RESEARCH HIGHLIGHT

Self-RNA *Rmrp* pre-dimerizes TLR3 for immune activation

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Endosomal Toll-like receptors (TLRs) are nucleic acid sensors that play an important role in innate immunity to combat viral infection; however, it is unclear how endogenous nucleic acids interact with endosomal TLRs. In a recent paper in *Cell Research*, Zhang et al. showed that self-RNA *Rmrp* primes TLR3 for pre-dimerization, which is essential for TLR3 activation.

Recognizing foreign nucleic acids by germline-encoded nucleic acid receptors is fundamental for antiviral immunity. TLR3, TLR7/8 and TLR9 are endosomal, membrane-bound sensors for double-stranded (ds)RNA, single-stranded (ss)RNA and unmethylated CpG DNA, respectively. While viral infection generates exogenous ligands for these nucleic acid receptors, endogenous nucleic acids can also engage TLRs and other nucleic acid receptors when they acquire 'foreign-like' features through modifications, conformational changes, or altered strand composition. Exploiting the ability of self-nucleic acids to activate immune response through nucleic acid receptors has been strategized in cancer therapy as "viral mimicry".[1] However, dysregulated recognition of self-nucleic acids can also lead to autoinflammatory diseases.[2] It is of broad interest to study the interactions between self-nucleic acids and nucleic acid sensors, given their crucial implications in human diseases.

Ligand-induced dimerization and conformational changes are essential for TLRs to activate proinflammatory signaling cascades. In the native cellular context, preformed homodimers without external ligand binding have been documented for endosomal TLR8[3] and TLR9,[4] while many other TLRs exist as monomers and require ligand binding to induce their dimerization. TLR3 ectodomain (ECD) in solution exists as monomers,[5] however, it remains unknown whether the membrane-bound TLR3 in vivo could exist as preformed dimers. Preventing TLR3 dimerization renders it incapable of binding to its ligand dsRNA,[6] suggesting that TLR3 pre-dimerization might be essential for its function. Upon dsRNA sensing, TLR3 interacts with Toll/Il-1R domain-containing adaptor-inducing IFN-β (TRIF) adaptor protein to initiate inflammatory response through IRF3-dependent type I interferon secretion and NF-κB-driven proinflammatory cytokine expression.[7] Although protein partners essential for TLR3 function have been documented,[8,9] it remains largely unknown whether and how endogenous RNA plays a role in TLR3 structural and functional regulation.

In a recent study published in *Cell Research*, Zhang et al.[10] profiled TLR3-interacting RNAs in Raw264.7 mouse macrophages and identified *Rmrp* as the top TLR3-binding endogenous RNA, that was also highly enriched in the endosome. *Rmrp*, a nuclear-encoded long non-coding (lncRNA) previously documented for mitochondrial RNA processing, telomerase function, and rRNA maturation, is mutated in humans with cartilage-hair hypoplasia, underscoring its physiological importance.[11] Zhang et al. transiently knocked down *Rmrp* in multiple human and mouse cell lines. With *Rmrp* deficiency, they observed abrogation of TLR3-dependent signaling. Furthermore, they generated myeloid-conditional *Rmrp*-deficient mice ($Rmrp^{fl/fl}Lyz2^{Cre}$) and established the essential role of *Rmrp* in TLR3-mediated anti-viral response in vivo using influenza A virus infection models. These experiments robustly showed that the endogenous lncRNA *Rmrp* not only interacts with TLR3 with high affinity but also is essential for TLR3-mediated innate immune signaling.

The molecular mechanisms whereby *Rmrp* regulates TLR3 have been further elucidated by the authors. Newly translated TLR3 needs to traffic from endoplasmic reticulum (ER) to endolysosomes[8] where acidic pH facilitates its cleavage by cathepsins[12] essential for its activation by exogenous ligands. The authors first found that *Rmrp* interacts with the non-cleaved TLR3 in the early endosome but not the cleaved form. Through both RIP-qPCR and RNA pull-down experiments, the authors showed that preventing the cleavage of TLR3 increased its interaction with *Rmrp*. Strikingly, they found that TLR3 exists as preformed dimers in cells, sharing the concept of pre-dimerization with endosomal TLR8[3] and TLR9.[4] Deficiency of *Rmrp* RNA prevented the dimerization of TLR3 and increased TLR3 monomers while overexpressing *Rmrp* induced TLR3 dimer formation. Notably, the authors also showed that TLR3 dimer formation in cells did not change with exogenous dsRNA ligand or viral infection, suggesting that endogenous RNA *Rmrp* enables TLR3 ligand binding by primming.

Zhang et al. also solved the cryo-EM structure of *Rmrp* bound to TLR3 ECD. At pH 6.0, full-length *Rmrp* formed heterogeneous complexes with TLR3, prompting analysis of a conserved catalytic domain (*Rmrp*-C) corresponding to nucleotides 1–81 and 203–275. *Rmrp*-C and TLR3 ECD formed two major conformations: The "elongated conformation" resembles the classical active TLR3 dimer, where the C-termini of the TLR3-ECD form protein–protein interactions. This elongated conformation stabilizes a pre-dimerized arrangement even after *Rmrp* degradation. The novel "lapped conformation" is driven by RNA–protein interactions exclusively, where the C-termini of the dimer remain separated. As shown by the authors, the RNA–protein interface I of *Rmrp*–TLR3 largely resembles that of dsRNA–TLR3 except for a specific lysine (K) residue K42 of TLR3 that seems to be unique for *Rmrp*–TLR3 interface. The functional importance of K42 was investigated by structure-guided mutagenesis. $TLR3^{-/-}$ cells reconstituted with TLR3 K42A did not respond to TLR3 agonists although TLR3 K42A could still bind to dsRNA. These results suggest that blocking the

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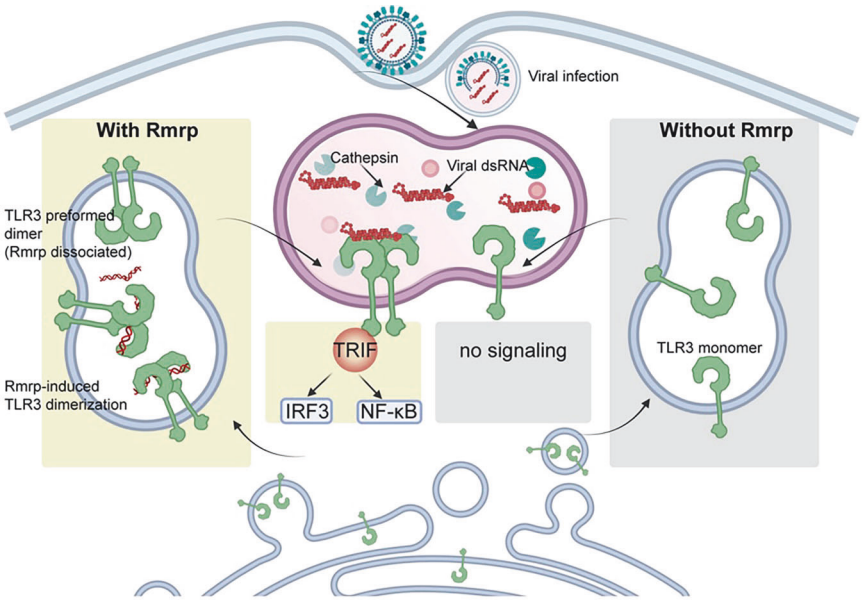

Fig. 1 *Rmrp* induces TLR3 pre-dimerization in early endosome, empowering its activation. As shown by Zhang et al., TLR3 is synthesized in the ER as monomers and trafficked to early endosome. At pH 6.0–6.5, *Rmrp* binds full-length TLR3, inducing its dimerization. With acidification of the endosome, *Rmrp* dissociates from TLR3 and is degraded. In endolysosome, TLR3 preformed dimer is capable of proinflammatory signaling after binding to its ligand dsRNA, unlike monomers that have not been primed by *Rmrp*.

TLR3–*Rmrp* interaction is sufficient to prevent TLR3-mediated inflammatory response.

Overall, this study provides molecular and structural insights into the interaction between self-RNA *Rmrp* and TLR3. The proposed model is: *Rmrp* in the early endosome interacts with full-length TLR3, inducing TLR3 dimerization in both elongated and lapped conformations, thereby priming the receptor for subsequent recognition of exogenous dsRNA internalized in lysosomes (Fig. 1). Together with previous findings showing that TLR3 signaling requires lysosomal trafficking,[8] cathepsins-mediated cleavage[12] and the involvement of co-receptors,[9] this study uncovers an additional layer of regulation through self-RNA. Excitingly, this work also identifies the *Rmrp*–TLR3 interaction as a potential therapeutic target for more selective inhibition of TLR3 in autoinflammatory diseases.

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COMPETING INTERESTS

R.A.F. is a consultant for Odyssey Therapeutics, GlaxoSmithKline, and a Founder and Scientific advisor for Ventus Therapeutics and Prelon Therapeutics.

ADDITIONAL INFORMATION

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