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Developmental Biology 299 (2006) 52–62
DEVELOPMENTAL BIOLOGY
www.elsevier.com/locate/ydbio

# Levels of mesenchymal FGFR2 signaling modulate smooth muscle progenitor cell commitment in the lung

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Received for publication 2 March 2006; revised 20 June 2006; accepted 5 July 2006
Available online 7 July 2006

**Abstract**

Fibroblast growth factor (FGF) signaling has been shown to regulate lung epithelial development but its influence on mesenchymal differentiation has been poorly investigated. To study the role of mesenchymal FGF signaling in the differentiation of the mesenchyme and its impact on epithelial morphogenesis, we took advantage of *Fgfr2c*$^{+/\Delta}$ mice, which due to a splicing switch express *Fgfr2b* in mesenchymal tissues and manifest Apert syndrome-like phenotypes. Using a set of in vivo and in vitro studies, we show that an autocrine FGF10–FGFR2b signaling loop is established in the mutant lung mesenchyme, which has several consequences. It prevents the entry of the smooth muscle progenitors into the smooth muscle cell (SMC) lineage and results in reduced fibronectin and elastin deposition. Levels of *Fgf10* expression are raised within the mutant mesenchyme itself. Epithelial branching as well as epithelial levels of FGF and canonical Wnt signaling is dramatically reduced. These defects result in arrested development of terminal airways and an "emphysema like" phenotype in postnatal lungs. Our work unravels part of the complex interactions that govern normal lung development and may be pertinent to understanding the basis of respiratory defects in Apert syndrome.


*Keywords:* FGF10; FGFR2b; Smooth muscle cells; Lung; Apert syndrome

## Introduction

Lung development is orchestrated by cross-talk between epithelial and mesenchymal cells. The epithelium and mesenchyme generate diverse cell types and this process is tightly coordinated with branching morphogenesis.

Very little is known about the origin and signals that regulate the differentiation of mesenchymal cell derivatives. We have previously reported that progenitors for parabronchial SMC lie at the most distal tips of each branch (Mailleux et al., 2005; Warburton et al., 2000). Soon after exiting this distal domain, a differentiation program is initiated likely in response to a tightly regulated combination of activating and inhibitory signals (Hogan, 1999). Increasing evidence suggests that the balance between these signals is critical to control the differentiation of the SMC progenitors.

Families of 22 FGFs and four FGF receptors (FGFRs 1–4) have been identified in mammals. Typically, FGFRs are comprised of an extracellular region composed of two or three immunoglobulin-like (Ig) domains, a transmembrane segment and an intracellular tyrosine kinase domain (Johnson and Williams, 1993; McKeehan et al., 1998). Alternative splicing of the exons that encode the carboxy terminal half of the third Ig domain in *Fgfrs-1*, *-2* and *-3* results in receptor isoforms termed 'IIIb' or 'IIIc', each with respectively distinct ligand-binding specificity and tissue distributions (Ornitz et al., 1996). The *Fgfr2* gene splice variant containing the IIIb exon (*Fgfr2b*) is expressed mainly in epithelial cells and is activated

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0012-1606/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.ydbio.2006.07.001

by FGFs-1, -3, -7, and -10, which are synthesized predominantly within the mesenchyme. By contrast, FGFR2-IIIc (FGFR2c) is expressed primarily in the mesenchyme and apart from FGF1, is activated by a different set of FGF ligands, namely, FGFs-2, -4, -6, -8, -9 and -18 (Bellusci et al., 1997; Mason, 1994; Ornitz et al., 1996; Orr-Urtreger et al., 1993; Peters et al., 1992; Yamasaki et al., 1996). FGF9 is known to be a key regulator of distal lung development. It is expressed initially in the mesothelium and epithelium but later becomes restricted to the mesothelium (Colvin et al., 1999). Sandwiched between these two cell layers is the lung mesenchyme and FGF9 is thought to act on the mesenchyme via FGFR2c (Ornitz et al., 1996). Inactivation of *Fgf9* results in reduced mesenchymal cell proliferation and consequently reduced secondary branching of the lung epithelium (Colvin et al., 2001). Treatment of embryonic lung mesenchymal explants with FGF9 inhibits SHH-induced differentiation of the mesenchyme into SMCs but does not affect mesenchymal cell proliferation (Weaver et al., 2003). Therefore, mesenchymal FGF signaling is seemingly crucial for maintaining lung mesenchymal progenitor cells in an undifferentiated state.

To further analyze the function of mesenchymal FGFR2 signaling in the differentiation of the lung mesenchymal progenitor cells and its impact on lung epithelial cells, we have studied the consequences of a gain-of-FGFR2 function mutation arising from the ectopic expression of *Fgfr2b* within the lung mesenchyme, alongside *Fgfr2c*. This genetic aberration takes places when one copy of *Fgfr2* exon 9 (IIIc exon) is deleted as in $Fgfr2c^{+/IIIc\Delta}$ mice (called $Fgfr2c^{+I\Delta}$ for simplification). This interferes with the normal balance of exon IIIb/IIIc alternative splicing (Hajihosseini et al., 2001), seemingly perturbing the tight molecular constraints placed on tissue-specific expression of IIIb and IIIc isoforms (Wagner et al., 2005). Co-expression of *Fgfr2b* and *Fgfr2c* in the lung mesenchyme would render these cells responsive to a broader range of FGF ligands, in particular FGF10, which is produced by mesenchymal cells themselves. Because the two receptor isoforms are otherwise identical in their intracellular sequence, mesenchymal cells perceive this as a net gain-of-FGFR2 function.

Using this in vivo approach, we determined how this gain-of-FGFR2 activity impinges on the cellular and molecular mechanisms underlying lung development and the interaction between mesothelial, mesenchymal and epithelial cells. Chief among these is the finding that increased mesenchymal signaling inhibits a progression of mesenchymal differentiation to the smooth muscle cell lineage.

## Results

### *$Fgfr2c^{+/\Delta}$ mice suffer from a complex lung phenotype*

E11.5 wild-type (WT) mouse lungs exhibit four primitive lobes on the right (cranial, medial, caudal and accessory) and one lobe on the left. At E11.5, $Fgfr2c^{+/\Delta}$ lungs also have four lobes, but one of these, the accessory lobe, consists solely of mesenchyme and eventually regresses by E12.5 (data not shown). At E12.5, epithelial branching is reduced as judged by the presence of fewer peripheral epithelial buds (4.6±0.5 versus 7.6±0.5 in WT, $n=5$, $P<0.05$; data not shown). By E14.5, $Fgfr2c^{+/\Delta}$ lungs continue to show a severe impairment in epithelial branching and absence of the accessory lobe (arrowheads in Figs. 1a–a′) and the remaining three right lobes are fused (Figs. 1a, a′). Moreover, $Fgfr2c^{+/\Delta}$ lungs show a more abundant mesenchyme (arrow in Fig. 1b′), as well as dilated distal airways (insets in Fig. 1b, b′). At Postnatal day 0 (P0) (data not shown) and P3, WT and $Fgfr2c^{+/\Delta}$ lungs are similar in size (Figs. 1c, c′), but mutant lungs show an "emphysema-like" arrested development of the terminal airways (Fig. 1d, arrow in d′) characterized by defects in the formation of secondary septa and thinner alveolar walls (insets in Figs. 1d, d′). $Fgfr2c^{+/\Delta}$ mice die by P7 because of growth retardation, bone and visceral defects (Hajihosseini et al., 2001) thus precluding analysis at later postnatal stages.

Phospho-histone H3 staining at E12.5, E13.5 and E14.5 WT and mutant lungs reveals that the observed branching defects involve perturbed rates of cell proliferation. Mutant E12.5 and E13.5 lungs contain significantly fewer mitotic epithelial cells compared to wild type (E12.5: 7.9±2% versus 20±2%, $n=3$, $P\leq0.05$; data not shown; E13.5: 13.5±3.0% versus 22.8±0.8%, $n=3$, $P\leq0.05$; data not shown), but no significant difference is seen in the proliferation of mesenchymal cells (E12.5: 16.3±2.% versus 16.5±1.5%, $n=3$, $P=0.5$; data not shown; E13.5: 14.8±3.8% versus 12.5±2.0%, $n=3$, $P=0.2$; data not shown). Hence, at E12.5 and E13.5, the more abundant mesenchyme likely reflects an imbalance in the epithelial to mesenchymal cell ratio resulting from reduced epithelial proliferation, rather than from mesenchymal hyperproliferation. However, at E14.5 when FGF10 expression has reached higher levels (Bellusci et al., 1997), increased mesenchymal proliferation is observed in the mutant lungs compared to wild type (18.7±1.2% versus 10.1±1.0%, $n=3$, $P\leq0.05$), while there are fewer mitotic epithelial cells compared to wild type (11.6±1.8% versus 22.4±2.3%, $n=3$, $P\leq0.05$) (Fig. 1e). In addition, at 14.5, the $Fgfr2c^{+/\Delta}$ mesenchyme shows an increase in the number of phospho-ERK (P-ERK)-positive cells compared to the wild-type mesenchyme (2.3±0.2% versus 1.1±0.2%, $n=3$, $P\leq0.05$), while there are less P-ERK-positive epithelial cells in $Fgfr2c^{+/\Delta}$ versus wild-type lungs (2.3±0.4% versus 3.7±0.3%, $n=3$, $P\leq0.05$) (Figs. 1f, g and g′). Elevated mesenchymal FGF signaling in $Fgfr2c^{+/\Delta}$ mice therefore correlates with an increase in P-ERK-positive cells in the mesenchyme of mutant versus WT lungs.

### *Ectopic activation of FGFR2b signaling within $Fgfr2c^{+/\Delta}$ mesenchyme*

Ectopically expressed *Fgfr2b* within the lung mesenchyme would render this tissue mostly responsive to FGF10, the major FGFR2b ligand during the pseudoglandular stage that is produced by mesenchymal cells themselves and which normally acts solely on the epithelium. To demonstrate ectopic expression of FGFR2b in the mesenchyme, we first compared the ability of mesenchymal cells isolated from E14.5 WT and