Tumor and Stem Cell Biology

FGF-2 Disrupts Mitotic Stability in Prostate Cancer through the Intracellular Trafficking Protein CEP57

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Abstract

Malignant tumors with deregulated FGF-2 expression such as prostate cancer are also frequently aneuploid. Aneuploidy can be caused by cell division errors due to extra centrosomes and mitotic spindle poles. However, a link between FGF-2 overexpression and chromosome missegregation has so far been elusive. Here, we show that FGF-2 rapidly uncouples centrosome duplication from the cell division cycle in prostate cancer cells through CEP57, an intracellular FGF-2-binding and trafficking factor. CEP57 was initially identified as a regulator of centriole overduplication in an RNA interference screen. We subsequently found that CEP57 rapidly stimulates centriole overduplication and mitotic defects when overexpressed and is required not only for FGF-2-induced centriole overduplication but also for normal centriole duplication. We provide evidence that CEP57 functions by modulating tubulin acetylation, thereby promoting daughter centriole stability. CEP57 was found to be overexpressed on the mRNA and protein level in a subset of prostate cancers, of which the vast majority also showed FGF-2 upregulation. Taken together, our results show an unexpected link between altered microenvironmental signaling cues such as FGF-2 overexpression and mitotic instability and provide a rationale for the therapeutic targeting of the FGF-2/FGFR1/CEP57 axis in prostate cancer. Cancer Res; 73(4); 1400–10. ©2012 AACR.

Introduction

Aneuploidy, that is, numerical whole chromosome imbalances, is a frequent finding in most human cancers including prostate cancer (1–3). Aneuploidy correlates with malignant progression, which is underscored by the finding that virtually all metastatic prostate cancers show such chromosomal aberrations. In addition, chromosomal imbalances have also been implicated in the development of castration resistance, a hallmark of lethal prostate cancer (4). Many aneuploid tumors harbor alterations in key signal transduction pathways but our knowledge of how aberrant signaling events contribute to chromosomal instability is incomplete (5).

Alterations of fibroblast growth factor (FGF) signaling are likewise very common in prostate cancer and other human malignancies. Numerous in vitro and in vivo results underscore a crucial role of FGFs and their receptors in carcinogenesis and malignant progression (6–8). The human FGF gene family consists of more than 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 phosphoinositide 3-kinase (PI3K)/AKT, mitogen-activated protein kinase (MAPK), phospholipase Cγ (PLCγ), and STAT signaling pathways. FGFs are mitogenic, they increase cell survival and migration and stimulate angiogenesis (9).

There is compelling evidence that upregulation of FGF-2 (basic FGF) plays an important role in prostate carcinogenesis and malignant progression (10). A major source of FGF-2 in the prostate is the stroma cell compartment, in which FGF-2 is secreted to function as a paracrine growth factor for prostate epithelial cells, which produce FGFFRs. A switch from paracrine to autocrine FGF-2 production by prostate cancer cells has been reported (6). Knockout of FGF-2 was found to delay tumor progression in transgenic adenocarcinoma of the mouse prostate (TRAMP) model (11). Whether and how aberrant FGF-2 expression can contribute to chromosomal instability in prostate cancer is not known.

Centrosomal protein 57 kDa (CEP57; also referred to as translokin) specifically interacts with the 18 kDa isoform of FGF-2 (FGFR-2). CEP57 is essential for the intracellular transport of FGF-2 to the nucleus and the perinuclear compartment in a microtubule-dependent manner after internalization of the FGFR1-bound protein (13). Although the precise function of nuclear FGF-2 is unclear, its nuclear localization was found to be required for its mitogenic activity. CEP57 contains a centrosomal localization and multimerization domain in its N-terminal half and a microtubule localization and stabilization...
domain in its C-terminal half (14). CEP57 binds microtubules and its ectopic expression was found to cause excessive microtubule bundling and the formation of basket-like microtubule structures (14). Recently, mutations in CEP57 have been identified as a cause of the autosomal-recessive mosaic variegated aneuploidy syndrome, which is characterized by mosaic aneuploidies, growth retardation, and cancer predisposition (15).

In most mammalian cells, microtubules are organized by cellular organelles known as centrosomes (16). Centrosomes normally consist of a pair of centrioles embedded in pericentriolar material (PCM). Their microtubule-organizing function involves the recruitment of γ-tubulin ring complexes to the PCM. Nondividing cells harbor a single centrosome, which must duplicate before mitosis. This process is tightly controlled as extra centrosomes can have detrimental effects on mitotic fidelity through multipolar mitoses or aberrant spindle–kinetochore attachment (5). On a molecular level, the formation of a new (daughter) centriole in proximity to the preexisting (maternal) centriole is initiated by CEP152-mediated recruitment of polo-like kinase 4 (PLK4) and involves the interactions of several additional centrosomal proteins including hSAS-6, CPAP, CEP135, and CP110 (17–25). A number of human oncogenes and tumor suppressor proteins have been implicated in centrosome aberrations and there is evidence that acute centrosome overduplication can drive malignant progression (26–29). Centrosome aberrations are a frequent finding in cancer including prostate cancer, in which centrosome defects increase with malignant grade and stage and correlate with aneuploidy (30, 31).

Here, we show that stimulation of prostate cancer cells with FGF-2 rapidly uncouples centriole duplication from the cell division cycle in a CEP57-dependent manner. We found that CEP57 itself promotes centriole overduplication and that CEP57 is a component of the PLK4-dependent centriole overduplication pathway. We furthermore provide evidence that CEP57 contributes to centriole amplification by stabilizing daughter centrioles. CEP57 was found to be overexpressed in a subset of primary prostate adenocarcinomas on the transcriptional and protein level and the majority of these tumors also overexpressed FGF-2. Taken together, our results provide evidence for a novel link between FGF-2 and mitotic stability that involves the FGF-2 trafficking factor CEP57. Our results warrant further exploitation for the development of improved prognostic biomarkers and FGF-2/CEP57–targeted therapies in prostate cancer.

Materials and Methods
Cell culture, transfections, and drug treatments
Human U-2 OS and LNCaP cells as well as mouse NIH 3T3 cells were obtained from American Type Culture Collection and maintained according to distributor’s recommendations. U-2 OS cells were manipulated to stably express centrin-1-GFP (kindly provided by Michel Bornens, Institut Curie, Paris, France; ref. 32). Human pCMV6-CEP57 was obtained from Origene. For transient transfections, plasmids encoding pCMV6-CEP57, mCEP57-Flag (kindly provided by Ko Momo-tani, University of Virginia, Charlottesville, VA), pcDNA3-PLK4-myc, or pcDNA-PLK4-D154-myc (both kindly provided by Erich Nigg, Biocenter, University of Basel, Basel, Switzerland) were used. Cells were transfected by lipofection using Fugene 6 (Roche) or the NEON transfection system (Life Technologies) and protein expression was monitored by immunoblotting. DsRED- or cyan fluorescent protein (CFP)-encoding plasmids (Clontech) were cotransfected as transfection markers. Cells were treated with Z-Lys/VS (Biomol) at a 1 μmol/L concentration or 0.1% dimethyl sulfoxide (DMSO) as solvent control. Cells were treated with nocodazole at a 2 μmol/L concentration dissolved in DMSO for time intervals indicated. Cells were treated with hydroxyurea at a 1 mmol/L concentration dissolved in dH2O for 48 hours. Cells were treated with cycloheximide at a 30 μg/mL concentration dissolved in dH2O for the time intervals indicated. LNCaP cells were treated with 10 ng/mL FGF-2 (Novus Biological) for the time intervals indicated with dH2O used as solvent control.

Immunostaining
For immunofluorescence microscopic analyses, cells were grown on 10-mm round coverslips, removed at subconfluent cell density and fixed in 4% parafomaldehyde/PBS for 15 minutes at room temperature, followed by washing in PBS and permeabilization with 1% Triton-X-100 in PBS for 20 minutes. After blocking in 10% normal donkey serum (Jackson ImmunoResearch), cells were incubated with primary antibodies overnight. The next morning, cells were washed at 37°C for 2 hours, washed in PBS, and incubated with secondary antibodies for 2 hours and mounted with 4′,6-diamidino-2-phenylindole (DAPI)-containing mounting media (Vectorshield; Vector Laboratories) to visualize nuclei. Cells were analyzed using an Olympus AX70 epifluorescence microscope or a Leica DM5000 epifluorescence microscope. Primary antibodies used were directed against γ-tubulin (Sigma-Aldrich), acetylated α-tubulin (Life Technologies), CAP350 (Bethyl), CEP57 (Sigma-Aldrich), CEP170 (Invitrogen), PLK4 (kindly provided by Erich Nigg, Biocenter, University of Basel, Basel, Switzerland), or hSAS-6 (Bethyl). Secondary antibodies used were conjugated with fluorescein isothiocyanate, Rhodamine Red, or aminomethylcoumarin acetate. Tissue microarrays (TMA) were obtained from US Biomax. Paraffin-embedded tissue specimens were deparaffinized and rehydrated by standard procedures (33). Antigen retrieval was conducted using citrate buffer (pH 6.0). Primary antibodies were directed against CEP57 (Abcam) or FGF-2 (Santa Cruz) and incubated overnight at 37°C. The Histostain Plus Kit (Life Technologies) was used for immunodetection of primary antibodies. Sections were counterstained with hematoxylin (Life Technology). Immunoblotting was conducted as previously described (34).

siRNA
An siRNA library targeting known centriolar proteins as described previously (35, 36) was obtained from Qiagen (2 different siRNAs per target; see Supplementary Table S1 for sequences). For the centriole overduplication screen, U-2 OS/centrin-GFP cells were grown on coverslips in 12-well tissue culture plates with 0.5 mL antibiotics-free Dulbecco’s Modified...
Eagle’s Medium. Cells were transfected with 3 μL of 20 μmol/L annealed RNA duplexes using oligofectamine (Life Technology) transfection reagent. Twenty-four hours posttransfection, cells were treated with 1 μmol/L of the proteasome inhibitor Z-L3VS and were microscopically analyzed 48 hours later. siRNA duplexes that yielded a more than 20% decrease of the proportion of cells with overduplicated centrioles normalized to Z-L3VS–treated, control siRNA-transfected cells in 2 independent rounds of screening were considered as positive hits. For confirmation of hits and further analyses, synthetic RNA duplexes were obtained from Qiagen and cells cultured in 60-mm dishes with 2 mL of antibiotics-free media were transfected with 12 μL of 20 μmol/L annealed RNA duplexes. Knockdown efficiency was monitored by immunoblotting and quantitative real-time PCR (qRT-PCR).

**Tissue qPCR analysis**

The TissueScan Prostate Cancer cDNA Array III (Origene) containing 39 prostate cancer and 9 normal prostate cDNA samples from total RNA obtained from pathologist-verified tissues, normalized and validated with β-actin in 2 sequential quantitative PCR (qPCR) analyses was used. cDNAs were resuspended according to manufacturer’s protocol. qPCR was conducted using specific primers to CEP57 (forward: 5′-AAAGCTGTTGGGATCCTTC-3′, reverse: 5′-GGGAGCCTGTCTAGGTACATATTA-3′: Integrated DNA Technologies) and measured using the SsoFast EvaGreen Kit (Bio-Rad) according to the manufacturer’s protocol. Cycling conditions were 95°C (10 seconds, activation), 95°C (5 seconds, denaturation), 60°C (10 seconds, annealing/extension) for 40 cycles on a Bio-Rad CFX96 Real-Time System run on a C1000 Thermal Cycler (Bio-Rad).

**Statistical analysis**

Student two-tailed t test for dependent or independent samples, Fisher exact probability test or the χ2 test were used wherever applicable. P values less than 0.05 were considered statistically significant.

**Results**

**CEP57 is a novel regulator of centrosome duplication**

To identify unknown regulators of centriole overduplication, we used an siRNA library including genes identified as part of the centrosome proteome by Andersen and colleagues (35; Supplementary Table S1). We identified 4 proteins that, when depleted, led to a reproducible reduction of the proportion of cells with centriole overduplication induced by Z-L3VS treatment (34). An at least 20% reduction in 2 consecutive independent screens was detected when cells were depleted of PLK4, hSAS-6, C4Orf15 (HAUS3), or CEP57. Whereas PLK4 and hSAS-6 have previously been implicated in centriole overduplication (17, 22), HAUS3 has recently been identified as an important factor for structural centrosome integrity in a similar RNA interference screen (37). We therefore focused on CEP57 and its potential role in centriole duplication control.

Confirmatory experiments showed a statistically significant 1.8-fold reduction of the proportion of cells with centriole overduplication (>4 per cell, >1 daughter at a single maternal centriole) from 62.7% in Z-L3VS–treated controls to 35.3% in CEP57-depleted Z-L3VS–treated U2-OS/centrin-GFP cells (P < 0.0001; Fig. 1A). Our definition of centriole overduplication ensures that only cells with aberrant synthesis of daughter centrioles are counted and not cells in which centrioles merely accumulate. Similar results were obtained with a different siRNA against CEP57.

A statistically significant 1.7-fold reduction of cells with abnormal centrosome numbers (>2 per cell as detected by immunofluorescence for the PCM marker γ-tubulin) was detected in murine CEP57 (mCEP57)–depleted NIH 3T3 fibroblasts treated with Z-L3VS from 40.9% in controls to 23.8% (P > 0.0001; Fig. 1B).

CEP57 was found to be present at the centrosome throughout the cell division cycle and to colocalize with a marker of mature maternal centrioles, CEP170, and PLK4 (Supplementary Fig. S1), respectively. We therefore tested next a possible role of CEP57 in PLK4-induced centriole overduplication. Ectopic expression of PLK4 has previously been shown to effectively stimulate centriole overduplication with a characteristic centriole multiplication or ”centriole flower” phenotype (21, 34), in which multiple daughter centrioles form concurrently at single mothers (Fig. 1C, left). This phenotype was significantly reduced by 1.6-fold from 47.8% in controls compared with 29.3% in PLK4-transfected cells in which CEP57 had been depleted (P < 0.0001; Fig. 1C, right). In contrast, knockdown of CEP57 in cells treated with hydroxyurea, which is believed to stimulate centriole overduplication through a...
different mechanism (centriole reduplication in early S-phase), had no effect on the proportion of cells with centriole overduplication (>4 centrioles per cell; Fig. 1D). To further corroborate that PLK4-induced centrin-GFP dots were in fact centrioles, we stained PLK4-transfected cells and controls for CEP57 and hSAS-6, which associates with nascent precentrioles (21). Centrin-GFP clearly colocalized with hSAS-6 dots underscoring a true centriole overduplication (Fig. 1E). These results suggest that CEP57 participates in PLK4-induced centriole overduplication but is not required for centriole overduplication during a prolonged S-phase arrest.

We next determined the effects of ectopically expressed CEP57 on centrosome numbers. Overexpression of mCEP57 in NIH 3T3 fibroblasts (Fig. 1F) led to a significant 3.9-fold increase of the proportion of cells with abnormal centrosome numbers (>2 per cell) from 5.1% to 20.1% when stained for γ-tubulin (P < 0.0001; Fig. 1G, left). This increase of centrosome aberrations was due to centriole overduplication as cells with aberrant numbers (>4) of FGFR1 oncogene partner (FOP) dots to visualize individual centrioles were significantly increased by 4.1-fold from 6% in controls to 24.8% (P < 0.0001; Fig. 1G, right).

To test whether overexpression of CEP57 also causes centriole overduplication in human cells, we ectopically expressed CEP57 in U2 OS/centrin-GFP cells. Both endogenous and overexpressed CEP57 were found to localize to the centrosome in these cells with an occasional formation of basket-like structures under conditions of overexpression as previously described (Supplementary Fig. S2). We found a statistically significant 11.0-fold increase of cells with genuine centriole overduplication (>4 per cell, >1 daughter at a single maternal centriole; Fig. 1H) from 0.7% in control-transfected cells to 7.7% in CEP57-transfected cells (P < 0.0001; Fig. 1H, middle). This increase was confirmed using FOP as centriolar marker with 3.2% of controls showing more than 4 FOP dots and 9.8% of CEP57-transfected U2 OS cells (3.1-fold; P < 0.001; Fig. 1H, right).

CEP57-induced supernumerary centrioles and centrosomes were functional as they were associated with a significant increase of cells with supernumerary mitotic spindle poles leading to either a pseudobipolar mitotic spindle pole arrangement or multipolar mitoses as evidenced by γ-tubulin staining (Fig. 1I, left and middle). CEP57-expressing cells with aberrant mitoses (pseudobipolar or multipolar) were in fact able to enter later stages of the cell division cycle as evidenced by a significant increase of cells with abnormal numbers of mitotic spindle poles in ana-/telophase (P < 0.05; Fig. 1I, right).

Finally, we determined the effect of CEP57 depletion on centrosome maintenance. We found that depletion of CEP57 in U2 OS/centrin-GFP cells by siRNA led to a reduction of centrioles to a significant increase of cells with only 1 centriole reaching 10.4% monocentriolar cells at 72 hours post-siRNA transfection (P < 0.0001). In addition, normal centriole duplication was found to be impaired in CEP57-depleted cells as the proportion of cells with fully duplicated centrioles (n = 4) was found to be significantly reduced (Fig. 1J).

Taken together, these results underscore that CEP57 is a novel regulator of centriole biogenesis and required for PLK4-induced centriole overduplication as well as normal centriole duplication.

**CEP57 stabilizes daughter centrioles**

We next sought to determine the mechanism through which CEP57 participates in centriole duplication control.

It has been shown that centriole assembly can be reverted by the microtubule-depolymerizing agent nocodazole without affecting the initiation of centriole duplication (38). To explore a possible role of microtubule stability control in CEP57-induced centriole overduplication, we transfected U2-OS/centrin-GFP cells with either PLK4 alone or PLK4 in combination with CEP57 (Fig. 2A, left). After 48 hours, cells were treated with nocodazole for 30 or 60 minutes and the percentage of cells in which centriole overduplication was detectable was assessed. We found that cells transfected with PLK4 alone showed a decline in the proportion of cells with centriole overduplication from 40.8% at t₀ to 25.5% at t₆₀ min nocodazole treatment (1.6-fold reduction; P < 0.0001; Fig. 2A, left). Cells transfected with PLK4 and CEP57 in combination showed more centriole overduplication at t₀ and the decrease of cells with centriole overduplication was less pronounced from 54.4 at t₀ to 47.6% at t₆₀ min (0.9-fold reduction; P < 0.005; Fig. 2A, left).

These results indicate that supernumerary daughter centrioles are more resistant to nocodazole-induced microtubule depolymerization in the presence of CEP57. We next wanted to confirm that the stabilized structures were in fact centrioles and in particular daughter centrioles. We stained U2-OS/centrin-GFP cells for the FOP-interacting protein CAP350, which localizes to centrosomes and has been reported to stabilize procentrioles. We found CAP350 to localize preferentially to daughter centrosomes surrounding a maternal centriole in untreated cells (Supplementary Fig. S3). After 60-minute nocodazole treatment, there was an increased abundance of CAP350 in CEP57-transfected cells in comparison with controls suggesting the presence of more aberrant daughter centrioles (Supplementary Fig. S3).

We next wanted to explore the effect of CEP57 on normal centriole duplication and we transfected U2-OS/centrin-GFP cells with empty vector or CEP57 and repeated the nocodazole experiment but now determined the proportion of cells with normally duplicated centrioles (3 or 4 per cell; Fig. 2A, right). We found that empty vector controls and CEP57-transfected cells showed the same proportion of cells with duplicated centrioles at t₀ (62%). Control cells showed a gradual decrease of cells with duplicated centrioles to 51% at t₆₀ min (P < 0.0001) and 33% at t₁₈₀ min (P < 0.001). In CEP57-expressing cells, however, this decrease was noticeably attenuated (58% at t₆₀ min P > 0.005; and 53% at t₁₈₀ min P < 0.005) suggesting that the stabilizing effect of CEP57 in the presence of nocodazole may be more pronounced on aberrantly synthesized rather than normal daughter centrioles, which is in line with a previous report (38).

To further explore how CEP57 may affect daughter centriole stability, we stained empty vector or CEP57-expressing U2-OS/centrin-GFP cells for acetylated α-tubulin after 180-minute treatment with nocodazole (Fig. 2B). CEP57-expressing cells...
were found to show a higher abundance of acetylated α-tubulin at centrioles after nocodazole treatment than controls (compare top right and bottom right in Fig. 2B at t = 180 min). This finding suggests that CEP57 increases tubulin acetylation to stabilize daughter centrioles.

To assess this increase more accurately, we conducted image quantification of both acetylated α-tubulin and centrin-GFP using the UN-SCAN-IT software (Silk Scientific) and calculated the ratio between the 2 values to account for differences in centriole numbers. We found that acetylated α-tubulin was significantly more abundant after 60-minute nocodazole treatment in cells transfected with PLK4 and CEP57 compared with PLK4 alone (2.2-fold; P < 0.0001; Fig. 2C, left). We repeated this experiment with U-2 OS/centrin-GFP cells transfected with empty vector or CEP57 alone and found a significant increase of the abundance of acetylated α-tubulin associated with daughter centrioles after 180-minute in CEP57-transfected cells compared with controls (Fig. 2C, right).

Finally, we sought to show biochemically that CEP57 enhances α-tubulin acetylation. Cells transfected with PLK4 alone or in combination with CEP57 were treated with nocodazole and analyzed by immoblotting for the abundance of acetylated α-tubulin. Expression levels of acetylated α-tubulin were higher in CEP57-expressing cells treated with nocodazole in comparison with controls (Supplementary Fig. S4). The finding that the detected increase was relatively modest is most likely related to the fact that CEP57 stabilizes mostly daughter centriolar α-tubulin and that microtubule bundling and basket formation was overall relatively rare in our experimental system (less than 5% of cells). These results show that CEP57-expressing cells can maintain α-tubulin acetylation in the presence of nocodazole as a mechanism of resistance to tubulin depolymerization.

Taken together, these results suggest a role of CEP57 in promoting daughter centriole stability through posttranslational modification of α-tubulin.

**FGF-2 disrupts centriole duplication control through CEP57**

Given the role of CEP57 as intracellular trafficking protein for FGF-2 (12), we sought to explore next whether FGF-2 itself has an effect on centrosome duplication control. Human LNCaP prostate cancer cells, which express FGFR1, were treated with recombinant FGF-2 (rFGF-2) followed by an analysis of centrosome numbers (Fig. 3A). We found that stimulation with 10 ng/mL of rFGF-2 led to a significant increase of cells with supernumerary centrosomes as evidenced by immunostaining for γ-tubulin from 0.5% to 6.7% after 8 hours (13.4-fold; P < 0.0001) and from 1.1% to 11% after 24 hours (10-fold; P < 0.0001; Fig. 3A). These aberrantly synthesized centrosomes were functional as rFGF-2 stimulation of U-2 OS/centrin-GFP cells also led to a modest increase of cells in S-phase (49.4% BrdUrd-positive cells vs. 56.7% BrdUrd-positive cells after 24 hours rFGF-2 stimulation). At the same time, we detected an increase of cells with genuine centriole overduplication, that is, aberrant daughter centriole assembly at maternal centrioles (Fig. 3C). Centriole overduplication increased from 0% to 2.2% after 8-hour stimulation with rFGF-2 (P < 0.0005), from 0.5% to 4.7% after 24-hour stimulation with rFGF-2 (P < 0.0001), and from 0.2% to 7.2% after 48-hour rFGF-2 stimulation (P < 0.0001), each in comparison with controls (Fig. 3C, right). These results show

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*Figure 2*. CEP57 stabilizes daughter centrioles. A, left, quantification of the percentage of U-2 OS/centrin-GFP cells with overduplicated (n > 4) centrioles after transfection with either PLK4 alone or CEP57 and PLK4 (48 hours) followed by treatment with nocodazole for the indicated time intervals. Right, quantification of the percentage of U-2 OS/centrin-GFP cells with normally duplicated centrioles (3 or 4) after transfection with empty control vector or CEP57 (48 hours) followed by treatment with nocodazole for the indicated time intervals. B, immunofluorescence microscopic analysis of U-2 OS/centrin-GFP cells for acetylated α-tubulin and centrin-GFP signals following the experimental procedure described in A. Each box/bar represents mean and SE of at least 3 independent experiments.
that FGF-2 can rapidly uncouple centriole duplication from the cell division cycle.

To determine the role of CEP57 in FGF-2–induced centrosome overduplication in LNCaP cells, we used an siRNA approach. Control and CEP57-depleted cells were treated with 10 ng/mL rFGF-2 for 24 hours. A significant increase of cells with abnormal centrosome numbers was detected in control cells from 0.5% to 8.3% (16.6-fold; \( P < 0.0001 \)), whereas FGF-2 was unable to stimulate centrosome overduplication in CEP57-depleted cells (1% vs. 2%; \( P > 0.05 \); Fig. 3D).

We next analyzed the role of FGFR1 in FGF-2–induced centrosome overduplication in LNCaP prostate cancer cells. We found that siRNA-mediated depletion of FGFR1 significantly impaired the ability of FGF-2 to stimulate centrosome overduplication (0.3% in control-treated vs. 2.7% in FGF-2–treated cells) in comparison with control-depleted cells (0.2% vs. 11.2%; Fig. 3E).

Taken together, our results show that FGF-2 rapidly uncouples the cell cycle from the centriole duplication cycle in a CEP57- and FGFR1-dependent manner.

**CEP57 is overexpressed in prostate cancer**

CEP57 expression has not been analyzed in human cancer so far. To explore whether prostate cancer, in which disruption of FGF-2 expression is a frequent event (6, 8), may also show altered CEP57 expression, we first conducted a qRT-PCR analysis of 9 normal and 39 cancerous prostate samples using a commercially available qPCR gene expression array.
containing normalized and validated cDNA samples (Fig. 4A). The mean $C_q$ for normal samples was 32.77 ± 0.36 (SD). Of 39 prostate cancer samples, 2 tumors showed a $C_q$ value greater than mean plus 3 times SD (33.85 cycles) indicating reduced mRNA expression in comparison with controls (5.1%). A total of 15 prostate cancers showed a $C_q$ below the mean minus 3 times SD (31.69 cycles) indicating increased mRNA expression (38.5%). These findings show CEP57 transcriptional deregulation, most prominently upregulation, in a subset of human prostate cancers.

To further corroborate and extend these results, we conducted an immunohistochemical analysis of normal ($n=10$), hyperplastic ($n=19$), and cancerous ($n=48$) prostate specimens using a TMA. In comparison with normal and hyperplastic tissue specimens, which showed a weak to moderate CEP57 staining (90% and 89.5%, respectively), an overexpression of CEP57 was detected in 12 of 48 of prostate adenocarcinomas (25%), whereas normal or hyperplastic tissue samples showed no overexpression of CEP57 ($P<0.01$). In a fraction of prostate cancer samples, we also detected a loss of CEP57 protein expression (27.1%). In addition, we conducted an immunohistochemical analysis of an adjacent section of the same TMA for FGF-2. Most normal and hyperplastic tissue samples showed a weak to moderate protein expression (79% and 63.2%, respectively), whereas 83.3% of the prostate adenocarcinomas showed FGF-2 overexpression ($P<0.0001$). FGF-2 was predominantly found to be expressed by carcinoma cells. Autocrine/paracrine production of FGF-2 has been reported for more advanced prostate cancer and most prostate cancers analyzed here had a T2 stage or higher. Importantly,

Figure 4. CEP57 is overexpressed in prostate cancer. A, qPCR analysis of 9 normal and 39 cancerous prostate specimens for CEP57 mRNA expression. Relative fluorescence units are given. Red amplification curves represent normal control tissue, blue amplification curves represent prostate cancer samples. B, immunohistochemical analysis of benign prostate specimens (left) and prostate adenocarcinomas (right) for CEP57 and FGF-2, respectively. Note the strong overexpression of both proteins in the prostate cancer specimens. Scale bar, 50 μm. C, overview of the immunohistochemical staining results for CEP57 and FGF-2 obtained from the TMA used in B. D, quantification of LNCaP cells with abnormal centriole numbers (>4 FOP dots per cell) after transfection with CEP57 or empty vector. E, quantification of LNCaP cells with abnormal centriole numbers (>4 centrin-GFP dots, plasmid was cotransfected) transiently transfected with CEP57 (24 hours) followed by stimulation with rFGF-2 (24 hours). Each bar represents mean and SE of at least 3 independent experiments.
11 of 12 prostate cancers with overexpression of CEP57 showed also an overexpression of FGF-2 (91.7%).

To directly test the ability of CEP57 to induce centriole aberrations in prostate cancer cells, we transiently transfected LNCaP cells with CEP57. Both endogenous and overexpressed CEP57 were found to show a centrosomal staining pattern in LNCaP cells, again, with an occasional formation of basted structures in cells with overexpressed CEP57 (Supplementary Fig. S5). When CEP57-transfected LNCaP cells were analyzed for centriole numbers by immunofluorescence microscopy for FOP, a significant increase from 5.2% of cells with more than 4 FOP dots per cell in controls to 10.0% was detected (1.9-fold; \( P < 0.05 \); Fig. 4D). In addition, we found that stimulation of CEP57-overexpressing LNCaP cells with rFGF-2 led to a further increase of cells with abnormal centriole numbers from 15.3% to 20.5% (\( P < 0.05 \); Fig. 4E).

Taken together, these data indicate that CEP57 is overexpressed in a fraction of prostate adenocarcinomas on the mRNA and protein level and, furthermore, that there is a high coincidence of CEP57 and FGF-2 overexpression. In vitro recapitulation of CEP57 overexpression and FGF-2 stimulation in LNCaP prostate cancer cells led to a high frequency of centriole aberration.

Discussion

There is compelling evidence from numerous in vitro as well as in vivo studies that aberrant FGF signaling plays a crucial role in prostate cancer development and progression (6, 8). Results presented here provide additional insights into the oncogenic activities of the FGF signaling axis. Our results provide the first evidence that a microenvironmental, secreted growth factor, FGF-2, can subvert mitosis, and hence promote chromosomal instability. They show that the FGF–2–binding and trafficking protein CEP57 is a critical link in this process. Our data furthermore provide the first evidence of CEP57 overexpression in a major human cancer, that is, prostate cancer. They also establish CEP57 as a novel regulator of centriole duplication through centriole stability control, an understudied but important aspect of centrosome duplication.

We initially identified CEP57 in an RNA interference screen for proteins involved in centriole overduplication. We subsequently discovered that CEP57 is critical for FGF–2–induced centriole overduplication as well as normal centriole duplication. Results showing the formation of extra centrioles at single maternal centrioles underscore that CEP57 causes a genuine centriole duplication defect and not simply an accumulation of centrioles. This is further corroborated by our finding that CEP57 is required for PLK4-induced centriole replication, a process in which multiple daughter centrioles form concurrently around single mothers (21, 34). Our results showing that FGF–2– and CEP57-induced centriole overduplication results in a significant increase of defective mitoses underscores that CEP57-induced extra centrioles are functional and can mature to form mitotic spindle poles.

CEP57 has previously been shown to bind and bundle microtubules and to localize to the centrosome through a C-terminal centrosomal localization domain (14). In the most simplistic model, FGF–2 stimulation would trigger increased FGF–2/FGFR1 internalization and CEP57–dependent transport of FGF–2 to the nuclear membrane (12), thereby shifting more CEP57 to the centrosomal pool. Increased centrosomal CEP57 would then lead to the stabilization of aberrant daughter centrioles. Our finding that CEP57 promotes the posttranscriptional modification of \( \alpha \)-tubulin by acetylation at daughter centrioles (Fig. 2 and Supplementary Fig. S4) supports this notion. Once internalized, additional FGF–2–dependent signaling events, for example MAPK activation, are likely to contribute to centrosome overduplication (39).

The question how a centriole-stabilizing protein may drive centriole overduplication remains to be elucidated. It has previously been shown that maternal centrioles can harbor more than one site of PLK4 recruitment without actual centriole formation (38, 40) and that the stability of elongating procentrioles is a highly regulated process (36, 38). It is hence possible that CEP57 stabilizes procentrioles that normally would not become daughter centrioles. The importance of procentriole stability for centriole growth is underscored by previous studies that implicate CPAP as well as CAP350 in this process (24, 38) and results shown here add CEP57 to this group of microtubule-stabilizing proteins required for proper centriole duplication. The fact that CEP57 overexpression results in an increase in the resistance of microtubules to nocodazole has also been observed for cytoplasmic microtubules (14). The precise molecular mechanisms of daughter centriole stabilization by CEP57 remain to be determined.

Our finding that CEP57 is overexpressed in prostate cancer is the first report to implicate CEP57 in a major human malignancy. However, mutations in CEP57 have recently been identified as a cause of a rare genetic disease associated with cancer predisposition, mosaic-variegated aneuploidy syndrome (MVA; ref. 15). The principal difference between the finding reported here and the implication of CEP57 in MVA is the loss of function in the germline mutation and the significant overexpression on the mRNA and protein level in prostate cancer. To reconcile these findings, it is important to emphasize that many proteins implicated in cancer can have both, oncogenic or tumor suppressive activities depending on the spectrum of their biologic functions, the cellular context and the timing of their expression. Inherited loss of CEP57 could promote cancer through the previously described role of CEP57 role in proper microtubule-kinetochore attachment and/or spindle pole integrity (41, 42). In contrast, its acute overexpression leads to a rapid uncoupling of the centrosome cycle from the cell division cycle and, subsequently, mitotic defects as shown here. Continuous adverse events such as the latter could ultimately cause chromosome missegregation in viable cells, aneuploidy and enhanced clonal evolution and tumor heterogeneity. Our data do not rule out that the massive overexpression seen in a fraction of prostate adenocarcinomas (Fig. 4) may also have growth-suppressive properties for example, through impaired microtubule-dependent transport due to aberrant microtubule bundling induced by CEP57 (14).

Collectively, our results provide a novel link between microenvironmental signaling cues such as FGF–2 and chromosomal instability in cancer. On the basis of the potentially oncogenic activities associated with acute FGF–2/CEP57 overexpression...
reported here, our results would argue in favor of FGF-targeted therapies, at least in a subset of patients with prostate cancer. Clearly, more detailed studies are warranted to dissect the role of CEP57 in prostate tumorigenesis and to fully exploit the FGF-2/FGFR1/CEP57 axis for the development of improved biomarkers and preventive as well as therapeutic approaches.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions

Conception and design: R. Cuevas, S. Duensing

Development of methodology: R. Cuevas, S. Duensing

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): N. Korzeniewski, Y. Tolstov, M. Hohenfellner, S. Duensing

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): R. Cuevas, N. Korzeniewski, S. Duensing

Writing, review, and/or revision of the manuscript: R. Cuevas, Y. Tolstov, M. Hohenfellner, S. Duensing

References


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