Pathogenetic Features of Hematopoiesis in MDS: Focus on Aging

Irving L. Weissman, MD
Stanford University School of Medicine
Director, Institute for Stem Cell Biology and Regenerative Medicine
Director, Stanford Ludwig Center for Cancer Stem Cell Research and Medicine
Professor of Pathology and Developmental Biology
Stanford, California
I have no relevant financial relationships to disclose.
Blood-forming stem cells make blood, and only blood
A Model of HSC Ontogeny

Cell intrinsic changes

Young adult → Mid-age → Old age

- **Self-renewal**
- **Myeloid potential**
- **Myeloid genes**
- **Leukemia genes**

- **Lymphoid potential**
- **Homing**
- **Lymphoid genes**

1) The cell-intrinsic functional and molecular properties of aging LT-HSC places the cellular and molecular rationale for the decreased immuno-competence that accompanies aging at the level of the LT-HSC.

2) Age-dependent upregulation of genes frequently activated as proto-oncogenes in leukemogenesis (17/32 highest.)

3) ...and quiescent aged HSC build up DNA DS breaks, repaired upon G0 to G1 to S transition

   *Rossi, Bryder, Weissman, Seita, Beerman*

4) Is this maturation of all HSC, or clonal selection of some HSC?
Clonal Selection or epigenetic programming of HSC with Aging

Hypothesis and data: Rossi, Eaves, Muller-Sieburg, Nakauchi, Bryder, Beerman, and Weissman
Leukemic cells in AML patients

LT STEM CELLS

CD34$^+$CD38$^-$Thy$^+$Lin$^-$

5-40%
AML-1/ETO$^+$

Normal colonies

MPP

CD34$^+$CD38$^-$Thy$^-$Lin$^-$

Leukemia Stem Cells [LSC]

Leukemic blast colonies

Miyamoto, Weissman
Akashi, PNAS 2000: 97: 6924
Cell of origin – progression to leukemia

We consider various events that lead to the emergence of a leukemic clone. These include:

1. Oncogenic lesions (e.g., BCR-ABL, AML1-ETO, JAK2-REAR translocations)
2. Antiapoptosis (1 or more events, e.g., BCL-2, FAS)
3. Loss of tumor suppression (e.g., p53, RB, PTEN)
4. Longevity assurance (e.g., TERT)
5. Evade adaptive immune system
6. Evade innate immune system
7. Deregulation of self-renewal (e.g., WNT)

The diagram illustrates the progression from stem cell compartment to mature effector cells, with specific events and their impact on the development of leukemia.
CML

- Fialkow: clonal disorder in G,M,E,B cells; Rowley/Nowell bcr-abl translocation; fusion protein in chronic, myeloproliferative phase; LSC proposed to be HSC or MPP; Jamieson and Weissman HSC.

- No increase in CD34+38lo90+Lin- frequency: competitive advantage vs nl HSC

- Myeloid blast crisis is at the stage of GMP, and overexpress activated β-catenin; axin inhibits them.

- 4/7 pts overexpress β-catenin by mis-splicing GSK3 the other inhibitor of β-catenin

Figure 3. Aberrant GSK3β Expression by Blast Crisis CML Progenitors

A. Progenitor FACS Analysis

Normal Progenitors  CML BC Progenitors

B. Q-PCR of GSK3β Expression

C. GSK3β FACS

D. Confocal Analysis of β-catenin Expression

Normal GMP  CML GMP

E. GSK3β cDNA Sequencing Analysis

Hematopoietic Stem Cells
Deletion of GSK3β exons 8 and 9

Progenitors
Deletion of GSK3β exons 8 and 9

Lineage-positive Cells
Deletion of GSK3β exon 9
Identification of Somatic Mutations by Exome Sequencing

AML Gene Annotation

<table>
<thead>
<tr>
<th>AML</th>
<th>Gene</th>
<th>Annotation</th>
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<tr>
<td>SU070</td>
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<td>V616I</td>
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<td></td>
<td>KALRN</td>
<td>S44P</td>
</tr>
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<td>Y1649stop</td>
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<td></td>
<td>TET2</td>
<td>T1884A</td>
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<tr>
<td></td>
<td>TMEM8B</td>
<td>nt G471A</td>
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<tr>
<td></td>
<td>NCRNA00200</td>
<td>nt G354A</td>
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<td></td>
<td>TMEM20</td>
<td>A143T</td>
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<td>ZRANB1</td>
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<td>GABARAP1</td>
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<td>DOCK9</td>
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<td></td>
<td>FLT3</td>
<td>599-610 ITD</td>
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</tbody>
</table>

Next generation exome sequencing

Max Jan
Thomas Snyder
Ryan Corces-Zimmerman
Steve Quake
Ravi Majeti
Irv Weissman

Science Translational Medicine
Analysis of Single HSC to Identify Pre-Leukemic Clones

Normal HSC

--- Stages of preleukemia progression in HSC clone---

LSC at progenitor stage

Max Jan
Thomas Snyder
Ryan Corces-Zimmerman
Steve Quake
Ravi Majeti
If you want to know which genes are likely oncogenes, ask the cancer.

Ryan corces –Zimmerman, Ravi Majeti, and IW
1 In AML, progression is in a blood stem cell clone, while the leukemia stem cells is at the progenitor stage: preleukemic clones of HSC compete with nl HSC

2 There are no leukemias we have found that are leukemias of HSC

3 If this is true for leukemia, it is probably true for all cancers in tissues that regenerate from tissue stem cells
CD47 was discovered as a marker of aging RBC by Oldenborg. We found it on m/h AML LSC.

**Hypothesis:** Increased expression of CD47 on myeloid leukemia cells contributes to pathogenesis by facilitating evasion of phagocytosis.

**Prediction:** Increased expression of CD47 on human AML is associated with a worse clinical outcome.

*Traver and IW 1998; Jaiswal, Majeti, Chao, and IW 2008.*
Anti-CD47 Antibodies Enable Phagocytosis of AML LSC

Human Macrophages

Mark Chao, Majeti et al
Anti-CD47 Antibody Depletes AML in the Bone Marrow

IgG Control  Anti-CD47  Anti-CD47

100X  100X  200X

200X  200X  200X

Inject Tumor Cells Into Peritoneal Cavity

Initiate Treatment

Evaluate Tumor Growth With Bioluminescence Imaging

2 Weeks

2.5 Months

Anti-CD47 Antibodies Inhibit Growth Of Xenotransplanted Patient Tumors

Ovarian Cancer
Anti-CD47 Antibodies Inhibit Growth Of Xenotransplanted Patient Tumors

Glialoblastoma

MITRA, JIANG, CHESHIER, RAVEH, IW

Inject Tumor Cells Into Left Hemisphere
Initiate Treatment
Monitor Tumor Growth With Bioluminescence Imaging
Evaluate Tumor Formation

2 Weeks
8 Weeks
### Investigation and Targeting of CD47 in Human Cancers

#### Monotherapy trials

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Condition</th>
</tr>
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<tbody>
<tr>
<td>Breast</td>
<td>Glioblastoma</td>
</tr>
<tr>
<td>Ovarian</td>
<td>Medulloblastoma</td>
</tr>
<tr>
<td>Bladder</td>
<td>Oligodendroglioma</td>
</tr>
<tr>
<td>Pancreatic</td>
<td>Hepatocellular Carcinoma</td>
</tr>
<tr>
<td>Colon</td>
<td>Gastric Cancer</td>
</tr>
<tr>
<td>Prostate</td>
<td>Multiple Myeloma</td>
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<tr>
<td>Lung</td>
<td>Chronic Myeloid Leukemia</td>
</tr>
<tr>
<td>Kidney</td>
<td>Acute Myeloid Leukemia</td>
</tr>
<tr>
<td>Leiomyosarcoma</td>
<td>Non-Hodgkin’s Lymphoma</td>
</tr>
<tr>
<td>Head &amp; Neck</td>
<td>T-Acute Lymphoblastic Leukemia</td>
</tr>
<tr>
<td>Melanoma</td>
<td>B-Acute Lymphoblastic Leukemia</td>
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</tbody>
</table>

In all cases tested with metastatic tumors, the metastases are eliminated.
Precancer cells express calreticulin, and emergent cancer clones overcome this with CD47

Increased expression of CD47 on myeloid leukemia cells contributes to pathogenesis by facilitating evasion of phagocytosis

Chao, Jaiswal, Weissman-Tsukamoto, Majeti, and IW Science Transl Med 2010. 2(63)
Toll like receptor agonist incubation of macrophages increases anti-CD47 mediated phagocytosis of cancer cells.

Mingye Feng, Volkmer, Weissman
TLR agonists synergize with anti-CD47 to eliminate a prostate cancer xenograft in vivo
BTK is expressed in many blood lineage cells, including macrophages.
Phagocytosis of tumor cells is inhibited by the BTK inhibitor, ibrutinib

Mingye Feng, Volkmer, Weissman
Anti calreticulin blocking antibody inhibits phagocytosis when incubated with macrophages, but not with tumor cells.
Precancer cells express calreticulin, and emergent cancer clones overcome this with CD47

Increased expression of CD47 on myeloid leukemia cells contributes to pathogenesis by facilitating evasion of phagocytosis

Calreticulin is an ER protein that is secreted following TLR to BTK signalling; BTK phosphorylates calreticulin, which in the ER is leaved to separate from KDEL domain. pY-calR binds to Lrp-1 or tumor cell. Mingye Feng, J. Volkmer, and Weissman: PNAS 2015
In MDS: HSCs are the disease initiating cell and exhibit defective hematopoiesis.

Pang et al, PNAS 2013
MDS is a pre-AML disease of older patients in which a cytopenia Precedes leukemia.

MDS HSC outcompete normal HSC in MDS patients and in transplanted NSG mice

Wendy Pang, John Pluvinage, Chris Park, IW, et al
Calreticulin expressed on MDS progenitors, but not HSC, causes programmed cell removal

Wendy Pang, John Pluvinage, Chris Park, IW, et al
Prospective identification and isolation of human erythroid lineage-committed progenitors (EP)

Mori et al, PNAS 2015
In MDS: EPs are reduced in frequency, with increased cell-surface calreticulin (CRT) expression.

* = p < 0.05
Conclusions

MDS HSC outcompete normal HSC in patient and xenotransplants

The MDS-initiating cell resides in the HSC compartment

High CRT predisposes MDS myeloid [GMP, MkP, EP] progenitors for programmed cell removal

Increased CD47 expression is a crucial step in the progression from MDS to AML

• Calreticulin and LRP1 are potential therapeutic targets

Wendy Pang, John Pluvinage, Chris Park, IW, et al
BCL2 blocks apoptosis, but not programmed cell removal of neutrophiles: Lagasse and Weissman JEM 1994

Table 1. Neutrophil Content in the Bone Marrow, Blood and Spleen of Control and Transgenic Mice

<table>
<thead>
<tr>
<th>Mice</th>
<th>Bone marrow</th>
<th>Blood</th>
<th>Spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nontransgenic</td>
<td>30.4 ± 4.0</td>
<td>5.8 ± 2.8</td>
<td>1.8 ± 0.4</td>
</tr>
<tr>
<td>Transgenic</td>
<td>30.3 ± 8.3</td>
<td>12.6 ± 3.8</td>
<td>2.2 ± 0.4</td>
</tr>
</tbody>
</table>

Neutrophils were counted by flow cytometric analysis of cells bearing Mac-1 and Gr-1 using two-color immunofluorescence. The results are expressed as arithmetic means (three mice) ± SD.
PCDeath and PCRemoval

- PCD is accompanied by PCR; blocking PCD with bcl2 does NOT block PCR [Lagasse and Weissman 1994, JEM]. PCR prevents inflammation.
- All cancers defeat PCD: p53, bcl2, bax,NFkB, etc
- All cancers defeat PCR: calreticulin, Ph-serine, asialoglycoprotein are ‘eat me’ signals countered by the CD47 ‘don’t eat me’ signal.
- Stimuli that induce PCD and/or PCR develop cell competition/selection in pre-cancer lineages that can result in cancer clones
In UAE: total MEPs are increased in frequency but have impaired erythroid differentiation.

Wendy Pang, Weissman, Schrier
UAE is not uniform:
E-MEP have impaired erythroid differentiation at different stages in liquid culture