, 1995; Wang et al., 2000). The rationale for using rSVM as a prostate inductor has been previously discussed in detail (Risbridger et al., 2001). Tissue recombinant grafts were allowed to grow in vivo for 8 weeks before being harvested.

The profound proliferation elicited in adult mouse prostatic epithelium by mesenchymal prostatic inductors (such as rSVM) can be further stimulated by serial transplantation of prostatic ducts combined with fresh mesenchyme (Cunha, unpublished). Therefore, second- and third-generation tissue recombinants of *Pten/Trp53* double heterozygous prostatic tissue were produced as follows: a duct was dissected from a tissue recombinant graft harvested after 8 weeks of growth and then combined with fresh rSVM. This second-generation tissue recombinant was grown on nutrient agar medium overnight and grafted under the kidney

capsule of a new set of intact male nude mice. Resulting second-generation grafts were harvested after 8 weeks. Third-generation tissue recombinants were produced similarly with a fragment of prostatic duct from second-generation *Pten/Trp53* double heterozygous grafts.

2.4. Histology and immunohistochemistry

All tissues were fixed in 10% neutral buffered formalin for 12 h at room temperature and then transferred to 70% ethanol until processing. For paraffin sections, tissues were dehydrated in graded ethanol and HistoClear (National Diagnostics, Atlanta, GA) and embedded. Tissues were cut 6 µm in thickness, deparaffinized, rehydrated, stained with hematoxylin and eosin, or used for immunohistochemistry.

For immunohistochemistry, antigens were unmasked by boiling sections in citrate buffer (Antigen Unmasking solution, Vector Laboratories, Burlingame, CA), pH 6, for 30 min in a microwave oven. Blocking solution (Superblock, Pierce, Rockford, IL) was applied to sections followed by the primary antibody (anti-androgen receptor, 1:50, Affinity Bioreagents, Golden, CO; anti-p63, 1:100, Santa Cruz Biotechnology, Santa Cruz, CA (#SC8431); anti-Ki67, 1:200, Novocastra Laboratories, Newcastle, UK; anti-E-cadherin, 1:200, BD Bioscience, San Jose, CA; anti-phospho-AKT (Ser473), 1:100, Cell signaling Technology, MA). Antibody binding was detected using secondary anti-rabbit or anti-mouse IgG antibodies conjugated to biotin (Amersham, Piscataway, NJ) and the ABC peroxidase method with 3',3'-diaminobenzidine (DAB) color reagent (Vector Laboratories).

2.5. Lesion classification

Prostatic lesions of *Pten* heterozygous and *Pten* conditional knockout mice have been described previously (Podsypanina et al., 1999; Wang et al., 2003). Definition and detailed description of prostatic lesions in genetically engineered mice, including benign hyperplasias, mouse prostatic intraepithelial neoplasia (PIN) and adenocarcinoma, have been published (Park et al., 2002; Shappell et al., 2004). Briefly, PIN I has 1 or 2 layers of atypical cells; PIN II has 3 or more layers of atypical cells; PIN III normally occupies the entire glandular lumen; and PIN IV fills and expands the glandular lumen, distorting the glandular profile and often bulging into the surrounding stroma. Although PIN IV lesions can be large and show cellular pleomorphism, they can be differentiated from adenocarcinomas due to the lack of stromal invasion. Nuclear atypia, defined by variation of nuclear size, shape or both, as well as the presence of abnormal chromatin patterns (chromatin clumping, excessive or abnormal nucleoli), was also graded in all samples. Grading was performed by evaluating a total of 3 slides taken at different depths in the block (one superficial and one deeper in the block) containing 2 repeat sections (6 µm). The absolute range of lesions was determined by noting whether each type of lesion or feature was present or not in each sample. Area occupied by each type of lesion or number of lesions per sample was not evaluated.

2.6. Laser microdissection of lesions and LOH analysis of *Pten* and *Trp53*

For laser microdissection, 10 µm paraffin sections were cut and placed on uncharged glass slides. Tissues were deparaffinized with xylene and stored in a dry place until use. An Arcturus PixCell II laser capture microdissection system (Arcturus Engineering, Inc., Mountain View, CA) built into an Olympus IMT 2 inverted microscope stand was used to collect epithelium from high-grade PIN lesions (PIN III and IV) from *Pten* heterozygous and from first- and third-generation *Pten/Trp53* double heterozygous tissue recombinants.

DNA from all samples was extracted using the HotSHOT (sodium hydroxide and Tris) solution as described previously (Truett et al., 2000). LOH analyses of *Pten* and *Trp53* were performed as described previously (Freeman et al., 2003; Ma et al., 2005). For *Pten* LOH 45 PCR cycles (94 °C—3 min, 94 °C—15 s, 64 °C—30 s, 72 °C—90 s, 72 °C—7 min, 4 °C) were run using

the following primers: TTG CAC AGT ATC CTT TTG AAG (WT allele—240 bp), GTC TCT GGT CCT TAC TTC C (null allele—320 bp) and ACG AGA CTA GTG AGA CGT GC (common). For *Trp53* LOH 35 PCR cycles (94 °C—3 min, 94 °C—15 s, 68 °C—90 s and 72 °C—7 min, 4 °C) were run using the following primers: ATA GGT CGG CGG TTC AT (WT allele, 550 bp), CCC GAG TAG CTG GAA GAC AG (null allele, 650 bp) and CCT CGT GCT TTA CGG TAT CGC (common). PCRs were run in duplicate and using low (DNA eluted in 150 µl of buffer) and high (DNA evaporated and resuspended in 6 µl of buffer) concentrations of template. Normal tissues from wild-type, *Pten*^{+/-}, *Pten*^{-/-}, *Trp53*^{+/-} and *Trp53*^{-/-} mice were used as controls.

2.7. Statistical analysis of results

The proportions of different lesion types were compared among groups using the chi-square test. The Fisher's exact test was used when expected counts in contingency tables were less than 5. The level of significance used in all analyses was 0.05.

3. Results

3.1. Lesional incidence among experimental groups

In this study, all tissues evaluated were obtained from rSVM+mPRE tissue recombinants constructed with prostatic epithelium from mice of different genotypes. For simplicity, we will, hereafter, use the genotype of the epithelium to refer to each tissue recombinant (i.e. rSVM+*Pten*^{+/-} mPRE will be referred to as *Pten*^{+/-}).

The incidence of benign hyperplasias and preneoplastic lesions (PIN) varied among the experimental groups (Table 1), with a much higher incidence of PIN in *Pten*/*Trp53* double heterozygous tissues when compared to *Pten*^{+/-}. PIN severity also varied according to the genotype of the prostatic epithelium used in tissue recombinants (Fig. 1A). Because PIN I was rare in this cohort, we combined PIN I and II (low-grade PIN) for the purpose of analysis. Invasive adenocarcinomas were not seen in our cohort. In addition to grading proliferative lesions, we also recorded the degree of nuclear atypia in all samples (Table 2). Nuclear atypia was the only significant change seen in *Trp53*^{-/-} prostatic epithelium in our tissue recombinants.

Prostatic tissues from *Trp53*^{-/-} mice showed mild hyperplasia and no evidence of PIN (Fig. 1B). On the other hand, 100% of the tissues in this group had moderate to severe nuclear atypia (Fig. 2A), which was the only feature that differentiated these *Trp53*^{-/-} tissue recombinants from those made with wild-type epithelium.

All of the *Pten*^{-/-} tissue recombinants had areas of PIN III and IV (Fig. 1C). In this group, epithelial cells in PIN lesions tended to be large and have abundant, finely vacuolated cytoplasm (Fig. 2B). Additionally, there was mild nuclear atypia seen in the epithelium of only 45% of the *Pten*^{-/-} tissue recombinants (Table 2). The difference in incidence and severity of nuclear atypia between the *Pten*^{-/-} and *Trp53*^{-/-} groups was highly significant ($p < 0.0001$).

In contrast to the *Pten*^{-/-} prostatic tissues, the incidence of PIN in *Pten*^{+/-} prostatic tissues was relatively low (17%, Table 1), and PIN IV was not seen. Cells with cytoplasmic vacuolation as described for *Pten*^{-/-} epithelium were also found in PIN lesions in the *Pten*^{+/-} group. Nuclear atypia was mild and infrequent in *Pten*^{+/-} prostatic epithelium.

3.2. Multi-generation *Pten*/*Trp53* double heterozygous tissue recombinants

The first generation of *Pten*/*Trp53* double heterozygous tissue recombinants had a majority of benign hyperplasias (69%, Table 1) and low-grade PIN, with less frequent PIN III and rare

PIN IV (Fig. 3). Overall, the lesions in this group were slightly more severe than those seen in *Pten*^{+/-} tissue recombinants ($p = 0.04$) and milder than those seen in *Pten*^{-/-} tissue recombinants ($p = 0.004$). Interestingly, the incidence of nuclear atypia (Fig. 3D) in the *Pten*/*Trp53* double heterozygous tissue recombinants of the first generation was 74% in comparison to only 11% seen in tissue recombinants made with *Pten*^{+/-} mPRE ($p < 0.0001$).

The second generation of *Pten*/*Trp53* double heterozygous prostatic tissue recombinants was prepared by combining a small epithelial fragment derived from a first-generation tissue recombinant with fresh rSVM. Tissues were harvested after 8 additional weeks of growth. In spite of the resulting 9–12 additional population doublings, lesions in this second generation of tissue recombinants were unexpectedly mild. The incidences of PIN III and IV in this group were slightly higher than those of the first generation of *Pten*/*Trp53* double heterozygous tissue recombinants (Fig. 3), but these trends were not statistically significant ($p > 0.04$). The proportion of nuclear atypia in the second-generation *Pten*/*Trp53* double heterozygous tissue recombinants was, however, significantly higher than that seen in *Pten*^{+/-} tissue recombinants ($p = 0.02$).

The third-generation *Pten*/*Trp53* double heterozygous tissue recombinants (prepared with epithelium from the second-generation grafts) had high incidences of both PIN III (64%) and PIN IV (59%). PIN lesions were significantly more severe in this group than in the *Pten*^{+/-} group ($p < 0.0001$), and more severe than in the first-generation *Pten*/*Trp53* double heterozygous tissue recombinants ($p = 0.0003$). Nonetheless, PIN lesions were still less severe in third-generation *Pten*/*Trp53*^{+/-} tissue recombinants than in *Pten*^{-/-} tissue recombinants ($p < 0.0001$). Profound nuclear atypia was seen in 100% of the PIN III and IV lesions in the third generation of *Pten*/*Trp53* double heterozygous tissue recombinants (Fig. 2C).

Overall, lesions tended to progress and become more severe as tissues were serially transplanted in second- and third-generation tissue recombinants. In addition, the simultaneous heterozygosity of *Pten* and *Trp53* in the serially transplanted recombinants seemed to amplify morphologic features attributable to each, namely nuclear atypia (*Trp53* loss) and the presence of large vacuolated cells in PIN (*Pten* loss).

3.3. Immunohistochemistry

Immunohistochemical staining patterns were identical in PIN lesions in tissue recombinants made with *Pten*^{-/-}, *Pten*^{+/-} and *Pten*/*Trp53* double heterozygous mPRE. PIN in these groups stained brightly for phospho-AKT, indicating the activation of the PI3K pathway (Fig. 4A). In our experimental groups E-cadherin expression was maintained by luminal epithelial cells in PIN, regardless of grade (Fig. 4B). Basal cell expression of E-cadherin was weaker or absent (data not shown). The marker Ki67 showed that proliferation was mostly confined to the basal region of the glands (Fig. 4C), which was the same area in which p63-positive cells were concentrated (Fig. 4D). Cells expressing p63 were rare towards the center or luminal aspects of PIN lesions in all groups. The majority of luminal and stromal cells and a smaller proportion of basal cells were positive for androgen receptor (AR) in PIN lesions (not illustrated).

Benign hyperplasias in *Trp53*^{-/-}, *Trp53*^{+/-}, wild-type and *Pten*^{+/-} tissue recombinants showed immunohistochemical results identical to normal glands and were negative for phospho-AKT and positive for epithelial membrane E-cadherin and nuclear AR (both epithelium and stroma). Proliferating cells, as shown by Ki67 positivity, were rare in normal glands and randomly distributed within basal and luminal aspects of hyperplastic glandular epithelium. The antibody p63 stained a discontinuous layer of basal cells in both normal and hyperplastic glands.

3.4. LOH of *Pten* and *Trp53* in tissue recombinants

LOH analysis of genomic DNA extracted into 150 μ l of buffer from PIN III and IV lesions (high-grade PIN) from first- and third-generation *Pten/Trp53* tissue recombinants revealed that 4/4 lesions in first-generation and 6/6 lesions in third-generation *Pten/Trp53* double heterozygous tissues had lost their wild-type *Pten* allele. In contrast, all 10 lesions examined retained their *Trp53* wild-type allele. Under the same conditions, LOH analysis of *Pten* in DNA extracted from high-grade PIN from *Pten*^{+/-} tissue recombinants showed loss of the wild-type allele in 2/4 samples. However, after the samples were concentrated (150 μ l to 6 μ l of template) we could detect the wild-type allele of *Pten* in all samples from *Pten*^{+/-} and from first- and third-generation *Pten/Trp53* double heterozygous samples. LOH analysis of *Trp53* in *Pten*^{+/-} tissues was also performed and showed a retained wild-type allele in all 4 samples of high-grade PIN tested.

4. Discussion

The role of the *PTEN* tumor suppressor gene in prostate cancer has been well documented in human prostate tumors and cell lines, as well as in several mutant mice ranging from *Pten* hypomorphs to *Pten* heterozygous and prostate conditional knockouts (Di Cristofano et al., 1998, 2001; Kwabi-Addo et al., 2001; Podsypanina et al., 1999; Stambolic et al., 2000; Trotman et al., 2003; Wang et al., 2003). Although the individual role of *Trp53* in prostate cancer has been controversial, the targeted deletion of both alleles of *Trp53* and *Pten* in the prostate was shown to result in faster prostate cancer progression (Chen et al., 2005). We showed in the present study that combined heterozygosity of *Pten* and *Trp53* can also accelerate PIN development.

These data are in agreement with an increasing body of literature pointing towards the functional cooperation between *Pten* and *Trp53* in mouse models of lymphoma and skin cancer (Komazawa et al., 2004; Mao et al., 2003; Freeman et al., 2003). Additionally, simultaneous LOH of *PTEN* and *TP53* mutations were identified in a large number of human gastric tumors (Oki et al., 2005). However, simultaneous loss of both alleles of *PTEN* and *TP53* is a rare occurrence in human cancers (Trotman and Pandolfi, 2003), suggesting that the role of these two genes in human disease may be more closely modeled in the context of heterozygosity.

Interestingly, our analysis revealed morphologic distinctions between lesions caused by *Pten* or *Trp53* disruption. In the absence or decreased function of *Pten* the AKT pathway is activated, leading to a series of anabolic, growth-promoting and life-maintaining events. A morphologic consequence of this pathway is that affected epithelial cells become larger and slightly vacuolated. This appearance is characteristic of *Pten* disruption in many tissues and is commonly noted in metabolically active cells, such as hepatocytes and renal tubular cells of *Pten*^{+/-} mice *in vivo*, as well as in hyperplasias or neoplasias originating in many organs of these mice, such as the thyroids glands, uterus and gallbladder. Additionally, similar cytomorphologic features can be seen in the prostate of AKT transgenic mice, which display low-grade PIN with large and vacuolated epithelial cells. This morphologic hallmark of *Pten* inactivation (AKT pathway) was noted in advanced PIN lesions in tissue recombinants prepared with *Pten*^{-/-}, *Pten*^{+/-} and *Pten/Trp53* double heterozygous prostatic epithelium.

On the other hand, prominent nuclear atypia was associated with tissues lacking *Trp53* function and may reflect polyploidy and/or genetic instability resulting from *Trp53* inactivation. This hallmark of *Trp53* disruption was seen in tissue recombinants prepared with prostatic epithelium derived from *Trp53*^{-/-} and from *Pten/Trp53* double heterozygous mice, and became more severe as the double heterozygous tissues were serially transplanted. These “gene- or pathway-specific” morphologic features allow the investigator to “visualize” the genetic make-up of the lesion to a certain extent and may provide insight into the pathogenesis

of prostate cancer. For example, the vacuolated epithelium in PIN lesions of *Pten*^{-/-} and *Pten*^{+/-} and *Pten/Trp53* double heterozygous prostatic tissues indicated activation of the AKT pathway and this was confirmed by positive phospho-AKT immunohistochemistry stain in these samples.

Since LOH of *Pten* has been shown to play a role in prostate lesion progression by many groups (Banach-Petrosky et al., 2006; Kim et al., 2002; Kwabi-Addo et al., 2001; Ma et al., 2005; Trotman et al., 2003), we hypothesized that earlier or more frequent loss of the wild-type allele of *Pten* in *Pten/Trp53* double heterozygous mice would help explain the increased PIN severity in this group. However, LOH analysis in our study showed retention of the wild-type allele of *Pten* in all high-grade PIN lesions examined. Interestingly, when PCR reactions were done with lower concentrations of template the wild-type allele of *Pten* could not be amplified in 50% of high-grade PIN lesions from the *Pten*^{+/-} group and in 100% of the first- and third-generation *Pten/Trp53* double heterozygous samples examined. These results suggest that accuracy of LOH analysis via PCR reaction is variable and seems to correlate with initial template concentration, which represents a problem in the study of laser microdissected samples. On the other hand, the apparent flaw of the first PCR experiment may suggest that there is a lower concentration of wild-type *Pten* in double heterozygous lesions when compared to *Pten*^{+/-} lesions. Unfortunately, we could not evaluate the exact dosage of *Pten* in this experiment due to the small quantity of DNA obtained and relatively poor quality of RNA generated from laser microdissected PIN lesions.

The increasing incidence and severity of nuclear atypia in third-generation *Pten/Trp53* double heterozygous mice mimicking *Trp53*^{-/-} samples suggested that the loss of the remaining wild-type *Trp53* allele might have taken place in the third-generation tissue recombinants. However, all PIN lesions in the first- and third-generation *Pten/Trp53* tissues maintained a wild-type *Trp53* allele, as detected by PCR analysis. An important consideration when interpreting these data is that LOH may be restricted to small groups of cells within larger lesions and may therefore be missed in the PCR analysis. This is likely since morphologically the appearance of cells within large PIN lesions was heterogeneous, and nuclear atypia was more severe in small and quite well-demarcated foci. Unfortunately, LOH analysis of such foci of increased nuclear atypia within PIN proved unrewarding due to the minute amount of DNA obtained from laser microdissected material from such small areas. In conclusion, one of the possible explanations for the lack of correlation between LOH of *Pten* or *Trp53* with PIN progression in this study is the low sensitivity of PCR-based LOH analysis.

Conversely, the retention of the wild-type alleles of both tumor suppressor genes in our model system suggests alternative downstream pathways of cooperation between *Pten* and *Trp53* at the pre-neoplastic phase of prostate carcinogenesis. This interaction may be at the protein level as is the case in lymphoma development in *Pten/Trp53* double heterozygous mice (Freeman et al., 2003). Double heterozygous animals develop lymphoma at a similar rate as *Trp53*^{-/-} mice, and much earlier than either *Pten*^{+/-} or *Trp53*^{+/-} control animals in spite of retention of the wild-type alleles and normal mRNA levels of both tumor suppressor genes. Although our results show that LOH does not explain the acceleration of PIN progression in *Pten/Trp53* double heterozygous mice, we cannot exclude other mechanisms of loss of function of either or both genes, such as point mutations or promoter methylation, which were not addressed in this study.

A previous study has shown that loss of *Rb* in the prostatic epithelium results in a *Trp53*-mediated tumor suppressor response in both epithelial and stromal compartments (Hill et al., 2005). Whereas loss of *Trp53* in the epithelium did not result in increased proliferation in this compartment, stromal loss of *Trp53* resulted in formation of Phyllodes-like stromal tumors in double mutants. These data suggest that oncogenic stress in the epithelium, via *Rb* loss, results

in negative selective pressure against *Trp53* in the stromal compartment and subsequent stromal proliferation. In the present experiments we did not observe stromal proliferation consistent with LOH of *Trp53* in the stroma. Furthermore, the use of rat mesenchyme in the tissue recombinant system makes the LOH analysis of the stroma difficult. Further studies utilizing *Pten*-deficient epithelium combined with *Trp53*-deficient mouse mesenchyme are granted in order to elucidate the paracrine regulation of these two tumor suppressor genes in the prostate.

It is important to mention that our results differ from those published in a recent study in which simultaneous heterozygosity of *Pten* and *Trp53* was considered to have no effect on lesion progression (Chen et al., 2005). The difference in our results could be explained in part by the fast induction of prostatic epithelial proliferation in rSVM+mPRE tissue recombinants, which facilitates the observation of lesions that may not occur in prostate glands *in vivo* due to long latency. However, other important factors could have contributed to the different outcomes in both studies, namely the deletion strategies used (Cre-Lox-induced prostate conditional knockout versus regular transgenics), as well as the mixed background strains of the mice evaluated. Furthermore, criteria used to judge lesion severity and progression in *Pten/Trp53* double heterozygous mice were not discussed in detail in the study mentioned above, making objective comparison of results difficult.

Background strain can play a significant role in lesion development in genetically engineered mice. For instance, it has been shown that FVB mice are more susceptible to both prostatic and skin neoplasia when compared to C57BL/6 mice, likely due to a defective sonic hedgehog signaling pathway in the C57BL/6 strain (Chiaverotti et al., 2008; Wakabayashi et al., 2007). Our cohort of double heterozygous mice was on a mixed C57BL/6J-129/SvJ background and this may help explain the absence of carcinoma progression in our tissue recombinants.

Also noteworthy is that immunohistochemical analysis revealed that proliferation in high-grade PIN lesions of all experimental groups seems to be concentrated along the p63-rich, basal region of the affected glands, which suggests a subpopulation of precursor stem cells in this location, as was described previously in *Pten* conditional knockout mice (Wang et al., 2006). Although decreased E-cadherin expression has been associated with prostate cancer progression in humans and in mouse models (Umbas et al., 1992, 1994, 1997; Wang et al., 2000), in our study E-cadherin expression remained unchanged in high-grade PIN lesions from all experimental groups.

Finally, the usefulness of the tissue recombinant technique in the study of slow-progressing prostate lesions in models that are otherwise extremely valuable should be underscored. Models such as the *Pten*^{+/-} and *Pten/Trp53* double heterozygous mice have long latency for lesion onset and slow lesion progression, which are related to the physiological growth-quiescence of the adult murine prostate. In adult mice, rates of prostatic epithelial proliferation and apoptosis are extremely low and in balance. Despite the absence of critical tumor suppressor genes such as *Pten* or *Trp53*, carcinogenesis still depends on the rate of cell proliferation, which can fix genetic lesions as they emerge.

By applying the tissue recombinant technique we take advantage of potent mesenchymal inductors, such as the rat seminal vesicle, which stimulate the mouse prostatic epithelium to undergo about 13 population doublings in 4 weeks. This high rate of prostatic epithelial proliferation exceeds the total number of prostatic epithelial population doublings that occur within the life-span of the adult mouse prostate *in situ*. When a very small fragment of normal murine prostatic duct from a *Pten*^{+/-} adult mouse is combined with rSVM, high-grade PIN is seen as early as 4 weeks (unpublished data) and found in relatively high frequency at 8 weeks. This means high-grade PIN lesions can be studied as early as 4 months after the mutant mouse

is born. In addition, the effects of the stroma in carcinogenesis can also be evaluated through the combination of epithelia and stroma of different genetic make-ups.

In short, we have shown that heterozygosity of *Pten* and *Trp53* is sufficient to accelerate PIN progression independently of LOH of either tumor suppressor gene. Furthermore, we emphasize in this study the advantages of the tissue recombinant technology as a tool to study prostate cancer initiation and progression.

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References

- Banach-Petrosky W, Ouyang X, Gao H, Nader K, Ji Y, Suh N, DiPaola RS, Abate-Shen C. Vitamin D inhibits the formation of prostatic intraepithelial neoplasia in Nkx3.1;Pten mutant mice. *Clin. Cancer Res* 2006;12:5895–5901. [PubMed: 17020998]
- Bookstein R. Tumor suppressor genes in prostatic oncogenesis. *J. Cell. Biochem* 1994;19:217–223.
- Bookstein R, MacGrogan D, Hilsenbeck SG, Sharkey F, Allred DC. p53 is mutated in a subset of advanced-stage prostate cancers. *Cancer Res* 1993;53:3369–3373. [PubMed: 8324747]
- Cairns P, Okami K, Halachmi S, Halachmi N, Esteller M, Herman JG, Jen J, Isaacs WB, Bova GS, Sidransky D. Frequent inactivation of PTEN/MMAC1 in primary prostate cancer. *Cancer Res* 1997;57:4997–5000. [PubMed: 9371490]
- Chen Z, Trotman LC, Shaffer D, Lin HK, Dotan ZA, Niki M, Koutcher JA, Scher HI, Ludwig T, Gerald W, Cordon-Cardo C, Pandolfi PP. Crucial role of p53-dependent cellular senescence in suppression of Pten-deficient tumorigenesis. *Nature* 2005;436:725–730. [PubMed: 16079851]
- Chi SG, deVere White RW, Meyers FJ, Siders DB, Lee F, Gumerlock PH. p53 in prostate cancer: frequent expressed transition mutations. *J. Natl. Cancer Inst* 1994;86:926–933. [PubMed: 7515114]
- Chiaverotti T, Couto SS, Donjacour A, Mao JH, Nagase H, Cardiff RD, Cunha GR, Balmain A. Dissociation of epithelial and neuroendocrine carcinoma lineages in the transgenic adenocarcinoma of mouse prostate model of prostate cancer. *Am. J. Pathol* 2008;172:236–246. [PubMed: 18156212]
- Costa-Pereira AP, Cotter TG. Molecular and cellular biology of prostate cancer—the role of apoptosis as a target for therapy. *Prostate Cancer Prostatic Dis* 1999;2:126–139. [PubMed: 12496822]
- Cunha GR, Donjacour A. Mesenchymal–epithelial interactions: technical considerations. *Progr. Clin. Biol. Res* 1987;239:273–282.
- Di Cristofano A, Pesce B, Cordon-Cardo C, Pandolfi PP. Pten is essential for embryonic development and tumour suppression. *Nat. Genet* 1998;19:348–355. [PubMed: 9697695]
- Di Cristofano A, De Acetis M, Koff A, Cordon-Cardo C, Pandolfi PP. Pten and p27KIP1 cooperate in prostate cancer tumor suppression in the mouse. *Nat. Genet* 2001;27:222–224. [PubMed: 11175795]
- Donjacour, AA.; Cunha, GR. *Development* (Cambridge, England). Vol. 121. Cambridge, England: Cambridge, England; 1995. Induction of prostatic morphology and secretion in urothelium by seminal vesicle mesenchyme; p. 2199–2207.
- Feng Z, Zhang H, Levine AJ, Jin S. The coordinate regulation of the p53 and mTOR pathways in cells. *Proc. Natl. Acad. Sci. USA* 2005;102:8204–8209. [PubMed: 15928081]
- Freeman DJ, Li AG, Wei G, Li HH, Kertesz N, Lesche R, Whale AD, Martinez-Diaz H, Rozengurt N, Cardiff RD, Liu X, Wu H. PTEN tumor suppressor regulates p53 protein levels and activity through phosphatase-dependent and -independent mechanisms. *Cancer Cell* 2003;3:117–130. [PubMed: 12620407]
- Gao H, Ouyang X, Banach-Petrosky WA, Gerald WL, Shen MM, Abate-Shen C. Combinatorial activities of Akt and B-Raf/Erk signaling in a mouse model of androgen-independent prostate cancer. *Proc. Natl. Acad. Sci. USA* 2006;103:14477–14482. [PubMed: 16973750]

- Gumerlock PH, Chi SG, Shi XB, Voeller HJ, Jacobson JW, Gelmann EP, deVere White RW. The Cooperative Prostate Network. p53 abnormalities in primary prostate cancer: single-strand conformation polymorphism analysis of complementary DNA in comparison with genomic DNA. *J. Natl. Cancer Inst* 1997;89:66–71. [PubMed: 8978408]
- Hill R, Song Y, Cardiff RD, Van Dyke T. Selective evolution of stromal mesenchyme with p53 loss in response to epithelial tumorigenesis. *Cell* 2005;123:1001–1011. [PubMed: 16360031]
- Kim MJ, Cardiff RD, Desai N, Banach-Petrosky WA, Parsons R, Shen MM, Abate-Shen C. Cooperativity of Nkx3.1 and Pten loss of function in a mouse model of prostate carcinogenesis. *Proc. Natl. Acad. Sci. USA* 2002;99:2884–2889. [PubMed: 11854455]
- Komazawa N, Suzuki A, Sano S, Horie K, Matsuura N, Mak TW, Nakano T, Takeda J, Kondoh G. Tumorigenesis facilitated by Pten deficiency in the skin: evidence of p53-Pten complex formation on the initiation phase. *Cancer Sci* 2004;95:639–643. [PubMed: 15298725]
- Kwabi-Addo B, Giri D, Schmidt K, Podsypanina K, Parsons R, Greenberg N, Ittmann M. Haploinsufficiency of the Pten tumor suppressor gene promotes prostate cancer progression. *Proc. Natl. Acad. Sci. USA* 2001;98:11563–11568. [PubMed: 11553783]
- Li DM, Sun H. PTEN/MMAC1/TEP1 suppresses the tumorigenicity and induces G1 cell cycle arrest in human glioblastoma cells. *Proc. Natl. Acad. Sci. USA* 1998;95:15406–15411. [PubMed: 9860981]
- Li J, Yen C, Liaw D, Podsypanina K, Bose S, Wang SI, Puc J, Miliareis C, Rodgers L, McCombie R, Bigner SH, Giovanella BC, Ittmann M, Tycko B, Hibshoosh H, Wigler MH, Parsons R. PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. *Science (New York, NY)* 1997;275:1943–1947.
- Ma L, Teruya-Feldstein J, Behrendt N, Chen Z, Noda T, Hino O, Cordon-Cardo C, Pandolfi PP. Genetic analysis of Pten and Tsc2 functional interactions in the mouse reveals asymmetrical haploinsufficiency in tumor suppression. *Genes Dev* 2005;19:1779–1786. [PubMed: 16027168]
- Mamillapalli R, Gavrilova N, Mihaylova VT, Tsvetkov LM, Wu H, Zhang H, Sun H. PTEN regulates the ubiquitin-dependent degradation of the CDK inhibitor p27(KIP1) through the ubiquitin E3 ligase SCF(SKP2). *Curr. Biol* 2001;11:263–267. [PubMed: 11250155]
- Mao JH, Wu D, Perez-Losada J, Nagase H, DelRosario R, Balmain A. Genetic interactions between Pten and p53 in radiation-induced lymphoma development. *Oncogene* 2003;22:8379–8385. [PubMed: 14627978]
- Mayo LD, Dixon JE, Durden DL, Tonks NK, Donner DB. PTEN protects p53 from Mdm2 and sensitizes cancer cells to chemotherapy. *J. Biol. Chem* 2002;277:5484–5489. [PubMed: 11729185]
- Meyers FJ, Gumerlock PH, Chi SG, Borchers H, Deitch AD, deVere White RW. Very frequent p53 mutations in metastatic prostate carcinoma and in matched primary tumors. *Cancer* 1998;83:2534–2539. [PubMed: 9874460]
- Oki E, Tokunaga E, Nakamura T, Ueda N, Futatsugi M, Mashino K, Yamamoto M, Watanabe M, Ikebe M, Kakeji Y, Baba H, Maehara Y. Genetic mutual relationship between PTEN and p53 in gastric cancer. *Cancer Lett* 2005;227:33–38. [PubMed: 16051030]
- Park JH, Walls JE, Galvez JJ, Kim M, Abate-Shen C, Shen MM, Cardiff RD. Prostatic intraepithelial neoplasia in genetically engineered mice. *Am. J Pathol* 2002;161:727–735. [PubMed: 12163397]
- Podsypanina K, Ellenson LH, Nemes A, Gu J, Tamura M, Yamada KM, Cordon-Cardo C, Catoretti G, Fisher PE, Parsons R. Mutation of Pten/ Mmac1 in mice causes neoplasia in multiple organ systems. *Proc. Natl. Acad. Sci. USA* 1999;96:1563–1568. [PubMed: 9990064]
- Radu A, Neubauer V, Akagi T, Hanafusa H, Georgescu MM. PTEN induces cell cycle arrest by decreasing the level and nuclear localization of cyclin D1. *Mol. Cell. Biol* 2003;23:6139–6149. [PubMed: 12917336]
- Risbridger G, Wang H, Young P, Kurita T, Wang YZ, Lubahn D, Gustafsson JA, Cunha G. Evidence that epithelial and mesenchymal estrogen receptor α mediates effects of estrogen on prostatic epithelium. *Dev. Biol* 2001;229:432–442. [PubMed: 11150243]
- Shappell, SB.; Thomas, GV.; Roberts, RL.; Herbert, R.; Ittmann, MM.; Rubin, MA.; Humphrey, PA.; Sundberg, JP.; Rozengurt, N.; Barrios, R.; Ward, JM.; Cardiff, RD. Prostate pathology of genetically engineered mice: definitions and classification. The consensus report from the Bar Harbor Meeting of the Mouse Models of Human Cancer Consortium Prostate Pathology Committee; *Cancer Res*; 2004. p. 2270-2305.

- Sheng X, Koul D, Liu JL, Liu TJ, Yung WK. Promoter analysis of tumor suppressor gene PTEN: identification of minimum promoter region. *Biochem. Biophys. Res. Commun* 2002;292:422–426. [PubMed: 11906179]
- Stambolic V, Tsao MS, Macpherson D, Suzuki A, Chapman WB, Mak TW. High incidence of breast and endometrial neoplasia resembling human Cowden syndrome in *pten*^{+/-} mice. *Cancer Res* 2000;60:3605–3611. [PubMed: 10910075]
- Stambolic V, MacPherson D, Sas D, Lin Y, Snow B, Jang Y, Benchimol S, Mak TW. Regulation of PTEN transcription by p53. *Mol. Cell* 2001;8:317–325. [PubMed: 11545734]
- Sugimura Y, Cunha GR, Donjacour AA. Morphological and histological study of castration-induced degeneration and androgen-induced regeneration in the mouse prostate. *Biol. Reprod* 1986;34:973–983. [PubMed: 3730489]
- Sun H, Lesche R, Li DM, Liliental J, Zhang H, Gao J, GavriloVA N, Mueller B, Liu X, Wu H. PTEN modulates cell cycle progression and cell survival by regulating phosphatidylinositol 3,4,5,-triphosphate and Akt/protein kinase B signaling pathway. *Proc. Natl. Acad. Sci. USA* 1999;96:6199–6204. [PubMed: 10339565]
- Suzuki H, Freije D, Nusskern DR, Okami K, Cairns P, Sidransky D, Isaacs WB, Bova GS. Interfocal heterogeneity of PTEN/MMAC1 gene alterations in multiple metastatic prostate cancer tissues. *Cancer Res* 1998;58:204–209. [PubMed: 9443392]
- Trotman LC, Pandolfi PP. PTEN and p53: who will get the upper hand? *Cancer Cell* 2003;3:97–99. [PubMed: 12620402]
- Trotman LC, Niki M, Dotan ZA, Koutcher JA, Di Cristofano A, Xiao A, Khoo AS, Roy-Burman P, Greenberg NM, Van Dyke T, Cordon-Cardo C, Pandolfi PP. Pten dose dictates cancer progression in the prostate. *PLoS Biol* 2003;1:E59. [PubMed: 14691534]
- Truett GE, Heeger P, Mynatt RL, Truett AA, Walker JA, Warman ML. Preparation of PCR-quality mouse genomic DNA with hot sodium hydroxide and tris (HotSHOT). *BioTechniques* 2000;29:52–54. [PubMed: 10907076]
- Umbas R, Schalken JA, Aalders TW, Carter BS, Karthaus HF, Schaafsma HE, Debruyne FM, Isaacs WB. Expression of the cellular adhesion molecule E-cadherin is reduced or absent in high-grade prostate cancer. *Cancer Res* 1992;52:5104–5109. [PubMed: 1516067]
- Umbas R, Isaacs WB, Bringuier PP, Schaafsma HE, Karthaus HF, Oosterhof GO, Debruyne FM, Schalken JA. Decreased E-cadherin expression is associated with poor prognosis in patients with prostate cancer. *Cancer Res* 1994;54:3929–3933. [PubMed: 7518346]
- Umbas R, Isaacs WB, Bringuier PP, Xue Y, Debruyne FM, Schalken JA. Relation between aberrant alpha-catenin expression and loss of E-cadherin function in prostate cancer. *Int. J. Cancer* 1997;74:374–377. [PubMed: 9291424]
- Wakabayashi Y, Mao JH, Brown K, Girardi M, Balmain A. Promotion of Hras-induced squamous carcinomas by a polymorphic variant of the Patched gene in FVB mice. *Nature* 2007;445:761–765. [PubMed: 17230190]
- Wang J, Ouyang W, Li J, Wei L, Ma Q, Zhang Z, Tong Q, He J, Huang C. Loss of tumor suppressor p53 decreases PTEN expression and enhances signaling pathways leading to activation of activator protein 1 and nuclear factor kappaB induced by UV radiation. *Cancer Res* 2005;65:6601–6611. [PubMed: 16061640]
- Wang S, Gao J, Lei Q, Rozengurt N, Pritchard C, Jiao J, Thomas GV, Li G, Roy-Burman P, Nelson PS, Liu X, Wu H. Prostate-specific deletion of the murine Pten tumor suppressor gene leads to metastatic prostate cancer. *Cancer Cell* 2003;4:209–221. [PubMed: 14522255]
- Wang S, Garcia AJ, Wu M, Lawson DA, Witte ON, Wu H. Pten deletion leads to the expansion of a prostatic stem/progenitor cell subpopulation and tumor initiation. *Proc. Natl. Acad. Sci. USA* 2006;103:1480–1485. [PubMed: 16432235]
- Wang SI, Parsons R, Iltmann M. Homozygous deletion of the PTEN tumor suppressor gene in a subset of prostate adenocarcinomas. *Clin. Cancer Res* 1998;4:811–815. [PubMed: 9533551]
- Wang Y, Hayward SW, Donjacour AA, Young P, Jacks T, Sage J, Dahiya R, Cardiff RD, Day ML, Cunha GR. Sex hormone-induced carcinogenesis in Rb-deficient prostate tissue. *Cancer Res* 2000;60:6008–6017. [PubMed: 11085521]

- Yuan ZQ, Sun M, Feldman RI, Wang G, Ma X, Jiang C, Coppola D, Nicosia SV, Cheng JQ. Frequent activation of AKT2 and induction of apoptosis by inhibition of phosphoinositide-3-OH kinase/Akt pathway in human ovarian cancer. *Oncogene* 2000;19:2324–2330. [PubMed: 10822383]
- Zheng HC, Sun JM, Li XH, Yang XF, Zhang YC, Xin Y. Role of PTEN and MMP-7 expression in growth, invasion, metastasis and angiogenesis of gastric carcinoma. *Pathol. Int* 2003;53:659–666. [PubMed: 14516315]

Fig. 1.
 (A) Schematic illustration of lesion progression from normal gland to PIN IV (left to right) in all rSVM+mPRE tissue recombinants. Note that PIN lesions in *Pten/Trp53* double heterozygous tissues are more severe than in *Pten*^{+/+}, but milder than in *Pten*^{-/-} tissues. (B) *Trp53*^{-/-} tissue recombinant graft under the kidney capsule. Note normal glands (black arrows) and mild hyperplasia (white arrow) but no PIN. (C) *Pten*^{-/-} tissue recombinant show high-grade PIN (III and IV), as well as hyperplasia (white arrow). Note the distortion and bulging of glandular outline in PIN IV (black arrow).

Fig. 2.

(A) *Trp53*^{-/-} tissues recombinant showing normal prostate gland with moderate nuclear atypia (arrows) and severe nuclear atypia (inset). (B) PIN III in *Pten*^{-/-} tissue recombinant showing large vacuolated cells with abundant cytoplasm characteristic of *Pten* disruption. (C) PIN IV in third-generation *Pten/Trp53* double heterozygous tissues showing severe nuclear atypia characteristic of *Trp53* disruption.

Fig. 3.
Histograms showing the incidence of PIN lesions and nuclear atypia in all rSVM+mPRE tissue recombinants.

Fig. 4.

(A) High- (III) and low-grade PIN (black arrow) stain brightly positive for phospho-AKT in *Pten*^{-/-} tissue recombination. Hyperplastic and normal gland (white arrows) do not express p-AKT. (B) PIN (III) as well as mildly hyperplastic glands (black arrows) stain brightly positive for E-cadherin. (C) In PIN III and IV, proliferating cells positive for Ki67 are concentrated at a basal location (arrow points to dotted line showing thin stromal projections). (D) p63-positive cells are also found more frequently at a basal location, where proliferating cells predominate (dotted line represents thin stromal projections).

Table 1

Proportion between benign prostatic hyperplasia (BPH) and prostatic intraepithelial neoplasia (PIN) in rSVM +mPRE tissue recombinants harvested 8 weeks post-grafting.

Prostate tissue recombinant grafts—mPRE genotypes	Sample size (<i>n</i>)	BPH (%)	PIN (%)
<i>Pten</i> ^{+/−} <i>Trp53</i> ^{+/−} 1st generation	35	69	34
<i>Pten</i> ^{+/−} <i>Trp53</i> ^{+/−} 2nd generation	25	20	32
<i>Pten</i> ^{+/−} <i>Trp53</i> ^{+/−} 3rd generation	22	18	77
<i>Pten</i> ^{+/−}	18	22	17
<i>Pten</i> ^{−/−}	20	0	100
<i>Trp53</i> ^{−/−}	19	58	0

Table 2

Degree of severity of nuclear atypia varying from none to severe in rSVM+mPRE tissue recombinants harvested 8 weeks post-graft.

Prostate tissue grafts—mPRE genotypes	Nuclear atypia (%)			
	None	Mild	Moderate	Severe
<i>Pten</i> ^{+/-} <i>Trp53</i> ^{+/-} 1st generation	26	57	17	0
<i>Pten</i> ^{+/-} <i>Trp53</i> ^{+/-} 2nd generation	56	36	8	0
<i>Pten</i> ^{+/-} <i>Trp53</i> ^{+/-} 3rd generation	0	9	27	64
<i>Trp53</i> ^{-/-}	0	0	16	84
<i>Pten</i> ^{-/-}	55	45	0	0
<i>Pten</i> ^{+/-}	89	11	0	0