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Research Article

# Pulmonary Expression of Non-Canonical Wnt Signaling is Upregulated in Pulmonary Hypertension Secondary to Left Ventricular Dysfunction

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## ABSTRACT

Pulmonary hypertension is a life-threatening progressive disease with few curable therapies due to its diversity and complexity. It results from increased pulmonary vascular tone and pulmonary vascular remodelling, with the aberrant Proliferation and Migration of Smooth Muscle Cells (PASMCs). The pulmonary vascular remodelling is more crucial than vasoconstriction in the development of human PH. Wnt is a family of secreted glycoproteins with varied expressions and functions. Its signalling, divided into the canonical and the non-canonical pathways, can regulate cell proliferation and cell migration pathways. Altered Wnt expressions can result in dysregulated cellular proliferation through extracellular receptors. The reactivation of fetal Wnt genes seen in embryonic development has been reported in a wide range of human pathologies, including cancers and lung disease. The exact mechanism of dysregulated Wnt expression on pulmonary vascular remodelling remains unresolved in pulmonary hypertension. We studied whether the disruption of the canonical or the non-canonical Wnt signalling played the greater roles in the development of PH by Aortic Banding for 42 days ($AOB_{42}$). Subsequently, pulmonary Wnt5a and Wnt11 protein levels were significantly higher in $AOB_{42}$ and significantly up-regulated pulmonary mRNA expressions of Wnt2 and Wnt11 were observed in $AOB_{42}$. In contrast, pulmonary mRNA expressions of the canonical specific Wnt3A and Wnt7A were decreased in $AOB_{42}$.

**Keywords:** Pulmonary hypertension; Wnt5a; Wnt11; B-catenin; Canonical Wnt signalling; Non-canonical Wnt signalling

## INTRODUCTION

Pulmonary Hypertension (PH), defined as having a mean Pulmonary Arterial Pressure (mPAP) of more than 20 mmHg, is a severely progressive disease. An unresolved increase in Right Ventricular (RV) afterload leads to premature death from right heart failure [1]. There are 5 clinical groups for pulmonary hypertension [2]. Pathophysiologically, it results from vasoconstriction, pulmonary vascular remodeling and perivascular inflammation [1]. The increased vascular tone results from an imbalance between vasodilatation and vasoconstriction [3]. Adaptive angiogenesis could result from response to tissue hypoxia injury, after which wound healing develops dysadaptive angiogenesis in chronic lung diseases including PH [4]. In PH, the altered expressions of various proteins of pulmonary vasoproliferative endothelium, including Bone Morphogenetic Protein Receptor II (BMPRII), and voltage-sensitive potassium channels et al., are similar to expression in cancer, except for metastasis [5,6]. The hypothesis of a cancer paradigm in PH developed, based on cancer-like alterations in Endothelial Cells (EC) which acquired an apoptosis-resistant, hyper-proliferative phenotype in the process of vascular remodeling in PH [7]. This could be related to the ineffective pharmacologic therapies, short survival and incurability. Further studies targeting related pathways may lead to more effective therapeutic strategies in PH patients, rather than reducing vascular tone.

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Wnt is derived from a fusion of the name of the Drosophila segment polarity gene wingless and the name of the vertebrate homolog, integrated or int-1 [8]. There are at least 19 secreted glycoproteins in the Wnt ligand family with varying expression patterns and many functions [9]. Wnt signaling is classified into canonical and non-canonical pathways. Both are crucial for the functions of nearly all progenitor cell functions, including self-renewal and differentiation, at homeostasis and during regeneration [10]. Wnt can induce transformation in mouse mammary epithelial C57MG cells [11]. There are a vast array of Wnt ligands and receptors expressed in specific cells and phases of lung development, and they promote epithelial-mesenchymal cross-talk with other signaling pathways, initiating key steps in the developmental process [12]. Dysfunctional Wnt pathways, canonical and non-canonical, are reported in cancers, modifying signal pathways [6,13,14]. Some authors recently further reported that the development of right ventricular remodeling in PH could be closely related to the reactivation of fetal Wnt genes in experiments [15]. Wnt pathways are recognized to control pulmonary angiogenesis but their role in PH is incompletely understood [16].

In this study, we tested the hypothesis that dysregulated expressions of Wnt/β-catenin signaling related proteins could be involved in pulmonary vascular remodeling during the development of PH secondary to left ventricular dysfunction after Aortic Banding for 42 days in rats ($AOB_{42}$). Pulmonary mRNA levels of selected Wnt ligands were evaluated to study whether canonical or non-canonical Wnt signaling pathways played a more important role in the development of PH.

## MATERIALS AND METHODS

### Animal model of PH

All protocols were approved by the Animal Research Committee of Kaohsiung Medical University (IACUC approval NO 101150). As previously described left parasternal thoracotomy in the 4th intercostal space was performed in male Wistar rats (6-week-old, weighing approximately 220 g), after they were anesthetized with sodium pentobarbital (20 mg/kg, i.p.) and ketamine (60 mg/kg, i.m.) and orotracheally ventilated using rodent respirators (Harvard, South Natick, MA) [17]. A blunt, sheathed hypodermic needle (19-gauge) was placed along the axis of the ascending aorta, and a length of 3-0 nylon suture was tied around the aorta. The sheathed hypodermic needle was then removed, leaving a stenosis in the ascending aorta approximately 1 cm distal to the aortic valve. The procedure took less than 30 min for each rat. Rats were then individually housed in a 12-h dark/light cycle-controlled room and fed regular rat diet. Effective aortic banding was indicated by a pressure gradient of around 40 mmHg, determined by transthoracic echocardiography (Hewlett-Packard, 4500 Model, 8-MHz transducer) on the first day. Sham-operated rats underwent the same operation, except that the aorta was not banded. On day 42 after banding or sham operation, the rats were sacrificed for further measurement. Totally, there were 5 Aortic Banded rats ($AOB_{42}$) and 5 Sham-operated rats (Sham42).

### Tissue preparation

Three pieces of lung tissue from different lobes of each sacrificed rat were exercised and immersed in 10% formalin for 24 hr. Hematoxylin-Eosin (H-E) staining was subsequently performed, and the areas around 100 μm in diameter of the pulmonary arterioles were evaluated for measurement of medial wall thickness under a microscope at magnification of 400X. For each arteriole, the medial wall thickness was expressed as follows: percent wall thickness=[(medial thickness × 2)/external diameter] × 100. One-half of the remaining lung tissue was homogenized for protein extraction, and the other half was frozen in liquid nitrogen and stored at -70oC for Western blot analysis, RNA study and for Mayer's hematoxylin counterstain.

### Western blot analysis

Tissues (100 mg) were homogenized in 1 ml RIPA buffer (1% Triton X-100, 15 mM HEPES-NaOH, pH 7.5, 0.15 mM NaCl, 1% sodium deoxycholate, 0.1% SDS, 1 mM sodium orthovanadate, 10 mM Ethylenediaminetetraacetic acid (EDTA), and 1% protease inhibitor cocktail) (Sigma, St. Louis, MO, USA) and centrifuged at 15,000 × g for 20 min at 4°C. Protein samples (100 μg) were subjected to 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto a Polyvinylidene Fluoride (PVDF) membrane (Pall, MI, USA). The membrane was blocked with 5% non-fat dry milk in Tris-Buffered Solution (TBS) and probed with anti-Wnt5a, anti-Wnt11, anti-Wnt2, (1:1000 dilution, Abcam, MA, USA), anti-β-catenin (1:2000, Millipore, MA, USA), or anti-actin (1:10000, Millipore, MA, USA) antibodies. Afterwards, it was then incubated with horseradish peroxidase-conjugated secondary antibody, and the signal was detected with the Western Lighting®Chemiluminescent kit (Millipore, MA, USA) according to the manufacturer's instructions.

### RNA extraction and reverse transcription-PCR for target genes

Total RNA was prepared from frozen tissue samples using a RNeasy Mini Kit (Qiagen). RNA (2 μg) was then reverse transcribed into cDNA using Super Script II Reverse Transcriptase (Invitrogen, Carlsbad, CA, USA). PCR was subsequently performed using 1 μL of the reverse transcription products in a total volume of 25 μL. Wnt11, Wnt3a, Wnt7a, FZD5, secreted Frizzle-Related Protein 1 (sFRP1), secreted Frizzle-Related Protein 2 (SFRP2), Wif1, Ltb, and Ltbr cDNA fragments were amplified using specific primer pairs (Genomics, New Taipei City, Taiwan) for rat. Rat β-actin was used as an internal control to normalize relative amount of cDNA used in each reaction. The reaction mixture contained 10 mM Tris-HCl, pH 8.3, 1.5 mM $MgCl_2$, 50 mM KCl, 200 mM dNTPs, 2 mM primer, and 1 U of Ex Taq Polymerase (Takara, Shiga, Japan). After an initial enzyme activation step for 10 min at 95°C, PCR was conducted for specific annealing temperature of each primer pairs, followed by a 10 min extension at 72°C (40 cycles). DNA fragments were then separated in 1.2% agarose gel and visualized by staining with ethidium bromide.

### Statistical analysis

The results obtained from Western blots and RNA extraction were analyzed by densitometry and expressed as mean ± standard error of the mean. Data from different groups were compared statistically using two-tailed T-test. A p-value of $<0.05$ was considered statistically significant.

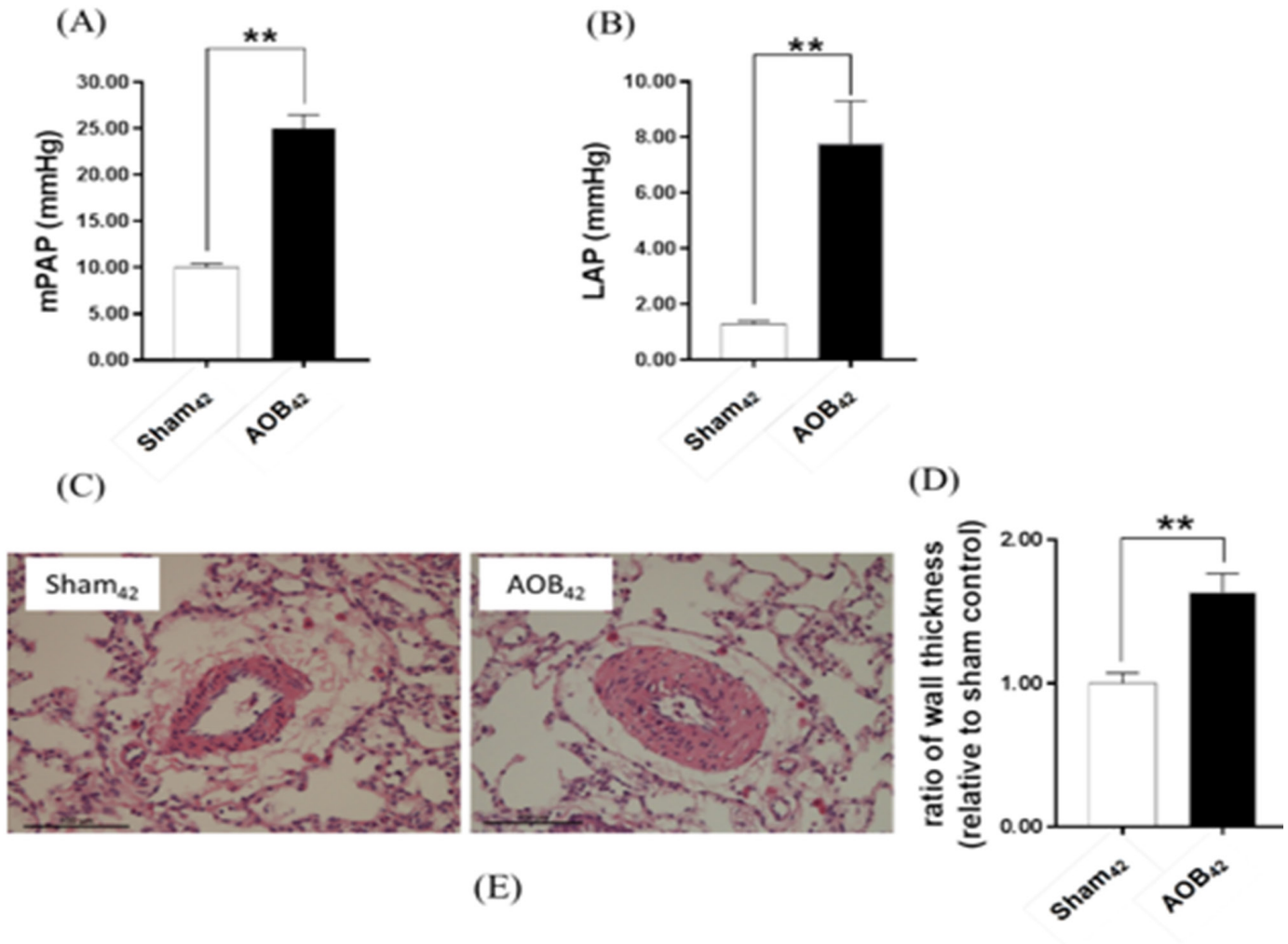
## RESULTS

There were four to six rats per group (Figure 1). There were significant increases in both the mean Pulmonary Arterial Pressure

(mPAP) and the Left Atrial Pressure (LAP) observed in $AOB_{42}$ when compared to Sham-operated rats (Sham42) (Figures 1A and 1B). The mean PAP and LAP values for the Sham42 group were 10.00 ± 0.41 mmHg and 1.28 ± 0.12 mmHg, respectively, while the corresponding values for the $AOB_{42}$ rat were 25.00 ± 1.47 mmHg (P< 0.01) and 7.75 ± 1.55 mmHg (P<0.01), respectively (Figure 1E). Likewise, H-E staining showed that the medial wall thickness of pulmonary arterioles in the $AOB_{42}$ rats was thicker than that of the Sham42 rats (ratio:1.64 ± 0.13 *vs.* 1 ± 0.07; P<0.01) (Figure 1C and 1D).

Compared with the Sham42 rats (Figure 2), there were significant increases in Wnt5a (Figure 2A) and Wnt11 (Figure 2B) protein expression in the $AOB_{42}$ group, while β-catenin protein levels were not altered (results not shown) (Figure 3). Significantly up-regulated mRNA expressions of Wnt2 (Figure 3A), and Wnt11 (Figure 3D) in the $AOB_{42}$ rats were observed. In contrast, mRNA expressions of Wnt3a (Figure 3B) and Wnt7a (Figure 3B) were significantly down-regulated in the lung of $AOB_{42}$ rats when compared with the Sham42 rats. No significant change in the pulmonary mRNA levels of Wnt5b was detected (results not shown) (Figure 4).

Compared with the Sham42 rats, significantly down-regulated pulmonary mRNA expressions of sFRP1 (Figure 4A), sFRP2 (Figure 4B), and Wif1 (Figure 4C), were found in the $AOB_{42}$ rats, while significant increases in pulmonary mRNA expression of FZD5 (Figure 4D), Lymphotoxin beta (Ltb) in Figure 5A, and Ltb receptor (Ltbr) (Figure 5B) were noted in the $AOB_{42}$ rats. In contrast, there were no significant differences in the pulmonary mRNA levels of LRP5 or DKK2 between the two groups of rats (results not shown) (Figure 5).




| Parameter | Shame42 | $AOB_{42}^{*}$ |
|---|---|---|
| mPAP (mmHg) | 10.00 ± 0.41 | 25.00 ± 1.47** |
| LAP (mmHg) | 1.28 ± 0.12 | 7.75 ± 1.55** |
| RWT | 1 ± 0.07 | 1.64 ± 0.13** |

**Figure 1:** Comparison of hemodynamic parameters and the thickness of pulmonary arterioles between Sham-operated rats (Sham42) and Aorta-Banded rats on day 42 ($AOB_{42}$). **Note:** (A) The mean Pulmonary Arterial Pressure (mPAP); (B) Left Atrial Pressure (LAP); (C) Micrographs of the pulmonary arterioles (200X); (D) Ratio of the medial wall thickness; (E) Summary table of the results. All measured parameters showed statistically significant higher values for the $AOB_{42}$ group than those of the Sham42 animals. Values represent mean ± SEM (n=5/group). **P<0.01. **Note:** (■) $AOB_{42}$, (▭) Sham42

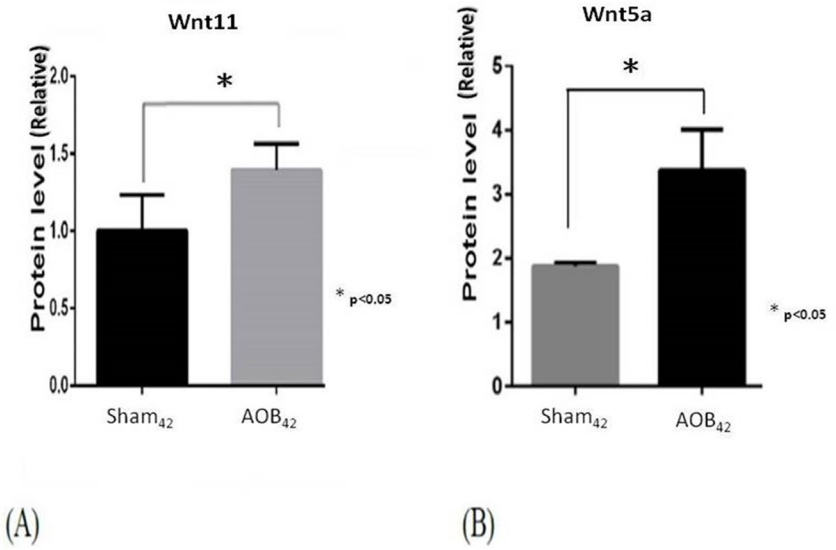



**Figure 2:** Comparison of pulmonary Wnt5a and Wnt11 protein expression between sham-operated rats (Sham42) and aorta-banded rats on day 42 ($AOB_{42}$) by Western blot analysis. Significantly higher pulmonary expressions of Wnt5a. **Note:** (A) Wnt11; (B) Aorta-banded rats on day 42 ($AOB_{42}$), compared with matched sham-operated rats (Sham42). Values represent mean ± SE. P<0.05.

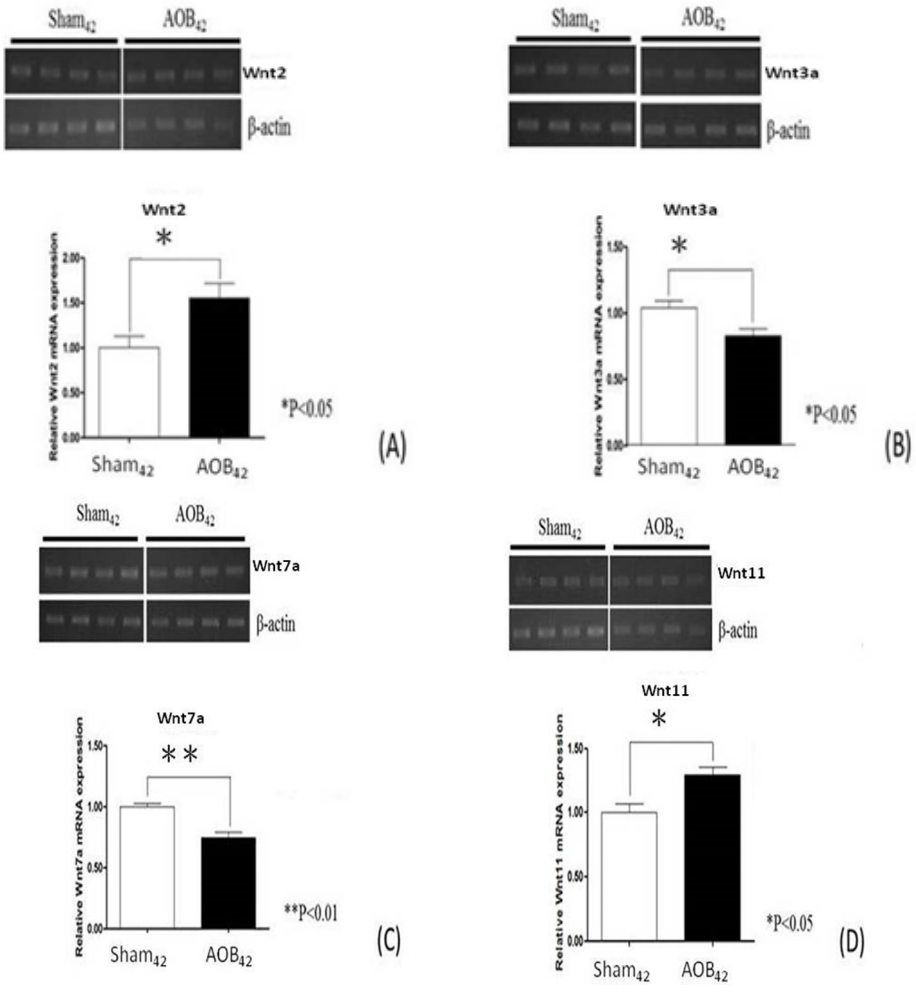



**Figure 3:** Comparison of pulmonary mRNA levels of Wnt2, Wnt3a, Wnt7a, and Wnt11 between sham-operated rats (Sham42) and aorta-banded rats on day 42 ($AOB_{42}$). Significantly higher pulmonary mRNA expressions of Wnt2. **Note:** (A) Wnt11. In contrast, pulmonary mRNA expressions of Wnt3a; (B) Wnt7a; (C) in the $AOB_{42}$ animals were significantly lower, compared with the Sham42 group; (D) were found in the $AOB_{42}$ group. Values represent mean ± SEM.*P<0.05 and **P<0.01.

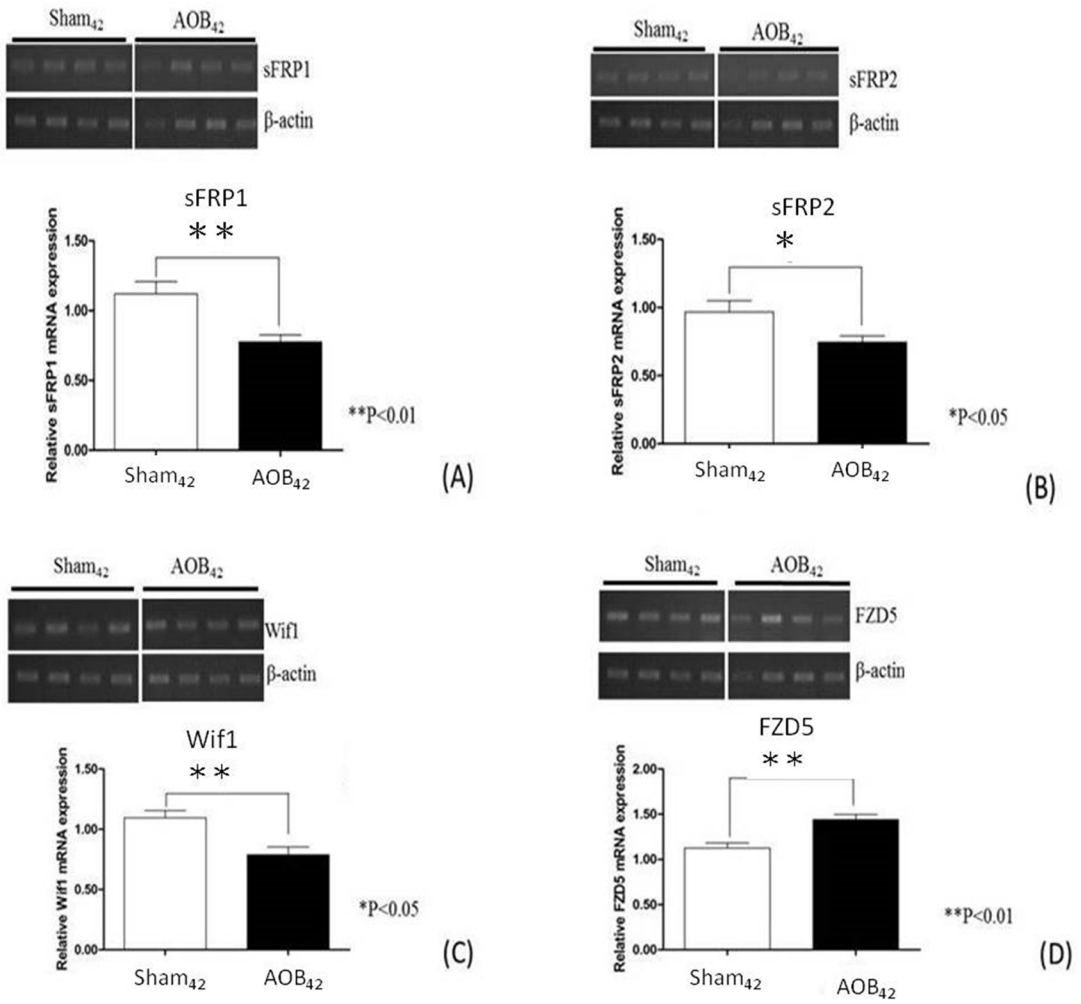



**Figure 4:** Comparison of pulmonary mRNA levels of sFRP1, sFRP2, Wif1, and FZD5 between sham-operated rats (Sham42) and aorta-banded rats on day 42 ($AOB_{42}$). Significant decreases in the pulmonary mRNA expressions of sFRP1. **Note:** (A), sFRP2; (B) Wif1; (C) $AOB_{42}$ animals when compared with the Sham42 rats. In contrast, pulmonary mRNA levels of FZD5; (D) were significantly higher in the $AOB_{42}$ group. Values represent mean ± SEM. *P<0.05 and **P<0.01.

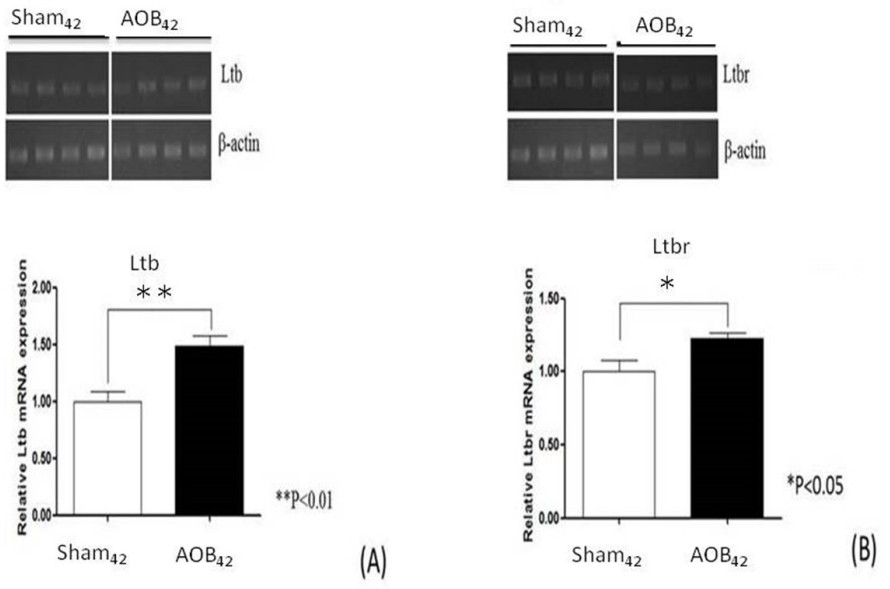



**Figure 5:** Comparison of pulmonary mRNA levels of Ltb and Ltbr between sham-operated rats (Sham42) and aorta-banded rats on day 42 ($AOB_{42}$). Significant increases in the pulmonary mRNA expressions of both Ltb (A) and Ltbr (B) were found in the $AOB_{42}$ animals when compared with the Sham42 rats. Values represent mean ±SEM. *P<0.05 and **P<0.01.

## DISCUSSION

The highly transforming Wnt family members are represented by Wnt1, Wnt3, Wnt3a, and Wnt7a, while the intermediately transforming or non-transforming members include Wnt2, Wnt4, Wnt5a, Wnt5b, Wnt6, Wnt7b, and Wnt11. Their signal transduction cascades include the canonical and non-canonical signaling pathways [18,19]. Wnt ligands are noted to have different degrees of selectivity towards the two pathways and can activate either of the two pathways depending on spatiotemporal parameters and receptor availability [20]. Lung Mesenchymal Vascular Progenitor Cell (MVPC) studies revealed that during the beginning of adaptive angiogenesis, Wnt can contribute to repair processes including angiogenesis, muscularization and normalized Microvascular (MV) patterns. Activation of Wnt signaling in dysadaptive angiogenesis includes MV dropout, collagen deposition and decreased pulmonary small vascular density during the persistent progress of chronic lung diseases [4]. Wnt signaling is involved in embryogeneis and development through the regulation of cell motility and cell polarization, and pathophysiologically associated with tumorigenesis and tissue metabolism [21]. The hallmark of the non-canonical signaling pathway is its β-catenin-independent actions [22]. It was reported that non-canonical signaling develops in early atherogenesis. Some authors reported that Wnt5a reduces insulin dependent endothelial Nitric Oxide Synthase (eNOS) activity, resulting in impaired vasodilatation in diabetic endothelial dysfunction [23]. In addition, Wnt5a/$Ca^{+2}$-dependent signaling can induce the release of inflammatory cytokines in endothelial cells and stimulate endothelial inflammation [17]. Reportedly Wnt5a could regulate left ventricular hypertrophy in an aortic constriction mouse model [24]. Also, it has been reported that the expressions of Wnt11, DSH and Rho-Kinase (ROCK) are up-regulated in endothelial cell of small pulmonary artery in experiments humans with Idiopathic Pulmonary Arterial Hypertension (IPAH) [25].

Similarly, we found higher pulmonary Wnt5a protein levels in the $AOB_{42}$ rats when compared with that of the Sham42 rats (Figure2A), without altered levels of β-catenin protein. Meanwhile, both pulmonary protein (Figure 2B) and mRNA (Figure 3D) levels of Wnt11 were significantly increased in $AOB_{42}$ rats compared with Sham42 rats. Together, these results seem to support that the non-canonical Wnt signaling pathway, β-catenin-independent, may play a dominant role during the development of PH in this animal model [26,27].

Wnt3a is one of the most highly studied canonical members, and Wnt/β-catenin pathway activation can enforce monocyte adherence to vascular endothelial cells, in which the nuclear localization of β-catenin may be noted in athero-prone regions of aortic endothelium [28]. In our study, pulmonary Wnt3a mRNA levels were significantly lower in the $AOB_{42}$ group (Figure 3B) when compared with the Sham42 animals. These results were consistent with the notion that Wnt3a may not be responsible for activating the non-canonical Wnt signaling pathway in our rat model of PH. Similar observations were made with Wnt7a, another ligand for the canonical pathway (Figure 3C). Similarly, it is reported that Wnt7a can promote Vascular Endothelial Growth Factor (VEGF) signaling in lung PMVECs and its loss is closely associated with an insufficient VEGF factor A angiogenic response. Down-regulated Wnt7a may contribute to progressive small vessel rarefaction in PH [29]. It is also reported that Wnt5a and Wnt11 could cooperate to attenuate canonical Wnt signaling in Second Heart Field (SHF) progenitor cells [30].

Other canonical Wnt proteins, such as Wnt2, were also found to promote Vascular Smooth Muscle Cell (VSMC) proliferation and migration [31,32]. In contrast to Wnt3a, pulmonary expression of Wnt2 mRNA was found to be significantly higher in the $AOB_{42}$ rats (Figure 3A). The reason for this discrepancy is unclear at present. It could be associated with the transitionally altered angiogenesis of Wnt activation on development of PH secondary to left ventricular dysfunction compared to other PH models. Some investigators have expressed concern about the complexity of and cross-talk between the canonical and non-canonical Wnt signaling pathways, and have tried to replace the binary classification of signaling by an integrated signaling pathway [33]. It is possible that signal transduction triggered by Wnt2 is model-specific as it has been shown to activate cardiac myocyte differentiation *via* the non-canonical pathway [34,35].

The regulators of Wnt signaling pathways are divided into two different groups: (A) secreted FZD-related proteins (sFRP) 1, 2, 4, and 5 as well as Wnt inhibitor factor 1(Wif1)35and (B) Dickkotf (DKK) proteins and Wise. The sFRP's are extracellular inhibitors of Wnt signaling that act by binding directly to Wnt ligands or to Frizzled receptors, whereas DKKs are negative regulators of Wnt signaling. Our results showed that pulmonary mRNA levels of sFRP1, sFRP2, and Wif1 were all significantly decreased (Figures 4A-4C) whereas that of Frizzled-5 (FZD5) was significantly increased (Figure 4D) in the $AOB_{42}$ rats. These conditions would further favor the activation of Wnt signaling during development of PH in the present animal model.

It has been reported that lymphotoxin beta receptor (Ltbr) signaling could reduce Wnt/β-catenin activity *via* non-canonical NF-κB signaling through the non-canonical NF-κB inducing kinase [36]. Our results also showed upregulated pulmonary mRNA expression of Lymphotoxin beta (Ltb) and lymphotoxin beta receptor (Ltbr) in the $AOB_{42}$ group (Figures 5A and 5B). We further inferred that activation of Ltbr-signaling is related to the downregulation of the Wnt/β-catenin pathway in PH secondary to left ventricular dysfunction in this animal model.

To our knowledge there have only been a few studies examining dysregulated Wnt signaling expression in humans or animals with PH [24]. Despite conflicting experimental data, a general picture is emerging that excessive stimulation of Wnt signaling adversely affects cardiovascular pathology. Our study demonstrated that Wnt signaling can be modulated either by inhibiting canonical pathways or activating non-canonical pathways during the development of PH of group II.

## CONCLUSION

Our data from this rat model of pulmonary hypertension secondary to left ventricular dysfunction suggest that non-canonical Wnt signaling, rather than canonical Wnt/β-catenin signaling, is mainly related to the development of PH. Thus, targeting non-canonical Wnt signaling and regulators may have beneficial effects for the treatment of PH. This indicates the activation of non-canonical Wnt pathways. Thus, targeting non-canonical Wnt signalling may have beneficial effects for the treatment of PH in pulmonary hypertension secondary to left ventricular dysfunction

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## CONFLICT OF INTEREST

The authors declare no conflicts of interest.

## DECLARATION OF COMPETING INTEREST

The authors declare no competing interest.

## REFERENCES

1. Goncharova EA, Kudryashova TV, Pullamsetti SS. Too hot? Too cold? Wnt signalling in pulmonary arterial hypertension: Can we treat it "just right"?. Eur Respir J. 2023;61(6):2300504.
2. Simonneau G, Montani D, Celermajer DS, Denton CP, Gatzoulis MA, Krowka M, et al. Haemodynamic definitions and updated clinical classification of pulmonary hypertension. Eur Respir J. 2019;53(1).
3. Budhiraja R, Tuder RM, Hassoun PM. Endothelial dysfunction in pulmonary hypertension. Circulation. 2004;109(2):159-165.
4. Summers ME, Richmond BW, Menon S, Sheridan RM, Kropski JA, Majka SA, et al. Resident mesenchymal vascular progenitors modulate adaptive angiogenesis and pulmonary remodeling *via* regulation of canonical Wnt signaling. FASEB J. 2020;34(8):10267-10285.
5. Corda G, Sala A. Non-canonical WNT/PCP signalling in cancer: Fzd6 takes centre stage. Oncogenesis. 2017;6(7):364.
6. Katoh M. Canonical and non-canonical WNT signaling in cancer stem cells and their niches: Cellular heterogeneity, omics reprogramming, targeted therapy and tumor plasticity. Int J Oncol. 2017;51(5):1357-1369.
7. Rai PR, Cool CD, King JA, Stevens T, Burns N, Winn RA, et al. The cancer paradigm of severe pulmonary arterial hypertension. Am J Respir Crit Care Med. 2008;178(6):558-564.
8. Nusse R. A new nomenclature for int-1 and related genes: The Wnt gene family. Cell. 1991;64:231-232.
9. He X, Semenov M, Tamai K, Zeng X. LDL receptor-related proteins 5 and 6 in Wnt/beta-catenin signaling: Arrows point the way. Development. 2004;131(8):1663-1677.
10. Raslan AA, Yoon JK. WNT signaling in lung repair and regeneration. Mol Cells. 2020;43(9):774-783.
11. Ishitani T, Kishida S, Hyodo-Miura J, Ueno N, Yasuda J, Waterman M, et al. The TAK1-NLK mitogen-activated protein kinase cascade functions in the Wnt-5a/$Ca^{2+}$ pathway to antagonize Wnt/β-catenin signaling. Mol Cell Biol. 2003;23(1):131-139.
12. Travaglini KJ, Nabhan AN, Penland L, Sinha R, Gillich A, Sit RV, et al. A molecular cell atlas of the human lung from single-cell RNA sequencing. Nature. 2020;587(7835):619-625.
13. Wang J, He T, Gao Q, Chang H, Dai X, Yang J, et al. The dysfunctional Wnt pathway down-regulates MLH1/SET expression and promotes microsatellite instability and immunotherapy response in colorectal cancer. Genes Dis. 2024;11(2):542-545.
14. Wang X, Luo L, Xu J, Lu Q, Xia H, Huang Y, et al. Echinatin inhibits tumor growth and synergizes with chemotherapeutic agents against human bladder cancer cells by activating p38 and suppressing Wnt/beta-catenin pathways. Genes Dis. 2024;11(2):1050-1065.
15. Edwards JJ, Brandimarto J, Hu DQ, Jeong S, Yucel N, Li L, et al. Noncanonical WNT Activation in Human Right Ventricular Heart Failure. Front Cardiovasc Med. 2020;7:582407.
16. Chakraborty A, Nathan A, Orcholski M, Agarwal S, Shamskhou EA, Auer N, et al. Wnt7a deficit is associated with dysfunctional angiogenesis in pulmonary arterial hypertension. Eur Respir J. 2023;61(6):2201625.
17. Gelfand BD, Meller J, Pryor AW, Kahn M, Bortz PD, Wamhoff BR, et al. Hemodynamic activation of beta-catenin and T-cell-specific transcription factor signaling in vascular endothelium regulates fibronectin expression. Arterioscler Thromb Vasc Biol. 2011;31(7):1625-1633.
18. Goodwin AM, D'Amore PA. Wnt signaling in the vasculature. Angiogenesis. 2002;5(1-2):1-9.
19. Villar J, Cabrera-Benitez NE, Ramos-Nuez A, Flores C, Garcia-Hernandez S, Valladares F, et al. Early activation of pro-fibrotic WNT5A in sepsis-induced acute lung injury. Crit Care. 2014;18(5):568.
20. Dijksterhuis JP, Petersen J, Schulte G. WNT/Frizzled signalling: Receptor-ligand selectivity with focus on FZD-G protein signalling and its physiological relevance: IUPHAR Review 3. Br J Pharmacol. 2014;171(5):1195-1209.
21. Logan CY, Nusse R. The Wnt signaling pathway in development and disease. Annu Rev Cell Dev Biol. 2004;20:781-810.
22. Gordon MD, Nusse R. Wnt signaling: Multiple pathways, multiple receptors, and multiple transcription factors. J Biol Chem. 2006;281(32):22429-22433.
23. Jin Y, Wang W, Chai S, Liu J, Yang T, Wang J. Wnt5a attenuates hypoxia-induced pulmonary arteriolar remodeling and right ventricular hypertrophy in mice. Exp Biol Med (Maywood). 2015;240(12):1742-1751.
24. Wang Y, Sano S, Oshima K, Sano M, Watanabe Y, Katanasaka Y, et al. Wnt5a-Mediated Neutrophil Recruitment Has an Obligatory Role in Pressure Overload-Induced Cardiac Dysfunction. Circulation. 2019;140(6):487-499.
25. Dai ZK, Wu BN, Chen IC, Chai CY, Wu JR, Chou SH, et al. Attenuation of pulmonary hypertension secondary to left ventricular dysfunction in the rat by Rho-kinase inhibitor fasudil. Pediatr Pulmonol. 2011;46(1):45-59.
26. Laumanns IP, Fink L, Wilhelm J, Wolff JC, Mitnacht-Kraus R, Graef-Hoechst S, et al. The noncanonical WNT pathway is operative in idiopathic pulmonary arterial hypertension. Am J Respir Cell Mol Biol. 2009;40(6):683-691.
27. Jones PL, Cowan KN, Rabinovitch M. Tenascin-C, proliferation and subendothelial fibronectin in progressive pulmonary vascular disease. Am J Pathol. 1997;150(4):1349-1360.
28. Zhang B, Abreu JG, Zhou K, Chen Y, Hu Y, Zhou T, et al. Blocking the Wnt pathway, a unifying mechanism for an angiogenic inhibitor in the serine proteinase inhibitor family. Proc Natl Acad Sci U S A. 2010;107(15):6900-6905.
29. Chakraborty A, Nathan A, Orcholski M, Agarwal S, Shamskhou EA, Auer N, et al. Wnt7a deficit is associated with dysfunctional angiogenesis in pulmonary arterial hypertension. Eur Respir J. 2023;61(6).

30. Gao L, Cui W, Kelting S, Woodroof J, Li H, Li L, et al. Canonical and non-canonical Wnt signal pathway in classic Hodgkin lymphoma and the prognostic significance of LEF1, beta-catenin, FZD6 and Wnt5a/b. Am J Blood Res. 2022;12(4):136-143.

31. Williams H, Mill CA, Monk BA, Hulin-Curtis S, Johnson JL, George SJ. Wnt2 and WISP-1/CCN4 Induce Intimal Thickening *via* Promotion of Smooth Muscle Cell Migration. Arterioscler Thromb Vasc Biol. 2016;36(7):1417-1424.

32. Tsaousi A, Williams H, Lyon CA, Taylor V, Swain A, Johnson JL, et al. Wnt4/β-catenin signaling induces VSMC proliferation and is associated with intimal thickening. Circ Res. 2011;108(4):427-436.

33. van Amerongen R, Nusse R. Towards an integrated view of Wnt signaling in development. Development. 2009;136(19):3205-3214.

34. Onizuka T, Yuasa S, Kusumoto D, Shimoji K, Egashira T, Ohno Y, et al. Wnt2 accelerates cardiac myocyte differentiation from ES-cell derived mesodermal cells *via* non-canonical pathway. J Mol Cell Cardiol. 2012;52(3):650-659.

35. Malinauskas T, Aricescu AR, Lu W, Siebold C, Jones EY. Modular mechanism of Wnt signaling inhibition by Wnt inhibitory factor 1. Nat Struct Mol Biol. 2011;18(8):886-893.

36. Conlon TM, John-Schuster G, Heide D, Pfister D, Lehmann M, Hu Y, et al. Inhibition of LTβR signalling activates WNT-induced regeneration in lung. Nature. 2020;588(7836):151-156.