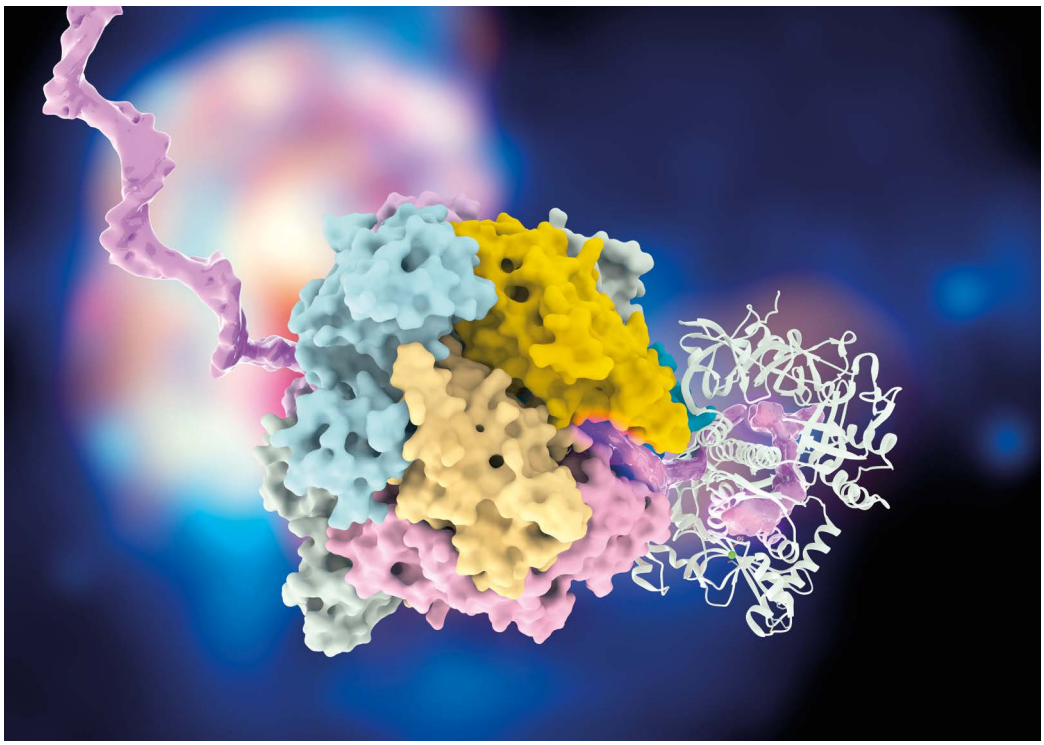
Research round-up

Highlights from extracellular RNA studies. By Elizabeth Svoboda



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Molecular model of an exosome complex.

Immune RNA drives heart disease

Small fragments of RNA that break away from immune cells might set people on the path to cardiovascular disease. Mast cells — immune cells produced in the bone marrow — are known to encourage the blood-vessel irritation and swelling that signal the onset of cardiovascular disease, but precisely how these cells initiate this inflammation has not been well understood. Now, a study led by researchers at Justus Liebig University in Giessen, Germany, has shown that fragments of RNA released by mast cells cause cellular changes that trigger inflammation.

Silvia Fischer and her colleagues grew mast cells from mouse bone marrow *in vitro*. They then treated the mast cells with chemicals that caused the cells to release RNA-containing particles called exosomes — a release that happens naturally inside the body. When the team added the RNA-filled vesicles to a culture of cells that line blood vessels, the cells expressed more inflammatory proteins called cytokines. The higher the concentration of RNA-containing vesicles that were introduced, the more cytokines the cells expressed.

The results suggest that extracellular RNA from mast cells spurs the inflammation that degrades vascular health. Circulating RNA molecules might also be involved in changes to blood-vessel walls related to conditions such as high blood pressure, hardening of the arteries and aneurysm, in which blood-vessel walls balloon outwards. The team is continuing to study how extracellular RNA interacts with blood vessels, and hopes to find out whether the RNA from mast cells promotes cell signalling that might affect disease processes.

FASEB J. **33**, 5457–5467 (2019)

Biomarkers for anxiety disorder

Biological markers for post-traumatic stress disorder (PTSD) have mostly proved elusive. Kai Wang at the Institute for Systems Biology in Seattle, Washington, and her colleagues are shifting the picture by showing that levels of some RNA fragments circulating in the blood of combat veterans with PTSD are different from the levels of those without the condition — a finding that could lead to a blood test for PTSD.

The researchers recruited 22 male combat veterans who served in Iraq or Afghanistan and an equal number of people without PTSD. They took a blood sample from each participant, extracted the fluid plasma portion and then sequenced the extracellular RNA found in the plasma. Compared with the control group, people with PTSD had unusual concentrations (sometimes higher than the control group, sometimes lower) of several RNA molecules circulating in their blood. These RNAs are involved in central nervous system development, inflammation and the control of the brain's neurotransmitter system. The team found a similar profile of circulating RNA in a validation group of ten additional veterans with PTSD.

The authors hope to confirm their proposed biomarkers in a larger experimental group that includes both men and women. If a biomarker test for PTSD proves reliable across the board, psychiatrists could also adopt it as a tool to monitor treatment effectiveness.

J. Clin. Med. **8**, 963 (2019)

Revealing early-stage Alzheimer's disease

RNA fragments in the bloodstream could allow physicians to detect Alzheimer's disease at an early stage, when treatments are more likely to be effective.

Many people experience years of cognitive decline before learning that they have

Alzheimer's. Other methods for detecting the disease early, such as brain imaging and cerebrospinal fluid analysis, are too expensive or invasive for use in widespread screening programmes. The experimental test developed by José Rodríguez-Álvarez at the Autonomous University of Barcelona in Spain and colleagues relies instead on a blood sample. The test detects snippets of circulating RNA that control proteins involved in forming synapses, the crucial connections between brain cells.

When the researchers ran the blood test on more than 100 people with a wide variation in cognitive performance, they identified three RNA molecules that were present at high levels in people with mild cognitive impairment (MCI) and early Alzheimer's disease, but not in people with healthy cognitive function. People with MCI who went on to develop Alzheimer's had higher levels of these three RNAs than had people who did not progress to Alzheimer's.

The researchers are planning large-scale trials of their blood test to validate their findings. If it proves to be a reliable indicator of cognitive impairment, the test could form the basis of population-wide screening programmes, because it can be performed easily at a lab or a physician's office. Clinicians could also use the test to predict who is most likely to develop Alzheimer's.

Alzheimers Res. Ther. **11**, 46 (2019)

RNA boost for kidney regeneration

Some stem cells emit tiny particles containing fragments of RNA that help damaged kidneys to regrow. Giovanni Camussi at the University of Torino in Italy and colleagues report that enriching these particles — known as extracellular vesicles, or EVs — with RNA molecules can elicit more-potent kidney regrowth than can non-engineered vesicles. This enrichment could allow for the development of drugs that use fewer EVs, potentially minimizing the drugs' risk and cost.

The team tested its approach on a group of mice with acute kidney injuries. Some of the mice were treated with naturally occurring EVs from mesenchymal stromal cells — a cell type known to regenerate injured tissue. Others received EVs from these stem cells that were treated with an electrical stimulus to open pores to pack more RNA molecules inside. The researchers enriched these vesicles with carefully selected RNA molecules that help cells in kidney ducts to grow more quickly.

A low dose of vesicles with the electrically added RNA cargo proved more effective at improving the mice's kidney function than did a similar dose of naturally occurring vesicles. Mice that received this dose of modified vesicles had less kidney damage, and their kidneys filtered toxins from the blood more efficiently. (Mice that received high doses of electrically treated EVs did not have significantly different outcomes from mice given high doses of untreated EVs.)

The study shows that vesicles can be made more therapeutically active by packing in a greater number of RNA molecules — a strategy that might spark the development of low-dose regenerative drugs for people with kidney injuries.

Int. J. Mol. Sci. **20**, 2381 (2019)

Predicting the course of multiple myeloma

The progression of multiple myeloma, a bone-marrow cancer typically affecting people over the age of 60, varies greatly from one person to the next. Andrew Spencer at Monash University in Melbourne, Australia, and colleagues report that levels of various RNA molecules in the bloodstream reveal key features of individual cases of the disease — an approach that could allow physicians to more accurately track patients' progress and to assess prognoses.

The researchers observed how 24 people with multiple myeloma that had returned or was resistant to treatment responded to a 28-day cycle of chemotherapy. They took blood samples from each participant several times over the course of the month and sequenced genetic information, including extracellular RNA, from each sample.

Participants with high levels of *CRBN* circulating RNA at the start proved to have a higher-than-normal risk of fast disease progression. Later in the month, people with increased blood levels of *IKZF1* RNA molecules, showed a better response to therapy and higher survival rates than did those with lower levels of this molecule. People with low levels of *CRBN* RNA at the outset and high levels of *IKZF1* RNA later responded the best.

The team plans to test a larger group of people with multiple myeloma to see if these circulating RNAs prove useful as biomarkers in a broader population. If so, its goal — a blood test that can be used to monitor each case and predict disease course with pinpoint precision — would move closer to clinical reality.

Leukemia **33**, 2022–2033 (2019)

Molecular 'backbone' increases efficiency

Extracellular RNA shows promise in correcting abnormal cell processes in conditions such as heart disease, cancer and brain injury. However, high doses of some circulating RNAs can be toxic — a roadblock that has ended some clinical trials. Derrick Gibbings at the University of Ottawa and colleagues have worked out a way to pack RNA molecules into a 'backbone' configuration that ensures more of the molecules affect target organs. This greatly reduces the amount of RNA needed for treatment and could lower the health risks.

The team made therapeutic sequences of RNA inside the nuclei of cells. Using enzymes, it integrated these sequences into an additional segment of RNA known as a backbone. This composite backbone then appeared in vesicles that the cells produced. The researchers injected the vesicles into mice and showed that they were widely distributed to organs, including the kidney, liver, intestine and lungs.

Importantly, a high percentage of the RNA molecules in the custom vesicles accumulated in target organs without being destroyed by the surrounding cells. By contrast, when the RNAs were delivered using lipid nanoparticles — a more established delivery technique — or with vesicles that lacked backbones, far fewer of the molecules reached their target. The custom vesicles probably perform best because of the efficient way that RNA is packaged.

The researchers say that their proof-of-concept study justifies further development of the backbone approach to see whether it is practical for safe drug delivery in clinical trials.

Nature Biomed. Eng. **4**, 52–68 (2020)

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