Mechanism of cytosolic receptor RIG-I pathways regulation by ligase Pellino3

Abstract

Receptors from the RLR family are a very important node of the innate immune response and are responsible for recognizing viral infection in the organism's cells. RIG-I and MDA5 are the only members of the RLR family that are able to initiate an antiviral signaling cascade. The main receptor on which this work focuses is RIG-I, which is responsible for recognizing double-stranded RNA present in the cytoplasm during infection with most viruses. As a result of RIG-I recognition of viral RNA, the signal is transmitted to the MAVS adapter protein. The signaling cascade then follows two pathways resulting in the activation of IRF3/7 and NF-κB transcription factors. This results in production of type I interferon and other cytokines such as IP-10 or TNFα.

The aim of the study was to determine the effect of Pellino3 protein on the regulation of the signaling pathway initiated by the RIG-I receptor. Pellino3 is a ubiquitin ligase which is involved in the regulation of many signaling pathways of innate immune responses including among others one activated by TLR3.

In the first stage of this work, it was established that the virus that most effectively stimulates the production of interferon in BMDM cells is VSIV. Then it was proved that in BMDM cells VSIV is recognized by receptors from the RLR family. The involvement of RLR receptors in the recognition of VSIV virus was then narrowed to the RIG-I receptor.

In the next stage, it was proved that the Pellino3 protein is involved in the regulation of the RIG-I activated cascade. Pellino3 has been shown to negatively regulate the activation path of IRF3/7 transcription factors and does not affect the NF-κB pathway. It has also been proven that the lack of Pellino3 protein in cells does not affect the expression level of RIG-I and MDA5 receptors and of the MAVS adaptor protein.

Further studies have shown that the lack of Pellino3 protein results in a lack of production of biologically active type I interferon and proinflammatory cytokines. In addition, it has been proven that in BMDM *Peli3*$^{-/-}$ cells, after VSIV infection, ERK1/2 kinases are not activated which activation is visible in wild-type cells. Deficiencies in cytokine production have also been associated with the lack of activation of one of the MAP (ang. mitogen-activated protein) kinases ERK1/2 by the use of the FR180204 inhibitor.

Searching for possible solutions to the problem of discrepancies between Pellino3 activation of transcription of *Ifnβ, Cxcl10* and *Tnfα* genes and the lack of production of these cytokines in the form of proteins was found on the ERK1/2-p90RSK signaling pathway which leads to activation of translation initiation factors. This led to the investigation of the level of p90RSK kinase activation. ERK1/2 phosphorylation disorder has been shown to result in a lack of p90RSK kinase activation. In addition, it has also been shown that the disruption of the ERK1/2-p90RSK signaling pathway results in the lack of activation of eIF4B and eIF4E translation initiation factors. The effect of the Pellino3 protein on the antiviral response was also confirmed in a mouse model. The results presented in the doctoral dissertation were used to propose a model in which the Pellino3 protein acts as a molecular switch between transcription and translation in the antiviral response initiated by the RIG-I receptor in BMDM cells.