Vascular E-selectin Protects Leukemia Cells from Chemotherapy by Directly Activating Pro-survival NF-kB Signalling

Therapeutic E-selectin Blockade with GMI-1271 Inhibits NF-kB Activation in AML


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Significance

Median survival for adults diagnosed with Acute Myeloid Leukemia (AML) remains only... years despite best practise. New strategies are needed to improve treatment.

Humanised mouse models show the leukemia repopulating cells that are in contact with... in the bone marrow are most likely to survive chemotherapy. Petrovsky et al., 2007.

We hypothesise that adhesion to vascular niche E-(endothelial)-selectin alone can promote leukemia stem cell (LSC) survival in vivo.

Introduction

The vascular adhesion molecule E-selectin has been shown to be a key component of the... role in facilitating HSC activation at the expense of self-renewal (Wakade et al., Nat Med. 2012).

Only ~3% of endothelial cells normally express E-selectin. However we find E-selectin to be greatly upregulated (~10-fold in mice engrafted with AML). This raises the question whether E-selectin-mediated signalling between HSC and AML LIC differs.

MECHANISM

RNA sequence analysis — NF-kB intracellular signalling pathway is dampened by E-selectin blockade with GMI-1271 in vivo

FACS purified BM leukemic blasts from mice administered ± GMI-1271 (4 days) were processed for RNA sequencing. NF-kB activation is frequent in AML. NF-kB activation is well known to promote survival to chemotherapy. Pommier, Galli et al., Frontiers in Oncology 2013.

To understand mechanisms of chemo-sensitisation, leukemic mice were administered the small molecule glycomimetic antagonist of E-selectin GMI-1271 (from GlycoMimetics), or saline vehicle control for 4 days & cytobane chemotherapy then analysed for AML survival. In some experiments NF-kb commercial small molecule inhibitor BMS-345541 was also used. Leukemia models used include murine mono-myelocytic leukemia induced by MLL-AF9 (13p23 translocation) and granulocytic leukemia induced by AML-ETO9a (12p21 translocation). Results also verified using human CD34+ leukemia cell line KG1a in vitro.

Reagents & Methods

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Fig 3a.. Fig 3b. These data are consistent with previous publications showing chemoresistance and re-entry thereby breaking the chemo resistance observed with these cells.

Fig 7. In mice E-selectin blockade (GMI-1271) is as efficacious as NF-kB blockade at chemo-sensitisation of bone marrow AML cells in vivo.

Fig 8. GMI-1271 administered together with chemotherapy significantly extends overall survival in mice.

Conclusions

Upstream blockade of E-selectin by GMI-1271 not only inhibits NF-kB activation but also mobilizes LSC out of the protective BM niche & prevents re-entry thereby breaking the chemo resistance observed with these cells.

Fig 2. In vitro chemo-sensitivity assay. Bone marrow leukemic blasts were cultured 4 days in wells pre-coated with immobilized recombinant adhesion molecules (E-selectin, VCAM-1) and controls (BSA, IgG) in presence of ± Cytarabine (Ara-C).

Fig 6. Blocking NF-kB signalling in AML cells in vitro reverses E-selectin-mediated chemoresistance.

Fig 4. E-selectin mediated adhesion upregulates NF-kB – GFP reporter in murine leukemia cell line. Readout by flow cytometry for GFP after 2hr adhesion to immobilised recombinant E-selectin compared to other vascular adhesion molecules in wells.

Fig 5. Adhesion to E-selectin leads to phosphorylation activation of NF-kB p65. Murine leukemia cells were cultured in contact with E-selectin for times (mins) specified, then cell lysates immunoblotted for NF-kB phospho p65 and actin loading control.

Fig 4. In vivo blockade of E-selectin and NK-kB similarly chemoresist AML LSC (CFC)

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A Phase I/II Clinical trial to study efficacy of GMI-1271 in combination with chemotherapy in AML patients (NCT02306293) is currently in progress.