Fibroblast growth factor receptor 3-IIIc mediates colorectal cancer growth and migration

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BACKGROUND: Deregulation of fibroblast growth factor receptor 3 (FGFR3) is involved in several malignancies. Its role in colorectal cancer has not been assessed before.

METHODS: Expression of FGFR3 in human colorectal tumour specimens was analysed using splice variant-specific real-time reverse transcriptase PCR assays. To analyse the impact of FGFR3-IIIc expression on tumour cell biology, colon cancer cell models overexpressing wild-type (WT-3b and WT3c) or dominant-negative FGFR3 variants (KD3c and KD3b) were generated by either plasmid transfection or adenoviral transduction.

RESULTS: Although FGFR3 mRNA expression is downregulated in colorectal cancer, alterations mainly affected the FGFR3-IIIb splice variant, resulting in an increased IIIc/IIIb ratio predominantly in a subgroup of advanced tumours. Overexpression of WT3c increased proliferation, survival and colony formation in all colon cancer cell models tested, whereas WT3b had little activity. In addition, it conferred sensitivity to autocrine FGF18-mediated growth and migration signals in SW480 cells with low endogenous FGFR3-IIIc expression. Disruption of FGFR3-IIIc-dependent signalling by dominant-negative FGFR3-IIIc or small interfering RNA-mediated FGFR3-IIIc knockdown resulted in inhibition of cell growth and induction of apoptosis, which could not be observed when FGFR3-IIIb was blocked. In addition, KD3c expression blocked colony formation and migration and distinctly attenuated tumour growth in SCID mouse xenograft models.

CONCLUSION: Our data show that FGFR3-IIIc exerts oncogenic functions by mediating FGF18 effects in colorectal cancer and may constitute a promising new target for therapeutic interventions.

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Pathogenesis of cancer results from progressive deregulation of cell homeostasis. It affects proliferation and cell death, differentiation, cell migration and tissue interactions (Hanahan et al, 1996) by deregulating intracellular signalling pathways. Such deregulation frequently involves receptor tyrosine kinases – a fact that makes those receptors suitable targets for cancer therapy (Zwick et al, 2001). Fibroblast growth factors (FGFs) and their tyrosine kinase receptors (FGFR) have significant roles in development, wound healing and angiogenesis. Consequently, their deregulation can contribute to tumour development at several levels by stimulating proliferation, survival, cell migration and vascularisation of tumours (Eswarakumar et al, 2005).

The human genome contains 22 genes coding for FGFs and four genes coding for functional FGFRs. The receptors in general possess an extracellular ligand-binding domain consisting of three immunoglobulin (Ig)-like loops, a short transmembrane domain and an intracellular kinase domain. Differential splicing creates multiple variants lacking IgG loops, the transmembrane domain or the cytoplasmic domain (Powers et al, 2000). Ligand binding occurs in Ig-like loop III, in which alternative splice variants result in IIIb and IIIc isoforms of FGFR1–3, with different ligand specificities (Powers et al, 2000; Ornitz and Itoh, 2001). IIIb forms are frequently expressed in epithelial tissues, whereas IIIc forms are observed mainly in the mesenchyme (Orr-Urtreger et al, 1993; Murgue et al, 1994; Scotet and Houssaint, 1995), where they mediate tissue interactions in normal development and wound healing, together with ligands expressed in adjacent tissues. Carcinomas have been observed to switch expression of FGFR to the mesenchymal isoform, enabling cells to receive signals usually restricted to the connective tissue, leading to more aggressive phenotypes (Yan et al, 1993; Carstens et al, 1997).

Fibroblast growth factor receptor 3 (FGFR3) has been described as a negative regulator of bone growth (L’Hote and Knowles, 2005) and is also involved in carcinogenesis. Epithelial cells of the pancreas, kidney or lung express the splice-variant FGFR3-IIIb, whereas splice-variant IIIc has been found, for example, in chondrocytes (Murgue et al, 1994; Scotet and Houssaint, 1995;
Among the FGFR3 ligands, FGF18 is upregulated in colon tumourigenesis (Shimokawa et al., 2003), allowing autocrine growth stimulation through FGFR3-IIIc. Accordingly, we have recently demonstrated strong autocrine effects of FGF18 on tumour cell growth and survival (Sonvilla et al., 2008). To investigate the role of FGFR3-IIIc in the pathogenesis of colorectal tumours, we have analysed expression of IIIb and IIIc variants in colorectal tumour tissue and examined the impact of FGFR3-IIIc signalling on growth, survival and migration of colorectal tumour cells in vitro and in vivo.

MATERIALS AND METHODS

Tissue specimen

Tissue specimens of colorectal carcinoma and normal mucosa were obtained from patients undergoing surgery for colorectal cancer after obtaining informed consent from all patients. Immediately after surgery, tissue specimens were frozen in liquid nitrogen until extraction of RNA. The tissue specimen has a tumour cell content of at least 70%, as judged from the histology of immediately adjacent tissue. The study had previous approval of the ethics committee of the Municipal Hospitals of Vienna.

Cell lines

SW480, Caco2 and HCT116 colon carcinoma cell lines were obtained from the American Type Culture Collection. The cell lines were kept under standard tissue culture conditions using Minimal Essential Medium containing 10% foetal calf serum (FCS). The Vaco235 colon adenoma cell line was a gift from JKV Willson (Ireland Cancer Centre, Case Western University, Cleveland, OH, USA) and was maintained as described in the study by Willson et al. (1987). LT97 adenoma cells were maintained as described by Richter et al. (2002). AKH14 and H64 colon carcinoma cell lines were established at our institute from a liver and brain metastasis, respectively, and cultivated in RPMI with 10% FCS.

Isolation of RNA and cDNA synthesis

Total RNA was isolated from subconfluent cultures or frozen colon tissue specimens using Trifast reagent according to the manufacturer’s instructions (PeqLab, Erlangen, Germany). First-strand cDNA was synthesised using RevertAid mouse Moloney leukemia virus (MMuLV) reverse transcriptase (Fermentas, Burlington, Ontario, Canada) and random hexamer primers (GE Healthcare, Piscataway, NJ, USA).

Quantitative RT–PCR analysis

Taqman assays from Applied Biosystems (Foster City, CA, USA) were used to determine expression of total FGFR3 (Hs00179829), FGFR3-IIIc (Hs00997397-m1) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Hs99999905-m1) mRNAs using quantitative reverse transcriptase PCR (RT–PCR). Probes specific for FGFR3-IIIb were custom designed by Applied Biosystems to recognise the exon 6/7 boundary specific for IIIb splice variants. Reactions were carried out on an ABI PRISM 7900 system (Applied Biosystems) using standard Taqman assay conditions. The FGFR3 gene expression in the cells and tumours was calculated as x-fold change compared with the corresponding normal mucosa using GAPDH as the control gene for normalisation using a standard curve method. In addition, selected RT–PCR experiments were performed using cycles of 40 s denaturation at 94°C, 40 s annealing at 56°C and 40 s extension at 72°C. Amplifications were carried out for 40 and 23 cycles in case of FGFR and GAPDH, respectively. PCR products were separated on 6% acrylamide gels. Bands were stained with ethidium bromide and quantified using a GelDoc system (Biorad, Hercules, CA, USA) and Image Quant 5.0 (GE Healthcare) software.

Overexpression of FGFR in colon carcinoma cells

Stable transfectants of SW480 and HCT116 cells were prepared with plasmids expressing wild-type FGFR3-IIIc (WT3c) or kinase-dead FGFR3-IIIc (KD3c, mutation K508R) driven by a CMV promoter (plasmids kindly provided by D. Donoghue; University of California, San Diego, CA, USA). The wild-type and kinase-dead FGFR3-IIIb constructs (WT3b, KD3b) were constructed by removing the exon 8 sequence in the respective FGFRIII-3c vectors and substituting it with exon 7. For that purpose, the respective sequence was amplified with primers forward 5'-CTGGGTCGTGGAGAACAAAG-3' and reverse 5'-CCGAGACGTCCCTCATT TG-3' and Pfu proofreading polymerase (Stratagene, La Jolla, CA, USA) from cDNA of Vaco235 cells, which predominantly expresses the IIIb form. The PCR product was digested with Eco47III and Xhol, and ligated into the similarly digested KD3c plasmid. The resulting KD3b plasmid was checked by sequencing. Cells that overexpressed FGFR3 were produced through transfection by electroporation. Controls received equal amounts of vector DNA (pCDNA3, Invitrogen, Lofer, Austria). Stably overexpressing cells were obtained by selection with 0.6 mg ml⁻¹ geneticin (G418; PAA, Pasching, Austria).

The dominant-negative FGFR3-IIIc adenoviral construct KD3-IIIcv was prepared using the KD3c plasmid and the AdEasy adenoviral system (Stratagene), following the manufacturer’s instructions. As controls, adenoviral constructs expressing GFP (GFPV) or a scrambled sequence (control virus, Cv) were used, all controlled by a CMV promoter. Cells were seeded at a density of 2 × 10⁵ in six-well plates in medium containing 10% FCS. Subsequently, they were infected at a multiplicity of infection of 1 for SW480 cells and 20 for Caco2 cells, yielding >90% of green fluorescent cells with the GFP virus.

Knockdown of gene expression

Small interfering RNAs (siRNAs) specifically targeting the IIIc-specific exon 8 or the IIIB-specific exon 7, but not the respective other exon, were custom designed by Ambion (Applied Biosystems). Cells were seeded at a density of 2 × 10⁵ cells per well into six-well plates in medium containing 10% serum. After 24 h, they were transfected with 3 μl Silentfect (BioRad) and 20 pmol of siRNA per well in culture medium without serum. A scrambled
siRNA without sequence homology to any known human gene and siRNA directed against GAPDH served as negative controls. After 24 and 48 h, RNA and protein were isolated to verify knockdown efficiency.

**Protein isolation and western blotting**

Total protein extraction and western blotting were performed as described (Sonvilla et al., 2008) using the following antibodies: FGFR3 (C-15) (#sc-123, recognises both FGFR3 splice variants; Santa Cruz Biotechnology, Inc., CA, USA), phospho-S6 (#2215) and S6 Ribosomal Protein 1:1000 (#2212, Cell Signalling, Boston, MA, USA), phospho-externally regulated kinase (ERK)/1/2 (sampler kit; phospho-p44/42 MAP Kinase.Thr202/Tyr204, Cell Signalling, ERK1/2: 1:5000 (#06-182, Upstate, Lake Placid, NY, USA), caspase 3: 1:1000 (H277, Santa Cruz Biotechnology, Inc.) and β-actin: 1:5000 (AC-15, SIGMA, Saint Louis, MO, USA).

**Cell viability and cell proliferation assays**

Cells were seeded at a density of 3 × 10⁴ cells per well into 96-well plates. For knockdown experiments, cells were transfected with siRNA directed against FGFR3 after 24 and 48 h, and viability was determined after an additional 48 h recovery period. For growth stimulation with recombinant growth factors, cells were serum starved for 24 h and then stimulated with recombinant FGF18 (hFGF18-25, Strathmann Biotec, Bovenaum, Germany) for 4 days. Cell viability was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) assay (EZ4U; Biomedica, Vienna, Austria). For determination of DNA synthesis, cells were incubated with 3H-thymidine (1 μCi·ml⁻¹) for 1 h, washed twice with phosphate-buffered saline and processed for scintillation counting.

**Colony formation assay**

SW480 and HCT116 cells were plated at a density of 100 or 200 cells per well, respectively, onto six-well plates in medium containing 10% FCS. After 10 days, the cells were washed with phosphate-buffered saline and fixed with methanol for 20 min. After washing with phosphate-buffered saline, cells were stained with 0.1% of crystal violet solution to assess colony formation.

**Cell migration assay**

Confluent monolayers of different cell lines were serum starved for 24 h and ‘scratch’-wounded using a yellow pipette tip. At each time point, four defined parts of each scratch were photographed and individual scratch widths (μm, mean ± s.d.) were measured using MetaMorph software (Meta Imaging Series 6.1, Universal Imaging Corporation, Downingtown, PA, USA). Migration distance was calculated from the difference of the scratch width.

For SW480 cells transfected with siRNA, as well as SW480 and Caco2 cells infected with adenoviral constructs expressing dominant-negative FGFRs, migration assays were performed using 8-μm-pore-sized PET track-etched membrane (BD-Falcon, Franklin Lakes, NJ, USA) in 24-well plates. Cells were seeded at a density of 0.5 × 10⁵ cells cm⁻². After a migration period of 24 and 48 h for SW480 and Caco2 cells, respectively, the nitrocellulose filters were removed and cells at the bottom of the membrane and in the lower chamber were fixed and stained with crystal violet. The number of cells or colonies was counted microscopically.

**Tumour growth**

SW480 cells were infected with 1 multiplicity of infection KD3-Illc or GFPv on cell culture plates. After 24 h, cells were harvested, washed with phosphate-buffered saline and suspended in Ringer’s solution. In all, 1 × 10⁶ cells in 50 μl Ringer’s solution were subcutaneously injected into the rear flanks of immunodeficient SCID/Balb/c recipient mice (female, aged 4 weeks, Harlan Winkelmann, Borchsen, Germany). Tumour formation was monitored periodically by palpation, and tumour size was determined using a vernier caliper. Tumour volume was calculated using the formula (smaller diameter² × larger diameter)/2. All experiments were performed in triplicate and carried out according to the Austrian and FELASA guidelines for animal care and protection. Tissue sections of experimental tumours were analysed using immunohistochemistry as previously described (Berger et al., 2005). Proliferation and apoptosis were evaluated by counting Ki67-positive cells and apopotic structures, respectively, from 3 to 5 random areas of each tumour section and calculated as a percentage of total cells.

**Cell death detection and caspase activity**

Cell death was determined by staining for 30 min with 5 μg ml⁻¹ Hoechst 33258 and 2 μg ml⁻¹ propidium iodide 5 days after siRNA transfection or adenoviral transduction. Alternatively, for determination of caspase activity, the medium was replaced by 50 μl rhodamine-labelled caspase substrate D³R (Alexis Biochemicals, Lausen, CH, USA) and cells were incubated for 30 min at 37°C. The fluorescent rhodamine cleavage product is released into the culture supernatant and measured in the fluorescein isothiocyanate channel at λex488/sem530 nm on a fluorometer (Synergy HT, Multi-Detection Microplate Reader, BIO-TEK Instruments, Inc., Winooski, VT, USA).

**Statistical analysis**

Tissue expression data were analysed by analysis of variance after obtaining a Gaussian distribution by transforming to log values. The relationship between tumour stage and shift in the FGFR3 IIIc/Illb ratio was determined in comparison with stage I using contingency tables and Fisher’s exact test. All cell biological results were analysed by Student’s t-test or Kruskal–Wallis test, depending on the results of normality testing.

**RESULTS**

**Expression of FGFR3 in colon carcinoma tumour tissues**

Total RNA was isolated from normal and tumour-derived tissues (N = 22) and expression of FGFR3 mRNA was measured by quantitative real-time RT–PCR relative to GAPDH. Expression of total FGFR3 mRNA was found to be downregulated in 20 of 22 tumour specimens, resulting in a significant overall reduction in tumours compared with non-malignant colon tissue specimens (Figure 1A, P = 0.0015). Expression of FGFR3 isoforms IIIb and IIIc was determined for a larger number of tumour specimens (n = 57) using isoform-specific real-time RT–PCR assays. The FGFR3-Illb was downregulated in 49 of these specimens. Expression of FGFR3-Illb was downregulated with increasing stage (Figure 1B; P = 0.0125, slope = −1.9). By contrast, FGFR3-Illc expression increased with stage (Figure 1C; P = 0.0303, slope = +0.84). As a consequence, the IIIb/Illc ratio, compared with normal mucosa, increased with stage at a slope of +2.622 (P = 0.0023). The fraction of tumours with a higher IIIb/Illc ratio also significantly increased with higher stages (Table 1).

Colorectal carcinoma cell lines also expressed the IIIb and IIIc variants of FGFR3 at different ratios, clearly showing FGFR3-Illc expression in the epithelial cell compartment. No FGFR3-Illc was detected in the adenoma cell lines, whereas the highest IIIb/Illc ratio was found in the H64 brain metastasis-derived cells (Figure 1D).
Impact of FGFR3-IIIc on cell growth and clonogenicity

SW480 and HCT116 cells were used to create cell line models stably overexpressing FGFR3. From the slowly growing Caco2 cells, stable transfectants could not be obtained. Plasmids expressing either wild-type FGFR3-IIIb or -IIIc receptors (WT3b and WT3c) or kinase-dead dominant-negative mutants of both FGFR3-IIIb and FGFR3-IIIc (KD3b and KD3c) were introduced by electroporation and stable transfectants were selected. Overexpression of FGFR3 in pools of stable clones was checked by RT–PCR using IIIb- or IIIc-specific primers on mRNA and with western blot analysis on protein level. In IIIc transfectants, the receptor subtype was found to be 15- to 20-fold at the RNA level, whereas expression of the IIIb variant was not affected. In IIIb transfectants, overexpression was 400-fold for IIIb at the RNA level. A distinct increase in total FGFR3 protein was found in all transfectants (for details see Supplementary Material).

Figure 1 Expression of fibroblast growth factor receptor 3 (FGFR3) mRNA in colorectal cancer. RNA was isolated from surgical specimens of colorectal tumours and adjacent normal mucosa and expression of FGFR3 was quantified by reverse transcriptase PCR (RT–PCR). Expression levels were calculated relative to glyceraldehyde 3-phosphate dehydrogenase (GAPDH), normalised relative to expression in normal mucosa. Repeated assays from each specimen yielded cycle threshold (Ct) values: <0.5 cycles apart. (A) Total FGFR; (B) FGFR3-IIIb; and (C) FGFR3-IIIc. (B and C) Results are grouped by stage of disease and each column shows the individual values with the mean of expression marked by a horizontal line. Differences between tumour and adjacent normal mucosa were analysed by analysis of variance from the log values of expression. P-values and linear trend are given in the inserts. (D) The FGFR3 splice-variant pattern expressed in colorectal tumour cell lines was quantified by real-time RT–PCR (right panel, mean ± s.d. of three independent experiments).

Table 1 FGFR3 subtype expression and staging in colorectal tumour specimen

| Stage | Total number of specimen (n) | Specimen with increasing IIIc/IIIb ratio (% of total) | Different from stage I (P-value) |
|-------|------------------------------|-----------------------------------------------------|---------------------------------|
| I     | 5                            | 0                                                   | 0                               |
| II    | 26                           | 12                                                  | 43                             |
| III   | 15                           | 9                                                   | 60                             |
| IV    | 6                            | 4                                                   | 67                             |

Abbreviation: FGFR3 = fibroblast growth factor receptor 3; NS, not significant. In all, 52 tumour specimens were classified according to the shift in the IIIc/IIIb ratio in comparison with the adjacent normal mucosa. Differences were assessed from contingency tables by Fisher’s exact test.

Impact of FGFR3-IIIc on cell growth and clonogenicity

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To determine the impact of the different FGFR3 constructs on cell growth, stable SW480 transfectants were seeded at equal density and kept in medium containing 10% FCS for up to 6 days. Cell viability was determined at the indicated time points. Cells expressing WT3c grew faster than control transfectants, reaching similar density after 6 days. Growth of WT3b cells was unaffected, but KD3c cultures showed decreased growth at all time points (Figure 2A, left panel). This effect became more severe with time in culture and KD3c transfectants lost viability after 3–4 passages. By contrast, vigorously growing cultures, the growth characteristics of which were identical to control transfectants, could be isolated from SW480-KD3b transfectants. DNA synthesis was measured by 3H-thymidine incorporation assays 24 h after plating. DNA synthesis was stimulated by WT3c but not by WT3b. Inhibition was observed in KD3c overexpressing cells, whereas KD3b again

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had no impact (Figure 2A, right panel). Stable HCT116 transfectants overexpressing FGFR3-IIIc were less sensitive to the impact of FGFR3-IIIc overexpression and was inhibited by FGFR3-IIIb overexpression (Figure 2B). Cell viability after 3 days in culture was not affected by the WT3c or KD3b construct, but was significantly decreased by WT3b and KD3c (70% of control).

3H-thymidine incorporation was stimulated by WT3c, reduced by WT3b and KD3c, and not affected by KD3b (Figure 2B right panel).

Differences in growth potential became even more obvious under the stringent conditions of colony formation assays. SW480 cells were plated at 100 cells per well and colony formation was determined after 10 days. Colony formation was increased about two-fold in WT3c and almost completely suppressed in KD3c.

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**Figure 2** Impact of fibroblast growth factor receptor 3-IIIc (FGFR3-IIIc) on growth and clonogenicity. (A) SW480 and (B) HCT116 transfectants were plated at 3 x 10^3 cells per well in 96-well plates and growth was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) assay at the indicated time points (A) or after 3 days (B). Data points represent the mean ± s.e.m. of 10 determinations from two independent experiments (left panels). In all, 2.5 x 10^4 cells were plated for assessment of DNA synthesis. After 24 h, cultures were incubated with 1 μCi ml⁻¹ 3H-thymidine for 1 h and incorporation of radioactivity into DNA was determined as described in Sonvilla et al (2008) (right panels). (C) Stable SW480 and HCT116 transfectants were plated at 100 and 200 cells per well, respectively, in six-well plates, fixed and stained with crystal violet 10 days later to show colony formation. Colonies were counted from two experiments using duplicate cultures (means ± s.e.m.). SW480 (left panel), HCT116 (right panel). (D) SW480 and Caco2 cells were infected with kinase-dead FGFR3 (KD3)-IIIcv or control virus (Cv). Cloning efficiency was determined from 200 cells per well and compared with untransduced control (UC). *, **, *** and #, ### indicate increase or a decrease, respectively, as compared with vector controls at P<0.05, P<0.01 or P<0.0001 (Student’s t-test). d, day.
transfectants. By contrast, KD3b increased plating of SW480 cells (Figure 2C, left panel). Colony formation of HCT116 cells was also enhanced by overexpression of WT3c, but the cells were less sensitive to inhibition by KD3c. KD3b caused a decrease in plating (Figure 2C, right panel). Impact of the dominant-negative construct was also assessed by transduction with an adenoviral vector in SW480 and Caco2 cells. Cv was used as control and stimulated the clonogenicity of SW480 but not of Caco2 cells, compared with uninfected cells (UC). KD3-IIIc virus severely inhibited attachment in both cell lines (Figure 2D).

Induction of apoptosis by blocking FGFR3-IIIc signalling

The impact of KD3-IIIcv transduction on cell survival was assessed and compared with Cv in SW480 (Figure 3; upper panels) and Caco2 cells (Figure 3; lower panels). Multiplicity of infections of 1 and 20 were used for transduction of SW480 and Caco2 cells, respectively. After 6 days, KD3-IIIcv reduced cell viability in both cell lines (Figure 3A). In SW480 cell cultures, a 17% cell loss was observed and the incidence of apoptotic cells was doubled. In Caco2 cultures, a similar cell loss (14%) was accompanied by an increase of only 15% in the incidence of apoptosis (Figure 3B). Although this effect was relatively minor, it was also highly reproducible and significant at P < 0.01. In addition, protein lysates were obtained on day 6 and procaspase 3 was analysed by western blotting (Figure 3C). The amount of procaspase was decreased in the KD3-IIIcv groups of both cell lines, indicating cleavage to produce active caspase 3 (Figure 3C). Accordingly, caspase activity increased in parallel (Figure 3D).

In addition, FGFR3-IIIc expression in SW480 cells was knocked down using siRNA that caused a reduction of both mRNA and protein levels (Figure 4A). The siRNA was most effective after double transfection 24 and 48 h after plating, reducing FGFR3-IIIc mRNA by 75%, but only minimally affecting FGFR3-IIIb (Figure 4A, left panel). The protein level was similarly reduced by 30 and 50%, respectively (Figure 4A, right panel). Cell viability was assessed another 48 h later and found to be reduced by 35% as compared with the scrambled control (Figure 4B). At the same time, incidence of apoptosis was increased 4.7- and 2.7-fold, respectively (Figure 4C). 3H-thymidine incorporation was inhibited at all cell densities, but was strongest in dense cultures (Figure 4D). 80 and 65% of control. To control for the subtype specificity of the effects, a similar series of knockdown experiments was performed for FGFR3-IIIb: siFGFR3-IIIb-1 caused a 90% reduction of FGFR3-IIIb mRNA and a 70% reduction of FGFR3 protein, but no alteration of FGFR3-IIIc expression. Knockdown of siFGFR3-IIIb2 was unreliable and was therefore not included in the growth assays (Figure 5A). Downregulation of FGFR3-IIIb did not result in either a reduction of viable cells in the culture or in a decrease of DNA synthesis (Figure 5B).

Impact of FGFR3-IIIc blockade on tumour growth in vivo

As in vitro growth assays strongly indicated an anti-tumourigenic effect of FGFR3 blockade, tumour growth was assessed in vivo by injection of KD3-IIIcv- and GFPv-transduced SW480 cells into SCID mice. Tumour growth was significantly retarded by FGFR3 blockade as compared with GFPv-infected controls (Figure 6A) and uninfected cells (data not shown). Tumour tissues were analysed by staining paraflin-embedded sections with either haematoxylin–eosin (Figure 6C) or antibodies recognising Ki67 (Figure 6D). Because of their larger size, GFP tumours contained significantly larger areas of necrotic tissue. Analysis of growth parameters was therefore restricted to non-necrotic tumour regions. In these viable tumour areas, the fraction of Ki67-positive cells was significantly decreased in KD3-IIIc tumours (Figure 6B,

![Figure 3](image_url)

**Figure 3** Induction of apoptosis by dominant-negative fibroblast growth factor receptor 3 (FGFR3). SW480 (upper panels) and Caco2 (lower panels) cells were infected with adenoviral constructs expressing kinase-dead FGFR3-IIIc (KD3-IIIcv) and control virus (Cv) at multiplicity of infections (MOIs) of 1 (SW480) or 20 (Caco2). (A) Cell viability was determined 6 days later by 3’-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) assay. The data points represent the mean ± s.e.m. of two independent experiments using quadruplet cultures. (B) Apoptotic cells were detected by staining with Hoechst dye and propidium iodide to visualise nuclear morphology and counted from 1000 cells, each in triplicate cultures. (C) To determine caspase activation, cell lysates were analysed by western blot using a monoclonal antibody recognising procaspase 3. (D) Caspase activity was determined using a substrate predominantly cleaved by caspase 3. The data points represent the mean ± s.e.m. of two independent experiments using quadruplet cultures. * indicate increase as compared with Cv at P < 0.05 and P < 0.01, respectively. #, ## indicate decrease as compared with Cv at P < 0.05 and P < 0.01 (Student’s t-test).
**Figure 4**  Impact of fibroblast growth factor receptor 3c (FGFR3c) knockdown on growth and viability. (A) Small interfering RNA (siRNA) directed against FGFR3-IIIc was introduced into SW480 cells by lipofection, and RNA (left panel) and protein (right panel) were assessed 48 h later. (B–D) For determination of growth and viability, cells were plated in 96-well plates at $2 \times 10^3$ cells per well and siRNA was delivered twice 24 and 48 h later. After 48 h, cell viability (B) and induction of apoptosis (C) and DNA synthesis (D) were determined as described in the Materials and methods section. *, ** and #, ## indicate increase or decrease, respectively, as compared with control at $P < 0.05$ or $P < 0.01$ (Student’s t-test).

**Figure 5**  Impact of fibroblast growth factor receptor 3b (FGFR3b) knockdown on growth and viability. (A) Small interfering RNA (siRNA) directed against FGFR3-IIIb was introduced into HCT116 cells by lipofection, and RNA (left panel) and protein level (right panel) were assessed 48 h later. (B) For determination of growth and viability, cells were plated in two-well plates at $2 \times 10^4$ cells per well and siRNA was delivered 48 h before cell viability was determined as described in the Materials and methods section. (C) DNA synthesis was measured by $^3$H-thymidine incorporation. All data represent the pooled results of at least three independent experiments and no statistically significant effect could be detected. ## indicates a decrease as compared with control at $P < 0.05$ (Student’s t-test).
left panel), although incidence of apoptosis was increased (Figure 6B, right panel), compared with control tumours.

Transduction of ligand-dependent signals

Previous experiments had shown that exogenous FGF18 did not stimulate growth under standard conditions in FGFR3-low SW480 cells (Sonvilla et al, 2008). To determine whether FGFR3-IIIc can confer FGF18 sensitivity to cells, receptor transfectants were kept in serum-free culture medium for 2 days before stimulation with recombinant FGF18 (Figure 7A). After 4 days, the cell number was measured by MTT assay and demonstrated dose-dependent growth stimulation in WT3c cells. KD3c cells were not stimulated (Figure 7A, left panel). As the data suggest that FGFR3-IIIc confers FGF18 responsiveness, the impact of FGFR3 inhibition on FGF18 sensitivity was determined by infecting cells with the dominant-negative KD3-IIIcv and Cv before exposure to the growth factor. This had no effect on FGF18-insensitive SW480 cells (data not shown). On the other hand, FGFR3-IIIc-high Caco2 cells responded to FGF18 with a 25% increase in cell viability when infected with control viruses, and KD3-IIIcv completely abolished the response (Figure 7A, right panel).

Impact of FGFR3-IIIc on cell migration

To investigate the impact of FGFR3-IIIc on FGF18-dependent downstream signalling, stable SW480-FGFR3 transfectants were serum starved for 24 h, stimulated with 10 ng ml⁻¹ recombinant FGF18 and, 15 min later, phosphorylation of ERK and S6 was determined by western blotting. Phosphorylation of both signalling molecules was stimulated by FGF18 only in WT3 cells to 132 ± 0.3% for ERK and to 123 ± 0.01% for S6. In KD3c cultures, control levels of ERK phosphorylation were lower than in controls or in WT3c transfectants, and the growth factor did not lead to pathway stimulation (Figure 7B).

Impact of FGFR3-IIIc on cell migration

To assess whether FGFR3-IIIc mediates migration signals, we performed migration assays with SW480 and Caco2 cells after infection with dominant-negative KD3-IIIcv and Cv. Uninfected cells and cells infected with Cv readily migrated through the filter, but the dominant-negative FGFR construct KD3c significantly reduced migration at comparable levels for both cell lines (Figure 8A).

In addition, we performed scratch assays with stably FGFR3-IIIc-transfected SW480 cells after 24 h serum starvation. Migration
of cells was followed up for up to 40 h (doubling time >48 h). In starvation medium without serum, all transfectants migrated 60–100 μm during the 40 h period without any significant differences between experimental groups (Figure 8B, left panel). Addition of 10 ng ml⁻¹ FGF18 stimulated WT3c cells to migrate 128 μm (P = 0.0005), whereas the pcDNA3 and KD3c groups remained within the 60–100 μm range (Figure 8B, center panel). A 10% FCS stimulated migration in all three groups. pcDNA3 and WT3c reached 274 and 246 μm, respectively (Figure 8B, right panel; not significantly different P = 0.052). KD3c inhibited migration as compared with the pcDNA3 control (Figure 8B, right panel; 197 μm, P = 0.01). However, KD3c cells were still stimulated by 10% serum (Figure 8B, left and right panels; P = 0.013).

DISCUSSION

The FGFR3 is a tyrosine kinase receptor, the constitutive activation of which is involved in skeletal malformations (Bellus et al, 2000) and drives tumour cell growth in bladder and cervix cancer (Cappellen et al, 1999; Hernandez et al, 2006; Mhawech-Fauceglia et al, 2006). Deregulation of FGFR3 signalling may be caused by mutation, transcriptional regulation or differential splicing. The latter mechanism most prominently affects the Ig-like loop III, producing IIIb and IIIc variants, which are expressed preferentially in epithelia and connective tissue, respectively (Murgue et al, 1994). In colon carcinomas, receptor mutations are rare (Jang et al, 2000), but aberrant splicing causes a decrease in functional FGFR3-IIIb, accompanied by a switch to FGFR1-IIIc (Jang, 2005).

Our results now confirm the downregulation of FGFR3-III, and also demonstrate differential effects on the IIIb and IIIc variants. Specifically, expression of the IIIc splice variant can be retained better so that the IIIc/IIIb ratio is higher in tumour tissue than in normal mucosa. This was observed in about 50% of the tumour specimens analysed and preferentially in more advanced tumours. The IIIc splice variant was also detected in colon carcinoma, but not in adenoma-derived cell lines, demonstrating (1) that expression of the splice variant is not only due to mesenchymal contaminants but also comes from the epithelial compartment of the tumour and (2) that it is upregulated during tumour progression.

Switching of FGFR expression to the IIIc isotypes has been shown for FGFR2 and FGFR1, and was described to be associated with tumour progression in prostate (Yan et al, 1993; Carstens et al, 1997; Feng et al, 1997) and bladder cancer (Chaffer et al, 2006). For FGFR3, mutation of the IIIc variant causes tumour growth in multiple myeloma cells (Chesi et al, 2001). Our results now demonstrate that overexpression of FGFR3-IIIc stimulates tumour cell growth, whereas its blockade inhibits cell growth and induced apoptosis in colorectal cancer cells. Overexpression or blockade of FGFR3-IIIb had little impact on cell growth. Only in...
HCT116-KD3b transfectants was colony formation also decreased by blockade of IIIb; however, this effect was transient, and 3 or more days after plating, no evidence of growth inhibition by the construct remained. This is in agreement with the observation of Jang (2005) that overexpression of WT3b in the human HCT116 colorectal carcinoma cell line has been shown to inhibit cloning efficiency so that loss of expression in colorectal cancer is cytotoxic in multiple myeloma cells in vitro and in xenograft models (Trudel et al., 2006; Xin et al., 2006). In bladder cancer cells, knockdown of FGFR3 was achieved by short hairpin RNA constructs, leading to decreased proliferation, reduced clonogenicity and soft agar growth (Tomlinson et al., 2007). Our results confirm the strong anti-tumourigenic effect in vivo due to disruption of FGFR3 signalling in colorectal cancer. Infection of SW480 colon carcinoma cells with a KD3c-expressing adenovirus reduced tumourigenicity in a SCID mouse model by about 40% by both inhibition of proliferation and increased incidence of apoptosis.

Using in vitro models, reduction of colony formation and clonogenicity by KD3c could be shown. At higher cell densities, the dominant-negative construct reduced viability and induced apoptosis by activating caspase 3 by a non-mitochondrial pathway, as indicated by a stable status of mitochondrial membrane potential (data not shown). An additional effect of KD3c mutant on other FGFRs by heterodimerisation cannot be ruled out at this point. However, growth inhibition and induction of apoptosis could also be achieved by FGFR3-IIIc but not by FGFR3-IIIb knockdown, demonstrating that the IIIc splice variant is actually crucial for malignant growth and survival. Even though the in vitro effects may be relatively minor in some cases, together they are believed to synergistically cooperate in the anti-tumourigenic effect observed in vivo. As, for example, blocking of FGFR3 disrupts growth factor signalling and colony formation, both these mechanisms may cooperate in inducing apoptosis by loss of colony formation (Gilmore, 2005).

Assessment of FGFR-targeting strategies in tumour therapy has to take into account its effect on cell migration, which constitutes
an important mechanism of invasion and metastasis (Dignass et al., 1994; Antoine et al., 2006). In neurons, FGFR1 associates with cell adhesion molecules, most importantly N-CAM, to stimulate neurite outgrowth and migration (Ronni et al., 2000). A similar configuration has been observed between FGFR4, N-CAM and N-cadherin in tumour cell migration (Cavallaro et al., 2001), providing a mechanistic explanation of the impact FGFR4 has on several tumour types (Stadler et al., 2006; Streit et al., 2006). Here, we demonstrate for the first time a major impact of FGFR3 on the migration of colon tumour cells. Although transfection of WT3c conferred sensitivity to FGF18-induced tumour cell migration, the dominant-negative KD3 virus reduced cell migration in both SW480 and Caco2 cells, but still permitted migration induced by 10% FCS. Together, these results indicate that autocrine stimulation by FGF18 is one, but not the only, mechanism inducing tumour cell migration. This indicates involvement of migratory signals unrelated to FGFRs, for example, c-met, which are also common in colorectal cancer (Otto et al., 2000).

REFERENCES

Antoine M, Wirz W, Tag CG, Gressner AM, Wycislo M, Muller R, Kieder P (2006) Fibroblast growth factor 16 and 18 are expressed in human cardiovascular tissues and induce on endothelial cells migration but not proliferation. Biochem Biophys Res Commun 346: 224 – 233

Barbieri MA, Ramkumar TP, Fernandez-Pol S, Chen PI, Stahl PD (2004) Receptor tyrosine kinase signaling and trafficking – paradigms revisited. Curr Top Microbiol Immunol 286: 1 – 20

Bellus GA, Spector EB, Speiser PW, Weaver CA, Garber AT, Bryke CR, Israel J, Rosengren SS, Webster MK, Donoghue DJ, Francanamo CA (2000) Distinct missense mutations of the FGFR3 cys650 codon modulate receptor kinase activation and the severity of the skeletal dysplasia phenotype. Am J Hum Genet 67: 1411 – 1421

Berger W, Setinek U, Hollaus P, Zidek T, Steiner E, Elbling L, Cantonati H, Carstens RP, Eaton JV, Krigman HR, Walther PJ, Garcia-Blanco MA (1997) Alternative splicing of fibroblast growth factor receptor 3 IIIb in intestinal epithelial cells. Nat Cell Biol 18: 167 – 177

Carstens RP, Eaton JV, Krigman HR, Walther PJ, Garcia-Blanco MA (1997) Alternative splicing of fibroblast growth factor receptor 2 (FGF-R2) in human prostate cancer. Oncogene 15: 3059 – 3065

Cavallaro U, Niedermeyer J, Fuxa M, Christofori G (2001) N-CAM modulates tumour-cell adhesion to matrix by inducing FGF-receptor signalling. Nat Cell Biol 3: 650 – 657

Chaffer CL, Brennan JP, Slavin JL, Blick T, Thompson EW, Williams ED (2005) Cellular signaling by fibroblast growth factor receptors. Cytokine Growth Factor Rev 16: 139 – 149

Feng S, Wang F, Matsubara A, Kan M, McKeehan WL (1997) Fibroblast growth factor receptor 2 limits and receptor 1 accelerates tumorigenicity of prostate epithelial cells. Cancer Res 57: 5369 – 5378

Gilmore AP (2005) Anoikis. Cell Death Differ 12(Suppl 2): 1473 – 1477

Hanahan D, Christofori G, Naik P, Armit J (1996) Transgenic mouse models of tumour angiogenesis: the angiogenic switch, its molecular controls, and prospects for preclinical therapeutic models. Eur J Cancer 32A: 2386 – 2393

Hernandez S, Lopez-Knowles E, Lloreta J, Kogevinas M, Amoros A, Tardon A, Carrato A, Serra C, Malats N, Real FX (2006) Prospective study of FGFR3 mutations as a prognostic factor in nonmuscle invasive urothelial bladder carcinomas. J Clin Oncol 24: 3664 – 3671

Jang JH (2005) Reciprocal relationship in gene expression between FGFR1 and FGFR3: implication for tumorigenesis. Oncogene 24: 945 – 948

Jang J-H, Shin K-H, Park Y-J, Lee RJ, McKeehan WL, Park J-G (2000) Novel transcripts of fibroblast growth factor receptor 3 reveal aberrant splicing and activation of cryptic splice sequences in colorectal cancer. Cancer Res 60: 4049 – 4052

Kanai M, Goke M, Tsunekawa S, Podolsky DK (1997a) Signal transduction pathway of human fibroblast growth factor receptor 3. Identification of a novel 66-KDa phosphoprotein, and activation of cryptic splice sequences in colorectal cancer. Oncogene 355 – 363

Kanai M, Rudolph I, Podolsky DK (1997b) Cytokine regulation of fibroblast growth factor receptor 3 IIIb in intestinal epithelial cells. Am J Physiol 272: G681 – G689

L’Hote CG, Knowles MA (2005) Cell responses to FGFR3 signalling: growth, differentiation and apoptosis. Exp Cell Res 304: 417 – 431

Mhawech-Fauceglia P, Cheney RT, Fischer G, Beck A, Herrmann FR (2006) FGFR3 and p53 protein expressions in patients with pTa and pT1 urothelial bladder cancer. Eur J Surg Oncol 32: 231 – 237

Murgue B, Tsunekawa S, Rosenberg I, deBeaumont M, Podolsky DK (1994) Identification of a novel variant form of fibroblast growth factor receptor 3 (FGFR3 IIIb) in human colonic epithelium. Cancer Res 54: 5206 – 5211

Ortiz DM, Ioh N (2001) Fibroblast growth factors. Genome Biol 2: REVIEW5000

Orr-Urtreger A, Bedford MT, Burakova T, Arman E, Zimmer Y, Yayon A, Ornitz DM (2001) Fibroblast growth factors. Genes Dev 15: 231 – 241

Otto JM, Schmitz F, Kiehne K, Stechlehu BU, Bansawischewitz T, Krekowicz W, Nakamura T, Folsch UA, Herzog K (2000) Functional expression of HGF and its receptor in human colorectal cancer. J Gastroenterol Hepatol 15: 165 – 197

Powers CJ, McLeskey SW, Wellstein A (2000) Fibroblast growth factors, their receptors and signaling. Endocr Relat Cancer 7: 165 – 197

Reilly JF, Mizukoshi E, Maher PA (2004) Ligand dependent and independent internalization and nuclear translocation of fibroblast growth factor (FGF) receptor 1. DNA Cell Biol 23: 538 – 548

In summary, our data demonstrate that FGFR3–IIIc exerts oncogenic effects in colorectal cancer cells by promoting in vitro tumour cell growth, survival, migration and responsiveness to oncogenic FGF ligands such as FGF8. Moreover, disruption of FGFR3–IIIc signalling distinctly attenuated colorectal cancer xenograft growth in SCID mice. This makes FGFR3–IIIc a promising candidate target for therapeutic interventions in colorectal cancer.

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Resat H, Ewald JA, Dixon DA, Wiley HS (2003) An integrated model of epidermal growth factor receptor trafficking and signal transduction. Biophys J 85: 730 – 743

Richter M, Jurek D, Wrba F, Kaserer K, Wurzer G, Karner-Hausch J, Marian B (2002) Cells obtained from colorectal microadenomas mirror early premalignant growth patterns in vitro. Eur J Cancer 38: 1937 – 1945

Ronn LC, Doherty P, Holm A, Berzin V, Bock E (2000) Neurite outgrowth induced by a synthetic peptide ligand of neural cell adhesion molecule requires fibroblast growth factor receptor activation. J Neurochem 75: 665 – 671

Scotet E, Houssaint E (1995) The choice between alternative IIb and IIIc exons of the FGFR-3 gene is not strictly tissue-specific. Biochim Biophys Acta 1264: 238 – 242

Shimokawa T, Furukawa Y, Sakai M, Li M, Miwa N, Lin Y-M, Nakamura Y (2003) Involvement of the FGFR1 gene in colorectal carcinogenesis, as a novel downstream target of the [beta]-catenin/T-cell factor complex. Cancer Res 63: 6116 – 6120

Sonvilla G, Allerstorfer S, Stattner S, Karner J, Klimpfinger M, Fischer H, Grasd-Kraupp B, Holzmann K, Berger W, Wrba F, Marian B, Grusch M (2008) FGF18 in colorectal tumour cells: autocrine and paracrine effects. Carcinogenesis 29(1): 15 – 24

Stadler CR, Knyazev P, Bange J, Ullrich A (2006) FGFR4 GLY388 isotype suppresses motility of MDA-MB-231 breast cancer cells by EDG-2 gene repression. Cell Signal 18: 783 – 794

Streit S, Mestel DS, Schmidt M, Ullrich A, Berking C (2006) FGFR4 Arg388 allele correlates with tumour thickness and FGFR4 protein expression with survival of melanoma patients. Br J Cancer 94: 1879 – 1886

Tomlinson DC, Hurst CD, Knowles MA (2007) Knockdown by shRNA identifies S249C mutant FGFR3 as a potential therapeutic target in bladder cancer. Oncogene 26: 5889 – 5899

Trudel S, Stewart AK, Rom E, Wei E, Li ZH, Kotzer S, Chumakov I, Singer Y, Chang H, Liang SB, Yayon A (2006) The inhibitory anti-FGFR3 antibody, PRO-001, is cytotoxic to t(4;14) multiple myeloma cells. Blood 107: 4039 – 4046

Vidrich A, Buzan JM, Ilo C, Bradley L, Skaar K, Cohn SM (2004) Fibroblast growth factor receptor-3 is expressed in undifferentiated intestinal epithelial cells during murine crypt morphogenesis. Dev Dyn 230: 114 – 123

Willson JK, Bittner GN, Oberley TD, Meisner LF, Weese JL (1987) Cell culture of human colon adenomas and carcinomas. Cancer Res 47: 2704 – 2713

Wuechner G, Nordqvist AC, Winterpacht A, Zabel B, Schalling M (1996) Developmental expression of splicing variants of fibroblast growth factor receptor 3 (FGFR3) in mouse. Int J Dev Biol 40: 1185 – 1188

Xin X, Abrams TJ, Hollembach PW, Rendahl KG, Tang Y, Oei YA, Embry MG, Svinarski DE, Garrett EN, Pryer NK, Trudel S, Jallal B, Mendel DB, Heise CC (2006) CHIR-258 is efficacious in a newly developed fibroblast growth factor receptor 3-expressing orthotopic multiple myeloma model in nude mice. Clin Cancer Res 12: 4908 – 4915

Yan G, Fukabori Y, McBride G, Nikolaropolous S, McKeehan WL (1993) Exon switching and activation of stromal and embryonic fibroblast growth factor (FGF)-FGF receptor genes in prostate epithelial cells accompany stromal independence and malignancy. Mol Cell Biol 13: 4513 – 4522

Zwick E, Bange J, Ullrich A (2001) Receptor tyrosine kinase signalling as a target for cancer intervention strategies. Endocr Relat Cancer 8: 161 – 173