AUTOIMMUNITY

Ro60-associated RNA takes its toll on disease pathogenesis

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A new study attributes the pathogenicity of anti-Ro60 autoantibodies and type I interferon in systemic lupus erythematosus and Sjögren syndrome to Alu retroelements, but is this RNA the complete story?

Refers to Hung, T. *et al.* The Ro60 autoantigen binds endogenous retroelements and regulates inflammatory gene expression. *Science* http://dx.doi.org/10.1126/science.aac7442

The 60 kDa SSA/Ro (Ro60) ribonucleoprotein is a clinically important member of the extractable nuclear antigen family and a frequent target of humoral autoimmunity in individuals with systemic lupus erythematosus (SLE) or primary Sjögren syndrome and in mothers of children with neonatal lupus. The striking association between anti-Ro60 autoantibodies and inflammatory sequelae in these diseases has led to the hypothesis that the RNA-binding properties of Ro60 give rise to aberrant Toll-like receptor (TLR) signalling. As such, identification of the Ro60-associated RNA that ligates the endosomal RNA-recognizing receptors TLR7 and TLR8 could provide novel therapeutic targets. A new study by Hung *et al.*[1] lends support to this hypothesis and suggests that endogenous retroelements could have a role in Ro60-related autoimmunity.

Hung *et al.*[1] catalogued Ro60-bound RNA in two human cell lines—an Epstein–Barr virus (EBV)-transformed B-cell line and an erythromyeloblastoid leukaemia cell line—and identified an extensive array of transcripts, which included common repetitive transposons termed Alu retroelements. Transfection of Alu elements into human peripheral blood mononuclear cells (PBMCs) stimulated secretion of proinflammatory cytokines. Moreover, Alu retroelement expression was increased in sera from patients with SLE relative to controls, particularly those with high interferon signature metric (ISM) scores[1]. Although these findings establish a link between Alu retroelements and type I interferon, relevance to anti-Ro60 autoantibody-mediated tissue injury is not as clear, and the potential for Alu elements and other Ro60-associated RNAs to stimulate TLR7 and TLR8 in a physiological setting warrants further investigation.

Ro60 is a ring-shaped protein that binds misfolded, defective small RNAs via the central cavity of the ring, raising the possibility that this autoantigen functions in RNA quality-control pathways[2]. The finding that depletion of Ro60 increased the expression of Alu transcripts[1] is consistent with such a role and implies that Ro60 targets Alu elements for degradation. Ro60 also binds a class of noncoding RNA termed Y RNA on the outer surface of the ring structure[2]. Four subsets of Y RNA occur in humans (hY1, hY3, hY4, hY5), with differing roles in regulating the subcellular distribution of Ro60 and interaction with other RNAs and proteins[2–4]. In the current study, Y RNA accounted for more than one-third of Ro60-bound RNA in one cell line[1].

Over the past decade, studies have focused on Y RNAs as the Ro60-associated RNA responsible for activating TLR7 and TLR8 and perpetuating inflammation in anti-Ro60 antibody-positive patients[4–7]. Specifically, Y RNA oligoribonucleotides transfected into primary human cells or delivered via an immune complex initiate TLR7/TLR8-dependent proinflammatory cytokine release. By contrast, Hung *et al.*[1] did not observe TNF, IL-6 or IFN-α secretion from human PBMCs treated with hY1 or hY3. This discrepancy might be attributable to their use of hY1 and hY3 cDNA sequences (noted in the supplementary data[1]), which contain thymine instead of uracil. A previous study

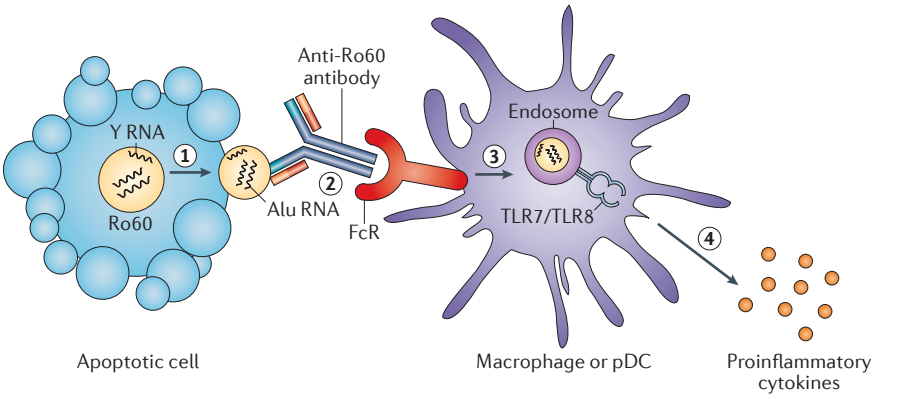

Figure 1 | **Model for anti-Ro60 autoantibody-mediated tissue injury in SLE, Sjögren syndrome and neonatal lupus.** (1) Apoptosis provides a mechanism for facilitating accessibility of the otherwise intracellular autoantigen to anti-Ro60 autoantibody. The exposure of Ro60 at the apoptotic cell membrane is dependent on Y3 RNA[4]. (2) Macrophages or pDCs engulf apoptotic cells opsonised with anti-Ro60 autoantibody or immune complexes released from apoptotic cells undergoing secondary necrosis via Fc gamma receptors. (3) Anti-Ro60 autoantibody, Ro60 protein and its associated RNA are delivered to endosomes where Ro60-bound RNA ligates TLR7/TLR8 and initiates inflammation. (4) Proinflammatory cytokines released from macrophages and pDCs increase expression of Y RNA and Alu transcripts[1], increasing exposure of Ro60 on apoptotic cells and RNA-ligand for TLR7/TLR8, thereby perpetuating inflammation and tissue injury. FcR, Fc receptor; pDC, plasmacytoid dendritic cell; SLE, systemic lupus erythematosus; TLR, Toll-like receptor.

reported that uracil-rich regions preceded by at least one cytosine or guanosine base in Y RNA were required to activate TLR7 and TLR8[5]. Indeed, similar sequences within Alu motifs stimulated these TLRs[1]. However, uracil-rich sequences can be found within the 3′ regions of many human small RNAs synthesized by RNA polymerase III[5], suggesting that numerous Ro60-bound transcripts, in addition to Alu elements and Y RNA, are capable of activating TLR7 and TLR8. Given the diversity of RNA bound by Ro60 (>50% of transcripts contained neither Y RNA nor Alu elements[1]) and the potential for many of these transcripts to ligate TLR7 and TLR8, are some specific RNA transcripts more relevant to disease—and hence more promising therapeutic targets—than others?

To determine the relevance of any Ro60-associated RNA to autoantibody-mediated disease, consideration of the cell type and conditions that facilitate accessibility of an otherwise intracellular autoantigen is critical. The diversity of Ro60-associated RNAs, including the Y RNA complement, varies between cell types and conditions[2]. This diversity is also evident in the study by Hung *et al.*[1], in which substantial differences in Ro60-bound transcripts were observed between the EBV-transformed B-cell line and the erythromyeloblastoid leukaemia cell line. Future studies should focus on cataloguing Ro60-bound RNAs in disease-relevant cells such as primary human keratinocytes, hepatocytes and fetal cardiomyocytes.

With regard to conditions, current models of anti-Ro60 antibody-mediated tissue injury posit apoptosis as a means for exposing Ro60 to extracellular autoantibody[4,7,8]. However, some Ro60-associated RNA are altered during apoptosis. For example, apoptosis truncates Y RNA, yet the degradation product preserves the uracil-rich TLR7/TLR8 ligand[5] and remains bound to Ro60 (REF. 9). Importantly, the exposure of Ro60 on the surface of apoptotic cells and subsequent proinflammatory cascade is dependent on Y3 RNA in murine cells[4]. The fate of Alu transcripts during apoptosis should be established. Additional studies comparing Ro60-associated RNA in apoptotic and viable cells would also be informative. Finally, the ability of Alu elements to engage TLRs should be evaluated in more-physiological conditions, such as immune complex delivery rather than transfection. Given that Ro60 binds multiple single-stranded RNAs inside its central cavity, some transcripts that ligate TLRs as naked RNAs might not be effective as part of Ro60-containing immune complexes.

The identification of increased levels of Alu transcripts in serum and purified IgG fractions from patients with SLE relative to controls, albeit in a small number of individuals, supports the idea that aberrant RNA processing contributes to disease pathogenesis. Levels of Alu retroelements were highest in SLE patients with high ISM scores regardless of whether they had anti-Ro60 autoantibodies[1]. One possibility is that an independent mechanism (for example, viral infection or other endogenous RNAs from delayed clearance of apoptotic cells) increases IFN-α expression, which, in turn, increases Alu expression. This concept is consistent with findings of increased levels of Alu transcripts in cells treated with IFN-α[1]. An alternative explanation is that immunosuppressive drugs used to control symptoms of SLE increase the expression of Alu elements. Further studies utilizing patient cohorts with documented medication use are critical to evaluate these possibilities.

In summary, Hung *et al.*[1] demonstrate that Alu retroelements activate TLR7 and TLR8 as oligoribonucleotides and associate with Ro60 in cell lines. These data support the hypothesis that the inflammatory sequelae associated with anti-Ro60 autoantibodies are due to the RNA-binding properties of Ro60. Current models of anti-Ro60 autoantibody-mediated tissue injury can now be expanded to include a potential role for Ro60-bound Alu transcripts (FIG 1). Future studies should evaluate the potential of Alu transcripts and other Ro60-associated RNAs to engage TLR7 and TLR8 in a physiological and disease-relevant setting, similar to previous studies evaluating Y RNA[4,7]. These studies will be informative for determining the therapeutic potential of targeting specific RNA molecules or broadly blocking TLR7/TLR8 signalling. A key challenge will be to break the continual turnover of Ro60-specific clones that seems to drive lifelong Ro60 humoral autoimmunity in patients with SLE and Sjögren syndrome[10]. This may entail a dual approach targeting both Ro60-associated RNAs (including Alu transcripts and Y RNAs) and Ro60-specific autoantibody clonotypes.

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Competing interests statement
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