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Modulation of the Extracellular Matrix in Advanced Atherosclerosis

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8 Discussion and Perspectives

8.1 Atherosclerosis-related disease and its socio-economical implications

The clinical manifestations of atherosclerosis depend upon the location, dimension and stability of critical lesions. Progressive narrowing of arteries combined with a dysfunctional endothelium results in reduced flow capacity towards downstream organs, such as the myocardium or the legs, possibly leading to clinical syndromes such as angina pectoris and intermittent claudication. Critical stenosis of the renal arteries may cause hypertension and chronic renal failure.[1] Vulnerable plaques are the principal substrate for acute arterial thrombosis, ensuing in acute ischemia and possibly infarction of downstream tissues.[2] Myocardial infarction not only is an important cause of death by itself, but also one of the primary causes for ventricular arrhythmias and malicious remodeling processes of the myocardium leading to chronic heart failure, a physically impairing and life threatening disease. Mural thrombi on ulcerated carotid plaques are notorious for their risk of embolizing and causing a transient ischemic attack (TIA) or even stroke, while intracranial aneurysms may rupture and lead to cerebral hemorrhage. The extensive remodeling of an atherosclerotic aorta may lead to rupture-prone aneurysms as well.

Taken together, atherosclerosis may give rise to a variety of impairing and possibly life-threatening clinical syndromes and therewith is one of the most important causes of death to this date. In 2001, 35% of total deaths in the Netherlands were attributable to cardiovascular maladies, the majority of which embracing ischemic heart (34%) or cerebrovascular disease (25%).[3] Although morbidity and mortality rates have decreased over the past three decades, an increasing number of people have been admitted to hospital for cardiovascular disease. During the eighties and early nineties, age-corrected cardiovascular-related hospitalization rates increased approximately 26% in the Dutch population.[3] The second half of the nineties preluded a downward trend in these figures, but that was largely attributable to a significant shift from hospital admission to day care and outpatient clinics. In 2001, a staggering 258,685 Dutch were admitted to hospital for cardiovascular disease, 18% of the annually hospitalized patients in the Netherlands. The majority of this group of patients, 49%, were suffering from ischemic syndromes, such as angina, myocardial infarction, cerebrovascular maladies and peripheral artery disease.[3]

Because of the impact on the general physical condition and endurance, atherosclerosis-related clinical syndromes can have a grave effect on the quality of life of both patients and their immediate social environment as well as on the overall economy. This cannot only be ascribed to costs for hospitalization and treatment, but also to the physical inability to contribute to the labour market and to costs for social benefits. Numerous governmental and non-governmental organizations world-wide that provide funds for prevention, health care and research programs acknowledge this socio-economical impact. Their joint effort, facilitating the design of prevention strategies and the development of novel methods for screening and diagnostic and therapeutic tools, contributes to the ongoing battle against atherosclerosis-related diseases. This can be illustrated by the recently reported mortality reduction as a consequence of primary prevention strategies, such as smoking cessation and lipid lowering, between 1981 and 2000 in England and Wales. The authors estimated 29,215 fewer deaths due to the 35% decline in smoking prevalence alone and stated that the relative mortality benefit from primary prevention is four-fold higher than that from secondary prevention.[4] The Association for the Eradication of Heart Attack recognizes the significance of early intervention and is predominantly focussing its attention and efforts to education and screening programs, development of prophylactic pharmaceuticals and immune modulation and vaccination strategies.[5]

8.2 Prevention and treatment of atherosclerosis

Notwithstanding the expectation that primary prevention is the most favourable approach to conquer atherosclerosis, we still face a large group of patients that suffer from this chronic, difficult to treat, disease today. Atherosclerosis is characterized by a complex, multifactorial pathogenesis that results in strong inter-individual variations in disease progression and response to treatment. This urges the need for a wide arsenal of therapeutic options and secondary prevention protocols, as well as accurate risk assessment tools, in order to provide the tailored approach in atherosclerosis management that the complexity of this disease demands.

At present, curative interventions principally target blood rheology, lipid metabolism and end organ damage. Reduced flow capacity, causing chronic or acute ischemia, is partly controlled by reducing the oxygen demand from downstream organs such as the heart. Nitrates, β-blockers and Ca^{++}-antagonists are designed to reduce the cardial workload and thus oxygen consumption. Aspirin and clopidogrel are anti-platelet drugs that are imperative in modern day management of acute coronary syndromes.[6-9] Also, GPIIbIIIa antagonists and LMW heparins cannot be omitted in the current treatment guidelines that deal with (risk of) acute thrombosis.[10] In addition, ACE inhibitors, AII-blockers and aldosterone antagonists can be used to prevent malicious myocardial remodeling processes, hence preventing ischemic heart failure.[11, 12]

Being a major risk factor, hypercholesterolemia has been subject to a considerable amount of research. Interestingly, the lipid-lowering statins have been proven remarkably successful not only by their effect on systemic lipid metabolism, but also by their direct anti-inflammatory effect on the arterial wall, therewith stabilizing atherosclerotic plaques.[13] For this reason statins are also recommended in secondary prevention protocols for normolipidemic patients.[14, 15]

In fact, these drugs are among the first to directly intervene in vascular biology. It is surprising that, while the pathobiology of atherosclerotic plaque may be regarded as the predominant culprit of acute ischemic events, our therapeutic arsenal contains so few instruments that are able to modulate specific pathogenic mechanisms in the arterial wall. While sirolimus- or paclitaxel-coated stents have been very successful in preventing restenosis, statins are probably the only plaque stabilizing agents today and yet this effect is non-specific and may be quite limited. In addition, a significant portion of patients is non-responsive to statin treatment.[16] This emphasizes the earlier mentioned need for a broader range of pharmacotherapeutics. Currently, ezetimibe, an inhibitor of cholesterol adsorption, has become available as an adjuvant treatment in cholesterol lowering[17, 18] and inhibitors of the acetylCoA-acyl transferase enzyme (ACAT) are now enrolled in phase III clinical trials.[19, 20] The latter are also believed to reduce plaque growth by promoting apoptosis of macrophage-derived foam cells. The detrimental effect that this effect might have on advanced plaques, however, is not clear.

In recent years, both basic and clinical sciences have greatly contributed to an increasing knowledge of the pathogenesis of atherosclerosis and plaque destabilization and defined several novel and promising targets. These include lipid metabolism within the plaque itself, inflammation, immune modulation, cell cycle regulation, matrix biology and neo-angiogenesis. Vaccination against pro-inflammatory cytokines, such as IL-12, may result in impaired plaque progression and enhanced plaque stability.[21] Various F(ab) fragments directed against the VEGF receptor 2 yielded promising results as an anti-angiogenic drug in cancer therapy.[22-24] It is conceivable that plaque stability can benefit from such a treatment as well,

although it should be kept in mind that angiogenesis is also of major importance in the formation of collateral vessels, a physiological adaptation to chronic ischemia.

In this respect, new plaque stabilizing therapies may require a lesion targeted strategy to prevent such, or any other systemic, side effects. Moreover, as discussed previously, biological processes that are beneficial at one stage of plaque progression, may be harmful at another. For example, inhibition of SMC migration may prevent restenosis and reduce plaque growth, but impairs fibrous cap stability as well. Vice versa, measures that improve resilience of the fibrous cap, could enhance constrictive arterial remodelling. A careful identification of high-risk lesions might avoid unwanted growth of otherwise stable plaques. Although several techniques to recognize unstable plaques are very promising, many have the disadvantage that they are invasive (e.g. IVUS, Raman spectrometry, near infrared spectroscopy, palpography). Non-invasive tools, such as multidetector CT, MR angiography and PET scanning, might open the way for a wider applicable screening strategy and thus to identifying more people with vulnerable plaques. For this, however, it is vital to uncover those targets that are highly specific not only for atherosclerotic burden, but also for advanced, unstable plaques that are at risk of causing acute occluding thrombosis.

8.3 Summary of this thesis

In this thesis all, but one, study involved the use of experimental animals in different mouse models of atherosclerosis. Although mice are very different from humans in many regards, they remain the principal testing ground for basic scientists in the field of vascular biology. Many genetic and interventional studies would not be practical or even ethical to perform in humans. Bone marrow transplantation, viral gene transduction, vaccination, creation of transgenes and the testing of lead compounds are only a few examples of experiments that can help to elucidate the contribution of a variety of gene products to disease progression and these require carefully controlled *in vivo* models.

The relevance of mouse models of atherosclerosis, however, should be carefully monitored and discussed for each study objective. Inherent mouse-human differences limit the extrapolation of observations in mice to the human situation. Apart from evident differences in vascular anatomy, murine lipid metabolism also differs considerably from humans, as it is deficient in, for instance, cholesteryl ester transfer protein (CETP) and apo(a).[25, 26] Other differences include inflammatory propensity and thrombotic tendency. Mice are relative resistant to thrombosis, complicating the design of adequate models for end stage atherosclerosis. Of particular interest when studying vascular matrix biology in mice, is the absence of MMP-1 in the murine vasculature. Numerous studies highlight MMP-1 as an important protease in atherogenesis, vessel remodeling and plaque stability. Although adult mice express two MMP-1 orthologues (e.g. mColA and -B), this is only the case in the uterus and the testes.[27]

In spite of these dissimilarities, the introduction of atherosclerosis-prone genetically modified mouse strains has indeed revolutionized the field of atherosclerosis research. While LDLr-/- and apoE-/- mice are the most commonly used mouse models for atherosclerosis today, the insertion of human CETP in apoE deficient mice resembles human lipid metabolism even better and thus could mimic human atherogenesis even closer.[28, 29] Moreover, the development of microsurgical

techniques to induce lesion formation, such as arterial ligation or collar placement and endothelial denudation, permitted intervention studies in lesions at a predefined site, in relatively uniform lesions and at any given stage of disease progression. The particular advantage of inducing atherogenesis within only a few weeks, as can be accomplished with the “collar model”, overcomes the relative short effect of gene transfer when applying the commonly used adenoviral vectors. Another benefit of these models can be found in the site of lesion induction. While the aorta for example is hardly available for lesion targeted intervention studies, the carotid plaques are easy to access for perivascular or transluminal treatment via the relatively redundant external carotid artery.[30, 31] Table 8.1 summarizes several commonly used models of atherosclerosis.

The following paragraphs provide a synopsis of the results that we obtained from such mouse models of atherosclerosis with respect to matrix biology. While the effects of IL-18, MMP-9 and TFPI-2 were studied in collar-induced carotid plaques of apoE-/- mice, the role of the cystein proteases cathepsin S and cathepsin K was specifically investigated in leukocytes by applying bone marrow transplantation in irradiated LDLr deficient recipients.

Table 8.1. Examples of mouse models of atherosclerosis and their characteristics

Model	Characteristics
Transgenics and knock-outs	
LDLr-/-	Hyperlipidemic on high-fat diet
	Lesions rich in macrophage foam cells
	Slow plaque progression
	No plaque formation in arteries accessible for intervention
ApoE-/-	Hyperlipidemic on chow diet
	Pro-inflammatory propensity
	Large, complex lesions with widespread distribution
	Plaque rupture in innominate artery
	Relatively slow plaque progression
	No plaque formation in arteries accessible for intervention
ApoE3*Leiden	Hyperlipidemic on chow
	Large, complex lesions with widespread distribution
	Relatively slow plaque progression
	No plaque formation in arteries accessible for intervention
	Co-expression of hCETP results in more human lipid profile with reduced HDL levels
Lesion induction	
Arterial ligation	Strongly reduced or interrupted blood flow
	Rapid induction of arterial lesion formation
	Accessible for perivascular treatment
Carotid collar/cast	Hemodynamic interference with altered shear stress
	Time controlled, rapid induction of atherosclerotic lesions
	Accessible for perivascular and transluminal treatment
Femoral cuff	Induction of adventitial damage
	Rapid formation of hypercellular and matrix-rich neointima
	Hardly accessible for local intervention studies
Denudation	Endothelial damage
	Rapid induction of intimal hyperplasia
	Accessible for perivascular and transluminal treatment
Venous graft	Rapid induction of intimal hyperplasia
	Accessible for perivascular and transluminal treatment
Intervention	
Oral gavage	Enteral administration of lead compounds
Osmotic pumps	Parenteral systemic administration of lead compounds
	Limited duration of treatment
Vaccination	Systemic immunomodulation
	Requires repeated vaccination
Perivascular pluronic gel	Topical administration of lead compounds
Perivascular drug eluting cuff	Topical administration of lead compounds with sustained release
Transluminal gene transfer	Directly targeted to the plaque
Bone marrow transplantation	Facilitates the investigation of cell specific effects
	Possibility of ex vivo gene transfer or siRNA treatment

8.3.1 Metalloproteinases

Generally, regulation of MMP activity has been observed from the perspective of the local (patho)biological environment. Gene expression and secretion of MMPs and their inhibitors are governed by auto- and paracrine immunomodulatory molecules and locally generated noxious agents such as oxLDL. In **Chapter 2** it is described that increased circulating plasma levels of the pro-inflammatory IL-18 can have an endocrine effect on MMP-13 activity with collagen degradation and subsequent plaque destabilization as a consequence. Although the role of IL-18 has been well established in mouse models of atherogenesis, its effect on plaque stability remained unclear. Earlier studies already reported increased IL-18 serum levels in patients with acute coronary syndromes and elevated IL-18 mRNA levels in unstable human plaques, but evidence for a direct causal relation between circulating IL-18 and plaque stability was still lacking.[32-34] Our observations causally link IL-18 to plaque vulnerability and also demonstrate that systemic inflammation can directly influence the plaques' matrix homeostasis. Hence contributing to the concept of the vulnerable patient in which a transient hyperinflammatory event, such as a viral infection, periodontal disease or surgery, can seriously affect plaque stability via endocrine pathways.[35, 36] In this study, IL-18 overexpression was found to activate MMP-13, a potent collagenolytic enzyme. This could, however, only be partially explained by enhanced gene expression. Further studies may be required to identify the mechanisms by which IL-18, directly or indirectly, might activate this enzyme, stimulate its secretion and/or influence its inhibitors in situ.

Chapter 3 demonstrates the biological significance of such a complex regulation of protease activity by showing a differential role for MMP-9 during plaque development. In spite of supposedly conflicting observations in recent years, MMP-9 does indeed appear to have an adverse effect on the stability of atherosclerotic lesions. However, it should be kept in mind that because its actions are pleiotropic and highly dependent upon the environmental context, this protease may function differently at various locations and progression stages of the lesion. This holds implications for the applicability of MMP inhibitors as a therapeutic agent in atherosclerosis. The prevention of adverse events by TIMP-1 co-overexpreesion suggests that MMP-9 inhibition may be a feasible therapy in selected, highly advanced plaques, but this benefit could not be shown for moderately progressed plaques. In fact, absence of MMP-9 activity could augment the degree of stenosis in intermediate plaques and might even be detrimental to the development of stable lesions during initial lesion formation as was observed in MMP-9 deficient apoE KO mice.[37]

Plaque destabilization in the above mentioned chapters was appreciated by morphologic staging and by the occurrence of intra plaque hemorrhage (IPH). With respect to studying plaque stability, it should be mentioned that the development of a proper substrate for plaque rupture has been proven to be extremely difficult, hampering the evaluation of new strategies for the treatment or prevention of vulnerable plaques.[38] Several models have been proposed thus far, but all have their specific disadvantages, particularly the lack of an unambiguous end-point. In the collar model for rapid atherogenesis a small proportion of plaques feature a vulnerable plaque phenotype. The introduction of the tumor-suppressor gene p53 by adenoviral transduction resulted in accelerated apoptosis within the fibrous cap and a hemodynamic challenge with phenylephrine caused plaque rupture in 40% of p53 overexpressing plaques.[31] Similar results have been described with the overexpression of the pro-apoptotic cytokine FasL[39] and the in chapter 3 discussed

metalloproteinase MMP-9. These models have been criticized for the low incidence of thrombotic events, but given the murine intrinsic resistance to thrombosis it is debatable if the latter would be a valid end-point in mouse models of plaque stability. Plaque morphology (including cap thickness and/or disruption) and composition or the occurrence of intraplaque hemorrhage may be more appropriate surrogate end-points for plaque destabilization in mice than the clinically more relevant thrombosis. Another proposed model for plaque vulnerability is the occurrence of spontaneous plaque disruption in the brachiocephalic artery of apoE deficient mice.[38, 40] Although still under debate, the often layered appearance of plaques at this site apparently originates from healed cap fractures as may be concluded from the presence of intimal fibrin residues and the inhibiting effect of pravastatin treatment on the accumulation of these "buried-caps".[41] As the brachiocephalic artery is very difficult to access for lesion targeted treatment, this model might be of particular benefit to study novel therapies by systemic intervention.

8.3.2 Metalloproteinase inhibitors

The intricate, context-dependent actions of the various MMPs necessitate a lesion targeted approach with a highly specific inhibitor. **Chapter 4** describes the early progress of the development of a specific MMP-9 inhibitor. Combinatorial peptide synthesis was applied to scan different amino acid arrangements, substitutions, terminal modifications and zinc-binders flanking the gelatinase inhibiting core peptide HWGF with regard to IC50 and MMP-9 specificity. At this stage we can conclude that the inhibiting capacity of these peptides is not so much dependent on their secondary structure (i.e. intramolecular cyclization and β-turns), as was suggested by the authors of the original paper that prompted us to modify their minimal essential motif TTCHWGFTLC, but appears to be directly related to the chemical characteristics of the individual amino acids. Also, we could find no evidence for the hypothesized zinc binding role of the histidine residue. In addition, hydroxamate zinc-binders, with or without PEG spacers, only marginally improved the rate of MMP inhibition, indicating that either the peptide does not bind near the metalloproteinase His-pocket or already contains an intrinsic and more potent zinc binding unit. Moreover, specificity for MMP-9 vs. MMP-2 appears to be reduced once zinc-binders are introduced. Additional experiments are required, and presently being conducted, to further optimize the affinity and specificity of the compounds and to study their pharmacological behaviour. The possible utilization of these peptides in *in vitro* MMP activity assays and in imaging techniques will also be explored.

In spite of a possible role for MMP-9 inhibition in the treatment of unstable plaques, the regulation of MMP activity in the context of atherosclerosis is not yet fully understood. Therefore, it is not yet possible to predict the consequences of selective MMP inhibition as a therapeutic strategy. A clear and thorough understanding of matrix biology is not possible without mapping all the physiologic inhibitors and determining their function in atherogenesis. TFPI-2 is an inhibitor of a wide variety of proteases, among which MMPs, and is expressed in the human endothelium and in atherosclerotic plaques.[42] It is speculated to have similar functions as the TIMPs. However, even the role of, for instance the relatively well characterized TIMP-1 in atherosclerosis is not unambiguous. While TIMP-1 overexpression has been described to attenuate plaque progression, TIMP-1

deficiency rendered similar results, highlighting the importance of context-dependency.[43, 44]

Being a potent MMP inhibitor in vitro, TFPI-2 may be of pathophysiological significance for human vascular disease. However, the observations in **chapter 5** may point to a slightly different role for TFPI-2 in mice, complicating the possibilities to unravel its function in human atherogenesis. Consistent with findings in human biology, murine TFPI-2 impairs plasma clotting time and both endothelial and SMC migration presumably via inhibition of protease activity. By contrast, mTFPI-2 was found to attenuate SMC proliferation and to promote the expression of MMP-9, while its human orthologue has been reported to act as a mitogen for VSMCs. Moreover, its expression in human plaques is inversely related to MMP expression levels.

In a mouse model of atherosclerosis, mTFPI-2 did not affect lesion size, but significantly reduced intimal collagen content and enhanced necrotic core formation, suggesting that mTFPI-2 compromises plaque stability, whereas human TFPI-2 has been proposed to stabilize atherosclerotic plaques.[42]

8.3.3 Cysteine proteinases

Similar to MMPs, cathepsins play a role in many different physiological processes. Being potent elastolytic and collagenolytic enzymes, they are involved in remodeling of the peri- or extracellular matrix, affecting not only matrix turnover, but also cellular motility, proliferation and behaviour. In addition, cathepsins have been demonstrated to modify lipoproteins, impinging on cholesterol efflux from macrophage-derived foam cells. However, in contrast to MMPs, cathepsins have also been reported to participate in intracellular processes. In fact, the first reported actions of these lysosomal proteases were degradation of phagocytosed proteins and processing of the invariant chain, important for antigen presentation to Th and NKT cells by dendritic cells.[45] Also, pro-caspases were found to be substrates for several cathepsins. Hence, these enzymes may, apart from their role in matrix homeostasis, also be involved in inflammation and cell death and survival.[46, 47]

In this thesis, both leukocyte cathepsin S and K appear to influence cell death within the atherosclerotic plaque. However, although both enzymes are thought to display similar substrate specificity, illustrated by their effect on elastic lamina degradation and intimal collagen content, they seem to have an opposite effect on macrophage survival. While leukocyte CatS deficiency indirectly reduced macrophage apoptosis (**chapter 6**) through its extracellular protease activity, the absence of leukocyte CatK resulted in larger necrotic cores and an enhanced susceptibility of monocytes for apoptotic and necrotic cell death (**chapter 7**) suggesting that CatK might function as an intracellular survival factor.

At this point it is not possible to conclude whether CatS or CatK are beneficial or detrimental for plaque stability. Due to their combined intra- and extracellular functionality, the effects of cathepsins are highly cell- and site-specific, rendering the search for an overall effect of cathepsins on plaque stability irrelevant. It is imperative to carefully dissect both cell specific as well as intra- vs extracellular functions of the various cathepsins within the arterial wall. However, the relevance of their concerted actions within a complex, continuously changing environment should not be omitted.

8.3.4 General conclusions

Taken together, it may be concluded that systemic inflammation can influence local MMP activity causing plaques to destabilize and that MMP inhibition

strategies should be investigated with care, paying special attention to their stage specific actions. The development of drug targeting techniques and/or selective inhibitors may enhance the therapeutic viability of MMP inhibition. However, while MMP-9 inhibition may benefit vulnerable plaques, the development of a highly specific MMP-9 inhibitor proves to be quite complicated.

In contrast to MMPs, cathepsins cannot be regarded as therapeutic target at this time. This thesis provides an initial contribution to the elucidation of the functional role of these proteases in atherosclerosis. Although cathepsin S and K appear to hold the same substrate specificity they show opposite effects on macrophage apoptosis and necrosis. This underlines the importance of the environmental context in which these proteases act and the need for further exploration of their pathobiological function. Especially because regulation of apoptotic cell death may be a valuable target for plaque stabilizing therapies.

8.4 Clinical implications and the role of translational medicine

Our findings that systemic inflammatory mediators can affect local processes in the arterial wall can be confirmed, at a clinical level, by many other studies that correlate circulating mediators of inflammation to acute ischemic events. Moreover, it is widely accepted that infections, surgery, mental stress or depression relate to acute ischemic events.[48-50] The concept of the “vulnerable patient” comprises the significance of a systemic influence from mental, behavioural, inflammatory and metabolic factors on both vascular biology and on hemostasis.[35 36, 49, 50] Viral infections, periodontal disease, surgery and mental stress can all result in a transient hyperinflammatory state that could evoke plaque destabilization. This means that modulation of these systemic factors, for instance by influenza vaccination, dental care or improving labour conditions, certainly have their place in the prevention and treatment of atherosclerosis.

Notwithstanding the significance of this more holistic view on the pathogenesis of atherothrombosis, plaque stability still is a very important aspect in this process and plaque stabilizing therapies may be able to dramatically reduce the incidence of acute thrombotic events. In chapter 3, MMP-9 is highlighted as a possible therapeutic target for intervention in advanced and complex lesions. Given the pleiotropic actions of many MMPs, it is vital to develop specific MMP inhibitors in order to prevent any adverse effects. With this thesis an initial progress has been made in designing a specific MMP-9 inhibitor. However, selective inhibitors alone are not sufficient. The need for lesion targeting shows that it is important to be able to identify those lesions that could benefit from MMP inhibition. Advanced, preferably non-invasive, imaging techniques to identify vulnerable plaques are essential tools that should precede such a therapy, but have yet to be developed.

Taken together, this demonstrates the need for intensive and extensive collaboration between both basic and clinical science on the one hand and technological innovation on the other. The unravelling of pathogenic mechanisms by basic science should contribute to clinical science by identifying novel diagnostic and therapeutic targets and designing drugs that modulate these targets. Vice versa clinical findings, particularly from genetics and epidemiology, can prompt basic science to investigate their causal relation with clinical outcomes. Furthermore, intensive dialogue between clinicians and basic scientists may clarify the problems that physicians face when managing atherosclerosis related disease, by allowing a

rapid feedback to basic research. It should be kept in mind, however, that scientific freedom is elementary to basic research and could definitely lead to interesting new insights that may benefit clinically relevant research. A third important player in this field is technological advance that may contribute for instance by designing drug targeting strategies, new materials such as drug eluting polymers and imaging techniques to improve basic research models as well as diagnostic tools and therapeutic strategies.

Although these three fields might be somewhat overlapping, they are also greatly professionalized and in order to pursue a common goal (i.e. treatment of atherothrombosis) communication and cooperation between the three is of utmost importance. Figure 8.1 depicts the relationship between the three major players in cardiovascular research and development and shows that their efforts could be very complementary. However, efficient and effective communication is only possible if all players involved have knowledge of one another's field of interest.

The Molecular Cardiology Project has made one step in the direction of achieving this by training MDs in molecular biology, but many more are necessary. Over the past four years, the Division of Biopharmaceutics in Leiden has gradually shifted its attention towards more integrated projects with the clinic, which are expected to directly contribute to individualized risk stratification and vulnerable plaque imaging. In conjunction with the ongoing efforts to elucidate the pathobiology of plaque destabilization and restenosis, these projects may lead to a better insight in disease progression, individual risk factors and thus a more tailored therapeutic approach.

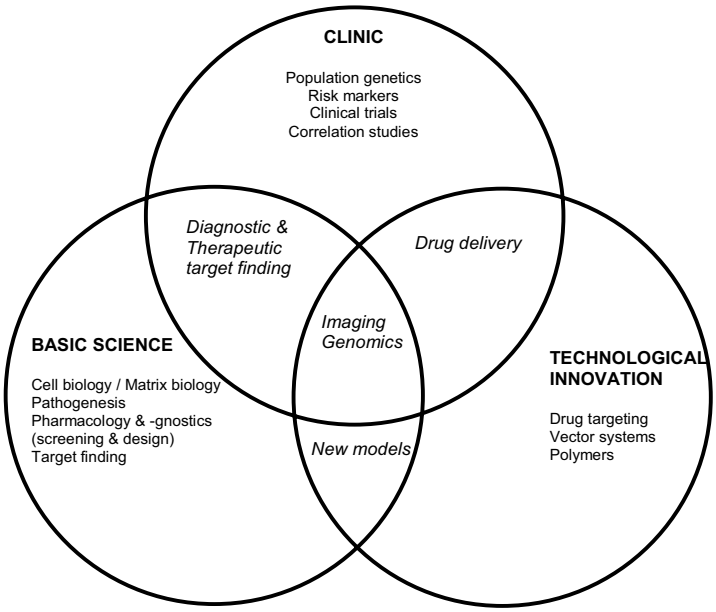

Figure 8.1. Interrelation between the three principal players in atherosclerosis research. The examples in each professional field illustrate the complementary character of the topics of interest.

8.5 References

1. D.E. Zipes PL, R.O. Bonow, E. Braunwald. *Braunwald's Heart Disease: A Textbook of Cardiovascular Medicine*. 7 ed: SAUNDERS W B CO; 2004.
2. Naghavi M, Libby P, Falk E, et al. From vulnerable plaque to vulnerable patient: a call for new definitions and risk assessment strategies: Part I. *Circulation.* Oct 7 2003;108(14):1664-1672.
3. H.L. Koek LATMvL, W.M.M. Verschuren, M.L. Bots. *Hart- en vaatziekten in Nederland 2003.* Den Haag: Nederlands Hartstichting; March 2003 2003.
4. Unal B, Critchley JA, Capewell S. Modelling the decline in coronary heart disease deaths in England and Wales, 1981-2000: comparing contributions from primary prevention and secondary prevention. *Bmj.* Aug 17 2005.
5. (AEHA) AfEoHA. *AEHA Mission Statement.* Bolsover, No 2005.
6. Maree AO, Fitzgerald DJ. Aspirin and coronary artery disease. *Thromb Haemost.* Dec 2004;92(6):1175-1181.
7. Manolis AS, Tzeis S, Andrikopoulos G, et al. Aspirin and clopidogrel: a sweeping combination in cardiology. *Curr Med Chem Cardiovasc Hematol Agents.* Jul 2005;3(3):203-219.
8. Doggrell SA. CLARITY about the use of clopidogrel in patients with acute coronary syndromes and myocardial infarction. *Expert Opin Pharmacother.* Aug 2005;6(10):1761-1764.
9. Sabatine MS, Cannon CP, Gibson CM, et al. Effect of Clopidogrel Pretreatment Before Percutaneous Coronary Intervention in Patients With ST-Elevation Myocardial Infarction Treated With Fibrinolytics: The PCI-CLARITY Study. *Jama.* Sep 4 2005.
10. Silber S, Albertsson P, Aviles FF, et al. Guidelines for percutaneous coronary interventions. The Task Force for Percutaneous Coronary Interventions of the European Society of Cardiology. *Eur Heart J.* Apr 2005;26(8):804-847.
11. St John Sutton M, Ferrari VA. Prevention of Left Ventricular Remodeling After Myocardial Infarction. *Curr Treat Options Cardiovasc Med.* Apr 2002;4(2):97-108.
12. Thohan V, Torre-Amione G, Koerner MM. Aldosterone antagonism and congestive heart failure: a new look at an old therapy. *Curr Opin Cardiol.* Jul 2004;19(4):301-308.
13. Bocan TM. Pleiotropic effects of HMG-CoA reductase inhibitors. *Curr Opin Investig Drugs.* Sep 2002;3(9):1312-1317.
14. Pfeffer MA, Sacks FM, Moye LA, et al. Cholesterol and Recurrent Events: a secondary prevention trial for normolipidemic patients. CARE Investigators. *Am J Cardiol.* Sep 28 1995;76(9):98C-106C.
15. Spin JM, Vagelos RH. Early use of statins in acute coronary syndromes. *Curr Cardiol Rep.* Jul 2002;4(4):289-297.
16. Pazzucconi F, Dorigotti F, Gianfranceschi G, et al. Therapy with HMG CoA reductase inhibitors: characteristics of the long-term permanence of hypocholesterolemic activity. *Atherosclerosis.* Oct 1995;117(2):189-198.
17. van Heek M, Compton DS, Davis HR. The cholesterol absorption inhibitor, ezetimibe, decreases diet-induced hypercholesterolemia in monkeys. *Eur J Pharmacol.* Mar 9 2001;415(1):79-84.
18. Kastelein JJ, Sager PT, de Groot E, et al. Comparison of ezetimibe plus simvastatin versus simvastatin monotherapy on atherosclerosis progression in familial hypercholesterolemia. Design and rationale of the Ezetimibe and Simvastatin in Hypercholesterolemia Enhances Atherosclerosis Regression (ENHANCE) trial. *Am Heart J.* Feb 2005;149(2):234-239.
19. Tardif JC, Gregoire J, L'Allier PL, et al. Effects of the acyl coenzyme A:cholesterol acyltransferase inhibitor avasimibe on human atherosclerotic lesions. *Circulation.* Nov 23 2004;110(21):3372-3377.
20. Kharbanda RK, Wallace S, Walton B, et al. Systemic Acyl-CoA:cholesterol acyltransferase inhibition reduces inflammation and improves vascular function in hypercholesterolemia. *Circulation.* Feb 15 2005;111(6):804-807.
21. Hauer AD, Uyttenhove C, de Vos P, et al. Blockade of interleukin-12 function by protein vaccination attenuates atherosclerosis. *Circulation.* Aug 16 2005;112(7):1054-1062.
22. Willett CG, Boucher Y, di Tomaso E, et al. Direct evidence that the VEGF-specific antibody bevacizumab has antivascular effects in human rectal cancer. *Nat Med.* Feb 2004;10(2):145-147.
23. Vosseler S, Mirancea N, Bohlen P, et al. Angiogenesis inhibition by vascular endothelial growth factor receptor-2 blockade reduces stromal matrix metalloproteinase expression, normalizes stromal tissue, and reverts epithelial tumor phenotype in surface heterotransplants. *Cancer Res.* Feb 15 2005;65(4):1294-1305.
24. Zhu XF, Xie BF, Zhou JM, et al. Blockade of vascular endothelial growth factor receptor signal pathway and antitumor activity of ON-III (2',4'-dihydroxy-6'-methoxy-3',5'-dimethylchalcone), a component from Chinese herbal medicine. *Mol Pharmacol.* May 2005;67(5):1444-1450.
25. Rubin EM, Smith DJ. Atherosclerosis in mice: getting to the heart of a polygenic disorder. *Trends Genet.* Jun 1994;10(6):199-203.
26. Nagashima M, McLean JW, Lawn RM. Cloning and mRNA tissue distribution of rabbit cholesteryl ester transfer protein. *J Lipid Res.* Dec 1988;29(12):1643-1649.
27. Nuttall RK, Sampieri CL, Pennington CJ, et al. Expression analysis of the entire MMP and TIMP gene families during mouse tissue development. *FEBS Lett.* Apr 9 2004;563(1-3):129-134.

28. Masucci-Magoulas L, Plump A, Jiang XC, et al. Profound induction of hepatic cholesteryl ester transfer protein transgene expression in apolipoprotein E and low density lipoprotein receptor gene knockout mice. A novel mechanism signals changes in plasma cholesterol levels. *J Clin Invest.* Jan 1 1996;97(1):154-161.
29. Plump AS, Masucci-Magoulas L, Bruce C, et al. Increased atherosclerosis in ApoE and LDL receptor gene knock-out mice as a result of human cholesteryl ester transfer protein transgene expression. *Arterioscler Thromb Vasc Biol.* Apr 1999;19(4):1105-1110.
30. von der Thusen JH, van Berkel TJ, Biessen EA. Induction of rapid atherogenesis by perivascular carotid collar placement in apolipoprotein E-deficient and low-density lipoprotein receptor-deficient mice. *Circulation.* Feb 27 2001;103(8):1164-1170.
31. von der Thusen JH, van Vlijmen BJ, Hoeben RC, et al. Induction of atherosclerotic plaque rupture in apolipoprotein E-/- mice after adenovirus-mediated transfer of p53. *Circulation.* Apr 30 2002;105(17):2064-2070.
32. Blankenberg S, Tiret L, Bickel C, et al. Interleukin-18 is a strong predictor of cardiovascular death in stable and unstable angina. *Circulation.* Jul 2 2002;106(1):24-30.
33. Mallat Z, Corbaz A, Scoazec A, et al. Expression of interleukin-18 in human atherosclerotic plaques and relation to plaque instability. *Circulation.* Oct 2 2001;104(14):1598-1603.
34. Mallat Z, Henry P, Fressonnet R, et al. Increased plasma concentrations of interleukin-18 in acute coronary syndromes. *Heart.* Nov 2002;88(5):467-469.
35. Naghavi M, Libby P, Falk E, et al. From vulnerable plaque to vulnerable patient: a call for new definitions and risk assessment strategies: Part II. *Circulation.* Oct 14 2003;108(15):1772-1778.
36. Meurman JH, Sanz M, Janket SJ. Oral health, atherosclerosis, and cardiovascular disease. *Crit Rev Oral Biol Med.* 2004;15(6):403-413.
37. Johnson JL, George S, Newby A, et al. Matrix metalloproteinases-9 and -12 have opposite effects on atherosclerotic plaque stability. *Atherosclerosis Supplements.* 2003;4(2):196.
38. Rosenfeld ME, Carson KG, Johnson JL, et al. Animal models of spontaneous plaque rupture: the holy grail of experimental atherosclerosis research. *Curr Atheroscler Rep.* May 2002;4(3):238-242.
39. Zadelaar AS, Thusen JH, LS MB, et al. Increased vulnerability of pre-existing atherosclerosis in ApoE-deficient mice following adenovirus-mediated Fas ligand gene transfer. *Atherosclerosis.* May 28 2005.
40. Johnson JL, Jackson CL. Atherosclerotic plaque rupture in the apolipoprotein E knockout mouse. *Atherosclerosis.* Feb 1 2001;154(2):399-406.
41. Johnson J, Carson K, Williams H, et al. Plaque rupture after short periods of fat feeding in the apolipoprotein E-knockout mouse: model characterization and effects of pravastatin treatment. *Circulation.* Mar 22 2005;111(11):1422-1430.
42. Herman MP, Sukhova GK, Kisiel W, et al. Tissue factor pathway inhibitor-2 is a novel inhibitor of matrix metalloproteinases with implications for atherosclerosis. *J Clin Invest.* May 2001;107(9):1117-1126.
43. Rouis M, Adamy C, Duverger N, et al. Adenovirus-mediated overexpression of tissue inhibitor of metalloproteinase-1 reduces atherosclerotic lesions in apolipoprotein E-deficient mice. *Circulation.* Aug 3 1999;100(5):533-540.
44. Silence J, Collen D, Lijnen HR. Reduced atherosclerotic plaque but enhanced aneurysm formation in mice with inactivation of the tissue inhibitor of metalloproteinase-1 (TIMP-1) gene. *Circ Res.* May 3 2002;90(8):897-903.
45. Riese RJ, Shi GP, Villadangos J, et al. Regulation of CD1 function and NK1.1(+) T cell selection and maturation by cathepsin S. *Immunity.* Dec 2001;15(6):909-919.
46. Li W, Yuan XM, Olsson AG, et al. Uptake of oxidized LDL by macrophages results in partial lysosomal enzyme inactivation and relocation. *Arterioscler Thromb Vasc Biol.* Feb 1998;18(2):177-184.
47. Yuan XM, Li W, Brunk UT, et al. Lysosomal destabilization during macrophage damage induced by cholesterol oxidation products. *Free Radic Biol Med.* Jan 15 2000;28(2):208-218.
48. Strike PC, Steptoe A. Psychosocial factors in the development of coronary artery disease. *Prog Cardiovasc Dis.* Jan-Feb 2004;46(4):337-347.
49. Rozanski A, Blumenthal JA, Davidson KW, et al. The epidemiology, pathophysiology, and management of psychosocial risk factors in cardiac practice: the emerging field of behavioral cardiology. *J Am Coll Cardiol.* Mar 1 2005;45(5):637-651.
50. Wolff B, Grabe HJ, Volzke H, et al. Relation between psychological strain and carotid atherosclerosis in a general population. *Heart.* Apr 2005;91(4):460-464.