Validation of a Novel Fgf10\textsuperscript{Cre–ERT2} Knock-in Mouse Line Targeting FGF10\textsuperscript{Pos} Cells Postnatally

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Fgf10 is a key gene during development, homeostasis and repair after injury. We previously reported a knock-in Fgf10\textsuperscript{Cre–ERT2} line (with the Cre-ERT2 cassette inserted in frame with the start codon of exon 1), called thereafter Fgf10\textsuperscript{Ki–v1}, to target FGF10\textsuperscript{Pos} cells. This line allowed fairly efficient and specific labeling of FGF10\textsuperscript{Pos} cells during the embryonic stage, it failed to target these cells after birth, particularly in the postnatal lung, which has been the focus of our research. We report here the generation and validation of a new knock-in Fgf10\textsuperscript{Cre–ERT2} line (called thereafter Fgf10\textsuperscript{Ki–v2}) with the insertion of the expression cassette in frame with the stop codon of exon 3. Fgf10\textsuperscript{Ki–v2/+} heterozygous mice exhibited comparable Fgf10 expression levels to wild type animals. However, a mismatch between Fgf10 and Cre expression levels was observed in Fgf10\textsuperscript{Ki–v2/+} lungs. In addition, lung and limb agenesis were observed in homozygous embryos suggesting a loss of Fgf10 functional allele in Fgf10\textsuperscript{Ki–v2} mice. Bioinformatic analysis shows that the 3’UTR, where the Cre-ERT2 cassette is inserted, contains numerous putative transcription factor binding sites. By crossing this line with tdTomato reporter line, we demonstrated that tdTomato expression faithfully recapitulated Fgf10 expression during development. Importantly, Fgf10\textsuperscript{Ki–v2} mouse is capable of significantly targeting FGF10\textsuperscript{Pos} cells in the adult lung. Therefore, despite the aforementioned limitations, this new Fgf10\textsuperscript{Ki–v2} line opens the way for future mechanistic experiments involving the postnatal lung.

Keywords: Fgf10, knock-in Cre line, lipofibroblast, adult lung, lineage tracing

INTRODUCTION

The fibroblast growth factor (FGF) family consisting of 22 members is divided into three groups: the paracrine FGF group signaling through FGFR and heparin-sulfate proteoglycans, the endocrine FGF group signaling through FGFR with Klotho family of proteins as co-receptors, and the intracellular FGF group involved in FGFR independent signaling (Ornitz and Itoh, 2001). The FGF7 subgroup which contains FGF3, 7, 10, 22 belongs to the paracrine FGF group. These growth
factors interact mostly with the FGFR2b receptor. FGFI0 in particular has been shown to play important roles during development, homeostasis and repair after injury (Yuan et al., 2018). In the lung, it plays a crucial role in regulating branching morphogenesis (Jones et al., 2020). Genetic deletion of either Fgf10 or its predominant receptor Fgfr2b leads to agenesis of both the limb and the lung, specific portions of the gut, the pancreas as well as the mammary, lacrimal and salivary glands (Min et al., 1998; Sekine et al., 1999; Ohuchi et al., 2000; Mailleux et al., 2002; Jaskoll et al., 2005; Parsa et al., 2008). During homeostasis, Fgfr2b signaling has been shown to be critical for the regeneration of the incisors in mice as well as for the maintenance of the terminal end buds in the mammary gland (Parsa et al., 2008, 2010). Lineage tracing of FGF10 in development indicated that these cells serve as progenitors for lipofibroblast as well as vascular and airway smooth muscle cells (El Agha et al., 2014).

In the context of the repair process, Fgf10 deletion in peribronchial mesenchymal cells leads to impaired repair following injury to the bronchial epithelium using naphthalene (Volckaert et al., 2011; Moiseenko et al., 2020). On the other hand, overexpression of Fgf10 reduces the severity of lung fibrosis in bleomycin-induced mice (Gupte et al., 2009). Given these diverse biological activities, it is important to generate and validate mouse knock-in lines allowing to monitor the localization, fate and status of FGF10 cells during development, homeostasis and repair after injury.

We have previously generated a Fgf10CreERT knock-in mouse line, called thereafter Fgf10KII−/v−/v mice, to monitor the fate of FGF10pos cells after tamoxifen (Tam) administration (El Agha et al., 2012). In these mice, the Tam-inducible Cre recombinase (Cre-ERT2-ires-YFP) was inserted in frame with the start codon of the endogenous Fgf10 gene. Fgf10KII−/v−/v corresponds to a loss-of-function allele for Fgf10 as evidenced by our observation that Fgf10KII−/v+/v−/v homozygous embryos die at birth from multi-organ agenesis, including the lung. In the Fgf10KII−/v+/v−/v lungs, the expression of Cre gradually decreases to almost undetectable levels postnatally, rendering the monitoring of FGF10pos cells postnatally impossible. This is likely due to the deletion of intronic sequences containing key transcription factor binding sites at the insertion site of the Cre-expression cassette.

In order to circumvent this problem, we therefore generated a new Cre-ERT2 knock-in line (named Fgf10KII−/v−/v) by targeting the 3′UTR of the endogenous Fgf10 gene. We here provide experimental evidence for the validation of these mice. Besides a PCR-based strategy to genotype the Fgf10KII−/v−/v allele, we have also established a qPCR-based approach to monitor the expression levels of Fgf10 and Cre at different developmental stages in the lung. Fgf10KII−/v−/v+ lines were crossed with the tdTomato pos mice to validate, at two distinct embryonic stages, the expression patterns of tdTomato in previously known domains of Fgf10 expression. Importantly, we validated the use of these mice in the adult stages to target FGF10 pos cells in the lung. Flow cytometry analysis and immunofluorescence staining were carried out to further characterize the contribution of these cells to the lipofibroblast lineage. Bioinformatic analysis of the insertion site of the Cre-ERT2 cassette in the 3′UTR was also carried out. Altogether, our results indicate that the new Fgf10CreERT line can be successfully used to target FGF10pos cells both in embryonic and adult stages.

MATERIALS AND METHODS

Genotyping

Two pairs of primers were used to determine the genotype of Fgf10KII−/v−/v knock-in mice. Primer P1 (′-AACACC TCTGCTACCTCTCC-T3′); and primer P2 (′-AGGTTCCAC TCCGCTTTTT-3′) were used to detect the knock-in allele (252 bp band) whereas primer P3 (′-GCAGGCAA TGTATGTTGGCA-3′) and primer P4 (′-TGCTTGGTGTTCT TACTGCT-3′) were used to detect the wild-type allele (580 bp band). The PCR program consists of a denaturation step at 94°C for 3 min, followed by 34 cycles of denaturation (94°C for 1 min), annealing (60°C for 30 s) and extension steps (72°C for 300 s). The program ends with a completion step at 4°C for infinity hold. Each PCR tube contains 4.3 µL of H2O, Taq DNA Polymerase in 5.5 µL of Qiagen Master Mix (QIAGEN, Hilden, Germany), 10 pmol of each primer, and 50 ng of genomic DNA in a final volume of 11 µL.

Mice and Tamoxifen Administration

All mice were kept under specific pathogen free (SPF) conditions with unlimited food and water. tdTomato pos reporter mice were purchased from Jackson lab (B6; 129S5-Gt(Rosa)26Sortm9(CAG-tdTomato)Hez/J, ref 007905). Embryonic day 0.5 (E0.5) was assigned to the day when a vaginal plug was detected. Animal experiments were approved by the Regierungspraesidium Giessen (approval number RP GI20/10- Nr. G47/2019). Tamoxifen stock solution was prepared by dissolving tamoxifen powder (Sigma, T5648-5G) in corn oil at a concentration of 20 mg/mL at room temperature and stored in −20°C. Adult mice received 3 successive intraperitoneal (IP) injections of tamoxifen (0.25 mg/g body weight) before analysis. Pregnant mice received a single IP injection of tamoxifen (0.1 mg/g body weight) and pups also received a single subcutaneous injection of tamoxifen (0.2 mg/pup) before analysis. Dissected mice were examined using Leica M205 FA fluorescent stereoscope (Leica, Wetzlar, Germany) and images were acquired using Leica DFC360 FX camera. Figures were assembled in Adobe Photoshop and Illustrator.

Quantitative Real-Time PCR and Statistical Analysis

Freshly isolated embryos and lungs were lysed, and RNA was extracted using RNeasy kit (74106, Qiagen, Hilden, Germany). One microgram of RNA was used for cDNA synthesis using Quantitect Reverse Transcription kit (205311, Qiagen). Primers and probes for Fgfl0, Cre, and β2-Microglobulin (B2M) were designed using NCBI Primer-BLAST1. More details about the used primers and probes can be found in Supplementary Table 1.

1https://www.ncbi.nlm.nih.gov/tools/primer-blast/
Quantitative real-time PCR (qPCR) was performed using LightCycler 480 real-time PCR machine (Roche Applied Science). Samples were run in doublets using B2M as a reference gene and the delta Ct method was used to calculate the relative quantification. GraphPad Prism 7.0 software was used to generate and analyze data. Statistical analyses were performed using Student’s t-test (for comparing two groups) or One-way ANOVA (for comparing three or more groups). Data were considered significant if \( P < 0.05 \).

Flow Cytometry
Freshly dissected lung were washed with Hanks’ balanced salt solution (HBSS, 14175-095, Thermo Fisher) and kept on ice. Sharp blades were used to cut the lung into small pieces and digested with 0.5% collagenase Type IV in HBSS (17104019, Life Technologies, Invitrogen) for 45 min at 37°C. Lung homogenates were then passed through 18, 21, and 24G needles followed by digestion with 0.5% collagenase Type IV in HBSS. 20 \( \mu \)L of sample is taken as an unstained control. Antibodies against CD45 (103114, APC-conjugated; 1:50), CD31 (102409, APC-conjugated; 1:50), EPCAM (APC-Cy7-conjugated; 1:50) and SCA1 (108120, Pacific blue-conjugated; 1:50) (all from Biolegend) as well as LipidTOX stain (FITC-conjugated, 1:200) were used to find the murine Cre in the lung mesenchyme (Herriges et al., 2012). Expression by qPCR at that stage indicated a drastic reduction in expression in lungs is comparable to the one observed in the \( Fgf10^{–/–} /J \) homozygous embryos (Figure 1E). Our initial design of the novel \( Fgf10^{K\text{i}2–v2} \) knock-in line targeting the 3' UTR was conceived to allow normal expression of \( Fgf10 \) in the lung of embryonic and postnatal mice isolated at different time-points (Figure 2A). Our results indicated that \( Fgf10 \) expression level in \( Fgf10^{K\text{i}2–v2/2} \) lungs is comparable to the one observed in the \( Fgf10^{+/+} \) lungs at all these time-points (Figure 2B). Next, we compared \( Fgf10 \) vs. \( Cre \) expression in \( Fgf10^{K\text{i}2–v2/2} \) lungs at different time-points (Figure 2C). Our results indicate a lower level of \( Cre \) compared to \( Fgf10 \) at all these time-points (Figure 2D). This difference between \( Cre \) and \( Fgf10 \) expression in \( Fgf10^{K\text{i}2–v2/2} \) lungs suggests that the insertion of the Cre-ERT2 cassette in the 3’UTR disrupted the expression of the endogenous \( Fgf10 \) gene produced from the recombined allele. Together with \( Fgf10 \) expression in \( Fgf10^{K\text{i}2–v2/2} /\text{WT} \) vs. \( Fgf10^{+/+} \) (WT), this result suggests that \( Fgf10 \) expression from the non-recombined allele in \( Fgf10^{K\text{i}2–v2/2} /\text{WT} \) lungs is increased to compensate the loss of \( Fgf10 \) expression from the recombined allele.

To determine whether the insertion of Cre-ERT2 in the endogenous \( Fgf10 \) locus led to loss of function of \( Fgf10 \), \( Fgf10^{K\text{i}2–v2} \) heterozygous animals were self-crossed and embryos were harvested at E15.5. \( Fgf10^{K\text{i}2–v2}/K\text{i}2–v2 \) homozygous embryos suffered from lung and limb agenesis, which is consistent with complete loss of function of \( Fgf10 \) (Figure 2E). Analysis of \( Fgf10 \) expression by qPCR at that stage indicated a drastic reduction in \( Fgf10 \) expression in \( Fgf10^{K\text{i}2–v2}/K\text{i}2–v2 \) embryo (\( n = 1 \)) compared to \( Fgf10^{+/+} \) or WT lungs (Supplementary Figure 1). We therefore conclude that the \( Fgf10^{K\text{i}2–v2} \) allele corresponds to a \( Fgf10 \) loss-of-function allele.

Validation of Cre Activity to Label FGF10\textsuperscript{Pos} Cells During Embryonic Development
In order to test the recombinase activity of Cre-ERT2, \( Fgf10^{K\text{i}2–v2/2} \) heterozygous mice were crossed with \( tdTomato^{+/\text{flx}} \) reporter mice. Pregnant mice received a single intraperitoneal (IP) injection of tamoxifen at E11.5 (Figure 3A)
FIGURE 1 | Generation and genotyping of the novel Fgf10\textsuperscript{Ki-v2} line. (A,B) Homologous recombination was carried out to insert the F2A-eYFP-T2A-Cre-ERT2-T2A-PA-NEO construct in frame with the stop codon of exon 3 of the mouse Fgf10 gene. Neomycin resistance coding gene was used for the positive selection. (C,D) Recombined ES cell clones were treated with flipase to remove the Neo cassette and blastocyst transfer of the selected ES cells was carried out to generate chimera animals. (E) PCR strategy to genotype mutant and wild type animals. Primers 1 and 2 were used for the detection of the mutant Fgf10\textsuperscript{Ki-v2} allele (252 bp) and Primers 3 and 4 were used for the detection of the wild type Fgf10\textsuperscript{+} allele (580 bp).

or E15.5 (Figure 4A). Embryos were harvested at E18.5. No fluorescent signal was observed in Fgf10\textsuperscript{+/-}, tdTomato\textsuperscript{flox/+} embryos (Figures 3B, 4B; n = 4) indicating absence of recombination in control embryos and lack of leakiness of the tdTomato\textsuperscript{flox} allele. By contrast, tamoxifen treatment at E11.5 led to a strong fluorescent signal in the limbs, stomach, cecum, colon and lungs of Fgf10\textsuperscript{Ki-v2+}, tdTomato\textsuperscript{flox/+} embryos (n = 3). In the limb, the labeled cells were more abundant in the digit tip
area, known to express high level of Fgf10 (Danopoulos et al., 2013). Along the gastro-intestinal tract, labeled cells were located in the anterior part of the stomach as well as in duodenum (data not shown) which are both reported to express high level of Fgf10 (Lv et al., 2019). A similar observation was made in the cecum and the distal colon (Lv et al., 2019). Throughout the lung, we found a robust tdTomato expression with a higher expression in the interlobular septa. This is similar to what was observed with the previously validated Fgf10LacZ reporter line and Fgf10\(^{KI-v1/+}\) line (Mailleux et al., 2005; El Agha et al., 2012, 2014). Interestingly, in the trachea, no labeled cells were observed in this experimental condition (Figure 3C).
Validation of the labeling of FGF10^Pos^ cells at E11.5. (A) Fgf10^{Ki-v2/+} were crossed with Fgf10^{+/+}; tdTom^{floxed/+} mice. Pregnant females received a single IP injection of tamoxifen when the embryos were at E11.5 and sacrificed at E18.5. (B) Head, limb, lung, and cecum of Fgf10^{+/+}; tdTom^{floxed/+} and Fgf10^{Ki-v2/+}; tdTom^{floxed/+} embryos are shown. Note the absence of fluorescence in the Fgf10^{+/+}; tdTom^{floxed/+} indicating that the non-recombined LoxP-Stop-LoxP-tdTomato allele is not leaky. (C) Higher magnification of lung and trachea showing enriched tdTomato expression in the interlobular septa and the lack of tdTomato expression between the cartilage rings, respectively. Scale bar in (B): head: 1.5 mm, Limb: 0.75 mm, Lung: 0.5 mm, cecum: 0.5 mm. Scale bar in (C): 125 µm.
Additionally, tamoxifen treatment at E15.5 revealed strong fluorescent signal in the pinna of the developing ear as well as in the trachea and in between the cartilage rings (Figure 4C). These two additional expression domains are consistent with sites of Fgfl0 expression (Sala et al., 2011; Zhang et al., 2020). We therefore conclude that Cre expression reflects Fgfl0 expression and that this line can be used to target FGF10Pos cells.

**FGF10Pos Cells Labeled After Birth Contribute to the Lipofibroblast Lineage but Not to the Smooth Muscle Cell Lineage**

Using the previously generated Fgfl0Kiv1/ line, we demonstrated that FGF10Pos cells labeled postnatally strongly...
FIGURE 5 | FGF10Pos cells labeled after birth do not contribute significantly to secondary crest myofibroblasts during alveologenesis. (A) FGF10Pos cells in Fgf10Kiy2/v2+/tdTomlox/−; tdTomlox/−; Fgf10Kiy1/v1+/tdTomlox/−; tdTomlox/− pups were labeled in vivo at P4 and analyzed at P21. We also used previously generated Fgf10Kiy1/v1+/tdTomlox/−; tdTomlox/− samples labeled between P2 and P14. (B) Whole-mount fluorescence images of Fgf10Kiy2/v2+/tdTomlox/− and Fgf10Kiy1/v1+/tdTomlox/− lungs showing more abundant labeled cells in Fgf10Kiy2/v2+/tdTomlox/− vs. Fgf10Kiy1/v1+/tdTomlox/− lungs. (C) ACTA2 IF on Fgf10Kiy2/v2+/tdTomlox/−; tdTomlox/− lungs shows little contribution of FGF10Pos cells to SCMF (ACTA2Pos/tdTomPos/ACTA2Pos). (D) Quantification of tdTomPos cells. Br: bronchi. Scale bar in (B): low magnification: 0.5 mm, High magnification: 50 µm. Scale bar in (D): low magnification: 50 µm, High magnification: 10 µm. Scale bar in (E): low magnification: 25 µm, High magnification: 10 µm. Scale bar in (F): Low magnification: 50 µm, High magnification: 10 µm. ****P ≤ 0.0001.
FIGURE 6 | Fgf10<sup>Ki-v1/+; tdTomato<sup>flox/flox</sup></sup>; Fgf10<sup>Ki-v2/+; tdTomato<sup>flox/flox</sup></sup> adult lungs display enhanced number of tdTom<sup>Pos</sup> cells compared with to the Fgf10<sup>Ki-v1/+; tdTomato<sup>flox/flox</sup></sup> adult lungs. (A) 2-months-old; Fgf10<sup>Ki-v1/+; tdTomato<sup>flox/flox</sup></sup> and Fgf10<sup>Ki-v2/+; tdTomato<sup>flox/flox</sup></sup> mice received 3 Tam IPs or oil at P61, P63 and P65 and were sacrificed at P67. (B) Whole-mount fluorescence images of oil-treated Fgf10<sup>Ki-v2/+; tdTomato<sup>flox/flox</sup></sup>, Tam-treated Fgf10<sup>Ki-v2/+; tdTomato<sup>flox/flox</sup></sup> and Tam-treated Fgf10<sup>Ki-v1/+; tdTomato<sup>flox/flox</sup></sup> lungs at P67. Higher magnification of the lungs are shown in the lower panel. (C) Flow cytometry analysis of Fgf10<sup>Ki-v2/+; tdTomato<sup>flox/flox</sup></sup> lung homogenate. Scale bar in (B): low magnification: 2.5 mm, High magnification: 0.75 mm.

Contribute to the lipofibroblast (LIF) lineage but not the smooth muscle cell (SMC) lineage. In particular, they do not contribute in a major way to the ACTA2<sup>Pos</sup> secondary crest myofibroblasts (SCMF) which are abundant during the first 2–3 weeks during alveologenesis which takes place from postnatal day 5 (P5) to P28 (El Agha et al., 2014). To confirm this observation with the new Fgf10<sup>Ki-v2/+</sup> line, we labeled FGF10<sup>Pos</sup> cells at P4 and examined the status of the labeled cells at P21, 1 week before the end of the alveologenesis phase (Figure 5A). Analysis of the whole lung by fluorescence stereomicroscopy indicated a much higher number of labeled cells in the Fgf10<sup>Ki-v2; tdTomato<sup>flox/+</sup></sup> lung compared with the Fgf10<sup>Ki-v1; tdTomato<sup>flox/+</sup></sup> lung (Figure 5B). Quantification of tdTom<sup>Pos</sup> cells indicated that a higher percentile of tdTom<sup>Pos</sup>/DAPI is observed on sections of Fgf10<sup>Ki-v2; tdTomato<sup>flox/+</sup></sup> vs. Fgf10<sup>Ki-v1; tdTomato<sup>flox/+</sup></sup> (4.7% ± 0.6% vs. 1.5% ± 0.2%, n = 2) thereby confirming the
fluorescence stereomicroscopy results (Figure 5C). LipidTOX staining of these lungs was used to visualize LIFs (Figure 5D). Quantification of this staining indicated that 62.6% ± 5.0% (n = 2) of the total tdTom^Pos are LT^Pos and that 27.0% ± 4.4% (n = 2) of the LT^Pos derive from tdTom^Pos cells. These data are in line with our results obtained with the previous Fgf10^K1/v1/+ line. Immunofluorescence (IF) for ACTA2 on these lungs was used to visualize LIFs (Figure 5D). Flow cytometry analysis was also conducted to quantify the total number of tdTom^Pos cells in both conditions as well as their identity (El Agha et al., 2014; Figure 6B). Flow cytometry analysis was also conducted to quantify the total number of tdTom^Pos cells in both conditions as well as their identity (El Agha et al., 2014; Figure 6B). Flow cytometry analysis was also conducted to quantify the total number of tdTom^Pos cells in both conditions as well as their identity (El Agha et al., 2014; Figure 6B). Flow cytometry analysis was also conducted to quantify the total number of tdTom^Pos cells in both conditions as well as their identity (El Agha et al., 2014; Figure 6B).

The New Fgf10^K1/v2 Line Allows More Efficient Labeling of FGF10^Pos Cells in the Adult Lung Compared With the Previous Fgf10^K1/v1 Line

Two months old Fgf10^K1/v2/+; tdTomato^floxed/floxed mice were treated with Tam IP or oil at day 1 (D61), 3 (D63), and 5 (D65) and the lungs were collected at day 7 (D67) (Figure 6A). No fluorescent signal was observed in oil-treated Fgf10^K1/v2/+; tdTomato^floxed/floxed mice, indicating that the line is not leaky. By contrast, a solid signal was found in Tam-treated Fgf10^K1/v2/+; tdTomato^floxed/floxed lungs. A weak signal was detected in Tam-treated Fgf10^K1/v1/+; tdTomato^floxed/floxed lungs as described in a previously study (El Agha et al., 2014; Figure 6B).

The 3'UTR Region of the Fgf10 Gene Contains Many Key Transcription Factor Binding Sites

The decrease in Fgf10 expression in Fgf10^K1/v2 mice (Figure 2D) suggested that important transcription factor binding sites (TFBS) were impacted by the genetic manipulation in the 3'UTR of the Fgf10 gene. We determined the identity of TFBS located at proximity of the 3'UTR of the Fgf10 gene using an online TFBS prediction tool. We compared these TFBS with previously published TF expressed in the lung

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**FIGURE 7** Bioinformatic analysis identified potential transcriptional factors binding sites in the 3’UTR of Exon 3. (A) The murine Fgf10 gene is made of 3 exons. (B) High magnification of Exon 3 and the associated 3’UTR. Several putative important transcriptional factor binding sites were found in this region.
FGF10 is an essential morphogen underlying the developmental process of multiple organs including the lung. FGF10 signaling is also crucial during homeostasis and in the process of injury/repair in the adult lung. FGF10 dysregulation in human has been implicated in some major respiratory diseases, such as bronchopulmonary dysplasia (BPD), Idiopathic pulmonary fibrosis (IPF) and chronic obstructive pulmonary disease (COPD) (Yuan et al., 2018). For example, increased FGF10 expression level in IPF patients has been found (El Agha et al., 2017). However, FGF10 expression is inversely correlated to the disease progression with higher levels in stable IPF vs. lower level in end-stage IPF. Higher FGF10 expression in the early, stable stage of IPF is most likely correlated with the repair process. Insufficient FGF10 level in prematurely newborn infants is associated with arrested lung development at the saccular stage (Prince, 2018). Fgf10 deficiency in a newborn mouse model of hyperoxia-induced BPD led to drastic increase in lethality associated with abnormal alveolar epithelial type 2 (AT2) cell differentiation as well as surfactant production (Chao et al., 2017).

FGF10 also performs a key function for the repair of the bronchial epithelium after injury (Volckaert et al., 2011). Our knowledge about the sources of FGF10 in this context has been evolving. FGF10 was first described to be expressed by airway smooth muscle cells (ASMCs) (Volckaert et al., 2011), whereas more recent work identified a peribronchiolar mesenchymal population capable of producing FGF10 during the repair process, which is not derived from the ASMCs (Moiseenko et al., 2020).

In COPD, the conducting airway epithelium undergoes massive remodeling causing an irreversible airway obstruction (Decramer et al., 2012). Interestingly, we have reported that the FGF10-HIPPO epithelial mesenchymal crosstalk also maintains and recruits lung basal stem cells in the conducting airways (Volckaert et al., 2017). When transient Fgf10 expression by ASMCs is critical for proper airway epithelial regeneration in response to injury, sustained FGF10 secretion by the ASMC niche, in response to chronic ILK/HIPPO inactivation, results in pathological changes in airway architecture resembling the abnormalities seen in COPD. The inhibition of FGF10/FGFR2b signaling may therefore be an interesting approach to treat chronic obstructive airway lung diseases. Conversely, the opposite situation might occur in the respiratory airways in that destruction of the alveolar compartments resulting in emphysema may be due to insufficient FGF signaling. Interestingly, recombinant FGF7 has been reported to induce de novo-alveologenesis in the elastase model of emphysema in mice (Yildirim et al., 2010).

The previous Fgf10<sup>Cre</sup>-V<sup>1</sup> model was mainly used to trace the FGF10<sup>pos</sup> cells during embryonic development. A near complete loss of the labeling capacity of FGF10<sup>pos</sup> cells during postnatal stages limited its utilization in the analysis of their cell fate in adult lung homeostasis and during the process of injury/repair. In order to overcome the limitations of the Fgf10<sup>Cre</sup>-V<sup>1</sup> line, we generated and validated this new knock-in Fgf10<sup>pos</sup>-LIF reporter line, we demonstrated that the tdTomato expression pattern lies between Fgf10<sup>Cre</sup>-ERT<sup>1</sup> and Fgf10<sup>Cre</sup>-ERT<sup>2</sup> mouse models, with the Fgf10<sup>pos</sup>-LIF expression pattern (El Agha et al., 2012), and a more robust labeling of FGF10<sup>pos</sup> cells was achieved in the postnatal stages in spite of a mismatch between Cre and Fgf10 expression, which could be explained by the disruption of critical TFBS located in the 3' UTR of the Fgf10 gene. Therefore, this line will be a valuable tool to further define critical mesenchymal cell populations in the adult lung contributing to the repair process after injury. Combined crosses with existing or novel Dre-ERT2 recombinase driver lines may allow to capture subpopulations of FGF10<sup>pos</sup> cells/lineages based on the expression of two markers (Jones et al., 2019). The main FGF10<sup>pos</sup> subpopulation is represented by the lipid-containing alveolar interstitial fibroblasts (lipofibroblasts or LIFs). More and more studies have acknowledged LIFs as an essential piece of the AT2 stem cell niche in the rodent lungs. Despite the fact that LIFs were initially believed to only assist AT2 cells in surfactant production during neonatal life, recent studies have shown that these cells are important for self-renewal and differentiation of AT2 stem cells during adulthood (Barkauskas et al., 2013). In spite of the increasing interests in lipofibroblast biology, little is known about their cellular origin or the molecular pathways that control their formation during embryonic development. We have shown that in the developing mouse lung, FGF10<sup>pos</sup> cells labeled at E11.5 or E15.5 are progenitors for LIFs (El Agha et al., 2014). In addition, FGF10 is also essential for the differentiation of these progenitors into the LIF lineage (Al Alam et al., 2015). We have also reported the existence of FGF10<sup>pos</sup>-LIF as well as FGF10<sup>pos</sup>-LIFs...
The difference between these two populations is still unclear and will require further studies. In the context of bleomycin-induced lung fibrosis, in vivo lineage tracing indicates that LIFs transdifferentiate into activated myofibroblast during fibrosis formation and that a significant proportion of the labeled activated myofibroblasts transdifferentiate back to LIFs during fibrosis resolution (El Agha et al., 2017).

In conclusion, we have successfully generated a new Fgf10CreERT2 line with enhanced labeling efficiency of FGF10\(^{\text{vOS}}\) cells postnatally. This line, which displays normal expression of Fgf10 in Fgf10\(^{\text{vOS} v+/+}\), avoids many developmental defects linked to deficient Fgf10 expression. Therefore, it paves the way for performing cell-autonomous based studies to investigate the role of these FGF10\(^{\text{vOS}}\) cells as well as associated signaling pathways during lung development and disease.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

Animal experiments were reviewed and approved by the Regierungsgpraedium Giessen (approval number RP GI/47-2019).

AUTHOR CONTRIBUTIONS

XC, ST, AIV-A, and LC performed the experiments. SH, CC, and J-SZ contributed to methodology. EEA and SB conceived the study. XC and SB wrote the manuscript. J-SZ, EEA, and SB edited the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcell.2021.671841/full#supplementary-material

Supplementary Figure 1 | Comparative Fgf10 expression in Fgf10\(^{+/+}\) and Fgf10\(^{vOS v/−}\) lungs vs. Fgf10\(^{v/−}\) livers at E15.5. qPCR was used to determine Fgf10 expression.

Supplementary Figure 2 | Co-expression of ACTA2 and tdTomato in FGF10\(^{vOS}\) cells. Fgf10\(^{v/−}\), tdTomato\(^{+/+}\) pups received one injection of Tam subcutaneously at P2 and were analyzed at P14.
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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.