Tumors of the lymphoid organs
- 7th most common cancer
- 74,680 new cases in 2017
- 19,910 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

Lymphomas
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%)

B-NHL

HODGKIN

T cell (15%)

T-NHL

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

V(D)J Recombination → IgV hypermutation → Ig isotype switch

Immature B-cells → Naive B-cells → Germinal Center B-cells

Bone Marrow → Mantle → Germinal Center

The Germinal Center and Lymphomagenesis

V(D)J Recombination → IgV hypermutation → Ig isotype switch

Immature B-cells → Naive B-cells → Germinal Center B-cells

Bone Marrow → Mantle → Germinal Center

Apoptosis

B-CLL

ALL MCL BL FL DLBCL

MM
Transcription factor networks regulating the GC

BCL6: the Germinal Center Master Regulator

Klein U and De Silva N, Nat Immunol Reviews 2015
The BCL6 protein

BCL6 is specifically expressed in GC B cells

BCL6

BCL6 CD20
BCL6 is required for GC formation and affinity maturation

Biological function of BCL6 in the Germinal Center

- Burkitt Lymphoma
- Follicular Lymphoma
- ABC-DLBCL
- GCB-DLBCL

Subset of LZ B cells

- Basso et al., Blood 2009
- Niu et al., J Exp Med 2003
- Phan et al., Nature 2004
- Ranuncolo et al., Nat Immunol 2007
- Tunyaplin et al., J Immunol 2004

Ci et al., Blood 2009

BCL6 is required for GC formation and affinity maturation.
Mechanisms of genetic lesion in B cell Lymphomas

<table>
<thead>
<tr>
<th>TUMOR</th>
<th>NUMERICAL (Aneuploidy Polyploidy)</th>
<th>STRUCTURAL</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Translocations</td>
<td>Amplifications</td>
<td>Deletions</td>
<td>Mutations</td>
</tr>
<tr>
<td>Epithelial</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Mesenchymal</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hematologic</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
Mechanisms of genetic lesion B-NHL

- non-random chromosomal translocations
  - due to DNA breaks during Class Switch Recombination
  - due to a malfunction of 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

Gene B (proto-oncogene)
Regulatory  Coding

Translocation

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>(3;other)(q27;other)</td>
<td>BCL6</td>
<td>Transcription Factor</td>
</tr>
</tbody>
</table>
Oncogene deregulation in the Germinal Center

- **MCL**: t(11;14) (IgH, BCL1, Bcl6, Cyclin D1), t(14;18) (Bcl2, cMyc)
- **FL**: t(3;other) (BCL6, other gene)
- **BL, DLBCL**: t(8;14) (IgH, BCL2, IgH)

**Physiology and Pathology of Somatic Hypermutation**

- **GC B cells**: Physiological
  - IgV
  - BCL6
  - Igα/β
  - Up to 10% of transcribed genes!
- **DLBCL**: Aberrant
  - IgV
  - BCL6
  - Igα/β
  - Up to 40% of transcribed genes!

*Pasqualucci et al., Nature 2001; Shen et al., Science 1998; Pasqualucci et al., PNAS 1998*
cMYC
- Proto-oncogene
- bHLH/LZ Transcriptional activator
- Control of cell progression, differentiation, apoptosis, metabolism, replication
- Deregulated expression associated with tumorigenesis


Model for the generation of genetic lesions in B-NHL

AID
Germinal center

CSR
SHM
Proliferation

Chromosomal Translocations (Ig-cMYC, Ig-BCL6)
Aberrant SHM (multiple oncogenes)

DLBCL/BL
DLBCL
B cell 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>
</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>
</tbody>
</table>

#### Diffuse Large B cell Lymphoma
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

Molecularly and clinically distinct subgroups of DLBCL
The DLBCL exome exhibits high genomic complexity compared to other hematologic malignancies.

DLBCL subtypes are associated with distinct genetic lesions.
Common and distinct pathways in DLBCL subtypes

- **Shared: Common and distinct pathways in DLBCL subtypes**
  - Altered Histone/chromatin modification
  - Deregulation of BCL6 activity
  - Escape from immune surveillance (CTL + NK)
  - FOXO1 deregulation

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

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

- **H3K27Ac, H3K18Ac**
- **H3K4me1** (enhancers)/**H3K4me3** (promoters)
- **H3K27me3**

Condensed chromatin state (repression)

Open chromatin state (activation)

Repressors

Activators

50-70% FL
25% DLBCL

80% FL
30% DLBCL

Pasquale et al., *Nature* 2011; Nat Genetics 2011
Morin et al., *Nature* 2010
Lohr et al., *PNAS* 2012

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Epigenetic mechanisms and transcriptional regulation

- **SUZ12**
- **EZH2**
- **COMPASS**
- **PRC2**

Closed chromatin state (repression)

Open chromatin state (activation)

Repressors

Activators

5-25% FL
20% GCB-DLBCL

10-25% FL
20% GCB-DLBCL

Morin et al., Nat. Genetics 2010
Mutations in epigenetic modifiers represent early events in the history of tumor evolution

+ BCL2 Tx Resistance to apoptosis
+ KMT2D M/CREBBP M Epigenetic reprogramming

+ CDKN2A/2B loss Cell cycle deregulation
+ TP53 loss DNA damage response
+ MYC deregulation Enhanced proliferation
+ B2M loss Immune evasion
+ ASHM Multiple consequences

- 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

CPC, common precursor cell

KMT2D (MLL2) and CREBBP mutations

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

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**CREBBP** opposes the activity of BCL6 in the GC

- GC B cells
- CREBBP/p300
- BCL6
- H3 acetylation
- Transcription
- No tumors
- FLDbLCL 80%

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Zhang et al., Cancer Discovery 2017
Pasqualucci et al., Nature 2011
Jiang et al., Cancer Discovery 2017

---

Pasqualucci and Dalla-Favera, Blood 2018
How can we translate this information for therapy?

Crebbp is a haploinsufficient tumor suppressor gene in lymphoma

Zhang et al., Cancer Discovery 2017
Pasqualucci et al., Nature 2011
Jiang et al., Cancer Discovery 2017

Pasqualucci and Dalla-Favera, Blood 2018

Crebbp$^{fl/fl}$
VavP-Bcl2
C$\gamma$1-Cre

a. Crebbp$^{fl/fl}$
VavP-Bcl2
C$\gamma$1-Cre

b. % GC B cell tumors

<table>
<thead>
<tr>
<th></th>
<th>FL</th>
<th>FLIs</th>
<th>DLBCL</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>0/20</td>
<td>3/22</td>
<td>0/24</td>
</tr>
<tr>
<td>HET</td>
<td>0/24</td>
<td>56%</td>
<td>16/20</td>
</tr>
<tr>
<td>KO</td>
<td>2/24</td>
<td>97%</td>
<td>3/22</td>
</tr>
</tbody>
</table>

p = 0.02

Zhang et al., Cancer Discovery 2017
Common and distinct pathways in DLBCL subtypes

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

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

### Multiple genetic alterations deregulate BCL6 activity in DLBCL

- **DNA damage response** (p53, ATR)
- **Cell Cycle arrest** (p21)
- **B cell activation** (CD80)
- **Programmed cell death** (Bcl2)
- **Plasma cell differentiation** (Blimp1)

- **BCL6 gene**
- **BCL6 protein**
- **NF-κB**

- **MEF2B**
- **IRF4**
- **CBP/EP300**

- Ying et al., Nature Immunol 2013
- Pasqualucci et al., Nature 2011
- Duan et al., Nature 2012
- Saito et al., Cancer Cell 2010
- Duan et al., Nature 2012
- Pasqualucci et al., Nature 2011
Common and distinct pathways in DLBCL subtypes

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- Deregulation of BCL6 activity
- Escape from immune surveillance (CTL + NK)
- FOXO1 deregulation

• BCL2 translocations 25%
• MYC translocations 10%
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• GNA13/S1PR2 mut 20%
• TNFRSF14 mutations 15%

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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GCB-DLBCL

Shared

ABC-DLBCL

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ABC-DLBCL
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- TNFRSF14 mutations 15%

Pharmacologic targeting of dysregulated pathways in ABC-DLBCL
Ibrutinib is effective in relapsed/refractory ABC-DLBCL (Wilson et al, Nat Med 2015)

**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.