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科学研究費助成事業　　研究成果報告書

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研究課題名（和文）High efficient myogenic differentiation of human pluripotent stem cells on New Generation Laminins towards regenerative medicine.

研究課題名（英文）High efficient myogenic differentiation of human pluripotent stem cells on New Generation Laminins towards regenerative medicine

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研究成果の概要（和文）：ヒトiPS細胞から骨格筋細胞および骨格筋幹細胞を高効率に分化誘導する系を、動物由来成分を含まないマトリックス（NGL：次世代ラミニン）を用いて確立する事を本研究の目的とした。我々の筋分化誘導プロトコルにおいてNGLは、動物由来のマトリゲルを使用した場合より、高効率に骨格筋前駆細胞を増加させるだけでなく、骨格筋細胞や骨格筋幹細胞も増加させる事がわかった。これらの結果は、NGLの特異的構造がFGF受容体シグナルを増幅させるためであるという事もわかった。この新しい筋細胞分化誘導系は、高品質な筋疾患モデルの作成を可能にしただけでなく、ゼノフリーの筋細胞は将来の臨床応用にも有用であると考えられる。

研究成果の学術的意義や社会的意義
Instead of animal derived matrix, using Xeno-free New Generation Laminin, we established a highly efficient differentiation system for myocytes and muscle stem cells induction from hiPSCs. This research will be very useful for disease modeling and cell therapy based on hiPSCs.

研究成果の概要（英文）：In this study, we aimed to use a xeno-free matrix establising a highly efficient differentiation system for inducing myocytes and muscle stem cells from hiPSCs. Instead of animal derived Matrigel, the NGL (New Generation Laminin) increases muscle progenitor cells, subsequently, increase the amounts of myocytes and muscle stem cells from hiPSCs in the stepwise differentiation protocol. Our results demonstrate that the unique crystal structure of NGL supports the highly efficient myogenic differentiation through FGF receptor signaling pathway regulated by heparan sulfates (HS). These novel effects of NGL can't be replaced by treating with high dose of FGF2 or coating the mixture of laminin421 E8 and perlecan. Using this NGL, we established a highly efficient differentiation system for myocytes and muscle stem cells induction from hiPSCs for disease modeling and clinical application.

研究分野：幹細胞生物学

キーワード：次世代ラミニン　iPS細胞　骨格筋幹細胞　再生医学

様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９、ＣＫ－１９（共通）

１．研究開始当初の背景

Duchenne muscular dystrophy (DMD), the most common and severe form of muscular dystrophy, accompanies with muscle degeneration that can not be combated until now. It may be treatable with stem cell therapy, if healthy muscle stem cells be generated from human induced pluripotent stem cells (hiPSCs).

Our group has established a strategy for generating skeletal muscle stem cells from hiPSCs. In this system, hiPSCs were developed into muscle stem cells which can regenerate dystrophin+ fibers in DMD model mouse. However, this approach shows low efficiency and requires Matrigel (MG) which is derived from mouse tumors. For clinical application, establishing high efficiency and xeno-free (animal derived substance-free) environment is necessary.

Laminins are a family of glycoproteins present in the basement membrane. All laminins are large heterotrimeric glycoproteins composed of α, β, and γ chains that assemble into a cross- shaped structure. Recombinant Laminin E8 fragments (LN-E8s), serving as a functionally minimal form, has been used as xeno-free coating substrata for in vitro cultures of hiPSCs in numerous studies. In previous study, we sought to replace Matrigel from our system by using LN-E8s (LN111E8, LN211E8, LN332E8, LN411E8, LN421E8 and LN511E8). Unfortunately, all of these LN-E8s provided relatively lower efficient myogenic differentiation comparing with Matrigel, indicating Matrigel contained heparan sulfate proteoglycan (Perlecan) supporting the differentiation in our system.

Perlecan is a multifunctional heparan sulfate proteoglycan that controls cell-signaling events by interacting with several growth factors, cytokines, and other signaling molecules. Cooperating with Dr. Sekiguchi in Osaka University, we designed new generation laminin (NGL) which combined perlecan to the C-termius of Laminin-421 E8 fragment (p421). We found that p421 provided highly efficient myogenic differentiation in our differentiation system.

However, the mechanism of p421 increasing myogenic differentiation is still unclear. The p421-dependent new differentiation system still needs well established.

２．研究の目的

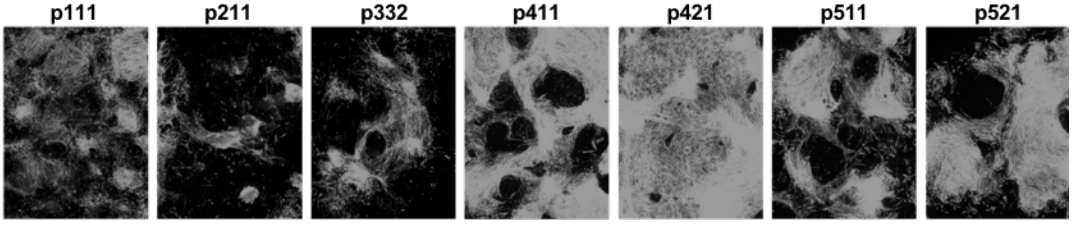
1. Using new generation laminin (NGL), establish a highly efficient differentiation system for myocytes and muscle stem cells induction from hiPSCs for disease modeling and cell therapy.
2. Elucidate the mechanism of NGL regulating myogenic differentiation from hiPSCs.

３．研究の方法

Compare p421 with other laminin-perlecan fragments which contain different α, β or γ chain, then establish a xeno-free system for myogenic differentiation from hiPSCs. Knock out heparin sulfate (HS) in p421 by heparitinase. Screen the cell signaling pathway which can be regulated by perlecan, then check the downstream of this pathway.

４．研究成果

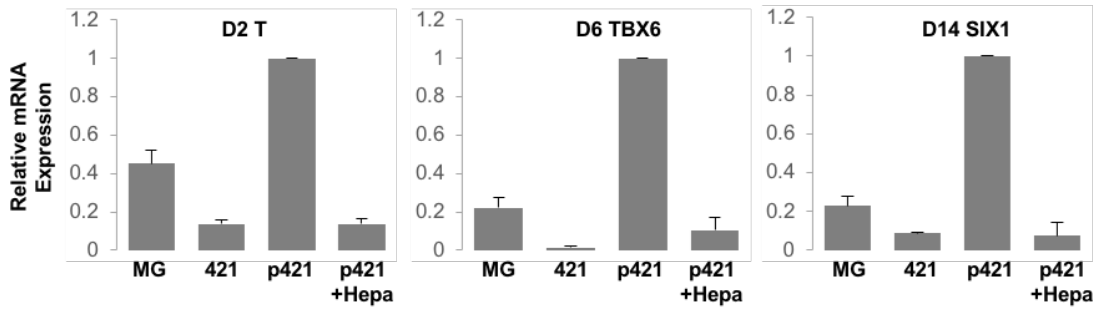
Fig. 1 p421 provided the most efficient myogenic differentiation



Immuno-staining of myosin heavy chain in D38 of differentiated myocytes derived from hiPSCs.

By screening all of the NGL (New Generation Laminin) isoforms which could be obtained, we revealed that p421 provided the most efficient myogenic differentiation [Fig.1]. Using p421 we successfully established a more efficient differentiation system for myocytes and muscle stem cells induction from hiPSCs. p421 promotes the development of paraxial mesoderm, dermomyotome, myogenic progenitors and muscle stem cells from hiPSCs in the stepwise differentiation protocol. The Heparan sulfates digestion experiment revealed that the unique effects of p421 is provided by HS (Heparan sulfate) [Fig.2].

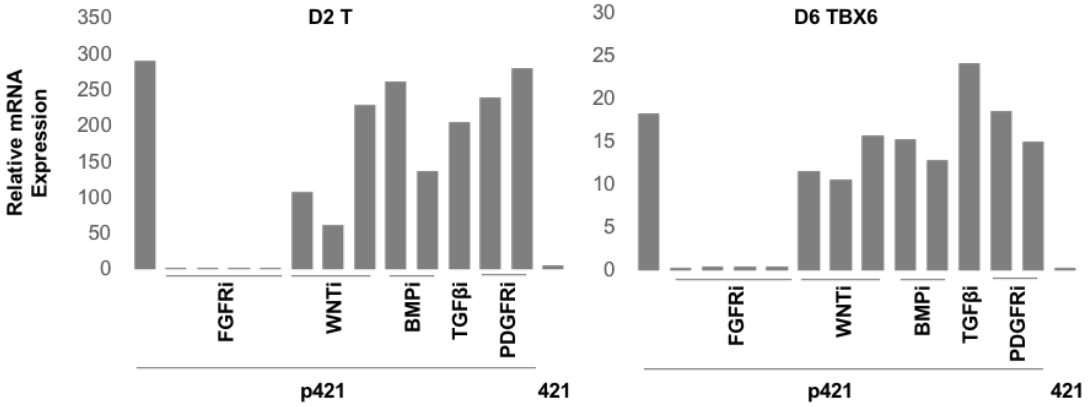
Fig. 2 p421 provided primitive streak, paraxial mesoderm and dermomyotome differentiation by HS



p421 increased the marker genes expression of T (day2), TBX6 (day6) and SIX1 (day21). The effects of p421 were significantly decreased by cutting HS by heparitinase.

By screening the pathways of FGF, WNT, BMP, TGFβ, PDGF signaling, we revealed that p421 regulating myogenic differentiation by regulating FGFR signaling pathway. HS bind to fibroblast growth factor-2 (FGF2), form a stable high affinity HS-FGF2-FGFR complexes on cell surface, then strongly stimulate FGFR signaling pathway. These effects depend on the unique structure of NGL, that could not be replaced by treating with high dose of FGF2 or coating the mixture of laminin421 E8 and perlecan.

Fig. 3 p421 provided myogenic differentiation though FGFR signaling pathway



Screen the pathways of FGFR, WNT, BMP, TGFβ, PDGF signaling pathway were screening by chemical inhibitors. Only FGFR inhibitors remarkably decrease gene expression of T (D2) and TBX (D6) in p421 provided myogenic differentiation.

Using this xeno-free matrix, we established a highly efficient differentiation system for hiPSCs induced paraxial mesoderm lineage, subsequently, paraxial mesoderm derived myocytes and muscle stem cells. It will be useful for iPSCs derived skeletal muscle disease modeling and clinical application.

５．主な発表論文等

〔雑誌論文〕（計　１件）

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② 趙明明, 次世代型ラミニン E8 フラグメントを活用した高効率分化誘導法の確立とメカニズム解明,　日本筋学会第 3 回学術集会,　2017 年-2018 年

③ Mingming Zhao, New generation laminin supports efficient myogenic differentiation of human induced pluripotent stem cells, 第 5 回骨格筋細胞研究会, 2017 年-2018 年

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〔図書〕(計　　件)

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○取得状況（計　　件）

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ホームページ等

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