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Abstract of Thesis

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Title	Kinetic model expressing the anomalous phenomenon in human induced pluripotent stem cell (hiPSC) culture (ヒト人工多能性幹細胞(hiPSC)培養におけるアノーマリーを表現する速度論モデルに関する研究)

Abstract of Thesis

Chapter 1-General Introduction: Modelling biological systems helps biologists to have new insights into particular problems, generate hypotheses, and design new experiments. Kinetic models can be applied to biological systems from subcellular scale (*e.g.* gene regulatory networks, cell signaling pathways), cellular scale, to tissue or whole organism scale. Recently, there is need for a kinetic model to understand underlying mechanism of an anomalous phenomenon in human induced pluripotent stem cell (hiPSC) culture which is called deviation from the undifferentiated state. Cells deviated from the undifferentiated state lose pluripotent characteristics and be negative for markers of the three germ cell layers. Deviated hiPSCs appear at the central and peripheral regions of colony in culture with SNL and MEF feeder cells, respectively. Later, a study found the spatial heterogeneity of cell migration in colony and suggested that anomalous cell migration at the central and peripheral region of colony triggered deviation from the undifferentiated state of hiPSCs in culture with feeder cells. However, many questions are still remained after all efforts of *in vitro* study due to technical limitation. The objective of this study is elucidation of those phenomena that occurred in hiPSCs culture by using a kinetic model.

Chapter 2-Understanding the spatial heterogeneity of cell migration in colony: The first target of this study is understanding the spatial heterogeneity of cell migration in hiPSC colony. With cellular automaton approach, the developed model described several fundamental cell behaviors including cell division, cell migration, cell-cell connection, and cell-substrate connection. All parameter values were estimated from *in vitro* data and the appropriateness of model was indicated by good agreement with *in vitro* results. Using the developed model, it was found that the cell division is the key factor that leads to the observed spatial heterogeneity of cell migration in hiPSC colony. Cells at the peripheral region of colony had faster migration than cells at the central region of colony due to the push generated from dividing cells. This result indicated that there is a need to separate components of *in vitro* cell migration before considering the actual trigger for the deviation from the undifferentiated state because each component contributes differently on the overall cell migration.

Chapter 3-Modelling the deviation from the undifferentiated state of hiPSCs: With suggestion from Chapter 2, not only apparent movement V_{app}, which had similar meaning to *in vitro* cell migration, but also constituent movements, including movement caused by active migration V_{act}, movement caused by passive migration V_{pas}, and movement caused by cell division V_{div} were analyzed to assess the potential triggers. *In silico* analyzation showed that V_{app} and V_{act} were possible triggers of deviation from the undifferentiated state at the central and peripheral region of colony, respectively. The anomalous cell migration-driven hiPSC deviation, then, was explained by two factors: a mechanical stimulus, represented by thresholds of V_{app} and V_{act}, and duration of the mechanical stimulus. This hypothesis was well validated by several *in vitro* properties of hiPSC deviation such as the position of deviated region in colony and the frequency of deviation in culture. *In silico* calculation showed that these triggers of deviation were ultra-rare (approximately 10^{-5}) following definition of ultra-rare diseases.

Chapter 4-General Conclusion and Perspectives: The spatial heterogeneity of cell migration in hiPSC colony was resulted from the push of dividing cells. Deviation from the undifferentiated state of hiPSCs cultured on SNL and MEF feeder cells was explained by continuous relatively low V_{app} or high V_{act}, respectively. This is the first kinetic model that describes the ultra-rare trigger that causes anomalous phenomenon in biological field. The developed model is expected to be a promising tool to optimize hiPSC culture conditions.

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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

本博士論文は，iPS 細胞の平面培養時における細胞挙動，未分化からの逸脱について，従来の正常・異常の考えから細胞挙動の外れ状態（アノーマリー）を設定した速度論モデルを構築し，これまで示すことができなかった十万分の一個以下の細胞の存在確率によって引き起こされる超レア現象を示している。

第 1 章では，細胞の培養における速度論モデルを分類し，本論文で構築するセルラーオートマトンの手法での方法の有効性を示している。

第 2 章では，本手法にて，個々の細胞の挙動エネルギー（遊走，細胞・細胞間接着，細胞・基質間接着）のバランスを表現できる速度論モデルを構築している。このモデルを，iPS 細胞の培養系へ適用することで，コロニー内の空間的遊走速度は，細胞分裂による細胞の押し出しに依存し，結果として，空間的不均一性を有することを示している。得られた計算結果は，実際の培養現象と一致しており，本モデルの有効性を証明している。

第 3 章では，正常な細胞挙動からの外れ状態を仮想し，アノーマリー指標を設定し，モデルに組み込むことで，細胞の未分化状態荒の逸脱の現象を表現している。その際，本指標に対し，設定値より大きくなると逸脱するという閾値を設定することで未分化状態から逸脱状態へと変化する現象を示している。これらの現象を，実際の培養と比較することで，本仮説を検証し，実際の培養系をよく反映していることを証明している。さらに，この逸脱現象は，10 万個の細胞の 1 個で生じることがわかり，希少疾患での表現と同じ頻度で，超レア（Ultra-rare）な現象であることが示されている．また，現象解明を促進することで，逸脱現象の抑制を可能としたことを報告している。

第 4 章では，総括ならびに将来展望を示し，本モデルの将来性を示している。

以上のように、本論文は，開発した細胞挙動を表現できる速度論モデルで，iPS 細胞の未分化培養の経過を表現することができ，さらに，超レア現象を表現できることで，実際の培養において防ぐことができなかった逸脱現象の理解を進め，結果として，逸脱現象の防止策を提案することに至っている実践的な報告である。よって本論文は博士論文として価値あるものと認める。