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The HIV-2 TAR RNA domain as a potential source of viral-encoded miRNA. A reconnaissance study.

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ABSTRACT

Recently, it has been reported that HIV-1 TAR RNA element releases functionally competent miRNAs upon processing by Dicer enzyme. Here, we extend the analysis of miRNA viral-encoding potential to the TAR RNA of the HIV-2. Using *in vitro* Dicer cleavages and computer-aided analysis we have found that the 124-mer TAR RNA domain, present at the 5' end of HIV-2 mRNAs, putatively encodes pre-miRNAs. When deduced sequences of the viral-encoded miRNAs were matched against the database of human mRNA 3'-UTRs, it appeared that two miRNA candidates may target a large number of cellular transcripts.

INTRODUCTION

RNA interference is a general mechanism for gene regulation guided by small RNA molecules. The mechanism also embraces an intrinsic host cell defense machinery against viruses. Not surprisingly, the therapeutic applications of RNA interference against HIV are studied intensively. On the other hand, it appeared that genomes of human viruses, such as Epstein-Barr virus (EBV) (1) and other DNA tumor viruses (2), also evolved to exploit the miRNA pathway for its replicative benefit. However, the question whether retroviruses such as the HIV-1 can supply functional miRNAs has raised a dispute (3-5).

The TAR RNA domain found at the 5' end of HIV mRNAs plays a key role in the trans-activation of viral transcription, as it is the target for the Tat protein and several cell factors. It is well known that latent cells, characterised by a low level of viral transcription, produce high levels of abortive RNA transcripts that contain TAR RNA structures. Very recently, two reports have shown that the HIV-1 TAR RNA element, a hairpin structure of ~50 nucleotides, is recognized by the RNAi machinery and processed by the Dicer enzyme to create viral-encoded miRNA (6,7). In the case of HIV-2, the TAR RNA domain (TAR-2) is much larger and encompasses 123 nucleotide residues.

Recently, we have reported (8) that the TAR-2 element exists *in vitro* in two global, alternative forms: a new, extended hairpin form with two conformers (E1 and E2) and the previously proposed branched hairpins form (B).

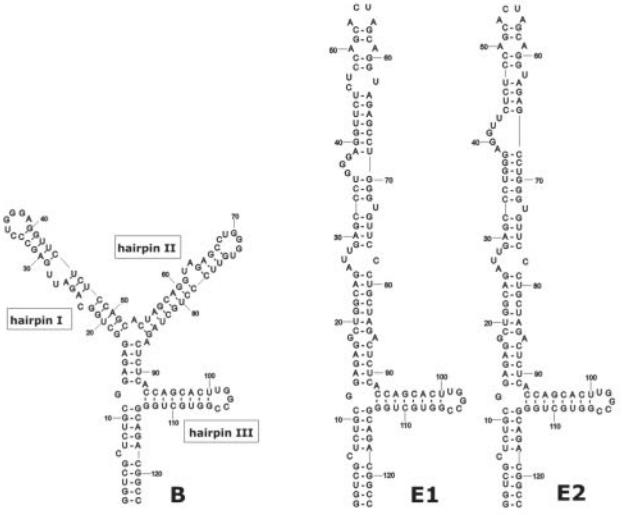

Fig. 1. Global forms of the HIV-2 TAR domain: branched (B) and extended E1 and E2 (see ref. 8).

We have been interested in examining whether TAR-2 RNA, due to the similarity of the 2D structure of its extended conformers to the pre-miRNAs, can also act as a source of viral-encoded miRNA. Here, we present the results of our reconnaissance study on the miRNA-encoding potential of the HIV-2 TAR RNA.

RESULTS AND DISCUSSION

To analyse the accessibility of the TAR-2 RNA structure to the Dicer enzyme, two 124-mer RNA transcripts were prepared as described (8): (i) of the TAR-2 (wt) sequence, which undergoes an equilibrium between the B and both E1 and E2 forms, and (ii) the TAR-2 mutant (A21) which prefers the extended form with two conformers: E1 and E2. Both transcripts bind the Dicer protein (Ambion) as revealed by EMSA on poliacrylamide gels at 4°C (data not shown). As presented on Fig. 2A, the 124-nt TAR-2 RNA (wt) is cleaved by Dicer to produce 23-25-nt duplexes as potential miRNA / miRNA*. Their positions, deduced from the cleavage patterns obtained for the 5′ end or 3′ end ^{32}P-labelled transcripts are well explained by the presence of E1 and E2 conformers (Fig. 2C). Surprisingly, strong cleavage at C33 is also present, most probably due to the TAR-2 wt in the B form. This finds its confirmation in the

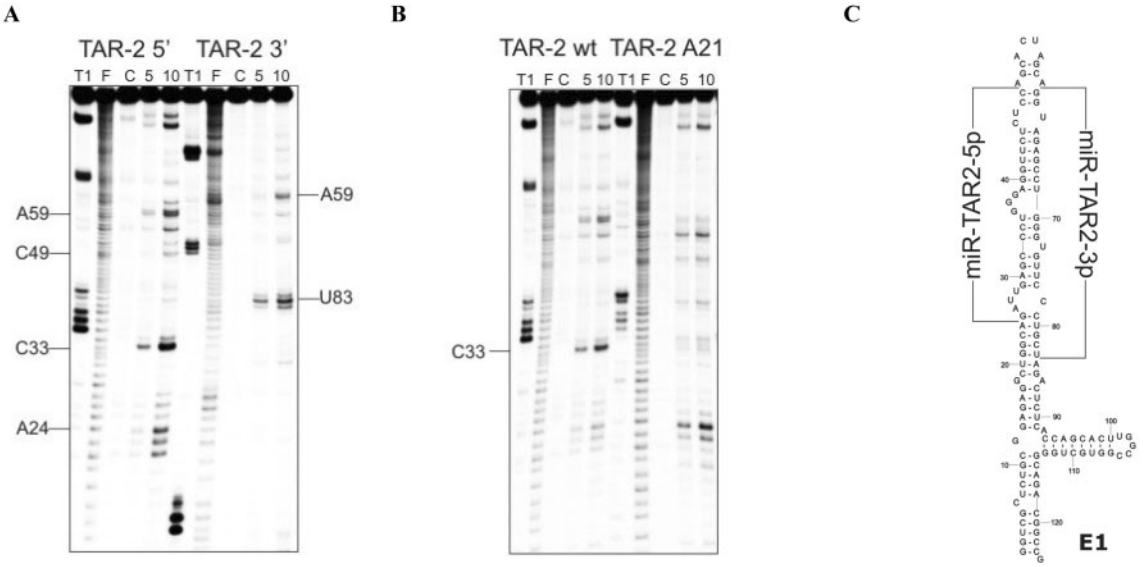

Fig. 2. Reactions of the 5′ or 3′ end-labelled TAR-2 wt (**A**) and 5′ end-labelled TAR-2 wt and A21 mutant (**B**) transcripts with Dicer (Ambion). RNA was incubated with Dicer for periods of time (min.) specified above the lines. Lane C represents the control sample with untreated RNA; lane F, formamide ladder; lane T1, limited hydrolysis with RNase T1. (**C**) Position of the viral-encoded miRNA / miRNA* candidate presented on the 2D structure of the TAR-2 RNA extended conformer E1.

fact that for the TAR-2 A21 mutant, Dicer cleavages in the proximity of this position were barely seen. The susceptibility of the TAR-2 branched form to Dicer requires further investigation.

Our experimental results were complemented with a computer-aided analysis focused on pre-miRNA and miRNA. Application of the MiRAlign web server (http://bioinfo.au.tsinghua.edu.cn/miralign) indicated a partial homology of the full-length TAR-2 sequence with two pre-microRNAs (hsa-mir-96 and cel-mir-272). A phylogenetic comparison of the TAR RNA sequences of different HIV-2 isolates comparable in length has shown that the seed sequence of the miRNA derived from the 3' arm of the TAR-2 extended form (abbreviated: miR-TAR2-3p; Fig. 2C) is highly conserved. For the miRNA strand derived from the 5' arm (abbreviated: miR-TAR2-5p; Fig. 2C) the seed sequence was less conserved. Subsequently, we have matched both candidate sequences against the database of 3'-UTRs from the human mRNAs using the miRTar prediction server (http://mirtar.mbc.nctu.edu.tw/). This search revealed that the miR-TAR2-3p exhibits typical seed complementarity with thousands of human UTR's. Importantly, many of the predicted targets have multiple matching regions. For the miR-TAR2-5p, there were only about 200 hits predicted and the accuracy was much lower.

In vivo studies will follow to verify experimentally the expression of our candidate miRNAs in HIV-2 infected cells.

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