

# Up-Regulation of Lamin A/C Expression in Epstein-Barr Virus Immortalized B Cells and Burkitt Lymphoma Cell Lines of Activated B Cell Phenotype

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## Abstract

Lamin A, B and C, the nuclear intermediate-filament proteins, play a role in epigenetic regulation. Lamin B could be detected in all nucleated cells studied, whereas the lamin A and lamin C isoforms (lamin A/C) encoded by the *LMNA* gene are co-expressed in most somatic cell types except mature B lymphocytes. Since Epstein-Barr virus (EBV), a human gammaherpesvirus, is associated with tumorigenic processes and is known to alter the epigenotype of its host cells, we studied the expression of the *LMNA* gene and its epigenetic marks in EBV-carrying human lymphoid cell lines. We observed a high lamin A/C mRNA expression in EBV-immortalized B lymphoblastoid cell lines (LCLs) and in a subset of Burkitt lymphoma (BL) lines characterized by an activated B cell phenotype and a unique latent EBV gene expression pattern (latency III). In these cells the first exon of *LMNA* was hypomethylated and associated with activating histone marks. In contrast, we observed a low level of lamin A/C mRNA expression in EBV negative BL lines and BL lines with a restricted expression of latent EBV products (latency I). Low *LMNA* promoter activity was associated with hypermethylation of the *LMNA* first exon. These data suggest a role for EBV latency products in switching on or upregulating the *LMNA* promoter (*LMNAP*) in EBV-infected activated B cells *in vitro*. Lamin A/C may contribute to the establishment of the activated B cell phenotype. Our data also imply a role of *LMNA* first exon methylation in the silencing of *LMNAP*.

**Keywords:** Lamin; Epstein-Barr virus latency; Transformation; Epigenetic regulation; Activated B cell

## Introduction

Lamins are intermediate-filament proteins forming a network called nuclear lamina between the inner nuclear membrane and the nucleoplasm that protects the chromatin from physical damage. They play a role in chromatin organization, too, and act as epigenetic regulators affecting the activity of promoters located to the lamin-associated domains (LADs) of the genome [1-7]. In addition, lamins may affect the temporal pattern of replication origin firing [8].

There are two types of lamins: A and B. The *LMNA* gene codes for the widely co-expressed major isoforms, lamin A and lamin C (lamin A/C) generated by alternative splicing [9]. Human B type lamins are expressed from *LMNB1* and *LMNB2*. At least one type B lamin was detected in all nucleated metazoan cells studied so far. In vertebrates lamin A/C is expressed predominantly in differentiated somatic cells; only a low level was found in pluripotent mouse embryonic stem cells [10-12]. Murine and human B cells don't express or express only low levels of lamin A/C [13-15].

Epstein-Barr virus (EBV), a human gammaherpesvirus, is regularly associated with human lymphomas of B cell origin and immortalizes human B cells with a high efficiency *in vitro* [16]. The expression of latent EBV genomes is highly restricted in Burkitt lymphoma (BL) cells that phenotypically resemble resting B cells: only EBNA1, an EBV-encoded nuclear antigen, and a set of non-translated viral RNAs can be detected [17]. The restricted EBV gene expression pattern characteristic of BLs *in vivo* and BL-derived cell lines that maintain the BL biopsy phenotype *in vitro* is called latency type I.

A less stringent gene expression pattern (latency type II) was described in nasopharyngeal carcinomas (NPCs): they typically express EBV-encoded latent membrane proteins (LMP1, LMP2A and LMP2B) in addition to the viral latency products detected in BLs. We note, however, that LMP1 is not expressed in a subset of NPCs [18].

The EBV-encoded latent membrane proteins are also expressed in latency type III, characteristic of post-transplant lymphoproliferative disease (PTLDs) developing in immunosuppressed patients and their *in vitro* counterparts, the *in vitro* immortalized B lymphoblastoid cell lines (LCLs). In addition to LMPs, however, six nuclear antigens (EBNAs) and three BHRF1 microRNAs are also expressed in latency type III [17,19,20]. It is worthy to note, that type III latency is also a characteristic of BL cells that acquired an activated B cell phenotype during *in vitro* cultivation.

Although it has been well documented that the host cell phenotype-dependent expression of viral oncoproteins could switch on or epigenetically silence a series of host cell promoters in EBV-positive lymphomas and carcinoma, the expression of *LMNA* in B lymphoid

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cells carrying latent EBV genomes has not been studied systematically [21,22]. The available data on B cell lines and NPCs are based on microarray experiments performed under variable experimental conditions [23,24].

Here we analyzed the expression of LMNA mRNA in well-characterized B lymphoid cell lines carrying latent EBV genomes. Unexpectedly, we observed an elevated LMNA transcription in LCLs and the majority of group III BL lines characterized by an activated B cell phenotype, but not in latency type I or EBV-negative B cells. The 5' LMNA promoter flanking sequences were hypomethylated in most of the cell lines analyzed and their methylation pattern did not correlate with promoter activity. We observed, however, that the first LMNA exon that partly overlapped with a CpG island was highly methylated, with one exception, at silent LMNA promoters, suggesting a role for first exon methylation in the regulation of LMNAp. Active LMNA promoters were enriched in euchromatic histone marks.

## Materials and Methods

### Cell lines and culture conditions

Well-characterized EBV-negative B lymphoma and BL lines, EBV-positive BL cell lines or clones, EBV-immortalized LCLs and carcinoma cell lines were maintained as described earlier [25,26] (Table 1). Cell lines were regularly tested for latency type-associated mRNA transcripts of LMP1 (EBV latency type II and III) and EBNA2 (EBV latency type III) using reverse transcription and real-time PCR to verify their originally described characteristics.

### Real-time quantitative PCR

Total RNA was isolated from the cells using TRI Reagent (Sigma), followed by DNase I treatment of 1 µg RNA and reverse transcription with Transcriptor High Fidelity cDNA Synthesis Kit (Roche) using lamin A/C (LMN-RT) and GAPDH-specific (GAPDH-RT) oligonucleotides. The relative levels of transcripts initiated at LMNAp (the LMNA promoter) were determined with real-time polymerase chain reaction (LightCycler 480; Roche) using LightCycler FastStart DNA Master SYBR Green I Kit (Roche) with primers complementary to lamin A/C and GAPDH coding sequences (Supplementary Table S1). The relative expression of lamin A/C mRNA was normalized to the level of GAPDH mRNA. To present reliable expression data we normalized Lamin A/C mRNA level to 18S rRNA and β-actin mRNA

amounts (not presented) as well, giving nearly identical results. Primers are designed to amplify cDNA from mRNA only by harboring splicing sites or surrounding large introns.

### CpG island search

We used the Methyl Primer Express v1.0 Software (Applied Biosystems), with the following parameters: minimum length of island, 400; maximum length of island, 2000; C+Gs/total bases >60%; CpG observed/CpG expected >0.65.

### Sequencing of control and sodium bisulfite-modified DNA samples

Control DNA sequences of the 5' regulatory region and part of the first exon of the LMNA gene were determined using the primers listed. GenBank accession numbers: BJAB: KF791133; BL41: KF791131; DG75: KF791134; Jijoye p79: KF791135; Rael: KF791136; LCL-721: KF791137; CB-M1-Ral-STO: KF791138; Raji: KF791139; Mutu-BL-I-CI-216: KF791142; Mutu-BL-III-CI-99: KF791143; C666-1: KF791144.

For bisulfite sequencing, we used the method of Frommer et al. [36] adapted for an automated DNA sequencer using primers listed [33,37]. The degree of cytosine methylation was estimated as described earlier [25,37].

### Chromatin immunoprecipitation (ChIP)

ChIP was performed according to Weinmann and Farnham [38] with some adaptations as described earlier using specific antibodies against Acetylated Histone 3 (Upstate, 06-599), Acetylated Histone 4 (Upstate, 06-598) and dimethylated lysine 4 of Histone 3 (Upstate, 07-030) or normal rabbit IgG (Santa-Cruz Biotechnology, sc-2027) [25,35,39]. Obtained DNA fractions were analyzed using primers specific for the 5' regulatory region and exon 1 of LMNA.

### Trichostatin A and 5-azacytidine treatment

The histone deacetylase-inhibitor trichostatin A (TSA; Sigma) was added to  $5 \times 10^6$  DG75 or BJAB cells in 150 nM end concentration. After treatment, LMNA transcripts were quantified as described above. The demethylating agent 5-azacytidine (AzaC; Sigma) was added to  $5 \times 10^6$  DG75, BJAB or Rael cells in 10 or 4 µM end concentration for 0, 1, 2 or 3 days, followed by lamin A/C mRNA analysis. The efficiency of AzaC treatment was monitored by assessing the level of the viral LMP1 mRNA that is known to be upregulated by AzaC in Rael cells [30,35,40].

Designation	Origin/EBV latency type	Notes	References
BJAB	B cell lymphoma/EBV-negative		[25,27]
DG75	Sporadic BL <sup>1</sup> /EBV-negative		[25]
BL41	Sporadic BL/EBV-negative		[25]
Rael	Endemic BL/latency type I		[28]
Mutu-BL-I-CI-216	Endemic BL/latency type I	Subclone of the BL line Mutu	[29]
BL41-E95B	<i>In vitro</i> EBV-converted BL/latency type III	BL41 cells were converted with the B95-8 EBV strain	[30]
Mutu-BL-III-CI-99	Endemic BL/latency type III	Subclone of the BL line Mutu	[29]
Raji	Endemic BL/latency type III	Deletion/truncation of EBNA3C, LMP2A, BALF1, BARF1, BZLF2, BALF2 and BERF5	[31]
Jijoye p79	Endemic BL/latency type III	Moderate expression of EBNA2	[25]
CB-M1-Ral-STO	LCL <sup>2</sup> /latency type III	Immortalized by the Rael EBV strain	[28]
LCL-721	LCL/latency type III	Immortalized by the B95-8 EBV strain	[32]
KR4	LCL/latency type III		[25]
C666-1	NPC <sup>3</sup> /latency type I	Established from an undifferentiated NPC	[33-35]

<sup>1</sup>BL: Burkitt Lymphoma; <sup>2</sup>LCL: Lymphoblastoid Cell Line; <sup>3</sup>NPC: Nasopharyngeal Carcinoma Cell Line

**Table 1:** Characteristics of the cell lines included in the study.

## Statistical analysis

All variables were tested for normal distribution using F probe to select the appropriate parametric or non-parametric statistical procedure. As a result, Mann-Whitney U test was used for the statistical evaluation of mRNA expression levels between groups of cell lines. Statistical significance was accepted at a p value of <0.01.

## Results

### Analysis of lamin A/C expression

The level of *LMNA* transcripts was high in the LCLs: CB-M1-Ral-STO, LCL-721 and KR4 and in C666-1 (an NPC line) and in latency type III BL cell line: Jijoye p79, moderate in latency type III BL cells (Mutu-BL-III-CI-99 and BL41-E95B) (Figure 1). A low level of *LMNA* transcripts was detected in EBV latency type I BL cells (Rael, Mutu-BL-I-CI-216), as well as in the latency type III Raji and the EBV-negative B cell and BL lines BJAB and DG75, BL41. The difference in *LMNA* mRNA levels between LCLs and other B cells was highly significant ( $p < 0.001$ ). In addition the expression was significantly higher in BLs carrying latency type III EBV genomes compared to other Burkitt lymphoma cells ( $p < 0.01$ ) (Figure 1).

### CpG methylation map of the *LMNA* promoter

Control sequencing of the 5' and 3' flanking sequences of the *LMNA* promoter revealed a C/A heterozygosity at position 4774 (-227bp to transcription start site: TSS) in Mutu-BL-I-CI-216 and Mutu-BL-III-CI-99 and a C-to-A change at position 5282 (+282bp to TSS) in C666-1 cells, compared with the reference sequence NG\_008692.1.

Using the Methyl Primer Express Software v1.0 (Applied

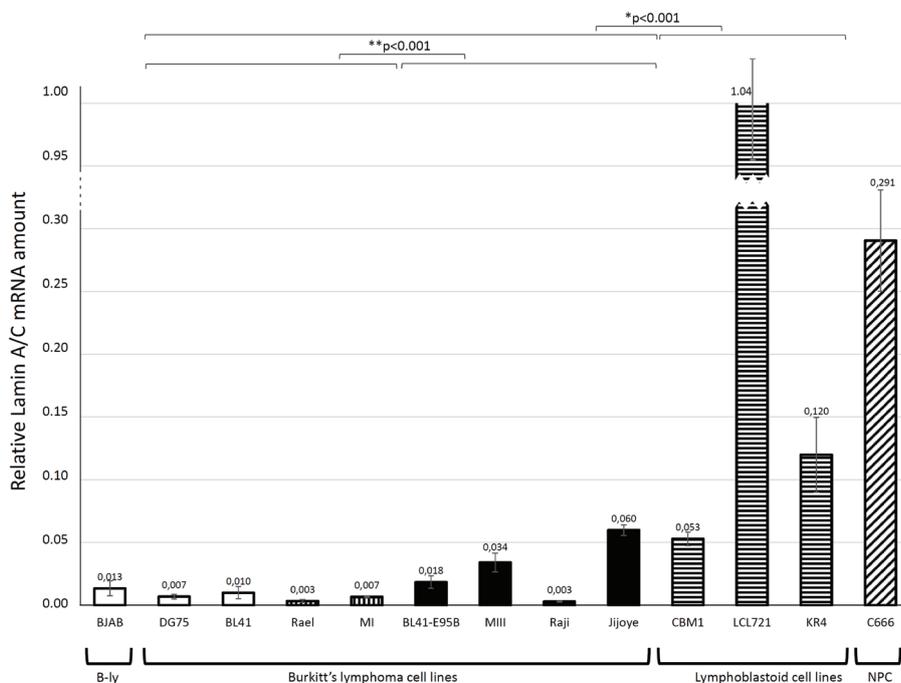
Biosystems) we identified a 739 bp long CpG island encompassing the 3' end of the regulatory region and the 5' end of the first exon of *LMNA* (CpG island: nucleotides -244 to +495, grey box (Figure 2).

In order to correlate lamin A/C expression with the CpG methylation pattern of *LMNA*, we performed bisulfite genomic sequencing (Figure 2). Independently of *LMNA* transcription level, the 5' regulatory region was hypomethylated with the exception of BL41-E95B, Mutu-BL-III-CI-99 and Raji, where highly or moderately methylated CpGs were detected. The situation was quite different at the 3' flanking sequences of the promoter: we found highly methylated CpG dinucleotides within the first *LMNA* exon in cell lines showing a low level of *LMNA* mRNA expression, except for BJAB that was hypomethylated in this region. In contrast, in LCLs and C666-1 nasopharyngeal carcinoma cells that expressed high levels of *LMNA* mRNA, exon 1 was either unmethylated or hypomethylated. We noticed that although the 5' regulatory sequences and exon 1 of *LMNA* were highly methylated in Mutu-BL-III-CI-99, this clone exhibited a high level of *LMNA* mRNA (Figure 2).

### Analysis of euchromatic histone marks at the *LMNA* promoter

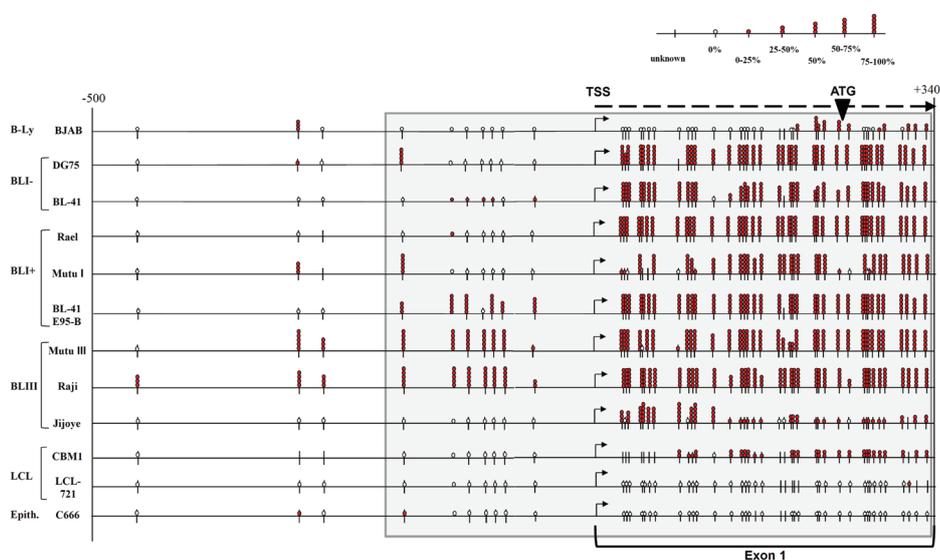
We analyzed the euchromatic, activating histone marks at *LMNA* using ChIP. Both the 5' regulatory region and exon 1 of *LMNA* were enriched in acetylated Histone 3 (AcH3) in all three LCLs (KR4, CB-M1-Ral-STO and LCL-721) expressing lamin A/C. In the lamin A/C-positive C666-1 cells, AcH3 was detected in high abundance only within the first *LMNA* exon (Figure 3).

In BL cells, the level of AcH3 was highly variable in the 5' regulatory sequences of *LMNA*: it was hardly detectable in Rael and Mutu-BL-I-CI-216 (low promoter activity) and in Mutu-BL-III-CI-99 (moderate promoter activity) and highly enriched in cell lines both with low



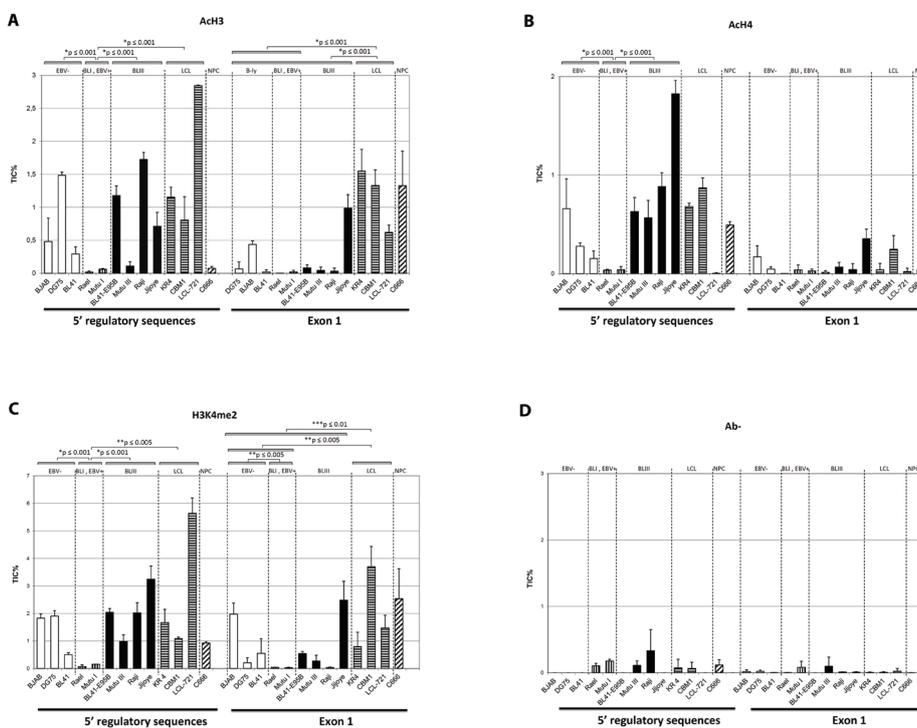
**Figure 1.** Expression of lamin A/C in B lymphoid and carcinoma cell lines.

In panel A, Relative lamin A/C mRNA expression was quantified by real-time RT-PCR normalized to GAPDH mRNA level. Results are average of three biological replicates. Abbreviations: MI: Mutu-BL-I-CI-216; MIII: Mutu-BL-III-CI-99; BL41-E: BL41-E95B; CBM1: CB-M1-Ral-STO; C666: C666-1; B-ly: B cell lymphoma; NPC: nasopharyngeal carcinoma cell line. The p value between groups of BL derived cells versus LCLs is indicated above the chart; similarly, the p value between a group of cell lines consisting of EBV-negative BLs and EBV-positive latency type I BLs versus EBV-positive latency type III BLs is also indicated.



**Figure 2:** CpG methylation map of the 5' regulatory sequence and exon 1 of LMNA.

The localization of the CpG island in the analyzed region is indicated by a grey box. Symbols: rightward arrow, transcription start site (TSS); Black triangle, (ATG): Start codon; thin vertical lines, positions of CpG dinucleotides. Cytosine methylation levels in cell lines and clones are indicated as follows: stick only, undetected; empty dot, 0%; full dot, 0%–25%; two dots, 25%–50%; three dots, 50%–75%; four dots, 75%–100%. Exon 1: the promoter proximal region of the 1st exon. Abbreviations: B-ly: B cell lymphoma; BL: EBV-negative Burkitt lymphoma; BLI: EBV-positive Burkitt lymphoma, latency I; BLIII: EBV-positive Burkitt lymphoma, latency III; LCL: lymphoblastoid cell line; NPC: nasopharyngeal carcinoma cell line; Mutu I: Mutu-BL-I-CI-216; Mutu III: Mutu-BL-III-CI-99; CBM1: CB-M1-Ral-STO; C666: C666-1 nasopharyngeal carcinoma line.



**Figure 3:** Levels of euchromatic histone modifications at the LMNA promoter.

Chromatin immunoprecipitation (ChIP) analysis of ACh3 (A), ACh4 (B) and H3K4me2 (C) levels. In D normal rabbit IgG was used. Results are average of three biological replicates expressed as the percentage of input DNA (TIC, total input chromatin). Antibodies specifically recognizing diacetylated histone H3 (ACh3), tetraacetylated histone H4 (ACh4) and histone H3 dimethylated at lysine 4 (H3K4me2), respectively, were used for ChIP analysis as described earlier [39]. Vertical dashed lines separate groups of cell lines as indicated on the top of the chart. Grey, thick horizontal lines represent groups that were analyzed for statistical significance. The p values between groups analyzed for significance are indicated on the top of the charts. Abbreviations: BL, EBV-negative Burkitt lymphoma; BLI: EBV-positive Burkitt lymphoma, latency I; BLIII: EBV-positive Burkitt lymphoma, latency III; LCL: lymphoblastoid cell line; NPC: nasopharyngeal carcinoma cell line; Mutu I: Mutu-BL-I-CI-216; Mutu III: Mutu-BL-III-CI-99; CBM1: CB-M1-Ral-STO; C666: C666-1 nasopharyngeal carcinoma line.

(DG75, Raji) or moderate (Jijoye p79) promoter activity. In exon 1, ACh3 was nearly undetectable in the examined cell lines except BJAB and Jijoye p79.

Acetylated Histone 4 (ACh4) was hardly detectable in the *LMNA* 5' regulatory region in Rael and Mutu-BL-I-CI-216, but it was abundant in all latency type III cells independently of promoter activity, with the exception of LCL-721. C666-1 cells were enriched in ACh4 in the *LMNA* 5' regulatory region. Exon 1 of *LMNA* was poor in ACh4 in all cell lines except BJAB, Jijoye p79 and CB-M1-Ral-STO.

Histone H3 dimethylated at lysine 4 (H3K4me2) was hardly detectable at the *LMNA* 5' regulatory region in Rael and Mutu-BL-I-CI-216 but it was moderately or highly enriched in all other cell lines examined. Exon 1 of *LMNA* showed an elevated H3K4me2 level in all latency type III cell lines with moderate or high *LMNA* promoter activity, except Mutu-BL-III-CI-99 (Figure 3).

The abundancy of all three histone modifications on the 5' regulatory sequence was significantly higher in latency type III BLs and LCLs compared to group I BL cell lines.

Immunoprecipitation with control normal rabbit IgG showed no significant enrichment in any cell lines.

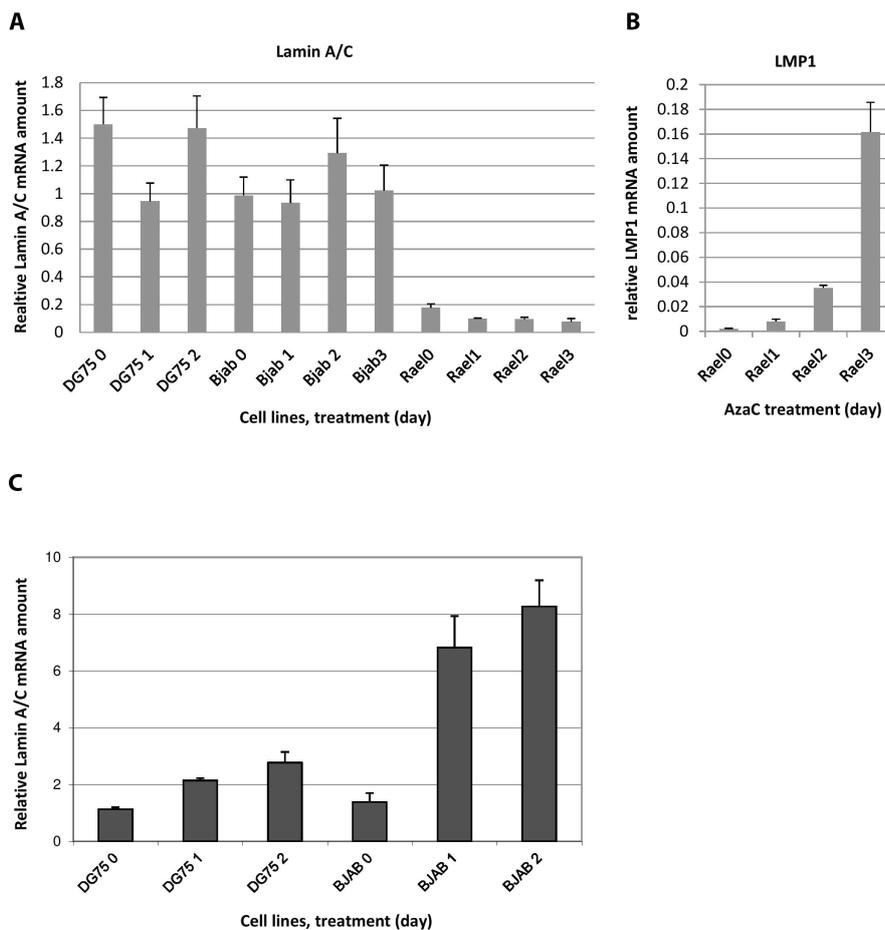
### Effect of AzaC and TSA treatment on *LMNA* transcription

The DNA hypomethylating agent 5-azacytidine did not affect the expression of *LMNA* in DG75, BJAB and Rael cells although it upregulated the expression of the methylated viral *LMP1* gene in Rael cells as observed earlier indicating that the demethylation was effective [30,35,40] (Figure 4).

To test whether increased histone acetylation indeed can activate *LMNA*, we treated EBV-negative B and BL lines with the histone deacetylase-inhibitor TSA. The *LMNA* 5' regulatory sequences were unmethylated in both cell lines, but they differed as to the methylation pattern of exon 1 that was methylated in DG75 cells but unmethylated in BJAB cells (Figure 2). TSA induced only a moderate elevation in *LMNA* gene expression in DG75 cells and a remarkably stronger increase in *LMNA* promoter activity in BJAB cells.

### Discussion

Type B lamins are widely expressed in metazoan cells [41,42]. Although both type A/C and B lamins are present in most murine and human somatic cells, hematopoietic cells express lamin B only [15,43,44]. Accordingly, B lymphoid cells (centrocytes and centroblasts)



**Figure 4:** Effect of 5-azacytidine and trichostatin A (TSA) treatment on *LMNA* transcription

A) DG75, BJAB and Rael cells were treated with 10 or 4  $\mu$ M 5-azacytidine for 0, 1, 2 or 3 days and analyzed for relative lamin A/C mRNA expression. B) Rael cells treated with 4  $\mu$ M 5-azacytidine for 0, 1, 2 or 3 days were analyzed for the expression of the methylation-sensitive EBV latency gene *LMP1*. C) DG75 and BJAB cells were treated with 150 nM TSA for 0, 1 or 2 days, respectively and analyzed for relative lamin A/C mRNA expression. Relative lamin A/C mRNA expression was quantified by real-time RT-PCR. Results are average of three biological replicates.

expressed lamin B1, whereas mantle zone lymphocytes were lamin B1 and B2 positive [14]. Cell lines derived from human neoplasms usually mirrored the lamin expression profile of the cell type they derived from [45-48].

Because EBV can alter the epigenotype and gene expression pattern of its target cells, and lamins are epigenetic regulators themselves, we did a systematic analysis of lamin A/C expression in EBV-positive and EBV-negative B lymphoid cell lines and analyzed the epigenetic marks of *LMNA* [1,16]. Unexpectedly, we observed that similarly to the carcinoma cell line of epithelial origin, there was a high level of *LMNA* transcription in LCLs and in the majority of group III BL lines characterized by an activated B cell phenotype. These cells express typically six nuclear antigens (EBNAs) and three latent membrane proteins (LMPs) encoded by the virus (EBV latency type III) [16]. In contrast, lamin A/C mRNA was present at a low level in EBV-negative B and BL lines and in EBV latency type I BL cell lines that expressed only EBNA1.

We noticed that there was a difference in lamin A/C mRNA expression between two clones of the BL line Mutu: Mutu BL-I-Cl-216 (latency I) expressed A-type lamins at a low level, compared to Mutu-BL-III-Cl-99 (latency III) (Figure 1). Because these clones carry the same EBV strain, one may speculate that one of the EBV-encoded oncoproteins or microRNAs expressed in type III latency may contribute to the activation of *LMNA*. Based on microarray data uploaded by Maier et al. [49] (<https://www.ncbi.nlm.nih.gov/geo/profile/24088892>) the EBV-encoded nuclear antigen EBNA2 is apparently not involved in *LMNA* activation, because expression of EBNA2 in EBV-negative BLs did not result in altered *LMNA* activity. Similarly to the Mutu clones, Rael cells and CB-M1-Ral-STO cells carry the same EBV strain but differ in latency type and expression of lamin A/C mRNA. In Rael cells (latency type I, EBNA1 only), lamin A/C mRNA was barely detected, whereas it was present in high level in CB-M1-Ral-STO cells expressing six EBNAs and three LMPs (Figure 1). This suggests a role for latent EBV gene products in the regulation of *LMNA* expression.

A low but variable level of lamin A/C mRNA expression was observed in EBV-negative B (BJAB) and BL cells (DG75; BL41), indicating that cellular factors may also modulate type A lamin expression.

Using bisulfite sequencing, we analyzed the CpG methylation pattern of *LMNA* located to a CpG island (Figure 2). A shorter stretch of the very same CpG Island was found to be unmethylated in nodal diffuse large B cell lymphomas actively using the *LMNA* promoter [45]. The CpG methylation pattern of the *LMNA* 5' regulatory region did not correlate with the activity of the *LMNA* promoter. Exon 1 was regularly hypermethylated in all BL lines, however, in the vicinity of low-activity *LMNA* promoters, in contrast to the B cell lymphoma BJAB, where it was hypomethylated (Figure 2). These data suggested a role for first exon methylation in the down-regulation of *LMNA* transcription in BL-derived cells. It is interesting to note that Brenet et al. [50] found a linkage between densely methylated first exon sequences and transcriptional silencing in a human acute myelogenous leukemia-derived cell line. Similarly, Yan et al. [51] found that DNA methylation progressively spread from the first exon to the promoter of *RASSF1A*, a tumor suppressor gene silenced in breast cancer. The *LMNA* CpG Island may certainly correspond to such a densely methylated first exon in cell lines with low *LMNA* activity. In cell lines with highly active *LMNA* promoters exon 1 was unmethylated or only partially methylated. However, in Mutu-BL-III-Cl-99 and Jijoye p79 that expressed moderate levels of *LMNA* mRNA, longer or shorter stretches

of highly methylated CpGs of exon 1 sequences flanked the promoter (Figure 2). These data indicated that in addition to DNA methylation, other regulatory mechanisms (e.g. histone modifications) also influence *LMNA* activity. This notion is supported by the observation that the euchromatic histone marks AcH4 and H3K4me2 were enriched in the 5' regulatory region of Mutu-BL-III-Cl-99 cells expressing a high level of lamin A/C mRNA, but not in Mutu BL-I-Cl-216 cells characterized by a low lamin A/C mRNA expression. In addition, the 5' regulatory sequences and exon 1 of *LMNA* were marked with the activating histone modifications AcH3, AcH4 and H3K4me2 in Jijoye p79 cells expressing a high level of lamin A/C mRNA. We observed that in EBV-immortalized LCLs (latency III) and an NPC cell line the sequences flanking the active *LMNA* promoter were enriched in euchromatic histone marks (Figure 3). Activating histone marks were absent, however, from low activity *LMNA* promoters in latency type I BLs. In addition, the histone deacetylase inhibitor TSA could upregulate the activity of *LMNA* in two EBV-negative BL lines, whereas the hypomethylating agent 5-azacytidine was ineffective (Figures 4). We observed that TSA treatment was apparently more effective in BJAB cells where the *LMNA*-associated CpG island was hypomethylated, than in DG75 cells, where the flanking sequences were highly methylated (Figure 4). Thus, in lymphoid cells, both CpG methylation and histone modifications seem to affect *LMNA* activity.

In latency type I BL lines and LCLs, there was a clear correlation between the low abundance of euchromatic histone modifications and low level of *LMNA* transcription. We found, however, that in the EBV-negative B and BL lines BJAB and DG75 enrichment of activating histone modifications at *LMNA* was insufficient to achieve a high level of lamin A/C transcripts (Figures 1 and 3). Thus, the presence of euchromatic histone marks at the promoter-flanking sequences could not upregulate *LMNA* transcription in EBV-negative BL cells, possibly due to the lack of a key viral or cellular activator.

Similarly to LCLs, latency type III BL cells (Mutu-BL-III-Cl-99, Jijoye p79) also expressed relatively high levels of lamin A/C mRNA, with the exception of Raji that does not express, however, the full set of EBV latent proteins: due to deletions in the viral genome, it lacks EBNA3C (EBNA6) and LMP2A [31] (Figure 1). In contrast to the low activity of *LMNA* in Raji cells, its flanking sequences were enriched in euchromatic histone modifications (Figures 1 and 3). Further studies may clarify the potential role of EBNA3C (EBNA6) or LMP2A in the regulation of *LMNA*.

In addition to genetic events, i.e. mutations, deletions, gene amplifications and chromosomal rearrangements, epigenetic events also contribute both to tumor initiation and tumor progression [52,53]. In general, malignant tumors are characterized by global genomic hypomethylation and simultaneous hypermethylation of CpG islands at tumor suppressor genes [54]. The latter phenomenon can be utilized for the treatment of tumors with epigenetic acting drugs, especially in myelodysplastic syndromes [55]. Moreover, tumor specific hypermethylated DNA sequences (the hypermethylome) appearing in biological fluids may also be exploited for early detection and staging of malignant tumors or may predict the prognosis of the patients [56-58]. Our finding that in EBV negative and EBV latency type I BL lines a low *LMNA* promoter activity was associated with hypermethylation of the *LMNA* first exon suggests that the *LMNA* first exon may belong to a hypermethylome characteristic for BL lines that stably retained the BL biopsy phenotype. It remains to be established, whether demethylation of *LMNA* first exon and its potential phenotypic consequences could be

exploited in epigenetic therapy of BLs, or whether the hypermethylated first exon of *LMNA* could be a suitable biomarker for BL.

In cancer cells, CpG island hypermethylation typically occurs at promoter regions [59]. Our finding suggests that the hypermethylome concept could also be extended to hypermethylated first exon sequences affecting promoter activity.

For the development of EBV-associated lymphomas and carcinomas, epigenetic dysregulation plays an outstanding role, with specific methylation profiles characterizing the different entities post-transplant lymphoproliferative disease, Burkitt lymphoma, Hodgkin lymphoma, nasopharyngeal carcinoma, and gastric carcinoma [60-62]. EBV-induced immortalization of B-cells resembling post-transplant lymphoproliferative disease caused extensive changes in the B-cell methylome affecting 2.18 GB of the cellular genome and about one-third of all genes [60]. Strikingly, overlapping hypomethylated blocks of 1.7 GB were also observed in the epigenomes of unrelated EBV-negative carcinomas, including colon, lung, breast and thyroid carcinomas and Wilms' tumors [54]. This implies that tumor-specific methylation changes may be used as biomarkers in methylation-specific diagnostic PCR assays from the blood for the early detection of occult tumors [63-65]. Furthermore, hypermethylation at specific tumor suppressor loci may serve as a predictor for the success of chemotherapy [66,67]. Epigenetic markers other than methylation, such as expression of repressive PcG proteins and the increased presence of the repressive histone mark H3K27me3 were indicators for a poor prognosis both for NPC, gastric carcinoma and B cell lymphomas [68-71].

## Conclusion

Our data showed that methylation of the first exon of *LMNA* was associated with the downregulation of *LMNA* expression whereas euchromatic histone marks were enriched at active *LMNA* promoters in EBV-immortalized LCLs. These data suggest that viral latency products or their combination may activate *LMNA* in EBV infected latency type III B cells *in vitro*. We speculate that expression of lamin A and lamin C may contribute to the establishment of activated B cell phenotype by providing new attachment sites for chromatin loops and thereby affecting the activity of promoters located to lamin-associated domains (LADs) of the genome. Further studies may reveal the contribution of lamin A and C upregulation to the epigenetic reprogramming of LCLs immortalized *in vitro* and PTLs, their *in vivo* counterparts.

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