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研究課題名（和文）Elucidation of tissue-repair response in lung fibrosis by focusing on the amino acid transporter SLC15A3

研究課題名（英文）Elucidation of tissue-repair response in lung fibrosis by focusing on the amino acid transporter SLC15A3

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研究成果の概要（和文）：SLC15A3は免疫細胞で高発現しており、PFにおいてその欠失がコラーゲン沈着の減少と呼吸機能の改善につながることが明らかになりました。SLC15A3欠損マクロファージではArg2の発現上昇が見られ、絶食はPF進行を抑制することが示されました。さらに、SLC15A3欠損はIL-11の減少を引き起こし、線維化に寄与する可能性があることが線維芽細胞およびPF肺で示されました。これらの結果は、SLC15A3標的化と食事介入が肺で抗線維化作用を生み出し、新しいPF治療法を提供する可能性があることを示唆しています。

研究成果の学術的意義や社会的意義
この研究はSLC15A3の欠損がPFに及ぼす影響を明らかにし、科学的な理解に貢献しています。SLC15A3の欠如によるコラーゲン減少や呼吸機能の改善、マクロファージでのArg2発現増加などの結果は、PF進行に関わる分子経路を明らかにしました。これにより、標的治療法の開発への可能性が広がりました。社会的には、この研究は困難な肺線維症の管理に重要な意義を持ちます。SLC15A3を治療対象とする新たなアプローチや食事介入によるArg2増強は、PF進行を抑制する可能性があり、標準的な医療治療を補完します。これらの成果は、肺線維症患者に希望をもたらし、生活の質の向上に向けた新たな展望を提供しています.

研究成果の概要（英文）：SLC15A3, an immune cell-expressed transporter, plays a significant role in pulmonary fibrosis (PF). Its deficiency leads to reduced collagen deposition, improved respiratory function, and increased expression of Arg2 in macrophages, indicating its involvement in the intricate molecular pathways of PF progression. Fasting has been shown to enhance Arg2 expression and suppress PF advancement. Furthermore, SLC15A3 deficiency has been associated with a decrease in IL-11 expression, potentially contributing to fibrosis. These findings highlight the potential of targeting SLC15A3 and implementing dietary interventions as innovative approaches for PF treatment, offering multiple anti-fibrotic changes in the lungs. The study expands our understanding of PF, holds promise for the development of novel therapies, and provides new perspectives for managing this challenging lung disease, ultimately improving the quality of life for individuals affected by PF.

研究分野：Immunology

キーワード：Pulmonary fibrosis　SLC15A3　macrophages　collagen deposition　Arg2 expression　fibroblasts　dietary intervention　therapeutic target

１．研究開始当初の背景

Pulmonary fibrosis (PF) is a lung disease characterized by progressive scarring and the buildup of certain substances, leading to a poor prognosis. The exact cause of PF is not well understood, but it often involves an inflammatory response triggered by infections or exposure to harmful substances like asbestos or silica. Unfortunately, there are limited treatment options available for PF, which highlights the urgent need to discover new targets for potential future therapies.

One potential target of interest is a protein called SLC15A3, which helps transport amino acids and small peptides. This protein is mainly found in innate immune cells such as macrophages and neutrophils in both humans and mice. Research has shown that SLC15A3 is strongly expressed in the lung tissues of both humans and mice, as well as in the lungs of PF patients, particularly in alveolar macrophages.

The presence of SLC15A3 protein has also been confirmed in the lungs of patients with a specific type of PF called idiopathic pulmonary fibrosis (IPF). Although we don't fully understand the function of SLC15A3, a similar protein called SLC15A4 has been associated with promoting symptoms of colitis and lupus by influencing certain inflammatory and metabolic signals within cells. These signals are often connected to fibrosis.

Based on these findings, we hypothesized that SLC15A3 might play a role in lung inflammation and further investigated its involvement in the development of PF using mouse models.

２．研究の目的

The purpose of this research is to investigate the role of a protein called SLC15A3 in the development of pulmonary fibrosis (PF), a lung disease characterized by scarring and matrix deposition. The researchers aim to understand the contribution of SLC15A3 to lung inflammation, particularly in the context of PF pathogenesis. By studying the expression and function of SLC15A3 in human and mouse lung tissues, as well as in PF patients, the researchers seek to identify potential novel targets for future treatments. This research is driven by the critical need to expand therapeutic options for PF, as current treatments are limited and the prognosis for patients with PF is generally poor.

３．研究の方法
The methods used in the study include:

Animal Models: Murine models of pulmonary fibrosis (PF) were employed to investigate the role of SLC15A3. These models represented different pathogenic routes for PF.

Gene Expression Analysis: Single-cell RNA sequencing (scRNAseq) analyses were performed to assess gene expression patterns in various cell subsets within fibrotic lungs, including macrophages, neutrophils, and fibroblasts.

Metabolic Analysis: Metabolic processes in macrophages were examined through metabolic profiling techniques to understand the impact of SLC15A3 deficiency on cellular metabolism.

In Vivo Experiments: Intratracheal (i.t.) administration of SLC15A3-deficient macrophages (Slc15a3−/− M□s) was performed to evaluate their therapeutic potential for PF alleviation.

Arg2 Manipulation: The role of Arg2, an enzyme involved in arginine metabolism, was investigated by manipulating its expression through genetic modifications (e.g., knockout and knockdown) in combination with Slc15a3−/− macrophages.

AMPK Activation: The activity of AMP-activated protein kinase (AMPK), a metabolic regulator, was assessed in macrophages to understand its involvement in the cellular responses associated with SLC15A3 deficiency.

Immunohistochemistry: Lung tissues from both mice and human patients with PF were analyzed using immunohistochemical staining to examine the expression of SLC15A3 protein.

Functional Assessments: Lung function and fibrosis severity were evaluated using various functional tests and histopathological analysis to assess the impact of SLC15A3 deficiency on pulmonary fibrosis.

Statistical Analysis: Statistical methods were employed to analyze the data and determine the significance of the findings.

These methods were utilized to investigate the role of SLC15A3 in the pathogenesis of pulmonary fibrosis and assess its potential as a therapeutic target.

４．研究成果
The purpose of this research is to investigate the potential therapeutic role of SLC15A3 deficiency in improving pulmonary function in cases of pulmonary fibrosis (PF). PF is a condition characterized by widespread organ failure caused by fibrotic scarring. It is becoming increasingly common due to factors such as environmental pollution (e.g., asbestos and PM2.5) and the long-term effects of the COVID-19 pandemic (i.e., long COVID).

The research findings suggest that SLC15A3 deficiency could offer an innovative approach to improving lung function in individuals with PF. The study used mouse models of PF with different disease pathways and found that the absence of SLC15A3 had a beneficial impact on lung function. The deficiency of SLC15A3 resulted in significant changes in gene expression patterns in various cell types within fibrotic lungs, including macrophages, neutrophils, and fibroblasts. Among these cell types, macrophages lacking SLC15A3 played a critical role in reducing fibrosis.

The study also revealed that the loss of SLC15A3 caused metabolic alterations in macrophages. Specifically, the deficiency led to metabolic abnormalities characterized by an enhanced catabolic response and increased AMPK activation. The sustained activation of AMPK appeared to be linked to continuous inflammation, contributing to the anti-fibrotic effects observed in the absence of SLC15A3.

Additionally, the research highlighted the importance of arginine metabolism in PF pathogenesis. The expression of Arg2, an enzyme involved in arginine metabolism, was significantly influenced by SLC15A3 deficiency. The study demonstrated that the induction of Arg2 had a positive impact on PF alleviation. Boosting Arg2 expression through dietary restriction (DR) offered a potential strategy for PF treatment. The scavenging functions of macrophages lacking SLC15A3 also played a role in promoting tissue regeneration by reducing fibrotic scarring.

Overall, the research findings suggest that targeting SLC15A3 or modulating arginine metabolism pathways may have therapeutic potential for the treatment of PF. Further investigations into the mechanisms of Arg2 induction and the effects of SLC15A3 deficiency on immune cells other than macrophages could provide valuable insights for developing novel therapeutic strategies to combat PF. Combinations of different approaches, such as SLC15A3 inhibition, dietary interventions, and other compounds to induce Arg2 expression, might yield additional benefits in preventing or treating PF.

５．主な発表論文等

〔雑誌論文〕　計2件（うち査読付論文　2件／うち国際共著　2件／うちオープンアクセス　2件）

１．著者名 Kobayashi Toshihiko、Nguyen-Tien Dat、Sorimachi Yuriko、Sugiura Yuki、Suzuki Takehiro、Karyu Hitomi、Shimabukuro-Demoto Shiho、Uemura Tatsuki、Okamura Tadashi、Taguchi Tomohiko、Ueki Kohjiro、Kato Norihiro、Goda Nobuhito、Dohmae Naoshi、Takubo Keiyo、Suematsu Makoto、Toyama-Sorimachi Noriko	４．巻 118
２．論文標題 SLC15A4 mediates M1-prone metabolic shifts in macrophages and guards immune cells from metabolic stress	５．発行年 2021年
３．雑誌名 Proceedings of the National Academy of Sciences	６．最初と最後の頁 1-10
掲載論文のDOI（デジタルオブジェクト識別子） 10.1073/pnas.2100295118	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

１．著者名 Nguyen-Tien Dat、Suzuki Takehiro、Kobayashi Toshihiko、Toyama-Sorimachi Noriko、Dohmae Naoshi	４．巻 3
２．論文標題 Identification of the interacting partners of a lysosomal membrane protein in living cells by BioID technique	５．発行年 2022年
３．雑誌名 STAR Protocols	６．最初と最後の頁 1〜15
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.xpro.2022.101263	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

〔学会発表〕　計3件（うち招待講演　1件／うち国際学会　3件）

１．発表者名 Toshihiko Kobayashi, Dat Nguyen-Tien, Noriko Toyama-Sorimachi
２．発表標題 Metabolic switches at endolysosomes; roles of lysosome-resident amino acid transporters in inflammatory responses
３．学会等名 The 50th Annual Meeting of the Japanese Society for Immunology（招待講演）（国際学会）
４．発表年 2021年〜2022年

１．発表者名 Noriko Toyama-Sorimachi, Dat Nguyen-Tien, Toshihiko Kobayashi
２．発表標題 A novel therapeutic strategy of pulmonary fibrosis based on arginine metabolism in macrophages
３．学会等名 The 50th Annual Meeting of the Japanese Society for Immunology（国際学会）
４．発表年 2021年〜2022年

１．発表者名 Dat Nguyen-Tien, Toshihiko Kobayashi, Shigeyuki Shichino, Satoshi Ueha, Masato Kubo, Kouji Matsushima, Noriko Toyama-Sorimachi
２．発表標題 A novel mechanisms of lung fibrosis mediated by SLC15A3
３．学会等名 The 50th Annual Meeting of the Japanese Society for Immunology（国際学会）
４．発表年 2021年～2022年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関