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Involvement of 5-lipoxygenase in survival of Epstein–Barr virus (EBV)-converted B lymphoma cells

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Abstract

Epstein–Barr Virus (EBV) is involved in the progression of lymphomas through still unknown mechanism involving increased resistance to induced apoptosis. We show here that in a set of apoptosis-resistant EBV-converted Burkitt's lymphoma clones, 5- and 12-lipoxygenases (LOXs) are over-expressed. Further investigations on 5-LOX showed that resistance to apoptosis increases parallely with the expression of 5-lipoxygenase (5-LOX). Inhibitors of 5-LOX: (a) decrease peroxides level, indicating that this enzyme promotes the generation of oxidative stress in EBV+ cells, and (b) potently induce apoptosis in the EBV resistant cell line E2R. 5- and 15-HETE, the products of the 5 and 15-LOXs, respectively, counteract 5-LOX inhibitor induced apoptosis, indicating that products of arachidonate metabolism, rather than peroxides, trigger a signal transduction that is required for survival of the EBV-converted cells. These findings suggest that 5- and, to a lesser extent, other LOXs, that are involved in tumor progression of several cell types, may also participate in lymphomagenesis, especially that EBV-mediated.
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1. Introduction

Many factors are involved in putative EBV tumorigenesis, among them important are de-regulation of cell cycle, increase in survival signals and augmented apoptosis resistance [1–3]. Multiple routes seem to be involved in EBV-mediated resistance to apoptosis, such as the expression of latent membrane proteins LMP-1 and LMP-2A which activate the PI3K/Akt [4,5] or NF-κB [6] survival/

Abbreviations: LOX, lipoxygenase; FLAP, 5-lipoxygenase activating protein; EBV, Epstein–Barr virus ; LMP1, latent membrane protein 1; COX, cyclo-oxygenase; NF-κB, nuclear factor kB; HETE, hydroxyeicosatetraenoic acid; 13-*S*-HODE, 13-*S*-hydroxyoctadecadienoic acid; BL, Burkitt's lymphoma; IAP, inhibitor of apoptosis protein; ROS, reactive oxygen species; NDGA, nordihydroguaiaretic acid; CAPE, Caffeic acid phenyl ether; DCFH-DA, 2',7′-dichloroflurorescein diacetate; NOS, nitric oxide synthase.

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antiapoptotic pathways and the small EBV-encoded RNAs (EBERs) which cause the over-expression of anti-apoptotic BCL2 protein [3].

In a previous work we have shown that in Epstein–Barr virus (EBV)-converted cell lines, the virus in the latent phase increases resistance to apoptosis and the endogenous cellular level of peroxides [7]. Persistent oxidative stress is characteristic of a wide spectrum of cancer cells [8]. High oxidative levels in cancers may play multiple roles: among them the activation of oncogenes via NF-κB in many systems [9,10] including lymphoid cells [9]; or signal transduction pathway such as JNK, p38 and Akt protein kinases. In addition a link between oxidative stress and viral infection is well documented [11].

Different possible modalities for the induction or production of reactive oxygen species (ROS) have been suggested. These studies consider the possibility that NADPH-oxidase, nitric oxide synthase (NOS), lipoxygenase (LOX) or cyclo-oxygenase (COX) may be involved in ROS generation [12–14].

It is known that modulation of arachidonate metabolism, for instance via interference with COX or LOX activity, may have dramatic effect on cell survival and apoptosis [15–17]. LOXs are a family of enzymes that process poly-unsaturated fatty acids, and are identified by substrate specificity (mostly arachidonic or linoleic acids) and/or the position (5, 12 or 15) of the double bond that it oxygenates. Accessory proteins may be involved in LOXs activation, such as FLAP, which is specific for 5-LOX [18].

Cancer cells may have an altered level of these enzymes [19], and the possibility to induce or sensitize to apoptosis various types of cancer cells with specific inhibitors of LOX was largely tested, indicating a promising approach to the problem [17,19,20]. Concerning human B-cells, EBV converted cell lines have been used to study the 5-lipoxygenase regulation and activation [21].

Starting from the correlation that we found in EBV positive cells between oxidative level and apoptosis resistance, we decided to investigate the origin and the role of ROS in our system, consisting of the EBV negative Burkitt's lymphoma cell line BL41 and the converted counterparts [7]. Here we provide evidence that specific high levels of ROS in the EBV-converted clones are due to over-expression of 5-LOX, and that LOXs products, rather than LOX-generated ROS, play a crucial role in the survival of EBV+ cells.

2. Materials and methods

2.1. Cell lines

BL 41 is an EBV negative B-cell line obtained from a Burkitt's lymphoma carrying a mutant form of p53 gene [22]. We obtained the, HS1 and E2b and E2R clones after infection of parental BL41 cell line with non-defective B95-8 EBV strain [7]. The EBV presence and the monoclonality was checked by "in situ" hybridization on metaphase spreads and by Southern analysis using the *Bam*HI "W" probe which represents the repeat unit of IR1 region [23,24]. Analysis with *Xho*I fragment of Nhet, *Eco*RI "I" and "J" and *Bam*HI "C" probes allowed to establish that all clones carry about one integrated and defective copy of EBV genome. The different clones are characterized by variable EBV genome deletions, being HS1 clones deleted in the LMP-1 encoding region. The cell lines were cultured in RPMI 1640 medium (Gibco-BRL) supplemented with 10% foetal calf serum (FCS) (Bio-Whittaker) and 2 mM L-glutamine, 100 IU/ml penicillin and streptomycin, and kept in a controlled atmosphere (5% CO_2) incubator at 37 °C.

2.2. Chemicals

Puromycin (solubilized in PBS), valinomycin (in DMSO), hydrogen peroxide, AA861 (in ethanol), allopurinol (in DMSO), indomethacine (in DMSO), nordihydroguaiaretic acid (NDGA) (in DMSO) were from Sigma; 5-*S*-HETE, 15-*S*-HETE, 12-HETE, 13-*S*-HODE (in ethanol) were purchased from BIOMOL; Caffeic acid phenethyl ester (CAPE) (in PBS), Trolox (in RPMI 1640), L-nitro-L-arginine methyl ester (L-NAME) (in H_2O), N^G-monomethyl-L-arginine monoacetate (L-NMMA) were from Alexis. Baicalein (in DMSO) and MK886 (in ethanol) were from Calbiochem.

2.3. Analysis and quantification of apoptotic cells

For morphological analysis of apoptosis, $2–6 \times 10^5$ cells were fixed in 4% (v/v) paraformaldehyde for 3–15 h, stained with DAPI fluorochrome and analyzed with Zeiss fluorescence microscopy. Apoptosis was quantified by scoring cells with condensed and fragmented nuclei according to Ghibelli et al. [25]; at least 500 cells in random fields were scored. Evaluation of apoptosis by morphological scoring of cells showing extensive nuclear condensation or fragmentation rules out any possible mis-interpretation.

2.4. Flow cytometric analysis of ROS

Intracellular peroxide levels were assessed using an oxidation-sensitive fluorescent probe DCFH-DA (Sigma–Aldrich). In presence of a variety of intracellular

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