TP53 and Cancer

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Cancer Biology Pathway
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Outline

1. Background into TP53 and the role *TP53* mutations play in cancer

2. Role of *TP53* mutations in the evolution of hematopoietic populations
   A. Chronic Lymphocytic Leukemia
   B. Therapy-related AML/MDS
   C. Hematopoiesis in AML patients following induction therapy

3. Dissecting the downstream effects of TP53
TP53 as a master sensor of cellular stresses

**TP53 mutations in human malignancies**

**TP53 mutation prevalence**

**Li-Fraumeni Syndrome**

**Table 2.** Observed and expected frequency of single-nucleotide substitutions within exons 5–8 of *TP53* gene

<table>
<thead>
<tr>
<th>Mutation type</th>
<th>Expected mutations</th>
<th>Observed mutations*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Missense</td>
<td>1150 (73.4%)</td>
<td>17,191 (87.9%)</td>
</tr>
<tr>
<td>Nonsense</td>
<td>58 (3.7%)</td>
<td>1435 (7.3%)</td>
</tr>
<tr>
<td>Silent</td>
<td>359 (22.9%)</td>
<td>932 (4.8%)</td>
</tr>
<tr>
<td>Total</td>
<td>1567</td>
<td>19,558</td>
</tr>
</tbody>
</table>

*Somatic mutations reported in the IARC TP53 Database (R13, November 2008).*

Majority of *TP53* mutations are loss of function
Many missense TP53 mutations may be gain of function/dominant negative.
Chronic Lymphocytic Leukemia (CLL)

Table 1. Rai and Binet staging systems

<table>
<thead>
<tr>
<th>Risk group</th>
<th>Criteria</th>
<th>Median survival (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rai stage(^1,2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>Low</td>
<td>Lymphocytosis(^a)</td>
</tr>
<tr>
<td>I</td>
<td>Intermediate</td>
<td>Lymphocytosis + lymphadenopathy</td>
</tr>
<tr>
<td>II</td>
<td>Intermediate</td>
<td>Lymphocytosis + splenomegaly or hepatomegaly</td>
</tr>
<tr>
<td>III</td>
<td>High</td>
<td>Lymphocytosis + anemia(^b)</td>
</tr>
<tr>
<td>IV</td>
<td>High</td>
<td>Lymphocytosis + thrombocytopenia(^c)</td>
</tr>
<tr>
<td>Binet stage(^d)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>Low</td>
<td>&lt;3 nodal sites(^d) involved</td>
</tr>
<tr>
<td>B</td>
<td>Intermediate</td>
<td>≥3 nodal sites involved</td>
</tr>
<tr>
<td>C</td>
<td>High</td>
<td>Anemia(^a) and/or thrombocytopenia(^c)</td>
</tr>
</tbody>
</table>

\(^a\) Absolute lymphocyte count in whole blood >5000/mm\(^3\).
\(^b\) Hemoglobin <110 g/l, with or without enlargement of lymph nodes, spleen or liver.
\(^c\) Platelets <100 x 10\(^3\)/l, with or without anemia or enlargement of lymph nodes, spleen or liver.
\(^d\) Five possible nodal sites: axillary, cervical, inguinal, spleen and liver.

Table 2. Most common cytogenetic markers in CLL

<table>
<thead>
<tr>
<th>Chromosomal aberration</th>
<th>Frequency (%)(^a)</th>
<th>Median overall survival (months)(^b)</th>
<th>Median treatment free survival (months)</th>
<th>Gene Involved</th>
<th>Particularity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deletion 17p13</td>
<td>~10</td>
<td>32</td>
<td>9</td>
<td>p53</td>
<td>Resistance to chemotherapy</td>
</tr>
<tr>
<td>Deletion 11q22–23</td>
<td>10–20</td>
<td>79</td>
<td>13</td>
<td>ATM ((r))</td>
<td>Extensive lymphadenopathy</td>
</tr>
<tr>
<td>Trisomy 12</td>
<td>15–30</td>
<td>114</td>
<td>33</td>
<td>Unknown</td>
<td>Better prognosis than normal karyotype</td>
</tr>
<tr>
<td>Deletion 13q14</td>
<td>40–60</td>
<td>133</td>
<td>92</td>
<td>miR15a and miR16-1 ((r))</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Based on Refs. [18,90,94–98]
\(^b\) Based on Ref. [18]
TP53 is frequently mutated in CLL
**TP53** mutations occur late in the evolution of CLL and are associated with a worse prognosis.

**TP53** mutations are often present at levels not detectable by traditional sequencing.

**TP53** mutations are associated with a poor prognosis even if present at very low levels.

CLL clones harboring TP53 mutations gain a fitness advantage after chemotherapy

These clones then become the dominant hematopoietic population.

Cytotoxic therapy shapes the clonal evolution of CLL, favoring leukemic cells harboring mutations in **TP53**

Therapy-related AML/MDS

- AML or MDS arising after the previous exposure to chemotherapy or radiotherapy (latency: 1-10 years)

- Clinically and biologically distinct from de novo AML/MDS
  - Worse prognosis (4 year RFS 24%)
  - Increased incidence of chromosomal abnormalities

Also reported by: Christiansen DH et al. JCO 19:1405, 2001.

Also reported by: Wong TN et al. Nature. 2015.
Hypothesis #1: Cytotoxic therapy directly induces genome-wide mutations leading to leukemia

HSCs \[\xrightarrow{\text{Chemotherapy Radiation}}\] Genome-wide mutations \[\xrightarrow{\text{mutated}}\] Mutated HSCs \[\xrightarrow{\text{1-5 years}}\] Additional mutations Clonal expansion \[\xrightarrow{\text{AML}}\]

T-AML exhibits a similar number and spectrum of somatic mutations as \textit{de novo} AML
HSPCs acquire pathogenic mutations as a function of aging

At Birth

After Aging

Individual

Could these somatic mutations also include mutations in TP53?

Hypothesis #2: After chemotherapy, HSPCs with age-related $TP53$ mutations gain a competitive advantage

**Prediction 1:** HSPCs with $TP53$ mutations will be present in some individuals never previously exposed to cytotoxic therapy.

**Prediction 2:** HSPCs harboring functional $TP53$ mutations will have a competitive advantage after chemotherapy exposure.

**Prediction 3:** In some patients developing $TP53$ mutated t-AML, an HSPC harboring the exact same $TP53$ mutation may be detectable before cytotoxic therapy exposure, later giving rise to the leukemic clone.
**TP53 mutations identified in hematopoietic cells from healthy elderly individuals**

Analyzed peripheral blood from 20 healthy cancer-free individuals (median age: 75)

- **TP53 mutations** identified in 9 of 19 evaluable cases (47%) at frequencies ranging from 1:270 to 1:9,000.
- Most of the **TP53 mutations** are known pathogenic mutations.

- **Variant Cosmic-associated**
  - D259A
  - G245S
  - V272M
  - R273H
  - V173M
  - A161T
  - Splicing
  - Intrinsic

- **Variant Not Cosmic-associated**
  - I195T
  - Y220C
  - R282W
  - R248G
  - R248W
Heterozygous loss of \( Tp53 \) provides a competitive advantage after chemotherapy

ENU = the alkylating chemotherapy
N-ethyl-N-nitrosoourea
Heterozygous loss of *Tp53* provides a competitive advantage after chemotherapy

Leukocyte Chimerism

![Graph showing the percentage of *Tp53* loss over time with and without ENU treatment](image)

Case 895681

Stage 1A DLBCL

Chemo/XRT 2 years

Autologous transplant 3.5 years

t-MDS

Bone marrow specimen

<table>
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<tr>
<th>Gene</th>
<th>Amino acid change</th>
<th>Variable allele frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP53</td>
<td>p.H179L</td>
<td>54.6%</td>
</tr>
<tr>
<td>ETV6</td>
<td>p.L341V</td>
<td>17.0%</td>
</tr>
</tbody>
</table>
A t-MDS TP53 mutation is present in a bone marrow sample banked prior to chemotherapy.
An HSPC harboring an aging-related pre-chemotherapy TP53 mutation later gives rise to the malignant t-MDS clone.
Early acquisition of TP53 mutations likely contributes to the following characteristic features of t-AML/t-MDS

1) Resistance to chemotherapy
2) High incidence of cytogenetic abnormalities
Hematopoietic populations following AML induction therapy

Identification of pre-leukaemic haematopoietic stem cells in acute leukaemia

Preleukemic mutations in human acute myeloid leukemia affect epigenetic regulators and persist in remission

Association Between Mutation Clearance After Induction Therapy and Outcomes in Acute Myeloid Leukemia
Assessing hematopoietic populations unrelated to the AML founding clone

Day = 0
Diagnosis

Day ≈ 30

Long-term follow-up

Anthracyline and Cytarabine

High Dose Cytarabine

Exome Sequencing
Bone Marrow
Presence of morphologic leukemic cells

Exome Sequencing
Bone Marrow
Morphologically normal

Exome Sequencing
Bone Marrow
Morphologically normal

Patients with VAFs of all AML-associated variants < 2.5% following induction therapy: 25

In 10 cases, no persistent non-leukemic clonal population emerged after induction therapy.

In 6 of 15 cases, hematopoietic populations related to the AML founding clone re-emerged at the time of follow-up.

4 of 15 cases had no evidence of oligoclonal hematopoiesis following induction therapy.
Expansion of a hematopoietic clone not related to the AML founding clone

In 5 of 15 cases, a hematopoietic population not related to the AML clone expanded after induction therapy.
Rare hematopoietic cells harboring rising clone variants are present at diagnosis.

Control

AML Diagnosis

Day ≈ 30

Long-term follow-up
1) Hematopoietic populations ("rising clones") unrelated to the initial AML clone frequently expand following AML treatment.
   - Induction therapy (7+3)
   - Epigenetic modification (decitabine); Welch J et al. (ASH 2015 abstract #689)
2) These rising clones frequently harbor mutations in genes recurrently mutated in AML (e.g. TP53).
3) Many of the somatic variants harbored by these rising clones are present at the time of AML diagnosis.
4) Rising clones often expand rapidly following induction therapy and persist long after the completion of chemotherapy.
5) The clinical significance of non-leukemic clonal expansion remains to be determined.
TP53 has many downstream targets and effects

Can the different activities of TP53 be separated?

Modulate TP53's activity to protect normal cells from the effects of cytotoxic therapy while maintaining TP53's tumor suppressor function.

Loss of specific effectors can suppress the apoptotic effects of TP53 while maintaining its tumor suppressor function

$p21^{-/-}; puma^{-/-}; noxa^{-/-}$

**Improved cell viability with cytotoxic therapy**

**Maintained tumor suppressor function**

Valente LJ et al. *Cell Reports*. 2013
Separating TP53’s DNA damage response from its tumor suppressor functions

TP53 levels can mediate the cell fate decision between growth arrest and apoptosis

TP53 level fluctuations can also mediate cell fate decisions

Distinct p53 Transcriptional Programs Dictate Acute DNA-Damage Responses and Tumor Suppression

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DOI 10.1016/j.cell.2011.03.035

Tumor Suppression in the Absence of p53-Mediated Cell-Cycle Arrest, Apoptosis, and Senescence

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