氏　　名	任（れん）　鮮英（しぇんいん）
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学位論文題目	A novel human artificial chromosome vector provides effective cell lineage-specific transgene expression in human mesenchymal stem cells （新規 HAC ベクターによる間葉系幹細胞の分化に伴う組織特異的遺伝子発現）
学位論文審査委員	（主査）　佐藤健三 （副査）　林眞一　　押村光雄

学位論文の内容の要旨

In cell-based gene therapy, human bone marrow-derived mesenchymal stem cells (MSCs) draw attention as a potential progenitor cell source for repair and regeneration of diverse adult tissues, including fat, cartilage, bone, marrow stroma and skeletal muscle. One of the major aims of stem cell-mediated gene therapy is to develop vectors that will allow appropriate levels of expression of therapeutic genes along differentiation under physiological regulation of the specialized cells. Human artificial chromosomes (HACs) are stably maintained as independent chromosomes in host cells and should be free from potential insertional mutagenesis problems of conventional transgenes. Therefore, HACs have been proposed as alternative implements to cell-mediated gene therapy. Previously, we constructed a novel HAC, termed as 21Δpq HAC, with a loxP site in which circular DNA can be reproducibly inserted by the Cre/loxP system. We here assessed feasibility of lineage-specific transgene expression by the 21Δpq HAC vector, utilizing *in vitro* differentiation system with a MSCs cell line, hiMSCs, which has potential for osteogenic, chondrogenic, and adipogenic differentiation.

Methods

The hiMSCs are capable of *in vitro* tri-directional differentiation into chondrocytes, adipocytes and osteocytes in response to appropriate culture stimuli. We made two types of osteopontin *(OPN)-EGFP* reporter constructs, with or without insulators in both sides of

OPN-EGFP expression units. These constructs are site-specifically inserted into the loxP site on the HAC via Cre-loxP system. Then, these HACs are introduced into the hiMSCs by microcell mediated chromosome transfer. FISH analysis is used to test the stability of HAC vector in hiMSCs. Expression status of the reporter gene in the hiMSCs hybrids containing these HAC vectors were tested before and after induction of osteogenic- and adipogenic -differentiation.

Results

HAC vector is successfully introduced into the hiMSCs and is stably maintained over 100 population doubling levels. In addition, the HAC does not affect the differentiation ability of hiMSCs. Two types of *OPN-EGFP* reporter construct, with or without insulators in both sides of *OPN-EGFP* expression units are site- specifically inserted onto the HAC in the CHO cells. Then, these HACs were introduced into hiMSCs via microcell mediated chromosome transfer. The *EGFP* gene was specifically expressed in the hiMSCs hybrid clones containing the HAC with Insulator-OPN-EGFP, that differentiated into osteocytes in coordination with the transcription of endogenous *OPN* gene, but was not expressed after adipogenic differentiation induction or in non-induction culture. However, the hiMSCs containing the HAC without the insulator in the OPN-EGFP reporter construct expressed the EGFP in non-induction culture.

Discussion

In the present study, we aimed at addressing whether the 21Δpq HAC vector could provide lineage-specific expression of a transgene, utilizing *in vitro* differentiation system in a MSCs cell line. Our results demonstrated that the 21Δpq HAC vector allowed inducible expression of EGFP gene driven by *OPN* promoter in hiMSCs differentiated into osteogenic lineage. These results suggest that use of the HACs vector is suitable for regulated expression of transgenes in stem cell-mediated gene therapy.

審 査 結 果 の 要 旨

本研究はヒト人工染色体（21Δpq HAC）により導入した遺伝子の分化誘導に伴う組織特異的な発現をヒト骨髄由来の間葉系幹細胞を用いて検討したものである。ヒト間葉系幹細胞の細胞株hiMSCsに21ΔpqHACベクターを移入し、未分化状態でのHACベクターの安定性とhiMSCsの多分化能を検討した。次いで MSCs から骨芽細胞への分化マーカーのひとつであるヒトOsteopontin(OPN)遺伝子プロモーターの制御下にEGFP遺伝子を繋いだレポーターコンストラクトを作製し、レポーター遺伝子の挿入された21ΔpqHAC ベクターを hiMSCs に移入し、骨が

細胞への分化誘導に伴うEGFPの発現を検討した。その結果、21ΔpqHACベクターがhiMSCsにおいて安定に保たれ、hiMSCsの多分化能に影響しないこと、さらにOPN-GFP/HACベクターを保持しているhiMSCsは骨芽細胞に分化した時のみGFPを発現し、脂肪細胞への分化あるいは未分化状態ではGFPを発現しなかったことを示した。本論文の内容は、従来のベクターとは全く違う新規のベクターとしてのヒト人工染色体の有用性を示したものであり、ヒト人工染色体の再生医療への応用などの研究分野において明らかに学術水準を高めたものと認める。