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The accumulation of the basic domain of HIV-1 Tat protein in the nuclei and the nucleoli is different from the accumulation of full-length Tat proteins

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Aim. Protein fragments coding for nuclear (NLS) and/or nucleolar (NoLS) localization signals are often used for the investigation of the mechanisms of protein accumulation inside the nuclei and the nucleoli, but it is possible that accumulation mechanisms in full-length proteins will be different. Methods. Here, we compared the nuclear and nucleolar accumulation of HIV-1 Tat protein and its basic domain containing both NLS and NoLS. Results. The pattern of accumulation of the basic domain of HIV-1 Tat protein in the nuclei and the nucleoli is different from that of full-length Tat proteins: the basic domain is accumulated weaker inside the nuclei, but stronger in the nucleoli as compared to the full-length protein. Conclusion. The molecular mechanism of nuclear and nucleolar accumulation of full-length Tat protein might be different from that of the Tat protein fragments.

Keywords: nucleus, nucleolus, NLS, NoLS, HIV-1 Tat.

Introduction

The human immunodeficiency virus-1 (HIV-1) produces a small transcriptional activator protein known as a trans-activator of transcription (Tat) which regulates the transcription of viral genes [1, 2] and modulates cellular processes by the interaction with different cellular structures, in particular, inside the cell nucleus [3]. The data concerning intranuclear localization of Tat protein are controversial. Tat protein expressed in animal cells is accumulated inside the nucleoli [4, 5], but, in stably transfected Jurkat cell, Tat protein was localized in the nucleoplasm [6]. It is possible that different localization depends on the nuclear concentration of Tat. Indeed, in stably transfected HeLa cells, Tat-GFP expressed at a low level was localized only in the nucleoplasm, whereas its overexpression resulted in the nucleolar accumulation [7].

The mechanisms of Tat protein accumulation in the nuclei and in the nucleoli are poorly understood. The cell nucleus contains a large number of substructures (nuclear bodies), within which various processes associated with the functioning of the genome are compartmentalized [8]. It was shown that the nuclear accumulation might depend on the presence of the short sequences in the protein, known as nuclear localization signals (NLSs) [9]. The nucleolar accumulation might be determined by the presence of nucleolar localization signals (NoLSs) [10]. The majority of experimental data concerning the mechanisms of Tat accumulation in the nucleus and the nucleolus are obtained using the chimeric pro-
The accumulation of the basic domain of HIV-1 Tat protein in the nuclei and the nucleoli is different from the accumulation of the proteins, which consist of the studied sequence and the marker protein (e.g., EGFP). It is possible that accumulation mechanisms in the full-length proteins will be different. In the present work, we have demonstrated that the pattern of accumulation of Tat protein in the nucleus and the nucleolus is different from that of its basic domain although this domain codes both NLS and NoLS.

Materials and Methods

To construct Tat-EGFP and EGFP-Tat, the pGST-Tat 1 86R plasmid obtained through the NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH from Dr. Andrew Rice [11] was used. Full-length Tat was PCR amplified with Pfu polymerase (Life Technology) using the following oligonucleotides: 5’-AGTCAA GCTTACCATGGAGCCAGTAGAT CCTAG-3’ and 5’-AGTCGGATCCGCTTCCTTCG GGCCTGTCGGGT-3’ (for Tat-EGFP); 5’-ACTG AAGCTTATA GGAGCCAGTAGATCCCTAGACT AG-3’ and 5’-A CTGGGATCCATTTCTCTCAGG CCTGTCGG-3’ (for EGFP-Tat). The amplified PCR products were digested with BamHI and HindIII and inserted into the EGFP-N1 or EGFP-C1 vector (Clontech). To construct plasmid coding for the basic domain of Tat protein fused to EGFP (EGFP-Tatbd), the oligonucleotides 5’-AGCTAGGAAGAGCGGAGACAG CGACGAAGAG-3’ and 5’-GAT CCTCTTCGGCCTGCGCTTTCTTTCTT-3’ were annealed and ligated into a HindIII- and BamHI-digested pEGFP-C1 vector (Clontech).

HeLa cells were grown in Dulbecco’s modified Eagle’s medium supplemented with L-glutamine, 8 % fetal calf serum (HyClone) and antibiotic/antimycotic solution (Gibco). Cellular transfection was performed using TurboFect reagent (Life Technology) according to the manufacturer’s instructions. Images of at least 50 living cells expressing fusion proteins were acquired in two different experiments using an LSM510 confocal laser scanning microscope (Carl Zeiss). To evaluate the nuclear and nucleolar accumulation efficiency, the mean fluorescence intensity in the nucleolus, in the nucleoplasm and in the cytoplasm was measured using ImageJ2 software. The ratio of nucleolar EGFP concentration to nucleoplasmic EGFP concentration has previously been referred to as the NoLS activity [12]. The ratio of cytoplasmic EGFP concentration to nucleoplasmic EGFP concentration is referred to as NLS activity.

Results and Discussion

In order to characterize the accumulation of Tat protein in the nucleus and nucleolus, plasmids encoding Tat protein fused with EGFP (Tat-EGFP and EGFP-Tat) were constructed. The basic domain of Tat protein (RKKRRQRRR) contains both NLS [4, 13–17] and NoLS [4, 17]. We constructed a plasmid coding for the basic domain of Tat protein fused to EGFP (EGFP-Tatbd). EGFP, a biologically inert protein weakly interacting with cell structures was used as a control. The plasmids encoding these proteins and the marker nucleolar protein (B23-TagRFP) were transfected into HeLa cells, and the protein localization was analyzed in vivo using a confocal microscope.

The images of cells expressing the chimeric proteins are shown in Figure 1. EGFP was distributed throughout the cell, with the exception of the nucleoli, in which the EGFP concentration was reduced. Tat-EGFP and EGFP-Tat were accumulated in the nuclei and the nucleoli; their concentration in cytoplasm was significantly reduced. EGFP-Tatbd was preferentially accumulated in the nucleoli as compared to the full-length Tat protein.

To estimate the accumulation, we measured the EGFP fluorescence levels in the cytoplasm, nucleoplasm and nucleoli, and determined the efficiency of accumulation of the studied proteins in the nucleoplasm (NLS activity) and the nucleolus (NoLS activity). A quantitative analysis indicates that both Tat-EGFP and EGFP-Tat were more intensively accumulated in the nucleoplasm as compared to EGFP-Tatbd (Fig. 2, A). In the case of nucleolar accumulation, the situation was reverse: EGFP-Tatbd was accumulated in the nucleoli stronger, compared with the full-length Tat protein (Fig. 2, B).

These differences may be connected with the mechanism of nuclear and nucleolar accumulation. It was described that Tat protein accumulates in the nucleus through the interactions with nuclear components, possibly with RNA [18]. This interaction may require the
Fig. 1. Localization of chimeric proteins (green) and nucleolar protein B23-TagRFP (red) in living HeLa cells. Bars – 5 μm. The distributions of coexpressed proteins were analyzed in living HaLa cells using confocal microscope. B23-TagRFP was used as a marker of nucleolus.
The accumulation of the basic domain of HIV-1 Tat protein in the nuclei and the nucleoli is different from the accumulation of the flanking sequences or the particular three-dimensional configuration of the basic domain that can occur only in the full-length protein.

Using the NoLS of Tat protein fused with EGFP, we have previously demonstrated that the electrostatic interaction of NoLS with the nucleolar components may lead to the nucleolar accumulation [19]. The charge of the basic domain can be partially compensated by the nearest amino acid residues within the full-length protein, or the basic domain can be less accessible to interact with components of the nucleoli.

Thus, the data obtained using the protein fragments might be different from those obtained by the analysis of full-length proteins. One cannot exclude that the mechanisms of nuclear or/and nucleolar accumulation might be also different in these two experimental systems, and some data indicate that the latter situation is quite possible. For example, it was described that nucleolar accumulation of the Tat protein may be due to its interaction with the nucleolar protein B23 [15], but NoLS of the Tat protein rather interacts with the nucleolar RNAs [19]. The reinvestigation of nuclear and nucleolar accumulation of proteins using full-length proteins might lead to principally different results and reveal novel molecular mechanisms.

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