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Original article

FGF-2 is a driving force for chromosomal instability and a stromal factor associated with adverse clinico-pathological features in prostate cancer

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Abstract

Background: There is mounting evidence to suggest that stromal cells play an integral role in the progression of prostate cancer (PCa). One of the most frequently altered growth factors in PCa is fibroblast growth factor-2 (FGF-2). It has previously been proposed that early stages of PCa are characterized by a primarily exogenous, that is, stromal cell-derived FGF-2 production, whereas advanced tumors rely more on an autocrine FGF-2 production. Prostate cancer progression is characterized by an increase of genomic instability including aneuploidy and structural chromosomal alterations. Herein, we address 2 problems that have not been comprehensively answered. First, we ask whether exogenous FGF-2 can directly drive genomic instability to promote PCa progression. Second, we investigate whether and to what extent stromal FGF-2 expression is maintained in advanced PCa and whether this influences tumor progression and patient prognosis.

Methods: In vitro experiments to investigate the role of FGF-2 in numerical and structural chromosomal instability were performed using immunofluorescence microscopy, fluorescence in situ hybridization and single cell electrophoresis. A human patient-derived xenograft mouse model recapitulating osteoblastic PCa bone metastasis was used for in vivo validation experiments. The prognostic role of stromal FGF-2 expression was analyzed using immunohistochemical staining of a tissue microarray with primary tumor specimens from 162 predominantly high-risk patients with PCa.

Results: Our results show that FGF-2 not only rapidly induces mitotic defects and numerical chromosomal imbalances but also an enhanced DNA breakage to promote chromosomal instability. Using the patient-derived xenograft model, we show that a deregulation of the FGF axis results in an increase of mitotic aberrations as well as DNA damage checkpoint activation in vivo. The FGFR inhibitor dovitinib was found to reduce numerical chromosomal instability as well as DNA breakage, thus underscoring the relevance of the FGF axis in promoting genomic instability. An overexpression of tumor cell-associated FGF-2 was detected in 52 of 162 patients (32.1%), whereas a stromal overexpression was found in 27 of 165 patients (16%). Remarkably, a strong stromal FGF-2 expression was associated with a significantly higher clinical stage and higher biochemical recurrence rate. Patients with strong stromal FGF-2 expression also had a significantly worse biochemical recurrence-free survival.

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Conclusions: Our results underscore that exogenous FGF-2 can shape PCa cell genomes and that stromal FGF-2 expression is detectable in a sizeable proportion of advanced PCa where it is associated with adverse clinico-pathological features. Our results highlight the impact of the tumor stroma on malignant progression and provide a rationale for a further exploration of components of the tumor stroma as therapeutic targets in PCa.

Keywords: Prostate cancer; FGF-2; Genomic instability; PDX model

1. Introduction

Prostate cancer is a leading cause of cancer-related morbidity and mortality in men in most industrialized countries [1]. Although the precise mechanisms of prostate cancer (PCa) progression and metastasis are still poorly understood, a number of findings suggest that these processes are not strictly tumor cell-specific but also shaped by strong influences of the tumor stroma. One growth factor family that has been extensively studied in this context are fibroblast growth factors (FGFs) [2–5].

Numerous in vitro and in vivo results underscore a crucial role of FGFs and their receptors in prostate carcinogenesis and tumor progression [3,6,7]. The human FGF gene family consists of over 20 members that encode secreted polypeptides. FGFs exert their activities mainly through 4 conserved transmembrane tyrosine kinase receptors (FGFR1–4). Downstream signaling events are complex and mainly consist of activation of the phosphatidylinositol 3-kinase (PI3K)/AKT, mitogen-activated kinase, phospholipase Cγ (PLCγ) and signal transducers and activators of transcription signaling pathways. FGFs are mitogenic, they increase cell survival and migration and stimulate angiogenesis [2].

There is compelling evidence that in particular upregulation of FGF-2 (basic FGF) plays an important role in prostate carcinogenesis [3,4]. A major source of FGF-2 in PCa is the stromal cell compartment as well as the extracellular matrix [6]. FGF-2 can also be produced by PCa cells to function in an paracrine or autocrine fashion [6]. A knock-out of FGF-2 was found to delay tumor progression in the transgenic adenocarcinoma of the mouse prostate model [8]. In addition, dovitinib, a small molecule multi-kinase inhibitor that exerts antitumoral activities through inhibition of the FGF and PDGF signaling axis, respectively, has shown promising clinical effects in FGFR-driven PCa patient-derived xenograft (PDX) models and in PCa patients with bone metastasis [9].

Prostate cancer progression is characterized by increasing genomic instability including numerical chromosomal aberrations (aneuploidy) as well as structural chromosomal changes [10,11]. Although aneuploidy is typically a result of mitotic defects, structural chromosomal instability requires DNA breaks that are left unrepaired or repaired in an erroneous fashion [12].

In the present report, we demonstrate that exogenous FGF-2 can provoke numerical chromosomal imbalances as well as DNA breakage both in vitro and in a PDX mouse model. In addition, we show that stroma-associated FGF-2 is a negative prognostic factor in high-risk PCa thus underscoring the important role of the tumor stroma in malignant progression. Our findings highlight that PCa cell genomes can be shaped by factors derived from the tumor stroma and that targeting these processes represents a promising therapeutic option [5].

2. Material and methods

2.1. Cell culture and drug treatments

LNCaP and PC-3 PCa cells were provided by CLS (Eppelheim, Germany) and maintained as recommended by the distributor. For experiments, cells were treated with 10 ng/ml biologically active recombinant FGF-2 (rFGF-2; NovActive, NBC1–21335, Novus Biologicals, Littleton, CO; migrating at 23 kDa and 26 kDa in SDS-PAGE) for 72 hour or dH_2O used as solvent control. Cells were treated with 1 μM dovitinib (TKI-258; Selleck Chemicals, Houston, TX) for 72 hour or 0.1% dimethyl sulfoxide (DMSO) as solvent control. Cells were also treated with rFGF-2 and dovitinib in combination where indicated.

2.2. Immunofluorescence microscopy

LNCaP or PC-3 cells were grown on 10 mm coverslips until they reached subconfluent density. Cells were then washed twice with cold PBS and fixed in methanol for 20 minute at room temperature and afterwards washed with cold PBS 3 times for 10 minute. After blocking in 5% BSA for 30 minute at 4°C and washing with cold PBS for 10 minute, the cells were incubated with an anti-γ-tubulin-antibody (GTU-88, Sigma-Aldrich, St. Louis, MO) or anti-53BP1-antibody (Novus Biologicals, NB100–304) at 4°C overnight. Subsequently, the cells were washed in cold PBS and then incubated with a secondary antibody labeled with a fluorescent dye (Life Technologies, Carlsbad, CA). Cells were washed in cold PBS and nuclei were counterstained with DAPI (Vectashield, Vector Laboratories, Burlingame, CA). At least 100 cells were analyzed per experiment using a Leica DM5000 B epifluorescence microscope (Wetzlar, Germany) and documented using Leica's LAS software package. The percentage of cells with either abnormal centrosome numbers, undergoing aberrant mitosis or