Available online at www.sciencedirect.com
ScienceDirect
Virology 371 (2008) 394–404
VIROLOGY
www.elsevier.com/locate/yviro

Feline immunodeficiency virus OrfA alters gene expression of splicing factors and proteasome-ubiquitination proteins

Magnus Sundstrom [a], Udayan Chatterji [b], Lana Schaffer [c], Sohela de Rozières [a], John H. Elder [a,*]

[a] *Department of Molecular Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, MB-14, La Jolla, CA 92037, USA*
[b] *Department of Immunology, The Scripps Research Institute, La Jolla, CA 92037, USA*
[c] *DNA Array Core Facility, The Scripps Research Institute, La Jolla, CA 92037, USA*

Received 10 August 2007; returned to author for revision 15 September 2007; accepted 28 September 2007
Available online 25 October 2007

Abstract

Expression of the feline immunodeficiency virus (FIV) accessory protein OrfA (or Orf2) is critical for efficient viral replication in lymphocytes, both *in vitro* and *in vivo*. OrfA has been reported to exhibit functions in common with the human immunodeficiency virus (HIV) and simian immunodeficiency virus (SIV) accessory proteins Vpr and Tat, although the function of OrfA has not been fully explained. Here, we use microarray analysis to characterize how OrfA modulates the gene expression profile of T-lymphocytes. The primary IL-2-dependent T-cell line 104-C1 was transduced to express OrfA. Functional expression of OrfA was demonstrated by *trans* complementation of the OrfA-defective clone, FIV-34TF10. OrfA-expressing cells had a slightly reduced cell proliferation rate but did not exhibit any significant alteration in cell cycle distribution. Reverse-transcribed RNA from cells expressing green fluorescent protein (GFP) or GFP + OrfA were hybridized to Affymetrix HU133 Plus 2.0 microarray chips representing more than 47,000 genome-wide transcripts. By using two statistical approaches, 461 (Rank Products) and 277 (ANOVA) genes were identified as modulated by OrfA expression. The functional relevance of the differentially expressed genes was explored by Ingenuity Pathway Analysis. The analyses revealed alterations in genes critical for RNA post-transcriptional modifications and protein ubiquitination as the two most significant functional outcomes of OrfA expression. In these two groups, several subunits of the spliceosome, cellular splicing factors and family members of the proteasome-ubiquitination system were identified. These findings provide novel information on the versatile function of OrfA during FIV infection and indicate a fine-tuning mechanism of the cellular environment by OrfA to facilitate efficient FIV replication.
© 2007 Elsevier Inc. All rights reserved.

Keywords: FIV; OrfA; Microarray; Spliceosome; Splicing factors; Proteasome; Ubiquitination; Affymetrix; Ingenuity

Introduction

FIV is a lentivirus associated with an AIDS-like syndrome in the domestic cat (Pedersen, 1993). Like HIV, FIV can be transmitted via mucosal exposure, blood transfer, and vertically via prenatal and post-natal routes (O'Neil et al., 1995, 1996; Obert and Hoover, 2000; Pedersen et al., 1987; Rogers and Hoover, 1998) and the primary target of infection is the $CD4^+$ T-cell. The overall genomic structure of FIV is markedly similar to HIV, although there are important distinctions (Olmsted et al., 1989; Phillips et al., 1990; Talbott et al., 1989). One such distinction is the lack of the transactivator gene, tat, and the presence of a short open reading frame termed OrfA. Translation of an approximate nine kDa protein encoded by this region occurs from a bicistronic mRNA that also encodes downstream Rev (de Parseval and Elder, 1999). The genomic location, size, and structural features of OrfA have many similarities to HIV Tat as well as to the L domain of visna virus, both of which demonstrate transactivating functions. In fact, OrfA has been shown to facilitate a net increase in translation of proteins whose expression is driven from the FIV long terminal repeats (LTRs) (de Parseval and Elder, 1999; Sparger et al., 1992; Waters et al., 1996). However, OrfA does not act via a TAR element, as is the case with HIV-1 Tat, and promotes a net increase in transcription/translation via mechanisms distinct from that of other lentiviruses (Chatterji et al., 2002; Gemeniano et al., 2003). Attempts to show direct interaction of OrfA with the FIV LTR proved negative (Chatterji et al., 2002) and the gene is dispensable for viruses adapted for propagation in

* Corresponding author. Fax: +1 858 784 2750.
E-mail address: jelder@scripps.edu (J.H. Elder).

0042-6822/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.virol.2007.09.039

adherent cell lines such as Crandell feline kidney cells (CrFK) and G355-5 cells (Phillips et al., 1990). However, OrfA is required for productive infection of the primary *in vivo* target cell, $CD4^+$ T-cells (Waters et al., 1996). Stable feline T-cell lines expressing OrfA can function *in trans* to complement an OrfA-defective FIV (this study; Gemeniano et al., 2003). Furthermore, cats inoculated with OrfA-mutated FIV clones had a greatly reduced plasma viremia (Pistello et al., 2002). Evidence has been presented that a 39-bp deletion in OrfA gives a 4-fold decrease in viral mRNA expression and a moderate decrease in Gag protein expression (Gemeniano et al., 2003). It has also been reported that OrfA may have relatedness to HIV-1 Vpr and is implicated in facilitating cell cycle arrest and virus release from the cell (Gemeniano et al., 2003, 2004). Overall, these findings suggest that OrfA may be a multi-functional protein, which would certainly be in keeping with the need for versatility, given the relatively small viral genome.

In the present report, we used genome array analysis to study the consequence of OrfA on pleiotropic cellular gene expression in T-cells. RNA was prepared from cells transduced with Mig-R1 vector expressing either green fluorescent protein (GFP) alone or both GFP and OrfA and analyzed by microarray analysis, using Affymetrix HU 133 Plus 2.0 chips. The results show many parallels with gene expression observed in HIV-infected cells (van 't Wout et al., 2003), with a down-regulated expression of factors reported to influence HIV-1 mRNA splicing. Furthermore, expressions of genes encoding ubiquitin-conjugating enzymes and proteasome subunits were identified as down-regulated in the OrfA-expressing T-cells.

Results

Generation and characterization of OrfA-expressing T-cells

In order to better understand the function of OrfA during FIV infection, we generated stable cell lines expressing GFP+/- FIV-PPR OrfA (Chatterji et al., 2002). These clones (termed 104-C1-GFP and 104-C1-GFP-OrfA) were established in the primary IL-2-dependent T-cell line, 104-C1. Flow cytometry analysis confirmed the GFP expression in 104-C1-GFP and 104-C1-GFP-OrfA to be >95% (data not shown). To assay for expression of functional OrfA, the cells were infected with FIV-34TF10, a strain deficient in functional OrfA protein (Phillips et al., 1990; Talbott et al., 1989). The presence of OrfA complemented FIV-34TF10 *in trans* and rescued viral replication in 104-C1-GFP-OrfA cells (Fig. 1A). In cells lacking OrfA (104-C1 and 104-C1-GFP), only background levels of reverse transcriptase activity was detected in the culture supernatant. In addition, gene expression of OrfA in 104-C1-GFP-OrfA cells, but not in 104-C1-GFP cells, was confirmed by Q-PCR (data not shown). The OrfA mutation at residue 44 in FIV-34TF10 changes the native Tryptophan (Trp) residue to a stop codon. Repair of this mutation restores wild-type infectivity on PBMCs and T-cell lines (Waters et al., 1996). Consistent with this finding, transduced 104-C1 cells expressing truncated OrfA-Stop failed to trans-complement FIV-34TF10 (Fig. 1B). An additional third base mutation was generated by alteration of the codon at residue 44 from Trp to Cys and the mutant OrfA-Cys was engineered into 104-C1 cells and FIV-34TF10. Interestingly, 104-C1-GFP-OrfA Cys was able to trans-complement the OrfA-defective FIV (Fig. 1B, left). However, placing this mutation in the context of FIV-34TF10 (termed 34TF10-Cys) did not generate wild-type levels of viral replication (Fig. 1B, right) until a back mutation to Trp appeared at approximately 16 days of culture (data not shown). These findings indicate that Cys at residue 44 is a less stable residue than Trp but that the latter residue is not mandatory for OrfA function. Low-level viral replication results in rapid reversion to Trp in the context of the virus as opposed to the genetically static state of the OrfA plasmid.

Further analyses of the stable 104-C1 clones revealed a small reduction in proliferation rate of the OrfA-expressing cells and in cells expressing only GFP (Fig. 2A). FIV-PPR OrfA has been reported to induce cell cycle arrest in the G2

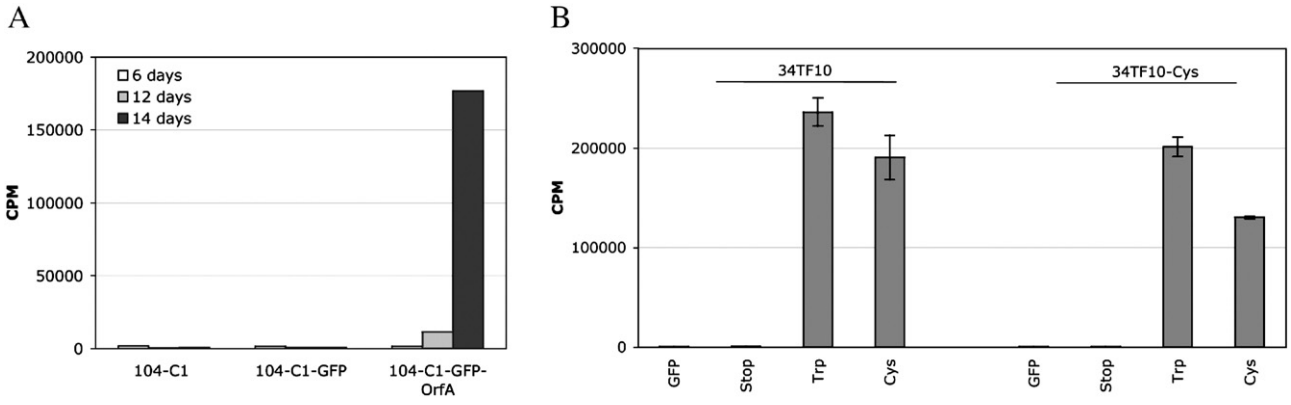

Fig. 1. Confirmation of functional OrfA expression by FIV-34TF10 infection of transfected 104-C1 cells and reverse transcriptase activity assay. (A) Expression of OrfA in 104-C1 cells (104-C1-GFP-OrfA) *trans* complements the OrfA-defective clone FIV-34TF10 and rescues viral replication. (B) 104-C1 cells were transfected to express GFP alone or GFP together with the following OrfA variants, stop: truncated OrfA due to a stop codon at position 44; Trp: OrfA with Trp at position 44; Cys: OrfA with a Cys at position 44. The transfected cells were infected with 34TF10 or 34TF10 with Cys at position 44 (34TF10-Cys) and analyzed for viral replication by RT activity assay at 14 days post-infection. Cells expressing OrfA with a Trp or Cys at residue 44 can rescue proliferation of 34TF10. However, 34TF10-Cys has a very low proliferation rate in 104-C1 cells unless OrfA-Trp or OrfA-Cys is expressed by the cell.

Download English Version:

https://daneshyari.com/en/article/3426337

Download Persian Version:

https://daneshyari.com/article/3426337

Daneshyari.com