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論文題目	Differentiation of Hypertrophic Chondrocytes from Human iPSCs for the *In Vitro* Modeling of Chondrodysplasias （ヒトiPSCから肥大軟骨細胞への分化誘導法の確立と軟骨異形成症の *in vitro* モデリング）		

（論文内容の要旨）

Chondrodysplasias are hereditary cartilage disorders causing skeletal abnormalities as a result of mutations in genes expressed in the growth plate, but due to large variations in patient phenotypes, the effects and precise mechanisms of individual disease-causing mutations are only poorly understood. This study aims to establish an *in vitro* model of chondrodysplasias using human iPSCs to analyze the roles of different mutations in the phenotype variations between patients.

For this purpose, iPSC lines bearing mutations in the extracellular matrix components *COL10A1* and *MATN3* were established using the CRISPR/Cas9 system. Specifically, COL10A1 G18E and COL10A1 L614Rfs*8 iPSC lines were established from MCDS (metaphyseal chondrodysplasia type Schmid) patients, and MATN3 T120M iPSC lines were established from an MED (multiple epiphyseal dysplasia) patient, together with isogenic controls. Additionally, MCDS-causing COL10A1 S600P and MED-causing MATN3 R209P mutations were introduced into wild type iPSCs.

To analyze the effect of these mutations on disease phenotypes, a hypertrophic chondrocyte induction protocol was established. It was found that after sclerotome induction, chondrocytes could be further differentiated and matured into hypertrophic chondrocytes in 3D pellet culture by adding the thyroid hormone T3. During the differentiation process, the cell morphology, the expression of chondrocyte genes, and the amount of dead cells were mostly similar between mutants and controls. However, in *COL10A1* and *MATN3* mutants, COL10 and MATN3 proteins were respectively detected in the ER, whose size was significantly increased, strongly suggesting that these mutated proteins are abnormally retained within the ER. This resulted in a mild to severe increase of ER stress markers and, in some mutants, an activation of different UPR (unfolded protein response) branches, indicating that the severity of the UPR may depend on the mutation.

Upon *in vivo* transplantation, sclerotome induced from mutant iPSCs developed into growth plate-like structures that showed zonal disorganization without changes in proliferation, cell death, or mineralization. In the MATN3 T120M mutant, cells with a hypertrophic morphology were observed throughout the growth plate-like structure, whereas the COL10A1 G18E mutant lacked a distinct hypertrophic zone, implying that *MATN3* mutations result in an acceleration of hypertrophy, while *COL10A1* mutations lead to a suppression of hypertrophy *in vivo*.

Transcriptomic changes in mutants were also strongly dependent on the mutation, and only the MATN3 T120M and COL10A1 S600P mutants showed a large number of changes apart from the UPR. In both mutants, angiogenesis- and bone homeostasis-related gene expression levels were affected, indicating that the skeletal abnormalities resulting from *MATN3* and *COL10A1* mutations stem from altered bone development. However, the way in which these alterations occur likely differ depending on the mutation, as the MATN3 T120M mutant had many changes in extracellular matrix and sterol synthesis gene expression, whereas in the COL10A1 S600P mutant, the integrin signaling pathways were affected.

Finally, this *in vitro* model was tested for its applicability to drug testing. Importantly, after treatment with the chemical chaperone TMAO, known to aid in restoring the normal configuration of misfolded proteins, the MATN3 T120M mutant showed greatly decreased ER stress and intracellular MATN3 accumulation. This result strongly suggests that this *in vitro* system is usable for screening compounds that ameliorate the effects caused by mutations.

Taken together, these results show that chondrodysplasia phenotypes can be recapitulated and analyzed *in vitro* using iPSCs. This study demonstrates that despite the common detection of ER stress in chondrodysplasia mutants, the severity of the phenotype varies depending on the mutation, and the effects of each mutation on cell behavior are highly diverse. This system will serve as a platform to examine rare chondrodysplasia mutations, better understand genotype-phenotype relationships, and perform drug screening in the future.

（論文審査の結果の要旨）

軟骨異形成症は成長板に発現する様々な遺伝子の変異により骨格異常をきたす遺伝性疾患であり、病態は極めて多様である。本研究では、ヒト iPS 細胞から肥大軟骨細胞への誘導系を確立し、シュミット型骨幹端軟骨異形成症（MCDS）と多発性骨端異形成症（MED）の原因遺伝子である *COL10A1* および *MATN3* 変異 iPS 細胞株を用いて、各変異と表現型との関連を解析した。その結果、細胞外マトリックスタンパク質である COL10 と MATN3 が小胞体（ER）で蓄積して ER ストレスが発生すること、ER ストレス応答の経路と度合いが変異によって異なることが明らかになった。トランスクリプトームの変化も変異に依存しており、特に ER ストレスの強い変異株では、骨代謝や血管新生に関連する遺伝子の発現変化が認められた。iPS 細胞由来硬節細胞をマウスに移植すると、*COL10A1* 変異株では成長板組織に明確な肥大軟骨層がなく、*MATN3* 変異株では全層において肥大軟骨細胞が観察されたことから、各変異が特有の軟骨分化異常をもたらすことが示唆された。また、*in vitro* で化学シャペロン TMAO を添加すると、MATN3 の小胞体内への蓄積と ER ストレスが抑制されたことから、本研究で確立した誘導系が軟骨異形成症の治療薬の探索にも有用であることが示された。

以上の研究は、軟骨異形成症の病態解明に貢献し、本疾患の治療薬の開発に寄与するところが多い。

したがって、本論文は博士（　医科学　）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 4 年 11 月 17 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