Burkitt Lymphoma Genome Sequencing Project:

Integrative Genomic and Transcriptomic Characterization of Burkitt Lymphoma


*,† Contributed equally
Burkitt lymphoma (BL) is an aggressive B-cell non-Hodgkin lymphoma

IG-MYC translocation is a genetic hallmark

Three clinical variants:

1) **Sporadic BL**: North America, Europe

2) **Endemic BL**: Africa, South America (malaria-endemic)

3) **Immunodeficiency-related BL**: global, mostly HIV+

Rare in sporadic, most common in children located in malaria-endemic regions such as equatorial Africa

Current challenges with treating Burkitt lymphoma

**Endemic BL**
- Late stage at presentation
- Poor response to therapy
- Treatment-related toxicity

**Sporadic BL**
- Therapy is less effective in adult and elderly patients

Figure source: Buckle et al. *Int J Cancer*. 2016;139(6):1231-40.

Figure source: Costa et al. *Blood*. 2013;121(24):4861-6.
Building a tremendous genomic resource for BL research

<table>
<thead>
<tr>
<th>Variable</th>
<th>BLGSP (N = 95)</th>
<th>ICGC (N = 17)</th>
<th>Total (N = 112)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>65</td>
<td>16</td>
<td>81</td>
</tr>
<tr>
<td>Female</td>
<td>30</td>
<td>1</td>
<td>31</td>
</tr>
<tr>
<td>Age Group</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Pediatric (0–20 yr)</td>
<td>92</td>
<td>17</td>
<td>109</td>
</tr>
<tr>
<td>Adult (21+ yr)</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Clinical Variant</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Endemic</td>
<td>71</td>
<td>0</td>
<td>71</td>
</tr>
<tr>
<td>Sporadic</td>
<td>20</td>
<td>17</td>
<td>37</td>
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<tr>
<td>HIV-positive</td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
</tbody>
</table>

Whole genome sequencing
- 80X tumors (ICGC at 40X)
- 40X matched normals

Ribo-depleted RNA sequencing
- 200 million reads per library

microRNA sequencing

Clinical metadata

Refining the mutational landscape in BL

Novel BL genes: SIN3A, CHD8, USP7, RFX7, HIST1H1E

Subtype-specific mutations:
More differences based on EBV status than clinical variant
Novel structural and non-coding mutations in *DDX3X*

Deletions and inversions (N = 5)

Predicted to disrupt open reading frame and truncate protein

Branch point mutations (N = 2)

Aberrant transcript splicing observed in RNA-seq data

Tumor

Normal

Figure source: Lim and Burge. *Proc Natl Acad Sci U S A.* 2001;98(20):11193-8.

Flanking sequence

Mutated

Branch point motif

Deep Deletion  Branch Point Mutation  Truncating Mutation  Missense Mutation
Potentially activating mutation hotspots in GNAI2

Mutated residues cluster in protein structure around GDP binding site

R179H is orthologous to gain-of-function R201H mutations in GNAS

Novel genes carry out functions relevant to BL biology

**Mutations in SIN3A (N = 16)**

- Known antagonist of Myc activity
- Induces histone acetylation of Myc responsive genes

**Mutations in USP7 (N = 7)**

- Encodes a deubiquitinase that counteracts Mdm2-mediated degradation of p53
Non-coding mutations form clusters in the genome

One cluster overlaps a validated PAX5 enhancer

Similar mutations found in:
- Chronic lymphocytic leukemia (CLL)
- Other B-cell lymphomas

PAX5 plays an important role in B-cell differentiation

Figure source: Puente et al. Nature. 2015;526(7574):519-24.
Aberrant somatic hypermutation is a feature of endemic or EBV-positive BL

Many non-coding mutations can be linked to somatic hypermutation
AID activity is significantly higher in EBV-positive tumors

**Number of mutated clusters**

<table>
<thead>
<tr>
<th>Clinical variant</th>
<th>EBV type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sporadic</td>
<td>0</td>
</tr>
<tr>
<td>Endemic</td>
<td>5</td>
</tr>
<tr>
<td>Negative</td>
<td>5</td>
</tr>
<tr>
<td>Type 1</td>
<td>10</td>
</tr>
<tr>
<td>Type 2</td>
<td>15</td>
</tr>
</tbody>
</table>

- N = 108
- Fold change = 2.7

- N = 108
- Fold change = 2.6

**AID mRNA expression**

- Germinal center
- Clinical variant
- EBV type

- N = 12
- Fold change = 10

- N = 91
- Fold change = 2.4

- N = 91
- Fold change = 2.6

Results summary

Landscape of coding and non-coding mutations was refined for both established and novel genes associated with BL.

Greater differences exist based on EBV status than geographic origin:
  - Number of significantly differentially mutated genes
  - Aberrant somatic hypermutation and AID expression

Possible therapeutic opportunities warrant further investigation:
  - Potential activating hotspot mutations in \textit{GNAI2}
  - Mdm2 inhibitors for \textit{USP7}-mutant, \textit{TP53}-wildtype tumors
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The patients and their families
Thank you for your attention

Any questions?