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Activating mutation (V617F) in the tyrosine kinase JAK2 is absent in locally-confined or castration-resistant prostate cancer

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Abstract. *Background*: Transcription factor Stat5a/b is highly critical for the viability of human prostate cancer cells *in vitro* and for prostate tumor growth *in vivo*. Stat5 is constitutively active in clinical prostate cancers but not in the normal human prostate epithelium. Moreover, Stat5a/b activation in prostate cancer is associated with high histological grade of prostate cancer. However, the molecular mechanisms underlying constitutive activation of Stat5a/b in prostate cancer are unclear. The receptor-associated tyrosine kinase Jak2 is a known key activator of Stat5a/b in prostate cancer cells in response to ligand stimulation. Recently, a single gain-of-function point mutation of *JAK2* was described in myeloproliferative diseases leading to constitutive Jak2 kinase activity, subsequent Stat5a/b activation and involvement of V617F Jak2 in the pathogenesis of myeloproliferative disorders.

Materials and methods: We determined whether *JAK2* undergoes the V617F activating mutation during clinical progression of human prostate cancer using a highly sensitive assay (amplification refractory mutation system) and a unique material of fresh specimens from organ-confined or castration-resistant prostate cancers.

Results: The *JAK2* V617F mutation was not found in any of the normal or malignant prostate samples analyzed in this study.

Conclusions: Future work should focus on determining the molecular mechanisms other than V617F mutation of Jak2 resulting in continuous Stat5 activation in clinical prostate cancers.

Keywords: *JAK2* V617 mutation, prostate cancer

1. Introduction

Transcription factor Stat5a/b is highly critical for the viability and growth of prostate cancer. Inhibition of Stat5 induces rapid apoptotic death of human prostate cancer cells [1–3], blocks human prostate cancer xenograft tumor growth in nude mice [3], and inhibits progression of prostate cancer in the TRAMP mouse prostate cancer model [5]. Moreover, Stat5a/b is constitutively active in human prostate cancer but not in normal prostate epithelium [1], and activation of Stat5a/b in prostate cancer epithelium is associated with lesions of high histological grades [7,8]. In primary organ-confined prostate cancer, activation of Stat5a/b predicts early disease recurrence [8]. In addition, Stat5a/b is activated in the majority of castration-resistant recurrent human prostate cancers [15], and active Stat5a/b synergizes with androgen receptor (AR) suggesting an involvement of Stat5a/b in the promotion of castration-resistant growth of prostate cancer [15]. The molecular mechanisms underlying constitutive activation of Stat5a/b in prostate cancer are unclear. The receptor-associated tyrosine kinase Jak2 is a known

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key activator of Stat5a/b in prostate cancer cells in response to ligand stimulation [7]. Recently, a single gain-of-function point mutation of *JAK2* was described in myeloproliferative diseases leading to constitutive Jak2 kinase activity, subsequent Stat5a/b activation and involvement of V617F Jak2 in the pathogenesis of myeloproliferative disorders [4,6,14]. This acquired somatic V617F *JAK2* mutation is located at amino acid position 617 within the JH2 autoinhibitory domain of Jak2 resulting in valine substitution to phenylalanine. Little is known about the direct involvement of Jak2 in prostate carcinogenesis. Specifically, it is not known whether Jak2 V617F is able to transform normal prostate epithelium. While constitutive Stat5a/b activation suggests hyperactivity of Jak2 in prostate cancer cells, no studies exist at present on Jak2 activation state in clinical prostate cancers. In this work, we determined whether *JAK2* undergoes the V617F activating mutation during progression of clinical human prostate cancer.

2. Materials and methods

2.1. Study subjects

Normal and malignant freshly frozen prostate tissues ($n = 78$) were obtained from the University of Turku (Turku, Finland): 19 benign prostate hyperplasias (BPH), 3 prostate intraepithelial neoplasias (PIN) and 57 primary organ-confined prostate cancers of different histological grades (Table 1). DNA was extracted using QIAamp DNA Micro kit (Qiagen Inc., Valencia, CA) according to the manufacturer's instructions.

Freshly frozen samples of 14 castration-resistant prostate cancers (Tampere University Hospital, Tampere, Finland) were from patients who had experienced a local progression of the disease during hormonal therapy (Table 1). The therapy modalities were: orchiectomy (4 cases), LHRH (luteinizing hormone-releasing hormone) analog (3 cases), estrogen (2 cases), orchiectomy and estrogen (2 cases), combined androgen blockade (CAB) (2 cases), and unknown (1 case). The mean time from the onset of the androgen ablation to the progression was 40 months (range: 15–68 months). DNA was amplified with GenomiPhi™ – DNA amplification kit (Amersham, GE Healthcare, UK) according to the manufacturer's instructions.

The use of de-identified archival tissue specimens in research was approved by the Ethics Committee of the Turku University Hospital, the Ethics Committee of the Tampere University Hospital and by the Thomas Jefferson University Institutional Review Board. All of the specimens were histologically examined for the presence of cancer (>60% of cells) using hematoxylin-eosin-stained slides (Table 1).

Table 1
Prostate cancer specimens

Prostate samples	No. of patients	%
Prostate intraepithelial neoplasia (PIN)	3	100
Benign prostate hyperplasia (BPH)	19	100
Organ-confined primary prostate cancer	56	100
Gleason grade 2	4	7
Gleason grade 3	13	23
Gleason grade 4	27	48
Gleason grade 5	8	14
Gleason grade 6	2	4
Gleason grade 7	2	4
Castration-resistant prostate cancer	14	100
Treatment		
Orchiectomy	4	29
Luteinizing hormone-releasing hormone (LHRH)	3	21
Estrogen	2	14
Orchiectomy and estrogen	2	14
Combined androgen blockage (CAB)	2	14
Unknown	1	8
Total	93	

2.2. JAK2 V617F mutation analysis

DNA (240 ng) of each sample was amplified in a 50 µl reaction using an amplification refractory mutation system (ARMS) [9,11] assay. Each reaction contained 400 ng of the *JAK2* forward primer (JAK2-FOR) (5′-tgaagcagcaagtatgatgag, TIB Molbiol, Adelphia, NJ), 400 ng of the *JAK2* mutation specific forward primer (JAK2-MFOR) (5-gcatttggttttaaattatggagt atatt, TIB Molbiol, Adelphia, NJ), which was nested with respect to 400 ng of the *JAK2* forward primer and 400 ng of the *JAK2* reverse primer (JAK2-REV) (5′-6FAM-tacactgacacctagctgtga, TIB Molbiol, Adelphia, NJ) [9]. In addition, each reaction contained 200 µM of each deoxynucleotide triphosphate (Roche, Indianapolis, IN), 1× Gene Amp PCR buffer with $MgCl_2$ and 1 U of AmpliTaq Gold DNA Polymerase (Applied Biosystems, Foster City, CA). The 174 bp fragment of the *JAK2* gene that was amplified with the JAK2-FOR primer and the JAK2-REV primers, served as an internal amplification control. This reaction was multiplexed with the amplification of the mutant sequence – if present – which rendered a 140 bp PCR amplicon using the mutation-specific JAK2-MFOR primer together with the JAK2-REV primer.

The cycling conditions for the prostate cancer DNA were 95°C for 10 min to activate the DNA polymerase, and 29 cycles of 95°C for 15 s, 58°C for 20 s and 73°C for 15 s on the GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA). The following controls were included: negative, 1% positive, 2% positive and 100% positive (Invivoscribe, San Diego, CA) as well as a no template control. For samples with small amounts of DNA, the cycle number was increased to 34 cycles. Each PCR product (1 µl) was mixed with 12 µl of deionized formamide (Applied Biosystems, Foster City, CA) and 0.5 µl of GeneScan ROX-500 (Applied Biosystems, Foster City, CA), heat-denatured and loaded on the ABI 3100 Avant Genetic Analyzer (Applied Biosystems, Foster City, CA) for a 6 second injection using POP4 polymer (Applied Biosystems, Foster City, CA).

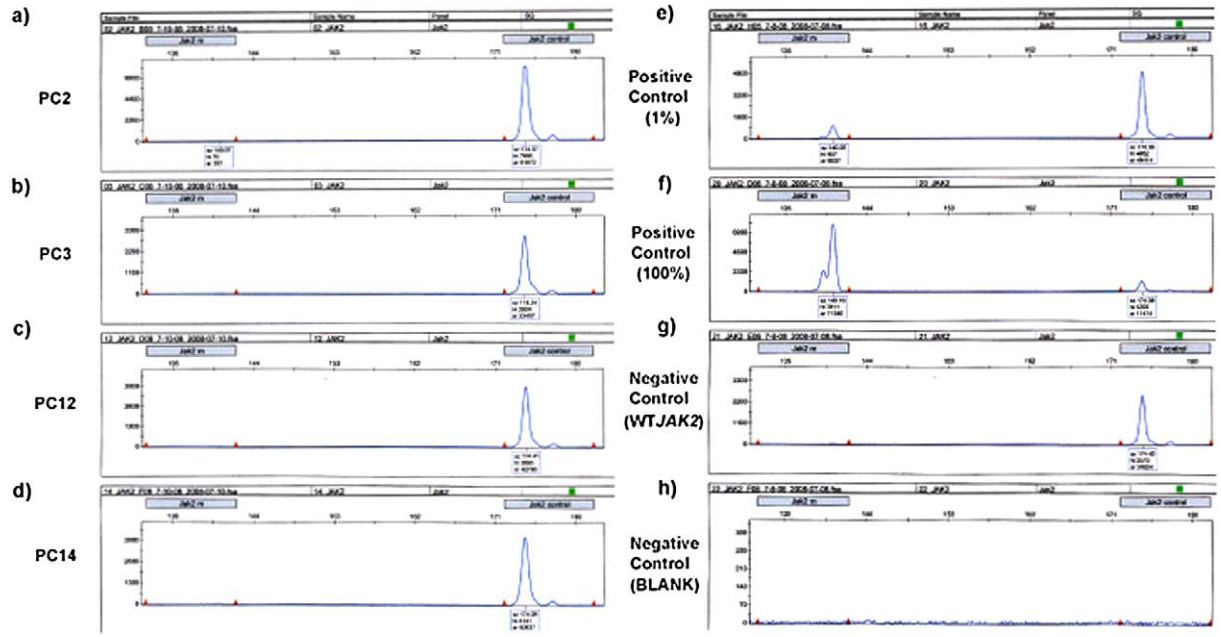

Fig. 1. Analysis of *JAK2* V617F mutation using an amplification refractory mutation system (ARMS) assay in benign prostate hyperplasias, prostate intraepithelial neoplasias, primary prostate cancers of different histological grades and castration-resistant prostate cancers. Selected cases of prostate cancers (PC2, PC3, PC12 and PC14) are presented showing amplification of the 174 bp internal control peak only (a–d). The positive controls were the following: (1) Template containing 1% of DNA with the *JAK2* V617F point mutation with 99% of DNA containing the wild type *JAK2* sequence (1%) (e) and (2) 100% of DNA containing the point mutation (100%) (f). The positive controls show both of the peaks which represent the mutation at 140 bp and the 174 bp internal control peak. The results for 5%, 10% and 30% positive controls are not shown. DNA with the wild type (WT) *JAK2* sequence showing the 174 bp internal control peak only was used as a negative control (g). An additional negative control which did not contain any template (BLANK) shows no amplification (f).

3. Results and discussion

Using a highly specific amplification refractory mutation system (ARMS) assay, we evaluated 19 benign prostate hyperplasias (BPH), 3 prostate intraepithelial neoplasia (PIN) lesions, 56 organ-confined primary prostate cancers and 14 castration-resistant prostate cancers (Table 1) for the activating V617F mutation in the tyrosine kinase Jak2. None of the benign or malignant prostate lesions possessed the *JAK2* V617F mutation. Since each cancer sample contained more than 60% of tumor cells and since the sensitivity of the assay is 1%, heterozygous mutation would have also been detected in our analysis. Of the 93 prostate samples analyzed (Table 1), representative cases of hormone-refractory prostate cancers are presented in Fig. 1(a–d). At the same time, the internal and external controls showed the expected amplicons (Fig. 1, e, f). While the number of cases analyzed is limited, the results of this work suggest that *JAK2* gene does not undergo the V617F somatic mutation in primary or castration-resistant prostate cancers, BPHs, or PIN lesions implicating other molecular mechanisms responsible for constitutive activation of Stat5a/b in prostate cancer. These mechanisms may include deactivated phosphatases, deregulated SOCS proteins [13], abnormal assembly of the receptor complexes and involvement of other peptide hormone receptors in the activation of Jak2 [10,12]. Based on the results of this study, future work should focus on determining the molecular mechanisms other than V617F mutation of Jak2 resulting in continuous Stat5 activation in clinical prostate cancers.

Conflict of interest

No potential conflicts of interest were disclosed.

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