Structural properties of MLKL protein and its role in cell death

Abstract

Necroptosis is a type of programmed cell death with morphological features of necrosis. After the loss of plasma membrane integrity, the cell content is released into the surroundings, triggering potent innate immune cell activation and inflammation. In case of severe necroptosis, over-activated immune response results in inflammatory diseases. MLKL (mixed lineage kinase-domain like) is a protein indispensible for necroptosis. It consists of the N-terminal domain and the C-terminal pseudokinase domain, which includes phosphorylating sites for MLKL activation. Two different isoforms of MLKL are known to exist in HT-29 cell lines, but their capacities to trigger cell death have not been compared directly. The mechanism of MLKL induced necroptosis, its oligomerization, a role of its structural parts and a role of MLKL in innate immune response is not yet fully understood. Here we have shown that MLKLN-154 consists of six distinct α-helices in solution, where it can be found as a monomer, dimer and oligomer. HEK293T cell lines were used to test the ability of different parts of the MLKL to trigger cell death. No cell death was observed for deletion mutants MLKLN-119, MLKLN-154 and MLKLN-166, whereas necroptotic activity was detected for MLKLN-126, MLKLN-139, MLKLN-201 and in the case of both full length MLKL isoforms. Given that amino acids 1-126 are able to initiate necroptosis, our results indicate that the helix that follows this region restrains necroptotic activity, which is again restored in longer constructs, suggesting that a region C-terminal to this helix (H6) is needed to facilitate pulling it away from the four helix bundle and unleashing MLKL activity. Higher necroptotic activity of MLKL-2 was observed, compared to MLKL-1. Models of both MLKL isoforms have shown that MLKL-2 lacks the majority of the pseudokinase domain, which in the case of MLKL-1 structurally supports the C-terminal α-helix and constrains the activity of MLKL-1, but not MLKL-2. That is not the case in c-Myc/His_6 tagged MLKL-1, where the tag disrupts this interaction and renders MLKL-1 fully active. Both MLKL isoforms were present in human monocyte derived macrophages, where the expression of MLKL-1 was 10-fold higher comparing to MLKL-2 on mRNA level. The expression of both isoforms were up-regulated by TLR4 and TLR3 agonists, LPS and poli(I:C). We have also confirmed the MLKL-1 up-regulation on protein level in human and mouse macrophages after LPS or poli(I:C) treatment. In the *Salmonella enterica* serovar Typhimurium infection assay, mouse macrophages succumbed to caspase related cell death, but when caspases were inhibited by zVAD inhibitor, macrophages died by RIP3 and MLKL dependent necroptosis. This once more demonstrated that necroptosis is an alternative pathway of regulated cell death, which is triggered when caspase dependent pathways are blocked.