Abstract

Chemoresistance is one of the main causes of failure of anticancer chemotherapy. Vacuolar-type ATPase (V-ATPase) is an ATP-dependent proton pump involved in the regulation of the pH in cells, cell organelles and the intracellular space. A significant acidification of the extracellular space and intracellular compartments occurs in connection with the metabolism of tumour cells (glucose metabolism, hypoxia, insufficient blood perfusion of the cancer tissue). Basic drugs are transferred into acidic organelles based on the pH gradient, where they are then protonated and accumulated. This mechanism is called lysosomal sequestration and is one of the mechanisms how tumour cells resist to applied drugs, which then do not reach their target site in cancer cell. An increased expression of V-ATPases has been described in relation to chemoresistance and the progression of tumours.

This dissertation is focused on observing the membrane subunit V_0d from the complex of V-ATPase and the changes in resistance to ellipticine caused by the silencing of this subunit's gene in human neuroblastoma cell lines UKF-NB-4 (sensitive) and UKF-NB-4^{ELLI} (resistant to ellipticine). The expression of the V_0d protein was first examined on mRNA level using real-time polymerase chain reaction (RT-PCR). The silencing of selected V-ATPase subunits genes was mediated by cell transfection with specific siRNA molecules (small interfering RNA) and its success was measured both on the protein level by using the Western blot method and on the mRNA level by RT-PCR. Confocal microscopy was used to observe the subcellular localisation of ellipticine in the transfected cell lines. The proliferation of transfected cells and their viability were evaluated in real-time using the xCELLigence system and PrestoBlue agent. It was proven that the expression of the V_0d subunit of V-ATPase is increased in the ellipticine-resistant neuroblastoma cell line. UKF-NB-4^{ELLI} cells transfected by siATP6V0D1 incubated with ellipticine showed significantly reduced proliferation and viability – the V_0d subunit can therefore significantly influence resistance to ellipticine in neuroblastoma cells. V_0d knock down alone had a negative effect on their viability. The combination of cells siRNA-mediated transfection and their incubation with ellipticine leads to the formation of vacuoles, the origin of which was not identified. [IN CZECH]

Keywords: neuroblastoma, ellipticine, cancer cells chemoresistance, V-ATPase, lisosomal sequestration of cytostatic drugs [IN CZECH]