Contribution ID : **42** Type : **Oral**

Understanding MLKL's molecular switch mechanism using two novel MLKL pseudokinase orthologue structures

Monday, 2 December 2019 15:45 (15)

Necroptosis is a form of programmed cell death characterized by lack of caspase activity and a loss of plasma membrane integrity. Morphologically similar to necrosis, during necroptosis, the plasma membrane is disrupted, causing release of cellular components to the extracellular fluid and an ensuing inflammatory response. Necroptosis proceeds via a regulated kinase cascade involving Receptor Interacting Protein Kinases RIPK1 and RIPK3. Mixed Lineage Kinase domain-Like protein (MLKL), a pseudokinase, is the final known obligate effector of necroptosis. The MLKL pseudokinase domain is incapable of catalysing phosphotransfer reactions, and is the site of RIPK3 phosphorylation. This phosphorylation event is thought to flip a molecular switch regulated by the pseudokinase domain, resulting in activation of MLKL. Upon activation, MLKL oligomerises, translocates to the plasma membrane, and destabilises it. Details of MLKL's molecular mechanism of action, including activation, oligomerisation and how it interacts with the plasma membrane, remain unknown.

To interrogate MLKL's molecular switch mechanism, we solved the structure of the pseudokinase domain of two MLKL orthologues; horse and rat, using X-ray crystallography at the Australian Synchrotron. By comparing the structure and sequence of the orthologues with previously published structures of mouse and human MLKL, we found that the novel conformations adopted in our structures may represent different stages of the molecular switch mechanism, with different conformations favoured for each orthologue due to sequence variation. We paired these findings with death assays in cells, to understand the commonalties between orthologue necroptotic signalling pathways and undertook mutational analysis to understand the significance of the conformations found in our structures.

Speakers Gender

Female

Travel Funding

Yes

Level of Expertise

Student

Do yo wish to take part in the poster slam

No

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Session Classification : Session 10

Track Classification : Structural biology and biological systems