Lymphoma as a Cancer Model

Cellular and Molecular Biology of Cancer
PATH G4500
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Columbia University
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Lymphomas

- Tumors of the lymphoid organs
- 7th most common cancer
- 72,580 new cases in 2016
  20,150 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 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
**Ratio between non-Hodgkin and Hodgkin’s lymphomas = 9:1**

- **B cell (90%)**
- **T cell (10%)**

**Thomas Hodgkin (1798-1866)**

**Hodgkin**

**Non-Hodgkin**

**Lymphoma**

**V(D)J Recombination**

- **IgV hypermutation**
- **Ig isotype switch**

**B cell Development and the Germinal Center reaction**

- **Bone Marrow**
- **Mantle**
- **Germinal Center**

**MCL, BL, FL, DLBCL, ALL, MM**

**Apoptosis**

**Histological**

**Plasma cells**

**Naive B-cells**

**Memory B-cells**

**T-Cell Lymphoma**

**B-Cell Lymphoma**

**B-NHL**

**T-NHL**

**Transcription factor networks regulating the GC**

- **Klein U and De Silva N, Nat Immunol Reviews 2015**

- Transcription factor networks regulating the GC
BCL6: the Germinal Center Master Regulator

The BCL6 protein

- Zinc Fingers (DNA binding)
- Trans-repression (phosphorylation/degradation, acetylation/inactivation)
- BTB/POZ (protein-protein interaction)

BCL6 is specifically expressed in GC B cells

- BCL6 protein interaction
- DNA binding motifs

BCL6 is specifically expressed in GC B cells
BCL6 is required for GC formation and affinity maturation

BCL6 is a key regulator in B-cell development and immune response. It is essential for germinal center (GC) formation and affinity maturation, which are critical steps in the generation of high-affinity antibodies. BCL6 regulates the expression of several genes involved in these processes, including those encoding Ig light chains and Ig heavy chains.

Biological function of BCL6 in the Germinal Center

In the germinal center, BCL6 plays multiple roles. It helps in the selection of B-cells that will undergo affinity maturation by promoting the expression of high-affinity Ig light chains. BCL6 also contributes to the survival and proliferation of GC B-cells, which are crucial for the development of memory B-cells and plasma cells.

Key references include:
- 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

Subset of LZ B cells

A subset of long-lived (LZ) B cells is marked by BCL6 expression. These cells are crucial for the generation of long-term immunity and memory.
Biological function of BCL6 in the Germinal Center

Mechanisms of genetic lesion in B cell Lymphomas

Pattern of chromosome aberrations in human malignancies

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Numerical</th>
<th>Structural</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epithelial</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Mesenchymal</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Hematologic</td>
<td>+</td>
<td>+++</td>
</tr>
</tbody>
</table>
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
- Gene B (proto-oncogene)
- Translocation
- Deregulated Expression
  - (Leukemia)
  - (Lymphoma)
### Most common chromosomal translocations in B cell Lymphoma

<table>
<thead>
<tr>
<th>Lymphoma Type</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>AP2/MLT</td>
<td>Anti-apoptosis</td>
</tr>
<tr>
<td>Burkitt</td>
<td>t(3;other)(q27;?)</td>
<td>cMYC</td>
<td>Transcription Factor</td>
</tr>
<tr>
<td>Diffuse Large B-Cell</td>
<td>t(1;other)(p27;other)</td>
<td>BCL6</td>
<td>Transcription Factor</td>
</tr>
</tbody>
</table>

### Oncogene deregulation in the Germinal Center

- MCL
  - t(11;14)
  - cyclin D1
  - Bcl6

- DLBCL
  - t(3;other)
  - cMYC

### Physiology and Pathology of Somatic Hypermutation

**Physiological**

- GC B cells
- IgV
- BCL6
- Ig

**Aberrant**

- DLBCL
- cMYC
- PAS
- IgV

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cMYC
• Proto-oncogene
• bHLH/LZ Transcriptional activator
• Control of cell progression, differentiation and apoptosis
• Deregulated expression associated with tumorigenesis


Model for the generation of genetic lesions in B-NHL

Germinal center

AID

CSR

Aberrant SHM
(multiple oncogenes)

Chromosomal Translocations
(Ig-cMYC, Ig-BCL6)

Proliferation

DLBCL/BL

DLBCL

B cell non-Hodgkin Lymphoma:
not a single entity, but a group of heterogeneous diseases
Historical background of lymphoma classification

<table>
<thead>
<tr>
<th>Classification</th>
<th>Year</th>
<th>Criteria</th>
</tr>
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<tbody>
<tr>
<td>Rappaport</td>
<td>'60</td>
<td></td>
</tr>
<tr>
<td>Kiel, Lukes &amp; Collins</td>
<td>'75</td>
<td></td>
</tr>
<tr>
<td>Working Formulation</td>
<td>'89</td>
<td></td>
</tr>
<tr>
<td>REAL</td>
<td>'90</td>
<td></td>
</tr>
<tr>
<td>WHO</td>
<td>2001</td>
<td></td>
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Classification Year Criteria

2016 WHO classification of mature lymphoid malignancies

Diffuse Large B cell Lymphoma

Result of an improved understanding in the biology and genetics of lymphoid cancers.
Diffuse Large B-cell Lymphoma (DLBCL)

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

- BCL2 translocations 25%
- MYC translocations 10%
- EZH2 mutations 22%
- GNA13/S1PR2 mut 20%
- TNFRSF14 mutations 15%

Common and distinct pathways in DLBCL subtypes:

- Altered Histone/chromatin modification
- Deregulation of BCL6 activity
- Escape from immune surveillance (CTL + NK)
- FOXO1 deregulation

Shared

ABC-DLBCL

GCB-DLBCL

Altered Histone/chromatin modification
Deregulation of BCL6 activity
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Epigenetic mechanisms and transcriptional regulation

**H3K27Ac**, **H3K18Ac**

**H3K4me1** (enhancers)**/H3K4me3** (promoters)

**H3K27me3**

**SUZ12**

**EZH2**

Condensed chromatin state (repression)

Open chromatin state (activation)

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Mutations in epigenetic modifiers represent early events in the history of tumor evolution

Resistance to apoptosis

Epigenetic reprogramming

- **BCL2** Tx
- **KMT2D** MCREBBP M

Cell cycle deregulation
DNA damage response
Enhanced proliferation
Enhanced migration
Immune evasion

Multiple consequences

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50-70% FL
25% DLBCL

80% FL
30% DLBCL

**HIST1H1E**

10-25% FL
20% GCB-DLBCL
Truncating and inactivating missense mutations in the enzymatic domains

- Monoallelic in 80% (CREBBP) and 60% (KMT2D) of DLBCL cases
- Wild type allele almost invariably expressed
- Pathogenic role of reduced CREBBP or KMT2D dosage demonstrated in other tissues (Rubinstein-Taybi syndrome, Kabuki syndrome)
- Transcriptional network regulated in GC B cells unknown

**KMT2D (MLL2) and CREBBP mutations**

**KMT2D**
- Monomeric
- Dimers
- Trimers

**CREBBP**
- Monomeric
- Dimers
- Trimers


- Pasqualucci et al., *Nat Genet* 2011; Morin et al., *Nature* 2011; Lohr et al., *PNAS* 2012

**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


**Role of CREBBP inactivation in B-NHL**

- Histones
- CBP/p300
- BCL6
- Inactivation
- Activation
- p53

- GC B cells
- 1FL, DLBCL

- Inhibits signaling
- B cell activation
- BCR/CDK signaling
- GC formation
- Normal GCs

- FL/BL/DLBC 80%
CREBBP opposes the function of BCL6 by facilitating transcription at its repressed targets

CBP-mediated acetylation may allow GC cells to rapidly reprogram in response to signals that promote differentiation and re-establish the expression of BCL6-repressed genes.

Zhang et al., Cancer Discovery 2017; Jiang et al., Cancer Discovery 2017

Loss of one Crebbp allele facilitates BCL2-driven follicular lymphoma development

Common and distinct pathways in DLBCL subtypes

Altered Histone/chromatin modification
Deregulation of BCL8 activity
Escape from immune surveillance (CTL + NK)
FOXO1 deregulation

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- MYC translocations 10%
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Zhang et al., Cancer Discovery 2017; Jiang et al., Cancer Discovery 2017
Multiple genetic alterations deregulate BCL6 activity in DLBCL

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- MYC translocations 10%
- EZH2 mutations 22%
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Common and distinct pathways in DLBCL subtypes

- Altered Histone/chromatin modification
- Deregulation of BCL6 activity
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- FOXO1 deregulation
- BCL2 translocations
- MYC translocations
- EZH2 mutations
- GNA13/S1PR2 mut
- TNFRSF14 mutations

B2M and HLA-I protein expression is lost in most DLBCL

- Loss of surface HLA-I

Disruptive Mutations
- Frameshift (n=9)
- Nonsense (n=3)
- Missense, start codon (n=5)
- Missense (n=8)

Challa-Malladi, Lieu et al., Cancer Cell 2011
Concurrent loss of surface B2M and CD58 favors escape from CTL and NK-mediated immune surveillance.

Common and distinct pathways in DLBCL subtypes:

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

FoxO1 deregulation

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Challa-Malladi, Lieu et al., Cancer Cell 2011
Loss of signalling via Gα13 impairs the S1PR2 and P2RY8 receptor ability to suppress AKT and migration

Common and distinct pathways in DLBCL subtypes

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The NF-κB-IRF4-BCL6-BLIMP1 pathway

Altered Histone/chromatin modification
Deregulation of BCL6 activity
Escape from immune surveillance (CTL + NK)
FOXO1 deregulation

Plasma cell
Bcl6-
Blimp1+
CD40
CD40L
TLR/IL1R
BLIMP1 KO
No plasma cells
LPD, DLBCL
Mandelbaum et al, unpublished

The NF-κB-IRF4-BCL6-BLIMP1 pathway

Plasma cell
Bcl6-
Blimp1+
CD40
CD40L
TLR/IL1R
BLIMP1 KO
No plasma cells
LPD, DLBCL
Mandelbaum et al, unpublished
Pathway lesions in ABC-DLBCL

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

Progression free survival

Overall survival

Influence of ABC mutations

Lenz et al., Science 2008
Compagno et al., Nature 2009
Kato 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

Pasqualucci et al., J Exp Med 2006
Saito et al., Cancer Cell 2007
Mandelbaum et al., Cancer Cell 2010
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 non-Hodgkin 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.