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journal or publication title	Oncolmmunology
volume	2
number	12
page range	1-3
year	2013-10-01
URL	http://hdl.handle.net/2297/37576

doi: 10.4161/onci.26853

Targeting inflammatory cytokines-androgen receptor (AR) signaling with ASC-J9[®] to better battle prostate cancer progression

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Abstract

Macrophages inflammatory cytokines/chemokines in prostate cancer (PCa) microenvironment may go through androgen receptor (AR) signaling to influence PCa progression: macrophages induce tumorigenesis by the alteration of AR-CCL4 signaling and can be interrupted by AR-degradation enhancer ASC-J9[®]. Androgen deprivation therapy with anti-androgens enhances CCL2-pSTAT3 signaling to promote metastasis and ASC-J9[®] can inhibit CCL2-pSTAT3 signaling to suppress PCa metastasis. Targeting inflammatory cytokines-AR signaling with ASC-J9[®] may become a promising therapy to battle PCa in future.

Introduction

Prostate cancer (PCa) is the most frequently diagnosed cancer in men and the second leading cause of cancer death in the United States (1). Although androgen deprivation therapy (ADT) is useful for advanced PCa, its effects are limited because PCa changes to a castration-resistant phenotype over 1-2 years of therapy. Early studies demonstrated that ADT with anti-androgens to prevent/suppress androgens binding to AR in whole body promoted the development of invasive PCa, suggesting therapeutic suppressing androgens binding to AR may elicit unwanted signals that may favor the progression of surviving PCa cells to the advanced stage (2). In another study, targeting AR signaling led to suppress the wound-healing process by modulating macrophage infiltration with alterations of cytokine expression profiles (3). Since gene signatures of wound healing responses are similar to genes identified in the progressive breast cancer with high metastatic potential (4), we studied the potential linkage of AR signaling and inflammatory responses from infiltrating macrophages and their impact on the PCa progression in this review.

Infiltrating macrophages promote prostate tumorigenesis

Infiltrating macrophages are a key component of inflammation during prostate tumorigenesis. Fang et al first demonstrated that co-culturing of immortalized prostate epithelial cells with macrophages induces prostate tumorigenesis (5). Clinical sample surveys confirmed the number of macrophages was significantly increased in high-grade prostatic intraepithelial neoplasia (PIN) or PCa lesions compared with those in benign prostate on human prostate tissue. Macrophages-induced prostate tumorigenesis involved the alteration of signaling of macrophage AR-inflammatory chemokine CCL4–STAT3 activation as well as epithelial-to-mesenchymal transition (EMT) and down-regulation of p53/PTEN tumor suppressors. PTEN^{+/-} mice lacking macrophage AR developed far fewer PIN lesions. CCL4-neutralizing antibody effectively blocked

macrophage-induced prostate tumorigenic signaling and targeting AR *via* a newly identified AR-degradation enhancer ASC-J9[®] reduced CCL4 expression and PCa tumor growth in xenografted mouse model. Importantly, CCL4 up-regulation was associated with increased snail expression and down-regulation of p53/PTEN in high-grade PIN and PCa.

Together, these results demonstrated that AR-CCL4 signaling in the prostate tumor microenvironment might become the potential therapeutic targets to effectively battle the inflammation-associated PCa initiation.

Suppression of AR *via* AR-siRNA induces CCL2 that leads to promote PCa metastasis

We next tested our hypothesis that suppressing AR function *via* AR-siRNA in PCa might simultaneously trigger undesirable inflammatory signals that could enhance the macrophage infiltration to stimulate progression of PCa (6). Targeting AR with AR-siRNA in macrophage THP-1 cells enhances the migration of macrophages towards PCa. Similarly, suppression PCa AR with AR-siRNA also enhances the recruitment of macrophage THP-1 cells. Mechanism dissection revealed that AR silencing *via* AR-siRNA in either macrophages or PCa cells induces the expression of CCL2 and CCL2-dependent STAT3 activation that may then enhance the EMT signaling to promote the PCa cell invasion. Pharmacologic interruption of the CCL2/CCR2-STAT3 signaling suppresses the EMT and PCa cell invasion, suggesting a newly identified signaling from CCL2/CCR2 to pSTAT3 to EMT may play key roles to promote the PCa cell invasion.

Knocking out macrophage AR in TRAMP PCa mouse model leads to promote the PCa metastasis *via* induction of CCL2 and macrophage infiltration. Combined targeting of PCa AR and anti-CCL2/CCR2 signaling results in better suppression of PCa growth with reduced metastasis than targeting PCa AR alone in a xenografted PCa mouse model.

Human PCa tissue microarray analysis also reveals that PCa patients' outcome may become much worse when their PCa were staining CCL2-positive as compared to those PCa patients with

CCL2-negative, suggesting that CCL2 may play key role in promotion the PCa progression.

Together, these results may provide a novel therapeutic approach to better battle PCa progression and metastasis at the castration resistant stage *via* the combination of targeting AR with AR-siRNA and anti-CCL2/CCR2-STAT3 signaling.

ADT with anti-androgens enhances PCa metastasis *via* enhanced the macrophage infiltration and STAT3-CCL2 signaling

Previous meta-analysis demonstrated that ADT using luteinizing hormone-releasing hormone (LH-RH) agonist plus anti-androgens improves overall survival rate of PCa patients compared with ADT using LH-RH agonist mono-therapy (7). Interesting, using Bicalutamide (Casodex), a currently used non-steroidal anti-androgen and/or Enzalutamide, formerly called MDV3100, a newly developed more powerful anti-androgen with better efficacy to suppress PCa at the castration-resistant stage (8), we found they could promote PCa cell invasion *via* increase the macrophage migration to PCa cells (9). Mechanism dissection revealed that bicalutamide and enzalutamide reduced the AR-mediated PIAS3 expression with enhanced the pSTAT3-CCL2 signaling. Suppression of pSTAT3-CCL2/CCR2 signaling reversed the bicalutamide- or enzalutamide-induced macrophage migration and PCa cell invasion. Importantly, ASC-J9[®] suppressed both macrophage migration and subsequent PCa cell invasion through regulation of pSTAT3-CCL2 signaling *via* an AR-independent pathway to directly suppress the STAT3 phosphorylation/activation. These *in vitro* cell lines findings were confirmed in the *in vivo* mouse model with orthotopically injected PCa cells.

Together, these results may raise the potential concern about the currently used ADT with anti-androgens that promotes PCa metastasis and may provide a new and better therapeutic approach *via* using ASC-J9[®] alone or a combinational therapy that simultaneously targets androgen/AR signaling and PIAS3-pSTAT3-CCL2 signaling to better battle PCa growth and

metastasis at castration-resistant stage.

Conclusions

Macrophages may play key roles from beginning of PCa initiation to later metastasis *via* regulating AR signaling and/or its modulated inflammatory cytokines/chemokines (**Figure 1**). ADT with anti-androgens may enhance macrophages-associated inflammatory cytokines-AR signaling to promote PCa metastasis. A combinational ADT therapy with additional drugs to target those macrophages-associated inflammatory cytokines may be needed to better battle PCa progression. Alternatively, using a newly developed AR degradation enhancer ASC-J9[®] to simultaneously suppress AR and those macrophages-associated inflammatory cytokines with less toxicity or side effects (10) may become a new therapy to better battle PCa in the future.

Acknowledgments

This work was supported by NIH grant CA156700 and George Whipple Professorship Endowment, and Taiwan Department of Health Clinical Trial and Research Center of Excellence grant DOH99-TD-B-111-004. ASC-J9[®] was patented by the University of Rochester, University of North Carolina, and AndroScience, and then licensed to AndroScience. Both the University of Rochester and C.C. own royalties and equity in AndroScience.

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Figure legend

Fig. 1. Vicious cycle between PCa cells and macrophages through chemokine activation:

Infiltrating macrophages and inflammatory cytokine, CCL4, play a key role during PCa initiation through the activation of AR-CCL4-STAT3 axis (arrows with broken line). As bicalutamide or enzalutamide reduce the AR-mediated PIAS3 expression and enhanced the pSTAT3-CCL2 pathway, these anti-androgens promote macrophage migration to PCa cells that consequently led to enhanced PCa cell invasion (white arrows). Suppressing macrophage AR function simultaneously triggers undesirable inflammatory signals that prompt macrophage infiltration and stimulate progression of PCa through the activation of the CCL2/CCR2-STAT3 axis (black arrows).

Prostate cancer progression

