C. (2009). Cancer Cell *15*, this issue, 255–269.

Takahashi, C., Bronson, R.T., Socolovsky, M., Contreras, B., Lee, K.Y., Jacks, T., Noda, M., Kucherlapati, R., and Ewen, M.E. (2003). Mol. Cell. Biol. *23*, 5256–5268.

Takahashi, C., Contreras, B., Bronson, R.T., Loda, M., and Ewen, M.E. (2004). Mol. Cell. Biol. *24*, 10406–10415.

Takahashi, C., Contreras, B., Iwanaga, T., Takegami, Y., Bakker, A., Bronson, R.T., Noda, M., Loda, M., Hunt, J.L., and Ewen, M.E. (2006). Nat. Genet. *38*, 118–123.

Zhang, Z., Wang, Y., Vikis, H.G., Johnson, L., Liu, G., Li, J., Anderson, M.W., Sills, R.C., Hong, H.L., Devereux, T.R., et al. (2001). Nat. Genet. *29*, 25–33.

Unraveling the Complexities of Androgen Receptor Signaling in Prostate Cancer Cells

Hannelore V. Heemers[1] **and Donald J. Tindall**[1,*]
[1]Department of Urology Research, Mayo Clinic, Rochester, MN 55905, USA
*Correspondence: tindall.donald@mayo.edu
DOI 10.1016/j.ccr.2009.03.009

Androgen signaling is critical for proliferation of prostate cancer cells but cannot be fully inhibited by current androgen deprivation therapies. A study by Xu et al. in this issue of *Cancer Cell* provides insights into the complexities of androgen signaling in prostate cancer and suggests avenues to target a subset of androgen-sensitive genes.

Prostate cancer (PCa) is the most frequently diagnosed cancer and the second leading cause of cancer-related death in Western men. Since the recognition of PCa as an androgen-sensitive disease in 1941, androgen deprivation strategies have been the principal treatment option for non-organ-confined PCa or PCa that recurs after initial surgery or radiation therapy. Androgen deprivation therapies (ADTs) target the action of the androgen receptor (AR), the transcription factor mediating the cellular effects of androgens, by reducing the circulating levels of its natural ligands and/or by administration of antiandrogens that compete for binding to the AR. Following initial remission, most PCas recur after ADT, giving rise to castration-recurrent PCa (CRPC), which is almost invariably lethal. Evidence from basic research and clinical studies indicates that the AR and AR-dependent transcriptional program remain activated in CRPC (Chen et al., 2008; Debes and Tindall, 2004; Mohler, 2008). This unexpected reactivation of the AR in CRPC, which highlights its validity as a therapeutic target also in CRPC, has been attributed to AR amplifications, gain-of-function mutations of the AR, and changes in the activity of regulators and signal transduction pathways that modulate AR activity. More recently, findings of local, intratumoral production of androgens in CRPC at levels sufficient to activate the AR have led to therapeutic approaches targeting in situ androgen biosynthesis (Attard et al., 2008; Mohler, 2008). While initial results from such clinical trials are encouraging, they also underscore the continued reliance of PCa on AR activation and the resourcefulness of PCa cells in evading ADT strategies. In-depth knowledge of the molecular mechanisms by which the AR regulates transcription of target genes that are critical to PCa disease may offer the rationale to design therapeutic alternatives targeting this critical regulator of PCa cell growth.

The AR is a member of the nuclear receptor superfamily of ligand-activated transcription factors. Its mechanism of action resembles that of other members of the steroid hormone receptor family. In the classical model of AR action, binding of androgens causes an inactive cytoplasmic AR to undergo a change in conformation, homodimerization, and relocation to the nucleus. There, the activated AR binds to specific recognition sequences known as androgen-responsive elements (AREs) located in or near androgen-regulated genes where it recruits the coregulators and the basal transcriptional machinery necessary to assemble a productive transcriptional complex and ultimately affect the transcription of target genes (Heemers and Tindall, 2007).

Over a decade of screening has identified approximately 200 proteins that interact with the AR and collaborate with it to execute its transcriptional program. At the same time, systems and bioinformatics approaches have identified hundreds of androgen-regulated genes and characterized genome-wide AR recruitment sites in PCa cells. The combined knowledge gained from these studies is starting to reveal a picture of daunting complexity underlying the activity of the AR transcriptional complex that far exceeds the simplicity of traditional models of androgen action.

Apart from general transcription factors, proteins recruited to DNA-bound AR can be divided into two classes: coregulators and specific transcription factors that interact with their consensus binding elements (Heemers and Tindall, 2007). The former class consists of proteins that associate either directly or indirectly with

the AR to enhance (coactivators) or repress (corepressors) its transcriptional activity without themselves necessarily binding to DNA. Recent efforts at cataloguing these regulators reveal a large number of functionally diverse proteins involved in a multitude of cellular processes and pathways, which take place in different cellular compartments. These classifications offer a first glance at the intricate level of protein-protein interaction associated with generating the appropriate AR transcriptional output (Heemers and Tindall, 2007). A significant proportion of AR-associated coregulators possess enzymatic properties that are able to modulate the acetylation, phosphorylation, methylation, ubiquitination, and SUMOylation status of the local histone environment, as well as that of the AR itself, several components of the general transcriptional machinery and cooperating coregulators (Heemers and Tindall, 2007). Such modifications have significant consequences for protein-protein interactions as well as protein localization, stability, and turnover and greatly affect the ability of the local DNA environment to support active transcription. Adding yet another means of control over the activity of the AR transcriptional complex, androgens, acting through the AR, regulate the expression levels of over a third of the coregulators in PCa cells. The kinetics and molecular mechanisms by which androgens exert their effects on coregulator expression are remarkably different (Heemers et al., 2009). In addition, a striking level of AR target gene specificity is observed among coregulators (Heemers et al., 2009). These observations suggest that temporal and quantitative changes in the composition of coregulator complexes exist at AR binding sites of different target genes.

Additional experimental evidence from tiling array studies mapping genome-wide AR recruitment (reviewed in Heemers and Tindall, 2007; Jia et al., 2008) supports the concept of a heavily context-dependent composition of the AR transcriptional complex at target genes. Characterization of the genomic composition of AR binding loci shows that they are selectively enriched in binding sites for several specific transcription factors and differ widely in the localization, composition, and clustering of AREs. Some AR binding sites appear even completely devoid of consensus motifs (5′-TGTTCT-3′-like) that are generally accepted to make up an ARE, suggesting that secondary transcription factors can recruit the AR as cofactor to convey androgen responsiveness over gene expression. The presence of these transcription factors at AR binding sites is critical for recruitment of the AR and RNA polymerase II, affects the reciprocal occupancy of these sites by other transacting factors, and is necessary for full and timely androgen responsiveness of the genes in question.

Overall, these observations support the concept of a selective and dynamic interplay between the AR, its associated coregulators, and specific transcription factors at regulatory sites in target genes. More importantly, they suggest that a better understanding of the events modulating the posttranslational modification status and expression levels of these regulators may offer novel approaches to more efficiently target AR action for PCa therapy.

The study by Xu et al. (2009) in this issue of *Cancer Cell* provides further evidence to support this hypothesis. The authors identify RNF6, a ubiquitin E3 ligase, as an AR-interacting protein and show that RNF6 induces ubiquitination of the AR. RNF6-dependent AR ubiquitination is enhanced by androgen treatment, promotes AR transcriptional activity, and selectively drives androgen-dependent expression of a subset of AR target genes. Key to these events is that RNF6 induces a K6/K27 polyubiquitination pattern at AR residues K845 and K847. This topology and branching pattern of ubiquitin chains is markedly different from other mono- or polyubiquitination modifications that have been described for the AR (Heemers and Tindall, 2007) and does not decrease AR stability. Instead, RNF6-dependent posttranslational modification of the AR appears to serve as a platform to selectively recruit coactivators such as ARA54 to AREs in the regulatory regions of a subset of AR target genes (Figure 1). Importantly, RNF6 is overexpressed in CRPC, and both RNF6 and K845 AR ubiquitination are critical for PCa growth under androgen-deprived conditions, indicating that targeting components of the ubiquitination machinery may hold promise for the treatment of CRPC. From a molecular perspective, the study by Xu et al. thus highlights the possibility that elucidation

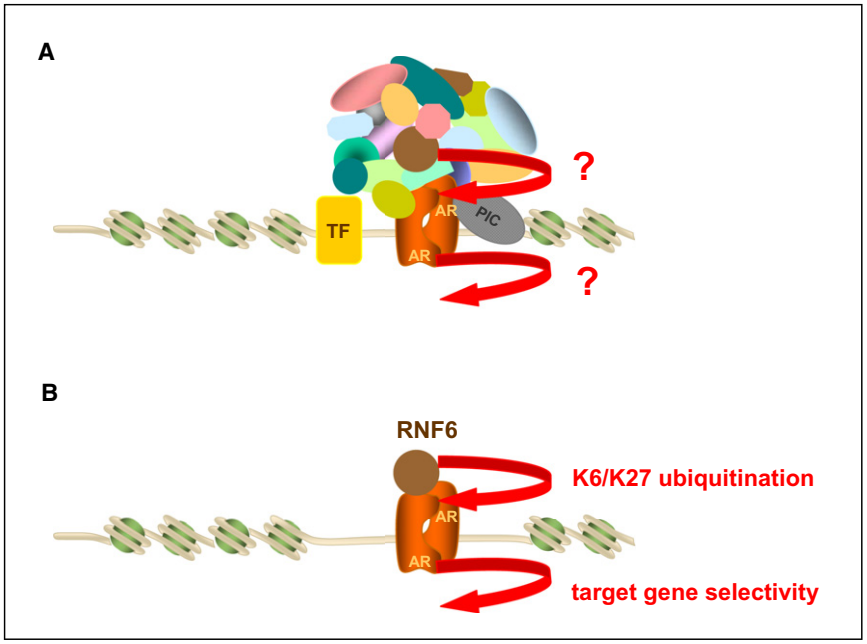

Figure 1. RNF6-Dependent Ubiquitination of the Androgen Receptor Selectively Regulates the Expression of a Subset of Target Genes

(A) Transcriptional activity of the androgen receptor (AR) at regulatory regions in target genes is mediated by a dynamic interplay between a large cohort of coregulators that function in multiple cellular pathways and specific transcription factors (TF) interacting with their cognate binding sites. PIC, preinitiation complex. (B) A new study by Xu et al. (2009) provides evidence that a K6/K27 ubiquitination of the AR by the ubiquitin E3 ligase RNF6 selectively regulates the expression of a subset of AR target genes.

of the composition of the AR transcriptional complex, and particularly unraveling of the ''modification codes'' of its critical components (e.g., Wu et al., 2004), may serve as a platform for rational design of PCa therapies.

REFERENCES

Attard, G., Reid, A.H., Yap, T.A., Raynaud, F., Dowsett, M., Settatree, S., Barrett, M., Parker, C., Martins, V., Folkerd, E., et al. (2008). J. Clin. Oncol. *26*, 4563–4571.

Chen, Y., Sawyers, C.L., and Scher, H.I. (2008). Curr. Opin. Pharmacol. *8*, 440–448.

Debes, J.D., and Tindall, D.J. (2004). N. Engl. J. Med. *351*, 1488–1490.

Heemers, H.V., and Tindall, D.J. (2007). Endocr. Rev. *28*, 778–808.

Heemers, H.V., Regan, K.M., Schmidt, L.J., Anderson, S.K., Ballman, K.V., and Tindall, D.J. (2009). Mol. Endocrinol. Published online January 22, 2009. 10.1210/me.2008-0363.

Jia, L., Berman, B.P., Jariwala, U., Yan, X., Cogan, J.P., Walters, A., Chen, T., Buchanan, G., Frenkel, B., and Coetzee, G.A. (2008). PLoS ONE *3*, e3645.

Mohler, J.L. (2008). Adv. Exp. Med. Biol. *617*, 223–234.

Wu, R.C., Qin, J., Yi, P., Wong, J., Tsai, S.Y., Tsai, M.J., and O'Malley, B.W. (2004). Mol. Cell *15*, 937–949.

Xu, K., Shimelis, H., Linn, D.E., Jiang, R., Yang, X., Sun, F., Guo, Z., Chen, H., Li, W., Chen, H., et al. (2009). Cancer Cell *15*, this issue, 270–282.

Glioma Stem Cells: Not All Created Equal

Howard A. Fine[1,*]
[1]Center for Cancer Research, Neuro-Oncology Branch, National Cancer Institute, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD 20892, USA
*Correspondence: hfine@mail.nih.gov
DOI 10.1016/j.ccr.2009.03.010

A growing body of evidence suggests that only a small subpopulation of malignant glioma cells have true tumorigenic potential. A study by Peñuelas et al. in this issue of *Cancer Cell* demonstrates that TGF-β can stimulate self-renewal and inhibit differentiation in a proportion of these glioma-initiating cells.

Primary central nervous system (CNS) malignancies represent an outstanding model system to explore the cancer stem cell hypothesis in that tumor- and glioblastoma-initiating/stem cells (GICs/GSCs) have been readily identified in both medulloblastomas and glioblastomas (GBMs) (Hemmati et al., 2003; Singh et al., 2004) and more is known about the normal development of embryonic and adult normal neural stem cells (NSCs) than any other tissue-specific stem cell except hematopoietic stem cells. In fact, GICs/GSCs have been shown to have significant similarities to NSCs through their ability to self-renew, expression of similar transcriptome profiles, and capability of differentiating along both glial and neuronal lines (Lee et al., 2006). Comparing the cellular, molecular, genetic, and epigenetic mechanisms that underlie basic stem cell properties such as self-renewal and differentiation should further elucidate the similarities and differences between NSCs and GICs/GSCs.

With this as a backdrop, Peñuelas et al. (2009) report in this issue of *Cancer Cell* on the effects of transforming growth factor beta (TGF-β) on GICs/GSCs. TGF-β has long been considered a potentially promising therapeutic target in malignant gliomas, as much through guilt by association as because of clear experimental and/or clinical evidence (Massagué, 2008). The overexpression of TGF-β commonly seen in malignant glioma has been variously implicated in glioma cell proliferation, migration, decreased apoptosis, and/or tumor-specific immunosuppression. In a new twist, Peñuelas and coworkers demonstrate that TGF-β induces GIC/GSC self-renewal in vitro and enhanced tumorigenicity in vivo. TGF-β mediates this activity through activation of and subsequent binding of a Smad2/3/4 complex to the promoter region of the leukemia inhibitory factor (*LIF*) gene. LIF then activates the JAK-STAT pathway, as demonstrated by phosphorylation of STAT3, leading to increased GIC/GSC tumorigenesis secondary to their increased self-renewal and decreased differentiation. Peñuelas et al. find that although LIF enhances NSC self-renewal and partially inhibits differentiation, TGF-β in contrast does not induce LIF expression and thus has no discernable effect on NSCs.

So does the different response of GICs/GSCs versus NSCs to TGF-β represent a corrupted TGF-β signaling pathway, as is often seen in other epithelial cancers? Although TGF-β signals through the Smad2/3/4 complex in both the GICs/GSCs and NSCs evaluated by Peñuelas et al., it is known that various cofactors associated with the Smad complex result in differential gene expression and repression in a cell context-dependent manner. It is therefore plausible that a different set of tumor-related Smad complex-associated cofactors accounts for the differential effects of TGF-β induction on the *LIF* promoter in GICs/GSCs compared to NSCs.

Alternately, it is possible that this TGF-β-mediated pro-self-renewal phenotype may not be tumor specific but may rather be representative of normal signaling within a particular NSC subtype not utilized in the Peñuelas et al. study. It is well known that the effects of TGF-β and TGF-β family members (BMPs, activins, inhibins) on CNS development are cell intrinsic, cell extrinsic, and context dependent. For example, TGF-β inhibits Wnt