E-Selectin Ligand Expression By Leukemic Blasts Is Associated with Prognosis in Patients with AML

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INTRODUCTION

The E-selectin ligand is a carbohydrate structure (sialyl Lewis x) expressed on myeloid cells and plays a role in cell adhesion and activation by binding specifically to E-selectin on endothelial cells. Acute myeloid leukemia (AML) blasts express the E-selectin ligand on glycoforms of CD44, CD62L and CD65. Interaction of AML blasts in the marrow microenvironment is believed to involve a number of receptors, including CKX4, VL4A, and CD44. E-selectin in the vascular niche regulates dormancy for hematopoetic stem cells (Winkler IG et al. Nat Med. 2012;18:1651-7), and chemoresistance and survival in AML. E-selectin inhibitors are now being studied in clinical trials in combination with chemotherapy for hematologic malignancies. Here, we evaluate the clinical significance of the expression of the E-selectin ligand using a panel of AML patient blood and bone marrow samples.

METHODS

We studied 89 serially acquired AML patient samples obtained with informed consent on an IRB approved protocol. Mononuclear cells were isolated from blood and bone marrow specimens after density depletion on Lymphocyte Separation Medium (Mediatech, Inc. Manassas, VA). We used multicolor flow cytometry to examine binding of an E-selectin-Fc chimeric protein or staining by HECA452 antibody that recognizes sialyl Lewis x/a carbohydrate epitopes. The blast population was gated by forward scatter vs. side scatter, then CD45 vs. side scatter. Leukemia stem cells (LSCs) were defined as CD34+CD138-CD123+. All antibodies were obtained from Becton Dickinson Biosciences. After labeling, the cells were washed with PBS and fixed with 2% paraformaldehyde. Flow cytometry analysis was performed using FACSsanto software (Tree Star, Inc.).

RESULTS

Correlations of E-selectin Ligand Expression with Clinical Parameters

Figure 1. Left. Percent E-selectin ligand functional expression (binding of Esel/Fc chimera) ranged from 0.4 to 99.4%, median 20.9%, mean 32.1%. Right. Percent staining by HECA452 antibody ranged from 0.9 to 99.6%, median 58%, mean 54.3%. These data suggest that the epitope recognized by the HECA452 antibody may be present, but not always functionally active. Correlation E-sel/Fc binding to HECA (Pearson’s) p-value = 1.54x10^-6

Figure 2. Left. Mean Fluorescence Intensity (MFI) for staining by HECA452 antibody ranged from 601 to 49,951, Median 4242, Mean 8853. Correlation Esel/Fc binding to HECA: 5.815e^-08

Figure 3. Left. Percent E-selectin ligand functional expression (binding of Esel/Fc chimera) by LSCs ranged from 0.6 to 100%, median 25%, mean 37.9%. Right. Percent staining by HECA452 antibody ranged from 1.9 to 98.8%, median 73%, mean 64%

Figure 4. Left. MFI for binding of Esel/Fc chimera by LSCs ranged from 338 to 29,980, Median 2340, Mean 5973. Right. MFI for staining by HECA452 antibody ranged from 3210 to 38,565, Median 3951, Mean 6680.

Figure 5. E-selectin ligand expression by blasts was highly correlated to expression by leukemia stem cells from the same patient, p=2.2e-16 (Pearson’s product-moment correlation) and correlation coefficient 0.90.

Figure 6. Left. Percent expression for new diagnosis vs. relapse. For percent binding of E-sel/Fc chimera (E-sel blast) new diagnosis (ND) mean 25% vs. relapse (R) 38% (*p=0.037). For percent HECA (Blast), ND 51% vs R 57% (p=0.40)

Right. The MFI was lower for E-sel/Fc chimera binding (Blast) and staining by HECA452 (Blast) for new diagnosis (ND) compared to relapse (R). MFI ND 2,884 vs R 1,794 for E-sel/Fc (*p=0.00074 Wilcoxon, p=0.0026 t-test), and MFI ND 3,387 vs R 8,994 by HECA staining (**p=0.0013 Wilcoxon, p=0.00038 t-test).

Figure 7. There was a significant difference for E-selectin ligand % expression by E-sel/Fc binding for favorable/intermediate (mean 24%) vs. unfavorable (mean 39%) cytogenetics. (p=0.0197) Wilch, p=0.053 Wilcoxon

Figure 8. E-selectin Ligand Expression with FLT3ITD or NPM1 Mutation Status

Figure 9. There was a significant difference for E-selectin ligand Blast MFI for FLT3ITD mutated (M) vs. wild type (WT), for E-sel/Fc binding, mean M 3885 vs WT 9919, *p=0.042, and for HECA M, S 300 vs WT 8733, *p=0.055. Right. For NPM1 mutated, E-sel/Fc binding (Blast) M 3597 M vs. 9839 WT, *p=0.038 and for HECA (Blast) M, 4562 and WT 8537, **p=0.049.

Statistical analysis was performed using the following: Welch Two Sample t test Wilcoxon rank sum test with continuity correction Pearson’s product-moment correlation

Patient Characteristics

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Acknowledgement/COI

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