The Epstein–Barr virus oncoprotein, latent membrane protein-1, reprograms germinal centre B cells towards a Hodgkin’s Reed–Sternberg-like phenotype

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Abstract

Although the latent membrane protein-1 (LMP1) of the Epstein–Barr virus (EBV) is believed to be important for the transformation of germinal centre (GC) B cells, the precise contribution of this viral oncoprotein to lymphoma development is poorly understood. In this study, we used a non-viral vector-based method to express LMP1 in primary human GC B cells. Gene expression profiling revealed that LMP1 induced in GC B cells transcriptional changes characteristic of Hodgkin’s lymphoma cell lines. Strikingly, LMP1 down-regulated the expression of B-cell-specific genes including B-cell receptor components such as CD79A, CD79B, CD19, CD20, CD22, and BLNK. LMP1 also induced the expression of ID2, a negative regulator of B-cell differentiation. Our data suggest that in EBV-positive cases, LMP1 is likely to be a major contributor to the altered transcriptional pattern characteristic of Hodgkin/Reed–Sternberg cells, including the loss of B-cell identity.

Keywords: Hodgkin’s lymphoma; Epstein–Barr virus; latent membrane protein-1; germinal centre B cells; gene expression

Introduction

Although the Epstein–Barr virus (EBV) usually establishes a harmless infection in human memory B cells, it is implicated in the development of germinal centre (GC) B-cell-derived malignancies, including Burkitt’s lymphoma (BL), classical Hodgkin’s lymphoma (cHL), and post-transplant lymphoma [1,2]. EBV is present in malignant Hodgkin/Reed–Sternberg (HRS) cells in approximately 50% of HLs, where there is restricted expression of a subset of virus genes, including the latent membrane proteins LMP1, LMP2A, and LMP2B [2]. LMP1 is the major EBV oncogene and is essential for B-cell immortalization [3]; it activates several signalling pathways (eg NF-κB, JAK/STAT) which are deregulated in HL and which contribute to HRS cell survival and proliferation [4–6].

HRS cells show characteristic transcriptional changes, which include the down-regulation of B-cell genes including the B-cell receptor (BCR) and its signalling components [7]. It has been suggested that loss of B-cell identity might contribute to the rescue of BCR-negative GC B cells which would usually undergo apoptosis. EBV can rescue BCR-negative GC B cells from apoptosis and down-regulates a limited number of B-cell lineage genes [8–11]. However, these effects have only been observed in immortalized B-cell lines, in tumour lines carrying an Ig-MYC translocation and in LMP2A-expressing transgenic mouse B cells [12–14]. The possible contribution of LMP1 to the global loss of B-cell identity has yet to be defined. Such a contribution seems plausible given that LMP1 partially mimics a constitutively active CD40 and that CD40 controls the exit of B cells from the GC, a process associated with the loss of B-cell identity [15,16]. In addition, LMP1 expression in mouse models induces extra-follicular B-cell differentiation in the absence of GC formation and is also associated with decreased BCL6 expression, suggesting that LMP1 interferes with the GC reaction [17,18]. In this study, we have investigated the effect of LMP1 on gene expression in normal human GC B cells using a non-viral vector-based system. We show that LMP1 induces many of the transcriptional changes characteristic of HRS
cells, including the down-regulation of B-cell lineage genes.

Materials and methods

Plasmids

The luciferase reporter gene under control of 3× NF-κB elements (p1242) and the LMP1-expressing pSG5-LMP1 expression vector were as described previously [19]. PRL-null vector was obtained from Promega and pMACSLNFGR from Miltenyi Biotec. The cloning procedure for CD79α promoter fragments and the pSG5HAID2 expression vector can be found in the Supplementary Material (available online at http://www.interscience.wiley.com/jpages/0022-3417/suppmat/path.2384.html).

Purification and transfection of GC B cells

Mononuclear cells were isolated by Ficoll-Isopaque centrifugation of tonsils from paediatric patients under informed consent and with ethical approval (ref No 06/Q2702/50). Briefly, CD10+ GC B cells were isolated by magnetic separation at 4°C with α-CD10-phycocerythrin (PE) (Becton Dickinson, BD) and α-PE microbeads with LS columns (Miltenyi Biotec). 4 × 10⁶ CD10+ cells in 100 µl of B-cell solution were nucleofected with 7 µg of pSG5 or pSG5-LMP1 expression vector with 3 µg of pMACSLNFGR using program U15 on the Nucleofector I, cultivated for 16 h, and stained with α-LNGRF-FITC or α-LNFR-allophycocyanin (APC) (Miltenyi Biotec) and propidium iodide (PI). FITC-labelled and PI-negative cells were collected by FACS on a MoFlo sorter (Dako Cytomation, Colorado, USA). For magnetic enrichment of ΔLNGFR-expressing cells, CD3+ cells were depleted from tonsillar mononuclear following the manufacturer’s instructions, using α-CD3 microbeads with LS columns. CD3+ cells were nucleofected as described above and ΔLNGFR-expressing cells were isolated using α-CDNGFR microbeads with MS columns (Miltenyi Biotec).

Microarray experiments

Total RNA was extracted from cell lines and biotinylated RNA prepared [20]. RNA from the FACS-sorted transfected GC B cells was amplified with the ExpressArt mRNA Amplification kit protocol (Ampotech). 10 µg of fragmented cRNA was hybridized to HG-U133Plus2 microarrays. Microarray chips were analysed using the GCOS Software from Affymetrix, Inc. Probe level quantile normalization and robust multiarray analysis were performed using the affy package of the Bioconductor (http://www.bioconductor.org) project. The gene expression profile of LMP1-transfected cells was compared with that of control vector-transfected GC B cells from three patients. One array per sample was used in the comparisons. The gene expression profile of four B-cell-derived HL cell lines (L428, L1236, L591, KMH2) was compared with that of GC B cells from three different patients. Differentially expressed genes were identified using significance analysis of microarrays (SAM) with a fold-change threshold of 1.5 and a q-value threshold of 5% [21]. Differentially expressed gene sets were identified using gene set analysis (GSA) with a FDR threshold of 5% from the C2 gene sets of MSigDB (http://www.broad.mit.edu/gsea/msigdb/index.jsp) [22,23]. The primary data are available from GEO (http://www.ncbi.nlm.nih.gov/geo/) under series accession Nos GSE10821and GSE10831.

Transfection and magnetic enrichment of transfected cell lines

Cell lines were cultivated as described previously [5,19]. BL2 and L428 cells were transfected by electroporation with 7 µg of pMACS4.1 and 14 µg of the expression vector pSG5, pSG5-LMP1 or pSG5-HAID2, pooled, and enriched as described previously [19,24]. Experiments were repeated in triplicate for each cell line. For dual luciferase reporter gene assays (Promega), cells were co-transfected with 10 µg of pGLCD79A-488, p1242 and 100 ng of pSG5 or pSG5-LMP1. 0.5 µg of pRLnull vector was co-transfected for internal control of the transfection efficiency for each probe. Transfected cells were harvested after 24 h and relative luminescence (rlu) was calculated as the ratio of firefly- to renilla-luciferase activity.

Immunohistochemistry

Cytospins of transfected GC B cells on Vectabond-coated slides (Vector Laboratories) were formaldehyde-fixed and incubated with LMP1-specific rabbit polyclonal antibody overnight at 4°C or for 1 h at room temperature in PBS [25]. Detection was by the Envision peroxidase system (DakoCytomation) and 3,3’-diaminobenzidine (DakoCytomation).

Immunofluorescence and western blot analysis

Cells were analysed for protein expression by SDS polyacrylamide gel electrophoresis and western blot analysis and for the detection of cell-surface antigen expression using flow cytometry as described previously [19]. Antibodies and sources were α-LMP1 (Dako), α-ID2 (Santa Cruz Biotecno), α-actin (Millipore), α-MCM7 (Sigma-Aldrich), α-LNFR FITC, α-LNFR-APC (Miltenyi Biotec), α-CD7 FITC, α-CD79A FITC (Acris), α-CD79B PE, and α-CD95 FITC (BD Pharmeden).

Details of real-time PCR can be found in the Supplementary Material (available online at http://www.interscience.wiley.com/jpages/0022-3417/suppmat/path.2384.html).
Results

LMP1 expression in purified human tonsillar CD10+ germinal centre B cells

We first expressed LMP1 in CD10+ GC B cells. Following magnetic separation, more than 95% of GC B cells were CD10+, and included centroblasts (CD77+) and centrocytes (CD77−), (Figure 1A) and less than 4% were CD3+ (data not shown). More than 90% of purified CD10+ B cells were viable and expressed CD38 and CD95, while only a small proportion expressed CD27, IgD or CD44 (Supplementary Figure 1A, available online at http://www.interscience.wiley.com/jpages/0022-3417/suppmat/path.2384.html, and data not shown). The transfection efficiency of living GC B cells was between 5% and 20% (Figure 1B). After 16 h cultivation, 40% of isolated and transfected cells were viable, and cultivation led to an approximately 50% reduction in the number of CD77-expressing cells (see Supplementary Figure 1B, available online at http://www.interscience.wiley.com/jpages/0022-3417/suppmat/path.2384.html). The purity of the FACS-sorted viable cells co-expressing CD10 and ΔLNGFR was greater than 95%. LMP1 expression in transfected cells was confirmed by immunoblotting and immunohistochemistry (Figure 1C).

Genes differentially expressed following LMP1 expression in GC B cells

SAM analysis revealed that of 20 551 named genes on the HG-U133 plus microarray, 9.37% (1926) were regulated by LMP1 in GC B cells; including 622 genes up-regulated and 1304 genes down-regulated (Figure 2; Supplementary Tables 1 and 2, available online at http://www.interscience.wiley.com/jpages/0022-3417/suppmat/path.2384.html). Many of the genes induced by LMP1 in GC B cells are known EBV, CD40 or NF-κB targets and are highly expressed in HL cell lines and in primary HL (eg EBI-1/CCR7 and CD54/ICAM1) [4,12,15,26–29]. Like HRS cells, LMP1-expressing GC B cells also showed increased expression of components of NF-κB signalling (eg NFKB1/p105, NFKB2/p100, NFKBIA/IκB-α, and REL), the AP-1/ATF pathway (JUNB, ATF5, BATF, SNF1), STAT signalling (STAT5A), and PI3-K signalling (PIK3CD). As described in other reports, LMP1 up-regulated FAS/CD95, anti-apoptotic genes (eg BCL2, BIRC2/CIAP1, BIRC3/CIAP2, BCL2A1/BFL1 TNFAIP3/A20, CLARF/CFLIP, and IER3), and down-regulated pro-apoptotic genes (eg CASP2, TNFSF10/TRAIL, CARD11/CARMA1, DIP, and HRK) [12,14,30].

LMP1-induced alterations in GC B cells resemble those observed in cultivated and primary Hodgkin/Reed–Sternberg cells

We next examined how often genes differentially regulated by LMP1 were also differentially expressed in HL cell lines. Using SAM with cut-offs of 5% FDR and 1.5-fold change, 5299 named genes were significantly changed in four HL cell lines compared with GC B cells. Of these, 881 named genes were common to the LMP1 and HL signature (Figure 2C and Supplementary Table 3, available online at http://www.interscience.wiley.com/jpages/0022-3417/suppmat/path.2384.html). We validated several of the LMP1 target genes contained within this overlap, including some known to be highly expressed.
Figure 2. Microarray analysis of LMP1-expressing and non-expressing CD10⁺ germinal centre B cells. (A) Hierarchical clustering of differentially expressed genes identified by SAM. Each row in the heat map represents a gene and each column represents a sample. The expression levels for each gene were standardized to a mean value of 0 and a standard deviation of 1, and are represented according to the colour scale; red and blue indicate high and low expression, respectively. (B) Subsets of LMP1-regulated genes are shown according to their function. For a complete list of genes, see Supplementary material, available online at http://www.interscience.wiley.com/pages/0022-3417/suppmat/path.2384.html. (C) Comparison of the LMP1 target gene signature with the Hodgkin lymphoma signature. The Affymetrix HG-U133 plus microarray contains 20,551 named genes, of which 1926 are LMP1-regulated and 5299 are differentially expressed in HL cell lines (KMH2, L428, L591, and L1236) compared with three different samples of GC B cells. 881 named genes are common to both signatures. 2198 named genes are up-regulated in HL cell lines, of which 9.2% (203/2198) are also up-regulated by LMP1. 3101 named genes are down-regulated in HL cell lines, of which 21.9% (678/3101) are also down-regulated by LMP1. (D) Selection of genes common to both signatures. An asterisk indicates that the gene was not significantly changed. (E) Validation of several LMP1-regulated genes in human GC B cells. Real-time RT-PCR of the relative quantity of IER3, NCK2, PTP4A3, SIRPA, CD274, and IRF4 in LMP1-expressing and non-expressing GC B cells. All samples were analysed in triplicate and are presented as 2⁻ΔΔCT values compared with vector control (pSG5).

Gene set analysis (GSA) was used to test whether LMP1-regulated genes were significantly associated with HL cell lines [22,23]. The sets of LMP1 up-regulated genes and down-regulated genes were analysed together with the 1892 C2 gene sets of the Molecular Signatures Database. The LMP1 down-regulated gene set was among the 70 significantly
down-regulated gene sets in the comparison of HL cell lines versus GC B cells with a FDR of less than 0.1%, while the set of genes up-regulated by LMP1 was not significantly changed in HL cell lines with a FDR threshold of 5%. Strikingly, in addition to the significant association between genes down-regulated by LMP1 and the HL cell lines, several gene sets involving BCR signalling pathways and lymphocyte differentiation were significantly changed in HL cell lines (see Supplementary Table 4, available online at http://www.interscience.wiley.com/jpages/0022-3417/suppmat/path.2384.html). Our data strongly suggest that LMP1 contributes to the altered transcriptional profile of EBV-positive HRS cells, including the characteristic loss of B-cell identity.

LMP1 mediates the down-regulation of B-cell-specific genes in GC B cells

The most striking observation was that LMP1 repressed in GC B cells, numerous B-cell-associated genes which are known to be down-regulated in primary and cultivated HL cells [27,31,32]; these included BCR signalling molecules (CD79A/Igα, CD79B/Igβ, BLNK/SLP65, VPREB3), BCR co-receptors (CD19, MS4A1/CD20 and CD22), downstream targets of the BCR (BRDG1, BANK1), and B-cell-associated transcriptional regulators (MYBL1, BCL6, POU2F2/OCT2). We confirmed the down-regulation by LMP1 of a number of these B-cell lineage genes (CD79A/Igα, CD79B/Igβ, POU2F2/OCT2, CD22, BLNK/SLP65, VPREB3, and PTPRC/CD45) in GC B cells and in the BL cell line, BL2 (Figures 3A–3C).

To ensure that the changes in gene expression were not an artefact of the technique used to separate GC B cells, we also enriched B cells from tonsils by CD3 depletion. The resulting population, which consisted of approximately 50% CD10+ cells, was transfected as described above. MACS separation was used to sort ΔLNGFR-expressing cells, which were predominantly found in the CD10+ cell population; this increase in CD10+ B cells found after transfection was most probably a consequence of our previous observation that proliferating GC B cells are more susceptible to transfection than are resting B cells (Supplementary Figure 2A, available online at http://www.interscience.wiley.com/jpages/0022-3417/suppmat/path.2384.html, and data not shown). FACs analysis was directly performed after the sorting of CD3-depleted transfected cells and confirmed several of the LMP1-induced changes that we had observed in the CD10-enriched and transfected GC B cells, including the down-regulation of CD79A expression and the up-regulation of CD95 expression (Figure 3D) [30]. We conclude that LMP1 can disrupt the B-cell phenotype in both normal GC B cells and GC-derived tumour cells.

ID2, a suppressor of B-cell-specific genes, is up-regulated by LMP1

Loss of B-cell identity is a hallmark of HRS cells and there is evidence that this might be mediated by aberrant ID2 expression [33,34]. ID2, an inhibitor of DNA binding, has been shown to cause global down-regulation of B-cell genes and to block B-cell differentiation [35]. We found that LMP1 expression in GC B cells and GC-derived tumour cell lines increased ID2 transcription and protein expression (Figures 4A and 4B). The small increase in ID2 expression by LMP1 is due to the fact that unsorted GC B cells were used for western blot analysis; thus, the up-regulation in sorted cells would be more obvious. We suggest that enhanced ID2 expression might be involved in down-regulation of B-cell-associated genes in GC B cells. We therefore compared the effects of LMP1 and ID2 on the down-regulation of CD79A, a B-cell marker gene that we had shown to be down-regulated by LMP1. When transfected into BL2 cells, LMP1 and ID2 both induced a pronounced decrease in CD79A protein expression (Figure 4C).

LMP1 regulates CD79A promoter activity

The proximal CD79A promoter contains a number of potential transcription factor binding sites, including E2A, EBF, and Pax5 sites, as described recently [36,37]. A reporter plasmid containing 488 bp of the human proximal CD79A promoter directing firefly-luciferase expression was co-transfected together with the LMP1 expression vector; the effect of LMP1 on a NF-κB-dependent luciferase reporter was analysed in parallel (Figure 5). In line with earlier studies, the NF-κB reporter gene was activated by LMP1 in BL2 cells (Figure 5, upper panel). CD79A promoter activity was inhibited in the presence of LMP1, indicating that LMP1 can regulate transcription of the CD79A gene (Figure 5, lower panel).

Discussion

Until now, the contribution of LMP1 to B-cell transformation has only been studied in EBV immortalized cells [14,38]. Because immortalization is already complete, these models provide little information on the contribution of LMP1 to the early stages of transformation. We therefore chose to study the transcriptional effects of LMP1 in GC B cells, the presumptive progenitors of HL. We show that many of the genes regulated by LMP1 in GC B cells are also differentially expressed in HL cell lines and are known CD40 and NF-κB targets [4,12,15]. We observed that LMP1 can down-regulate many B-cell identity genes including the transcription factor POU2F2/OCT2, the BCR signalling molecules CD79A and CD79B, the BCR co-receptors CD19 and CD22, and downstream targets of the BCR. Some of the B-cell-associated genes down-regulated by LMP1 have also been reported to
Figure 3. Down-regulation of B-cell-associated genes in LMP1-expressing normal and transformed germinal centre B cells. Real-time RT-PCR of the relative quantity of B-cell lineage genes in LMP1-expressing and non-expressing CD10+ GC B cells in two different transfected tonsil samples (A) and in LMP1-expressing and non-expressing BL2 cells (B). Relative mRNA levels were determined using real-time fluorometric measurement of cDNA using a comparative CT method according to the TaqMan protocol. All samples were analysed in triplicate and are presented as 2^{-ΔΔCT} values compared with vector control (pSG5). (C) Flow cytometric analysis of CD79A and CD79B expression in BL2 cells transfected with pSG5 (black) or LMP1 (grey). (D) Flow cytometric analysis of CD3− tonsillar B cells transfected with pSG5 (left) or LMP1 (right) together with pMACSLNGFR after enrichment of ΔLNGFR-expressing cells. Cells were stained for CD10 and NGFR together with CD79A or CD95.

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Figure 4. LMP1 and ID2 mediate down-regulation of CD79A in normal and transformed germinal centre B cells. (A) Real-time RT-PCR of the relative quantity of ID2 mRNA in LMP1-expressing and control GC B cells (left panel) and GC-derived tumour cells, BL2, and L428 (right panel). (B) Detection, by immunoblot analysis, of LMP1, ID2, and MCM7 or β-actin protein (loading control) in LMP1-positive and LMP1-negative GC B cells (left panel) and L428 cells (right panel). (C) Flow cytometric analysis of CD79A expression in BL2 cells transfected with pSG5 (grey) or LMP1 (black) (upper panel), or pSG5 (grey) or ID2 (black) (lower panel).

be suppressed after CD40 stimulation (BCL6, SYK, CD27, and IGJ) [15]. Although it has been suggested that down-regulation of the B-cell-related transcriptional programme is secondary to the loss of BCR expression [31], our results show that LMP1 can simultaneously down-regulate all aspects of B-cell identity, including the expression of BCR components. GC B cells lacking a functional BCR usually die from apoptosis, as they do not receive a survival signal that is provided by CD40 activation. If HRS progenitors arise from such pre-apoptotic GC B cells, they must have escaped apoptosis. Down-regulation of B-cell identity and resistance to apoptosis are hallmarks of cHL. Given the suggestion that the down-regulation of B-cell identity could allow HRS cells or their progenitors to escape recognition by the cell’s apoptotic machinery, LMP1-mediated loss of B-cell identity could facilitate the survival of GC B cells with non-functional BCR [7,32,39]. In addition, LMP1 could provide a direct anti-apoptotic signal; we have shown in the present study that LMP1 modulates a variety of anti-apoptotic and pro-apoptotic genes (e.g., BCL2, BCL2A1/BFL1, CLARF/CFLIP, and TNFSF10/TRAIL) that are able to protect GC cells from apoptosis [40,41].

Loss of B-cell identity occurs during the normal differentiation of a GC B cell into plasma or memory B cells, while other cellular markers are up-regulated [15]. Comparison of the LMP1-induced expression profile in GC B cells with the transcriptional profiles

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of different tonsillar B-cell subtypes from previous studies revealed an overlap with memory and plasma cells, suggesting that LMP1 drives B-cell differentiation towards the post-GC stage [42,43]. Amongst others, we observed up-regulation of IRF4 and STAT5 and the chemokines CCL17 and CCL22 along with the down-regulation of BCL-6, which is critical for the exit of B cells from the germinal centre. This finding is in line with earlier studies, where it was shown that LMP1 can induce extra-follicular B-cell differentiation in the absence of GC formation and even inhibits GC formation [17]. Therefore, EBV could access the memory B-cell pool following an extra-follicular B-cell maturation programme which is driven by LMP1, and not through a conventional GC stage. If LMP1 drives but fails to complete GC B-cell differentiation, an LMP1-expressing post-GC cell may emerge from which the HRS cell develops. This would be consistent with the finding that HRS cells often express some of the markers of plasma cell differentiation, and also with the observation that the completion of plasma cell differentiation can result in the activation of viral replication with disruption of viral latency [44,45].

How LMP1 represses B-cell-associated gene expression is unclear, but it might involve ID2, an inhibitor of DNA binding that has been shown to cause global down-regulation of B-cell genes and to block B-cell differentiation [35]. ID2 is strongly and uniformly expressed in EBV-positive and EBV-negative HRS cells, and amplification or genomic gain of the ID2 locus has been reported in 50% of patients with HL [33,34].

Although we have shown here that LMP1 up-regulates ID2, and that LMP1 and ID2 can inhibit CD79A expression, our microarray data suggest that other mechanisms might also be involved in the down-regulation of B-cell identity in these cells, such as down-regulation of Runx1 and EBF [36,37]. Epigenetic silencing has been shown to contribute to the loss of B-cell markers (eg CD79B, CD19) in HRS cells [46]. However, although LMP1 induces the expression of DNA methyltransferases in epithelial cells [47], DNMT1 was down-regulated by LMP1 in our study.

In summary, our data provide a unique snapshot of the transcriptional effects of a major viral oncogene in normal human GC B cells. Because we have shown that LMP1 can down-regulate B-cell lineage genes, it is tempting to conclude that this phenotype is critical for viral persistence. We anticipate that the model that we have described will also prove useful when studying the contribution of other viral genes (eg EBNA1 and LMP2) and cellular genetic events (eg constitutive NF-κB activation) to the pathogenesis of HL and other GC-derived tumours.

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Supplementary material

Supplementary material may be found at the web address http://www.interscience.wiley.com/jpages/0022-3417/suppmat/path.2384.html

References

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13. Portis T, Dyck P, Longecker R. Epstein–Barr virus (EBV) and the Epstein–Barr virus oncoprotein LMP1 expression in GC B cells 91


