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Androgen receptor, ccl2, and epithelial-mesenchymal transition

A dangerous affair in the tumor microenvironment

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High levels of chemokine (C-C motif) ligand 2 (CCL2) promote the metastatic dissemination of prostate cancer by recruiting macrophages to neoplastic lesions. We have recently discovered that inhibiting the androgen receptor (AR) in prostate cancer cells or tumor-infiltrating macrophages results in the upregulation CCL2 and promotes disease progression by activating signal transducer and activator of transcription 3 (STAT3) and by favoring the epithelial-to-mesenchymal transition. Our results indicate that the sole inhibition of AR as a therapeutic intervention against prostate cancer is intrinsically destined to failed.

Introduction

The macrophages that infiltrate neoplastic lesions play a key role in the survival, proliferation, and progression of malignant cells.[1] Unlike traditional macrophages, tumor-associated macrophages (TAMs) have been reprogrammed by the tumor microenvironment to mediate limited cytotoxic and antigen-presenting functions, hence allowing for tumor growth and progression.[2] One elegant study has demonstrated that interleukin-1β (IL-1β) mediates a crosstalk between TAMs and prostate cancer cells that promotes the resistance of the latter to hormonotherapy as it alters the sensitivity of the androgen receptor (AR) to specific inhibitors.[3] Thus, TAMs also modulate the biological activity of the AR in prostate cancer cells. Conversely, androgen withdrawal in patients with prostate cancer promotes tumor infiltration by various immune cell subsets, including T cells and macrophages,[4] which may underlie the inflammatory responses developing within prostate malignancies upon such a therapeutic intervention. However, little is known on how the AR, a major driver of prostatic oncogenesis and tumor progression, crosstalk with inflammatory signals in this setting. Interestingly, androgen ablation has been shown to elicit the recruitment of various leukocyte subsets into prostate neoplasms, eventually resulting in the development of castration resistance upon the secretion of lymphotoxin by B cells.[5] Macrophages were also identified within prostate cancers in this study, but it was not clear whether TAMs or their cytokines were involved in the development of castration resistance. These findings indicate that castration may promote the recruitment of macrophages to the prostate tumor microenvironment, perhaps resulting in the emission of inflammatory signals that could be important for the development of resistance. However, it remains to be determined if the AR is specifically involved in this process, since androgen deprivation therapy (ADT) may have a global impact on a variety of cells of the tumor microenvironment. We believe that understanding the molecular mechanisms whereby the inhibition of AR signaling regulates the inflammatory tumor infiltrate may assist the design of novel strategies to maximize the clinical benefits obtained by prostate cancer from ADT.

AR Downregulation Promotes the Secretion of CCL2 and the Accumulation of M2 Macrophages

Recently, we have determined if the inhibition of the AR in the tumor microenvironment would result in the activation of specific inflammatory signaling pathways that could support the growth and progression of prostate cancer. By means of western blot-based cytokine arrays, we identified chemokine (C-C motif) ligand 2 (CCL2) as a downstream target of silencing the AR by small-interfering RNAs in prostate cancer cells or macrophages.[4] Robust CCL2 expression positively correlates with tumor infiltration by TAMs, which drive the progression of prostate cancer as they release, among various mediators, potent pro-angiogenic factors.[6] Moreover, CCL2 supports the survival of M2 monocytes/macrophages.[7] Thus, high levels of CCL2 within prostate

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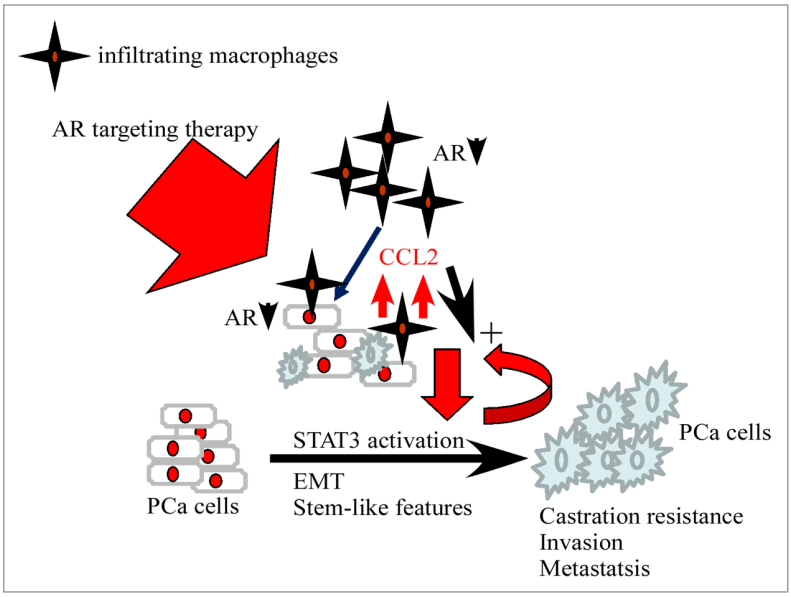

Figure 1. Disease-promoting effects of androgen receptor inhibition in prostate cancer. Targeting the androgen receptor (AR) in prostate cancer cells promotes the expression of chemokine (C-C motif) ligand 2 (CCL2), the recruitment of macrophages, the activation of signal transducer and activator of transcription 3 (STAT3), hence the epithelial-to-mesenchymal transition. Altogether, these alterations promote disease progression.

cancers may favor the recruitment of macrophages, support their differentiation toward an M2 phenotype, and support their ability to establish an immunosuppressive microenvironment. Interestingly, we observed increased levels of M2 markers and CCL2 in macrophages subjected to AR silencing co-cultured with prostate cancer cells. These findings suggest that the AR inhibits M2 macrophage polarization as well as the secretion of CCL2.

Another key result of our study is that the lineage-specific ablation of *AR* in myeloid cells (based on the Cre recombinase expressed under the control of the *Lys2* promoter) accelerates the growth and metastatic dissemination of prostate tumors in transgenic adenocarcinoma of the mouse prostate (TRAMP) mice. This results from the activation of a CCL2-signaling pathway mediated by signal transducer and activator of transcription 3 (STAT3) that drives the epithelial-to-mesenchymal transition (EMT), indicating that the AR can modulate the function of TAMs in the course of prostate tumor progression. This finding is particularly important as therapeutically targeting AR is often focused on prostate cancer cells instead of non-malignant cells such as TAMs or stromal cells that are also important for oncogenesis and tumor progression. Thus, globally targeting the AR in the tumor microenvironment with ADT could mediate immunosuppressive effects on tumor-infiltrating macrophages, hence favoring the escape of prostate cancer cells from immunosurveillance through the production of CCL2. Our study suggests that the ADT-elicited upregulation of CCL2 in the tumor microenvironment may support the survival of prostate cancer cells and facilitate the establishment of an immunosuppressive environment favorable to disease progression. The identification of this regulatory circuitry may have important implications for any therapeutic interventions targeting the AR in prostate cancer, since all these strategies might promote CCL2 expression and hence antagonize their own antineoplastic activity.

The CCL2-STAT3-EMT Axis is Responsible for Prostate Cancer Progression

Most importantly, we found that silence the AR in prostate cancer cells promotes the phosphorylation-dependent activation of STAT3, hence driving the EMT. It had previously been shown that STAT3 can stimulate the secretion of CCL2 by cancer cells and support the acquisition of stem cell-like features that may resistance to ADT.[8,9] It remains to be determined if the activation of STAT3 by CCL2 truly promotes stem cell-like features among prostate cancer cells, hence favoring metastatic dissemination and hormonal resistance. Our results suggest that CCL2 and STAT3 may engage in a positive feedback loop in AR-depleted prostate cancer cells. The major finding of our study is that the CCL2-STAT3-EMT axis may serve as a means for prostate cancer cells to escape androgen deprivation. We postulate that STAT3 could constitute a point of convergence between signaling pathways that are crucial for the development of the castration resistance and metastasis. In summary, our study indicates that ADT might have both local and global effects that promote prostate cancer progression. Our findings increase our understanding of the molecular mechanisms linking CCL2, tumor infiltration by macrophages and the pathogenesis of castration-resistance prostate cancer (**Fig. 1**). The underlying signal transduction cascade represents a candidate target for the development of new therapeutic agents to be tested in combination with ADT against prostate cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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