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Brief Communication

The agnoprotein of polyomavirus JC is released by infected cells: Evidence for Its cellular uptake by uninfected neighboring cells

Onder Otlu [1], Francesca Isabella De Simone [1], Yolanda-Lopez Otalora, Kamel Khalili, Ilker Kudret Sariyer *

Department of Neuroscience, Center for Neurovirology, Temple University School of Medicine, 3500 North Broad Street, 7th Floor, Philadelphia, PA 19140 USA

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ABSTRACT

Poliomavirus JC replicates in glial cells in the brain, and causes the fatal demyelinating disease, progressive multifocal leukoencephalopathy (PML). PML is usually seen in patients with underlying immunocompromised conditions, notably among AIDS patients and those on chronic immunosuppressive regimens. The late leader sequence of JC virus contains an open reading frame encoding a small regulatory protein called agnoprotein. Agnoprotein contributes to progressive viral infection by playing significant roles in viral replication cycle. Here, we demonstrate that agnoprotein can be detected in cell-free fractions of glial cultures infected with JCV, transfected with expression plasmids or transduced with an adenovirus expression system. We also provide evidence that extracellular agnoprotein can be taken up by uninfected neighboring cells. These studies have revealed a novel phenomenon of agnoprotein during the viral life cycle with a potential of developing diagnostic and therapeutic interventions.

Introduction

JC virus (JCV) is a human polyomavirus that infects greater than 50% of the human population during childhood, and establishes a latent/persistent infection for the rest of the life in healthy individuals (Weber, 2008; Moens and Johannessen, 2008). Replication of the neurotropic strain of JCV in glial cells causes the fatal demyelinating disease of the central nervous system, progressive multifocal leukoencephalopathy (PML), which is seen in patients with underlying immunocompromised conditions, notably HIV-1/AIDS (Safak et al., 2005; Berger and Concha, 1995; Miller et al., 1982). PML is the only viral demyelinating disease of the human brain characterized by lytic infection of oligodendrocytes (Safak et al., 2005; Berger and Concha, 1995; Padgett et al., 1971). Over the past few years, exogenous immunosuppressive treatments such as natalizumab, efalizumab, and rituximab have also been associated with PML in patients with autoimmune diseases, including Multiple Sclerosis, Crohn's Disease, Psoriasis, and Lupus (Ferenczy et al., 2012; Tavazzi et al., 2011). Like other polyomaviruses, the genome of JCV is composed of a double-stranded circular DNA genome of approximately 5 kb in size with a bi-directional non-coding control region that is located between the early and late coding sequences (Ferenczy et al., 2012). The early coding region is responsible for the expression of large T antigen (T-Ag), small t antigen (t-Ag), and a group of T′ proteins, which are produced upon alternative splicing of the early primary transcript. Similarly, alternative splicing of the late transcript results in production of the viral capsid proteins VP1, VP2, and VP3 which are essential for completion of the viral lytic cycle and formation of viral particles.

In addition to the capsid proteins, JCV encodes a small (71 aa long), regulatory, phosphoprotein, agnoprotein, from the late viral transcript. Agnoprotein forms highly stable dimers and oligomers (Saribas et al., 2011, 2013) and has an important role in viral DNA replication by enhancing T-Ag binding to the origin of replication (Saribas et al., 2012). The expression pattern of agnoprotein in tissue sections from PML shows localization to the cytoplasmic and perinuclear regions of infected glial cells (Okada et al., 2002). Recent observations also suggest that agnoprotein localizes to the endoplasmic reticulum, interacts with lipid membranes and may function as a viroporin (Suzuki et al., 2010, 2013). Furthermore, agnoprotein expression is required for the successful completion of JC virus life cycle, because mutant JC virus with a deletion in the agno gene is unable to propagate (Ellis et al., 2013, Sariyer et al., 2006, 2011a).

Because of its highly basic structure, co-localization with endoplasmic reticulum at the perinuclear area and its association with

* Corresponding author. Tel.: +1 215 707 6337; fax: +1 215 707 4888.
E-mail address: isariyer@temple.edu (I.K. Sariyer).
[1] These authors contributed equally.

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intracellular lipid membranes, we sought to investigate possible release of agnoprotein by infected cells. Our results have revealed the presence of extracellular agnoprotein in cell free supernatant fractions of infected cultures as well as in glial cell lines expressing agnoprotein in the absence of viral lytic infection.

Results

To determine the possible secretion of JC virus agnoprotein from infected cells, we first infected SVG-A human glial cell line with Mad-1 strain of JC virus. SVG-A cells were transfected with viral genome to initiate a uniform infection cycle and whole cell protein lysates were collected at 24 h intervals up to 10 days post-infection (dpi). Protein samples were processed for SDS-PAGE, transferred to nitrocellulose membranes and expression of VP1 and agnoprotein were determined by Western blot. As shown in Fig. 1A and B, VP1 expression was started at the second day post-infections, reached a peak at 4 dpi, and showed a dramatic decrease at 6 and 7 dpi that corresponded to the time of the completion of the first replication cycle (Sariyer et al., 2009). Similar to the VP1, agnoprotein expression was detectable as early as 2 dpi, reached a peak at 4 dpi, and stayed high until 6 dpi. Consistent with the expression of VP1, agnoprotein levels were barely detectable at 6 and 7 dpi. Interestingly, agnoprotein expression levels came back to peak levels at 9 dpi, followed by another sharp reduction in expression at 10 dpi. These experiments suggested that unlike the VP1 expression, agnoprotein showed a dynamic expression pattern during the replication cycle of the virus in glial cells. Next, we asked whether agnoprotein could be released by infected cells and its expression in the cells correlate with its release pattern during the course of viral replication cycle. The growth media from the cells were collected from the same infection studies presented in Fig. 1A, and processed for agnoprotein detection by immunoprecipitation followed by Western blot. Whole cell protein lysates from 9 dpi and 0 dpi (uninfected) were loaded as positive and negative controls of agnoprotein expression. As shown in Fig. 1C (upper panel), unlike to its cellular expression, agnoprotein was not detected in growth media earlier than 4 dpi (compare Fig. 1A and C). Surprisingly, a significant amount of agnoprotein was present in the growth media at 4 dpi when there were still peak levels of cellular expression (compare Fig. 1A lane 5 with C lane 6). At 6 and 7 dpi when agnoprotein cellular expression levels started to decrease, agnoprotein was still present in the growth media in significant amounts (compare Fig. 1A lanes 7 and 8 withC lanes 8 and 9). Interestingly, there were no detectable agnoprotein in growth media at 8 dpi when agnoprotein expression began to reappear in the cells. Agnoprotein was again present in the growth media in parallel to its peak expression at 9 dpi and stayed at a constant level in contrast to a drop in its cellular expression level at 10 dpi. To determine the time of viral release, the major capcid protein VP1 was also immunoprecipitated from the growth media of infected cells and analyzed by Western blotting (Fig. 1C lower panel). Unlike agnoprotein, VP1

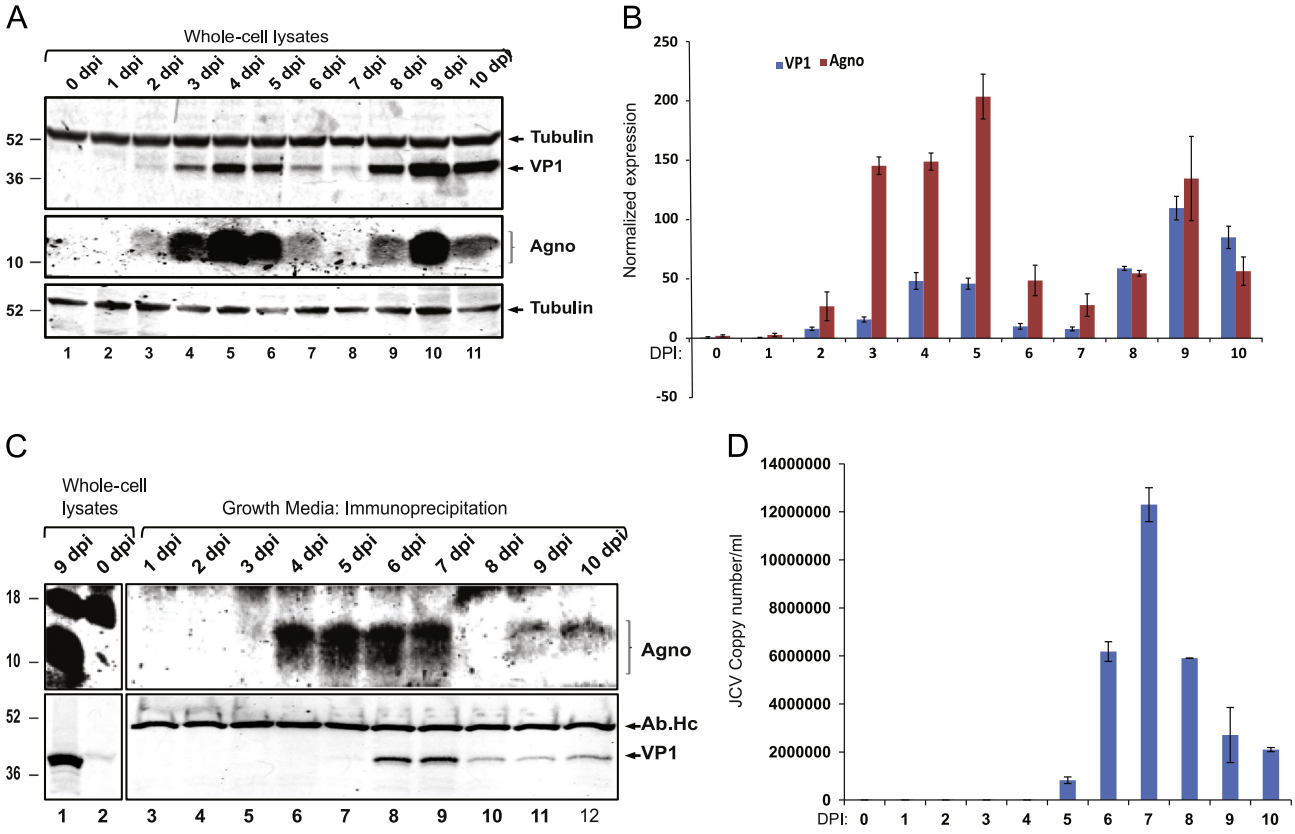

Fig. 1. Agnoprotein is detected in cell-free fractions of glial cells infected with JCV. A. SVG-A cells were transfected/infected with Mad-1 JCV as described previously (Sariyer and Khalili, 2011b; Uleri et al., 2013). Whole cell protein samples were prepared from cellular pellets with 24 h intervals up to 10 dpi. Expression of VP1 and agnoprotein were analyzed by Western blot. In lane 1, protein samples from uninfected SVG-A cells were loaded as negative control (0 dpi). Membranes were stripped and re-probed for β-tubulin as a loading control. dpi is an abbreviation for "days post-infection". B. Bar graph representation of normalized expression of VP1 and agnoprotein during JCV infection in glial cells. **C.** Immunoprecipitation of agnoprotein and VP1 from the growth medium of cells infected with JC virus. Immune complexes were separated by SDS-PAGE and processed for Western blot of agnoprotein and VP1. In lanes 1 and 2, whole-cell protein lysates from JCV-infected SVG-A cells (9 dpi) and from uninfected cells (0 dpi) were loaded as positive and negative controls of agnoprotein and VP1 expression, respectively. D. Q-PCR analysis of JCV copy number in growth medium. The growth medium of JCV-infected cells used in IP studies (panel C) were also subjected to Q-PCR analysis for the detection of viral loads as described in Materials and Methods. All experiments were carried out in triplicate. Images depict representative data.