様式３

論　文　内　容　の　要　旨

氏　　名　（　　東　真里奈　　）	
論文題名	Development of Stem Cell Medium and Parthenogenote Chimeras for Mammalian Development Studies （哺乳類発生研究のための幹細胞培地および単為発生キメラ法の確立）

論文内容の要旨

Development of a Chemically Disclosed Serum-Free Medium for Mouse Pluripotent Stem Cells

Mouse embryonic stem cells (mESCs) have traditionally been cultured in medium supplemented with fetal bovine serum (FBS). However, the inconsistency in the chemical composition of FBS results in batch variability and reproducibility challenges in research. Commercial serum-free media, such as those supplemented with KSR or B27, are widely used for culturing pluripotent stem cells (PSCs), but their precise components remain undisclosed. To address this issue, I developed a chemically defined serum-free medium, DA-X-modified medium for robust growth of PSCs (DARP). DARP medium supports normal transcriptomes and the differentiation potential into all three germ layers, as demonstrated by teratoma formation assays. DARP medium also facilitated the derivation of mESCs from blastocysts and supported cells capable of contributing to all three germ layers and germ cells in chimeric embryos. Additionally, DARP medium effectively cultured mouse epiblast stem cells (mEpiSCs), uncovering a key distinction: cholesterol is essential for the maintenance of naïve mESCs but is dispensable for primed mEpiSCs. These results establish DARP as a reliable and reproducible culture system for various types of PSCs, advancing research and applications in stem cell biology.

Parthenogenote-Derived Brain Unveils the Critical Role of Paternal Genome in Neuronal Development

Studies using pronuclear transfer to create uniparental embryos have exhibited distinct phenotypes, revealing that maternal and paternal alleles have unique and non-redundant roles. These insights were pivotal in defining the genomic imprinting as an epigenetic mechanism in eutherian mammals that governs parent-of-origin-specific gene expression. However, the lethality of parthenogenetic (Pg) embryos, which lack a paternal genome, has limited the investigation of these roles in later stages and postnatal life. To address this limitation, I developed Cell Replacement with Parthenogenote-derived cells (CReP) mice by creating chimeras of Pg embryos with *Wnt1*-knockout (KO) fertilized embryos. By using *Wnt1*-KO embryos as recipient, Pg-derived cells compensated for the midbrain and cerebellum defects characteristic of *Wnt1*-KO embryos, demonstrating their capacity to contribute to late-stage brain development. The body weight of CReP mice was lower than that of Cell Replacement with Fertilized embryo-derived cells (CReF) mice, used as controls, and most CReP mice failed to survive beyond one week. RNA sequencing of Pg-derived cells revealed up-regulation of maternally imprinted genes (e.g., *H19, Igf2r*) and significant down-regulation of paternally imprinted genes (e.g., *Rasgrf1, Peg10*). Additionally, 626 novel candidate imprinted genes, including *Per3, Slc2a1, CD93, Gal, Tbxas1,* and *Trim14* were identified. Single-nucleus RNA sequencing further identified 26 cell clusters in CReP brains, with Pg-derived cells showing particularly lower proportions in neural stem cell clusters compared to fertilized embryo-derived donor cells in CReF. CReP brain-derived cells exhibited reduced proliferation and increased apoptosis in culture. Notably, these cells displayed activation of the Notch signaling pathway, impairing neural stem cell maintenance and driving excessive differentiation into oligodendrocytes. These findings demonstrated that paternal alleles play a key role in regulating neural stem cell differentiation and proliferation during brain development, at least partly by modulating Notch signaling. This study highlights the indispensable roles of the paternal genome in neuronal development and the compensatory function of the parental genome in eutherian mammals.

These studies introduce two novel methods to tackle key challenges in mammalian development, offering opportunities for new insights in developmental biology and regenerative medicine.

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

東真里奈さんは、DARP培地およびCReP法というほ乳類の発生研究のための新たな手法を開発した。

DARP培地の開発においては、ES細胞の未分化性維持と多能性評価に関する一連の実験を行い、キメラ実験を通じて高品質な幹細胞の樹立を実証した。この成果は2024年にFront. Bioengineering Biotechnol.誌に共同筆頭著者として掲載された。

CRePマウスの開発においては、Pg胚由来細胞の脳発生への寄与を詳細に解析し、これまで不明であった後期発生期の単為発生胚由来細胞の異常を解析した。単為発生胚由来脳細胞での、Notchシグナルの異常活性化が神経幹細胞の動態に与える影響を明らかにすることで、父親由来ゲノムの神経発生における重要性を示した。この研究は現在論文執筆中である。

上記の研究は、幹細胞培養の再現性向上およびゲノムインプリンティングの生理的意義の解明に大きく貢献するものであり、博士の学位を授与するに値すると判断した。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る。