Can you summarise your work on cardiac therapies and explain the importance of improving treatments?

In the past decade, we have tried to decipher the molecular workings of microcirculation in disease. One example is chronic ischaemia, which may affect the lower limbs or the heart. In the latter case, the gradual blockage of coronary arteries causes reversible loss of function in the hypoperfused myocardial region (hibernating myocardium). Surgeons have long noted that revascularisation via aorto-coronary bypass restores pump function. Unfortunately, some vessels are not accessible to current revascularisation techniques. Here, therapeutic neovascularisation via molecular signalling may step in. However, the complex processes of microvessel sprouting, maturation and collateral growth (arteriogenesis) must be taken into account.

How have neovascularisation therapies evolved in recent years?

Therapeutic neovascularisation is a longstanding concept in cardiovascular medicine, but the first generation of biologics aimed at angiogenesis (including proteins, complementary DNAs and vectors) did not yield clear cut success. This failure was not the fault of the agents used, rather that the biological processes involved in building new vessels turned out to be more complex than previously thought.

A second generation of neovascular agents were then developed, considering the complex processes of arteriogenesis. Novel proteins were also screened for endothelial proliferation and sprouting, pericyte and smooth muscle cell attraction, as well as collateral growth induction.

Does thymosin β4 signalling differ from other factors used in clinical studies, such as vascular endothelial growth factor A (VEGF-A) and granulocyte macrophage colony stimulating factor (GM-CSF)?

VEGF-A and GM-CSF signalling are not yet fully understood; their recruitment of pro-angiogenic monocyte/macrophages has only recently been reported and information on how they reprogramme target cells is still incomplete. Fundamentally, both vasoactive ligands bind to receptors that act as kinases and signal through phosphorylation.

Thymosin β4 does not bind to any receptor yet known and its kinase activation may be indirect. However, its activation of the myocardin-related transcription factor (MRTF)/serum response factor (SRF) pathway – via translocation of MRTFs to the nucleus and subsequent co-activation of SRF, leading to induction of pro-angiogenic and maturation factors – appears to rely on G-actin sequestration, a feature not known for receptor tyrosine (VEGF-A) or serine/threonine kinases (GM-CSF).

What have been the previous successes of gene therapies, and why are they an important tool in combating cardiac diseases?

Gene therapy has been extensively studied experimentally but the transition to the clinic has rarely been achieved. One notable exception is gene therapy for heart failure, using a combination of adeno-associated virus 1 (AAV1) and SERCA2a, a gene encoding a pump that moves calcium from the cytosol to the sarcoplasmatic reticulum during diastole. The results of a phase IIb study (CUPID 2) are imminent. Thus, AAV-gene therapy is promising, despite only being in the early clinical stages. We hope to complement this approach by offering a vascular-oriented gene therapy with similar vectors, since it may provide added value and target a different patient population.
Mending a broken heart

Ischaemic heart disease is the most common cause of death in the Western world. Researchers from Technische Universität München are trying to reverse this trend by targeting signalling proteins that promote the return of blood to the heart and subsequent recovery of function.

THE WORLD HEALTH ORGANIZATION (WHO) estimates that by 2020 more than 23 million people will die each year from cardiovascular diseases. The most common form is ischaemic heart disease. A large proportion of these deaths are due to ischaemia in the heart muscle, where cells receive insufficient blood and oxygen, most often due to fatty deposits in the arteries. Although restoring blood flow by coronary artery bypass surgery can return function to the heart, conventional strategies are becoming exhausted and the number of so-called no-option patients is growing.

There is a clear need for more innovative techniques, such as those involving neovascularisation – the de novo formation of microvascular networks (including arterioles, capillaries and venules) to supply blood to the heart. However, this is no simple feat, as arteriogenesis – the development of collateral blood vessels that bypass obstructions – is essential at the same time, and the required balanced growth process is inherently complex.

Embracing this challenge, Dr Christian Kupatt is an expert in the signalling processes underlying arteriogenesis. Professor of Cardiology at the Technische Universität München, Germany, Kupatt is leading the search for factors that aid the proliferation of vessel endothelial cells. He is part of a new movement to isolate factors that promote the sprouting of these cells and the pericytes that surround them, thereby facilitating tissue recovery following ischaemia.

By studying the molecular basis of a process crucial to both pathogenesis and reversal of ischaemia, Kupatt’s team could open the door to a new generation of gene therapy – a treatment that has long shown promise in molecular cardiology but has not yet reached its full potential.

PROMOTING PROLIFERATION

During arteriogenesis, the enlarged vascular walls are covered by the induced proliferation of existing endothelial cells, and a growing body of evidence suggests that circulating cells secrete factors that have a key role in this growth. Kupatt began his search for these factors in endothelial progenitor cells. He honed in on one particular highly expressed factor, thymosin β4, an actin-sequestering protein involved in cytoskeleton polymisation and the proliferation, migration and differentiation of cells. This was a promising line of enquiry as thymosin β4 had previously been shown to repair damaged heart tissue following a heart attack.

In a paper published in Nature Communications in June 2014, Kupatt’s team shed new light on the details of thymosin β4’s action. Based on knowledge of its actin-binding domain, the researchers investigated a number of actin-mediated transcription factors – proteins that bind to DNA to alter gene expression – including myocardin-related transcription factors (MRTFs) and the co-activated serum response factor (SRF). By forcing the expression of MRTF-A, Kupatt revealed the induction of two matrix proteins – connective tissue growth factor 1 and 2 (CCN1/2) – that promote capillary proliferation and pericyte recruitment, respectively.

ANIMAL EVIDENCE

Kupatt’s group has used mice and pig models to show that either MRTF-A or thymosin β4 can induce functional neovascularisation and enhance the contractile function of damaged heart muscle. “To our surprise and delight, gene therapy with a vector encoding thymosin β4 was at least as effective as the existing gold-standard therapy,” Kupatt expounds. Having shown the clinical benefits, the team set out to elucidate the molecular origin of the process, finding that upon binding actin, thymosin β4 prompts the translocation of MRTF-A to the nucleus, where SRF is activated and CCN1/2 transcription initiated.

Taken together, Kupatt has shown that MRTF-A promotes both microvessel growth (via CCN1) and maturation (via CCN2), enhancing blood flow and enabling the functional recovery of ischaemic muscle tissue. This demonstrates the critical role of vessel maturation and growth in recovery and paves the way for new therapeutic routes. In particular, thymosin β4 represents a novel mediator of neovascularisation, able to induce vascular regeneration after prolonged ischaemia. “The growing population of cardiovascular no-option patients might be targeted by this treatment, and we view thymosin β4/MRTF-based gene therapy as a translational and revolutionary approach to the care of patients with chronic ischaemic heart or muscle disease,” Kupatt concludes.

THE WORLD HEALTH ORGANIZATION (WHO)