Form 3

Abstract of Thesis

Name （ LATIEFA BINTI KAMARULZAMAN ）	
Title	Single-cell proteome analysis based on 3D single-molecule fluorescence imaging （３次元１分子蛍光イメージングを用いた単一細胞プロテオーム解析　）

Abstract of Thesis

Heterogeneity exists at multiple levels of molecular biology and plays an important role in many biological processes. For years, conventional bulk analyses have been used to study the central dogma of biology, yet they often provide only average measurements across cell populations. Rare subpopulations of cells with unique features are frequently masked at the bulk level. To address this problem, single-cell technologies were developed, with single-cell RNA sequencing emerging as a powerful tool for studying cellular heterogeneity. While this technology offers high throughput and resolution for profiling single-cell transcriptomes, RNA expression alone cannot reliably predict protein abundance due to factors like protein degradation and post-transcriptional/translational modifications. Therefore, direct protein measurements at the single-cell level are essential for a comprehensive understanding of biological processes. Recent advances in shotgun proteomics and immunoassays have driven the development of single-cell proteomics technologies. At present, mass spectrometry (MS) and antibody-based analysis are the primary methods for quantifying protein expression in single cells. While these methods excel in many aspects like throughput and multiplexity, they bear sensitivity constraints which make quantification of low-abundance proteins (10^1–10^3 copies per cell) at the single-cell level challenging.

In this study, I introduced single-cell PAGE-PISA, a novel strategy for ultra-sensitive single-cell proteome analysis that integrates polyacrylamide gel electrophoresis (PAGE) for protein separation based on molecular sizes, with a custom-built light-sheet microscope (PISA) for 3D single-molecule imaging. By performing an 'all-in-one' sample preparation in PCR tubes, including cell lysis and protein labelling with fluorescent dyes, followed by electrophoresis and single-molecule counting, single-cell PAGE-PISA enables 3D imaging of all target molecules within a sub-millimeter polyacrylamide gel (~0.3 mm). As a result, this technique quantified over 10^7 protein copies from a single mammalian cell with the sensitivity to detect proteins present at low copy numbers, as few as 100 copies per species. Furthermore, single-cell PAGE-PISA effectively classified different cell types based on their proteomic signatures and revealed strong correlation between protein expression profiles with predicted developmental states during cardiomyocyte differentiation. With the ability to track developmental trajectories from as few as 49 cells, single-cell PAGE-PISA shows great promise for studying rare cell populations or limited samples, while also opening new possibilities in developmental biology, regenerative medicine, and disease modeling. Potentially serving as a powerful complementary approach to single-cell RNA sequencing, single-cell PAGE-PISA bridges the gap between transcriptome and proteome, enabling a comprehensive, sensitive, and precise analysis of the single-cell proteome, and advancing our understanding of complex biological processes from single-cell insight.

様式７

論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

本論文は、近年注目される単一細胞プロテオミクスにおいて、従来法では困難だった高感度・高網羅的なタンパク質定量を可能にする新技術「single-cell PAGE-PISA」を開発・実証したものである。本手法は、単一細胞の全タンパク質を蛍光標識し、電気泳動で分離後、３次元１分子蛍光イメージングを基に１分子単位で検出・定量を行う。これにより、１つの哺乳類細胞あたり約１０の７乗個のタンパク質の検出とその分析を実現した。さらに、心筋細胞分化過程における各細胞の測定では、細胞状態と強く相関するタンパク質群を検出し、トランスクリプトーム解析を上回る相関が得られることを示した。以上より、本論文は生命機能科学における高度な学識、優れた研究能力、新たな知見を示しており、博士（理学）の学位論文として十分に価値あるものと認めた。さらに、2025年5月21日に口頭試問を行い、合格と判定した。なお、チェックツール“iThenticate 2.0”を使用し、剽窃、引用漏れ、二重投稿等のチェックを終えていることを申し添えます。