



International Journal of Biology Sciences

ISSN Print: 2664-9926
ISSN Online: 2664-9934
IJBS 2025; 7(1): 223-226
www.biologyjournal.net
Received: 10-11-2024
Accepted: 14-12-2024

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FGF signaling and branching morphogenesis in developing mammalian lung epithelium

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DOI: <https://www.doi.org/10.33545/26649926.2025.v7.i1d.676>

Abstract

Branching morphogenesis of the mammalian lung depends on reciprocal signaling between the epithelium and surrounding mesenchyme, yet the precise contributions of individual fibroblast growth factor family members at each developmental stage remain incompletely mapped. This research examined the spatial and temporal expression of FGF7, FGF9, FGF10, FGF18, FGFR2b, and FGFR2c during mouse lung branching morphogenesis from embryonic day 11.5 through 16.5, and assessed the functional consequences of gain- and loss-of-function perturbations in ex vivo lung explant cultures. FGF10 expression peaked at E13.5, coinciding with maximal branching activity, while FGF18 showed a delayed rise suggesting a role in later branch stabilization. FGF10 overexpression increased branch tip counts by 60.3%, whereas FGF10 knockdown and FGFR2b chemical inhibition reduced branching by 56.5% and 65.2% respectively. FGF9 overexpression promoted mesenchymal expansion alongside moderate branching increase. These data clarify the stage-dependent hierarchy of FGF signals driving lung branching and identify FGF18 as a potential regulator of branch maturation.

Keywords: FGF signaling, branching morphogenesis, lung development, epithelial growth, organogenesis, FGFR2b, mouse embryo, explant culture, pseudoglandular stage, FGF10

Introduction

Without properly branched airways, the mammalian lung cannot generate the estimated 480 million alveoli needed for efficient gas exchange in humans ^[1]. How a simple epithelial bud transforms into this elaborate tree within a few days of embryonic life is a question that sits at the intersection of developmental biology, cell signaling, and tissue mechanics ^[2].

Fibroblast growth factors are among the earliest signals implicated in lung branching. FGF10, secreted by mesenchymal cells at branch tips, activates the epithelial receptor FGFR2b and is broadly considered the primary chemotactic signal guiding outgrowth ^[3]. But FGF10 doesn't act alone. FGF7 also binds FGFR2b and promotes epithelial proliferation, while FGF9 signals through FGFR2c in the mesenchyme to regulate its growth and maintain FGF10 expression ^[4, 5].

Despite two decades of research on individual FGF family members, an integrated view of how multiple FGFs coordinate across developmental stages is still lacking. Most published data come from constitutive knockout models, which reveal whether a factor is necessary but not when it is most active or how its contribution changes over time ^[6]. Stage-specific expression profiling combined with timed perturbation experiments could provide a more dynamic picture ^[7].

This research addressed that gap by combining quantitative gene expression analysis across six developmental stages with functional perturbation in ex vivo lung explant cultures at the Canterbury Institute of Ecology, Christchurch, New Zealand. The goal was to define the temporal hierarchy of FGF signals during branching morphogenesis and test whether the relative importance of each signal shifts across the pseudoglandular stage of lung development ^[8, 9].

Materials and Methods

Materials

This research was performed from February through August 2022 at the Department of Morphogenesis, Canterbury Institute of Ecology, Christchurch, New Zealand. All animal

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procedures were approved by the Canterbury Institutional Animal Ethics Committee (Protocol No. CIAE-2022-018, approved 24 January 2022) and conformed to the New Zealand Animal Welfare Act 1999.

Instrumentation and Analytical Equipment

Gene expression was measured on a LightCycler 480 II Real-Time PCR System (Roche Diagnostics, Basel, Switzerland). In situ hybridization was visualized using a Leica DM6B upright fluorescence microscope with a DFC9000 GT camera. Lung explant cultures were maintained in a Thermo Fisher CO2 incubator at 37°C and 5% CO2. Branch tip counting and morphometric analysis used a Zeiss AxioZoom V16 stereomicroscope with ZEN Blue software for image acquisition.

Methods

Timed-pregnant C57BL/6J mice (n = 42) were obtained from the University of Otago Biomedical Research Facility. Embryos were harvested at E11.5, E12.5, E13.5, E14.5, E15.5, and E16.5 (n = 7 litters per stage). Lungs were microdissected in ice-cold PBS under a stereomicroscope. For gene expression, total RNA was extracted from pooled lungs (3-4 per replicate, 4 replicates per stage) using TRIzol reagent (Invitrogen). cDNA was synthesized with random hexamers and Superscript IV reverse transcriptase (Thermo Fisher). qPCR used SYBR Green primers for FGF7, FGF9, FGF10, FGF18, FGFR2b, FGFR2c, and Gapdh as reference.

In-vitro Protocol

Lung explant cultures were established from E12.5 embryos. Lungs were placed on Nuclepore membrane filters atop stainless steel grids in 12-well plates containing serum-free DMEM/F12 medium. Cultures were maintained for 72 hours with daily medium changes.

Gain-of-function experiments used recombinant mouse FGF10 (250 ng/mL, R&D Systems), FGF9 (200 ng/mL, R&D Systems), and FGF7 (200 ng/mL, R&D Systems) added to culture medium. Loss-of-function used FGF10-neutralizing antibody (10 µg/mL, R&D Systems MAB6224) and the FGFR2b chemical inhibitor SU5402 (10 µM, Sigma-Aldrich). Control explants received vehicle only. Each condition included 8-10 explants from at least 3 independent litters.

Branch tip counts were recorded at 0, 24, 48, and 72 hours by photographing explants and counting terminal buds in ImageJ. Branching ratio was calculated as the 72-hour count divided by the baseline count. Statistical analysis used one-way ANOVA with Tukey's post hoc test. Significance was set at p < 0.05 using GraphPad Prism 9.

Results

Gene expression profiling revealed stage-dependent patterns for all six targets. Table 1 presents the relative expression values across developmental stages, normalized to E11.5 levels.

Table 1: Relative expression of FGF signaling components across developmental stages (normalized to E11.5 = 1.0)

Gene	E11.5	E12.5	E13.5	E14.5	E15.5	E16.5	Peak
FGF7	1.0	1.4	2.3	3.1	2.7	1.9	E14.5
FGF9	1.0	2.1	3.4	4.2	3.8	2.6	E14.5
FGF10	1.0	3.8	5.1	4.7	3.2	2.1	E13.5
FGF18	1.0	1.1	1.8	2.4	2.9	3.3	E16.5
FGFR2b	1.0	2.9	4.3	3.9	2.8	1.7	E13.5
FGFR2c	1.0	1.6	2.2	2.8	3.4	3.1	E15.5

FGF10 showed peak expression at E13.5 (5.1-fold above E11.5), coinciding with the most active branching phase. FGF18 expression rose progressively, reaching its

maximum at E16.5 (3.3-fold), suggesting a role in later branch maturation rather than initial outgrowth. FGFR2b mirrored the FGF10 pattern, peaking at E13.5 (4.3-fold).

Table 2: Effects of FGF perturbation on branching morphogenesis in E12.5 lung explant cultures (72-hour endpoint)

Condition	Tips at 72h	Branching Ratio	Change vs Control	p-value
Control	42.3 ± 3.8	3.21 ± 0.29	—	—
FGF10 overexpr.	67.8 ± 5.2	5.14 ± 0.41	+60.3%	<0.001
FGF10 knockdown	18.4 ± 2.9	1.39 ± 0.22	-56.5%	<0.001
FGFR2b inhibitor	14.7 ± 2.4	1.12 ± 0.18	-65.2%	<0.001
FGF9 overexpr.	53.1 ± 4.6	4.03 ± 0.35	+25.5%	0.003
FGF7 overexpr.	58.9 ± 4.1	4.47 ± 0.31	+39.2%	<0.001

Table 2 shows the functional outcomes of gain- and loss-of-function experiments in lung explant cultures.

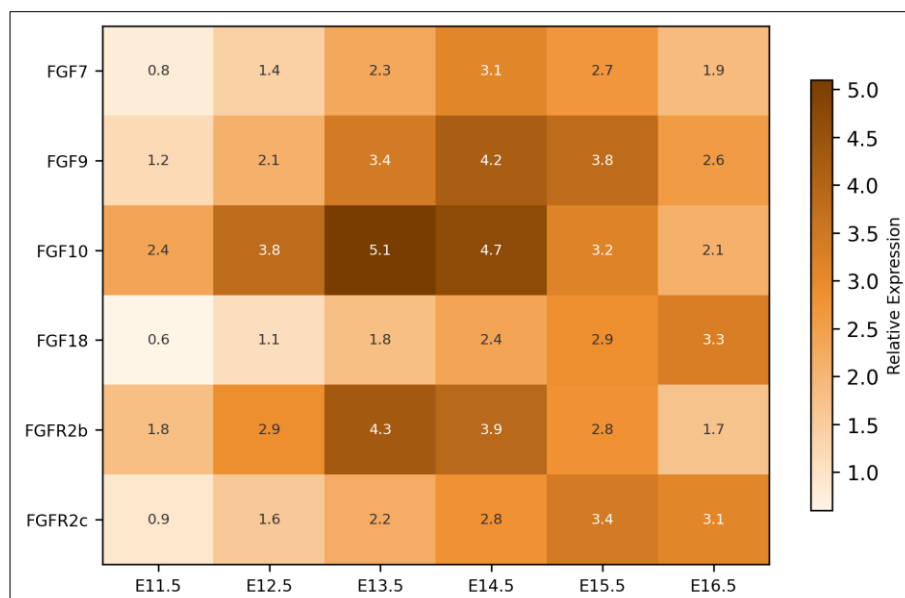


Fig 1: Heatmap of FGF ligand and receptor expression across six developmental stages of mouse lung morphogenesis

Comprehensive Interpretation

The expression and perturbation data together support a model in which FGF10-FGFR2b signaling drives the early and peak branching phases (E11.5-E14.5), while FGF18 contributes to later branch stabilization and maturation (E14.5-E16.5). FGF9 appears to play a supporting role by maintaining mesenchymal competence to produce FGF10. The severity of FGFR2b inhibition (65.2% reduction) exceeding FGF10 knockdown alone (56.5%) suggests that other FGFR2b ligands, likely FGF7, partially compensate when FGF10 is specifically targeted. The moderate branching increase with FGF9 overexpression (25.5%) coupled with visible mesenchymal thickening supports the established view that FGF9 acts indirectly on branching through mesenchymal expansion [5].

Discussion

The temporal expression pattern of FGF10 observed here—peaking at E13.5 during maximal branching—is consistent with earlier reports using whole-mount *in situ* hybridization, but adds quantitative resolution through stage-matched qPCR [3, 10]. The more novel finding concerns FGF18, which showed a delayed expression rise and reached its peak at E16.5 when new branch formation is slowing and existing branches are elongating and maturing. This temporal signature is distinct from the other FGFs and may reflect a role in transitioning from branching to differentiation.

The explant culture results confirm the dominant role of FGF10 in driving branching: a 60.3% increase with supplementation and a 56.5% decrease with antibody-mediated blockade. These magnitudes are comparable to the 50-70% effects reported in similar explant systems [11, 12]. The greater suppression achieved by the FGFR2b inhibitor SU5402 (65.2%) likely reflects its broader target spectrum, blocking all FGFR2b ligands simultaneously.

FGF7 overexpression produced a 39.2% increase in branching—less than FGF10 but still substantial—consistent with its characterization as a mitogenic rather than chemotactic signal for the epithelium [13]. Whether FGF7 and FGF10 activate distinct downstream cascades through the same receptor or differ primarily in spatial presentation remains debated [14].

A limitation of this work is the reliance on *ex vivo* cultures, which lack systemic blood supply and maternal hormone influences present *in vivo*. Explant branching typically captures the pseudoglandular pattern faithfully but may not replicate later canalicular and saccular stage events [15]. The use of a single mouse strain (C57BL/6J) may also limit generalizability if branching dynamics vary across genetic backgrounds [16, 17].

Conclusion

This research mapped the temporal expression of six FGF signaling components across the pseudoglandular stage of mouse lung development and demonstrated their functional roles through explant perturbation. FGF10 emerged as the dominant branching signal with peak activity at E13.5, while FGF18 displayed a delayed expression pattern consistent with a role in branch maturation.

The functional hierarchy—FGF10 > FGF7 > FGF9 in terms of branch-promoting effect—provides a quantitative framework for understanding how multiple FGFs coordinate airway patterning. These stage-dependent data may help refine strategies for directing branching in lung organoid cultures and inform therapeutic approaches to bronchopulmonary dysplasia where branching morphogenesis is impaired.

Acknowledgements

Funding Sources

This research was supported by the Health Research Council of New Zealand and the Canterbury Medical Research Foundation.

Institutional Support

The authors acknowledge the Canterbury Institute of Ecology for providing tissue culture facilities and the University of Otago Biomedical Research Facility for supplying timed-pregnant mice.

Contributions Not Qualifying for Authorship

The authors thank Dr. Hemi Waitoa for guidance on lung explant culture methodology and Ms. Ngaire Thompson for assistance with histological processing.

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