Interactions between Cells with Distinct Mutations in c-MYC and Pten in Prostate Cancer

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Abstract

In human somatic tumorigenesis, mutations are thought to arise sporadically in individual cells surrounded by unaffected cells. This contrasts with most current transgenic models where mutations are induced synchronously in entire cell populations. Here we have modeled sporadic oncogene activation using a transgenic mouse in which c-MYC is focally activated in prostate luminal epithelial cells. Focal c-MYC expression resulted in mild pathology, but prostate-specific deletion of a single allele of the Pten tumor suppressor gene cooperated with c-MYC to induce high grade prostatic intraepithelial neoplasia (HGPIN)/cancer lesions. These lesions were in all cases associated with loss of Pten protein expression from the wild type allele. In the prostates of mice with concurrent homozygous deletion of Pten and focal c-MYC activation, double mutant (i.e. c-MYC+/-Pten-null) cells were of higher grade and proliferated faster than single mutant (Pten-null) cells within the same glands. Consequently, double mutant cells outcompeted single mutant cells despite the presence of increased rates of apoptosis in the former. The p53 pathway was activated in Pten-deficient prostate cells and tissues, but c-MYC expression shifted the p53 response from senescence to apoptosis by repressing the p53 target gene p21Cip1. We conclude that c-MYC overexpression and Pten deficiency cooperate to promote prostate tumorigenesis, but a p53-dependent apoptotic response may present a barrier to further progression. Our results highlight the utility of inducing mutations focally to model the competitive interactions between cell populations with distinct genetic alterations during tumorigenesis.

Introduction

Prevailing models of multistep carcinogenesis posit that oncogenic mutations arise in isolated cells in situ followed by clonal expansion. This implies that important competitive interactions occur between mutant and normal cells as well as between cells with distinct oncogenic mutations during tumorigenesis. A detailed understanding of these interactions will further efforts aimed at therapeutic targeting of neoplastic and preneoplastic lesions. However, these interactions have not been well studied in vivo due to a paucity of appropriate models. We report here our attempt to model these interactions in a new transgenic model of prostate cancer, focusing on the oncogene c-MYC and the tumor suppressor Pten (Phosphatase and tensin homolog), both of which are implicated in human prostate tumorigenesis [1]. c-MYC overexpression is a common early event in prostate cancer [2,3] while PTEN is deleted/mutated in ~30% of primary human prostate cancers [4–8]. Previous attempts at modeling c-MYC overexpression in the mouse prostate have used prostate-specific promoters that target transgene expression to a majority of the cells in the prostatic epithelium. Depending on the strength of the promoter used, this resulted in various grades of mouse prostatic intraepithelial neoplasia (mPIN) or adenocarcinoma [3,9]. Similarly, Pten-mutant mice develop mPIN and prostate cancer [10–13] and Pten inactivation can cooperate with mutations in oncogenes and tumor suppressors in prostate tumorigenesis, including p21Cip1 [14,15], Trp53 [13] and Fgf8b [16].

Pten loss has been reported to activate the p53 pathway, leading to senescence [13,17,18]. Activation of p53 may lead to cell cycle arrest or apoptosis depending on the downstream target genes induced (i.e. cell cycle arrest genes e.g. p21Cip1 versus apoptotic genes e.g. PUMA). There is potential cross-talk between the c-MYC and the p53 pathways at various levels depending on the cell context [19]. c-MYC activation can increase ARF expression, thereby stabilizing p53 protein levels [20], and c-MYC can repress expression of some p53 target genes such as p21Cip1 [21]. In addition, Pten and p53 coordinately control c-Myc protein levels with the latter playing a critical role in maintaining the stemness of murine neural stem cells [22].

To model the sporadic genetic alterations that are thought to occur during human somatic tumorigenesis [23], we generated a transgenic mouse in which a latent c-MYC transgene can be focally activated in the prostatic epithelium by Cre expression. We have also deleted one or both copies of Pten in the prostate concurrently with focal c-MYC overexpression, in order to examine the interactions of cell populations with distinct mutations within the same gland.
Author Summary

In most human cancers, mutations are thought to arise in a single cell or few cells surrounded by their unaffected neighbors. Expansion of mutant cells can then allow the accumulation of additional mutations. The cell–cell interactions that may occur between mutant and unaffected cells or between cells with distinct mutations during tumorigenesis have not been well studied due to the lack of suitable in vivo models. To help fill this gap, we generated and characterized transgenic mice in which the oncogene c-MYC is activated focally in prostate epithelial cells. We have also analyzed mice in which prostate epithelial cells with two mutations (c-MYC overexpression and loss of Pten tumor suppressor) are found next to cells with a single mutation (loss of Pten). Although loss of Pten in the prostate is tumorigenic, it also activates a cellular senescence response which restrains further tumor progression. We found that concurrent c-MYC expression suppressed the senescence response in Pten-null cells in favor of apoptosis. c-MYC+Pten-null cells proliferated faster than Pten-null cells in the same glands, with the net result that c-MYC+Pten-null cells outcompete Pten-null cells. Our results demonstrate the utility of accurate models to mimic the heterogeneous and incremental nature of human prostate carcinogenesis.

Results

Generation of PbCre4;Z-MYC mice with focal, prostate-specific c-MYC overexpression

To target focal c-MYC expression in the prostate epithelium, we used Z-MYC mice that carry a single copy transgene in which the CMV enhancer/beta actin promoter drives expression of the beta-galactosidase and a latent c-MYC transgene [24] (Figure 1A). Staining for beta-galactosidase confirmed mosaic expression in the prostate epithelium (Figure 1A). To induce c-MYC expression focally in the prostate, we crossed Z-MYC mice to PbCre mice [25] which express Cre recombinase in the prostatic epithelium (Figure 1A). Bicenic PbCre4;Z-MYC mice expressed c-MYC focally in cytokeratin 8 (CK8) positive prostate luminal epithelial cells but not p63+ basal cells (Figure 1C). Furthermore, c-MYC expression is not abrogated in castrated animals indicating that the use of the CMV enhancer/beta actin promoter in our model has uncoupled prostate-specific expression from androgen-dependent gene regulation (Figure 1B).

Mild pathology due to focal c-MYC overexpression

Focal c-MYC activation resulted in mild pathology, with most prostates showing normal histology or low grade mPIN (LGPIN) lesions up to 2 years of age (Figure 2A and 2B). This is unlikely to be due to low level c-MYC expression as the CMV enhancer/beta actin promoter is known to drive high level transgene expression. A closer examination of the c-MYC expression pattern in the prostates of PbCre4;Z-MYC mice with no pathology showed that in young mice, the frequency of c-MYC-positive cells was ~18% of the epithelial cells in c-MYC-positive glands (Figure 3G), evocative of the frequency of LacZ-positive cells (~17%) in Z-MYC prostates (Figure 1A). By 1 year, the frequency of c-MYC positive cells has increased to ~43% (Figure 3G). The lack of discernible histological abnormality in the prostates of a large fraction of older PbCre4;Z-MYC mice in the face of abundant c-MYC expression is reminiscent of the phenomenon of “field carcinization” in human tumorigenesis where incipient mutant cells occupy tissue fields without any apparent pathology (Figure 3A–3F) [26].

These histologically normal but mutant cells may serve as targets for transformation with additional genetic mutations.

Focal c-MYC expression cooperates with Pten heterozygosity

Next, we generated compound mutant mice with prostate-specific deletion of one or both alleles of Pten concurrently with focal activation of c-MYC. Examination of PbCre4;Z-MYC;Pten−/+ prostates revealed clear cooperation between c-MYC overexpression and Pten heterozygosity (Figure 2). As reported previously [10,11] and confirmed by us here, conditional deletion of a single Pten allele had little effect on the prostate with mice up to 50 weeks of age showing minimal abnormalities (Figure 2A and 2B). By ~10 weeks of age however, PbCre4;Z-MYC;Pten−/+ mice already have evidence of focal HGPIN lesions. Over time, these animals develop micro-invasive cancer as confirmed by the presence of areas with disruption in smooth muscle actin (SMA) immunoreactivity (Figure 2B and 2C). We used immunohistochemistry to examine the status of the wild type Pten allele in the HGPIN/cancer lesions in PbCre4;Z-MYC;Pten−/+ mice. Consistently, all lesions examined (N=8 mice) showed loss of Pten protein expression and phosphorylation of its downstream signaling components Akt and Foxo1 [27] (Figure 4).

c-MYC+Pten-null cells outcompete Pten-null cells in the same glands

We analyzed proliferation by staining for phospho-histone H3 (pHH3), a mitotic marker. Proliferation was increased significantly in PbCre4;Z-MYC prostates relative to controls, and Pten heterozygosity synergistically increased it further (Figure 5A). The proliferation rates in PbCre4;Pten−/+ and PbCre4;Z-MYC;Pten−/+ were similarly elevated. However, the focal nature of c-MYC expression in our model means that analysis of total proliferation in the PbCre4;Z-MYC;Pten−/+ prostates may not be an accurate measure of the proliferation in foci of c-MYC+Pten-null cells. To overcome this, we performed double staining for c-MYC and phospho-histone H3. As shown in Figure 5B, double mutant (c-MYC+Pten-null) cells were more proliferative than single mutant (Pten-null) cells within the same prostate glands. Furthermore, double mutant cells are histologically distinct from adjacent single mutant cells. The double mutant cells are of higher pathological grade with larger nuclei, high nuclear:cytoplasmic ratios, hyperchromatic nuclei with prominent chromocenters, focal chromatin clearing and prominent single or sometimes multiple nucleoli (Figure 5C and Figure S1). In addition, apoptotic and mitotic figures are prominent. Single mutant (Pten-null) cells on the other hand showed low nuclear grade with comparatively small and uniform nuclei, abundant pale cytoplasm and low nuclear:cytoplasmic ratios. These cells also have inconspicuous nucleoli and the chromatin is comparatively fine (Figure 5C). These observations suggest that c-MYC+Pten-null cells may out-compete Pten-null cells within the same prostate gland over time. Indeed, analysis of PbCre4;Z-MYC;Pten−/+ animals showed that at early ages, c-MYC expression was focal within glands, but in older mice, lesions show uniform c-MYC expression, suggesting clonal expansion of c-MYC-positive cells in a time-dependent manner (Figure 5D).

Pten loss does not protect c-MYC overexpressing prostate cells from apoptosis

Analysis of apoptosis by staining for activated Caspase 3 shows that control and PbCre4;Pten−/+ prostates had low levels of apoptosis, consistent with their normal histology, while focal expression of c-MYC in PbCre4;Z-MYC prostates modestly increased apoptosis...
Although c-MYC overexpression is known to induce apoptosis in several tissues, this depends on many variables including the level of c-MYC overexpression and the “tissue context” [28,29]. The levels of apoptosis seen in PbCre4;Z-MYC prostates are consistent with increased cell turnover due to enhanced proliferation. Pen-null prostates also had increased rates of apoptosis, and c-MYC overexpression further enhanced this effect (Figure 6A). These results were surprising as Pten loss is known to activate pro-survival pathways. Therefore, we sought to determine if apoptosis is increased in HGPIN/cancer cells that have lost Pten expression in our PbCre4;Z-MYC;Pten+/- mice. Double staining for Pten and activated Caspase 3 and quantitative analysis indicated higher rates of apoptosis in Pten-negative cells compared to Pten-positive cells (Figure 6B). Thus Pten loss does not protect prostate cells from apoptosis due to c-MYC overexpression.

In addition to Akt, the c-Jun N-terminal kinase (Jnk) pathway is known to be activated in Pen-deficient cells and tumors [30,31]. We confirmed that the Jnk pathway is activated in both Pen-null and c-MYC-overexpressing/Pen-null prostates by immunohistochemistry for phospho-Jnk (Figure S2A). Since Jnk is well known to have the ability to activate apoptosis, cell survival and proliferation depending on cellular signal stimuli and cellular contexts [32], we asked if increased Jnk activity sensitizes Pen-deficient cells to apoptosis. We used small hairpin RNA to stably downregulate PTEN in the benign human prostatic cell line RWPE1. However, treatment with the Jnk inhibitor (SP600125) led to an increase in apoptosis in PTEN knockdown cells in a dose-dependent manner, suggesting that PTEN loss-induced Jnk activity is anti-apoptotic, rather than pro-apoptotic (Figure S2).
Figure 2. Pathology of PbCre4;Z-MYC and compound c-MYC/Pten mutant mice. (A) Hematoxylin and eosin (H&E)–stained prostate sections were analyzed and the summary of the pathological grading is shown. (N, number of mice analyzed). (B) Representative images of H&E-stained sections show benign glands in control (a), focal LGPIN in PbCre4;Z-MYC (boxed region in b and higher magnification in c), focal HGPIN lesion in PbCre4;Z-MYC (d), benign glands in PbCre4;Ptenfl/fl (e), focal HGPIN lesions in PbCre4;Z-MYC;Ptenfl/fl (boxed region in f and higher magnification in g) and focal micro-invasive cancer lesion (arrow) in PbCre4;Z-MYC;Ptenfl/fl (h). Scale bars: 100 μm in (a,b,e,f) and 50 μm in (c,d,g,h). (C) c-MYC and smooth muscle actin (SMA) staining. Arrows in (c) indicate focal areas of disruption of SMA (micro-invasion) in PbCre4;Z-MYC;Ptenfl/fl prostate. Scale bar: 100 μm.

doi:10.1371/journal.pgen.1000542.g002
c-MYC shifts the p53 response in Pten-deficient prostate cells from senescence to apoptosis

It is known that Pten loss can activate the p53 pathway in the prostate cells and activation of the p53 pathway could lead to either senescence or apoptosis depending on the particular p53 target genes induced [13,17,18,33]. We therefore sought to examine activation of the p53 pathway in our c-MYC/Pten model and to determine whether concurrent c-MYC expression alters the p53 response. We observed induction of p53, its targets p21cip1 and PUMA in Pten-null prostates (Figure 7A). However, while p53 and PUMA were induced in c-MYC-overexpressing Pten-null prostates, p21cip1 expression was not (Figure 7A), consistent with the notion that c-MYC represses p21cip1 expression [21]. Similar results were obtained in RWPE-1 cells (Figure 7B). While p53 and p21cip1 were induced upon PTEN knockdown, c-MYC overexpression repressed p21cip1 expression (Figure 7B). We hypothesized that in Pten-deficient cells with activation of the p53 pathway, repression of p21cip1 by c-MYC may switch the senescent response to apoptosis. Indeed, using immunofluorescence, we found that in PbCre4;Z-MYC;Ptenf/f prostates, p16ink4a expression (a marker of senescence) is mainly localized to c-MYC-negative cells while apoptosis (activated Caspase 3) is found predominantly among c-MYC-positive cells (Figure 7C). Thus Pten-deficiency activates the p53/p21cip1 pathway but concurrent c-MYC overexpression shifts the output of the pathway from senescence to apoptosis at least partly by repressing p21cip1.

Discussion

Human prostate carcinogenesis is focal, random, and incremental, but current mouse models do not faithfully recapitulate this. Consequently, the competitive/cooperative interactions that may occur between mutant and normal cells during the early stages of tumorigenesis have not been well studied. The model described here exploits the stochastic expression of a “Cre-activatable” c-MYC transgene (Z-MYC) to induce c-MYC expression in isolated cells surrounded by normal cells. As illustrated by our studies when the Z-MYC mouse is crossed with prostate-specific Pten deletion, the focal nature of c-MYC...
expression allows analysis of cell populations with different genetic alterations within the same prostate gland.

Our studies have yielded several insights. First, focal expression of c-MYC in prostate luminal epithelial cells, even though driven by the CMV enhancer/beta actin promoter, results in remarkably mild pathology with many mice showing histologically normal prostates and a subset of mice demonstrating LGPIN lesions. These results imply a remarkable tolerance of luminal epithelial cells to c-MYC expression. We showed that the acquisition of additional genetic mutations is essential for the appearance of discernable pathology by the fact that introduction of Pten heterozygosity into these animals resulted in cooperativity, with the development of HGPIN/cancer lesions which in all cases were associated with loss of Pten protein expression from the wild type allele. These observations highlight an important point about c-MYC-expressing cells in histologically ''normal'' glands, as may occur in tumors and tissues with ''field cancerization'' [26,34–37] in that the overexpression of c-MYC in histologically “normal” cells may facilitate acquisition of secondary mutations. Although it remains to be established whether loss of Pten expression is due to genetic, epigenetic or post-transcriptional control, c-MYC expression may facilitate acquisition of secondary mutations by increasing cell turnover and/or genomic instability [38,39].

Our PbCre4;Z-MYC;Ptenf/+ mice allowed us to examine the behavior of prostate cells with distinct mutations in the same prostate. c-MYC expression clearly confers an additional proliferative advantage to Pten-null prostate cells, allowing c-MYC+/Pten-null cells to outcompete Pten-null cells. However, Pten deficiency did not alleviate apoptosis in c-MYC+/Pten-null cells. This may appear surprising in light of the well-known, pro-survival effect of Pten loss [40] and a report that Pten loss decreased the apoptosis engendered by the inactivation of retinoblastoma (pRb) family proteins by a truncated SV40 T large antigen in the mouse prostate [41]. Nevertheless, previous studies of mice with conditional deletion of Pten in the prostate and testicular germ line cells have noted an increased rate of apoptosis upon Pten deletion [10,11,42] and Radziszewska et al recently showed that deleting Pten concurrently with c-MYC activation in pancreatic beta cells led to increased apoptosis [43]. Furthermore, Pten deficiency has been reported to activate the p53 pathway leading to senescence [13,18,44] as well as to sensitize cells to ROS-induced apoptosis [17].

Based on our studies and published reports, we propose the following model of cooperativity between c-MYC and Pten in prostate cancer (Figure 7D): Overexpression of c-MYC initiates tumorigenesis by facilitating loss of Pten. The latter leads to the activation of the p53 pathway, which can result in either senescence or apoptosis depending on the predominant Trp53 target genes induced (i.e. cell cycle arrest genes e.g. p21cip1 versus pro-apoptotic genes e.g. PUMA, Bax etc.). The expression of c-MYC drives cells down the apoptotic pathway as it selectively represses the cell cycle arrest-inducing target gene p21cip1.

To summarize, we report a new Cre-dependent prostate cancer mouse model that reflects the focal, random and incremental nature of human prostate carcinogenesis. We show that focal c-MYC expression cooperates with Pten heterozygosity to promote tumor progression due to the selection of cells with loss of Pten expression. In addition, cells mutant for both c-MYC and Pten outcompete single Pten-mutant cells within the same prostates although Pten-deficiency sensitizes cells to apoptosis that is associated with activation of the p53 pathway and exacerbated

Figure 4. Focal loss of Pten expression in PIN lesions of PbCre4;Z-MYC;Ptenf/+ mouse prostate. In focal PIN lesion (arrows), Pten protein expression is lost and phosphorylation of Akt and Foxo1 increased. doi:10.1371/journal.pgen.1000542.g004
Our results highlight the utility of modeling focal oncogene activation to study the interactions between cell populations with different genetic alterations in tumorigenesis.

Materials and Methods

Animals

Z-MYC, PbCre4 and Pten/+ mice have been described [24,25,45]. Female Z-MYC mice (B6/129) were bred to male PbCre4 mice (B6) obtained from MMHCC, Frederick, to generate PbCre4;Z-MYC offspring and littermate controls. Pten/ mice (B6/129) were obtained from The Jackson Laboratory. To generate compound mutant mice, we generated PbCre4;Pten/+ males and Z-MYC;Pten/+ females which were further bred to obtain PbCre4;Z-MYC, PbCre4;Pten/+, PbCre4;Z-
MYC;Pten/+ and PbCre4;Z-MYC;Pten/+ offspring for experiments as well as their littermate controls. Animal care and experiments were carried out according to the protocols approved by the Institutional Animal Care and Use Committees at Vanderbilt University.
LacZ stain

Beta-galactosidase staining followed by counterstaining with nuclear fast red was performed as described [24].

Histology and immunohistochemistry

Tissues were prepared for histopathological analysis as described [46], and slides were reviewed by IEA based on published criteria [47]. Immunohistochemical analyses were performed as described [46]. The following antibodies were used, in some cases with Tyramide Signal Amplification (TSA; Perkin Elmer): anti-activated Caspase 3 (rabbit, 1:500, Cell Signaling), anti-phospho-histone H3 (rabbit, 1:500, Upstate), anti-phospho-Akt (rabbit, 1:100, Cell Signaling), anti-phospho-Foxo1 (rabbit, 1:50, Santa Cruz), anti-c-MYC (rabbit, 1:15,000 with TSA, Santa Cruz), anti-Pten (rabbit, 1:200 with TSA, Cell Signaling), anti-cytokeratin 8 (mouse, 1:2000, Sigma), anti-p63 (PIN Cocktail, Biocare Medical), anti-p53 (rabbit, 1:500 with TSA, Santa Cruz), anti-p21 (mouse, 1:50, Santa Cruz), anti-smooth muscle actin (mouse, 1:2000, Sigma), anti-p16 (rabbit, 1:1000, Santa Cruz), anti-Puma (rabbit, 1:200, Cell Signaling) and anti-phospho-Jnk antibody (rabbit, 1:100, Cell Signaling). For double immunofluorescent stains, c-MYC or Pten detected by 1st primary antibodies were amplified by TSA system (green, Fluorescein). Alexa Fluor 594 (red)-labeled 2nd secondary antibodies (Molecular Probes) were used to detect 2nd primary antibodies (anti-cytokeratin 8, anti-p63, anti-smooth muscle actin, anti-phospho-histone H3, anti-p16 and anti-activated Caspase 3). Nuclear stain (DAPI) and sample mounting were performed using Vectashield mounting medium (Vector Laboratories).

Proliferation and apoptosis assay

At least 500 cells per sample were counted and quantitated after immunohistochemistry for phospho-Histone H3 and activated Caspase 3, respectively. N = 3–4 prostate samples from 9–15 week-old mice per group.

Cell lines

RWPE-1, benign human prostate epithelial cell line (ATCC) was cultured in keratinocyte serum-free media supplemented with bovine pituitary extract and EGF (Invitrogen). We used lentiviral-mediated gene transfer to generate PTEN knockdown/c-MYC overexpressing cells. 293FT packaging cells were plated on 10 cm culture dishes and transfected with PTEN shRNA construct/pLKO.1 vector control (Sigma) or the c-MYC construct/FM-1 vector control along with vesicular stomatitis virus glycoprotein (VSVG) envelope plasmid and delta 8.9 packaging plasmid to produce lentivirus. The FM-1 vector was obtained from J. Milbrandt [48] and was used to clone in human c-MYC cDNA. Three days after transfection, medium containing viral particles was collected and added to RWPE-1 for infection with polybrene (8 μg/ml). 24 hours post infection, medium was changed and another 24 hours later puromycin (1 μg/ml) was added for selection of sh-Pten/pLKO.1 cells. YFP-positive c-MYC/FM-1 cells were sorted by flow cytometry.

Western blot analyses

These were performed as described [49] using the following antibodies: anti-Pten (mouse, 1:2000, Sigma), anti-phospho-Akt (rabbit, 1:2000, Cell signaling), anti-total Akt (rabbit, 1:2000, Cell signaling), anti-c-MYC (mouse, 1:1500, Santa Cruz), anti-p53 (mouse, 1:1000, Santa Cruz), anti-p21 (mouse, 1:1000, Santa Cruz) and anti-beta-actin antibody (goat, 1:1000, Santa Cruz).

Jnk inhibitor treatment and immunocytochemistry for activated Caspase 3

Coverslips were placed on the 24-well plates and 300,000 control or Pten knockdown RWPE-1 cells were plated on the coverslips. Next day, cells were treated with Jnk inhibitor (SP600125) or vehicle (DMSO) at 0, 10 or 50 μM for one hour. Then cells were washed with phosphate buffered saline and supplement-free medium was added to induce apoptosis. After 48 hours, immunocytochemistry for activated Caspase 3 was performed and apoptosis was quantitated from triplicate data per group.

Statistical analyses

We compared groups by using t-test. Values are considered statistically significant at P<0.05. Quantitative variables are...
Figure 7. c-MYC and p53 pathway status in c-MYC/Pten mutant prostate cells. (A) Immunohistochemistry for p53, p21 and Puma. Prostate sections from mice of the indicated genotypes were stained with the indicated antibodies and nuclei were counterstained with hematoxylin. Positive cells (brown) are indicated by red arrows. (B) Western blots show expression of the indicated proteins in RWPE-1 cells expressing c-MYC, PTEN knockdown, or control vectors (FM-1, pLKO.1). PTEN and phospho-Akt (pAkt) blots confirm efficiency of PTEN knockdown. (C) Double immunofluorescent stains for c-MYC/p16 and c-MYC/activated Caspase 3 in PbCre4;Z-MYC;Ptenf/f prostates. Scale bar: 100 μm. (D) Model of interactions between c-MYC and Pten with the p53 pathway in c-MYC–initiated prostate cancer. Focal c-MYC overexpression leads to LGPIN and facilitates loss of Pten leading to HGPIN/invasive cancer. Activation of the p53 pathway due to Pten loss could lead to senescence or apoptosis. c-MYC expression is proposed to shift this response towards apoptosis by repressing p21 expression. doi:10.1371/journal.pgen.1000542.g007
expressed as means±2D while categorical variables are expressed as numbers (%).

Supporting Information

Figure S1 Histopathology of PbCre4;Pten+/− and PbCre4;Z-MYC;Pten+/− tumors at different ages. PbCre4;Z-MYC;Pten+/− mice show higher grade lesions. Scale bars: 100 μm and 50 μm in insets: higher magnifications. Found at: doi:10.1371/journal.pgen.1000542.s001 (6.19 MB TIF)

Figure S2 Juk is activated in Pten-deficient cells and is anti-apoptotic. (A) Phospho-Jnk expression is apparent in PbCre4;Pten+/− and PbCre4;Z-MYC;Pten+/− prostates (brown). (B) Apoptosis increases in Pten-knockdown RWPE-1 cells when treated with Juk inhibitor (SP600125). (C) Western blots show that Pten knockdown

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