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# ADAR1 is a novel multi targeted anti-HIV-1 cellular protein

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ABSTRACT

We examined the antiviral activity of ADAR1 against HIV-1. Our results indicated that ADAR1 in a transfection system inhibited production of viral proteins and infectious HIV-1 in various cell lines including 293T, HeLa, Jurkat T and primary CD4+ T cells, and was active against a number of X4 and R5 HIV-1 of different clades. Further analysis showed that ADAR1 inhibited viral protein synthesis without any effect on viral RNA synthesis. Mutational analysis showed that ADAR1 introduced most of the A-to-G mutations in the *rev* RNA, in the region of RNA encoding for Rev Response Element (RRE) binding domain and in *env* RNA. These mutations inhibited the binding of *rev* to the RRE and inhibited transport of primary transcripts like *gag, pol* and *env* from nucleus to cytoplasm resulting in inhibition of viral protein synthesis without any effect on viral RNA synthesis. Furthermore, ADAR1 induced mutations in the *env* gene inhibited viral infectivity.
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## Introduction

ADAR1 (Adenosine Deaminase Acting on RNA 1), an RNA editing enzyme, catalyzes hydrolytic deamination of adenosine to inosine (A-to-I) in completely or partially double-stranded RNA (Hough and Bass, 1994; Kim et al., 1994; O'Connell and Keller, 1994; Patterson and Samuel, 1995). This has a number of effects. The A-to-I substitution affects pre-mRNA splicing by changing the splicing site and decreases the binding of RNA to protein resulting in functional changes of the protein (Bass, 2002; Samuel, 2003). Furthermore, during mRNA translation, Inosine is recognized as Guanosine and therefore it incorporates incorrect amino acid sequence (Bass, 2002; Samuel, 2003). Because of these pluripotent properties of ADAR1, it disrupts replication of a number of RNA viruses. ADAR1 inhibits replication of hepatitis delta virus by editing of adenosine to inosine in pre-mRNA of hepatitis delta virus which eventually affects splicing of untranslated regions (Patterson and Samuel, 1995; Polson et al., 1996; Sato et al., 2001). ADAR1 was also known to edit double-stranded RNA found in the measles virus, which inhibited virus assembly and release from cells, leading to a persistent infection and development of fatal neuropathic measles infection (Horikami and Moyer, 1995). Taylor et al. (2005) showed that ADAR1-induced viral RNA editing inhibited Hepatitis C viral replication (Taylor et al., 2005). Recently Suspene et al. have shown ADAR1 induced mutation in seasonal influenza and attenuated measles viruses.

Since HIV-1 genome has several putative double stranded secondary RNA structures throughout its genome, HIV-1 RNA was considered a potential target for ADAR1. Therefore, in this report we investigated the antiviral effect of ADAR1 on HIV-1. We provided evidence that ADAR1 inhibited HIV-1 protein synthesis and viral infectivity in a variety of cells and against HIV-1 of different tropisms and different clades. We further demonstrated that such antiviral activity was at the post transcriptional stage of HIV-1 replication and that ADAR1-induced mutation at the *rev* and *env* RNA may be responsible for such posttranscriptional inhibition of viral protein synthesis. In elucidating the mechanism of ADAR1 induced inhibition of HIV-1 protein synthesis we found that ADAR1 induced A-to-G mutations in *rev* inhibited its transport activity of primary transcripts *gag, pol* and *env* from the nucleus to cytoplasm and thereby inhibited viral protein synthesis without any effect on viral RNA synthesis. ADAR1-induced mutations in the *env* gene further attenuate viral infectivity.

## Results

### ADAR1 inhibits HIV-1 protein synthesis and viral infectivity

In order to evaluate the effect of ADAR1 on HIV-1 production, 293 T cells were co-transfected with 0.1 μg pNL4.3 HIV-1 DNA and different amount of ADAR1 DNA. In each transfection assay (this one and subsequent ones), cells were also co-transfected with a luciferase-expressing plasmid DNA to control transfection efficiency. The expression of ADAR1 p150 from transfected ADAR1 DNA was analyzed by Western Blot and normalized against β-actin loading control to show the relative intensity of ADAR1 p150 expression (Supplemental

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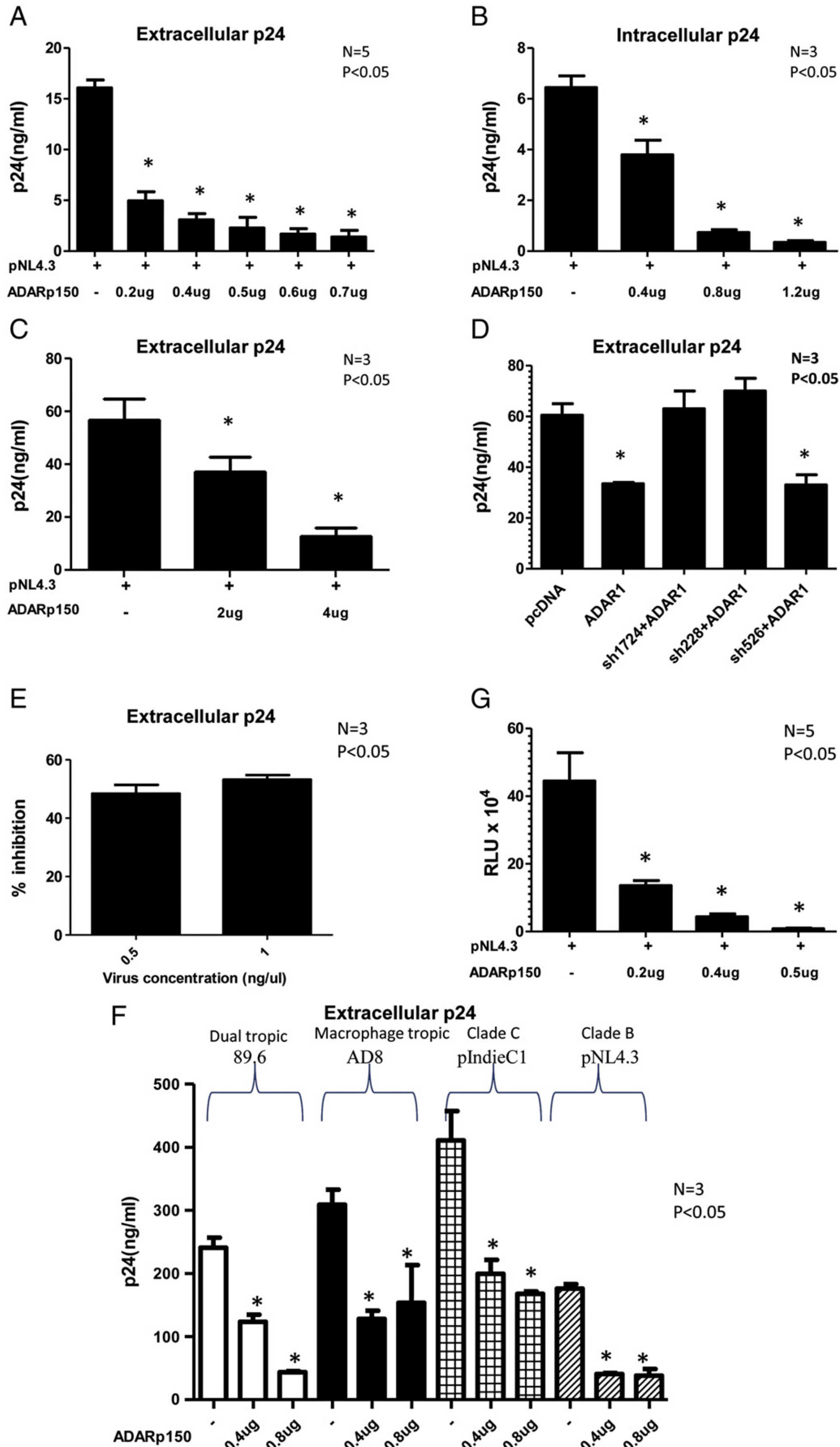
Fig. 1A). Following 48 h of transfection, viral protein synthesis was quantified by measuring HIV-1 p24 in culture supernatant and intracellular HIV-1 p24 production in a cell extract by ELISA. ADAR1 inhibited extracellular (Fig. 1A) and intracellular (Fig. 1B) HIV-1 p24 production in a dose dependent manner. With 0.7 μg of ADAR1 containing plasmid there was an 8 fold reduction of extracellular HIV-1 p24 production in



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