Vascular grafts: biomolecules to prevent blood vessels from reclosing

Obstructed blood vessels can be operated on and adequate blood flow restored. However, stents and bypasses are often subject to high reclosure rates. Excessive immune reactions close to where the intervention took place might prevent the regeneration of adjacent vessel walls and even lead to reclosure. New interventions involving RNA interference seem to be a way out of this dilemma.

Cardiovascular diseases are the number one cause of death in Germany. According to the WHO, roughly 12 million people worldwide die of cardiovascular diseases every year. Controllable heart disease factors include excess weight, smoking and physical inactivity. Despite comprehensive information and prevention campaigns, the problem stands to increase among ageing Western populations.

Constricted and obstructed blood vessels are the most common direct cause of cardiovascular diseases. Numerous treatment methods to restore adequate blood flow are now available, including sophisticated coronary artery bypass surgery. For bypass surgery, superficial veins are taken from the patient's legs, inserted between the aorta and joined to a part of the artery below the obstructive lesion. Another possibility is the use of stents or artificial vascular grafts made of a polymer reinforced by a metal mesh. Less extensive vascular obstructions can be treated using a balloon dilatation catheter to inflate a peripheral blood vessel and improve the blood flow in the compromised artery. These interventions are usually carried out in combination with medicinal therapy and, together with a change in lifestyle, are directed towards restoring a normal blood flow.

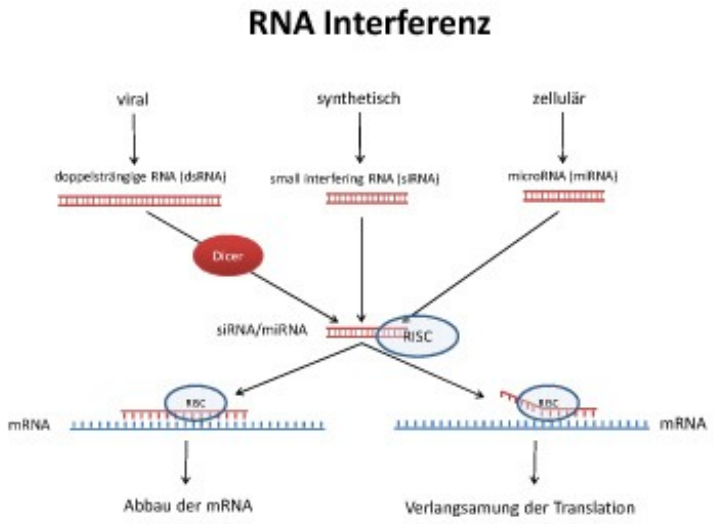
Almost one third of all surgically treated blood vessels are at risk of reclosure

The team of researchers from Tübingen (from left to right): PD Dr. Tobias Walker, Dr. Andrea Nolte and Prof. Dr. Hans Peter Wendel are aiming to use siRNA for the regeneration of vascular walls.

However, it is still difficult to prevent new blockages, and bypass grafts can close again and fail due to a process known as restenosis. Numerous studies have been carried out to assess the risk of restenoses, and some studies have identified a frequency of 30 per cent and more. Coated drug-eluting stents that slowly release a drug to block cell proliferation have been on the market for quite some time. However, the so-called ideal stent is still lacking. A major cause of restenosis is the surgical intervention itself, which can lead to an excessive immune reaction in the treated area. Leukocytes attach to the vessel wall and mediate inflammatory processes that eventually lead to the reclosure of the artery.

Dr. Andrea Nolte, PD Dr. Tobias Walker and Prof. Dr. Hans Peter Wendel from the Department of Thoracic, Cardiac and Vascular Surgery (Medical Director: Prof. Dr. Dr. Schlensak) at the University Hospital of Tübingen have been focusing on these mechanisms for several years and hope to be able to prevent them from occurring by using innovative regenerative methods. In a BMBF-funded project carried out in cooperation with industrial partners and researchers from the NMI Natural and Medical Sciences Institute in Reutlingen, Nolte, Walker and Wendel have developed "gene-silencing stents". These stents have been shown to improve the outcome of intravascular interventions. The implanted stents elute specific small interfering RNAs (siRNAs) that silence specific genes involved in inflammatory signalling cascades. The method does not lead to changes in the genome and the silencing effect is only transient. This is why this technique is not considered to be gene therapy.

RNA interference prevents inflammatory reactions



The schematic shows how siRNA slows down the translation and final degradation of mRNA

A mechanism known as RNA interference has proven to be key in the researchers' efforts to improve the regeneration of the vascular wall and prevent the reclosure of the blood vessels. This mechanism was not invented by molecular biologists but is a natural process within living (animal, plant and human) cells that moderates the activity of their genes. In 2006, Andrew Z. Fire and Craig C. Mello were awarded the Nobel Prize in Physiology or Medicine for their work on RNAi

interference. The two researchers discovered how short RNA fragments (siRNA) bind to complementary mRNA (messenger RNA) and decrease its activity. The siRNA interferes with the mRNA, blocks and eventually destroys it. The mRNA is thus prevented from producing a protein. In 2004, Walker and Wendel started to focus on the development of siRNA. They were looking for sequences that impaired the production of proteins that are key in vascular biology. "The fact that we have been working in this field from the early days has given us a lead. We believed in our findings and ensured that we protected our results with patents," said Walker. In order to increase the compatibility of vascular grafts, the team has been investigating numerous proteins that are needed by the leukocytes to enter and attach to the endothelial wall. "We have investigated numerous adhesion receptor molecules and found siRNAs that prevented the formation of receptors," Wendel explains. Something that sounds quite simple actually involved a great deal of work. The researchers tested a huge number of siRNA sequences in order to find the one that had the best "gene knock-down" effect, i.e. silenced the genes involved in the pathway of intimal hyperplasia, which is the universal response of a vessel to injury and one of the complications involved in reconstruction procedures. In the hope of obtaining a knock-down effect that was as comprehensive as possible (ed. note: transfection of exogenous siRNA can be problematic as the gene knock-down is only transient), the researchers from Tübingen initially concentrated on proteins such as E-selectin that play a pivotal role in leukocyte-endothelial interactions during the early phases of the inflammatory cascade.

Challenge: the functional and local delivery of siRNAs

Metal mesh of a classic stent that is implanted into blood vessels in order to keep them open

The development of suitable siRNA was an important step on the researchers' path towards developing a therapy that enabled better vascular wall regeneration. However, the researchers were faced with many more challenges. For example, they had to find a suitable transfection mechanism that enabled the functional and local delivery of double-stranded RNA into the cytosol

of endothelial cells located in the vicinity of the stent. This was necessary as RNA is only enzymatically cleaved into smaller siRNA fragments inside cells. “Many means of transfection that are suitable for robust cancer cells such as HeLa cells kill endothelial cells and were therefore unsuitable for our purposes,” said Nolte. Another obstacle was the local administration of the siRNAs: “We were confronted with numerous technical difficulties. For example, we had to find a way to encapsulate the RNA in order to prevent it from being destroyed by the RNAse enzymes. We also had to find a solution that enabled us to immobilize the capsules with the polymer which we used to coat the stent.” And last not least, the researchers also had to deal with the release kinetics as the siRNA must only exert its effect for as long as the tissue is affected by the surgical intervention.

“The interaction of the siRNA with the components of the vascular wall proved to be the major challenge; we had to find the right combination of effect and compatibility,” said Wendel summarizing their research. This is now something the researchers have succeeded in doing. The BMBF project is drawing to a close and the researchers are specifically concentrating on validating the new technology. “One of our industrial partners is a stent manufacturer, and this company will be placing our “gene-silencing stents” on the market in the not-too-distant future,” said Wendel referring to the commercially relevant success of the project.

The researchers are nevertheless not sitting on their laurels and have plans to transfer the principle to other applications, for example the knock-down of genes that are upregulated under different pathological conditions. “We have plans to apply the encapsulated RNA to special medicinal products”, said Wendel who can even imagine applying the method to neuronal stents in the brain.

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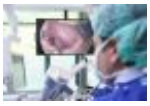

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