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論文題目	Selective vulnerability of human-induced pluripotent stem cells to dihydroorotate dehydrogenase inhibition during mesenchymal stem/stromal cell purification ジヒドロオロト酸デヒドロゲナーゼ阻害剤による間葉系幹／間質細胞からの未分化 iPS 細胞の選択的除去		

（論文内容の要旨）

Human induced pluripotent stem (iPS) cells have emerged as an innovative tool in stem cell regenerative therapy and disease modeling due to their ease of derivation and ethical sourcing from donor tissues. While offering vast potential for differentiation into various stem cell types, the clinical application of iPS cells presents challenges such as potential tumorigenicity and the heterogeneity of induced differentiation.

In response to these concerns, this research delves into addressing the issues associated with iPS cells by investigating the selective elimination of undifferentiated cells and the induction of regionally specific cells. The study utilizes Brequinar (BRQ), which is a potent inhibitor of dihydroorotate dehydrogenase (DHODH), a key enzyme in the de novo pyrimidine biosynthesis pathway. Its primary applications revolve around cancer therapy, immunosuppression, antiviral activity, and the treatment of inflammatory diseases. By disrupting pyrimidine nucleotide synthesis, BRQ exhibits anti-cancer properties, inhibiting the proliferation of tumor cells. This study utilizes BRQ to target and eliminate undifferentiated iPS cells while sparing other cell types. Undifferentiated iPS cells, with their high proliferative capacity, heavily rely on de novo pyrimidine synthesis for DNA and RNA production during rapid cell division. BRQ disrupts this pathway, selectively inhibiting DHODH and impeding nucleotide synthesis. Therefore, undifferentiated iPS cells experience a pronounced reduction in their ability to replicate and maintain pluripotency.

In contrast, differentiated cells, having undergone specialization into specific cell types, often exhibit a slower rate of cell division and reduced reliance on de novo pyrimidine synthesis. Thus, these differentiated cells are comparatively less vulnerable to the inhibitory effects of BRQ. The selectivity of BRQ for undifferentiated cells makes it a promising agent for targeted elimination of pluripotent cells within a mixed cell population.

The application of BRQ activates apoptosis and downregulates the MYC pathway, effectively reducing the tumorigenic potential of iPS cells. Furthermore, BRQ-treated iPS cell aggregates, acting as an in vitro teratoma-like model, exhibited a significant decrease in size and a reduction in pluripotent marker gene expression. Furthermore, the application of BRQ to induce apoptosis in undifferentiated iPS cells did not adversely affect the survival, differentiation potential, or gene expression of induced mesenchymal stem cells (iMSCs).

This research highlights the promising role of BRQ as an effective agent for purifying iMSCs from potentially undifferentiated iPS cell populations, a critical step in ensuring the success of iMSC-based therapy to achieve safe and effective regenerative medicine.

（論文審査の結果の要旨）

間葉系幹／間質細胞（mesenchymal stem/stromal cells、MSC）は、あらゆる組織に存在し、免疫調整や組織再生等の機能がある細胞で、既に移植片対宿主病への投与で臨床応用もされている。

しかし、生体から MSC を得る場合、侵襲性、増殖限界、採取ごとの生物学的特性の違いなど、いくつか課題がある。iPS 細胞から作製した MSC（iMSC）はこれらの課題を解決可能であるが、移植治療使用には未分化な iPS 細胞の残存による奇形腫形成のリスクがある。よって、移植時に不要な iPS 細胞を完全に除去する方法の開発は、安全な iMSC 再生医療の実現のために重要なステップである。

本研究は、増殖の速い細胞が de novo ピリミジン生合成経路に強く依存していることに注目し、iPS 細胞除去に対するピリミジン新規合成経路酵素の DHODH の阻害剤の有効性を検証した。その結果、DHODH 阻害剤 BRQ がヒト iPS 細胞に細胞死、細胞周期停止、分化誘導効果を示す一方で、iMSC には作用しないことを見出した。さらに、ヒト iPS 細胞と iMSC の混合培養実験からも、未分化 iPS 細胞への BRQ の選択的除去効果を示した。他方、BRQ 処理は、iMSC の分化能には影響を与えないことを確認した。

以上の研究は、iMSC を使った再生医療に有効な基盤技術の開発に貢献し、将来的な iMSC を使った再生医療の成功に寄与するところが多い。

したがって、本論文は博士（　医学　）の学位論文として価値あるものと認める。

なお、本学位授与申請者は、令和　６年　２月　２９日実施の論文内容とそれに関連した試問を受け、合格と認められたものである。

要旨公開可能日：　　年　　月　　日　以降