intellicyt
screening solutions for life

Application Note

Myeloid Progenitor Cell Differentiation

Screening for Compounds that Induce Myeloid Progenitor Cell Differentiation Using the HyperCyt® High Capacity Flow (HCF) Screening Platform

Thomas D. Duensing[1] and David B. Sykes[2,3]

[1]IntelliCyt Corporation, 317 Commercial Street, Suite G100, Albuquerque, NM 87102
[2]Massachussetts General Hospital, Center for Regenerative Medicine, 185 Cambridge St. CPZN 4400, Boston, MA 02114
[3]Contact info: dbsykes@partners.org

Key Features & Benefits

FEATURES	BENEFITS
• Suspension cell screening assay using primary bone marrow derived myeloid progenitor cells	• Optimized detection technology for a biologically relevant cell system
• Simultaneous measurement of cell viability, cell number, and green fluorescence (GFP+) in each well	• Improved productivity by direct identification of active compounds and simultaneous elimination of cytotoxic compounds
• Condensed format in a multiplex experiment	• Reduces reagent use and assay variability

Introduction

Scientific advances in the field of stem and progenitor cell biology over the past decade have stimulated strong interest in the pursuit of new therapeutic options for clinically devastating diseases such as acute myeloid leukemia. One powerful approach to finding new treatments for such diseases is through the use of expanded small molecule and biologics screens that target both known and unknown pathways. Using this approach, small molecules which manipulate cellular processes such as proliferation and differentiation can be identified. This case study describes how Dr. David Sykes, a scientist at the Massachusetts General Hospital is incorporating IntelliCyt's high capacity flow screening platform into his efforts to understand the biology of acute myeloid leukemia.

Acute myeloid leukemia (AML) in adults is a devastating disease, with a 5-year survival of 25%. Development of therapies that overcome differentiation arrest and trigger the normal differentiation of leukemic stem cells is an attractive therapeutic target for AML. However, screening for compounds that target differentiation has been hindered by the availability of inadequate model systems.

This report describes a high throughput screening method based on the HyperCyt high capacity flow cytometry platform for identifying biologically relevant compounds that overcome differentiation arrest in primary myeloid progenitor cells. By incorporating this platform into the screen, we were able to easily differentiate compounds that stimulated differentiation of stem cells while eliminating compounds that exhibited toxic effects on the cells.

Materials & Methods

Cell lines were derived from the primary bone marrow cells of a transgenic mouse expressing green fluorescent protein (GFP)

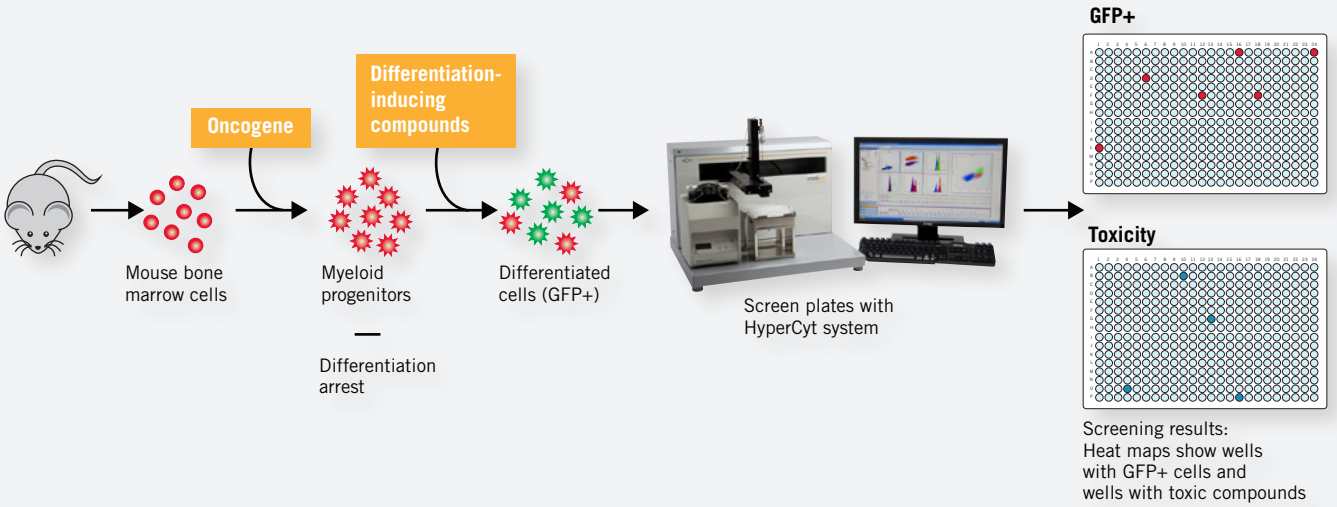

Figure 36. Workflow used in screening for small molecule inducers of stem cell differentiation using the HyperCyt high capacity flow screening system.

under the control of the lysozyme promoter. These cell lines were GFP-negative in their undifferentiated state and GFP-positive in their differentiated state. The cells were plated in 384 well plates (2500 cells/well in 50 µL) and cultured in the presence of test compounds (final 5 µM in DMSO). After incubation for 4 days, cells were analyzed using the HyperCyt high-capacity flow screening system connected to a flow cytometer (LSRII, BD Biosciences). The readout for the assay was the percentage of GFP positive cells in each well. Measurement of forward scatter, side scatter, and cell number in each well enabled the simultaneous identification of toxic compounds without the addition of viability dyes to the system.

Results & Discussion

In this report we have demonstrated the feasibility of using the HyperCyt high capacity flow screening system to identify compounds that overcome differentiation arrest in primary myeloid progenitor cells (**Figure 1**). The sensitivity of the system allowed robust detection of green fluorescence and the ability to distinguish GFP-negative from GFP-positive cells. The assay demonstrated a two log dynamic range in fluorescence intensity between positive and negative controls. In addition, the system simultaneously differentiated live cells from dead cells on the basis of forward and side scatter properties without requiring the addition of a viability or nuclear dye. Using the system's HyperView data analysis software, non-toxic compounds that released differentiation arrest were easily identified (**Figure 2**).

In this pilot screen, a library consisting of 1920 compounds was tested for their ability to release differentiation arrest. Negative and positive control compounds were run on separate plates. Both control and test compounds were tested in duplicate plates for a total of 14 x 384-well plates. The average time to run each plate through the HyperCyt system was 30 minutes. The screen identified two compounds active in triggering an increase in green fluorescence as a surrogate for differentiation in this non-adherent primary cell model of acute myeloid leukemia.

This pilot screen confirmed the feasibility of using IntelliCyt's high capacity flow screening platform to screen compounds using a GFP reporter based assay for differentiation using primary stem cells. By taking full advantage of the multiplexing capabilities of the HyperCyt system, this study demonstrates the power of using a flow cytometry based detection in a high throughput screening setting. By enabling studies on primary suspension cells using multiplexed screening assays, we believe the incorporation of this high capacity flow platform will lead to significant advances in the understanding of stem cell biology.

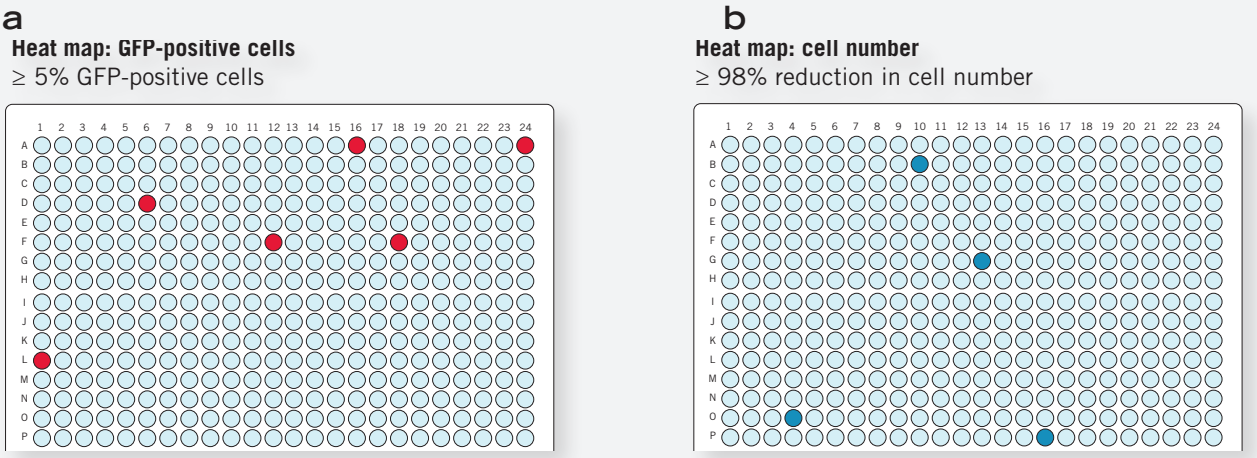

Figure 37. HyperView heat maps representing one plate from the screen. The heat maps enable simultaneous investigation of compounds that induced differentiation indicated by the presence of GFP positive cells (**a**) and toxicity manifested by the loss of cells (**b**).

References

40. Knoepfler PS, Sykes DB, Pasillas M, Kamps MP. HoxB8 requires its Pbx-interaction motif to block differentiation of primary myeloid progenitors and of most cell line models of myeloid differentiation. *Oncogene.* 2001; 20:5440–5448.

41. Calvo KR, Sykes DB, Pasillas MP, Kamps MP. Nup98-HoxA9 immortalizes myeloid progenitors, enforces expression of Hoxa9, Hoxa7 and Meis1, and alters cytokine-specific responses in a manner similar to that induced by retroviral co-expression of Hoxa9 and Meis1. *Oncogene.* 2002 Jun 20; 21(27):4247–56.

intellicyt

IntelliCyt Corporation
9620 San Mateo Blvd NE
Albuquerque, NM 87113
505.345.9075
www.intellicyt.com

© 2011–2012 IntelliCyt Corporation. All rights reserved. HyperCyt, HyperView, HTFC, and IntelliCyt are registered trademarks and ForeCyt is a trademark of IntelliCyt Corporation. HyperCyt is protected under US patents: 6878556, 6890487, 7368084, 7842244 and Patents Pending.

IC-AB-003 Rev A