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Expression of STAT3 in Prostate Cancer Metastases

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Abstract

STAT3 and its upstream activator IL6R have been implicated in the progression of prostate cancer and are possible future therapeutic targets. We analyzed 223 metastatic samples from rapid autopsies of 71 patients who had died of castration-resistant prostate cancer (CRPC) to study protein and gene expression of pSTAT3 and IL6R. Immunohistochemical analysis revealed that 95% of metastases were positive for pSTAT3 and IL6R, with varying expression levels. Bone metastases showed significantly higher expression of both pSTAT3 and IL6R in comparison to lymph node and visceral metastases. *STAT3* mRNA levels were significantly higher in bone than

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in lymph node and visceral metastases, whereas no significant difference in *IL6R* mRNA expression was observed. Our study strongly supports the suggested view of targeting STAT3 as a therapeutic option in patients with metastatic CRPC.

Keywords

Castration-resistant; Metastases; Prostate cancer; STAT3; Tissue microarray

Treatment of locally advanced and metastatic prostate cancer (PCa) is currently hampered by drug resistance, and there is an urgent need to identify new therapeutic targets. A recently explored candidate target for the treatment of aggressive PCa is the transcription factor STAT3 [1,2]. STAT3 is an important oncogenic-associated protein that is constitutively activated in PCa [3,4]. After activation via phosphorylation, STAT3 is translocated to the nucleus and acts on the transcription of several genes involved in anti-apoptosis, proliferation, and metastasis (eg, BCL2, MCL1, cyclin D1, and MMP9) [1]. Phosphorylation of tyrosine residue 705 in the STAT3 protein is correlated with aggressive PCa and high Gleason score [4]. It has been shown that IL6 is a main driving force in pSTAT3 activation, and is highly expressed in PCa patients with poor prognostic outcome [5]. Cell-surface IL6R has also been implicated in cancer progression [3]. As the expression of pSTAT3 in PCa metastases is not fully clarified, we immunostained a unique cohort of 223 tumor samples obtained from 71 patients with castration-resistant PCa from the Prostate Cancer Donor Rapid Autopsy Program at the University of Washington (Seattle, WA) [6] for both pSTAT3 and its upstream activator IL6R. All patients were on androgen deprivation therapy (ADT) and most had been treated with chemotherapy. Patient samples were available from bone, lymph node and visceral metastases (Supplementary Tables 1 and 2). Immunostaining for pSTAT3 was nuclear, and was evaluated using a scoring index whereby both the staining intensity (0, 1, 2, and 3; Fig. 1A–D) and the percentage of tumor cells stained (0–10%, 1; 11–75%, 2; and >75%, 3) were recorded. These scores were then multiplied to create an expression score that ranged from 0 to 9. Immunostaining for IL6R was cytoplasmic, membranous, and ubiquitous, leading to scoring based solely on intensity (0, 1, 2, and 3; Fig. 1E–H).

We found that 99% of bone, 96% of lymph node, and 86% of visceral metastases expressed pSTAT3 (Fig. 1I). The average pSTAT3 expression score was highest in bone (5.23 ± 2.40), followed by lymph node (3.78 ± 2.42) and visceral (2.59 ± 2.11) metastases. Matched samples of all three tissue types were available for 24 patients, for which we found similar results to those observed for the entire group of tumors, with significant differences in pSTAT3 expression observed between bone and lymph node and visceral metastases (Fig. 1J).

Some 99% of bone, 88% of lymph node, and 92% of visceral metastases expressed IL6R (Fig. 1K). The average IL6R expression score was significantly higher in bone (2.20 ± 0.66) compared to lymph node (1.68 ± 0.94) but not visceral (1.96 ± 0.88) metastases. Among 23 patients with matched tissues sample, bone expression was higher than among patients with

non-matched samples, leading to significantly higher IL6R expression in bone compared to both lymph node and visceral metastases (Fig. 1L).

Coexpression of pSTAT3 and IL6R was observed in 98% of bone, 84% of lymph node, and 78% of visceral metastases (data not shown).

A subset of 63 patients with 149 unique tumor samples was available for transcript abundance analysis of *STAT3* and *IL6R* mRNA expression. *STAT3* mRNA levels were significantly higher in bone, mirroring the immunostaining results for pSTAT3. There was no significant difference in *IL6R* mRNA expression among the different metastatic sites (Fig. 2). Recently published data indicate that the location of metastasis plays a role in overall survival among patients with metastatic disease [7]. We observed significantly higher pSTAT3 levels in bone compared to lymph node and visceral metastases, suggesting that tissue location may dictate pSTAT3 expression levels. When looking specifically at patients for whom tissue sample for all three metastasis sites were available, it was apparent that pSTAT3 expression levels were heterogeneous within each patient, with highest expression in bone, similar to previous results for downstream targets of STAT3 in a similar cohort [8].

Bone metastases can be either osteolytic or osteoblastic, with the majority of bone lesions in PCa being the latter. Osteoblasts express IL6, supporting the theory of a tissue-specific microenvironment that stimulates activation of pSTAT3 [9]. Moreover, a recent study showed that IL6 expression in bone is derived from endothelial cells in blood vessels and not tumor cells themselves [10]. However, further investigation is needed to better understand why pSTAT3 is highly expressed in bone metastases in CRPC. It is unlikely that our findings are related to an artifact caused by the decalcification process, as this process did not affect the immunostaining intensity in soft tissue (Supplementary Fig. 1). Our antibodies have been validated, as described in the Supplementary material.

It is interesting to note that more than 95% of our patient cohort expressed some level of pSTAT3, suggesting that phosphorylation of STAT3 is a common event in metastatic CRPC, in line with current hypotheses that STAT3 activation is commonly found in aggressive PCa cells. Furthermore, we observed higher *STAT3* mRNA levels in bone tissue than in lymph node and visceral tissue, mirroring our results for pSTAT3 immunostaining. Correlation analysis did not reveal strong associations between pSTAT3 and IL6R at the immunohistochemical level or *STAT3* and *IL6R* at the mRNA level, but coexpression of pSTAT3 and IL6R in IHC was observed in a high number of samples (Supplementary Table 3), indicating that pSTAT3 expression levels may be influenced by other signaling pathways in addition to IL6R, such as EGF or other cytokines [1]. These results need to be further evaluated in larger cohorts.

Our data suggest that pSTAT3 is a potential therapeutic target in metastatic CRPC. Bone metastases exhibited the highest expression of both pSTAT3 and *STAT3* mRNA, suggesting an environmental niche created by bone tissue that favors high pSTAT3 expression. Follow-up studies are needed to better understand what regulates pSTAT3 expression in metastases in different organs. Nevertheless, owing to its widespread presence in metastatic CRPC,

direct targeting of STAT3 represents a viable future treatment for patients with late-stage prostate cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Bishop JL, Thaper D, Zoubeidi A. The multifaceted roles of STAT3 signaling in the progression of prostate cancer. *Cancers*. 2014; 6:829–59. [PubMed: 24722453]
2. Canesin G, Evans-Axelsson S, Hellsten R, et al. The STAT3 inhibitor galiellalactone effectively reduces tumor growth and metastatic spread in an orthotopic xenograft mouse model of prostate cancer. *Eur Urol*. 2016; 69:400–4. [PubMed: 26144873]
3. Tam L, McGlynn LM, Traynor P, Mukherjee R, Bartlett JM, Edwards J. Expression levels of the JAK/STAT pathway in the transition from hormone-sensitive to hormone-refractory prostate cancer. *Br J Cancer*. 2007; 97:378–83. [PubMed: 17595657]
4. Mora LB, Buettner R, Seigne J, et al. Constitutive activation of Stat3 in human prostate tumors and cell lines: direct inhibition of Stat3 signaling induces apoptosis of prostate cancer cells. *Cancer Res*. 2002; 62:6659–66. [PubMed: 12438264]
5. Shariat SF, Andrews B, Kattan MW, Kim J, Wheeler TM, Slawin KM. Plasma levels of interleukin-6 and its soluble receptor are associated with prostate cancer progression and metastasis. *Urology*. 2001; 58:1008–15. [PubMed: 11744478]
6. Roudier MP, True LD, Higano CS, et al. Phenotypic heterogeneity of end-stage prostate carcinoma metastatic to bone. *Hum Pathol*. 2003; 34:646–53. [PubMed: 12874759]
7. Halabi, S., Kelly, WK., Ma, H., et al. Meta-analysis evaluating the impact of site of metastasis on overall survival in men with castration-resistant prostate cancer. *J Clin Oncol*. In press <http://dx.doi.org/10.1200/JCO.2015.65.7270>
8. Akfirat C, Zhang X, Ventura A, et al. Tumor cell survival mechanisms in lethal metastatic prostate cancer differ between bone and soft tissue metastases. *J Pathol*. 2013; 230:291–7. [PubMed: 23420560]
9. Lu Y, Zhang J, Dai J, et al. Osteoblasts induce prostate cancer proliferation and PSA expression through interleukin-6-mediated activation of the androgen receptor. *Clin Exp Metastasis*. 2004; 21:399–408. [PubMed: 15672864]
10. Yu S-H, Zheng Q, Esopi D, et al. A paracrine role for IL6 in prostate cancer patients: lack of production by primary or metastatic tumor cells. *Cancer Immunol Res*. 2015; 3:1175–84. [PubMed: 26048576]

Patient summary

We studied the levels of two proteins (pSTAT3 and IL6R) in metastases from patients who died from castration-resistant prostate cancer. We found high levels of pSTAT3 and IL6R in bone metastases, suggesting that these proteins could be used as targets for new anticancer drugs.

Fig. 1.

– Examples of immunohistochemical (IHC) staining and distribution of IHC expression scores for pSTAT3 and IL6R. (A–D) Examples of pSTAT3 staining in metastases. (A) Negative (0) liver staining. (B) Low (1) lymph node staining. (C) Medium (2) lymph node staining. (D) High (3) bone staining. (E–H) Examples of IL6R staining in metastases. (E) Negative (0) liver staining. (F) Low (1) lymph node staining. (G) Medium (2) lymph node staining. (H) High (3) bone staining. Scale bar, 100 μ m. (I–L) Expression in bone compared to lymph node and visceral metastases. (I) Overall tissue sample distribution for pSTAT3 staining, with significant differences observed between bone ($n = 113$), lymph node ($n = 51$),

and visceral ($n = 51$) metastases. (J) Matched tissue samples for pSTAT3 staining ($n = 24$), with significant differences observed between bone and both lymph node and visceral metastases. (K) Overall tissue samples for IL6R staining, with significant differences observed only between bone ($n = 117$) and lymph node ($n = 50$) metastases, and no significant difference between bone and visceral metastases ($n = 50$). (L) Matched tissue samples for IL6R staining ($n = 23$), with significant differences observed between bone and both lymph node and visceral metastases. (I,J) Scoring index: zero = 0; low = 1, 2; medium = 3, 4; high = 5–9. (K,L) Scoring index: zero = 0; low = 1; medium = 2; high = 3. Asterisks denote significant differences between groups according to a Wilcoxon signed-rank test (** $p < 0.01$, *** $p < 0.001$).

Fig. 2.

Comparison of mRNA expression scores (log2 signal intensity of Cy3) for bone, lymph node, and visceral metastases. Asterisks denote significant differences between groups according to a Wilcoxon signed-rank test (***) $p < 0.001$. Significant differences in *STAT3* levels were observed between bone and both lymph node and visceral metastases. No significant differences were observed in *IL6R* expression between the different metastasis sites.