

The Role of RNF216/TRIAD3 in Neuroinflammation Through Interactions with Toll-Like-Receptors

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Ubiquitin E3 ligases are enzymes that mark certain substrates with ubiquitin proteins which leads to different cellular fates. Ring finger protein 216 (RNF216) is a ubiquitin E3 ligase that is involved in synaptic plasticity, inhibiting cellular autophagy, and the immune response in the peripheral nervous system. Previous literature has demonstrated that RNF216 participates in various aspects of inflammation by regulating ubiquitination and proteasomal degradation of receptor-interacting serine-threonine kinase 1 (RIPK1), toll-interleukin 1 receptor domain containing adaptor protein (TIRAP), and TIR-domain-containing adapter-inducing interferon- β (TRIF), targeting TNF receptor associated factor 3 (TRAF3) for degradation. TLRs initiate signal transduction pathways which can lead to cytokine production, proliferation, survival, adaptive immunity, phagocytosis, or apoptosis. One of the RNF216 isoforms, TRIAD3A, controls the intensity and duration of TLR signaling, specifically TLR4 and TLR9, through ubiquitination and subsequent proteolytic degradation. The role of RNF216 in regulation of factors that contribute to inflammation has never been studied in the central nervous system. The goal of this work is to examine the extent to which interactions of RNF216 with TLRs and their downstream effectors is involved in neuroinflammatory pathways. GT1-7 mouse hypothalamic cells have been used by researchers to study neuroinflammation involving TLRs. To study the role of RNF216 in these neuroinflammatory pathways, we created a GT1-7 cell line with RNF216 deleted using the Crispr-Cas9 system. Using quantitative polymerase chain reaction (qPCR), we measured expression of TLRs that regulate immune function and neuroinflammation. In the future, we will also determine if overexpression of RNF216 can rescue alterations in gene expression. Our data suggests that RNF216 functions as a positive regulator in TLR signaling pathways in GT1-7 cells.