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RESEARCH HIGHLIGHT

The ubiquitin ligase Siah2 is revealed as an accomplice of the androgen receptor in castration resistant prostate cancer

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The androgen receptor (AR) remains the primary molecular target for prostate cancer (PCa) treatment and for development of novel therapies. Profiling and other analyses of human prostate adenocarcinoma have shown that the AR is still functional during late-stage disease in the absence of circulating hormone following castration therapy. The molecular mechanisms that operate during this 'castration resistant' phase are still not well understood. Qi *et al.* have now implicated the ubiquitin ligase Siah2 as an important mediator of AR action in castration resistant prostate cancer (CRPC). Siah2 was found to target repressed AR chromatin complexes for degradation, resulting in activation of AR-regulated genes involved in tumor cell proliferation, cell motility and lipid metabolism. The authors show a requirement for Siah2 activity for PCa cell growth under conditions of low androgen, and also that targeting Siah2 results in tumor growth suppression under castrate conditions. These findings identify a new mechanism of AR regulation in progressing disease as well as a novel enzymatic target for therapeutic intervention.

The AR is a well-studied hormone receptor that belongs to the large nuclear receptor gene superfamily. AR is activated by androgen binding (principally 5α-dihydrotestosterone, 5α-DHT), which elicits changes in AR-resident cytosolic complexes, translocation of the receptor to cell nuclei, formation of multiprotein transcriptional complexes on chromatin, and activation or repression of gene expression. Experiments with *in vivo* model systems have provided evidence that virtually all physiologic processes affected by androgens require the AR as a mediator of molecular effects at the gene level.[1] AR is widely expressed beyond the reproductive systems and is believed to play important roles in several non-reproductive tissues, including muscle and brain. The fact that PCa growth is initially dependent on the presence of androgens in the circulation, and that prostate tumors will regress temporarily with castration, has been recognized for over half a century.

Molecular cloning of the AR in the 1980s was initially felt to be a dispositive step toward pharmacological interventions that would greatly improve treatment outcomes for PCa. However, despite many advances since that time, there is currently no effective therapy for disease that has become unresponsive to treatment with hormone ablation. The 'hormone refractory' phase of the disease is intriguing from a biochemical perspective because the AR still appears to play a critical role under conditions where androgen concentrations in the blood are extremely low. RNA and gene profiling of many human PCa tumors has shown that hormone suppression provides a strong selection pressure that results in overexpression and/or amplification of the AR during metastatic dissemination.[2] Molecular and biochemical studies of the AR have revealed a bewildering level of complexity involving over 150 protein partners. The AR is also post-translationally modified by phosphorylation, sumoylation and acetylation, and AR expression can be controlled at transcriptional and post-transcriptional levels, adding additional layers of regulatory complexity.

One important locus of AR activity lies in the ubiquitin/proteasome pathway, which controls the specificity and rate of protein degradation. AR stability was shown previously to be regulated by ubiquitin ligases,[3] proteins that form complexes with ubiquitin-conjugating enzymes to catalyze attachment of the small protein ubiquitin to lysines on a protein target, thereby directing the modified protein to the proteasome for degradation. Siah1 and Siah2 are RING finger E3 ubiquitin ligases that regulate ubiquitination-mediated degradation of a range of signaling proteins, resulting in diverse biological effects such as resistance to apoptosis and effects on mitochondrial function. Qi *et al.*[4] previously showed that knockout of the Siah2 gene in the TRAMP transgenic mouse model of PCa, a system that rapidly produces aggressive autochthonous prostate tumors that progress to metastasis, resulted in suppression of tumor formation. In a recent paper in *Cancer Cell*,[5] the same group has now gone on to uncover the mechanism of this surprising effect.

Further experiments in TRAMP mice indicated that loss of Siah2 decreased prostate size, an indication of a loss of AR signaling, and also increased the sensitivity to castration, suggesting the possibility that Siah2 may operate under low androgen conditions. Knockdown of Siah2 in PCa cell lines indicated that Siah2 controls a subset of AR-regulated genes, including the gene encoding the important clinical biomarker, prostate-specific antigen. Global transcriptional profiling identified a Siah2-regulated gene network consisting of almost 1000 genes, about 100 of which were found to be AR regulated. The AR- and Siah2-dependent genes were mostly associated with lipid, sterol and cholesterol metabolism. Analysis of human PCa profiling

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data indicated that a large fraction of this subnetwork, as well as Siah2 itself, is upregulated in CRPCs.

Biochemical studies demonstrated that Siah2 binds AR directly and acts as an E3 ubiquitin ligase for the receptor. Notably, AR targeting occurs largely at chromatin sites in which AR is bound to the transcriptional corepressor NCOR1. The selective degradation of these repressed AR complexes results in their replacement with AR bound to coactivators, resulting in up-regulation of the AR- and Siah2-dependent genes. Thus, Siah2 mediates reconditioning of chromatin regions that govern AR-dependent transcription in a manner that turns up the 'volume' of gene expression controlling androgen and lipid metabolism. Biological studies also showed that the Siah2/AR network of genes is directly involved in characteristic malignant behaviors such as anchorage-independent growth, cell motility, and growth of castration-resistant prostate tumors.

Translation to the clinic of these new insights into how AR is regulated will require further analysis of the mechanisms of formation and selective degradation of transcriptional complexes, with particular attention paid to the relationship to other AR co-regulators, including other ubiquitin ligases. The findings of Qi *et al.*[5] contribute to a growing body of literature that the spatiotemporal regulation of AR, the tumor microenvironment, and the genetic and epigenetic landscape of the tumor cell all contribute significantly to the extent to which AR drives the disease in the CRPC phase.[6]

Most PCa cells seem to carry along with them the intrinsic dependence on the AR that constrained their normal progenitors. Experiments with model systems have suggested that total ablation of the AR might cure PCa, even CRPC, in some patients. Challenges with emulating experimental conditions in the clinic arise from the pharmacological and technical limitations of our present arsenal of agents that target the AR, the vagaries of human physiology, and the ability of tumors to evolve under selective pressure. But evidence suggests that if the AR could be definitively inhibited in a sustained manner, dramatic clinical effects against CRPC might be achieved. The exciting results described by Qi and colleagues have uncovered a new molecular target that acts as an essential AR companion in the lethal phase of disease progression. Clever minds will now turn to this critical new player in the incurable condition of CRPC and seek to decipher what its own vulnerabilities might be.

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