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The mechanism of myoblast deformation in response to cyclic strain – A cytomechanical study

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Abstract

Mechanical strain is one of the important epigenetic factors that cause deformation and differentiation of skeletal muscles. This research was designed to investigate how myoblast deformation occurs after cyclic strain loading. Myoblasts were passaged three times and harvested; various cyclic strains (2.5 kPa, 5 kPa and 10 kPa) were then loaded using a pulsatile mechanical system. The adaptive response of the myoblasts was observed at different time points (0.5 h, 1 h, 6 h and 12 h) post-loading. At the early stage of cyclic strain loading (<1 h), almost no visible morphological changes were observed in the myoblasts. The actin cytoskeleton showed a disordered arrangement and a weak fluorescence expression; there was little expression of talin. At 6 h and 12 h post-loading, the myoblasts changed their orientation to parallel (in the 2.5 kPa and 5 kPa groups) or perpendicular (in the 10 kPa group) to the direction of strain. Fluorescence expression of both the actin cytoskeleton and talin was significantly increased. The results suggest that cyclic strain has at least two ways to regulate adaptation of myoblasts: (1) by directly affecting actin cytoskeleton at an early stage post-loading to cause depolymerization; and (2) by later chemical signals transmitted from the extracellular side to intracellular side to initiate repolymerization.

Keywords: Deformation; Cyclic strain; Actin cytoskeleton; Actin binding protein; Talin

1. Introduction

Deformation is a fundamental cellular function, particularly when cells confront a mechanical ecto-stimulus. The actin cytoskeleton, composed of double chain F-actin and existing in the cytoplasm as a kind of complicated net structure (Li et al., 2003; Hizume et al., 2007), plays a significant role in these processes because it is a major determinant of cell shape. Under mechanical strain conditions, the cytoskeleton displays a dynamic state. Only when the external strain exceeds the limits that the cytoskeleton can resist, cell deformation may occur (Byers et al., 1984; Are et al., 1999). Furthermore, interactions of the actin filaments and the cell membrane are necessary both for changes in, and maintenance of, cell morphology. Our understanding of membrane–cytoskeleton linkages has been advanced significantly with the recognition of various actin binding proteins as adaptors between the integral membrane and the actin cytoskeleton. Proteins of the band 4.1 superfamily – band 4.1, talin, ezrin/radixin/moesin (ERM), and merlin, the product of the neurofibromatosis type 2 tumor suppressor gene – perform this function in many cell types (Tsukita et al., 1997). Talin plays an essential role in integrin-mediated cell matrix adhesion and in integrin-mediated signaling by

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binding to integrin cytoplasmic domains, focal adhesion kinase (FAK), actin and the actin binding protein vinculin (DeMali et al., 2002; Di Paolo et al., 2002; Grose et al., 2002).

Functional therapy is extensively used in orthodontic practice and includes rebuilding the mandible, joint and muscles of mastication in mechanical circumstances (Moss and Rankow, 1986; Ruf and Pancherz, 1999). Recent studies have focused on how hormone and growth factors regulate the growth of mandible and joint (Bresin and Kiliaridis, 2002). For the muscles of mastication, many researchers have placed their interests in electrophysiology, anatomy and imaging, with little focus on cytomechanics (Lipker et al., 1977; Wang et al., 2001).

In this study, we investigate the effect of cyclic strain on myoblasts in the following regard: morphologic change; alteration of the actin cytoskeleton; and distribution of talin, a crucial actin binding protein required for integrin binding to dominate actin cytoskeleton during repolymerization. In addition, we evaluate the relationship between these factors in order to understand the mechanism of strain transmission at the level of cytomechanics.

2. Materials and methods

2.1. Preparation of the myoblasts

Neonatal SD rats were anesthetized by an intravenous injection of ketamine (1 mg/kg, North China Company, China) and later sacrificed. All animals in this study were handled according to the experiment protocol approved by the Animal Research Committee of Sichuan University in China. Skeletal muscle was carefully obtained from the buccinator muscle of rats and washed with PBS; this was then digested in 0.2% collagenase I solution containing EDTA (1 mM) while continuously stirring at 37 °C. After serial trypsinization (successive 10 min periods until all tissues were dispersed) and centrifugation for 5 min at 500*g*, pellets were collected and pre-plated for 20–30 min to reduce the number of fibroblasts. The myoblasts were diluted with growth medium (83% DMEM-high glucose, 15% horse serum and 2% chick embryo extract) to a concentration of 0.8×10^6 cells/ml. The cells were then seeded in 10-cm plastic tissue culture dishes (10 ml per dish) coated with a mixture of collagen–gelatin (1:1). The cells were grown in a water-saturated atmosphere incubator (95% air, 5% CO_2, 37 °C), and the medium was changed every 2 days. Ninety percentage of confluent myoblasts were regularly passaged. Myoblasts in the third passage were harvested and identified by Desmin staining.

2.2. Strain loading protocol

The cyclic strain in this study was achieved with a pulsatile mechanical system (developed by Biodynamics Institution of Sichuan University, China). The incubator in the pulsatile mechanical system was composed of two air-tight ventricles – an upper-culture chamber and a lower hydraulic pressure chamber – separated by a piece of elastic silicon rubber membrane (Fig. 1). Myoblasts were seeded at 2×10^5 cells/well in a six-well collagen-coated plate adherent to the upper side of the silicon membrane. Various cyclic strains were obtained by deformation of membrane, which was controlled by

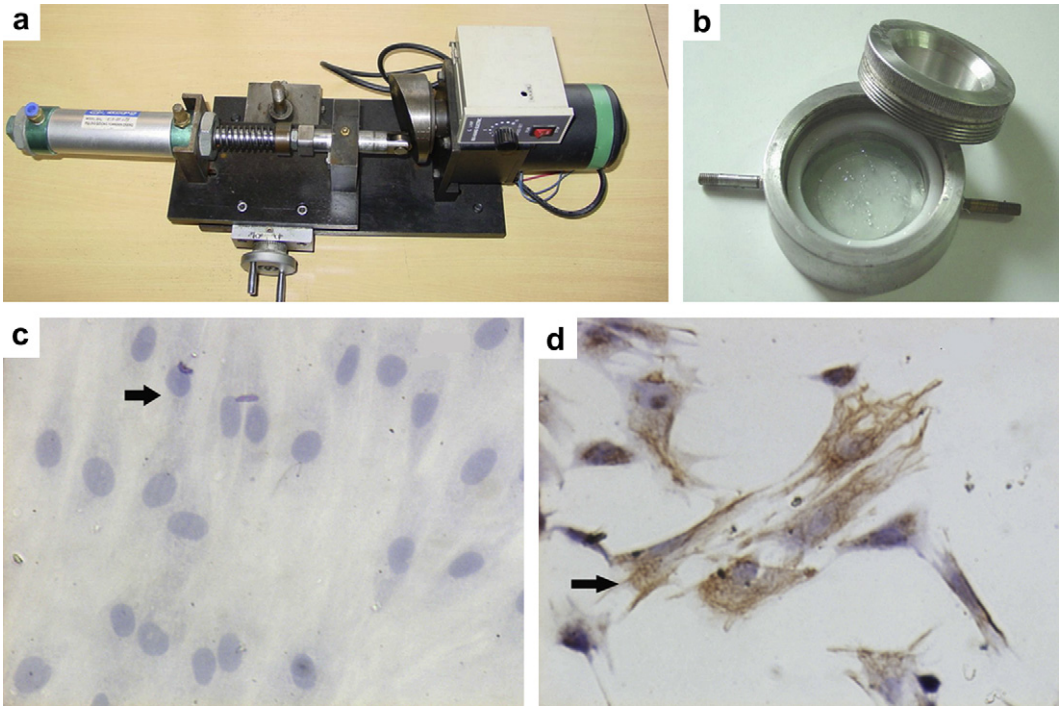

Fig. 1. The pulsatile mechanical system can provide cyclic uniaxial compressive or tensile strain on adherent cells *in vitro*. (a) Control part derived by alternating current, (b) culture chamber; Desmin stain to distinguish myoblasts from fibroblasts (200×), (c) fibroblasts (arrow) expressed negative, and (d) myoblasts expressed positive (arrow).