„Role of the endoplasmic reticulum (ER) membrane channels in the transport of ricin from the ER to the cytosol”
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The endoplasmic reticulum (ER) forms an irregular network of cisternae and tubules that are isolated from the cytoplasm by biological membranes. A distinctive structure characterised by the presence of numerous ribosomes is the rough endoplasmic reticulum (RER), which is responsible, among others, for the biosynthesis of proteins secreted outside the cell and a sizable proportion of intracellular proteins. Proteins that have not reached their native structure in the ER are transported to the cytosol, where they undergo proteasome-dependent degradation. This specialized process of substrate recognition in the ER, transport to the cytosol and subsequent degradation is referred to as endoplasmic reticulum-associated protein degradation (ERAD). Proteins are transported from the ER to the cytoplasm through special translocation channels present in the ER membrane. Several types of channels are currently known. These are formed by the membrane proteins Sec61, Derlin and the ER-associated ubiquitin ligase HRD1, among others. The Sec61 complex is a heterotrimer consisting of α (found in two isoforms: Sec61A1 and Sec61A2), β and γ subunits. The process of selection of these channels by substrates of the ERAD process is very poorly understood. It is not only misfolded proteins that are transported through ER membrane channels. Some viruses and toxins that are active in the cytoplasm use ERAD in their transport from the endoplasmic reticulum to the cytosol. One of the unusual substrates of the ERAD process is the plant-derived toxin, ricin.

The main objective of the present study was to characterize Sec61α protein isoforms in different cell lines and to understand the mechanisms of selection of endoplasmic reticulum membrane channels, i.e. the Sec61 complex and Derlin family proteins by ricin.

The study showed that in all cell lines studied, that is, HEK293, HeLa, HDFa and Vero, the genes encoding isoforms 1 and 2 of the Sec61α protein are expressed. In the HEK293, HeLa and HDFa lines, the *SEC61A1* gene encoding isoform 1 of the Sec61α protein is expressed at a significantly higher level than that of isoform 2 of this protein. In the Vero cell line, isoform 2 of the Sec61α encoded by the *SEC61A2* gene was found to be dominant.

To downregulate gene expression of individual Sec61α protein isoforms, cells were transfected with specific siRNA. It was proven that the downregulation of Sec61α protein levels did not significantly affect the viability of HEK293 and Vero cells.

Studies of the transport of the enzymatically active ricin toxin A chain (RTA) from the ER to the cytosol showed that downregulation of isoform 1 and both isoforms of Sec61α did not affect the transport of RTA from the ER to the cytosol in HEK293 cells. In these cells, under conditions of efficient silencing of the expression of the gene encoding isoform 1 of the Sec61α protein, there is a downregulation of the transcript of the gene encoding isoform 2 and an upregulation of the expression of the genes encoding the Derlin family proteins, that is, Derlin-1, Derlin-2, Derlin-3. This suggests a potential involvement of Derlin proteins in RTA transport, particularly in cells with an inactive Sec61 translocon. These suggestions formed the basis for further research, which showed that overproduction of Derlin-1 and Derlin-2 proteins did not alter the cytotoxicity of ricin or the retrotranslocation of RTA from the ER to the cytosol. Overproduction of the Derlin-3 protein, on the other hand, affects the reduction of RTA transport from the ER to the cytosol.

The analyses presented in this study have shown that, in contrast to the other cell lines studied, in Vero cells, the Sec61α protein isoform 2 gene is expressed at a higher level than the isoform 1 gene, and Sec61α protein isoform 2 can be considered dominant. The results of experiments investigating the transport of RTA from the ER to the cytosol in Vero cells showed that downregulation of isoform 2 and both isoforms of the Sec61α protein reduced the retrotranslocation of RTA from the ER to the cytosol. These results indicate that RTA transport from the ER to the cytosol is dependent on isoform 2 of the Sec61α protein.

In conclusion, studies in HEK293 and Vero cells revealed the complexity of the mechanism of RTA transport from the ER to the cytosol. In HEK293 cells, the Sec61 complex and the Derlin-1 and Derlin-2 proteins do not play a key role in this process, whereas overproduction of Derlin-3 may impair RTA transport to the cytosol. In Vero cells, RTA transport was found to be specifically dependent on isoform 2 of the Sec61α protein, highlighting its key role in RTA retrotranslocation.

The study provides valuable information on the role of ER translocation channels, such as Sec61 and Derlin, in the context of the ERAD process. In the present study, ricin, an atypical substrate of this process, was used to thoroughly investigate its targeting to retrotranslocons and to better understand their function.

Ricin can be used in the treatment of cancer due to its cytotoxic effect, but it can also serve as a biological weapon requiring the development of a suitable drug. Understanding its mechanisms of action, including the modes of transport from the ER to the cytosol that have a direct bearing on the toxic effects of ricin, may help to better understand the processes involved in this toxin.