Molecular Pathology of Lymphoma (Part 1)

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# Lecture outline

<table>
<thead>
<tr>
<th>Time</th>
<th>Content</th>
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<tbody>
<tr>
<td>10:00 – 11:00</td>
<td>• Introduction to lymphoma</td>
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<tr>
<td></td>
<td>• Review of lymphocyte biology</td>
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<tr>
<td>11:15 – 12:10</td>
<td>• <em>IGH</em> and <em>TCR</em> gene rearrangement studies</td>
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<tr>
<td>12:20 – 13:15</td>
<td>• Advanced molecular pathology tests for lymphoma</td>
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Introduction to lymphoma
Definition

Lymphoid neoplasms

- Clonal tumours of mature and immature B, T or NK cells at various stages of differentiation.
2008 WHO Classification of lymphoid neoplasms

- Precursor lymphoid neoplasms
- Mature B-cell neoplasms
- Mature T- and NK-cell neoplasms
- Hodgkin lymphoma

Total 64 different types

Used to be called non-Hodgkin lymphoma
Normal blood cells

The Elements of Blood

- Erythrocytes
- Monocyte
- Eosinophil
- Lymphocyte
- Neutrophil
- Platelets
- Basophil
Normal haemopoiesis

Picture: CanStockPhoto
Normal haematopoiesis

Lymphoid neoplasms
The lymphoid organs

- Lymphatics
- Lymph nodes
- Waldeyer ring (tonsils)
- Spleen
- Thymus
- Mucosa-associated lymphoid tissue (MALT)
Architecture of a normal lymph node

- Cortex & lymphoid follicles
- Paracortex
- Medulla
The ‘B symptoms’

- Fever
- Night sweats
- Weight loss

Cause of ‘B symptoms’: Cytokines secreted by the lymphoma cells
Lymphoma: Gross morphology

- Tongue and larynx infiltrated by lymphoma
- A few enlarged lymph nodes around the larynx
Lymphoma: Gross morphology

- Multiple tumour nodules in spleen
- Several enlarged lymph nodes around spleen hilum
Normal lymph node
Diagnosis of lymphoma

- Clinical symptoms and signs
- Morphology
- Immunophenotype
- Molecular pathology
Lymphoma example
• Chronic lymphocytic leukaemia/small lymphocytic lymphoma
Lymphoma example

- Mantle cell lymphoma
Lymphoma example

- Diffuse large B-cell lymphoma
Lymphoma examples
- Hodgkin lymphoma
Lymphoma example

- Nasal type extranodal NK/T-cell lymphoma
Lymphoma example

- Peripheral T-cell lymphoma of the small intestine
Is this a lymphoma?

- Diagnosis: Reactive lymphoid follicles
Is this a lymphoma?

- Diagnosis: Kimura disease of the skin (reactive process)
Is this a lymphoma?

- Diagnosis: Kikuchi disease (another reactive condition)
Is this a lymphoma?

- Diagnosis: Burkitt’s lymphoma (a high grade B-cell lymphoma)
Is this a lymphoma?

- Diagnosis: Follicular lymphoma (a low grade B-cell lymphoma)
Is this a lymphoma?
Diagnosis: Reactive lymphoid follicle
• It is sometimes difficult to distinguish lymphomas from reactive lymph nodes on morphology and immunohistochemistry
• Molecular pathology techniques are hence needed to arrive at the correct diagnosis
Review: Lymphocyte biology

- LYMPHOCYTE BIOLOGY
# Lymphocytes

<table>
<thead>
<tr>
<th>Type</th>
<th>Function</th>
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</thead>
<tbody>
<tr>
<td><strong>B-cells</strong></td>
<td>• Produce immunoglobulins (antibodies)</td>
</tr>
<tr>
<td></td>
<td>• CD20-positive</td>
</tr>
<tr>
<td><strong>Helper T-cells</strong></td>
<td>• Assist development of B and T-cells</td>
</tr>
<tr>
<td></td>
<td>• CD3 and CD4-positive</td>
</tr>
<tr>
<td><strong>Cytotoxic T-cells</strong></td>
<td>• Kill infected cells by inducing apoptosis</td>
</tr>
<tr>
<td></td>
<td>• CD3 and CD8-positive</td>
</tr>
<tr>
<td><strong>Natural killer (NK) cells</strong></td>
<td>• Kill abnormal cells, but part of innate immunity system (not adaptive)</td>
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<tr>
<td></td>
<td>• CD56-positive</td>
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</table>
Normal haematopoiesis

Lymphoid neoplasms
B-cells and immunoglobulins

HUMORAL IMMUNITY
Helper (CD4) T-cells
Cytotoxic (CD8) T-cells

Cytotoxic T cell Activation and Action

1. Antigen recognition
   - Antigen
   - MHC-I
   - TCR
   - Immature cytotoxic T cell

2. Clonal selection
   - Activated cytotoxic T cells
   - Memory T cells

3. Lethal hit
   - Infected/cancer target cell
   - Target cell dies
Normal lymph node
Normal lymph node – CD3

T-cells mostly situated in the interfollicular areas and paracortex
Normal lymph node – CD20

B-cells situated in lymphoid follicles
Immunoglobulin (Ig) molecule

- Two heavy chains and two light chains
- Variable region: binds to antigens, **highly variable** amino acid sequence among different
- Constant region: For structure, and also activates complements. This part can be recognized by other white cells
Immunoglobulin classes

- IgG: Most common Ig circulating in plasma
- IgM: First antibody produced after infection
- IgA: Secreted in mucous membranes
- IgE: Associated with hypersensitivity reaction
- IgD: On surface of immature B-cells, function unknown
Normal B-cell development

From immunoglobulin (IG) genes to IG molecules
IGH gene (chromosome 14q32)

Blue boxes: The variable (V<sub>H</sub>) segments
Grey boxes: Non-functional segments

Picture: Leukaemia. 2003; 17:2257-317
IGK gene (chromosome 2p11)

Blue boxes: The variable (V_κ) segments
Grey boxes: Non-functional segments
Kde: Kappa deleting element
IGL gene (chromosome 22q11)

Blue boxes: The variable (V_\lambda) segments
Grey boxes: Non-functional segments

Picture: Leukaemia. 2003; 17:2257-317
**V(D)J recombination**

V: Variable  
D: Diversity  
J: Joint  
C: Constant  

(Also called ‘somatic recombination’)
Somatic hypermutation

- Germinal centre B-cells can further modify the V region of the immunoglobulin gene to increased the affinity of the Ig molecule to the antigen
- AID: Activation-induced (cytidine) deaminase
**Class-switch recombination**

- B-cells originally manufacture IgM
- Germinal centre B-cells can modify the Constant region of their Ig gene further
- Result: IgG, IgA or IgE is manufactured
- This process is called 'class-switch recombination'

![Diagram of class-switch recombination](Picture: Wikipedia)
Normal T-cell development

Cell-mediated immune response

**CELL-MEDIATED IMMUNE RESPONSE**

- **Infect cell**
- **Macrophage**
- **Helper T cell**
- **Killer T cell**
- **Memory T cell**
- **Cytokines**
- **Mitosis**

**T-cell receptor**
The T-cell receptor (TCR) gene

Two types of TCR: TCR\(\alpha\beta\) and TCR\(\gamma\delta\). Each T-cell expresses either TCR\(\alpha\beta\) or TCR\(\gamma\delta\) but not both.

Picture: Leukaemia. 2003; 17:2257-317
Significance of the gene rearrangements

- All cells from a lymphoma descended from one mutated cell, which has undergone VDJ rearrangement in the IG/TCR genes
  - All tumour cells in a lymphoma share the same VDJ recombination (i.e. marker of clonality)
  - Detectable using Southern blot or polymerase chain reaction (PCR)
Significance of the gene rearrangements

- The genetic lesions in lymphomas most probably arises during the VDJ recombination, somatic hypermutation, and/or class-switch recombination.
- These reactions are the major source of ‘chromosomal instability’ in lymphoma genesis.
- They are also the source of ‘recurrent chromosomal translocations’ in certain types of lymphomas.
# Molecular tests in lymphoma diagnosis

<table>
<thead>
<tr>
<th>Clinical Question</th>
<th>Molecular pathology answer</th>
<th>Available Techniques</th>
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<tr>
<td><strong>Is this biopsy a lymphoma?</strong></td>
<td>Demonstration of clonality</td>
<td>• Southern blot</td>
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<tr>
<td></td>
<td></td>
<td>• Polymerase chain reaction (PCR)</td>
</tr>
<tr>
<td><strong>Which type of lymphoma is this?</strong></td>
<td>Detection of recurrent chromosome translocation</td>
<td>• Southern blot</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• PCR</td>
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<td></td>
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<td>• Fluorescence in-situ hybridization (FISH)</td>
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| How will this lymphoma affect my patient (prognosis and/or therapy)? | • Detection of chromosomal lesions  
• Gene expression levels | • FISH  
• Gene expression profiling |
Summary of Part 1

After listening to this part of the lecture, you should be able to:

1. Define what is lymphoma
2. Name the major categories of lymphoma
3. Describe the normal development of B and T-cells
4. Describe the genetic modifications of the antigen receptors/immunoglobulin molecule during B/T-cell development
5. Name the major indications for molecular pathology tests in lymphoma diagnostics