Epstein-Barr Virus Latency in B Cells Leads to Epigenetic Repression and CpG Methylation of the Tumour Suppressor Gene Bim

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*Plos Pathogens, 5(6): e1000492, 2009*

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10/06/2009
Burkitt’s lymphoma (BL)

- Described in 1958 by Dr. Burkitts as a prevalent lymphosarcoma of young children in east Africa.
- Most prevalent in a central band of sub-Saharan Africa (lymphoma belt).
- EBV was discovered in 1964 by Epstein, Barr and Achong.
- The first virus that has ever been linked to a cancer.
- Oral transmission
Epstein-Barr Virus (EBV)

- A double strand DNA, \( \gamma \)-herpesvirus
- Latent (B cells) – Lytic (epithelial cells) cycle
- Infects approximately 95% of the world’s population

- Pathogenicity:
  Nasopharyngeal carcinoma, Berkitt’s lymphoma, Hodgkin’s lymphoma, gastric carcinoma, infectious mononucleosis, multiple sclerosis(?)

*Nature Reviews Cancer, 2004, Lawrence S. Young and Alan B. Rickinson*
Latent-Lytic switch

Latency

EX: EBNA1, 2, 3A, 3C

Stress

EX: gp350, gp110
Bim

- Bcl-2 interacting mediator
- Apoptotic Bcl-2 family member
- BH3-only subfamily
- Tumor suppressor gene
- Haploinsufficient

Nature Reviews Immunology, 2002, Annette R. Khaled and Scott K. Durum
Bim promoter
CpG dinucleotide

- Cytosine nucleotide occurs next to a guanine nucleotide in the linear sequence of bases (CGCGCGCG....).
- The "p" in CpG notation refers to the phosphodiester bond between the cytosine and the guanine.
- CpG island: At least 200 bp and with a GC percentage that is greater than 50%; observed/expected CpG ratio that is greater than 60%
- Methylation of CpG sites within the promoters of genes can lead to their silencing.
EBV vs Bim

- EBNA3A & EBNA3C repress Bim
Epigenetic regulation

- Inheritable
- DNA methylation (DNA methyltransferase, DNMT)
- Histone code: acetylation, methylation, phosphorylation, ubiquitination, ADP-ribosylation
Methylation regulates gene expression

- DNA methylation: through CpG methylation

*Placenta*, 2008, J. Ohgane a, S. Yagi a, K. Shiota
Histone modification

- Histone acetylation: HAT (histone acetyltransferase)
- Histone deacetylation: HDAC (histone deacetylase)
- Histone methylation: HMT (histone methyltransferase)

H3K4 & H3K36 → transcriptional activation
H3K9 & H3K27 → transcriptional repression
Interplay between histone methylation & DNA methylation

MBP: methyl-CpG binding protein
HDAC: histone deacetylase
HMT: histone methyltransferase
Dnmt: DNA methyltransferase

Int. J. Dev. Biol., 2009,
NOBUHIRO SASAI and PIERRE-ANTOINE DEFOSSEZ
Specific aim

B lymphocyte

EBV

_immortalization_

M M M M
(CGCGCGCG)

X
Bim
1. Bim mRNA 

2. HDAC
   DNMT

3. H3K27-Me3

Acetylated histone CpG methylation
H3K27-Me3
CpG methylation
1. Bim mRNA  
   → Bim  or  
   → Bim mRNA

2. HDAC  
   ↓  
   → Bim  
   → Acetylated histone
   → CpG methylation

3. H3K27-Me3  
   ↑  
   → H3K27-Me3  
   → CpG methylation
Bim is not suppressed by proteolysis nor unstabilizing mRNA

Western blot

\[ \text{Bim EL} \quad \text{p21} \quad \text{\(\gamma\)-tubulin} \]

<table>
<thead>
<tr>
<th>MG-132</th>
<th>No treatment</th>
<th>5(\mu)M</th>
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<th>15(\mu)M</th>
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<tr>
<td>BL31 WT</td>
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</tbody>
</table>

E2KO: EBNA2-deleted

→ No increase in Bim protein after introducing MG-132.

→ No difference in degradation rate of mRNA.

qRT-PCR

\[ 0 \quad 2 \quad 4 \quad 6 \quad 8 \]

Time of Act D treatment (hours)
Framework

1. Bim mRNA
2. HDAC
   DNMT
   Acetylated histone
   CpG methylation
3. H3K27-Me3
   CpG methylation
Up-regulation of Bim by treating HDAC & DNMT inhibitor

Western blot

<table>
<thead>
<tr>
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<th>0h</th>
<th>24h</th>
<th>48h</th>
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<tr>
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<tr>
<td>BL31 WT</td>
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<tr>
<td>LCL-CH</td>
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TSA

AZA

Na But.

qRT-PCR

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<tr>
<td>LCL-CH</td>
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</table>

TSA

AZA

Na But.
HDAC and DNMT might have something to do with the mechanism EBV uses.
Reduction in the amount of acetylated histone H3 & H4

ChIP assay
Bim promoter

Two experiments can check CpG methylation:
Bisulphite sequencing, Methylation specific PCR (MSP)
Bisulphite sequencing

Allele 1 (methylated)

---ACTCCACCGG---TCCATCGCT---
---TGAGTGGCC---AGCTAGCGA---

Allele 2 (unmethylated)

---ACTCCACCGG---TCCATCGCT---
---TGAGTGGCC---AGCTAGCGA---

Bisulphite treatment
Alkylation
Spontaneous denaturation

---AUTUAUGG---YUQATUGCT---
---TGAGTGGCJU---AGCTAGCGA---

Non-methylation-specific PCR
Methylation-specific PCR

Differentiation of bisulphite-generated polymorphisms
Methylation specific PCR (MSP)
EBV increases DNA methylation of the Bim promoter

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<thead>
<tr>
<th>Cell Type</th>
<th>Cell Line</th>
<th>Primer sets</th>
<th>LCL-CH (early)</th>
<th>LCL-BF (early)</th>
<th>Otis (early)</th>
<th>IB4 (late)</th>
<th>JAC (late)</th>
<th>X50-7 (late)</th>
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<td>U</td>
<td>U</td>
<td>U</td>
<td>U</td>
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<td>Purified B cells</td>
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<td>M</td>
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<td>U</td>
<td>U</td>
<td>M</td>
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<td>BL EBV -ve</td>
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<td>U</td>
<td>U</td>
<td>M</td>
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<td>M</td>
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</table>

[Table showing DNA methylation data with primer sets and cell lines]
EBV increases DNA methylation of the Bim promoter.

Bisulphite sequencing
# Results from BL biopsy samples

<table>
<thead>
<tr>
<th>Type</th>
<th>Cells/ Biopsy number</th>
<th>Primer sets</th>
<th>Biopsy information</th>
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</thead>
<tbody>
<tr>
<td>Jurkat meth.</td>
<td>Meth. Control</td>
<td>U U U U U</td>
<td>EBV -ve lymphoid infiltrate, non-BL</td>
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<tr>
<td>Primary cells</td>
<td>Purified B cells</td>
<td>U U U U U</td>
<td>EBV -ve lymphoid infiltrate, non-BL</td>
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<tr>
<td></td>
<td>PBMC</td>
<td>U U U U U</td>
<td>EBV +ve BL</td>
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<tr>
<td>DNA from tumor biopsies</td>
<td>26</td>
<td>M M M M M</td>
<td>EBV +ve BL</td>
</tr>
<tr>
<td></td>
<td>78</td>
<td>U U U U U</td>
<td>EBV +ve BL</td>
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<tr>
<td></td>
<td>217</td>
<td>M U M M M</td>
<td>EBV +ve BL</td>
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<td></td>
<td>248</td>
<td>M U M M M</td>
<td>EBV +ve BL</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>U U M M M</td>
<td>EBV +ve BL (weak EBER)</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>U U U U U</td>
<td>EBV +ve BL (with large epithelial field)</td>
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<tr>
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<td>31</td>
<td>U U M U U</td>
<td>EBV +ve BL (weak EBER)</td>
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<td>63</td>
<td>M U M U U</td>
<td>EBV +ve BL</td>
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<td>64</td>
<td>M U M U M</td>
<td>EBV +ve BL (weak EBER)</td>
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<td>67</td>
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<td>EBV +ve BL</td>
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<td>73</td>
<td>M U U M U</td>
<td>EBV +ve BL (weak EBER)</td>
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<td></td>
<td>74</td>
<td>U U M M M</td>
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<td>204</td>
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<td>EBV +ve BL</td>
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<td>EBV +ve BL</td>
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<td>27</td>
<td>M M M M M</td>
<td>EBV +ve BL</td>
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<tr>
<td></td>
<td>61</td>
<td>M M U M U</td>
<td>EBV +ve BL</td>
</tr>
</tbody>
</table>
Results from BL biopsy samples
Methylation at CpG sites happens when EBV infects.
Framework

1. Bim mRNA

2. HDAC
   DNMT

3. H3K27-Me3
Trimethylation of histone H3 lysine 27

- In prostate cancer, 5% of promoters (16% CpG islands and 84% non-CpG islands) were enriched with H3K27-Me3.
- Mechanism of tumor-suppressor gene silencing.
H3K27-Me3 increases at the Bim promoter

Histone H3K27-Me3 is more abundant on the Bim promoter of EBV infected cells.
H3K27-Me3 may occur before DNA methylation
H3K27-Me3 may occur before DNA methylation

Judge by the accumulation levels of each two phenomena, trimethylation of histone H3 K27 happens earlier than CpG methylation.
Summary

No EBV infection

With EBV infection

Properly conduct apoptosis

Cell immortalized
Thanks for your listening.
Latency

- 6 nuclear antigens: EBNA1, EBNA2, EBNA3A, EBNA3B, EBNA3C, EBNA-LP
- 3 membrane associated proteins: LMP1, LMP2A, LMP2B
- Transforming essential: EBNA1, 2, 3A, 3C, LP, LMP1
- **EBNA1**: Responsible for replicating and tethering the viral genome to ensure its segregation.
- **EBNA2**: Transcription factor that is responsible for transactivating the promoters of the viral genes expressed in the growth programme. Upregulates *MYC* expression.
- **EBNA3A & EBNA3C**: Negative regulators of EBNA2 that are thought to be involved in the transition from the growth programme to the default programme through the epigenetic silencing of the C and W promoters. suppress Bim