PRACTICAL APPLICATIONS OF MOLECULAR BIOLOGY IN SURGICAL PATHOLOGY

P. Heimann MD
Dpt of Medical Genetics
Erasme Hospital - ULB
Tumor types

- Lymphoma
- G.I. tract
I. Lymphoma

• B and T cell monoclonalities
  - Rearrangement of immunoglobulin and TCR genes.

• Identification of non-random chromosomal abnormalities detectable by PCR
  - \( t(14;18) \) or \( t(11;14) \) translocations in FL and MCL respectively.
B and T cell monoclonality

- Genotype does not correspond to phenotype!

Lineage infidelity of Ig and TCR gene rearrangements ("Illegitimate rearrangements"):
- 50-60% of lymphoblastic B cell malignancies.
- 20-30% of lymphoblastic T cell malignancies.
- ~10% of mature B and T cell malignancies.

Therefore, Ig and TCR gene rearrangements cannot be used as markers for B and T cell lineages, respectively.
B and T cell monoclonality

- Monoclonality is not always equivalent to malignancy!

- Clinically benign lymphoproliferative may consist of clonal cell populations.

- Although this pitfall is encountered in B cells, it is mainly observed in T cell monoclonality (cf limited combinatorial diversity of TCR-γ and -δ genes)
B and T cell monoclonality

Molecular tools

- Southern Blot
- PCR
Schematic diagram of IgH gene rearrangements

- : indicate the JH probe location for Southern Blot method
- : indicate the primers location for PCR method
B cell monoclonality - Southern Blot

Illustration

Patients

1  2  3  4  5  6  1  2  3  4  5  6

Restriction Enzymes

Hind III  Hind III/BamH1  EcoRI
Southern Blot

Advantages:
- Very good qualitative sensitivity since ~100% of B and T cell malignancies are detectable by Southern Blot

Disadvantages:
- time-consuming
- requires relatively large amounts of pathological material
- low quantitative sensitivity (~ 5 %)
Schematic representation of mono and polyclonal populations detected by PCR.

1) Clonal population: monoallelic rearrangement
2) Clonal population: biallelic rearrangement
3) Polyclonal lymphoid population
4) Non lymphoid population
B and T cell monoclonalities - PCR
Illustration on paraffin embedded tissue

B cells

Tcells

*? oligoclonality
B cell monoclonality - PCR
Illustration (Genescan)

polyclonality

monoclonality
PCR

Advantages: (vs Southern Blot)
- simple and faster
- requires much less amounts of pathological material
- greater quantitative sensitivity
- can be applied on DNA paraffin-embedded tissue

Disadvantages: (vs Southern Blot)
- lower qualitative sensitivity
- need to use several different PCR strategies in order to increase the overall detection rate.
PCR strategies

• Necessity to use several sets of primers in order to increase the overall detection rate (~90%) of the PCR method: FR3-JH, FR1c-JH, FR1f-JH,...

• This detection rate varies according to the underlying disorders
Detection rates by PCR according to pathological subtypes

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Detection Rate</th>
</tr>
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<tbody>
<tr>
<td>SLL</td>
<td>~100 %</td>
</tr>
<tr>
<td>MCL</td>
<td>~100 %</td>
</tr>
<tr>
<td>SNCL</td>
<td>~80 %</td>
</tr>
<tr>
<td>PCN</td>
<td>~70 %</td>
</tr>
<tr>
<td>DLCL</td>
<td>~60 %</td>
</tr>
<tr>
<td>LC (IBL)</td>
<td>~50-60 %</td>
</tr>
<tr>
<td>LF</td>
<td>~50 %</td>
</tr>
</tbody>
</table>
PCR - Pitfalls

False negative:
- chromosomal translocations into the IgH locus (in FL or DLCL)
- Somatic mutation (in FL and DLCL)
- partial D-J rearrangements (in immature malignancies)
- no VDJ rearrangement produced (in immature malignancies)
- failure of the IgH primers to recognize the VH segment involved

False positive:
- very weak amount of DNA
- reactive lymphoid populations
B and T cell monoclonalities

- Some cases of unequivocal B-cells lymphoma do not generate a clonal signal by PCR despite a demonstrated clonality by Southern Blot.

- Any result must be interpreted in view of other findings and clinical informations
Recurrent molecular abnormalities in lymphoma

- t(14;18) / Bcl2 - J_H in follicular lymphoma
- t(11;14) / Bcl1 - J_H in Mantle Zone lymphoma
- t(3;14) / Bcl6 - J_H in Diffuse Large Cell lymphoma
- t(8;14) / cMyc - J_H in Burkitt lymphoma
- t(2;5) / ALK-NPM in Anaplastic Large Cell Lymphoma
Two distinct types of chromosomal translocations at molecular level

A. Quantitative changes
Bcl2-J_H, Bcl1-J_H,…

B. Qualitative changes
ALK-NPM,…
Bcl2 in Follicular lymphoma

- t(14;18) detectable by cytogenetic in ~ 90 % of cases

- this translocation gives rise to an overexpression of the antiapoptotic Bcl2 protein

- four different breakpoints on Bcl2 gene

  mbr         in ~ 45 % of cases
  mcr         in ~ 7 % of cases
  3’UTR       in ~ 10 % of cases
  icr         in ~ 10% of cases
PCR Bcl2-JH in follicular lymphoma
Illustration

M \ H_2O - + + + + + +

mbr breakpoint
## Bcl2 in Follicular lymphoma

<table>
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<tr>
<th>Method</th>
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<tr>
<td><strong>FISH</strong></td>
<td>&gt;95%</td>
</tr>
<tr>
<td>Cytogenetic</td>
<td>~80-90%</td>
</tr>
<tr>
<td>S.B.</td>
<td>65-80%</td>
</tr>
<tr>
<td>PCR Bcl2 / mbr-JH</td>
<td>40-50%</td>
</tr>
<tr>
<td>PCR Bcl2 / mcr-JH</td>
<td>~10%</td>
</tr>
</tbody>
</table>
Bcl2 in Follicular lymphoma

False positivity in normal patients (~23%) and in benign follicular hyperplasia where a very low percentage of positive cells ($10^{-3} - 10^{-4}$) are detectable by nested PCR → need to use a less sensitive method (standard PCR) to avoid false positive cases
Bcl2 in Diffuse Large Cell Lymphoma

Bcl2-JH rearrangement is detectable in ~35% of DLCL and seems to be associated with a better prognosis.
Bcl1 in Mantle Cell lymphoma

- t(11;14) detectable in ~ 100 % of MCL
  observed also in MM (~ 20 %)
  in SLVL (~ 20 %)

- this translocation gives rise to an overexpression of
  the Bcl1 gene encoding the cyclin D1 protein (positive
  cell cycle regulatory protein)

- the majority of the Bcl1 breakpoints are clustered in the
  MTC region
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</tr>
<tr>
<td>PCR Bcl1/MTC - J&lt;sub&gt;H&lt;/sub&gt;</td>
<td>40 %</td>
</tr>
<tr>
<td>Northern Blot</td>
<td>100 %</td>
</tr>
<tr>
<td>RT-PCR (cyclinD1 overexpression)</td>
<td>100 %</td>
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BCL1 Overexpression in MCL

Competitive RT-PCR:
Comparison of the different expression profiles of the three cyclins D1, D2 and D3

This method avoids any false positive results