P22 & FP – **NLRP3 lacking leucine-rich repeat domain can be fully activated by the canonical inflammasome pathway**

Iva Hafner-Bratkovič[1,2], Petra Sušjan[1], Duško Lainšček[1], Ana Tapia-Abellan[3], Kosta Cerović[1], Lucija Kadunc[1], Diego Angosto-Bazarra[3], Pablo Pelegrín[3], Roman Jerala[1,2]

[1]National Institute of Chemistry, Ljubljana, Slovenia

[2]EN-FIST Centre of Excellence, Ljubljana, Slovenia

[3]Biomedical Research Institute of Murcia (IMIB-Arrixaca), University Clinical Hospital "Virgen de la Arrixaca", Murcia, Spain

Iva.Hafner@ki.si

NLRP3 inflammasome is a multiprotein complex mediating inflammatory response in a variety of metabolic and degenerative diseases. Upon activation with diverse triggers NLRP3 oligomerizes, recruits adaptor protein ASC (apoptosis-associated speck-like protein containing a CARD), and the effector protein pro-caspase-1. Inflammasome assembly leads to autoproteolytic activation of caspase-1, which processes IL-1β and IL-18 cytokines to their mature form and gasdermin D to a pore-forming protein that induces pyroptotic cell death. Although the role of NLRP3 in various pathologies has been described, not much is known about the molecular mechanism of NLRP3 inflammasome activation.

In order to define the role of particular domains of NLRP3 in inflammasome trigger sensing, assembly and autoregulation systematic truncation of NLRP3 and reconstitution of NLRP3 variants in NLRP3-deficient macrophages was performed. We demonstrate that LRR domain is dispensable for NLRP3 activation and self-regulation. A minimal NLRP3 truncation variant was found fully responsive to various canonical NLRP3 activators. Using bioluminescence resonance energy transfer we showed that further truncation led to the variant that still changed conformation in response to canonical NLRP3 inflammasome trigger yet failed to form a functional inflammasome, even when a disease-causing point mutation was introduced. Substitution of the pyrin domain of NLRP3 with the CARD domain of NLRC4 or ASC led to a constitutive activation, demonstrating that the pyrin domain restricts NLRP3 in an inactive conformation.

NLRC4 inflammasome is formed by self-catalytic polymerization of NLRC4 initiated with bacterial ligand/NAIP complex. We were interested whether similar process is involved in NLRP3 activation. We show that pathological mutations of NLRP3 failed to engage wild-type NLRP3 in a self-catalytic oligomerization, demonstrating that the activating signal is not enhanced at the level of NLRP3 oligomerization, representing an additional level of NLRP3 regulation.

These results contribute to the understanding of the molecular basis of NLRP3 inflammasome activation and demonstrate the versatility of recognition and regulation mechanisms of the innate immune receptors.