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Translin modulates mesenchymal cell proliferation and differentiation in mice

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ABSTRACT

Translin, a highly conserved DNA/RNA binding protein that forms a hetero-octamer together with Translin-associated factor X (TRAX), possesses a broad variety of functions, including RNA processing and DNA repair. Recent studies have reported that Translin is involved in mesenchymal cell physiology. Thus, here we analyzed the intrinsic role of Translin in mesenchymal cell proliferation and differentiation. Translin-deficient E11.5 mouse embryonic fibroblasts showed enhanced growth. Translin-deficient bone marrow-derived mesenchymal stem cells showed substantial expansion *in vivo* and enhanced proliferation *in vitro*. These cells also showed enhanced osteogenic and adipocytic differentiation. Histological analyses showed adipocytic hypertrophy in various adipose tissues. *Translin* knockout did not affect the growth of subcutaneous white adipose tissue-derived stem cells, but enhanced adipocytic differentiation was observed *in vitro*. Contrary to previous reports, *in vitro*-fertilized Translin-null mice were not runted and exhibited normal metabolic homeostasis, indicating the fragility of these mice to environmental conditions. Together, these data suggest that Translin plays an intrinsic role in restricting mesenchymal cell proliferation and differentiation.

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1. Introduction

Translin, a nuclear protein that is phylogenetically conserved in eukaryotes, constitutes a hetero-octamer together with Translin-associated factor X (TRAX) [reviewed in Refs. [1,2]]. Translin was originally discovered as a protein that binds to consensus DNA sequences at breakpoint junctions of chromosomal translocations in lymphoid neoplasms and was postulated to have a biological role in genome stability [3]. Indeed, the role for Translin/TRAX complex in DNA repair especially in relation with the action of ATM, a major component for double-stranded DNA repair, is now highlighted [[4], reviewed in Refs. [1,2]]. On the other hand, elucidation of the structural biology of the Translin/TRAX complex has led to the discovery of its greater affinity for single-stranded RNA/DNA and its endoribonuclease activity, which mediates processing of microRNAs and tRNAs [5–7]. However, the biological significance of Translin has not been fully documented.

Genetic ablation of murine *Translin* has allowed us to uncover the role Translin plays in a variety of biological phenomena, including cell growth [8], neurological development [9], and spermatogenesis [10]. Translin's role in mesenchymal cell physiology is of particular interest; *Translin* knockout (KO) mice have previous been shown to exhibit obesity (suggesting adipocytic hypertrophy), proliferation of osteoblasts, endothelial cells, adipocytes, and osteocytes in bones and bone marrow (BM), altered endochondral ossification of long bones, and proliferation of fibroblasts in the liver [1,11,12]. All of these phenotypes appear to be related to mesenchymal cell dysfunction, indicating that Translin plays a pivotal role in mesenchyme physiology. However, it remains unknown whether they reflect Translin's intrinsic activities at a cellular level.

In order to define Translin's inherent role in mesenchymal cell proliferation and differentiation, we conducted cell biology

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analyses by using mice-derived mesenchymal cells. Additionally, we re-defined the runt phenotype previously found in *Translin* KO mice [13], using *in vitro*-fertilized mice. We propose that Translin is a physiological restrictor of early-stage mesenchymal cell proliferation and differentiation and provides protection from the environment.

2. Materials and methods

2.1. Mice

Translin KO mice, backcrossed at least 10 times with C57BL6 [11], were kindly provided by Masataka Kasai. For genotyping, crude genomic DNA was used for PCR analysis (primer sequences available upon request). Male mice were analyzed unless otherwise specified. All animal experiments were performed according to the institutional guidelines set forth by Tokyo Medical University, and Kobe BM Laboratory, Oriental Bioservice, Inc.

For *in vitro*-fertilized mice, two-cell embryos obtained by *in vitro* fertilization of *Translin*$^{-/-}$ sperms and *Translin*$^{+/-}$ ova were transferred into pseudo-pregnant ICR mice (Sankyo Labo Service, Tokyo, Japan). Pups were housed with their individual surrogate mothers until weaning had been achieved.

2.2. Cell culture and colony-forming units fibroblast (CFU-Fs)

Mouse embryonic fibroblasts (MEFs) were prepared from E11.5 embryos that were obtained via heterozygous crosses, and were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS). MEFs at passage 2 were used for studies.

BM-derived CFU-Fs were obtained by cultures of BM cells (1.0×10^6 per 6-well plates) derived from femora and tibiae of 39-week-old males in MesenCult MSC Basal Medium supplemented with MesenCult Mesenchymal Stem Cell Stimulatory Supplements (StemCell Technologies). After 13 days of culture, May-Grünwald Giemsa staining was performed and CFU-Fs were counted. For BM-MSCs, BM-derived CFU-Fs were trypsinized, harvested and cultured in DMEM with 10% FBS.

For adipose-derived stem cells (ADSCs), subcutaneous white adipose tissues (WATs) were minced and agitated in DMEM with 0.04% collagenase (FUJIFILM Wako) for 1 h at 37 °C, after which sediment was removed. Precipitated ADSCs were then cultured in MEM α, GlutaMAX™ Supplement, no nucleosides (Life Technologies), with 10% FBS.

2.3. Cell growth and DNA synthesis

For cell growth, cells (2×10^3 or 2×10^4) were plated on 24-well plates and counted after trypsinization. For DNA synthesis, bromodeoxyuridine (BrdU) was added to the cells on 24-well plates. After purging for 6 h, the incorporation of BrdU was measured as previously described [14].

2.4. Osteogenic differentiation of BM-MSCs

When cells reached 30–50% confluency, osteogenic differentiation was induced by exchanging the media for StemXVivo Human/Mouse Osteogenic Base Media (R&D Systems) supplemented with 5% StemXVivo Mouse Osteogenic Supplement (R&D Systems) by volume. After induction, cells were subjected to staining or extraction of total RNA with Isogen II (Toyobo) and quantification. Staining was performed with one of two stains; for alkaline phosphatase, cells were fixed with 4% paraformaldehyde for 15 min and stained with VECTOR Red Alkaline Phosphatase Substrate Kit (Vector Laboratories) according to the manufacturer's protocol. For Alizarin Red S, cells were fixed in 95% ethanol, washed with H_2O, and stained with 1% Alizarin Red S (FUJIFILM Wako) for 30 min.

2.5. Adipocytic differentiation of BM-MSCs and ADSCs

Adipocytic differentiation was induced using AdipoInducer Reagent (TaKaRa) according to the manufacturer's protocol when cells reached 80–90% confluency. Medium was exchanged twice a week. After induction, the cells were either stained for lipid droplets with Oil Red O (FUJIFILM Wako), or total RNA was extracted. After Oil Red O staining, the dye was extracted by incubating cells with isopropanol for 30 min, and quantified by measuring the optical density at 450 nm (OD_{450}).

2.6. Histological analysis

Tissues were stored in 10% neutral formalin and embedded in paraffin blocks; each section was deparaffinized, rehydrated, and stained with hematoxylin and eosin.

2.7. Quantitative RT-PCR

Total cellular RNA (0.5 μg) was reverse-transcribed with ReverTra Ace qPCR RT Master Mix with a gDNA Remover kit (Toyobo). Quantitative PCR (StepOnePlus Real-Time PCR system; Thermo Fisher Scientific) was performed for quantification of mRNA. Mouse glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used to normalize results. The sequences of the primers used in amplification are available upon request.

2.8. Statistical analyses

All numerical results were expressed as means ± SD. Data were compared using Student's t-test (two groups) or one-way analysis of variance (ANOVA) (more than two groups). Two-way ANOVA was used for studies over a prolonged time and, when significant, values for each time point were evaluated by Student's t-test or one-way ANOVA. We considered $P < 0.05$ as significant. $P < 0.05$ and $P < 0.01$ were represented by * and **, respectively.

3. Results

3.1. Translin restricts growth of MEFs

In previous studies, *Translin*$^{-/-}$ mice were reported to be smaller [13] and *Translin*$^{-/-}$ E14.5 MEFs were found to grow more slowly [8] than *Translin*$^{+/+}$ and *Translin*$^{+/-}$ controls, suggesting that Translin intrinsically promotes cell growth. By contrast, in another study, *Translin*$^{-/-}$ mice displayed obesity in addition to increased numbers of osteoblasts and osteocytes, adipocytes, endothelial cells, and fibroblasts in aged *Translin*$^{-/-}$ bones and liver [11], indicating that Translin restricts mesenchymal cell growth. To resolve these discrepancies, we hypothesized that Translin restricts the growth and differentiation solely of immature mesenchymal cells. To test this hypothesis, we studied the effects of *Translin* KO on MEFs of a more primitive stage, namely, E11.5.

Indeed, contrary to the slow growth of *Translin*$^{-/-}$ E14.5 MEFs [8], the growth rates of *Translin*$^{+/+}$, *Translin*$^{+/-}$ and *Translin*$^{-/-}$ E11.5 MEFs were inversely proportional to *Translin* levels (Fig. 1A). The BrdU incorporation assay to measure MEF mitogenicity revealed enhanced DNA synthesis in *Translin*$^{-/-}$ and *Translin*$^{+/-}$ MEFs compared with that in *Translin*$^{+/+}$ MEFs (Fig. 1B). These results indicated that Translin specifically restricts early-stage mesenchymal precursor cells.

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