PML/RARA FUSION RESULTING FROM A CRYPTIC INSERTION OF RARA GENE INTO PML GENE WITHOUT THE RECIPROCAL RARA/PML FUSION; CLINICAL, CYTOGENETIC AND MOLECULAR CHARACTERIZATION AND PROGNOSIS

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Acute Promyelocytic Leukemia (APL)

- A distinct subtype of acute myeloid leukemia (AML)
  M3 and M3v by FAB
  AML with recurrent genetic abnormalities by WHO
- Comprises 5-8% of AML cases
  With high incidence among Latin Americans
- With accurate diagnosis and proper treatment
  Favorable prognosis in > 80% of cases
- Highly sensitive to all-trans retinoic acid (ATRA) therapy
  First ever molecular targeting therapy
  Designed to reverse the effects of leukemogenesis

Pathogenesis of APL

- Involves two key processes:
  Self-renewal of leukemia initiating cells (LIC)
  Transcription repression / Differentiation arrest
- Direct consequence of the PML-RARA fusion product
  Characteristic genetic hallmark of APL
Reciprocal Translocation [t(15;17)]

- PML-RARA fusion protein results from the reciprocal exchange of genetic material between chromosomes 15 and 17.

PML-RARA Fusion

- PML is mapped to three breakpoint cluster regions (bcr): bcr1 (long form), bcr2 (variable form) and bcr3 (short form).

| bcr1 | 3 | 1 | 0 | 1 | 3 | 48% Long |
| bcr2 | 3 | 1 | 6 | 2 | 1 | 0% Variable |
| bcr3 | 3 | 3 | 3 | 3 | 48% Short |

- Bcr1 (~293bp) and bcr3 (~103bp) are reported in 95% of APL cases.
- Only 5% harbor bcr2 breakage, producing variant transcripts.
- Long form tends to have longer remission than short form.

PML Gene Region

- PML translocation product is transcribed in almost all patients.
RARA Gene Region

- RARA translocation product is not transcribed in all patients.

Case Report

- July 2012
  61 year old female noticed bruising on left arm

- September 2012
  Noticed bruising on arms, legs and abdomen with no history of trauma
  Presented to clinic where labs showed low blood counts
  Presented to ER where peripheral smear showed blasts and promyelocytes with Auer rods
  Peripheral blood and bone marrow sent for cytogenetics

Methods

- Peripheral Blood (PB)
  Cytogenetics
  D-FISH with PML-RARA / No Chromosome Analysis
  Molecular Studies
  Qualitative Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

- Bone Marrow (BM)
  Cytogenetics
  D-FISH with PML-RARA / Chromosome Analysis
  Molecular Studies
  Qualitative Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)
**D-FISH Results**

**Bone Marrow**

Atypical abnormal signal pattern
1F1O2G
\#2% fusions

**Metaphase**

Fusion on chromosome 15
No fusion on chromosome 17

Orange = PML (Chromosome 15)
Green = RARA (Chromosome 17)

**RT-PCR Results (PML-RARA)**

Typical PML-RARA transcripts seen on peripheral blood and bone marrow.

**BM Karyotype Results**

Shows T8 and normal chromosomes 15 and 17, discordant with FISH and molecular studies.
**Metaphase FISH Results**

- **Hypothesis**
  - PML-RARA fusion gene was the result of a cryptic insertion

  Orange = WCP Chromosome 15
  Orange = PML (Chromosome 15)
  Green = RARA (Chromosome 17)

  Metaphase FISH utilizing Cytocell (LPH023, LPP15-R)

- **BM RT-PCR Results (RARA-PML)**
  - Reciprocal RARA-PML transcripts not detected in patient sample.

- **Atypical Cryptic Insertions**
  - Trisomy 8 as a secondary sole anomaly is very rare
    - Karyotype without overt t(15;17) is suggestive of cryptic insertion
  - Response to ATRA is favorable as seen in typical pattern
    - Due to presence of the PML-RARA fusion protein
  - Rare and most often seen with interstitial insertion of RARA into PML
    - Insertion of RARA on other chromosomes reported
      - (Do not respond to ATRA)

**All-trans Retinoic Acid (ATRA)**

- Induces PML-RARA degradation by:
  - Decelerating self-renewal of LIC
  - Transcriptional activation and differentiation
- Molecular mechanism of ATRA not fully understood until recently.

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**Molecular Mechanism of APL & ATRA**

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**Follow-up Studies**

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<tr>
<th>November 2012</th>
<th>FISH</th>
<th>KARYOTYPE</th>
<th>RT-PCR (PML-RARA)</th>
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<td>Normal</td>
<td>98%</td>
<td>46,XX[20]</td>
<td>Negative</td>
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- PML breakpoint at bcr3 indicating favorable prognosis.
- Continue to monitor treatment and remission in our patient.
Summary

- Our case supports previous reports of atypical APL expressing non-reciprocal PML-RARA transcripts while lacking the t(15;17).
- Research proves that FISH and molecular analysis are essential tools in further characterizing clinically diagnosed APL and monitoring treatment.
- Atypical cryptic insertions of RARA into PML respond favorably to ATRA therapy like the typical translocations in APL, therefore, early detection of presence of PML-RARA fusion transcripts is critical.
- Literature suggests that identification of the breakpoint cluster region on PML may predict possible response to ATRA and likelihood of relapse.
- Based on this information, a diagnosis of APL should not be ruled out on conventional cytogenetics alone.

Acknowledgements

Ikeda, et al., 1993; Choppa, et al., 2003


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