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BIOCHEMISTRY, BIOPHYSICS, AND MOLECULAR BIOLOGY

Relationship between the Expression Level of PSMD11 and Other Proteasome Proteins with the Activity of Ricin and Viscumin

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Abstract —The role of proteasome proteins and proteins of the ERAD system in the cytotoxicity of type II ribosome-inactivating proteins ricin and viscumin was investigated. For this, the cell line of colorectal adenocarcinoma HT29, as well as the HT29-sh002 line obtained on its basis, were used. On the basis on the proteome analysis of these lines and the estimation of the proportion of inactivated ribosomes, it was shown that the contribution of the proteasome to the degradation of the catalytic subunits of toxins is different. The role of the Cdc37 co-chaperone in maintaining the stability of A subunit of viscumin in the cytoplasm is shown.

Keywords: Cdc37, ERAD, HT29, MLI, PSMD11, degradation, proteasome, ribosome inactivating protein, ricin, viscumin

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A characteristic feature of type II ribosome-inactivating proteins (RIP-II) is the presence of A and B subunits connected through a disulfide bond [1]. The B subunit ensures the binding of RIP-II to the cell membrane and penetration into the endoplasmic reticulum (ER) via retrograde transport. The A subunit ensures the toxic effect of RIP-II, exhibits catalytic *N*-glycosidase activity, and irreversibly modifies ribosomes. For the realization of the toxic effect, the disulfide bond connecting the subunits must be reduced in ER. In this case, the A subunit partially unfolds, and the flexibility of the protein globule increases, which is a signal for its translocation through the ER membrane, mediated by the ERAD system—an ER-associated protein quality control and degradation system [1–3]. The degradation of partially unfolded proteins, endogenous for ER, includes the following main steps: (1) detection and sorting, (2) translocation through the ER membrane and transfer of the protein to the cytoplasm, and (3) degradation in the proteasome. The RIP-II A subunits, unlike the endogenous proteins, after translocation into the cytoplasm are able to fold back, thereby avoiding degradation in the proteasome [1]. The cytotoxic effect of RIP-II is largely determined by the efficiency of this process, as well as the lifetime of the catalytically active refolded A subunit in the cytoplasm [4].

In this work, we studied the effect of changes in the expression of proteins involved in the process of protein degradation by the ERAD system on the cytotoxicity of ricin and viscumin. These RIP-II are structural homologs and have the same mechanism of action [5, 6]. Despite the fact that the A subunits of ricin (RTA) and viscumin (VTA) are characterized by similar catalytic activity [7], the toxic effects of ricin and viscumin are significantly different [8–10]. Apparently, this is due to the difference in the stability of RTA and VTA protein globules and the ability of their unfolded polypeptide chains to fold back into a catalytically active form after translocation into the cytoplasm [3]. We derived a modified colorectal adenocarcinoma line HT29 (HT29-sh002) [11]. The pro-

Abbreviations: ERAD, endoplasmic reticulum-associated protein quality control and degradation system, RTA, ricin toxin subunit A, VTA, viscumin toxin subunit A, real-time PCR, polymerase chain reaction with real-time product detection, RIB, type II ribosome-inactivating protein, ER, endoplasmic reticulum.

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Table 1. Changes in protein expression in HT29-sh002 cells compared to HT29 cells

Protein designation	Change in protein expression, fold *
Adrm1	2.5
Cdc37 (p50)	2.6
PSMD6	2.1
PSMD11	9.1

* For all indicated FDR values, p-values < 0.05.

teome analysis performed as described in [11] revealed changes in the expression of a number of proteins involved in ERAD-associated degradation, including proteasome proteins, in the cells of this line (Table 1). Among the proteasome proteins, the largest increase in expression was detected for the regulatory subunit of the PSMD11 proteasome. Earlier, it was shown that PSMD11 plays the key role in the 26S proteasome assembly, and knockdown of the gene encoding this protein increases the sensitivity of cells to ricin [12]. Thus, an increase in the PSMD11 expression, as well as other proteasome components, should lead to a decrease in the sensitivity of cells to ricin.

The advantage of work with RIP-II is the possibility of quantifying the proportion of ribosomes specifically inactivated by them by real-time PCR using the procedure developed by us earlier [9]. The appearance of inactivated ribosomes is direct evidence of getting the catalytically active A subunits of RIP-II into the cytoplasm. Cells of the studied lines were treated with ricin or viscumin for 1 h, after which the medium was removed, and the cells were washed and incubated in RIP-II-free medium for 1 h. Then, the cells were lysed, RNA was isolated, the quality and quantity of RNA samples were evaluated, and the proportion of inactivated ribosomes was determined as described in [9, 13]. Indeed, as it was assumed, the proportion of inactivated ribosomes in HT29-sh002 cells was lower than in the original HT29 line for all ricin concentrations used (Table 2).

However, when the cells were treated with viscumin, the proportion of inactivated ribosomes in HT29-sh002 cells was, conversely, higher than in HT29 cells (Table 2). In contrast to ricin, for which the 26S proteasome-dependent RTA degradation was shown [1], data on the VTA degradation are currently quite scanty. The obtained results indicate that the 26S proteasome plays a less important role in the degradation of VTA as compared to RTA. This is correlated with the absence of lysine residues in the VTA amino acid sequence [3].

The increase in the proportion of inactivated VTA ribosomes in HT29-sh002 cells may be associated with an increase in the expression of the Cdc37 co-chaperone (Table 1). The latter is involved in the folding of proteins by the Hsp90 chaperone and maintenance of their stability in the cytoplasm [14, 15]. Cdc37 may contribute to more efficient VTA refolding after translocation into the cytoplasm and maintain its stability between catalytic events during the interaction with ribosomes.

Thus, the results obtained in this study indicate that the degradation of VTA by the 26S proteasome is, apparently, less characteristic than that of RTA. In the case of RTA, the expression level of the regulatory subunit of the PSMD11 proteasome seems to be important in this process. The role of the ubiquitin-indepen-

Table 2. Ribosome inactivation by viscumin and ricin in HT29 and HT29-sh002 cells

RIP-II, M	Proportion of inactivated ribosomes, % *		Ratio of the proportion of inactivated ribosomes in HT29-sh002 and HT29 cells **
	HT29	HT29-sh002	
Viscumin			
1×10^{-9}	<0.001	0.003	3.0 (↑)
1×10^{-8}	0.02	0.075	3.7 (↑)
1×10^{-7}	0.4	1.5	3.7 (↑)
Ricin			
1×10^{-9}	0.8	0.05	16.0 (↓)
1×10^{-8}	6.1	0.6	10.2 (↓)
1×10^{-7}	30	6.0	5.0 (↓)

* For all indicated values, the determination error (SD) did not exceed 10%.

** The arrow indicates the direction of change in the proportion of inactivated ribosomes in HT29-sh002 cells compared to HT29 cells.

dent degradation of A subunits of ricin and viscumin by the 20S proteasome requires additional studies.

ADDITIONAL INFORMATION

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COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflict of interest. This article does not contain any studies involving animals or human participants performed by any of the authors.

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