The role of transforming growth factor-β in suppression of hepatic metastasis from colon cancer

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Abstract

Background: The role of transforming growth factor-β (TGF-β) in the development of hepatic metastasis from colon cancer is not clearly elucidated. The aim of this study was to determine the role of TGF-β in the development of such metastasis.

Methods: Two human colon cancer cell lines were utilized: FET-α cells (intact TGF-β inhibitory response), and CBS cells (defects in TGF-β inhibitory response caused by a deficiency in type II receptor activity). The ability of these cell lines to metastasize was analysed in an orthotopic colon cancer mouse model.

Results: FET-α cells did not metastasize to the liver, but showed lung metastasis in 10% of the animals, whereas CBS cells gave rise to metastasis in 65%. Following the elimination of TGF-β activity by transfection and overexpression of dominant negative type II receptor, FET-α cells demonstrated liver and lung metastasis in 70% of the animals. Similarly, after the restoration of type II receptor activity by ectopic expression, CBS cells formed metastasis in fewer (10%) animals.

Conclusions: The results of our study demonstrate for the first time that TGF-β displays selective metastasis suppressor activity. These abnormal pathways can serve as selective targets for future development of targeted therapies.

Keywords
colorectal metastases < liver, basic science < liver

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Introduction

Successful metastatic colonization involves several processes. These include invasion at the primary site, intravasation into the circulation, escape from immune surveillance, extravasation into a distal organ and establishment of metastatic colonies at this secondary organ site. The molecular mechanisms involved in the individual steps of the process are not well characterized.1 The development of aberrant cell survival signalling is required for each step of the process and there is evidence that different molecular mechanisms for aberrant survival signalling can occur at each step.2 The determination of these survival mechanisms could potentially provide a basis for the development of therapeutic interventions to address the high death rates associated with metastatic disease in major cancers. Aberrant cell survival mechanisms arise in response to stress.3 Evasion of apoptosis can occur as a result of loss of tumour suppressor activity or enhanced oncogenic activity, which shifts the balance of stress response toward resistance to cell death.

The transforming growth factor-β (TGF-β) signalling pathway is involved in the control of several biologic processes, including cell proliferation, differentiation, migration and apoptosis, and is one of the most commonly altered pathways in human cancers.4 The TGF-β ligand family consists of three members: TGF-β1, TGF-β2 and TGF-β3. TGF-β signalling is transduced by the serine/threonine type I TGF-β receptor (TGF-β RI) and type II...
TGF-β receptor (TGF-β RII) found in most cell types. TGF-β ligand stimulation results in activation of TGF-β RII serine/threonine kinase activity followed by receptor heteromerization with TGF-β RI and the activation of this component by trans-phosphorylation. Activated TGF-β RI receptor serine/threonine kinase then transmits signals through phosphorylation of receptor-activated Smads (R-Smads) to regulate transcription of target genes as well as Smads transcription-independent signalling mechanisms.5

The general consensus is that TGF-β is a multi-functional cytokine that plays a dichotomous role in tumour progression.6–10 TGF-β signalling is tumour-suppressive in early carcinogenesis, but becomes tumour-promoting during later stages of cancer, which leads to the development of metastasis. TGF-β signalling through Smad activation is regarded as tumour-suppressive during the early stages of cancer and pre-cancerous lesions as it has been shown that loss of TGF-β tumour suppressor signalling, at either the ligand or receptor level, has been associated with tumour initiation and the progression of several types of tumours, including colon cancer. Epigenetic transcriptional repression and DNA methylation of TGF-β RII or TGF-β RII has been observed in a variety of tumour types,11,12 and TGF-β RII is inactivated by mutation in human colon cancers with microsatellite instability.13 By contrast, it has been reported that increased TGF-β1 expression by tumour cells correlated with tumour progression in non-small cell lung cancer (NSCLC), colorectal cancer and gastric carcinomas.14–17 Similarly, other authors have demonstrated the tumour progression and metastasis-enhancing potential of TGF-β.18–20 TGF-β signalling has also been shown to prime breast tumour cells for metastasis by promoting their extravasation from blood vessels to distal organ sites.21

Therefore, the exact roles of the pleiotropic TGF-β functions in the development of metastasis in colorectal cancer are not clear. A direct association of TGF-β signalling with the initiation of colon cancer metastasis has not yet been identified. The aim of our study was to investigate the role of TGF-β in the development of hepatic metastasis from colorectal cancer in an orthotopic mouse model.

Materials and methods

Cell lines and reagents

The FET colon cancer cell line does not normally form tumours in athymic mice,22 but becomes highly tumorigenic after transfection (FET-α) with transforming growth factor-α (TGF-α).23 FET-α cells are highly tumorigenic, exhibit growth factor independence and are highly invasive (100%) at the orthotopic primary site in vivo, but do not metastasize.24 It has been shown that FET cells have robust autocrine TGF-β signalling that inhibits cell proliferation and contributes to apoptosis in response to stress such as growth factor deprivation stress (GFDS).7 Although FET-α cells have an aberrant oncogenic pathway constitutive (TGF-α/epidermal growth factor receptor [EGFR]) signalling, they retain an intact TGF-β tumour suppressor signalling pathway which prevents them from forming metastasis.

FET-α-DN cells were developed by repressing TGF-β activity by transfection with a dominant negative (DN) receptor II construct. Repression of TGF-β activity has been shown to lead to vigorous progressive expansion of subcutaneous implants.24 The CBS colon cancer cell line is also highly invasive (100%) at the orthotopic primary site, but is associated with a high metastatic potential ranging up to 65%.

FET-α and FET-α-DN colon carcinoma cells were cultured at 37 °C in a humidified atmosphere of 5% CO2 in SM medium (McCoy’s 5A serum-free medium [Sigma-Aldrich, Inc., St Louis, MO, USA] with pyruvate, vitamins, amino acids and antibiotics) supplemented with 10 ng/ml epidermal growth factor, 20 μg/ml insulin, and 4 μg/ml transferrin. Antibodies for Akt and phosphorylated Akt (Ser473) were obtained from Cell Signaling Technology (Danvers, MA, USA). Actin and tubulin antibodies were purchased from Sigma-Aldrich, Inc. pSmad2 antibodies were obtained from the Chemicon Division of Millipore Corp. (Bedford, MA, USA) and Abcam, Inc. (Cambridge, MA, USA), respectively. PI3K inhibitor LY294002, and TGF-β were obtained from the Calbiochem Division of EMD Chemicals, Inc. (Gibbstown, NJ, USA). ApopTag Plus Peroxidase In Situ Apoptosis Detection Kit was sourced from the Chemicon Division of Millipore Corp. and both the Dako Envision System HRP and the monoclonal anti-human KI-67 antigen (Clone Mib-1) were obtained from Dako Corp. (Carpinteria, CA, USA). An Annexin V-FITC Apoptosis Detection Kit (including propidium iodide) was sourced from BD Biosciences Pharmingen (San Jose, CA, USA) and a Cell Death Detection ELISAPLUS Kit was sourced from Roche Diagnostics, Inc. (Indianapolis, IN, USA). Haematoxylin was obtained from Protocol and eosin was purchased from Sigma-Aldrich, Inc.

Ectopic expression of dominant negative TGF-β RII receptor

The DN RII expression vector has been described previously.24 The truncated TGF-β RII encoded amino acid residues 1–283 of the human RII; thus, most of the serine/threonine kinase domain and COOH-terminal tail of the normal human RII is absent from DN RII protein. The truncated cDNA was subcloned into an MX-IV retroviral vector. The 293GP packaging cells (Clontech Laboratories, Inc., Mountain View, CA, USA) were co-transfected with the truncated construct and pSVS-G. The viruses were harvested 48 h later and used to infect FET-α cells. Puromycin (3.0 μg/ml) was used to select infected cells for 8 days, after which cells were pooled.

Immunoblot analysis

Cells were lysed in TNESV lysis buffer (50 mmol/l Tris [pH 7.5], 150 mmol/l NaCl, 1% NP40, 50 mmol/l NaF, 1 mmol/l NaVO4, 25 μg/ml h-glycerophosphate, 1 mmol/l phenylmethane-sulfonyl fluoride, one protease inhibitor cocktail tablet [Roche Diagnostics, Inc.] per 10 ml) for 30 min on ice. The supernatants were then collected by centrifugation for 15 min. Protein was deter-
mained by the Pierce BSA (bovine serum albumin) method. Protein samples were dissolved in 1× sample buffer (50 mM Tris [pH 6.8], 1% SDS, 10% glycerol, 0.03% bromophenol blue and 1% β-mercaptoethanol). Protein (10–50 µg) was fractionated on a 10% acrylamide denaturing gel and transferred onto a nitrocellulose membrane (Amersham Life Science, Inc., Arlington Heights, IL, USA) by electroblotting. The membrane was blocked with 5% non-fat dry milk in TBST (50 mmol/l Tris [pH 7.5], 150 mmol/l NaCl, 0.05% Tween 20) for 1 h at room temperature or overnight at 4 °C and washed in TBST. The membrane was then incubated with primary antibodies at 1 : 1000 dilutions for 1 h at room temperature or overnight at 4 °C. The membranes were washed with TBST for 30 min and then incubated with peroxidase-conjugated goat anti-mouse or anti-rabbit IgG (Jackson Immunoresearch Laboratories, Inc., West Grove, PA, USA) at a 1 : 100 dilution for 1 h at room temperature and washed again in TBST for 30 min. Proteins were then detected by the enhanced chemiluminescence (ECL) system (Amersham Life Science, Inc.).

**MTT assay**

Cells were grown to 80% confluence, after which MTT (3-[4, 5-dimethylthiazoll-2-yl]-2, 5-diphenyltetrazolium bromide) was added to the medium, which was then incubated at 37 °C for 2 h. The medium was aspirated to visualize stained cells. Dimethyl sulphoxide (DMSO) was added and the plate was covered with foil and shaken for 15 min. Duplicate volumes (150 µl) were added to a 96-well plate and absorbance was observed at 570 nm.

**TGF-β growth inhibition assay**

[3H]Thymidine incorporation was used to determine growth inhibition of FET-α and FET-α-DN cells after TGF-β treatment. The cells were seeded in six-well tissue culture plates and grown to 60% confluence. At 48 h after TGF-β treatment, the cells were labelled with [3H]thymidine (7 µCi; 46 Ci/mmoll [Amersham Corp.]) for 1 h. DNA was then precipitated with 10% trichloroacetic acid and solubilized in 0.2 mol/l NaOH. The amount of [3H]thymidine incorporated was analysed by liquid scintillation counting in a Beckman LS7500 scintillation counter.

**Annexin V-PI staining**

An Annexin V-FITC Apoptotic Kit (BD Biosciences Pharmingen) was used to assess apoptosis. Cells were grown to 80% confluence and harvested or growth factor-deprived for 24 h or 48 h. Cells were washed with phosphate-buffered saline (PBS) and then re-suspended in binding buffer (10 mmol/l Hepes/NaOH [pH 7.4], 140 mmol/l NaCl, 2.5 mmol/l CaCl2) to a cell density of 1 × 10^6 cells/ml. Cell suspensions (100 µl: 10^6 cells) were stained with annexin V-FITC and propidium iodide (PI) at room temperature for at least 15 min in the dark. Cells were analysed in an FACS Calibur flow cytometer within 1 h of staining. The data were analysed with CellQuest Pro (BD Biosciences, Inc., San Jose, CA, USA).

**Immunohistochemistry**

Primary tumours established from the FET-α and FET-α-DN cells were harvested and placed in 10% neutral buffered formalin fixative for 12–24 h and then embedded in paraffin. Deparaffinized tissue specimens were subjected to immunohistochemical staining for the detection of pAkt-S473 using an indirect detection method according to Sharkey et al.26 The catalysed signal amplification system was used for the phosphospecific antibodies (Dako Corp.). Antibody staining was accompanied by a negative control in which slides were incubated with a matching blocking peptide (obtained from same company as the primary antibody) to the primary antibody. Specimens were processed on the same day to eliminate any variability in conditions. Slides were digitally photographed using the same settings.

**TUNEL assay**

Slides were cut from paraffin-embedded blocks and stained according to the ApopTag TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling) Assay Kit (Oncor, Inc., Gaithersburg, MD, USA). The apoptotic rate was quantitatively determined by counting the number of positively stained apoptotic bodies per 75-µm² field at magnification ×20. Twelve and 15 histological slides for the FET-α and FET-α-DN tumours, respectively, were analysed. Three histologically similar fields viewed at magnification ×20 were randomly selected from each slide for analysis. This procedure was performed by two blinded observers. The ratio of the average number of apoptotic cells to the total number of cells counted was used to represent apoptotic rates.

**KI-67 staining**

Haematoxylin and eosin (H&E) staining was performed on tissue blocks used for immunohistochemical characterizations. Serial sections were cut to complement the H&E sections and were stained with an IgG1 rabbit polyclonal antibody for KI-67 (Dako Corp.). KI-67 is a non-histone nuclear antigen present in late G1, G2 and S phases of the cell cycle, but not in G0. The optimal dilution of 1 : 100 was used. Sections of 3–4 µm were cut, deparaffinized in xylene, and rehydrated in descending grades of ethanol. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide in water. Immunostaining was carried out using a variation of the avidin-biotin-peroxidase method. Slides were counterstained with methyl green. The proliferation rate was determined quantitatively using NIH Image J (public domain software). Image settings used a threshold range of 10–192 and a pixel size of 20–5000. Twelve slides from FET-α and FET-α-DN were analysed. Three histologically similar fields viewed at magnification ×20 were randomly selected for analysis. The mean proliferation was determined for each group.

**Orthotopic implantation**

Orthotopic implantation was performed as previously described.25 Briefly, green fluorescent protein (GFP)-labelled FET-α and FET-
α-DN cells were subcutaneously injected onto the dorsal surfaces of separate BALB/c nude male mice and allowed to grow to 300 mm³. When the xenografts were established, they were excised and minced into 1-mm³ pieces. Two of these pieces were then orthotopically implanted into other BALB/c nude mice. Forty-four animals were implanted with FET-α cells and 30 animals with FET-α-DN cells. For operative procedures, animals were anaesthetized with isoflurane inhalation. A 1-cm laparotomy was performed and the cecum and ascending colon were exteriorized. Using magnification ×7 and microsurgical techniques, the serosa was disrupted in two locations. Pieces of xenograft (1 mm³) were subserosally implanted using an 8–0 nylon suture at the disrupted serosal locations. The bowel was then returned to the peritoneal cavity and the abdomen was closed with a 5–0 vicryl suture. Fluorescence imaging was carried out weekly on the animals to follow tumour growth and progression (Lightools Research, Encinitas, CA, USA). The animals were killed at days 50 (mice implanted with FET-α) and 63 (mice implanted with FET-α-DN) after implantation. Organs were explanted, imaged and immediately placed in buffered 10% formalin. Tissues were then processed and embedded in paraffin. Slides were cut for H&E staining.

Results

TGF-β suppresses metastasis in vivo

Our laboratory has shown that constitutive activation of EGFR by ectopic expression of TGF-α results in tumour formation in both xenograft and orthotopic implantation in in vivo models of the FET colon carcinoma cell line.25 Orthotopic implantation of FET-α generates invasion at the primary tumour site. These cells retain a robust inhibitory response to TGF-β, suggesting that TGF-β tumour suppressor signalling does not suppress tumour initiation or progression to malignancy in this model (as reflected by the acquisition of an invasive capability). As the FET-α cells do not readily metastasize, we hypothesized that TGF-β signalling was acting as a suppressor of metastasis. To test this hypothesis, we stably co-transfected FET-α cells with a truncated kinase domain DN RII receptor construct (nucleotides 1–283) and pVSV-G, and denoted these cells as FET-α-DN. Stably transfected cell pools were obtained by puromycin selection and compared with pooled FET-α cells. Abrogation of TGF-β signalling was confirmed by growing FET-α and FET-α-DN cells to 70% confluence and then treating the cells with varying concentrations of TGF-β (0 ng/ml, 5 ng/ml, 10 ng/ml) for 2 h. Cells were harvested for antigenic solubilization. The amount of [3H]thymidine was analysed by liquid scintillation counting. Changes in growth were assessed by normalizing results to FET-α in the absence of exogenous TGF-β. The results (Fig. 2) showed that FET-α cells exhibited a 60% decrease in tritiated thymidine incorporation into newly synthesized DNA, whereas no change was observed in FET-α-DN cells in the absence or presence of exogenous TGF-β. Thus, the growth inhibitory effects of TGF-β, as well as TGF-β receptor-mediated Smad signalling, were eliminated from FET-α cells by transfection of the DN RII construct, as expected.

The comparison of FET-α and FET-α-DN cells by orthotopic implantation was used to assess the effect of loss of TGF-β inhibitory signalling on malignant progression and metastasis. Exponentially growing GFP-labelled FET-α and FET-α-DN cells (5 × 10⁶) were subcutaneously inoculated into athymic nude mice and allowed to grow to 300 mm³ as determined by two-dimensional caliper measurements. When the xenografts were established, they were excised and minced into 1-mm³ pieces. Pairs of these pieces were then implanted onto the colons of other BALB/c nude mice. A total of 44 animals were implanted with...
FET-α cells and 30 animals were implanted with FET-α-DN cells. Weekly fluorescence imaging with GFP was used as a gross means of following tumour growth and progression. Figure 3 shows GFP imaging of primary colon carcinoma during xenografting and orthotopic implantation (open and closed abdomen). The lungs and liver were excised, imaged and immediately placed in buffered 10% formalin. Imaging with GFP also facilitated the identification of metastatic disease. Metastatic colony formation was seen in lung tissue from FET-α-DN orthotopically implanted animals, but not in lung tissue from FET-α orthotopically implanted animals. There was 100% primary tumour growth at the site of implantation in all animals, indicating that the FET-α-DN cells, like FET-α cells, were fully invasive. Invasion at the primary tumour site and metastatic colonization were evaluated by H&E staining, which confirmed that FET-α-DN cells were highly invasive. A significant difference was observed in the metastatic potential between the two FET transfected cell lines. The group of animals with FET-α implants showed a 5% metastatic rate (two of 44 animals with orthotopic implants) as assessed by H&E staining of five histological slides bread-loafed from each lung and each lobe of the liver. This can be compared with the 77% metastatic rate (23 of 30 animals) observed in animals with FET-α-DN implants. The presence of metastatic colonies in animals with FET-α-DN implants suggests that these cells may have acquired enhanced survival capabilities that enable them to extravasate and form new colonies at a distal organ site as a result of loss of TGF-β inhibitory signalling.

Proliferation and survival were assessed by KI-67 immunohistochemistry and TUNEL assays, respectively. KI-67 is a non-histone nuclear antigen present in late G1, S, G2, and M phases of the cell cycle, but not in G0.26,27 It is used as an indicator of cells undergoing proliferation. The TUNEL assay is used to assess DNA fragmentation, an indicator of apoptosis, by using the terminal nucleotidyl transferase (TDT) enzyme to catalyse the addition of dUTPs that are secondarily labelled with a marker that allows visualization.26 Immunohistochemical staining of KI-67 showed that both FET-α and FET-α-DN cells had positive staining for Ki-67 antigen. KI-67 staining indicated no difference in proliferation rates between animals with FET-α implants and those with FET-α-DN implants. However, TUNEL staining was ~2.5-fold higher in tumours from mice with FET-α implants than in those from mice with FET-α-DN, which indicates a significantly higher rate of cells undergoing apoptosis in FET-α tumours. Taken together, these results indicate that TGF-β signalling in this colon carcinoma cell line is not capable of suppressing tumour initiation or invasion at the primary site, but is capable of suppressing the progression of an invasive carcinoma with 100% penetrance from evolving to a robust metastatic capability. Thus, shifting the tumour suppressor/oncogenic balance toward oncogenesis by constitutive EGFR activation allows for malignancy, but not a fully metastatic phenotype because of continued metastasis suppressor signalling by TGF-β.
Abrogation of TGF-β tumour suppressor signalling in vitro results in enhanced survival during GFDS

The ability of FET-α cells to invade the primary site, but not to carry out subsequent aspects of the metastatic cascade as a result of TGF-β signalling suggests that this tumour suppressor activity is strong enough to shift the balance of tumour suppressor/oncogenesis toward cell death when these cells encounter the stresses associated with circulation and/or colonization in the foreign microenvironment of distant organs. However, the loss of the tumour suppressor activity associated with TGF-β would be expected to shift this balance toward a higher capacity for cell survival in FET-α-DN cells. To test the hypothesis that the loss of TGF-β tumour suppressor signalling results in a higher capacity for cell survival, we utilized growth factor deprivation as a cell survival stress model to compare FET-α and FET-α-DN cells. Apoptosis, or programmed cell death, is associated with a set of morphological characteristics which include translocation of membrane phospholipids, caspase activation, plasma membrane blebbing, cell shrinkage, chromatin condensation and fragmentation. Cells were deprived of growth factors for 48 h, after which assays directed at different characteristics of apoptosis were performed. We utilized annexin V conjugated to FITC to ascertain cell membrane integrity (the loss of which is an indicator of apoptosis). An early indicator of apoptosis is the translocation of the membrane phospholipid phosphatidylinerine from the inner to the outer leaflet of the plasma membrane. Once phosphatidylinerine is exposed to the extracellular environment it can be bound by annexin V (a phospholipid binding protein) conjugated to FITC, allowing fluorescence visualization and quantification of positive cells. Annexin V-FITC was used in combination with PI to distinguish cells that were in early apoptosis from cells that had lost membrane integrity completely (indicated by PI staining of DNA). Figure 4A shows the results of annexin V-FITC/PI staining of FET-α and FET-α-DN cells that were grown to 80% confluence and then subjected to GFDS for 48 h. The upper left quadrant shows cells that stained positive for PI (indicative of cells undergoing necrosis). The upper right quadrant shows cells that stained positive for both annexin V-FITC and PI, which indicates cells in late apoptosis. The lower left quadrant shows cells that did not stain for either annexin V-FITC or PI and thus represent viable cells. The lower right quadrant shows cells that stained for annexin V-FITC, representing cells in early apoptosis. We compared annexin V staining for early apoptosis in FET-α and FET-α-DN cells (Fig. 4B). Controls for this experiment were unstained cells and cells stained with either annexin V-FITC or PI only. The results show that 10-fold more FET-α cells stained positive for annexin V-FITC compared with FET-α-DN cells after 48 h of GFDS. Similar results were obtained from PARP (poly [ADP-ribose] polymerase) cleavage as assessed by immunoblot analysis.

Increased Akt activation through repression of TGF-β signalling contributes to cell survival

Based on the observation that TGF-β signalling can repress Akt activation in these cells, we determined whether Akt activation was responsible for the enhanced cell survival that resulted from the loss of TGF-β inhibitory signalling in FET-α-DN cells. Cells were grown to 80% confluence and pelleted or deprived of growth factors for 48 h, after which immunoblot analysis to probe for Akt phosphorylation with total Akt as a loading control was conducted. The results showed that phosphorylation of Akt decreased in FET-α cells during GFDS, whereas Akt phosphorylation was persistent in FET-α-DN cells under the same conditions (Fig. 5A). To confirm that Akt was linked to cell survival in FET-α-DN cells, we used LY294002, a potent inhibitor of PI3K which is upstream of the activation of Akt. To verify that LY294002 could inhibit Akt activity, cells were grown to 80% confluence and then deprived of growth factors in the presence of DMSO (the drug carrier) or 25 μM LY294002 for 48 h, after which immunoblot analysis for phosphorylated Akt was performed. The results showed that

Figure 4 Abrogation of TGF-β tumour suppressor signalling in vitro results in enhanced survival during growth factor deprivation stress (GFDS). (A) Annexin V-FITC/PI staining of FET-α and FET-α-DN cells. (B) Comparison of apoptotic cell rates between FET-α and FET-α-DN cells in response to GFDS.
LY294002 effectively inhibited phosphorylation of Akt and did not change total Akt expression (Fig. 5B). The effect of LY294002 inhibition on cell survival was determined by growing cells to 80% confluence and depriving them of growth factors for 48 h in the presence or absence of 25 μM LY294002, a PI3K inhibitor. Cells were then harvested and immunoblot analysis was performed with pAkt-S473 or total Akt antibodies.

Collectively, these data suggest that increased Akt activation resulting from the abrogation of TGF-β tumour suppressor signalling contributes to enhanced cell survival, as reflected by the lower apoptotic rates observed in TUNEL staining of FET-α-DN tumours relative to FET-α tumours.

The results with the FET cell model indicate that TGF-β signalling is a metastasis suppressor, as demonstrated by the acquisition by FET-α of an invasive and highly metastatic capability following the elimination of tumour suppressive TGF-β RII signalling. We extended these results by using the reverse strategy to inhibit metastatic capability in another native colon carcinoma cell line (CBS4) by the genetic rescue of TGF-β Smad signalling through transfection of TGF-β receptors in an additional human colon cancer orthotopic model. CBS4 is a human colon carcinoma cell line that has a compromised TGF-β signalling pathway as a result of reduced expression of TGF-β receptor type II.24 TGF-β sensitivity was restored to native CBS4 cells through the reintroduction of TGF-β type II receptor (designated CBS4-RII). We noted 100% invasiveness at the primary site in both CBS4 and CBS4-RII, but the rate of metastasis decreased from 65% in CBS4 to 10% in CBS4-RII. Taken together with the FET-α-DN studies, these results introduce a novel role for TGF-β signalling as a metastasis suppressor, in conjunction with that as a suppressor of tumour initiation.

**Discussion**

Better understanding of the molecular changes that drive the metastasis responsible for most cancer-related deaths is vital. TGF-β tumour suppression involves cytostasis through the induction of cell cycle regulators, differentiation promotion, suppres-
The dichotomous role of TGF-β signalling is problematic to understanding the consequences of TGF-β signalling and how to effectively target aberrant TGF-β signalling in cancer. Currently, the development of TGF-β inhibitors in treating cancers has increased as a result of growing clinical evidence that TGF-β acts as a tumour-derived immunosuppressor, an inducer of mitogens and a promoter of carcinoma invasion, and is able to trigger prometastatic cytokine secretion. However, our results now show that inhibition of TGF-β signalling can promote metastasis in invasive cancer cells that have not yet progressed to metastatic colonization. Therefore, inhibiting TGF-β signalling in primary tumours may enhance the metastatic capability of the tumour cells.

Conflicts of interest
None declared.

References


