Literature Review

RNA circles with minimized immunogenicity as potent PKR inhibitors

HPLC Column, Sepax, SRT SEC-2000 PEEK, 5um, 2000 A
4.6 x 300 mm

Part Number: 215980P-4630

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Liu et al., RNA circles with minimized immunogenicity as potent PKR inhibitors, Molecular Cell (2021), https://doi.org/10.1016/j.molcel.2021.11.019.

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Abstract

Exon back-splicing-generated circular RNAs, as a group, can suppress double-stranded RNA (dsRNA)-activated protein kinase R (PKR) in cells. We have sought to synthesize immunogenicity-free, short dsRNA-containing RNA circles as PKR inhibitors. Here, we report that RNA circles synthesized by permuted self-splicing thymidylate synthase (td) introns from T4 bacteriophage or by Anabaena pre-tRNA group I intron could induce an immune response. Autocatalytic splicing introduces ~74 nt td or ~186 nt Anabaena extraneous fragments that can distort the folding status of original circular RNAs or form structures themselves to provoke innate immune responses. In contrast, synthesized RNA circles produced by T4 RNA ligase without extraneous fragments exhibit minimized immunogenicity. Importantly, directly ligated circular RNAs that form short dsRNA regions efficiently suppress PKR activation 103- to 106-fold higher than reported chemical compounds C16 and 2-AP, highlighting the future use of circular RNAs as potent inhibitors for diseases related to PKR overreaction.

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https://www.sciencedirect.com/science/article/pii/S1097276521010091?via%3Dihub

Sample

Broad Sample Type	DNA/RNA/OLIGO
Sample	circRNA
Sample Notes	circRNA
Molecular Weight of Sample	-circPOLR2A_Ana (522 nt) -circPOLR2A_Lig, which contains two imperfect dsRNA modules (blue shadows, 32 and 21 bp) -circRNAs in cells tend to form 16–26 bp imperfect dsRNA regions -15 nt single-stranded RNA (ssRNA)

"PKR is an IFN-inducible Ser/Thr protein kinase that is directly activated by long dsRNA (>33 bp, with 79 bp achieving maximal activation) and plays a central role in the innate immune response to dsRNAs, whereas short dsRNAs of 16–33 bp in length can bind PKR monomers and block activation in vitro (Bou-Nader et al., 2019; Nallagatla et al., 2011; Zheng and Bevilacqua, 2004)"

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Sample (con't)

Sample Prep	***"In vitro RNA transcription, circularization and purification*** *RNAs were in vitro transcribed from T7 expression vector prepared by RiboMax large RNA production system (Promega) according to the manufacturer's protocol with slight modifications. Briefly, circRNA precursors synthesized by method I (T4 RNA ligase 1) were transcribed from 1 µg PCR-amplified T7-DNA fragments, incubated with 2 µL T7 RNA polymerase enzyme in the presence of rATP, rCTP, rUTP, and GMP (each at 5 mM), rGTP (1 mM) for 3.5 hours at 37°C. Linear RNAs or circRNA precursors synthesized for self-splicing (T4 thymidylate synthase (td) gene and permuted Anabaena pre-tRNA, method II and method III, respectively) were synthesized in the same way by in vitro transcription in the presence of 5 mM rNTPs. In vitro transcription was followed by DNase I (Promega) treatment for 30 min at 37°C to remove DNA templates. Transcribed RNAs were column purified using a MEGAclear Transcription Clean-up kit (Invitrogen). For T4 RNA ligase 1 circularization system (Method I), 50 µg linear RNAs were incubated with T4 RNA ligase 1 (NEB) in 1mL reaction for 3 hours at 37°C according to the manufacturer's protocol. For group I intron autocatalytic splicing system (method II and method III), we used the group I intron of phage T4 thymidylate synthase (td) gene or permuted Anabaena pre-tRNA (Wesselhoeft et al., 2018, 2019), in which the td gene is different from the sequences from Chen et al. (2017) with a 34 nt extra spacer. 50 µg circRNA precursors were heated to 70°C for 3 min and immediately placed on ice for 2 min, then GTP was added to a final concentration of 2 mM with a buffer that has the same components as the T4 RNA ligase buffer (NEB) for 8 min at 55°C.* *Circularized or linear RNAs were then concentrated by ethanol precipitation, resolved on denaturing urea polyacrylamide gel or agarose gel and visualized by Ethidium bromide staining. Of note, we routinely run circularized RNAs shorter than 1,500 nt on denaturing urea polyacrylamide gel; while circularized RNAs larger than 1,500 nt on native agarose gel with formamide and heat denaturing of the RNA before loading. Corresponding bands on denaturing urea polyacrylamide or native agarose gels were excised for circular or linear RNA purification (Figures S1 and S2). Purified circular or linear RNAs were validated by RNase R treatment normally for 45 min. Of note, different batches of RNase R were pre-determined for the use of concentrations (Zhang et al., 2016b). Primers for circularization were listed in Table S2."*

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Article Overview

LR2022021101

Experimental conditions

Column	**SRT SEC-2000 PEEK, 5um, 2000 A 4.6 x 300 mm**
Mobile Phase	Nuclease-free TE buffer (pH = 6.0)
Flow Rate	0.35 mL/minute
Instrument	HPLC; UV
Instrument Notes	260

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Literature Reference
CircRNA on Sepax Analytical SEC

LR2022021101

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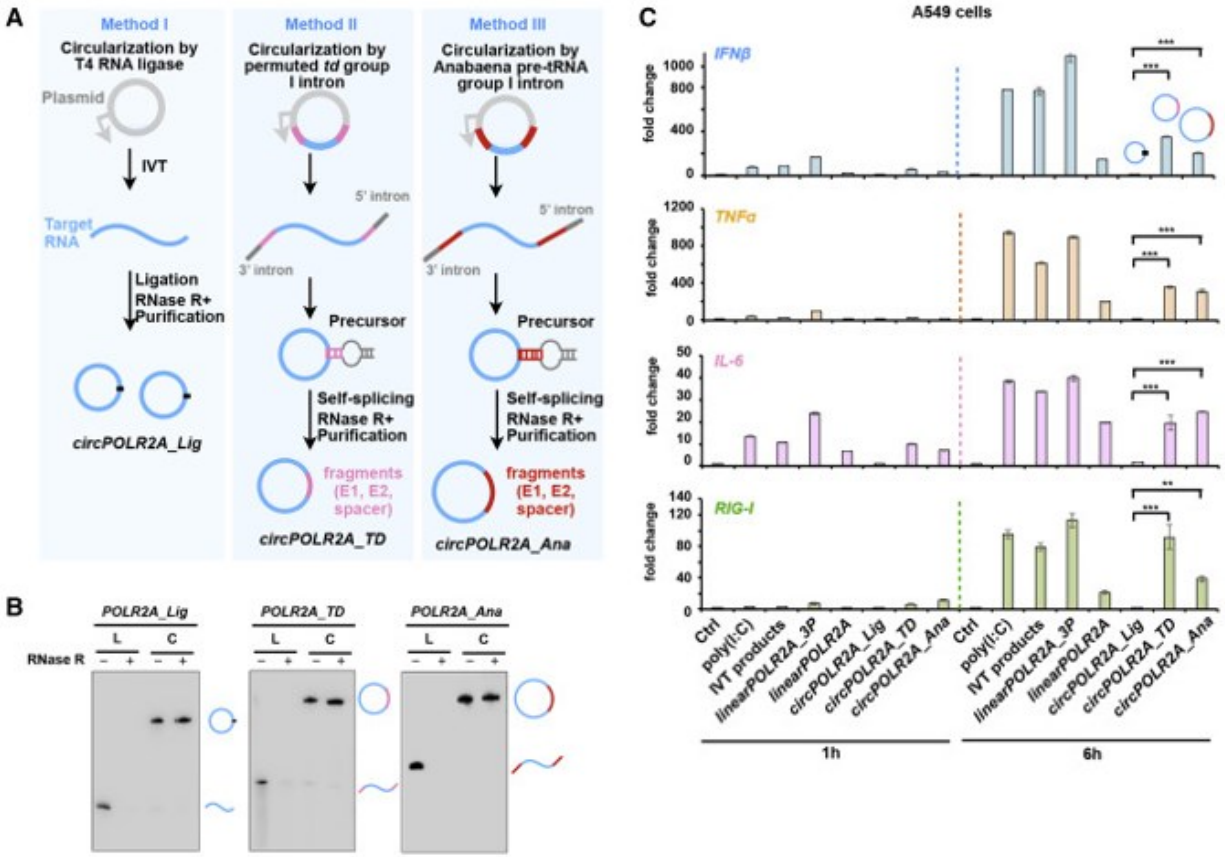

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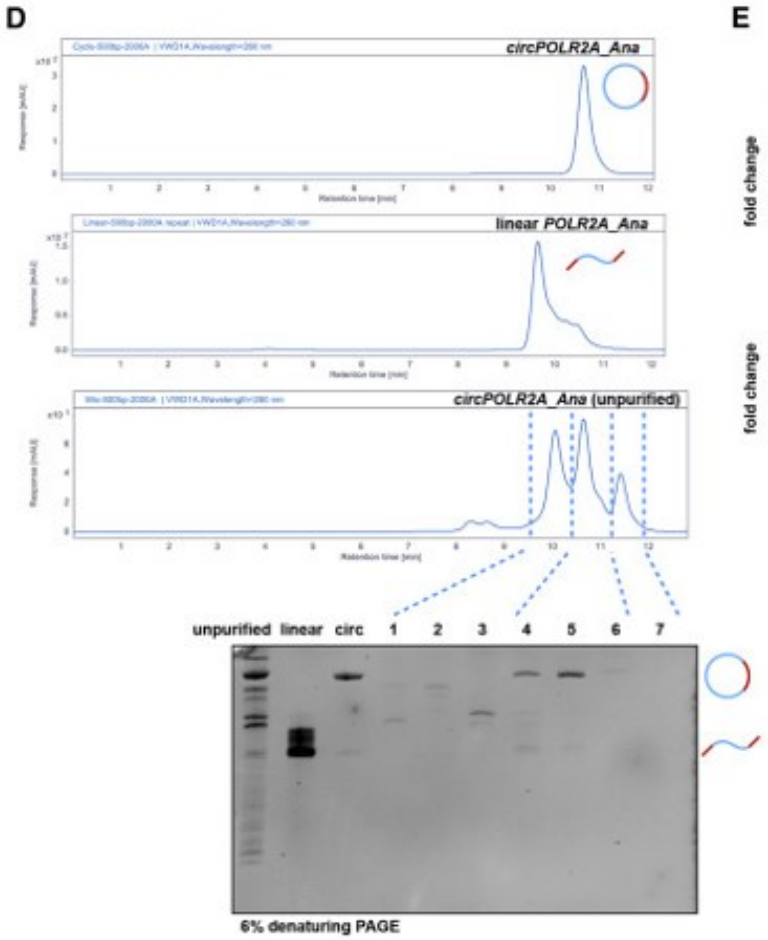

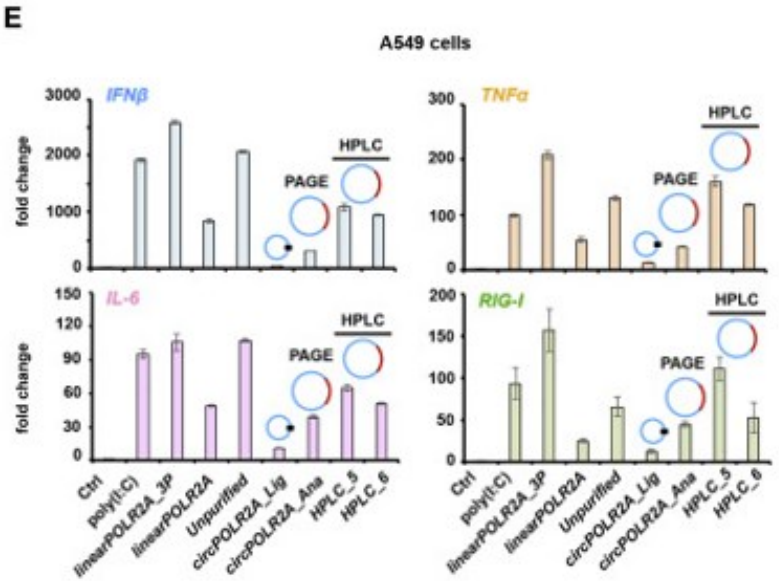

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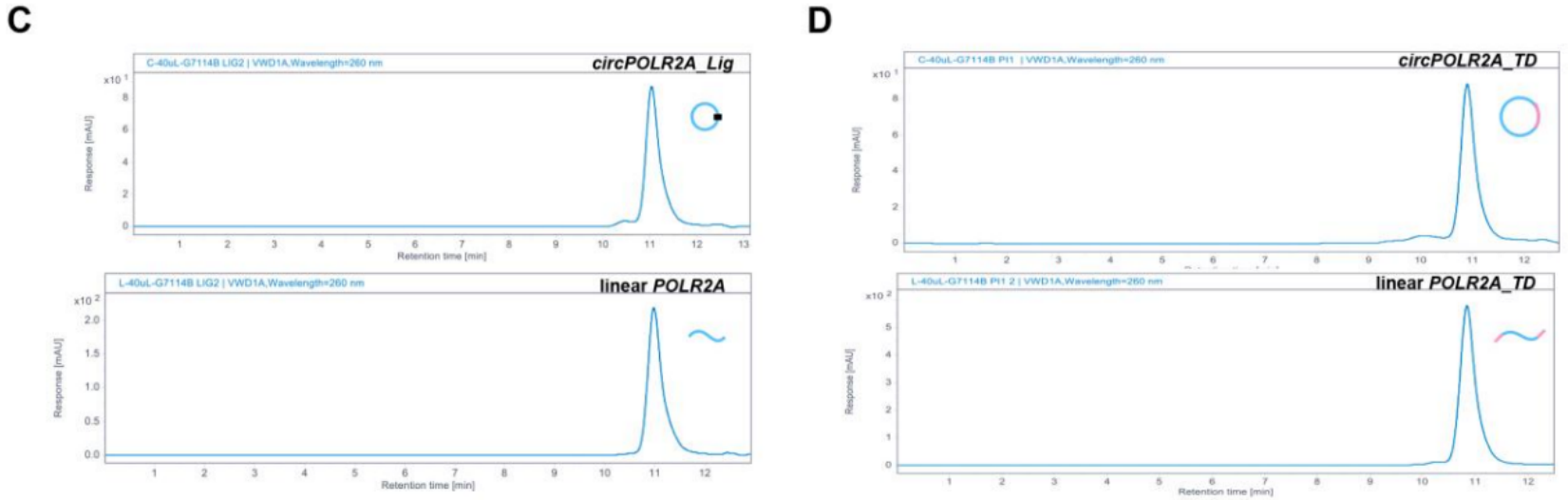

Figure S4. Circular RNAs produced from different *in vitro* circularization strategies induce distinct immune responses in human cells. (Related to Figure 1)

circPOLR2A_TD was first purified by RNase R digestion, gel excision and purification, followed by subjected to HPLC analyses. The corresponding linear RNA was also gel purified prior to HPLC analysis. Of note, the peaks of gel purified circular and linear circPOLR2A_Lig or circPOLR2A_TD products in HPLC were totally overlapped.

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