Engineering of Ribosome-inactivating Proteins for Improving the anti-HIV Efficacy

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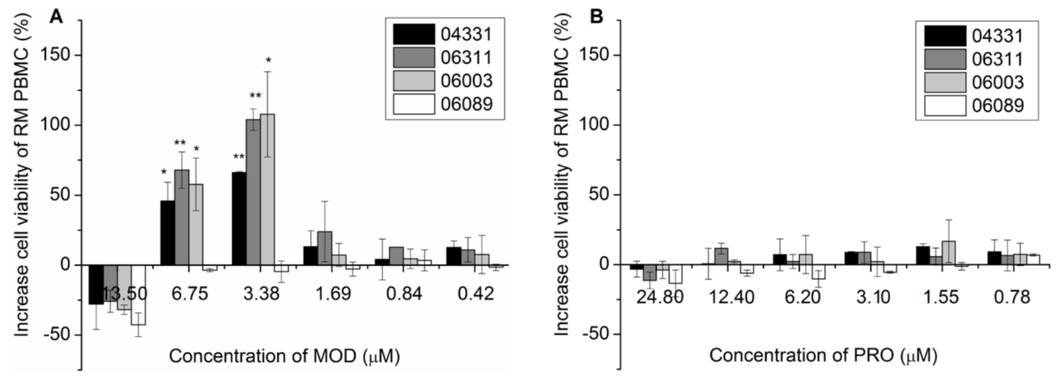
Introduction

Ribosome-inactivating proteins (RIPs) are N-glycosidases. They depurinate A-4324 in rat 28S ribosomal RNA in the conserved α-sarcin/ricin loop (α-SRL) and cease protein synthesis. Our group has shown that the internal peptide of the maize RIP precursor reduced the anti-HIV activity of the protein in infected macaque peripheral blood mononuclear cells (PBMC) and SHIV 89.6-infected Chinese rhesus macaque. We made use of the switch-on mechanism of maize RIP to incorporate HIV-1 protease recognition sequences to its internal inactivation region. Upon activation of this engineered maize RIP by HIV-1 protease in HIV-infected cells, the N-glycosidase activity and inhibitory effect on p24 antigen production in vitro and in infected human T cells were enhanced. This switch-on mechanism can also be applied to ricin A chain (RTA). RTA variants with HIV-1 protease recognition sequence at the C-terminus can be cleaved both in vitro and in HIV-infected cells. Furthermore, its antiviral effect was enhanced and the cytotoxicity towards uninfected cells was reduced. Our study provides a platform technology in creating protein toxin derivatives with increased pathogen-specific cytotoxicity.

Results

Table 1. Cytotoxicity and antiviral activities of maize ribosome inactivating protein (RIP) variants tested on rhesus macaque peripheral blood mononuclear cells (PBMC).

RIP variants	Cytotoxicity CC_{50} (μM)	p27 Antigen reduction EC_{50} (μM)	
	Uninfected	SHIV89.6	SIVmac239
His-TAT-Pro	>15	>24.85	>24.85
His-TAT-MOD	8.98 ± 0.36	5.53	11.23



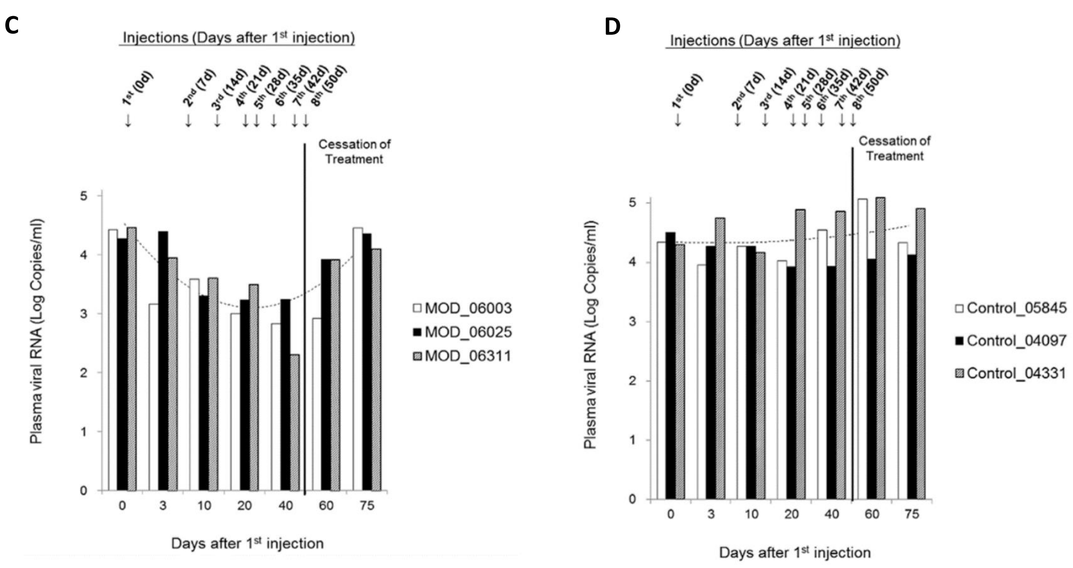
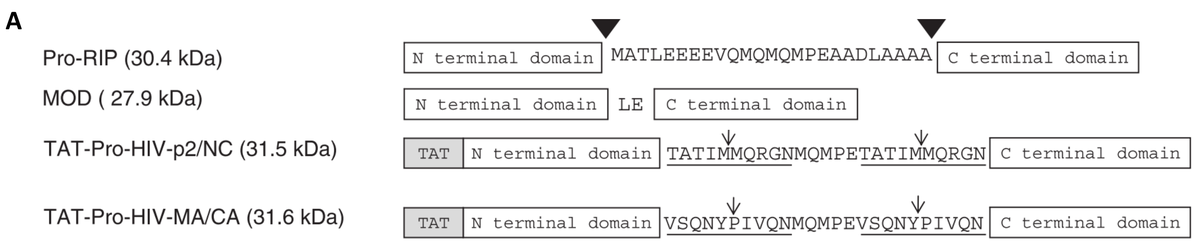


Figure 1 Antiviral Activities of Maize RIP in Rhesus Macaques

Protection for simian-human immunodeficiency virus (SHIV)-induced lysis on SHIV89.6-infected macaque PBMC upon treatment of (A) His-TAT-MOD; and (B) His-TAT-Pro. The experiment was repeated three times and mean ± SD was calculated for graphic presentation. Paired T-test was used for statistical analysis (* $p < 0.05$, ** $p < 0.01$). Plasma SIV viral load in rhesus macaques treated with (C) His-TAT-MOD; and (D) normal saline as negative control. Each group consisted of three macaques and "---" represented trend-line plotted using the average value from three individuals.



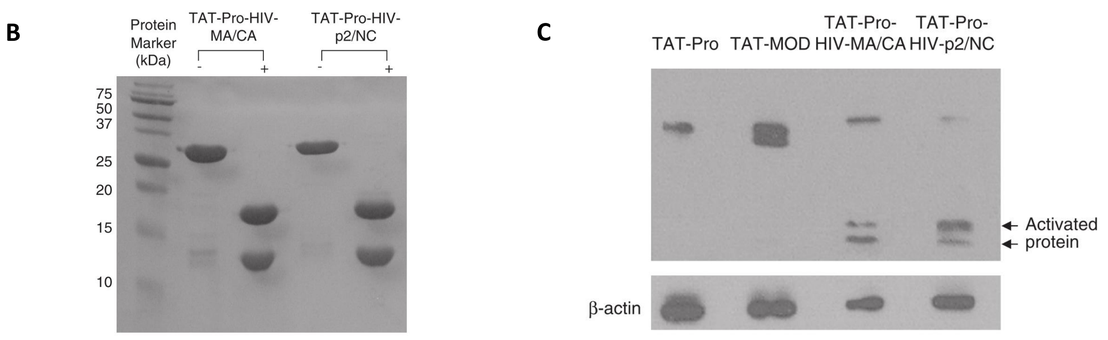


Figure 2 A switch-on mechanism to activate maize ribosome-inactivating protein for targeting HIV-infected cells

(A) Schematic diagram of the maize RIP and recombinant variants. **(B)** Cleavage of TAT-fused maize RIP variants by HIV-1 protease. TAT-Pro-HIV-MA/CA and TAT-Pro-HIV-p2/NC were completely cleaved by HIV-1 protease in vitro. **(C)** HIV-1IIIB acutely infected C8166 cells (1×10^6) were incubated with protein samples (0.4 mg in a volume of 2 ml) for 72 h and immunoblotted with anti-MOD polyclonal antibodies specific for Pro-RIP and its cleavage fragments (~11 and 17 kDa).

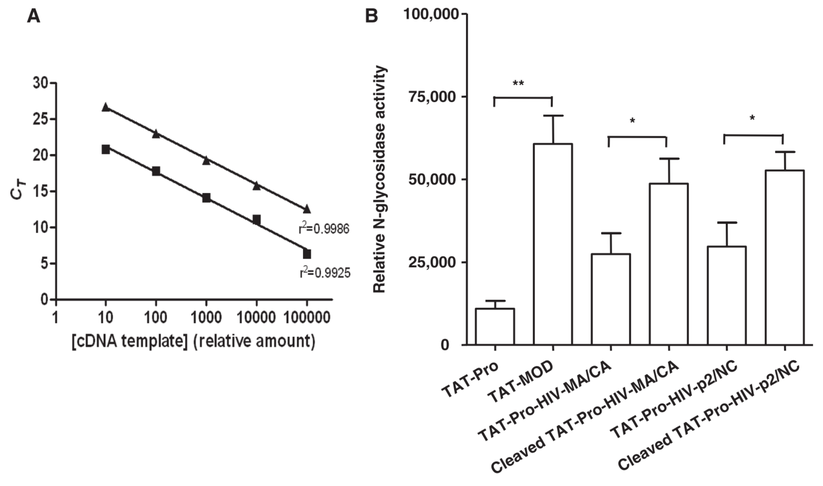


Figure 3 *In vivo* N-glycosidase activity of TAT-fused maize RIP and variants in mouse macrophage 28S rRNA (J774A.1)

During first-strand cDNA synthesis, reverse transcriptase preferentially inserts an adenine at the site of depurination, resulting in a T to A transversion in sequencing reads. N-glycosidase activity was determined by qPCR using primers that target the modified site. **(A)** qRT-PCR efficiency test for primer pairs. **(B)** Relative N-glycosidase activity of the TAT-fused maize RIP and variants. The relative N-glycosidase activity was calculated as the relative amount of altered rRNA of sample-treated cells over the untreated cells and mean±SEM was calculated for the graphic presentation. Unpaired t-test was performed for statistical analysis (n=6) (*P<0.05, **P<0.01).

A

RTA-WT	RTA-WT			
RTA-C10	RTA-WT	VSQNY/PIVQN	His	
RTA-C10V	RTA-WT	VSQN**HH**IVQN	His	
RTA-C25	RTA-WT	VSQNY/PIVQN *MQMPE* VSQNY/PIVQN	His	
RTA-C25V	RTA-WT	VSQN**HH**IVQN *MQMPE* VSQN**HH**IVQN	His	

B

	Uninfected	HIV-1_{IIIB}-infected	
	Cytotoxicity CC_{50} (μM)	P24 antigen reduction EC_{50} (μM)	Syncytial reduction EC_{50} (μM)
RTA-WT	6.56 ± 0.75	0.46 ± 0.02	0.51 ± 0.05
RTA-C10	>10	1.27 ± 0.11	4.03 ± 0.09
RTA-C10V	>10	3.63 ± 0.44	7.09 ± 0.07
RTA-C25	>10	0.97 ± 0.10	3.82 ± 0.24
RTA-C25V	>10	3.09 ± 0.15	6.11 ± 0.08

Figure 4 Engineering a switch-on peptide to ricin A chain for increasing its specificity towards HIV-infected cells

(A) Design and preparation of RTA variants. RTA-C10 and RTA-C25 were generated by inserting one and two HIV-1 specific sequences respectively at C-terminus of RTA in prior to His-tag. RTA-C10V and RTA-C25V are the corresponding non cleavable counterparts with two middle residues of sequence modified to HH (bolded). **(B)** Cytotoxicity and antiviral activity of C-terminal variants in uninfectedandHIV-1IIIB acutely infected C8166 cells. The values are presented as means ± SD (n = 6).

Conclusion

In this study, we found that the recombinant active maize RIP protected chimeric simian-human immunodeficiency virus (SHIV) 89.6-infected macaque peripheral blood mononuclear cells from lysis *ex vivo* and transiently reduced plasma viral load in SHIV89.6-infected rhesus macaque model. Besides, a switch-on strategy was applied on maize RIP and RTA based on the incorporation of HIV-1 protease recognition sequences to their internal inactivation region for activating the enzymatic activity of maize RIP in target cells, which provides a platform for combating pathogens with a specific protease.

References

Wang, R.R., et al. The recombinant maize ribosome-inactivating protein transiently reduces viral load in SHIV89.6 infected Chinese Rhesus Macaques. Toxins (Basel) 2015, 7, 156-169