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Long-term maintenance of mouse embryonic stem cell pluripotency by manipulating integrin signaling within 3D scaffolds without active Stat3

Seung Tae Lee [a,b], Jung Im Yun [a,b], Andre J. van der Vlies [a], Stephan Kontos [a], Mi Jang [c], Seung Pyo Gong [d], Dae Yong Kim [e], Jeong M. Lim [c], Jeffrey A. Hubbell [a,*]

[a] Institute of Bioengineering, School of Life Sciences and School of Engineering, Ecole Polytechnique Fédérale de Lausanne (EPFL), Station 15, Lausanne CH-1015, Switzerland
[b] Department of Animal Biotechnology, Kangwon National University, Chuncheon 200-701, Republic of Korea
[c] WCU Biomodulation Program, Seoul National University, Seoul 151-921, Republic of Korea
[d] Department of Marine Biomaterials and Aquaculture, Pukyong National University, Busan 608-737, Republic of Korea
[e] College of Veterinary Medicine, Seoul National University, Seoul 151-742, Republic of Korea

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ABSTRACT

We engineered an acellular biomimetic microenvironment to regulate stem cell fate and applied it to maintain mouse embryonic stem (ES) cell self-renewal. In the 3D environment formed using hydrogel scaffolds in which specific integrin ligation was provided, Stat3 activation by exogenous leukemia inhibitory factor (LIF) no longer acted as a limiting factor for stem cell self-renewal. Instead, simultaneous stimulation of integrins $\alpha_5\beta_1$, $\alpha_v\beta_5$, $\alpha_6\beta_1$ and $\alpha_9\beta_1$ within the 3D scaffold greatly increased Akt1 and Smad 1/5/8 activation, which resulted in prolonged self-renewal of the ES cells. The ES cells exposed to the combined stimulation of the integrins for 4 wk in LIF-free 3D scaffolds maintained the spherical morphology of cell colonies without losing any activity of pluripotency. In conclusion, cell niche-specific integrin signaling within the 3D environment supported mouse ES cell self-renewal, and the resulting integrin signaling replaced Stat3 with Akt1 and Smad 1/5/8 as critical signals for mouse ES cell self-renewal.

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1. Introduction

Improvements in conventional culture systems are urgently needed to reduce chromosomal and functional aberrancy of stem cells maintained *in vitro*. We have focused on establishing biomimetic, defined microenvironments for supporting stem cell self-renewal *in vitro*; we recently reported the possibility of maintaining mouse ES cell phenotype and cellular activity without feeder cells through the use of poly(ethylene glycol) (PEG)-based 3D scaffolds by optimizing integrin ligation within these scaffolds [1]. Since the cell niche and its physical environment greatly influences resulting stem cell characteristics [2–9], elucidation of critical signals for self-renewal in a specific cell niche is very important for designing an optimal biomimetic microenvironment.

Leukemia inhibitory factor (LIF), which induces Stat3 signaling, is widely known as a critical factor for maintaining the stemness characteristics of mouse ES cells cultured in conventional 2D systems. Within a 3D environment, we have found that stemness gene expression in mouse ES cells could be prolonged by optimizing ligation of integrins via scaffold-bound peptide integrin ligands. Here, we explored how different integrin ligation environments in 3D scaffolds could influence critical signals for mouse ESC self-renewal under LIF-free conditions.

2. Materials and methods

2.1. ES cell culture

E14tg2a ES cells purchased from ATCC (Manassas, VA) were maintained on 10 μg/ml mitomycin C (Sigma–Aldrich, St. Louis, MO)-treated MEFs in standard ES cell culture medium consisting of Dulbecco's modified Eagle's medium (DMEM; Gibco Invitrogen, Grand Island, NY) supplemented with 15% (v/v) heat-inactivated fetal bovine serum (FBS; HyClone, Logan, UT), 0.1 mM β-mercaptoethanol (Gibco Invitrogen), 1% (v/v) nonessential amino acids (NEAA; Gibco Invitrogen), 1 mM sodium pyruvate (Sigma–Aldrich), 2 mM L-glutamine (Gibco Invitrogen), a 1% (v/v) lyophilized mixture of penicillin and streptomycin (Gibco Invitrogen) and 1000 units/ml mouse LIF (Chemicon International, Temecula, CA). Moreover, unless otherwise noted, the ES cells were subpassaged every 3 d and medium change was performed daily during subculture.

* Corresponding author. Tel.: +41 21 693 9681; fax: +41 21 693 9685.
E-mail address: jeffrey.hubbell@epfl.ch (J.A. Hubbell).

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