Loss of family with sequence similarity 13, member A exacerbates pulmonary hypertension through accelerating endothelial-to-mesenchymal transition

Rinastiti, Pranindya

(Degree)
博士（医学）

(Date of Degree)
2020-03-25

(Resource Type)
doctoral thesis

(Report Number)
甲第7811号

(URL)
https://hdl.handle.net/20.500.14094/D1007811

（課程博士関係）

学 位 論 文 の 内 容 要 旨

Loss of family with sequence similarity 13, member A exacerbates pulmonary hypertension through accelerating endothelial-to-mesenchymal transition

Family with sequence similarity 13, member A の欠損は内皮—間葉転換を促進する結果、肺高血圧症を増悪させる

神戸大学大学院医学研究科医科学専攻
循環器内科学
（指導教員：平田　健一　教授）

PRANINDYA RINASTITI

Background and purpose:

Pulmonary hypertension is a progressive and fatal lung disease diagnosed by a sustained elevation of pulmonary arterial pressure more than 20 mmHg. Pulmonary hypertension is characterized by pathological pulmonary artery remodeling such as intimal and medial thickening of muscular arteries, vaso-occlusive lesions, and fully muscularized small diameter vessels that are normally non-muscular peripheral vessels. These vascular remodeling is a result from endothelial cell dysfunction, smooth muscle cell and endothelial cell proliferation, and also cellular transdifferentiation. Many pathogenic pathways in pulmonary arterial hypertension have been revealed; TGF-β signaling, inflammation, pericyte-mediated vascular remodeling, iron homeostasis, and endothelial-to-mesenchymal transition (EndMT). Nonetheless, detailed molecular mechanisms underlying pulmonary hypertension remain to be elucidated, especially to develop more effective therapeutic strategies to treat this life-threatening disease.

Recent genome-wide association studies identified family with sequence similarity 13, member A (*FAM13A*) gene as a genetic locus associated with chronic pulmonary disease including chronic obstructive pulmonary disease (COPD), asthma and pulmonary fibrosis. However, the role of *FAM13A* in the development of pulmonary hypertension remained to be unknown. The aim of this study is to investigate the possible role of *FAM13A* in the pathogenesis of pulmonary hypertension.

Methods:

For *in vivo* studies, wild-type (WT) and *Fam13a*$^{-/-}$ mice at 6-7 weeks old were put in the chamber with non-recirculating gas mixture of 10% O_2 and 90% N_2 for 3-6 weeks to induce pulmonary hypertension. Before the hemodynamic assessments, heart rate, fractional shortening, cardiac output, and pulmonary artery acceleration time were evaluated by echocardiography. Mice were anesthetized with ~2% isoflurane, and RVSP was measured by inserting catheter transducer into right ventricle through right jugular vein. Mice were then sacrificed, lung samples were collected for histological analysis, western blot, and quantitative PCR. Hearts were dissected and weighed to evaluate the right ventricular hypertrophy.

For *in vitro* studies, human pulmonary artery endothelial cells (PAECs) were cultured in Humedia-EG2. To induce endothelial-to-mesenchymal transition, PAECs were treated with 10 ng/mL TGF-β1 and 10 ng/mL IL-1β for 6 days in the medium supplemented with 2% FBS. The medium was changed every other day. For retrovirus infection, PAECs were grown to 70% confluency, and incubated with medium containing retrovirus carrying GFP or *FAM13A* gene in the presence of polybrene (8 μg/mL) for 24 hours. The medium was then replaced with a fresh growth medium, and cells were treated or used for functional assays 48 hours after initial infection.

Results:

In the lungs of mice with pulmonary hypertension induced by chronic exposure to hypoxia, *Fam13a* expression was remarkably reduced comparing to that in the control mice. We have generated mice with target deletion of *FAM13A* (*Fam13a*$^{-/-}$) in which LacZ cassette

was inserted into the intron of the *Fam13a* gene locus. By using LacZ-staining, the expression of *Fam13a* was found in endothelial cells of lung vasculatures.

The role of *FAM13A* in pulmonary hypertension was then further explored by using *Fam13a*$^{-/-}$ mice. Under normoxic condition, there was no significant difference in lung structures, hemodynamic, and pulmonary arterial pressure between wild-type (WT) and *Fam13a*$^{-/-}$ mice. When exposed to chronic hypoxia, *Fam13a*$^{-/-}$ mice showed deteriorated pulmonary hypertension assessed by higher right ventricular systolic pressure and augmented right heart ventricular hypertrophy. Histologically, fully muscularized small diameter vessels were increased, while peripheral capillaries decreased in the lungs of *Fam13a*$^{-/-}$ mice. Mechanistically, mesenchymal markers and transcription factors expression were found to be significantly increased both in mRNA and protein levels in *Fam13a*$^{-/-}$ mice. These data suggested the enhanced endothelial-to-mesenchymal-transition (EndMT) in the lungs of *Fam13a*$^{-/-}$ mice exposed to chronic hypoxia. Furthermore, EndMT assessed by the emergence of cells double positive for endothelial and mesenchymal marker was apparently enhanced in the lungs of *Fam13a*$^{-/-}$ mice compared with that in WT mice. These data strongly suggest that loss of *FAM13A* promotes EndMT, resulting in the deteriorated pulmonary vascular remodeling and consequent pulmonary hypertension.

To further analyze the role of *FAM13A* in EndMT, *in vitro* study was done by utilizing PAECs. When EndMT was induced by IL-1β and TGF-β1 treatment, *FAM13A* expression was significantly reduced in PAECs. By using retrovirus-mediated gene transfection, *FAM13A* was overexpressed in PAECs, and subsequently treated with IL-1β and TGF-β1 to induce EndMT. Overexpression of *FAM13A* inhibited the induction of mesenchymal markers, whereas reduction of endothelial markers was not affected. In contrast, endothelial angiogenic capacities such as tube-formation, migration, proliferation, and apoptosis were not affected by *FAM13A*-overexpression. Of note, overexpression of *FAM13A* significantly reduced the non-phosphorylated active β-catenin and its nuclear accumulation in PAECs overexpressing *FAM13A* as compared to the control cells. Considering a crucial role of β-catenin in promoting EndMT, *FAM13A* decelerates the EndMT process at least partially through inhibiting the β-catenin signaling.

Discussion:

In this manuscript, the previously undescribed role of *FAM13A* in the development of pulmonary hypertension was finally revealed. Given that *Fam13a* was reduced in the lungs of mice with pulmonary hypertension, and genetic loss of *FAM13A* exacerbated pulmonary hypertension, enhancing and/or preserving *FAM13A* in the lungs might have a therapeutic potential.

All forms of pulmonary arterial hypertension are characterized by vascular remodeling and dysfunction, of which EndMT is one of the potential factors. EndMT is a cell transdifferentiation process in which endothelial cells lose endothelial specific markers and acquire mesenchymal properties. It has been reported that EndMT is involved in a variety of cardio-pulmonary diseases such as atherosclerosis, cardiac fibrosis, pulmonary fibrosis, and pulmonary hypertension. In the remodeled vasculatures in pulmonary arterial hypertension, α-smooth muscle actin-expressing mesenchymal-like cells accumulate, especially in obstructive pulmonary vascular lesions, and EndMT derives significant number of these mesenchymal-like cells.

Previous genome-wide association studies strongly suggested a role of *FAM13A* in chronic lung diseases. *FAM13A* is expressed in various types of tissues and cells, including airway and alveolar epithelial cells in the lung, pulmonary vascular cells, and mature adipocytes in adipose tissue. Accordingly, *FAM13A* has been involved in multiple biological processes such as epithelial cell regeneration, tumor cell proliferation and survival, and insulin signaling. In the current study, a protective role of *FAM13A* in the progression of pulmonary hypertension have been identified by utilizing mice in which *Fam13a* was genetically deleted. To our knowledge, this is the first report that identifies *Fam13a* expression in the lung vasculature and *FAM13A* negatively regulates β-catenin activity in endothelial cell. β-catenin signaling has been involved in epithelial-to-mesenchymal transition in pulmonary disease and cancer. Also, β-catenin has been reported to promote EndMT through nuclear accumulation and subsequent activation of TCF/Lef transcription factors. In the current study, overexpression of *FAM13A* decelerates the EndMT process in association with reduced active β-catenin levels and its nuclear accumulation in endothelial cells. These data strongly suggest that *FAM13A* negatively regulates EndMT process at least partially through inhibiting β-catenin signaling.

Because *FAM13A* is expressed in variety types of cells in the lungs, other *FAM13A*-mediated cellular processes might be involved in the pathogenesis of pulmonary hypertension. Nonetheless, our *in vivo* data using *Fam13a*$^{-/-}$ mice clearly showed that loss of *FAM13A* exacerbated pulmonary hypertension, and thus *FAM13A* is an attractive pharmacotherapeutic target for the treatment of pulmonary hypertension.

神戸大学大学院医学(系)研究科（博士課程）

論文審査の結果の要旨

受付番号	甲 第２９５８号	氏　名	PRANINDYA RINASTITI
論文題目 Title of Dissertation	Family with sequence similarity 13, member A の欠損は内皮—間葉転換を促進する結果、肺高血圧症を増悪させる Loss of family with sequence similarity 13, member A exacerbates pulmonary hypertension through accelerating endothelial-to-mesenchymal transition		
審査委員 Examiner	主　査 Chief Examiner　中村 俊一 副　査 Vice-examiner　西村 善博 副　査 Vice-examiner　古屋敷智之		

（要旨は１，０００字～２，０００字程度）

【目的】

肺高血圧症は肺動脈圧が持続的に上昇し、右心不全を来して死に至る予後不良な疾患である。エンドセリン受容体拮抗薬を含む新規の治療薬が開発された結果、肺高血圧の予後は顕著に改善したが、その治療効果は未だ不十分であり、新しい治療法の開発が喫緊の課題である。

血管の病的な肥厚、狭窄、閉塞を伴う肺動脈のリモデリングが持続的な肺動脈圧上昇の原因であるが、その背後にある分子メカニズムについては不明な点が多い。Family with sequence similarity 13, member A (Fam13a)は人疾患のゲノムワイド関連解析により、閉塞性肺疾患や肺線維症などの慢性・進行性肺疾患と密接に関わる遺伝子座として報告されてきた。しかしFam13a が肺高血圧の病態に関与するのか、またその分子機構については全く知られていなかった。

そこで本研究者は、肺高血圧症の発症・進展における Fam13a の関与とその分子メカニズムの解析を行った。

【方法および結果】

本研究者は最初に Fam13a の肺における発現様式の検討を行い、Fam13a は低酸素暴露により肺内で発現が上昇すること、および肺動脈、特に肺動脈血管内皮細胞が Fam13a を発現していることを見出した。

次に、Fam13a を欠損した Fam13a-KO マウスを作出し、肺血管の構造および肺動脈圧の解析を行った。通常酸素下で飼育した Fam13a-KO マウスでは肺血管の構造異常や肺動脈圧の上昇は認められなかった。一方、これらマウスを低酸素に３週間暴露すると Fam13a-KO マウスでは野生型マウスと比べて肺動脈圧が有意に上昇し、肺高血圧が増悪することが明らかとなった。低酸素に暴露した Fam13a-KO マウスでは肺小血管の筋性化が進行し、また肺小動脈が減少していた。

更に低酸素に暴露した Fam13a-KO マウスの肺では、血管内皮細胞マーカー遺伝子の発現が減少・間葉系細胞のマーカー遺伝子発現が増加し、加えて間葉系マーカー遺伝子を発現する血管内皮細胞が散見されることを見出した。これら結果は Fam13a-KO マウスの肺で内皮-間葉転換が進行していることを示唆していた。内皮-間葉転換は内皮細胞が間葉系細胞へと形質転換を

来し、高い遊走能と増殖能を獲得して血管内外で異常な細胞活動を起こす現象であり、肺動脈リモデリングに関与することが報告されている。そこで本研究者は、Fam13a の内皮-間葉転換における役割を解明するための細胞実験を行った。ヒト肺動脈内皮細胞に Fam13a を過剰発現させた後に内皮-間葉転換を誘導したところ、Fam13a を過剰発現した内皮細胞では内皮-間葉転換が抑制されることがわかった。加えて、Fam13a を過剰発現する内皮細胞では内皮-間葉転換に関わるシグナル経路の一端を担うβカテニンの活性が低下していることも明らかとなった。

【考察】

本研究では、Fam13a が肺動脈血管内皮細胞の内皮-間葉転換を阻害し、その結果、肺高血圧の発症・進展を抑制することを明らかとした。肺動脈内皮細胞における Fam13a の機能調節は肺高血圧に対する新たな治療標的として大いに期待される。

【結論】

本研究では、Fam13a が肺高血圧の発症・進展に抑制的に関与することを動物実験・細胞実験を通じて明らかにした。これらの成果は、肺動脈の血管生物学的な意義解明とともに肺高血圧症に対する将来の治療標的の候補探索につながる臨床的意義を有する。以上より、本研究者は博士（医学）の学位を得る資格があると認める。