Cytogenetics: Nomenclature and Disease

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Overview

• Normal Chromosomes
  – Structure
  – Genes

• Chromosomal Disruptions
  – Types of Chromosomal Changes

• Disruptions and Disease
Structural Overview

- DNA forms a double helix
- Double helix structure is wound around histones
- DNA/histone complex then forms the chromosome structure

Cell Division and Cytogenetics

- Tissue cells of interest are grown in culture
- Cell must be “frozen” at metaphase
  - Mitotic inhibitor added
  - Chromosomes condensed
  - Cells harvested
Human Chromosome Basics

- 22 pairs plus 2 sex chromosomes (diploid number: 46): (46, XX)
- Composed of DNA plus infrastructure (histones, proteins, RNA, sugars)
- 3 groups of shapes based on centromere position, arm length

Example of G-Banding: Chromosome 11

- GTL stain: Giemsa/Trypsin/Leishman
- Chr 11 is submetacentric
- Representative ideogram
- Stained to distinguish denser and less dense areas
- Unique staining patterns for each chromosome
- Many genes coded
- Banding ≠ genes

How Do You Define a Gene?

• DNA sequence begins with a start codon; ends with a stop codon
• Amino acids (each with a 3-character code) then join to form a protein which then has a function
• There is “filler” DNA that codes for other stuff
So Many Genes...


URL: http://AtlasGeneticsCytogenetics.OncolOncology.org/indexbychrom/idxa_11..html

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What can go wrong with a gene?

- The correct sequence is critical to coding the right protein/protein structure.
- If the chromosome carrying a particular gene is altered, then the resulting mutated protein or control elements may cause problems.
What can go wrong with a chromosome?

- Constitutional vs acquired abnormalities

- Numerical abnormalities
  - Monosomy: loss of a whole chromosome
  - Trisomy: gain of a whole chromosome

- Structural abnormalities
  - Deletions
  - Inversions
  - Translocations
Monosomy X: Turner Syndrome Constitutional Loss
Trisomy 21: Down Syndrome
Constitutional Gain

[Diagram of chromosome with trisomy 21 highlighted]
Deletion

Deletion 5q
Acquired Loss

• Interstitial losses of the long arm of chromosome 5
• These losses result in large numbers of genes being lost
• Often associated with myelodysplastic syndromes and acute myeloid leukemia

From: http://atlasgeneticsoncology.org/Educ/Images/GeneticCancerFig7.jpg
Inversions

Inversion (3)(q24q27)
Acquired Abnormality

- Interstitial segment inverts

From: http://members.aol.com/chroninfo/images/inv3ideo.gif
Translocations

Translocation t(9;22) Acquired Abnormality

- Material is exchanged between chromosomes 9 and 22, creating a new fusion gene: \textit{bcr/abl}
- Breakpoint may vary a bit such that the newly created fusion protein may be of several lengths
  - p190 (kDa)
  - p210

\text{http://atlasgeneticsoncology.org/Anomalies/CML.html}
Dicentric Chromosome

Isochromosomes

Ring Chromosomes

Duplication

Recap of Basic Abnormalities

- Loss or gain of entire chromosomes
  - Monosomy
  - Trisomy
- Structural
  - Deletions
  - Inversions
  - Translocations
- Plus more uncommon types of abnormalities
  - Derivative chromosome (der)
    - Used when only one chromosome of a translocation is present or
    - One chromosome has two or more structural abnormalities
  - Dicentric chromosome (dic) [chromosome has two centromeres]
  - Duplicate (dup) [duplication of a portion of a chromosome]
  - Insertion (ins)
  - Isochromosomes (i) [both arms are the same]
  - Marker chromosome (mar) [unidentifiable piece of chromosome]
  - Ring chromosome (r)
  - Hyperdiploidy: greater than 48 chromosomes
Interpreting Cytogenetic Reporting

• In sequence:
  – the overall number of chromosomes identified
  – sex chromosomes
  – affected chromosomes
  – type of abnormalities described in shorthand
  – chromosomal band location
  – In brackets, the number of cells with a given karyotype

• Examples
  – 46, XX; t(9;22)(q13;q22) [20]
  – 47, XY; +21 [12]
  – 46, XX; inv 16(q13; q21) [20]
  – 45, XY; -5 [18]; 46, XY [2]
  – 46, XY; -5 (q13) [4]; 46, XX [16]
Cytogenetic Pioneers

Barbara McClintock
- First genetic map of maize
- Genetic and physical characteristics correlated
- Her work helped explain how cells that share the same genome can have different functions
- Nobel Prize for transposons in 1983

Janet Rowley
- Hypothesized that leukemias might contain non-random genetic abnormalities
- 1972: Showed that recurring chromosomal abnormalities occurred in leukemia and sometimes defined the disease’s characteristics
Leukemias and Cytogenetics

• Certain morphologic subtypes were known to have distinct prognoses and/or clinical syndromes (M0-M7)

• Examples:
  – acute promyelocytic leukemia (M3)
    • High bleeding risk due to coagulopathy but favorable prognosis
    • t(15; 17)(q22;q12)
  – AML, subtype M4eo
    • Favorable prognosis
    • inv(16)(p13;q22)
  – Chronic myeloid leukemia
    • t(9;22)(q11;q34)
  – Some myelodysplastic patients—typically older women—had a pattern of normal platelet counts and a favorable prognosis
    • 5q- syndrome
Risk Stratification for Acute Leukemias Using Cytogenetics

- Previous to Janet Rowley and others’ observations about cytogenetics and prognosis, leukemias were only categorized by morphology under the microscope

- AML
  - Favorable Risk
    - inv (16)
    - t (8;21)
    - t (15;17)
  - Intermediate Risk
    - All abnormalities not in favorable or high risk categories, plus normal
  - Poor Risk
    - Monosomy 5 or 7; 5q-; 7q-
    - t (9;22)
    - Complex (3 or more abnormalities)

- ALL
  - Favorable risk
    - Hyperdiploidy
  - High Risk
    - t (1;19)
    - t (9;22)
    - t (4;11)
### Pre-TED Form (1): Cyto data

<table>
<thead>
<tr>
<th>Item</th>
<th>Description</th>
</tr>
</thead>
</table>
| 174  | - AML with t(8;21)(q22;q22), (AML1/ETO) (281)  
- AML with abnormal BM eosinophils and inv(16)(p13q22) or t(16;16)(p13;q22), (CBFβ/MYH11) (282)  
- APL with t(15;17)(q22;q12), (PML/RARα) and variants/ M3 (283)  
- AML with 11q23 (MLL) abnormalities (284) |
| 186  | - Precursor B-cell ALL {L1/L2} (191)  
- If known, indicate subtype:  
  - t(9;22)(q34;q11); BCR/ABL+ (192)  
  - t(v;11q23); MLL rearranged (193)  
  - t(1;19)(q23;p13) E2A/PBX1 (194)  
  - t(12;21)(p12;q22) ETV/CBF-α (195)  
  - Precursor T-cell ALL (195)  
  - ALL, NOS (190) |

- First three AML cyto abnormalities are associated with favorable prognosis (AML/ETO, e.g., refers to the two genes involved in the leukemia)
- AML with 11q23: often associated with previous topoisomerase inhib.-based chemotherapy (MLL gene is located at 11q23); usually t(9;11) (p22;q23)
In the pre-TED example above, Ph+ refers to the cytogenetics; bcr refers to the detection of bcr/abl gene product, usually by PCR or FISH
Form 2100 Chimerism Studies

Valid Method Codes

<table>
<thead>
<tr>
<th>Method Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Conventional (standard) cytogenetics</td>
</tr>
<tr>
<td>2</td>
<td>Fluorescent in situ hybridization (FISH)</td>
</tr>
<tr>
<td>3</td>
<td>Restriction fragment-length polymorphisms (RFLP)</td>
</tr>
<tr>
<td>4</td>
<td>HLA typing</td>
</tr>
<tr>
<td>5</td>
<td>VNTR or STR, micro or mini satellite</td>
</tr>
</tbody>
</table>

90 - Other, specify: ____________________________________________________________

- Note that for collection of chimerism data, PCR is not an option and should not be recorded under “other”
Disease Status: FISH

- For data purposes, FISH is a subset of cytogenetics (cellular level)
- Molecular evidence would be PCR and similar
The Future of Prognosticating Outcomes in Acute Leukemia

- May be based on the molecular biology of the leukemia as ascertained by:
  - PCR
  - FISH
  - Microarray data/gene profiling

- More and more critical to understand the molecular basis as more targeted therapies become available:
  - Anti-bcr/abl drugs: imatinib and 2nd generation drugs
  - Anti flt3 etc.
Summary

• A variety of chromosomal abnormalities can be characterized and described using cytogenetics.
• Non-random chromosomal alterations occur, can define the disease (e.g. APML), and can have important prognostic value.
• Not all genetic abnormalities can be seen using cytogenetic techniques (e.g. normal cytogenetics in AML).
• Newer techniques (polymerase chain reaction [PCR], fluorescent in-situ hybridization [FISH]) can assist in searching for occult genetic aberrations.
Web References