Can gene therapy cure broken hearts?

Professor Patrick Most explains his new S100A1 gene-based cardiac disease therapy, which holds the promise of being able to cure cardiomyocytic disease through the control of calcium levels and cell regeneration

Can you begin by explaining what attracted you to medicine, and more specifically to cardiology?

It was a combination of altruism and a growing passion for science that led me to medicine. I was always very much intrigued by molecular models that could actually explain the underlying causes of diseases that we have seen clinically. At the beginning of my clinical training, I worked in an intensive care cardiology unit which spurred me on to specialise in heart disease with the hope of meeting medical needs and positively changing outcomes.

Could you outline some of your most important research results to date, and explain how these discoveries will be used in the treatment of heart disease?

We are developing new therapies to tackle cardiovascular disease at the molecular level; research which has led us to the calcium-binding S100AI protein. Exploring its role in regulating cardiovascular functions, we have made two major discoveries. Firstly, S100A1 controls heart rhythm and improves contractile forces by regulating calcium levels in the cardiomyocytes. Based on a sound molecular understanding, we have developed clinically applicable molecular tools and technologies to selectively deliver the human DNA that encodes S100A1 to cardiomyocytes in the diseased heart. With the support of the Gene Therapy Resource Program of the National Institutes of Health (NIH), we hope to enter first clinical safety studies soon.

Secondly, the C-terminal S100A1 peptide fragment emulates most of the base protein functions upon calcium binding and exposure to the molecule surface. The peptide has remarkable physical and biochemical properties allowing for the permeation of cellular biomembranes. The discovery of this so-called biologic lays the foundation for the development of an alternative and more classical pharmacological approach to exploit S100A1's therapeutic profile. Essentially, by restoring S100A1 protein levels and/or its function, dysfunctional myocardial tissue can be repaired at the molecular level. We have filed patents for the synthesis of these peptide fragments and are looking for commercial partners to produce suitable therapies.

How does this unique therapy work and what sets it apart from beta blockers and ancillary drugs?

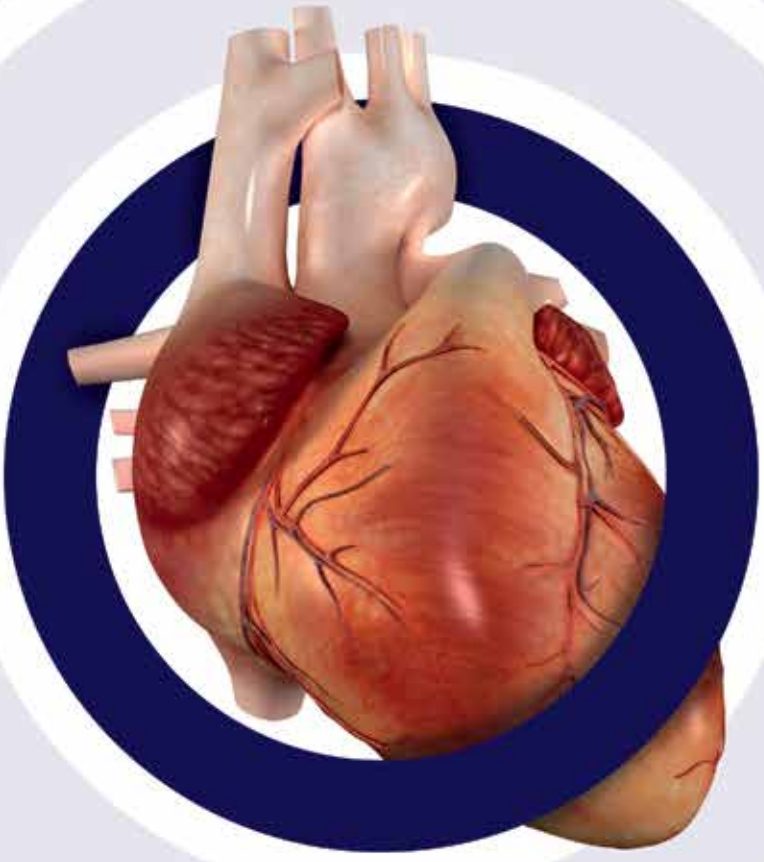
S100A1 has a subset of proteins that regulate calcium flows at the intracellular level, controlling functions such as energy homeostasis and several other interdependent cardiac functions. In this, they are superior to beta blockers which impact on the symptoms, but not the causes of tachyarrhythmias. S100A1 allows the heart to overcome ischaemic muscle tissue loss and helps restore heart function to nominal levels. S100A1 and beta blockers, which are positively and negatively inotropic, respectively, can surprisingly work in tandem without side-effects. The therapeutic effect, however, might be limited by the amount of residual myocardium that is functionally compromised but somehow still viable.

It has been 22 years since cardiac gene therapy was first tested in humans. Why is there still no government-approved product?

There are a number of factors at play here. Scientists are only human and sometimes we are overenthusiastic about results, which can lead to a feed-forward cycle that blurs thinking. This seems to have happened in the gene therapy field, with researchers failing to grasp the complexity of the topic, skipping over details, failing to make progress and then leaving their research under clouds of frustration. Unfortunately, at least one human death has been caused by inadequate biohazard safety protocols which set the research back years. It is clear that gene therapy is incredibly complicated, far more so than was first thought.

What does the future hold for cardiac gene therapy, and how do you see your own research progressing over the next five to 10 years?

I truly believe that cardiac gene therapy has the potential to change the landscape of heart failure treatment. S100A1 is not a universal panacea, but it offers the promise of actually repairing hearts damaged by infarctions; perhaps not in the most advanced cases, but certainly in those cases in which there is enough viable heart muscle left to be strengthened and regenerated. In one sense this gene therapy follows pharmaceutical therapeutics, in which doses are adjusted to reflect the severity of the disease; therefore, dose control systems will become very important for gene therapies.



To the core of **cardiac disease**

Hope for improved gene therapy in the treatment of heart failure is emerging from the **University of Heidelberg**, where researchers are exploring the therapeutic potential of a new candidate protein

CARDIAC DISEASE IS the subject of extensive research globally. As the largest single cause of death by disease worldwide, the importance of this research is clear. Professor Patrick Most from the University of Heidelberg in Germany is making a significant contribution in this field. The current focus of his team is the investigation of cardiac disease at the molecular level, aiming to understand the causative defects. Most's group has arrived at an intriguing possibility; that of using the body's own mechanisms to combat the effects of the condition on damaged cardiomyocyte, and in doing so providing a new therapy that is more effective than those currently available. The development of this approach has led Most's investigations to two main areas of focus: first and foremost, understanding the molecular mechanisms of the disease, as well as the various proteins and genes involved; and secondly, developing an effective therapy alongside a suitable means of delivering it.

A PROMISING PROTEIN

In 1996, Most's starting point was the clinical observation of diminished S100A1 expression/abundance in end-stage human heart failure, but Most's team did not know if it was a meaningful alteration. The only 'fact' that nourished their idea of potential relevance to the heart was the almost luxurious exclusive cardiac S100A1 expression pattern that gradually emerged from normal human and other mammalian tissue expression studies.

In this respect, Most's work focused on developing strategies to overexpress S100A1 both in normal cardiac myocytes and hearts of mice by the means of viral and genetically based manipulation. Applying a broad series of different methodologies, *in vitro* and *in vivo* models revealed the inotropic effect of the protein. Crucially, S100A1-mediated inotropy is independent of and additive to the beta-adrenergic system. It is a different form of cardiac performance enhancement that bypasses the typical upstream signalling components that beta-adrenergic signalling relies on, combining inotropy with protection from cardiac tachyarrythmias. The latter happens when the heart is chronically exposed to beta-adrenergic stimulation but not to chronic S100A1 overexpression. Over time, thr group understood that the protein itself acts through/orchestrates the function of a set of key regulators of calcium handling, myofilament function, as well as energy metabolism and cell death in the cardiomyocyte – S100A1 can be thought of as a 'conductor' who unifies the important players, forces them to play together and therefore sets both the tone and rhythm. If it is absent, there is literally 'disharmony' that drives the downhill course of the disease. Surprisingly, to elicit the effect, only a twofold increase in S100A1 is typically needed.

CAUSAL LINKAGE

While there remains work to be done to fully elucidate this mechanism, the team has been able to prove a causal linkage between the expression of calcium in the heart muscles and the regulation of various heart functions including: the contractile function; the control of heart rhythms; energy homeostasis; and apoptosis of the cardiomyocyte cells. This marks S100A1 with a unique set of properties, all based on a number of target proteins used by S100A1 to regulate these activities in cardiomyocytes. Essentially (and surprisingly), by restoring S100A1 levels within diseased heart muscles, their dysfunctional state can be reversed and they begin to recover their natural functions and efficiency.

Furthermore, the group has discovered that S100A1 contains peptide fragments that mirror most of the inherent S100A1 protein functions, in other words mimicking the molecule in its fully active state, but not playing any part in restoring full expression of S100A1. It is these peptide fragments that offer the possibility of synthesising a clinically suitable therapy.

INVESTIGATING CARDIOMYOCYTES

Cardiomyocytic cells are also subject to performance fluctuations, directly related to S100A1 expression. In his 2008 paper, Most demonstrated that endothelial cells from both diseased hearts and associated arteries display reduced S100A1 expression in a similar way to that exhibited by cardiomyocytes. S100A1 has a powerful and positive inotropic effect and enhances the contractile forces of the atrias and ventricles by regulating the supply of calcium to those muscles. Because it controls calcium levels, S100A1 also prevents calcium-based tachyarrhythmias.

This is in contrast to other commonly used inotropic therapies, which are at best pro-arrhythmogenic and can only be employed

INTELLIGENCE

CARDIAC S100A1 GENE TRANSFER: ANALYSIS OF THE EFFICACY OF A NEW CANDIDATE PROTEIN FOR SOMATIC GENE THERAPY OF HEART FAILURE

OBJECTIVES

To develop and clinically implement novel therapies that target the underlying molecular defects causing cardiovascular diseases.

KEY COLLABORATORS

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FUNDING

Deutsche Forschungsgemeinschaft (DFG) • National Institute of Health (NIH) • Bundesministerium für Bildung und Forschung (BMBF)

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for relatively short periods of time, lest they induce potentially lethal arrhythmias. For beta blockers, this rings particularly true because they are negatively inotropic, and only relieve the symptoms of myocardial dysfunction, not the cause. In yet another twist to this tale, S100A1 and beta blockers can be used in concert to stabilise any ischaemic loss of myocardium without any apparent long-term side-effects.

NITRIC OXIDE

Most also explored the role of S100A1 in endothelial cells, and their ability to produce nitric oxide (NO). NO production by the vascular endothelium is particularly important in the regulation of blood flow and, because of this, abnormal NO production as a result of disease can adversely affect blood flow and other vascular functions.

The role played by NO in endothelial cells came to light during the course of two mice-based studies; one in 2008, which looked at hypertension, and the other in 2012, in which retarded postnatal angiogenesis was observed in mice suffering from a lack of S100A1. This lack of S100A1 was shown to cause endothelial cells to become incapable of producing NO, in turn impacting upon vasodilation, vasorelaxation and the formation of new capillaries in ischaemic tissue.

Chronic limb ischaemias are also known to cause very low NO levels in the associated skeletal muscles and arterial endolethial cells, and this in turn inhibits neovascularisation from taking place in the affected limbs. The possibility exists, therefore, that direct application of S100A1 therapies my cause neovascularisation. At this juncture in the research programme, it became clear that atherosclerosis and thromboses occur more often in vasculature which is NO deficient, though the exact mechanism will be the subject of separate research.

By restoring S100A1 levels within diseased heart muscles, their dysfunctional state can be reversed and they begin to recover their natural functions and efficiency

FIXING A BROKEN HEART

In order to establish how S100A1 can be delivered as a therapy – in other words, how the dose can be matched to the severity of the disease – Most conducted a long series of tests to provide proof of concept for an S100A1 DNA-based therapy in isolated failing human cardiomyocytes and small animal models of the disease, such as mice and rats, which eventually led to pre-clinical trials on domestic pigs; whose cardiovascular systems and physiologies come close to those of human. Molecular tools and delivery technologies were developed with the sole aim of homogenously delivering the therapeutic S100A1 DNA to diseased myocardium.

These trials will help to establish the sort of mechanism that can be used to administer gene therapy. Currently, the easiest method of administering drugs is through ingestion of pills or powders, or injections. Most expects the target to emulate this, and is aiming to produce a pill that can induce increased expression of S100A1 in the target cell or organ by means of a chemical activator. He has a patented process, based on the synthesis of very short peptide fragments derived from S100A1, and delivery mechanisms are being developed by a US partner company. Most is truly at the heart of an exciting area of research with S100A1 seeming to offer a means of repairing damaged myocardium and restoring the heart to its former glory.