Virology 443 (2013) 161–176

Contents lists available at SciVerse ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

Essential roles of Leu/Ile/Phe-rich domain of JC virus agnoprotein in dimer/oligomer formation, protein stability and splicing of viral transcripts

A. Sami Saribas [a], Magid Abou-Gharbia [b], Wayne Childers [b], Ilker K. Sariyer [a], Martyn K. White [a], Mahmut Safak [a,*]

[a] Department of Neuroscience, Laboratory of Molecular Neurovirology, MERB-757, Temple University School of Medicine, 3500 N. Broad Street, Philadelphia, PA 19140, United States

[b] School of Pharmacy, Temple University, Moulder Center for Drug Discovery Research-Room 515, 3307 N. Broad Street, Philadelphia, PA 19140, United States

ARTICLE INFO

Article history:
Received 18 January 2013
Returned to author for revisions
21 March 2013
Accepted 3 May 2013
Available online 6 June 2013

Keywords:
Polyomavirus JCV
SV40
BKV
Merkel cell carcinoma virus
Large T antigen
Agnoprotein
Transcription
DNA replication
Progressive multifocal leukoencephalopathy
Splicing
Stable dimer formation
Stable oligomer formation

ABSTRACT

Agnoprotein is one of the key regulatory proteins of polyomaviruses, including JCV, BKV and SV40 and is required for a productive viral life cycle. We have recently reported that agnoprotein forms stable dimer/oligomers mediated by a predicted amphipathic α-helix, spanning amino acids (aa), 17 to 42. Deletion of the α-helix renders a replication incompetent virus. Here, we have further characterized this region by a systematic deletion and substitution mutagenesis and demonstrated that a Leu/Ile/Phe-rich domain, (spanning aa 28–39) within α-helix is indispensable for agnoprotein structure and function. Deletion of aa 30–37 severely affects the dimer/oligomer formation and stable expression of the protein. Mutagenesis data also indicate that the residues, 34–36, may be involved in regulation of the splicing events of JCV transcripts. Collectively, these data suggest that the Leu/Ile/Phe-rich domain plays critical roles in agnoprotein function and thus represents a potential target for developing novel therapeutics against JCV infections.

Introduction

Proteins fulfill many complex functions through highly specific interactions with their binding partners. These interactions can occur between two identical proteins (homo-dimerization/oligomerization); or between two or more different proteins (hetero-dimerization/oligomerization). Such interactions provide the participant proteins specificity and diversity to perform their functions successfully in many different biological processes including DNA replication, gene transcription and regulation of different pathways.

Viruses are obligatory intracellular parasites and rely heavily on the mechanisms and functions provided by the host cells for their propagation. Some viruses encode a limited number of regulatory proteins and yet successfully complete their replication cycle, suggesting a multi-functional nature for these proteins. This multi-functionality mostly stems from their ability to form diverse but higher ordered dimeric, heteromeric or oligomeric structures. There are many examples of such structures among the viral proteins that have been reported in the literature, including human immunodeficiency virus 1 (HIV-1) proteins (Rev, Vpr, Vif and Vpu) (Bernacchi et al., 2011; Bourbigot et al., 2005; Daugherty et al., 2010a, 2010b; Lu et al., 2010), Hepatitis C virus nonstructural protein 4B (Gouttenoire et al., 2010), Ebola virus VP40 (Hoenen et al., 2010), and large T-antigen (LT-Ag) of polyomaviruses (Cuesta et al., 2010; Foster and Simmons, 2010). Recently, agnoprotein of polyomaviruses was also shown to operate in this fashion. We have reported the formation of highly stable dimeric/oligomeric structures by the agnoprotein of JC virus (JCV), BK virus (BKV) and simian virus 40 (SV40), and roughly mapped the region responsible for this phenomenon to amino acids (aa) spanning from 17 to 42 for JCV agnoprotein (Saribas et al., 2011).

JCV agnoprotein is a small (71 aa long), multifunctional, regulatory protein encoded by the late coding region of the viral

* Corresponding author. Fax: +1 215 707 4888.
E-mail address: msafak@temple.edu (M. Safak).

0042-6822/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.virol.2013.05.003

genome and was previously shown to interact with a number of cellular and viral proteins, including p53 (Darbinyan et al., 2002), YB-1 (Safak et al., 2002), FEZ1 and HP1-α (Okada et al., 2005), JCV small t-antigen (Sm t-Ag) (Sariyer et al., 2008) and JCV large T-antigen (LT-Ag) (Safak and Khalili, 2001). It has been implicated in many different aspects of the JCV life cycle, including viral transcription (Safak and Khalili, 2001), replication (Safak and Khalili, 2001), functioning as viroporin (Suzuki et al., 2010), encapsidation (Sariyer et al., 2006) and interfering with the process of exocytosis (Johannessen et al., 2011). In addition, this protein was found to deregulate cell cycle progression, where cells that stably express agnoprotein largely accumulate at the G2/M phase of the cell cycle (Darbinyan et al., 2002). Despite these previous reports and intense research, the regulatory function of agnoprotein in viral replication is not fully understood.

JCV agnoprotein shows a high degree of sequence identity and similarity to that of SV40 and BKV (Safak et al., 2001). For example, there is a 60% identity and a 79% similarity between JCV and SV40 agnoprotein sequences respectively, and 82% and 93% respectively between JCV and BKV agnoprotein (Altschuler, 1999). SV40 and BKV agnoprotein, like JCV agnoprotein, have also been previously implicated in different aspects of viral replication cycle (Alwine, 1982; Haggerty et al., 1989; Hay et al., 1982; Hou-Jong et al., 1987; Johannessen et al., 2008; Margolskee and Nathans, 1983; Moens et al., 2011; Myhre et al., 2010; Ng et al., 1985; Unterstab et al., 2010).

Expression of agnoprotein is required for the successful propagation of Orthopolyomaviruses, including JCV, BKV and SV40, because an agnoprotein null mutant of each virus is unable to sustain the replication cycle although mutant viruses were shown to be successfully released from the infected cells (Myhre et al., 2010; Sariyer et al., 2011). Most importantly, even the constitutive expression of LT-Ag, required for polyomavirus DNA replication, is not sufficient to sustain an efficient viral propagation cycle in the absence of agnoprotein, which further emphasizes the importance of agnoprotein in viral life cycle (Sariyer et al., 2011). Agnoprotein mostly resides in cytoplasm with high concentrations accumulating in the perinuclear region of the infected cells. However, a small amount of the protein is also consistently detected in the nucleus, indicating a possible nuclear function for agnoprotein. In fact, our recent DNA binding studies supported this idea demonstrating that agnoprotein stimulates the DNA binding activity of LT-Ag without directly interacting with DNA (Saribas et al., 2012). In addition, we have also recently reported that agnoprotein forms highly stable, SDS-resistant homodimers and oligomers; and the 17–42 aa region of the protein is responsible for this property (Saribas et al., 2011). While the 3D structure of agnoprotein has yet to be determined, computer modeling studies predict that the 17–42 aa, which is important for stable dimer/oligomer formation, is involved in forming an amphipathic α-helical structure (Saribas et al., 2011; Unterstab et al., 2010). This α-helical region contains a Leu/Ile/Phe-rich domain between aa 28 and 39. All three Phe residues (Phe31, Phe35 and Phe39) of JCV agnoprotein localize to this Leu/Ile/Phe-rich domain along with two negatively charged residues, Glu34 and Asp38. The Phe residues of JCV agnoprotein appear to be involved in viral DNA replication (Saribas et al., 2012). With respect to the function of Phe residues, the protein distribution studies with confocal microscopy showed that Phe39 residue of BKV agnoprotein may play a role in localization of the protein to the lipid droplets in infected cells (Unterstab et al., 2010). In addition to in vitro stable dimer/oligomer formation, we have recently reported to dimer formation in vivo in the infected cells (Saribas et al., 2011). Suzuki et al. (2010) have also demonstrated homodimer, homo-oligomer formation in vivo by JCV agnoprotein, by employing intermolecular fluorescence resonance energy transfer (FRET) and chemical cross-linking techniques.

All these studies strongly suggest that the role of agnoprotein in JCV replication cycle is critical and therefore in progressive multifocal leukoencephalopathy (PML), where JCV lytically infects the oligodendrocytes in the central nervous system (CNS). This ubiquitous human polyomavirus, JCV, infects a majority of the human population worldwide (70–80%). Initial infection by this virus is followed by a latency period. The reactivation of the virus mainly occurs in patients with immunosuppressive conditions (1–5%), including in those with cancer, organ transplant, and HIV-1/AIDS (Berger, 2011; Berger and Concha, 1995; Major, 2010; Major et al., 1992). In addition, a small percentage of patients with autoimmune diseases such as multiple sclerosis (MS) and severe psoriasis (Crohn's disease) who underwent an immunosuppressive therapy with specific monoclonal antibodies (such as natalizumab, rituximab and efalizumab) developed PML (Kleinschmidt-DeMasters and Tyler, 2005; Langer-Gould et al., 2005; Van Assche et al., 2005), suggesting that JCV is an important risk factor in these patients.

In this study, we further examined the region of JCV agnoprotein responsible for formation of highly stable dimers and oligomers using mutagenesis analysis. Our data demonstrate that aa from 30 to 37 are sufficient to confer the dimer/oligomer formation property of agnoprotein. Consistent with our in vitro mapping studies, functional studies demonstrated that the deletion mutants, partially or completely missing the Leu/Ile/Phe-rich domain, showed a significant decrease in viral replication compared to wild-type. In addition, transcriptional analysis studies with the mutants revealed interesting defects in splicing patterns of the viral late transcripts, suggesting a new novel function for agnoprotein in JCV gene regulation at the post-transcriptional level. Collectively, these findings demonstrate that the Leu/Ile/Phe-rich domain is critically important for the dimer/oligomer formation, stability and function of agnoprotein and thus represents a potential target for developing novel therapeutic agents for progressive multifocal leukoencephalopathy.

Results

Structural features of JC virus agnoprotein

Agnoprotein is one of the few regulatory proteins of JCV. It possesses a highly basic aa composition with dominant Arg (R) and Lys (K) residues, which are located at the amino and carboxy ends of the protein (Fig. 1A). However, the middle portion of the protein, encompassing aa from 28 to 39, is composed of mostly hydrophobic residues, including Ile, Leu and Phe. The two negatively charged residues Glu34 (E34) and Asp38 (D38) are also located within this region. It is also interesting to note that all three Phe residues (Phe31, Phe35 and Phe39) of JCV agnoprotein are evenly dispersed and confined within the Leu/Ile/Phe-rich domain (Fig. 1A). We have recently analyzed the functional importance of these Phe residues by site-directed mutagenesis and the results showed that they have a combinatorial effect on viral replication cycle (Saribas et al., 2012). The only cysteine residue of agnoprotein (Cys40) is found towards the middle portion of the protein right after the Leu/Ile/Phe-rich domain. The three dimensional (3D) structure of agnoprotein is yet to be resolved, however, computer modeling studies shown in Fig. 1B using the I-TASSER progam (Roy et al., 2010) suggest that the Leu/Ile/Phe-rich domain is involved in forming an amphipathic α-helix (Fig. 1B and Fig. 1C). The Leu/Ile/Phe-rich domain is highly conserved between agnoprotein of JCV, SV40 and BKV (Fig. 1D). Three D modeling studies also suggest that carboxyl terminal half of the protein encompassing aa from 40 to 71 as well as a small portion of the amino terminal region of the protein may adopt an