様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

令和　６ 年　 ６ 月 ２０ 日現在

機関番号：１５４０１
研究種目：若手研究
研究期間：2021～2023
課題番号：２１Ｋ１５０８８

研究課題名（和文）Specificities of cellular uptake of primary cilium-derived extracellular vesicles for intercellular communication

研究課題名（英文）Specificities of cellular uptake of primary cilium-derived extracellular vesicles for intercellular communication

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交付決定額（研究期間全体）：（直接経費）　　3,600,000円

研究成果の概要（和文）：本研究では、野生型NIH/3T3細胞の培養上清由来の細胞外生理活性因子が、創傷後の一次繊毛欠損NIH/3T3-Kif3a-KO細胞の移動および増殖能に及ぼす影響を検討した。野生型NIH/3T3細胞の培養上清由来の因子は、一次繊毛欠損NIH/3T3-Kif3a-KO細胞の培養上清由来の因子と比較して、標的細胞の細胞移動/増殖速度を増加させた。さらに、培養上清のオミックス解析により、一次繊毛欠損細胞の培養上清中に高濃度を示す特定の分子成分を同定した。これらの知見は、線維芽細胞が一次繊毛依存的に、液性生理活性因子によって一次繊毛欠損標的細胞の細胞移動/増殖プロセスを制御していることを示唆している。

研究成果の学術的意義や社会的意義
繊毛症における繊毛欠損細胞の細胞内因性決定因子の解明は大きく前進しましたが、繊毛欠損細胞からの細胞外因性因子がこれらの病態に及ぼす影響については、まだ比較的未開拓の領域です。この研究は、繊毛症が初めて細胞外因性の観点から研究されたという意味で重要です。これは、将来、繊毛症を治療するための治療法を見つける道を開くものでもあります。

研究成果の概要（英文）：In this study, we examined the influence of extracellular bioactive factors derived from the culture supernatant of wildtype NIH/3T3 cells on the migratory and proliferative capacities of primary cilium-deficient NIH/3T3-Kif3a-KO cells after wounding. The factors derived from the culture supernatant of wildtype NIH/3T3 cells increased the rate of cell migration/proliferation in target cells compared to the factors derived from the culture supernatant of primary cilium-deficient NIH/3T3-Kif3a-KO cells. Additionally, through omics analysis of the supernatants, we identified a specific molecular constituent exhibiting elevated concentrations in the culture supernatant of primary cilium-deficient cells. These findings suggest that fibroblasts regulate the cellular migration/ proliferation process in primary cilium-deficient target cells by humoral bioactive factors in a primary cilium-dependent manner.

研究分野：Cell Biology

キーワード：primary CIlium

様　式　Ｃ－１９、Ｆ－１９－１（共通）

１．研究開始当初の背景

The primary cilium, a microtubule-based, hair-like protrusion on the cell surface in mammalian organisms, functions as a sensory organelle enabling cells to sense and respond to their environment. It assumes a pivotal role in the regulation of diverse cellular processes, including cell proliferation, differentiation, and developmental pathways. Notably, perturbations in primary cilium structure and functionality are associated with a spectrum of clinical disorders termed ciliopathies, encompassing a wide array of pathological manifestations.

２．研究の目的

While significant advances have been made in elucidating the cell-intrinsic determinants of cilia-deficient cells in ciliopathies, the contribution of cell-extrinsic factors from cilia-deficient cells to these pathologies remains a relatively unexplored domain. The purpose of this study is to explore the cell extrinsic factors associated with ciliopathies.

３．研究の方法

To confirm if the previous phenomenon we discovered about Increase in migration rates of target cells after wildtype derived-conditioned medium treatment is primary cilium dependent, another primary cilium defective cell line was made by knockout of Dync2h1 gene through CRISPR gene editing. Scratch wound assay experiments were done to confirm the results obtained in Kif3a-KO cells. Next, to identify the conditioned medium components behind this effect, conditioned medium was fractionated into extracellular vesicle and supernatant fractions. Then, target cells were treated with these fractions.

We also screened for broader changes in gene expression and cellular pathways in response to supernatant treatment. The NIH/3T3-*Kif3A*-KO cells were exposed to supernatant derived from wild-type cells. Following exposure to supernatant, total RNA was extracted for transcriptomics analysis.

Finally, to identify the humoral factor present in the supernatant, which is responsible for this effect, omics analysis was performed on the supernatants derived from wildtype, Kif3a-KO and dync2h1-KO cells. The identified molecule was confirmed through rescue experiments.

４．研究成果

Previously we detected the increase in cell migration in target cells treated with conditioned medium derived from wildtype NIH/3T3 cells compared to primary cilium deficient NIH/3T3 Kif3a-KO cells. These results were confirmed in another ciliopathy cell line DynC2h1-KO with very few and short primary cilia. Similar to Kif3a-KO cell derived conditioned medium, cells treated with Dync2h1-KO cells derived conditioned medium showed decrease in cell migration rates compared to wild type treatment (Fig1A).

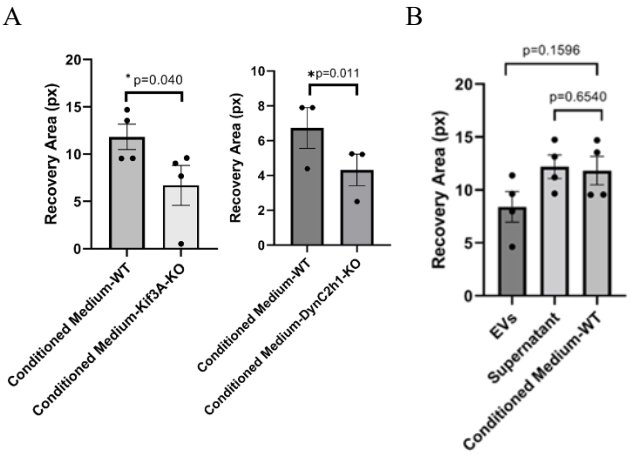

Fig 1. Conditioned medium derived from wild-type cells enhance migration in a primary cilium-dependent manner (A). WT-derived supernatant enhances target cells migration (B).

Next, we checked which fraction of the conditioned medium is responsible for this increase and found out that supernatant fraction obtained after separating the EVs showed maximum increase in the cell migration compared to EVs fraction and like control that is conditioned medium derived from wildtype cells (Fig 1B).

To screen for broader changes in gene expression and cellular pathways in response to supernatant fraction, transcriptomics analysis of the target cells after treatment with the supernatant fractions was done. Gene expression changes to cell migration were observed in cells at 24 hrs and 48 hrs after treatment. The highest increase was observed in thrombospondin-2 and osteoglycan gene expression in cells wildtype-derived supernatant compared to Kif3a-KO cells derived supernatant (Fig 2).

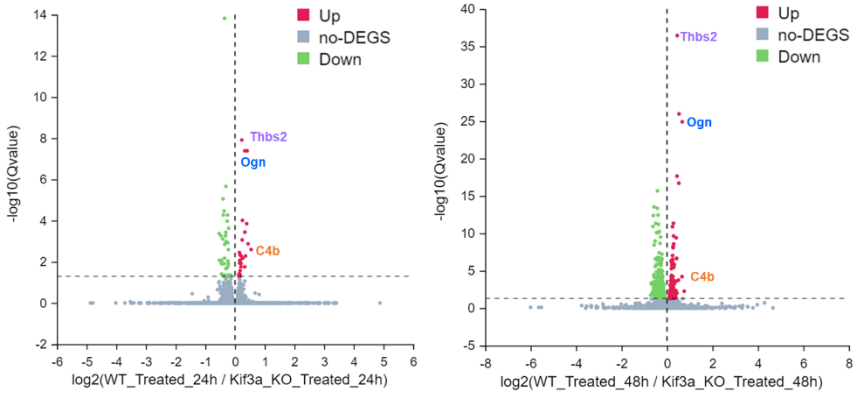

Fig 2. Volcano plot of differentially expressed proteins in target cell after supernatant treatment at 24 hours and 48 hours.

Next, to identify which biomolecules in the wildtype derived supernatant are causing the increase in migration rate in target cells we performed the omics analysis of the supernatant fractions from the NIH/3T3 cells, Kif3a-KO cells and DynC2h1-KO cells. We identified molecule X which expression was decreased in both Kif3a-KO cells and DynC2h1-KO cells derived supernatant compared to NIH/3T3-derived supernatant. To confirm if the molecule X is responsible for increase in cell migration rates in target cells, we performed rescue experiments where the target cells were treated with the Kif3a-KO derived supernatant supplemented with molecule X. After treatment, the rate of cell migration was similar to that of wildtype control (Fig 3).

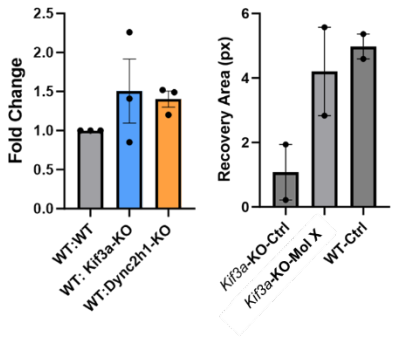

Fig 3. Molecule X present in the wildtype derived supernatant increases the rate of migration in target cells.

These findings suggest that fibroblasts regulate the cellular migration/ proliferation process in primary cilium-deficient target cells by humoral bioactive factors in a primary cilium-dependent manner.

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　1件）

１．著者名 Faryal Ijaz, Ryota Nakazato, Mitsutoshi Setou & Koji Ikegami	４．巻 12
２．論文標題 A pair of primers facing at the double-strand break site enables to detect NHEJ-mediated indel mutations at a 1-bp resolution	５．発行年 2022年
３．雑誌名 Scientific Reports	６．最初と最後の頁 1-12
掲載論文のDOI（デジタルオブジェクト識別子） 10.1038/s41598-022-15776-5	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 Ryota Nakazato, Hiroshi Otani, Faryal Ijaz, Koji Ikegami	４．巻 175
２．論文標題 Time-lapse imaging of primary cilium behavior with physiological expression of fluorescent ciliary proteins	５．発行年 2023年
３．雑誌名 Methods in Cell Biology	６．最初と最後の頁 45-68
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/bs.mcb.2022.10.003	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

〔学会発表〕　計3件（うち招待講演　0件／うち国際学会　2件）

１．発表者名 Faryal Ijaz
２．発表標題 Double-strand break Site-Targeted PCR coupled with high concentration TBE-PAGE enables to detect NHEJ-mediated indel mutations at a 1-bp resolution
３．学会等名 The 74th Annual Meeting of the Japan Society for Cell Biology（国際学会）
４．発表年 2022年

１．発表者名 Faryal Ijaz
２．発表標題 Primary Cilium-dependent Humoral Bioactive Factors Control Fibroblast Cell Migration and Proliferation
３．学会等名 The 74th Annual Meeting of the Japan Society for Cell Biology（国際学会）
４．発表年 2023年

１．発表者名 Faryal Ijaz
２．発表標題 Primary Cilium-dependent Control of Fibroblast Cell Migration and Proliferation by Humoral Bioactive Factors
３．学会等名 13th Cilia Society Meeting
４．発表年 2023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