Arch. Immunol. Ther. Exp. (2012) 60:431–442
DOI 10.1007/s00005-012-0192-z

REVIEW

Pathophysiological Mechanisms of Carotid Plaque Vulnerability: Impact on Ischemic Stroke

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Received: 5 April 2012 / Accepted: 6 August 2012 / Published online: 4 September 2012

Abstract Stroke is among the major causes of mortality and disabilities in the world. About 80 % of all strokes in the anterior circulation are ischemic and up to 20 % of all ischemic strokes are caused by extracranial atherosclerotic carotid artery stenosis. The prevalence of a cervical internal carotid artery stenosis increases with age and can be found in 6.9 % of the elderly population (>65 years). Atherosclerotic changes of the carotid vessel wall can lead to plaque vulnerability and may result in arterio-arterial embolism, which frequently underlie carotid-related cerebrovascular ischemic events. Carotid atherosclerosis is characterised by inflammation, extensive degradation of extracellular matrix components, neovascularization, and as recently recognised is also affected by epigenetic changes. These factors accelerate the progression of atherosclerosis towards vulnerable plaques and increase the risk of ischemic stroke. In this review, the main pathophysiological mechanisms leading to rupture-prone carotid artery plaques and successive ischemic stroke are considered. It is important to recognise the heterogeneity of atherosclerosis and that various pathophysiological processes dissected in this review are not acting individually, but rather in a complementary manner. The identification and careful integration of all relevant factors will be required for the development of future diagnostic and therapeutic strategies.

Keywords Carotid atherosclerosis · Vulnerable carotid plaque · Ischemic stroke

Abbreviations

ADAM	A disintegrin and metalloprotease domain
ADAMTS	ADAM-related metalloproteases with a thrombospondin domain
AGEs	Advanced glycation end products
Ang-1, -2	Angiopoietins-1 and -2
CEA	Carotid endarterectomy
CKD	Chronic kidney disease
DM	Diabetes mellitus
ECM	Extracellular matrix
Eph	Ephrins
FGF	Fibroblast growth factor
ICA	Internal carotid artery
HDL	High density lipoproteins
HIF-1	Hypoxia-inducible growth factor
IL	Interleukin
INF-γ	Interferon-gamma
LDL	Low density lipoproteins
oxLDL	Oxidised low density lipoproteins
LDL(-)	Electronegatively charged low density lipoproteins
MP	Metalloprotease
MMP	Matrix metalloprotease
PDGF	Platelet-derived growth factor
PGs	Proteoglycans
RAGE	AGE receptor
TNF-α	Tumour necrosis factor-alpha
VSMCs	Vascular smooth muscle cells

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VEGF	Vascular endothelial growth factor
VEGFR	Vascular endothelial growth factor receptor

Introduction

Stroke is among the major causes of mortality and permanent disabilities worldwide, accounting for at least 5.5 million deaths and over 44 million disabilities every year (Mukherjee and Patil 2011). In Germany, stroke was found to be the most common manifestation of cardiovascular disease with 6.1 % of all cases of death in 2010. Moderate to high grade extracranial carotid artery stenosis (>50 %) can be detected in 1–3 % of all adults (Eckstein 2012). With ageing (>65), the mean prevalence increases up to 6.9 % (Eckstein 2012). Ruptured plaques, embolised thrombotic material, and acute carotid occlusions are responsible for cerebral ischemia initiating ischemic stroke. This cerebrovascular event leads to a loss of brain function and is often accompanied by the permanent damage of neuronal cells due to the lack of blood supply caused by obstruction of brain vessels either by thrombosis (local obstruction) or by embolism (obstruction from elsewhere). About 80 % of all strokes in the anterior circulation are ischemic and at least 20 % of the ischemic strokes are caused by carotid artery atherosclerosis (Rothwell 2000 Mughal et al. 2011; Eckstein 2012).

Atherosclerotic changes within the vessel wall of the internal carotid artery can lead to plaque vulnerability, which constitute the main reason for carotid-related cerebrovascular ischemic events (Rothwell 2000; Virmani et al. 2006; Pelisek et al. 2009; Heider et al. 2009). Atherosclerosis is commonly described as a chronic inflammatory disease of the vessel wall, characterised by lipid accumulation, inflammation, and extensive degradation of extracellular matrix components (Stary et al. 1992, 1994, 1995; Newby et al. 1994; Newby 2005; Libby et al. 2009). Furthermore, neovascularization, and disease often associated with atherosclerosis, such as diabetes mellitus (DM) or chronic kidney disease (CKD), and as recently recognised also epigenetic changes markedly contribute to disease progression and vulnerability (Turunen et al. 2009; Lund and Zaina 2011; Pelisek et al. 2010, 2011, 2012a, b). In this review, we focus on the main pathophysiological mechanisms leading to rupture-prone carotid artery plaques and consequent ischemic stroke.

Vulnerable Plaque

Atherosclerotic plaque development is a rather slow process and can take several decades. Early atherosclerotic plaque formation is initiated by the sub-endothelial accumulation of lipids, cholesterol, and other blood components within intimal layer of the vessel wall. Modified LDL, especially oxidised LDL (oxLDL) and often coined "bad cholesterol", is well known to be associated with the onset and progression of atherosclerosis. OxLDL induces the expression of various adhesion molecules on ECs and leads to the recruitment of monocytes into the intima of vessel wall. Furthermore, oxLDL is taken up by macrophages via scavenger receptors, resulting in transformation of these cells to macrophage-derived foam cells (Ross 1999). OxLDL in addition stimulates the migration and proliferation of SMCs and increases the production of reactive oxygen species, which in turn further facilitate atherosclerosis by augmenting inflammation (Shen et al. 2001). In addition, Avogaro et al. (1988) found a sub-fraction of electronegatively charged oxLDL (Chen et al. 2003; Lu et al. 2009; Mello et al. 2011), subsequently termed minimally oxidised LDL or LDL(−), based on their electric mobility. Similar to oxLDL, also LDL(−) is increased in patients with hypercholesterolemia, diabetes mellitus, and coronary artery disease (Fabjan et al. 2001; Tomasik et al. 2003; Mello et al. 2011). The role of LDL(−) seems to be complex and includes activation of inflammatory and immune responses, which promote the progression of atherosclerosis. The accumulation of lipids is tightly correlated by endothelial dysfunction, infiltration of monocytes, and immigration of other inflammatory cells (Libby et al. 2009; Halvorsen et al. 2008). These processes, together with an activation of vascular smooth muscle cells (VSMCs) and their excessive production of extracellular matrix (ECM) in the arterial wall, result in vessel wall thickening and a usually asymmetrical narrowing of the vessel. Although mainly affecting the intimal vessel wall layer, as also outlined in the definition of atherosclerosis (American Heart Association) (Stary et al. 1992, 1994, 1995), also media and adventitia are critically involved in disease development and progression (Rey and Pagano 2002; Langheinrich et al. 2007). While early atherosclerotic lesions mainly consist of an excess of lipids, cholesterol, and ECM components, especially collagen I and III, elastin, and progeoglycans (PGs), and initially encompass a thickening of the vessel wall without any vessel narrowing, an extensive vessel wall remodelling and a proteolytic degradation of ECM, and lumen narrowing occurs at later stages. Inflammatory processes within the plaque together with infiltrated leukocytes and activated macrophages have been shown to be involved in driving lesion progression and contributing to plaque vulnerability (Halvorsen et al. 2008; Libby et al. 2009). On this account, e.g. endocannabionic signalling is often triggered as a response to inflammation and its deregulation may contribute to the development of atherosclerosis (Montecucco

et al. 2012). In addition, the majority of atherosclerotic lesions are vascularised by a large network of small vessels that originate from vasa vasorum of the adventitia (Langheinrich et al. 2007; Pelisek et al. 2012a, b). These neovessels play also an important role in plaque growth and can likewise contribute to plaque instability (Virmani et al. 2005; Chen and Walterscheid 2006; Sluimer and Daemen 2009; Di Stefano et al. 2009; Pelisek et al. 2012a, b). Finally, epigenetic changes have recently been associated with cardiovascular disease and atherosclerosis (Dong et al. 2002; Egger et al. 2004; Ingrosso and Perna 2009; Turunen et al. 2009; Bayarsaihan 2011; Radford et al. 2011).

In the literature the terms "vulnerable plaque" or "rupture-prone plaque" are frequently used in order to define the pathophysiological mechanisms leading to plaque instability. Plaque vulnerability was first mentioned by Muller and Tofler (1992), describing an atherosclerotic plaque that became "vulnerable to rupture". Naghavi et al. (2003a, b) describe "vulnerable plaques" to define lesions susceptible to complications. Furthermore, the alternative term "high-risk plaque" has recently been proposed. So what do we know about the criteria that specify a rupture-prone plaque, and how is its morphology? Virmani et al. defined coronary artery plaques to be vulnerable if atherosclerotic lesions with large necrotic/lipid cores were covered by a "thin fibrous cap" of less than 65 μm (Burke et al. 1997; Virmani et al. 2000). This definition was deduced from histological analyses of 41 ruptured plaques, in which 95 % of the fibrous caps measured <64 μm and the mean plaque thickness was 23 ± 19 μm (Burke et al. 1997). Vulnerable plaques were furthermore characterised as follows: (1) thin fibrous cap with an extensive loss of smooth muscle cells and extracellular matrix within the cap, (2) fibrous cap is usually overlying large necrotic/lipid core, (3) atherosclerotic plaques compose often of haemorrhage and/or calcification, (4) intraplaque neovascularization is abundant. Naghavi et al. (2003a, b) on the other hand recommend that all thrombosis-prone plaques and plaques with high probability of undergoing rapid progression should be defined as "vulnerable". The authors furthermore propose to also classify vulnerable myocardium as ischemic or non-ischemic, and with or without atherosclerosis-induced myocardial damage. The outcome of these entities after plaque rupture seems to be mainly determined by the autonomic nervous activity and by the form of cardiomyopathy.

Results derived from the analyses of coronary arteries, however, do not necessarily apply to other atherosclerotic vessels such as the carotid artery. On this account, Redgrave et al. (2008) performed a detailed histological analysis of 526 carotid plaques of symptomatic patients undergoing carotid endarterectomy (CEA) and determined the thickness of the fibrous cap in correlation with plaque rupture. In the ruptured plaques, mean cap thickness was observed to be 300 μm, with a minimum cap thickness of 150 μm. For non-ruptured plaques, cap thickness averaged 500 μm and 250 μm, respectively. In consequence, the optimum cut-offs to discriminate between ruptured and non-ruptured plaques were set at <200 μm for minimum cap thickness and <500 μm for mean cap thickness. Interestingly, these values were much greater than the cap thickness reported for coronary plaques. Combining the both pioneering works of Virmani et al. and Redgrave et al., we propose to define vulnerable carotid plaques as atherosclerotic lesions with a thin fibrous cap of <200 μm overlying large necrotic/lipid core, which often contain intraplaque haemorrhage and/or calcification and neovascularization. In addition, an extended term "cardiovascular vulnerable patient" is proposed, which integrates both intraplaque and systemic factors to assess the cumulative risk of cardiovascular events, including various serum markers, lipid profile, state of inflammation, and changes in coagulation (Naghavi et al. 2003a, b; Montecucco et al. 2010). Such a composite risk score may be most suitable for approaching risk stratification.

Nevertheless, the question still remains: what pathophysiological mechanisms lead to plaque vulnerability?

Inflammation

Inflammation has been recognised as a crucial force driving the development and progression of atherosclerosis throughout all stages (Ross 1999; Halvorsen et al. 2008; Libby et al. 2002, 2009; Weber et al. 2008; Hansson 2005; Plutzky 2003). The onset of atherosclerosis is initiated by endothelial dysfunction and induction of inflammation, accompanied by the increased expression of adhesion molecules that trigger the recruitment of leukocytes to the vessel wall. Recruited inflammatory cells, but also cells of the vessel wall, produce a plethora of different cytokines, chemokines, adhesion molecules, and perpetuate leukocyte accumulation (Hansson 2005, Weber et al. 2008; Zernecke and Weber 2005, 2010; Zernecke et al., 2008). E.g. TNF-α produced by endothelial cells, smooth muscle cells, and macrophages stimulates the synthesis of plethora of other inflammatory factors (Sundström and Vasan 2006; Ferri et al. 2006). IL-8 (CXCL8) contributes to the recruitment of different leukocyte subsets and can furthermore amplify inflammation by the induction of IFN-γ secretion (Zernecke et al. 2008). While monocytes and macrophages constitute the predominant cell population in atherosclerosis, also other inflammatory cells, such as T and B cells and dendritic cells accumulate in atherosclerotic lesions. Besides these cells, also neutrophils can be detected within

unstable lesions and were identified at sites of plaque rupture (Naruko et al. 2002). The functions of neutrophils in atherosclerotic plaques encompass endocytosis of cell debris and the secretion of proteolytic enzymes such as elastase, myeloperoxidase, and neutral endopeptidase. These enzymes may further contribute to plaque vulnerability by degrading ECM and modulating inflammatory responses. In line with this notion, Naruko et al. (2002) observed neutrophil infiltration to be actively associated with acute cardiovascular events, and suggested that the presence of neutrophils points to differences in the underlying pathophysiology of ruptured plaques. The secretion of pro- and anti-inflammatory cytokines and modulation of specific immune mechanisms further contribute to the disease development (Weber et al. 2008; Hansson and Hermansson 2011; Manthey and Zernecke 2011). Many of these pro-inflammatory mediators, including various interleukins, interferon (IFN)-γ, and tumour necrosis factor (TNF)-α are powerful stimulators of multiple responses in vascular- and atheroma-associated cells (Libby 2002; Plutzky 2003). We have demonstrated increased levels of TNF-α and IL-8 in blood of patients with vulnerable carotid plaques in comparison to the stable carotid lesions. Furthermore, a strong correlation between levels of IL-8 in blood and the accumulation of macrophages within carotid artery plaques in these patients was observed (Pelisek et al. 2009). In addition, we have also noted that in human atherosclerotic aneurismal specimen activated inflammatory cells, especially macrophages are able to synthesise various matrix metalloproteases (MMPs), thus directly contributing to the proteolytic destabilisation of vessel wall (Reeps et al. 2009).

Degradation of Extracellular Matrix

Degradation of extracellular matrix components, particularly by MMPs, together with the above mentioned inflammatory responses, are the main forces driving plaque progression towards rupture (Newby et al. 1994; Newby 2005; Virmani et al. 2006). MMPs proteolytically degrade the basement membrane around VSMCs, promoting a change from the quiescent/contractile to the proliferating/synthetic phenotype. These VSMCs can then also migrate from the media into the intima and start to produce new ECM components. This process can be seen as an adaptive repair response to inflammatory stimuli acting in the vessel wall (Hansson 2005; Virmani et al. 2006; Libby et al. 2009).

ECM consists of proteoglycans (PGs), collagen, and elastin. PGs build large molecule complexes, which in the vessel wall mainly constitute of hyaluronan, versican, chondroitin sulphate, dermatan sulphate, heparan sulphate, biglycan, and perlecan (Labat-Robert et al. 1990; Kinsella et al. 2004; Kunz 2007). Important functions of vascular PGs are the regulation of permeability, interactions with other ECM components, influence on cell behaviour, and steaming of mechanical pressure (Kunz 2007). Elastin and several collagens, especially types I and III and to less extent also types IV, V and VI (Murata et al. 1986), ensure the elasticity and stability of the vessel. All these ECM components are synthesised predominantly by VSMCs. Hence, VSMCs, their viability and synthetic potential are essential for the maintenance of vessel wall integrity.

Ezymatic proteolysis of ECM by extracellular proteases is an important pathophysiologic process in tissue repair, chronic inflammatory diseases, and atherosclerosis. Especially metalloproteinases (MPs) play a crucial role in these pathophysiological processes. There are three classes of MPs (Table 1): (1) matrix metalloproteinases (MMPs) that have already been recognised to contribute to plaque vulnerability and rupture (Galis et al. 1994, 1995; Halpert et al. 1996; Nikkari et al. 1995; Newby et al. 1994; Newby 2005; Loftus et al. 2000; Formato et al. 2004; Garcia-Touchard et al. 2005; Dollery and Libby 2006; Lemaitre and D'Armiento 2006), (2) the ADAM family of metalloproteases (a disintegrin and metalloprotease domain) (Millichip et al. 1998; Seals and Courtneidge 2003; Sahin et al. 2004; Blobel 2005; Roy et al. 2006; Mochizuki and Okada 2007; Duffy et al. 2009; van Goor et al. 2009), and (3) the ADAMTS family of ADAM-related metalloproteases with a thrombospondin domain (Tang 2001; Apte 2004; Porter et al. 2005; Wight 2005; Jones and Riley 2005; Roy et al. 2006). MMPs and ADAMTS are secretory proteases; ADAMs are cell surface bound proteolytic enzymes termed sheddases. Currently at least 25 MMPs are described, of which 14 have already been detected in the vessel wall (Newby 2005; Dollery and Libby 2006; Lemaitre and D'Armiento 2006; Heider et al. 2009). According to their substrate specificity and structure, MMPs can be divided into several subgroups including collagenases (MMP-1,-8,-13), gelatinases (MMP-2,-9), stromelysins (MMP-3,-10), matrilysins (MMP-7), membrane-type MMPs (MMP-14,-15,-16,-17) and others (MMP-11,-12). Dollery and Libby described expression of MMP-1, -2, -3, and -9 in shoulder regions of carotid plaques associated with macrophages (Galis et al. 1994, 1995; Dollery and Libby 2006). Nikkari et al. (1995) localised MMP-1 within intraplaque haemorrhage, usually indicating lesion instability. Other groups correlated levels of MMP-2 and MMP-9 with the inflammatory mediators IL-6 and IL-8 in carotid artery lesions (Dollery and Libby 2006). Higher levels of MMP-9 were detected in unstable plaques and in symptomatic patients following embolic stroke (Loftus et al. 2000; Formato et al. 2004). Furthermore, a plethora of MMPs were found in inflammatory cells within

Table 1 Summary of metalloproteases that are potentially involved in atherosclerosis

	Substrate	Tissue distribution	Function and disease involvement
MMP-1	Collagen I, II, III, VII, VIII, X, gelatin, fibronectin, versican, perlecan	Ubiquitous, pathological remodelling, tissue repair, vessel wall, heart, lung	Atherosclerosis, aneurysm, breast cancer
MMP-2	Collagen I, IV, V, VII, X, XI, gelatin, elastin, fibronectin, aggrecan, versican, laminin	Ubiquitous, vessel wall, heart	Cardiovascular disease, myocardial infarction, aneurysm
MMP-3	Collagen II, IV, IX, X, gelatin, elastin, fibronectin, laminin, perlecan, versican	Ubiquitous, vessel wall, heart	Atherosclerosis, myocardial infarction, aneurysm, cancer
MMP-7	Collagen I, II, III, IV, V, X, gelatin, elastin, aggrecan, laminin	Mucosal and exocrine gland epithelial cells, vessel wall	Colorectal cancer, atherosclerosis
MMP-8	Collagen I, II, III, V, VII, VIII, X, gelatin, aggrecan, laminin	Ubiquitous, remodelling, inflammatory disease, vessel wall	Atherosclerosis, aneurysm, breast cancer
MMP-9	Collagen IV, V, VII, X, gelatin, fibronectin, laminin, versican	Ubiquitous, vessel wall, heart	Atherosclerosis, myocardial infarction, aneurysm, cancer
MMP-10	Collagen III, IV, V, gelatin, fibronectin, laminin		Various carcinomas, wound healing
MMP-11	Laminin, fibronectin	Embryonic tissue	Embryonic development, wound healing, various carcinomas
MMP-12	Elastin, fibronectin, laminin	Macrophages, inflammatory cells	Aneurysm
ADAM8	Myelin basic protein, CD23	Granulocytes, monocytes, macrophages	Leukocyte infiltration, atherosclerosis
ADAM9	HB-EGF, fibronectin, gelatin, collagen XVII, (TNFα)	Ubiquitous, somatic	Sheddase, migration, breast cancer, atherosclerosis
ADAM10	HB-EGF, TNFα, Notch, ephrin-A2, delta L1, collagen IV and XVII, gelatin, e-cadherin	Ubiquitous, somatic	Sheddase, cell fate, cancer, neuroblastoma
ADAM12	HB-EGF, IGF binding protein, gelatin, coll IV, fibronectin	Ubiquitous, somatic	Sheddase, myoblasts fusion, atherosclerosis
ADAM15	Collagen IV, gelatin, CD23	Ubiquitous, somatic	Cell–cell binding, neovascularization, prostate cancer, atherosclerosis
ADAM17	TNFα, HB-EGF, Notch, Fas lig., fractacline, L-selectin, TNFRI and II, IL-1RII, IL-6R	Ubiquitous, somatic	Sheddase, breast cancer
ADAMTS1	Aggrecan, versican, brevican/inflammatory response, anti-angiogenic	Heart, placenta, liver, muscle, kidney, aorta, colon, prostate, cartilage	Antiangiogenic, stabilising tissue, colon and pancreas cancer, atherosclerosis
ADAMTS2	Procollagen I, II and III	Aorta, bone, skin, kidney, lung, liver, muscle, cartilage	
ADAMTS3	Procollagen II (and I)	Placenta, lung, brain, heart, skin, cartilage	
ADAMTS4	Aggrecan, versican, cartilage matrix protein, fibromodulin, decorin, transferrin	Bladder, brain, heart, muscle, uterus, cartilage	Stabilising tissue, arthritis
ADAMTS5	Aggrecan	Bladder, cervix, placenta, uterus, cartilage	Stabilising tissue, arthritis
ADAMTS8	Aggrecan/anti-angiogenic	Lung, heart, placenta, brain	Antiangiogenic, stabilising tissue
ADAMTS9	Aggrecan, versican	Heart, placenta, lung, muscle, kidney, pancreas, colon	Stabilising tissue, embryonic development, breast cancer
ADAMTS13	Von Willlebrand factor	Kidney, brain, liver, muscle, heart, cartilage	Breast carcinoma

atherosclerotic lesions (Dollery and Libby 2006, Reeps et al. 2009). However, the patterns of MMP expression vary markedly. MMP-7 and -12 occur along the perimeter of the lipid core and are adjacent to but not associated with cells of the fibrous cap (Labat-Robert et al. 1990; Dollery and Libby 2006). In contrast, MMP-1,-2, and -9 are localised predominately within the plaque shoulder and fibrous cap. We have observed an increased expression of MMP-1,-2,-3, and -9 in human carotid lesions (Pelisek et al. 2009, 2012a, b).

The ADAM family of metalloproteases is involved in various biological functions including cell adhesion, migration, activation of cell surface markers, and proteolysis (Seals and Courtneidge 2003; Blobel 2005; Roy et al. 2006; Duffy et al. 2009). Twenty-nine human members have been identified to date, many of them with so far

unknown functions (Seals and Courtneidge 2003). ADAM10 and 17 have been shown to activate TNF-α (Sahin et al. 2004; Blobel 2005; Roy et al. 2006). ADAMs are also able to degrade ECM components (Millichip et al. 1998; Sahin et al. 2004; Mochizuki and Okada 2007; van Goor et al. 2009; Duffy et al. 2009). However, still very little is known about the function of ADAMs in atherosclerosis. We have observed a high expression level of ADAM 8, 9, 12, and 15 in the vessel wall of diseased carotid arteries but also in the healthy vessels (J.P., unpublished observation). Thus, ADAM proteases seem to be involved also in normal vessel function.

Nineteen members of the family of ADAMTS of metalloproteases have been so far identified (Tang 2001; Apte 2004; Porter et al. 2005; Wight 2005; Jones and Riley 2005; Roy et al. 2006). Of these proteolytic enzymes ADAMTS1, 2, 3, 4, 5, 8, 9, and 13 are supposed to be expressed within the vessel wall. Most ADAMTS are able to degrade proteoglycans, especially aggrecan and versican, but ADAMTS2 and 3 are, e.g. also able to digest procollagen I, II, and III (Porter et al. 2005; Jones and Ripley Jones and Riley 2005We have observed a relatively low expression of ADAMTS in carotid arteries, compared to the expression of ADAMs and MMPs (J.P., unpublished observation); the role of ADAMTS in atherosclerosis remains to be elucidated.

Regarding the role of proteolytic enzymes in vessel wall, it is to mention that these proteases are not only the "villains" attacking and destroying the vessel wall. They are also beneficial and necessary for normal artery function, e.g. responsible for degradation of ECM components surrounding the cells, enabling their proliferation and migration. The migration of cells through the ECM is likely the major function of MMPs (Dollery and Libby 2006). MMP-2 and -9 facilitate migration of VSMCs, MMP-1 promotes cell growth and invasion and is playing an important role in tissue remodelling (Thakore et al. 2007; Pelisek et al. 2009). MMP-7 and partially MMP-3 are the most active enzymes degrading proteoglycanes (Lemaitre and D'Armiento 2006; Pelisek et al. 2009). Furthermore, MMP-1,-3, and -9 are abundantly expressed in diseased vessels, especially in ECs, VSMCs, macrophages, and other inflammatory cells (Reeps et al. 2009). In contrast, MMP-2 is produced mainly by VSMCs (Reeps et al. 2009). MMPs have been so far implicated in intimal thickening (increased ECM synthesis and deposition) and plaque rupture (ECM degradation). During plaque progression, an increased expression and activity of MMPs occur, in consequence leading to an increased degradation of ECM. This is in imbalance with the production of new ECM components, especially within the fibrous cap. Of note, in contrast to predominantly VSMC-driven synthesis of collagen, almost all cells within the atherosclerotic plaque, including macrophages and other inflammatory cells, are able to express and release proteases (Reeps et al. 2009). In addition, in high grade carotid artery stenosis, reduced numbers of VSMCs are being observed, and some MMPs, such as MMP-7, have been also involved in the induction of VSMC apoptosis (Clarke et al. Clarke and Bennett 2006a, Clarke and Bennett 2006b; Williams et al. 2010; Pelisek et al. 2011).

In consequence, the production of ECM components is continuously decreasing, whereas a high activity of proteolytic enzymes degrading ECM is maintained or even increased during the progression of atherosclerosis. These pathophysiological mechanisms inevitably lead to plaque vulnerability, rupture, and thus can finally result in ischemic stroke.

Neovascularization

The majority of atherosclerotic plaques are vascularised by a large network of small vessels (Langheinrich et al. 2007). These neovessels play an important role in plaque progression and stability (Carmeliet 2003; Virmani et al. 2005; Chen and Walterscheid 2006; Sluimer and Daemen 2009; Pelisek et al. 2012a, b). Neovascularization is induced by hypoxia and ongoing inflammatory reaction within the atherosclerotic plaque. Both biological processes can activate angiogenic factors such as hypoxia-inducible growth factor (HIF-1), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), angiopoietins (Ang), ephrins (Eph), and others (Heilmann et al. 2002; Shimizu et al. 2002; Carmeliet 2003; Pelisek et al. 2004). In addition to the formation of neovessels in atherosclerotic plaques from pre-existing vasa vasorum, a processes termed angiogenesis, also arteriogenesis, which describes the growth and widening of already existing vessels, occurs in parallel (Schaper and Scholz 2003; Pelisek et al. 2004; Chen and Walterscheid 2006). Both processes complement each other to form and stabilise the newly developing vessels. In healthy organs, angiogenesis is activated by VEGF and its receptors VEGFR-1 and -2 (Heilmann et al. 2002; Cross et al. 2003; Shibuya 2008; Ferrara 2009). Following the onset of the first endothelial tubes, pericytes and smooth muscle cells are recruited by PDGF to stabilise the new vessels. Ang-1 reduces vascular permeability and increases vessel stability; Ang-2, an antagonist of Ang-1, undermines the effect of Ang-1 and leads to vessel destabilisation (Armulik et al. 2005; Karamysheva 2008). Interestingly, an imbalance in the expression of Ang-1 and -2 was observed in atherosclerotic lesions (Lim et al. 2005). Furthermore, we have detected the expression of VEGF, VEGFR-2, PDGF, Ang-1 and -2 in advanced carotid lesions (Pelisek et al. 2012a, b).

Although neovessels are present in most atherosclerotic lesions, there is so far only limited evidence of causal relationships between progression of atherosclerosis, plaque vulnerability, and neovascularization. Furthermore, the role of angiogenic factors in atherosclerosis has not yet been sufficiently addressed (Lim et al. 2005; Karamysheva 2008). However, neovascularization may indeed affect plaque stability and angiogenic factors, such as VEGF or Ang-1, which could serve as possible biomarker of patients at risk of ischemic stroke (Pelisek et al. 2012a, b). First, significant differences in the number of neovessels between vulnerable and stable lesions were observed. Second, the number of inflammatory cells correlated significantly with the extent of neovascularization. Third, the majority of the neovessels were immature (>97 %), without the stabilising layer of VSMCs, independent of the vessel size. Such microvessels are unstable and highly permeable (Pries and Kuebler 2006; Pelisek et al. 2012a, b). This may allow inflammatory cells to pass the layer of endothelial cells and to accumulate deep within the plaque. In line, disrupted neovessels are often surrounded by a large number of inflammatory cells and accompanied by intra-plaque bleeding. Thereby, neovascularization and the accumulation of inflammatory cells may propagate plaque progression and destabilisation, and contribute to lesion vulnerability. Interestingly, expressions of MMP-1,-2,-3,-7, and -9 and also ADAM 12 and 15 were detected in neovessels as well (Pelisek et al. 2012a, b; J.P., unpublished observations). Thus, neovascularization may furthermore also directly contribute to plaque instability by degradation of ECM components.

Accompanying Disease

Carotid plaque vulnerability depends on various pathological mechanisms such as inflammation, excessive degradation of ECM by proteases, and neovascularization, as described above. But it may also depend on accompanying diseases that are able to influence plaque composition. For instance, cardiovascular complications and the risk of ischemic stroke are markedly increased in patients with chronic kidney disease (CKD), with a higher prevalence of premature arterial plaque formation and a 20-fold increased cardiovascular mortality compared to subjects with normal kidney function (Rossi et al. 1996; Foley et al. 1998; Taylor et al. 2000; Raggi et al. 2000; Schmermund and Erbel 2001; Leskinen et al. 2003; Russo et al. 2009; Pelisek et al. 2011, 2012a, b). Atherosclerotic plaques of CKD patients are frequently calcified, and increased artery stiffness is observed (Savage et al. 1998; Goodman et al. 2000; London et al. 2008). The role of calcification in atherosclerotic lesions and its contribution to plaque vulnerability, however, is still controversial. While some studies demonstrated stabilising effects of calcium deposits in atherosclerotic lesions, rendering vessels are less prone to rupture (Cheng et al. 1993; Alderman et al. 1993; Huang et al. 2001), others have observed a destabilising effect and an increased risk of plaque disruption (Savage et al. 1998; London et al. 2008). CKD has been shown to be associated with an elevation of some MMPs (Perco et al. 2006; Pelisek et al. 2011, 2012a, b). We have observed that CKD is associated with considerable changes in the composition of carotid plaques in patients with high-grade carotid artery stenosis (Pelisek et al. 2011). CKD patients had significantly more calcified plaques, and these were more frequently vulnerable or ruptured compared to plaques of individuals with normal kidney function. Of particular interest was a significant difference in the prevalence of remote strokes or silent infarcts; neurological events were significantly more common in CKD patients (84 %) compared to individuals without CKD (26 %). In line with our data, also Kobayashi et al. (2009) showed an independent association of the CKD stage with the prevalence of silent brain infarction.

Another common accompanying disease of patients with advanced carotid atherosclerotic lesions is diabetes mellitus (DM), often associated with hyperglycaemia (Plutzky 2003). Despite growing evidence that DM is associated with accelerated atherosclerosis, underlying mechanisms remain unclear (Ginsberg and Huang 2000; Plutzky 2003). In patients with DM, chronically elevated plasma glucose levels stimulate the production of advanced glycation end products (AGEs), a heterogeneous group of proteins, lipids and nucleic acids, irreversibly cross-linked with sugars, which cause adverse vascular responses (Schmidt and Stern 2000; Plutzky 2003). In addition, the lipid profile, and in particular the LDL/HDL ratio, is frequently abnormal in DM patients, and characterised by decreased plasma HDL cholesterol levels and an elevated proportion of LDL, which exhibits increased susceptibility to oxidation (Tomkin and Owens 2001). An important mechanism by which hyperglycaemia contributes to vascular injury is through the binding of AGE to their receptor [RAGE]. The AGE/RAGE interaction promotes the release of pro-inflammatory mediators and accelerates inflammation in atherosclerotic lesions. Furthermore, an increased level of oxidative stress is induced in numerous cell types, leading to injurious effects and an accelerated progression of diabetic atherosclerotic lesions towards vulnerable plaques.

Epigenetics

Epigenetic modifications enable cells to rapidly respond to environmental changes, providing a link between genes

and environment. Epigenetic changes most frequently comprise DNA methylation and histone modifications, which result in altered chromatin organisation (Serman et al. 2006; Halvorsen et al. 2008, Turunen et al. 2009; Ordovás and Smith 2010). Importantly, epigenetic changes solely encompass DNA and chromatin modifications without alternations in the genetic code; thus, all these changes are in principle reversible.

Epigenetics has emerged as one of the most promising approaches to further improve our knowledge of inherited traits. Several reviews have recently discussed possible roles of epigenetics in cardiovascular disease. However, original articles in this field are still scarce unlike in cancer research, where the important role of epigenetics has been well conceived. Epigenetic processes have been described in the initiation of cancer, its progression and metastasising (Boumber and Issa 2011; Herceg and Vaissière 2011; Caffarelli and Filetici 2011; Lahtz and Pfeifer 2011). E.g. silencing of specific genes is an important regulatory mechanism to protect cells against oncogenic changes (Herceg and Vaissière 2011; Caffarelli and Filetici 2011). The role of epigenetics as a potential mechanism to control genes has also been proposed in cardiovascular disease (Halvorsen et al. 2008; Ordovás and Smith 2010).

Kim et al. (2007) investigated DNA methylation in the promoter of oestrogen receptor beta gene in cardiovascular atherosclerotic disease. Plaques showed consistently higher methylation levels than non-diseased regions. This activity was augmented by histone deacetylase inhibition. These findings provide evidence of epigenetic dysregulation of oestrogen receptor beta in atherosclerosis. Stenvinkel et al. (2007) on the other hand demonstrated in a study including three different patient groups of CKD and healthy subjects that global DNA hypermethylation in peripheral blood monocytes is associated with inflammation and increased mortality in CKD patients. Another study suggested that aberrant DNA methylation in atherosclerosis is affecting transcription of various pro-atherogenic genes (Castillo-Díaz et al. 2010). We have observed that epigenetic modifications also occur in carotid atherosclerotic lesions, and that the degree of global DNA hypomethylation is significantly associated with the severity of atherosclerosis. These results could also be recapitulated in the blood of patients with high-grade carotid artery stenosis compared to healthy individuals (J.P., unpublished observations).

Epigenetic changes have also been associated with inflammation (Libby 2002; Bayarsaihan 2011), expression of MMPs (Newby 2005; Pelisek et al. 2009), neovascularization (Virmani et al. 2005; Pelisek et al. 2012a, b), and disease accompanying atherosclerosis, such as CKD (Stenvinkel et al. 2007). Thus, epigenetic modifications may be associated with numerous pathophysiological factors related to atherosclerosis, and are predicted to play an important role in the development and progression of atherosclerotic lesions. Further, studies are clearly warranted to evaluate the relevance of epigenetic changes in atherosclerosis and their value as possible biomarkers, e.g. for the identification of patients at risk of ischemic stroke.

Conclusions

In summary, different pathophysiological mechanisms are responsible for carotid atherosclerotic plaque vulnerability, including inflammation, excessive degradation of ECM by various proteolytic enzymes, neovascularization, associated diseases, and also epigenetic changes (Fig. 1). These atherosclerotic processes cannot be regarded as separate entities driving the disease, but rather act in concert to promote the progression of atherosclerosis towards vulnerable plaques and an increased risk of ischemic stroke. The main mediators leading to unstable plaques and rupture are proteolytic enzymes that degrade the ECM, a mechanism especially relevant in the fibrous area overlying the necrotic core, precipitating a "thin fibrous cap" and vulnerable atherosclerotic lesions. Expression of proteases is further stimulated by inflammation and inflammatory cytokines. Neovascularisation, abundantly present in atherosclerotic lesions, may in addition facilitate the accumulation of inflammatory cells and promote plaque progression. Inflammatory cells, macrophages, and also endothelial cells of neovessels are furthermore able to express proteolytic enzymes themselves, thus directly contributing to the degradation of ECM. Finally, epigenetic changes seem to play an important role in all pathophysiological processes leading to the progression of atherosclerosis and plaque vulnerability. These features mark atherosclerosis as a highly heterogeneous and complex vascular disease.

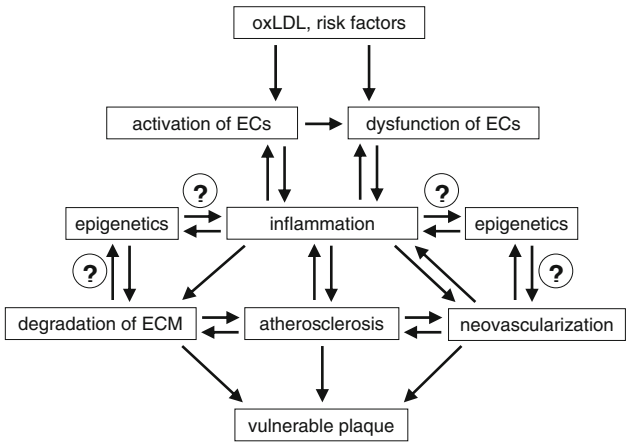

Fig. 1 Schematic summary of various pathophysiological mechanisms involved in atherosclerosis leading to vulnerable plaques and their potential interactions

Consequently, all relevant factors should be carefully considered and integrated for the development of future diagnostic and therapeutic strategies.

References

Alderman EL, Corley SD, Fisher LD, Chaitman BR, Faxon DP, Foster ED, Killip T, Sosa JA, Bourassa MG, CASS Participating Investigators and Staff (1993) Five-year angiographic follow-up of factors associated with progression of coronary artery disease in the Coronary Artery Surgery Study (CASS). J Am Coll Cardiol 22:1141–1154

Apte SS (2004) A disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motifs: the ADAMTS family. Int J Biochem Cell Biol 36:981–985

Armulik A, Abramsson A, Betsholtz C (2005) Endothelial/pericyte interactions. Circ Res 97:512–523

Avogaro P, Bon GB, Cazzolato G (1988) Presence of a modified low density lipoprotein in humans. Arteriosclerosis 8:79–87

Bayarsaihan D (2011) Epigenetic mechanisms in inflammation. J Dent Res 90:9–17

Blobel CP (2005) ADAMs: key components in EGFR signalling and development. Nature 6:32–43

Boumber Y, Issa JP (2011) Epigenetics in cancer: what's the future? Oncology 25(220–6):228

Burke AP, Farb A, Malcom GT, Liang YH, Smialek J, Virmani R (1997) Coronary risk factors and plaque morphology in men with coronary disease who died suddenly. N Engl J Med 336: 1276–1282

Caffarelli E, Filetici P (2011) Epigenetic regulation in cancer development. Front Biosci 17:2682–2694

Carmeliet P (2003) Angiogenesis in health and disease. Nat Med 9:653–660

Castillo-Díaz SA, Garay-Sevilla ME, Hernández-González MA, Solís-Martínez MO, Zaina S (2010) Extensive demethylation of normally hypermethylated CpG islands occurs in human atherosclerotic arteries. Int J Mol Med 26:691–700

Chen CH, Walterscheid JP (2006) Plaque angiogenesis versus compensatory arteriogenesis in atherosclerosis. Circ Res 99:787–789

Chen CH, Jiang T, Yang JH, Jiang W, Lu J, Marathe GK, Pownall HJ, Ballantyne CM, McIntyre TM, Henry PD, Yang CY (2003) Low-density lipoprotein in hypercholesterolemic human plasma induces vascular endothelial cell apoptosis by inhibiting fibroblast growth factor 2 transcription. Circulation 107:2102–2108

Cheng GC, Loree HM, Kamm RD, Fishbein MC, Lee RT (1993) Distribution of circumferential stress in ruptured and stable atherosclerotic lesions: a structural analysis with histopathological correlation. Circulation 87:1179–1187

Clarke M, Bennett M (2006a) Defining the role of vascular smooth muscle cell apoptosis in atherosclerosis. Cell Cycle 5:2329–2331

Clarke M, Bennett M (2006b) The emerging role of vascular smooth muscle cell apoptosis in atherosclerosis and plaque stability. Am J Nephrol 26:531–535

Cross MJ, Dixelius J, Matsumoto T, Claesson-Welsh L (2003) VEGF-receptor signal transduction. Trends Biochem Sci 28:488–494

Di Stefano R, Felice F, Balbarini A (2009) Angiogenesis as risk factor for plaque vulnerability. Curr Pharm 15:1095–1106

Dollery CM, Libby P (2006) Atherosclerosis and proteinase activation. Cardiovasc Res 69:625–635

Dong C, Yoon W, Goldschmidt-Clermont PJ (2002) DNA methylation and atherosclerosis. J Nutr 132:2406S–2409S

Duffy MJ, McKiernan E, O'Donovan N, McGowan PM (2009) The role of ADAMs in disease pathophysiology. Clin Chim Acta 403:31–36

Eckstein HH (2012) Evidence-based management of carotid stenosis: recommendations from international guidelines. J Cardiovasc Surg (Torino) 53:3–13

Egger G, Liang G, Aparicio A, Jones PA (2004) Epigenetics in human disease and prospects for epigenetic therapy. Nature 429: 457–463

Fabjan JS, Abuja PM, Schaur RJ, Sevanian A (2001) Hypochlorite induces the formation of LDL(−), a potentially atherogenic low density lipoprotein subspecies. FEBS Lett 499:69–72

Ferrara N (2009) Vascular endothelial growth factor. Arterioscler Thromb Vasc Biol 29:779–789

Ferri N, Paoletti R, Corsini A (2006) Biomarkers for atherosclerosis: pathophysiological role and pharmacological modulation. Curr Opin Lipidol 17:495–501

Foley RN, Parfrey PS, Sarnak MJ (1998) Clinical epidemiology of cardiovascular disease in chronic renal disease. Am J Kidney Dis 32:S112–S119

Formato M, Farina M, Spirito R, Maggioni M, Guarino A, Cherchi GM, Biglioli P, Edelstein C, Scanu AM (2004) Evidence for a proinflammatory and proteolytic environment in plaques from endarterectomy segments of human carotid arteries. Arterioscler Thromb Vasc Biol 24:129–135

Galis ZS, Sukhova GK, Lark MW, Libby P (1994) Increased expression of matrix metalloproteinases and matrix degrading activity in vulnerable regions of human atherosclerotic plaques. J Clin Invest 94:2493–2503

Galis ZS, Sukhova GK, Libby P (1995) Microscopic localization of active proteases by in situ zymography: detection of matrix metalloproteinase activity in vascular tissue. FASEB J 9:974–980

Garcia-Touchard A, Henry TD, Sangiorgi G, Spagnoli LG, Mauriello A, Conover C, Schwartz RS (2005) Extracellular proteases in atherosclerosis and restenosis. Arterioscler Thromb Vasc Biol 25:1119–1127

Ginsberg HN, Huang LS (2000) The insulin resistance syndrome: impact on lipoprotein metabolism and atherothrombosis. J Cardiovasc Risk 7:325–331

Goodman WG, Goldin J, Kuizon BD, Yoon C, Gales B, Sider D et al (2000) Coronary-artery calcification in young adults with end-stage renal disease who are undergoing dialysis. N Engl J Med 342:1478–1483

Halpert I, Sires UI, Roby JD, Potter-Perigo S, Wight TN, Shapiro SD, Welgus HG, Wickline SA, Parks WC (1996) Matrilysin is expressed by lipid-laden macrophages at sites of potential rupture in atherosclerotic lesions and localizes to areas of versican deposition, a proteoglycan substrate for the enzyme. Proc Natl Acad Sci USA 93:9748–9753

Halvorsen B, Otterdal K, Dahl TB, Skjelland M, Gullestad L, Øie E, Aukrust P (2008) Atherosclerotic plaque stability—what determines the fate of a plaque? Prog Cardiovasc Dis 51:183–194

Hansson GK (2005) Inflammation, atherosclerosis, and coronary artery disease. N Engl J Med 352:1685–1695

Hansson GK, Hermansson A (2011) The immune system in atherosclerosis. Nat Immunol 12:204–212

Heider P, Pelisek J, Poppert H, Eckstein HH (2009) Evaluation of serum matrix metalloproteinases as biomarkers for detection of neurological symptoms in carotid artery disease. Vasc Endovascular Surg 43:551–560

Heilmann C, Beyersdorf F, Lutter G (2002) Collateral growth: cells arrive at the construction site. Cardiovasc Surg 10:570–578

Herceg Z, Vaissière T (2011) Epigenetic mechanisms and cancer: an interface between the environment and the genome. Epigenetics 6:804–819

Huang H, Virmani R, Younis H, Burke AP, Kamm RD, Lee RT (2001) The impact of calcification on the biomechanical stability of atherosclerotic plaques. Circulation 103:1051–1056

Ingrosso D, Perna AF (2009) Epigenetics in hyperhomocysteinemic states. A special focus on uremia. Biochim Biophys Acta 1790:892–899

Jones GC, Riley GP (2005) ADAMTS proteinases: a multi-domain, multi-functional family with roles in extracellular matrix turnover and arthritis. Arthritis Res Ther 7:160–169

Karamysheva AF (2008) Mechanisms of angiogenesis. Biochemistry (Mosc) 73:751–762

Kim J, Kim JY, Song KS, Lee YH, Seo JS, Jelinek J, Goldschmidt-Clermont PJ, Issa JP (2007) Epigenetic changes in estrogen receptor beta gene in atherosclerotic cardiovascular tissues and in vitro vascular senescence. Biochim Biophys Acta 1772:72–80

Kinsella MG, Bressler SL, Wight TN (2004) The regulated synthesis of versican, decorin, and biglycan: extracellular matrix proteoglycans that influence cellular phenotype. Crit Rev Eukaryot Gene Expr 14:203–234

Kobayashi M, Hirawa N, Yatsu K, Kobayashi Y, Yamamoto Y, Saka S, Andoh D, Toya Y, Yasuda G, Umemura S (2009) Relationship between silent brain infarction and chronic kidney disease. Nephrol Dial Transplant 24:201–207

Kunz J (2007) Matrix metalloproteinases and atherogenesis in dependence of age. Gerontology 53:63–73

Labat-Robert J, Bihari-Varga M, Robert L (1990) Extracellular matrix. FEBS Lett 268:386–393

Lahtz C, Pfeifer GP (2011) Epigenetic changes of DNA repair genes in cancer. J Mol Cell Biol 3:51–58

Langheinrich AC, Kampschulte M, Buch T, Bohle RM (2007) Vasa vasorum and atherosclerosis—Quid novi? Thromb Haemost 97:873–879

Lemaitre V, D'Armiento J (2006) Matrix metalloproteinases in development and disease. Birth Defects Res C Embryo Today 78:1–10

Leskinen Y, Lehtimaki T, Loimaala A, Lautamatti V, Kallio T, Huhtala H, Salenius JP, Saha H (2003) Carotid atherosclerosis in chronic renal failure—the central role of increased plaque burden. Atherosclerosis 171:295–302

Libby P (2002) Inflammation in atherosclerosis. Nature 420:868–874

Libby P, Ridker PM, Hansson GK (2002) Progress and challenges in translating the biology of atherosclerosis. Nature 473:317–325

Libby P, Ridker PM, Hansson GK, Leducq Transatlantic Network on Atherothrombosis (2009) Inflammation in atherosclerosis: from pathophysiology to practice. J Am Coll Cardiol 54:2129–2138

Lim HS, Lip GY, Blann AD (2005) Angiopoietin-1 and angiopoietin-2 in diabetes mellitus: relationship to VEGF glycaemic control endothelial damage/dysfunction and atherosclerosis. Atherosclerosis 80:113–118

Loftus IM, Naylor AR, Goodall S, Crowther M, Jones L, Bell PR, Thompson MM (2000) Increased matrix metalloproteinase-9 activity in unstable carotid plaques. A potential role in acute plaque disruption. Stroke 31:40–47

London GM, Marchais SJ, Guérin AP, Boutouyrie P, Métivier F, de Vernejoul MC (2008) Association of bone activity, calcium load, aortic stiffness, and calcifications in ESRD. J Am Soc Nephrol 19:1827–1835

Lu J, Yang JH, Burns AR, Chen HH, Tang D, Walterscheid JP, Suzuki S, Yang CY, Sawamura T, Chen CH (2009) Mediation of electronegative low-density lipoprotein signaling by LOX-1: a possible mechanism of endothelial apoptosis. Circ Res 104:619–627

Lund G, Zaina S (2011) Atherosclerosis: an epigenetic balancing act that goes wrong. Curr Atheroscler Rep 13:208–214

Manthey HD, Zernecke A (2011) Dendritic cells in atherosclerosis: functions in immune regulation and beyond. Thromb Haemost 106:772–778

Mello AP, da Silva IT, Abdalla DS, Damasceno NR (2011) Electronegative low-density lipoprotein: origin and impact on health and disease. Atherosclerosis 215:257–265

Millichip MI, Dallas DJ, Wu E, Dale S, McKie N (1998) The metallodisintegrin ADAM 10 from bovine kidney has type IV collagenase activity in vitro. Biochem Biophys Res Commun 245:594–598

Mochizuki S, Okada Y (2007) ADAMs in cancer cell proliferation and progression. Cancer Sci 98:621–628

Montecucco F, Lenglet S, Gayet-Ageron A, Bertolotto M, Pelli G, Palombo D, Pane B, Spinella G, Steffens S, Raffaghello L, Pistoia V, Ottonello L, Pende A, Dallegri F, Mach F (2010) Systemic and intraplaque mediators of inflammation are increased in patients symptomatic for ischemic stroke. Stroke 41:1394–1404

Montecucco F, Di Marzo V, da Silva RF, Vuilleumier N, Capettini L, Lenglet S, Pagano S, Piscitelli F, Quintao S, Bertolotto M, Pelli G, Galan K, Pilet L, Kuzmanovic K, Burger F, Pane B, Spinella G, Braunersreuther V, Gayet-Ageron A, Pende A, Viviani GL, Palombo D, Dallegri F, Roux-Lombard P, Santos RA, Stergiopulos N, Steffens S, Mach F (2012) The activation of the cannabinoid receptor type 2 reduces neutrophilic protease-mediated vulnerability in atherosclerotic plaques. Eur Heart J 33:846–856

Mughal MM, Khan MK, DeMarco JK, Majid A, Shamoun F, Abela GS (2011) Symptomatic and asymptomatic carotid artery plaque. Expert Rev Cardiovasc Ther 9:1315–1330

Mukherjee D, Patil CG (2011) Epidemiology and the global burden of stroke. World Neurosurg 76:S85–S90

Muller JE, Tofler GH (1992) Triggering and hourly variation of onset of arterial thrombosis. Ann Epidemiol 2:393–405

Murata K, Motayama T, Kotake C (1986) Collagen types in various layers of the human aorta and their changes with the atherosclerotic process. Atherosclerosis 60:251–262

Naghavi M, Libby P, Falk E, Casscells SW, Litovsky S, Rumberger J, Badimon JJ, Stefanadis C, Moreno P, Pasterkamp G, Fayad Z, Stone PH, Waxman S, Raggi P, Madjid M, Zarrabi A, Burke A, Yuan C, Fitzgerald PJ, Siscovick DS, de Korte CL, Aikawa M, Juhani-Airaksinen KE, Assmann G, Becker CR, Chesebro JH, Farb A, Galis ZS, Jackson C, Jang IK, Koenig W, Lodder RA, March K, Demirovic J, Navab M, Priori SG, Rekhter MD, Bahr R, Grundy SM, Mehran R, Colombo A, Boerwinkle E, Ballantyne C, Insull W Jr, Schwartz RS, Vogel R, Serruys PW, Hansson GK, Faxon DP, Kaul S, Drexler H, Greenland P, Muller JE, Virmani R, Ridker PM, Zipes DP, Shah PK, Willerson JT (2003a) From vulnerable plaque to vulnerable patient: a call for new definitions and risk assessment strategies: part I. Circulation 108:21664–21672

Naghavi M, Libby P, Falk E, Casscells SW, Litovsky S, Rumberger J, Badimon JJ, Stefanadis C, Moreno P, Pasterkamp G, Fayad Z, Stone PH, Waxman S, Raggi P, Madjid M, Zarrabi A, Burke A, Yuan C, Fitzgerald PJ, Siscovick DS, de Korte CL, Aikawa M, Airaksinen KE, Assmann G, Becker CR, Chesebro JH, Farb A, Galis ZS, Jackson C, Jang IK, Koenig W, Lodder RA, March K, Demirovic J, Navab M, Priori SG, Rekhter MD, Bahr R, Grundy SM, Mehran R, Colombo A, Boerwinkle E, Ballantyne C, Insull W Jr, Schwartz RS, Vogel R, Serruys PW, Hansson GK, Faxon DP, Kaul S, Drexler H, Greenland P, Muller JE, Virmani R, Ridker PM, Zipes DP, Shah PK, Willerson JT (2003b) From vulnerable plaque to vulnerable patient: a call for new definitions and risk assessment strategies: part II. Circulation 108:1772–1778

Naruko T, Ueda M, Haze K, van der Wal AC, van der Loos CM, Itoh A, Komatsu R, Ikura Y, Ogami M, Shimada Y, Ehara S, Yoshiyama M, Takeuchi K, Yoshikawa J, Becker AE (2002) Neutrophil infiltration of culprit lesions in acute coronary syndromes. Circulation 106:2894–2900

Newby AC (2005) Dual role of matrix metalloproteinases (matrixins) in intimal thickening and atherosclerotic plaque rupture. Physiol Rev 85:1–31

Newby AC, Southgate KM, Davies M (1994) Extracellular matrix degrading metallo-proteinases in the pathogenesis of arteriosclerosis. Basic Res Cardiol 89:59–70

Nikkari ST, O'Brien KD, Ferguson M, Hatsukami T, Welgus HG, Alpers CE, Clowes AW (1995) Interstitial collagenase (MMP-1) expression in human carotid atherosclerosis. Circulation 92:1393–1398

Ordovás JM, Smith CE (2010) Epigenetics and cardiovascular disease. Nat Rev Cardiol 7:510–519

Pelisek J, Shimizu M, Nikol S (2004) Differential developmental origin of arteries: impact on angiogenesis and arteriogenesis. Med Chem Rev 1:317–326

Pelisek J, Rudelius M, Zepper P, Poppert H, Reeps C, Schuster T, Eckstein HH (2009) Multiple biological predictors for vulnerable carotid lesions. Cerebrovasc Dis 28:601–610

Pelisek J, Assadian A, Sarkar O, Eckstein HH, Frank H (2010) Carotid plaque composition in chronic kidney disease: a retrospective analysis of patients undergoing carotid endarterectomy. Eur J Vasc Endovasc Surg 39:11–16

Pelisek J, Hahntow IN, Eckstein HH, Ockert S, Reeps C, Heider P, Luppa PB, Frank H (2011) Impact of chronic kidney disease on carotid plaque vulnerability. J Vasc Surg 54:1643–1649

Pelisek J, Reeps C, Ockert S, Zimmermann A, Zepper P, Poppert H, Eckstein HH (2012) Evaluation of serum biomarkers for patients at increased risk of stroke. Int J Vasc Med [Ahead of print]

Pelisek J, Well G, Reeps C, Rudelius M, Kuehnl A, Culmes M, Poppert H, Zimmermann A, Berger H, Eckstein HH (2012) Neovascularization and angiogenic factors in advanced human carotid artery stenosis. Circ J [Epub, ahead of print]

Perco P, Pleban C, Kainz A, Lukas A, Mayer B, Oberbauer R (2006) Protein biomarkers associated with acute renal failure and chronic kidney disease. Eur J Clin Invest 36:753–763

Plutzky J (2003) The vascular biology of atherosclerosis. Am J Med 115:55S–61S

Porter S, Clark IM, Kevorkian L, Edwards DR (2005) The ADAMTS metalloproteinases. Biochem J 386:15–27

Pries AR, Kuebler WM (2006) Normal endothelium. Handb Exp Pharmacol 176:1–40

Radford EJ, Ferrón SR, Ferguson-Smith AC (2011) Genomic imprinting as an adaptative model of developmental plasticity. FEBS Lett 585:2059–2066

Raggi P, Callister TQ, Cooil B, He ZX, Lippolis NJ, Russo DJ, Zelinger A, Mahmarian JJ (2000) Identification of patients at increased risk of first unheralded acute myocardial infarction by electron-beam computed tomography. Circulation 101:850–855

Redgrave JN, Gallagher PJ, Lovett JK, Rothwell PM (2008) Critical cap thickness and rupture in symptomatic carotid plaques: the Oxford Plaque Study. Stroke 39:1722–1729

Reeps C, Pelisek J, Seidl S, Schuster T, Zimmermann A, Kuehnl A, Eckstein HH (2009) Inflammatory infiltrates and neovessels are relevant sources of MMPs in abdominal aortic aneurysm wall. Pathobiology 76:243–252

Rey FE, Pagano PJ (2002) The reactive adventitia: fibroblast oxidase in vascular function. Arterioscler Thromb Vasc Biol 22:1962–1971

Ross R (1999) Atherosclerosis: an inflammatory disease. N Engl J Med 340:115–126

Rossi A, Bonfante L, Giacomini A, Calabro A, Rossi G, Saller A, Abbruzzese E, Baldo G, Mastrosimone S, Beccari A, Baiocchi MR, de Silvestro L, Roncali D, Bolzonella R, Giardin C, Antonello A, Normanno M, Crepaldi G, Borsatti A (1996) Carotid artery lesions in patients with nondiabetic chronic renal failure. Am J Kidney Dis 27:58–66

Rothwell PM (2000) Carotid artery disease and the risk of ischaemic stroke and coronary vascular events. Cerebrovasc Dis 10:21–33

Roy R, Zhang B, Moses MA (2006) Making the cut: protease-mediated regulation of angiogenesis. Exp Cell Res 312:608–622

Russo D, Morrone LF, Brancaccio S, Napolitano P, Salvatore E, Spadola R, Imbriaco M, Russo CV, Andreucci VE (2009) Pulse pressure and presence of coronary artery calcification. Clin J Am Soc Nephrol 4:316–322

Sahin U, Weskamp G, Kelly K, Zhou HM, Higashiyama S, Peschon J, Hartmann D, Saftig P, Blobel CP (2004) Dictinct roles for ADAM 10 and ADAM 17 in ectodomain shedding of six EGFR ligands. J Cell Biol 164:769–779

Savage T, Clarke AL, Giles M, Tomson CR, Raine AE (1998) Calcified plaque is common in the carotid and femoral arteries of dialysis patients without clinical vascular disease. Nephrol Dial Transplant 13:2004–2012

Schaper W, Scholz D (2003) Factors regulating arteriogenesis. Arterios Thromb Vasc Biol 23:1143–1151

Schmermund A, Erbel R (2001) Unstable coronary plaque and its relation to coronary calcium. Circulation 104:1682–1687

Schmidt AM, Stern D (2000) Atherosclerosis and diabetes: the RAGE connection. Curr Atheroscler Rep 2:430–436

Seals DF, Courtneidge SA (2003) The ADAMs family of metalloproteaes: multidomain proteins with multiple functions. Genes Dev 17:7–30

Serman A, Vlahović M, Serman L, Bulić-Jakus F (2006) DNA methylation as a regulatory mechanism for gene expression in mammals. Coll Antropol 30:665–671

Shen CM, Mao SJ, Huang GS, Yang PC, Chu RM (2001) Stimulation of smooth muscle cell proliferation by ox-LDL- and acetyl LDL-induced macrophage-derived foam cells. Life Sci 70:443–452

Shibuya M (2008) Vascular endothelial growth factor-dependent and -independent regulation of angiogenesis. BMB Rep 41:278–286

Shimizu M, Pelisek J, Nikol S (2002) Vasculogenesis and angiogenesis depend on the developmental origin in the arterial tree. Curr Med Chem 9:1619–1630

Sluimer JC, Daemen MJ (2009) Novel concepts in atherogenesis: angiogenesis and hypoxia in atherosclerosis. J Pathol 218:7–29

Stary HC, Blankenhorn DH, Chandler AB, Glagov S, Insull W Jr, Richardson M, Rosenfeld ME, Schaffer SA, Schwartz CJ, Wagner WD et al (1992) A definition of the intima of human arteries and of its atherosclerosis-prone regions. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. Circulation 85:391–405

Stary HC, Chandler AB, Glagov S, Guyton JR, Insull W Jr, Rosenfeld ME, Schaffer SA, Schwartz CJ, Wagner WD, Wissler RW (1994) A definition of initial, fatty streak, and intermediate lesions of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. Circulation 89:2462–2478

Stary HC, Chandler AB, Dinsmore RE, Fuster V, Glagov S, Insull W Jr, Rosenfeld ME, Schwartz CJ, Wagner WD, Wissler RW (1995) A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. Circulation 92:1355–1374

Stenvinkel P, Karimi M, Johansson S, Axelsson J, Suliman M, Lindholm B, Heimbürger O, Barany P, Alvestrand A, Nordfors L, Qureshi AR, Ekström TJ, Schalling M (2007) Impact of inflammation on epigenetic DNA methylation—a novel risk factor for cardiovascular disease? J Intern Med 261:488–499

Sundström J, Vasan RS (2006) Circulating biomarkers of extracellular matrix remodeling and risk of atherosclerotic events. Curr Opin Lipidol 17:45–53

Tang BL (2001) ADAMTS: a novel family of extracellular matrix proteases. Int J Biochem Cell Biol 33:33–44

Taylor AJ, Burke AP, O'Malley PG, Farb A, Malcom GT, Smialek J, Virmani R (2000) A comparison of the Framingham risk index, coronary artery calcification, and culprit plaque morphology in sudden cardiac death. Circulation 101:1243–1248

Thakore AH, Guo CY, Larson MG, Corey D, Wang TJ, Vasan RS, D'Agostino RB Sr, Lipinska I, Keaney JF Jr, Benjamin EJ, O'Donnell CJ (2007) Association of multiple inflammatory markers with carotid intimal medial thickness and stenosis (from the Framingham Heart Study). Am J Cardiol 99:1598–1602

Tomasik A, Jacheć W, Skrzep-Poloczek B, Widera-Romuk E, Wodniecki J, Wojciechowska C (2003) Circulating electronegatively charged low-density lipoprotein in patients with angiographically documented coronary artery disease. Scand J Clin Lab Invest 63:259–265

Tomkin GH, Owens D (2001) Abnormalities in apo B-containing lipoproteins in diabetes and atherosclerosis. Diabetes Metab Res Rev 17:27–43

Turunen MP, Aavik E, Ylä-Herttuala S (2009) Epigenetics and atherosclerosis. Biochim Biophys Acta 1790:886–891

van Goor H, Melenhorst WB, Turner AJ, Holgate ST (2009) Adamalysins in biology and disease. J Pathol 219:277–286

Virmani R, Kolodgie FD, Burke AP, Farb A, Schwartz SM (2000) Lessons from sudden coronary death: a comprehensive morphological classification scheme for atherosclerotic lesions. Arterioscler Thromb Vasc Biol 2012:62–1275

Virmani R, Kolodgie FD, Burke AP, Finn AV, Gold HK, Tulenko TN, Wrenn SP, Narula J (2005) Atherosclerotic plaque progression and vulnerability to rupture: angiogenesis as a source of intraplaque hemorrhage. Arterioscl Thromb Vasc Biol 25:205420–205461

Virmani R, Burke AP, Farb A, Kolodgie FD (2006) Pathology of the vulnerable plaque. J Am Coll Cardiol 47:C13–C18

Weber C, Zernecke A, Libby P (2008) The multifaceted contributions of leukocyte subsets to atherosclerosis: lessons from mouse models. Nat Rev Immunol 8:802–815

Wight TN (2005) The ADAMTS proteases, extracellular matrix, and vascular disease: waking the sleeping giant(s)! Arterioscler Thromb Vasc Biol 25:12–14

Williams H, Johnson JL, Jackson CL, White SJ, George SJ (2010) MMP-7 mediates cleavage of N-cadherin and promotes smooth muscle cell apoptosis. Cardiovasc Res 87:137–146

Zernecke A, Weber C (2005) Inflammatory mediators in atherosclerotic vascular disease. Basic Res Cardiol 100(2):93–100

Zernecke A, Weber C (2010) Chemokines in the vascular inflammatory response of atherosclerosis. Cardiovasc Res 86:192–201

Zernecke A, Shagdarsuren E, Weber C (2008) Chemokines in atherosclerosis: an update. Arterioscler Thromb Vasc Biol 28:1897–1908