Available online at www.sciencedirect.com

SCIENCE DIRECT

Experimental Cell Research 303 (2005) 47–55

Experimental Cell Research

www.elsevier.com/locate/yexcr

Epstein-Barr virus-encoded EBNA-5 binds to Epstein-Barr virus-induced Fte1/S3a protein

Elena Kashuba[a,*], Mariya Yurchenko[b], Krisztina Szirak[c], Joachim Stahl[d], George Klein[a], Laszlo Szekely[a]

[a]*Microbiology and Tumor Biology Center (MTC), S-171 77 Stockholm, Sweden*
[b]*Institute of Experimental Pathology, Oncology and Radiobiology (IEPOR) NAS of Ukraine, 03022 Kiev, Ukraine*
[c]*University of Debrecen, Medical and Health Science Center, Department of Human Genetics, H-4012 Debrecen, Hungary*
[d]*Max Delbruck Center of Molecular Medicine, D-13125 Berlin, Germany*

Received 2 April 2004, revised version received 13 July 2004
Available online 18 September 2004

Abstract

Epstein-Barr virus (EBV) transforms resting human B cells into immortalized immunoblasts. EBV-encoded nuclear antigens EBNA-5 (also called EBNA-LP) is one of the earliest viral proteins expressed in freshly infected B cells. We have recently shown that EBNA-5 binds p14ARF, a nucleolar protein that regulates the p53 pathway. Here, we report the identification of another protein with partially nucleolar localization, the v-*fos* transformation effector Fte-1 (Fte-1/S3a), as an EBNA-5 binding partner. In transfected cells, Fte-1/S3a and EBNA-5 proteins showed high levels of colocalization in extranucleolar inclusions. Fte-1/S3a has multiple biological functions. It enhances v-*fos*-mediated cellular transformation and is part of the small ribosomal subunit. It also interacts with the transcriptional factor CHOP and apoptosis regulator poly(ADP-ribose) polymerase (PARP). Fte-1/S3a is regularly expressed at high levels in both tumors and cancer cell lines. Its high expression favors the maintenance of malignant phenotype and undifferentiated state, whereas its down-regulation is associated with cellular differentiation and growth arrest. Here, we show that EBV-induced B cell transformation leads to the up-regulation of Fte-1/S3a. We suggest that EBNA-5 through binding may influence the growth promoting, differentiation inhibiting, or apoptosis regulating functions of Fte-1/S3a.

Keywords: Epstein-Barr virus; EBNA-5; Fte-1/S3a ribosomal protein; Cell transformation; Yeast two-hybrid system

Introduction

Epstein-Barr virus (EBV) is a lymphotropic gamma-herpes virus that infects more than 90% of the human population and targets B cells for infection. EBV causes B cell lymphomas in immunosuppressed hosts, such as transplant recipients (posttransplant lymphoproliferative disease, PTLD) and AIDS patients. EBV is associated with several malignancies, particularly with Burkitt lymphoma (BL) and nasopharyngeal carcinoma (NPC).

EBV infection transforms B cells into large immunoblasts that express six EBV-encoded nuclear antigens (EBNA-1-6), three membrane proteins (LMP-1, -2A, -2B) and two small non-polyadenylated RNAs (EBER-1-2). Five of them—EBNA-2, -3, -5, and -6 and LMP-1—are required for immortalization of B cells (for review, see Ref. [1]).

EBNA-5 (also called EBNA-LP) is, together with EBNA-2, the earliest viral protein expressed in freshly infected B cells [2,3]. These two proteins together can induce G_0 to G_1 transition in resting B cells [4]. It was shown that coexpression of EBNA-5 with EBNA-2 increases the transactivating power of EBNA-2 [5,6].

EBNA-5 is a nuclear phosphoprotein that is tightly associated with the nuclear matrix [7,8]. Several interaction partners of EBNA-5 have already been identified. The

* Corresponding author. Microbiology and Tumor Biology Center (MTC), Karolinska Institute, Nobels vag 16 Box 280, S-171 77 Stockholm, Sweden. Fax: +46 8 330498.
E-mail address: Elena.Kashuba@mtc.ki.se (E. Kashuba).

0014-4827/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.yexcr.2004.08.025

soluble, detergent-eluable fraction of EBNA-5 was found to be coimmunoprecipitated with the heat shock protein Hsp70 [9,10]. Flag-labeled EBNA-5 could precipitate HA95 (AKAP95, A-kinase anchoring protein 95), small ATP-independent chaperone Hsp27, prolyl 4-hydroxylase alpha subunit, alpha-tubulin, and beta-tubulin from the lymphoblastoid cell lysates [11]. Yeast two-hybrid screening identified the cytoplasmic Hax-1 protein [12,13] and HERR-1 (human estrogen-related receptor 1) [14] as binding partners.

We have recently found that EBNA-5 also binds to the nucleolar p14ARF protein, an upstream regulator of the p53 pathway [15]. Co-transfection of p14ARF and EBNA-5 led to accumulation of these proteins in the nuclear precipitates, where they were colocalized. EBNA-5 prolonged the survival of p14ARF-transfected cells.

We now report the identification of another human protein with partially nucleolar localization, Fte-1/S3a, as an EBNA-5 binding partner. This protein was first identified as v-*fos* transformation effector (Fte-1) [16]. Later, it was shown that Fte-1 is identical to the protein S3a, an important component of the small ribosome subunit. S3a is directly involved in eukaryotic protein synthesis by interacting with 3′ region of 18S rRNA, mRNA, initiation factors eIF-2 and -3, and elongation factors EF-1 and -2 (for review, see Ref. [17]). In this way, Fte-1/S3a plays a central role in the regulation of translation. In addition, the overexpression of Fte-1/S3a is associated with a rapid cell proliferation, maintenance of undifferentiated state, and proneness for apoptosis [18].

Materials and methods

Plasmids

Construction of the plasmids in GAL4-binding domain containing vector, BD (Clontech) BD-EBNA-1, BD-EBNA-3, BD-ΔEBNA-4, BD-EBNA-5 was described previously [19]. Cloning of GST2TK-EBNA-5, CMV-EBNA-5, and pBabe-puro-EBNA-5 was discussed in [20]. Construction of GFP-EBNA-5 was mentioned in Ref. [21]. All EBNA-5 constructs contained four W_1W_2 repeats and the unique C-terminal domain (Y). GST2TK-EBNA-5 mutants that contained the Y domain and a varying number of W_1W_2 repeats (1, 2, and 4), as well as a construct that lacked the Y but contained the four W_1W_2 repeats, were described in [20].

Mouse p53, lacking N-terminus, in pGBT9 (pVA3) and SV40 Large T-antigen in pGAD10 (pTD1, both from Clontech) were used as positive interaction controls.

The GFP-Fte-1/S3a was generated by inserting of the 835 bp long PCR product, digested in *Bam*H1/*Eco*RI sites into GFP vector, cleaved with *Bgl*I/*Eco*RI. GST2TK-Fte-1/S3a was created similarly, except that GST2TK was digested in *Bam*H1/*Eco*RI site. Primers for cloning and cloning strategy are described below.

Yeast strains and cDNA library screening

The *Saccharomyces cerevisiae* HF7c strain was used for library screening. SFY526 strain was used to confirm the interaction. Human lymphocyte MATCHMAKER cDNA library in pACT GAL-4 transcriptional activation domain vector and the yeast strains were obtained from Clontech. Library screening was run according the Clontech protocol. Interacting clones were selected on SD plates lacking His, Leu, and Trp. The fastest growing clones were further tested for β-galactosidase activity by ONPG test as described [22]. Specific activity of the given clones was calculated as percentage of β-galactosidase units of the positive control. The samples were incubated with ONPG at 30°C for 2 h.

Sequencing

Sequencing was done using capillary Apply BioSystem sequence machine (Perkin-Elmer).

PCR

PCR was carried using Idahotech thermocycler. 5′ primer for Fte-1/S3a cloning, sequence (5′ to 3′): TAATTGGATCCATGGCGGTTGGCAAGAACAAGCGCCTTACGAAAGGCGGCAAAAA 3′ primer for Fte-1/S3a cloning, sequence (5′ to 3′): CGTGAATCCGCCACTATTTGGAGTCTGAACTTTA.

Primers were obtained from GIBCO BRL.

First cycle was the following: 94°C for 4′, 65°C for 20′, and 72°C for 2′; 35 cycles of 94°C for 10″, 65°C for 20″, and 72°C for 40″ were run, ending with 72°C for 7′.

Cells and cell culture

MCF7 breast carcinoma cell line was cultured at 37°C, in Iscove medium containing 10% fetal bovine serum. Periodic staining with Hoechst 33258 (bisbenzimide) monitored the absence of mycoplasma. The cells were grown on the cover glass. We transfected cells with GFP-Fte-1/S3a, pBabe-EBNA-5, GFP-EBNA-5, and CMV-EBNA-5 constructs using Lipofectamine Plus Reagent (Life Technology) according to the manufacturer's protocol.

Tonsil B cells were isolated from human tonsils obtained from routine tonsillectomy (Karolinska Hospital, Stockholm). The tonsils were cut into the fragments and passed through a metal mesh. Mononuclear cells were isolated on Lymphoprep gradients. The two subsequent rounds of E-rosetting removed T cells. Cells were anchored onto glass slides using Cytospin cytocentrifuge.

GST pull-down assay

GST pull-down assay was performed as described [23]. GST-fusion proteins on beads were treated with RNAse A and DNAse. All of the cell lysates contained 0.5% of BSA