

“Cytogenetics Rocks”

Cytogenetics in Pathology

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M2 Pathology Course
Lecture 14
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9:15 AM

Reading assignment: Robbins Pathologic Basis of Disease, 6th Edition, Chapter 6, pp. 165-176; Chapter 8, p 285

Goals and Objectives

1. Outline how chromosomes are obtained from tissue
2. Describe the normal human chromosome complement
3. Interpret standard chromosome nomenclature
4. Diagram how aneuploidy occurs by nondisjunction
5. List the clinical features of trisomy 21, 18, and 13
6. Explain how Down syndrome can recur in families
7. List the clinical features of sex chromosome abnormalities
8. Categorize several types of structural chromosome abnormalities as balanced or unbalanced
9. Know the common karyotypes in spontaneous abortions
10. Explain how FISH analysis works and what it can be used for, including an example of interphase FISH and of metaphase FISH
11. Describe the uses of cytogenetic and FISH analysis using chronic myelogenous leukemia as an example
12. Be able to order FISH or cytogenetics analysis as appropriate for the specimen and patient situation

Outline

WHAT IS CYTOGENETICS AND WHY DO IT?

- Cytogenetics is the study of chromosomes
 - Chromosomes are nuclear structures containing DNA and proteins
- Chromosome abnormalities are important mechanism of disease, for example:
 - Etiology of 50% of spontaneous abortion
 - Chromosome abnormality in 1:160 liveborns
 - Chromosome abnormalities occur in most cancers

ETIOLOGY OF BIRTH DEFECTS—Pie Chart

TWO MAIN TESTS

- Routine cytogenetic analysis
 - In use since 1960s
 - Looks at all chromosomes

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- Serves as general screen
- Fluorescence in situ hybridization (FISH)
 - In clinical use for about 10 years
 - Only looks at chosen region(s) of the genome
 - Come back to this later

ROUTINE APPLICATIONS OF CYTOGENETIC ANALYSIS

- Constitutional analysis--examines chromosome content of all body's cells
 - Prenatal analysis
 - Postnatal analysis of babies, kids, and adults for explanation of abnormal phenotype
 - Reproduction issues in adults
- Acquired abnormalities--anomalies found only in tumor tissue
 - Diagnosis, prognosis, and disease monitoring

STEPS IN CHROMOSOME PREPARATION

Tissue—Culture—Harvest—Slide Making—Banding & Staining—Exam on Scope

WHAT CHROMOSOMES LOOK LIKE

- Chromosomes look like this down a microscope after preparation at metaphase
- Each rod is a duplicated structure with constriction called centromere
 - Site of spindle attachment to assort chromosomes to daughter cells

NORMAL HUMAN COMPLEMENT

- Must know normal to tell what is abnormal
- 23 pairs of chromosomes
 - 22 autosomes and 1 pair of sex chromosomes
 - Normally get 1 of each pair from each parent
- Normal person is diploid--46,XX female or 46,XY male
 - Sperm and egg cells are haploid (23,X or 23,Y)

CHROMOSOME ANALYSIS

- Each chromosome is unique
- Chromosomes are organized into a karyotype on basis of:
 - Size from largest to smallest
 - Position of centromere (constriction where microtubules attach)
 - Pattern of light and dark bands induced by trypsin treatment
- 400 to 1200 bands per haploid set depending on stage of mitosis

NORMAL MALE KARYOTYPE—46,XY

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STANDARD NOMENCLATURE

- International standing committee agrees on nomenclature to describe abnormalities and identify each band (based on drawings or ideograms)
- Standard nomenclature allows every cytogeneticist to understand exactly what abnormalities are present regardless of language

X CHROMOSOME DISEASE MAP--FIGURE 6-21

CHROMOSOME 18 IDEOGRAMS

- Bands coalesce as they progress through metaphase
- 400-550 bands are most common levels used for routine analysis

NOMENCLATURE EXAMPLES

- Number of chromosomes first, then sex chromosome constitution, followed by any abnormalities in numerical order
 - Each part separated by commas
- 46,XX is a normal female karyotype
- 47,XY,+21 is a male with extra chromosome 21
- 46,XX,t(9;22)(q34;q11.2) is a female karyotype with a translocation between chromosomes 9 at band q34 and chromosome 22 at band q11.2

PLOIDY

- Euploidy is $23n$ --23, 46, 69, 92
- Aneuploidy is any other number
 - Extra and missing chrs occur mostly by nondisjunction
- Failure of chromosomes to divide properly at meiosis
- Aneuploidy most commonly occurs in maternal meiosis
 - 80% (70% at MI, 10% at MII)
 - Frequency increases with increasing maternal age

CONSTITUTIONAL NUMERICAL ABNORMALITIES

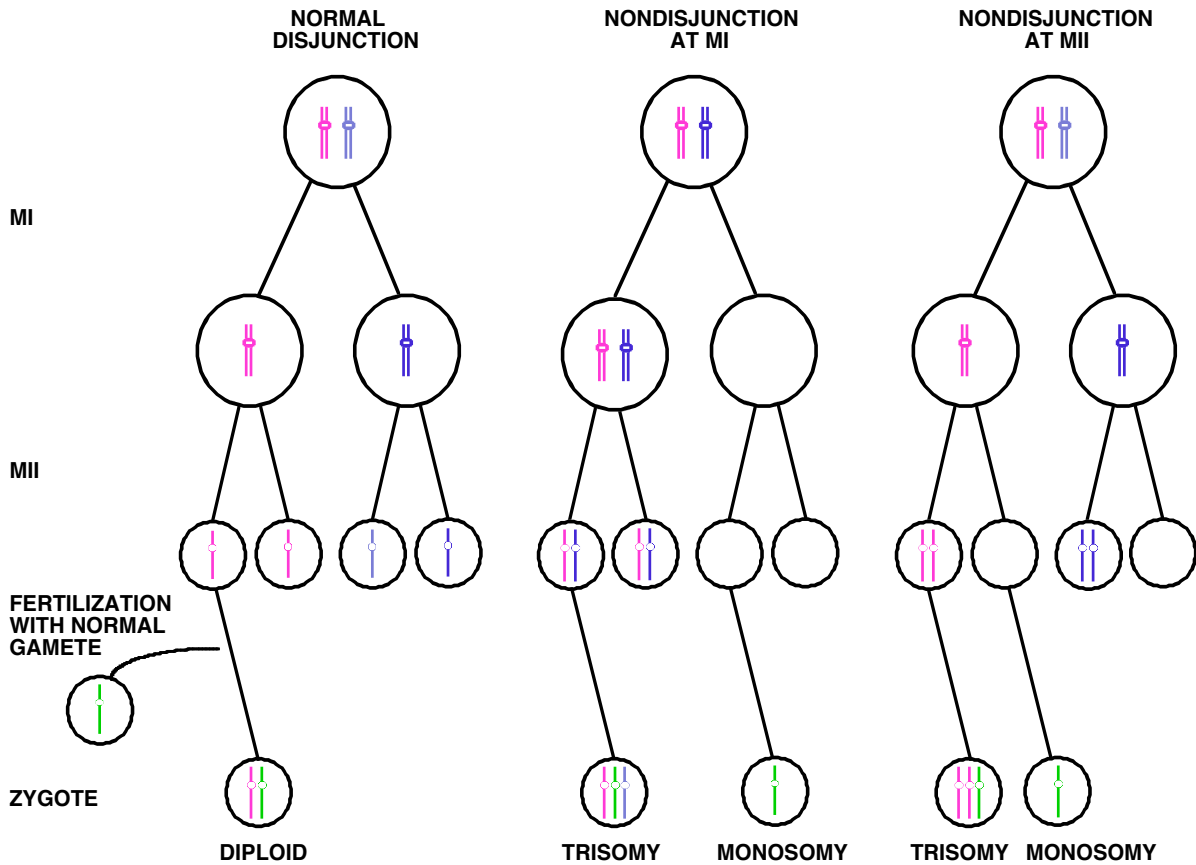
- Disomy is 2 copies of a chromosome (normal #)
- Trisomy is 3 copies of a chromosome
 - Example: 47,XX,+21 (Down syndrome)
- Monosomy is 1 copy of a chromosome
 - Example: 45,X (Turner syndrome)
- Polyploidy
 - Triploidy, 3 sets of chromosomes
 - 2 from 1 parent and 1 from the other
 - Example: 69,XXY or 69,XXX (spontaneous abortions)
 - Tetraploidy, 4 sets of chromosomes

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- Example: 92,XXXX or 92,XXYY
- Spontaneous abortions or some specialized cells in body

GENERATION OF ANEUPLOIDY BY NONDISJUNCTION



STRUCTURAL ABNORMALITIES

- Instead of involving copy numbers of whole chromosomes, structural anomalies involve breakage within a chromosome or chromosomes & rejoining ends in new way
- Fall into 2 categories
 - Balanced
 - All genetic material present, just in different spot
 - Usually normal phenotype
 - Unbalanced
 - Loss or gain of genetic material

TYPES OF STRUCTURAL ABNORMALITIES—FIGURE 6-26

ANEUPLOIDY VIABILITY

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- Only viable aneuploidies are +21, 45,X, 47,XXY, and other sex chromosome variations
- Trisomy 13 and trisomy 18 also occur in newborns, but these babies usually die within days after birth, rarely live 1 year
- Mosaic trisomy 8 and 9 are also viable
- No other non-mosaic aneuploidies are found in liveborns

47,XX,+21 KARYOTYPE

DOWN SYNDROME--47,XX,+21

- Most common abnormality in man (1:660 newborns)
- Most common clinical features include:
 - Hypotonia, floppy
 - Flat face
 - Upslanting eye slits
 - Excess neck skin
 - Heart disease
 - Short stature
 - IQ 30-60
 - 10 X increased risk of acute leukemia

KAROTYPIC VARIATION IN DS

- About 95% are trisomy 21
 - Increased maternal age is greatest risk factor
- About 1% are mosaics
 - Mixture of cells with normal karyotype and cells with extra chr 21 (47,X-,+21/46,X-)
 - Mitotic error in embryo
- About 4% are translocation DS
 - Higher risk of recurrence if carried by parent

ROBERTSONIAN TRANSLOCATIONS

- Some members of normal population carry Robertsonian translocations
 - Special type of translocation
 - Robertsonian translocations involve 2 of chromosomes 13, 14, 15, 21, and 22
- Even though normal person who carries an RB has only 45 chromosomes, considered balanced abnormality in such a person
- Acrocentric chromosomes (13, 14, 15, 21, and 22) have only repeated DNA on short arms

der(14;21)

- Translocation is not completely reciprocal since short arms are lost and 2 long arms are joined together

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- Individual who carries Rb t has 45 chromosomes, but is normal

TRANSLOCATION DOWN SYNDROME

- Normal individuals who carry Rb ts are at greater risk of having child with:
 - DS if Rb t involves 14 and 21
 - +13 if it involves 13 and 14
 - Reason all DS and trisomy 13 cases have to be karyotyped, even if clinically evident, because of risk of recurrence for parents

TRISOMY 18

- Clinical features include:
 - Clenched fists
 - Rocker bottom feet
 - Cardiac defects
 - Severe MR
 - Limited survival
 - Can look quite normal
- Incidence 1 in 7500

TRISOMY 13

- Clinical features include:
 - Midline facial defects such as cleft palate and lip, microphthalmia or cyclopia
 - Cardiac defects
 - Rocker bottom feet
 - Polydactyly
 - Severe MR
 - Limited survival
- Karyotypes include +13 and Robertsonian der(13;14)
- Frequency 1 in 15-20,000

SEX CHROMOSOME ABNORMALITIES

- Abnormalities of X and Y chromosome have milder effects than analogous abnormalities of autosomes
 - X chromosome inactivation (lecture 2)
 - Small # of genes on Y (lecture 2)
- Effects largely related to sexual development and reproduction
- Diagnosis is often at puberty or in adulthood

45,X KARYOTYPE

TURNER SYNDROME--45,X

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- Clinical features include:
 - Short stature
 - Gonadal dysgenesis
 - Webbed neck, lymphedema
 - Normal IQ, some LD
- Frequency about 1:5000 female births
 - Detected at birth or puberty
- Very lethal in utero
- Cytogenetically variable
 - Only 55% are 45,X

47,XXY KARYOTYPE

KLINFELTER SYNDROME--47,XXY

- Clinical features include:
 - Long limbs
 - Small genitalia
 - Sterility due to azoospermia
 - Some have enlarged breasts (gynecomastia)
 - Normal IQ, some LD
- Often detected as adults in infertility clinics
- Frequency is 1:1000 males
- Other karyotypes include:
 - 47,XXY/46,XY
 - 47XXY/48,XXX
 - More severe

XYY

- Frequency of 1:1000
- Normal phenotype
 - May be tall
- Normal IQ
- Low frequency of behavior difficulties

ABNORMALITIES IN SPONTANEOUS ABORTIONS

- Monosomies other than 45,X are rarely found
 - Loss of material is more severe wrt phenotype
- All trisomies are found in SABs or pre-implantation studies
- Most common findings in SABs
 - 45,X
 - 69,XXX or XXY (triploidy)
 - Trisomy 16

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TRIPLOIDY--69,XXY

- Common in spontaneous abortion, rarely liveborn
- Common origin is 2 sperm fertilizing 1 egg

DELETIONS

- All deletions are unbalanced
- Classic deletions of >4 MB
 - Visible by routine cytogenetics
 - Cri du chat and Wolf Hirshhorn syndromes
- Microdeletions of ~1-5 Mb
 - Are difficult or impossible to see cytogenetically
 - FISH is required to document
 - Prader Willi, Angelman, and DiGeorge syndromes (more later)

DELETIONS AND DUPLICATION IDEOGRAMS

WOLF HIRSCHHORN SYNDROME

- Deletion of distal end of chromosome 4 short arm (4p16)
- Clinical features include:
 - Severe growth retardation
 - Severe MR
 - Microcephaly
 - "Greek warrior helmet"
- Usually detectable cytogenetically but may require FISH

RING CHROMOSOME 20 PARTIAL KARYOTYPE

- Breaks at each end and rejoining of portion connected to centromere; loose ends lost
- Very little material missing
- Phenotype in this girl was uncontrolled seizures
- About 20 similar cases in literature

LIMITS OF CYTOGENETICS

- Cytogenetics is a good general screen for abnormalities if you don't know what you're looking for
- Can't analyze non-dividing cells
- One chromosome band contains at least 3-5 million base pairs
 - Even alterations of this size can be difficult to see or diagnose with confidence

HOW BIG IS A CHROMOSOME BAND?

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3 X 10⁶ base pairs, ~30 genes

FISH ANALYSIS

- Technique of fluorescence in situ hybridization circumvents some of limitation of routine chromosome analysis
- Spans part of the resolution gap between cytogenetic analysis and DNA methods, also called molecular cytogenetics
- FISH works on metaphase or interphase cells

FISH PROCEDURE

- Much like Southern blot
 - Target is interphase or metaphase cells on a slide rather than blot
 - Probe is any DNA sequence available commercially (or home brew), labeled with fluorescent tag
 - Denature probe and target, then hybridize
 - Detect on fluorescent microscope

PROBE TYPES

- Centromere probes (alpha satellite, repeated sequence)
- Chromosome paint
 - Probes from an entire chromosome or probe from each chromosome to label all chromosomes, (latter is mainly research)
- Unique sequence
 - Known gene
 - Anonymous sequence

X/Y FISH PROBES ARE REPEATED SEQUENCES

XY FISH

- Using *DXZI* (X centromere, red) and *DYZ1* (Yq12 heterochromatin, green)
- Interphase cells (left) identified as male (1R1G) or female (2R)
- Metaphase cell (right) shows location of X and Y signals

FETAL TRANSLOCATION—t(3;9)

INHERITED TRANSLOCATION

- Found a translocation of chromosome 9 material to chromosome 3 in a fetus
- Studied parents' blood and found that mother had same translocation
- Implies translocation does not involve loss of material or a break in a gene
- Predict child will be normal

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CHROMOSOME PAINT

- Use of a probe from sequences of chromosome 9 “paints” that chromosome
- Proves origin of material

SPECTRAL KARYOTYPING

CHROMOSOME 22 MICRODELETION

- Various phenotypes, different manifestation of same genetic defect
- 2 Syndromes
 - DiGeorge syndrome
 - Calcium and thymus defects
 - Velocardiofacial syndrome
 - Prominent nose with square nasal root
 - Cleft palate
- Isolated heart defects
 - 5% of all congenital cases

CHROMOSOME 22 MICRODELETIONS

- Variable expressivity
 - Some individuals with deletion may have little manifestation
 - Children’s risk to inherit 50%, can be more severe
- Very important to diagnose
- Difficult to impossible cytogenetically
- FISH is gold standard for diagnosis

DIGEORGE FISH PROBES

D22S275 DELETION BY FISH IN DIGEORGE SYNDROME

- Single copy sequence from chromosome 22 and control probe marking distal end of 22
- One chr 22 lacks *D22S275* signal

SNRPN LOCUS—15Q11.2

SNRPN HYBRIDIZES TO EXTRA MARKER CHROMOSOME

- *SNRPN* is a single copy gene on chr 15 labeled in red
 - Chromosomes stained with DAPI
- FISH identifies extra chromosome as 15 derived
- Note 3 copies in interphase nucleus
- Phenotype—autism

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CANCER IS A GENETIC DISEASE

- Abnormal accumulation of cells occurs in cancer because of imbalance in genes involved in cell proliferation and death
- Chromosome alterations reflect those changes
- Specific abnormalities occur in specific tumors
 - True for all tumor types, but hematologic disorders most studied for technical reasons

ACQUIRED ABNORMALITIES

- Since specific anomalies occur in specific neoplastic tissues, identification of abnormality can aid in:
 - Diagnosis
 - Prognosis
 - Disease monitoring (remission and relapse)
 - Gene identification and mapping

CANCER CYTOGENETICS

- Same basic types of anomalies occur as in constitutional abnormalities
 - Numerical and structural, but often multiple and complex
- First changes to occur are thought to be primary and later ones secondary or noise
- Changes are clonal--occur in a single cell
- Disappear in remission and recur in relapse

CHRONIC MYELOGENOUS LEUKEMIA

- Usually disease of middle age, incidence of 1-2 per 100,000
- Clonal expansion of hematopoietic progenitor cells that increases myeloid and erythroid cells and platelets
- Has chronic, accelerating, and acute phases

BCR-ABL REARRANGEMENT

- *ABL* (Abelson) is a proto-oncogene encoding tyrosine kinase (signal transducer) on chromosome 9; *BCR* (breakpoint cluster region) is phosphoprotein on 22
- Bringing these 2 sequences together is primary event in generation of CML

BCR-ABL REARRANGEMENT

- Rearrangement accomplished by breaks in *ABL* and in *BCR* and rejoining to opposite chromosome, translocation
- Translocation [t(9;22)] of *ABL* to *BCR* generates new fusion protein with increased tyrosine kinase activity

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t(9;22) OF CML—IDEOGRAM AND KARYOTYPE

DIAGNOSIS OF CML

- Demonstration of BCR/ABL rearrangement required for diagnosis of CML
- Cytogenetic demonstration of t(9;22)
- FISH using probes for *BCR* and *ABL* demonstrates fusion

BCR/ABL INTERPHASE FISH

THERAPY BASED ON MOLECULAR ABNORMALITY

- 1st cancer chromosome abnormality discovered--Philadelphia chromosome (1960); appreciated as translocation [t(9;22)] in 1973
- First “designer drug” therapy for CML based on that abnormality--Gleevec
 - Signal transduction inhibitor
 - Some patients on Gleevec show reduction to absence of *BCR/ABL* fusion on FISH and/or chr analysis
 - May turn CML into a truly chronic disease

CYTOGENETICS VS FISH

CYTOGENETICS	FLUORESCENCE IN SITU HYBRIDIZATION
Good general screen	Must know possible abnormality
Requires technical expertise and time	Easier and faster technically
Can't analyze non -dividing cells	Works on interphase cells
Alteration of 3 -5 million bps is difficult to see and diagnose	Bridges gap between cytogenetics and DNA methodology