Update on animal models for MDS

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Approaches to model MDS *in vivo*

- Xeno-graft human MDS cells into immuno-deficient mice
- Transplant of modified bone marrow (most commonly, use of retrovirus to introduce candidate oncogenes).
- Genetically-engineered mice (GEM)
  - knock-out
  - knock-in
  - transgenic
  - conditional
MDS Xenografts

• Nilsson et al (Jacobsen lab)
  – 7 del5q patients into NOD/SCID mice
  – 1/7 engrafted 12% human cells, no evidence for clinical MDS

• Benito et al (Deeg lab)
  – 61 MDS pts (RA-RAEBt) into NOD/SCID
  – 48 evaluable, 34 engrafted, no mice with clinical MDS, no evidence for MDS clone engraftment
  – F/U paper demonstrated engraftment (0.7-58%) of a del(5q) clone in NOD/SCID/B2 null mice, but no clinical MDS

• Thanopoulou et al (Eaves lab)
  – 7 MDS (RA-RAEBt) into NOD/SCID/B2 null tg huIL3/GM-SCF/SCF
  – Engrafted MDS cells with trisomy8 or del5q, but low level (<1%), no evidence for clinical MDS
Koch’s postulates

- The microorganism must be found in abundance in all organisms with the disease.
- The microorganism must be isolated from a diseased organism and grown in culture.
- The cultured microorganism should cause disease when introduced into a healthy organism.
- The microorganism must be re-isolated from the inoculated, diseased experimental host.

Koch’s postulates (adapted for cancer)

- The (cancer gene) must be found in abundance in all organisms suffering from the (particular subtype of cancer).
- The (cancer gene) must be isolated from a diseased organism.
- The (cancer gene) should cause disease when introduced into a healthy organism.
- The (cancer gene) must be re-isolated from (expressed in) the inoculated, diseased experimental host.
## Mouse models for MDS

<table>
<thead>
<tr>
<th>Gene</th>
<th>Clinical Findings</th>
<th>Technique</th>
<th>Mouse Findings</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evi1</td>
<td>Translocated in some patients with MDS</td>
<td>Retroviral transduction</td>
<td>Anemia, thrombocytopenia, variable leukopenia, hypercellular BM, BM dysplasia, uniformly fatal (14 mos), no leukemia.</td>
<td>Buonamici JCI 114:713 2004</td>
</tr>
<tr>
<td>RUNX1</td>
<td>15% MDS patients have point mutation</td>
<td>Retroviral transduction (S291fs)</td>
<td>Anemia, macrocytosis, leukopenia, thrombocytopenia, hyper/normocellular BM, dysplasia, survival 10% at 14 mos, 60% leukemic transformation.</td>
<td>Watanabe-Okochi Blood 111:4297 2008</td>
</tr>
<tr>
<td>SALL4B</td>
<td>Over-expressed in some patients with AML</td>
<td>Transgenic (CMV promoter)</td>
<td>Mild anemia, mild neutropenia, variable thrombocytosis, BM dysplasia and hypercellularity, survival 12% at 24 mos, 50% leukemic transformation (AML).</td>
<td>Ma Blood 108:2726 2006</td>
</tr>
</tbody>
</table>

- 5q- syndrome
- Dicer deletion
- **NUP98-HOX fusions**
5q- syndrome

- 10% of all MDS.
- Characterized by macrocytic anemia, thrombocytosis, uncommon transformation to AML (<10%), and deletions of 5q; CDR at 5q33.1.
- Candidates; *miR145/miR146a, RPS14*.
- Deletion of *Rps14* (floxed region; *Lmo2-Cre*) leads to erythrodysplasia, macrocytic anemia, mild thrombocytopenia.  (Barlow et al., Nat Med 2010)
- *miR145* targets *Fli-1*; overexpression of *Fli-1* leads to thrombocytosis (Kumar, ASH #947, 2009)
- *miR145/146a* target *TIRAP/TRA6*. Suppression of *miR145* or *146a* leads to increased megakaryocytic colony formation; overexpression of *TRA6* leads to thrombocytosis, erythroid/megakaryocytic dysplasia, and transformation to AML.  (Starczynowski et al., Nat med 2010).
MDS caused by abnormal hematopoietic niche

• Mesenchymal stromal cells that express *osterix* are important for HSC niche formation.
• *Dicer* is an RNA endonuclease essential for miRNA processing.
• Delete *dicer* specifically in bone progenitors by using *osterix* promoter to direct Cre expression in mice with a floxed *dicer* allele. (OCD^{fl/fl}; OCD^{fl/+}).

Peripheral blood cytopenia, dysplasia, and apoptosis

MDS in OCDfl/fl mice is induced by bone marrow microenvironment.

~2% of mice develop AML (chloromatous presentation) with clonal chromosomal abnormalities
# NUP98 Translocations and Hematologic Diseases

<table>
<thead>
<tr>
<th>Translocation</th>
<th>Partner</th>
<th>Homeodomain?</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>t(7;11)(p15;p15)</td>
<td><em>HOXA9, 11, 13</em></td>
<td>Yes</td>
<td>MDS, AML, CML</td>
</tr>
<tr>
<td>t(11;12)(p15;q13)</td>
<td><em>HOXC11,13</em></td>
<td>Yes</td>
<td>MDS, AML</td>
</tr>
<tr>
<td>t(2;11)(q31;p15)</td>
<td><em>HOXD11, 13</em></td>
<td>Yes</td>
<td>MDS, AML, CML</td>
</tr>
<tr>
<td>t(1;11)(q23;p15)</td>
<td><em>PMX1 (PRRX1)</em></td>
<td>Yes</td>
<td>MDS, AML</td>
</tr>
<tr>
<td>t(9;11)(q34;p15)</td>
<td><em>PMX2 (PRRX2)</em></td>
<td>Yes*</td>
<td>MDS, AML</td>
</tr>
<tr>
<td>t(10;11)(q23;p15)</td>
<td><em>HHEX</em></td>
<td>Yes*</td>
<td>AML</td>
</tr>
<tr>
<td>t(11;17)(p15;p13)</td>
<td><em>PHF23</em></td>
<td>Yes*</td>
<td>AML</td>
</tr>
<tr>
<td>t(11;12)(p15;p13)</td>
<td><em>JARID1A</em></td>
<td>Yes*</td>
<td>AML</td>
</tr>
</tbody>
</table>

Can NUP98-HOX fusions be used to model MDS?

**FG**=phenylalanine/glycine repeats  
**HD**=homeodomain
**vavNHD13 mice display peripheral blood cytopenia and bone marrow dysplasia/hypercellularity**

<table>
<thead>
<tr>
<th></th>
<th>WBC (10^9/L)</th>
<th>NE (10^9/L)</th>
<th>Ly (10^9/L)</th>
<th>Hb (g/dl)</th>
<th>MCV (fl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NHD13 (n = 22)</td>
<td>1.83 ± 0.54</td>
<td>0.36 ± 0.22</td>
<td>1.24 ± 0.36</td>
<td>11.9 ± 2.1</td>
<td>54.0 ± 5.0</td>
</tr>
<tr>
<td>Control (n = 7)</td>
<td>6.50 ± 1.84</td>
<td>1.38 ± 0.95</td>
<td>4.77 ± 0.90</td>
<td>14.2 ± 0.7</td>
<td>52.5 ± 3.2</td>
</tr>
<tr>
<td><strong>p value</strong>d</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.901</td>
</tr>
</tbody>
</table>

- Micro-megakaryocytes
- Multinucleate erythroblasts
- Hypercellularity
  - WT: 21 - 23 x 10^6 cells
  - NHD13: 45 - 60 x 10^6 cells
Impaired differentiation and increased apoptosis of *NHD13* cells *in vitro*

Immature lineage negative (lin^neg^) cells cultured with IL3, IL6, SCF

Apoptosis of lin^neg^ cells with IL3/6/SCF

Yang-Jo

Chul Won
Over 60% of *NHD13* mice transform to AML

Progression of MDS to erythroleukemia

40% of AML mice have spontaneous, acquired mutations in *Kras, Nras, or Cbl*

Peripheral blood

Bone marrow
Increased expression of interferon-induced and homeodomain genes in *NHD13* BM

- 10 genes 3-fold increased in whole BM from *NHD13* mice with MDS (MEEBO/Stanford 38K)
- *Oas2, Ifit1, Ifi44, Hoxa9, Hoxa7, Klhl4, Pbx3, Lin28b, Hoxc6, Stfa2.*  
  *Interferon induced*  
  *Homeodomain*
- RQ-PCR demonstrated 3-10 fold increased expression of *Hoxa* cluster genes (*Hoxa5, 7, 9, 10*). Similar results in lin- and lin+ BM fraction.
Is MDS transplantable (Yang Jo Chung)?
(Attempts to xenograft MDS by numerous labs have been unsuccessful)
(Why - cross-species barrier, inability to transplant pre-malignant lesion?)
Can an MDS Stem (Initiating) Cell (M-IC) be identified?

\[
\text{NHD13} + \quad \text{Donor (CD45 Ly5.2)} \quad \text{NHD13} - (\text{WT})
\]

\[\pm \text{WT competitor BM (CD45 Ly5.1)}\]

1000rad 1000rad

Recipient mice (CD45 Ly5.1)

Assay for hematologic engraftment and differentiation.

**Cell number**

- \(1 \times 10^6\) NHD13 or WT (Ly5.2) + \(1 \times 10^5\) WT competitor (Ly5.1)/ mouse
- \(1 \times 10^5\) NHD13 or WT (Ly5.2) + \(1 \times 10^6\) WT competitor (Ly5.1)/ mouse

(Collaborator: T. Fry) (Chung et al., PNAS, 2008)
Peripheral blood cytopenias and normocellular BM = ineffective hematopoiesis

Mice transplanted with 1x10^6 Donor (Ly5.2) and 1x10^5 WT competitor (Ly5.1) cells

<table>
<thead>
<tr>
<th></th>
<th>HGB (g/dL)</th>
<th>MCV (fL)</th>
<th>PLT (K/uL)</th>
<th>WBC (K/uL)</th>
<th>Polys (K/uL)</th>
<th>BM cellularity</th>
<th>% Ly5.2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>6 week</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT e6/5</td>
<td>13.37 ± 0.29</td>
<td>48.43 ± 0.28</td>
<td>813.0 ± 34.9</td>
<td>8.27 ± 0.49</td>
<td>2.09 ± 0.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NHD13 e6/5</td>
<td>11.53 ± 0.26</td>
<td>55.70 ± 0.67</td>
<td>932.8 ± 89.9</td>
<td>2.36 ± 0.15</td>
<td>0.51 ± 0.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>16 week</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT e6/5</td>
<td>13.27 ± 0.27</td>
<td>45.80 ± 0.12</td>
<td>864.3 ± 3.2</td>
<td>12.00 ± 1.55</td>
<td>1.80 ± 0.33</td>
<td>6.53X10^7 ± 0.7 X 10^7</td>
<td>80.49 ± 1.73</td>
</tr>
<tr>
<td>NHD13 e6/5</td>
<td>8.40 ± 1.57</td>
<td>57.13 ± 2.37</td>
<td>540.3 ± 259.1</td>
<td>3.70 ± 0.75</td>
<td>0.53 ± 0.18</td>
<td>5.33X10^7 ± 0.5 X 10^7</td>
<td>82.87 ± 2.57</td>
</tr>
</tbody>
</table>
NHD13 BM cells outcompete WT cells
Mice transplanted with 1x10^5 Donor (Ly5.2) and 1x10^6 WT competitor (Ly5.1) cells

<table>
<thead>
<tr>
<th>16 week</th>
<th>HGB (g/dL)</th>
<th>MCV (fL)</th>
<th>PLT (K/uL)</th>
<th>WBC (K/uL)</th>
<th>Polys (K/uL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT e5/6</td>
<td>13.43 ± 0.22</td>
<td>44.97 ± 0.38</td>
<td>899.0 ± 62.5</td>
<td>10.39 ± 0.89</td>
<td>2.40 ± 0.25</td>
</tr>
<tr>
<td>NHD13 e5/6</td>
<td>12.05 ± 0.22</td>
<td>53.55 ± 0.26</td>
<td>863.8 ± 57.7</td>
<td>5.05 ± 0.44</td>
<td>0.87 ± 0.13</td>
</tr>
</tbody>
</table>

Peripheral Blood engraftment

* AML at 48+ wks

BM engraftment

* 73.6%

* 6.0%
Mice transplanted with *NHD13* BM showed:

- Ineffective hematopoiesis
- Peripheral blood cytopenia (in the face of a normocellular BM)
- BM dysplasia (binucleate cells, intranuclear bridging, bizarre mitosis)
- Transformation to acute leukemia
- MDS-IC is Lin⁻; Lin⁻ fractionation demonstrates it is in the L⁻S⁺K⁺ compartment
Decitabine Rx of NHD13 transplanted mice

NHD13+ Donor Engraftment (% Ly5.2)

% Ly5.2 (NHD13 Donor cells)

N = 5

weeks

DAC

HGB

ANC

6 12 16 22 27 32 37 42

6 12 16 22 27 32 37 42

Hgb (g/dL)

U/Trm/M (K/mL)

Sheryl
Summary

- Xeno-transplantation of MDS has proven to be quite difficult. ¿ immune rejection, deficient micro-environment?
- Several mouse models of MDS are now available.
- NUP98-HOX translocations are associated with MDS.
- NUP98-HOXD13 translocations are rare, but lead to HOXA (5,7,9,10) cluster gene activation; HOXA activation is common in AML and MDS.
- NHD13 MDS to AML transformation accompanied by complementary mutations (Meis1, Mn1, mir29a, Cbl, ras).
- MDS can be initiated by abnormal hematopoietic microenvironment (OCD fl/fl), independent of HSC.
- Potential target for microenvironment deficiency is Sbds (Schwachman-Diamond syndrome) gene; SDS patients have skeletal abnormalities and are predisposed to MDS/AML.