Post-interventional vascular remodeling: novel insights and therapeutic strategies

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CHAPTER 10

General Summary & Future Perspectives

GENERAL SUMMARY

Post-interventional vascular remodeling is a delicate process that requires tight balance of both inflammation and proliferation. On the one hand, the acute inflammatory response that is induced by surgery that is needed to initiate proliferation of endothelial cells (ECs) to facilitate reendothelialization of the injured vessels. Rapid reendothelialization prevents thrombosis and limits excessive inflammation when the endothelial barrier is restored. Moreover, the acute inflammatory response after surgery triggers vascular smooth muscle cell (VSMC) proliferation. This, in turn, is required to alleviate the hemodynamic (flow-related) and biomechanical (pressure-related) stress on the vessel wall and instigate outward remodeling. On the other hand, chronic inflammation and excessive proliferation in the vessel wall can result in a transition from an adaptive to a maladaptive response after surgery, which leads to inward rather than outward remodeling. An uncontrolled inflammatory and myofibroproliferative response will lead to excessive thickening of the vessel wall, in which new atherosclerotic plaques may develop. Consequently, the lumen will narrow leading to diminished blood flow and reappearance of symptoms for which patients initially underwent (endo)vascular interventions.

The exact molecular and cellular mechanisms that drive adverse post-interventional vascular remodeling are only partly understood and therapies to abrogate the maladaptive response after surgery are limited, leading to high number of patients demanding re-interventions. The aim of this thesis was to further elucidate the biological processes that control post-interventional vascular remodeling, specifically on the interaction between leukocytes and ECs, and to utilize this knowledge to design novel therapeutic strategies to promote beneficial vascular remodeling after surgery.

In this thesis, we have focused on the role of the endothelium and the interaction between ECs and leukocytes as well as their effect on atherosclerotic cardiovascular disease (ASCVD) and post-interventional vascular remodeling. The endothelium lines the lumen of all blood vessels and acts as a semi-permeable barrier that controls migration of leukocytes from the lumen into the vessel wall. Extravasation of leukocytes requires concerted action between ECs and immune cells. Despite variations in the specific mechanisms utilized by different leukocytes to cross different vascular beds, there is a general principle of capture, rolling, slow rolling, arrest, crawling, and ultimately diapedesis via a paracellular or transcellular route, without vascular leakage.

Under pathophysiologic conditions, for example after vascular interventions, the barrier function of the endothelium is impaired, which leads to uncontrolled leukocyte migration and vascular leakage. The immune cells that enter the vessel wall trigger an inflammatory and proliferative response that initiates post-interventional vascular remodeling and can initiate formation of an atherosclerotic plaque. During the initial

stages of plaque development, leukocyte infiltrate into the vessel wall via the luminal endothelium, whilst intraplaque neovessels become the major entry route as the plaque continues to grow.

In **chapter 2**, we summarize the molecular processes involved maintaining endothelial integrity and review current knowledge on leukocyte transendothelial migration (TEM). We emphasize the central role of the (dysfunctional) endothelium in ASCVD as well as post-interventional vascular remodeling. Additionally, we highlight the importance of cell-cell junctions and accentuate the role of intraplaque angiogenesis in atherosclerotic plaque progression and adverse vascular remodeling. The association between neovascularization and intraplaque hemorrhage is discussed as well as the effects of the different leukocyte subsets, including macrophages and T cells, on atherogenesis are discussed. Furthermore, we underline restoration of the endothelial barrier function as a potential therapeutic target to reduce ASCVD burden.

In **chapter 3**, we investigated how the endothelial monolayer matures over time and its relation to actin cytoskeleton remodeling. Human umbilical vein ECs (HUVECs) were cultured *in vitro* and at different timepoints analyzed to assess how stable intercellular junctions develop. We discern a crucial role for junctional F-actin bundles, which recruit various junction proteins (such as claudin-1/5) to the cell border to regulate endothelial integrity. Increasing junctional F-actin bundles through overexpression of RhoGEF Trio resulted in decreased vascular leakage *in vitro*. Additionally, inducing ectopic expression of Trio in murine vein grafts through local application of a pluronic gel loaded lentiviral vectors immediately post-operatively resulted in decreased intraplaque hemorrhage *in vivo*, whilst intraplaque angiogenesis was unaffected. In **chapter 4**, we employed Trio to assess the effect of improved endothelial integrity on immune cell TEM. Interestingly, paracellular, but not transcellular TEM of both polymorphonuclear neutrophils, $CD4^+$ and $CD8\ T^+$ cells was reduced by TrioN.

The interaction between immune cells, specifically macrophages, and ECs in post-interventional vascular remodeling and atherosclerotic plaque formation are further investigated in **chapter 5**. To assess the crosstalk between macrophages and ECs as well as the role of hypoxia / hypoxic signaling on this interaction, we performed vein graft surgery on a myeloid specific PHD2 conditional knock-out mouse. The deletion of PHD2 evokes pseudo-hypoxic signaling and we observe that this increases excretion of vascular endothelial growth factor (VEGF) by macrophages *in vitro*. Additionally, VEGF was increased *in vivo* which coincided with increased neovascularization. Interestingly, the maturation of these neovessel was increased, whilst the number of extravasated erythrocytes was decreased which indicates a reduction in intraplaque hemorrhage. Furthermore, vessel wall remodeling and atherosclerotic plaque stability was improved upon myeloid PHD2 knock-out compared to wildtype. Overall, this demonstrates that intraplaque angiogenesis and intraplaque hemorrhage are two separate processes. Moreover, the quality (maturation) of the neovessels rather than

their number determines atherosclerotic plaque fate, highlighting that intraplaque angiogenesis in itself does not necessarily have to instigate plaque growth, when uncoupled from intraplaque hemorrhage.

In this thesis, we also assessed the efficacy of therapeutic interventions that target the pre-, peri- and post-operative period to improve post-interventional vascular remodeling. The therapeutic potential and importance of the pre-operative period are highlighted in **chapter 6**. In this chapter, we demonstrate that short-term preoperative sulfur amino acid restriction (methionine and cysteine), known as Methionine Restriction (MetR), reduces adverse vein graft and arterial remodeling. The dietary regimen, which constitutes an isocaloric diet (compared to high-fat diet, which served as control in this study) with adequate levels of all macronutrients, upregulates endogenous production of hydrogen sulfide, a gaseous molecule that has vasoprotective effects. Just one week of the MetR intervention prior to surgery protected from arterial intimal hyperplasia in a murine focal stenosis model, and protected from adverse remodeling after vein graft surgery.

Specifically for venous bypass surgery, we established that the protective effect of the dietary intervention depends on presence of the perivascular adipose tissue (PVAT), which undergoes phenotypic switching from negative to protective phenotype in response to MetR. Mice that were fed control diet who received a vena cava with intact PVAT had exacerbated vein graft disease, in contrast to MetR-fed mice, which had reduced adverse vein graft remodeling. Mechanistically, we found that MetR induces browning of PVAT and yields an anti-inflammatory phenotype of macrophages. Through bulk and nuclear RNA-sequencing, we revealed upregulation of several pathways (AMPK- and PPAR-signaling as well as thermogenesis) that indicated browning of caval vein PVAT upon MetR. Interestingly, we observed that the browning and anti-inflammatory response were sustained until 28 days after surgery. Overall, this work highlights the therapeutic potential of both the pre-operative period and PVAT in vascular interventions.

The association between the endothelium (intraplaque angiogenesis) and immune cells, specifically T cells, is further explored in **chapter 7**. We assessed the T cell immune landscape of vein grafts by flow cytometry during different stages of vascular remodeling. A progressive increase in the number of $CD4^+$ and $CD8^+$, specifically effector-memory, T cells over time was observed, whilst predominantly $CD8^+$ T cells are activated rapidly after surgery. Furthermore, we identify CD137-CD137L signaling as a key regulator of, predominantly $CD8^+$, T cell activation in the vein graft wall. CD137 expression was only increased on T cells early after surgery (days) in the vein graft wall, but not in other tissue, thus serving as an attractive target for immunomodulation during the peri- / early post-operative period.

Therapeutic activation of CD137 by agonistic antibodies at post-operative day 1, improved vein graft remodeling by inducing outward remodeling, whilst inhibition of CD137 signaling accelerated lesion development. Moreover, agonistic CD137

treatment induced effector-memory T cell differentiation both systemically and locally, i.e. in the vein graft wall. Systemic IFN-γ levels were shortly increased after agonistic CD137 treatment, whilst expression of IFN-γ on lesional T cells remained elevated compared to control, which suggests a local effect on $CD137^+$ T cells in the vein graft that translated into a systemic effect. Furthermore, agonistic CD137 treatment improved plaque stability by inhibiting intraplaque angiogenesis, whilst concomitantly improving neovessel maturation. In contrast, antagonistic CD137 treatment decreased neovessel maturation. Altogether, these results underline the importance of early immune activation as a critical regulator of post-interventional vascular remodeling and demonstrate that early immunomodulation by activating or inhibiting CD137-CD137L signaling has long-lasting effects that regulate vein graft remodeling.

In **chapter 8** we investigated the therapeutic potential of PARP1 inhibition to improve post-interventional vascular remodeling in three mouse models, for arterial, arteriovenous and venous remodeling. PARP1 is an enzyme that orchestrates cellular responses to (DNA) damage and oxidative stress. Extensive PARP activation results in cellular dysfunction, inflammation, proliferation and necrosis. We demonstrate that PARP1 is increasingly expressed over time after surgery in vein grafts in predominantly macrophages and, to a lesser extent, VSMCs. We, therefore, hypothesized that PARP1 closely associates with the (chronic) inflammatory and myofibroproliferative response after surgery that drive adverse post-interventional vascular remodeling. Consequently, we assessed the efficacy of a newly developed, highly selective small molecule PARP1-inhibitor to promote beneficial vascular remodeling. Administration of this compound dose-dependently decreased adverse vein graft remodeling. Similarly, we observed that PARP1 inhibition prevents neointima formation after arteriovenous fistula (AVF). Furthermore, arterial restenosis and intimal hyperplasia was also reduced upon PARP1 inhibition. Mechanistically, we observed that the PARP1-inhibitor mainly affected the inflammatory response after surgery. Overall, our data highlight the therapeutic potential of this PARP1 inhibitor to induce beneficial arterial, arteriovenous as well as venous remodeling following surgery, warranting further investigations in larger animals models.

The inflammatory and (myofibro)proliferative response in the vessel wall after surgery are intrinsically correlated, largely as a result of chronic hemodynamic and biomechanical alterations. Cells residing in the vessel wall can sense and respond to these mechanical stimuli. In **chapter 9**, we describe how saphenous vein progenitors, cells residing in the adventitia, are activated upon mechanical stretch (as seen after vascular interventions). This induces nuclear translocation of YAP, which results in differentiation into myofibroblasts, proliferation and migration. This myofibroproliferative response upon mechanical strain was abrogated by the FDA-approved YAP-TAZ inhibitor Verteporfin. *Ex vivo* cultured saphenous veins that have been exposed to arterial flow also exhibited increased expression of YAP target genes, which was diminished by Verteporfin. *In vivo*, expression and nuclear translocation of

YAP increased over time in both pig and murine vein grafts. In murine vein grafts, YAP was not only expressed cells residing in the medial and adventitial layer of the vein graft wall, but also by ECs, both luminal as well as adventitial/neovessel. This might imply a role for YAP-TAZ in intraplaque angiogenesis. Administration of Verteporfin inhibited intraplaque angiogenesis and reduced adverse vein graft remodeling, thus emphasizing YAP-TAZ inhibition as a potential therapeutic strategy to improve post-interventional vascular remodeling.

FUTURE PERSPECTIVES

Clinical success following invasive interventions in cardiovascular disease patients is suboptimal as a result of adverse post-interventional vascular remodeling. The mechanisms that drive this phenomenon, however, remain to be fully understood. In this thesis, we reviewed and investigated the role of the endothelium and immune cells as well as their interaction in ASCVD and post-interventional vascular remodeling.

Intimal hyperplasia and buildup of an atherosclerotic plaque are the major drivers of adverse post-interventional vascular remodeling. Despite subtle differences, these processes are reminiscent of primary atherogenesis. Currently, lipid-lowering drugs are cornerstone therapy to prevent (progression of) ASCVD. Despite significant reductions in serum cholesterol as a result of high-dose statin therapy, a large number of number patients still develop major adverse cardiovascular events (MACE), indicating that other factors are involved in the development and progression of ASCVD.

The strong association between biomarkers of inflammation, such as high-sensitivity C-reactive protein (hsCRP) and IL-6, and increased risk of MACE – independent of cholesterol levels – in both men[1] and women[2] established the current paradigm of atherosclerosis as a chronic inflammatory disease. Specifically in apparently healthy persons, with relatively low lipid levels, hsCRP predicts cardiovascular disease risk.[1-3] Interestingly, statins appear to decrease hsCRP levels, independent of their cholesterol lowering effect, suggesting an anti-inflammatory effect of statin therapy.[4, 5] The CANTOS trial unequivocally proved that anti-inflammatory therapy reduces MACE in patients with previous myocardial infarction and hsCRP. Furthermore, colchicine treatment also reduced MACE in patients with a recent myocardial infarction.[6, 7]

Inflammation also affects post-interventional vascular remodeling. In patients undergoing PCI[8] or CABG[9, 10], preoperative CRP was associated with restenosis or mortality, whilst high postoperative CRP was also associated with MACE.[11] Reducing inflammation before or during surgery, therefore appears a promising technique to improve clinical outcomes. Currently, however, there is conflicting evidence whether perioperative statin therapy (that reduces hsCRP) can mitigate MACE after cardiac surgery.[11-13] On the one hand, these studies underline the importance of early, and even pre-operative, inflammation in post-interventional vascular remodeling. On the

other hand, these studies (as well as the CANTOS and colchicine trials) demonstrate that designing therapies to achieve immunomodulation and improve outcomes of vascular interventions remains a challenge. The relative benefit in these clinical trials, which was only 15-20%, only became apparent after approximately one year, thus leaving room for improvement when designing anti-inflammatory therapies. Moreover, it poses the question whether the anti-inflammatory therapy affects existing, unstable plaques or prevents development of 'new' unstable plaques. Furthermore, it is unknown whether greater efficacy of treatment could be achieved, when started immediately after vascular interventions. In its current setup, the excessive inflammatory response to surgery was not diminished, leaving the door open to investigate a potential therapeutic benefit of current anti-inflammatory therapy, when administered early – or even before – vascular interventions.

In the case of post-interventional vascular remodeling, the **pre-operative** period provides an unique opportunity for surgical preconditioning, due to the planned nature of a large body of vascular interventions. Healthcare providers should utilize this period for therapeutic interventions to optimize outcomes. Short-term preoperative dietary interventions, such as the MetR diet used in **chapter 6**, represent a highly attractive approach to precondition patients for planned stressors such as surgery. Dietary restriction regimens have enormous health benefits, including improved cardiometabolic health and restored the immune balance. The beneficial effects of short-term preoperative dietary restriction have been reported in renal[14-16], hepatic[14, 17] and vascular injury[18] as well as venous bypass surgery[19] models. Moreover, the safety and feasibility in patients has been demonstrated in vascular[20, 21] and cardiac surgery.[22] Interestingly, one week of MetR prior to cardiothoracic surgery did reduce the length of stay on the intensive care and total hospitalization, whilst the trial was too small to assess its effects on surgery outcomes.[22]

The clinical translation of dietary interventions, however, might prove to be difficult due to adherence problems. Adding to that, the large variety in interpatient responses to dietary alterations [23] can hinder clinical success and implementation of dietary interventions, even after perfect patient compliance. In the case of MetR, for example, cardiometabolic responses are highly sexually dimorphic. It is, therefore, an illusion to find an 'one-size-fits-all' approach. Recent advances in understanding cardiometabolic health might pave the way towards tailored dietary interventions in individual patients. Meanwhile, a better understanding of the molecular pathways and cellular effectors that convey the protective effects of dietary restriction will prove to be crucial to design new pharmacological therapies, especially for patients that cannot adhere to dietary interventions. Identification of browning of PVAT as a result of MetR, for example, and its positive effect on post-interventional vascular remodeling warrants research into the preoperative use of compounds (such as resveratrol, PPARα-agonists and β3-adrenoceptor agonists) to pharmacologically

mimic dietary interventions. Whether these drugs will yield protective effects to the same extent as the dietary interventions, remains a topic of future research.

Peri-operative

In addition to the possibility for surgical preconditioning, the invasive nature of the vascular interventions allows for local rather than systemic administration of a drug during surgery. A clinical trial investigating whether transfection of saphenous veins with TIMP3, in a brief *ex vivo* incubation protocol with viral vectors, before implementation is currently ongoing (G1001147/1). Previous trials using a similar set-up but then targeting transcription factor E2F[26, 27], were unsuccessful despite sufficient transgene expression[28, 29], thus accentuating the importance of target selection. Preventing excessive extracellular matrix turnover and consequent MMP-mediated cell migration, by overexpression of TIMP3 is most likely a better target than inhibiting VSMC proliferation, which only becomes problematic in the months and years after surgery. Additionally, the experimental set-up is crucial. In **chapter 3**, we used a pluronic gel loaded with lentiviral vectors, which are known to achieve transgene expression for a longer period of time compared to adenoviruses. Specifically for vein grafts and AVFs, the cells that will ultimately populate the vessel wall are both of venous as well as arterial origin. Using *ex vivo* incubation might therefore hamper the therapeutic efficacy of viral transfection, since only venous cells will have transgene expression.

Although excessive vessel wall thickening was not prevented in **chapter 3**, the use of a pluronic gel to achieve local compound delivery should not be discarded. Combining anti-inflammatory medication (such as canakinumab or dexamethasone) with a pluronic gel remains an attractive approach to locally diminish the acute inflammatory response after surgery, since the gel remains in place for approximately 5-7 days. To achieve early and local immunomodulation, it is key to identify (inflammatory) targets that are locally expressed in the vein graft in this peri-operative period and assess the kinetics of this expression. As discussed in **chapter 7**, CD137 represents an attractive target for early immunomodulation due to its local expression in the vessel wall rapidly after surgery. Systemic administration of CD137 agonistic antibodies resulted in beneficial vein graft remodeling, which raises the question whether local administration would result in a similar effect without potential side-effects. Furthermore, one could imagine a therapeutic approach in which $CD8^+$ T cells, collected from blood, will be treated *ex vivo* with agonistic CD137 and injected after surgery. Whether, and to what extent, this would result in the same beneficial effects after vascular interventions, remains to be investigated. Concomitantly, it is imperative to closely monitor oncology patients that receive immune-checkpoint inhibitors for accelerated development of cardiovascular disease. The activation of immune cells as a result of immunotherapy has dramatically increased survival in cancer patients since 2011.[33] The use of these drugs will undoubtedly increase, whilst the first reports of increased atherogenesis as a result of immune activation

also indicate serious long-term side-effects.[34, 35] In the coming years, the field should investigate whether this also translates into MACE. Non-invasive imaging methods such as ultrasound, MRI or photoacoustic imaging could provide an opportunity to closely monitor patients receiving cardiovascular therapy and, for example, identify large, unstable atherosclerotic plaques. Especially the combination of ultrasound and photoacoustic imaging will yield information on both plaque size as well as plaque composition. Applying artificial intelligence for multi-modal imaging analysis could guide clinical decision making in the future and improve patient selection for surgical intervention and/or (aggressive) medical therapy.

In addition to pharmacotherapy, external support devices could also improve (arterio-)venous post-interventional vascular remodeling. Theoretically, these devices should hamper the pathologic response to perturbed hemodynamics. Their use in venous bypass surgery and AVFs, however, has remained controversial. Clinical trials assessing the efficacy of external support devices after venous bypass surgery have initially been unable to reduce vein graft failure after one year, although in-stent intimal hyperplasia was reduced, confirming the preclinical evidence.[36] Nevertheless, bypass failure within 12 months is mostly derived from technical failure and thrombosis, which external stents do not primarily aim to prevent. Analysis of these vein grafts after 4.5 years revealed that neointima formation was significantly reduced in the stented bypasses,[37] indicating that the beneficial effects of external support devices are maintained for longer period of time. Whether this reduction translates into clinically meaningful results remains a topic of future research. For AVFs, external vascular support is feasible, whilst reducing reintervention rates, compared to traditional fistulas.[38] Especially for AVFs, configuration, and consequent direction of blood flow, is a critical determinant of clinical outcomes.[39] It is to be expected that the effects of external support devices would be larger compared to venous bypasses. Clinical evidence from large cohort studies or randomized controlled trials regarding the efficacy of external support devices for AVFs, however, is currently lacking. Nevertheless, the current (pre-)clinical evidence vindicates additional research.

Similar to the continuous evolution of ‘internal’ stents, which has led to the development of drug-eluting stents, one could envision a comparable process for external stents. Preclinical evidence already suggests superior performance of a rapamycin-coated external stent to prevent intimal hyperplasia after venous bypass surgery compared to a non-coated stent.[40] Although drug-eluting stents have reduced neointima formation, there have been increased (late) thrombotic events due to delayed reendothelization.[41, 42] It will be interesting to observe whether coated, external stents will prevent neointima formation without increased risk of thrombosis due to the external release of anti-proliferative therapy which might not affect EC proliferation. Additionally, the use of biodegradable stents appears promising, even though there are significant challenges to be addressed. Optimal

timing for degradation, especially after (arterio-)venous reconstructions, is yet to be determined to provide mechanical resistance and prevent acute and chronic recoil, whilst also allowing moderate vessel wall thickening before complete degradation.[43]

Post-operative period

The acute, hyper-inflammatory response to vascular interventions is often only partly resolved and reappears at later stages of post-interventional vascular remodeling in a chronic form that is similar to the inflammation observed in naïve atherosclerosis. This instigates neointima formation and development of an atherosclerotic plaque. In addition to VSMCs, other cells such as macrophages, dedifferentiated ECs, mesenchymal stem cells and and fibroblast(-like) cells also actively contribute to plaque growth.

In **chapter 9**, we describe how saphenous vein progenitor cells can sense mechanical stress and respond by inducing cellular proliferation through activation of YAP-TAZ signaling, indicating a potential contribution to post-interventional vascular remodeling. Blocking YAP-TAZ signaling using Verteporfin, prevented proliferation and migration of these progenitor cells. Moreover, Verteporfin limited adverse vein graft remodeling, demonstrating the therapeutic potential of YAP-TAZ inhibition in post-interventional vascular remodeling. The ubiquitous expression of YAP-TAZ in various cell types hampers direct clinical use due to increased risk of unwanted side-effects and warrants further research into the effects of YAP inhibition in leukocytes, for example. The systemic decrease in pro-inflammatory cytokines also makes one wonder to what extent the beneficial effects were derived from local effects on the vein graft wall compared to the systemic effects. Nevertheless, the desensitization to mechanical stimuli by Verteporfin calls for research in other models of post-interventional vascular remodeling that experience perturbed biomechanics. Assessing efficacy of Verteporfin after, for example, AVF surgery will also indicate whether the beneficial effects relied on abrogation of the myofibroproliferative response or also (partly) on the inhibition of intraplaque angiogenesis.

Although the angiogenic response to hypoxia is initially physiologic, over time it becomes pathologic due the lack of pericyte coverage and formation of proper intercellular junctions. Anti-angiogenic therapy aimed at preventing plaque neovascularization has demonstrated to reduce vein graft atherosclerosis, although not every strategy has yielded similar results. Taking into account **chapter 3 and 5**, we provide compelling evidence that the quality, rather than the quantity, of these neovessels determines atherosclerotic plaque fate. Systemic administration of anti-angiogenic therapy, however, is unlikely to translate into clinical practice. The atherosclerotic plaque itself, when symptomatic, yields hypoxia downstream of the lesion. ASCVD patients, therefore, require neovascularization in the hypoxic tissue, which highlights the paradoxical role of angiogenesis in atherogenesis. Local delivery of anti-angiogenic treatment during surgery is unlikely to achieve meaningful

results, since plaque neovascularization occurs at later stages of post-interventional vascular remodeling. The leakiness of these neovessels, however, allows for targeted therapy using, for example, (lipid) nano-particles. When loaded with VEGFR2-inhibitor, for example, the nano-particles could be injected intra-arterially upstream of the atherosclerotic lesion, in which they would locally accumulate due to the defective endothelial barrier. Theoretically, this would limit systemic side-effects. Furthermore, the local effect could be enhanced when combined with ultrasound-pulsed destruction of microbubbles, in which a laser could locally destroy the nano-particles leading to controlled release of the compound, that can also, for example, be immunomodulatory.

PVAT

The neovessels that grow into the hypoxic plaque, often arise from the vasa vasorum of the adventitial layer as well as the PVAT. In patients with coronary artery disease, inflamed PVAT (as measured by CT angiography, and termed PVAT attenuation) associated with plaque instability.[46] **In chapter 6**, we identified that PVAT can be therapeutically targeted to reduce adverse post-interventional vascular remodeling. Assessing PVAT attenuation in ASCVD patients could therefore potentially identify high-risk plaque and improve patient selection for anti-inflammatory therapy. Moreover, it could also be used to monitor treatment efficacy and, as a result, guide clinical decision making. Failure to reduce PVAT attenuation after canakinumab therapy, for example, could point towards limited effects and direct a clinician to switch or discontinue current medication. Current investigations have focused arterial PVAT, resulting in lack of knowledge whether CT-based attenuation can be applied to venous PVAT. Assessing PVAT attenuation prior to venous bypass or AVF surgery could assist vessel selection and identify patients that might benefit from surgical preconditioning by, for example, dietary restriction (mimetics) or anti-inflammatory therapy. Furthermore, it could influence surgical decision making and justify removal of PVAT, in case of attenuation, given the negative effects of inflamed PVAT on vascular remodeling.

Therapeutic angiogenesis

The notion that intraplaque neovessels are mainly derived from existing adventitial vessels and PVAT, implies that venous cells are capable of formation of arterial sprouting. Recent evidence suggests that in developing zebrafish, venous cells indeed are capable of arterial sprouting[47], whilst this has not yet unequivocally been demonstrated for non-developing venous ECs. The concept of venous sprouting could be a new approach to stimulate therapeutic angiogenesis. To this end, the signaling pathways involved in venous sprouting should be identified using RNA sequencing and (phospho)proteomics. The pathways that drive venous ECs to form angiogenic sprouts are most likely different compared to arterial cells, thus allowing new therapeutics avenues to be explored.

In the past years, most investigations for therapeutic angiogenesis have aimed to increase VEGF-levels, yielding conflicting results.[48-50] In peripheral artery disease (PAD) patients, there is controversial clinical evidence suggesting that VEGF-gene therapy stimulates angiogenesis, resulting in a significant increase in pain-free walking distance[51-53], although limb amputation was not prevented[51] nor was there any improvement in exercise performance or quality of life.[54] Moreover, serious adverse events (spider angiomata and edema) were observed, probably due to VEGF-induced neovessel leakiness.[51, 54] Interestingly, more recent studies have demonstrated elevated levels of both circulating and intramuscular VEGF in PAD patients.[55-58] Furthermore, VEGF levels correlate with disease severity and are associated with poor treatment outcomes.[56] This, therefore, points towards inadequate VEGF-signaling, rather than deficient VEGF-production and challenges the concept of VEGF gene therapy to reduce PAD. Overall, it vindicates selection of other targets, that can also be upstream or downstream (such as Trio) of VEGF, for therapeutic angiogenesis. Integration of new RNA sequencing data (preferentially also ones that include venous sprouting) with existing data-sets should guide target identification by artificial intelligence. Simultaneously, target validation should occur on protein level in patients and matched controls. Preferably, a distinction between circulating, unaffected and ischemic tissue should be made before proceeding to (pre-clinical) investigations. Patients with CAD would be an interesting group due to increasing clinical demand as well as relatively small area of ischemic tissue (compared to PAD patients) that can also be directly targeted through intramyocardial injection.

Overall, the work described in this thesis has focused on understanding as well as improving post-interventional vascular remodeling to improve surgical outcomes. The (mechanistic) insights have shed new light on the cellular and molecular mechanisms involved in vascular disease and have, hopefully, paved the way for new therapeutic avenues to be explored. To further enhance our understanding of cardiovascular disease and ultimately improve medical care, clinicians and scientists from various backgrounds should closely collaborate, whilst not forgetting the patient perspective. Only then will these international, inter-disciplinary and inter-institutional efforts be successful on the long term. In the near future, integrating various existing techniques into multi-modal imaging platforms, that are analyzed and supported by artificial intelligence, can guide clinical decision making and augment clinical care, by improving patient selection for current medical interventions. Integrating novel and existing RNA as well as multi-omic data sets should serve as the basis for identification of novel therapeutic targets by artificial intelligence as well as clinician-scientists, after careful target validation in human samples on both gene and protein level.

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