H3K9 methylation drives resistance to androgen receptor–antagonist therapy in prostate cancer

Mehdi Baratchiana, Ritika Tiwaria, Sirvan Khalighib, *et al.*

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(1) 簡述論文的重大發現

晚期的攝護腺癌主要是以 androgen-deprivation therapy 治療，但治療一段時間後，攝護腺癌細胞會對 androgen-deprivation therapy 治療有抗性。雖然後續還有藥物可以使用（如 enzalutamide），一旦進展為 castration-resistant prostate cancer (CRPC)，預後便會隨之變差。

研究團隊為找出攝護腺癌對 androgen-deprivation therapy(本篇用 enzalutamide)有抗性的原因，使用 Validation-based insertional muta- genesis (VBIM) screening 抗性與 repeat elements 的 epigenetic modification 有關。然後進一步的實驗發現，在 androgen-deprivation therapy 後，本來會受 enzalutamide active 的 repeat elements，攝護腺癌細胞會增加 H3K9me3 的合成，從而抑制 repeat elements 對癌細胞抑制，而對 enzalutamide 有抗性。

(2) 對論文內容的疑問

本研究證實在 in vitro, in vivo 的有些實驗結果有出入，只有題出假設的原因，未有對假設的原因作驗證。

(3) 論文的缺點與評論

控制攝護腺癌最主要的階段是還在原位的時候，延緩轉移出去的時間，所以新藥的使用時間也是一直被提前（如 enzalutamide），雖然現階段還是使用在已轉移的攝護腺癌。本篇研究的細胞用轉移到淋巴結的攝護腺癌細胞，基因為表現是否跟還在原位的攝護腺癌細胞相同？如果研究團隊後續使用還在原位的攝護腺癌細胞，研究在中期的時候，如何對 androgen-deprivation therapy 產生抗性，可對攝護腺癌的治療更有幫助。

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H3K9 methylation drives resistance to androgen receptor–antagonist therapy in prostate cancer

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Antiandrogen strategies remain the prostate cancer treatment backbone, but drug resistance develops. We show that androgen blockade in prostate cancer leads to derepression of retroelements (REs) followed by a double-stranded RNA (dsRNA)-stimulated interferon response that blocks tumor growth. A forward genetic approach identified H3K9 trimethylation (H3K9me3) as an essential epigenetic adaptation to antiandrogens, which enabled transcriptional silencing of REs that otherwise stimulate interferon signaling and glucocorticoid receptor expression. Elevated expression of terminal H3K9me3 writers was associated with poor patient hormonal therapy outcomes. Forced expression of H3K9me3 writers conferred resistance, whereas inhibiting H3K9-trimethylation writers and readers restored RE expression, blocking antiandrogen resistance. Our work reveals a drug resistance axis that integrates multiple cellular signaling elements and identifies potential pharmacologic vulnerabilities.

prostate cancer | hormonal therapy | androgens | enzalutamide | epigenetics

Significance

This study reveals that antiandrogen therapy induces viral mimicry responses that are crucial for antitumor activity. H3K9 trimethylation to silence endogenous repeat elements is essential for regaining heterochromatin stability and progression to antiandrogen resistance in prostate cancer. We found that the H3K9 trimethylation machinery is linked to poor outcomes in men with prostate cancer. Blockade of this epigenetic axis can resensitize drug-resistant tumors and elicit cytotoxic interferon responses. Antiandrogen timing and regulation of the H3K9 methylation–endogenous repeat elements– interferon axis should be considered in the development of novel epigenetic therapies and immunotherapeutic strategies for prostate cancer.

Targeting androgen receptor (AR) signaling constitutes the backbone of treatment strategies for advanced prostate cancer (1). Despite the initial response to androgen-deprivation therapy (ADT), achieved by medical or surgical castration, patients eventually progress to castration-resistant prostate cancer (CRPC), frequently through mechanisms reinstating AR activity (2, 3). Next-generation antiandrogens such as the androgen synthesis inhibitor, abiraterone, or the potent AR blocker, enzalutamide (Enz), have significantly improved the overall survival of CRPC patients; however, resistance to these agents eventually occurs and leads to lethality (4–6). Among various mechanisms, elevated levels of glucocorticoid receptor (GR) compensates for the reduced AR-signaling and confers resistance to antiandrogen therapies. In order to bypass androgen blockade, GR is thought to partially take over the transcriptional landscape of AR and regulate gene expression to promote tumor progression (7).

Unbiased genetic and chemical screening approaches have the potential to identify unexpected underlying mechanisms of drug resistance and can enable the discovery of clinically targetable drug-resistance pathways (8, 9). Validation-based insertional mutagenesis (VBIM) is one such genetic screening tool, which, by use of lentiviral vectors (LVs), inserts a cytomegalovirus (CMV) promoter in random genomic locations in a population of cells, generating libraries of millions of cells, each with a unique integration site (10). By isolating cells harboring a mutant phenotype and identifying the VBIM insertion sites, novel genes can be linked with the phenotype of interest. Here, we applied this rigorous screening strategy to identify mechanisms of Enz resistance and found that epigenetic modification of repeat elements (REs) is important for the progression to antiandrogen resistance in CRPC.

Transcriptional silencing of REs in normal somatic cells is achieved through DNA methylation (5-methylcytosine) and repressive histone marks (methylation of H3K9 and H4K20 residues) that are enriched in constitutive heterochromatin (11). Among these, H3K9me1 and H3K9me2 are dynamically regulated by H3K9 dimethyltransferases, euchromatic histone-lysine *N*-methyltransferase 1 and 2 (EHMT1 and EHMT2). Mono- and dimethylation of the H3K9 also helps in the deposition of the DNA methylation marks at the CpG islands, further aiding the gene silencing (12). H3K9me3 modifications are catalyzed by histone methyltrasferases SUV39H1, SUV39H2, and SETDB1. Aberrant RE regulation leads to their transcriptional activation and formation of dsRNAs that elicit IFN-mediated viral mimicry responses. Tumor cells employ endogenous or acquired strategies to evade treatment-induced viral mimicry states that cause immune responses or undermine their genomic stability and fitness (13). A variety of these repeat elements are differentially expressed in prostate cancer (14, 15); however, there are no previous studies to support the notion of RE perturbation in

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