An FGFR1-SPRY2 Signaling Axis Limits Basal Cell Proliferation in the Steady-State Airway Epithelium

Graphical Abstract

Highlights
- FGFR1 signaling is important for inhibiting airway stem cell proliferation
- FGFR1 post-translationally activates SPRY2, which inhibits activity of other RTKs
- Balancing the activity of different RTKs tunes airway stem cell proliferation rates
- Ciliated cell fate specification also requires FGFR1 but does not require SPRY2

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In Brief
Airway basal cells are highly quiescent at homeostasis. Rawlins et al. show that this quiescence is actively maintained, in part by an FGFR1-SPRY2 signaling mechanism. This FGFR1 signaling limits steady-state basal cell proliferation by repressing an RTK-mediated pro-proliferative signal.

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An FGFR1-SPRY2 Signaling Axis Limits Basal Cell Proliferation in the Steady-State Airway Epithelium

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SUMMARY
The steady-state airway epithelium has a low rate of stem cell turnover but can nevertheless mount a rapid proliferative response following injury. This suggests a mechanism to restrain proliferation at steady state. One such mechanism has been identified in skeletal muscle in which pro-proliferative FGFR1 signaling is antagonized by SPRY1 to maintain satellite cell quiescence. Surprisingly, we found that deletion of Fgfr1 or Spry2 in basal cells of the adult mouse trachea caused an increase in steady-state proliferation. We show that in airway basal cells, SPRY2 is post-translationally modified in response to FGFR1 signaling. This allows SPRY2 to inhibit intracellular signaling downstream of other receptor tyrosine kinases and restrain basal cell proliferation. An FGFR1-SPRY2 signaling axis has previously been characterized in cell lines in vitro. We now demonstrate an in vivo biological function of this interaction and thus identify an active signaling mechanism that maintains quiescence in the airway epithelium.

INTRODUCTION
The airway epithelium of both mouse and humans is essentially quiescent at steady state, with an extremely low rate of stem cell proliferation (Cole et al., 2010; Kauffman, 1980; Teixeira et al., 2013). Nevertheless, airway basal cells (BCs) can rapidly enter the cell cycle in response to luminal cell loss (Hong et al., 2004; Pardo-Saganta et al., 2015; Rawlins et al., 2007). Several paracrine signaling pathways that promote airway stem cell proliferation following injury have been characterized (reviewed in Hogan et al., 2014). In addition, autocrine signaling mechanisms can initiate airway proliferation in response to local damage (Vermeer et al., 2003). A critical question remains: are there also mechanisms which actively inhibit airway proliferation at homeostasis and therefore function to maintain quiescence?

In overall organization the mouse trachea is very similar to human smaller airways (Hackett et al., 2011; Rock et al., 2010; Teixeira et al., 2013). The adult mouse tracheal epithelium comprises three main cell types. BCs include both slowly dividing stem cells and committed luminal precursors (Mori et al., 2015; Rock et al., 2009; Watson et al., 2015). Luminal secretory cells can self-renew and produce luminal ciliated cells, while ciliated cells are terminally differentiated (Rawlins and Hogan, 2008; Rawlins et al., 2007, 2009). In vitro and in vivo evidence suggests that airway BC proliferation requires epidermal growth factor receptor (EGFR) activity (Brechbuhl et al., 2014; You et al., 2002). Moreover, inhibition of EGFR signaling via contact inhibition is necessary to restrain BC proliferation following injury (Lu et al., 2013). WNT and Notch signaling can also promote BC proliferation in some contexts (Giangreco et al., 2012; Paul et al., 2014; Rock et al., 2011). By contrast, YAP prevents differentiation of BCs (Mahoney et al., 2014; Zhao et al., 2014). However, no specific signaling pathways that actively inhibit BC proliferation at steady state have been identified.

In other organs, stem cell quiescence is actively maintained by feedback inhibition. For example, in the satellite cells of skeletal muscle steady-state quiescence requires the function of specific receptor tyrosine kinase (RTK) inhibitors, SPRY proteins, to antagonize pro-proliferative fibroblast growth factor receptor 1 (FGFR1) signaling (Chakkalakal et al., 2012; Shea et al., 2010). We speculated that similar mechanisms would operate in the steady-state airway epithelium.

FGFR signaling has been extensively studied in lung development and the smaller conducting airways (e.g., Abier et al., 2009; Volckaert et al., 2011, 2013; Yin et al., 2011) where, similar to its role in muscle, it has been found to have a pro-proliferative function. However, the role of FGFR signaling in airway BCs remains undetermined. We therefore tested whether antagonism of FGFR1 activity by SPRY proteins is required for BC quiescence. Surprisingly, we found that deletion of either Fgfr1 or Spry2 resulted in increased levels of BC proliferation. We demonstrate that in airway BCs, SPRY2 is post-translationally modified downstream of FGFR1, allowing SPRY2 to antagonize signaling from other RTKs, most likely EGFR, and maintain quiescence.

There is a well-documented in vitro relationship between FGFR1-mediated modification of SPRY2 and RAS-ERK inhibition (Lao et al., 2006, 2007). However, a role for this interaction has never previously been identified in vivo.
RESULTS

FGFR1 Signaling Is Required for Normal Tracheal Cellular Homeostasis

FGFR signaling pathway components are readily detected in the steady-state adult mouse trachea by RT-PCR (Figure S1A). Fgfr1 and Spry2 mRNA were also detected in purified BC, secretory, and ciliated cell populations by qRT-PCR (Figures 1A, S1B, and S1C) and by single-cell qRT-PCR (Watson et al., 2015). Moreover, FGFR1 protein and mRNA were detected in BCs and luminal cells in the intact mouse trachea (Figures S1D and S1F). We conditionally deleted Fgfr1 and activated a GFP reporter in tracheal BCs using Tg(KRT5-CreER); Rosa26R-fGFP; Fgfr1+/fx (Fgfr1 conditional knockout, cKO) and control

Figure 1. Deletion of Fgfr1 in BCs Results in Altered Tracheal Epithelial Homeostasis

(A) Relative expression of Fgfr1 and Spry2 mRNA in sorted wild-type basal, secretory, and ciliated cells. (B) Schematic of Fgfr1 conditional knockout experiment. (C) Relative expression of Fgfr1 mRNA in sorted GFP+ BCs from control and Fgfr1 cKO mice 3 weeks after tmx administration. (D) Representative confocal sections from control Tg(KRT5-CreER); Rosa26R-fGFP+ mice. Green, GFP (reporter); red, KRT5 (BCs); blue, DAPI (nuclei). Increased labeling of luminal cells is visible in the Fgfr1 cKOs by 5 weeks (arrowheads), becoming large patches of labeled cells (brackets) by 13 and 24 weeks. (E) Percentage of the total T1α+ BCs that are also GFP+. (F) Percentage of total T1α+ luminal cells that are also GFP+. Error bars denote SEM. Scale bars, 50 μm. See also Figures S1 and S2.

Tg(KRT5-CreER); Rosa26R-fGFP mice. Four doses of tamoxifen (tmx) were administered to adult (>8 weeks old) mice (Figure 1B). To confirm deletion of Fgfr1fx in the GFP+ BCs, we performed qRT-PCR on pools of isolated GFP+ BCs and showed that the level of Fgfr1 mRNA in the Fgfr1 cKOs was reduced to 20% of the control level (Figure 1C). This result showed that widespread co-recombination of the Fgfr1fx and Rosa26RfGFP loci was occurring, and we used GFP+ cells as a surrogate marker for Fgfr1+/fx cells in further experiments. However, it is important to note that Rosa and Fgfr1 co-recombination is <80%, and we therefore underestimate the extent of the Fgfr1 phenotype using this scoring method.

Tracheae were harvested at intervals and processed for histology to assess the contribution of GFP+, Fgfr1+/fx BCs to the epithelium during normal homeostatic turnover. At 1.5 weeks after tmx administration, ~30% of total BCs were GFP+ in both Fgfr1 cKOs and controls. In control animals this percentage increased modestly to 40%–50% at 5–13 weeks before dropping to initial levels by 24 weeks. This change was much more marked in the Fgfr1 cKO mice, in which the percentage of labeled BCs peaked at 60% at 13 weeks before returning to initial levels (Figures 1D and 1E). In both genotypes, labeled BCs produced labeled luminal cells as expected. However, luminal cell production was greater in the Fgfr1 cKO mice, with levels of GFP+ luminal cells reaching 38% ± 2% of the total luminal cell population by 24 weeks, compared with 21% ± 4% in the controls (Figure 1F). Tg(KRT5-CreER); Rosa26R-fGFP; Fgfr1+/fx (Fgfr1 conditional heterozygous) mice displayed a phenotype similar to that
of the cKOs (Figures S2A and S2B). Thus, greater numbers of both basal and luminal cells are produced by the Fgfr1\(^{-/-}\) BCs than controls, showing that FGFR1 signaling influences the rate of epithelial cell turnover in the steady-state trachea.

**Increased Levels of Basal Cell Proliferation in the Fgfr1 cKO Tracheae**

We assessed proliferation rates using KI67 antibody staining. This showed that in vivo rates of proliferation were >3-fold higher in the Fgfr1\(^{-/-}\) BCs than in controls (Figures 2A and 2B). Moreover, the level of KI67 mRNA in GFP\(^+\) BCs isolated from Fgfr1 cKO tracheae was increased 11-fold compared with controls (Figure 2C). Proliferation levels were intermediate in the Fgfr1 conditional heterozygotes (Figure S2C). We confirmed the increase in BC proliferation in vitro using a limiting dose of Cre recombinase adenovirus (Ad-Cre) to induce recombination in small numbers of cells from Rosa26R-fGFP; Fgfr1\(^{-/-}\) and control Rosa26R-fGFP tracheae (Figure 2D). The Fgfr1\(^{-/-}\) cells produced larger GFP\(^+\) colonies than the controls, and these cultures also contained higher numbers of KI67\(^+\) cells (Figures 2E–2G).

We predicted that if deletion of Fgfr1 results in BC proliferation, activating FGFR1 signaling in vitro would inhibit growth of BC colonies. FGF2 is expressed in the homeostatic trachea (Figure S1A), is known to activate FGFR1 preferentially in vitro, and is strongly linked to FGFR1 activation in multiple in vivo situations (Arai et al., 2015; Chakkalakal et al., 2012; Garmy-Susini et al., 2004; Hackett et al., 2011; Hadjab et al., 2013; Kraneburg et al., 2005; Ornitz et al., 1996). We therefore tested the effects of the addition of exogenous FGF2 protein on the proliferation of wild-type primary BCs in culture. We plated wild-type BCs at low density and added FGF2 on culture day 2 after plating. Red, E-cadherin (lateral cell membrane); blue, DAPI (nuclei).

(F) Fgf1 deletion in vitro results in increased BC proliferation. Red, KI67 (proliferating cells); blue, DAPI (nuclei).

(G) Percentage of proliferating (KI67\(^+\)) cells in vitro.

(H) Schematic for in vitro experiment. Wild-type (WT) BCs plated at low density (3 \( \times \) 10\(^4\) cells/insert) and exposed to 100 ng/ml FGF2 from day 2. (I) FGF2-treated and control wild-type BCs at day 4 after plating. Red, E-cadherin (lateral cell membrane); blue, DAPI (nuclei).

(J) Number of DAPI\(^+\) cells per colony in control and FGF2-treated cells.

Error bars denote SEM. Scale bars represent 50 \( \mu\)m in (A), (E), and (F), and 20 \( \mu\)m in (I). See also Figure S2.
Figure 3. Loss of FGFR1 Signaling in BCs Leads to an Increase in Phosphorylation of Downstream Effector Proteins and a Decrease in Levels of a SPRY2 Isoform

(A) Schematic of in vitro experiment.

(B) Representative western blots from control and Fgfr1 cKO day-5 BCs showing pERK1/2, total ERK1/2, pAKT, total AKT, SPRY2, and histone H3.

(C) Quantification of protein in (B).

(D) Model. A major function of FGFR1 in BCs is to post-translationally modify SPRY2, resulting in a SPRY2 isoform which can negatively regulate the ERK/MAPK and AKT/PI3K pathways downstream of other RTKs.

(E) Confocal images of control Tg(KRT5-CreER); Rosa26RfGFP/+ and cKO Tg(KRT5-CreER); Rosa26RfGFP/++; Fgfr1 Δfx tracheal sections at 1.5 weeks after tmx administration showing an increase in pERK1/2 levels in the Fgfr1 cKO BCs. Green, GFP (reporter); red, pERK1/2 (active ERK1/2); blue, DAPI (nuclei). Scale bar, 25 μm.

(legend continued on next page)
opposite effect to removal of Fgfr1 and significantly reduced colony size (Figures 2I and 2J).

**Fgfr1**/+/− BCs Have Increased Levels of MAPK and AKT Pathway Activity

To identify the mechanism by which FGFR1 influences BC proliferation, we assessed the levels of activity of well-characterized downstream intracellular signaling pathways. Given that we had successfully replicated the proliferation phenotype in primary cell culture (Figures 2D–2F), we used primary cultures of Fgfr1+/−/− cells with a high dose of Ad-Cre and harvested cells 3 days after infection for western analysis of the whole cell lysate (Figure 3A). Genotyping of the cultures suggested that many cells were Fgfr1+/−/− in these conditions (Figure S3A). These experiments showed that there was a 2.2-fold increase in levels of phosphorylated ERK1/2 in the Fgfr1+/−/− cells. In addition, there was a 3.5-fold increase in total AKT protein and a 1.3-fold increase in the fraction of phosphorylated AKT (Figures 3B and 3C). The change in MAPK pathway activity was confirmed in vivo by immunostaining for pERK1/2, which showed higher levels in Fgfr1+/−/− BCs than controls at 1.5 weeks after tmx administration (Figure 3E). Thus, the loss of an RTK surprisingly resulted in increased levels of signaling through the RAS-ERK and PI3K intracellular pathways, which are typically associated with active RTK signaling. We therefore analyzed negative regulators of intracellular pathways, which are typically associated with active RTK signaling.

Stimulating BCs with FGF2 Results in Increased Levels of the Faster-Migrating SPRY2 Isoform

We tested whether activating FGF signaling is sufficient to modulate levels of the SPRY2 isoforms in wild-type cells (Figure 3F). A 10-min or 24-h FGF2 stimulation resulted in a reproducible small decrease in pERK1/2 compared with control cells, particularly at the 10-min harvest time (Figures 3G, 3H, and S5A–S5C). Following FGF2 stimulation we observed no change in total SPRY2 levels but a 1.6-fold increase in levels of the faster-migrating SPRY2 isoform (Figures 3G and 3H). These changes never reached statistical significance. However, the experiments were performed in primary cells that have multiple inputs into the ERK pathway in the presence of serum and the data were reproducible, supporting our hypothesis that manipulating FGFR1 signaling results in post-translational changes in SPRY2.

**Fgfr1**/−/− BCs Cannot Produce Ciliated Cells

It has previously been reported that FGFR1 signaling in zebrafish and *Xenopus* embryos can regulate cilia assembly and length (Hong and Dawid, 2009; Neugebauer et al., 2009). We therefore examined ciliogenesis in the Fgfr1 cKO tracheae. At 24 weeks after tmx administration, Fgfr1 cKO tracheae had an excess of GFP+ secretory cells and fewer GFP+ ciliated cells than the controls (Figures 4A and 4B), suggesting that ciliated cell production was impaired in the mutant. Indeed, the only GFP+ ciliated cells that could be detected in the Fgfr1 cKO tracheae still retained GFP+ FOXJ1+ cells that could be detected in the Fgfr1 cKO tracheae. At 24 weeks after tmx administration, Fgfr1 cKO tracheae had an excess of GFP+ secretory cells and fewer GFP+ ciliated cells than the controls (Figures 4A and 4B), suggesting that ciliated cell production was impaired in the mutant. Indeed, the only GFP+ ciliated cells that could be detected in the Fgfr1 cKO tracheae still retained GFP+ FOXJ1+ cells that could be detected in the Fgfr1 cKO tracheae.
zebrafish and *Xenopus* systems (Hong and Dawid, 2009; Neu-gebauer et al., 2009). This may be a true cross-species difference or may reflect the limitations of the different experimental systems used.

Homeostatic production of new ciliated cells occurs at an extremely slow rate in vivo (Rawlins and Hogan, 2008). To confirm the ciliated cell differentiation phenotype in the Fgfr1
d

cells, we grew air-liquid interface (ALI) epithelial cultures from Rosa26R-fGFP; Fgfr1
and control Rosa26R-fGFP; Fgfr1
animals 24 weeks after tmx administration.

In summary, the Fgfr1 cKO BCs have an increased proliferation rate from 1.5 weeks after injection. They are also unable to produce mature ciliated cells, likely due to a fate-choice defect. We focused on BC proliferation as the primary phenotype and, based on our observation that levels of the fast-migrating SPRY2 isoform were decreased in the Fgfr1 cKO cells (Figures 3B and 3C), we hypothesized that a major role of FGFR1 signaling in the tracheal BCs is to modulate SPRY2 activity and, moreover, that the function of SPRY2 in these cells is to inhibit signaling activity downstream of RTKs other than FGFR1, for example EGFR, which is necessary for tracheal BC proliferation (Brehchuhl et al., 2014; Lu et al., 2013). To test this hypothesis, we deleted Spry2 in BCs of the adult mouse trachea.

**Loss of Spry2 Results in Increased Levels of Tracheal Basal Cell Proliferation**

Spry2 is widely expressed in basal and luminal cells of the homeostatic mouse trachea (Figures 1A and S1). We generated...
Tg(KRT5-CreER); Rosa26R-GFP; Spry2 \textsuperscript{-/-} (Spry2 cKO) and control Tg(KRT5-CreER); Rosa26R-GFP adult mice and administered tmx to activate GFP and delete Spry2 in a subset of BCs (Figure 5A). During normal turnover there was an increase in the proportion of GFP\(^+\), Spry2 \textsuperscript{-/-} luminal cells also increased (Figures 5B and 5D). These changes most likely resulted from the \(4\)-fold increase in rates of BC proliferation observed in the Spry2 cKOs compared with controls (Figures 5E and 5F). Of epithelial stratification, and levels of E-cadherin protein (Figure 5G) and apical-basal polarity (Figure S6C) appeared normal. These data suggest that the observed change in cell density may result from an altered threshold for contact inhibition in the Spry2 cKO cells.

We tested whether we could recapitulate the Spry2 cKO phenotypes using Ad-Cre in primary tracheal cell culture (Figure 6A). Deletion with a high titer of Ad-Cre resulted in loss of all detectable SPRY2 protein (Figure 6E) and failure to amplify the Spry2\textsuperscript{fx} allele from the genomic DNA (Figure S3B), suggesting that we...
Figure 6. Rapidly Proliferating Spry2 Conditional Knockout BCs Have Elevated Levels of RAS-ERK and PI3K Signaling

(A) Schematic of in vitro experiment.
(B) Spry2 cKO (Rosa26R<sup>GFP</sup>/++; Spry2<sup>Dfx</sup>) cells proliferate more rapidly than controls (Rosa26R<sup>GFP</sup>/GFP) in vitro and are more densely packed at culture day 5. Note that not all cKO cells are GFP<sup>+</sup>, even though SPRY2 protein is undetectable. Green, GFP (reporter); red, KRT5 (BCs); blue, DAPI (nuclei). Arrowheads indicate similar regions of the cultures.
(C) Spry2 cKO cells still undergo some contact inhibition in vitro at day 7 after ALI induction. Their proliferation rate slows following induction of ALI and there are no regions of multi-layering, although KI67<sup>+</sup> cells are still visible (arrowhead). Green, GFP (reporter); red, KI67 (proliferating cells); blue, DAPI (nuclei).
(D) Spry2 cKO primary cells continue to proliferate faster in vitro following passage at day 2 after replating. Green, GFP (reporter); red, KI67 (proliferating cells, e.g., arrowhead); blue, DAPI (nuclei).
(E) Representative western blots from control and Spry2 cKO day-5 BCs showing SPRY2, pAKT, total AKT, pERK1/2, total ERK1/2, SOX2, and histone H3.
(F) Quantification of protein levels. Error bars denote SEM.
(G) Confocal images of control Tg(KRT5-CreER<sup>T2</sup>); Rosa26R<sup>GFP</sup>/++; Spry2<sup>Dfx</sup> and cKO Tg(KRT5-CreER<sup>T2</sup>); Rosa26R<sup>GFP</sup>/GFP, Spry2<sup>Dfx</sup> tracheal sections at 1.5 weeks after tmx administration showing an increase in pERK1/2 levels in the Spry2 cKO BCs. Green, GFP (reporter); red, pERK1/2 (active ERK1/2); blue, DAPI (nuclei).

Scale bars, 25 μm. See also Figure S3.
achieved complete recombination of the Spry2<sup>x<sup>a</sup> allele. In spite of this there were large patches of GFP<sup>+</sup> cells in our cultures that had not recombined the Rosa reporter (Figure 6B). This indicates that the Spry2<sup>x<sup>a</sup> allele recombines more efficiently than the Rosa26R-fGFP reporter and supports the conclusion that most of the GFP<sup>+</sup> cells observed in vivo were co-recombinants with Spry2<sup>x<sup>a</sup></sup>. Therefore, it is likely that scoring GFP<sup>+</sup> cells in the Spry2 cKO tracheae results in a good approximation to the Spry2<sup>2<sup>a</sup>/2<sup>a</sup></sup> phenotype (compare with the Fgr1 cKO tracheae, where scoring GFP<sup>+</sup> cells results in an underestimate of the Fgr1<sup>2<sup>a</sup>/2<sup>a</sup></sup> phenotype).

The Spry2<sup>2<sup>d</sup>/2<sup>d</sup></sup> cultures had a greater rate of proliferation than the controls and also reached a higher cell density (Figures 6B and 6C). Proliferation of the Spry2<sup>2<sup>d</sup>/2<sup>d</sup></sup> cells slowed after the cells reached confluence, but did continue (Figure 6D). Moreover, confluent cultures could easily be passaged and continued to show a faster proliferation rate (Figure 6D). Importantly, although the Spry2<sup>2<sup>d</sup>/2<sup>d</sup></sup> cells reached a greater density than controls, we never observed phenotypes consistent with a complete loss of contact inhibition, such as multi-layering or cell detachment. This is again consistent with an altered threshold for, but not complete loss of, contact inhibition.

Similar to the Fgr1<sup>2<sup>d</sup>/2<sup>d</sup></sup> cells, the Spry2<sup>2<sup>d</sup>/2<sup>d</sup></sup> cells had a 3.5-fold increase in pERK1/2 and a 5.2-fold increase in pAKT levels compared with the controls (Figures 6E and 6F). These changes were highly reproducible, although not always statistically significant. The increase in MAPK pathway activity was confirmed in vivo by immunostaining for pERK1/2, which showed higher levels in Spry2<sup>2<sup>d</sup>/2<sup>d</sup></sup> BCs than controls at 1.5 weeks after tamoxifen administration (Figure 6G). We also observed that the Spry2<sup>2<sup>d</sup>/2<sup>d</sup></sup> cells had a 3.3-fold increase in levels of EGFR protein (Figures 6E and 6F). This is consistent with the published function of the slower-migrating SPRY2 isoform (which is absent in the Spry2<sup>2<sup>d</sup>/2<sup>d</sup></sup> cells, but is unaffected in the Fgr1<sup>2<sup>d</sup>/2<sup>d</sup></sup> cells) in EGFR trafficking/degradation (Gao et al., 2012; Kim et al., 2007; Walsh and Lazzara, 2013).

**DISCUSSION**

The tracheal epithelium is normally quiescent, with a minimal amount of cell turnover. By combining in vivo genetic analysis and primary cell culture, we have shown that BC proliferation is actively repressed under steady-state conditions by an FGFR1-SPRY2 signaling axis that functions to inhibit signaling through the ERK and AKT intracellular pathways. This result argues that airway quiescence does not result simply from the absence of a proliferative signal or from feedback inhibitory loops. Rather, we propose a model in which quiescence is maintained by a balance between signaling mechanisms that either promote proliferation or repress it. This is an emerging theme in the stem cell biology of quiescent organs. For example, a recent publication has found a similar tonic Hedgehog signal that inhibits steady-state proliferation in the smaller mouse airways (Peng et al., 2015), but it is not known whether this Hedgehog-based mechanism applies in the BC-containing trachea.

SPRY2 was previously characterized in vitro as a protein with two isoforms, which can either inhibit ERK/AKT signaling (faster-migrating SPRY2 isoform) or modulate the subcellular trafficking/degradation of EGFR protein (slower-migrating isoform) (Guy et al., 2009). However, the relative roles of these SPRY2 isoforms in vivo and how they function downstream of RTK receptors have not previously been explored. Several lines of evidence presented here support a model in which the faster-migrating SPRY2 functions downstream of FGFR1 to control cell proliferation and tracheal epithelial homeostasis (Figure 7A). First, following the loss of FGFR1 protein in airway BCs we observe reduced levels of the faster-migrating (ERK/AKT-inhibiting) SPRY2 isoform, as well as increased levels of dERK and pAKT, increased rates of proliferation, and increased resistance to EGFR inhibition. By contrast, the slower-migrating SPRY2 isoform is unaffected (Figure 7B). Second, exposing wild-type BCs to FG2 ligand results in increased levels of...
faster-migrating SPRY2, a decrease in dpERK, and inhibition of BC proliferation. Third, loss of all SPRY2 results in increased ERK/AKT signaling and BC proliferation (Figure 7C). Taken together, our data suggest that active FGFR1 signaling in the steady-state trachea maintains a pool of SPRY2 protein, which functions to antagonize ERK/AKT signaling and limit cell proliferation downstream of other RTKs, most likely EGFR (Figure 7A).

A relationship between FGFR1 and post-translational modification of SPRY2 protein has previously been documented in cell lines in vitro (Lao et al., 2006, 2007). We now demonstrate its importance in vivo, clearly illustrating that the context in which a cell interprets EGFR/RTK signaling can be highly dependent on the activity of other RTKs present. This has clear implications for cancer development and the evolution of drug-resistant tumors.

Another important finding of our work is that FGFR1 and SPRY2 can also function independently in airway epithelial homeostasis. FGFR1 is required for multi-ciliated cell differentiation (Figure 4). Moreover, our data suggest that secretory cells are produced at the expense of ciliated cells by the Fgr1 cKO BCs. A role for FGFR1 in ciliated cell differentiation is also consistent with previous reports from other model organisms (Hong and Dawid, 2009; Neugebauer et al., 2009), but the details of the cellular requirements remain to be worked out. SPRY2 regulates EGFR protein levels in BCs (Figure 6). Similar observations have previously been made in vitro or in cancer models, but we demonstrate this SPRY2-EGFR relationship for the first time in a homeostatic tissue (Gao et al., 2012; Kim et al., 2007; Walsh and Lazzara, 2013). SPRY2 thus emerges as a highly flexible switch that can be modulated by FGFR1 signaling to control different aspects of RTK signaling. In excess of ten phosphorylation sites and multiple binding partners have been documented for SPRY2 in vitro (DaSilva et al., 2006; Edwin et al., 2009; Lao et al., 2007). These data suggest that SPRY2 is extensively and flexibly modified in many different cellular settings in vivo and that our observations may be the tip of the iceberg for the control of cellular homeostasis by SPRY2.

A key question raised by our model concerns what activates FGFR1 in vivo. We observed that FGF2 is transcribed in the tracheal mesenchyme in vivo at steady state, and that recombinant FGF2 inhibits tracheal BC proliferation and modulates SPRY2 in vitro. These data are consistent with a role for FGF2 signaling downstream of other RTKs, partly EGFR. We have thus demonstrated a specific in vivo function for an FGFR1-SPRY2 signaling axis that was previously documented in vitro. This illustrates the complexity of RTK signaling networks and the importance of further investigation in both homeostatic tissues and developing malignancies.

**EXPERIMENTAL PROCEDURES**

See Supplemental Experimental Procedures for a full description of all materials and methods used.

**Mice**

Experiments were performed under Home Office licenses PPL80/2326 and 70/812. Fgf10tmx (Xu et al., 2002), Tg(KRT5-CreERT2) (Rock et al., 2009), Rosa26R-fGFP (Rawlins et al., 2009), Spry2tmx (Shim et al., 2003) were described previously. Fgf10tmx and Spry2tmx were generated by crossing floxed alleles to Zp3-Cre (de Vries et al., 2000). All strains were maintained on a C57Bl/6J background. Males and females >8 weeks old were used. Wild-type mice were C57Bl/6J.

**Tamoxifen**

Animals were injected intraperitoneally with tmx, 0.2 mg/g body weight, four times every other day.

**Tracheal Epithelial Cell Culture**

Tracheae were cut into small pieces and incubated in 50% Dispase II (Gibco, 16 U/ml). Epithelial sheets were isolated and dissociated to single cells. Unless otherwise stated, 5 × 10⁶ cells in 0.5 ml of MTEC+/medium (You et al., 2003) were plated on collagen-coated 12-well tissue culture inserts (BD Falcon, 353180). Differentiation was induced in confluent cultures by removal of insert medium and addition of MTEC serum-free medium to the outer chamber. Ad-Cre (University of Iowa, Gene Transfer Vector Core) MOI 7 was used for scattered recombination and MOI 2,500 for complete recombination; 1 × 10⁶ vector plaque-forming units, 100 ng/ml recombinant mouse FGF2, and 0.25 μM erlotinib (Sigma).

**Immunostaining**

Cryosections (6 μm) or whole-mount primary cultures were stained with ACT (mouse, 1:3,000, Sigma, T7451), E-cadherin (rat, 1:3,000, Invitrogen, 13-1900), pERK1/2 (rabbit, 1:200, Cell Signaling Technology, 4695), FGFR1 (rabbit, 1:200, Novus Biologicals, NB1-20067), FOXJ1 (mouse, 1:200, eBioscience, clone 2A5, 14-9965-80), GFP (chick, 1:1,000, Abcam, Ab13970), KRT5 (rabbit, 1:500, Covance, PRB-160P), Ki67 (mouse, 1:200, Becton Dickinson, 550609), SCGB1A1 (goat, 1:400, Santa Cruz Biotechnology, sc-13972), SPRY2 (rabbit, 1:200, Abcam, Ab50317), and Tuj1 (1:1,000, DSHB, 8.1.1). Antigen retrieval was in 10 mM boiling sodium citrate for Ki67 (pH 8), pERK1/2 (pH 6.5), and FOXJ1 (pH 6). Conjugated secondary antibodies were Phalloidin-568 and Alexa Fluor (Life Technologies, 1:2,000).
mRNA In Situ Hybridization
Cryosections (10 μm) were hybridized with DIG-labeled anti-sense mRNA probes to Fgfr1 (EST: AW495528) or Spry2 (Minowada et al., 1999) (DIG RNA labeling kit, Roche, 11175025910), and visualized using alkaline phosphatase-coupled anti-DIG (1:2,000, Roche, 129300223) and BCIP/NBT (Roche, 1382313).

Microscopy and Image Scoring
Slides were imaged on a Zeiss Axiosimager compound microscope or a Leica SP5 confocal microscope as stated. Cells were scored manually in Fiji (ImageJ), or using the Gurdon Institute Imaging Facility’s plugin ObjectScan. For cryosections, every epithelial cell along the entire proximal to distal length of a longitudinal section from the center of the trachea was scored. BCS were T1+ or KRT5+ with the apical surface not reaching the lumen. For cultured cells, at least three random fields of view per insert were scored.

RT-PCR
Samples were enriched for epithelium or mesenchyme using Disparse digestion and manual peeling. Total RNA was extracted using TRIzol reagent.

qRT-PCR
Primary tracheal epithelial cells were isolated and sorted using a fluorescence-activated cell sorting MoFlo flow cytometer. Wild-type cells were gated as basal EpCAM, GSI/I4 lectin; secretory EpCAM, SSEA1; and ciliated EpCAM. CD24. GFP+ BCs from control and Fgfr1 cKO tracheae were sorted as GFP-, GSI/I4 lectin. Total RNA was extracted using a QiaGen RNEasy Mini Kit, and Taqman gene expression assays (Life Technologies) were used.

Immunoblot
Cells were collected in Cell Extraction Buffer (Innitrogen, FNN0011) with protease inhibitor (Roche, 04693116001) and PMSF (Sigma, P7626). Proteins were separated on 10% or 12% SDS-PAGE gels before transfer to Millipore PVDF Membrane (Merck Millipore, IPVH00010). Membranes were probed with pAKT/S473 (Cell Signaling, 3787, 1:3,000), pan-AKT (Cell Signaling, 4691, 1:1,000), dpERK1/2 (Cell Signaling, 4370, 1:300), ERK1/2 (Cell Signaling, 4969, 1:300), SPRY2 (Abcam, Ab50317, 1:300), SOX2 (Abcam, Ab97959, 1:3,000), Histone H3 (Abcam, Ab39655, 1:10,000), and EGFR (Millipore, 04-290, 1:300). Detection was performed with horseradish peroxi-dase-conjugated secondaries (Abcam, ab87051, ab87023, 1:10,000) and enhanced chemiluminescence (Thermo Scientific, PI-32109). Quantitation is based on analysis of protein from three biological replicates separated on the same polyacrylamide gel. Band intensity was analyzed in Fiji normalized to loading control.

Statistics
Statistics were calculated using Student’s two-tailed t test with unequal variance.

SUPPLEMENTAL INFORMATION
Supplemental Information includes Supplemental Experimental Procedures and six figures and can be found with this article online at http://dx.doi.org/10.1016/j.devcel.2016.03.001.

AUTHOR CONTRIBUTIONS
G.B. designed and performed experiments, analyzed data, and edited the manuscript. J.J. performed experiments. M.A.B. provided mice and trachea samples and assisted with experimental design. E.R. conceived and led the project, performed experiments, analyzed data, and wrote and edited the manuscript.

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REFERENCES
Abler, L.L., Mansour, S.L., and Sun, X. (2009). Conditional gene inactivation reveals roles for Fgf10 and Fgfr2 in establishing a normal pattern of epithelial branching in the mouse lung. Dev. Dyn. 238, 1999–2013.
Arai, D., Hegab, A.E., Soejima, K., Kuroda, A., Ishioka, K., Yasuda, H., Naoki, K., Kagawa, S., Hamamoto, J., Yin, Y., et al. (2015). Characterization of the cell of origin and propagation potential of the fibroblast growth factor 9-induced mouse model of lung adenocarcinoma. J. Pathol. 235, 593–605.
Blatt, E. N., Yan, X. H., Wuerfel, M. K., Hamilos, D. L., and Brody, S. L. (1999). Forkhead transcription factor FHF-4 expression is temporally related to cilio-genesis. Am. J. Respir. Cell Mol. Biol. 21, 168–176.
Brechbuhl, H. M., Li, B., Smith, R. W., and Reynolds, S. D. (2014). Epidermal growth factor receptor activity is necessary for mouse basal cell proliferation. Am. J. Physiol. Lung Cell Mol. Physiol. 307, L800–L810.
Chakkalakal, J. V., Jones, K. M., Basson, M. A., and Brack, A. S. (2012). The aged niche disrupts muscle stem cell quiescence. Nature 490, 355–360.
Cole, B. B., Smith, R. W., Jenkins, K. M., Graham, B. B., Reynolds, P. R., and Reynolds, S. D. (2010). Tracheal basal cells: a facultative progenitor cell pool. Am. J. Pathol. 177, 362–376.
Dahanay, H., Hessotti, A. D., Coote, J., Montgomery, B. E., Xia, D., Wilson, A., Yang, H., Wang, Z., Bevan, L., Thomas, C., et al. (2015). Notch2 is required for inflammatory cytokine-driven goblet cell metaplasia in the lung. Cell Rep. 10, 239–252.
DaSilva, J., Xu, L., Kim, H. J., Miller, W. T., and Bar-Sagi, D. (2008). Regulation of sprouty stability by Mnk1-dependent phosphorylation. Mol. Cell Biol. 26, 1898–1907.
de Vries, W. W., Binns, L. T., Fancher, K. S., Dean, J., Moore, R., Kemler, R., and Knowles, B. B. (2000). Expression of Cre recombinase in mouse oocytes: a means to study maternal effect genes. Genesis 26, 110–112.
Dutt, A., Ramos, A. H., Hammerman, P. S., Mermel, C., Cho, J., Shafirnia, T., Chande, A., Tanaka, K. E., Stransky, N., Greulich, H., et al. (2011). Inhibitor-sensitive FGFR1 amplification in human non-small cell lung cancer. PLoS One 6, e20351.
Edwin, F., Anderson, K., Ying, C., and Patel, T. B. (2009). Intermolecular interactions of Sprouty proteins and their implications in development and disease. Mol. Pharmacol. 76, 679–691.
Gao, M., Patel, R., Ahmad, I., Fleming, J., Edwards, J., McCracken, S., Sahadevan, K., Seywright, M., Norman, J., Sansom, O., et al. (2012). SPRY2 loss enhances ErbB trafficking and PI3K/AKT signaling to drive human and mouse prostate carcinogenesis. EMBO Mol. Med. 4, 776–790.
Garmy-Susini, B., Delmas, E., Gourdy, P., Zhou, M., Bossard, C., Bugler, B., Bayard, F., Krust, A., Prats, A. C., Doetschman, T., et al. (2004). Role of fibroblast growth factor-2 isoforms in the effect of estradiol on endothelial cell migration and proliferation. Circ. Res. 94, 1301–1309.
Giangreco, A., Lu, L., Vickers, C., Teixeira, V. H., Groot, K. R., Butler, C. R., Abler, L. L., Mansour, S. L., and Crystal, R. G. (2011). The human airway epithelial cell lineage disrupts muscle stem cell quiescence. Nature 490, 355–360.
Guy, G. R., Jackson, R. A., Yusoff, P., and Chow, S. Y. (2009). Sprouty proteins: modified modulators, matchmakers or missing links? J. Endocrinol. 203, 191–202.
Hackett, N. R., Shaykhiev, R., Walters, M. S., Wang, R., Zwick, R. K., Ferris, B. R., Witover, B., Salit, J., and Crystal, R. G. (2011). The human airway epithelial basal cell transcriptome. PLoS One 6, e18378.
Hadjab, S., Franck, M. C., Wang, Y., Sterzenbach, U., Sharma, A., Emfors, P., and Lallemend, F. (2013). A local source of FGF initiates development of the unmyelinated lineage of sensory neurons. J. Neurosci. 33, 17656–17666.
Hogan, B.L., Barkauskas, C.E., Chapman, H.A., Epstein, J.A., Jain, R., Hsieh, C.C., Niklason, L., Calle, E., Le, A., Randell, S.H., et al. (2014). Repair and regeneration of the respiratory system: complexity, plasticity, and mechanisms of lung stem cell function. Cell Stem Cell 15, 123–138.

Hong, S.K., and Dawid, I.B. (2009). FGF-dependent left-right asymmetry patterning in zebrafish is mediated by Ier2 and Fip1. Proc. Natl. Acad. Sci. USA 106, 2230–2235.

Hong, K.U., Reynolds, S.D., Watkins, S., Fuchs, E., and Stripp, B.R. (2004). In vivo differentiation potential of tracheal basal cells: evidence for multipotent and unipotent subpopulations. Am. J. Physiol. Lung Cell Mol. Physiol. 286, L643–L649.

Huang, T., You, Y., Spoor, M.S., Richer, E.J., Kudva, V.V., Paige, R.C., Seiler, M.P., Liebler, J.M., Zabner, J., Plopper, C.G., et al. (2003). Fox1 is required for apical localization of ezrin in airway epithelial cells. J. Cell Sci. 116, 4935–4945.

Kauffman, S.L. (1980). Cell proliferation in the mammalian lung. Int. Rev. Exp. Pathol. 22, 131–191.

Kim, H.J., Taylor, L.J., and Bar-Sagi, D. (2007). Spatial regulation of EGFR signaling by Sprouty2. Curr. Biol. 17, 455–461.

Kranenburg, A.R., Willems-Widyastuti, A., Mooi, W.J., Saxena, P.R., Sterk, P.J., de Boer, W.I., and Sharma, H.S. (2005). Chronic obstructive pulmonary disease is associated with increased bronchial expression of FGF-1, FGF-2, and FGF-R1. J. Pathol. 206, 28–38.

Lao, D.H., Chandramouli, S., Yusoff, P., Fong, C.W., Saw, T.Y., Tai, L.P., Yu, C.Y., Leong, H.F., and Guy, G.R. (2006). A Src homology 3-binding sequence on the C terminus of Sprouty2 is necessary for inhibition of the Ras/ERK pathway downstream of fibroblast growth factor receptor stimulation. J. Biol. Chem. 281, 29993–30000.

Lao, D.H., Yusoff, P., Chandramouli, S., Philip, R.J., Fong, C.W., Jackson, R.A., Saw, T.Y., Yu, C.Y., and Guy, G.R. (2007). Direct binding of PPA2 to Sprouty2 and phosphorylation changes are a prerequisite for ERK inhibition downstream of fibroblast growth factor receptor stimulation. J. Biol. Chem. 282, 9117–9126.

Lu, L., Teixeira, V.H., Yuan, Z., Graham, T.A., Endesfelder, D., Kolluri, K., Al-Jurfali, N., Hamilton, N., Nicholson, A.G., Falcon, M., et al. (2013). LRG1 regulates cadherin-dependent contact inhibition directing epithelial homeostasis and pre-invasive squamous cell carcinoma development. J. Pathol. 229, 608–620.

Mahoney, J.E., Mori, M., Szymaniak, A.D., Varelas, X., and Cardoso, W.V. (2014). The hippo pathway effector Yap controls patterning and differentiation of airway epithelial progenitors. Dev. Cell 30, 137–150.

Minowada, G., Jarvis, L.A., Neubuser, A., Sun, X., Hacohen, N., Krasnow, M.A., and Martin, G.R. (2005). Vertebrate Sprouty genes are induced in diverse epithelia. Nature 433, 184–197.

Pardo-Saganta, A., Lam, B.M., Tata, P.R., Villoria, J., Saeg, B., Mou, H., Zhao, R., and Rajagopal, J. (2015). Injury induces direct lineage segregation of functionally distinct airway basal stem/progenitor cell subpopulations. Cell Stem Cell 16, 184–197.

Paul, M.K., Bisht, B., Darmawan, D.O., Chiou, R., Ha, V.L., Wallace, W.D., Chon, A.T., Hegab, A.E., Grogan, T., Elashoff, D.A., et al. (2014). Dynamic changes in intracellular ROS levels regulate airway basal stem cell homeostasis through NFκB-dependent notch signaling. Cell Stem Cell 15, 199–214.

Peng, T., Frank, D.B., Kadzick, R.S., Morley, M.P., Rathi, K.S., Wang, T., Zhou, S., Cheng, L., Lu, M.M., and Morrisey, E.E. (2015). Hedgehog actively maintains adult lung quiescence and regulates repair and regeneration. Nature 526, 578–582.

Rawlins, E.L., and Hogan, B.L. (2008). Ciliated epithelial cell lifespan in the mouse trachea and lung. Am. J. Physiol. Lung Cell Mol. Physiol. 295, L231–L234.

Rawlins, E.L., Ostrowski, L.E., Randell, S.H., and Hogan, B.L. (2007). Lung development and repair: contribution of the ciliated lineage. Proc. Natl. Acad. Sci. USA 104, 410–417.

Rawlins, E.L., Okubo, T., Xue, Y., Brass, D.M., Auten, R.L., Hasegawa, H., Wang, F., and Hogan, B.L. (2009). The role of Sgcβ4a1+ Clara cells in the long-term maintenance and repair of lung airway, but not alveolar, epithelium. Cell Stem Cell 4, 525–534.

Rock, J.R., Onaitis, M.W., Rawlins, E.L., Yu, Y., Clark, C.P., Xue, Y., Randell, S.H., and Hogan, B.L. (2009). Basal cells as stem cells of the mouse trachea and human airway epithelium. Proc. Natl. Acad. Sci. USA 106, 12771–12775.

Rock, J.R., Randell, S.H., and Hogan, B.L. (2010). Airway basal stem cells: a perspective on their roles in epithelial homeostasis and remodeling. Dis. Model. Mech. 3, 545–556.

Rock, J.R., Gao, X., Xue, Y., Randell, S.H., Kong, Y.Y., and Hogan, B.L. (2011). Notch-dependent differentiation of adult airway basal stem cells. Cell Stem Cell 8, 639–648.

Rubin, C., Zwang, Y., Vaisman, N., Ron, D., and Yarden, Y. (2005). Phosphorylation of carboxyl-terminal tyrosines modulates the specificity of Sprouty-2 inhibition of different signaling pathways. J. Biol. Chem. 280, 9735–9744.

Shia, K.L., Xiang, W., LaPorta, V.S., Licht, J.D., Keller, C., Basson, M.A., and Brack, A.S. (2010). Sprouty1 regulates reversible quiescence of a self-renewing adult muscle stem cell pool during regeneration. Cell Stem Cell 6, 117–129.

Shim, K., Minowada, G., Koling, D.E., and Martin, G.R. (2006). Sprouty2, a mouse deafness gene, regulates cell fate decisions in the auditory sensory epithelium by antagonizing FGF signaling. Dev. Cell 8, 553–564.

Teixeira, V.H., Nadarajan, P., Graham, T.A., Pipnikas, C.P., Brown, J.M., Falcon, M., Nye, E., Poulsom, R., Lawrence, D., Wright, N.A., et al. (2013). Stochastic homeostasis in human airway epithelium is achieved by neutral competition of basal cell progenitors. Elife 2, e00966.

Vermeer, P.D., Einwarter, L., Moninger, T.O., Rokhina, T., Kern, J.A., Zabner, J., and Welsh, M.J. (2003). Segregation of receptor and ligand regulates activation of epithelial growth factor receptor. Nature 422, 332–326.

Volckaert, T., Dill, E., Campbell, A., Tiozzo, C., Majka, S., Bellusci, S., and De Langhe, S.P. (2011). Parabronchial smooth muscle constitutes an airway epithelial stem cell niche in the mouse lung after injury. J. Clin. Invest. 121, 4409–4419.

Volckaert, T., Campbell, A., Dill, E., Li, C., Minoo, P., and De Langhe, S. (2013). Localized Fgf10 expression is not required for lung branching morphogenesis but prevents differentiation of epithelial progenitors. Development 140, 3731–3742.

Walsh, A.M., and Lazzara, M.J. (2013). Regulation of EGFR trafficking and cell signaling by Sprouty2 and Mig6 in lung cancer cells. J. Cell Sci. 126, 4339–4348.

Watson, J.K., Rulands, S., Wilkinson, A.C., Wuidart, A., Ousset, M., Van Keymeulen, A., Goggens, B., Blanpain, C., Simons, B.D., and Rawlins, E.L. (2015). Clonal dynamics reveal two distinct populations of basal cells in slow-tumorove airway epithelium. Cell Rep. 12, 90–101.

Weiss, J., Sos, M.L., Seidel, D., Pelfer, M., Zander, T., Heuckmann, J.M., Ulrich, R.T., Menon, R., Maier, S., Sottermann, A., et al. (2010). Frequent and focal FGFR1 amplification associates with therapeutically tractable FGFR1 dependency in squamous cell lung cancer. Sci. Transl. Med. 2, 62ra93.

Wynes, M.W., Hinz, T.K., Gao, D., Martini, M., Marek, L.A., Ware, K.E., Edwards, M.G., Bohm, D., Perner, S., Helfrich, B.A., et al. (2014). FGFR1
mRNA and protein expression, not gene copy number, predict FGFR TKI sensitivity across all lung cancer histologies. Clin. Cancer Res. 20, 3299–3309.
Xu, X., Qiao, W., Li, C., and Deng, C.X. (2002). Generation of Fgfr1 conditional knockout mice. Genesis 32, 85–86.

Yin, Y., Wang, F., and Ornitz, D.M. (2011). Mesothelial- and epithelial-derived FGF9 have distinct functions in the regulation of lung development. Development 138, 3169–3177.

You, Y., Richer, E.J., Huang, T., and Brody, S.L. (2002). Growth and differentiation of mouse tracheal epithelial cells: selection of a proliferative population. Am. J. Physiol. Lung Cell Mol. Physiol. 283, L1315–L1321.
Zhao, R., Fallon, T.R., Saladi, S.V., Pardo-Saganta, A., Villoria, J., Mou, H., Vinarsky, V., Gonzalez-Celeiro, M., Nurra, N., Hariri, L.P., et al. (2014). Yap tunes airway epithelial size and architecture by regulating the identity, maintenance, and self-Renewal of stem cells. Dev. Cell 30, 151–165.