(blebbistatin) or an inhibitor of LIMK, a kinase that acts downstream of ROCK to promote actin assembly, blocked skin thickening and reduced β-catenin levels in K14-ROCK:ER mice. Similarly, the increase in collagen deposition was found to be reduced.

To test whether ROCK activation would impact tumor growth and progression, skin papillomas were induced by two-step chemical tumorigenesis using dimethylbenz[a]anthracene (DMBA) and 12-O-tetradecanoylphorbol-13-acetate (TPA). Through a mechanism involving mutation of HRAS and subsequent MAP-kinase activation, skin papillomas appear and a small proportion progress to invasive carcinomas. When the inducible ROCK:ER mice were used in the two-stage chemical carcinogenesis protocol, induction of ROCK activity increased total papilloma burden and accelerated progression to carcinomas compared with controls. Moreover, total and nuclear β-catenin levels were significantly increased in papillomas in the ROCK:ER mice, indicating that the pathways regulating skin thickening are also important during tumor progression. Interestingly, the authors showed that simultaneous treatment of mouse skin with DMBA/TPA and the ROCK inhibitor Y-27632 resulted in a significantly lower papilloma burden and a lower conversion rate. It will be of great interest to determine the effects of blocking ROCK activity on established papillomas and carcinomas. Importantly, Samuel et al. (2011) provide evidence that ROCK signaling is frequently upregulated in human skin carcinomas. Further work will be required to provide a detailed picture of the levels of ROCK and ROCK signaling to tumor grade and to extend these studies to other tumor types.

Without doubt, Samuel et al. (2011) have provided strong evidence for ROCK-mediated intracellular contractility driving tumorigenesis by affecting ECM deposition, remodeling, and tissue stiffness. Importantly, they show that increased tissue stiffness results from increased collagen deposition and crosslinking following increased actomyosin contractility (Figure 1B). Why there is an increased collagen deposition remains unclear, whereas the mechanism for an increase in levels of β-catenin and its transcriptional activity may depend on increased integrin signaling (Figure 1B). In the experimental system used in this study, elevated actomyosin contractility is generated in the tumor cells themselves; however, other work suggests that tumor-associated cells such as carcinoma-associated fibroblasts can generate actomyosin contractility for extracellular matrix remodeling (Gaggioli et al., 2007). Furthermore, there is evidence that some cancers may harbor mutations that reduce actomyosin contractility (Brognard et al., 2011). Thus, the study of the roles of actomyosin contractility is likely to generate many new insights into tumor biology.

REFERENCES

Brognard, J., Zhang, Y.W., Puto, L.A., and Hunter, T. (2011). Cancer Res. *71*, 3152–3161.

Gaggioli, C., Hooper, S., Hidalgo-Carcedo, C., Grosse, R., Marshall, J.F., Harrington, K., and Sahai, E. (2007). Nat. Cell Biol. *9*, 1392–1400.

Levental, K.R., Yu, H., Kass, L., Lakins, J.N., Egeblad, M., Erler, J.T., Fong, S.F., Csiszar, K., Giaccia, A., Weninger, W., et al. (2009). Cell *139*, 891–906.

Paszek, M.J., Zahir, N., Johnson, K.R., Lakins, J.N., Rozenberg, G.I., Gefen, A., Reinhart-King, C.A., Margulies, S.S., Dembo, M., Boettiger, D., et al. (2005). Cancer Cell *8*, 241–254.

Samuel, M.S., Munro, J., Bryson, S., Forrow, S., Stevenson, D., and Olson, M.F. (2009). Genesis *47*, 440–446.

Samuel, M.S., Lopez, J.I., McGhee, E.J., Croft, D.R., Strachan, D., Timpson, P., Munro, J., Schröder, E., Zhou, J., Brunton, V.G., et al. (2011). Cancer Cell *19*, this issue, 776–791.

Sanz-Moreno, V., and Marshall, C.J. (2010). Curr. Opin. Cell Biol. *22*, 690–696.

Vicente-Manzanares, M., Newell-Litwa, K., Bachir, A.I., Whitmore, L.A., and Horwitz, A.R. (2011). J. Cell Biol. *193*, 381–396.

Turning Reciprocal Feedback Regulation into Combination Therapy

Timothy C. Thompson[1,*]
[1]Department of Genitourinary Medical Oncology–Research, Unit 18-3, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030-4009, USA
*Correspondence: timthomp@mdanderson.org
DOI 10.1016/j.ccr.2011.05.022

Two recent *Cancer Cell* articles report the discovery of reciprocal feedback regulation between androgenic and *PTEN* loss/PI3K-AKT signaling in prostate cancer. Both studies link endocrine regulation with a common oncogenic pathway, which led to the development of a combination therapeutic approach with immediate application in prostate cancer.

The exquisite sensitivity of the prostate gland to androgenic steroids has provided a foothold for the development of systemic prostate cancer therapy for more than seventy years (Huggins and Hodges, 1941). A sustained strategic approach that focused on inhibiting this unique signaling pathway led to the use of androgen-deprivation and antiandrogenic therapies for

advanced prostate cancer. These therapies continue to serve as the standard of care, although, unfortunately, antiandrogenic therapies are not curative; new approaches are needed. With the advent of targeted therapies for cancer, antiandrogenic agents have continued to form the base on which combination therapies—including those that target common oncogenic signaling activities—can be developed. In the case of prostate cancer, this has proved particularly challenging because of the extremely heterogeneous nature of the genetic alterations that underlie this disease.

A prominent molecular target for prostate cancer therapy is the PI3K-AKT signaling pathway. A recent study of 218 prostate cancer tumors showed that 42% of the primary tumors and 100% of the metastases harbored genomic aberrations in that pathway (Taylor et al., 2010). The best-characterized genetic alteration in this pathway is in *PTEN*, which has been shown to be mutated and/or exhibit loss of heterozygosity in approximately 15% of localized prostate cancer and 30% of metastatic disease (Sarker et al., 2009). Multiple small-molecule inhibitors of PI3K-AKT signaling have been developed and tested clinically. Although the results of early clinical trials are inconclusive, the therapeutic activities of PI3K-AKT inhibitors as single agents have generally been modest in patients with advanced prostate cancer. Thus, there is considerable effort to rationally integrate PI3K-AKT inhibitors into combination therapy protocols.

In recent issues of *Cancer Cell*, both Carver et al. (2011) and Mulholland et al. (2011) report on having identified reciprocal feedback regulation between AR and *PTEN* loss/PI3K-AKT signaling in prostate cancer. By making effective use of the PB-*Cre*;*Pten*$^{lox/lox}$ mouse model and carefully annotated human prostate cancer tissue samples, these two groups of investigators have made a seminal contribution to our understanding of the regulation of growth and survival signaling in prostate cancer cells and, by extension, to the rationale for use of specific combination therapy for advanced prostate cancer.

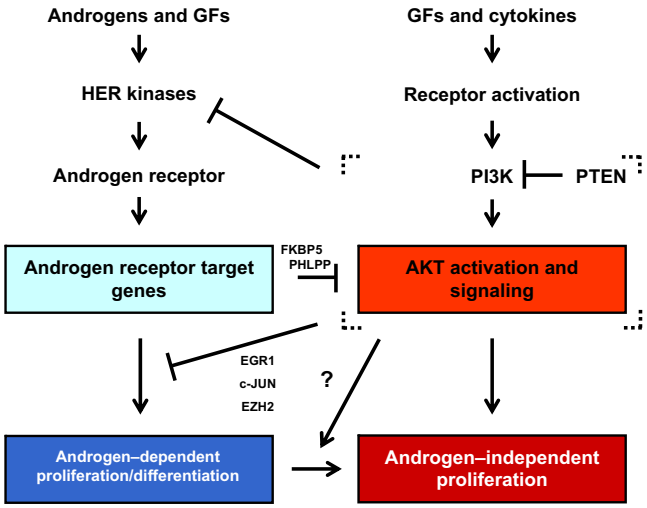

Figure 1. Reciprocal Inhibition Feedback Links AR- and *PTEN* Loss/PI3K-AKT Signaling Pathways in Prostate Cancer

As reported in Carver et al. (2011) and Mulholland et al. (2011), reciprocal negative feedback underlies the oncogenic activities of *PTEN* loss/PI3K-AKT signaling (depicted as the area inside the dotted "corners"). Activation of PI3K-AKT leads to suppression/subversion of AR signaling through suppression of HER kinases; upregulation of EGR1 and c-JUN transcriptional coregulators; and upregulation of the Polycomb group protein EZH2. Reciprocal negative feedback is established, in part, through AR-stimulated, FKBP5-mediated activation of AKT phosphatase PHLPP. GFs, growth factors.

Using similar experimental approaches, Carver et al. (2011) and Mulholland et al. (2011) demonstrated that loss of PTEN function sets into motion a series of molecular events that establish a linkage between two expansive signaling networks that exert control over the growth, survival, and differentiation of prostatic epithelial cells. Activation of PI3K-AKT signaling as a result of *Pten* mutation in the PB-*Cre*;*Pten*$^{lox/lox}$ mouse leads to suppression of AR signaling. Transcriptome analysis revealed substantial overlap of up- and downregulated genes between intact male *Pten*$^{-/-}$ mice and castrated wild-type mice and also demonstrated that *PTEN* loss is associated with reduced AR signaling in *PTEN*-deficient human prostate tumors. These results, together with those of previous studies (Gao et al., 2006; Jiao et al., 2007), show that the loss of PTEN function and activation of PI3K-AKT signaling plant the seeds for androgen-independent prostate cancer growth by establishing a castrate genetic program.

Using both pharmacologic and genetic approaches, Carver et al. (2011) and Mulholland et al. (2011) showed that different mechanisms contribute to the repression of AR output. Carver et al. (2011) demonstrated that PI3K-AKT, but not MEK signaling, is responsible for inhibiting AR signaling, and that this inhibition depends on upstream HER kinase inhibition. Using a PTEN re-expression approach, Mulholland et al. (2011) showed that PTEN loss may suppress androgen-responsive genes through upregulation of Egr1 and c-Jun transcriptional coregulators and the catalytic subunit of Polycomb repressive complex 2, Ezh2. Thus, *PTEN* loss can lead to repression of AR signaling on two levels: upstream suppression of MAPK-stimulated HER kinase, and suppression/subversion of AR-mediated transcription through increased expression of transcriptional coregulators and a histone methyltransferase (Figure 1).

Probing the castration response in PB-*Cre*;*Pten*$^{lox/lox}$ mice, PB-*MYC* mice, and androgen-sensitive prostate cancer cells (Carver et al., 2011) and analyzing a double-knockout mutant, PB-*Cre*;*Pten*$^{lox/lox}$;*Ar*^{lox}/Y, mouse and human prostate cancer samples (Mulholland et al., 2011) led to the second crucial surprising finding—that castration or AR loss increased AKT phosphorylation. An important note is that these two experimental approaches independently led to the identification of a reciprocal negative-feedback signal in the PB-*Cre*;*Pten*$^{lox/lox}$ model and in androgen-sensitive human prostate cancer cell lines; that signal is AR-stimulated, FKBP5-mediated activation of the AKT phosphatase PHLPP, which suppresses AKT activities (Figure 1).

On the basis of their results, both groups hypothesized that prostate cancers in a castrate state (or with low AR levels) have greater dependency on *PTEN* loss/PI3K-AKT signaling. To test this hypothesis in vivo, in scientific synchrony, Carver and colleagues showed that a combination of BEZ235 (a dual PI3K and mTOR inhibitor) and castration resulted in dramatic reductions in tumor volume, in contrast to no effect of single-pathway therapy, in LNCaP

xenografts and near-complete pathologic responses in the PB-*Cre*;*Pten*$^{lox/lox}$ model; Mulholland and colleagues demonstrated that rapamycin (an mTOR inhibitor) treatment of castrated PB-*Cre*;*Pten*$^{lox/lox}$; *Ar*^{lox}/Y mice harboring prostate cancer resulted in significantly reduced proliferation and tumor burden when compared with castration alone.

The reciprocal negative feedback that links the AR and *PTEN* loss/PI3K-AKT signaling networks is intriguing on many levels. The inhibitor studies of Carver et al. (2011) directly link PI3K-AKT signaling with HER kinase inhibition. However, the gene expression analysis of Mulholland et al. (2011) does not exclude PI3K-AKT-independent, PTEN loss-mediated signaling as a mechanism underlying upregulation of EGR1, c-JUN, and EZH2, extending the linkage between the androgenic and *PTEN* loss/PI3K-AKT signaling. It is well established that AR signaling promotes the growth and differentiation of prostate epithelial cells. The precision and coordination involved in androgenic regulation of prostatic growth, morphogenesis, and cytodifferentiation depends to a large extent on AR target gene activities, which are modulated by numerous coregulators (Lamont and Tindall, 2010). A recent article showed that the *TMPRSS2-ERG* gene fusion product can disrupt androgenic signaling in prostate cancer cells through multiple mechanisms, including binding to AR target genes and induction of EZH2 expression, which in turn can suppress prostate cell differentiation (Yu et al., 2010). In addition, under some conditions, PI3K-AKT signaling can enhance AR activities and induce AR target genes, such as *p21*$^{WAF/CIP}$, which is associated with androgen-independent growth of prostate cancer (Lu et al., 2006). In light of the new knowledge about this mechanistic framework that has resulted from the discovery of reciprocal negative feedback linking the AR and PI3K-AKT signaling networks, it may be possible to better characterize and delineate additional signaling pathways and identify additional transcriptional coregulators and chromatin modifiers that underlie specific AR target gene functions related to androgen-dependent prostatic growth and/or differentiation and to androgen-independent growth in prostate cancer.

The inexorable process of selection through which cancer cells develop resistance to all types of anticancer agents presents research and clinical oncologists with a daunting task. Through their discovery of important reciprocal negative feedback involving AR and *PTEN* loss/PI3K-AKT signaling in prostate cancer, Carver et al. (2011) and Mulholland et al. (2011) have not only set the stage for rapid clinical testing of combination therapies aimed at these two signaling networks, but they have also unmasked the potential for future genetic and pharmacologic approaches to understanding AR target gene functions and for identifying new targets for prostate cancer therapy.

ACKNOWLEDGMENTS

The author thanks Karen F. Phillips, ELS, for her expert editorial assistance.

REFERENCES

Carver, B.S., Chapinski, C., Wongvipat, J., Hieronymus, H., Chen, Y., Chandarlapaty, S., Arora, V.K., Le, C., Koutcher, J., Scher, H., et al. (2011). Cancer Cell *19*, 575–586.

Gao, H., Ouyang, X., Banach-Petrosky, W.A., Shen, M.M., and Abate-Shen, C. (2006). Cancer Res. *66*, 7929–7933.

Huggins, C., and Hodges, C.V. (1941). Cancer Res. *1*, 293–297.

Jiao, J., Wang, S., Qiao, R., Vivanco, I., Watson, P.A., Sawyers, C.L., and Wu, H. (2007). Cancer Res. *67*, 6083–6091.

Lamont, K.R., and Tindall, D.J. (2010). Adv. Cancer Res. *107*, 137–162.

Lu, S., Ren, C., Liu, Y., and Epner, D.E. (2006). Int. J. Oncol. *28*, 245–251.

Mulholland, D.J., Tran, L.M., Li, Y., Cai, H., Morim, A., Wang, S., Plaisier, S., Garraway, I.P., Huang, J., Graeber, T.G., and Wu, H. (2011). Cancer Cell *19*, this issue, 792–804.

Sarker, D., Reid, A.H.M., Yap, T.A., and de Bono, J.S. (2009). Clin. Cancer Res. *15*, 4799–4805.

Taylor, B.S., Schultz, N., Hieronymus, H., Gopalan, A., Xiao, Y., Carver, B.S., Arora, V.K., Kaushik, P., Cerami, E., Reva, B., et al. (2010). Cancer Cell *18*, 11–22.

Yu, J., Yu, J., Mani, R.-S., Cao, Q., Brenner, C.J., Cao, X., Wang, X., Wu, L., Li, J., Hu, M., et al. (2010). Cancer Cell *17*, 443–454.