Lewis x/CD15 expression in human myeloid cell differentiation is regulated by sialidase activity

Samah Zeineb Gadhoum[1, 2] and Robert Sackstein* [1, 2, 3, 4]

From the Departments of Dermatology[1] and Medicine[3], Brigham and Women's Hospital, Harvard Skin Disease Research Center[2], Harvard Medical School, and Department of Medical Oncology[4], Dana-Farber Cancer Institute, Boston, Massachusetts 02115

***Corresponding author:**

Robert Sackstein, M.D.,Ph.D., Harvard Institutes of Medicine, 77 Ave. Louis Pasteur, Rm. 671, Boston, MA 02115. Tel.: 617-525-5604; Fax: 617-525-5571; E-mail: rsackstein@rics.bwh.harvard.edu.

Supplementary Figure 1

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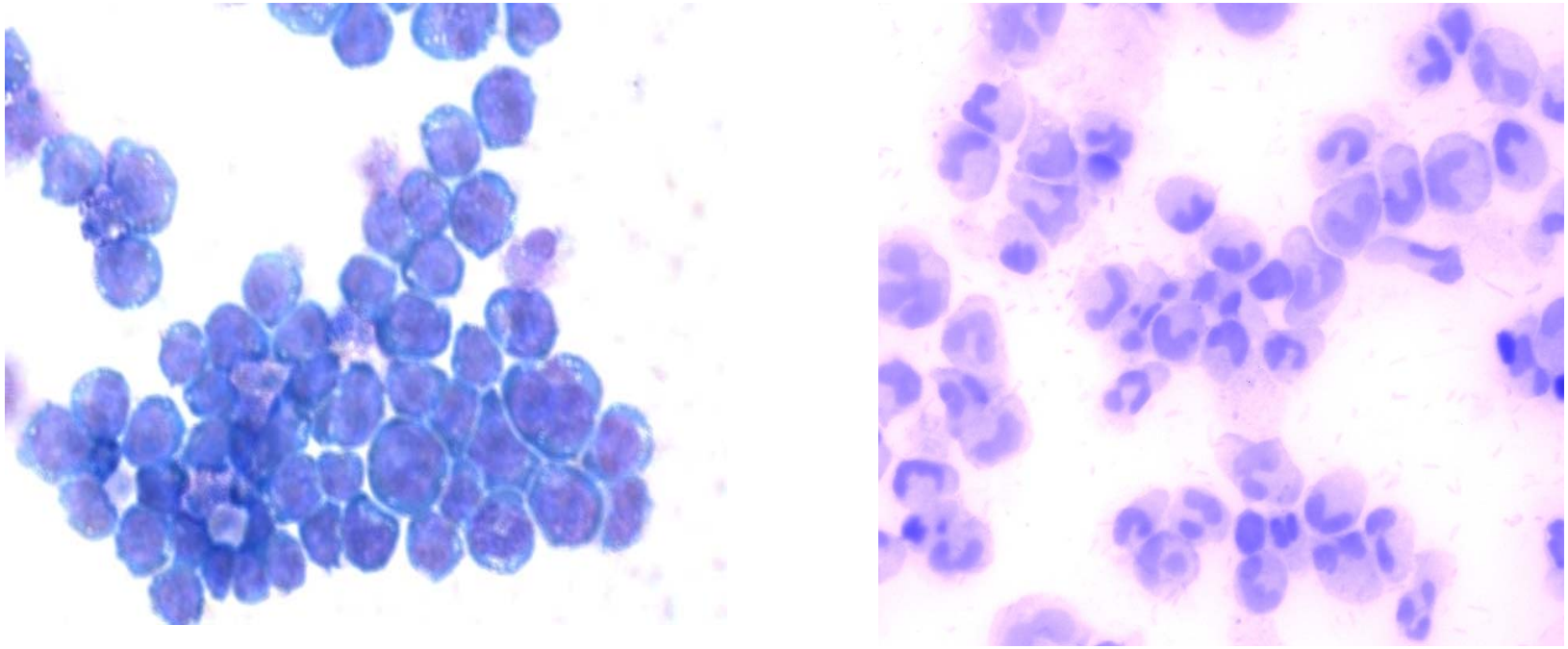
Control

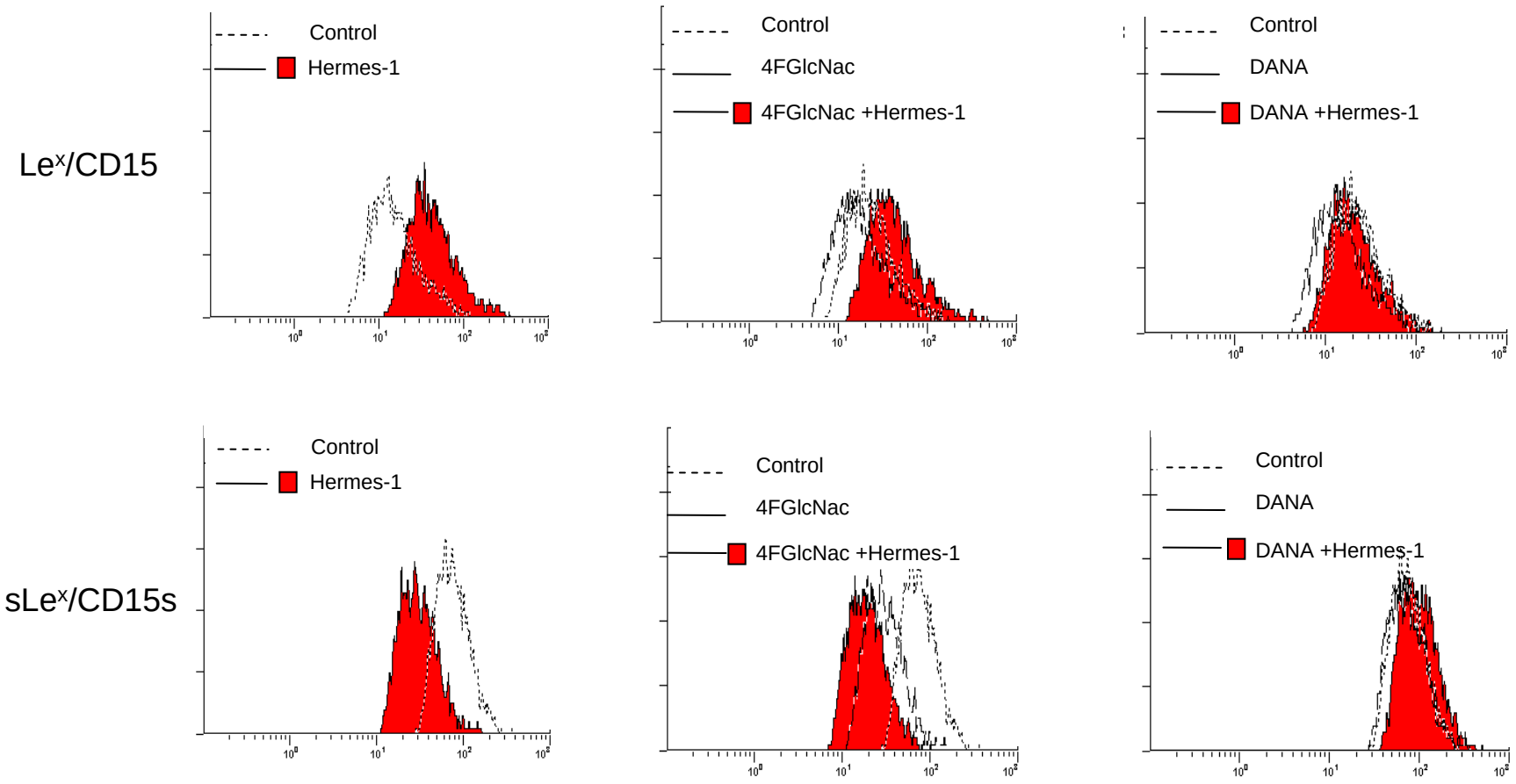
Hermes-1



CD44 ligation-induced changes in morphology

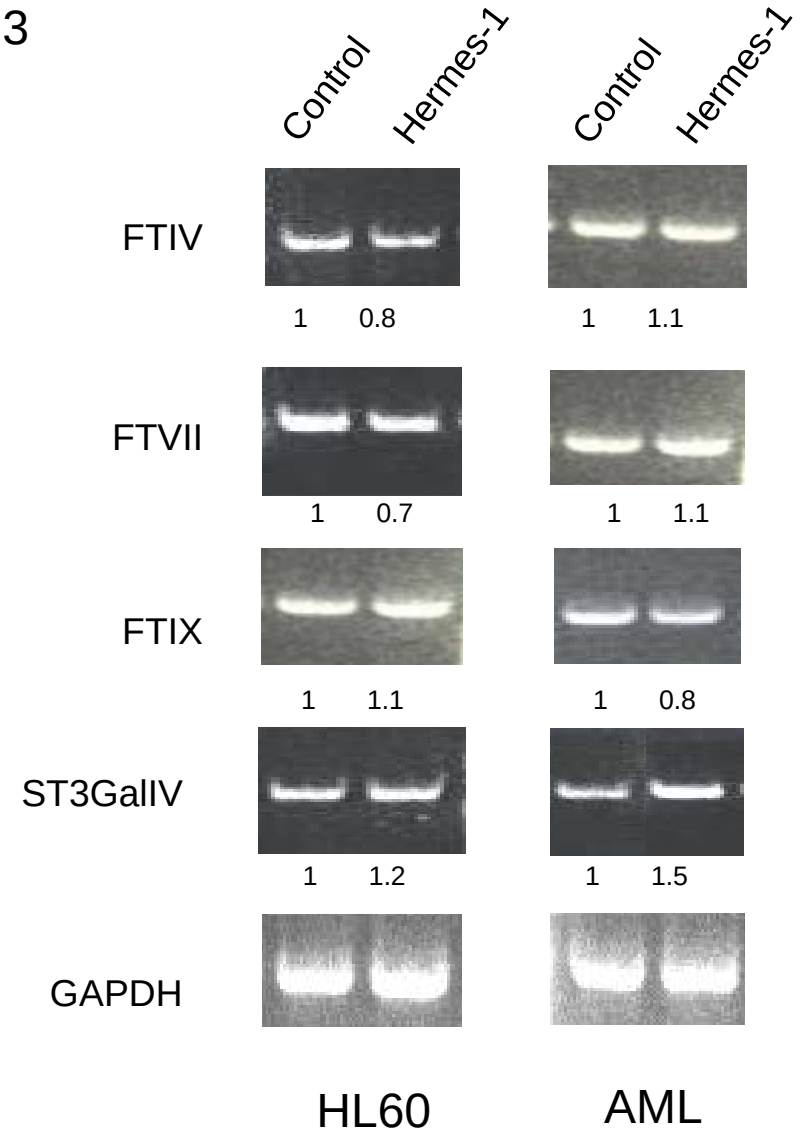
May-Grunwald Giemsa stained cytosmears of HL60 cells after 72h of treatment with Hermes-1 or isotype control mAb (control). Cells treated with anti-CD44 mAb show characteristic features of differentiated granulocytes, including a multilobular nucleus, condensed chromatin and a low nuclear:cytoplasmic ratio.

Supplementary Figure 2



CD44 ligation-induced changes in expression of sLex/CD15s and Lex/CD15
This figure is a representative histogram of Figure 1a.

Supplementary Figure 3



Changes in glycosyltransferase expression after anti-CD44 treatment
Representative ethidium bromide–stained gels of PCR-amplified RNA encoding glycosyltransferases from HL60 cells and AML blasts treated with isotype-matched mAb (control) or Hermes-1. Numbers indicate the relative expression of RT-PCR product normalized against GAPDH control.

Supplementary Table 1: Primers used for RT-PCR reactions

Gene	Sens	Antisens
ST3Gal IV	CTC TCC GAT ATC TGT TTT ATT TTC CCA TCC CAG AGA GAA GAA GGA G	GAT TAA GGT ACC AGG TCA GAA GGA GGT GAG GTT CTT
FucT-IV	TGG ACG CGT GGC GAG CCG CGG TGG CCA CTC GTG GA	AAC ACG CGT AGT ACC AGC GCC TTA TCC GTG CGT TC
FucT-VII	CCC ACC GTG GCC CAG TAC TAC CGC TTC T	CTG ACC TCT GTG CCC AGC CTC CCG T
FTIX	CAT TGA AAT CCA TAC CTA CGG GCA AG	AAA TCT CCA CCA AAA TAT ACA CGT TAC C
Neu-1	ACT GCC ACT GCC GAA TTG	GGT TGC CAG GGA TGA ATA GCC
Neu-3	GAG ACT GGC CCT GAG TCG ACA	AAC AGG CGG AAG GCA ATC
GAPDH	CCT CTG ACT TCA ACA GCG ACA	CAT GAC AAG GTG CGG CTC CC

Supplementary Methods

Antibodies and Reagents

The rat IgG2a anti-human CD44 mAb Hermes-1 was a gift from Dr Brenda Sandmaier (Fred Hutchinson Cancer Research Center, Seattle, WA). Neuraminidase was from Roche Diagnosis (Manneheim, Germany), bromelain, 4-methyllumbelliferyl-α-D-N-acetylneuraminic acid (4-MU-NANA) ammonium salt and 2,3-dehydro-deoxy-N-acetylneuraminic acid (DANA) were from Sigma Chemical Co (Saint-Louis, MO), proteinase K was from Boerhinger Mannheim Biochemical Co (Indianapolis, IN) and 4-F-GlcNAc was purchased from Toronto Research Chemicals Inc (Toronto, Canada). Murine anti-human Le^x (CD15) mAb (80H5, IgM) was purchased from Beckman Coulter (Miami, FL). Murine anti-human sLe^x (CD15s) mAb KM93 (IgM) was from Calbiochem (San Diego, CA) and CSLEX1 (IgM), mouse anti-human PSGL-1 mAb KPL-1 (IgG1), mouse anti-human CD43 mAb 1G10 (IgG1), rat anti-human CLA (HECA- 452, IgM) and Rat IgG2a were purchased from BD Biosciences (San Jose, CA). Anti-Neu1 rabbit polyclonal Ab (H-300) was purchased from Santa Cruz biotechnology (Santa cruz, CA) and Anti-Neu3 (mIgG1) was from MBL (Japan).

Human cells

All human samples were obtained in accordance with the procedures approved by the Human Experimentation and Ethics Committees of the Partners Cancer Care Institutions (Massachusetts General Hospital, Brigham and Women's Hospital and Dana Farber Cancer Institute). Primary AML blasts were isolated by Ficoll-hypaque (1.077

g/ml; Sigma Aldrich) density centrifugation of peripheral blood of patients with AML. The human leukemic cells HL60 and KG1a were from ATCC (Manassas, VA). Cells were isolated from normal human bone marrow. Red cells were separated using the dextran sedimentation method. Immature cells were separated from mature granulocytes by discontinuous Percoll (Amersham Pharmacia Biotech) density gradient centrifugation (1.065 g/ml and 1.080 g/ml; 1000*g* for 20 min at 4 °C).