Ribosome dysfunction as a cause of bone marrow failure

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Belgian Hematological Society
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Genetics of bone marrow failure

**Telomerase**
- Dyskeratosis congenita
- Aplastic anemia

**DNA damage repair**
- Fanconi anemia

**Ribososome**
- Diamond Blackfan anemia
- Del(5q) myelodysplastic syndrome
- Shwachman-Diamond syndrome
Ribosome biology

Eukaryotic ribosome

28S, 18S, 5.8S rRNAs (Pol I)
80 proteins (Pol II)
5S rRNA (Pol III)

40S subunit (RPS proteins)
60S subunit (RPL proteins)

Mature 80S ribosome
Diamond Blackfan anemia

Clinical features:
- Severe macrocytic anemia
- Short stature
- Craniofacial abnormalities, tri-phalangeal thumb
- Cancer predisposition

Diagnosis:
- Macrocytic anemia usually detected within a year of birth
- Paucity of erythroid precursors in the bone marrow
- Increased adenosine deaminase in the red blood cells

Treatment:
- Corticosteroids
- Bone marrow transplantation

Narla and Ebert, *Blood* 2010
## Diamond Blackfan anemia genetics

### Mutations

<table>
<thead>
<tr>
<th>Gene</th>
<th>% of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPS19</td>
<td>25%</td>
</tr>
<tr>
<td>RPS26</td>
<td>6%</td>
</tr>
<tr>
<td>RPS10</td>
<td>3%</td>
</tr>
<tr>
<td>RPS7</td>
<td>2%</td>
</tr>
<tr>
<td>RPS24</td>
<td>2%</td>
</tr>
<tr>
<td>RPS17</td>
<td>2%</td>
</tr>
<tr>
<td>RPL11</td>
<td>10%</td>
</tr>
<tr>
<td>RPL5</td>
<td>7%</td>
</tr>
<tr>
<td>RPL35a</td>
<td>2%</td>
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</tbody>
</table>

**Total** 50 - 60%

### Deletions

- Total 10-20%

*Universally heterozygous*

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Farrar et al., *Blood* 2011
Myelodysplastic syndrome: 5q- syndrome

Distinct haematological disorder with deletion of long arm of No. 5 chromosome


Phenotype:
- Macrocytic anemia
- Normal/elevated platelets
- Hypolobated micromegakaryocytes
- Low rate of progression to AML
RNA interference screen of del(5q)

Anatomy of del(5q)
## Acquired and germline ribosomal disorders

<table>
<thead>
<tr>
<th>5q- syndrome</th>
<th>Diamond Blackfan Anemia</th>
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</thead>
<tbody>
<tr>
<td>• Acquired, somatic deletion</td>
<td>• Congenital disorder</td>
</tr>
<tr>
<td>• Refractory anemia</td>
<td>• Refractory anemia</td>
</tr>
<tr>
<td>• Macrocytosis</td>
<td>• Macrocytosis</td>
</tr>
<tr>
<td>• Predisposition to leukemia</td>
<td>• Predisposition to leukemia</td>
</tr>
<tr>
<td>• RPS14 allelic insufficiency</td>
<td>• RPS19, RPS24, RPS17, RPS7, RPL5, RPL11, RPL35A, etc.</td>
</tr>
<tr>
<td></td>
<td>• allelic insufficiency</td>
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</table>
Case report: del(5q) MDS

Age 5: female with severe macrocytic anemia
  • Bone marrow biopsy: deficiency of erythroid progenitor cells
  • Cytogenetics: 46, XX
  • Diagnosis: Diamond Blackfan anemia

Age 24: continued red blood cell transfusion dependence
  • Bone marrow biopsy: marked decrease in erythroid lineage, < 5% blasts

Adrianna Vlachos et al., ASH 2010
Ribosomal proteins required for pre-rRNA processing
RPS14 required for 5’ processing of 18S rRNA

RPS14 required for 40S subunit formation

The diagrams show the absorbance at 260 nm (A_{260}) for luciferase (Luc) and RPS14 constructs. Peaks at 40S, 60S, and 80S indicate the formation of ribosomal subunits.
• Strong genetic evidence that heterozygous inactivation of ribosomal genes impairs erythropoiesis

• Ribosomal protein genes play specific roles in ribosome biogenesis

• How does ribosomal protein gene haploinsufficiency cause the disease phenotype?
Activation of p53 by ribosomal haploinsufficiency

MDM2 is a ubiquitin ligase that regulates p53 levels

MDM2 binds RPL5, RPL11, RPL23a

Fumagalli, Nature Cell Biol 2009
p53 induction by RPS14 or RPS19 deficiency

Dutt et al., *Blood* 2010
p53 target gene induction

**Enrichment plot: KANNAN_P53_UP**

*P21*  Cyclin-dependent kinase inhibitor 1A (p21, Cip1)
*MDM2*  Mdm2, transformed 3T3 cell double minute 2
*DDB2*  Damage-specific DNA binding protein 2, 48kDa
*MAN2B1*  Mannosidase, alpha, class 2B, member 1
*GADD45A*  Growth arrest and DNA-damage-inducible, alpha
*BAX*  BCL2-associated X protein
*BTG2*  BTG family, member 2
*ADFP*  Adipose differentiation-related protein
*NINJ1*  Ninjurin 1
*CES2*  Carboxylesterase 2 (intestine, liver)
*BCL6*  B-cell CLL/lymphoma 6 (zinc finger protein 51)
*DGKA*  Diacylglycerol kinase, alpha 80kDa
*CSPG2*  Chondroitin sulfate proteoglycan 2 (versican)
*FHL2*  Four and a half LIM domains 2
*FEZ1*  Fasciculation and elongation protein zeta 1 (zygin I)
*PLK3*  Polo-like kinase 3 (Drosophila)

**Normalized fold expression**

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<tr>
<th></th>
<th>Luc</th>
<th>G4</th>
<th>G8</th>
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<tr>
<td><strong>p21 mRNA expression</strong></td>
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<td>18</td>
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<tr>
<td><strong>RPS14 shRNA</strong></td>
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<td><strong>1</strong></td>
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**Normalized fold expression**

<table>
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<th>Luc</th>
<th>C12</th>
<th>D3</th>
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<tr>
<td><strong>p21 mRNA expression</strong></td>
<td>0</td>
<td><strong>2.5</strong></td>
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<tr>
<td><strong>RPS19 shRNA</strong></td>
<td>0.5</td>
<td><strong>2</strong></td>
<td><strong>2.5</strong></td>
</tr>
</tbody>
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*Dutt et al., Blood 2011*
p53 induction is specific to the erythroid lineage

Dutt et al., *Blood* 2011
Activation of p53 in 5q- patient samples

Dutt et al., *Blood* 2011
Animal models of ribosomopathies
Murine model of RPS19 mutation

Positional cloning:
• Dsk3 mice have mutations in RPS19
• Dsk4 mice have mutations in RPS20

Hyperpigmented mice from large scale mutagenesis screen

Mice heterozygous for ribosomal mutations activate p53

Mice with ribosomal haploinsufficiency have decreased hematocrit and macrocytosis. Blood defects are rescued by crossing mice to p53 null mice

McGowan et al., Nat Genetics 2008
Murine model of RPS19 mutation

Jaako et al., Blood 2011
• Deletion including RPS14 causes macrocytic anemia

• Hematopoietic defects reversed in p53 null background

Barlow et al., Nat Med 2010
Other ribosomopathies

Narla and Ebert, Blood 2010
Shwachman Diamond syndrome

**Phenotype**
- Neutropenia
- Short stature
- Pancreatic insufficiency

**Genetic basis**
- *SBDS* homozygous mutation

**Mechanism**

![Diagram showing the mechanism of Shwachman-Diamond syndrome](image)
Treacher Collins syndrome

• Phenotype: craniofacial defects

• Genetics: heterozygous mutation in *TCOF1* gene

• Diamond Blackfan anemia: similar craniofacial defects
Craniofacial abnormalities are mediated by p53

\[ Tcof^{+/+} \quad Tcof^{+-} \quad Tcof^{-/-} \; Trp^{-/-} \]

Jones et al., Nat Med 2008
Ts +/- mice have Rpl38 mutations

- Ts (tail short) phenotype is caused by heterozygous Rpl38 mutations
- Rpl38 is differentially expressed
- Rpl38 regulates Hox mRNA translation, causing homeotic transformations

Kondrashov et al., Cell 2011
Treatment for ribosomopathies
Dexamethasone and lenalidomide increase erythroid colony formation

Narla et al., Blood 2011
Leucine improves hemoglobinization in RPS19 deficient zebrafish

mTor pathway senses amino acid levels and regulates translation

Response of a Diamond Blackfan anemia patient to leucine (Pospisilova et al., Haematologica 2007)
Leucine rescues the developmental and growth defects in Rps19 deficient zebrafish embryos
Ribosomopathies

Mutations and deletions of ribosomal protein genes
• Cause a severe macrocytic anemia
• Diamond Blackfan anemia
• 5q- syndrome
• MDM2-dependent induction of p53 plays a central role

Other disorders of ribosome function
• Shwachman Diamond syndrome
• Treacher Collins syndrome

Proactive animal models illustrate the complexity of ribosome function

Therapy
• Corticosteroids for Diamond Blackfan anemia
• Lenalidomide for del(5q) MDS
• Potential for therapeutic targeting of ribosome function
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