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研究課題名（和文）ヒト多能性幹細胞からの腱・靭帯誘導法の開発

研究課題名（英文）Induction of tendon/ligament progenitor cells from human iPSCs

研究代表者

ＡＬＥＶ　ＣＡＮＴＡＳ（ALEV, CANTAS）

京都大学・iPS細胞研究所・特定拠点助教

研究者番号：３０７２６４７７

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研究成果の概要（和文）：我々はin vitroでiPS細胞から、ヒトの腱/靱帯前駆細胞(TLPC)の段階的な誘導法を確立した。この目標を達成するために、我々はTLPCの胚発生時の段階的な分化を模倣した。iPS細胞からのヒト未分節中胚葉や体節中胚葉の段階的誘導法を利用し、続いてTLPC特異的転写因子であるscleraxis (SCX)やmohawk (MKX)を発現している靱帯節細胞の誘導を行った。最終的に、我々の定義したフィーダーフリーのiPS細胞を基にした誘導法は、in vitroで腱靱帯付着部様のTLPCを誘導することに成功した。これらの細胞は疾患モデルの構築や細胞補充療法に非常に有益であると考えられる。

研究成果の概要（英文）：We succeeded to establish a step-wise in vitro induction protocol of human tendon/ligament progenitor cells (TLPCs) from iPSCs. To this end we aimed to mimic the emergence of TLPCs during embryonic development. We utilised a step-wise chemically defined feeder-free iPSC-based induction protocol of human pre-somitic and somitic mesoderm from iPSCs, followed by the in vitro induction of human syndetome cells expressing the TLPC-specific transcription factors scleraxis (SCX) and mohawk (MKX). In addition TLPCs with enthesis-like features could be also derived. These in vitro induced human tendon/ligament progenitor cells have the potential to be used for disease modelling and cellular replacement therapy.

研究分野：　Stem Cell Biology, Developmental Biology

キーワード：　Tendon Progenitor Cell　iPSCs　Mesoderm Differentiation

１．研究開始当初の背景

Background:

Tendons and ligaments, strong extracellular matrix (ECM) rich but still elastic dense fibrous connections between designated skeletal elements (e.g. bones and muscles), have limited regenerative capacity and injuries of these important anatomical structures are currently difficult to treat. Furthermore tendons and ligaments are the targets of several debilitating diseases, which largely lack adequate therapeutic options as well as appropriate cellular model systems suitable to study these diseases *in vitro*. The successful and efficient *in vitro* differentiation of tendon/ligament progenitor cells from human iPSCs would thus provide medical and basic researchers with an invaluable tool and novel option for the treatment and study of tendon/ligament related injuries and diseases.

So far, there has been no published report regarding the successful *in vitro* induction of tendon and/or ligament progenitors from human ES/iPS cells. Our proposed *in vitro* differentiation protocol follows and mimics the step-wise emergence of tendon/ligament progenitor cells during development. Tendon and ligament progenitor cells (of the axial skeleton) arise hereby from the so called syndetome, located between the dermo-myotome and sclerotome compartments of forming somites. We utilize hereby our expertise in embryology and knowledge about the *in vivo* active signaling cascades and specific growth/differentiation conditions associated with mesoderm and syndetome development.

２．研究の目的

Purpose:

The core aim of the research project was the efficient step-wise *in vitro* induction of tendon/ligament progenitor cells (TLPCs) from human induced pluripotent stem cells (iPSCs), while mimicking their emergence during embryonic development. We focused hereby initially on the *in vitro* induction of tendon/ligament progenitor cells of the axial skeleton. Molecular analysis of the induced TLPCs as well as establishment of fluorescent reporter-lines of TLPC-specific genes, were additional aims of this research project.

３．研究の方法

Method of research:

Human induced pluripotent stem cell (iPSC) based *in vitro* induction and differentiation protocols were applied in order to derive in a step-wise fashion, the desired tendon/ ligament progenitor cells, mimicking their emergence during embryonic development. Our chemically-defined feeder-free two- dimensional TLPC induction protocol was based on the step-wise and successive induction of human primitive streak, pre-somitic mesoderm and somitic mesoderm cells, with the latter ones being used as starting material for the subsequent induction, proliferation and maturation of human syndetome cells. CRISPR/Cas9- mediated genome editing technology was further

utilized for the establishment of TLPC-specific fluorescent reporter lines. Fluorescent and selection cassettes are hereby being targeted via suitable guide RNAs into the 3'UTR regions adjacent to the stop-codon of the genes of interest (e.g. SCX (*scleraxis*)).

４．研究成果

Research result: We succeeded with the establishment of a fully-defined feeder-free step-wise protocol for the *in vitro* induction of human paraxial mesoderm and somitic mesoderm from iPSCs, which was further used as the starting material for the *in vitro* derivation of human syndetome cells containing tendon/ligament progenitor cells of the axial skeleton. SCX or MKX positive human syndetome cells could be induced from iPSC-derived somitic mesoderm via timely exposure to combination of small molecules and recombinant human growth factors. Initial experiments for the *in vitro* maturation of induced TLPCs into enthesis-like cells were performed, yielding promising preliminary results. The induced cell populations were further analyzed and validated via qPCR, FACS and antibody staining. Preliminary results were also obtained towards CRISPR/Cas9-based generation of fluorescent reporter lines of TLPC-specific transcription factors (e.g. SCX (*scleraxis*)).

We have in summary succeeded with the establishment of a so-far elusive method to induce tendon & ligament progenitor cells from human iPSCs. These *in vitro*-derived TLPCs could be used to study and model debilitating diseases affecting tendons and ligaments, for which currently no *in vitro* disease model systems are available. In the mid-to-long term these cells might be also utilized for advanced cellular and regenerative therapeutic approaches aiming to treat acute injuries or chronic diseases of tendons and ligaments such as *Ossification of the posterior longitudinal ligament* (*OPLL*).

Our research provides furthermore invaluable insights into the development of human tendon/ligaments progenitor cells (of the axial skeleton), which is due to ethical and practical reasons otherwise not accessible.

５．主な発表論文等

（研究代表者、研究分担者及び連携研究者には下線）

〔雑誌論文〕（計 0 件）

なし

〔学会発表〕（計 5 件）

① Alev C., Matsuda M., Yamanaka Y., Uemura M., Osawa M., Saito M., Yamamoto T., Yoshitomi H., Toguchida J., Woltjen K., Ebisuya M. CRISPR/Cas9 mediated genome editing in human pluripotent stem cells for modeling human skeletal development and diseases. The 9th Takeda Science Symposium on Pharma-Sciences, "Genome Editing Towards Medicinal Applications", Osaka, Japan, February 7 – 8, 2018

② Matsuda M., Uemura M., Yamanaka Y., Saito M., Osawa M., Ikeya M., Yamamoto T., Yoshitomi H., Toguchida J.,

Woltjen K., Ebisuya M., Alev C. Modeling the human segmentation clock with pluripotent stem cells. 15th Annual Meeting of the International Society for Stem Cell Research (ISSCR), Boston, MA, USA, June 14-17, 2017

③ Matsuda M., Uemura M., Yamanaka Y., Saito M., Osawa M., Ikeya M., Yamamoto T., Yoshitomi H., Toguchida J., Woltjen K., Ebisuya M., Alev C. Modeling the segmentation clock with pluripotent stem cells. Annual 50th Annual Meeting of the Japanese and Society for Developmental Biology (JSDB), Tokyo, Japan, May 10-13, 2017

④ Matsuda M., Uemura M., Yamanaka Y., Saito M., Osawa M., Ikeya M., Yamamoto T., Yoshitomi H., Toguchida J., Woltjen K., Ebisuya M., Alev C. Establishment of a pluripotent stem cell based model of the segmentation clock. RIKEN Center for Developmental (CDB) Symposium 2017 “Towards Understanding Human Development, Heredity, and Evolution”, Kobe, Japan, March 27-29, 2017

⑤ Matsuda M., Uemura M., Yamanaka Y., Saito M., Osawa M., Ikeya M., Yamamoto T., Yoshitomi H., Toguchida J., Woltjen K., Ebisuya M., Alev C. Modeling the segmentation clock with pluripotent stem cells. Joint Meeting of the German and Japanese Societies of Developmental Biologists, Zoological Institute, Kiel, Germany, March 15-18, 2017

〔図書〕（計　0 件）

なし

〔産業財産権〕

○出願状況（計 0 件）
なし

○取得状況（計 0 件）
なし

６．研究組織
(1)研究代表者
アレブ　ジャンタッシュ（ALEV, Cantas）
京都大学・iPS 細胞研究所・特定拠点助教
研究者番号：３０７２６４７７

(2)研究分担者
池谷　真（IKEYA, Makoto）
京都大学・iPS 細胞研究所・准教授
研究者番号：２０４４２９２３

(3)連携研究者
ウォルツェン　クヌート（WOLTJEN, Knut）
京都大学・iPS 細胞研究所・准教授
研究者番号：５０５８９４８９

(4)連携研究者
戸口田　淳也（TOGUCHIDA Junya）
京都大学・ウイルス・再生医科学研究所・教授
研究者番号：４０２７３５０２

(5)連携研究者
シェン　ゴージュン（SHENG Guojun）
熊本大学・国際先端医学研究機構・教授
研究者番号：２０３９９４３９