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Enhanced aerobic glycolysis of nasopharyngeal carcinoma cells by Epstein-Barr virus latent membrane protein 1

Wei-Wen Sung[a,b], Peir-Rong Chen[c], Ming-Hui Liao[a,*], Jeng-Woei Lee[d,**]

[a] Department of Veterinary Medicine, National Pingtung University of Science and Technology, Pingtung, Taiwan
[b] Bio-Innovation Center, Buddhist Tzu Chi Medical Foundation, Taiwan
[c] Department of Otolaryngology, Buddhist Tzu Chi General Hospital, Hualien, Taiwan
[d] Department of Life Sciences, Tzu Chi University, Hualien, Taiwan

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ABSTRACT

Latent membrane protein 1 (LMP1) is a principal viral oncoprotein in Epstein-Barr virus (EBV)-associated malignancies, including nasopharyngeal carcinoma (NPC), which acts through regulating tumorigenesis and metabolic reprogramming of cancers. In the presence of oxygen, we demonstrated that glucose consumption, lactate production and lactate dehydrogenase (LDH) activity were significantly increased upon LMP1 expression in NPC cells and in a LMP1 variant derived from NPC patients-transformed BALB/c-3T3 cells. The amounts of the α subunit of hypoxia-inducible factor-1 (HIF-1α), a key regulator of aerobic glycolysis, and its targets, pyruvate dehydrogenase kinase 1 (PDK1) and the pyruvate kinase M2 (PKM2) isoform, were also consistently elevated by LMP1. Moreover, in parallel with reductions in the oxygen consumption rate and mitochondrial membrane potential in cells, an augmented extracellular lactate concentration was observed due to LMP1 induction. In conclusion, our results proved facilitation of the Warburg effect by LMP1 through alteration of mitochondrial function in NPC cells.

1. Introduction

Accumulating evidence has revealed an intimate relationship between virus infection and viral carcinogenesis. A common human virus, the Epstein-Barr virus (EBV), has been identified as an etiological factor in several human malignancies, including Burkitt's lymphoma (BL), nasopharyngeal carcinoma (NPC), Hodgkin lymphoma (HL), post-transplantation lymphoproliferative disease and gastric cancer [1–4]. During EBV infection, a limited set of latent viral products is expressed in tumor cells, and notably, evidence has revealed that latent membrane protein 1 (LMP1) plays an important role, giving rise to oncogenesis. LMP1 is a 63-kDa integral membrane protein that consists of a 23-amino-acid N-terminal cytoplasmic tail, six transmembrane domains and 200-amino-acid C-terminal activating regions (CTARs) [5]. Of interest, via oligomerization and lipid-raft partitioning, LMP1 activity is similar to that of tumor necrosis factor receptor (TNFR) CD40, in a ligand-independent manner. Through CTARs engagement with adaptor proteins (tumor necrosis factor receptor-associated factor (TRAF) and TNFR-associated death domain protein (TRADD)), LMP1 recruits a multitude of cellular signaling transduction pathways, such as phosphatidylinositol 3-kinase (PI3K)/Akt, nuclear factor-κB (NF-κB), mitogen-activated protein kinases (MAPKs)/ATF2, Janus-activated kinase 3 (JAK3)/signal transducers and activators of transcription (STATs), to stimulate cell proliferation, anti-apoptosis, angiogenesis, and invasion, as well as metastasis [6,7].

In contrast to normal cells, cancer cells rewire anabolic and catabolic pathways to maintain infinite proliferation and nutrient acquisition. Intriguingly, an increased glucose uptake and facilitation of glycolysis from glucose to lactate are observed in tumor cells in spite of functional mitochondrial oxidative phosphorylation (OXPHOS),

Abbreviations: EBV, Epstein–Barr virus; HPV, human papilloma virus; HCV, hepatitis C virus; LMP1, latent membrane protein 1; NPC, nasopharyngeal carcinoma; BL, Burkitt's lymphoma; HL, Hodgkin lymphoma; HIF-1α, hypoxia-inducible factor-1α; TNFR, tumor necrosis factor receptor; TRAF, TNFR-associated factors; TRADD, TRAF-associated death domain protein; PI3K, phosphatidylinositol 3-kinase; NF-κB, nuclear factor-κB; MAPKs, mitogen-activated protein kinases; JAK3, Janus-activated kinase 3; STATs, signal transducers and activators of transcription; OXPHOS, oxidative phosphorylation; LDH, lactate dehydrogenase; PDK1, pyruvate dehydrogenase kinase 1; PKM2, pyruvate kinase M2 isoform; PEP, phosphoenolpyruvate; GLUT1, glucose transporter; PDH, pyruvate dehydrogenase; HKs, hexokinases; PFKFB2, 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 2; F2, 6BP, fructose-2,6-bisphosphate; PFK1, phosphofructokinase 1; OGDH, oxoglutarate dehydrogenase; TCA cycle, tricarboxylic acid cycle; FGF2, fibroblast growth factor 2; FGFR1, FGF receptor 1; Hox, homeobox genes; COX4, cytochrome oxidase 4 isoform

* Correspondence to: Department of Veterinary Medicine, National Pingtung University of Science and Technology, 1, Shuefu Rd, Neipu, Pingtung 91201, Taiwan.
** Correspondence to: Department of Life Sciences, Tzu-Chi University, No. 701, Zhongyang Rd., Sec 3, Hualien 97004, Taiwan.
E-mail addresses: mhliao@mail.npust.edu.tw (M.-H. Liao), jwlee@mail.tcu.edu.tw (J.-W. Lee).

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even under normoxic conditions. This phenomenon is known as the Warburg effect [8]. In addition, tumor cells also metabolize glutamine to synthesize nucleotides and fatty acids in response to nutrient stress [9–11]. Remarkably, multiple glycolytic enzymes are involved in aerobic glycolysis (the Warburg effect) and tumor development. For instance, the pyruvate kinase M2 (PKM2) isoform, a rate-limiting enzyme, converts phosphoenolpyruvate (PEP) to pyruvate by transferring a phosphate group from PEP to ADP, manifesting as an elevated expression and an enhanced Warburg effect in tumor cells [12,13]. It is worthy of note that PKM2 is a downstream target of hypoxia inducible factor-1 (HIF-1), and interacts with HIF-1 to reprogram glucose metabolism via transactivation of the expressions of other glycolytic genes, such as glucose transporter (GLUT1), which enhances glucose uptake and increases lactate dehydrogenase (LDH), leading to lactate production from pyruvate conversion. In addition, pyruvate dehydrogenase kinase 1 (PDK1) prevents oxidative decarboxylation of pyruvate by repression of pyruvate dehydrogenase (PDH) activity [12–14]. Consequently, amplification of HIF-1-mediated aerobic glycolysis in cancer cells has a profound effect in terms of reprogramming metabolism-related requirements for tumor growth and proliferation.

NPC is a prevalent EBV-associated malignancy in Southern China, Hong Kong and Taiwan [1,3]. As mentioned above, LMP1 is a principal EBV oncoprotein, and therefore, in this study, we assessed the influence of LMP1 on the Warburg effect in NPC cells.

2. Materials and methods

2.1. Specimens, cell lines and plasmids

Specimens from 3 NPC patients were collected, and the study protocol was approved by the Research Ethics Committee at Buddhist Tzu Chi General Hospital, Hualien, Taiwan. EBV-negative human NPC cell line NPC-TW01, E2-plus cells, a cell line established from N-LMP1 (clone 1510), a LMP1 variant derived from Taiwanese NPC patients-transformed BALB/c-3T3 stable clone (3T3/N-LMP1) E2-induced tumor cells, and 3T3/Neo (Neo) cells containing a vector control, as well as established Tet-on-expressing normal human foreskin keratinocytes (RHEK-1)-derived cell lines RHEK/Tet-LMP1 and RHEK/Tet-On, were gifts from Dr. Ching-Hwa Tsai (National Taiwan University, Taiwan), Dr. Kai-Ping N Chow (Chang Gung University, Taiwan) [15] and Dr. Won-Bo Wang (National Taiwan University, Taiwan), and were cultured as described previously [16]. Plasmid pSG5Flag-LMP1 was kindly provided by Dr. Kenneth Izumi (National Institute of Health, USA). asHIF-1α/pcDNA3.1(−) plasmid was generated for antisense RNA expression. Briefly, an isolated HIF-1α fragment in an antisense orientation from asHIF-1α/yTA plasmid, which was obtained by PCR subcloning with forward primer 5′-CTCCATCTCCTACCCACATAC-3′ and reverse primer 5′-ATCCATTGATTGCCCCAGC-3′ using pHA-HIF-1α plasmid (a gift from Dr. Kou-Juey Wu, China Medical University, Taiwan) as a template and T & A cloning vector (Yeastern Biotech, Taipei, Taiwan), was inserted into *Kpn*I/*Bam*HI restriction sites of pcDNA3.1(−) vector (Invitrogen, Carlsbad, CA, USA).

2.2. Transient transfection, $CoCl_2$ treatment and Western blotting analysis

Fifty to sixty percent confluent cells were transfected with plasmid mixture using jetPrime reagent (Polyplus-transfection, Illkirch, France) according to the manufacturer's instructions. After transfection, cells were harvested and subjected to protein extraction. For $CoCl_2$ treatment, 80–90% confluent cells were treated with 800 μM $CoCl_2$, a hypoxia-mimetic agent, for the indicated time period, and total protein was then collected. For Western blot analysis, mouse anti-LMP1 antibody was purchased from Dako (Glostrup, Denmark). Rabbit anti-HIF-1α antibody, anti-PDK1 antibody and anti-PKM2 antibody were purchased from Novus Biologicals (Littleton, CO, USA), Enzo Life Sciences (Plymouth meeting, PA, USA) and Epitomics, Inc. (Burlingame, CA, USA), respectively. The densities of the bands were measured using ImageJ software, and values were normalized to the densitometric values of the internal control in each sample. The relative fold changes of protein amounts were then calculated for the experimental set as compared with the mock or vector control.

2.3. Biochemical analysis

Cells were incubated with culture medium supplemented with (CM) or without fetal bovine serum (FBS) (SFM). When cells were grown to 70–80% confluence, conditional medium was collected and subjected to measurement of glucose and lactate levels, as well as LDH activity, using a Siemens Dimension RXL Max Integrated Chemistry System at the Department of Laboratory Medicine, Tzu Chi Medical Center (Hualien, Taiwan).

2.4. Oxygen consumption rate and lactate production assay

Cells were seeded into 96-well plates and then grown to 70–80% confluence. Measurements of oxygen consumption rate and lactate production were carried out using an Oxygen Consumption/Glycolysis Dual Assay Kit (Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturer's instructions.

2.5. Measurement of mitochondrial membrane potential ($\Delta\Psi m$)

Seventy to eighty percent confluent cells were stained with cationic lipophilic fluorescent dye, 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimi-dazolylcarbocyanine iodide (JC-1), using a JC-1 Mitochondria Membrane Potential Assay Kit (Caymen Chemical) according to the manufacturer's instructions. Cells were then harvested and subjected to flow cytometry. Healthy cells with functional mitochondria (high ΔΨm) containing red JC-1 aggregates were detected in the FL2 channel; however, unhealthy cells with collapsed mitochondria (low ΔΨm) containing JC-1 green monomers were detected in the FL1 channel.

2.6. DNA microarray analysis

Total RNA was extracted from cells with or without LMP1 expression plasmid transfection. The RNA samples were then labeled with Cy3- or Cy5-CTP using a Low Input Quick-Amp Labeling Kit (Agilent Technologies, Palo Alto, CA, USA). Subsequently, fluorescent-labeled cRNA probes were mixed and subjected to competition hybridization using an Agilent SurePrint G3 Human GE 8×60 K Microarray. Images were scanned using an Agilent Microarray Scanner and analyzed using Agilent Feature Extraction 10.5.1.1 software. After quantification and normalization by the rank-consistency-filtering LOWESS method, the expression fold change was calculated as the intensity of the test (LMP1 transfectant) in comparison with the intensity of the mock control.

2.7. Animal and microPET/CT imaging analysis

Congenic/NOD.CB17-Prkdc-scid/JTcu mice of 5 weeks of age were purchased from the Laboratory Animal Center, Tzu Chi University (Hualien, Taiwan), and underwent subcutaneous injection of 10^7 3T3/Neo cells or E2-plus cells in 100 μL of PBS into the flank region of the right or left hind limb. The animal experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee, Tzu Chi University (Hualien, Taiwan). Subsequently, tumor-bearing mice were intravenously injected with ^{18}F-fluorodeoxyglucose (^{18}F-FDG) under isoflurane anesthesia. High-resolution positron emission tomography (micro-PET) and X-ray computed tomography (micro-CT) images were obtained using a FLEX Triumph PET/SPECT/CT preclinical imaging system (Gamma Medica-Ideas, Northridge, CA, USA)

at the Department of Nuclear Medicine, Taipei Veterans General Hospital (Taipei, Taiwan).

2.8. Statistical analysis

Statistical differences were analyzed using the paired *t*-test and were considered to be significant at values of $P < 0.05$.

3. Results

3.1. LMP1 induces aerobic glycolysis in NPC cells

Because accumulated lactates, the end product of glycolysis, are released outside cells via monocarboxylate transporter 4 (MCT-4) [17], extracellular lactate levels can serve as a marker of intracellular

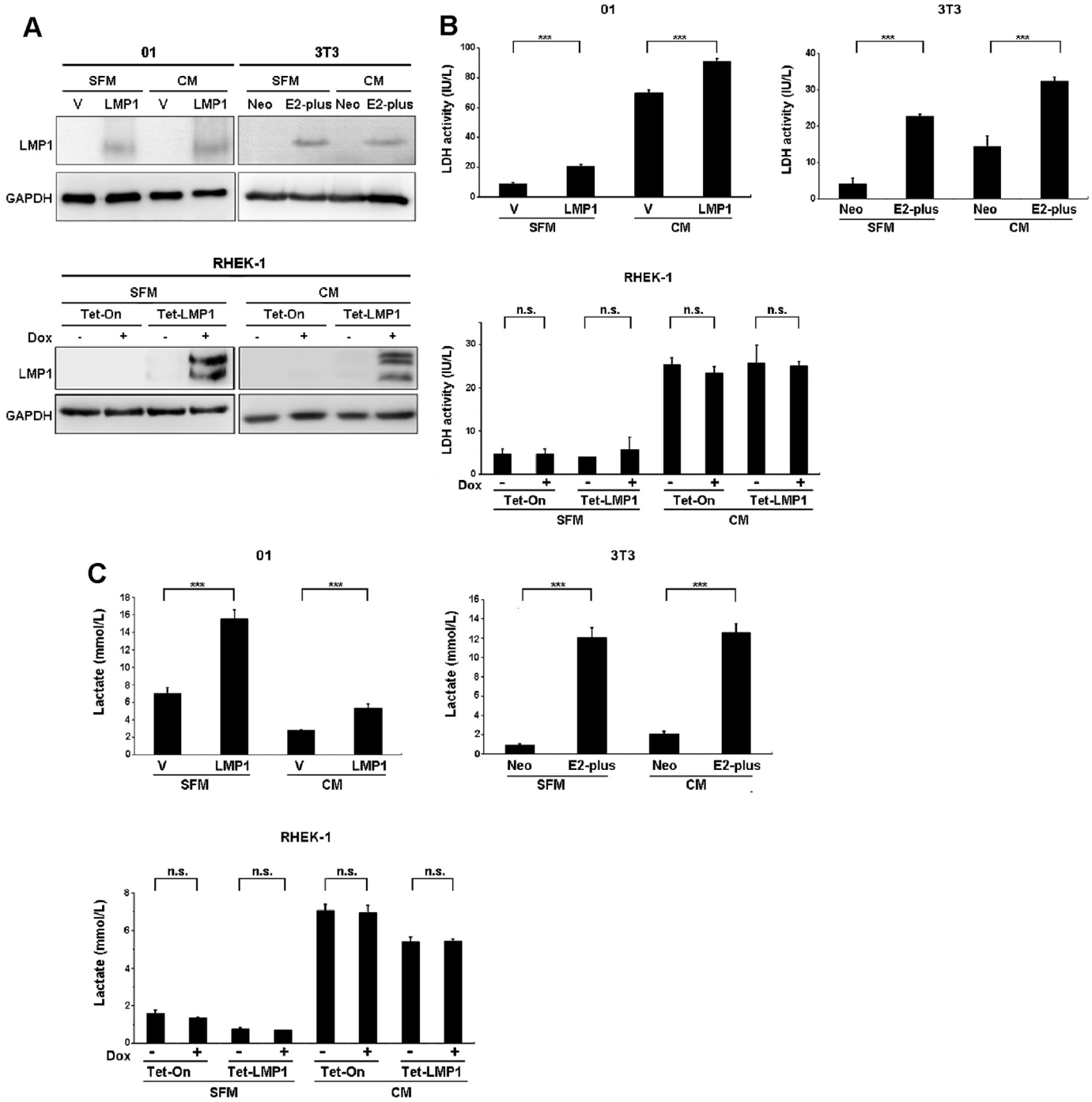

Fig. 1. LMP1 facilitated aerobic glycolysis in NPC cells. LMP1-expressing NPC-TW01, E2-plus or 1 μg/mL of doxycycline (Dox)-induced RHEK/Tet-LMP1 cells were incubated with medium in the presence (CM) or absence (SFM) of serum supplement for 48 h. Cell lysates were then extracted and subjected to Western blot analysis for LMP1 detection (A). Conditional medium was also collected in order to measure LDH activity (B), lactate concentration (C), and glucose concentration (D), as described in Section 2, which are presented as the mean ± SD of three independent experiments. Transfection with pSG5 was utilized as the vector control. Asterisks indicate significance at ***$P < 0.001$; n.s., non-significant. (E) MicroPET/CT images of tumor-bearing mice. High ^{18}F-FDG uptake (red) in an E2-plus tumor is indicated by the arrowhead. 3T3/Neo cells were also s.c. injected into another site in the same mouse to serve as a control (arrow), as described in Section 2. *Left panel:* transverse sections (upper: microPET, lower: CT scan). *Lower panel*: coronal sections (left: microPET, right: CT scan). All Western blot analysis data shown are representative of three independent experiments. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

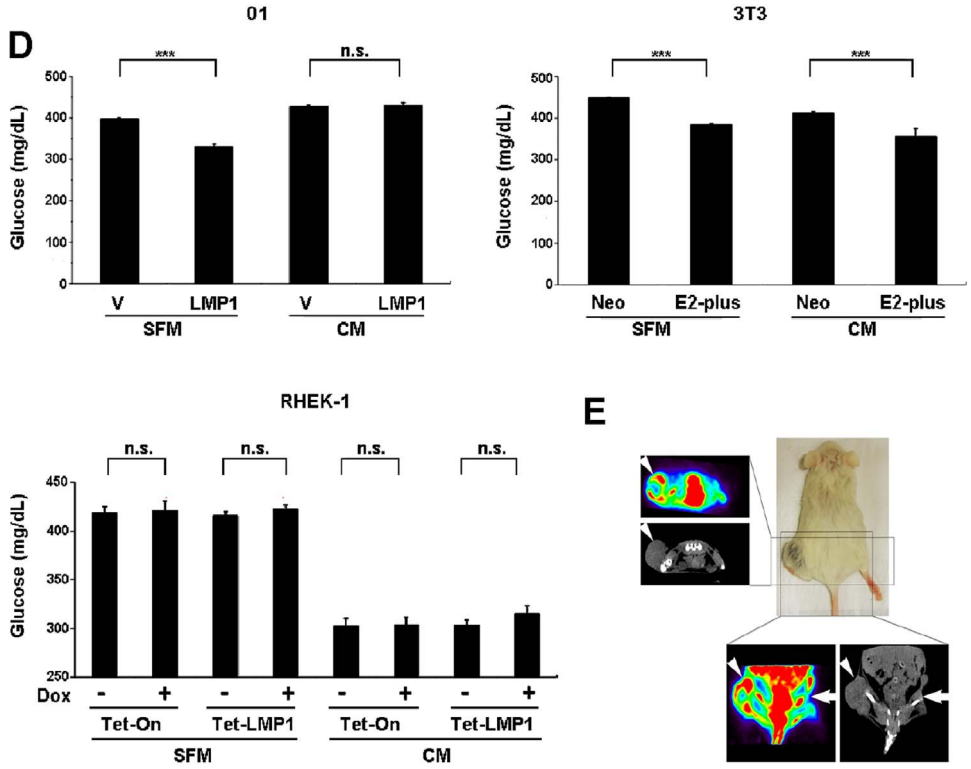

Fig. 1. (*continued*)

glycolytic activity. As mentioned above, to assess the reprogramming of glycolysis in NPC cells by LMP1, a LMP1 expression plasmid was transfected into an EBV-negative NPC cell line, NPC-TW01, under normoxic conditions with measurement of LDH activity and lactate and glucose concentrations either in serum-free (SFM) or complete culture medium (CM) (Fig. 1). In contrast to the vector control, the LDH activity, lactate secretion and glucose consumption in the NPC cells were significantly elevated upon LMP1 introduction, regardless of culture medium (SFM or CM) (Fig. 1B, C and D). Intriguingly, the disparities in LDH activity and lactate and glucose concentrations between the vector set and the LMP1 transfectants were higher following culture in SFM than in CM, indicating a glucose addiction in NPC cells due to insufficient glucose fuel provision in SFM.

Rewiring of glycolysis by LMP1 was also consistently observed in LMP1 variant N-LMP1 (clone 1510)-transformed 3T3 cells, E2-plus cells [15] (Fig. 1B, C and D), which paralleled the high intensity of ^{18}F-FDG in E2-plus tumor-bearing mice identified using a microPET/CT scanner (Fig. 1E), although physiologically high uptakes were observed in the bladder and pelvic cavity. Remarkably, via utilization of LMP1-inducible immortalized RHEK-1 cells (Tet-LMP1), unchanged LDH activity and lactate and glucose concentrations were observed in comparison with the vector control in spite of doxycycline induction (Fig. 1B, C and D). Consequently, these results established a connection between LMP1 oncogenicity and reprogramming of tumor cell glycolysis under normoxic conditions.

In line with multiple glycolytic enzymes, such as hexokinases (HKs), LDH, PKM2 and PDK1, being direct targets of transactivator HIF-1 [12–14,18,19], supporting evidence revealed that in hypoxia-mimetic agent-treated NPC cells (Fig. 2A), in the presence of oxygen, the expressions of HIF-1α, PKM2 and PDK1 were clearly enhanced by LMP1 in comparison with the vector control (Fig. 2B), yet the increased PKM2 and PDK1 levels were mitigated upon knock-down of expression of HIF-1α (Fig. 2C). In concert with our findings, concomitant expressions of PKM2, PDK1 and HIF-1α were also identified in NPC biopsies, although an undetectable or low level of LMP1 was observed by immunoblot analysis (Fig. 2D). Considering a positive regulatory role of LMP1 on the expression of HIF-1α under normoxic conditions [16,20,21], these results suggested that LMP1-mediated HIF-1α induction was involved in elevation of glycolytic enzymes in NPC cells. Besides, via examination of global gene expressions, the transcription levels of HKs, which catalyze the first step of glycolysis, as well as 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 2 (PFKFB2), which is involved in the regulation of fructose-2,6-bisphosphate (F2,6BP), an allosteric activator of phosphofructokinase 1 (PFK1) that catalyzes the conversion of fructose-6-phosphate into fructose-1,6-bisphosphate in the glucose metabolism [22], were observed to have increased due to LMP1 induction (Table 1). Notably, the expression of mitochondrial oxoglutarate dehydrogenase (OGDH), which is responsible for the conversion of α-ketoglutarate to succinyl-CoA and produces NADH in the tricarboxylic acid (TCA) cycle [23], was reduced by LMP1 (Table 1). Collectively, these data indicated a pro-Warburg effect activity of LMP1 through up-regulation of glycolytic enzymes as well as concomitant interference in TCA cycle progression.

3.2. Reduction of mitochondrial OXPHOS and membrane potential by LMP1

We next assessed whether altered mitochondrial activity contributed to the dominant role of LMP1 in aerobic glycolysis. Of interest, the oxygen consumption rate (OCR), an indicator of mitochondrial OXPHOS, was significantly diminished by LMP1 as compared with the vector control, whereas the extracellular lactate level, a marker of glycolysis, was augmented (Fig. 3A and B), implying a metabolic shift from malfunction of the oxidative metabolism to high glycolysis flux under LMP1 induction. Furthermore, to assess the correlation between mitochondrial OXPHOS and mitochondrial activity, the mitochondrial membrane potential (ΔΨm) was measured using a mitochondrial-specific dual-fluorescence probe, JC-1, by flow cytometric analysis. Notably, expression of LMP1 remarkably led to mitochondrial dysfunction via an increased fluorescence ratio of green monomer to red

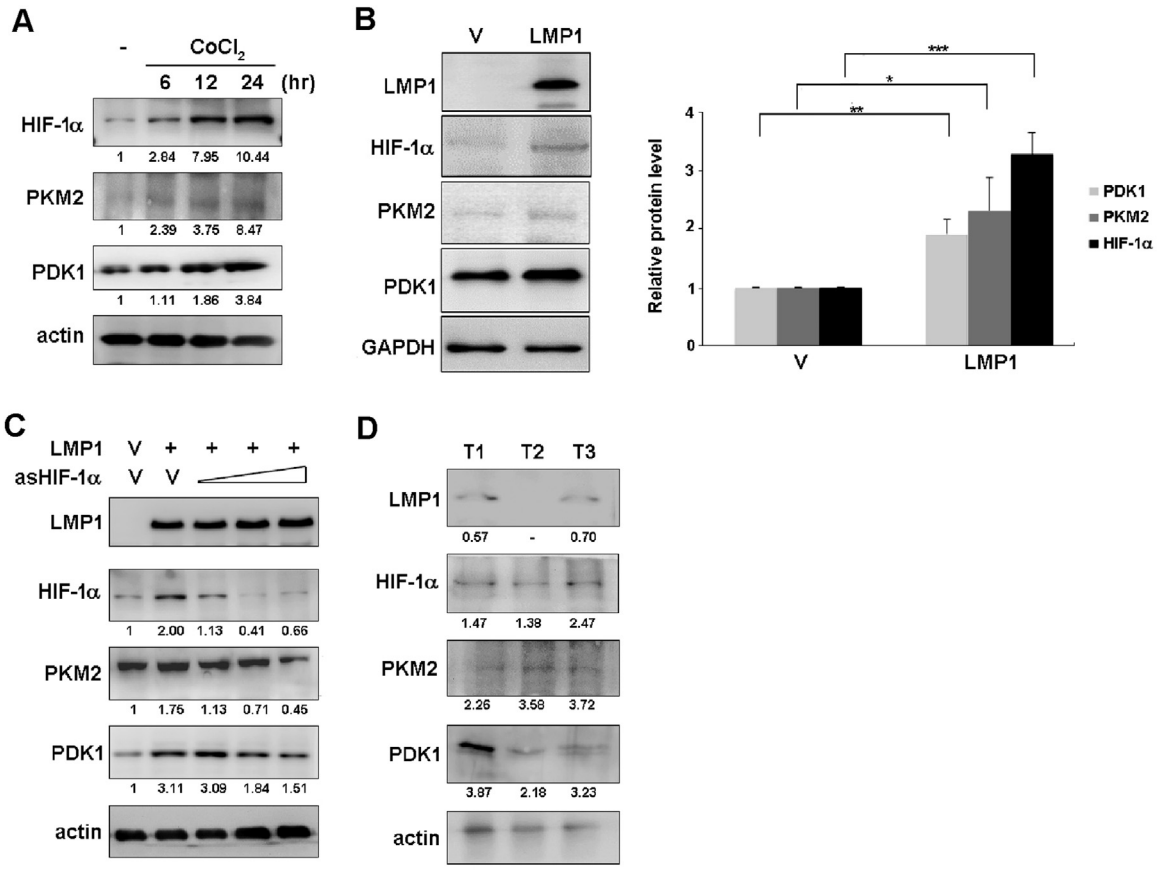

Fig. 2. Expressions of glycolytic enzymes were enhanced by LMP1 through HIF-1α activation. Cell lysates were extracted from NPC-TW01 cells under $CoCl_2$-induced hypoxic conditions (A), transfected with LMP1 expression plasmid (B) or also with asHIF-1α expression plasmid (C), or were obtained from OCT-embedded NPC biopsies (T1-T3) (D), and then subjected to Western blot analysis for LMP1, HIF-1α, PKM2 and PDK1, respectively. pSG5 (B) or plus pcDNA3.1(−) (C) transfection served as the vector control. The protein expression fold change was calculated as described in *Materials and Methods*, and is shown as the mean ± SD of three independent experiments. Asterisks indicate significance at $^{*}P < 0.05$, $^{**}P < 0.01$ or $^{***}P < 0.001$. All Western blot analysis data shown are representative of three independent experiments.

aggregate, indicating elicitation of mitochondrial depolarization (Fig. 3C). Taken together, these results suggested that in the presence of LMP1, enhanced glycolytic flux in cancer cells could be a consequence of adaption to lower mitochondrial activity under normoxic environments.

4. Discussion

In addition to modulation of cellular signaling pathways, intriguingly, impacts of tumor-associated viruses, such as the human papilloma virus (HPV), hepatitis C virus (HCV) and EBV, on glucose metabolism through facilitating glucose uptake and glycolytic flux have also been demonstrated, which not only corroborate viral carcinogenesis, but also establish circumstances favorable to virus infection [24]. Consequently, via up-regulation of c-myc, HIF-1 and NF-κB, as well as the PI3K/Akt and fibroblast growth factor 2 (FGF2)/FGF receptor 1 (FGFR1) pathways, or through repression of homeobox (Hox) genes, the pivotal oncoprotein LMP1 can excite aerobic glycolysis and further promote proliferation, transformation, invasion and apoptosis resistance in EBV-associated lymphoma and NPC cells, respectively [25–28].

In this study, we employed an assay model distinct from those used in previous studies to evaluate the relationship between LMP1 and glycolysis reprogramming in NPC cells. Using a NPC cell line to mimic host cell circumstances in terms of expression of LMP1, and employing NPC patients-derived LMP1 variant-transformed mouse 3T3 cells to manifest oncogenic activity of LMP1 in vitro, in addition to mouse PET/CT imaging analysis, we showed that LMP1-mediated transformation and tumorigenesis were congruent with the Warburg effect (aerobic glycolysis), but this evidence was absent in inducible LMP1-expressing immortalized RHEK-1

Table 1
Changes in glycolysis or TCA cycle-related gene expression in NPC cells under expression of LMP1.

Gene name	Accession number	Function	Fold change
Hexokinase 2 (HK2)	NM_000189	Glycolysis (glucose→glucose-6-phosphate)	1.73
Hexokinase 3 (HK3)	NM_002115	Glycolysis (glucose→glucose-6-phosphate)	1.94
6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 2 (PFKFB2)	NM_006212	Glycolysis (both the synthesis and degradation of fructose-2,6-bisphosphate)	1.66
Oxoglutarate dehydrogenase (OGDH)	NM_001003941	TCA cycle (α-ketoglutarate→succinyl-CoA)	0.43

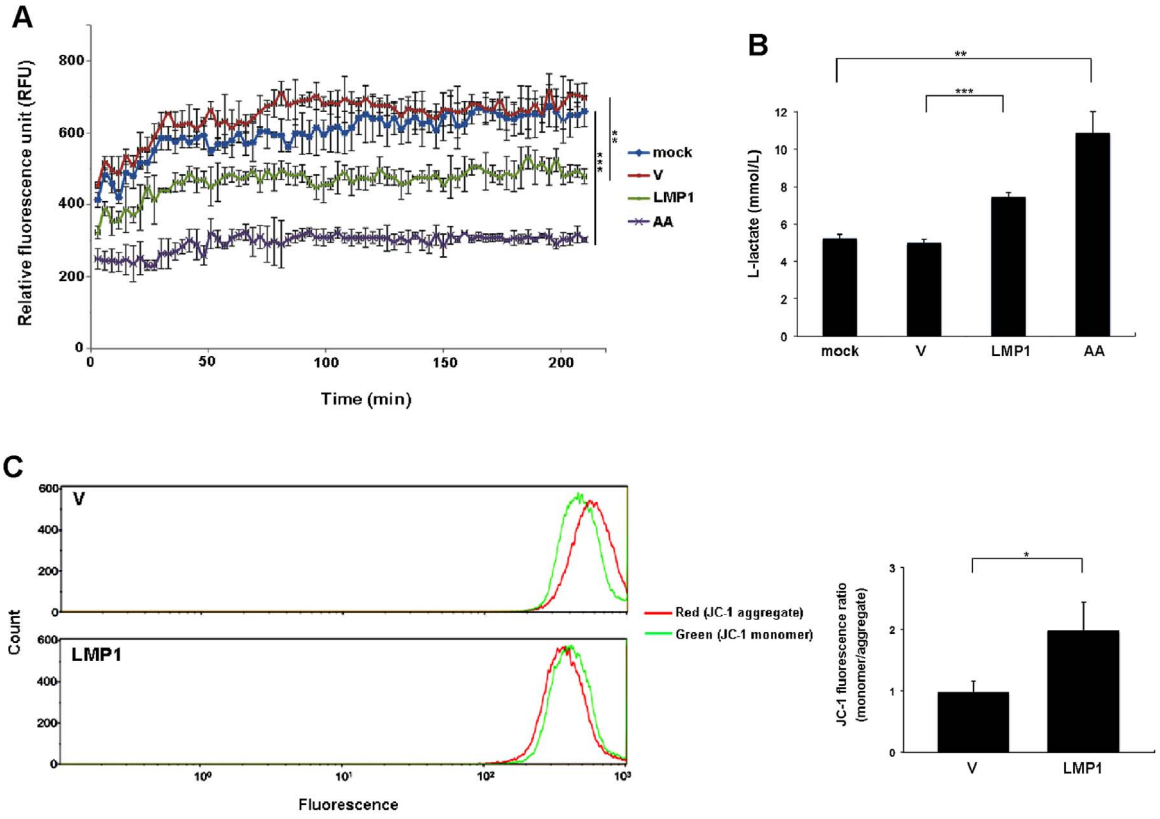

Fig. 3. Mitochondrial OXPHOS and membrane potential (ΔΨm) were interrupted by LMP1. NPC-TW01 cells were transfected with LMP1 expression plasmid. Transfection with pSGF5 was used as the vector control. The oxygen consumption rate (A) and extracellular lactate production (B) of cells were measured as described in Section 2, and are presented as the mean ± SD of three independent experiments. Antimycin A (AA) treatment served as the positive control due to its inhibition of mitochondrial electron transport. (C) The mitochondrial membrane potential (ΔΨm) of cells was also determined by JC-1 staining, as described in Section 2. *Left*: representative histograms of flow cytometric analysis. *Right*: ratio of JC-1 green fluorescence to red fluorescence in each sample. Data are shown as the mean ± SD of three independent experiments. Asterisks indicate significance at $^{*}P < 0.05$, $^{**}P < 0.01$ or $^{***}P < 0.001$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

cells (Fig. 1), which could be due to the short cultivation period for measurement of lactate and glucose concentrations and LDH activity, a delayed glycolytic metabolism response, or a non-permissive (immortalized cells) environment for LMP1 action. In addition, owing to discrepancies in cell property and the analytic system between NPC cells with LMP1 transfection and a cell line established from mice bearing LMP-transformed 3T3 tumors, the LDH activity and the extracellular lactate level in LMP1-expressing NPC were not equivalent to those in E2-plus cells, despite similar alterations of glycolysis in these cell models. In general, the observation of glycolytic flux induction further illustrated the dominant role of LMP1 in tumor development.

Obviously, the expressions of a number of glycolytic enzymes, which are frequently increased in tumor cells [22], were also elevated by LMP1 (Fig. 2 and Table 1). Although comprehensive studies have shown normal function of mitochondrial OXPHOS and ATP synthesis in tumor cells concomitant with aggressive aerobic glycolysis, however, our results indicated mitochondrial dysfunction under LMP1 expression (Fig. 3), which also coincided with the original assumption of Otto Warburg and observations in other tumor cells [29]. Of interest, HIF-1 may be one possible mechanism of disrupted mitochondrial function in cancer cells through interruption of the TCA cycle and mitochondria respiration, such as via decreased conversion of pyruvate to acetyl-coA by transactivation of expressions of PDK1, LDH and PKM2, as well as regulation of the expression of the cytochrome oxidase 4 (COX4) isoform [12–14,29–32]. Importantly, HIF-1 induction and transactivation are not limited to hypoxic conditions (Fig. 2A). In NPC cells, LMP1 can directly induce HIF-1α gene expression under normal oxygen tension [16,20,21] (Fig. 2B and C), although co-expression of LMP1 and HIF-1α in NPC biopsies is still controversial (Fig. 2D) [33,34]. It is worthy of note that 20–60% of NPC cases have positive signals for LMP1 detection by immunoblotting or immunohistochemistry [35,36]. This varying percentage of NPC samples expressing LMP1 could be a consequence of insufficient sensitivity of detection techniques or negative regulation of the LMP1 level by EBV-encoded BART microRNAs [37]. Notably, in compliance with transactivation of HIF-1 on glycolytic enzyme genes (Fig. 2B and C), parallel expressions of PKM2, PDK1 and HIF-1α were also found in NPC biopsies (Fig. 2D). Accordingly, LMP1-mediated HIF-1 expression might have contributed to aerobic glycolysis and malfunction of mitochondria, despite the limitations in terms of detection of LMP1 in tumor tissues.

Collectively, our results not only confirmed the positive role of LMP1 on the Warburg effect in NPC cells via reduced mitochondrial function under normoxic conditions, but also provided suggestions as to the adaption of the tumor metabolism for clinical application in EBV-associated malignancy.

Conflict of interest

The authors declare no conflict of interest.

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