Translational regulation of Myeloid Cell Differentiation: Novel Mechanisms and Players PDCD4, DAP5 and eIF2α

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LEUKEMIA

- 33,440 new cases of leukemia will be diagnosed in the US this year
- 21,700 individuals will die of the disease
LEUKEMIA

↑ Immature malignant progenitor cells in circulation
BM infiltration & failure:
↓ RBC- Anemia
↓ WBC- Infections
↓ Platelets- Hemorrhage

Acute Leukemia

AML

% 20-30 survival with Standard Rx

Chronic Leukemia

CML

Gleevec
(STI-571)

ALL

CLL
Acute Promyelocytic Leukemia (APL)

- Type of Acute Myeloid Leukemia (M3-AML)
- The incidence is 3-fold higher in Hispanic/latinos
- Characterized by translocation (15;17) (q22;q21) that leads to expression of PML-RARα fusion receptor

Lin et al, TAG 1999
LEUKEMIA

Differentiation block

Leukemic blasts

Normal Hematopoiesis

HSC

G-CSF, GM-CSF, ATRA, IL-3

Granulocytes (Neutrophils, Basophils, Eosinophils)

Monocytes / Macrophages

RBC

Platelet

Mature Blood Cells

Mature Blood Cells

Granulocytes

Monocytes / Macrophages

RBC

Platelet

HSC
All-trans Retinoic Acid (ATRA) is used for the Differentiation therapy of APL

- A natural derivative of Vitamin A (Retinol)
- induces differentiation
- First line standard therapy in APL
ATRA treatment of Acute Promyelocytic leukemia (APL)

- ATRA alone induces Complete remission in 90-95% APL patients
Molecular mechanisms of ATRA induced differentiation is not well understood.

- 1300 increased
- 1700 decreased

Expression of ~3000 genes is altered.

- 85% inhibition in protein expression
**GOAL:**

Determine the molecular mechanism of ATRA induced terminal differentiation of myeloid cells.
ATRA induces differentiation in APL (NB4) cells

**CD11b**

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>0</th>
<th>12</th>
<th>24</th>
<th>48</th>
<th>72</th>
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<tbody>
<tr>
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<td>0</td>
<td>10</td>
<td>20</td>
<td>30</td>
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**CD11c**

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<th>ATRA (μM)</th>
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<th>0.01</th>
<th>0.1</th>
<th>1</th>
<th>10</th>
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<tr>
<td>% Differentiation (CD11c⁺)</td>
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<td>20</td>
<td>30</td>
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Differentiation in maturation resistant APL cells (NB4.R1) cannot undergo maturation by ATRA.
ATRA suppresses translational regulators during terminal differentiation of APL cells by Proteomics analysis

Harris & Ozpolat, Blood. 104 (5):1314-23. 2005
## ATRA downregulates expression of translational regulators in APL cells

<table>
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<tr>
<th>Accession Number</th>
<th>Protein Name</th>
<th>Functional Classification</th>
<th>Mr (kDa) (theor)</th>
<th>pI</th>
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<td>2.1* 2.9 2.5</td>
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</table>

*All values are statistically significant (non-treated versus ATRA-treated), p < 0.05*
Central hypothesis:

Translational suppression plays a role in ATRA-induced differentiation
Deregulation of mRNA translation can contribute to cell transformation and the malignant phenotype.

Translational factors are overexpressed in different cancer types:
- **eIF4E**: CML, breast, melanoma, neuroblastoma, colon, prostate, lung cancer
- **eIF4A**: Melanoma
- **eIF4G**: Melanoma
- **hnRNPK**: CML, Lung cancer (SCLC)
- **eIF2**: CML, colon
- **eEF1α**: Pancreas and prostate

Transfection of cells with eIFs leads to cell transformation:
- **eIF4E** (De Bendetti *al*, 1997)
- **eIF4G** (Fukuchi *et al*, 1997)
- **eIF2α** (Donze *et al*, 1995)
- **eEF1α** (Tatsuka *et al*, 1992)
Signaling pathways activated by Cytokines, Growth Factors or Stress Regulate Translational control

Mitogens, Cytokines, Growth Factors

MAPKK
- MEK
- ERK
- p38

MAPK
- Mnk
- Ras

PI3K
- AKT
- mTOR

Rapamycin

4E-BP1
- eIF4E

p70S6K
- S6K
- Ribosomal S6
- eIF4G
- eIF4B
- eIF4A

eEF2
- PKR

Translation ———> Growth & Proliferation
Hypothesis

Leukemia
APL

Translational activation
proliferation

Differentiation

ATRA

Translational Inhibition

?
Hypothesis:

ATRA suppresses translation by inducing translational inhibitors (PDCD4, DAP5 and p-eIF2$\alpha$) during the differentiation.
PDCD4 tumor suppressor and DAP5 are novel inhibitors of translational initiation.

Translation “on”

Translation “off”

DAP5
PDCD4 (Programmed Cell Death 4)

• A novel tumor suppressor protein (Yang et al, 2003)
• Inhibits neoplastic transformation
• Specific inhibitor of translation initiation
  - inhibits helicase activity of eIF4A
• Its expression is downregulated in many tumors
ATRA induces PDCD4 expression during granulocytic differentiation of APL cells

<table>
<thead>
<tr>
<th>Time (h):</th>
<th>0</th>
<th>6</th>
<th>12</th>
<th>24</th>
<th>48</th>
<th>72</th>
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<tr>
<td>ATRA (1 μM)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATRA (0.1 μM)</td>
<td>6</td>
<td>12</td>
<td>24</td>
<td>48</td>
<td></td>
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</table>

**ATRA-induced PDCD4 expression**

- **ATRA (1 μM)**
  - PDCD4 (time points: 0, 6, 12, 24, 48, 72)
  - β-Actin (constant)

- **ATRA (0.1 μM)**
  - PDCD4 (time points: 6, 12, 24, 48)
  - β-Actin (constant)

**Graph showing relative PDCD4 expression**

- X-axis: Time (h) from 0 to 72
- Y-axis: Relative PDCD4 protein expression
- ATRA (1 μM) data points: 0, 6, 12, 24, 48, 72
ATRA fails to induce PDCD4 in differentiation-resistant APL cells
ATRA induces marked PDCD4 expression in primary APL cells isolated from APL patients.
Knockdown of PDCD4 by siRNA inhibits ATRA-induced granulocytic differentiation of APL

CD11c inhibition

- TR
- Control siRNA
- PDCD4 siRNA

CD11b inhibition

- TR
- Control siRNA
- PDCD4 siRNA

P<0.05
DAP5
(Death Associated protein 5)
ATRA induces DAP5 in APL and AML cells

<table>
<thead>
<tr>
<th>Time (h):</th>
<th>0</th>
<th>24</th>
<th>48</th>
<th>72</th>
</tr>
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<tbody>
<tr>
<td>ATRA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATO</td>
<td></td>
<td></td>
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</table>

**DAP5/p97**

**β-Actin**

---

**NB4**

**HL60**

<table>
<thead>
<tr>
<th>Time (h):</th>
<th>0</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATRA</td>
<td></td>
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</table>

**DAP5/p97**

**β-Actin**

**Control**

**ATRA**
ATRA-resistant APL (NB4.R1) cells do not express and fail to upregulate DAP5

<table>
<thead>
<tr>
<th></th>
<th>NB4</th>
<th>NB4.R1</th>
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<tbody>
<tr>
<td></td>
<td>ATRA</td>
<td>ATO</td>
</tr>
<tr>
<td>0</td>
<td>24  48</td>
<td>24  48</td>
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</table>

**DAP5/p97**

**Actin**
Downregulation of DAP5 partially inhibits ATRA-induced granulocytic differentiation of APL cells
eIF2α
(Eukaryotic Initiation Factor 2 alpha)
ATRA induces phosphorylation (Ser51) of eIF2α during the granulocytic differentiation of APL cells.

**ATRA (1 μM) vs. ATRA (0.1 μM)**

- **Time (h):** 0 6 12 24 48 72 48 72
- **p-eIF2α**
- **eIF2α**
- **β-Actin**

**ATO (0.4 μM)**

- **Time (h):** 0 6 12 24 48 72
- **p-IF2α**
- **IF2α**
- **β-Actin**

**NB+ATRA (1 μM)**

- **Time (h):** 0 6 12 24 48 72
- **p-eIF2α/Actin**
- **p-eIF2α/eIF2α**

**Relative p-eIF2α expression**

- **Time (h):** 0 6 12 24 48 72
- **p-eIF2α/Actin**
- **p-eIF2α/eIF2α**
Knockdown of eIF2α blocks ATRA-induced differentiation of APL cells

% Differentiation (CD11b+)

- NT
- TR
- eIF2α siRNA
- PKR siRNA
- Control siRNA

ATRA
ATRA inhibits translation initiation during terminal granulocytic differentiation in APL

<table>
<thead>
<tr>
<th>Time (h):</th>
<th>0</th>
<th>24</th>
<th>48</th>
<th>72</th>
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<tr>
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<td>DAP5</td>
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<td>p(Ser51)-IF2α</td>
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<td>p-4EB-P1</td>
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ATRA (1 μM)
ATRA-Induced translational control involves multiple mechanisms regulating cap-dependent and global translational control

PDCD4
DAP5
4E-BP1
Summary

• ATRA induces translational suppression by PDCD4, DAP5, 4E-BP1 and p-eIF2α during the granulocytic differentiation

• Induction of PDCD4 tumor suppressor protein is involved in granulocytic but not monocytic differentiation

• Inhibition of PDCD4, DAP5, eIF2 block ATRA-induced granulocytic differentiation

• ATRA-resistant cells are not able to upregulate translational inhibitors (PDCD4 and DAP5) and cannot translocate them into nucleus

• PI3K/Akt/mTOR pathway negatively regulates PDCD4 and DAP5 expression in AML and solid tumors
Conclusions

ATRA suppresses cap-dependent and global translational through multiple mechanisms during terminal granulocytic differentiation

PDCD4, DAP5 and eIF2α play a role in terminal differentiation program towards granulocytic lineage

Lack of or altered of translational control may be involved in disease pathogenesis (leukomogenesis) and resistance/relapses in APL patients
Future Goals:

1. To determine the role of translational regulators (PDCD4, DAP5, eIF2a) in response to therapy, resistance/relapse and survival in APL and AML patients.

2. To identify downstream molecular targets of ATRA-induced differentiation in APL patients samples.

3. To determine molecular mechanisms of ATRA resistance and relapses in APL patients using proteomics:
   - NB4 cell lines (Sensitive vs. ATRA-resistant)
   - Patient APL cells (Before treatment vs. the relapse)
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