The Use of SC1 (Pluripotin) to Support Mouse ES Cell Self-Renewal in the Absence of LIF

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SUMMARY
Leukemia inhibitory factor (LIF) is routinely used to maintain self-renewal of mouse embryonic stem (ES) cell cultures, however, high prices of LIF may deter researchers from pursuing avenues of study on mouse ES cell cultures. SC1 may be an economical alternative to LIF for the maintenance of self-renewal of mouse ES cell cultures. This small molecule functions through dual inhibition of Ras-GAP and ERK1. Illustration of its mechanism of action makes it a useful tool to study the fundamental molecular mechanism of self-renewal. Here we test the toxicity and function of SC1 in supporting mouse ES cell self-renewal in the absence of LIF and show that SC1 is able to maintain self-renewal of mouse ES cell cultures.

INTRODUCTION
Mouse embryonic stem (ES) cells are conventionally cultured with leukemia inhibitory factor (LIF)\(^1\). However, activation of LIF/JAK/Stat3 pathway is not absolutely required to maintain mouse ES cells in their self-renewal state. It is postulated that the ES cell self-renewal state can be achieved if a few critical internal signaling molecules reach a ground state\(^2\). Researchers have indicated that mouse ES cells can self-renew in the absence of LIF\(^2\). In this case, mouse ES cells remain in a long-term self-renewal state when Wnt signaling is turned on by inactivation of GSK3-beta with CHIR99021; MEK/ERK is inhibited by PD0325901; and the bFGF pathway is inactivated by the FGFR inhibitor PD173074. Long-term self-renewal of mouse ES cells via direct modulation of the internal signaling pathways with small molecules demonstrate that the ground state for self-renewal is independent of extracellular stimuli. However, this suggests there could be multiple ways to reach a ground state through modulation of different signaling pathways. The discovery of SC1 indicated that activation of PI3K instead of Wnt and inhibition of ERK could be another ground state of self-renewal\(^3\). Instead of three known molecules targeting multiple signaling pathways, the single novel molecule SC1 can replace the function of LIF, providing another efficient method for mouse ES cell culturing. Concurrently it can also reveal a novel mechanism for self-renewal and can be further applied to investigate the fundamental mechanisms for ES cell self-renewal.

Here we show that *Stemolecule™ SC1 (Pluripotin)* (Cat. No. 04-0011) can maintain self-renewal of mouse ES cells in the absence of LIF. Expression of typical pluripotency markers (Oct4, Sox2, Nanog, and SSEA1) was sustained after five passages in the presence of SC1. Furthermore, SC1 caused no overt toxicity on mouse ES cells. Although SC1 concentrations of 100 nM and 300 nM worked well in this study, it is highly recommended that the optimal concentration of SC1 be determined by end users after testing on their specific cell types and culture conditions.

RESULTS
Toxicity Evaluation
As shown in Figure 1, no overt toxicity was observed on mouse ES cells when concentrations of SC1 were 1 µM or less. A strong toxicity effect was observed when SC1 concentrations exceeded 10 µM.

![Figure 1. Toxicity of SC1 on mouse ES cells. No toxicity was observed when SC1 concentrations were 1 µM or less.](image)

Maintenance of Self-Renewal as Shown by Morphology and Expression of Pluripotency Markers
*Stemolecule SC1* was added to the culture medium at varying concentrations to assess whether it could be used as a replacement for LIF in the maintenance of mouse ES cell self-renewal. LIF (1000 U/ml) was used as a control. Cells were also maintained in medium only or medium with 0.1% DMSO.
The mouse ES cell colonies maintained in medium without LIF or SC1 began to differentiate by the second passage and were completely differentiated by the third passage. Cells maintained in medium supplemented with LIF maintained mouse ES cell colony morphology for five passages; the course of the experiment. The cells maintained in medium supplemented with 300 nM or 100 nM of SC1 also maintained normal mouse ES cell colony morphology for five passages. When 1 µm of SC1 was used, cell death was observed after four passages (data not shown).

Figure 2 shows the cell morphology observed when medium was supplemented with LIF compared to Stemolecule SC1 (100 nM). There is no difference in morphology between the two cultures; both colonies exhibit typical mouse ES cell colony morphology.

Expression of Pluripotency Markers
Mouse ES cells maintained for five passages in medium supplemented with 100 nM of Stemolecule SC1 or 1000 U/ml of LIF were fixed and examined by immunocytochemistry (ICC) for SSEA1, Oct4, Nanog and Sox2 (Figures 3 and 4). Colonies for both SC1 and LIF showed expression for these pluripotency markers; further proof that colonies maintained under these conditions will not differentiate.

Figure 2. Mouse ES cell morphology in the presence of Stemolecule SC1 or LIF. No difference in morphology was observed between cells maintained in SC1 and cells maintained in LIF.
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July 21, 2012
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EXPERIMENTAL PROCEDURES

Preparation of SC1
Stemolecule SC1 (Pluripotin) (Cat. No. 04-0011) was dissolved in DMSO and serially diluted to various concentrations.

Cell toxicity assay
Mouse ESC R1 ES cells were plated in 96 well plates at 8 x 10^3 and 4 x 10^3 cells per well in Growth Medium (Knockout™ DMEM supplemented with 15% Knockout Serum Replacement, 4 mM L-glutamine, 0.1 mM non-essential amino acids, and 1X β mercaptoethanol) supplemented with Stemfactor™ LIF, Mouse Recombinant (Cat. No. 03-0011). After an overnight incubation, medium was replaced with Growth Medium supplemented with one of the following: 1 nM, 10 nM, 100 nM, 1 µM, 10 µM, 100 µM, or 1 mM of SC1. 0.1% DMSO was used as a control. Cells were incubated for an additional six hours then cytotoxicity was assessed using the CytoTox-Glo™ Cytotoxicity Assay.

Cell culture
Mouse ESC R1 ES cells were seeded at a density of 5 x 10^3 cells per well on irradiated E14.5 CF-1 MEFs in 12-well plates in Growth Media supplemented with 1000 units/ml LIF. Cells were cultured overnight.

The next day the medium was replaced with Growth
Medium supplemented with one of the following: LIF (1000 U/ml), 1 µM of SC1, 300 nM of SC1, or 100 nM SC1. Cells were also maintained in Growth Medium with no supplement.

Cells were cultured for 3 to 4 days in each passage. Medium was replaced every 48 hours. After the initial seeding in the first passage, cells were constantly cultured in their corresponding media. Cells were split as needed with 0.05% trypsin at 1:10 to 1:15 to maintain a similar density as the initial seeding density.

**Immunocytochemistry and Flow Cytometry**

Immunocytochemistry was performed using standard procedures (reference Protocol: Immunocytochemistry online at [www.stemgent.com/support/protocols](http://www.stemgent.com/support/protocols)).

**REFERENCES**


**ADDITIONAL READING**


The authors wish to thank Dr. Sheng Ding of The Scripps Research Institute and Dr. Hongkui Deng of Peking University for their assistance and direction.