MYC Drives Pten/Trp53-Deficient Proliferation and Metastasis due to IL6 Secretion and AKT Suppression via PHLPP2


ABSTRACT

We have recently recapitulated metastasis of human PTEN/TP53-mutant prostate cancer in the mouse using the RapidCaP system. Surprisingly, we found that this metastasis is driven by MYC, and not AKT, activation. Here, we show that cell–cell communication by IL6 drives the AKT–MYC switch through activation of the AKT-suppressing phosphatase PHLPP2, when PTEN and p53 are lost together, but not separately. IL6 then communicates a downstream program of STAT3-mediated MYC activation, which drives cell proliferation. Similarly, in tissues, peak proliferation in Pten/Trp53-mutant primary and metastatic prostate cancer does not correlate with activated AKT, but with STAT3/MYC activation instead. Mechanistically, MYC strongly activates the AKT phosphatase PHLPP2 in primary cells and prostate cancer metastasis. We show genetically that Phlpp2 is essential for dictating the proliferation of MYC-mediated AKT suppression. Collectively, our data reveal competition between two proto-oncogenes, MYC and AKT, which ensnars the Phlpp2 gene to facilitate MYC-driven prostate cancer metastasis after loss of Pten and Trp53.

SIGNIFICANCE: Our data identify IL6 detection as a potential causal biomarker for MYC-driven metastasis after loss of PTEN and p53. Second, our finding that MYC then must supersede AKT to drive cell proliferation points to MYC inhibition as a critical part of PI3K pathway therapy in lethal prostate cancer.

Cancer Discov; 5(6); 636–51. © 2015 AACR.
INTRODUCTION

Prostate cancer is one of the most prevalent cancers among men, and it is estimated that in 2015 there will be 220,800 new prostate cancer cases, making up 14% of all new cancer cases and causing an estimated 27,540 deaths in the United States alone (1). Although mortality rates have been decreasing, prostate cancer is still among the most common causes of cancer-related death and malignancy in men in developed countries (2). The prognosis for men with metastatic prostate cancer is worst, especially due to resistances that arise during treatment (3, 4). Although a large-scale recent study suggested that early (PSA-based) detection reduces mortality, the screening-associated interventions have negative side effects that challenge the low efficiency of the screening effort and can result in overtreatment of the disease (5). Thus, there is an unmet need for causative biomarkers of potentially lethal prostate cancer and for more effective treatment options.

Recent advances in the genomic analysis of prostate cancer have revealed a wealth of data about gene alterations in the disease. Commonly observed events include TMPRSS2–ERG gene fusions (6), deletions of 8p and gains in 8q chromosomal arms (7), and point mutations in the SPOP gene (8). Regarding progression to lethal prostate cancer, a most notable and potentially actionable principle that has emerged is the observed increase and predictive value of copy-number alterations in disease progression (9). Metastatic prostate cancer commonly harbors MYC and AR amplifications, and deletions of PTEN, RB1, and TP53 tumor-suppressor genes (9, 10).

To perform functional analyses of lethal metastatic prostate cancer, we recently generated the RapidCaP mouse model, which allowed us to demonstrate that loss of Pten and Trp53 genes suffices to cause prostate cancer metastasis at near-complete penetrance (11). These data strongly suggested that, in Pten-deficient mouse prostate cancer, the progression from indolent to metastatic disease requires disruption of the p53 senescence response (12). Although there has been much progress in understanding the role that genetic alterations play within the metastatic prostate cancer cell, it has remained less well understood how these aberrations affect cell–cell communication. Emerging evidence supports the idea that inflammation contributes to the initiation and progression of prostate cancer (13), and that inflammatory chemokines and interleukins affect cell motility and proliferation in the transition from normal to benign prostatic hyperplasia (BPH) to prostate cancer (reviewed in ref. 14).

Thus, we set out to define hallmarks of cell secretion that are associated with the metastatic gene signature of Pten and Trp53 loss. Our findings reveal that IL6 secretion is integral to metastasis of Pten-deficient prostate cancer because it promotes the MYC proto-oncogene to drive proliferation and disease progression.
RESULTS

Codeletion of Pten and Trp53 Triggers IL6 Secretion

Functional analysis in genetically engineered mouse (GEM) models of prostate cancer has confirmed the need for alterations in Trp53 (the murine TP53 ortholog) in disease progression after Pten loss (12, 15). Moreover, we have recently shown that Pten/Trp53 codeletion in only a few prostate cells can trigger highly penetrant endogenous metastasis in the RapidCaP mouse model (11). Collectively, these observations emphasized the need for a transition to “post-senescence” in Pten-mutant prostate cancer metastasis. Our analysis of human Pten and TP53 deletions using three recently published whole-genome studies on metastatic prostate cancer (Supplementary Fig. S1A–S1C) confirmed that Pten/TP53 codeletion is significantly associated with metastasis (9, 16, 17), thus underscoring the value of studying the biology of this specific genetic setting.

To investigate the effect of Pten/Trp53 loss on secretion, we used PtenloxP/Trp53loxP/loxP; loxP-stop-loxP (Isl-tldTom; Supplementary Fig. S1D) primary mouse embryonic fibroblasts (MEF), and infected them with Cre recombinase–expressing adenovirus (AdCre). This approach yielded over 95% infection of MEFs, and infected them with Cre recombinase–expressing adenovirus (AdCre). This approach yielded over 95% infection rate (see Supplementary Fig. S2A). Cells were analyzed up to day 6 after infection without antibiotic treatment to minimize selection for spontaneous gene alterations. As shown in Fig. 1A, Pten–/– MEFs grew significantly more slowly than wild-type (WT) MEFs due to induction of senescence (Supplementary Fig. S2B), consistent with previous results (12, 18, 19). Deletion of Trp53 alone caused only slightly increased growth compared with WT MEFs. In contrast, combined loss of Pten and Trp53 led to significant growth acceleration (Fig. 1A).

To study the differences in secreted proteins between post-senescent cells (Pten+/Δ, Trp53+/Δ–double mutant) and the other three genotypes (WT, Pten+/Δ, and Trp53+/Δ), we collected conditioned medium and analyzed secreted cytokines and chemokines using mouse-specific cytokine profiling arrays (Fig. 1B). Among 40 profiled cytokines and chemokines, IL6 stood out by being secreted specifically in the Pten+/Δ,Trp53+/Δ cells. Other proteins detected in the conditioned medium included CCL5 (RANTES), CXCL1 (KC), and CXCL10 (IP-10); however, these did not respond to Pten status and were also upregulated by loss of Trp53+/Δ alone. The heatmap for hierarchical clustering analysis of quantified data from multiple cytokine arrays confirmed induction of IL6, specifically after codeletion of Pten and Trp53 (Fig. 1C). We next validated these results using IL6-specific ELISA assays on supernatant from the cells (Fig. 1D). Using RT-qPCR analysis, we found that Il6 upregulation occurred at the RNA level (Fig. 1E). Taken together, our results revealed that IL6 secretion is a specific response to combined Pten and Trp53 loss.

IL6 Secretion Drives Cell Proliferation through STAT3/MYC after Loss of Pten/Trp53

We next studied Pten and p53 signaling pathway components. As shown (Fig. 2A, left), deletion of Pten alone activated AKT and p53/p21/p16 signaling pathways, which cause the senescence phenotype (Supplementary Fig. S2B; refs. 12, 15, 18, 19). Accordingly, upon codeletion of Pten and Trp53, the p21 and p16 tumor suppressors were no longer activated. Furthermore, we observed increased STAT3 phosphorylation and significantly increased expression of its transcriptional target, MYC, when compared with deletion of Pten or Trp53 alone (Fig. 2A, densitometric quantification). Note that we did not detect p44/p42 activation, which was shown previously to increase MYC protein stability through phosphorylation (20).

Intracellular signaling downstream of PI3K has previously been implicated in STAT3 activation through the TEC kinase family member BMX, which contains a PIP2-sensitive PH domain (reviewed in ref. 21). Therefore, we used shRNA against Il6 to test whether its secretion contributed in an autocrine/paracrine fashion to proliferation of Pten/Trp53–lacking cells as scored in MEFs with conditional tdTomato alleles (Pten+/Δ,Trp53+/Δ–tdTom+/Δ MEFs). As shown in Fig. 2B (left), the addition of sensor-screened miR-E–based shRNA (22, 23) against Il6 decreased proliferation of the double-mutant MEFs. Molecular pathway analysis furthermore confirmed that shIl6-positive cells significantly impaired STAT3 activation and MYC expression (Fig. 2B, right). In a concentration-dependent manner, IL6-neutralizing antibodies also decreased proliferation of the double-mutant MEFs (Supplementary Fig. S3A) and suppressed STAT3 and MYC activation (Supplementary Fig. S3B). Activation of STAT3 in the Pten+/Δ,Trp53+/Δ cells depended, at least in part, on PI3K activity, as the pan-PI3K inhibitor LY294002, but not rapamy- cin, suppressed STAT3 activation (Supplementary Fig. S3C). Taken together, these data suggested that, in addition to intracellular PI3K signaling through AKT, autocrine/paracrine IL6 signaling significantly contributed to STAT3 activation and growth of Pten/Trp53-deficient cells.

Pharmacologic targeting of STAT3 activation also suppressed proliferation: The STAT3 inhibitor WP1066 (24) reduced Pten–/–,Trp53+/Δ–tdTom+/Δ cell proliferation by over 62.5% compared with DMSO treatment (Fig. 3A, top). Western blot analysis confirmed WP1066-dependent reduction of phosphorylated (p) STAT3 levels, and decreased phosphorylation of STAT3 correlated with a marked reduction of its transcriptional target MYC (Fig. 3A, bottom). STAT3 was previously shown to bind the MYC promoter (25) and to affect the activity of MYC (reviewed in ref. 26). Next, we targeted STAT3 genetically by overexpressing a dominant-negative isoform, STAT3DN, which harbors a point mutation in a tyrosine phosphorylation site (Y705F) that is critical for its activation and nuclear translocation (27, 28). The PtenloxP/loxP;Trp53loxP/loxP; Isl-tldTom MEFs were first infected with AdCre, and then with a lentiviral plasmid containing green fluorescent protein and STAT3DN or with a control lentivirus containing green fluorescent protein alone (29). Double-infected cells thus expressed both red and green fluorescent proteins and were counted by flow cytometry (Supplementary Fig. 3D). Our results (Fig. 3B, top) have shown that overexpression of the dominant-negative STAT3DN significantly reduced proliferation of the Pten/Trp53-deleted cells compared with the control plasmid. Western blotting analysis confirmed the reduction of both STAT3 phosphorylation and MYC levels. Note that...
IL6 Drives MYC in PTEN-Mutant Prostate Metastasis

Figure 1. IL6 secretion is a hallmark of postsenescence. A, combined loss of the Pten and Trp53 genes causes primary MEFs to proliferate more aggressively when compared with the single genotypes. Error bars, SD; n ≥ 3; *, P < 0.05 with ANOVA, Dunnett post hoc test on all genotypes versus WT at days 4 and 6. B, IL6 is secreted specifically after simultaneous loss of Pten and Trp53 genes. A threshold of 3-fold was used to filter upregulated proteins specific for Trp53Δ/Δ, PtenΔ/Δ, and PtenΔ/Δ;Trp53Δ/Δ cells. CCL5 (RANTES), CXCL1 (KC), and CXCL10 (IP-10) proteins were upregulated in both Trp53Δ/Δ and PtenΔ/Δ;Trp53Δ/Δ cells. C, protein secretion represented as log 2 -transformed fold expression relative to WT and normalized to total cellular protein content. Note that there are no proteins specifically secreted in PtenΔ/Δ MEFs that exceed the 3-fold cutoff. n = 3. Note that we cannot exclude stimulation of protein secretion in senescence (i.e., in the Pten-null cells) below the threshold criteria. D, ELISA results confirm increased expression of IL6 in conditioned medium from PtenΔ/Δ, Trp53Δ/Δ MEFs. ANOVA, Dunnett post hoc test, **, P < 0.01, versus WT; error bars, SD, n = 3. E, increased transcription of IL6 is observed in PtenΔ/Δ, Trp53Δ/Δ MEFs compared with WT. ANOVA, Dunnett post hoc test, **, P < 0.01, versus WT; error bars, SD, n = 4.
the constitutively active STAT3C mutant showed only minor activation of STAT3/MYC beyond the observed activation in the Pten/Trp53-deficient background (Fig. 3B, bottom), and, accordingly, it had no effect on cell proliferation (Fig. 3B, top). Collectively, our data revealed that STAT3 activation after loss of Pten and Trp53 significantly contributes to cell proliferation, and suggested that, in this setting, paracrine signaling through IL6 can mediate activation of MYC in cell culture.

Next, we asked whether MYC activation contributes to proliferation of the Pten/Trp53-deficient cells. First, we used the BRD4 inhibitor JQ1, which, among other targets, suppresses MYC transcription (30, 31). As shown (Fig. 3C), the drug indeed suppressed MYC activation and suppressed proliferation of the primary Pten/Trp53-null MEFs. Similarly, two separate shRNAs targeting Myc also strongly reduced proliferation of the double-mutant Pten/Trp53-deficient MEFs (Fig. 3D). Importantly, RNA interference selectively blocked MYC, but not STAT3, activation, consistent with MYC being downstream of STAT3 action.

Taken together, our results using primary MEFs with defined genetic alterations suggested that activation of MYC via IL6/STAT3 (i) is a hardwired response to codeletion of Pten and Trp53 genes, (ii) is critical for driving cell proliferation in this genotype (see also cartoon, Supplementary Fig. S7), and (iii) presents a targeting opportunity for drugs.

**Pten**<sup>pc−/−;Trp53</sup><sup>pc−/−</sup> Prostate Epithelia Secrete IL6 and Activate Stromal Proliferation through STAT3/MYC

Next, we tested the relevance of our findings to prostate tumorigenesis in vivo. As previously described (12, 32, 33), we used the probasin promoter to generate prostate conditional knockout mice by prompting Cre recombination in the prostate epithelium (Pten<sup>pc−/−;Trp53</sup><sup>pc−/−</sup>;B-Cre4). First, we
IL6 Drives MYC in PTEN-Mutant Prostate Metastasis

**Figure 3.** STAT3/MYC signaling is downstream of IL6 signaling in Pten\textsuperscript{−/−},Trp53\textsuperscript{−/−} cells and is responsible for proliferation. **A,** treatment with the STAT3 inhibitor WP1066 decreases proliferation of Pten\textsuperscript{−/−},Trp53\textsuperscript{−/−} primary MEFs compared with DMSO treatment and decreases phosphorylation of STAT3. ANOVA, Dunnett post hoc test; \( ^{**} P < 0.01, ^{*} P < 0.05 \) versus DMSO; error bars, SD; \( n = 3 \). **B,** overexpression of dominant-negative STAT3DN blocks proliferation of Pten\textsuperscript{−/−},Trp53\textsuperscript{−/−} primary MEFs; dominant-negative STAT3DN inhibits the STAT3 transcriptional target MYC. ANOVA, Dunnett post hoc test; \( ^{**} P < 0.01, ^{*} P < 0.05 \) versus GFP at prospective day; error bars, SD; \( n = 3 \). **C,** JQ1, a BRD4 inhibitor, blocks proliferation of Pten\textsuperscript{−/−},Trp53\textsuperscript{−/−} primary MEFs as measured using crystal violet (top) and MYC expression (bottom), the Student \( t \) test; \( ^{***} P < 0.001 \); error bars, SD; \( n = 3 \). **D,** short hairpins against MYC decreased proliferation of Pten/Trp53-negative cells, and Western blot analysis confirms efficient knockdown of MYC and decrease in activation of STAT3/MYC signaling; PCNA Western blot analysis confirms decreased proliferation but to a lesser extent than IL6. ANOVA, Dunnett post hoc test; \( ^{***} P < 0.001 \), versus Ren.713 at prospective day; error bars, SD; \( n = 4 \).

harvested prostates from these mice at 11 weeks (4 weeks after Pten/Trp53 recombination) to test STAT3 status in vivo. Western blot analysis of Pten\textsuperscript{−/−},Trp53\textsuperscript{−/−} prostates (see Fig. 4A, each lane corresponds to a different animal) confirmed strong AKT activation as well as increased STAT3 phosphorylation compared with normal prostate, fully consistent with our observations in MEF cells (Fig. 2A). Using prostate immunohistochemistry (IHC) analysis, we observed markedly elevated IL6 levels in Pten\textsuperscript{−/−},Trp53\textsuperscript{−/−} prostate epithelial cells (Fig. 4B, gland), consistent with their role as sender cells, analogous to the Pten\textsuperscript{−/−},Trp53\textsuperscript{−/−} MEFs identified earlier. Notably, we were able to detect significantly higher IL6
Figure 4. Secretion of IL6 and STAT3/MYC signaling is specific to the in vivo Ptenpc−/−;Trp53pc−/− genotype. A, Western blot analysis of anterior prostate tissue from Ptenpc−/−;Trp53pc−/− mice at 11 weeks shows lack of PTEN, activation of AKT, and STAT3. B, hematoxylin and eosin (H&E) analysis of 11-week-old prostates shows expansion of stroma, and high cytoplasmic IL6 levels in Pten/Trp53-deleted prostate but not in WT prostate. Scale bar, 100 μm. C, IL6 levels are increased in blood serum taken from Ptenpc−/−;Trp53pc−/− animals (n = 9) reaching almost 20 pg/mL levels, in contrast to serum from WT animals (n = 4) that was below the detection level (<7.8 pg/mL). Mann–Whitney test, P = 0.0112 versus WT; error bars, SEM.

levels in the blood of the Ptenpc−/−;Trp53pc−/−;Pb-Cre4 mice than in WT mice (see Fig. 4C), suggesting that a systemic readout of the Pten/p53–mutant state might be possible.

As seen in Fig. 4B [hematoxylin and eosin (H&E)], Ptenpc−/−;Trp53pc−/− prostates showed a massive expansion of stromal fibroblasts compared with the few stromal cells between prostate glands of WT animals (Fig. 4B, IL6, far right). This striking response was absent from Pten- or Trp53-deficient prostate glands (Supplementary Fig. S4A). Furthermore, IHC analysis of the proliferation marker Ki67 revealed strong staining of stromal fibroblasts specifically surrounding Ptenpc−/−;Trp53pc−/− mutant epithelia (Fig. 5A, Ki67). Next, we tested whether stromal cells also had activated STAT3. As shown (Fig. 5A, pSTAT3Y705), stromal fibroblasts were pSTAT3Y705 positive and also showed high levels of MYC. In contrast, stromal fibroblasts were negative for pAKT5473, consistent with loss of Pten/Trp53 being restricted to the prostate epithelium (see PTEN staining in Supplementary.
IL6 Drives MYC in PTEN-Mutant Prostate Metastasis

Figure 5. Prostate stroma and gland analysis show specific interactions between MYC and pAKT<sup>S473</sup> activation. A, IHC analysis of stroma reveals paracrine activation of MYC/STAT3 signaling in Pten<sup>−/−</sup>;Trp53<sup>−/−</sup> prostate. Note that AKT is not activated in the stroma. B, epithelial Pten<sup>−/−</sup>;Trp53<sup>−/−</sup> glands show correlation of the proliferation marker Ki67, MYC, and pSTAT3<sup>Y705</sup> in the periphery of the glands, where phosphorylated AKT is weak. Scale bar (A and B), 50 μm. C, target plot analyses visualize the average distance of IHC-positive epithelial cells to stroma and confirm the correlation between Ki67, pSTAT3<sup>Y705</sup> and MYC, and their inverse correlation with pAKT<sup>S473</sup>. D, schematic of target plot generation from IHC data (note that only entire glands were recorded and analyzed). Track 1 represents epithelial cells along the epithelial border defined as 0 to 25 μm from the stroma/fibromuscular layer; Track 2: cells within 25% of the maximum possible distance of a cell in a particular gland from the border; Track 3: cells that are within 25% to 50% of this maximum possible distance; Track 4: cells that are within 50% to 75% of this maximum possible distance; and Track 5: cells within 75% to 100% of the maximum possible distance. Color intensity represents the relative number of stained cells.

Fig. S4B). These data revealed that the activation of STAT3 and MYC is conserved in Pten<sup>−/−</sup>;Trp53<sup>−/−</sup> mutant prostate epithelium and strongly suggested that <i>in vivo</i> IL6 secretion acts in a paracrine fashion on the surrounding stromal cells to trigger proliferation in the tumor environment in the absence of AKT activation and Pen loss. Note that this stromal activation phenotype is unusual in humans, yet is a characteristic of the Pten<sup>−/−</sup>;Trp53<sup>−/−</sup> mutant prostate model (34) and lacking when disease initiation is more focal, as in the RapidCaP system (11). Examination of cell proliferation in the Pten<sup>−/−</sup>;Trp53<sup>−/−</sup> mutant prostate epithelium revealed that it was strongest in the periphery of glands (Fig. 5B, Ki67). This high proliferation zone also had most of the phosphorylated STAT3 and MYC staining (Fig. 5B, pSTAT3<sup>Y705</sup>, MYC). To our surprise, however, IHC staining for phosphorylated AKT revealed an inverse correlation with Ki67 staining: Inside the gland, where Ki67 staining was relatively infrequent, there was a strong pAKT<sup>S473</sup> signal. Conversely, the gland’s outermost cell layer showed the weakest pAKT<sup>S473</sup> staining, where proliferation and pSTAT3<sup>Y705</sup>/MYC were strongest. Quantification of the radial distribution of IHC signal in the glands confirmed these results (Fig. 5C and D; see also Methods).
MYC and pSTAT3[T308S] correlated with the distribution of the proliferation marker Ki67, whereas pAKT[473S] was inversely correlated with Ki67 (Fig. 5C). In Pten-deficient prostate glands, however, Ki67 and pAKT[473S] staining did correlate: both were found throughout the gland, consistent with a lack of IL6/STAT3/MYC signaling activation when Pten is deleted alone and not together with Trp53 (Supplementary Fig. S4C). Western blot analysis also consistently revealed these results: pAKT[473S] staining was weaker when MYC was activated in Pten[Δ/Δ]Trp53[Δ/Δ] MEFs than in the Pten[Δ/Δ] cells (Fig. 2A, see pAKT[473S] and MYC, Western and densitometry).

Next, we probed the mechanism responsible for this suppression of AKT phosphorylation. We have previously shown that the AKT phosphatase PHLPP2 (35, 36), but not the PHLPP1 homolog, is specifically induced by AKT pathway activation to limit the signaling output (ref. 15, reviewed in ref. 37). As shown in Fig. 6A, we confirmed these results in Pten-deficient MEFs, but found that combined loss of Pten/Trp53 resulted in even higher PHLPP2 protein levels, which correlated inversely with AKT[473S] phosphorylation and thus could mechanistically explain AKT suppression in this genotype. Because the increase in PHLPP2 protein correlated with the increase in MYC in Pten[Δ/Δ]Trp53[Δ/Δ] MEFs, we next tested whether MYC causes these elevated PHLPP2 levels. As shown in Fig. 6B, the CRISPR/Cas9-based knockout of MYC led to decreased expression of PHLPP2 in the Pten[Δ/Δ], Trp53[Δ/Δ] MEFs. The overexpression of constitutively active AKT1 (Myc-AKT1) in the Pten/Trp53−/− MEFs led to reduced, not increased, expression of the proliferating cell nuclear antigen (PCNA) and AKT1-DN did not affect levels of PCNA (Fig. 6C). This experiment shows that proliferation is not increased even by high levels of active AKT in the context of Pten/Trp53 loss and spontaneous activation of MYC. Conversely, the overexpression of MYC led to increased expression of PHLPP2 in the Pten[Δ/Δ]/Trp53[Δ/Δ] MEFs (Fig. 6D) and a corresponding suppression of pAKT[473S]. Importantly, cell proliferation, measured by cell count (Fig. 6E) or PCNA levels, in both of these cases correlated with MYC levels, and not those of pAKT. Therefore, these data confirmed that MYC dictates proliferation and is necessary and sufficient for suppression of AKT activation. Subsequently, we tested whether PHLPP2 is essential for suppression of AKT downstream of MYC. To this end, we transduced Cre into Pten[Δ/Δ]LoxP/LoxP, Trp53[Δ/Δ]LoxP/LoxP, PHLPP2LoxP/LoxP, Pten[Δ/Δ]LoxP/LoxP, PHLPP2LoxP/LoxP, triple-mutant MEFs (generated from PHLPP2-mutant mice; see Methods). As shown in Fig. 6F, overexpression of MYC in these cells no longer suppressed AKT phosphorylation, consistent with the loss of its phosphatase, PHLPP2. To our surprise, however, MYC overexpression did not trigger increased cell proliferation in these triple-mutant MEFs, as shown by PCNA staining and cell number counts in proliferation assays (Fig. 6F and G).

Collectively, these data strongly suggested that activation of MYC and the resulting PHLPP2-mediated suppression of pAKT are critical for the proliferation of Pten/Trp53−/−-deficient cells.

**MYC Suppresses AKT in Prostate Metastasis via the AKT Phosphatase PHLPP2**

We next tested the relevance of these findings to prostate cancer metastasis by using our recently developed RapidCaP GEM model, which features endogenous Pten/Trp53−/−-deficient prostate cancer metastasis at 50% penetration within 4 months. MYC can drive the initiation and maintenance of metastasis, as revealed by treatment with the BRD4-antagonizing bromodomain inhibitor JQ1 (31, 38) and by using MYC as a transgenic driver of metastasis in RapidCaP. Castration of RapidCaP mice suppresses primary and metastatic disease. However, this invariably results in lethal relapse, which presents with even stronger MYC expression, Myc gene amplification, and with growth at much faster rates than the precastration lesions (11). As shown in Fig. 7A, metastatic prostate cancer nodules, which can be found in the lungs of untreated or castrated RapidCaP mice, are positive for the NXX3.1 prostate marker and show high levels of MYC, yet no or very faint staining for pAKT[473S]. Importantly, we validated that the metastatic nodules consistently presented with elevated levels of the PHLPP2 phosphatase, which inversely correlated with the low/absent pAKT[473S] staining. These patterns were not observed in WT lungs (Supplementary Fig. S5A). We also confirmed increased expression of STAT3/IL6 in these Pten/Trp53−/−-deficient metastatic nodules and their proliferative potential using PCNA (Fig. 7B). IL6 staining was present in all metastatic nodules, with the extent typically varying between 20% and 70% positive cells per nodule (Supplementary Fig. S5B). We found no IL6 activation in the Trp53-deficient prostate and the Pten-null glands, which were free of hyperplasia/neoplasia, whereas the proliferating Pten-deficient prostate glands showed IL6 staining, consistent with spontaneous breaking of the p53 response in parts of the tissue, as published previously (Supplementary Fig. S5C; ref. 12). These results were in agreement with our in vitro (see Fig. 1) and in vivo ELISA analysis, where Pten/Trp53 double-mutant probasin-driven animals had high IL6 levels in blood and tissue (Fig. 4A and B). Note that we found no IL6 changes in blood from Trp53-null animals and either undetectable or strong IL6 increase in the Pten-null animals (not shown), consistent with their variable spontaneous evolution mentioned above.

IHC analysis of the androgen receptor (AR) status in RapidCaP lesions showed no detectable (nuclear) AR staining (Supplementary Fig. S6A) but positive staining for NXX3.1, as published previously (see Fig. 7A; ref. 11). In contrast, the primary prostates from both RapidCaP and Probasin-Cre animals showed strong AR staining (Supplementary Fig. S6B). Note that we have previously shown that spontaneous loss of AR can also be found in some prostate epithelial cells of the RapidCaP mice (11) and that some metastases strongly respond to castration, suggesting that both IHC-positive and IHC-negative AR cells can metastasize. It is important to note that the histopathology-based IHC analysis requires lesions that are large enough to be unambiguously identified by pathology. These advanced IHC-negative AR cells in metastatic lesions are very likely resistant to castration, similar to the IHC-negative AR cells of the castration-resistant prostate lesions (11).

Collectively (see Supplementary Fig. S7), these data are consistent with a model in which Pten/Trp53−/−-deficient prostate metastasis progresses through an AKT-independent pathway, which requires the activation of MYC by IL6, and PHLPP2-mediated suppression of AKT, to drive proliferation and castration resistance.
IL6 Drives MYC in PTEN-Mutant Prostate Metastasis

**Figure 6.** MYC decreases phosphorylation of pAKT S473 via activation of the PHLPP2 phosphatase. **A,** activation of the AKT phosphatase PHLPP2 is found after MYC activation and pAKT reduction after Pten/Trp53 loss. **B,** knockout of MYC using the CRISPR/Cas-9 system leads to decrease in PHLPP2 expression and reduced levels of the proliferation marker PCNA. **C,** the overexpression of constitutively active AKT1 (Myr-AKT1) in the Pten/Trp53–recombined MEF cells prompts increased PCNA levels, whereas AKT1-DN does not. **D,** overexpression of MYC triggers an increase in PHLPP2 and in proliferation (PCNA), but reduces AKT phosphorylation. **E,** overexpression of MYC in Pten/Trp53-negative cells causes a sharp increase in proliferation compared with the eGFP control (*, P < 0.05, Student t test; n = 4). **F,** deletion of Phlpp2 uncouples MYC from causing increased proliferation (ns, not significant; P > 0.05, Student t test; n = 4).
**DISCUSSION**

Our findings identify a critical role for IL6/STAT3 signaling in promoting MYC as a driver of Pten/Tp53-mutant metastasis. The MYC gene is frequently amplified in prostate cancer (as curated at the cBio portal; ref. 39) and functional validation established it as a driver of prostate cancer (40) and its metastasis, as seen using the RapidCaP system (11). Human genome analysis revealed that MYC alterations usually stem from broad amplifications on chromosome 8q and correlate with metastatic prostate cancer (57% in metastasis vs. 15% in primary prostate cancer; ref. 9). Codeletion of PTEN and TP53 is also a hallmark of prostate cancer metastasis, as previously published and shown in Supplementary Fig. S1A, by using additional recent prostate cancer metastasis genome studies.

Our data support two novel concepts for metastatic prostate cancer (Supplementary Fig. S7). First, they present a molecular mechanism that links MYC activation to loss of both PTEN and TP53, but not to loss of either separately. The combined loss marks a genetic condition that is tightly linked to metastatic prostate cancer, as discussed above. This regulatory signaling to MYC occurs within 4 days of Pten/Tp53 loss in our *in vitro* experiments, thus likely preceding MYC gene amplification. If validated in humans, this could indicate a therapeutic window of opportunity before MYC gene amplification is detected as a driver of late-stage disease at a point when it may respond less well to anti-MYC therapy.

These data suggest that cell–cell communication is intimately linked to genetic changes of PTEN and TP53. It is known that chemokines and interleukins change their expression patterns during prostate cancer progression (14). Now, our *in vitro* and *in vivo* data specifically link the gene loss of PTEN and TP53 to IL6 secretion. The Probasin-Cre–driven *Pten*Δ/Δ, *Tp53*Δ/Δ model illustrates the effect that this communication can have on normal stroma, by triggering MYC expression and proliferation (and possibly a sarcoma phenotype of this classic model; ref. 34). Our data suggest that the IL6/STAT3/MYC module shown in Supplementary...
IL6 Drives MYC in PTEN-Mutant Prostate Metastasis

Fig. S7 becomes independent of pAKT in metastasis. It remains to be seen whether the MYC module still depends on PI3K activity in metastasis, as it has been shown that the PI3K family member TEC-kinases, such as BMX, can activate STAT3 downstream of PI3P (reviewed in ref. 21). Alternatively, IL6/STAT3 may be increased through a PI3K pathway autonomous mechanism such as gene amplification (IL6 amplification is observed in 38% of metastatic samples of the published Memorial Sloan Kettering Cancer Center cohort). It remains to be seen whether IL6 signaling of prostate cells at the metastatic site is strictly needed to enhance fitness and proliferation of the cancer cells or also helps disease progression by landscaping the microenvironment and immune response. ELISA-based detection of IL6 in the blood of Pten<sup>loxP/loxP;</sup>Trp53<sup>loxP/loxP;</sup>C cells points to a potential avenue for identifying patients with PTEN/TP53-mutant metastasis. Elevated levels of IL6 have been associated with advanced tumor stages of various cancers, including multiple myeloma, non-small cell lung carcinoma, and prostate, colorectal, renal, breast, and ovarian cancers. Anti-IL6 therapies use a neutralizing antibody (e.g., siltuximab) or anti-JAK/STAT3 signaling (e.g., ruxolitinib). Siltuximab has shown mixed results in metastatic castration-resistant prostate cancer (41–44). We hope that future trials on IL6/STAT3 pathway inhibition may benefit from our modeling results by pointing to MYC for patient selection and monitoring of drug efficacy.

Second, our results surprisingly suggest that both MYC activation and AKT suppression are required to drive proliferation in Pten<sup>loxP/loxP</sup>;Trp53<sup>loxP/loxP</sup>–deficient cells. It is particularly intriguing that a bona fide tumor-suppressing AKT phosphatase, PHLPP2, is co-opted by MYC to drive proliferation by suppressing AKT activity. Several factors could explain why metastatic and castration-resistant disease favors MYC over AKT activity. Apart from its role in promoting ribosome biogenesis, MYC has been implicated in the dedifferentiation of cells (reviewed in ref. 45), which may explain the observed loss of epithelial cell characteristics in the MYC-positive Pten<sup>loxP/loxP</sup>;Trp53<sup>loxP/loxP</sup>–deficient RapidCaP lung metastases and in the castration-resistant prostate cancer (41–44). Furthermore, several studies have previously identified isoform-specific antimigratory and cancer-suppressing roles for AKT, suggesting several mechanisms by which AKT may impede prostate metastasis (refs. 46–51; reviewed in ref. 52). Indeed, this switch may be critical for resistance to hormone therapy. Using the RapidCaP system, we consistently observed the highest levels of MYC protein and highest percentage of Myc gene amplification (32%) in relapsed castration-resistant prostate tumors, and activation of MYC also correlated with loss of AR staining (11). Thus, enhanced reliance of lethal prostate cancer lesions on MYC could create novel tumor sensitivity to drugs that target MYC.

METHODS

Mouse Embryonic Fibroblasts

Animals with different combinations of loxP alleles (Pten<sup>loxP/loxP;</sup>Trp53<sup>loxP/loxP</sup>;Phlpp2<sup>flp/flp</sup> or Pten<sup>loxP/loxP;</sup>Trp53<sup>loxP/loxP;</sup>MadTm1Cre) were used. At 13.5 days, embryos were harvested by sacrificing a female using CO2. Embryos were minced and trypsinized for 15 minutes in a 37°C in water bath. Cells were plated on 10-cm dish and cultured in 10% DMEM medium and 1% penicillin-streptomycin; this was called passage 0. Three days after plating, cells were split to 10-cm dish at 900,000 cells per dish per 15 mL volume. Cells were cultured for the next 3 days, collected, frozen in 20% dimethyl sulfoxide, 80% FBS, and stored in batches of 1,000,000 cells per cryovial in liquid nitrogen. All protocols for mouse experiments were in accordance with the institutional guidelines and were approved by the Institutional Animal Care and Use Committee at Cold Spring Harbor Laboratory (CSHL).

Mutant Mice

Mutant mice were generated using animals containing homozygous alleles for Pten<sup>loxP/loxP</sup> (33) and Trp53<sup>loxP/loxP</sup> (53). Female Pten<sup>loxP/loxP;</sup>Trp53<sup>loxP/loxP</sup> mice were crossed with a male mouse carrying a heterozygous allele for Phlpp2<sup>flp/flp</sup>. Embryonic stem cells were purchased from the Mouse Phenotype Consortium (PHLPP2–IKMC Project: 47191). The Phlpp2 gene gives rise to three WT transcripts, of which two are protein coding. Initially, mice were crossed to a flipase mouse to remove the lacZ/loxP/neo cassette, which gave rise to loxp conditional mice. Following removal of the floxed region, two transcripts were produced to produce truncated protein products, which may be subject to nonsense-mediated decay (NMD). Our expression analysis confirmed knockouts of PHLPP2 at the protein level. For genotyping, DNA from the tail was extracted, and PCR analyses were performed as previously described (53). Pten<sup>loxP/loxP;</sup>Trp53<sup>loxP/loxP</sup> Mutant mice were generated by intraprostatic delivery of virus and the prostate cancer metastases to lung analyzed as previously described (11).

Cell Proliferation Assays

Total cell counts were done using the Guava System (Millipore). Briefly, cells were trypsinized and mixed with medium in a 1:5 ratio, then 150 μL was loaded into 96-well plates and analyzed using the Guava easyCyte 8HT Benchtop Flow Cytometer (Millipore). Cell proliferation was also measured using the crystal violet method. Briefly, cells were fixed in 10% formalin for 15 minutes, washed with sterile water, and stained with 0.1% crystal violet solution. Solutions were aspirated and washed with water, and then plates were air-dried overnight and 10% acetic acid was added and mixed with water in a 1:3 ratio. Absorbance was measured at 590 nm using a plate reader (Synergy H4 Hybrid Multi-Mode Microplate Reader, Biotek).

Cell Treatments

For IL6-neutralizing experiments, IL6-neutralizing antibody (R&D Systems; AB-406-NA) and control IgG antibody (R&D Systems; AB-108) were used to assess effects of secreted IL6 on the proliferation of MEFs. The STAT3 inhibitor WP1066 (Selleckchem; S2796), PI3K inhibitor LY294002 (Cell Signaling Technology; #9901), rapamycin (Cell Signaling Technology; #9904), and JAK inhibitor 1 (Calbiochem; 420099) were resuspended in DMSO. JQ1 was a kind gift from Dr. J. Bradner (Harvard Medical School, Boston, MA) and was used as described previously (31). Note that the IC<sub>50</sub> value for inhibition of proliferation was 1.4 μmol/L for Pten<sup>loxP/loxP</sup>–deleted cells, and 3.3 μmol/L for WT cells. These results are in a similar range to earlier reported WP1066 effects on cell survival, proliferation, and STAT3 phosphorylation (54–56).

cDNA Plasmids

The adenoviral plasmid AdSCMVcre was obtained from the Gene Transfer Core, University of Iowa (Iowa City, IA). FUGW backbone lentiviral plasmids were purchased from Addgene (plasmid numbers in parentheses): GFP (14883), STAT3C (24983), STAT3DN (24984; ref. 29), AKT1-DN (53597), Myr-AKT1 (53583), and Myr-AKT3 (53596; ref. 57). Retroviral plasmids were kind gifts from Dr. C. Vakoc (CSHL, Cold Spring Harbor, NY): control (GFP) and MYC
that were cloned into pMSCV-PGK-Puro-IRE-GFP plasmid as published previously (31).

shRNA Plasmids

Retroviral short hairpins against Il6 and Myc are using the optimized “mir-E” backbone and were identified using a high-throughput RNAi Sensor assay and expressed from the optimized “mir-E” backbone that improves pri-miRNA processing efficiency (23). The following 97-mer sequences were used:

sh_Il6.277:TGCTGTACGGAGTCGTAG TAAGTTTGAGGCAGTTGCCTACTGCCTCGGA
sh_Myc.1891:7GTCTGTTGAGTCGTAG CAGTTGGAAACATAGTGAAGCCACAGATGTATCAGATTGTTTCAACTCGC
sh_Myc.2105:7GCTCTGTTGAGTCGTAG CAAGTGCTGCCTACTGCCTCGGA

CRISPR/Cas9 Plasmids

The guide RNAs (gRNA) were designed using the R package CRISPRseek (58). The three highest-ranking gRNAs were chosen. Among these was guide Myc-87 (5′-CTGGGAGATGAGGATCCGG-3′), a gRNA that targets Myc in the first exon. The gRNA oligonucleotides were purchased from Sigma-Aldrich, and phosphorylated and annealed according to previously published protocols (59). The duplex oligonucleotides were subsequently cloned into the lentCRISPR_V1 plasmid (Addgene #49535), replacing the 2-kb filler.

Retrovirus Production

Phoenix cells were plated at a density of 10^6 cells per 10-cm dish and 2 μl of 100 mmol/L chloroquine. Medium was changed after 14 hours and viral supernatants were collected at 48 and 72 hours after transfection. Supernatants were then centrifuged and filtered with a 0.45-μm filter before infection. The 293FT cells were plated at a density of 10^6 cells per 10-cm dish and treated with 6.7-μM chloroquine. Medium was changed after 14 hours, and viral supernatants were collected at 48 and 72 hours after transfection. Supernatants were then centrifuged and filtered with a 0.45-μm filter before infection of cells. The 293FT cells were a kind gift from Dr. Scott W. Lowe (Memorial Sloan Kettering Cancer Center, New York, NY). They were not authenticated.

Lentivirus Production

The 293FT cells were plated at a density of 10 × 10^6 cells per 10-cm dish the day before calcium chloride transfection. Plasmid DNA (15.5 μg) was combined with 5-μg helper constructs and 2 μl/M calcium chloride. The mixture was then added dropwise to plates after treatment with 2.5 μl of 100 mmol/L chloroquine. Medium was changed after 14 hours and viral supernatants were collected at 48 and 72 hours after transfection. Supernatants were then centrifuged and filtered with a 0.45-μm filter before infection of cells. The 293FT cells were a kind gift from Dr. Scott W. Lowe. They were not authenticated.

Antibody Arrays

Medium was collected and snap-frozen in liquid nitrogen and stored at −20°C until analyzed. Expression of cytokines and chemokines was measured using the Mouse Cytokine Array Panel A (R&D Systems; ARY006), which enables simultaneous profiling of multiple secreted proteins. For data analysis, the signal intensity was determined using the ImageJ 1.38x software, backgrounds were subtracted using the intensity from negative control spots on the strip. All genotypes were normalized to WT and these ratios were log2-transformed in order to determine relative secretion levels.

Western Blotting

To determine protein expression in our system, we used SDS-PAGE (6%, 8%, and 12% reducing gels, 5% 2-μl mercaptoethanol). Gels were loaded with 20-μg protein per well (Bradford assay). The following antibodies were used: β-actin (1:3,000, Sigma), pAKT (1:473; 193H12, 1:2,000; Cell Signaling Technology), total AKT (40D4, 1:300; Cell Signaling Technology), pSTAT3 (Tyr705; 9145; 1:2,000; Cell Signaling Technology), PCKA (Santa Cruz Biotechnology; 1:6,000), PTEN (612H1; Cascade Bioscience; 1:1,000), total STAT3 (9139; 1:2,000; Cell Signaling Technology), and p16 (M-156; Santa Cruz Biotechnology; 1:1,000; p21 (sc-397; 1:200; Santa Cruz Biotechnology), and p53 (MX25; 1:100; Leica). Enhanced chemiluminescence (ECL) was used with Amersham Hyperfilm ECL (Amersham Bioscience). For data analysis, the signal intensity was determined using the ImageJ 1.38x software.

Histology and IHC Analysis

Tissues were fixed in 10% buffered formalin for 24 hours before transfer to PBS. Paraffin-embedded tissues were sectioned 6 μm thick, placed on charged glass slides, and stained with H&E or the appropriate immunohistochemical stains. Antigen retrieval was performed by incubating the slides in 0.01 mol/L citric acid buffer (pH 6.0) at 95°C for 15 minutes. Slides were then cooled to room temperature for 20 minutes in citric acid buffer. The slides were transferred to PBS (pH 7.4) for 5 minutes after a wash with deionized water. The following detection and visualization procedures were performed according to the manufacturer’s protocol. Slides were counterstained in Mayer’s hematoxylin, dehydrated, cleared, and cover slipped. Negative control slides were stained with secondary antibody only. Antibodies: AR (5153, 1:200), pAKT^S473 (4060; 1:800; Cell Signaling Technology), total AKT (40D4, 1:300; Cell Signaling Technology), MYC (14721-1; 1:50; Cell Signaling Technology), pSTAT3^Tyr705 (9145; 1:800; Cell Signaling Technology), total STAT3 (9139; 1:400; Cell Signaling Technology), Ki67 (1:2,000; Novus), IL6 (1:50; Cell Signaling Technology), PCNA (1:1,000; Cell Signaling Technology), and PHLPP2 (1:200, Bethyl). Pretreated sections were blocked with 5% normal horse serum and 1% BSA (in PBS) for 1 hour at room temperature. Primary antibodies were diluted as suggested by the manufacturer and incubated overnight at 4°C. Following three 10- minute washes with PBS, sections were incubated with biotinylated secondary antibody for 30 minutes at room temperature and rinsed three times with PBS for 10 minutes. Sections were then treated with diaminobenzidine for 5 minutes, rinsed with distilled water, mounted on gelatin-coated slides, air-dried, dehydrated with 70% to 100% alcohol, cleared with xylene, and then cover-slipped for microscopic observation. Stained slides were digitally scanned using Aperio ScanScope software (Vista).

Target Plots for IHC Staining Patterns

“Target plots” were used to quantify and summarize the distance of positively stained cells in a gland from the stroma, using ImageJ. The first circle layer represents epithelial cells along the epithelial border, defined as being within 0 to 25 μm from the stroma/fibromuscular layer. The second layer represents cells that are within 25% of the maximum possible distance of a cell in a particular gland from the border. The third layer represents cells that are within 25% to 50% of this maximum possible distance, and the fifth layer represents cells within 75% to 100% of the maximum possible distance.
IL6 Drives MYC in PTEN-Mutant Prostate Metastasis

In Vitro ELISA

Medium was collected and snap-frozen in liquid nitrogen, and then stored at -20°C until analysis. IL6 concentration was measured using the mouse Quantikine IL-6 ELISA (R&D Systems; ARY006) according to the manufacturer’s protocol. IL6 levels were normalized to total protein concentration from the same well.

In Vivo ELISA

Around 0.8 mL of blood was collected from mouse, and blood samples were left to clot for 2 hours at room temperature. After 2 hours of incubation, samples were centrifuged for 20 minutes at 2,000 × relative centrifugal force at 4°C and serum was delicately removed and aliquoted 200 μL in 1 mL cryovials and was snap-frozen in liquid nitrogen. Samples were stored as serum at -20°C until used. The concentration of IL6 was measured using mouse Quantikine IL-6 ELISA (R&D Systems, ARY006) according to the manufacturer’s protocol.

RT-qPCR Analysis

Total RNA from MEFs: WT, Pten<sup>loxP/loxP</sup>; Il6<sup>+/+</sup> and Pten<sup>loxP/loxP</sup>; Trp5<sup>loxP/loxP</sup> was isolated using the RNeasy Plus Mini Kit (Qiagen). cDNA was reverse transcribed using the SuperScript VILO cDNA Synthesis Kit (Life Technologies), and RT-PCR for the Il6 mRNA was performed using a LightCycler 480 SYBR Green I Master (Roche). The forward primer used for mouse Il6 was 5′-TAGGTGGTCTTTGACCCATTCAC-3′ and the reverse primer was 5′-TTGTTGTCCTTAGGCCACTCCTC-3′. Hprt mRNA was measured as an internal control; the oligos used were: forward primer, 5′-TACTGTCACAGGGGCACTAAA-3′; reverse primer, 5′-GGGCTGTACTGCTTACACCG-3′.

Data Analysis

For multiple comparisons, statistical analyses were carried out on raw data using the one-way ANOVA test (Dunnnett post-test) and P < 0.05 was considered statistically significant. To compare two groups, the Student t test or the Mann–Whitney test was used, and P < 0.05 was considered statistically significant. Values are expressed as the means (SD) unless otherwise stated. Graphs and heatmap analyses were performed using the R software and ggplot2 and heatmap.2 packages (60) on Apple Macintosh computers.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors’ Contributions

Conception and design: D.G. Nowak, H. Cho, S. Senturk, R. Sordella, L.C. Trotman


Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): T. Herza, K. Watrud, D.V. DeMarco, D. Ding, T. Beinortas, D. Kleinman, M. Chen, M. Castillo-Martin, C. Cardon-Cardo, B.D. Robinson

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): D.G. Nowak, V.M.Y. Wang, S. Senturk, D. Kleinman, J.E. Wilkinson, M. Castillo-Martin, C. Cardon-Cardo, B.D. Robinson, L.C. Trotman

Writing, review, and/or revision of the manuscript: D.G. Nowak, V.M.Y. Wang, J.E. Wilkinson, M. Castillo-Martin, C. Cardon-Cardo, B.D. Robinson, L.C. Trotman

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): H. Cho, V.M.Y. Wang, L.C. Trotman

Study supervision: D.G. Nowak, L.C. Trotman

Acknowledgments

The authors thank Mikala Egeblad, Tim Kees, Daniel Ferrante, Chris Vakoc, Junwei Shi, and Jay Bradtner and members of the Trotman laboratory for valuable discussions and help with experimental protocols and reagents; the CSHL Animal Resources team, Lisa Bianco, Lotus Altholtz, Jodi Coblenz, and Michael Cahn for help with animal work; Aigoul Noruyanova, Raisa Puzis, Denise Hoppe, Afsar Barlas, and Katia Manova for help with histopathology procedures; Pamela Moody and Jordan Ratcliff for cell sorting and FACs advice; and Dorothy Tsang for help with the article. The authors are also very thankful for the generous donations from local foundations Long Island Cruisin’ for the Cure and Glen Cove Cares.

Grant Support

L.C. Trotman is a Young Investigator of the Pershing Square Sohn Cancer Research Alliance and a Research Scholar of the American Cancer Society. The authors are grateful for the generous funding of this work through grants to L.C. Trotman from the NIH (CA137050), the Pershing Square Sohn Cancer Research Alliance, the Department of Defense (W81XWH-14-1-0247), the STARR Foundation (18-AB-112), and the Robertson Research Fund of CSHL, and from the NIH to the CSHL Cancer Center through Support Grant 5P50CA045508 for funding of animal shared resources, histology, flow cytometry, and gene sequencing.

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 September 22, 2014; revised March 25, 2015; accepted March 26, 2015; published OnlineFirst March 31, 2015.

REFERENCES


IL6 Drives MYC in PTEN-Mutant Prostate Metastasis


MYC Drives Pten/Trp53-Deficient Proliferation and Metastasis due to IL6 Secretion and AKT Suppression via PHLPP2

Dawid G. Nowak, Hyejin Cho, Tali Herzka, et al.


Updated version
Access the most recent version of this article at:
doi:10.1158/2159-8290.CD-14-1113

Supplementary Material
Access the most recent supplemental material at:
http://cancerdiscovery.aacrjournals.org/content/suppl/2015/04/07/2159-8290.CD-14-1113.DC1

Cited articles
This article cites 56 articles, 22 of which you can access for free at:
http://cancerdiscovery.aacrjournals.org/content/5/6/636.full#ref-list-1

Citing articles
This article has been cited by 8 HighWire-hosted articles. Access the articles at:
http://cancerdiscovery.aacrjournals.org/content/5/6/636.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.