Transplant Arteriosclerosis and In-Stent Restenosis
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General discussion
Introduction

The aim of the studies described in this thesis was to gain more insight into the (cellular and molecular) mechanisms underlying the development of transplant arteriosclerosis (TA) and in-stent restenosis (ISR). TA and ISR represent two major macrovascular occlusive diseases for which no effective preventive and treatment strategies currently exist. Both TA and ISR are processes of inward vascular remodeling that develop long-term after allogeneic solid organ transplantation (particularly in heart and kidney) and after endovascular stenting, respectively. The vascular occlusions (*i.e.* neointima [NI]) in TA and ISR primarily consist of α-smooth muscle actin-expressing myofibroblasts or vascular smooth muscle cells (VSMCs). Both TA and ISR presumably result from vascular wall damage although the inciting damage-inducing events clearly differ. TA is the result of alloantigen- and non-alloantigen-mediated damage to graft vasculature whereas ISR results from mechanically-induced vascular damage. The foremost target for initial damage resulting in TA and ISR is most likely the endothelium, which plays an important role in the onset and progression of these diseases.1-3 Insults to the endothelium lining the luminal surface of arteries prime the successive cascade of inflammatory, migratory and proliferative events culminating in obstructive NI formation. Endothelial cell (EC) loss must be associated with an efficient mechanism of concomitant regeneration in order to reestablish vessel integrity. This can be achieved by resident vascular EC proliferation (when sufficient reservoirs are available) and by recruitment of circulating and/or vascular wall-resident endothelial progenitor cells (EPCs). Although it is well established that proliferation and migration of VSMCs is a crucial event in intimal expansion, the anatomical origin of neointimal cells is still a matter of debate. In addition to medial (donor) origin of neointimal cells (as proposed in the “response to injury” hypothesis), also recruitment of recipient-derived cells ([recirculating] bone marrow [BM]-derived or vascular wall residing precursor cells) might contribute to NI formation. These recipient-derived cells are considered to originate from a population of Smooth Muscle Progenitor Cells (SMPCs). Diverse sources for neointimal VSMCs been described4-6 including donor medial VSMCs, fibroblast-myofibroblast transdifferentiation (donor or recipient origin), adjacent recipient medial VSMCs (recipient origin), BM-derived SMPCs (recipient origin), and non-BM-derived SMPCs residing in the vascular wall (donor or recipient origin). Regardless of their exact origin, chemotactic factors are required to recruit the neointimal VSM ancestral cells towards the developing neointima. A range of chemokine pathways including SDF-1-CXCR4, fractalkine-CX3CR1, and MIPα/RANTES/MCP3-CCR1 are known to be involved in mobilization of different subtypes of pro-angiogenic cells, with direct influence on NI formation. Despite the advances made in understanding the cellular and molecular mechanisms involved in NI formation, the biology of progenitor cell recruitment needs to be further explored. Focus of this thesis was therefore to obtain more insight on post-injury endothelium regeneration, neointimal cell origin, their recruitment and proliferation, as well as the therapeutic potential of a peroxisome proliferator-activated receptor γ agonist in inhibiting NI formation. The results of the studies performed are described in detail in the previous chapters. Below, the data obtained will be discussed and put in a broader context.
Chapter 1 provided an overview of various aspects of vascular remodeling processes involved in macrovascular complications following solid organ transplantation (TA) and stenting (ISR). Similarities and differences in the pathogenetic mechanisms of TA and ISR development are presented. Special attention is paid to the central role of endothelium in the initiating phase of TA and ISR development, VSMC proliferation and the potential role of vascular progenitor cells in NI formation. Chapter 2 shortly introduced the experimental work described in the subsequent chapters.

Part I: Transplant Arteriosclerosis (TA)

Susceptibility for TA correlates with neointimal VSMC proliferation and fibrocyte frequency

Development of TA is primarily the result of immune-mediated vascular damage in transplanted organs. TA is considered as the general histologic hallmark of chronic rejection. Despite major progress achieved in preventing and treating acute rejection episodes relatively early after transplantation (<1 year post transplantation), long-term outcome in solid organ transplantation remains unsatisfactory. Among transplant recipients transplanted with similar HLA-incompatible grafts, receiving similar immunosuppression and exposed to the same known risk factors, variation exists in both the rejection rate and long-term outcome. This variation has not been fully explained yet, but data indicate that both immune and non-immune-related factors might contribute in determining the different transplant outcomes in different individuals. In addition to differences in preservation, ischemia-reperfusion, cytokine status of the recipient (due to e.g. infections, surgical trauma, use of blood-related products), clinical data suggest that different recipients might display different immune responses against an allograft (i.e. a different immunological responder status). Both a donor and a recipient origin of neointimal cells have been described. In the rat aorta transplant model, we and others showed that the cells forming the neointima are recipient-derived. Based on this observation in Chapter 3 the hypothesis that (genetically-determined) differences in neointimal VSMC proliferative capacity and recipient progenitor cell frequency are correlated with TA susceptibility was tested. To this end, host MHC and non-MHC-encoded determinants, intrinsic neointimal VSMC proliferative capacity, and the VSMC progenitor cell availability were analyzed in different donor-recipient combinations using the aortic transplant model in rats. Using Lewis (Lew) and Brown Norway (BN) rat strains as donors and recipients, the development of TA in time was analyzed. In contrast to Lew hosts, which gradually developed TA with time, BN hosts developed maximal TA as early as 4 weeks after transplantation compared with 24 weeks in Lew hosts and this difference was dependent on the presence of BN non-MHC encoded determinants. The severity of TA in both strains was similar eventually, indicating that the rate of TA development rather than the severity per se was strain dependent. The high TA responder status of BN recipients was clearly correlated with the intrinsic proliferative capacity of neointimal VSMCs. Neointimal VSMCs isolated from Lew allografts transplanted in BN recipients (VSMCs of BN origin) displayed markedly increased proliferative responses \textit{in vitro} compared with neointimal
VSMCs isolated from BN allografts transplanted in Lew recipients (VSMCs of Lew origin). This increased proliferation rate of BN-derived neointimal VSMCs was associated with increased endothelial and smooth muscle cell-derived neuropilin-like protein (ESDN) mRNA expression levels. This observation is in line with the reported temporal and spatial ESDN expression pattern described in vascular cell proliferation during vascular remodeling. A few years ago, ESDN was reported as a marker for neointimal VSMC proliferation, being minimally expressed in normal arteries and significantly upregulated following alloimmune or mechanical injury. ESDN overexpression in vitro leads to a decrease in growth VSMC rate. Conversely, knocking down ESDN in VSMCs leads to an increase in number of platelet-derived growth factor (PDGF, a potent VSMC mitogen) binding sites (PDGFRβ), favoring PDGF-induced VSMC proliferation. ESDN affects the number of PDGF receptors on the VSMC surface by modulating PDGFRβ ubiquitination. As such, PDGFRβ ubiquitination, as a negative regulator of PDGFRβ signaling, is reduced by ESDN down-regulation, resulting in increased PDGFRβ signaling. Therefore, ESDN might play a role as a negative regulator of growth-inducing signals and its upregulation in high TA-responders can be considered in this respect as an attempt to control proliferation by reducing the response to growth factors in a negative feedback loop. ESDN upregulation may be a downstream effect in the signaling events that lead to cell proliferation or, alternatively, may be directly or indirectly induced by cell proliferation. Further studies are necessary to establish the role of ESDN on neointima formation in vivo and evaluate its therapeutic potential controlling pathological states where excessive cell proliferation plays a central role. Furthermore, the high TA responder status of BN recipients was correlated with the frequency of circulating fibrocytes which are considered as putative neointimal VSMC ancestral cells also known as SMPCs. Although SMPCs have been described in various vascular (circulating SMPCs, vascular wall resident SMPCs) and extravascular sites (bone marrow, adipose tissue), it is likely that there is a continuous movement between compartments, and many resident and extravascular non-BM derived SPMCs were once derived from the BM during ontogeny. Efforts are being made for further characterization of specific SMPC antigens to allow their identification. Various progenitor and stem-derived surface and cytoplasmic SMPC antigens like CD14, CD34, CD105, flt1, c-kit, sca-1 were described. Several chemokine pathways promoting mobilization of neointimal cells are already known. Intimal, but not medial, VSMCs were shown to express higher levels of functional CC chemokine receptor-1 (CCR1), essential for neointimal recruitment, in human and murine TA. CX3CR1 mononuclear cell population was shown to provide a source of SMPCs and the CX3CR1-fractalkine interaction in vivo is essential for SMPC differentiation of BM-derived progenitor cells at the vessel wall level. Stromal cell-derived factor 1α (SDF-1α)/CXCR4 is another SMPC-mediated vascular repair axis, shown to influence cell migration towards injured organs.

In our model, the alloreactive immune response causes massive apoptosis of the medial VSMCs, and promotes recipient-derived SMPC recruitment to the place of vascular injury, leading to NI formation. The increased fibrocyte frequency in BN rats was detected in both naïve and transplanted rats indicating that these differences are endogenously present.
The observation that BN rats are high TA responders compared with LEW rats is in line with previous findings obtained in a balloon injury model showing that BN rats develop more severe restenosis compared with Lew rats.31 These data indicate that the high TA responder status of BN rats may reflect a general susceptibility of BN rats to develop more severe occlusive vascular disease. These results thus indicate that in the process of NI formation following transplantation both increased progenitor cell availability, and possibly also their recruitment to the site of vascular damage, as well as increased neointimal VSMC proliferation are positively correlated with the rate of TA development. Before transplantation, screenings of transplant recipients for high frequency of SMPC circulating levels could therefore potentially reveal the subjects with increased risk for neointima development in grafted organs.

Endothelial injury is considered the initiating event in TA development1 and the presence of an intact endothelial monolayer, either by preserving the donor endothelial cells or by reendothelialization with host-derived cells, supposedly leads to reduced TA development and prolonged graft survival. Therefore, we analyzed host-mediated endothelial repair, i.e. host EC-chimerism in the early post-transplant period. In line with the studies showing that increased levels of host EC-chimerism were associated with more severe vascular damage in renal allografts32,33, we showed that the high BN TA responder-status was associated with enhanced host EC-chimerism when compared with the low Lew TA responder-status. These data indicate that enhanced EC-chimerism is associated with worse outcome, which might reflect an earlier and more aggressive acute vascular rejection and therefore a more severe vascular damage. In addition, reduced necessity for an accelerated reendothelialization in BN grafts transplanted in Lew recipients can reflect a lower vulnerability of BN endothelium to transplantation-related events like ischemia/reperfusion injury. Thus, therapies aiming at preservation of graft endothelium, by reducing damage in the peri-operative period, rather than inducing host EC-chimerism might be an efficacious strategy to reduce TA development.

Rosiglitazone attenuates TA development, but impairs systemic VSMC function
Immunosuppressive therapy intends to target the recipient's immune system to dampen the alloreactive response and to preserve the transplanted organ, but while it succeeded to prevent the acute rejection episodes, little advances were made in preventing and treating TA. Beside strategies aiming at influencing the immune response, direct modulation of VSMC as well as endothelial and adventitial cell responses to both immunologic and non-immunologic assaults might be considered as an alternative strategy to intervene in the development of TA.34 Therefore, in Chapter 4 the efficacy of the thiazolidinedione rosiglitazone (RSG), a synthetic peroxisome proliferator-activated receptor-γ (PPARγ) agonist, to attenuate TA development was studied as well as mechanisms involved. Using the aorta transplant model in rats, RSG was shown to significantly reduce the development of TA. The underlying mechanism appeared to be plural since RSG was shown to 1) reduce alloreactive T cell responses, which was however not mediated through upregulated frequency and function of CD4+CD25+FoxP3+ regulatory T cells, and 2) reduce intragraft
expression of SDF-1α and PDGFRβ (a receptor for PDGF). Reduced intragraft expression together with the observed reduced PDGF-induced proliferation of medial and neointimal VSMCs in vitro in the presence of RSG favor for a direct effect of RSG on VSMC proliferation. Furthermore, reduced expression levels of SDF-1α, a potent chemoattractant for progenitor cells, within the neointima following in vivo treatment with RSG, favors for reduced recruitment and homing of SMPCs into the injured allograft vascular wall as a putative mechanism for reduced TA development.

Endothelial dysfunction is a key player in cardiovascular pathology as endothelium has a crucial role in maintaining normal vessel wall function by its ability to inhibit thrombus formation, leukocyte adhesion, VSMC proliferation and by regulation of vascular tone. Endothelial dysfunction is the triggering event in the proliferative and fibrotic processes involved in atherosclerosis and associated complications and it is an independent predictor of future cardiovascular events.35,36 In the transplantation setting, beside the vascular tree inside the transplanted organ, the recipient vasculature outside the graft is affected, supposedly due to transplant-associated systemic inflammatory status which will have deleterious effects on systemic endothelial cell function37,38, and thereby increasing the risk for cardiovascular events after transplantation.39 Therefore, in Chapter 5 the effect of in vivo RSG treatment on the development of systemic EC dysfunction was studied. Alike clinical solid organ transplantation, experimental allogeneic aorta transplantation resulted in the development of transplantation-induced systemic EC dysfunction. RSG treatment resulted in an overall improvement of EC-dependent relaxation in response to metacholine as measured ex vivo using isolated thoracic aorta rings in an organ-bath setup. However, in vivo exposure to RSG decreased vasorelaxation indicative of an impaired dilatory response of VSMCs in response to the EC-independent vasodilator sodium nitrite. Although the direct negative effect of RSG on VSMC function was compensated by the general improvement of EC-function and there is an overall RSG-induced improvement of vasodilator function, nonetheless, the long-term effects of RSG treatment on endothelial-independent relaxation and therefore, on the risk for cardiovascular events subsequent to allografting remain to be reported. Clinical evaluations by meta-analysis reported that RSG treatment in patients with type 2 diabetes is associated with increased incidence of myocardial infarction and death from cardiovascular causes.40,41 A possible explanation for these observations could be based on its effect to increase fluid retention and precipitate heart failure.40 Our data on RSG-induced VSMC impaired dilatory response might offer a mechanistic explanation on increased risk for cardiovascular events in diabetic patients which, in addition to their changes in the structure of microvessels with hypertrophic remodeling of small vessels42, present an altered VSMC dilatory response. This might aggravate the abnormal myogenic responsiveness already altered in type 2 diabetic patients43, thereby contributing to increased wall stress for a given intraluminal pressure, which may further stimulate vascular hypertrophy. Although the aforementioned meta-analysis has its limitations44, more studies actually suggest harmful effects of RSG usage. Caution is therefore required when prescribing RSG to patients who are already at risk for development of cardiovascular diseases including diabetics and renal transplant recipients. Following the results of the meta-analysis published in 2007, the Food
and Drug Administration (FDA) issued a boxed warning that RSG may increase the risk for myocardial ischemic events, including myocardial infarction. Since then, a consensus regarding the risk posed by RSG has emerged among experts and in September 2010 the FDA decided to restrict access to RSG. Nonetheless, our results presented in Chapter 4 and Chapter 5 as well as a rapidly increasing number of publications have underscored the importance of PPARγ in cardiovascular diseases. Although contra-intuitive based on clinical data, results from in vitro and in vivo animal models suggest that PPARγ and its selective agonists have a vascular protective role via their beneficial effects on inflammatory response, neointimal VSMC proliferation, progenitor cell recruitment factors, and endothelial function. However, the controversial clinical outcomes indicate an insufficient knowledge of the cardiovascular biology of the nuclear receptors and the necessity for the development of more effective agonists. Further in-depth studies are necessary to uncover the cardiovascular functions of PPARγ and address its potential and safety use in treatment of occlusive cardiovascular diseases.

Part II: In-Stent Restenosis (ISR)

Neointimal VSMCs and ECs in ISR are non-bone marrow-derived

Another major vascular pathological remodeling process is represented by neointima formation after stent implantation. Development of ISR after endovascular stenting still is a major side-effect resulting in the need for re-revascularization interventions. Although the use of drug-eluting stents have resulted in a decrease in the incidence of ISR, development of ISR can still not be prevented. The phenotype and the origin of vascular cells as well as the molecular pathways involved in vascular cell recruitment, differentiation and proliferation need to be clarified in order to design novel effective strategies aiming at further attenuating ISR development. Alike TA, it is generally accepted that the main constituents of the neointima in ISR are α-actin-positive VSMCs or myofibroblasts. Their anatomical origin and recruiting mechanisms are still a matter of debate. Recent findings obtained in our lab and by others favor for a role of recirculating vascular progenitor cells, which may be partly derived from the BM as demonstrated in the development of TA and restenosis in experimental models in rodents. The involvement of BM-derived vascular cells in the development of ISR is, however, as yet not clear, although the presence of BM-derived cells in human atherectomy samples has been suggested. However, from this study it remains unclear whether the BM-derived cells detected in the neointima were already present in the atherosclerotic lesion before stenting. Therefore, in Chapter 6, the contribution of BM-derived VSMCs in the development of ISR without established atherosclerosis was analyzed using an experimental abdominal aorta stenting model in transgenic (hPAP) BM-chimeric rats. This model allowed identification of BM-derived vascular cells recruited in response to stenting. However, results obtained indicated that virtually all neointimal VSMCs were of non-BM origin and only infiltrating CD45+ leukocytes turned out to be derived from the BM compartment, in line with results in a rat model.
balloon injury model with a small population of neointimal cells coming from BM-derived progenitors. In wire-induced injury of the femoral artery in mice, Daniel et al. recently showed that most of the BM-derived cells found in the NI were monocytes/macrophages, and there was no apparent substantial long-term contribution of these cells to the cellular mass of the NI. Moreover, their results provide evidence that the definite differentiation of BM-progenitor cells into VSMCs or ECs is only an exceedingly rare event. These findings were confirmed by another recent study showing that even though BM-derived α-smooth muscle actin-expressing cells do infiltrate injured vessels in several models of vascular injury, they do not fully differentiate in VSMCs. These new findings favor for a local origin of VSMCs in NI formation. The precise anatomical origin of neointimal VSMCs in ISR remain to be determined and may include (recirculating) vascular progenitor cells residing the vascular wall. Vascular wall resident progenitor cells where recently isolated from vasculogenic niches in the media and the adventitia of the vascular wall. Although BM-derived cells seem to play a minor role in neointima formation in experimental ISR, this does not exclude the possibility that early after stenting BM-derived cells are recruited to the injured vascular wall and create a microenvironment in which local progenitor cells niches are activated and mobilized by BM-derived cells in a paracrine fashion. Rodriguez-Menocal et al. showed in a rat balloon injury model that BM-derived monocytes/macrophages were abundantly present in the media and adventitia of injured vessels in early stages but their number declined in the vascular wall with time.

Long-term experimental Type 1 diabetes enhances ISR

The incidence of macrovascular disease (i.e. atherosclerosis), including peripheral and coronary artery disease is increased in diabetic patients in comparison to nondiabetic patients. Hyperglycemia can promote vascular complications by multiple mechanisms resulting in diabetes-associated vascular disease, characterized by systemic endothelial cell dysfunction and structural changes of large and small arteries leading to tissue hypoperfusion and hypoxia. Moreover, diabetic patients present worsened clinical outcome and repeated re-vascularizations after percutaneous coronary intervention with stent placement and as yet the precise underlying mechanism is unknown. In order to get more insight into the mechanism underlying enhanced development of ISR in diabetes, reliable long-term diabetic models are necessary. Therefore, in Chapter 7 a novel rat model for long-term hyperglycemia was established and used to study the development of ISR following stenting in the abdominal aorta using age-matched non-diabetic rats as controls. This model was developed in diabetes prone BioBreeding (BBDP) rats with long-term impaired glycemic control. BBDP rats develop immune-mediated diabetes resembling human Type 1 diabetes since it develops during adolescence and involves a disorder of the immune system. Poorly controlled diabetes in BBDP rats was maintained by suboptimal treatment with insulin pellets resulting in chronically elevated blood glucose and HbA\textsubscript{1c} levels. After stent implantation, the diabetic rats presented increased neointima formation compared with control rats. This novel experimental model reflecting poorly controlled Type 1 diabetes can therefore be used to study the augmented severity of ISR allowing more
in-depth analyses on the mechanism(s) involved. In addition, this model might constitute a useful tool in investigating the profile of circulating vascular progenitor cells associated with a poor glycemic control and offers the possibility to determine the effect of the diabetic metabolic environment on progenitor cell mobilization after vascular injury. Increased N1 hyperplasia in diabetics might be caused by a disequilibrium between various classes of progenitor cells. While SMPCs might contribute to neointima hyperplasia as decreased SMPC number is associated with reduced neointima formation, EPCs might prevent it, replacing the damaged endothelium. Increased SMPCs is the sign of a deleterious profibrotic status resulting in adverse remodeling of damaged tissue in diabetes. In a mouse model of type 1 diabetes increased number of SMPCs with the involvement of TGF-β/BMP-6 axis as an important modulator for these cells was described. Moreover, Nguyen et al. showed that blood from diabetic patients yielded higher numbers of myofibroblast progenitor cells than blood from control subjects, presenting increased proliferation and decreased apoptosis.

Long-term experimental Type 1 diabetes impairs angiogenic potential of the vascular wall
The long-term diabetic model described above was then used to investigate the presence of progenitor cells in the vascular wall and the effect of the hyperglycemic status on their function. As shown in Chapter 6, BM-derived cells had none or a limited contribution to ISR. Possible mechanisms by which progenitor cells may contribute to intimal hyperplasia would be to directly adhere to the vascular wall and building up neointima “inside-in” or entering vascular wall via the perivascular vasa vasorum, migrate to the neointima from the “outside-in.” Therefore, in Chapter 8, the presence of angiogenic cells residing in the vessel wall was investigated and the long-term effect of diabetes on their function was analyzed. Using ex vivo cultured aorta rings obtained from long-term hyperglycemic rats as well as from their age-matched controls, the angiogenic potential of the cells residing in the vessel wall was assessed. We showed that sprouting in long-term diabetic rats was significantly impaired compared with non-diabetic age-matched control animals. These results from isolated vessels show that the effects on neovascularization include deficient angiogenic sprouting of vascular wall resident cells, independent of effects of recirculating cells or their secreted pro- or anti-angiogenic factors. We already showed that non-BM-derived progenitor cells contribute to neointima formation in ISR, and at least some part of them might originate from the vasculogenic zone of vascular wall itself. The vascular wall was described to harbor both smooth muscle and endothelial progenitor cells. An impaired ability of vascular wall resident EPCs to migrate and proliferate, along with the already proven deficiency in number and function of circulating EPCs in diabetic condition would lead to impaired reendothelialization, favoring the neointima formation. In addition to direct participation to reendothelialization, these local angiogenic cells might have a paracrine function by promoting the homing and proliferation of circulating EPCs. The deficient angiogenic potential of diabetic vascular wall cells would also impair the generation of neo-vessels by angiogenesis, thereby worsening the recovery from an ischemic event. Local delivery of agents that could influence the number and the quality of vascular wall resident
EPCs, along with a systemic double edged therapy in order to increase circulating EPC and reduce SMPC frequency, would be a possible approach to reduce neointima formation.

**TA and ISR: a matter of a disturbed EPC and SMPC balance?**

Mobilization and recruitment of circulating and/or tissue-resident progenitor cells that can give rise to ECs and VSMCs play an important role in neointima formation. Maintaining the fine balance between the “beneficial” EPCs and “deleterious” SMPCs in favor of proliferation, migration and homing of the EPCs and diminishing the number of SMPCs would be an efficient approach in decreasing the rate and the extent of vascular remodeling in both TA and ISR. In end-stage renal disease patients who have an increased incidence of atherosclerotic cardiovascular disease, a decreased number of EPCs was found, whereas the number of SMPCs remained unaffected, suggesting that an imbalance between the two population could negatively affect vascular remodeling. Nonetheless, caution should be taken when considering progenitor cell manipulation, as there is no definitive “good” or “bad” vascular progenitor cell as both endothelial and smooth muscle progenitors may act as a double-edged sword in the pathogenesis of arteriosclerosis. Moreover, both EPCs and SMPCs may even derive from a common precursor and EPCs can transdifferentiate in SMPCs which may complicate cell-based therapies. Ideally one drug would control the microenvironment in which progenitor cells reside and/or are recruited in such a way that their subsequent differentiation into either ECs or VSMCs would eventually lead to reduced neointima formation. Moreover, such a drug would promote both effects by simultaneously targeting two different signal pathways within same tissue microenvironment resulting in opposite and biologically complementary effects. Several drugs have beneficial effects on EPCs like PPARγ agonists and statins, and in the same time inhibit VSMC proliferation and therefore reduce neointimal formation.

**Concluding perspectives**

Figure 1 summarizes several contributors to the pathogenetic mechanism(s) involved in TA and ISR development as revealed in the experimental work discussed in this thesis. They all represent possible interventional areas in controlling neointima hyperplasia. Neointima formation is a complex pathological process with both humoral and cellular participants, influenced by local and systemic factors. Injury of endothelium is the initiating event in both TA and ISR development and the severity of EC damage correlated with the magnitude of TA formation. Preventing endothelial damage and preserving its function in the first place (improved transplantation and stenting procedures) would be the ideal approach in preventing NI development. Nonetheless, with all caution taken, EC damage appears and the need to reduce activation (and further damage) following transplantation as well as to enhance reendothelialization of mechanically-injured vessels is warranted. Both circulating and vascular resident wall EPC might contribute in different phases and
General discussion

Figure 1. Possible interventional targets to prevent and treat neointima hyperplasia addressed in this thesis. In-stent restenosis (ISR, top left): neointima formation after revascularization by stent placement in an atherosclerotic plaque with a focal distribution in the vascular tree. Transplant arteriosclerosis (TA, top right): characteristic concentric intimal thickening with a diffuse distribution of neointima hyperplasia throughout the vascular tree. Systemic interventional strategies: (a) control risk factors (diabetes), (b) improve systemic EC function, (c) diminish the pro-fibrotic status (decreasing circulating smooth muscle progenitors). Local interventional strategies: (d) modulate progenitor cell recruitment (decreased SMPCs and increased EPCs), (e) reduce inflammation (reducing endothelial and medial damage), (f) reduce VSMC proliferation, (g) maintain endothelium integrity or favor reendothelialization (enhance local angiogenic potential). Figure was produced using Servier Medical Art (www.servier.com).
proportion to recover the endothelial lining. Therefore, potentially efficacious therapies might include modulation of vascular progenitor cell subsets to facilitate vascular repair by increasing EPC number and homing capacity to the site of injury. Also, controlling debilitating factors like diabetes (PPARγ agonists) will improve the attempts to maintain a healthy endothelium.

VSMCs are the major players in NI formation and therefore their recruitment, migration and proliferation form all potential targets for therapeutic interventions. Some of currently used immunosuppressive drugs like rapamycin, mycophenolic acid, cyclosporin, calcium channel blockers, and statins are known to possess anti-proliferative properties. As inhibiting VSMC proliferation does not succeed to prevent NI, therapies aimed at earlier stages (i.e. before their homing and proliferation is mounted) might be a more efficient approach. Targeting SMPCs recruitment and homing (e.g. by SDF-1/CXCR4 blockade) in conjunction with local control of proliferative factors (like PPARγ or ESDN), may offer a more efficient preventive and therapeutic strategy for controlling NI development.

Although much progress was made in the last decade regarding vascular progenitor cell biology and their different sources, their efficient use in therapeutic schemes is just at early stages. To specifically target the vascular progenitor cell and modulate their behavior in order to limit NI, a more complex understanding of the multitude of factors regulating their biology is required. There are still a lot of unanswered remaining issues/questions like: 1. It is not yet fully established what the frequency and the degree of contribution of these cells is in NI formation, 2. What is the percentage of cells that are derived from different sources, 3. What factors do actually determine their recruitment, 4. What is the role of mature endothelial and smooth muscle cell interaction with progenitor cells, 5. What are the mechanisms that influence the recruitment of either a common progenitor cell for ECs and VSMCs and/or of separate progenitors for the different cells lineages, and 6. What molecular mechanisms determine the direction of their differentiation.

Both TA and ISR represent major macrovascular complications in treating end-stage organ failure and occlusive atherosclerotic diseases, respectively. Both diseases are characterized by EC damage, inflammation and NI hyperplasia. The additional cardiovascular risk factors, like diabetes, influencing these impaired responses to vascular injury, require multifactorial and multi-level complex therapies. At present there are no efficacious therapeutic measure to control their onset and evolution. The lack of adequate prevention and therapeutic protocols impels for further research aiming at unraveling their pathogenetic mechanisms. Knowledge on mechanisms will enable the development of more efficacious interventional strategies specific for various stages of the diseases meant for inhibiting or limiting their evolution. Knowing how they arise, is knowing how to control them.
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