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

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
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

Inversion (3)(q24q27)
Acquired Abnormality

- Interstitial segment inverts

Translocation t(9;22)
Acquired Abnormality

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

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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]
  – Duplication (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 46 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 (AML-M7)
- Examples:
  - acute promyelocytic leukemia (M3)
  - high bleeding risk due to coagulopathy but favorable prognosis
  - t(15;17)(q22;q12)
  - AML, subtype M4eco
  - Favorable prognosis
  - inv(16)(p13q22)
  - Chronic myeloid leukemia
  - t(9;22)(q34;q34)
  - Some myelodysplastic patients—typically older women—had a pattern of normal platelet counts and a favorable prognosis
  - 5q syndromes

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
      - t(19;22)
      - Complex (3 or more abnormalities)
- ALL
  - Favorable Risk
    - Hyperdiploidy
    - t(1;19)
    - t(12;22)
  - Intermediated Risk
    - 5q-
    - 7q-
    - t(9;22)
  - Poor Risk
    - Monosomy 5 or 7; 5q-
    - t(9;22)
    - Complex (3 or more abnormalities)

Pre-TED Form (1): Cyto data

- 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 II-based chemotherapy (MLL gene is located at 11q23); usually t(9;11) (p22;q23)

Pre-TED Form (2)

17q CHRONIC MYELOGENOUS LEUKEMIA (CML)
Philadelphia chromosome+, Ph+, t(9;22) (q34;q11), or variant OR bcr/abl+

- 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

- 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