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Identification and detection of murine leukemia blasts by flow cytometry

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Method Article

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Abstract

Introduction

Human leukemia has been determined and classified with the help of flow cytometry for the past two decades. Past attempts to detect leukemia blasts relied on both forward and side scatter \(FSC and SSC) based on cell size and granularity. However, this technique failed to show a clean separation of blasts from normal lineage cells. In 1993, Borowitz, et al developed flow cytometric analysis to distinguish human leukemia blasts from other normal lineage cells by using fluorescence-conjugated CD45 antibodies \(1). On CD45-SSC plots, the blasts are distinctly located below granuocytes in human acute myeloid leukemia. This technical advance has significantly improved diagnosis and classification of human leukemia. Animal models mimicking human leukemia help us make a deeper understanding of leukemia progression and develop more effective therapeutic interventions in human leukemia. Recently, two groups of researchers published Bethesda proposals for classification of nonlymphoid and lymphoid hematopoietic neoplasms in mice \(2,3). Similar to the French-American-British \(FAB) criteria for human leukemia, the proposed classification depends on idenfication of leukemia blasts and their lineage identities. However, lack of a simple and sensitive methodology to identify leukemia blasts prevents us from characterizing leukemia in animal models and conducting subsequent translational research. Here we adopt the CD45-SSC analysis and characterize blasts and lineage cell populations of WT and Pten null T cell leukemia mice on CD45-SSC plots \(4). The blast population is distinct from normal lineage cells, but its location is different from that of human blasts \(4). The method is potentially useful for other murine leukemia models.

Reagents

1. HBSS+: 1x HBSS, 2% Fetal calf serum, 10mM Hepes buffer \(pH 7.2), 1% Penicillin/streptomycin 2. Bone marrow harvesting medium: 1x DMEM, 2% Fetal calf serum, 10mM Hepes buffer \(pH 7.2), 1% Penicillin/streptomycin 3. CD45 antibody \(rat monoclonal clone, 30-F11) 4. 7-AAD soluction \(BD Pharmingen)

Equipment

BD FACScan, FACSCalibur or any other FACS analyzer with more than three-color capability.

Procedure

1. Prepare single cell suspension. a) Harvest bone marrow cells by flushing femur with ice-cold bone marrow harvesting medium. b) Filter the suspended cells through 70um cell strainer \(BD Cat. 352350) while keeping cells on ice. c) Count the cell concentration. d) centifuge the filtered cells at 1500rpm for 5-10 min. e) Resuspend the cells with HBSS+ staining buffer \(HBSS+, ~$2x10^7$ cells/mL if possible). 2. Stain cells with appropriate antibodies. a) Aliquot 100ul single suspension cells for staining. b) Prepare

controls including unstained sample and samples stained with single color fluorescence antibody \(antibodies) for FACS compensation. c) Add 2uL CD45 antibody and other antibodies to 100uL cells and slowly rock in 4ºC for 15 min. d) Spin down the cells at 1500rpm for 7 min and resuspend cell pellets with 200ul ice-cold HBSS+. e) Add 5-7ul 7-AAD solution into 200ul cells to exclude dead cells during FACS analysis. 3. Perform FACS analysis a) Adjust voltage. b) Run compensation. c) Run samples and collect FACS data. d) Analyze your FACS data on CD45-SSC plots.

Timing

It takes 3 hours to harvest cells, stain with antibodies, and run stained cell samples on a FACS analyzer.

Critical Steps

1. To include control samples unstained and stained with single color fluorescence-conjugated antibody\(antibodies) for proper FACS compensation. 2. To include some leukemia cells into FACS compensation controls to ensure proper location of granulocytes and blasts by adjusting voltage of the CD45 color channel on a FACS analyzer. 3. To include a wild-type sample to aid in determining the blast gate on CD45-SSC plots.

Troubleshooting

1. The CD45 antibody conjugated with different fluorescence dyes shows slightly different locations for myeloid cells and leukemia blasts on FACS plots, but the cell population patterns are very similar.

Anticipated Results

1. The method is verified for T cell leukemia in Pten null leukemia mice \(4). The T-ALL blasts are located on the right side of granulocytes on CD45-SSC plots as shown in Figure 1,2. This blast region is consistent for cells from bone marrow, spleen and thymus, but different from that of human leukemia blasts, which is located below granulocytes \(1). 2. The blast region for other murine leukemia remains to be determined.

References

1. Borowitz, M. J., Guenther, K. L., Shults, K. E., and Stelzer G. T. Immunophenotyping of acute leukemia by flow cytometric analysis. Use of CD45 and right-angle light scatter to gate on leukemic blasts in three-color analysis. _Am J Clin Pathol_ **100**, 534-540 \(1993). 2. Kogan, S.C., _et. al_. Bethesda proposals for classification of nonlymphoid hematopoietic neoplasms in mice. _Blood_ **100**, 238-245 \(2002). 3. Morse, H.C., _et. al_. Bethesda proposals for classification of lymphoid neoplasms in mice. _Blood_ **100**, 246-58 \(2002). 4. Guo, W, et al, Multi-genetic events collaboratively contribute to Pten-null leukaemia stem-cell formation. _Nature_ 2008.

Acknowledgements

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Figures

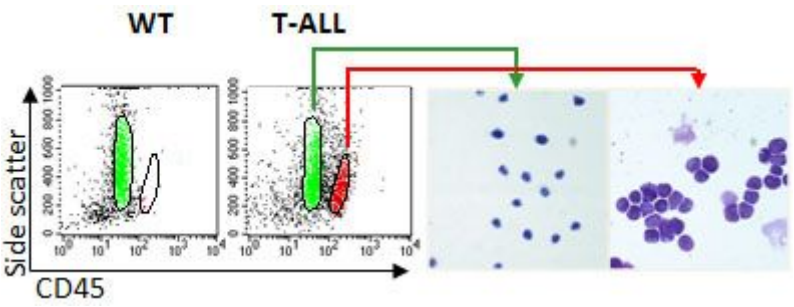

Figure 1

Figure 2 Characterization of cell populations on CD45-SSC plots The cell populations on CD45-SSC plots have been characterized for WT and Pten mutant mice at different disease stages: wild-type (WT), chronic phase of myeloproliferative disorder (CP), and T cell leukemia during blast crisis (BC). Note: The lineage cells determined with surface markers are highlighted in pink and their percentages are denoted on FACS plots.

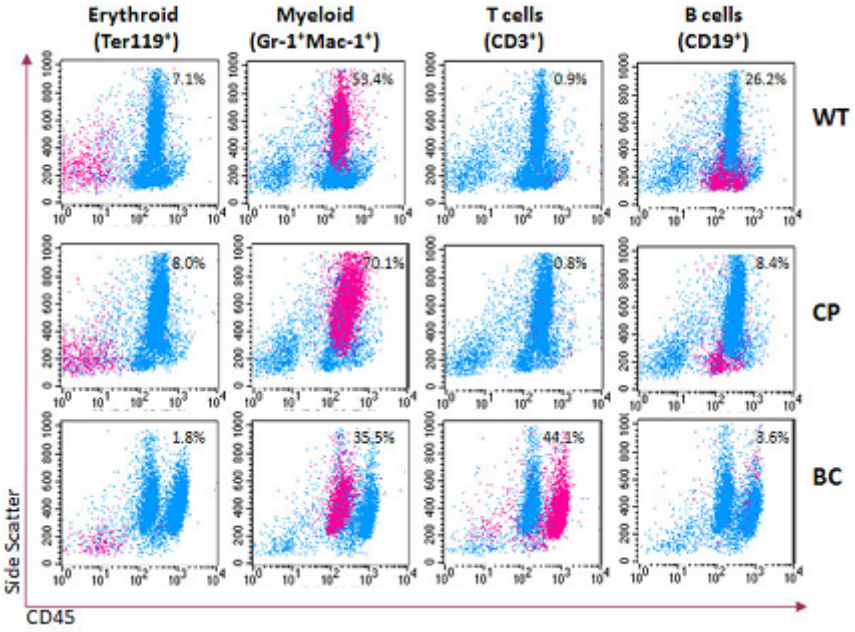

Figure 2

Figure 1 Identification of leukemia blasts by CD45-SSC analysis Bone marrow samples from both WT and leukemia mice were analyzed by CD45-SSC FACS analysis (4). An abnormal blast population was detected in the leukemia mouse and constituted more than 20% of the total leukocytes (left panel), satisfying the FAB criteria for human acute leukemia. The cells in the blast and neutrophil regions were

sorted and stained with Giemsa-Wright solution(Fisher, right panel). The sorted blast cells were large, immature, and morphologically distinct from normal lineage cells but similar to human leukemic blasts (right panel).