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BIOCHEMISTRY, BIOPHYSICS, AND MOLECULAR BIOLOGY

Intracellular Transport of Ribosome-Inactivating Proteins Depends on Annexin 13

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Abstract– In the present study, we assessed the role of annexin 13 membrane-binding protein (ANXA13) in the intracellular transport of vesicles containing type II ribosome-inactivating proteins (RIP-IIs). A modified human intestinal epithelial cell line HT29 was used, in which the expression of ANXA13 was significantly reduced. The cytotoxic effect of ricin and viscumin was evaluated by modification of 28S ribosome RNA. The observed differences in the activity of toxins on the parental and modified HT29 lines indicate that ANXA13 plays a different role in the intracellular transport of vesicles containing the RIP-IIs.

Keywords: ANXA13, HT29, MLI, annexin 13, endocytosis, intestinal epithelium, intracellular transport, ribosome-inactivating protein, ricin, viscumin

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Endocytosis, the mechanism of vesicular transport of various molecules from the plasma membrane of eukaryotic cells into the cytoplasm, is involved in a wide range of physiological processes [1]. In eukaryotic cells, several different pathways of endocytosis have been described. However, despite the long-term research, many details of this process remain unclear [1]. Type II ribosome-inactivating proteins (RIP-II) are a classic tool for identifying new and deeper understanding of already known pathways of intracellular transport [1, 2]. The widely used RIP-IIs include ricin and viscumin, which have the same mechanism of action and are structural homologs [2–4], but their cytotoxic activity significantly differs [5–8]. Both proteins consist of two chains, catalytic (A chain) and binding (B chain), which are connected through a disulfide bond. The B chain binds to glycosylated proteins and lipids containing the terminal galactose residue on the cell surface. Part of the RIP-II molecules bound to the membrane undergo endocytosis in components of membrane vesicles and subsequent retrograde transport to the endoplasmic reticulum (ER), where the disulfide bond is reduced and the A chain is translocated to the cytoplasm. The A chain is an *N*-glycosidase and irreversibly modifies the ribosomal 28S RNA, which leads to termination of protein synthesis. An important feature of work with RIP-II is the possibility to quantify the proportion of inactivated ribosomes in the cell by real-time PCR (qPCR) [5].

The aim of this study was to evaluate the role of annexin 13 (ANXA13) in the intracellular transport of vesicles containing ricin and viscumin. ANXA13 belongs to the family of membrane-binding proteins annexins, which are involved in intracellular vesicular transport and are a specific marker of intestinal epithelial cells [9]. Recently, using a stable knockdown of the gene encoding the extracellular matrix protein α5 of the laminin chain, we obtained a modification of the colorectal adenocarcinoma cell line HT29 (HT29-mod) [10], which was characterized by a decreased expression of ANXA13 at the level of both mRNA (9.3 and 6.3 times according to microarrays and qPCR, respectively) and protein (30.6 times) (Fig. 1). The gene expression was analyzed using microarrays as described in [11], using qPCR as described in [12], and proteome analysis as described in [10].

Cells were treated with ricin or viscumin for 1 h. Then, the culture medium was replaced with a fresh RIP-II-free medium. The fraction of inactivated ribosomes was estimated 1 h after the medium replacement by qPCR using the procedure developed by us earlier [5]. The main events that underlie this method

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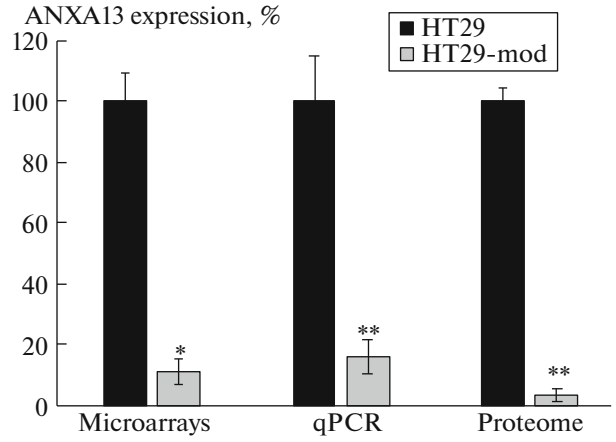

Fig. 1. Comparison of ANXA13 expression in HT29 and HT29-mod cells according to microarray, qPCR, and proteome data; * $p < 0.05$, ** $p < 0.01$.

are as follows: (1) the RIP-II A chain hydrolyzes the *N*-glycosidic bond of adenosine at position A4324 of the α-sarcin-ricin loop in 28S rRNA, forming the AP site; (2) reverse transcriptase during cDNA synthesis inserts adenine opposite to the AP site, as a result of which thymine is replaced by adenine in the sequence; (3) using specific primers whose 3' end is complementary to either the original or modified sequence, the accumulation of the signal of the modified 28S rRNAs can be detected; and (4) normalization is performed using the accumulation of the signal of the 28S rRNA fragment remote from the modification site. The obtained results showed that, 2 h after the treatment of HT29-mod cells with viscumin at all concentrations used, the proportion of inactivated ribosomes was 2–3.5 times higher as compared to the parental line (Table 1). However, when the cells were treated with ricin, the proportion of the inactivated ribosomes in HT29-mod cells was, conversely, lower.

Table 1. Ribosome inactivation by viscumin and ricin in HT29 and HT29-mod cells

RIP, M	Proportion of inactivated ribosomes, % *	
	HT29	HT29-mod
Viscumin		
1×10^{-9}	<0.001	0.002
1×10^{-8}	0.02	0.07
1×10^{-7}	0.4	1.2
Ricin		
1×10^{-9}	0.8	0.07
1×10^{-8}	6.1	1.1
1×10^{-7}	30	8.4

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

One of the known functions of ANXA13 in intestinal epithelial cells is the involvement in the anterograde transport of vesicles from the Golgi apparatus to the plasma membrane. The obtained results indicate that a decrease in the ANXA13 expression has an opposite action on the effects of viscumin and ricin. ANXA13 preferentially binds to the membrane regions enriched in sphingolipids and cholesterol [9], which may determine the specificity of transport of intracellular vesicles containing the studied RIP-IIs [6, 13]. Annexins, by binding to the membrane, determine the radius of curvature of the forming vesicles [9], which affects the rate of their transport in the cytoplasm [14]. A decrease in the ANXA13 expression leads to an increase in the size of the forming vesicles and deceleration of their transport. In the case of viscumin, this leads to a decrease in the anterograde transport of vesicles and an increase in the proportion of the toxin molecules that reached the ER and cytoplasm.

In the case of ricin, ANXA13 apparently plays a minor role in the anterograde transport of toxin-containing vesicles; however, it may also be involved in retrograde transport. A change in the size of ricin-containing vesicles leads to a decrease in the proportion of the toxin molecules that reached the ER and cytoplasm. It is important to note that, in HT29-mod cells, the expression of proteins involved in the intracellular transport such as Rab1a, Rab21, and TMED2 also decreases (5.6, 4.4, and 11.5 times, respectively) according to the proteome data. Knockdown of the genes encoding these proteins leads to a decrease in the sensitivity of cells to ricin [15]. We have shown for the first time that, in the case of viscumin, a change in the expression of these proteins apparently plays a minor role in the transport of toxin-containing vesicles.

Summarizing the obtained data, we can conclude that the anterograde transport of viscumin and ricin is different. The effect of ANXA13 on the transport of membrane vesicles is associated both with its structural (vesicle size) and receptor properties (sorting of vesicles). A decrease in the expression of ANXA13, apparently, decelerates the anterograde transport of viscumin-containing vesicles, which determines the increase in the proportion of inactivated ribosomes in cells. In the case of ricin, the role of ANXA13 in anterograde transport seems to be minor. The involvement of ANXA13 in the retrograde transport of the ricin-containing vesicles requires additional research. It cannot also be ruled out that a decrease in the ANXA13 expression leads to compensatory activation of an alternative pathway of intracellular vesicular transport, which is more specific for the viscumin-containing vesicles.

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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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