Poster Presentation

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Immune response regulation by paralogous endoribonucleases: ZC3H12C and N4BP1

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Inflammation is a protective response by the body to ensure removal of detrimental stimuli, as well as a healing process for repairing damaged tissue. Different innate pattern recognition receptors like Toll-like receptors (TLR) and RIG-1 like receptors sense the infection and activate complex signaling networks, which induces expression of various pro-inflammatory cytokines such as tumor necrosis factor (TNF), interleukin (IL)-1 and IL-6 (1). Cytokine levels are tightly controlled at transcriptional and post-transcrip¬tional levels by various regulatory proteins, because a cytokine storm may lead to immunodeficiency and autoimmune disorders (2).

Recent studied have revealed the involvement of endoribonucleases in TLR induced cytokine expression regulation. Specifically, the MCPIP (macrophage chemotactic protein-induced proteins) family (also known as ZC3H12 family) member MCPIP1/Regnase-1 has been shown to regulate IL-6 and other cyto¬kines by targeting a specific-stem loop in the 3' untranslated region (UTR) of translationally active mRNAs by utilizing the helicase activity of UPF1 (3). Similar to Regnase-1, the paralogous ribonucleases MCPIP3/ZC3H12C and N4BP1 also show similar overexpression profiles in TLR mediated macrophage activation, and a knockout mouse model also confirms their involvement in immune response regulation as it shows severe defects in B-lymphocytes and development of autoimmune disease, respectively.

ZC3H12C and N4BP1 share similarities in harboring a homologous PIN-like RNase domain, while they differ in containing different RNA recognition motifs, a CCCH type Zn-finger and tandem KH domains, respect¬ively. We present the 1.9 Å crystal structure of the ZC3H12C RNase domain and the 1.8 Å crystal structure of the N4BP1 tandem KH domains. The RNase domain displays a Rossmann-fold like structure, and biochemical analysis shows the importance of the Zn finger in RNA substrate recognition in a Mg2+ ion dependent catalytic reaction, while the tandem N4BP1 KH domains suggest a non-canonical mode of RNA recognition by the ribonuclease. Moreover, a cell based analysis reveals that ZC3H12C and N4BP1 are localized in cytoplasm and nucleus, respectively, and target cytokine mRNA at two different levels to regulate their expression and protect against autoimmune condition.

Overall, we aim to understand the structure-function relation of the TLR induced immune response by endoribonucleases acting in different sub-cellular localizations in macrophages. Future work will focus on co-crystallization of cognate RNA with the endoribonucleases to understand its target recognition and enzymatic mechanism.

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