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Nanotopography as modulator of human mesenchymal stem cell function

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ABSTRACT

Nanotopography changes human mesenchymal stem cells (hMSC) from their shape to their differentiation potential; however little is known about the underlying molecular mechanisms. Here we study the culture of hMSC on polydimethylsiloxane substrates with 350 nm grating topography and investigate the focal adhesion composition and dynamics using biochemical and imaging techniques. Our results show that zyxin protein plays a key role in the hMSC response to nanotopography. Zyxin expression is downregulated on 350 nm gratings, leading to smaller and more dynamic focal adhesion. Since the association of zyxin with focal adhesions is force-dependent, smaller zyxin-positive adhesion as well as its higher turnover rate suggests that the traction force in focal adhesion on 350 nm topography is decreased. These changes lead to faster and more directional migration on 350 nm gratings. These findings demonstrate that nanotopography decreases the mechanical forces acting on focal adhesions in hMSC and suggest that force-dependent changes in zyxin protein expression and kinetics underlie the focal adhesion remodeling in response to 350 nm grating topography, resulting in modulation of hMSC function.

1. Introduction

Tissue regeneration and cell-based therapies often require a supporting scaffold to optimize cell function. Interestingly substrate nanotopography has been shown to influence the differentiation or maintain the stemness of human mesenchymal stem cells (hMSC) depending on the topographical features [1–3]. Recent literature presents many interesting findings on how nanotopography facilitates cell adhesion, alters cell morphology, affects proliferation, initiates intracellular signaling, provides contact guidance and mediates stem cell differentiation [4]. However little is known about the molecular mechanisms underlying the cellular response to substrate topography. Namely, how is nanotopography sensed by the cell, what molecules are necessary in this process, and most importantly how does this influence the cellular functions? Understanding the molecular mechanism is crucial to the better design of a scaffold for a given application or to combine substrates with biochemical cues to enhance the modulatory effect of cell–topography interactions.

Human mesenchymal stem cells (hMSCs) are marrow-derived, self-renewing cells with multipotent differentiation potential. They give rise to various anchorage-dependent cell types, including adipocytes, chondrocytes, myoblasts, and osteoblasts [5]. Their differentiation potential is influenced by substrate elasticity [6], geometrical confinement [7],[8], and substrate topography [1–3].

Cell-substrate or cell-extracellular matrix (ECM) adhesions are mediated by dynamic multiprotein structures called focal adhesions (FA). They are important for force transmission, cytoskeletal regulation and signaling. At these sites the cell establishes a transmembrane connection between elements of the ECM and the actin cytoskeleton [9]. The transmembrane integrin proteins orchestrate these events [10]. The integrins, heterodimers containing one α- and one β– subunit, bind with their extracellular domain to the ECM proteins fibronectin, laminin, and vitronectin.

The cytosolic domain of integrins binds to a large number of proteins such as paxillin and zyxin either directly or via scaffolding proteins [11]. Some of these proteins are implicated in strengthening the linkage between the extracellular matrix and the cytoskeleton, others play a role in adhesion-mediated signaling [12]. Cellular adhesions can be classified into three categories: Focal complexes (FX), FA and fibrillar adhesions [13]. The FX along the leading lamella of migrating cells are early adhesions, which transform into focal adhesion upon RhoA activation [14,15] or as a result of external mechanical perturbation [16,17]. Fibrillar adhesions develop from FAs following actomyosin contraction [18,19]. Recruitment of zyxin protein has been proposed as a molecular marker for mature FAs [20]. Zyxin facilitates actin polymerization in response to mechanical forces [21] and dissociates from focal adhesions upon force dissipation [22].

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