Chemotherapy resistance in acute myeloid leukemia: the role of adhesion

Joe C. Huang, M.D.
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Mentor: Pamela S. Becker, M.D., Ph.D.
Associate Professor of Medicine/Hematology
Institute for Stem Cell and Regenerative Medicine
University of Washington
Acute myeloid leukemia (AML)

- Most common adult leukemia
- Incidence in 2007: 13,410 cases
- Deaths in 2007: 8,990
- For patients <60 yrs old, 30-35% survive >5 years
- For patients >60 yrs, <10% survive long-term
AML

• Principle cause of treatment failure is chemotherapy resistance despite initial complete remission rate of 65%
• Survival of AML cells in bone marrow following chemotherapy = minimal residual disease
• Minimal residual disease results in emergence of chemotherapy-resistant leukemia
• What role does the bone marrow provide in chemotherapy resistance?
Environment mediated drug resistance

- Bone marrow microenvironment promotes survival and proliferation of malignant cells, with adhesion sequestering malignant cells in a protective niche – *de novo* drug resistance independent of genetic changes caused by selective pressure of drug exposure (acquired drug resistance)
Role of CXCR-4

• Chemokine receptor expressed by normal and malignant hematopoietic cells – including NHL, myeloma, chronic and acute leukemias

• Ligand: stromal-derived factor-1 (SDF-1)

• SDF-1 is constitutively secreted by bone marrow cells
CXCR-4

- SDF-1 acts on normal and malignant hematopoietic progenitor cells that express its receptor
- Critical to homing and retention of malignant cells to the bone marrow microenvironment
- CXCR-4 expression correlated to prognosis in AML (low expression with longer relapse-free and overall survival)
Role of VLA-4

- Very late antigen-4, an $\alpha 4\beta 1$ integrin
- Ligands: vascular cell adhesion molecule-1 (VCAM-1) and alternatively spliced fibronectin
- Migration of CD34+ cells and AML cells beneath marrow stroma
- Altered integrin expression involved in solid tumor metastasis (prostate, lung, breast) to bone and lodgment in bone marrow
VLA-4

• Integrin-mediated signaling protects against chemotherapy toxicity through activation of survival/anti-apoptotic pathways (PI3K/Akt/bcl-2, Wnt pathways) and induces quiescence

• Function of VLA-4 correlated to AML survival

• CXCR-4 enhances VLA-4 mediated adhesion and therefore both are potential therapeutic targets
Leukemia stem cells

- AML a disease of myeloid stem/progenitor cells
- Often CD34+ CD38- (variable) CD123+ CD96+ quiescent population
- Functional capacity of transplanted human LSC to re-populate lymphoid and myeloid lines of NOD/SCID mice
- Cells capable of self-renewal and propagation of the leukemia (theoretical)
Cytarabine (Ara C)

- Ara C – the most active nucleoside inhibitor in inducing remission in patients with AML
- S-phase specific, therefore activity dependent on cell cycling
- Ara C resistance likely due mechanisms that lead to decreased intra-cellular Ara CTP levels
Ara C Metabolism

- **1 = hENT1**, human equilibrative nucleoside transporter 1, transports ara C into cell
- **2 = dCK**, deoxycytidine kinase, rate-limiting step in ara C metabolism
- **3 = PN-1**, pyrimidine nucleotidase 1, inhibits dCK, therefore decreases ara CTP
- **4 = CDA**, cytidine deaminase, de-activating enzyme for ara CTP
- **5 = dCPMD**, deoxycytidylate deaminase, de-activating enzyme for ara CTP
Hypotheses

• Adhesion between primary AML cells and stromal cells protect leukemia cells from chemotherapy in vitro

• Disruption of adhesion interactions between AML cells and stromal cells enhance chemosensitivity of AML cells to Ara C

• Adhesion interactions between leukemia stem cells and stromal cells alters chemotherapy metabolism
Methods

- Primary AML samples from bone marrow or peripheral blood obtained from patients with newly-diagnosed AML and cryopreserved in liquid nitrogen.
- Samples thawed and incubated in media supplemented by serum and cytokines.
- Ficoll-hypaque separation of live cells.
Methods

• Stromal cells obtained from HS-5 and HS27a cell lines and maintained in tissue culture
• Stromal cells plated and allowed to form monolayer prior to addition of AML cells in tissue culture plates
• Retronectin used as a recombinant ligand for VLA-4
Methods

• AML cells separated by magnetic bead column enrichment/depletion – CD34+, CD38-, CD14+
• CD34+CD38- AML stem cells sorted by flow cytometry and serially transplanted into NOD/SCID mice
• AML cells fluorescently labeled with CFSE prior to adhesion with stroma
• AML cells co-cultured with stromal cells prior to addition of chemotherapy
Methods

- Ara C tested at 5, 10, 20 μM
- AMD3100 (Plerixafor) for CXCR-4 inhibitor at 1 and 15 μM
- Anti-CD49d for anti-VLA-4 inhibitor at 10 μg/mL
- Cell viability determined via MTT reduction assay, trypan blue exclusion and CFSE-labeling of leukemia cells with cell counting on hemocytometer following trypsinization 24 – 72 hrs following exposure to chemotherapy
Methods

• RNA extraction, RT-PCR and DNA gel electrophoresis to evaluate expression of enzymes in Ara C metabolism on CD34+ vs. CD34-14+ cells and the effect of adhesion with retromectin

• Primers and probes for enzymes in Ara C metabolism obtained from Applied Biosystems Inc.
CFSE-labeled AML cell on stroma
CFSE-labeled AML cell on stroma
CFSE-labeled AML cells/trypan blue exclusion on hemocytometer
AML026 CD34+ bone marrow cells cultured on HS5 stroma following exposure to Cytarabine (Ara C, µM) +/- AMD3100 (15 µM) +/- VLA-4 inhibitor (anti-CD49d, 10 µg/mL) at 72h
AML037 CD34+ bone marrow cells cultured on HS27a stroma after exposure to Cytarabine (Ara C, µM) +/- AMD3100 (1 µM) +/- VLA-4 inhibitor (anti-CD49d, 1 µM) x 24h
NOD/SCID Mouse 2095 (engrafted from CD34+38- cells of AML037) CD34+ bone marrow cells cultured on HS27a stroma after exposure to Cytarabine (Ara C, µM) +/- AMD3100 (1 µM) +/- VLA-4 inhibitor (anti-CD49d, 1 µM) x 24h

Cell survival (% of control)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Cell Survival %</th>
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<tbody>
<tr>
<td>Control</td>
<td>100</td>
</tr>
<tr>
<td>Ara C 20</td>
<td>80</td>
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<tr>
<td>Ara C 20 + AMD3100</td>
<td>70</td>
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p = 0.70

p = 0.12
AML029 CD34+ peripheral blood cells (with and without AMD3100 1 µM exposure) cultured on HS27a stroma and exposed to cytarabine (Ara C) and/or clofarabine at varying concentrations (µM) with survival at 72h.

Cell survival (% control)

- Control
- Ara C 4
- Ara C 4 + Clofar 2
- Ara C 4 + Clofar 4
- Clofar 4
- Ara C 2 + Clofar 4
- Ara C 7 + Clofar 3

p = 0.13
AML022 CD14+ peripheral blood cells (with and without AMD3100 1 µM exposure) cultured on HS27a stroma and exposed to cytarabine (Ara C) and/or clofarabine at varying concentrations (µM) with survival at 72h.
AML012 CD34+ vs CD34+38- peripheral blood cells cultured on retronectin following exposure to Cytarabine (Ara C) [µM] with cell viability by MTT assay

Control Ara C 5 Ara C 10 Ara C 20

Optical Density

CD34+ cells
CD34+38- cells

p = 0.01  p = 0.16  p = 0.28  p = 0.43
Adhesion and Ara C metabolism

- Do leukemia stem cells (CD34+) differ from more mature cells (CD34-14+) in expression of enzymes for Ara C drug metabolism?
- Does adhesion lead to altered chemotherapy metabolism?
- CD34+ cells vs CD34-14+ cells cultured on heat-inactivated 2% BSA or retronectin with and without Ara C at 1 μM
- RNA extraction with RT-PCR and DNA gel electrophoresis performed to evaluate expression of hENT1, dCK, CDA, dCMPD, PN-1 with GAPDH as control
DNA Electrophoresis
RT-PCR Results

• CD34+ and CD34-14+ cells with greater expression of de-activating enzymes (CDA, dCMPD, PN-1) when cells are cultured on retronectin compared to BSA

• CD34-14+ cells with greater expression of de-activating enzyme dCMPD compared to CD34+ cells – CD34+ cells with likely decreased uptake of Ara C and therefore less expression of enzymes involved in Ara C inactivation
Conclusions

• Adhesion of CD34+ AML cells to HS5 or HS27a stroma promotes survival from chemotherapy
• Leukemia stem cells appear more resistant to chemotherapy
• For some patients with newly-diagnosed AML, disruption of adhesion between stromal cells and CD34+ AML cells with a combination of CXCR-4 and VLA-4 inhibitors appear to enhance sensitivity to Ara C
• Adhesion-mediated interactions may protect AML cells from chemotoxicity due to altered Ara C metabolism by increased expression of enzymes that degrade Ara C
Future Directions

• Characterizing the critical adhesion interactions between leukemia cells and stroma
• Use of multiple adhesion inhibitors with chemotherapy to improve killing of leukemia stem cells
• Measurement of Ara-CTP levels and the effect of adhesion on Ara-CTP accumulation rates
• Real-time RT-PCR of leukemia stem cells to evaluate the effect of adhesion on a variety of signaling pathways involved in chemotherapy uptake, metabolism, apoptosis, growth and differentiation
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References


