Introduction to Cytogenetics

Part 2

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Introduction to Cytogenetics II

• Structural Chromosome Abnormalities
  – Underlying Mechanisms
  – Nomenclature
  – Deletions and Duplications
  – Translocations and Segregation Mechanisms
  – X-chromosome Abnormalities
  – Inversions and Recombinant Chromosomes

• Cytogenetics in Cancer
  – Hematologic malignancies overview
  – Cytogenetic abnormalities and nomenclature
  – Genetic basis of cancer: oncogenes, tumor suppressors
Structural Abnormalities

• Definition: Breakage and rejoining of chromosomes or chromosome segments
• May be either balanced or unbalanced
• Breakpoints can disrupt gene expression (within a gene or regulatory element)
• Can create gene fusions or affect gene expression (↑↓) by position effect
  – Common in cancer
Mechanisms Underlying Structural Rearrangements- Errors in...

• Recombination: exchanges between homologous, non-allelic sequences via non-allelic homologous recombination (NAHR)
• Repair: double-stranded breaks that are repaired incorrectly by non-homologous end-joining (NHEJ)
• Replication: discontinuous replication of the lagging strand leads to invasion into other replication forks: fork stalling and template switching (FoSTes)
Structural abnormalities
(Abnormal is on the right)

Deletions
- Terminal
- Interstitial

Duplications

Insertions

Reciprocal Translocations
- Balanced
- Unbalanced

Robertsonian Translocations
- Balanced
- Unbalanced
Structural abnormalities
(Abnormal is on the right)

Inversions

Pericentric inversion

Paracentric inversion

Recombinant chromosomes

Ring chromosomes

Isochromosomes
Normal variable chromosomal features/
Heteromorphisms
(NOTE: generally, these are not included in the karyotype)

Variation in length (+ or -)
• 1qh+
• 9qh-
• 16qh+
• Yqh+
• 13ps+
• 21pstk-

Variation in position
• inv(2)(p11.2q13)
• inv(9)(p12q13)
• Yqs
Designation of Regions, Bands, Sub-bands

Example: 4p15.3

Idiogram
## Differences in level of resolution by sample type

<table>
<thead>
<tr>
<th>Type</th>
<th>Resolution Range</th>
<th>Image 1</th>
<th>Image 2</th>
<th>Image 3</th>
<th>Image 4</th>
<th>Image 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>BM</td>
<td>350</td>
<td><img src="3-350-BM.png" alt="Image" /></td>
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<td><img src="3-350-BM.png" alt="Image" /></td>
<td><img src="3-350-BM.png" alt="Image" /></td>
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</tr>
<tr>
<td>AF</td>
<td>400-425</td>
<td><img src="3-400-425-AF.png" alt="Image" /></td>
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<td><img src="3-400-425-AF.png" alt="Image" /></td>
<td><img src="3-400-425-AF.png" alt="Image" /></td>
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<tr>
<td>POC</td>
<td>550-700</td>
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<td><img src="3-550-700-PB.png" alt="Image" /></td>
<td><img src="3-550-700-PB.png" alt="Image" /></td>
</tr>
</tbody>
</table>
Standard Nomenclature for Karyotype Designation

General designation includes:
- Chromosome number (count)-based on #centromeres
  - Expressed relative to the ploidy level
- Sex chromosome constitution
  - Use +/- for acquired sex chromosome aneuploidy only
- List of abnormalities present
  - Ordered by chromosome number (sex chromosomes, then autosomes 1-22) and abnormality type (numerical abnormalities/aneuploidies, then structural abnormalities, listed alphabetically and by arm/band, low to high)
- Multiple cell lines
  - Mosaicism: List abnormal clone(s) first, list multiple abnormal clones from largest to smallest in size
  - Chimerism: List recipient (individual’s karyotype) first
Common symbols and abbreviated terms  
**constitutional studies**

- **+**  additional normal or abnormal chromosome (trisomy)
- **-**  loss of a chromosome (monosomy)
- **add**  added material of unknown origin, typically resulting in a loss of material distal to breakpoint
- **del**  deletion
- **der**  derivative chromosome, due to structural rearrangement(s)
- **dic**  dicentric chromosome
- **dup**  duplication
- **dn**  de novo (not inherited)
- **i**  isochromosome (composed of two identical chromosome arms)
- **idic**  isodicentric chromosome (isochromosome w/ two centromeres)
- **ins**  insertion
- **inv**  inversion
- **mar**  marker chromosome, unknown origin
- **mat**  maternal origin
- **mos**  mosaic (multiple cell lines/clones present)
- **pat**  paternal origin
- **r**  ring chromosome
- **rob**  Robertsonian translocation, a whole arm translocation between acrocentric chromosomes
- **t**  translocation
- **/**  separates clones (for mosaic karyotypes)
- **///**  separates clones (for chimeric karyotypes)
- **[]**  indicate number of cells (for mosaic or chimeric karyotypes)
Structural Abnormalities Description
(Illustrated by Examples)

• Terminal vs interstitial
  – add(11)(q23)
  – del(4)(p16.3)
  – dup(17)(p11.2p13)
• Interchromosomal vs intrachromosomal
  – t(9;22)(q34;q11.2)
  – inv(3)(q21q26.2)
  – ins(2)(q13p11.2p14)
• Whole chromosome arm rearrangements
  – i(12)(p10)
  – der(1;7)(q10;p10)
  – rob(13;14)(q10;q10)
• Combination of abnormalities
  – 47,XY,+8,t(8;14)(q24;q32)
  – der(7)del(7)(p11.2)del(7)(q22)
  – mos 45,X[12]/46,X,idic(X)(p11.22)[8]
Nomenclature Practice: Structural Abnormalities
Abnormal, constitutional

Female

p13
p11.2
Abnormal, constitutional

46,XX,del(11)(p11.2p13)

Female
Abnormal, constitutional

Male with Klinefelter syndrome
Abnormal, constitutional

47,XXY,ins(13;12)(q32;q22q24.1)

Male with Klinefelter syndrome
Abnormal, constitutional
Abnormal, constitutional

45,XX,rob(14;15)(q10;q10)
Abnormal, constitutional

Female

11

q23.3

q11.2

22
Abnormal, constitutional

47,XX,+der(22)t(11;22)(q23.3;q11.2)

Female
Structural abnormalities

Deletions
- Terminal
- Interstitial

Duplications

Insertions

Reciprocal Translocations
- Balanced
- Unbalanced

Robertsonian Translocations
- Balanced
- Unbalanced
Some recurrent deletions and duplications

- 1p36 del
- 2q37 del BDMR
- 7q11.23 del (WBS)/dup
- 13q14 del RB1
- 15q11-13 del pat/mat & dup mat PWS/AS
- 16p13.3 del Rubenstein-Taybi
- 17p13.3 del Miller-Dieker
- 17p11.2 del/dup
- 18p- 18q- 20p12 del Alagille
- 22q11 del (VCFS)/dup
- * Cat-eye Phelan-McDermid
- Cat-eye Inv dup 15
- * Pallister-Killian
- 11q24 del Jacobsen
- 11p13 del Pallister-Killian
- 11p11.2 del
- 11p15 dup pat/mat WAGR
- 11p13 del
- 15q11-13 del pat/mat & dup mat PWS/AS
- 15q11-13 del pat/mat & dup mat PWS/AS
- 17p11.2 del/dup
- 17p13.3 del Miller-Dieker
- 20p12 del Alagille
- 22q11 del (VCFS)/dup
- * Cat-eye Phelan-McDermid
- 22q13 del
- Xp22.31 STS/KAL del
- * Tetrasomy
## Incidence of Recurrent Deletion and Duplication Syndromes

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Incidence</th>
<th>Cause</th>
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<tbody>
<tr>
<td>1p36 deletion</td>
<td>1:7500</td>
<td>Terminal deletion</td>
</tr>
<tr>
<td>1q21.1 deletion (distal)</td>
<td>1:500</td>
<td>Interstitial deletion (SD)</td>
</tr>
<tr>
<td>4p-/-Wolf-Hirschhorn</td>
<td>1:50,000</td>
<td>Terminal deletion</td>
</tr>
<tr>
<td>5p-/-Cri du chat</td>
<td>1:50,000</td>
<td>Terminal deletion</td>
</tr>
<tr>
<td>7q11.23/Williams</td>
<td>1:7500</td>
<td>Interstitial deletion (SD)</td>
</tr>
<tr>
<td>15q11q13/Prader willi</td>
<td>1:20,000</td>
<td>Interstitial deletion (pat)/mUPD/Me defect/mutation</td>
</tr>
<tr>
<td>22q11.2/DiGeorge/VCFS</td>
<td>1:5000</td>
<td>Interstitial deletion (SD)</td>
</tr>
</tbody>
</table>
Low copy repeats (LCRs) mediate many recurrent genomic rearrangements via NAHR.
Segmental duplication (low-copy repeat, LCR) architecture mediates recurrent CNVs/rearrangements

Emanuel and Saitta, Nat Rev Genet 2007
NAHR: misalignment and exchange occurs between non-allelic homologous sequences (LCRs)

DxD=allelic HR

Balanced recombinants

DxA=non-allelic HR

Unbalanced recombinants

Duplicated
Deleted

Emanuel and Saitta, Nat Rev Genet 2007
NAHR underlies many recurrent genomic rearrangements

Liu et al., 2012
Multiple techniques are employed for the detection of different cytogenetic abnormalities

<table>
<thead>
<tr>
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<th></th>
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<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>G-banded chromosomes</td>
<td>3-5 Mb (550 bands)</td>
<td>10-15%</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Metaphase FISH</td>
<td>100’s kb</td>
<td>n/a</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Interphase FISH</td>
<td>100’s kb</td>
<td>1-5%</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>GMA</td>
<td>10-100’s kb</td>
<td>10-20%</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

➢ Sizes: kb=1x10³, Mb=1x10⁶
Structural abnormalities

Deletions
- Terminal
- Interstitial

Duplications

Insertions

Reciprocal Translocations
- Balanced
- Unbalanced

Robertsonian Translocations
- Balanced
- Unbalanced
Incidence of chromosome abnormalities detected in newborns

<table>
<thead>
<tr>
<th>Abnormality</th>
<th>Rate/1000</th>
<th>Rate (1/n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autosomal Trisomy</td>
<td>1.62</td>
<td>617</td>
</tr>
<tr>
<td>Sex Chromosome Aneuploidies (All)</td>
<td>2.70</td>
<td>375</td>
</tr>
<tr>
<td>Balanced Structural Rearrangements</td>
<td>2.04</td>
<td>490</td>
</tr>
<tr>
<td>Translocations, insertions</td>
<td>0.97</td>
<td>1,028</td>
</tr>
<tr>
<td>Inversions</td>
<td>0.16</td>
<td>6,331</td>
</tr>
<tr>
<td>Robertsonians</td>
<td>0.91</td>
<td>1,099</td>
</tr>
<tr>
<td>Unbalanced Structural Rearrangements</td>
<td>0.63</td>
<td>1,587</td>
</tr>
<tr>
<td>Translocations, insertions, inversions</td>
<td>0.09</td>
<td>10,935</td>
</tr>
<tr>
<td>Robertsonians</td>
<td>0.07</td>
<td>13,366</td>
</tr>
<tr>
<td>Deletions, rings</td>
<td>0.06</td>
<td>17,184</td>
</tr>
<tr>
<td>+Markers (e.g. isochromosomes)</td>
<td>0.41</td>
<td>2,455</td>
</tr>
</tbody>
</table>

Data from: Milunsky and Milunsky, Genetic Disorders of the Fetus, 6th Ed. (2010). Benn, Chp. 6

➢ ~1/500 is a carrier of a balanced rearrangement
Effects of Translocations

• Constitutional carriers are at risk for infertility, recurrent miscarriage and/or birth of a child with a congenital anomaly syndrome
  – Most risk figures fall into the range of 0-30% for a liveborn child with an abnormality (higher end if previous child)
• May disrupt gene expression (breakpoint within a gene or regulatory element by position effect)
  – In prenatal setting and de novo, risk ~6% (Warburton ‘91)
• Create gene fusions and affect gene expression by position effect
  – Esp. in cancer ex. t(9;22) BCR-ABL1 chimeric transcript or t(11;14) CCND1 upregulation by translocation near the IGH locus regulatory region
Pachytene configuration (quadrivalent) in the balanced translocation carrier/translocation heterozygote

A, B: Normal chromosomes  
A’, B’: Derivative chromosomes
Modes of Segregation During Gametogenesis in the Balanced Translocation Carrier

Only 2:2 alternate segregation will result in normal/balanced gametes.

All other modes of segregation result in unbalanced gametes.

Chromosome Abnormalities and Genetic Counseling. 4th ed. Gardner, Sutherland and Shaffer. 2012
Predicting clinical outcomes for the balanced translocation carrier

Factors that influence segregation and outcomes

- Location of the breakpoints, relative to chromosome size and the centromere
- Relative size of chromosomes involved

See also Table 5-4 in Gardner, Sutherland and Shaffer 2012
Tertiary trisomy in the t(11;22)(q23;q11) carrier

46,t(11;22)

47,+der(22),t(11;22)
(Emanuel syndrome)

Tertiary trisomy 3:1 segregation

Gardner, Sutherland and Shaffer. 2012
Predicting clinical outcomes for the balanced translocation carrier

Factors that influence segregation and outcomes
- Location of the breakpoints, relative to chromosome size and the centromere
- Relative size of chromosomes involved
- Biological consequence of associated monosomy/trisomy
  - Least imbalanced, least monosomic is most likely to produce a viable conceptus

See also Table 5-4 in Gardner, Sutherland and Shaffer 2012

FIGURE 5-5  Prediction of likely viable segregant outcomes by pachytene diagram drawing and assessment of the configuration of the quadrivalent.
Pedigree of a family carrying a translocation with a large centric segment

FIGURE 5–16  No unbalanced product viable. (a) Pedigree of a kindred in which mother and daughter have had multiple miscarriages, each having (b) the karyotype 46,XX,t(4;6)(q25;p23). (Case of A. J. Watt.) The presumed pachytene configuration during gametogenesis in the heterozygote would be as in Figure 5–5d (chromosome 4 chromatin, open; chromosome 6 chromatin, crosshatched) and, with large centric and translocated segments, the translocation has none of the features that enable viability of any unbalanced segregant combination.
Structural abnormalities

Deletions
- Terminal
- Interstitial

Duplications
- 8

Insertions
- 12
- 13

Reciprocal Translocations
- Balanced
- Unbalanced

Robertsonian Translocations
- Balanced
- Unbalanced
Robertsonian translocations

- Frequency \( \sim 1/1000 \), 95% are nonhomologous
  - \( \text{rob}(13;14) \) is most common (1:1300)
- Homology and orientation of sequences in p-arm stalks of chrs 13, 14 and 21 likely explain relative prevalence of \( \text{rob}(13;14) \) and \( \text{rob}(14;21) \) amongst carriers (via NAHR)

<table>
<thead>
<tr>
<th>TRANSLOCATION</th>
<th>LITERATURE REVIEW</th>
<th>UNBIASED ASCERTAINMENT</th>
</tr>
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<tbody>
<tr>
<td>13q13q</td>
<td>3%</td>
<td>2%</td>
</tr>
<tr>
<td>13q14q</td>
<td>33%</td>
<td>74%</td>
</tr>
<tr>
<td>13q15q</td>
<td>2%</td>
<td>2%</td>
</tr>
<tr>
<td>13q21q</td>
<td>2%</td>
<td>1%</td>
</tr>
<tr>
<td>13q22q</td>
<td>1%</td>
<td>2%</td>
</tr>
<tr>
<td>14q14q</td>
<td>½%</td>
<td>-</td>
</tr>
<tr>
<td>14q15q</td>
<td>2%</td>
<td>5%</td>
</tr>
<tr>
<td>14q21q</td>
<td>30%</td>
<td>8%</td>
</tr>
<tr>
<td>14q22q</td>
<td>1%</td>
<td>2%</td>
</tr>
<tr>
<td>15q15q</td>
<td>2%</td>
<td>-</td>
</tr>
<tr>
<td>15q21q</td>
<td>3%</td>
<td>½%</td>
</tr>
<tr>
<td>15q22q</td>
<td>½%</td>
<td>1%</td>
</tr>
<tr>
<td>21q21q*</td>
<td>17%</td>
<td>3%</td>
</tr>
<tr>
<td>21q22q</td>
<td>2%</td>
<td>½%</td>
</tr>
<tr>
<td>22q22q</td>
<td>1%</td>
<td>-</td>
</tr>
</tbody>
</table>

Gardner, Sutherland and Shaffer. 2012
Robertsonian translocations: Meiotic segregation

Modified from Gardner, Sutherland and Shaffer. 2012
Imprinted chromosomes and human disease due to uniparental disomy (UPD)

![Image of chromosomes with annotations]

<table>
<thead>
<tr>
<th>Chromosome UPD and Inheritance</th>
<th>Associated Genetic Disease or Abnormalities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paternal UPD 6</td>
<td>Transient neonatal diabetes mellitus</td>
</tr>
<tr>
<td>Maternal UPD 7</td>
<td>Silver-Russell syndrome</td>
</tr>
<tr>
<td>Paternal UPD 11</td>
<td>Beckwith-Wiedemann syndrome</td>
</tr>
<tr>
<td>Maternal UPD 14</td>
<td>Hypotonia, motor development delay, mild dysmorphic facial features, low birth weight, growth abnormalities</td>
</tr>
<tr>
<td>Paternal UPD 14</td>
<td>Severe mental and musculoskeletal abnormalities</td>
</tr>
<tr>
<td>Maternal UPD 15</td>
<td>Prader-Willi syndrome</td>
</tr>
<tr>
<td>Paternal UPD 15</td>
<td>Angelman syndrome</td>
</tr>
<tr>
<td>Maternal UPD 16</td>
<td>Intrauterine growth retardation</td>
</tr>
<tr>
<td>Maternal UPD 20</td>
<td>Intrauterine growth retardation and/or postnatal growth retardation</td>
</tr>
</tbody>
</table>

Image from: http://carolguze.com/text/442-10-nontraditional_inheritance.shtml

Velissarioiu, Balkan J Med Gen
Risk for uniparental disomy (UPD)

- Risk for expression of clinical phenotype if rob chromosome contains imprinting genes (differentially expressed genes based on parent of origin) (chrs. 14 and 15)

- Heterodisomy: two homologous copies or segments from the same parent

Images modified from from Shaffer et al., 2001, Genetics in Medicine
Risk for uniparental disomy (UPD)

- Risk for expression of clinical phenotype if rob chromosome contains imprinting genes (differentially expressed genes based on parent of origin) (chrs. 14 and 15)

- Isodisomy: two identical copies or segments from the same parent
  - Risk for expression of two recessive alleles with isodisomy

Images from Shaffer et al., 2001, Genetics in Medicine
Empiric risk estimates for offspring of Robertsonian translocation carrier

- Risk to have unbalanced is greater for females
  - 10-15% for chromosomes 21
- Risk for UPD is the same
- The risk to homologous rob carriers is ~100%
  - Very rare instances of post-zygotic correction are reported

Table 7–2. Estimates of Risks to Have a Child with Aneuploidy or with a Uniparental Disomy Syndrome, for the Heterologous rob Carrier

<table>
<thead>
<tr>
<th>rob</th>
<th>MOTHER UNBAL.</th>
<th>MOTHER UPD*</th>
<th>FATHER UNBAL.</th>
<th>FATHER UPD*</th>
</tr>
</thead>
<tbody>
<tr>
<td>13q14q</td>
<td>1%</td>
<td>&lt;1½%</td>
<td>&lt;1%</td>
<td>&lt;1½%</td>
</tr>
<tr>
<td>13q15q</td>
<td>1%</td>
<td>&lt;1½%</td>
<td>&lt;1%</td>
<td>&lt;1½%</td>
</tr>
<tr>
<td>13q21q</td>
<td>10%–15%</td>
<td>–</td>
<td>&lt;1%</td>
<td>–</td>
</tr>
<tr>
<td>13q22q</td>
<td>1%</td>
<td>–</td>
<td>&lt;1%</td>
<td>–</td>
</tr>
<tr>
<td>14q15q</td>
<td>–</td>
<td>½%</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>14q21q</td>
<td>10%–15%</td>
<td>&lt;1½%</td>
<td>&lt;1%</td>
<td>&lt;1½%</td>
</tr>
<tr>
<td>14q22q</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>15q21q</td>
<td>10%–15%</td>
<td>&lt;1½%</td>
<td>&lt;1%</td>
<td>&lt;1½%</td>
</tr>
<tr>
<td>15q22q</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>21q22q</td>
<td>10%–15%</td>
<td>–</td>
<td>&lt;1%</td>
<td>–</td>
</tr>
</tbody>
</table>

Note: Estimates for the uncommon rob translocations are extrapolated from data for the common robs. Unbal, unbalanced, with a full aneuploidy for chromosome 13 or 21; UPD, uniparental disomy; UPD*, abnormal child with syndrome of UPD 14 or UPD 15.

Gardner, Sutherland and Shaffer. 2012
Modes of X-inactivation

- Most X-inactivation occurs randomly
  - Random X-inactivation often protects against (masks) pathogenic (recessive) mutations in females
- Non-random (skewed) X-inactivation may occur by chance (primary) or through cell selection (secondary)
  - Can lead to expression of X-linked recessive mutations in females
  - Can protect against an otherwise dominant-acting mutation

Morey and Avner, 2001
Non-random X-inactivation can rescue effects of X-chromosome abnormalities in females

- Most structural abnormalities and some mutations lead to non-random inactivation

Key
- Active X = White
- Inactive X = Gray
- * = XIST

Leppig and Disteche, Semin Reprod Med, 2001
Translocation X;A in females-balanced carriers may also be affected, dependent on X-inactivation

- There is an inherent risk to the balanced female carrier if X inactivation is not skewed to preferentially inactivate the normal X
  - Risk for functional disomy (double expression of X-linked genes relative to their normal level) of the translocated X segment on the der(A)
  - Risk for functional monosomy of the translocated autosomal segment on the der(X)

Key
- Active X = White
- Inactive X = Gray
- Autosomal material = hashed
- * = XIST

Leppig and Disteche, Semin Reprod Med, 2001
Structural abnormalities

Inversions
- Pericentric inversion
- Paracentric inversion
- Recombinant chromosomes

Ring chromosomes
- Isochromosomes

Ring chromosomes
- 7
- 8
- Paracentric inversion
- Isochromosomes
- 10
- 11
- 12
- 13
- 14
- 15
- 16
- 17
- 18
- 19
- 20
- 21
- 22
Recombinant chromosome arises from a parental pericentric inversion.

```
rec(8)dup(8q)inv(8)(p23.1q23.1)
```
Cytogenetics in Cancer

• Information from cytogenetic testing is used to:
  – Establish diagnosis
  – Guide therapy
  – Predict outcome
  – Monitor response to therapy or engraftment post-bone marrow transplant (BMT)
Basic terminology for classifying hematologic malignancies

- **Leukemia**: cancer of the blood and/or bone marrow
- **Lymphoma**: cancer in the lymphatic tissue (nodal or extranodal)
- **Myeloid**: cells that arise and differentiate in the bone marrow (RBC’s, platelets, WBCs: granulocytes)
- **Lymphoid**: cells that arise in the bone marrow and differentiate and/or function in the lymphatic system (WBC types: B-cells, T-cells, NK cells)
Blood Cell Lineages

Myeloid-type diseases
- AML
- CML
- MDS
- MPD

Lymphoid-type diseases
- ALL
- CLL
- MM
- Lymphomas

Types of Chromosome Abnormalities in Cancer

• Numerical
  – Aneuploid: 2n - or + chromosomes
    • Monosomy or trisomy
  – Polyploid: 1n, 2n, 3n, 4n, etc. where n=23 chr.

• Structural
  – Deletions
  – Duplications/amplifications
  – Translocations: balanced or unbalanced
  – Inversions

• Copy-neutral loss of heterozygosity (LOH)
  – Mitotic recombination
  – Mitotic malsegregation: uniparental disomy
Comparing technologies...

Aberrations of copy number, structure

Aberrations of genotype

<table>
<thead>
<tr>
<th></th>
<th>Polyploid</th>
<th>Aneuploid</th>
<th>Balanced</th>
<th>Unbalanced</th>
<th>Amplification (double minutes)</th>
<th>Amplification (HSR)</th>
<th>Amplification (distributed insertions)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Karyotyping</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>FISH</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CMA (SNP)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Image modified from Albertson et al., 2003, Nature Genetics
Defining clonality/acquired changes in oncology studies

• Karyotyping:
  – At least two metaphase cells with the same extra chromosome, structural abnormality
  – At least three metaphase cells with the same chromosome loss

• FISH:
  – Abnormality observed in a percentage of cells (usually >1-5%), 200 interphase FISH cells are examined

• Genomic microarray:
  – Evidence of mosaicism in the sample as shown by the copy number and/or SNP-containing probes
  – Cannot determine whether multiple mosaic abnormalities represent different clones/evolution (clonal diversity)
Karyotyping in Cancer
e.g. Clinical Utility of Karyotype in ALL

Cytogenetic subtype distribution by age

Harrison. ASH Education Program (2013) 118-125
The Genetic Basis of Cancer
Types of genes involved in cancer

Calvert and Frucht, 2002, Ann Int Med
Types of genes in cancer

- **Oncogenes**: mutant forms of genes (proto-oncogenes) that positively regulate cell proliferation and survival
  - Dominant, gain-of-function type mutations

Image source: http://www.scq.ubc.ca/images/oncogeneformation.gif
Mechanisms of oncogene activation

• Chromosomal rearrangements (translocations, inversions)
  – A gene fusion creating a chimeric protein
  – Upregulation of gene expression by position effect

• Copy number gains
  – Trisomy, tetrasomy, etc.
  – Gene amplification

Image modified from Albertson et al., 2003, Nature Genetics
Oncogene Activation by Gene Fusion
t(9;22) in chronic myelogenous leukemia (CML)

- First chromosomal abnormality associated with cancer, discovered in 1960
- Abnormal Chr. 22 named the Philadelphia (Ph) chromosome

Image source: http://atlasgeneticsoncology.org/Anomalies/t0922CML.html
The t(9;22)(q34;q11) reciprocal translocation

(Proto-oncogene)
BCR/ABL1 protein is a constitutively active tyrosine kinase

- The N-terminal cap regulates controlled ABL kinase activity
- Fusion to 5’ BCR
  - Increases cell proliferation
  - Inhibits programmed cell death
  - Increases invasiveness
  - Inhibits DNA repair

Goldman and Melo, NEJM, 2003
Targeted Therapy: Inhibitors of tyrosine kinase (TKIs)

- Imatinib mesylate (Gleevec) was the first TKI approved by the FDA in 2001
- Mechanism: Competes with ATP for binding sites
- Inhibits progression of CML in the majority of patients
- Drug resistance can develop over time

Image source: http://upload.wikimedia.org/wikipedia/commons/c/ca/Bcr_abl_STI_1IEP.png

BCR-ABL1 kinase inhibited by Imatinib
Oncogene Activation by Position Effect
c-MYC rearrangements in Burkitt lymphoma

- Cell of origin is a peripheral memory B-cell
- c-MYC at 8q24 is a proto-oncogene is a transcription factor that induces cell proliferation
- Immunoglobulin genes are strongly expressed in B-cells
- Translocation juxtaposes c-MYC with IG enhancers
- t(8;14)(q24;q32) in 75-85% cases
- t(8;22)(q24;q11) in ~10% cases
- t(2;8)(p12;q24) in ~5% cases

Image source: http://atlasgeneticsoncology.org/Anomalies/t0814ID1050.html
C-Myc influences the transcription of a variety of proteins involved in the cell cycle.
## Selected Rearrangements in Cancer

<table>
<thead>
<tr>
<th>Neoplasm</th>
<th>Translocation</th>
<th>Percentage of Cases</th>
<th>Oncogene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic myelogenous leukemia</td>
<td>t(9;22)(q34;q11)</td>
<td>100% (includes variant fusions)</td>
<td>BCR-ABL1</td>
</tr>
<tr>
<td>Acute lymphocytic leukemia</td>
<td>t(9;22)(q34;q11)</td>
<td>10-15%</td>
<td>BCR-ABL1</td>
</tr>
<tr>
<td>Acute lymphocytic leukemia</td>
<td>t(4;11)(q21;q23)</td>
<td>5-10%; 40% &lt;1y</td>
<td>KMT2A-AFF1</td>
</tr>
<tr>
<td>Acute promyelocytic leukemia</td>
<td>t(15;17)(q22;q21)</td>
<td>100%</td>
<td>PML-RARA</td>
</tr>
<tr>
<td>Acute myeloid leukemia</td>
<td>t(8;21)(q22;q22)</td>
<td>5-10%</td>
<td>RUNX1T1-RUNX1</td>
</tr>
<tr>
<td>Acute myeloid leukemia</td>
<td>inv(16)(p13.3q22) or t(16;16)(p13;q22)</td>
<td>5-10%</td>
<td>CBFB-MYH11</td>
</tr>
<tr>
<td>Burkitt lymphoma</td>
<td>t(8;14)(q24;q32)</td>
<td>75-85%</td>
<td>MYC</td>
</tr>
<tr>
<td></td>
<td>t(8;22)(q24;q11)</td>
<td>10-15%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>t(2;8)(q11;q24)</td>
<td>2-5%</td>
<td></td>
</tr>
</tbody>
</table>
Types of genes in cancer

- **Tumor suppressors**: genes that block tumor development by negatively regulating cell growth and proliferation

- Recessive, loss-of-function type mutations

Knudson’s Two-Hit Hypothesis

Image modified from UW Cytogenetics Lab
Mechanisms of tumor suppressor inactivation

• **Copy number losses**
  - Monosomy
  - Deletions
  - Note: copy number loss may in itself be pathogenic or may unmask a recessive mutant allele

• **Loss of heterozygosity (LOH)**
  - Somatic recombination
  - Uniparental disomy

Image modified from Albertson *et al.*, 2003, Nature Genetics
Nomenclature in Cancer
Common symbols and abbreviated terms

- +  additional normal or abnormal chromosome (trisomy)
- -  loss of a chromosome (monosomy)
- add  added material of unknown origin, typically resulting in a loss of material distal to breakpoint
- c  constitutional
- cp  composite (clonal, but variable across cells)
- del  deletion
- der  derivative chromosome, due to structural rearrangement(s)
- dic  dicentric chromosome
- dmin  double minute chromosome
- dup  duplication
- i  isochromosome (composed of two identical chromosome arms)
- idic  isodicentric chromosome (isochromosome w/ two centromeres)
- ins  insertion
- inv  inversion
- mar  marker chromosome, unknown origin
- r  ring chromosome
- sl  stemline (used with clonal evolution)
- sdl  sideline (used with clonal evolution)
- t  translocation
- ?  designates uncertainty (used in place of, or in front of a finding)
- /  separates clones (for mosaic karyotypes)
- //  separates clones (for chimeric karyotypes)
- [ ]  indicate number of cells (for mosaic or chimeric karyotypes)
Case 1: CHR BM for a patient after treatment for AML shows disease persistence

46,XY,t(6;11)(p21.1;q23)[2]/46,XY[18]

Rearrangement involving 11q23 (MLL/KMT2A) associated w/ a poor prognosis in AML
Case 2: AML, CHR BM reveals complex karyotype with multiple related abnormal clones, shows clonal evolution

46,XX,add(5)(q15),del(9)(q31),del(20)(q11.2q13.1)[4]/46-47,sl,+8,ins(11;?)(q13;?),2-12dmin[cp13]/46,XX[3]

Complex karyotypes are associated w/ a poor prognosis in AML
Case 3: CHR BM reveals trisomy 21 in a newborn male with pancytopenia (uncertain if patient has Down syndrome)

- Careful with abnormalities present in every cell?constitutional
- DS patients have an increased risk of transient myeloid disease and ALL
  - Trisomy 21 is a recurrent acquired change in hematologic disease
- Test PB lymphocytes to see whether abnormality is constitutional/clonal
Case 4: CHR BM on a patient with multiple myeloma (MM) reveals a complex karyotype

44-45,XY,+3,-13,-14,der(16)t(16;17)(q11.2;q21),-17[5]/46,XY[19]

- Loss of Chromosome 17 (TP53 gene) is associated with unfavorable prognosis in MM (and virtually all other cancers)
Principles of Cytogenetics
Categorical Course
Introduction to Cytogenetics 2

Erica Andersen, PhD
Medical Director, Cytogenetics and Genomic Microarray, ARUP Laboratories
Assistant Professor, Department of Pathology, University of Utah