Molecular Pathogenesis Human Leukemia

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The Hemopoietic System

- Stem Cell
  - Lymphoid Progenitor
    - Pro-B (cIg^-)
      - Pre-B (cIg^+)
        - Lymph. B (sIg^+)
          - Plasma cell
  - Myeloid Progenitor
    - Proerythroblast
    - Myeloblast
    - Megakaryoblast
      - Pro-T
      - Pre-T
      - Lymph. T
        - Erythroblast
          - Erythrocyte
        - Myelocyte
          - Granulocyte
          - Monocyte
          - Platelet
        - Promonocyte
        - Promegakaryocyte
Chromosomal Translocations in Human Leukemias

- **Fusion oncogenes**: chimeric proteins containing functional domains that disrupt normal properties and result in dominant negative or constitutive active factors.

- **Overexpressed factors**: normal proteins under control of highly active promoters result in aberrant (deregulated and out of context) expression.
Stem Cell

Promegakaryocyte

Promonocyte

Myelocyte

Erythroblast

Pre-T

(cIg+)

Pro-T

(cIg-)

B-cell Precursor ALL

Lymphoid Progenitor

Myeloid Progenitor

Lymph. T

Lymph. B (sIg+)

Plasma cell

Erythrocyte

Granulocyte

Monocyte

Platelet

Pro-T

Promonocyte

Promegakaryocyte

Erythroblast

Myeloblast

Myeloid Progenitor

Erythrocyte

Proerythroblast

Megakaryoblast

Stem Cell

B-cell Precursor ALL

Lymphoid Progenitor

Myeloid Progenitor

Erythrocyte

Granulocyte

Monocyte

Platelet
Pediatric ALL

• Acute lymphoblastic leukemia (ALL) is the most common pediatric tumor accounting for 25% of all childhood cancer.

• There are approximately 3,100 children and adolescents younger than 20 years diagnosed with ALL each year in the United States.

• Cancer in children and adolescents is rare, although the overall incidence of childhood cancer, including ALL, has been slowly increasing since 1975.

• Between 1975 and 2010, the 5-year survival rate for ALL has increased from 60% to approximately 90% for children younger than 15 years and from 28% to more than 75% for adolescents aged 15 to 19 years.

• A sharp peak in ALL incidence is observed among children aged 2 to 3 years (>90 cases per 1 million per year), with rates decreasing to fewer than 30 cases per 1 million by age 8 years.

• The incidence of ALL appears to be highest in Hispanic children and is substantially higher in white children than in black children.
Charles G. Mullighan, Genomic Characterization of Childhood Acute Lymphoblastic Leukemia, Seminars in Hematology Volume 50, Pages 314–324, (2013),
A

Hyperdiploid
>50 chromosomes
30%

No recurrent chromosomal abnormalities
25%

TCR translocations
7q35/TCRβ
3%
14q11/TCRαδ
4%

MYC
2%

Ig translocations
t(8;14), t(2;8), t(8;22)

E2A-PBX1
t(1;19)
5%

E2A-HLF
t(17;19)
1%

Near-haploid/very hypodiploid
<38 chromosomes

TEL-AML1
t(12;21)
20%

MLL fusions
t(4;11), t(1;11), t(11,19)
5%

BCR-ABL
t(9;22)
4%

t(1;19)

E2A-PBX1

5%

t(4;11), t(1;11), t(11,19)

MLL fusions

<38 chromosomes
t(12;21) TEL-AML1 in early pre-B ALL.

- Most common genetic lesion in pediatric: 20% to 25% of pre-B-ALL, rare in adults
- Difficult to detect in cytogenetics requires FISH or molecular testing by RT-PCR
- TEL-AML1:
  - Helix-loop-helix (HLH) domain of TEL fused to nearly all of AML1,
  - TEL-AML1 represses the expression of AML1 regulated genes.
- Frequent association with deletion of the non-translocated copy of TEL
- Excellent prognosis: 90% long term survival
t(1;19)(q23;p13) E2A-PBX1 in pre-B ALL.

- t(1;19) and E2A-PBX1:
  - 3-5% of all B-lineage ALLs
  - 25% of pre-B (cytoplasmic immunoglobulin-positive) phenotype.
- E2A-PBX:
  - Fusion of basic HLH transcription factor E2A gene to a PBX1 homeobox gene
  - E2A-PBX contains trans-activation domains of E2A plus homeobox DNA-binding domain of PBX1 → interaction with homeobox genes and strong trasactivation
- E2A-PBX1 expression induces AML, T-ALL and B-lineage ALL in transgenic mice
- Poor prognosis with conventional antimetabolite-based therapy but improved survival with intensive therapy.
Activation of MYC in B-cell ALL.

- Characteristic of B-cell acute leukemia and Burkitt’s lymphoma
- Aggressive tumors with mature (Ig+) phenotype and good response to chemotherapy
- Translocations of MYC (chr 8) to the vicinity of an IG gene leads to dysregulation of MYC expression
- MYC: basic helix-loop-helix/leucine zipper (bHLHZip)
- Overexpression of MYC protein transactivates target genes by the formation of heterodimers with MAX.
- Activated MYC targets: cell division, growth, death, metabolism, adhesion, and motility genes.
NOTCH1 in T-ALL.

- *NOTCH 1* is required for T-lineage commitment and thymocyte development.
- Ligand activation induces proteolytic cleavage of NOTCH1, translocation of intracellular domains to the nucleus and transcriptional activation.
- Rare translocations to the TCR loci activate NOTCH1 by truncating the NOTCH1 gene.
- Activating mutations in NOTCH1 are present in >50% of T-ALLs.
- The gamma secretase protease complex responsible for NOTCH1 processing can be inhibited with small molecules blocking NOTCH1 signaling: potential therapeutic use.
Activating Mutations in NOTCH1 are present in T-ALL

Mutations in Extracellular NOTCH1
- Amino Acid Substitutions
- In Frame Insertions and Deletions
- Ligand Independent Activation

Mutations in intracellular NOTCH1
- Truncating mutations
  (Point mutations Insertions and Deletions)
- Increased Transactivation
Ligand binding
ADAM10 cleavage
NOTCH1 HD and JME mutations
γ-secretase cleavage
γ-secretase inhibitors
NOTCH1 PEST mutations
FBXW7 mutations
Proteasome
Translocated Transcription Factor Oncogenes in T-ALL

Transcription Factor Oncogenes in Pediatric T-ALL

- **TAL1**: 60%
- **HOX11**: 25-30%
- **HOX11L2**: 20%
- **MLL-ENL**: 2%
- **CALM AF10**: 5%
- **LYL1**: 10%

Transcription Factor Oncogenes in Adult T-ALL

- **TAL1**: 45%
- **HOX11**: 25-30%
- **Unknown**: 3%
- **MLL-ENL**: 2%
- **CALM AF10**: 5%
- **LYL1**: 7%
Late Cortical Markers:
- CD34
- BCL2
- CD7
- LSP1
- SELL
- IL7R
- TCRD
- TCRG
- CD10
- CD1A
- CD1B
- CD1C
- CD45
- CD6
- LCK
- CD3D
- CD3E
- TCRB
- TCR A

Early Cortical Markers:
- CD34
- BCL2
- CD7
- LSP1
- SELL
- IL7R
- TCRD
- TCRG
- CD10
- CD1A
- CD1B
- CD1C
- CD45
- CD6
- LCK
- CD3D
- CD3E
- TCRB
- TCR A

γδ Thymocyte Markers:
- γδ
- MLL
- ENL

Double Negative Thymocytes:
- LYL1 +

Early Cortical:
- HOX11 +

Late Cortical:
- TAL1 +

Mature T-cell:
- CD8
- CD4
Gene expression profiling defines distinct oncogenic groups in ALL related to the presence of different fusion oncogenes.

Yeoh et al Cancer Cell 2002
LOH and copy number variations identify novel tumor suppressors and define genes in the B-cell differentiation pathway as key players in the pathogenesis of preB-ALL

Mullighan et al Nature 2007
LOH and copy number variations identify novel tumor suppressors and define genes in the B-cell differentiation pathway as key players in the pathogenesis of preB-ALL

Mullighan et al Nature 2007
Chronic Myeloid Leukemia (CML)

• CML is a clonal myeloproliferative disorder that involves hematopoietic stem cells, affecting all cell lineages.

• Clinically CML shows a increase in the white cell count in peripheral blood with immature myeloid forms, and a hypercellular bone marrow.

• Hepatomegaly and particularly splenomegaly are frequently present.

• CML usually presents in an indolent chronic phase, which may last 4-6 years.

• Chronic phase invariably progresses to an acute (blastic) phase that behaves as a very poor prognosis AML.
CML Myeloid Hyperplasia Without Differentiation Block

Stem Cell → Lymphoid Progenitor → Pro-B (cIg-) → Pre-B (cIg+) → Lymph. B (sIg+) → Plasma cell

Stem Cell → Myeloid Progenitor → Pro-T → Pre-T → Lymph. T → Plasma cell
t(9;22) Philadelphia chromosome, BCR-ABL

• The t(9;22) is characteristic of CML (>95% of cases) and adult ALL (25% of cases)

• t(9;22) results in the fusion of the CML gene in chromosome 22 with the ABL1 tyrosine kinase in chromosome 9

• CML-ABL fusions in CML generate the p210 and ALL p190 tyrosin kinases respectively.

• Both p210 and p190 are transforming and seem to have similar oncogenic properties

• BCR-ABL affects apoptosis, differentiation and cell adhesion.

• An important effect of BCR-ABL is the induction of cellular resistance to DNA damage agents.
Imatinib inhibition of BCR-ABL
the new paradigm of targeted cancer therapies

• Imatinib mesilate is a tyrosine kinase inhibitor of ABL, KIT and FGFRB
• Mice deficient in ABL develop a wasting syndrome and die soon after birth.
• Treatment of CML patients in chronic phase induces hematologic remissions.
• Additional applications of imatinib in cancer therapy:
  - Hypereosynophilic syndrome: FIP1L1-PDGFRA
  - Gastro intestinal stromal tumors: KIT mutations
  - Myeloproliferative syndromes: TEL-PDGFRB
• Resistance to the drug frequently occurs from mutations in ABL
Acute Myeloid Leukemia (AML)

- Stem Cell
  - Lymphoid Progenitor
    - Pro-B (cIg⁻)
    - Pre-B (cIg⁺)
    - Lymph. B (sIg⁺)
      - Plasma cell
  - Myeloid Progenitor
    - Pro-T
    - Pre-T
    - Lymph. T
      - Lymph. B (sIg⁺)
      - Lymph. T

- Megakaryoblast
- Erythroblast
- Myeloblast
- Promonocyte
- Promegakaryocyte
- Platelet
Core Binding Factor

- Transcriptional complex that regulates differentiation
- Alpha subunits (RUNX1/AML1, RUNX2, RUNX3) binds DNA, beta subunit (CBFB) dimerizes with alpha subunit and enhances transcriptional activity
- Targeted by more than 12 chromosomal translocations
- RUNX1 and CBFB are essential for definitive hematopoiesis
Structure of CBF transcription fusion factor oncogenes in human leukemia

Nature Reviews Cancer 2; 502-513 (2002);
t(8;21) AML1-ETO

- Associated with M2 AML with prominent single Auer rods, large granules and vacuoles and confers relative good prognosis

- AML1-ETO is a transcriptional corepressor that acts as a dominant negative form of CBF recruiting corepressor complex and chromatin remodeling machinery to CBF promoters

- AML1-ETO knock-in mice show no definitive hematopoiesis similar to AML1 null mice

- Expression of AML1-ETO is NOT enough to induce leukemia in mice but confers some proliferative, self renewal and survival advantage to myeloid cells.
AML1-ETO acts as dominant negative form of CBF
inv(16) CBFB–MYH11

- Associated with M4 AML with eosynophilia and confers relative good prognosis
- This intrachromosomal rearrangement fuses CBFB with the gene for smooth muscle myosin heavy chain MYH11.
- The CBFB–MYH11 fusion contains most domains of CBFB and the C terminal dimerization domain of MYH11
- CBFB–MYH11 locates in the cytoplasm forming high molecular complexes that sequester AML1, blocking the activity of CBFB.
- Similar to AML1-ETO, CBFB–MYH11 knock-in mice show no definitive hematopoiesis similar to AML1 null mice and expression of CBFB–MYH11 is NOT enough to induce leukemia.
t(15;17) and Acute Promyelocytic Leukemia, M3

- M3 AML is characterized by a differentiation block at the promyelocyte stage of differentiation and clinically by presenting severe coagulation problems.

- Once associated with very bad prognosis is now the prototype of molecularly targeted therapy and has a favorable prognosis.

- The t(15;17) results in fusion of the retinoic acid receptor alpha (RARA) with PML.
PML-RARA inhibits Retinoic acid differentiation of myeloid cells

• RARA is a nuclear receptor that binds retinoic acid and activates the expression of genes involved in myeloid differentiation.

• PML-RARA is a dominant negative repressor that recruits histone deacetylases and repressor complex factors to retinoic acid sensitive promoters.

• PML-RARA also interferes with the function of normal PML which is a candidate tumor suppressor gene
ATRA induces differentiation of APL cells

• PML-RARA fusion retains the retinoic acid binding domain of RARA and pharmacologic doses of all trans retinoic acid (ATRA) effectively revert the dominant negative activity of the fusion protein inducing terminal differentiation and apoptosis of leukemic cells.

• Treatment with ATRA rapidly reverts the coagulopathy associated with APL, is able to induce complete remission (including long term remissions) and improves the outcome of APL patients.
(a) Diagram of proteins and mutations:

1. PML protein chain:
   - N-terminus (H2N)
   - RBBBCC at position 394-552
   - COOH-terminus

2. RARα protein chain:
   - NH2-terminus
   - ABCDEF at position 394-552
   - COOH-terminus

3. PML-RARα fusion protein:
   - N-terminus (H2N)
   - RBBBCC at position 394-552
   - COOH-terminus

(b) Mechanism of action:

1. HDAC and co-repressors binding to PML-RARα

2. PML-induced dimerization: enhanced co-repressor binding and repression through histone deacetylation

3. RA (10^-6 M) binding to RARα

4. Co-activators and HAT activation

5. RARα targets are activated

6. Differentiation

7. Clinical remission

Source: Nat Rev Cancer © 2010 Nature Publishing Group
Loss of function mutations in AML1, C/EBPA and GATA1

- Loss of function mutations of AML1 are responsible for the inherited leukemia syndrome FAP/AML (familial platelet disorder with AML).
- Somatic mutations in AML1 are present in 3-5% of AML samples with higher frequency in MO AML (25%)

- C/EBPA is a transcription factor required for myeloid development
- Point mutations resulting in dominant negative forms of C/EBPA are found in a fraction of AML M2 cases with favorable prognosis.

- GATA1 is a transcription factor required for the development of the erythroid lineage
- GATA1 mutations are found in AML M7 (megacarioblastic) leukemias occurring in patients with trisomy 21 (Down's syndrome)
Activation of tyrosine kinases in AML

• Activating mutations in FLT3 (30%) and KIT (5%) receptor tyrosine kinases are found in AML

• FLT3 kinase mutations include:
  - Internal tandem duplications in the juxtamembrane domain (20-25%)
  - Point mutations in the activation loop (5-10%)

• FLT3 mutations are associated with poor prognosis

• FLT3 mutations frequently occur in conjunction with AML1-ETO, PML-RARA, CBF-MYH11 of MLL rearrangements

• Kinase inhibition with sorafenib targets active FLT3 mutant AML
Location and mechanism of FLT3 mutations in AML

Nature Reviews Cancer 3; 650-665 (2003);
Epigenetic deregulation in AML

-Methylation profiling reveals deregulated epigenetic landscape in AML

-Mutations in DNMT3a are common in AML

-TET2, an enzyme responsible for clearance of DNA methylation marks is commonly mutated in myeloid tumors

-IDH mutations generate 2HG, an oncometabolite that inhibits TET family of enzymes
Cooperating mutations and the multistep pathogenesis of AML

- More than one mutation is necessary to develop AML
  - Progression of CML from chronic phase to acute phase is accompanied by additional chromosomal abnormalities
  - Treatment with mutagenic agents is necessary for the leukemic phenotype in AML1-ETO and CBFB-MYH11 mice

- Activating mutations in tyrosine kinase genes (FLT3, KIT, BCR-ABL) and RAS are present in 50% of cases but are rarely found together in the same patient
  → PROLIFERATION HIT

- Translocations involving transcription factors (AML1-ETO, CBFB-MYH11...) are never observed together in the same leukemia
  → DIFFERENTIATION HIT
The Leukemic Stem Cell

- Leukemic clones are self perpetuating and as such must have self renewal capacity
- Only rare leukemic cells can effectively engraft in immunodeficient mice
- These leukemic stem cells share immunophenotypic features with the normal hematopoietic stem cells (CD34$^+$ CD38$^-$)
- Stem cell is the target for mutations or mutations confer self renewal capacity to progenitor cells.
Normal granulopoiesis

Stem Cell → Myeloid Progenitor → Myeloblast

AML

INITIATING LEUKEMIA ONCOGENE

Stem Cell → Myeloid Progenitor

SECOND HIT
Clonal hematopoiesis

- Age related clonal hematopoiesis:
  - Increased frequency with age
  - Associated with increased leukemia risk
  - Frequent mutations in epigenetic tumor suppressors (DNMT3A, TET2) implicated in stem cell self renewal

Aplastic anemia clonal hematopoiesis

Autoimmune destruction of stem cells

High prevalence of clonal hematopoiesis following immunosuppressive therapy

High risk of AML/MDS

Mutations promote immune escape (6pLOH, PIGA) or increased self renewal (DMT3A, ASXL1)

Seishi Ogawa
Blood 2016 128:337-347;
Prenatal origin of childhood ALL

- In some cases of childhood ALL, the initial genomic alteration appears to occur in utero.
- **Immunoglobulin or T-cell receptor antigen rearrangements** that are unique to each patient’s leukemia cells can be detected in blood samples obtained at birth.
- In ALL characterized by specific chromosomal abnormalities, some patients have blood cells that carry at least one leukemic genomic abnormality at the time of birth, with additional cooperative genomic changes acquired postnatally.
- Genomic studies of identical twins with concordant leukemia show common genetic alterations indicative of a prenatal clone shared through placental circulation.
- Some children who never develop ALL are born with very rare blood cells carrying a genomic alteration associated with ALL such as the ETV6-RUNX1 translocation.
Unsolved clinical challenges in ALL
The mutational landscape of relapsed ALL
Clonal evolution patterns in relapsed ALL
NT5C2 mutations are relapse-specific
NT5C2 mutations in relapsed ALL
NT5C2 dephosphorylates and inactivates the active metabolites of 6-MP and TG
NT5C2 mutant proteins have altered responses to ATP allosteric activation
Relapse associated NT5C2 mutations induce resistance to 6-mercaptopurine in ALL

6-mercaptopurine
NT5C2 mutations associate with early relapse and progression under treatment

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