Molecular Diagnosis

Oncologic Diagnosis

Oncology: Molecular Targets

- Detection of Clonal Populations
- Antigen Receptor Gene Rearrangement
- EBV clonality
- Southern Blotting Methods
- PCR methods
- Identification of Tumor-specific genetic alterations
- Translocations (Chromosomal rearrangements):
  - w/ fusion genes
  - Oncogene mutations/amplifications
- Tumor suppressor gene mutations/deletions
- Specific chromosomal losses
- Alterations in genomic methylation
- Gene Expression Profiling

Antigen Receptor Gene Rearrangement

Ig heavy chain

Gene Expression Profiling

Antigen Receptor Gene Rearrangement
DH-JH rearrangement

PCR paraffin vs frozen

Clonality PCR vs Southern

Translocations w/o gene fusion

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Translocation</th>
<th>Activated Gene</th>
<th>Mechanism of Activation</th>
</tr>
</thead>
<tbody>
<tr>
<td>B-ALL/Burkitt</td>
<td>t(8;14)(q24;q32)</td>
<td>MYC</td>
<td>Relocation to IgH locus</td>
</tr>
<tr>
<td>Large Cell Lymphoma</td>
<td>t(14;19)(q32;q12)</td>
<td>BCL6</td>
<td>Relocation to IgH locus</td>
</tr>
<tr>
<td>Mantle Cell Lymphoma</td>
<td>t(11;14)(q13;q32)</td>
<td>Cyclin D1</td>
<td>Relocation to IgH locus</td>
</tr>
<tr>
<td>Follicular B-cell lymphoma</td>
<td>t(14;18)(q32;q21)</td>
<td>BCL2</td>
<td>Relocation to IgH locus</td>
</tr>
<tr>
<td>T-cell ALL</td>
<td>t(8;14)(q24;q11)</td>
<td>MYC</td>
<td>Relocation to TCR α/δ locus</td>
</tr>
<tr>
<td>T-cell ALL</td>
<td>t(11;14)(q32;q11)</td>
<td>TAL1</td>
<td>Relocation to TCR α/δ locus</td>
</tr>
</tbody>
</table>

BCL2-IgH gene rearrangement
Translocations w/fusion product: hematologic tumors

<table>
<thead>
<tr>
<th>TUMOR</th>
<th>Translocation</th>
<th>Gene fusion</th>
</tr>
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<tbody>
<tr>
<td>Chronic myelogenous leukemia</td>
<td>t(9;22)</td>
<td>BCR-ABL(p210)</td>
</tr>
<tr>
<td>Acute promyelocytic leukemia</td>
<td>(15;17); (11;17)q23;q21;</td>
<td>PML-RAR, PLZF-RAR, NPM-RAR, NUMA-RAR, STAT5b-RAR</td>
</tr>
<tr>
<td>AML</td>
<td>t(8;21)q22;p22</td>
<td>AML1-ETO</td>
</tr>
<tr>
<td>AML and ALL (esp. infants and post-Rx)</td>
<td>11q23, 12p;q23</td>
<td>MLL(30 partners)</td>
</tr>
<tr>
<td>Anaplastic large cell lymphoma (pediatric)</td>
<td>t(1;13); t(2;13)</td>
<td>PAX7/FKHR, PAX3/FKHR</td>
</tr>
<tr>
<td>ALL</td>
<td>t(9;22)</td>
<td>API2-MLT</td>
</tr>
</tbody>
</table>

Detection of Gene Fusions

- **RT-PCR:**
  - Detection of fusion transcripts.
  - Present only in tumor cells, not in normal cells.
  - Marker of tumor volume.
  - “Real-time” methods used to follow change in tumor burden.
    - Sensitivity approximately 1:10K
  - “Nested” RT-PCR to detect minimal residual disease
    - Sensitivity approximately 1:1M

Oncogene Mutations/amplification

- **HER-2 amplification in breast CA**
  - Poor prognosis
  - Response to Herceptin (Trastuzumab)

- **KIT mutations in Gastrointestinal Stromal Tumor**
  - Diagnostic of tumor
  - Specific mutations associated with sensitivity/resistance to Gleevec (Imatinib).

- **nMYC amplification in Neuroblastoma**
  - Poor prognosis, important criterion in classification of risk category.

- **EGFR mutations in Lung Carcinoma**
  - Predict response to gefitinib (Iressa), but NOT (so far) Erlotinib (Tarceva)
  - Seen more commonly in females, East Asians, and never smokers.

Loss of Heterozygosity in tumors

- **Tumor suppressor genes:**
  - Need to lose two copies. Often one copy lost by mutation, and the second by loss of the chromosomal locus, or by gene conversion (“loss of heterozygosity”).

- **Oligodendrogliomas:**
  - Loss of Chromosome 1p and 19q
    - Diagnostic value
    - Predicts chemosensitivity.
**HER2 amplification: FISH**

**Ewing’s Sarcoma: FISH**

**EGFR mutation: 15bp deletion**

**Gene Expression Profiling**

- Label total RNA from a tumor
- hybridize to chip w/ ≥25,000 cDNAs/oligonucleotides.
  - Expression profile unique to tumor type.
  - Predict behavior
  - Identify origin of mets
  - Identify targets for therapy.