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Self-renewal of embryonic stem cells through culture on nanopattern polydimethylsiloxane substrate

Kilsoo Jeon [a,1], Hyun-Jik Oh [b,c,1], Hyejin Lim [a], Jung-Hyun Kim [a], Duk Hyun Lee [d], Eung-Ryoung Lee [a], Bae Ho Park [d], Ssang-Goo Cho [a,*]

[a] Department of Animal Biotechnology (BK21), Animal Resources Research Center and SMART-IABS, Konkuk University, Seoul 143-702, Republic of Korea
[b] Department of Biomedical Engineering, College of Health Science, Korea University, Seoul 136-100, Republic of Korea
[c] Seoulin Bioscience Institute, Gyeonggi-do 463-400, Republic of Korea
[d] Division of Quantum Phases & Devices, Department of Physics, Konkuk University, Seoul 143-701, Republic of Korea

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ABSTRACT

Embryonic stem (ES) cells can undergo continual proliferation and differentiation into cells of all somatic cell lineages *in vitro*; they are an unlimited cell source for regenerative medicine. However, techniques for maintaining undifferentiated ES cells are often inefficient and result in heterogeneous cell populations. Here, we determined effects of nanopattern polydimethylsiloxane (PDMS) as a culture substrate in promoting the self-renewal of mouse ES (mES) cells, compared to commercial plastic culture dishes. After many passages, mES cells efficiently maintained their undifferentiated state on nanopattern PDMS, but randomly differentiated on commercial plastic culture dishes, as indicated by partially altered morphologies and decreases in alkaline phosphatase activity and stage-specific expression of embryonic antigen-1. Under nanopattern PDMS conditions, we found increased activities of STAT3 and Akt, important proteins involved in maintaining the self-renewal of mES cells. The substrate-cell interactions also enhanced leukemia inhibitory factor (LIF)-downstream signaling and inhibited spontaneous differentiation, concomitant with reduced focal adhesion kinase (FAK) signaling. This reduction in FAK signaling was shown to be important for promoting mES cell self-renewal. Thus, our data demonstrates that nanopattern PDMS contributes to maintaining the self-renewal of mES cells and may be applicable in the large-scale production of homogeneously undifferentiated mES cells.

1. Introduction

Embryonic stem (ES) cells are pluripotent cells derived from the inner cell mass of blastocysts and are able to differentiate into all derivatives of the three germ layers [1,2]. They have the dual ability to self-renew and to differentiate into multiple cell types. Therefore, growth and expansion of pluripotent ES cells are regulated by a balance between survival, cell death, self-renewal, and differentiation signals [3,4]. Although various signaling pathways have been shown to regulate the proliferation and self-renewal of ES cells [5–10], the detailed mechanisms involved in maintaining the self-renewal of ES cells is not clearly characterized. For mouse ES (mES) cells, the addition of leukemia inhibitory factor (LIF) is needed for their long-term self-renewal [11,12]. LIF binds to a heteromeric receptor that consists of gp130 and the low-affinity LIF receptor; this association induces the activation of STAT3, phosphatidylinositol 3-kinase (PI3K), and the mitogen-activated protein kinase (MAPK) family members ERK1 and ERK2. Activated STAT3 plays an essential role in the maintenance of self-renewal and pluripotency in mES cells [13,14]. However, the cellular microenvironment has also long been known to influence the maintenance of mES cells and to regulate their self-renewal and differentiation [15,16]. Spontaneous differentiation of mES cell cultures occurs through seemingly random pathways, even under normal cell culture conditions [17,18]. Thus, self-renewal of mES cells seems to require a balance between extrinsic and intrinsic signaling pathways. Specifically, self-renewal and differentiation of ES cells are regulated by microenvironmental stimuli such as cell–cell, cell-extracellular matrix, and cell-soluble factor interactions [19]. That is, components of the microenvironment are critical for maintaining ES cell self-renewal. The interaction between the extracellular environment and integrin proteins plays

* Corresponding author. Tel.: +82 2 450 4207; fax: +82 2 455 1044.
E-mail address: ssangoo@konkuk.ac.kr (S.-G. Cho).
[1] These authors contributed equally to the work.

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