Lymphoma as a Cancer Model

Cellular and Molecular Biology of Cancer
PATH G4500
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Lymphomas

- Tumors of the lymphoid organs
- 6th most common cancer
- 82,310 new cases in 2019
- 20,970 cancer-related deaths
- Annual incidence rates doubled between 1975 and 2008 (but stable over the past 10 years)
- Not a single disease: over 40 distinct types recognized by the WHO classification
Outline of today’s lecture

- The Germinal Center and B cell Lymphomagenesis
- BCL6: the Germinal Center Master Switch
- Mechanisms of Genetic Lesion in lymphoma
- Lymphoma Classification
- Genetic Basis of Diffuse Large B cell Lymphoma
- Therapeutic Implications

A Historical View of Two Lymphomas

LYMPHOMA

NON-HODGKIN

B cell (85%)

T cell (15%)

B-NHL

T-NHL

HODGKIN

B cell (100%)

Ratio between non-Hodgkin and Hodgkin’s lymphomas = 9:1 (74K vs 8K cases/yr)
B cell development and the germinal center reaction

“a disaster waiting to happen”
Transcription factor networks regulating the GC reaction

Pasqualucci L, Immunological Reviews 2019
BCL6: the Germinal Center Master Regulator

The BCL6 protein

(protein-protein interaction) (phosphorylation/degradation) (DNA binding) (acetylation/inactivation)
BCL6 is specifically expressed in GC B cells

BCL6 is required for GC formation and affinity maturation
Biological function of BCL6 in the Germinal Center

- BCL6
- Naive B cell
- Memory B cell
- Subset of LZ B cells
- Burkitt Lymphoma
- Follicular Lymphoma
- ABC-DLBCL
- GCB-DLBCL
- Plasma cell

Mechanisms of genetic lesion in B cell Lymphomas

- Ci et al., Blood 2009
- Basso et al., Blood 2009
- Niu et al., J Exp Med 2003
- Phan et al., Nature 2004
- Phan et al., Nat Immunol 2005
- Ranuncolo et al., Nat Immunol 2007
- Saito et al., Proc Natl Acad Sci USA 2009
- Tunyaplin et al., J Immunol 2004
### Pattern of chromosome aberrations in human malignancies

<table>
<thead>
<tr>
<th>TUMOR</th>
<th>NUMERICAL (Aneuploidy Polyploidy)</th>
<th>STRUCTURAL</th>
<th>Translocations</th>
<th>Amplifications</th>
<th>Deletions</th>
<th>Mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epithelial</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Mesenchymal</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hematologic</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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### DNA Remodeling Processes in the Germinal Center

- **SHM**: High affinity antigen binding
- **CSR**: Change in the effector function

Küppers et al., NEJM 1999
Mechanisms of genetic lesion B-NHL

- non-random chromosomal translocations
  - due to DNA breaks during
    - V(D)J recombination
    - Class Switch Recombination
    - Somatic Hypermutation

- aberrant somatic hypermutation (in DLBCL)
  - due to a malfunction of SOMATIC HYPERMUTATION

- mechanisms common to other cancer types
  - Deletions
  - Amplifications
  - Point mutations

NHL-associated Chromosomal Translocations

**Key Features**

- Balanced
- Reciprocal
- Clonal
- Recurrent
Consequences of Chromosomal Translocations

Gene A
Regulatory  Coding

Translocation

Gene B (proto-oncogene)
Regulatory  Coding

Fusion Protein (Leukemia)

Deregulated Expression (Lymphoma)

Most common chromosomal translocations in B cell Lymphoma

<table>
<thead>
<tr>
<th>LYMPHOMA</th>
<th>TRANSLOCATION</th>
<th>GENE</th>
<th>FUNCTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>MANTLE CELL</td>
<td>t(11;14)(q13;q32)</td>
<td>BCL1</td>
<td>Cell cycle Regulator</td>
</tr>
<tr>
<td>LYMPHOPLASMACYTIC</td>
<td>t(9;?)(p13;?)</td>
<td>PAX5</td>
<td>Transcription Factor</td>
</tr>
<tr>
<td>FOLLICULAR</td>
<td>t(14;18)(q32;q11)</td>
<td>BCL2</td>
<td>Anti-apoptosis</td>
</tr>
<tr>
<td>MALT LYMPHOMA</td>
<td>t(11;18)(q21;q21)</td>
<td>API2/MLT</td>
<td>Anti-apoptosis</td>
</tr>
<tr>
<td>BURKITT</td>
<td>t(8;14)(q24;q32)</td>
<td>cMYC</td>
<td>Transcription Factor</td>
</tr>
<tr>
<td>DIFFUSE LARGE B-CELL</td>
<td>t(3;other)(q27;other)</td>
<td>BCL6</td>
<td>Transcription Factor</td>
</tr>
</tbody>
</table>
Oncogene deregulation in the Germinal Center

MCL
- IgH
- BCL1
- t(11;14)
- Cyclin D1
- Bcl6
- t(14;18)
- BCL2
- IgH
- t(3;other)
- t(8;14)

DLBCL
- other gene
- BCL6
- t(11;14)
- cMyc
- t(3;other)
- IgH
- BCL2
- IgH
- cMYC

FL
- BL, DLBCL

Physiology and Pathology of Somatic Hypermutation

physiological
- GC B cells
- BCL6
- IgV
- Igα/Igβ

aberrant
- DLBCL
- PIM1
- cMyc
- IgV
- TTF

Up to 10% of transcribed genes!
Model for the generation of genetic lesions in B-NHL

**AID**

**CSR**

**SHM**

**Germinal center**

**Physiologic**

**Pathologic**

**Chromosomal Translocations**

(Ig-cMYC, Ig-BCL6)

**Aberrant SHM**

(multiple oncogenes)

**DLBCL/BL**

**DLBCL**

**Proliferation**

**B cell Lymphoma:**

not a single entity, but a group of heterogeneous diseases
2016 WHO classification of mature lymphoid malignancies

### 2016 WHO classification of mature lymphoid malignancies

- DLBCL: 41%
- Follicular: 29%
- Burkitt: 8%
- Mantle cell: 1%
- MALT: 1%
- CLL: 9%
- LPL: 1%

Historical background of lymphoma classification

<table>
<thead>
<tr>
<th>Classification</th>
<th>Year</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rappaport</td>
<td>'60</td>
<td>morphology</td>
</tr>
<tr>
<td>Kiel, Lukes &amp; Collins</td>
<td>'70</td>
<td>morphology, phenotype</td>
</tr>
<tr>
<td>Working Formulation</td>
<td>'80</td>
<td>morphology, phenotype, clinical</td>
</tr>
<tr>
<td>REAL</td>
<td>'90</td>
<td>morphology, phenotype, clinical, genetics</td>
</tr>
<tr>
<td>WHO</td>
<td>2001</td>
<td>Result of an improved understanding in the biology and genetics of lymphoid cancers</td>
</tr>
<tr>
<td></td>
<td>(2008, 2016)</td>
<td></td>
</tr>
</tbody>
</table>
Diffuse Large B cell Lymphoma

- Most common aggressive subtype of B-NHL in adults (~40% of all diagnoses)
- De novo origin or clinical evolution of indolent diseases (FL, CLL)
- Poor response to therapy, with ~30% of the cases not cured by currently available therapeutic strategies
- Marked heterogeneity in phenotypic, molecular, and clinical features
- Several phenotypic subgroups recognized by gene expression profile analysis
Molecularly and clinically distinct subgroups of DLBCL


Putative Cells of Origin for DLBCL Subgroups

Molecular subgroups of DLBCL-NOS are associated with shared and specific genetic lesions

Genetic classification of DLBCL based on coordinated genetic events

~50% of cases not assigned

The DLBCL exome exhibits high genomic complexity compared to other hematologic malignancies

modified from Lawrence et al., Nature 2013
The road to the clinic is not free from hurdles...

Beyond the usual suspects:
- Which are the drivers?
- Which are the passengers?

- Look at Recurrence
- Conceptually organize genes into biological programs

Common and distinct pathways in DLBCL subtypes

Altered Histone/chromatin modification

Deregulation of BCL6 activity
Escape from immune surveillance (CTL + NK)

FOXO1 deregulation

- BCL2 translocations 25%
- MYC translocations 10%
- EZH2 mutations 22%
- GNA13/S1PR2 mut 20%
- TNFRSF14 mutations 15%
Epigenetic mechanisms and transcriptional regulation

Mutations in epigenetic modifiers represent early events in the history of tumor evolution
**KMT2D loss perturbs GC formation and cooperates with BCL2 deregulation in lymphoma**

- Major writer of H3K4me1/2 at enhancers
- Increased GC formation upon early deletion
- Tumor suppressor in mice

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**Common and distinct pathways in DLBCL subtypes**

**Altered Histone/chromatin modification**

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Multiple genetic alterations deregulate BCL6 activity in DLBCL

Ying et al., Nature Immunol 2013
Brescia et al., Cancer Cell 2018

Duan et al., Nature 2012
Schneider et al., Blood 2016

Saito et al., Cancer Cell 2010
Pasqualucci et al., Nature 2011

Common and distinct pathways in DLBCL subtypes

Altered Histone/chromatin modification
Deregulation of BCL6 activity
Escape from immune surveillance (CTL + NK)

shared

- BCL2 translocations 25%
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- TNFRSF14 mutations 15%

GCB-DLBCL

ABC-DLBCL
Concurrent loss of surface B2M and CD58 favors escape from CTL and NK-mediated immune surveillance

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Common and distinct pathways in DLBCL subtypes

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**Escape from immune surveillance (CTL + NK)**

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### Shared

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The NF-κB-IRF4-BCL6-BLIMP1 pathway

Pathway lesions in ABC-DLBCL

A20 25%
TRAF2 4%
TRAF3 3%
TRAF5 4%
RANK 5%

≥80%
≥25%

MITOCHONDRIA

Apoptosis
DNA damage response
differentiation
cell cycle arrest

NFκB
IRF4
BCL6
BLIMP1

NFκB (A20 KO)
IRF4 KO
BCL6 Knock in
BLIMP1 KO

Plasma cells

KO Blimp1

Ag
CD40
CD40L
TLR/IL1R

ABC-DLBCL

NFκB-A20

MAPK/ERK
PI3K/AKT

Ag
BCR

CD79A,B
CARD11

20% 9%

≥25%

MYD88 30%

Lenz et al., Science 2008
Compagno et al., Nature 2009
Davis et al., Nature 2010
Ngo et al., Nature 2010
Pasqualucci et al., J Exp Med 2006
Saito et al, Cancer Cell 2007
Mandelbaum et al., Cancer Cell 2010
Pharmacologic targeting of dysregulated pathways in ABC-DLBCL

Ibrutinib is effective in relapsed/refractory ABC-DLBCL (Wilson et al, Nat Med 2015)

Overall response rate

Influence of ABC mutations

Progression free survival

Overall survival
Learning Objectives

After attending this class, participants should be able to:

- Describe the relationship between B cell lymphomas and normal B cell developmental stages

- Illustrate the major mechanisms of genetic lesion that are associated with mature B cell lymphomas

- Define the most common targets of structural alterations in major lymphoma subtypes

- Explain how these lesions can favor malignant transformation

- Identify ways to utilize this information for diagnostic and therapeutic purposes.