Mutational Activation of *FGFR3* is Not Involved in the Development of Prostate Cancer

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Key Words

Prostate cancer • *FGFR3* • Incidental prostate cancer

Abstract

Objective: The mutational constitutive activation of *FGFR3* has been discovered in several malignancies but only limited data on *FGFR3* mutations in prostate cancer are available. Most recently, activating *FGFR3* mutations were described as being associated with low-grade prostate tumors. Therefore, we investigated the *FGFR3* mutation status in a comprehensive series of prostate tumors. **Methods:** 102 archival formalin-fixed paraffin-embedded prostate tumors of patients treated with radical prostatectomy [with a low-grade subgroup (Gleason score ≤ 6) of 29 patients] as well as 29 incidental prostate tumors [low-grade tumors (Gleason score ≤ 6); $n = 22$] and 16 benign prostatic hyperplasia samples obtained by transurethral resection of the prostate were investigated. After microdissection and DNA isolation, all *FGFR3* mutation hotspots discovered in human malignancies

were analyzed using the SNaPshot[®] approach or restriction fragment length polymorphism (RFLP) analysis. **Results:** All cases could successfully be analyzed by SNaPshot; 80 cases were investigated using RFLP. No mutation in *FGFR3* could be detected in any of the analyzed cases. **Conclusion:** The most recently reported *FGFR3* mutations in low-grade prostate tumors could not be verified in our series. There were also no mutations in prostate tumors from patients with concomitant bladder tumors as reported previously. These data suggest that the mutational activation of *FGFR3* plays no important role in prostate carcinogenesis, which is in accordance with previous studies performed on smaller tumor cohorts.

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Introduction

Fibroblast growth factors (FGF) and their receptors (FGFR) have been demonstrated to be involved in multiple biological processes, e.g. differentiation, motility, or

proliferation, and this ligand-receptor system plays an important role in tumorigenesis [1]. A deregulation of the FGF/FGFR pathway was also described in prostate cancer (PCa). A closer functional evaluation of this pathway even suggested this system to be a potential target for therapy against human PCa [2]. An overexpression of various members of the FGF/FGFR system was detected in PCa (e.g. FGFR1 and FGFR4 proteins), and a link between the FGF/FGFR system and an aggressive biological phenotype of PCa could be demonstrated recently (FGF8 protein expression in bone metastasis of PCa) [3, 4].

In recent years, research on the FGF/FGFR system has spotlighted 1 member of the *FGFR* gene family. Alterations of *FGFR3* were identified in various malignant (e.g. bladder cancer and cervix cancer) [5] and benign (e.g. seborrhic keratoses and epidermal nevi) [6, 7] tumors, and these alterations could also be linked to a favorable course of the disease in noninvasive papillary bladder cancer [8]. Additionally, in bladder cancer *FGFR3* mutations were linked to protein overexpression, but overexpression without concomitant mutations was especially found in advanced tumors [9]. In PCa the role of *FGFR3* is still not clear. Studies investigating *FGFR3* expression at the protein and mRNA levels could not find *FGFR3* overexpression in benign prostatic hyperplasia (BPH) or PCa [2, 3, 10]. The mutation status of *FGFR3* in PCa is a controversial topic of discussion at the moment. Three studies investigated *FGFR3* mutations showing no evidence for *FGFR3* mutations in PCa [11–13]. Most recently, Hernández et al. [14] reported a remarkable *FGFR3* mutation frequency in low-grade PCa and in PCa in patients with other associated malignancies.

To further this discussion we investigated the *FGFR3* mutation status in a comprehensive series of prostate tumors.

Material and Methods

Tissue Samples

Overall, 102 unselected archival formalin-fixed paraffin-embedded prostate tumors obtained by radical prostatectomy [among them low-grade tumors (Gleason score ≤ 6); $n = 29$], as well as 29 incidental prostate tumors [among them low-grade tumors (Gleason score ≤ 6); $n = 22$], and 16 BPH samples obtained by transurethral resection of the prostate, were analyzed. Classification of the tumors was performed according to the TNM classification (International Union Against Cancer, Geneva, Switzerland) [15] and the World Health Organization (WHO) classification of prostate tumors [16]. The clinical and histopathological characteristics of patients and tumors are shown in table 1.

Table 1. Clinical and histopathological characteristics of the analyzed patients and tumors

	Radical prostatectomy group	n	Incidental prostate cancer group	n
Number	102		29	
Age, years				
Median (range)	66 (46–80)		73 (60–83)	
Mean $\pm$ SD	64.6 $\pm$ 6.1		72.4 $\pm$ 6.1	
Stage	pT1–3a	81	pT1a–1c	27
	pT3b–3c	21	pT2a–pT2b	2
Gleason score	4–6	29	3–6	22
	7	48	7	7
	8–10	23	–	–
	no data available	2	–	–

DNA Isolation

Serial sections (5 μ m) from the tissue blocks were deparaffinized and rehydrated for microdissection, and areas with $\geq 80\%$ tumor cells were marked on an H&E-stained section by an experienced surgical pathologist before microdissection. The marked tissue sections were then used as templates for microdissection which was performed manually with sterile needles after methylene blue staining. DNA was isolated using a High Pure PCR Template Preparation Kit[®] (Roche, Mannheim, Germany) according to the manufacturer's instructions. A previously described highly sensitive SNaPshot[®] multiplex assay, based on the SNaPshot Multiplex System assay (Applied Biosystems, Foster City, Calif., USA), was used to screen for activating *FGFR3* point mutations. Using this assay, screening could be performed for 11 known mutations found in bladder tumors and other epithelial tumors (R248C, S249C, G372C, S373C, Y375C, G382R, A393E, K652E, K652M, K652Q, and K652T) [7, 17]. Extended primers were separated by capillary electrophoresis in an automatic sequencer, and the presence or absence of a mutation was indicated by the incorporated wild-type or mutant-labeled dideoxynucleotide. When a mutation is present, a second peak originating from the mutated nucleotide will appear next to the wild-type peak in the electropherogram. In order to analyze a previously reported additional activating *FGFR3* mutation in codon 697 (G697C) [18], we used the restriction fragment length polymorphism (RFLP) approach which was described in detail previously [19].

Results

The *FGFR3* SNaPshot analysis could successfully be performed on all 131 PCa samples and on all 16 BPH samples. In all samples only the wild-type sequence of *FGFR3* was found, and none of the analyzed samples showed an *FGFR3* mutation (fig. 1a). For the RFLP analysis a higher amount of DNA was necessary compared to the SNaPshot analysis. Therefore, only a subset of prostate tumors

Fig. 1. a Representative *FGFR3* analysis by SNaPshot assay. DNA from a prostate tumor (pT3aG3, Gleason score = 7) showing the wild-type sequence in the known *FGFR3* mutation hotspots. **b** RFLP analysis of *FGFR3* codon 697. Polymerase chain reaction fragments (209 bp) containing the wild-type sequence in codon 697 could be digested with *MspI* (100 + 109 bp; both fragments appear as 1 band on the gel). In case of a mutation, the *MspI* site is destroyed. 1–4 = DNA from PCa samples; 5 = DNA from the urothelial cell line UROtsa (representing normal urothelium) after *MspI* digestion; 6 = UROtsa DNA without *MspI* digestion; 7 = negative control (H₂O); St. = Size standard. All analyzed samples showed the wild-type sequence for codon 697.

was available for the *FGFR3* codon 697 analysis. All investigated samples showed the wild-type sequence for codon 697, and no mutation was detected (fig. 1b).

Discussion

Our results are contrary to those of the most recent study of Hernández et al. [14] which reported up to 18% *FGFR3* mutations in low-grade PCa (combined Gleason score = 6). We analyzed a larger cohort of low-grade PCa than the aforementioned study but did not find a mutation. We also screened a larger group of cases with a combined Gleason score of 7 which showed a mutation frequency of at least 3% in the study of Hernández et al. but, again, we only found the wild-type sequence in the *FGFR3* mutation hotspots.

One explanation for the lack of *FGFR3* mutations in our cohort could be a strong contamination of the material with nonneoplastic cells, which could make the detection of mutations impossible. However, great care was taken during microdissection to avoid the analysis of mixed cell populations. In addition, the SNaPshot assay used is very robust and highly sensitive, detecting 1 mutant allele in a background of 97% wild-type alleles [17]. Therefore, contamination with normal cells is a very unlikely explanation for the lack of *FGFR3* mutations in our study.

The lack of detection of the rare G697C mutation was also unlikely to be due to technical limitations because this mutation has only been reported in oral squamous

cell carcinomas to date, and has not been analyzed in other tumor types so far. Therefore, the presented study is the first to report a lack of this rare mutation in PCa.

The highest number of *FGFR3* mutations reported by Hernández et al. [14] was found in patients with additional tumors apart from PCa, i.e. bladder, skin, and colon tumors. We also screened our cohort for secondary tumors and found only 2 cases with bladder cancer (1× pTa low-grade without an *FGFR3* mutation and 1× pT2 high-grade with an *FGFR3* S249C mutation) and 1 case with colon adenoma. This showed that a secondary tumor is a rare event in PCa patients. None of the prostate tumors from these cases showed an *FGFR3* mutation. Although it is known that patients with PCa or a family history of PCa have a higher risk of bladder and other secondary cancers [20, 21], the large PCa patient group with secondary bladder, colon, or skin tumors presented by Hernández et al. [14] did not reflect the general population. This highly selected patient group with up to 3 tumors in different locations might represent a population with a high overall risk of developing a malignancy. As stated in the aforementioned study, there was a remarkably high percentage of *FGFR3* mutations in PCa patients with secondary bladder and skin tumors, both of which are known to be associated with *FGFR3* mutations [5, 22]. Hernández et al. [14] also analyzed the *FGFR3* mutation status in these secondary tumors and the corresponding normal tissue in some cases, but a mutation was only detected in 1 bladder tumor and never in the normal tissue. Nevertheless, it could not completely be ruled out that these patients might present a mosaicism of *FGFR3* mutations as has already been

shown for epidermal nevi [7]. This might explain the high frequency of *FGFR3*-associated tumors in the subjects from the patient cohort of Hernández et al. [14], although the lack of *FGFR3* mutation in the secondary tumors would argue against this possibility.

Interestingly, *FGFR3* mutations affecting codon 650 in spermatocytic seminomas were recently described [23]. These tumors are a rare variant of testicular germ cell tumors and show a later mean age of onset (approx. 55 years) than common testicular germ cell tumors (approx. 25–35 years). This study also described increased levels of *FGFR3* mutations in sperm from healthy donors at an advanced age. One might speculate that *FGFR3* mutations could occur in specific cells or a subset of tissues which are under constant hormonal influence at an advanced age. But this aspect remains highly speculative and lacks any experimental evidence.

In conclusion, the results of our study revealed no evidence of an involvement of the *FGFR3* mutation in pros-

tate carcinogenesis. Our data are in line with previous published *FGFR3* analyses on smaller prostate cancer cohorts and are strengthened by the inconspicuous *FGFR3* expression in prostate tumors compared to nonmalignant prostate tissue. The high frequency of *FGFR3* mutations in low-grade PCa reported by Hernández et al. [14] could not be confirmed in our cohort and awaits urgent validation.

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