Researchers discover a new type of RNA that inhibits a broad range of viral infections

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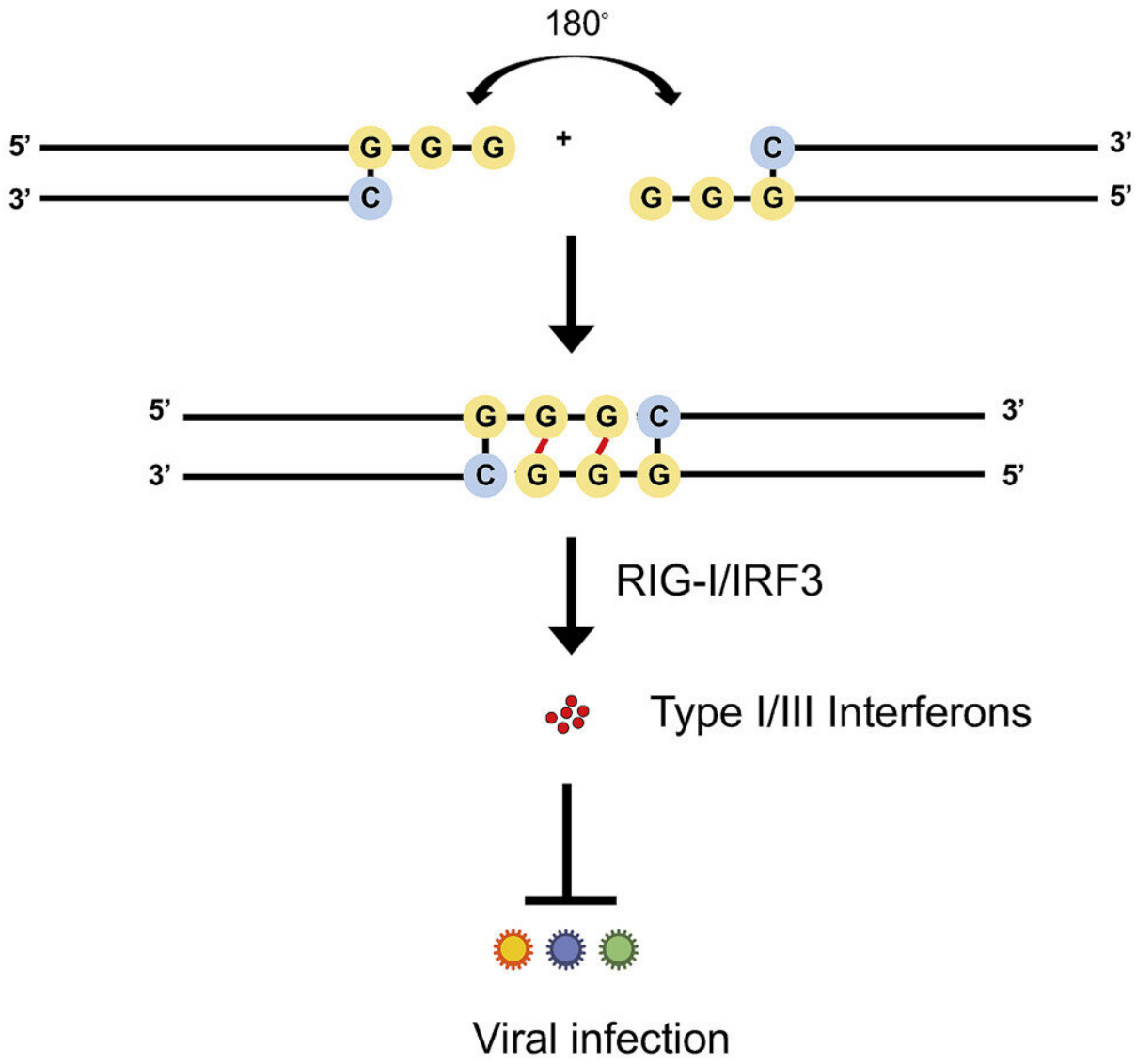

Graphical abstract. Credit: *Molecular Therapy - Nucleic Acids* (2022). DOI: 10.1016/j.omtn.2022.08.031

RNA is often described as the single-stranded cousin of DNA, the double-stranded molecule that makes up the genomes of all living organisms. Many types of non-living viruses, however, carry their genetic information in a double-stranded form of RNA (dsRNA). When the human immune system detects the presence of this foreign RNA, it sounds the alarm by increasing the production of protective cytokines called interferons (IFN), which activate the innate immune response against viral invaders.

Scientists have attempted to harness this natural antiviral response by creating therapeutic dsRNAs that mimic features of viral genomes. But meddling with the innate immune system is a double-edged sword. Viral RNA analogs can also activate molecular pathways that lead to excessive inflammation in the body, which could cause more harm than their antiviral benefits.

Now, a group of researchers at the Wyss Institute for Biologically Inspired Engineering at Harvard University has discovered a new class of immunostimulatory dsRNAs that potently induces the production of two forms of IFN (IFN-I and IFN-III) while limiting the inflammation commonly observed with previous types of RNA-based immunostimulants.

The new dsRNA molecules dramatically inhibited the ability of many viruses with pandemic potential—including SARS-CoV, SARS-CoV-2, MERS-CoV, and multiple influenza A strains—to infect human cells, in both traditional cell culture as well as in complex human Organ Chip models of the lung. In a mouse model of COVID-19, the dsRNA

reduced the amount of the virus in the animals' bodies by more than 1,000-fold. The results are published today in *Molecular Therapy—Nucleic Acids*.

"These new dsRNAs are an attractive treatment option for COVID-19 because SARS-CoV-2 infection produces an imbalanced immune response in which the protective IFN reaction is suppressed while the inflammation reaction is elevated. By preferentially increasing IFN-I, our dsRNA has the potential to correct this imbalance, and could be used to treat many other viral diseases as well," said co-first author Haiqing Bai, Ph.D., a former Wyss Technology Development Fellow who is now Director of Preclinical Development at Xellar Biosystems.

Serendipitous discovery, systematic sleuthing

For years, Bai and other members of Wyss Founding Director Don Ingber's lab have been developing new treatments for viral diseases using their human Organ Chip platform. Prior to 2020, the team was working on identifying genes in human lung cells that regulate their responses to influenza A infection, which at the time was the virus of highest pandemic concern. They started by using CRISPR-Cas9 screening to identify host genes that might inhibit the proliferation of the influenza virus. One of their top candidates from the screening was a gene called DGCR5, which produces a long, noncoding RNA sequence rather than a protein. To see how DGCR5 impacted infection rates, they "knocked down" the gene by designing three different double-stranded small interfering RNAs (siRNAs) to interfere with the gene's expression, then delivered the siRNAs into cultured lung cells.

The researchers found that this mixture reduced DGCR5 levels by more than 80% and inhibited influenza A infection by more than 90%. Excited by these results, they started testing the siRNAs individually as well as some additional DGCR5-specific siRNA sequences. To their

surprise, while nine of the ten siRNAs they created significantly reduced DGCR5 gene expression, only one of them inhibited viral infection. It seemed that DGCR5 activity might not be the driver of viral infection rates.

When they investigated further, they confirmed that this particular siRNA caused the upregulation of multiple genes that are involved in the IFN signaling pathway. Additional studies revealed that the siRNA exclusively activated one of three signaling pathways that can trigger IFN production, known as the RIG-I pathway, which is an essential component of the innate immune response. The researchers began to suspect that they had discovered a new class of dsRNAs that stimulated the immune system via a previously unknown mechanism of action.

They got to work systematically generating and testing more than 200 variations on the original dsRNA sequence to tease out that mechanism. They identified a particular string of nucleotides, called a "motif," that was consistently present on one end of dsRNAs with high IFN-stimulating activity: a cytosine (C) on one strand and three guanines (GGG) on the other strand. Because the C binds to one G of the GGG sequence in the motif, there is an "overhang" of two Gs at the end of each dsRNA. When many copies of the dsRNA are present, the overhanging Gs of one molecule can bind to those of another molecule via an unusual phenomenon called G-G Hoogsteen base pairing. The resulting dsRNA dimers then directly bind to RIG-I very effectively, causing its activation and the subsequent IFN response.

"That was very interesting to us, because previous studies had shown that RIG-I can be activated by 'blunt-ended' duplex dsRNAs that lack overhangs, and that almost any type of overhang can prevent RIG-I binding. But the unique G-G binding behavior we observed effectively creates blunt-ended dsRNAs that can potently activate RIG-I, so in this case, the overhang is crucial to the activity of our novel dsRNA," said co-

first author Longlong Si, Ph.D., a former Wyss Technology Development Fellow who is now a Professor at the Shenzhen Institute of Advanced Technology in China.

From flu to COVID and beyond

To see how effective their newly discovered dsRNAs were in living cells, the researchers tested one of them head-to-head against a synthetic immunostimulant that mimics viral infections called poly(I:C). When they applied the two treatments to human epithelial cells, they found that the dsRNA produced a less inflammatory antiviral response, while the more inflammatory poly(I:C) caused much broader changes in gene expression and impacted other biological processes that are essential for normal cell function.

Taking it one step further, the team then tested the dsRNA in human Lung Airway and Alveolus Chips previously developed at the Wyss Institute to replicate complex human organ tissue and functions in vitro. They introduced the dsRNA into healthy chips and saw that IFN-I expression increased between 12- to 40-fold. When they then added influenza A to the Organ Chips, they found that the dsRNA suppressed infection by 80-90%.

Then the COVID-19 pandemic hit, and the team pivoted their studies from influenza to SARS-CoV-2 and the related coronaviruses SARS-CoV, MERS-CoV, and the common cold virus HCoV-NL63. Their dsRNA took the change in stride, inhibiting MERS-CoV and HCoV-NL63 infection of a mammalian cell line derived from monkeys by more than 90%, and SARS-CoV by more than 1,000-fold. It also inhibited SARS-CoV-2 infection of a human epithelial cell line by a stunning 99.99%.

These studies were completed in collaboration with Matthew Frieman's

group from the University of Maryland School of Medicine and Benjamin tenOever from the Icahn School of Medicine at Mount Sinai (now the Grossman School of Medicine at New York University).

Finally, they tested the dsRNA in a mouse model of COVID-19 with collaborators Dong Yang and Colleen Johnsson, Ph.D. at the University of Tennessee Health Science Center. When they infected the treated mice with SARS-CoV-2, the dsRNA reduced the viral load in the animals' lungs by more than 1,000-fold compared to animals treated with a scrambled dsRNA sequence.

"The COVID-19 pandemic has made it painfully clear that we need broad-spectrum therapeutics that are capable of attenuating infection by a wide variety of viruses, rather than developing a bespoke treatment for each individual disease as it arises. I'm hopeful that this RNA therapeutic technology, whose chemical and physical properties make it easily manufacturable at large scales, will become a widely used approach for fighting future pandemics," said Don Ingber, M.D., Ph.D., who is also the Judah Folkman Professor of Vascular Biology at Harvard Medical School (HMS) and Boston Children's Hospital, and Hansjörg Wyss Professor of Bioinspired Engineering at the Harvard John A. Paulson School of Engineering and Applied Sciences.

In addition to treating viruses, these novel dsRNAs could treat bacterial, fungal, and parasitic infections, as well as conditions like cancer and autoimmune diseases that could benefit from increased IFN production. They could also be used as an adjuvant to enhance the activity of other vaccines.

The authors say that it is important for future studies to establish the optimal timing of treatment, as activating IFN too late in an infection may exacerbate inflammation, as well as to investigate the possibility of administering the dsRNA directly to the upper airway (for example, via

an inhaler) to minimize systemic immune activation.

More information: Longlong Si et al, Self-assembling short immunostimulatory duplex RNAs with broad-spectrum antiviral activity, *Molecular Therapy—Nucleic Acids* (2022). DOI: 10.1016/j.omtn.2022.08.031

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