

Canonical Wnt/ β -Catenin Signaling Modulates C/EBP α Abundance and Alveolar Epithelial Differentiation Marker Expression Under Hyperoxia: An *In Vitro* Model of Bronchopulmonary Dysplasia

Tao Shen¹, Zhen Jia¹, Qinqin Niu¹, Zhen Wang^{1,*}

¹Department of Pediatrics, Suzhou Hospital of Integrated Traditional Chinese and Western Medicine, 215101 Suzhou, Jiangsu, China

*Correspondence: zhwong@petalmail.com (Zhen Wang)

Submitted: 4 August 2026 Revised: 19 August 2026 Accepted: 16 September 2026 Published: 23 October 2026

Background: Bronchopulmonary dysplasia reflects an alveolar epithelium in which the differentiation program remains incomplete. CCAAT/enhancer-binding protein α (C/EBP α) is indispensable for alveolar type II (AT2) maturation, yet the mechanisms governing its abundance under oxidant stress remain unexamined. Because canonical Wnt/ β -catenin signaling has been reported to reduce C/EBP α in other lineages, we asked whether β -catenin signaling modulates C/EBP α in hyperoxia-exposed alveolar epithelium and whether this is accompanied by changes in differentiation marker expression.

Methods: Rat AT2-like RLE-6TN cells were exposed to 95% O₂ for 48 h. Wnt output was manipulated bidirectionally with ICG-001 (10 μ M) under hyperoxia and lithium chloride (20 mM) under normoxia, while β -catenin was silenced under hyperoxia and overexpressed under normoxia. The contribution of C/EBP α was examined by silencing it during Wnt inhibition and by restoring it under continued hyperoxia without manipulating the pathway. Pathway activity (active β -catenin, *Axin2*), C/EBP α , differentiation markers (pro-SP-C/*Sftpc*, AQP5/*Aqp5*) and apoptosis were assessed.

Results: Hyperoxia increased active β -catenin and *Axin2* and reduced C/EBP α , pro-SP-C and AQP5, with increased apoptosis (all $p < 0.001$). ICG-001 reduced *Axin2* without altering active β -catenin and increased C/EBP α , pro-SP-C and AQP5, whereas pharmacological glycogen synthase kinase-3 β (GSK-3 β) inhibition with lithium chloride reproduced the hyperoxic pattern under normoxia. β -catenin silencing under hyperoxia increased C/EBP α , *Sftpc* and *Aqp5*, whereas β -catenin overexpression under normoxia reduced them (all $p < 0.001$). Silencing C/EBP α markedly attenuated the ICG-001-induced increases in *Sftpc* and *Aqp5*, while C/EBP α overexpression alone increased both despite persistent hyperoxia.

Conclusions: Canonical Wnt/ β -catenin activity and C/EBP α abundance were inversely associated across perturbation experiments in hyperoxia-exposed AT2-like cells, and C/EBP α appears to contribute substantially to the effect of Wnt inhibition on differentiation marker expression. These findings support a functional β -catenin-C/EBP α relationship that may contribute to hyperoxia-associated changes in alveolar differentiation marker expression, pending mechanistic and *in vivo* confirmation.

Keywords: bronchopulmonary dysplasia; hyperoxia; Wnt/ β -catenin signaling; C/EBP α ; alveolar epithelial differentiation markers

Introduction

At the margins of viability, survival commonly depends on ventilatory support and supplemental oxygen, yet the structurally and functionally immature lung is particularly vulnerable to ventilator-induced injury and oxygen toxicity [1,2,3]. Bronchopulmonary dysplasia (BPD) is a frequent consequence [4,5,6]. Whereas the disorder first described by Northway and colleagues [7] was dominated by airway injury and fibrosis in relatively mature lungs [8], contemporary BPD affects infants born during the late canalicular or early saccular stages of lung development and is characterized histologically by fewer, enlarged and simplified distal airspaces, together with a reduced and dysmorphic pulmonary capillary bed [9,10]. Because supplemental oxygen remains indispensable in neonatal care but

can itself injure the immature lung, neonatal hyperoxia has become the most widely used experimental paradigm for modelling BPD [11,12].

Viewed at cellular resolution, airspace simplification reflects an alveolar epithelium whose differentiation program remains incomplete [13,14]. Two functionally interdependent epithelial lineages populate the mature alveolus: alveolar type I (AT1) and type II (AT2) cells [15,16]. AT2 cells synthesize, store and secrete pulmonary surfactant, with pro-surfactant protein C, which is encoded by SFTPC in humans and *Sftpc* in mice, serving as a canonical marker of mature AT2 identity [15,17]. They also act as resident facultative progenitors capable of self-renewal and of generating AT1 cells [15,17]. AT1 cells, identified here by aquaporin 5 (AQP5) [18], spread into the thin barrier across

which gas exchange actually occurs [15]. Completion of alveolarization therefore requires not merely the survival of AT2 cells after oxidant injury, but preservation of the temporally regulated epithelial fate transitions that establish an appropriate balance of AT2 and AT1 cells [14,19]. Interventions aimed only at cell death leave this second requirement untouched, which may help explain why antioxidant and anti-inflammatory strategies have translated poorly.

Among the transcription factors implicated, CCAAT/enhancer-binding protein α (C/EBP α) occupies an unusually decisive position [20]. Conditional deletion of *Cebpa* from fetal respiratory epithelium is lethal at birth [21,22]. Glycogen-laden pre-type II cells fail to mature, surfactant lipid and protein synthesis collapses, and differentiated cells of both alveolar lineages are lacking [21,22]. Deletion of C/EBP α from the post-natal respiratory epithelium produces no overt baseline phenotype, yet renders mice strikingly intolerant of 95% oxygen, with defective SP-B and SP-C maturation and disruption of gene programs governing surfactant and redox homeostasis [23]. Consistent with this, hyperoxia lowers C/EBP α together with SP-C in cultured AT2 cells, and forced C/EBP α expression blunts the injury [24]. In neonatal mice, hyperoxia has instead been reported to increase C/EBP α , with its silencing improving alveolar architecture [25]. This discrepancy across developmental stages and models itself argues for defining, in a controlled setting, what governs C/EBP α abundance under oxidant stress. That upstream question has received little attention.

Ligand engagement of Frizzled and LRP5/6 disables the AXIN-APC-GSK-3 β destruction complex, sparing β -catenin from degradation [26]. The stabilized protein enters the nucleus, partners with T-cell factor/lymphoid enhancer-binding factor (TCF/LEF) family members and transactivates targets such as *Axin2*, whose transcript therefore serves as an endogenous readout of pathway output [27]. During alveologenesis, this pathway behaves as a fate-holding signal rather than a differentiation signal: a secondary wave of Wnt activity sustains AT2 proliferation and stemness, whereas experimental dampening of the pathway shifts AT2 cells toward AT1 conversion [28,29]. Differentiation, in other words, requires Wnt to be relieved at the right moment. Hyperoxia appears to prevent precisely this transition. Elevated β -catenin and increased expression of pathway components have been reported in hyperoxia-exposed newborn rat lungs [30]. Although model-dependent, evidence implicates aberrant Wnt signaling across experimental and clinical BPD. Hyperoxia increases nuclear β -catenin and AXIN2 expression in human developmental organotypic cultures and Wnt5a expression in neonatal mouse lung, while increased WNT5A and AXIN2 expression is detectable in lung tissue from infants with BPD [31]. Consistent with a pathogenic role in selected contexts, pharmacological inhibition of β -catenin-dependent transcription with ICG-001 improves alveolarization and reduces pul-

monary vascular remodeling and pulmonary hypertension in hyperoxia-exposed neonatal rats [32].

Canonical Wnt signaling maintains adipogenic progenitors in an undifferentiated state, in part by suppressing the adipogenic transcriptional program centered on *Cebpa* and *Pparg*. Similarly, activated β -catenin-TCF signaling reduces CEBPA expression in liver cancer cells [33,34]. Whether the same regulatory relationship operates in hyperoxia-exposed alveolar epithelium remains unknown, and correlation alone cannot establish it. Coincident changes are equally compatible with C/EBP α acting downstream of β -catenin, with both responding independently to oxidant stress, or with C/EBP α feeding back on Wnt signaling. Distinguishing among these possibilities requires bidirectional perturbation of each node, together with testing whether C/EBP α is required for the effects of manipulating Wnt output.

We therefore addressed this question using rat AT2-like RLE-6TN cells exposed to 95% O₂ for 48 h. Wnt output was manipulated bidirectionally: it was suppressed during hyperoxia with the β -catenin/CREB-binding protein (CBP; CREB, cAMP response element-binding protein) interaction inhibitor ICG-001 and activated under normoxia with the GSK-3 β inhibitor lithium chloride as a supportive pharmacological probe. This approach tested whether altering pathway activity was accompanied by corresponding changes in C/EBP α , pro-SP-C and AQP5, while genetic manipulation of β -catenin provided a specificity test beyond pharmacology alone. β -catenin itself was silenced under hyperoxia and overexpressed under normoxia to examine the direction of the functional association genetically. Central to the argument, C/EBP α was knocked down while Wnt was inhibited and, conversely, restored under continued hyperoxia without pharmacological or genetic manipulation of the pathway, to test whether increasing C/EBP α alone could alter differentiation marker expression. The results support a model in which C/EBP α is likely involved downstream of β -catenin signaling and contributes, though not exclusively, to hyperoxia-associated changes in alveolar differentiation marker expression.

Materials and Methods

Cell Line

RLE-6TN cells (rat alveolar type II-like epithelium) were the sole cellular system used in this study. This spontaneously immortalized line, derived from alveolar type II cells of an adult male F344 rat, grew as an adherent monolayer with a polygonal, cobblestone-like epithelial morphology, clear intercellular borders and a high nuclear-to-cytoplasmic ratio, forming tightly packed islands that coalesced as confluence was approached. This appearance was retained throughout the passage range used, and no spindle-shaped or fibroblast-like subpopulations were observed. The cell line was purchased from SUNNCELL

(SNL-277, Wuhan, China), and was used between passages 5 and 15 after receipt. Growth conditions were DMEM/F-12 base medium with 10% fetal bovine serum, 2 mM glutamine and penicillin-streptomycin, held at 37 °C under 5% CO₂/21% O₂. Subculture was performed at 80–90% density. Every experiment began with cells plated 24 h earlier and treated once they had reached 60–70% density. Cultures were screened for Mycoplasma contamination. Every experiment reported here was performed on cells that had tested negative, and no anti-Mycoplasma agent was used at any stage.

Oxygen Exposure

Hyperoxia was delivered in a sealed modular chamber. Before each 48-h exposure, the chamber was purged for 10 min with humidified 95% O₂/5% CO₂ at 3–5 L/min, then closed and returned to a 37 °C incubator. Purging was repeated at 24 h to prevent drift in oxygen tension, and the achieved concentration was verified using an in-line analyzer. Matched control plates were maintained for the same 48 h at 21% O₂/5% CO₂. Medium was renewed in all groups immediately before exposure to ensure that nutrient status did not differ between experimental arms.

Drug Treatments

ICG-001 (HY-14428, MedChemExpress, Monmouth Junction, NJ, USA) was diluted from DMSO stock into growth medium (final 10 μM), and LiCl (HY-Y0649, MedChemExpress) was diluted from a sterile PBS stock (final 20 mM). Both compounds were added at the start of the 48-h exposure and remained in the medium throughout. DMSO never exceeded 0.1% (v/v), and vehicle at this concentration was added to all arms not receiving ICG-001. LiCl was dissolved in sterile PBS, and an equivalent volume of PBS was added to the corresponding control arms. No iso-osmolar salt control, such as NaCl or KCl at a matched concentration, was included; therefore, the contribution of the osmotic and ionic load imposed by 20 mM LiCl cannot be distinguished from that of GSK-3β inhibition in this arm. Accordingly, this arm is treated as supportive pharmacological evidence rather than as a specificity test (see Limitations).

Gene Silencing and Forced Expression

Duplex siRNAs targeting the rat *Ctnnb1* transcript (si-β-catenin, sense 5'-AGUAAUCAAUUGACAACUG-3', antisense 5'-GUUGCAAUUUGAUUAACUAU-3') and rat *Cebpa* (si-C/EBPα, sense 5'-UUCUUAAUACUAAAACUGCC-3', antisense 5'-CAGUUUUAGUAUUAAGGAAGU-3'), plus a scrambled non-targeting duplex (non-targeting negative control siRNA [si-NC], sense 5'-UUCUCCGAACGUGUCACGUTT-3', antisense 5'-ACGUGACACGUUCGGAGAATT-3'), were obtained from GenePharma (Shanghai, China). Overexpression

constructs were designed by the authors and custom-synthesized and cloned by GenePharma (Shanghai, China). The complete coding sequence of rat *Ctnnb1* (RefSeq NM_053357, encoding 781 amino acids) or rat *Cebpa* (RefSeq NM_012524, encoding 358 amino acids) was preceded by a Kozak consensus sequence, carried no epitope tag, and was inserted into pcDNA3.1(+) (V79020, Invitrogen, Carlsbad, CA, USA) under the control of the cytomegalovirus (CMV) promoter, yielding the overexpression constructs designated OE-β-catenin and OE-C/EBPα, respectively, with the empty backbone serving as the matched control (empty-vector control [OE-NC]). Each construct was verified across the full length of the insert and across both insert-vector junctions by Sanger sequencing, and the reads were identical to the corresponding RefSeq entries. Endotoxin-free plasmid DNA was prepared and quantified spectrophotometrically before use.

Transfection used Lipofectamine 3000 (L3000015, Invitrogen) in six-well plates containing 2 mL of medium per well. Duplex siRNA was delivered at a final concentration of 50 nM per well with 5 μL of Lipofectamine 3000 reagent, and plasmid DNA at 2.5 μg per well with 5 μL of Lipofectamine 3000 reagent and 5 μL of P3000 enhancer. Total siRNA concentration and plasmid mass were kept constant across all arms within each experiment. Complexes were formed in serum-free Opti-MEM and applied to 60–70% confluent monolayers. After 6 h, the medium was replaced with complete medium, and oxygen exposure began 24 h post-transfection. Efficiency was re-verified by immunoblot in every independent run rather than only once at the outset. Protein was used as the primary measure of efficiency because β-catenin abundance is regulated chiefly by post-translational stability and because transcripts derived from the CMV-driven constructs, which lack the native untranslated regions, cannot be distinguished from endogenous transcripts using primers located within the coding sequence. Throughout the manuscript, gene symbols (*Ctnnb1*, *Cebpa*, *Axin2*, *Sftpc*, *Aqp5*) denote transcripts, whereas β-catenin and C/EBPα denote the corresponding proteins. The si- and OE- prefixes designate experimental arms, which are named after the protein affected rather than the transcript targeted.

Transcript Quantification

TRIzol lysis (DP424, TIANGEN, Beijing, China) yielded total RNA, and only preparations with an A260/A280 ratio between 1.8 and 2.1 were carried forward. One μg per sample was reverse-transcribed, and the resulting cDNA was amplified with a SYBR Green chemistry (FP205, TIANGEN, Beijing, China). Product identity was confirmed by melt-curve inspection at the end of each run. Gapdh served as the internal reference, and relative abundance was calculated using the 2^{−ΔΔCt} method, with the mean of the relevant control arm defined as unity. Each

Table 1. Primers (rat).

Gene	Forward (5'-3')	Reverse (5'-3')
<i>Axin2</i>	CGAGCCTGGCTTCAGAAGAT	CTCCTTGAAGTGGCCCAGAG
<i>Cebpa</i>	TACCGAGTAGGGGAGCAAA	TGGTCCCAGTGCTTCATCC
<i>Sftpc</i>	GGAGCACCGGAAACTCAGAA	CTTATAGGCGGTCCAGGAGCC
<i>Aqp5</i>	CTGGCGGCCATCCTCTATTT	CCCCAGCTGAGAGGATGTTG
<i>Gapdh</i>	ACGGGAAACCCATCACCATC	CTCGTGGTTCACACCCATCA

Axin2, axis inhibition protein 2; *Cebpa*, CCAAT/enhancer-binding protein α ; *Sftpc*, surfactant protein C; *Aqp5*, aquaporin 5; *Gapdh*, glyceraldehyde-3-phosphate dehydrogenase.

Table 2. Antibodies.

Target	Source	Dilution
Non-phospho (active) β -catenin	8814, Cell Signaling Technology, Danvers, MA, USA	1:1000
β -catenin, total	ET1601-5, HUABIO, Woburn, MA, USA	1:1000
C/EBP α	bs-1630R, Beijing Biosynthesis Biotechnology Co., Ltd., Beijing, China	1:1000
pro-SP-C	bsm-54814R, Beijing Biosynthesis Biotechnology Co., Ltd., Beijing, China	1:1000
AQP5	20334-1-AP, Proteintech, Rosemont, IL, USA	1:1000
GAPDH	60004-1-Ig, Proteintech, Rosemont, IL, USA	1:5000
HRP anti-rabbit/anti-mouse IgG	ZB-2301/2305, ZS Golden Bridge, Beijing, China	1:2000

C/EBP α , CCAAT/enhancer-binding protein α ; SP-C, surfactant protein C; HRP, horseradish peroxidase; IgG, immunoglobulin G.

biological sample was analyzed in three technical wells, which were averaged before statistical treatment. Primer sequences are listed in Table 1.

Immunoblotting

Monolayers were rinsed twice in chilled PBS and solubilized for 30 min on ice in RIPA buffer (P0013J, Beyotime, Shanghai, China) fortified with protease and phosphatase inhibitors (ST506, Beyotime, Shanghai, China). Cleared lysates (12,000 $\times$ g, 15 min, 4 °C) were quantified by bicinchoninic acid (BCA) assay, and 20–30 μ g of protein per lane was heat-denatured (95 °C, 5 min), resolved on 10–12% polyacrylamide gels (P0015, Beyotime, Shanghai, China), and transferred to 0.45 μ m PVDF (IPVH00010, Millipore, Burlington, MA, USA). Blocking was performed with 5% (w/v) skimmed milk in TBST for 1 h at ambient temperature. Primary antibodies (Table 2) were applied overnight at 4 °C, followed by HRP-conjugated secondary antibodies for 1 h at ambient temperature. Signals were then developed using enhanced chemiluminescence. Densitometry was performed using ImageJ (version 1.54b, National Institutes of Health, Bethesda, MD, USA). Each target band was first normalized to GAPDH on the same membrane and then scaled to the control arm of that experiment.

Pathway activation was read with an antibody recognizing non-phospho (active) β -catenin at Ser33/Ser37/Thr41, whereas knockdown and overexpression efficiency was assessed using a total β -catenin antibody.

Apoptosis Measurement

At the end of the 48-h exposure, cells were harvested using ethylenediaminetetraacetic acid (EDTA)-free trypsin, washed twice with cold PBS (G4202, Servicebio, Wuhan, China), and resuspended at approximately 1×10^6 /mL in binding buffer. Annexin V-FITC and propidium iodide (5 μ L each; AP101, LIANKE, Hangzhou, China) were added, and the suspensions were incubated for 15 min in the dark at ambient temperature before acquisition within the hour. Annexin V signal was collected in the FITC-A channel and PI in the ECD-A channel (CytoFLEX, Beckman Coulter, Inc., Brea, CA, USA). At least 10,000 events were recorded per tube. Quadrant boundaries were set using unstained and single-stained tubes prepared in parallel. The apoptotic index is the sum of the Annexin V⁺/PI⁻ and Annexin V⁺/PI⁺ fractions. Apoptosis was quantified in the normoxia and hyperoxia arms only. Viability and proliferation were not assessed in the pharmacological or genetic intervention arms.

Statistical Analysis

Three independent replicates underlie every value, and results are presented as mean $\pm$ SD. Distributional assumptions were assessed before statistical testing using the Shapiro-Wilk test for normality and Levene's test for homogeneity of variances. Comparisons involving three or more groups were performed by one-way ANOVA with Tukey's correction. Two-arm comparisons used an unpaired, two-tailed *t*-test. Analyses were performed using GraphPad Prism (version 10.1.2, GraphPad Software, Boston, MA, USA), with two-tailed *p* < 0.05 considered statistically significant.

Results

Hyperoxia Is Associated With Increased Canonical Wnt Signaling Readouts and Reduced C/EBP α and Alveolar Differentiation Marker Expression

Rat AT2-like RLE-6TN cells were maintained under 95% O₂ for 48 h to model hyperoxic epithelial injury *in vitro*. Active (non-phospho) β -catenin protein accumulated relative to normoxic cultures ($p < 0.001$; Fig. 1A,B), and transcription of the pathway target *Axin2* increased in parallel ($p < 0.001$; Fig. 1C).

Differentiation-related marker expression moved in the opposite direction. C/EBP α decreased under hyperoxia at both protein and transcript levels (both $p < 0.001$; Fig. 1A–C). The alveolar markers pro-SP-C (AT2-associated) and AQP5 (AT1-associated) also declined (both $p < 0.001$; Fig. 1A,B). Annexin V-FITC/PI staining confirmed that the model also exhibited the expected cytotoxic component, with the apoptotic fraction being higher after hyperoxia than under normoxia ($p < 0.001$; Fig. 1D).

The functional association between Wnt activity and changes in differentiation marker expression was evaluated pharmacologically in both directions. Adding the β -catenin/CBP interaction inhibitor ICG-001 throughout the hyperoxic exposure left the pool of active β -catenin essentially unchanged (no significant difference from hyperoxia alone; Fig. 1A,B) yet reduced pathway output, as indicated by *Axin2* expression ($p < 0.001$ vs. hyperoxia; Fig. 1C). This dissociation is consistent with the point at which the compound acts: the transcriptional complex rather than β -catenin stabilization itself. Against this background, ICG-001 increased C/EBP α protein and *Cebpa* mRNA, and likewise increased pro-SP-C and AQP5, in each case significantly above levels in the untreated hyperoxia group (all $p < 0.001$; Fig. 1A–C).

Exposing normoxic cells to the GSK-3 β inhibitor lithium chloride, without an oxidant challenge, increased active β -catenin and *Axin2* while simultaneously reducing C/EBP α protein, *Cebpa* mRNA, pro-SP-C and AQP5 relative to untreated normoxic controls (all $p < 0.001$; Fig. 1A–C). Because no iso-osmolar salt control was included, this arm establishes that pharmacological GSK-3 β inhibition reproduces the hyperoxic pattern but does not by itself demonstrate that the effect is Wnt-specific. That question is addressed genetically below.

Hyperoxic activation of Wnt/ β -catenin signaling was associated with reduced C/EBP α and AT2/AT1 differentiation marker expression. The pharmacological data are consistent with increased pathway activity contributing to this pattern, which was further examined in the genetic experiments that follow.

β -Catenin Perturbation Is Associated With Inverse Changes in C/EBP α and Alveolar Differentiation Marker Expression

Pharmacology alone cannot establish the order of the two events, so β -catenin itself was manipulated genetically by silencing it in hyperoxia-exposed cells (si- β -catenin vs. si-NC) and overexpressing it in normoxic cells (OE- β -catenin vs. OE-NC). Immunoblotting confirmed that the intended change in β -catenin protein was achieved in each case, with levels reduced after silencing and increased after transfection (both $p < 0.001$; Fig. 2A,B). *Axin2* changed in the same direction as β -catenin in both arms (both $p < 0.001$; Fig. 2C), indicating that transcriptional output tracked β -catenin levels. C/EBP α responded inversely, and did so in both directions. Knocking down β -catenin under hyperoxia increased C/EBP α protein and *Cebpa* mRNA relative to their respective controls, whereas β -catenin overexpression under normoxia reduced both (all $p < 0.001$; Fig. 2A–C). *Sftpc* and *Aqp5* transcripts likewise increased after β -catenin silencing under hyperoxia and decreased after β -catenin overexpression under normoxia (all $p < 0.001$; Fig. 2C). These reciprocal perturbations therefore produced mirror-image outcomes, supporting a model in which C/EBP α is likely involved downstream of β -catenin signaling, with corresponding changes in AT2- and AT1-associated marker expression.

C/EBP α Contributes to Wnt Inhibition Associated Changes in Differentiation Marker Expression Under Hyperoxia

Hyperoxia-exposed cells received ICG-001 together with either control siRNA or siRNA targeting C/EBP α . As before, ICG-001 increased C/EBP α protein relative to hyperoxia plus control siRNA ($p < 0.001$), whereas C/EBP α -targeting siRNA abolished this increase, returning C/EBP α to the level observed in the untreated hyperoxia group ($p < 0.001$ vs. hyperoxia + si-NC + ICG-001). This also confirmed effective knockdown (Fig. 3A).

ICG-001 increased *Sftpc* and *Aqp5* above the hyperoxia + si-NC group (both $p < 0.001$), and silencing C/EBP α markedly attenuated both increases (both $p < 0.001$ vs. hyperoxia + si-NC + ICG-001; Fig. 3B). The attenuation was, however, incomplete. Transcript levels in the si-C/EBP α arm remained slightly above those in the hyperoxia + si-NC group, suggesting that a component of the ICG-001 effect may be independent of C/EBP α . It is also important to consider where the si-C/EBP α arm came to rest. Knockdown did not reduce C/EBP α below its hyperoxic baseline, but instead returned the protein to the level observed in hyperoxia + si-NC (Fig. 3A). The hyperoxia + si-NC arm therefore serves as a reference in which C/EBP α is low and ICG-001 is absent, whereas the hyperoxia + si-C/EBP α + ICG-001 arm represents a condition in which C/EBP α is similarly low but ICG-001 is present. *Sftpc* and *Aqp5* differed only marginally between these two arms, indicat-

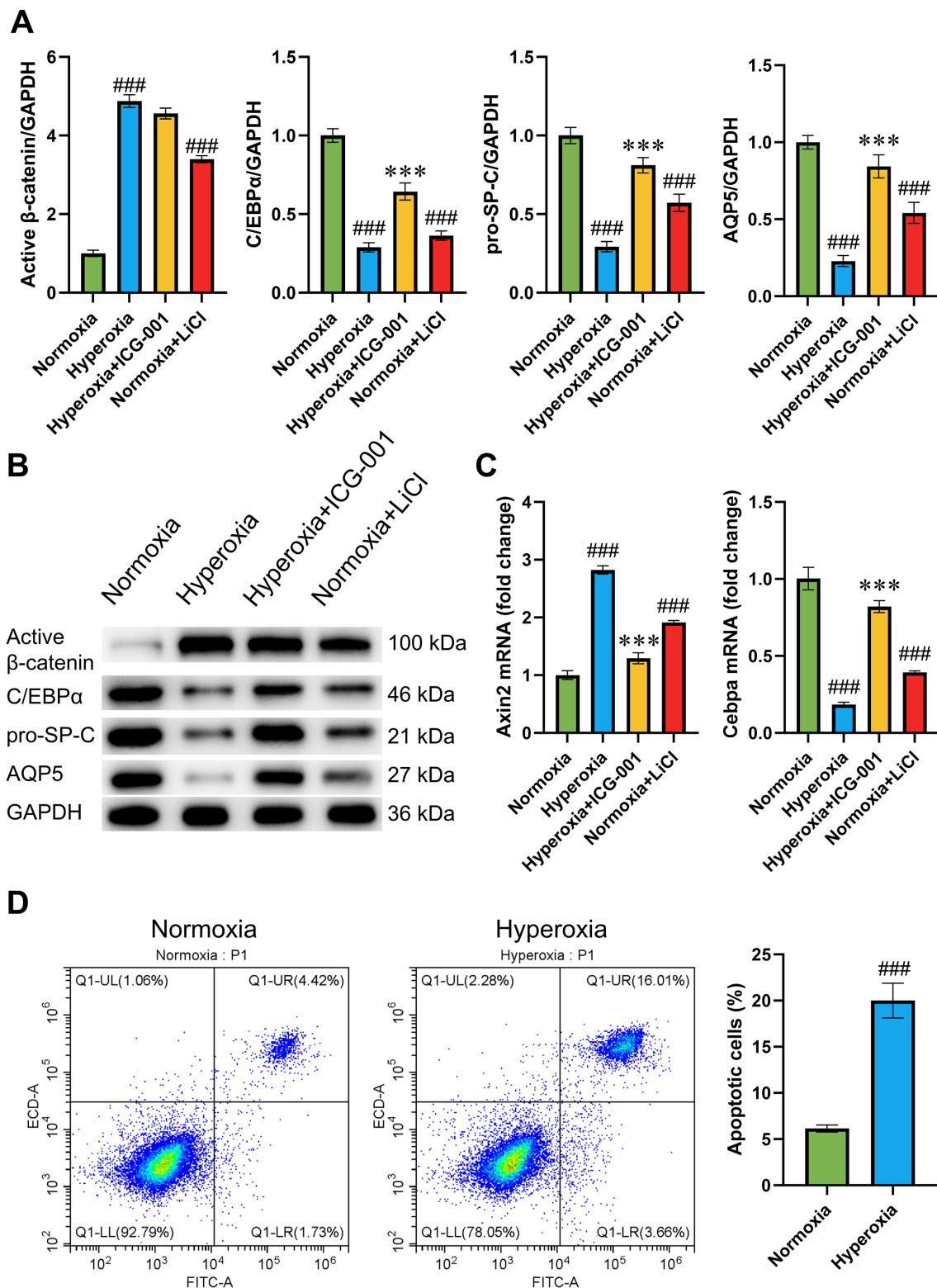


Fig. 1. Hyperoxia is associated with increased Wnt/ β -catenin signaling readouts and reduced C/EBP α and alveolar epithelial differentiation marker expression in RLE-6TN cells. (A) Densitometric quantification of active β -catenin, C/EBP α , pro-SP-C and AQP5, normalized to GAPDH and expressed relative to normoxia. (B) Representative western blots. (C) qRT-PCR analysis of *Axin2* and *Cebpa*, normalized to *Gapdh*. (D) Representative Annexin V-FITC/PI flow cytometry dot plots for the normoxia and hyperoxia groups and quantification of the total apoptotic fraction. Data are expressed as the mean $\pm$ SD of three independent experiments. ### p < 0.001 vs. Normoxia, *** p < 0.001 vs. Hyperoxia. Wnt, wingless-related integration site; qRT-PCR, quantitative reverse transcription polymerase chain reaction; FITC, fluorescein isothiocyanate; PI, propidium iodide; SD, standard deviation.

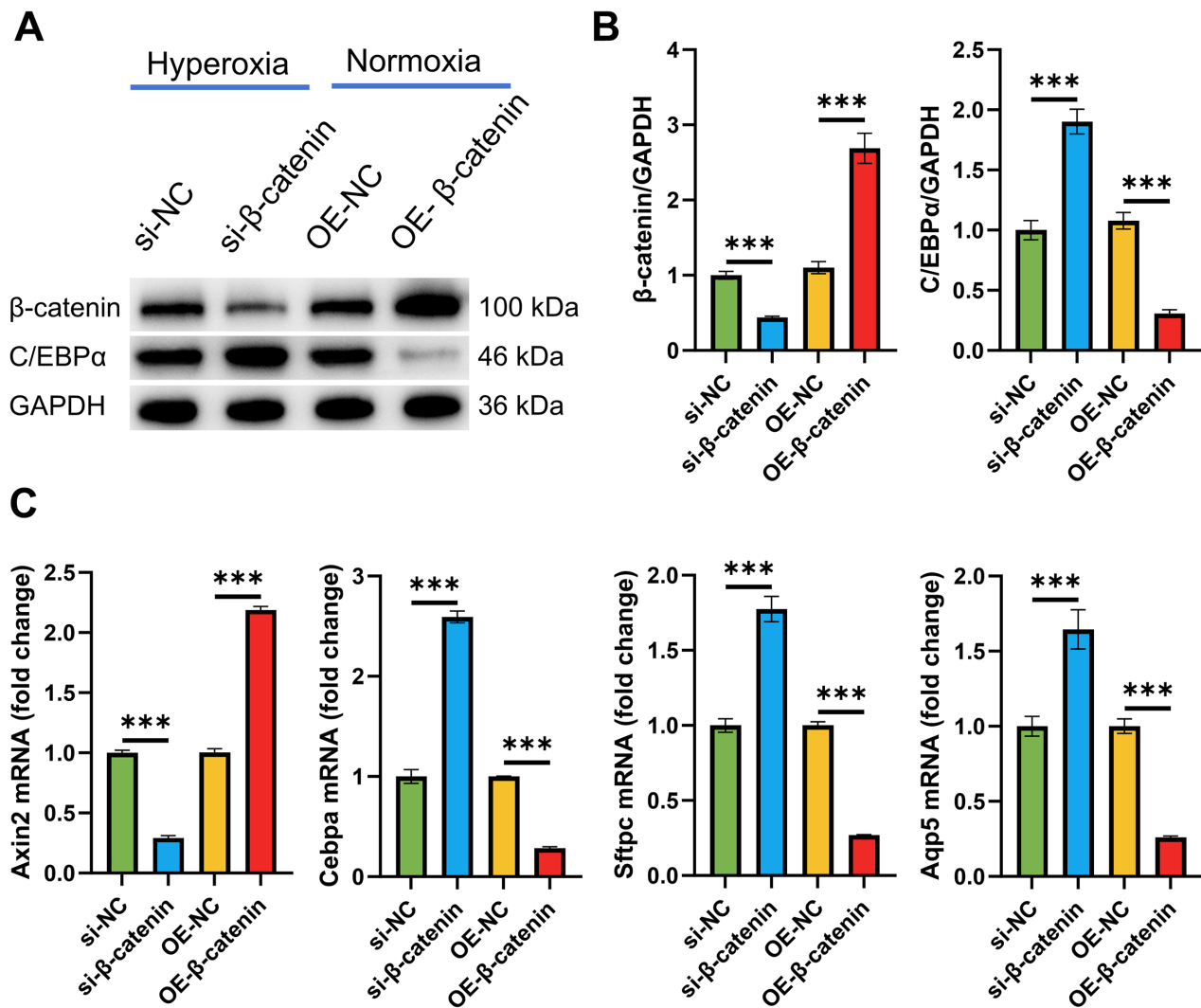


Fig. 2. β-catenin perturbation is associated with inverse changes in C/EBPα and alveolar differentiation marker expression. β-catenin was silenced in hyperoxia-exposed cells (si-β-catenin versus si-NC) and overexpressed in normoxic cells (OE-β-catenin versus OE-NC). (A) Representative immunoblots for β-catenin and C/EBPα. (B) Corresponding densitometry, normalized to GAPDH. (C) qRT-PCR for *Axin2*, *Cebpa*, *Sftpc* and *Aqp5*. Values are expressed as the mean ± SD from three independent experiments. *** $p < 0.001$. si-NC, non-targeting control siRNA; OE-NC, empty-vector control.

ing that the transcriptional benefit of ICG-001 was largely lost once C/EBPα was maintained at its hyperoxic level. This is consistent with C/EBPα contributing substantially to the ICG-001-associated increase in marker expression, although the comparison is indirect. A complementary overexpression experiment tested whether increasing C/EBPα alone could alter marker expression without pharmacological or genetic manipulation of the pathway. Transfection of the C/EBPα overexpression construct (OE-C/EBPα) into hyperoxia-exposed cells increased C/EBPα protein relative to empty vector ($p < 0.001$; Fig. 3C) and increased *Sftpc* and *Aqp5* transcripts in the same cells (both $p < 0.001$; Fig. 3D), despite continued hyperoxic exposure. Pathway activity was not measured in this arm, so the possibility that increased C/EBPα itself altered Wnt output cannot be ex-

cluded. This result therefore shows that C/EBPα overexpression is associated with increased differentiation marker expression under hyperoxia. It does not establish that pathway activity remained unchanged or determine C/EBPα's position within the pathway.

Considered together with the loss-of-function data, these findings support the interpretation that C/EBPα is likely involved downstream of β-catenin signaling and contributes to a substantial proportion of the effect of Wnt inhibition on alveolar epithelial differentiation marker expression. The present design does not permit a formal test of mediation, so this interpretation remains provisional.

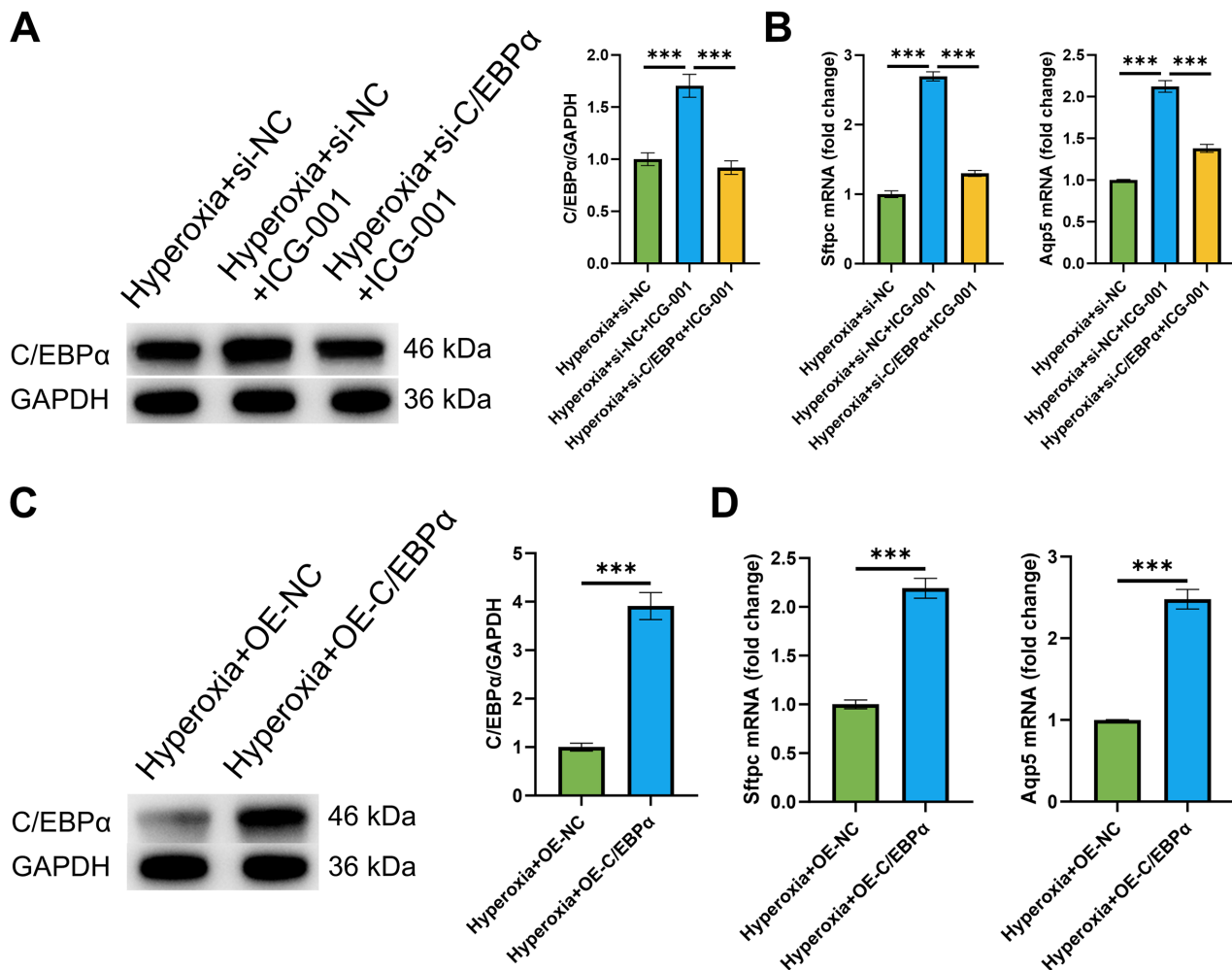


Fig. 3. C/EBPα contributes to Wnt inhibition-associated changes in differentiation marker expression under hyperoxia, and C/EBPα overexpression increases these markers. (A) Immunoblot and densitometry of C/EBPα across hyperoxia + si-NC, hyperoxia + si-NC + ICG-001 and hyperoxia + si-C/EBPα + ICG-001. (B) *Sftpc* and *Aqp5* transcripts in the same three arms. (C) Immunoblot and densitometry of C/EBPα in hyperoxia-exposed cells carrying an empty vector (OE-NC) or the C/EBPα overexpression construct (OE-C/EBPα). (D) *Sftpc* and *Aqp5* transcripts in the same two arms. Values are expressed as the mean ± SD from three independent experiments. *** $p < 0.001$.

Discussion

The transcriptional output of canonical Wnt signaling and the abundance of C/EBPα moved in opposite directions, regardless of which node was manipulated or in which direction. Hyperoxia increased active β-catenin and *Axin2* while reducing C/EBPα, pro-SP-C and AQP5. Interrupting β-catenin/CBP-dependent transcription during hyperoxia reduced *Axin2* and returned all three of these readouts part of the way toward control values. β-catenin overexpression under normoxia reproduced the hyperoxic pattern in the absence of oxidant exposure, and pharmacological GSK-3β inhibition with lithium chloride produced a concordant result, which we regard as corroborative rather than as independent proof of Wnt specificity. When C/EBPα was silenced, the increase in *Sftpc* and *Aqp5* accompanying Wnt

inhibition was largely lost, whereas restoring C/EBPα alone increased both transcripts under continuing hyperoxia without direct manipulation of the pathway. Taken together, these observations support a model in which C/EBPα is likely involved downstream of β-catenin signaling and contributes substantially to Wnt inhibition-associated changes in differentiation marker expression. We regard this as the parsimonious interpretation of the present dataset rather than as a settled mechanism. We wish to be explicit about what the present design can and cannot support. Because C/EBPα was silenced only in the presence of ICG-001, the experiment does not provide a formal test of mediation between Wnt inhibition, C/EBPα and marker expression, and no interaction term between Wnt inhibition and C/EBPα silencing could be estimated. What the data show is that the effect of Wnt inhibition on differentiation marker expres-

sion is largely absent when C/EBP α is held at its hyperoxic level, and that C/EBP α overexpression alone increases the same transcripts under continuing hyperoxia. These observations are consistent with a contributory role for C/EBP α , but they do not demonstrate mediation. A second constraint concerns the direction of the relationship. Neither active β -catenin nor *Axin2* was measured in cells overexpressing C/EBP α , so a reciprocal effect of C/EBP α on pathway output cannot be excluded. We therefore describe C/EBP α as likely involved downstream of β -catenin signaling, while acknowledging that reciprocal effects cannot be excluded. The directional evidence in this study comes from the β -catenin gain- and loss-of-function experiments, in which *Axin2* tracked β -catenin while *Cebpa* moved inversely in both directions, rather than from the C/EBP α overexpression arm. The same caution applies to the interpretation of the markers themselves. What was measured was the abundance of *Sftpc*, *Aqp5*, pro-SP-C and AQP5 in the surviving adherent population, and changes in their abundance are not equivalent to changes in differentiation state. We have therefore framed the results throughout as changes in differentiation marker expression.

That C/EBP α is important for alveolar epithelial maturation is not in dispute. Epithelium-restricted deletion of *Cebpa* in fetal mice arrests AT2-cell maturation and prevents the emergence of both differentiated AT1 cells and mature, surfactant-secreting AT2 cells [21]. Adult mice in which the gene is deleted after birth tolerate 95% oxygen poorly, with disordered surfactant protein processing [23]. In cultured adult human AT2 cells, hyperoxia reduced both C/EBP α and SP-C expression, whereas forced C/EBP α expression partially restored SP-C expression and cell proliferation while limiting apoptosis [24]. Our data show a similar pattern. What has not been examined, to our knowledge, is what contributes to reduced C/EBP α abundance when the epithelium is exposed to oxygen. The present work addresses this question at the level of functional association.

The literature is not internally consistent regarding the direction of the C/EBP α response. In neonatal mice, 72 hours of hyperoxia has been reported to increase C/EBP α , with its silencing improving alveolar architecture through enhanced epithelial proliferation [25], the opposite of what we observed. Several differences distinguish the two settings: an intact developing lung containing mesenchymal, endothelial and immune compartments versus a single epithelial cell line in isolation; a developmental window of active alveolarization versus a cell line derived from adult rat lung; whole-organ homogenate versus a single cell type; and architectural outcomes versus marker abundance. We cannot adjudicate between these findings, and we do not interpret our data as contradicting the *in vivo* report. One plausible reconciliation is that C/EBP α supports maturation while suppressing proliferation, such that the same change in its abundance can appear protective or harmful depending on whether the dominant tissue phenotype re-

flects maturation-related changes or cell number. Testing this possibility would require the two systems to be compared side by side.

That ICG-001 lowered *Axin2* without measurably changing the amount of active β -catenin is consistent with the compound's mechanism rather than being an inconsistency. ICG-001 disrupts the interaction between β -catenin and CBP downstream of the point at which β -catenin escapes degradation, while leaving the alternative β -catenin-p300 complex comparatively intact. Pathway output therefore falls without a corresponding reduction in the pool of stabilized protein. The dissociation between these two readouts is thus best regarded as internal evidence that the drug acted at the expected point in the pathway, and it is one reason why the genetic experiment was necessary (see Limitations).

The direction we observed is compatible with what is known about Wnt activity during normal alveolarization. A postnatal wave of canonical Wnt signaling maintains AT2 identity, proliferation and progenitor capacity, whereas reduced Wnt exposure permits AT2-lineage cells to adopt an AT1 fate [29]. On this view, differentiation requires the signal to be withdrawn on schedule, and sustained Wnt activity would be expected to hold cells short of a fully mature state, consistent with the pattern produced by our hyperoxia model. A precedent for this antagonism also exists outside the lung: canonical Wnt signaling restrains adipogenic commitment and differentiation, in part by suppressing the C/EBP α -peroxisome proliferator-activated receptor gamma (PPAR γ) transcriptional program, while β -catenin-TCF signaling reduces CEBPA expression in liver cancer cells [33,34]. Whether the same regulatory logic operates at the rat *Cebpa* locus in alveolar epithelium was not tested here. The relationship observed here should therefore be understood as a functional association supported by perturbation experiments, not as evidence of direct physical or transcriptional regulation (see Limitations).

Limitations

These findings should be interpreted in light of several limitations. First, the cellular system is narrow. RLE-6TN cells are an immortalized line of adult rat origin studied in isolation, whereas bronchopulmonary dysplasia is a disorder of the developing lung in which epithelial maturation is coordinated with mesenchymal, endothelial and immune compartments and is further influenced by inflammation and mechanical stretch. An adult-derived cell line also cannot reproduce the developmental window during which alveolarization occurs. Nothing in these experiments addresses alveolar architecture, and no inference regarding clinical management or the therapeutic use of Wnt inhibitors follows from them.

Second, the C/EBP α silencing experiment was not a full factorial design. C/EBP α was silenced only in the presence of ICG-001, and a hyperoxia plus si-C/EBP α arm with-

out ICG-001 was not included. Consequently, no formal interaction term could be estimated, and the independent contribution of C/EBP α knockdown under hyperoxia was not measured directly. This experiment therefore cannot provide a formal test of mediation by C/EBP α , and we do not describe it as an epistasis test. Our inference rests instead on the observation that silencing returned C/EBP α to, rather than below, its hyperoxic level, together with the independent overexpression result obtained under hyperoxia.

Third, C/EBP α is not the only route. Silencing C/EBP α reduced but did not eliminate the effect of ICG-001 on *Sftpc* and *Aqp5*, suggesting that other effectors may also contribute to the effect of Wnt inhibition. C/EBP α should therefore be viewed as one component of the response rather than as the sole link between Wnt inhibition and marker expression.

Fourth, the readouts are asymmetrically distributed across the experiments. Differentiation markers were assessed at the protein level only in the pharmacological arms, whereas the genetic arms relied on transcript data alone. Discordance between transcript and protein levels cannot be excluded in those experiments.

Fifth, the relationship may not be strictly unidirectional. Neither active β -catenin nor Axin2 was measured in cells overexpressing C/EBP α , so an effect of C/EBP α on pathway output cannot be excluded, and the two nodes may be mutually rather than unidirectionally coupled. We therefore describe C/EBP α as likely involved downstream of β -catenin signaling while acknowledging possible reciprocity, and interpret the overexpression result only as evidence that increasing C/EBP α can increase marker expression under continuing hyperoxia, not as evidence of its position within the pathway.

Sixth, the mechanism at the *Cebpa* locus was not resolved. Promoter reporter assays and chromatin immunoprecipitation were not performed, and the present data cannot distinguish direct transcriptional repression by β -catenin-TCF from an indirect mechanism involving intermediate factors.

Seventh, the pharmacological tools have important limitations. ICG-001 disrupts the β -catenin-CBP interaction while leaving the alternative β -catenin-p300 complex comparatively intact, so this arm interrogated one branch of β -catenin-dependent transcription rather than the pathway in its entirety. Lithium chloride inhibits GSK-3 β but is not a selective Wnt agonist, and no iso-osmolar salt control was included. Osmotic, ionic and GSK-3 β -independent effects of 20 mM LiCl therefore cannot be excluded. We accordingly do not treat this arm as evidence of Wnt-specific activation. Rather, it is presented as supportive pharmacological evidence, with the corresponding conclusion resting primarily on the genetic gain-of-function experiment, in which β -catenin overexpression under normoxia reproduced the same pattern without pharmacological intervention.

Eighth, marker abundance is not equivalent to lineage identity, and differences in cell survival provide a competing explanation. Higher *Aqp5* expression in a type II-like cell line is consistent with a shift toward a type I program, but in the absence of lineage tracing or functional readouts, it cannot be equated with completed transdifferentiation. Hyperoxia increased the apoptotic fraction approximately threefold, while apoptosis, viability and proliferation were not assessed in any of the pharmacological or genetic intervention arms. Therefore, we cannot exclude the possibility that differences in the size or composition of the surviving population contributed to the observed changes in marker expression. Transcript and protein measurements were normalized to *Gapdh* and GAPDH, respectively, in surviving adherent cells, so a uniform reduction in cell number would not by itself alter normalized abundance; a selective loss of differentiated cells would be required. That possibility nevertheless remains open. Throughout the manuscript, we therefore describe the interventions as altering the expression of differentiation-related markers rather than as promoting, restoring or inhibiting differentiation itself.

Future Directions

Enthusiasm for pathway blockade should be tempered by the fact that Wnt signaling sustains type II cell self-renewal and contributes to epithelial repair. Its suppression may therefore trade maturation against regenerative capacity, making the timing and degree of any intervention important. Within the present system, the most informative next steps would be to complete the C/EBP α silencing experiment as a full factorial design, crossing hyperoxia $\pm$ ICG-001 with $\pm$ si-C/EBP α and including a formal interaction term; to add an Axin2 measurement to the C/EBP α overexpression arm; and to pair marker measurements with viability and proliferation assays in every intervention arm. These additions would allow the independent and combined contributions of Wnt inhibition and C/EBP α silencing to be better resolved and would more directly test whether C/EBP α contributes to the effect of Wnt inhibition. Beyond this system, primary type II cells or alveolar organoids would test whether the relationship persists outside a transformed cell line. A neonatal rodent hyperoxia model with morphometric endpoints would establish whether it operates during alveolarization and translates into changes in lung architecture. Epithelium-specific manipulation of β -catenin would reduce the ambiguity inherent in systemic pharmacology, while reporter and chromatin assays at the *Cebpa* locus would determine whether the β -catenin-C/EBP α relationship involves direct transcriptional regulation.

Conclusions

In hyperoxia-exposed rat alveolar type II-like cells, canonical Wnt/ β -catenin activity and C/EBP α abundance

were inversely associated across the perturbation experiments, and C/EBP α appears to contribute substantially to Wnt inhibition-associated increases in type II and type I differentiation marker expression. These findings support a functional β -catenin-C/EBP α relationship that may contribute to hyperoxia-associated changes in alveolar differentiation marker expression. Confirmation of pathway order, directness and relevance in developing lung tissue will require additional mechanistic and *in vivo* studies.

Availability of Data and Materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions

TS and ZW designed the research study. TS and QQN performed the research. ZW and ZJ analyzed the data. TS drafted the article and all authors contributed to the important editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

Not applicable.

Funding

This work was supported by the Suzhou Science and Technology Program Development Project (Grant No. SKYD2023074).

Conflict of Interest

The authors declare no conflict of interest.

References

- [1] Zhang EY, Bartman CM, Prakash YS, Pabelick CM, Vogel ER. Oxygen and mechanical stretch in the developing lung: risk factors for neonatal and pediatric lung disease. *Frontiers in Medicine*. 2023; 10: 1214108. <https://doi.org/10.3389/fmed.2023.1214108>
- [2] Kalikkot Thekkevedu R, El-Saie A, Prakash V, Katakam L, Shivanna B. Ventilation-Induced Lung Injury (VILI) in Neonates: Evidence-Based Concepts and Lung-Protective Strategies. *Journal of Clinical Medicine*. 2022; 11: 557. <https://doi.org/10.3390/jcm11030557>
- [3] Norman M, Jonsson B, Wallström L, Sindelar R. Respiratory support of infants born at 22-24 weeks of gestational age. *Seminars in Fetal & Neonatal Medicine*. 2022; 27: 101328. <https://doi.org/10.1016/j.siny.2022.101328>
- [4] Moreira A, Noronha M, Joy J, Bierwirth N, Tarriela A, Naqvi A, et al. Rates of bronchopulmonary dysplasia in very low birth weight neonates: a systematic review and meta-analysis. *Respiratory Research*. 2024; 25: 219. <https://doi.org/10.1186/s12931-024-02850-x>
- [5] Cosgrove E, Boel L, Kotecha S. Recent Advances in the Management of Bronchopulmonary Dysplasia. *Current Treatment Options in Pediatrics*. 2025; 11: 20. <https://doi.org/10.1007/s40746-025-00334-8>
- [6] Yang X, Luo G, Lou F. Nesfatin-1 Suppresses Inflammation in Bronchopulmonary Dysplasia by Regulating HMGB-1/TLR4/p65/NLRP3 Signaling Pathway. *Discovery Medicine*. 2025; 37: 933–945. <https://doi.org/10.24976/Descov.Med.202537196.83>
- [7] Northway WH, Jr, Rosan RC, Porter DY. Pulmonary disease following respirator therapy of hyaline-membrane disease. Bronchopulmonary dysplasia. *The New England Journal of Medicine*. 1967; 276: 357–368. <https://doi.org/10.1056/NEJM.196702162760701>
- [8] Bush A, Hilgendorff A. Editorial: Bronchopulmonary Dysplasia: Past, Current and Future Pathophysiologic Concepts and Their Contribution to Understanding Lung Disease. *Frontiers in Medicine*. 2022; 9: 922631. <https://doi.org/10.3389/fmed.2022.922631>
- [9] Ambalavanan N, Deutsch G, Pryhuber G, Travers CP, Willis KA. The evolving pathophysiology of bronchopulmonary dysplasia. *Physiological Reviews*. 2026; 106: 197–237. <https://doi.org/10.1152/physrev.00042.2024>
- [10] Hadchouel A, Delacourt C. The pathogenesis of bronchopulmonary dysplasia: “It is never the heart, it is always the lung” - myth or maxim? *Breathe (Sheffield, England)*. 2025; 21: 240102. <https://doi.org/10.1183/20734735.0102-2024>
- [11] Ding KL, Smith C, Seedorf G, Abman SH. Nintedanib preserves lung growth and prevents pulmonary hypertension in a hyperoxia-induced lung injury model. *Pediatric Research*. 2025; 97: 1676–1683. <https://doi.org/10.1038/s41390-024-03562-0>
- [12] Sotiropoulos JX, Oei JL. The role of oxygen in the development and treatment of bronchopulmonary dysplasia. *Seminars in Perinatology*. 2023; 47: 151814. <https://doi.org/10.1016/j.sempri.2023.151814>
- [13] Cui X, Fu J. Reinitiating lung development: a novel approach in the management of bronchopulmonary dysplasia. *Respiratory Research*. 2024; 25: 384. <https://doi.org/10.1186/s12931-024-02996-8>
- [14] Xia S, Vila Ellis L, Winkley K, Menden H, Mabry SM, Venkatraman A, et al. Neonatal hyperoxia induces activated pulmonary cellular states and sex-dependent transcriptomic changes in a model of experimental bronchopulmonary dysplasia. *American Journal of Physiology. Lung Cellular and Molecular Physiology*. 2023; 324: L123–L140. <https://doi.org/10.1152/ajplung.00252.2022>
- [15] Adams TS, Marlier A, Kaminski N. Lung Cell Atlases in Health and Disease. *Annual Review of Physiology*. 2023; 85: 47–69. <https://doi.org/10.1146/annurev-physiol-032922-082826>
- [16] Shiraiishi K, Shah PP, Morley MP, Loebel C, Santini GT, Katzen J, et al. Biophysical forces mediated by respiration maintain lung alveolar epithelial cell fate. *Cell*. 2023; 186: 1478–1492.e15. <https://doi.org/10.1016/j.cell.2023.02.010>
- [17] Guild J, Juul NH, Andalon A, Taenaka H, Coffey RJ, Matthay MA, et al. Evidence for lung barrier regeneration by differentiation prior to binucleated and stem cell division. *The Journal of Cell Biology*. 2023; 222: e202212088. <https://doi.org/10.1083/jcb.202212088>
- [18] Dasgupta Q, Jiang A, Wen AM, Mannix RJ, Man Y, Hall S, et al. A human lung alveolus-on-a-chip model of acute radiation-

- induced lung injury. *Nature Communications*. 2023; 14: 6506. <https://doi.org/10.1038/s41467-023-42171-z>
- [19] Toth A, Steinmeyer S, Kannan P, Gray J, Jackson CM, Mukherjee S, et al. Inflammatory blockade prevents injury to the developing pulmonary gas exchange surface in preterm primates. *Science Translational Medicine*. 2022; 14: eabl8574. <https://doi.org/10.1126/scitranslmed.abl8574>
- [20] Hassan D, Chen J. CEBPA restricts alveolar type 2 cell plasticity during development and injury-repair. *Nature Communications*. 2024; 15: 4148. <https://doi.org/10.1038/s41467-024-48632-3>
- [21] Bassères DS, Levantini E, Ji H, Monti S, Elf S, Dayaram T, et al. Respiratory failure due to differentiation arrest and expansion of alveolar cells following lung-specific loss of the transcription factor C/EBPα in mice. *Molecular and Cellular Biology*. 2006; 26: 1109–1123. <https://doi.org/10.1128/MCB.26.3.1109-1123.2006>
- [22] Martis PC, Whitsett JA, Xu Y, Perl AKT, Wan H, Ikegami M. C/EBPα is required for lung maturation at birth. *Development* (Cambridge, England). 2006; 133: 1155–1164. <https://doi.org/10.1242/dev.02273>
- [23] Xu Y, Saegusa C, Schehr A, Grant S, Whitsett JA, Ikegami M. C/EBPα is required for pulmonary cytoprotection during hyperoxia. *American Journal of Physiology. Lung Cellular and Molecular Physiology*. 2009; 297: L286–L298. <https://doi.org/10.1152/ajplung.00094.2009>
- [24] Lu H, Chen X, Lu Y, Zhu H, Tang W, Wang Q. Effects of C/EBPα overexpression on alveolar epithelial type II cell proliferation, apoptosis and surfactant protein-C expression after exposure to hyperoxia. *BMC Pulmonary Medicine*. 2019; 19: 142. <https://doi.org/10.1186/s12890-019-0911-x>
- [25] Yang G, Hinson MD, Bordner JE, Lin QS, Fernando AP, La P, et al. Silencing hyperoxia-induced C/EBPα in neonatal mice improves lung architecture via enhanced proliferation of alveolar epithelial cells. *American Journal of Physiology. Lung Cellular and Molecular Physiology*. 2011; 301: L187–L196. <https://doi.org/10.1152/ajplung.00082.2011>
- [26] Qin K, Yu M, Fan J, Wang H, Zhao P, Zhao G, et al. Canonical and noncanonical Wnt signaling: Multilayered mediators, signaling mechanisms and major signaling crosstalk. *Genes & Diseases*. 2023; 11: 103–134. <https://doi.org/10.1016/j.gendis.2023.01.030>
- [27] Fröhlich J, Rose K, Hecht A. Transcriptional activity mediated by β-CATENIN and TCF/LEF family members is completely dispensable for survival and propagation of multiple human colorectal cancer cell lines. *Scientific Reports*. 2023; 13: 287. <https://doi.org/10.1038/s41598-022-27261-0>
- [28] Li C, Peinado N, Smith SM, Zhou J, Gao F, Kohbodi G, et al. Wnt5a Promotes AT1 and Represses AT2 Lineage-Specific Gene Expression in a Cell-Context-Dependent Manner. *Stem Cells* (Dayton, Ohio). 2022; 40: 691–703. <https://doi.org/10.1093/stmcls/sxac031>
- [29] Negretti NM, Son Y, Crooke P, Plosa EJ, Benjamin JT, Jetter CS, et al. Epithelial outgrowth through mesenchymal rings drives lung alveologenesis. *JCI Insight*. 2025; 10: e187876. <https://doi.org/10.1172/jci.insight.187876>
- [30] Zhu Y, Li Y, Jin W, Li Z, Zhang L, Fang Y, et al. Hyperoxia exposure upregulates Dvl-1 and activates Wnt/β-catenin signaling pathway in newborn rat lung. *BMC Molecular and Cell Biology*. 2023; 24: 4. <https://doi.org/10.1186/s12860-023-00465-6>
- [31] Sucre JMS, Vickers KC, Benjamin JT, Plosa EJ, Jetter CS, Cutrone A, et al. Hyperoxia Injury in the Developing Lung Is Mediated by Mesenchymal Expression of Wnt5A. *American Journal of Respiratory and Critical Care Medicine*. 2020; 201: 1249–1262. <https://doi.org/10.1164/rccm.201908-1513OC>
- [32] Alapati D, Rong M, Chen S, Hehre D, Hummler SC, Wu S. Inhibition of β-catenin signaling improves alveolarization and reduces pulmonary hypertension in experimental bronchopulmonary dysplasia. *American Journal of Respiratory Cell and Molecular Biology*. 2014; 51: 104–113. <https://doi.org/10.1165/rcmb.2013-0346OC>
- [33] Yang Loureiro Z, Joyce S, DeSouza T, Solivan-Rivera J, Desai A, Skritakis P, et al. Wnt signaling preserves progenitor cell multipotency during adipose tissue development. *Nature Metabolism*. 2023; 5: 1014–1028. <https://doi.org/10.1038/s42255-023-00813-y>
- [34] Nakagawa S, Yamaguchi K, Takane K, Tabata S, Ikenoue T, Furukawa Y. Wnt/β-catenin signaling regulates amino acid metabolism through the suppression of CEBPA and FOXA1 in liver cancer cells. *Communications Biology*. 2024; 7: 510. <https://doi.org/10.1038/s42003-024-06202-9>