See related article on page 2505

COMMENTARY

Stat5a/b in Prostate Cancer Metastasis

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Invasion and metastasis are critical hallmarks of cancer progression.¹ Although the overall survival of patients with localized prostate cancer (CaP) has been significantly increased because of prostate-specific antigen screening and improved treatment modalities, the survival rate for patients who develop metastatic CaP remains unchanged.² The potential mechanisms of the acquired ability of CaP cells to invade and to disseminate at distant sites remain largely unknown and represent a challenging problem in metastatic CaP research. In the current issue of *The American Journal of Pathology*, Talati et al³ describe a novel mechanism for Stat5a/b signaling in promoting metastasis in CaP *in vitro* and *in vivo*.³

Stat5a/b in CaP Progression

Stat proteins are a family of transcription factors that mediate cytokine and growth factor responses.⁴ The 94-kDa Stat5a and 92-kDa Stat5b are highly homologous isoforms that are critical cytoplasmic signaling proteins and nuclear transcriptional factors.⁵ Stat5a/b are phosphorylated and activated by upstream kinases, such as Jak2, in response to different cellular ligands, such as prolactin (Prl).⁶ Activated Stat5a/b dimerize and rapidly translocate to the nucleus, where they bind to the γ -interferon activation sequence element and induce Stat5a/b target genes, including the antiapoptotic protein Bcl-XL and the cell-cycle-mediating protein cyclin D1, as well as other factors that are critical in cell survival and growth.⁷ Furthermore, activated Stat5a/b regulates CaP viability, and inhibition of Stat5a/b induces cell apoptotic death of CaP cells in *in vitro* and *in vivo* models.

Evidence suggests that activated Stat5a/b is elevated in >60% of CaP metastasis,⁸ implicating a role for Stat5a/b in clinical CaP progression. Furthermore, Stat5a/b status is used to predict early CaP recurrence and early CaP-specific death.⁹ Introducing constitutively activated forms of Stat5a/b into CaP DU145 cells increases the formation of lung metastases compared with parental DU145 cells¹⁰; however, the mechanism(s) remain unknown.

In the current study, Talati et al³ proposed that active Jak2-Stat5a/b signaling induces epithelial-to-mesenchymal transition (EMT) and cancer stem cell (CSC) properties in CaP, processes critical in cancer metastasis. By using different models, they demonstrate that active Stat5a/b induced EMT and CSC markers, and promoted metastasis formation of CaP cells *in vivo*.³

EMT-Associated Changes in CaP

EMT is a physiological process by which epithelial cells gain mesenchymal characteristics. EMT is involved in a spectrum of both normal physiology and pathological processes and can be activated during the course of invasion and metastasis of CaP. During EMT, the epithelial cells lose epithelial phenotypes, such as cell polarity and cell-cell adhesion, and instead develop mesenchymal phenotypes, such as increased migration, ability to invade, and disorganized extracellular matrix.¹¹ A decrease in epithelial biomarkers (eg, E-cadherin and β -catenin) and an increase in mesenchymal markers (eg, N-cadherin and vimentin) are associated with EMT and tumor invasion. Different signaling pathways have been reported to stimulate EMT in CaP, including the growth factor receptor tyrosine kinase-activated pathway,¹² Pten-related pathway,¹³ Wnt, Notch, and Hedgehog signaling pathways.¹⁴

Jak2-Stat5a/b Signaling Is Critical for Prl Induction of EMT in CaP Cells

Talati et al³ reported that activation of Stat5a/b by cellular ligand Prl was associated with a decrease in E-cadherin expression and a substantial increase in N-cadherin and

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other mesenchymal markers, such as vimentin, fibronectin, and Twist1, in different CaP cell lines independent of the status of androgen receptor. Jak2 kinase inhibitor AZD1480 and knockdown of Stat5a/b by shRNA in CaP cells blocked the Prl-induced changes in the expression of EMT markers. These results were further validated using different animal models, CaP xenograft of patient tumors, and patient samples cultured *ex vivo*.

A significant advantage of this study is the use of CWR22Pc cells, which mimic the clinical course of CaP patients. Mice bearing CWR22Pc tumors were treated with Stat5a/b signaling inhibitor AZD1480 during both primary growth in the presence of androgens and CRPC growth. Inhibition of Stat5a/b up-regulated E-cadherin expression and down-regulated Twist1 expression in CWR22Pc tumors during different time points. Data confirmed that the Jak2-Stat5a/b is critical for Prl induction of EMT in CaP cells.

Twist1 Is the Key Mediator of the Stat5a/b-Induced EMT in CaP Cells

EMT is a crucial process during cancer cell migration and metastasis. Talati et al³ analyzed several critical end points associated with EMT in CaP cells. Use of Boyden chamber assays, epithelial monolayer growth versus anchorage-independent survival of small CaP cell clusters, and adhesion of CaP cells to fibronectin confirmed that activated Jak2-Stat5a/b signaling induces EMT markers and is accompanied by the EMT-associated changes in CaP. Talati et al³ further identified Twist1 as the key mediator of the Stat5a/b-induced EMT in CaP. Twist 1 expression was required for EMT markers and the EMT-associated changes.

EMT Promotes the Induction of CSC Phenotype in CaP Cells

CSCs are a small subset of stem cell–like cancer cells, which have the capability of self-renewal and differentiation. The CSC theory has received increasing attention recently because preclinical models have provided evidence of CSCs contributing to cancer proliferation, relapse, and metastasis. In addition, CSCs generally have slow growth rates and are resistant to chemotherapy and radiotherapy.¹¹ Stem cell markers are up-regulated in CaP xenografts and mouse CaP models on androgen-deprivation therapy,^{15–17} suggesting that the CSC subpopulation might contribute to castration resistance progression in CaP. Interestingly, EMT is linked with the acquisition of stem cell–like characteristics under the regulation of many cancer-relevant pathways, such as Wnt, Notch,¹⁸ and Hedgehog signaling.¹⁹

Talati et al³ demonstrated that, in CaP cells, EMT promoted the induction of a CSC phenotype. The induction of EMT by activation of Jak2-Stat5a/b signaling was accompanied with increased sphere formation of CaP cells concurrent with induction of markers associated with cancer stem–like

properties, such as CD44, Sox2, and BMI1. The study was next expanded to demonstrate that the Jak2-Stat5a/b axis concomitantly drives a stemness phenotype in the CaP cells, an effect also mediated through induction of BMI1. These observations suggest that BMI1 is critical and is required by the Jak2-Stat5a/b axis to promote stem-like cell properties in CaP cells. By using a human *PRL* knock-in genetically engineered mouse model, Talati et al³ demonstrated, for the first time, that the Prl–Jak2-Stat5a/b axis drives CaP lung metastasis, which is accompanied with increased levels of Twist1 and BMI1 in the metastatic sites, which further confirmed the results from the *in vitro* study.

Conclusions

The current work³ suggests that Stat5a/b instigates EMT through inducing the expression of Twist1. The induction of BMI1 by active Stat5a/b is also through Twist1 suppression, which leads to induction of the CSC phenotype. Results from Talati et al³ provide an important piece of evidence for the link between EMT and cancer stem–like properties in CaP. This study also reports, for the first time, that Stat5a/b-induced Twist1 plays an important role in EMT and the induction of cancer stem–like property in CaP, both of which increase the metastatic capability of CaP cells to the lungs of mice. Further studies are needed to understand the mechanisms of regulation of Twist1 expression by Stat5a/b. Such findings, with the development of pharmacological inhibitors of the Jak2-Stat5a/b–Twist1–BMI1 signaling axis, may present a novel and effective therapeutic strategy to prevent metastasis and dissemination of CaP.

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