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The effects of actin cytoskeleton perturbation on keratin intermediate filament formation in mesenchymal stem/stromal cells

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ABSTRACT

F-actin plays a crucial role in composing the three-dimensional cytoskeleton and F-actin depolymerization alters fate choice of mesenchymal stem/stromal cells (MSCs). Here, we investigated differential gene expression and subsequent physiological changes in response to F-actin perturbation by latrunculin B in MSCs. Nineteen genes were down-regulated and 27 genes were up-regulated in the first 15 min after F-actin depolymerization. Functional enrichment analysis revealed that five genes involved in keratin (KRT) intermediate filaments clustering in the chromosome 17q21.2 region, i.e., KRT14, KRT19, KRT34, KRT-associated protein (KRTAP) 1-5, and KRTAP2-3, were strongly up-regulated. Transcription factor prediction identified NKX2.5 as the potential transcription factor to control KRT19, KRT34, KRTAP1-5, and KRTAP2-3; and indeed, the protein level of NKX2.5 was markedly increased in the nuclear fraction within 15 min of F-actin depolymerization. The peak of keratin intermediate filament formation was 1 h after actin perturbation, and the morphological changes showed by decrease in the ratio of long-axis to short-axis diameter in MSCs was observed after 4 h. Together, F-actin depolymerization rapidly triggers keratin intermediate filament formation by turning on keratin-related genes on chromosome 17q21.2. Such findings offer new insight in lineage commitment of MSCs and further scaffold design in MSC-based tissue engineering.

1. Introduction

Actin filaments, microtubules and intermediate filaments are the three major types of protein filaments that form the cytoskeleton in mammalian cells. Formation of cytoskeletal filaments is dynamic in eukaryotic cells, and the cytoskeletal arrangement needs to be reorganized to attain different physical properties [1] Polymerized actin filaments (F-actins) are composed of two-stranded helical polymers of actin monomers (G-actin). The self-assembly and dynamic structures of cytoskeletal filaments contribute to the control of cell migration, proliferation, and shape stability [2]. The organization of the F-actin network maintains the 3-dimensional cell structure that delivers signals from the extracellular matrix (ECM) via integrin-actin connections [3] and signals from the cell–cell junctions via cadherin-actin complexes [4]. F-actins are sensitive to mechanical forces [5], and patterning of the actin cytoskeleton is crucial for morphological events during development such as tissue orientation, vasculogenesis, and stem cell differentiation [6].

Engineering of the biomaterials in the scaffold to guide the fate determination of stem cells through cell–matrix interaction by regulating integrin signals and actin cytoskeleton organization has been an attractive approach to offer proper cues for the cells seeded into the scaffold constructs [7,8]. Actin cytoskeleton reorganization can be achieved by F-actin depolymerization first

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Table 1
Primers for real-time reverse transcription-polymerase chain reaction.

Gene	Primer sequence		Produce (bp)
Runx2	F: 5′-GTGCCTAGGCGCATTTCA-3′	R: 5′-GCTCTTCTTACTGAGAGTGGAAGG-3′	78
C/EBPβ	F: 5′-CGCTTACCTCGGCTACCA-3′	R: 5′-ACGAGGAGGACGTGGAGAG-3′	65
PPARr	F: 5′-TCCATGCTGTTATGGGTGAA-3′	R: 5′-TGTCAACCATGGTCATTTC-3′	113
MMP2	F: 5′-TGAAGCACAGCAGGTCTCAG-3′	R: 5′-GTGTTCAAACCAGGCACCTC-3′	71
MMP9	F: 5′-TGTACCGCTATGGTTACACTCG-3′	R: 5′-GCCCCAGAGATTTCGACTC-3′	60
ERK1	F: 5′-CCCTAGCCCAGACAGACATC-3′	R: 5′-GCACAGTGTCCATTTTCTAACAGT-3′	94
ERK2	F: 5′-CAAAGAACTAATTTTTGAAGAGACTGC-3′	R: 5′-TCCTCTGAGCCCTTGTCCT-3′	81
RhoA	F: 5′-CAGAAAAGTGGACCCCAGAA-3′	R: 5′-TGCCTTCTTCAGGTTTCACC-3′	147
ROCK1	F: 5′-CCCTCGAACGCTTTCTACA-3′	R: 5′-CACAGGGCACTCAGTCACAT-3′	104
ROCK2	F: 5′-TCAGTGGCATTGGGATAACAT-3′	R: 5′-TGCTGTCTATGTCACTGCTGAG-3′	74
KRT14	F: 5′-TGGATCGCAGTCATCCAGAG-3′	R: 5′-ATCGTGCACATCCATGACCTT-3′	73
KRT19	F: 5′-CAGCTTCTGAGACCAGGGTT-3′	R: 5′-GACTGGCGATAGCTGTAGGA-3′	70
KRT34	F: 5′-TTAACCGCAGGGAAGTGGAGC-3′	R: 5′-GCTGGATACCACCTGCTTGTT-3′	71
KRTAP1-5	F: 5′-CCACCTCTGGACCACTAACAA-3′	R: 5′-GTCCCCAGTGAAGGGTCAAG-3′	126
KRTAP2-3	F: 5′-AGCTGATCCTCAAGCACGAA-3′	R: 5′-GAGAGGGCCAGGATTAGCTG-3′	80
18S rRNA	F:5′-ATGGCCGTTCTTAGTTGGTG-3′	R:5′-AACGCCACTTGTCCCTCTAA-3′	132

Runx2, runt-related transcription factor 2; C/EBPβ, CCAAT-enhancer-binding protein beta; PPARγ, peroxisome proliferator-activated receptor gamma; MMPs, matrix metalloproteinases; ERKs, extracellular signal-regulated kinases; RhoA, Ras homolog gene family, member A; ROCKs, Rho-associated, coiled-coil containing protein kinases; KRTs, keratins; KRTAPs, keratin-associated proteins.

and subsequent re-arrangement. Latrunculin B (LAB) is a G-actin sequestering molecule, which is membrane permeable and binds to monomor G-actin to prevent F-actin assembly [9,10]. LAB causes the concentration-dependent disruption of the F-actin, and enables the investigation of the effects of F-actin depolymerization. However, F-actin depletion-induced cell death may be a consequence of high concentration treatment of LAB [11].

Mesenchymal stem/stromal cells (MSCs) are fibroblast-like adherent cells that possess multiple differentiation capacities [12,13]. It is well known that MSCs undergo spontaneous

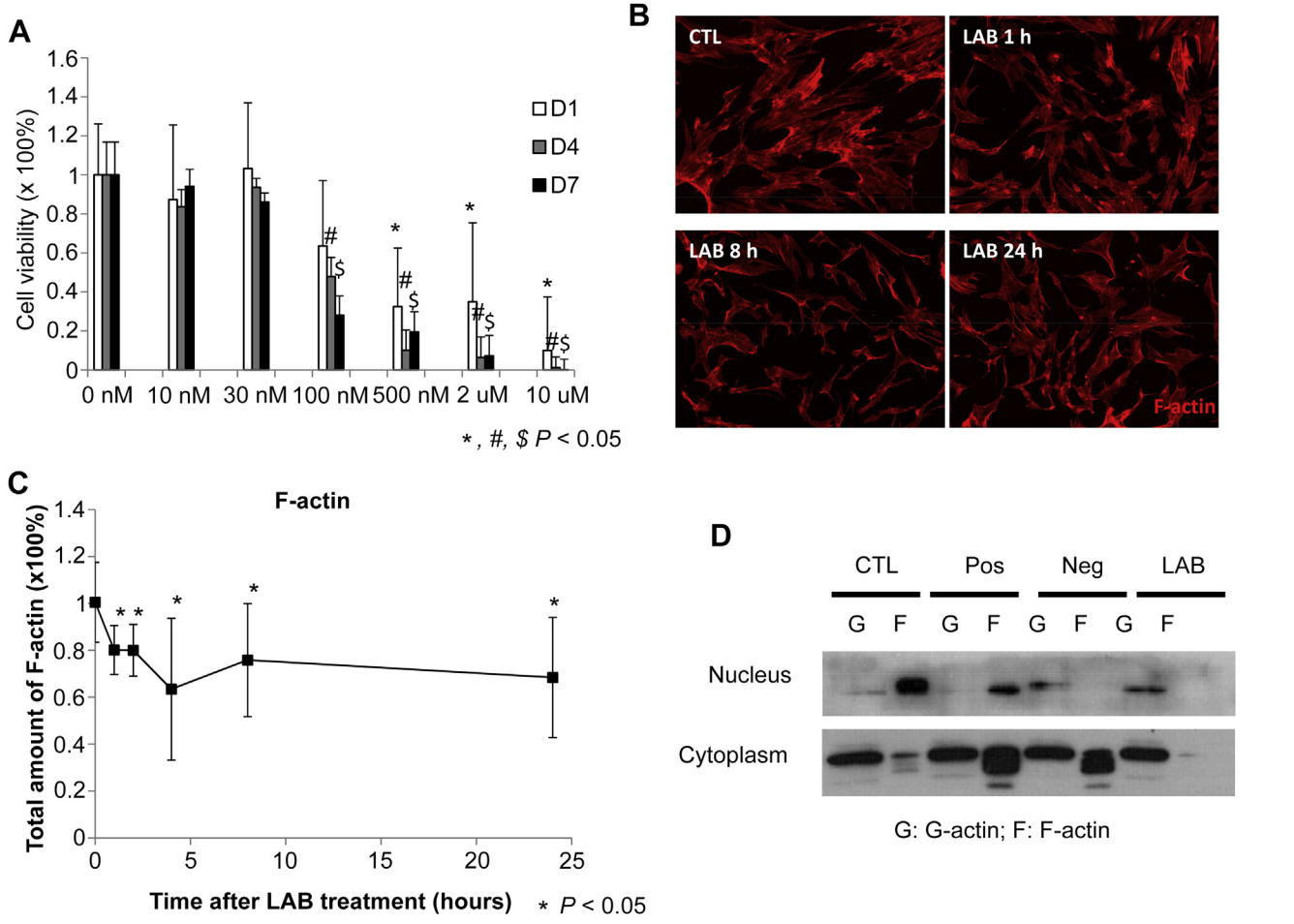

Fig. 1. Depolymerization of F-actin by latrunculin B (LAB). (A) 24-h treatment with LAB at up to 100 nM did not affect the viability of mesenchymal stem cells (MSCs), and 30 nM of LAB and below did not alter MSC viability in the first 7 days. (*, #, $ $p < 0.05$, $n = 3$) (B) LAB (30 nM) altered the shape of MSCs and the arrangement of F-actin in fibroblast-like MSCs. (C) Total amount of F-actin in MSCs was significantly decreased after 1 h of LAB (30 nM) treatment. (*$p < 0.05$, $n = 6$) (D) LAB (30 nM for 1 h) significantly disrupted F-actin in both the nucleus and cytoplasm.