Pathobiology of Hodgkin lymphoma

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Hodgkin lymphoma

• One of the most frequent lymphomas in Western world

• Tumor cells are called Hodgkin and Reed/Sternberg (HRS) cells in classical HL

• Derivation of HRS cells from germinal center B cells

• HRS cells usually represent <1% of cells in tissue

• HRS cells always CD30-positive

• Activation of numerous signaling pathways
Scenario for HRS cell generation from crippled GC B cells

Clonal Expansion
Somatic Hypermutation

Selection

Disadvantageous mutations:
- nonsense mutation
- reduced affinity
- gain of autoreactivity

Advantageous mutations

Differentiation

Additional transforming events

Rescue from apoptosis

Memory B cell

Apoptotic B cell

HRS cell

Küppers & Rajewsky; Annu Rev Immunol. 1998
• Lost B cell phenotype

• Genetic lesions

• Generation of multinuclear cells

• Normal CD30+ B cells and their relationship to HRS cells

• The role of BATF3 in Hodgkin lymphoma and ALCL
The lost B cell identity of HRS cells of classical HL:
Deregulated transcription factor networks
Lost B-cell identity of HRS cells

- Global gene expression analysis of HRS cell lines with microarrays
- Nearly complete loss of B cell specific gene expression program
- Retained expression of several B cell transcription factors (PAX5, E2A, EBF) and genes involved in interaction with CD4+ T cells
- Validated by immunohistochemistry and GEP of primary HRS cells

Schwering et al., Blood, 2003; Tiacci et al., Blood 2012
Factors contributing to the downregulation of B cell genes in HRS cells

Kreher et al., PNAS, 2014

NK cell factor

T cell factor

Myeloid receptor

CSF1R

LMP2a
LMP1
STAT5
BMI-1
IRF5
ID2
E2A
Deltex1
NOTCH1-IC
ABF-1
EBF
PAX5
E2A
Oct-2
Pv.1
BoB1

Kreher et al., PNAS, 2014
The lost B cell phenotype of HRS cells
- conclusions-

• Multiple factors contributing to lost B cell program:
  - downregulation of key B cell transcription factors
  - aberrant expression of master regulators of non-B cells
  - epigenetic silencing of B cell genes
• Reexpression of B cell program toxic for HRS cells

The lost B cell phenotype of HRS cells
- conclusions-

- Multiple factors contributing to lost B cell program:
  - downregulation of key B cell transcription factors
  - aberrant expression of master regulators of non-B cells
  - epigenetic silencing of B cell genes

- Reexpression of B cell program toxic for HRS cells

→ Loss of B cell phenotype critical pathogenetic event
→ Needed for HRS precursor cells to escape from apoptosis as crippled germinal center B cells?
Genetic lesions in HRS cells
NF-κB pathway
Multiple genetic lesions in the NF-κB pathway in HRS cells

- **TNFAIP3 mutations**: 40%
- **NFKBIA and NFKBIE mutations**: 10-20%
- **REL amplification**: 40%
- **EBV infection (40%**: LMP1
- **NIK gains**: 20%
- **IKKα, IKKβ**: NEMO
- **CYLD mutations**: 5%
- **BCL3 gains or translocations**: rare

**Pathway Diagram**

- **CD30, CD40, RANK, CD40, BCMA, TACI**
- **NIK**: gains
- **IKKα, IKKβ**: NEMO
- **CYLD**: mutations
- **TNFAIP3**: mutations
- **TRAF3 mutations**: 5%
- **p100, RELB**: nuclear translocation
- **IkBα, IkBε**: proteasomal degradation
- **BCL3**
- **REL amplification** (40%)
- **cytoplasm**
- **nucleus**
Genetic lesions in the JAK-STAT pathway in HRS cells
Genetic lesions in the JAK-STAT pathway in HRS cells

- Inactivating mutations in SOCS1 (40% of cases)
- Gains in JAK2 (30% of cases)
- Inactivating mutations in PTPN1 (20% of cases)
- Gains of STAT6 (30% of cases)

Gains of JAK2 often affect further pathogenetically relevant genes: PD-1 ligands 1 and 2 and JMJD2C.
Further recently identified genetic lesions in HRS cells

- Tanslocations affecting the MHC class II transactivator in ca. 15% of cHL (Steidl et al., 2011)
- Mutations in the MHC class I component β2 microglobulin in >50% of cHL (Reichel et al., 2015)
- Frequent mutations in CD58 gene in HL cell lines (3/7) and deletions in primary cHL cases (3/13) (Schneider et al., 2015)

-> Immune evasion strategies of HRS cells?
Generation of bi- und multinuclear Reed-Sternberg cells from mononuclear Hodgkin cells
Relationship between Hodgkin and Reed-Sternberg cells

Key feature of classical HL:
Tumor cell population composed of mononuclear Hodgkin and bi- or multinucleated Reed-Sternberg cells

Mechanism?
- Fusion of two independent cells unlikely (Küppers et al., Blood 2000; Re et al., 2001)
- Mitosis without cell division (acytokinetic mitosis)?
- Other mechanism?

Experiment:
long-term time-lapse microscopy of HL cell lines
Time lapse microscopy of HL cell lines

Rengstl, .., Rieger, Hansmann, PNAS, 2014
Complete or incomplete cytokinesis before refusion?

Time-lapse experiment with tubulin-RFP labeled HL cell lines

83% of refused cells with detectable persistent microtubule bonds
RS cell generation
- Summary and Conclusions -

• Refusion as major route of RS cell generation from Hodgkin cells

• Refusion mostly if not always based on incomplete cytokinesis

• Mechanisms for failure to complete cytokinesis unknown
Gene expression profiling analysis of CD30-positive B cells and their relationship to HRS cells of Hodgkin lymphoma
Extrafollicular and GC CD30-positive lymphocytes

blue = CD30

J Mol Histol 2005;36:249
Human CD30⁺ B cells: open questions

- Cellular origin of CD30⁺ NGC B cells?
- Normal GC reaction of CD30⁺ GC B cells?
- Close relationship of CD30⁺ B cells to HRS cells?
- CD30⁺ B cells distinct B cell subsets?
## VH gene mutation analysis of CD30+ GC and non-GC B cells

<table>
<thead>
<tr>
<th>Donor</th>
<th>CD30+ cells</th>
<th>Mutated sequences (%)</th>
<th>Aver. mutation frequency* (%)</th>
<th>R/S ratios FRs#</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>GC</td>
<td>30 / 30 (100)</td>
<td>5.9</td>
<td>1.4</td>
</tr>
<tr>
<td>2</td>
<td>GC</td>
<td>23 / 23 (100)</td>
<td>8.2</td>
<td>1.9</td>
</tr>
<tr>
<td>3</td>
<td>GC</td>
<td>28 / 28 (100)</td>
<td>4.6</td>
<td>2.3</td>
</tr>
<tr>
<td>4</td>
<td>GC</td>
<td>38 / 41 (93)</td>
<td>6.2</td>
<td>1.5</td>
</tr>
<tr>
<td>3</td>
<td>non-GC</td>
<td>16 / 20 (84)</td>
<td>3.9</td>
<td>1.6</td>
</tr>
<tr>
<td>4</td>
<td>non-GC</td>
<td>36 / 41 (88)</td>
<td>5.6</td>
<td>1.6</td>
</tr>
<tr>
<td>5</td>
<td>non-GC</td>
<td>22 / 26 (79)</td>
<td>8.6</td>
<td>1.4</td>
</tr>
<tr>
<td>6</td>
<td>non-GC</td>
<td>9 / 20 (42)</td>
<td>2.2</td>
<td>1.9</td>
</tr>
</tbody>
</table>

* of mutated sequences; # of productive rearrangements
Unsupervised hierarchical clustering of normal B cell subsets

Manhattan distance
Average linkage
SD > 1
407 probesets
High MYC activity in CD30+ GC B cells

CD30+ GC B cells versus bulk GC B cells

<table>
<thead>
<tr>
<th>Gene set</th>
<th>MYC targets</th>
<th>NES</th>
<th>Nominal p-value</th>
<th>FDR q-value</th>
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<tbody>
<tr>
<td>Seitz; 2011 PLoS One</td>
<td></td>
<td>2.105</td>
<td>0.0</td>
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</table>

26 MYC gene sets among most significantly enriched gene sets in normal CD30+ vs. bulk GC B cells
Differential gene expression between CD30+ B cells and HRS cells

Higher expression in CD30+ B cells
- typical B cell genes
- signatures for strong proliferation (MYC, E2F)
- genes with roles in regulation of mitosis and DNA stability

Higher expression in HRS cells
- regulators of extracellular matrix
- many chemokines and cytokines
- non-B-cell genes (CD3D, granzyme B, ID2,..)
- several transcription factors: MAF, MAFB, STAT1, BATF3

→ Key features of HRS cells are disease-associated
Potential mechanisms for genetic instability and disturbed cytokinesis in HRS cells

Supervised analysis of genes differentially expressed between HRS cells and CD30+ GC B cells

Among 207 genes downregulated at least 5-fold in HRS cells, there were 41 genes with functions in mitosis, cytokinesis, genomic stability, DNA repair

Such a downregulation is less pronounced in DLBCL and FL

Potential cause for the genomic instability of HRS cells and generation of multinucleated RS cells
Gene expression profiling and V gene mutation analysis of CD30+ B cells

- Identification of distinct CD30+ GC and non-GC B cell subsets with specific gene expression patterns.

- High MYC activity in CD30+ GC B cells -> GC B cells at transition from centrocytes back to centroblasts

- CD30+ non-GC B cells mostly post-GC B cells; highly activated and proliferating memory B cells

- HRS cells show significant similarities to normal CD30+ B cells (a CD30 activation signature or indication for cell of origin?).

- Key features of HRS cells are disease-associated
The AP-1 transcription factor BATF3 is constitutively expressed in classical Hodgkin lymphoma and contributes to tumor cell survival and proliferation.
Gene expression profiling studies of HRS cells revealed high expression of BATF3 in primary HRS cells.

RT-PCR of microdissected or sorted cells

<table>
<thead>
<tr>
<th>Samples</th>
<th>BATF3 positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>HRS cells</td>
<td></td>
</tr>
<tr>
<td>case 1</td>
<td>3/3</td>
</tr>
<tr>
<td>case 2</td>
<td>2/3</td>
</tr>
<tr>
<td>case 3</td>
<td>3/3</td>
</tr>
<tr>
<td>case 4</td>
<td>2/3</td>
</tr>
<tr>
<td>Non-HRS cells</td>
<td>0/12</td>
</tr>
<tr>
<td>GC B cells</td>
<td>0/6</td>
</tr>
</tbody>
</table>


Affymetrix HG-U133-Plus2.0
Tiacci et al, Blood, 2012
Frequent BATF3 protein expression in classical HL, PMBCL and ALCL

- Frequent BATF3 protein expression in all three types of lymphomas
- EBV+ HRS cells rarely positive
- Lymphomas share CD30 positivity of tumor cells & JAK/STAT activity

<table>
<thead>
<tr>
<th>Lymphoma</th>
<th>BATF3 positive</th>
</tr>
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<tbody>
<tr>
<td>classical Hodgkin lymphoma</td>
<td>EBV- 12 /20</td>
</tr>
<tr>
<td>EBV+</td>
<td>1 / 10</td>
</tr>
<tr>
<td>PMBCL</td>
<td>8 / 9</td>
</tr>
<tr>
<td>ALCL*</td>
<td>40 / 40</td>
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</tbody>
</table>

*Eckerle et al., Leukemia, 2009
Basic facts about BATFs

- Members of the AP-1 family
- form heterodimers with other AP-1 family members
- form composite elements with IRFs
- Can have inhibitory and activating functions
- BATF3 mainly expressed in TH1 cells and subsets of dendritic cells

Proposed inhibitory function of BATF

Interaction at AP-1-IRF composite elements

Murphy, Nat Rev Immunol, 2013
High expression of AP-1 and IRF factors in classical HL and ALCL cell lines

<table>
<thead>
<tr>
<th>HL cell lines</th>
<th>NHL cell lines</th>
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<tbody>
<tr>
<td>BATF3</td>
<td>BATF3</td>
</tr>
<tr>
<td>JunB</td>
<td>JunB</td>
</tr>
<tr>
<td>c-Jun</td>
<td>c-Jun</td>
</tr>
<tr>
<td>IRF4</td>
<td>IRF4</td>
</tr>
<tr>
<td>IRF8</td>
<td>IRF8</td>
</tr>
<tr>
<td>α-Tub</td>
<td>α-Tub</td>
</tr>
<tr>
<td>NLP HL</td>
<td>cHL</td>
</tr>
<tr>
<td>HDLM2</td>
<td>L428</td>
</tr>
<tr>
<td>KMH2</td>
<td>UHO1</td>
</tr>
<tr>
<td>L1236</td>
<td>UHO1</td>
</tr>
<tr>
<td>L428</td>
<td>K299</td>
</tr>
<tr>
<td>SUPHD1</td>
<td>SR786</td>
</tr>
<tr>
<td>SUDHL1</td>
<td>SUDHL1</td>
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<tr>
<td>HDLM2</td>
<td>BL</td>
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<td>KMH2</td>
<td>GCBBL</td>
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<td>AB</td>
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<tr>
<td>L428</td>
<td>PMBL</td>
</tr>
<tr>
<td>SUPHD1</td>
<td>MEDB-1</td>
</tr>
<tr>
<td>SUDHL1</td>
<td>U266</td>
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</table>
Inhibition of JAK2 leads to downregulation of BATF3 in 4/5 cHL cell lines

BATF3 downregulation upon JAK2 inhibition in a PMBCL line (Rui et al., Cancer Cell 2010)

TG101348/ Fedratinib (Jak2 inhibitor)
STAT factors bind to the BATF3 promoter

Chromatin immunoprecipitation and quantitative PCR to study binding of pSTAT3, pSTAT5 and pSTAT6 to a putative STAT binding site in the BATF3 promoter in the cHL cell lines L-428 and U-HO1.

Binding of pSTAT3 and pSTAT6 to the BATF3 promoter in cHL cell lines
The AP-1 family members JUN and JUNB form heterodimers with BATF3 (co-immunoprecipitation studies)

<table>
<thead>
<tr>
<th>HDLM-2</th>
<th>Immunoprecipitation:</th>
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<tbody>
<tr>
<td></td>
<td>5% INPUT</td>
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<tr>
<td>IB: BATF3</td>
<td>[Image]</td>
</tr>
<tr>
<td>IB: JUN</td>
<td>[Image]</td>
</tr>
<tr>
<td>IB: JUNB</td>
<td>[Image]</td>
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<table>
<thead>
<tr>
<th>SR-786</th>
<th>Immunoprecipitation:</th>
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<tr>
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<td>5% INPUT</td>
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<tr>
<td>IB: BATF3</td>
<td>[Image]</td>
</tr>
<tr>
<td>IB: JUN</td>
<td>[Image]</td>
</tr>
<tr>
<td>IB: JUNB</td>
<td>[Image]</td>
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<table>
<thead>
<tr>
<th>K-299</th>
<th>Immunoprecipitation:</th>
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<tbody>
<tr>
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<td>5% INPUT</td>
</tr>
<tr>
<td>IB: BATF3</td>
<td>[Image]</td>
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<tr>
<td>IB: JUN</td>
<td>[Image]</td>
</tr>
<tr>
<td>IB: JUNB</td>
<td>[Image]</td>
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</tbody>
</table>

IB – Immunoblot
shRNA-mediated down-regulation of BATF3 leads to growth disadvantages of HRS and ALCL cells

A) cHL

<table>
<thead>
<tr>
<th>BATF3</th>
<th>β-actin</th>
<th>BATF3</th>
<th>β-actin</th>
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</thead>
<tbody>
<tr>
<td>L-428</td>
<td>U-HO1</td>
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</table>

ALCL

<table>
<thead>
<tr>
<th>BATF3</th>
<th>β-actin</th>
<th>BATF3</th>
<th>β-actin</th>
</tr>
</thead>
<tbody>
<tr>
<td>SR-786</td>
<td>K-299</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

B)

B) sh#1BATF3

% shRNA+ cells (normalized to day 3 and shNT)

- Daudi
- SUDHL4
- HDLM2
- UHO1
- L428
- Karpas 299
- SR786

Days: 3 6 7 9 10 11 12 13 14 15 17 18 20 21
Downregulation of positive MYC target genes in L428 HRS cells upon downregulation of BATF3
Downregulation of BATF3 causes downregulation of MYC

L-428 (cHL)  SR-786 (ALCL)

<table>
<thead>
<tr>
<th></th>
<th>BATF3</th>
<th>MYC</th>
</tr>
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<tbody>
<tr>
<td>shNT</td>
<td><img src="image" alt="" /></td>
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<tr>
<td>sh#4</td>
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</tr>
<tr>
<td>sh#5</td>
<td><img src="image" alt="" /></td>
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</tr>
</tbody>
</table>

β-actin

- BATF3
- Myc

Downregulation of BATF3 causes downregulation of MYC.
BATF3 and JUN bind to the MYC promoter, indicating direct regulation of MYC expression.

JUN and JUND binding known (Iavarone et al., J Biol Chem 2003)
BATF3 in Hodgkin lymphoma and ALCL
- Summary and Conclusions -

- IL13
  - JAK2
  - STAT3/6
  - highly expressed in cHL and ALCL
  - STAT3/6
  - BATF3 gene
    - upregulated by STAT3/6 activity

- BATF3
  - forms heterodimers with JUN and JUNB
  - BATF3 target genes
  - BATF3
    - JUN(B)
  - JUN
  - MYC gene
    - binds to MYC promoter and upregulates MYC expression
  - Proliferation, survival
    - promotes survival and proliferation of HRS and ALCL cells

- (cHL)
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