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Adhesion Of Acute Myeloid Leukemia Blasts To E-Selectin In The Vascular Niche Enhances Their Survival By Mechanisms Such As Wnt Activation

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Background: Adhesion within the bone marrow microenvironment enhances leukemia survival and chemoresistance. Both normal hematopoietic stem cells and cancer cells are known to express E-selectin ligands, and adhesion of colon carcinoma cells to E selectin activates survival pathways such as NFκB (Porquet et al., BMC Cancer 11:285, 2011). E-selectin within the bone marrow vascular niche induces proliferation of normal hematopoietic stem cells (HSC), and a selectin inhibitor enhances HSC quiescence and self-renewal (Winkler et al., Nat Med 18:1651, 2012). We therefore initiated a study of E-selectin ligand expression and function by acute myeloid leukemia (AML) blasts to elucidate the potential role of E-selectin in AML biology and chemotherapy resistance.

Methods: Primary AML blasts and leukemia stem cells (LSCs) ($CD34^+CD38^-CD123^+$) obtained with informed consent from 40 patients were analyzed for E-selectin ligands by flow cytometry for binding of E-selectin-Fc chimera or by labeling with the HECA452 antibody. Primary AML blasts were engrafted in NODscid IL2Rgc-/- mice for studies of E-selectin inhibitors in combination with chemotherapy. Human Stem Cell Signaling, Leukemia, Apoptosis, and NFκB PCR Arrays (SA Biosciences) were used to analyze gene expression by quantitative RT-PCR after adhesion of primary AML patient blasts to E-selectin coated plates, compared to bovine serum albumin (BSA) coated plates. The activation of the Wnt pathway was studied by luciferase reporter assay.

Results: We find that the majority of primary patient acute myeloid leukemia blasts and leukemia stem cells express an E-selectin ligand, as demonstrated by flow cytometry by binding of E-selectin-Fc chimera and by staining with HECA-452 antibody [that recognizes hematopoietic cell E-/L-selectin ligand (HCELL) and cutaneous lymphocyte antigen (CLA)], as well as by binding to E-selectin coated plates. Flow cytometry analysis reveals that the mean percent binding of E-selectin-Fc chimera is 28% ± 24% (SD) by AML blasts, the mean % staining by the HECA-452 antibody 51% ± 35% (SD). De novo patients tended to have smaller mean fluorescence intensity (MFI) values than relapsed/refractory patients, as follows: for blasts, de novo mean 1441 ± 1127 (SD) vs. relapsed/refractory 4488 ± 4920 (SD) (Wilcoxon p=0.024), and for LSCs, de novo mean 1578 ± 1560 (SD) vs. relapsed/refractory 6601 ± 8498 (SD) (Wilcoxon p=0.061), suggesting upregulation of expression of E-selectin ligand for relapsed as compared to newly diagnosed patients. The specific E-selectin small molecule inhibitor GMI-1271 is able to overcome adhesion mediated chemotherapy resistance of AML in vitro and reduce the leukemia burden of primary AML engrafted NODscid $IL2Rgc^{-/-}$ mice in combination with chemotherapy agents daunorubicin and cytarabine. Addition of GMI1271 to chemotherapy in this xenograft model reduced the spleen burden at 2 weeks post treatment from 17.1 ± 10.4 X 10^6 $hCD45^+$ cells/spleen to 7.6 ± 5.8 (p=0.04).

To assess the molecular mechanism by which adhesion to E-selectin might protect AML blasts, we first screened with quantitative RT-PCR arrays. We found that adhesion to E-selectin caused upregulation of members of the Wnt and sonic hedgehog pathways for primary AML patient blasts grown on E-selectin vs. BSA coated plates, as well as members of other pathways critical to leukemia such as GM-CSF and IL-3 receptors and Fos. We then confirmed adhesion to E-selectin by AML blasts from 4 different patients enhanced activity of Wnt target genes by Wnt reporter assays. The Wnt reporter assay demonstrated 2-3 fold enhanced activity of Wnt target genes for AML blasts on E-selectin as compared to those on BSA, which increased to 3.3-4.5 fold with addition of Wnt3a. The inhibitor GMI-1271 reduced Wnt activity to 1.4-2.5 fold, similar to XAV939, an inhibitor of the Wnt/β catenin pathway that reduced activity to 1.1-1.8 fold. Similar reduction of Wnt pathway gene expression by GMI-1271 was also observed by the quantitative RT-PCR assay.

Conclusion: These data support a critical role for E-selectin, likely in the vascular bone marrow niche, that promotes survival of AML, that can be targeted with therapeutic intent, and suggests that GMI-1271 should be explored as a treatment for AML in combination with chemotherapy.