Regulatory role of Pellino3 ligase in RIG-I signaling pathways activated by influenza B virus

RIG-I-like receptors (RLRs) are an important family of pattern recognition receptors that are involved in recognition of viral infection. These cytosolic receptors are responsible for recognizing double-stranded RNA (dsRNA) present in a cell during viral infection. The RLRs family consists of a three receptors: RIG-I receptor (retinoic-acid inducible gene), MDA5 receptor (melanoma differentiation-associated protein) and LGP2 (laboratory of genetics and physiology 2), but only RIG-I and MDA5 have the ability to induce antiviral signaling cascades. Upon dsRNA recognition, receptors interact with MAVS (mitochondrial antiviral signaling protein) through the CARD domains (caspase activation and recruitment domain) present in the RIG-I and MDA5 receptors and in the MAVS protein. Subsequently, downstream cytosolic proteins are recruited to the signaling complex, which leads to the activation of transcription factors, such as NF-κB (nuclear factor kappa-light-chain) and IRF3/7 (interferon regulatory factor), resulting in the production of proinflammatory cytokines and interferons. RLR signaling pathways are regulated by posttranslational modifications such as ubiquitination. Ubiquitination of the appropriate proteins may promote the formation of signaling complexes which activates subsequent signaling cascades or direct proteins to degradation, which consequently results in the inhibition of proinflammatory cytokines and interferons production.

The aim of this study was to investigate the role of Pellino3 ubiquitin ligase in the regulation of the RIG-I receptor signaling pathway activated by infection with influenza B virus. So fat, the role of this ligase has been described in the signaling pathways of TLR3 (Toll-like receptor), TLR2, TLR4, NOD2 (nucleotide-binding oligomerization domain-containing protein 2), TNFR (tumor necrosis factor receptor 1) and RIG-I. These studies were conducted using mouse macrophages or human monocytic cells. However, epithelial cells also play an extremely important role in immune processes induced by viral infection. Given the different functions of immune cells and epithelial cells, a human cell line derived from lung epithelium was used in this study.

In the first stage of this work, it was observed that the lack of Pellino3 ligase in cells resulted in increased expression and production of type I interferons in response to influenza B virus (IBV) infection. Subsequently, it was established that the RIG-I receptor plays a pivotal role in the IBV recognition. Using a specific ligand for the RIG-I receptor, it was confirmed that the observed differences in the interferon secretion induced by viral infection are RIG-I receptor dependent. It has also been shown that the lack of the Pellino3 protein does not affect the expression level of the RIG-I receptor and the MAVS adapter protein.

The aim of the next stage of research was to determine which of the signaling pathways dependent on the RIG-I receptor are regulated by the Pellino3 ligase. It has been shown that IBV infection causes activation of ERK 1/2 and p38 kinases, however, this activation is independent of Pellino3 ligase. It has been observed that IBV infection does not activate the NF-κB pathway but leads to activation of the transcription factor IRF3. Furthermore, Pellino3 ligase has been shown to be a negative regulator of the activation of this transcription factor. It

has also been observed that infection with IBV leads to activation of the AP-1 complex factors (ATF-2 and c-Jun), however, this activation is independent of the Pellino3 ligase.

Further studies showed that in cells with knockout of *PELI3* gene, the level of TRAF3 increases during infection. In the next steps, it was found that the Pellino3 ligase interacts with the TRAF3 and is responsible for the K48-linked ubiquitination of this protein.

In the final stage of the study, it was shown that the presence of Pellino3 ligase influences other aspects of the antiviral response induced by the production of type I interferons, such as activation of the transcription factor STAT1 and production of CXCL10.

The results presented in this dissertation allowed to propose a model in which Pellino3 ligase regulates the level of TRAF3 protein by K48-linked ubiquitination, leading to proteasomal degradation. This prevents the interaction of TRAF3 with the IRF3, and thus its activation and translocation to the cell nucleus, which results in inhibited expression of type I interferons. Consequently, reduced production of type I interferons leads to reduced activation of the transcription factor STAT1 and inhibition of transcription activation of ISGs (interferon-stimulated gene), including CXCL10. In the proposed signaling pathway, Pellino3 ligase acts as a limiting factor in the activation of the signaling cascade, preventing overstimulation of the cell by viral infection.