FGFR2 Isoforms Support Epithelial–Stromal Interactions in Thyroid Cancer Progression

Miao Guo1,2,4, Wei Liu1,3,5, Stefano Serra1,3,5, Sylvia L. Asa1,3,5, and Shereen Ezzat1,2,4

Abstract
Alternate splicing yields two distinct isoforms of the fibroblast growth factor (FGF) receptor FGFR2-IIIb and FGFR2-IIIc varying their extracellular structure in human thyroid cancer, in which FGFR expression is commonly dysregulated. In this study, we characterized the function of these variants in modulating thyroid cancer behavior. Enforced expression of either FGFR2-IIIb or FGFR2-IIIc in thyroid epithelial cancer cells reduced expression of fibronectin, MAGE-A3 and MMP9, while increasing p21 and enhancing Rb dephosphorylation. Consistent with these tumor-suppressive properties, FGFR2-IIIb and FGFR2-IIIc each diminished invasive behavior in vitro and reduced tumor growth and metastasis in vivo. Notably, these effects contrasted with those produced by expression of these FGFR isoforms in fibroblasts, in which they both stimulated cell growth. Moreover, in xenograft tumors generated by coimplantation of epithelial and fibroblast cells expressing that same isoform, there was no significant effect on tumor progression. Conversely, FGFR2-IIIb expression in epithelial cells yielded higher FGF4/FGF7 expression that, in the presence of FGFR2-IIIc–expressing fibroblasts, enhanced tumor progression. Together, our findings highlight the importance of cellular context in assigning growth properties to growth factor receptor isoforms. More specifically, they show how alternative splicing of FGFR2 yields heteroisoforms critical to the growth-promoting actions of FGFs that exert distinct epithelial–stromal effects in thyroid cancer.

Introduction
Thyroid cancer is the most common endocrine malignancy and is increasing in incidence (1). The majority of thyroid carcinomas derives from follicular epithelial cells and shows a spectrum of differentiation from indolent well-differentiated papillary and follicular carcinomas to more aggressive poorly differentiated carcinomas and highly lethal anaplastic carcinomas (2, 3). This spectrum of progression has been linked with a pattern of cumulative genetic defects that correlates with tumor differentiation, aggressiveness, and metastatic potential (4). Growth factors and their receptors that are upregulated in thyroid tumors include MET, epidermal growth factor receptor, platelet-derived growth factor, and VEGF (4).

Fibroblast growth factors (FGF) and FGF receptors (FGFR) are also implicated in regulating endocrine neoplasia including thyroid carcinoma (5, 6). FGFs comprise a family of heparin-binding proteins that currently includes 23 members. They signal through 4 high-affinity tyrosine kinase receptors (FGFR 1–4). Following FGF binding and receptor dimerization, several signal transduction pathways are activated, mainly involving FRS2 and PLCγ. Activated FRS2 recruits the GRB2/SOS complex and ultimately mitogen-activated protein kinase (MAPK or ERK1/2; ref. 7). Combinations of FGFs, FGFR isoforms, and adaptor proteins are involved in complex signaling networks that play fundamental roles in development, organogenesis, cell differentiation, angiogenesis, and tumor progression (6, 8).

We have previously shown that FGFR expression is dysregulated in human thyroid tumors and cell lines (5). In particular, FGFR2, which is situated on chromosome 10q26, was the FGFR consistently detected in normal primary thyroid tissue. In contrast, its expression was diminished in some thyroid carcinoma cell lines (5).

Each of the FGFRs is composed of 3 immunoglobulin (Ig)-like extracellular domains, 2 of which are involved in ligand binding, a single transmembrane domain, a split tyrosine kinase, and a C-terminal tail with multiple autophosphorylation sites (9). Alternative splicing results in variants of FGFRs 1, 2, and 3 that have different third Ig-like domains with variable ligand affinities. Such alternative splicing of the FGFR2 mRNA generates the FGFR2-IIIb isoform, which selectively binds FGF7 with high affinity (10, 11), or the FGFR2-IIIc isoform that selectively binds FGF4 but not FGF7 (12, 13). The importance of FGFR2-IIIb in development is supported by the fact that targeted genetic disruption of...
FGFR2-IIIb causes agenesis of several organs including the thyroid gland (14).

In this study, we focused on characterizing FGFR2 signaling actions of the 2 alternatively spliced variants in modulating thyroid cancer behavior. As FGFR2-IIIb expression is mainly in epithelial cells, we forced its expression in WRO and TPC-1 thyroid cancer cells. Because FGFR2-IIIc is typically expressed in mesenchymal cells (15), we expressed it in NIH-3T3 fibroblast cells. We compared the independent signaling properties and effects in vivo when the 2 FGFR2 isoforms were coexpressed in the same or different cell types. Our data highlight the importance of FGFR2 hetero-isoforms in mediating distinct epithelial–stromal interactions in supporting thyroid cancer progression.

Materials and Methods

Cell lines and cell culture

The human follicular thyroid carcinoma-derived cell line WRO-82 (established by Dr. G. Juillard, UCLA, Los Angeles, CA) was maintained in RPMI-1640 (Gibco) supplemented with 10% FBS, 2 mmol/L L-glutamine, 1 mmol/L sodium pyruvate, 1x nonessential amino acids, streptomycin sulfate (100 U/mL), and penicillin (100 U/mL). The human papillary thyroid carcinoma-derived cell line TPC-1 (established by Dr. S. M. Jhiang, Ohio State University, Columbus, OH) was cultured in Dulbecco’s modified Eagle’s medium (DMEM) with 5% FBS and 2 mmol/L L-glutamine. These human-derived cell lines were authenticated by DNA short-tandem repeat analysis 2 years ago. Mouse fibroblast NIH-3T3 cells were cultured in DMEM with 10% FBS and antibiotics.

Vector constructs and stable gene expression

The cDNA encoding human FGFR2-IIIb, also known as Ksam-IC1 (established by Drs. M. Terada and T. Yoshida, National Cancer Institute, Tokyo, Japan) in pcDNA1/Neo expression vector (Invitrogen) and an empty vector (control) were kindly provided by Dr. F. Radvanyi (Center National de la Recherche Scientifique, Paris, France). The cDNA encoding human FGFR2-IIIc was inserted in pcDNA3.1/V5-His-TOPO vector (Invitrogen). Sequence fidelity was confirmed before transfection into cells using Lipofectamine (Invitrogen). Stable clones were selected and maintained in a growth medium containing 1 mg/mL of Geneticin or G418 (Invitrogen). FGFR2-IIIb and FGFR2-IIIc plasmids were also used as templates for PCR to generate the respective cDNA for insertion into the MSCV-PIG viral vector (kindly provided by S. Muthuswamy, Ontario Cancer Institute, Toronto, Ontario, Canada). In all experiments, FGFR2 expression was confirmed by Western blotting. At least 2 independent clones with comparable levels of expression of each isoform were used for all studies.

Growth factor stimulation

After 24 hours of starvation in serum-free medium, cells were treated with the nonselective ligand FGF1 (25 ng/mL; Sigma), the FGFR2-IIIb-avid FGF7 (25 ng/mL; Sigma), or the FGFR2-IIIc-binding FGF4 (25 ng/mL; Sigma), each with 10 U/mL of heparin (Sigma) in serum-free medium for 15 minutes at 37°C. Identical volume of vehicle served as control.

RNA extraction, RT-PCR, and real-time PCR

Total RNA was isolated from cells using RNeasy kit (Qiagen), followed by DNase I treatment. Six hundred nanograms of total RNA were reverse transcribed in 30 μL reaction mixture containing 500 μmol/L of each deoxynucleotide triphosphate, 12 U ribonuclease inhibitor, and MultiScribe reverse transcriptase using TaqMan reverse transcription reagents (Applied Biosystems, Life Technologies). The reaction mixture was incubated at 25°C for 10 minutes, 48°C for 30 minutes, and 95°C for 5 minutes. The specific PCR primers for MAGE-A3, the housekeeping gene PGK-1, FGFR2-IIIb, and FGFR2-IIIc, and the PCR conditions are listed in Supplementary Table S1. Amplification was designed to cross exon–intron boundaries to exclude genomic DNA contamination. Amplification was done using HotStarTag DNA polymerase kit (Qiagen). Real-time PCR was done on cDNA samples in triplicate using an ABI PRISM 7900HT sequence detection system (Applied Biosystems). SYBR Green PCR Master Mix (Applied Biosystems) was used according to the manufacturer’s protocol. The primers for amplification of human MAGE-A3 and an endogenous control glyceraldehyde-3-phosphate dehydrogenase were provided by Real Time Primers Company (LLC) and the sequences are listed in Supplementary Table S1. PCR reactions were carried out in a 20 μL volume containing 0.5 μmol/L primers and 1 μL cDNA using the following conditions: 50°C for 2 minutes, 95°C for 10 minutes, 40 cycles of 95°C for 15 seconds, and 60°C for 1 minute. The results were analyzed using a comparative method. The amount of target, normalized to the endogenous reference, is given by the formula 2−ΔΔCt, in which Ct represents the threshold cycle, indicating the fractional cycle number at which the amount of amplified target reaches a fixed threshold.

Protein isolation and Western blotting

Mouse xenografted tissues were homogenized using a polytron homogenizer in radioimmunoprecipitation assay (RIPA) lysis buffer with protease inhibitors (1x PBS, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, 1 mmol/L phenylmethylsulfonylfluoride, 12 μg/mL aprotinin, and 1 mmol/L sodium orthovanadate) and 5 μL of phosphatase inhibitor cocktail 1 and 2 (Sigma). Cultured cells were lysed in the same RIPA lysis buffer with protease inhibitors and phosphatase inhibitor cocktails 1 and 2.

Equal amounts of protein (50 μg) solubilized in sample buffer were separated on 8% or 10% SDS polyacrylamide gels and transferred electrophoretically onto nitrocellulose membranes. Membranes were blocked in TBS containing 0.1% Tween 20 (TBS-T) plus 5% nonfat dried milk for 1 hour at room temperature, probed with primary antibodies at 4°C overnight. Primary antibodies were used at the specified dilutions: anti-FGFR2 (1:1,000; Santa Cruz Biotechnology), anti-phospho-MAPK (1:1,000; Cell Signaling), anti-total MAPK (1:4,000; Sigma), anti–phospho-akt, anti–total-Akt (1:1,000; Cell Signaling), anti-p21 (1:500; BD Biosciences), anti-p27 (1:1,000; Transduction Laboratories, BD Biosciences), anti-p53 (1:1,000; Santa Cruz Biotechnology), anti–phospho-Rb
were incubated at 37°C.

Thyroids of 5- to 6-week-old female severe-combined immunodeficient (SCID) mice were used to generate orthotopic tumors as previously described (16). Tumor volume was monitored every 2 days. Mice were sacrificed after 14 days following cell implantation when tumor volumes and weights were recorded, and full autopsies were conducted to identify and quantitate metastases. Half of excised tumor tissue was fixed in 10% formalin and embedded in paraffin for light microscopy and immunohistochemical staining. The other half was snap-frozen in liquid nitrogen and stored at −70°C for RNA and protein extraction. Lungs, liver, and other tissues with potential metastases were fixed in formalin and embedded in paraffin for morphologic evaluation. The mouse protocol was approved by the Ontario Cancer Institute Animal Care and Utilization Committee.

Statistical analysis
Data are presented as mean ± SEM. Statistical analysis was done using the Student t test. P values of 0.05 or less were considered statistically significant. For tissue analyses, the statistical software package SPSS 18.0 was used for comparisons.

Results

**Forced FGFR2 expression in thyroid epithelial cancer cells and NIH-3T3 fibroblasts**

To investigate the functions of FGFR2 isoforms in human thyroid cancer progression and based on endothogenous FGFR expression profiling (5), we selected the human follicular thyroid carcinoma cell line WRO and the papillary thyroid carcinoma cell line TPC-1 that do not express detectable FGFR2 for forced expression of FGFR2-IIIb and FGFR2-IIIC. Clones stably expressing pcDNA3.1 (TPC-1 control and WRO control), FGFR2-IIIb (TPC-1-FGFR2-IIIb and WRO-FGFR2-IIIb) or FGFR2-IIIC (TPC-1-FGFR2-IIIC and WRO-FGFR2-IIIC) were identified and verified by Western blotting (Fig. 1A). To validate the functionality of the introduced FGFR2 isoforms, we stimulated these cells with the nonselective ligand FGF1, with FGF7 that selectively activates FGFR2-IIIb, or with FGF4 that selectively activates FGFR2-IIIC. Consistent with the ability of FGFR2 to sequester FRS2 away from other endogenous FGFRs (17), cells expressing this receptor show lower basal levels of pMAPK and pAKT than controls (Fig. 1A). In contrast, FGF stimulation results in FGFR2 isoform-selective responses in TPC-1 and WRO cells transfected with FGFR2 isoforms (Fig. 1B and C).

NIH-3T3 fibroblasts express endogenous FGFR2-IIIC, but clones were generated to force overexpression of FGFR2-IIib (NIH-3T3-FGFR2-IIib), FGFR2-IIIC (NIH-3T3-FGFR2-IIIC), or the empty vector (NIH-3T3 control; Fig. 1D).

**FGFR2-IIib and FGFR2-IIIC independently inhibit thyroid epithelial cancer cell growth**

To examine the effects of FGFR2 isoforms on thyroid cancer epithelial cell proliferation, control, FGFR2-IIib- and FGFR2-IIIC-expressing synchronized cells were monitored by direct cell counting. Both FGFR2 isoforms in TPC-1 and WRO cells reduced cell numbers compared with their controls (Fig. 2A and B). Unexpectedly, FGFR2-IIIC inhibited epithelial cell...
proliferation more potently than FGFR2-IIIb in these cells (Fig. 2A and B). Measures of apoptosis, including caspase-3 cleavage and PARP degradation, were not influenced by either FGFR2 isoforms (data not shown). In contrast, FGFR2-IIIb enhanced cell proliferation when introduced into NIH-3T3 fibroblasts (Fig. 2C). Colony formation in soft agar was also examined in WRO cells. Both FGFR2 isoforms inhibited colony formation. After 10 days of growth, there was an average 61% ± 3 decrease in colony number in FGFR2-IIIb cells and 39% ± 2 reduction in FGFR2-IIIc cells compared with controls. TPC-1 cells did not

Figure 1. Forced expression of FGFR2 isoforms in thyroid epithelial cancer cells and fibroblasts. A, clones overexpressing FGFR2-IIIb or FGFR2-IIlc of human thyroid papillary carcinoma TPC-1 (left) and follicular carcinoma WRO cells (right) are confirmed by Western blotting. The functionality of FGFR2-IIIb and FGFR2-IIlc in TPC-1 cells and WRO cells was shown by treatment with FGFs. FGF7 selectively activates FGFR2-IIIb and FGF4 selectively activates FGFR2-IIlc leading to phosphorylation of MAPK (B) and AKT (C). Bar graphs represent mean densitometric values derived from 3 independent experiments. D, FGFR2 isoforms were characterized using site-specific enzymatic digestion in which AvaI restricts the FGFR2-IIIb and EcoRV restricts the FGFR2-IIlc isoform. Only the FGFR2-IIlc isoform is detected endogenously in NIH-3T3 cells (left). Western blotting (right) shows overexpression of FGFR2-IIIb and FGFR2-IIlc compared with control empty vector in NIH-3T3 cells.
Develop colonies in soft agar in the absence or presence of FGFR2.

Furthermore, FGFR2-IIIb or FGFR2-IIIc significantly inhibited invasion without affecting cell migration in WRO and in TPC-1 epithelial thyroid cancer cells (Fig. 3A and B). In contrast, both FGFR2 isoforms significantly promoted NIH-3T3 cell invasion but not cell migration (Fig. 3C).

To examine FGFR2 isoform actions in vivo, we used a xenograft mouse model that showed a significant reduction in tumor volume and tumor weight of WRO cells expressing FGFR2-IIIb or FGFR2-IIIc compared with control cells (Fig. 4A). In contrast to epithelial cells, both FGFR2-IIIb and FGFR2-IIIc promoted NIH-3T3 fibroblast tumor growth compared with controls (Fig. 4B) in mouse xenografts. Examination of cell-cycle control elements in xenografted tissue showed that both FGFR2 isoforms increased p21 and inhibited Rb phosphorylation without in vivo effects of thyroid cancer TPC-1 (A), WRO (B), or NIH-3T3 (C) fibroblast cells stably expressing FGFR2-IIIb, FGFR2-IIIc, or their control vector were grown for 24 and 48 hours. Direct cell counting shows reduction of TPC-1 and WRO cells expressing either FGFR2-IIIb or FGFR2-IIIc compared with control cells. In contrast, both FGFR2 isoforms increased the number of NIH-3T3 cells compared with controls. Data shown represent mean ± SEM obtained from 3 independent experiments, each conducted in triplicate.

FGFR2 isoforms limit thyroid cancer metastasis

To determine the potential impact of FGFR2 isoforms on metastatic growth, we examined the spread of tumor in the orthotopic mouse model of thyroid carcinoma. Whereas 5 of 20 control mice bearing xenografts of WRO control cells developed lung metastases, none of 20 mice with xenografts of WRO cells expressing FGFR2-IIIb or FGFR2-IIIc developed lung metastasis ($P = 0.047$; Supplementary Fig. S1a). This arrest of metastasis development was accompanied by inhibition of the metalloproteinase MMP-9 (Supplementary Fig. S1b). Furthermore, we confirmed by Western blotting that the thyroid cancer metastasis–promoting MAGE-A3 (16) was strongly downregulated by FGFR2-IIIb and FGFR2-IIIc (Supplementary Fig. S1c). Reverse transcriptase PCR (RT-PCR) and real-time PCR confirmed that both FGFR2 isoforms decreased MAGE-A3 mRNA levels compared with controls (Supplementary Fig. S1d).

Coexpression of FGFR2-IIIb and FGFR2-IIIc results in distinct cell-specific effects on tumor progression in vivo

To investigate the potential interactions between FGFR2-IIIb and FGFR2-IIIc in modulating tumor growth in vivo, we carried out mouse xenograft studies in which the 2 FGFR2 isoforms were expressed in the same or different cell types. When we introduced equal proportions of epithelial WRO cells, the concomitant expression of FGFR2-IIIb and FGFR2-IIIc resulted in inhibition of tumor progression intermediate to that of cells expressing one receptor isoform or the other (Fig. 6A).

As FGFR2-IIIb expression has been described mainly in epithelial cells and FGFR2-IIIc is predominantly in stromal fibroblasts, we tested whether the in vivo interactions of the 2 FGFR2 isoforms were cell-type dependent. As there are currently no thyroid-derived fibroblast cell lines available, we selected NIH-3T3 cells for coinoculation with thyroid epithelial carcinoma cells. First we compared the growth of NIH-3T3-FGFR2-IIIb cells when mixed with an equal number of epithelial WRO cells expressing FGFR2-IIIb or FGFR2-IIIc (Fig. 6B). Expression of FGFR2-IIIc in both epithelial WRO and in NIH-3T3 fibroblasts was not sufficient to enhance tumor growth compared with controls (Fig. 6B). Indeed, the only combination that resulted in enhanced tumor growth was NIH-3T3-FGFR2-IIIc with WRO-FGFR2-IIIb (Fig. 6B). This prompted us to examine the production of dedicated FGFs by the 2 cell types in the xenograft studies described. We focused on FGF4 and FGF7 as the 2 FGFs that we had tested (Fig. 5A). As FGFR2-IIIb expression is increased mainly in epithelial cells and FGFR2-IIIc is predominantly in stromal fibroblasts, we tested whether the in vivo interactions of the 2 FGFR2 isoforms were cell-type dependent. As there are currently no thyroid-derived fibroblast cell lines available, we selected NIH-3T3 cells for coinoculation with thyroid epithelial carcinoma cells. First we compared the growth of NIH-3T3-FGFR2-IIIb cells when mixed with an equal number of epithelial WRO cells expressing FGFR2-IIIb or FGFR2-IIIc (Fig. 6B). Expression of FGFR2-IIIc in both epithelial WRO and in NIH-3T3 fibroblasts was not sufficient to enhance tumor growth compared with controls (Fig. 6B). Indeed, the only combination that resulted in enhanced tumor growth was NIH-3T3-FGFR2-IIIc with WRO-FGFR2-IIIb (Fig. 6B). This prompted us to examine the production of dedicated FGFs by the 2 cell types in the xenograft studies described. We focused on FGF4 and FGF7 as the 2 FGFs that we had tested in vitro and were expressed in our tumor xenografts. These examinations identified stable FGF4 expression in all xenografts, but FGFR2-IIIb in epithelial cells diminished expression of its own ligand FGF7 (Fig. 6C). Thus, the FGF4/FGF7 ratio was increased by FGFR2-
Figure 3. FGFR2-IIIb and FGFR2-IIIc independently inhibit thyroid cancer cell invasion but stimulate fibroblast invasion in vitro. Forced expression of FGFR2-IIIb or FGFR2-IIIc significantly inhibits invasion by TPC-1 cells (A) and by WRO cells (B); migration is not affected. C, in contrast, forced expression of FGFR2-III isoforms significantly enhances NIH-3T3 cell invasion without affecting cell migration. Data are expressed as the mean ± SEM from 4 independent experiments, each conducted in duplicate.
IIIb in epithelial cells (Fig. 6C). We propose that epithelial FGFR2-IIIb promotes a positive FGF4/FGF7 balance that, in the presence of stromal FGFR2-IIIc, couples epithelial signal- 
ing with stromal expansion to support in vivo tumor progres-
sion. Consistent with this model, histologic examination from 
these xenografts identi 
fi-
ced both epithelial and stromal con-
tribution to the enhanced growth of the WRO-FGFR2-IIIb/
NIH-3T3-FGFR2-IIIc tumors (Fig. 7). These 
findings under-
scored the importance of the interaction between the 2 FGFR2 
isofoms when expressed in different admixed cell types.

Discussion

Situated on chromosome 10q26, FGFR2 is at the heart of a 
genomically unstable region associated with increased cancer 
risk including that of the breast (18). The role of FGFR2 isoforms 
as tumor suppressors or oncogenic signals continues to be 
controversial. Here we show contrasting actions for FGFR2 
isofoms in epithelial thyroid carcinoma cells and fibroblasts. 
Indeed, when introduced separately in epithelial cells, FGFR2-
IIIb and FGFR2-IIc display signi 
fi-
cant and consistent growth 
in vitro 
and in vivo. In contrast, when expressed in 
NIH-3T3 fibroblasts, each FGFR2 isoform promoted cell growth 
and enhanced tumor progression in mouse xenografts. Inter-
estingly, forced expression of the same isoform in epithelial cells 
and fibroblasts together did not support maximal tumor pro-
gression, indicating that interactions between FGFRs in differ-
ent cell types alone is not sufficient to explain differing tumor 
behaviors. In this context, enhanced tumor progression was 
most evident when epithelial cells and fibroblasts expressed 
alternate FGFR2 isoforms, suggesting cross-talk between the 2 
cellular compartments through the isoforms of this receptor.

FGFR2-IIIb and FGFR2-IIc isoforms are expressed in nor-
mal mouse and human tissues, including pituitary (19), thyroid 
(17, 20), and breast (21). However, downregulation of FGFR2 
has also been reported in a variety of tumors including 
asastrocytomas, bladder (22, 23), prostatic carcinoma (24), 
pituitary tumors (14, 19, 25), and thyroid carcinomas (5). Missense 
mutations of FGFR2 occur in endometrial cancer, ovarian 
cancer, breast cancer, lung cancer, and gastric cancer (26– 
28). Eight single-nucleotide polymorphisms within intron 2 of 
the FGFR2 gene have been associated with increased risk of 
breast cancer (18, 29–31). Copy number gain of FGFR2 has also 
been described in breast and gastric cancers (32, 33). These 
divergent expression pro 
fi-
files have further confounded inter-
pretation of the actions of FGFR2, especially FGFR2-IIc.

We have previously reported that forced expression of 
FGFR2-IIIb can impose on the RAS/BRAF/MAPK pathway to 
modulate thyroid cancer progression in mouse xenografts (17). 
However, the role of FGFR2-IIc alone or in the concomitant 
presence of FGFR2-IIIb in epithelial tumors or thyroid cancer 
has not been previously examined. In this study, we forced 
expression of FGFR2-IIc in 2 distinct thyroid epithelial carcino-
ama cell lines (TPC-1 and WRO) and noted that like FGFR2-
IIIb, FGFR2-IIc can significantly inhibit proliferation of these 
cells. This was noted in vitro by direct cell counting, by colony 
formation in soft agar, and in mouse xenografts. Consistent 
with these findings, both FGFR2-IIb and FGFR2-IIc increased 
p21, decreased Rb phosphorylation, and reduced EMT, as

Figure 4. FGFR2-IIIb and FGFR2-
IIc independently inhibit thyroid 
cancer cell growth but stimulate 
fibroblast growth in vivo. A, forced 
expression of FGFR2-IIIb or FGFR2-
IIc significantly inhibits tumor growth 
in mouse xenografts of WRO cells in 
6-week-old SCID mice. The 
indicated tumor volumes and 
weights represent the mean ± SEM 
of 20 mice in each group. B, in 
contrast, forced expression of 
FGFR2-IIIc promotes tumor growth in xenografts of NIH-
3T3 cells in the same model. The 
indicated tumor volumes and 
weights represent the mean ± SEM 
of 10 mice in each group.
evidenced by decreased fibronectin and cytokeratin 14 expressions. Furthermore, both FGFR2 isoforms independently inhibited MMP9, cell invasion, and lung metastasis. We also noted that the 2 FGFR2 isoforms each profoundly inhibit the metastasis-promoting MAGE-A3 (16). These findings build on our previous report showing FGFR2-IIIb inhibition of MAGE-A3 through enhanced promoter methylation (34). Consistent with these findings, forced expression of FGFR2-IIIb has been reported to retard cancer cell proliferation in several models (22, 35–37).

FGFR2-IIIb expression is typically restricted to epithelial cells, whereas FGFR2-IIIc is characteristic of mesenchymal lineages (10, 38). Furthermore, it has been suggested that the dedicated ligands for FGFR2-IIIb are usually expressed by mesenchymal cells and the ligands for FGFR2-IIIc by epithelial cells. Moreover, a switch from FGFR2-IIIb to FGFR2-IIIc has been reported with malignant progression in prostate and bladder cancers (39–41). Thus, to investigate the functions of FGFR2 isoforms in nonepithelial cells, we used mouse NIH-3T3 fibroblasts. We confirmed that these cells endogenously express low levels of FGFR2-IIIc but not FGFR2-IIIb. This feature allowed us to compare the cell type–specific actions of each of the isoforms. Specifically, forced FGFR2-IIIb, FGFR-IIIc, or both resulted in growth inhibition when expressed exclusively in WRO epithelial cells. In contrast, overexpression of FGFR2-IIIc in NIH 3T3 fibroblasts promoted tumor progression. These data further highlight the differential functions of FGFR2 isoforms in epithelial cells and fibroblasts.

To further clarify the effects of FGFR2-IIIc in epithelial and mesenchymal cells on tumor growth, we introduced...
mixtures of the same number of WRO epithelial and NIH-3T3 fibroblasts expressing different combinations of each of the FGFR2 isoforms. As noted in vitro, FGFR2-IIIc showed a clear inhibitory effect on WRO tumor xenografts. In marked contrast, introduction of either FGFR2-IIIb or FGFR2-IIIc alone promoted NIH-3T3 xenograft growth, highlighting the cell-type dependency of FGFR2 action. These findings indicate that cell type contributes only partially to the differential actions of FGFR2 isoforms. Indeed, the most striking neoplastic growth was noted when the 2 FGFR2 isoforms were expressed in different cell types. These findings represent the earliest data showing distinct actions for the FGFR2 isoforms in different cell types, particularly when coexpressed in epithelial and fibroblast combinations.

Neoplastic growth is composed of both cancer cells and stromal cells, including fibroblasts. There is increasing evidence of the importance of stromal cells in several aspects of cancer biology, including transformation and progression (42). In this study, we show the importance of FGFR2-IIIc in mediating epithelial–stromal interactions in thyroid cancer progression. In the mouse orthotopic model, we found that FGFR2-IIIc expression in epithelial cells inhibits, whereas in fibroblasts it stimulates, tumor growth. We speculate that FGFR2 isoforms regulate multiple cytokines that can selectively stimulate the alternate isoform. Consistent with this model, we noted that epithelial FGFR2-IIIb diminishes expression of its own ligand FGF7, without influencing FGF4. In this model, the expression of FGFR2-IIIb by epithelium results in a relatively higher FGF4/FGF7 ratio, creating an environment that also supports the growth of mesenchymal cells expressing FGFR2-IIIc. This provides a mechanism by which tumor growth engages growth of supporting stroma. Of course, we cannot exclude the
involvement of other FGFs including FGF8 and FGF10 that can also activate FGFR2.

In summary, this study shows the differential functions of FGFR2 isoforms in epithelial thyroid cancer and fibroblasts and the importance of cellular context in assigning growth-promoting or growth-suppressive functions to this receptor. We propose that alternative splicing of FGFR2 yields isoforms of functional importance to epithelial and stromal interactions in promoting neoplastic progression.

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

Authors' Contributions
Conception and design: S.L. Asa and S. Ezzat
Development of methodology: M. Guo, W. Liu, S.L. Asa, and S. Ezzat
Acquisition of data: M. Guo, W. Liu, S.L. Asa, and S. Ezzat
Analysis and interpretation of data: M. Guo, W. Liu, S. Serra, S.L. Asa, and S. Ezzat
Writing, review, and/or revision of the manuscript: M. Guo, W. Liu, S.L. Asa, and S. Ezzat
Administrative, technical, or material support: S.L. Asa
Study supervision: S. Ezzat

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