NEIL WEBB

HEART DISEASE

RNA therapy sets sail for the heart

Researchers hope that understanding the many roles of non-coding RNA in heart health and cardiovascular disease could deliver a therapeutic breakthrough.

BY CHRIS WOOLSTON

When Stefanie Dimmeler isolates strands of RNA in her laboratory, she's not just handling an interesting piece of genetics. She's searching for a possible advance to fight the world's number one cause of death.

In the past decade, scientists such as Dimmeler have shown that certain types of RNA help to regulate the basic mechanisms of cardiovascular disease, including the formation of cholesterol, the build-up of plaques and the death of cells after a heart attack.

By learning more about the roles of RNA in cardiovascular disease, researchers hope to harness the molecules to prevent and even reverse key steps in the process.

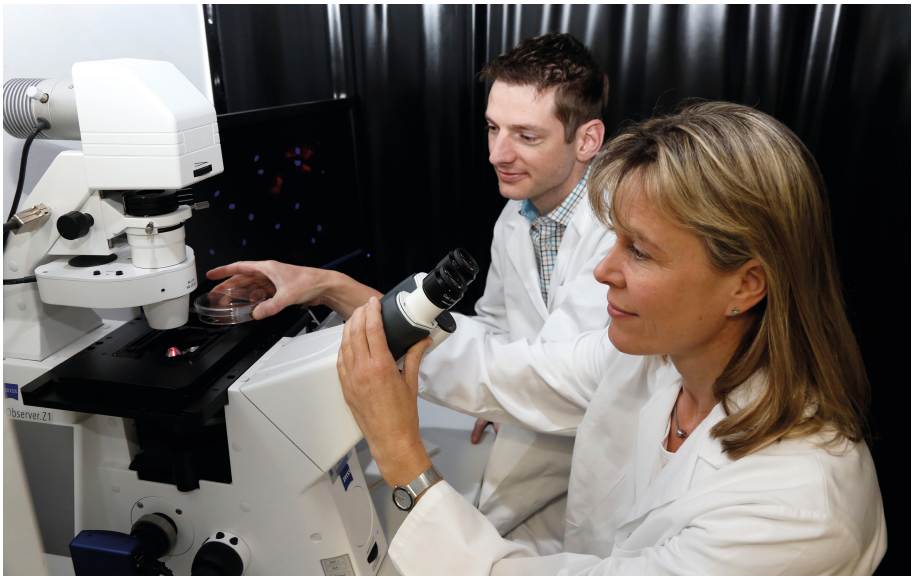
Dimmeler, a specialist in cardiovascular regeneration at Goethe University Frankfurt in Germany, describes RNA as a "biologically and mechanistically attractive target".

BENEFITS AND RISKS

Because cardiovascular disease is often caused by a complex process that occurs slowly over decades, any therapy targeting RNA would have to deliver long-lasting results with minimal side effects, a difficult balance to strike. The near ubiquity of heart disease also raises the stakes. Researchers have to think carefully about who — if anyone — could benefit from this potentially risky treatment. "If we can get something going with RNA, the impact could be major," she says.

Most of the focus is on a class of RNA that doesn't directly code for proteins. Once thought of as meaningless genetic noise, such non-coding RNA is now known to regulate the expression of proteins throughout the body, including many of the key proteins involved in heart disease. In particular, non-coding microRNAs — strands of 21–23 nucleotides — are thought to affect the protein output of more than 60% of human genes, says Carlos Fernandez-Hernando, a pathologist at Yale University in New Haven, Connecticut.

Some of the non-coding RNA molecules that have already been isolated, identified and tested in the laboratory have created



COURTESY OF STEFANIE DIMMELER

Stefanie Dimmeler at Goethe University Frankfurt, Germany, investigates RNA that affects heart health.

real cause for optimism, Fernandez-Hernando says. Long non-coding RNA, which is made up of more than 200 nucleotides and can both promote and suppress the expression of proteins, intrigues researchers with its diversity and flexibility. But when it comes to therapeutic potential in the short term, scientists such as Fernandez-Hernando have focused mainly on microRNAs. These strands have just one main function: gumming up the production of proteins by binding to the transcription machinery in a cell.

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Fernandez-Hernando's lab is especially interested in suppressing miR-33, a type of microRNA that has an important role in cholesterol metabolism. Among other functions, the molecule slows the production of high density lipoprotein (HDL) cholesterol, known as 'good' cholesterol because it helps transport low density lipoprotein (LDL) cholesterol to the liver, where it can be broken down and eliminated. Fernandez-Hernando and his colleagues have shown[1] that levels of HDL cholesterol in mice surge when the animals are treated with DNA sequences called antisense oligonucleotides that target and destroy miR-33.

Fernandez-Hernando explains that miR-33 inhibits at least ten genes involved in producing HDL cholesterol. The molecule's effect on any particular gene is fairly mild, he says, reducing the expression of a protein by just 5–10%. But because the microRNA affects different parts of the same biological process, suppressing the molecule can have a substantial impact on the final outcome. "MicroRNA is wonderful because you ought to be able to target an entire pathway," he says. "The effect on the output could be lot stronger than if you targeted a single gene. There's a lot of excitement for a potentially powerful technique."

CARDIAC AGEING

That same excitement has spurred Dimmeler and her team in Frankfurt to investigate a suite of microRNAs that threaten heart health at the most fundamental levels. The microRNA miR-34, for example, promotes cardiac ageing and cell death after a heart attack. Just as Fernandez-Hernando hopes to boost good cholesterol by suppressing miR-33, Dimmeler thinks that lowering the production of miR-34 could promote healing after a heart attack.

Another molecule in Dimmeler's sights is miR-92A, a microRNA that induces cell death in cells deprived of oxygen after a heart attack. Dimmeler and her team showed that inhibiting miR-92A significantly improved heart function in mice and pigs with sudden insufficient blood supply to the heart, or acute ischaemia[2]. "There's also unpublished data showing that miR-92A can improve heart function in diabetic pigs with chronic ischaemia," she says. "It could be a very interesting target for heart failure."

Other microRNAs work the other way, by encouraging regeneration of heart tissue. Instead of suppressing these targets, researchers are hoping to make them more abundant. In 2012, scientists showed that over expression of miR-199 enhanced the proliferation of heart cells in mice[3]. In practice, such an approach could trigger an unhealthy overload of heart cells. Dimmeler says that any treatment that uses miR-199 to encourage regeneration of damaged tissue would have to be carefully titrated and given for a short time.

Other obstacles might prove tougher to overcome. Because microRNAs can attach to many different genes, unintended consequences are often a serious concern, says Andrew Baker, a molecular biologist at the University of Edinburgh, UK. Studies of miR-21, a microRNA associated with plaque build-up in arteries, have found it is seven times more abundant in peripheral arteries clogged with atherosclerosis than in healthy arteries[4]. But broad, unfocused attempts to suppress the molecule will almost certainly hit other targets with unpredictable results. Baker says "miR-21 is expressed in virtually every cell". "You need to target the therapy to a particular cell type," he adds.

Scientists are exploring several strategies for delivering RNA therapies to the intended cardiovascular targets, including piggybacking microRNA onto an adenovirus vector — commonly used to insert genes into cells — and using catheters to deliver the package to specific tissues. Another option is using microRNA inhibitors that could be activated by precisely aimed beams of light. Dimmeler notes that using light can be effective for surface tissues but is much more challenging for internal organs such as the heart. Baker and his colleagues are exploring ways to deliver miRNA-based therapies directly to veins exposed during surgical grafts. "Having access to the tissue is a real bonus," Baker says. "It also presents some challenges because you don't have access to the tissue for very long."

If and when such technical hurdles are overcome, the central question remains: who might benefit from cardiovascular therapy that targets RNA? Baker suspects that it could be most helpful for people at very high risk of heart disease, such as those with unstable plaques or early-stage heart failure. Targeting a process such as the slow build-up of plaque in arteries, a nearly universal occurrence that develops throughout a lifetime, would require further breakthroughs and a deeper understanding of the risks of manipulating RNA for decades at a time. "There are a lot of unknowns about such long-term treatment," Baker says. "That's not to say that it can't be done."

The uncertainties should help to spur more basic research in the future. In addition to fine-tuning approaches using known microRNAs, Baker says that researchers should continue looking for new candidates. It's quite possible, he says, that the best targets for cardiovascular disease have yet to be discovered. "There are a lot of non-coding RNAs in the human genome that nobody knows anything about," he says. Until all of RNA strands at the core of heart disease have been identified, the full potential of the RNA approach will remain unknown.

It might turn out that RNA-based therapies never add much firepower to the fight against cardiovascular disease, Baker says. But with so many potential targets and so much on the line, it's clearly worth a shot. ■

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1. Rotllan, N. *et al. Arter. Thromb. Vasc. Biol.* **33**, 1973–1977 (2013).
2. Hinkel, R. *et al. Circulation* **128**, 1066–1075 (2013).
3. Eulalio, A. *et al. Nature* **492**, 376–381 (2012).
4. Hung, J. *et al. Front. Physiol.* https://doi.org/10.3389/fphys.2018.01655 (2018).