Title	Study on Effects of Isotropic Gravity on Neurogenic Differentiation Potential in Human Mesenchymal Stem Cells
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Citation	大阪大学, 2020, 博士論文
Version Type	VoR
URL	https://doi.org/10.18910/76511
rights	
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Abstract of Thesis

Name	(Koaykul Chaiyong)
Title	Study on Effects of Isotropic Gravity on Neurogenic Differentiation Potential in Human Mesenchymal Stem Cells (等方性重力場における間葉系幹細胞の神経分化能に対する研究)

Abstract of Thesis

Human mesenchymal stem cells (hMSCs) are considered to be capable of responding to environmental changes induced by gravity. In cell expansion process, the maintenance of differentiation potential is one of the most important consideration for the efficacy of cell-based therapy. Although reactions to unidirectional gravity culture have been intensively studied in conventional culture conditions, little is known about the cellular adaptation to isotropic gravity culture conditions. Thus, this study investigated the effects of isotropic gravity on hMSCs using a 3D-clinostat (Gravite®). This study focused on changes of neurogenic differentiation capacity in passaged hMSCs between the cells cultured under different gravitational conditions. Moreover, this study also examined alterations in dynamic cell behavior, mechanotransduction, and histone methylation in hMSCs in order to understand the mechanism of cellular modification involved in their differentiation potential changes.

Chapter 1 shows the influence of isotropic gravity on modulated behavior mechanism and structural components relating to migration and mechanotransduction. Time-lapse observation revealed that cells cultured under unidirectional gravity conditions had unidirectional migration, while cells cultured under isotropic gravity conditions had multidirectional migration with active extension of leading edge and partially contraction of cell rear. Cells cultured under unidirectional gravity presented the maintenance of their spindle-shape via fibronectin fibril formation in their bodies as well as stabilization of focal adhesions with enriched apical actin stress fibers. However, cells cultured under isotropic gravity conditions had more intense of phosphorylated paxillin in regions of leading and trailing edges together with the up-regulation of MT1-MMP expression. Moreover, cells in isotropic gravity conditions showed fibronectin mainly as aggregate structure with less focal contacts and few apical actin stress fibers. Lastly, cells cultured under unidirectional gravity conditions mostly presented a basal-to-apical polarization of mechano-sensitive nuclear lamin A/C, but cells cultured under isotropic gravity conditions mainly showed the non-polarization of lamin A/C. Therefore, this chapter demonstrates that isotropic gravity-driven fibronectin assembly affects nuclear lamina organization through the spatial reorganization of actin cytoskeleton influencing on changes in cell behavior and mechanotransduction.

Chapter 2 presents the effects of isotropic gravity on nucleoskeleton, epigenetics, and neurogenic differentiation potential of passaged hMSCs. During serial cultivation with hMSC growth medium, the analysis of lamin organization and histone modifications at promoters of neurogenic lineage genes indicated that cells passaged under isotropic gravity conditions sustained the ratio between lamin A/C to lamin B together with preservation of histone methylation during passage culture. However, the lamin ratio and H3K27me3 enrichment significantly increased in cells grown under unidirectional gravity conditions against their increasing passage numbers. In neurogenic induction culture, differentiated cells from the cells cultured under isotropic gravity conditions expressed higher neurogenic levels than those cultured under unidirectional gravity conditions. The levels of neurogenic markers in the cells from isotropic gravity conditions were consistent during an increase in their passage numbers, while cells from unidirectional gravity conditions exhibited a reduction of the neurogenic levels against the increased passage numbers. These results demonstrate that isotropic gravity-coordinated lamin organization leads to the suppression of histone modification associated with maintenance of neurogenic differentiation potential in passaged hMSCs.

Taken together, this study is the first time to provide an insight into the effects of isotropic gravity on neurogenic differentiation potential in hMSCs. The pioneered data of cell behavior, mechanotransduction, lamin organization, and histone modification promote the directivity of neurogenic differentiation in hMSCs during cell expansion that would be the supportive information for applications in cell-based therapy.

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

本博士論文は，幹細胞の平面培養時における細胞挙動，エピジェネティック，分化能について，従来の単方向重力負荷条件下と等重力分散負荷条件下で比較を行っており，メカノトランスダクションについて議論を行っている．

第 1 章では，等重力分散負荷条件が，ヒト間葉系幹細胞の細胞遊走とメカノトランスダクションに関連する細胞挙動と骨格形成へ及ぼす影響について示されている．単方向重力負荷条件下で培養された細胞は，単方向遊走が見られ，一方，等重力分散負荷条件下では，細胞先端での伸展と細胞尾部の収縮が活性化されることにより生じる多方向遊走が観察されている．これらは，細胞遊走にかかわるパキシリンのリン酸化およびプロテアーゼの活性化によることを明らかにしている．さらに，骨格形成では，細胞上部側のストレスファイバーの未発達が見られ，その結果，核膜タンパクのラミン A/C の局在性が現れ，これは，メカノトランスダクションによることを示している．

第 2 章では，等重力分散負荷条件下での間葉系幹細胞の継代培養において，細胞核の骨格構造，エピジェニック，神経系分化能について検討を行っている．ラミン形成及び神経系分化にかかわるプロモータに関係するヒストン修飾について検討を行い，メチル化を阻止していることが示されている．3 回の継代を経た細胞を用い，神経系分化誘導を行ったところ，高い分化能を維持していることが示されている．これらのことより，等重力分散負荷条件下で培養は，細胞におけるラミンの形成が神経系分化能を低下させるヒストン修飾を阻止することを示している．

以上のように、本論文は，等重力分散負荷条件下でヒト間葉系幹細胞を平面培養した時，細胞挙動，核膜タンパク形成，エピジェネティック，分化能について，従来の一方向への重力負荷条件下での培養と比較することで，メカノトランスダクションと神経系分化能力の維持について報告を行っている．よって本論文は博士論文として価値あるものと認める。