**„Role of EDEM family proteins in the regulation of amyloid precursor protein (APP) metabolism”**

**Jowita Nowakowska-Gołacka, MSc.**

The endoplasmic reticulum-associated degradation (ERAD) process is part of the endoplasmic reticulum quality control (ERQC) system, which involves identifying and directing misfolded proteins to the cytosol, where they undergo proteasomal degradation. The ER degradation-enhancing α-mannosidase-like proteins (EDEM) family lectins: EDEM1, EDEM2 and EDEM3 are involved in substrate recognition of the ERAD process. Several studies indicate a connection between disruption of the ERAD process and Alzheimer's disease (AD). β-amyloid (Aβ), a product of proteolytic processing of the amyloid precursor protein (APP), plays an important role in the pathophysiology of AD. The three most common isoforms of the APP protein are: $APP_{695}$, $APP_{770}$ and $APP_{751}$. APP undergoes N-glycosylation in the ER and subsequent maturation in the Golgi apparatus. The ER is known to play a significant role in APP metabolism at the protein folding stage and in regulating the amount of APP associated with degradation during the ERAD process.

The main aim of this study was to investigate the role of EDEM family proteins in regulating APP protein metabolism and Aβ production. The study showed that increased expression of genes encoding EDEM family proteins reduced the amount of both endogenous forms of APP protein and its overproduced isoform $APP_{695}$ in human embryonic kidney cells – HEK293. The most significant reduction of more than 80% in the amount of the immature, N-glycosylated form of $APP_{695}$ was observed in cells in which amounts of EDEM1 and EDEM3 proteins were elevated. The use of aproteasome inhibitor at increased amounts of EDEM proteins abolished the previously observed APP protein reduction effect. The findings indicate that EDEM proteins regulate the amount of endogenous APP protein and its overproduced $APP_{695}$ isoform through proteasomal degradation. The study also demonstrated that reduced expression of genes encoding EDEM1 and EDEM3 proteins increased the amount of APP protein. Reduced level of EDEM1 protein resulted in a more than 20-fold increase in the amount of endogenous immature form of $APP_{695}$ in HEK293 cells. This effect was also confirmed on another cell model, SH-SY5Y – neuroblastoma cells. EDEM1 and EDEM3 proteins have also been shown to affect the retrotranslocation of $APP_{695}$ from the endoplasmic reticulum to the cytosol in HEK293 cells. In cells with increased amounts of EDEM1, the relative amount of the immature form of $APP_{695}$ transported from the ER to the cytosol was more than twice as high as in the control variant. In cells overproducing EDEM3, a 30% increase in the amount of $APP_{695}$ transported from the ER to the cytosol was observed compared to the control variant.

The study also showed the interaction of EDEM1 and EDEM3 proteins with $APP_{695}$ and the effect of EDEM1 and EDEM3 proteins on a significant increase in the amount of Aβ secreted, both $A\beta_{40}$ and $A\beta_{42}$.

Based on the results presented in this thesis, it can be concluded that EDEM family proteins, in particular EDEM1 and EDEM3, significantly affect APP metabolism and Aβ production. The studies are also crucial for a better understanding of the overall mechanisms of protein transport and degradation in the ERAD process.