ORIGINAL RESEARCH

Hypoxia Upregulates the Notch3 Signaling Pathway to Promote Epithelial-Mesenchymal Transition in Pulmonary Artery Cells

Yongli Tan, MM; Yemu Zhu, MM

ABSTRACT

Objective • To investigate the role and mechanism of Notch3 in a hypoxia-induced model of pulmonary hypertension, specifically pulmonary artery hypertension.

Methods • A pulmonary artery hypertension rat model was induced using monocrotaline, and hepatic encephalopathy staining was used to observe the pathomorphological changes in pulmonary artery tissue. Primary isolation and extraction of rat pulmonary artery endothelial cells were performed, and a pulmonary artery hypertension cell model was established through hypoxia induction. Notch3 overexpression lentivirus (LV-Notch3) was used for intervention, and the expression of the Notch3 gene was detected using a real-time polymerase chain reaction. Western blotting was conducted to assess the expression of vascular endothelial growth factor, matrix metalloproteinase-2, and matrix metalloproteinase-9 proteins. Cell proliferation levels were measured using a medical training therapy assay.

Results • Compared to the control group, the model group showed significant thickening of the pulmonary artery membrane, increased pulmonary angiogenesis, and endothelial cell damage. After Notch3 overexpression, the LV-Notch3 group showed further thickening of the pulmonary artery tunica media, increased pulmonary angiogenesis, and significantly improved endothelial cell injury. Compared to control cells, the model group showed a significant decrease in Notch3 expression ($P<.05$), while the expression levels of vascular endothelial growth factor, MMP-2, and MMP-9 proteins and cell proliferation ability increased significantly ($P < .05$). Following Notch3 overexpression, there was a significant increase in Notch3 expression ($P<.05$), and the expression levels of vascular endothelial growth factor, MMP-2, and MMP-9 proteins, as well as cell proliferation ability decreased significantly ($P<.05$).

Conclusions • Notch3 can potentially reduce angiogenesis and proliferation in pulmonary artery endothelial cells and improve hypoxia-induced pulmonary artery hypertension in rats. (*Altern Ther Health Med.* 2023;29(6):158-163).

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INTRODUCTION

Pulmonary arterial hypertension, also known as pulmonary artery hypertension, is a severe vascular disease characterized by a high incidence rate and mortality.[1] In medicine, connective tissue disease encompasses conditions such as lupus erythematosus, scleroderma, dermatomyositis, rheumatoid arthritis, and rheumatic fever rather than being a single disease entity.[2] Connective tissue disease is recognized as a significant contributing factor in the development of pulmonary artery hypertension.[1,2]

Research studies have indicated that connective tissue disease-related pulmonary artery hypertension shares clinical and pathological characteristics with primary pulmonary artery hypertension. These features include intravascular hyperplasia, smooth muscle hypertrophy, and thickening of the arterial wall's middle layer, implying common underlying mechanisms and therapeutic targets.[3-5]

It has been reported that endothelial cell injury induced by hypoxia may contribute to the development of connective tissue disease-related pulmonary hypertension. Pulmonary vascular remodeling under hypoxic conditions is a major factor leading to right ventricular failure and mortality.[6,7] Autoimmune and inflammatory responses leading to pulmonary vascular damage play a significant role, particularly in patients with early-stage disease and high disease activity. In addition to general therapy and targeted drug interventions, the administration of high-dose glucocorticoids in combination with immunosuppressants can promptly alleviate and stabilize

the underlying condition, potentially leading to substantial improvements or even complete resolution.[8] Therefore, enhancing angiogenesis holds significant therapeutic implications for the management and prognosis of both pulmonary artery hypertension and connective tissue disease.

Connective tissue disease-related pulmonary artery hypertension is a complex condition associated with a particularly poor prognosis. Currently, there is no specific treatment regimen available to effectively improve and stabilize this condition. Consequently, investigating the underlying mechanisms of connective tissue disease-related pulmonary artery hypertension becomes of paramount importance. In recent years, studies have revealed the involvement of Notch3 in various pathophysiological processes, including cell proliferation, differentiation, apoptosis, immune response, and tumorigenesis.[8-10] Additionally, Notch3 plays a crucial role in regulating the signal network of the vascular system.[11] However, limited research is available on the role and mechanism of Notch3 in connective tissue disease-related pulmonary artery hypertension.

This study aims to investigate the role and mechanism of Notch3 in hypoxia-induced connective tissue disease-related pulmonary artery hypertension. Polycyclic aromatic hydrocarbons (PAHs) are organic compounds widely distributed in nature. Due to their persistence, bioaccumulation, long-distance migration, and high biotoxicity, they have been classified as priority pollutants by the United States Environmental Protection Agency.[8] These compounds pose potential harm to human health and ecosystems. Biodegradation of PAHs in the pulmonary artery hypertension field offers advantages such as being environmentally friendly, fast, safe, and cost-effective, making it a practical approach for pollution remediation.[9-10] The biodegradation of PAHs can be categorized into aerobic respiration and anaerobic respiration based on the different final electron acceptors. Aerobic metabolism has gained popularity due to its ease of microbial cultivation and high degradation efficiency.[8-10] However, it has limitations, such as generating toxic intermediate metabolites and challenges associated with subsequent degradation, potentially leading to severe secondary pollution.

MATERIALS AND METHODS

Study Design

The study design involves a combination of in vivo and in vitro approaches to investigate the role and mechanism of Notch3 in hypoxia-induced connective tissue disease-related pulmonary artery hypertension. In the in vivo component, a rat model of pulmonary artery hypertension was established using monocrotaline, and Notch3 overexpression lentivirus was used for intervention. Pathomorphological changes in pulmonary artery tissue were observed through hepatic encephalopathy staining.

Main Reagents Used

Monocrotaline (microwave coagulation therapy; Shanghai Aladdin Biochemical Technology Co., Ltd.), trypsin (Sigma Aldrich Company), primary antibody to vascular endothelial growth factor, primary antibody to matrix metalloproteinase, primary antibody to MMP-9, β-Actin primary antibody, whole protein extraction kit, BCA protein concentration determination kit (Shenyang Wanlei Biotechnology Co., Ltd.), MTT cell proliferation detection kit (Shanghai Biyuntian Biotechnology Co., Ltd.), and enzyme labeling instrument (BioTek Company of the United States) were used as the main reagents in this study. Other reagents mentioned in the text were obtained from Beijing Solebo Company.

Animal Selection and Experimental Protocol

Forty healthy male rats with stable diseases, aged 4-6 weeks and weighing 180-220 g, were obtained from Liaochangsheng Biotechnology Co., Ltd. The rats were maintained under standard conditions with a 12-hour light/12-hour darkness cycle, constant temperature (22 ± 1) °C, and constant humidity (45-55%). They were provided with standard feed and free access to drinking water. The rats were allowed to adapt to the environment for one week before the experiment, during which no signs of mental abnormalities, abnormal feeding, or other diseases were observed. The experimental procedures involving the animals were conducted in compliance with the guidelines of the National Research Council for the care and use of laboratory animals or as per the relevant national laws.

Establishment of Pulmonary Artery Hypertension Rat Model

A total of 40 healthy male rats with stable diseases were randomly divided into four groups: control group, model group, LV-NC group, and LV-Notch3 group, with ten rats in each group. The pulmonary artery hypertension model was induced in the model group, LV-NC group, and LV-Notch3 group by subcutaneous monocrotaline injection (MCT) at a 60 mg/kg dose. In the LV-Notch3 group, the rats received nasal drip administration of LV-Notch3 (2×10^8 TU) 48 hours before modeling and 7 and 14 days after modeling (obtained from American Addgene company) for intervention. The LV-NC group received the same dose of LV-NC (obtained from American Addgene company) nasal drip as a control, while the control group was injected with an equivalent amount of normal saline. After 21 days of model establishment, all rats in each group were euthanized simultaneously, and lung tissues were collected and fixed in 4% neutral paraformaldehyde for subsequent HE staining.

Primary Culture of Rat Pulmonary Artery Endothelial Cells

Fresh rat pulmonary artery tissue was thoroughly rinsed and longitudinally cut into pieces measuring 1.5 mm × 1.5 mm. The inner membrane surface was placed onto a sterile culture dish and incubated in a 37°C incubator for 2 hours, allowing the tissue to dry and adhere to the dish's bottom. Subsequently, 4 ml of complete medium was added, and the cells were cultured at 37°C and 5% CO2 for 72 hours. The arterial tissue was then removed from the culture dish. Primary rat pulmonary artery endothelial cells were cultured in DMEM medium supplemented

with 10% FBS. The isolated cells were randomly divided into four groups: control group, model group, LV-NC group, and LV-Notch3 group. Cells in the model group, LV-NC group, and LV-Notch3 group were cultured under 5% O_2 for 24 hours to establish the pulmonary artery hypertension cell model. The LV-Notch3 group cells were treated with LV-Notch3 infection, while the LV-NC group cells were synchronously infected with LV-NC as a control.

Real-Time Polymerase Chain Reaction (PCR) Detection

Total RNA was extracted from primary rat pulmonary artery endothelial cells and reverse transcribed into cDNA. The cDNA served as the template for PCR amplification using specific primers for Notch3 and the SYBR Green dye. The forward primer sequence for Notch3 was cgggcaacattcaacgctgt, and the reverse primer sequence was gtgcagggtccgaggtattc. The PCR reaction conditions were as follows: initial denaturation at 94°C for 5 minutes, followed by denaturation at 94°C for 10 seconds, annealing at 60°C for 30 seconds, and 40 cycles of amplification. A melting curve analysis was performed from 60°C to 94°C for determination and analysis. The PCR results were analyzed using the $2^{-\Delta CT}$ method.

Hematoxylin and Eosin (H&E) Staining of Lung Tissue

The rat lung tissue was fixed with 4% neutral paraformaldehyde, followed by dehydration using an ethanol gradient and xylene for transparency. The tissue was then embedded in paraffin, and 5 μm thick sections were prepared. Hematoxylin and eosin staining were performed, and the sections were observed under a microscope at a magnification of ×200 after undergoing ethanol gradient dehydration and xylene transparency.

Western Blotting Detection

Total protein was extracted from primary rat pulmonary artery endothelial cells, and the quantitative protein content was determined using the BCA method. Electrophoresis was performed using 11% and 15% separation gels and a 5% stacking gel. The proteins were then transferred to a PVDF membrane using the wet transfer method. The membrane was blocked with 5% skimmed milk powder and incubated overnight at 4°C with primary antibodies against vascular endothelial growth factor (VEGF) (1:500), MMP-2 (1:500), MMP-9 (1:500), and β-actin (1:1000). After washing with TBST, the membrane was incubated with a horseradish peroxidase (HRP)-conjugated secondary antibody (1:5000) for 45 minutes at 37°C. The protein bands were visualized using the ECL method, and the optical density of the target bands was analyzed using gel image processing software (Gel-Pro-Analyzer).

Cell Proliferation Assay Using MTT Method

Primary rat pulmonary artery endothelial cells in the logarithmic growth stage were selected for the assay. After lentivirus infection and hypoxia induction, the cells were treated with a working solution of MTT (0.5 mg/ml) and incubated in a constant temperature incubator (37°C, 5% CO_2) for 4.5 hours. Following the incubation period, the supernatant was removed, and 150 μL of DMSO was added to each well. The plate was then kept in the dark for 10 minutes to dissolve the formazan crystals. The optical density (OD) value at 460 nm was measured using a microplate reader.

Figure 1. Lung Histomorphology Comparison in Each Group (magnification ×200).

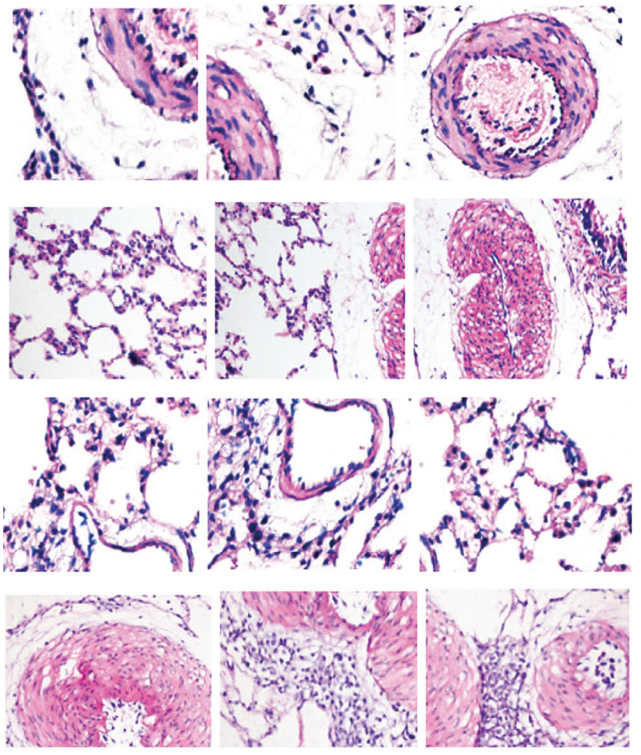


Figure 2. Expression of VEGF, MMP-2, And MMP-9 In Cells of Each Group (*$P < .05$).

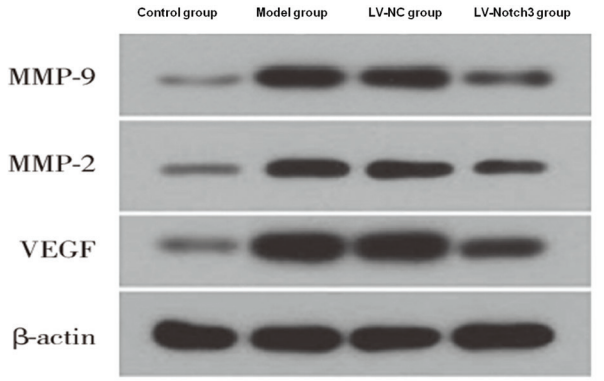


Figure 3. Relative Expression of VEGF, MMP-2, and MMP-9 mRNA in Cells of Each Group (*$P < .05$).

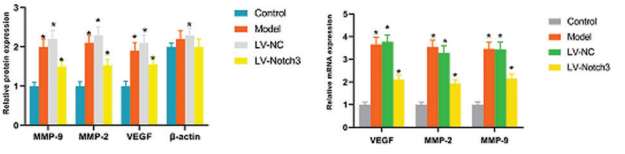


Figure 4. Protein Sequence Coverage Distribution.

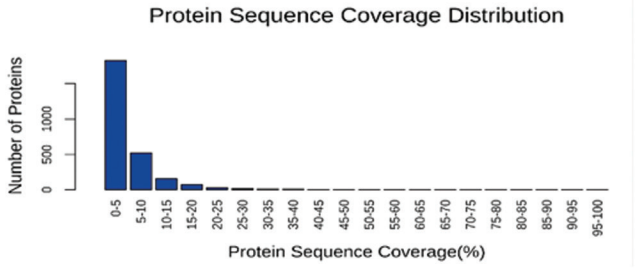


Figure 5. Quantitative Distribution of Identified Peptides.

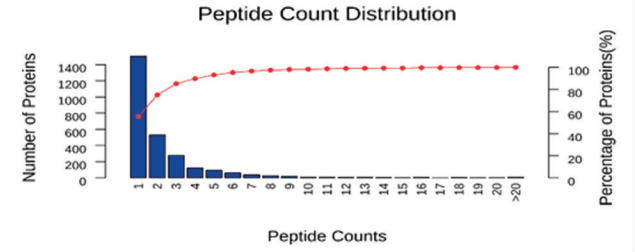
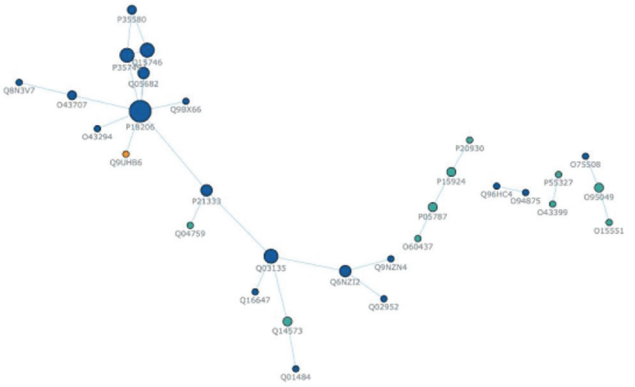


Figure 6. Direct Interaction Network of Target Proteins.



Statistical Analysis

Statistical analysis was performed using SPSS 21.0 software. The data were presented as mean ± standard deviation (x ± S). Inter-group comparisons were conducted using one-way ANOVA, and the Bonferroni method was applied for post-hoc corrections. A significance level of $P < .05$ was considered statistically significant.

RESULTS

Observation of Lung Histomorphology in Each Group

The lung histomorphology of rats in each group was examined. Compared to the control group, the model group showed a significant thickening of the tunica media in the pulmonary artery, along with notable pulmonary angiogenesis, endothelial cell injury, and detachment. In contrast, compared to the model group, the LV-Notch3 group demonstrated a significant improvement in pulmonary artery media thickening, pulmonary angiogenesis, endothelial cell injury, and detachment. No significant differences were observed in media thickening, pulmonary angiogenesis, endothelial cell injury, and detachment in the LV-NC group (Figure 1).

Expression of Notch3 in Pulmonary Artery Endothelial Cells of Rats in Each Group

The expression of Notch3 in the pulmonary artery endothelial cells was analyzed using real-time polymerase chain reaction. The results showed that the expression levels of Notch3 in the control group, model group, LV-NC group, and LV-Notch3 group were 1.00 ± 0.03, 0.31 ± 0.03, 0.29 ± 0.02, and 1.34 ± 0.04, respectively. Compared to the control group, the model group exhibited a significantly lower expression level of Notch3 ($P < .05$). In contrast, the LV-Notch3 group showed a significantly higher expression level of Notch3 compared to the model group ($P < .05$). There was no significant difference in the expression level of Notch3 in the LV-NC group ($P > .05$).

Expression of VEGF and MMP Proteins in Pulmonary Artery Endothelial Cells

The expression levels of vascular endothelial growth factor (VEGF), MMP-2, and MMP-9 proteins in pulmonary artery endothelial cells were assessed by Western blotting. The results demonstrated that compared to the control group, the model group exhibited significantly higher expression levels of VEGF, MMP-2, and MMP-9 proteins ($P < .05$). Conversely, in the LV-mir-181a-5p group, the expression levels of VEGF, MMP-2, and MMP-9 proteins were significantly lower than those in the model group ($P < .05$). No significant differences were observed in VEGF, MMP-2, and MMP-9 protein expression levels in the LV-NC group ($P > .05$). Please refer to Figure 1 and Table 1 for more details.

Proliferation of Pulmonary Artery Endothelial Cells in Each Group

The results of medical training therapy showed that the cell proliferation activity in the control group, model group, LV-NC group, and LV-Notch3 group was 0.581 ± 0.080, 0.997 ± 0.102, 0.931 ± 0.079, and 0.603 ± 0.085, respectively. Compared to the control group, the model group exhibited a significant increase in proliferation ability ($P < .05$). Conversely, the LV-Notch3 group demonstrated a significant decrease in proliferation ability compared to the model group ($P < .05$). No significant difference was observed in the proliferation ability of the LV-NC group ($P > .05$).

DISCUSSION

Connective tissue disease is an autoimmune condition frequently associated with pulmonary artery hypertension. Pulmonary artery hypertension is recognized as a severe complication in patients with connective tissue disease, and it is known to have a poor prognosis. Research indicates that early detection and treatment of pulmonary artery hypertension in patients with connective tissue disease can significantly enhance patient prognosis.[12,13]

Pulmonary artery hypertension is a cardiovascular disease characterized by vasoconstriction, vascular remodeling, and elevated vascular resistance. Excessive cell proliferation leading to vascular remodeling is a critical clinicopathological feature and a significant factor contributing to disease progression.[14] Consequently, delaying the process of pulmonary vascular remodeling becomes crucial in improving the prognosis and overall survival of pulmonary artery hypertension patients.

VEGF is a potent angiogenic factor that plays a crucial role in angiogenesis and endothelial cell biology. Its involvement in various diseases is mediated through complex signal transduction pathways. VEGF exhibits specific effects on endothelial cells, including angiogenesis, vascular permeability, and endothelial cell growth.[15] In this study, we observed a high expression of VEGF in hypoxia-induced pulmonary artery hypertension, accompanied by significant endothelial cell angiogenesis. Inhibition of VEGF expression led to a notable decrease in endothelial cell angiogenesis and improvement in hypoxia-induced pulmonary artery hypertension. These findings indicate the important regulatory role of VEGF-induced endothelial cell angiogenesis in connective tissue disease-related pulmonary artery hypertension, which aligns with previous studies.[16,17]

Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases that play critical roles in various physiological and pathological processes, including embryonic development, wound healing, angiogenesis, arthritis, cardiovascular disease, and cancer.[18] Among the MMPs, MMP-2 and MMP-9 are considered key gelatinases involved in lung development and have a superior ability to degrade basement membrane components compared to other MMPs. They participate in angiogenesis by regulating the migration and proliferation of endothelial cells. Studies have reported that hypoxia can activate MMP-2 and MMP-9, which are associated with tumor invasion, leading to vascular abnormalities and promoting the development of pulmonary artery hypertension.[19]

In our study, we observed a significant increase in the expression levels of MMP-2 and MMP-9 in pulmonary artery endothelial cells after hypoxia induction, accompanied by enhanced proliferation and angiogenesis of endothelial cells. These findings suggest a close association between connective tissue disease-related pulmonary artery hypertension and the proliferation and angiogenesis of endothelial cells induced by MMP-2 and MMP-9. Furthermore, when the expression of MMP-2 and MMP-9 was inhibited, we observed a notable decrease in the proliferation and angiogenesis of endothelial cells, along with an improvement in hypoxia-induced pulmonary artery hypertension. These results are consistent with previous research, further supporting the role of MMP-2 and MMP-9 in the pathogenesis of pulmonary artery hypertension.

Notch3 plays a crucial regulatory role in cell differentiation, proliferation, and apoptosis. Its involvement in endothelial cell proliferation and angiogenesis has been well documented. Studies have demonstrated that Notch3 acts as a suppressor of cell proliferation and angiogenesis in breast and colon cancer, significantly impacting the progression of these diseases. Additionally, Notch3 has been found to inhibit cell proliferation, impeding the development of glioma, lung cancer, and other conditions.[20,21]

In our study, we observed a significant decrease in the expression level of Notch3 in hypoxia-induced pulmonary artery hypertension. However, when Notch3 was overexpressed, a substantial improvement in hypoxia-induced pulmonary artery hypertension was observed. This improvement was closely associated with reduced expression levels of vascular VEGF, MMP-2, and MMP-9, as well as a decrease in endothelial angiogenesis and proliferation. These findings align with previous reports highlighting the impact and mechanisms of Notch3.

Study Limitations

Several limitations should be acknowledged in this study. Firstly, the experiment used a rat model, which may not fully reflect the complex pathophysiology of pulmonary artery hypertension in humans. Further studies involving human subjects are needed to validate these findings. Secondly, the sample size in each group was relatively small, which may limit the generalizability of the results. Increasing the sample size in future studies could enhance the statistical power and reliability of the findings. Additionally, the study primarily focused on the role of Notch3 and its downstream targets, and other potential contributing factors to pulmonary artery hypertension were not investigated. Exploring the comprehensive mechanisms underlying this condition could provide a more comprehensive understanding.

CONCLUSION

Our findings demonstrate that Notch3 plays a critical role in regulating the expression of VEGF, MMP-2, and MMP-9, thereby influencing the proliferation and angiogenesis of endothelial cells in hypoxic pulmonary artery hypertension. By modulating these factors, Notch3 can potentially ameliorate hypoxia-induced pulmonary arterial hypertension associated with connective tissue diseases. These results contribute to identifying novel therapeutic targets for managing hypoxia-induced connective tissue disease-related pulmonary artery hypertension, offering theoretical and experimental foundations for future research in this field.

Future Research Directions

Future research in pulmonary hypertension and connective tissue disease should focus on improving clinical management and exploring novel treatment strategies. Collaboration among rheumatology and immunology physicians and researchers from international and domestic medical institutions is crucial for advancing our understanding of this condition. Promising treatment strategies should be further investigated and published in reputable journals in medicine and pharmacology. It is important to assess the

impact of these strategies on disease progression, patient symptoms, and quality of life. As pulmonary hypertension remains a significant vascular complication with high morbidity in patients with connective tissue disease, effective treatments and interventions are essential to prevent stagnation in the field and mitigate the potential consequences for affected individuals. This calls for continued efforts from medical professionals and researchers to improve patient outcomes and address the challenges posed by these diseases.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

AUTHORS' CONTRIBUTIONS

All authors contributed equally to this work.

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REFERENCES

1. Shi R, Zhu D, Wei Z, et al. Baicalein attenuates monocrotaline-induced pulmonary arterial hypertension by inhibiting endothelial-to-mesenchymal transition. [J]. *Life Sci*. 2018;207:442-450. doi:10.1016/j.lfs.2018.06.033
2. Jin L, Piao ZH, Sun S, et al. Gallic acid attenuates pulmonary fibrosis in a mouse model of transverse aortic contraction-induced heart failure. [J]. *Vascul Pharmacol*. 2017;99:74-82. doi:10.1016/j.vph.2017.10.007
3. Luo L, Hong X, Diao B, Chen S, Hei M. Sulfur dioxide attenuates hypoxia-induced pulmonary arteriolar remodeling via Dkk1/Wnt signaling pathway. Biomed Pharmacother. 2018 Oct;106:692-698. doi: 10.1016/j.biopha.2018.07.017. Epub 2018 Jul 11. PMID: 29990860.
4. Hines EA, Sun X. Tissue crosstalk in lung development. [J]. *J Cell Biochem*. 2014;115(9):1469-1477. doi:10.1002/jcb.24811
5. Lu Chang, Xie Chengmao, Wei Wei. Genetic analysis of iron death related diagnostic genes and therapeutic targets in endometriosis [J]. *Progress in modern obstetrics and gynecology*, 2023, 32 (3):188-195. DOI: 10.13283 / j.carol carroll nki XDFCKJZ. 2023.03.002.
6. Hines EA, Sun X. Tissue crosstalk in lung development. [J]. *J Cell Biochem*. 2014;115(9):1469-1477. doi:10.1002/jcb.24811
7. Hong Cuiping, Hu Xiaoxia, Lu Shan. Greater omentum endometriosis with large amounts of bloody ascites 1 case [J]. *Chinese journal of obstetrics and gynecology clinical*, 2023, 24 (02) : 198-199. DOI: 10.13390 / j.i SSN. 1672-1861.2023.02.026.
8. Fu Ying Wei, Wang Yating, Yu Yanling. The research progress of endometriosis [J]. *Chinese and foreign medical research*, 2023, 21 (7) : 162-165. DOI: 10.14033 / j.carol carroll nki CFMR. 2023.07.041.
9. Zeng M, Chen S, Li H, et al. The role of β-catenin in pulmonary artery endothelial-mesenchymal transformation in rats with chronic thromboembolic pulmonary hypertension. [J]. *J Thromb Thrombolysis*. 2021;52(2):454-465. doi:10.1007/s11239-020-02356-5
10. Fujii A, Inoue K, Nagai T, et al. Clinical Utility of Atrial Electromechanical Conduction Time Measured with Speckle Tracking Echocardiography after Catheter Ablation in Patients with Atrial Fibrillation: A Validation Study with Electroanatomical Mapping. [J]. *Echocardiography*. 2016;33(9):1317-1325. doi:10.1111/echo.13259
11. Klára S, Nikolett G, József F, Tünde V, Patrícia N, József Á. Balog, László G,Puskás, Gábor J, Szebeni. Chronic Obstructive Pulmonary Disease: Epidemiology, Biomarkers, and Paving the Way to Lung Cancer. [J]. *J Clin Med*. 2021;10(13).
12. Bryce E. Montané, Andrew M, Fiore, Emily C, Reznicek, Vardhmaan, Jain, Christine, Jellis, Haala, Rokadia, Manshi, Li, Xiaofeng, Wang, Raed, Dweik, Eileen, Loh, A Claire,Watkins, Francois, Haddad, Myriam, Amsallem, Roham T, Zamanian, Vinicio Jesus, Perez, Gustavo A, Heresi. Optimal Tricuspid Regurgitation Velocity to Screen for Pulmonary Hypertension in Tertiary Referral Centers. [J]. *Chest*. 2021;160(6):2209-2219.
13. Ahmad K, Khangoora V, Nathan SD. Lung Disease-Related Pulmonary Hypertension. [J]. *Cardiol Clin*. 2022;40(1):77-88. doi:10.1016/j.ccl.2021.08.005
14. Vlahos I, Jacobsen MC, Godoy MC, Stefanidis K, Layman RR. Dual-energy CT in pulmonary vascular disease. [J]. *Br J Radiol*. 2022;95(1129):20210699. doi:10.1259/bjr.20210699
15. Hayashi T, Ono H, Kaneko Y. Association of Preoperative Mixed Venous Oxygen Saturation with Postoperative Segmental Pulmonary Hypertension in Pulmonary Atresia with Ventricular Septal Defect and Major Aortopulmonary Collaterals. [J]. *Pediatr Cardiol*. 2020;41(8):1689-1696. doi:10.1007/s00246-020-02428-6
16. Moni SS, Murthy S. Pulmonary Hypertension in Pregnancy. Clin Obstet Gynecol. 2020 Dec;63(4):868-877. doi: 10.1097/GRF.0000000000000577. PMID: 33060373.
17. Lai C, Savale L, Boytchev I, et al. Risks and outcomes of gastrointestinal endoscopy with anaesthesia in patients with pulmonary hypertension. [J]. *Br J Anaesth*. 2020;125(6):e466-e468. doi:10.1016/j.bja.2020.09.017
18. Li Y, Zhang Y, Wang J, et al. Pulmonary hypertension in end-stage renal disease patients on dialysis and predialysis patients. [J]. *Clin Invest Med*. 2020;43(3):E44-E48. doi:10.25011/cim.v43i3.34631
19. Horinouchi K, Ueno K, Nakae K, Kawamura J, Kawano Y. Successful treatment of pulmonary hypertension with unilateral absent pulmonary artery. Pediatr Int. 2020 Sep;62(9):1117-1118. doi: 10.1111/ped.14262. Epub 2020 Sep 7. PMID: 32893928.
20. Colin GC, Verlynde G, Pouleur AC, et al. Pulmonary hypertension due to left heart disease: diagnostic value of pulmonary artery distensibility. [J]. *Eur Radiol*. 2020;30(11):6204-6212. doi:10.1007/s00330-020-06959-7
21. Ruoss JL, Rios DR, Levy PT. Updates on Management for Acute and Chronic Phenotypes of Neonatal Pulmonary Hypertension. [J]. *Clin Perinatol*. 2020;47(3):593-615. doi:10.1016/j.clp.2020.05.006