CELLULAR AND MOLECULAR MECHANISMS OF SACCULAR INTRACRANIAL ANEURYSM FORMATION AND RUPTURE

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ABSTRACT

Subarachnoid hemorrhage from ruptured saccular intracranial aneurysms (sIAs) comprises a significant clinical burden with high mortality rates. The invasive therapeutic procedures come with a risk of severe complications often equaling or exceeding the risk of sIA rupture. Therefore, the assessment of sIA rupture risk is of absolute importance. To realize this, a thorough understanding of the underlying cellular and molecular mechanisms of sIA formation and rupture is pivotal. Up to date these mechanisms remain poorly understood. A growing body of evidence from human and animal studies supports the theory that hemodynamic stress causes endothelial dysfunction which subsequently induces inflammation and remodeling in the vascular wall, thus leading to sIA formation. Ruptured sIAs are reported to have higher degrees of inflammation, mural cell death, extracellular matrix breakdown and oxidative stress compared to unruptured sIAs. This is evident on morphological, molecular and gene expression level. However, due to the complex and unknown interplay between the cellular and molecular mechanisms, together with methodological issues, reasons as to why some sIAs rupture and other do not are difficult to assess. Based on the reviewed data, it is hypothesized that the balance of inflammation and remodeling in the vessel wall induces adaptive changes leading to unruptured sIAs, while maladaptive changes result in sIAs prone to rupture.

INTRODUCTION

Subarachnoid hemorrhage (SAH) results from a leakage of blood from a cerebral artery into the subarachnoid spaces of the brain, in some cases penetrating and affecting the brain parenchymal tissue.[1] It is an acute event with a sudden and rapid onset of symptoms. Typically, SAH is represented by an explosive headache with differing degrees of decline in conscious level and neurological status.[1] SAH comprises a significant clinical and socio-economic burden despite recent diagnostic and prognostic advances. In most Western populations the incidence of SAH is approximately 10.5 per 100.000 person-years, with large regional variations.[2] Based on meta-analysis, mortality rates have been reported to lie between 27% and 44%, not accounting patients who die from undiagnosed SAH. Furthermore, about 50% of the surviving patients are left with significant disabilities.[3] Together with a peak incidence reported to be between 40 to 60 years of age, SAH causes a significant loss of productive life years.[3] The majority of non-traumatic SAH arise from the rupture of intracranial aneurysms (IAs) with the remaining cases being caused by arteriovenous malformations or an unidentified cause.[4] Aneurysmal SAH account for approximately 5% of all strokes.[5-7]

IAs are pathological dilatations of intracranial arteries. Although there are several forms of IAs, this literature study will focus on saccular intracranial aneurysms (sIAs), the most common type of IA and the leading cause of aneurysmal SAH.[6] Other types of IA pathologies are associated with dissection and hemorrhage within the vessel wall, neoplasms, infections and immunodeficiencies and will not be further discussed.[8] sIAs, often referred to as classical or 'berry-like' aneurysms, are lobed focal outpouchings of the arterial wall.[7] With a prevalence of approximately 3.2 % in the general population, unruptured sIAs are a common vascular abnormality found during cerebral angiography and autopsy.[9] Rare exceptions excluded, sIAs are non-congenital and typically arise at or near bifurcations of major arteries in the Circle of Willis (Figure 1), however sidewall sIAs are also observed.[10] The most frequent site being the internal carotid artery, followed by the anterior communicating and middle cerebral arteries.[11] Despite discrepancies between studies, many genetic and external risk factors for sIA formation and rupture have been identified. Family history of sIA and SAH with two or more members affected and autosomal hereditary diseases such as polycystic kidney disease and fibromuscular dysplasia have been associated with higher incidence of sIA.[12-14] Also, several loci have been found to be associated with the development of sIAs based on genome-wide association analyses.[15] However, the association of external factors such as increasing age, female gender, smoking, alcohol consumption and hypertension is much stronger than the highest reported genetic association.[6] Similarly, the rupture risk of sIAs is influenced in great extent by the geometry and anatomical location of the aneurysm. Higher incidences of rupture have been

associated with sIAs larger than 5-10 mm, dome-to-neck ratio >1.6, locations in the posterior communicating and middle cerebral arteries, right sidedness and multiplicity.[16,17] However, external factors age >60 years, female gender, smoking and untreated hypertension have been shown to be the most important risk factors for sIA rupture.[18, 19] Only a minority of formed sIAs actually rupture.[20] An overall risk of rupture of 1.2% in Western populations has been reported based on meta-analysis of other published studies.[21] Although the overall risk of sIA rupture is relatively low, due to the devastating outcomes, many sIAs are treated before they rupture. They are isolated from the cerebral circulation by endovascular therapy or microsurgical methods.[17, 22] However, these invasive procedures come with the risk of severe complications which often equal or exceed the risk of rupture.[17] Currently, all risk assessment strategies concerning sIA rupture are mainly based on the size, shape and location of the aneurysm.[23, 24] This approach is inaccurate as it fails to differentiate between sIAs with similar morphologic features, but different natural histories and outcomes as smaller sized sIAs also do rupture.[17] However, the assessment of sIA rupture risk is of absolute importance considering the risk of both operative and conservative clinical management strategies. To realize this, a thorough understanding of the underlying mechanisms of sIA formation is necessary.

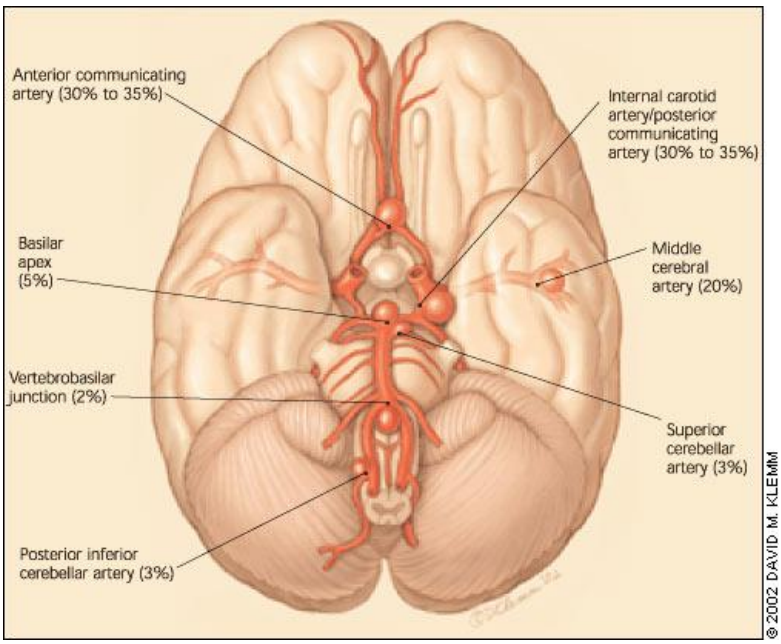


Figure 1. Common locations of sIAs. A schematic representation of sIAs at bifurcations of the Circle of Willis is shown with approximate incidences. Figure adapted from Vega *et al*., 2002.[F1]

The pathogenesis of sIA formation and rupture has been subject of debate for many years and still remains poorly understood.[6, 7, 10] It has been suggested that sIA formation and the rupture of an existing sIA are separate processes and that the pathobiology of unruptured and ruptured IAs is distinct. This is supported by reported prevalence of ruptured sIAs and rupture rates far below 100%.[3, 20] Also, some risk factors seem to affect sIA formation in larger extent than they do sIA rupture.[6] Furthermore, the induction of sIAs in animal models does not lead to spontaneous rupture.[25] Due to the lack of knowledge, no reliable risk stratification methods and safe and non-invasive therapies have yet been implemented or identified. Currently published studies suggest roles for hemodynamic and oxidative stress, inflammation, extracellular matrix (ECM) degradation and vascular remodeling in sIA pathobiology.[6, 8] In this literature study, existing data accumulated from experimental reports, human and animal studies is critically reviewed in an attempt to elucidate the underlying cellular and molecular mechanisms of sIA formation and rupture. In particular, differences between unruptured and ruptured sIAs are addressed on morphologic, cellular, molecular and genetic level wherein the roles of inflammatory processes and vascular remodeling are highlighted. Based on the findings a hpothesis for sIA formation and rupture is proposed and discussed.

PATHOGENESIS OF SIA FORMATION

Observations from early histopathology studies have characterized sIA walls as lacking the normally highly organized intima-media-adventitia structure (Figure 2A). A prominent loss or fragmentation of the internal elastic lamina (IEL) and endothelial lining has been observed (Figure 2B). Some sIA walls were found to be abundant with disorganized mural cells i.e. vascular smooth muscle cells (VSMCs) and (myo)fibroblasts (Figure 2B), while in other walls the majority of mural cells has been lost. Additionally, the presence of inflammatory cells has been reported, however these cells were merely characterized as plasma cells and polymorphonuclear leukocytes, as reviewed by Frösen *et al*.)[6] Changes in ECM components have also been observed. Type 1 collagen and fibronectin expression are spread throughout the entire sIA wall, while they are normally restricted to the adventitia and media respectively. In contrast, type III and IV collagen and laminin expression are decreased in the sIA wall compared to healthy vessels.[29] Results from human specimens and animal models have implicated some important mechanisms underlying these vascular wall changes associated with sIA formation. As mentioned before, these mechanisms are thought to involve coupling of hemodynamic stress to endothelial dysfunction and inflammation associated with sustained abnormal vascular remodeling and subsequent cell death. These mechanisms are suggested to be a common pathway in the formation of sIAs.[30] In this section, the pathogenesis of sIAs is discussed without addressing potential sIA rupture. This will be discussed in a later stage.

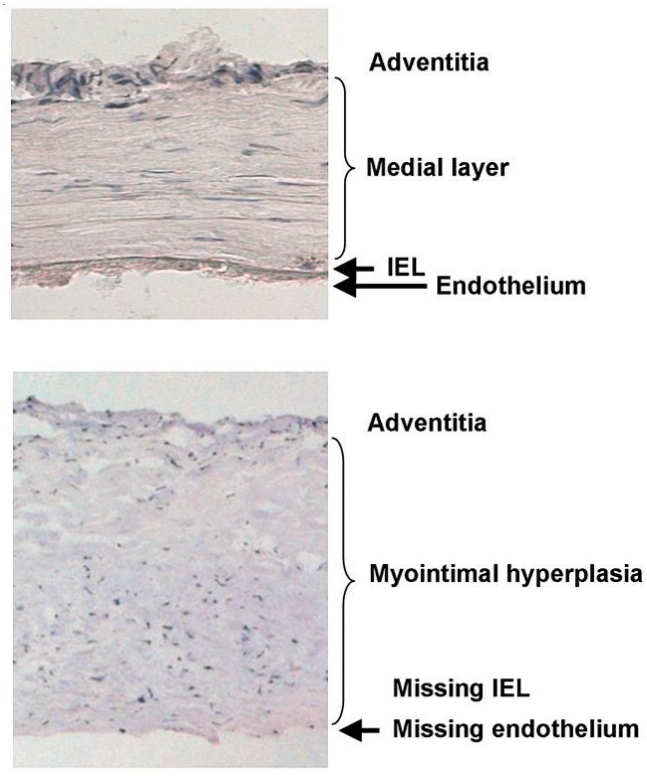


Figure 2. Histology of the healthy human cerebral arterial vessel wall (A) and sIA wall (B). Intimal layer: endothelial cells and IEL. Medial layer: VSMCs. Adventitial layer: fibroblasts and collagen fibers. **(A)** Cerebral arteries are characterized by a well-developed internal elastic lamina, a scarceness of elastic fibers in the media, little adventitial tissue and the lack of an external elastic lamina in contrast to systemic vessels. The sIA wall often lacks or has a disrupted IEL and endothelium and is often disorganized **(B).** Figure adapted from Tulamo *et al*., 2010.[F2]

Hemodynamic stress and endothelial dysfunction: effects on vascular remodeling and inflammation

It is generally believed that disruptions in hemodynamic homeostasis lie at the base of sIA pathogenesis. This notion is supported by the increased association of sIA with arterial bifurcations and arterial anatomical anomalies; sites where complex and dangerous patterns in blood flow arise.[31,32] Under physiologic circumstances, a delicate balance exists between blood flow patterns, hemodynamic stress and the arterial wall integrity and response. [7] Wall shear stress (WSS) is one of the most important pathologic factors of hemodynamic stress.[33] Two highly discussed theories are proposed for the role of hemodynamic stress in sIA formation. The high flow effect theory focuses on the effects of high WSS, which causes damage and localized dilatation of the artery wall by the triggering of endothelial cell dysfunction through physical forces.[33, 34] The low-flow theory suggests that lowered WSS can create stagnation zones which promote endothelial dysfunction by up-regulation of leukocyte adhesion to intracellular adhesion molecule-1 (ICAM-1) and vascular adhesion molecule-1 (VCAM-1). Thus, platelet adhesion and leukocyte transmigration are increased and subsequent inflammatory cascades are initiated.[33] Based on computational fluid dynamic (CFD) simulations, Mantha *et al.* have hypothesized that low WSS-induced stagnation zones may be caused by cancellation of randomly oriented hemodynamic force vectors in three dimensions.[35] A majority of the cerebral vessels exhibiting lowered WSS in the CFD simulations resulted in the formation of sidewall sIAs rather than sIAs near arterial bifurcations (Figure 3).[35] Thus, it has been suggested that lowered WSS plays a role in the formation of sidewall sIAs and elevated WSS plays a role in the formation of sIAs at arterial bifurcations.[33] Regardless of the hemodynamic mechanism by which endothelial dysfunction is induced, it seems to trigger remodeling and inflammation in the vascular wall to adapt to the newly exerted WSS.[36] From other vascular pathologies, it has been recognized that remodeling and inflammation are regulated by endothelial factors such as nitric oxide (NO), prostaglandins and various growth factors.[37] Furthermore, inflammatory cascades may be initiated by the increasingly migrated leukocytes into the vessel wall, thereby stimulating ECM breakdown and cell death.[36]

The interplay between WSS, endothelial dysfunction and subsequent remodeling and inflammation in human sIA formation is difficult to assess. Obtaining hemodynamic data from the small human cerebral vessels is difficult, thus large part of the knowledge considering blood flow and WSS is acquired from non-biological, CFD simulations.[33] The biologic effects of altered WSS in the vascular wall associated with sIA formation are based on observations in animal models.[38] In an angiotensin-II and carotid ligation-induced rat model of sIA formation, the increased WSS was shown to trigger higher signaling of prostaglandin E synthase (PGES) and prostaglandin E receptor-2 (EP2) pathway, a mediating pathway of inflammation. Inflammation was amplified through a positive feedback loop

consisting of EP2, NF-κB and cyclooxygenase-2 (COX-2), resulting in a higher incidence of aneurysm formation.[39]

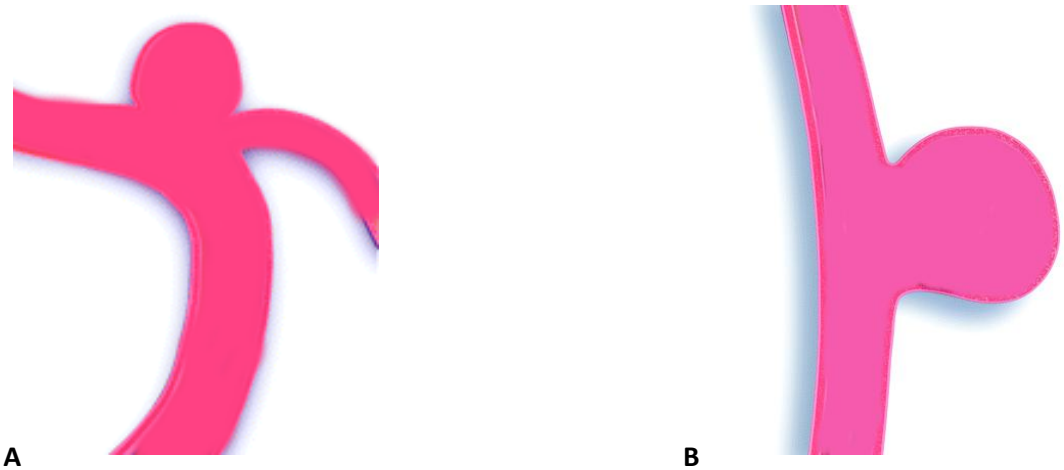


Figure 3. Schematic representation of a branchpoint sIA (A) and a sidewall sIA (B). It has been suggested that the formation of branchpoint sIAs is initiated by elevated WSS whereas sidewall sIAs are initiated by lowered WSS and subsequent stagnation zones in the blood flow. Figure adapted from Pierot *et al*., 2012.[F3]

In a rabbit model, flow in the basilar artery was increased by carotid artery ligation, IEL was lost in the regions close to the bifurcation, i.e. regions which experienced increased WSS.[40] Although the endothelium was still mainly present, IEL loss was associated with an increased expression of matrixmetalloproteinases (MMPs) 2 and 9 and localized apoptosis.[40] Environments of high WSS increase NO and transforming growth factor-beta (TGF-β) secretion from endothelial cells which inhibit VSMC proliferation, thus weakening the wall and allowing physical flow forces to locally dilate the artery.[41] A study by Fukuda *et al*. has demonstrated that NO decrease, caused by inhibition of inducible nitric oxide synthase (iNOS), suppressed sIA formation in conditions of increased WSS.[42] It was hypothesized that the down-regulation of available NO inhibited sIA formation by the inhibition of the anti-proliferative effects on VSMCs. In contrast, endothelial nitric oxide synthase (eNOS) protects arterial walls from vascular inflammation by relieving hemodynamic force through NO production.[43] In a hypertension-induced sIA rat model, eNOS expression has been shown to be decreased at sites of sIA formation.[43] When sIAs were induced in eNOS knockout mice, the incidence of sIA formation was similar to that in WT mice. However, the expression of neuronal nitric oxide synthase (nNOS) was up-regulated compared to WT mice, suggesting a compensatory mechanism of nNOS. eNOS and nNOS knockout mice exhibited an increased incidence of sIA formation.[43] Thus, NO seems to play a dual role in sIA formation.

Another hemodynamic factor that has not been extensively studied yet, but may prove to be important in the pathogenesis of sIAs, is mechanical stretch.[33] Recently it has been shown that an increased mechanical stretch up-regulates endothelin-1 B receptors (ETBR), a very potent vasoconstrictor.[44] ETBR activation has been associated with VSMC apoptosis and ETBR antagonists have shown to decrease sIA formation in hypertension-induced rats models.[45]

After sIA formation, the irregular shape of the aneurysm further drives abnormal blood flow patterns and WSS. Consequently, mechanisms of sIA formation are thought to be similar to those of progression, thereby continuing the remodeling process in the sIA wall.[33]

Vascular remodeling in the sIA wall

Vascular remodeling in sIA walls is characterized by changes in ECM constituents and mural cells.[27] As mentioned before, growth factors and mediator proteins contributing to vascular remodeling are mainly secreted from activated endothelial cells and infiltrated leukocytes.[37]

Most published studies focus on the roles of MMPs, which have been shown to directly enable vascular remodeling by breakdown of the ECM, and indirectly by activating other proteinases and angiogenic factors.[46] In a rat model, it has been demonstrated that MMP levels are higher in sIAs compared to control arteries. MMP-2 expression was high in most sIAs while MMP-9 was expressed primarily in sIAs with atherosclerotic changes.[47] Furthermore, it has been demonstrated that a broad-spectrum inhibitor of MMPs suppresses sIA formation.[47] In a hypertension-induced mouse model, MMP-2 was not shown to be critical for the formation of sIAs, while mice lacking MMP-9 had a reduced sIA incidence.[48] Recently it has been demonstrated in a rat model of sIA that MMP-9 inhibitor Imidapril significantly reduced sIA size and medial thinning.[49] These results suggest a prominent role for MMP-9 in sIA progression, at least in experimentally induced sIAs. In human studies, MMP expression levels have shown to be elevated in serum of sIA patients as well as the sIA walls.[50] This could indicate a possible role for MMPS to serve as an easily obtainable biomarker for the presence of sIAs. As a reaction to the increased MMP expression and activity, a switch in VSMC phenotype occurs from a contractile to a proliferative, pro-inflammatory, ECM remodeling phenotype.[51] This process strongly resembles myointimal hyperplasia and neointima formation and is supported by histological findings.[52] Proliferation and matrix synthesis by VSMCs are likely to increase tensile strength in the vascular wall and presumably thereby protecting it against sIA formation.[51] However, several studies have demonstrated decreased collagen biosynthesis in the sIA wall. Synthesis of collagen types I and III was found to be significantly reduced at the transcriptional

and posttranscriptional enzymatic modification level in human[29, 53] and experimentally-induced[53, 53, 54] sIA walls compared to healthy vessel walls. As mentioned before, IEL disruption and degeneration have been observed in histopathologic studies of sIA walls.[26, 27] Furthermore, a common means of inducing experimental sIAs in animals is the incubation of elastase in arterial walls, thus degenerating the IEL.[25] However, the hereditary Marfan syndrome wherein abnormal elastin fibers are formed, does not seem to increase the risk of sIA formation.[55]

Growth factors are key regulators of vascular remodeling and thus could play an important role in the formation of sIAs.[56] Only a few studies have been published focusing on growth factor profiles in sIA tissues. In human sIA walls, the angiogenic growth factors basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF) were shown to be up-regulated compared to normal arteries, while transforming growth factor-alpha (TGF-α) was down-regulated in sIA tissue.[29] bFGF-R1, 3 and 4 were shown to be up-regulated in sIA tissue compared to controls[52] and bFGF has been shown to stimulate myointimal hyperplasia in rat sIAs.[57] Besides regulation of angiogenesis, VEGF is also involved in the maintenance of structural integrity of the vessel wall and could possibly play an important role in sIA formation.[58] In a preliminary study by Maderna *et al*., a differential expression pattern of VEGF and its receptors VEGF receptor-1 and 2 (VEGFR1 and VEGFR2) was found in sIA wall tissues compared to superficial temporal artery (STA) control walls.[59] It has previously been suggested by Bussolati *et al*. that VEGFR1 and VEGFR2 have different roles in angiogenesis; VEGFR1 as a promoter of endothelial cell differentiation and VEGFR2 as a promoter of migration and proliferation.[58] While no clear trend in VEGFR expression patterns could be observed, it was proposed that an imbalance of VEGF and its receptors and down-stream NO release by eNOS play a role in sIA formation.[59] Furthermore, VEGF-R1 activation has been demonstrated to increase MMP activity in SMCs,[60] and may likely increase MMP activity and remodeling in the sIA wall as well. As the balance growth factors and their receptors is very important for the outcome of vascular remodeling, it is interesting to investigate the roles of factors influencing this balance more extensively.

Roles of inflammatory cells in sIA formation

Numerous studies have shown infiltration of inflammatory cells, especially macrophages, into sIA walls.[30] Leukocytes enter the sIA wall through transmigration over the endothelium via an interaction of chemoattractants and adhesion proteins.[56] The expression of endothelial leukocyte adhesion molecule (E-selectin) was shown to be significantly up-regulated in human sIA tissue samples compared to control STA tissue.[61] Also, in an angiotensin-II induced rat model, the expression of tight junction structural proteins occludin and zona occludens-1 (ZO-1) in endothelial cells regresses

during the course of aneurysmal progression.[62] This regression was correlated with macrophage transmigration.

The role of macrophages seems to be critical for sIA formation based on observations from animal models. By liposome-induced macrophage depletion in a mouse model of combined elastin and angiotensin II-induced IA formation, a significantly lower incidence of sIAs was observed in mice with macrophage depletion compared to WT mice.[63] Similarly, mice lacking monocyte chemotactic protein-1 (MCP-1), a macrophage chemoattractant, exhibited lower incidence of IAs compared to WT mice.[53, 63]

Macrophages are an important source of proteinases, pro-inflammatory cytokines and growth factors, all of which have been shown to contribute to sIA wall degradation.[38] In hypertension-induced sIA walls in rats, macrophages have been reported to be an important source of MMP-2 and 9.[47] Additionally, macrophages may induce fibrosis through secretion of TGF-β which promotes transcription of pro-collagens I and III.[30] A prominent role is reserved for tumor necrosis factor-α (TNF-α) and interleukins (IL) 1β and 6.[64, 65] TNF-α is a pro-inflammatory and pro-apoptotic cytokine. It is up-regulated in sIA tissue and is associated with a higher expression of MMPs, Toll-like receptors (TLRs) and a down-regulation of TIMP-1.[66] Furthermore, TNF-α polymorphysms in the Japanese population have been associated with an increased incidence of sIA.[67] IL 1β has been reported to induce VSMC apoptosis in animal models of sIA. Although IL 1β knockout mice did not develop less sIAs compared to WT mice, the aneurysms were far less developed.[68] In cultured human and rat VSMCs, biosynthesis of collagens I and III as well as lysyl oxidase, needed for collagen cross-linking, is reduced by IL-1β.[69] An IHC study by Frösen *et al*. in 2004 has reported that a large part of the macrophages in human sIA walls are CD163+.[27] CD163+ acts as scavenger receptors for hemoglobin-haptoglobin complexes and CD163+ macrophages are recruited in antioxidant defense mechanisms. Subsequently CD163+ macrophages induce the expression of anti-inflammatory pathways.[70] These observations suggest that a part of the macrophage infiltration in sIA walls is a reaction to elevated oxidative stress.[6]

Besides macrophages, the presence of mast cells and T-cells has been observed in human sIA walls in greater extent compared to healthy vessel walls.[27] Mast cell infiltration was significantly higher in IA walls compared to healthy vessels in rat models and mast cell degranulation inhibitors diminished the chronic inflammatory processes in the IA wall, as assessed by decreased NFκB activation, MCP-1 expression and subsequent macrophage infiltration and MMP and IL-1β expression.[71] In cultured VSMCs from rat intracranial arteries, mast cell degranulation resulted in increased MMP-2 and 9

expression and activation and the induction of iNOS. These results suggest that mast cells play a critical role in aneurysm formation through the induction of inflammation, at least in experimental rat models. Although T-cells have been found in the sIA wall,[27] no studies thus far have been published concerning their profiling and activity.

Molecular mediators of inflammation

The role of MCP-1, Ets-1 and NFκB is suggested to be pivotal in sIA formation by the induction of an inflammatory response. Pro-inflammatoy transcription factor Ets-1 has been shown to regulate vascular inflammation and remodeling in physiological and pathological conditions.[72] In hypertension-induced sIAs in rats and in human sIAs, Ets-1 is expressed and activated mainly in VSMCs.[73] CHIP assays revealed that Ets-1 activates MCP-1 expression in VSMC in sIA walls. Inhibition of Ets-1 activity with decoy oligodeoxynucleotides (ODNs) one month after sIA formation resulted in regression of sIA size and decreased expression of MCP-1 and macrophage infiltration in sIA walls.[54, 73] Another key modulator in macrophage recruitment and activation is NF-κB, a nuclear transcription factor which regulates pro-inflammatory gene expression.[74] Treatment with NF-κB decoy ODNs have been shown to prevent the enlargement of IAs and the thinning of the media in IA walls.[74] Recently, it has been demonstrated that chimeric NF-κB and Ets-1 decoy ODNs regressed preexisting sIAs through a synergistic inhibitory effect on inflammation in sIA walls.[54] Furthermore, MCP-1 expression in sIA walls was decreased as well as subsequent macrophage infiltration.[54] Based on these results, these chimeric decoy ODNs have a promising potential as drug for sIA regression.

The humoral inflammatory response has also found to be active in sIA walls.[6] Complement activation in the human sIA wall has been shown to occur via the classical pathway, evident by the presence of activators IgG, IgM and C-reactive protein (CRP).[75] The outer sIA wall has been reported to lack complement inhibitors, in contrast to the luminal part of the sIA wall, thus allowing complete complement activation.[75, 76] The complement system in sIA walls is possibly activated by degenerative mechanisms such as fibrinolysis and exerts its chemotactic effects on inflammatory cells, thus increasing inflammation in the sIA wall.[6, 77]

Gene expression profiling in sIA formation and progression

Gene expression studies may indicate involved and altered genes and molecular pathways in sIA formation. Currently, only five genome-wide microarray gene expression studies have been published analyzing human sIA tissue.[78] Roder *et al*. have recently performed a meta-analysis of all

published microarray studies on sIA tissues obtained from patients after microsurgical clipping, analyzing a total of 60 sIA tissues.[78] Between the five studies, 507 differentially expressed genes were reported between ruptured and unruptured sIAs combined, compared to healthy control tissue (middle meningeal artery (MMA) or STA). Of these genes, 57 have shown to be differentially expressed in more than two independent microarray studies and seven genes in more than three independent studies, making it very likely that these genes are involved in sIA pathobiology. Up-regulated genes are collagen type I and collagen type V. Down-regulated genes are those encoding for anti-apoptotic protein B-cell lymphoma 2 (Bcl-2), tissue inhibitor of metalloproteinase 4 (TIMP-4) and the extracellular matrix glycoprotein tenascin C. Collagen type 3α and the lymphocyte chemokine stromal cell-derived factor 1 (SDF-1) were differentially expressed.[78] These results mostly coincide with the previously discussed findings from histological human studies and from animal models. Most of the analyzed gene expression studies have reported an up-regulation of different types of collagen genes in sIA tissue, possibly as an indicator of increased collagen turnover in the remodeling process. Regarding MMPs, the meta-analysis did not substantiate the previously discussed observations. However, the decreased expression of TIMP 4, potent of inhibiting all MMPs, might results in their increased activity as observed in human and animal sIA walls. While no reports have been made about glycoprotein tenascin C in sIA pathogenesis thus far, previous studies have shown that tenascin C is significantly up-regulated in tissues undergoing remodeling, neovascularization and inflammation.[79] Thus, altered tenascin C expression might be linked to sIA pathogenesis by deregulation of these processes. Gene expression studies have limitations, as will be discussed in the next chapter, thus further research is necessary to validate the results.

To study temporal changes of potentially important pathways in the pre-clinical stages of sIA pathobiology, Kardivel *et al*. developed a rabbit-specific gene-chip for microarray analysis of 209 genes related to cell signaling, adhesion and growth, apoptosis, inflammation, oxidative stress and remodeling in elastase-induced sIAs.[80] Two weeks post-procedure, 75% of the genes and 12 weeks post-procedure, 42% of the genes exhibited significant differential expression between aneurysm and control artery tissue.[80] Most genes encoding transcription factors, inflammatory pathways and structural proteins were down-regulated at both time-points in sIA tissue. In contrast, expression of collagen type III, MMP-2 and 9 and their inhibitors TIMP-1, 2 and *3* was higher at both time points in sIA tissue compared to control arteries, indicating increased ECM turnover. Continuing on this study, a five year follow-up was performed. [81] After five years, 13 of the 25 rabbits died of causes unrelated to the aneurysms. Between the induced aneurysms and control arteries, 0.33% of the genes were differentially expressed. Among these genes were those coding for molecular inflammatory mediators, growth factors, cell adhesion molecules, and ECM components.[81] As the difference in

gene expression was much smaller compared to the time points in the initial study, it was hypothesized that structure and function of the sIA wall changes over time, at least in experimental aneurysms.

CHARACTERISTIC DIFFERENCES BETWEEN UNRUPTURED AND RUPTURED SIAS

A key step in the process of deciphering why some sIAs rupture and others do not, is the comparison between characteristics of unruptured and ruptured sIA tissue. However, thus far few studies have been published using this approach. Also, studies that are published are difficult to interpret since some operate under the assumption that ruptured and unruptured sIAs share a pathogenesis to some extent, while others assume they have distinct pathogeneses. Most of the available data is obtained from patient material collected after surgical clipping or autopsy, thus only providing end-point data.[27] In the currently available animal models of sIA, rupture is very difficult to assess due to the low incidence of spontaneous rupture.[25] Observations from the published human studies are discussed in detail below.

Hemodynamic differences

sIA rupture occurs when hemodynamic stress exceeds the strength of the sIA wall. There is much controversy regarding the roles of the high and low-flow theories of sIA formation in regard to eventual rupture.[82] Thus, it is possible that the differences between unruptured and ruptured sIAs originate from the base of their pathogeneses. Circle of Willis anomalies have been demonstrated to be found more commonly in patients with ruptured as opposed to unruptured sIAs, presumably leading to differences in blood flow patterns and hemodynamic stresses.[32] Recently, it has been shown that ruptured and unruptured sIAs have statistically different qualitative hemodynamic characteristics. Using CFD simulations based on patient-specific sIA geometries and correlation to rupture history, it has been shown that ruptured sIAs were more likely to have complex and unstable flow patterns and increased WSS. No correlation was found between rupture and areas in the sIA subjected to abnormally low WSS.[34] These results seem to favor the high-flow theory of sIA formation leading to rupture. Most CFD studies rely on an assumption that the aneurysm anatomy and thus WSS patterns are changed insignificantly by rupture.[34] However, during and immediately after a hemorrhage they may undergo a variety of changes, thus the observed WSS may be a cause as well as a consequence of rupture.

Differences in wall structure

Only a few studies have been published focusing on the specific cell populations in ruptured and unruptured sIA walls. Two studies, published in 1999 by Kataoka *et al*. and in 2004 by Frösen *et al.,* in 2004, compared the walls of ruptured and unruptured sIAs by immunohistochemistry.[26, 27] Both groups of sIA did not significantly differ in dimensions.[27] In both studies, a significant loss of

endothelial cells and subsequent disruption of the endothelium was observed in ruptured sIAs. This was accompanied by the loss of mural cells (Figure 4A). Moreover, Frösen *et al.* have found a clear association of endothelial damage and subsequent luminal thrombosis with degeneration and rupture of the sIA wall (Figure 4A). As mentioned before, unruptured sIA walls tended to exhibit processes resembling neo-intima formation and myointimal hyperplasia, which are stages of arterial wound healing. (Figure 4B) Walls of ruptured sIAs are reported to be associated with ECM breakdown and partial hyalinization (Figure 4C).[26, 27] Most likely, due to the loss of mural cells, matrix-synthesizing capability is lost progressively, leading to decreased tensile strength and an increased susceptibility for sIA rupture.[27] Accordingly, serum levels and mRNA expression of MMP-2 and 9 were shown to be significantly higher in ruptured sIA compared to unruptured. Furthermore, the expression ratios of MMP-2 and 9 to their respective inhibitors TIMP-1, 2 and 3 were higher in ruptured sIA walls compared to unruptured.[50] In ruptured sIA walls, fibronectin is more highly expressed compared to unruptured sIAs, wherein fibronectin expression is similar to that in healthy vessel walls. Laminin expression was reported to be almost equally expressed between ruptured and unruptured sIAs.[29] Receptors of growth factors associated with remodeling, namely TGF-β receptor 2 and VEGFR1, were found to be down-regulated in ruptured sIA walls compared to unruptured, while VEGFR2 expression was unchanged.[52] Furthermore, VEGFR-2 expression was associated with myointimal hyperplasia in sIA walls, while VEGFR-1 was associated with T-cell and macrophage infiltration, luminal thrombosis and minor leaks.[52] However, VEGF signaling has complex and largely unknown interactions with other signaling pathways, making it difficult to deduce its role in sIA rupture.

Significantly higher inflammatory cell invasion was observed in ruptured compared to unruptured sIA walls in both studies. Inflammatory cells have been characterized as macrophages, T-cells and mast cells.[26, 27, 83] However, no studies have been published concerning the differences in expression of cellular adhesion molecules in unruptured and ruptured sIAs. It is not completely clear whether different subsets of leukocytes are predominant in ruptured over unruptured sIAs. Recently it has been demonstrated that in unruptured sIAs, M1 and M2 macrophages are present in equal proportions while M1 macrophages are significantly predominant over M2 macrophages in walls of ruptured sIAs. In STA control tissue few M1 or M2 macrophages are present.[83] M1 activated macrophages are immune effector cells and secrete pro-inflammatory chemokines while M2 activated macrophages function in tissue repair and secrete anti-inflammatory chemokines.[84] It is hypothesized that a balance between M1 and M2 macrophage populations leads to the formation of stable sIAs while an increased population of M1 macrophages together with an up-regulation of mast cells biases sIAs to

rupture. [83] A significant correlation of the overall integrity of the wall structure with influx of inflammatory cells was found (Figure 5).[26]

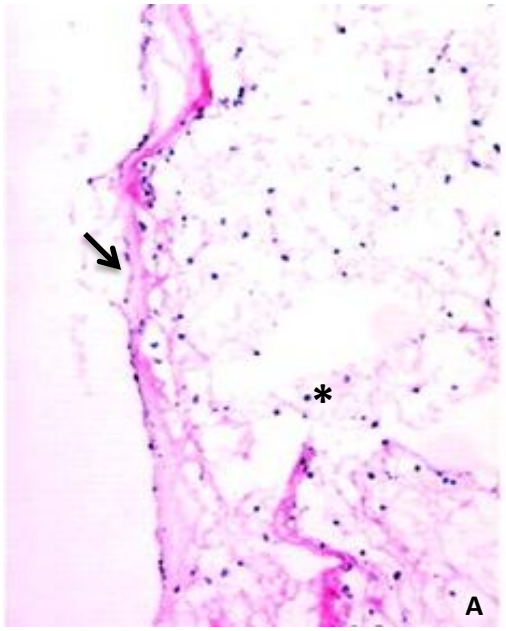
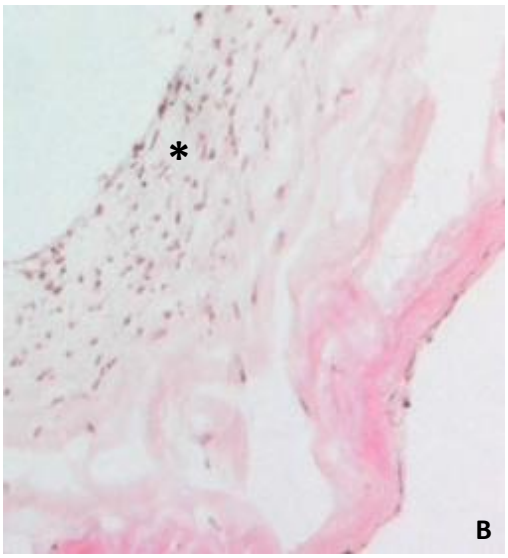
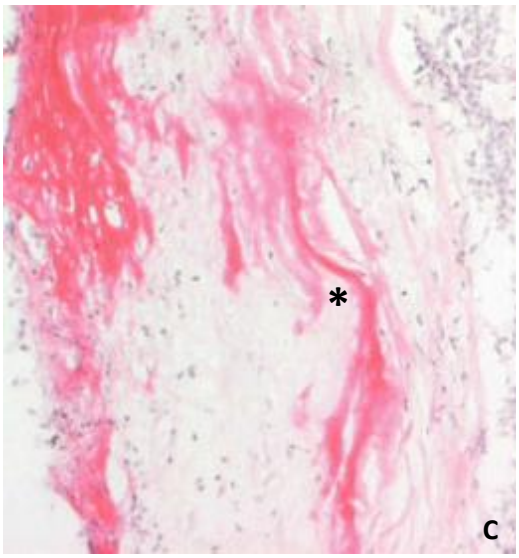


Figure 4. Histology of wall degeneration in human ruptured (A & C) and unruptured (B) sIAs. A: Loss of mural cells (asterisk) and thrombosis-lined endothelium (arrow)**. B:** Myointimal hyperplasia(asterisk). **C:** ECM breakdown in hypocellular wall areas (asterisk). Hematoxylin-Eosin staining. Figures adapted from Frosen *et al*., 2004 & 2012.[F4, F5]





Based on the histopathological studies, four types of walls have been described which are associated with rupture. From endothelialized walls with organized VSMCs, thickened walls with myointimal hyperplasia to decellularized walls with an organized thrombus to extremely fragile and hypocellular walls lined with thrombosis. In all the subgroups described, at least 40% of the sIAs that represented them, were ruptured.[27] This suggests that there are no specific sIA wall phenotypes that are not

prone to rupture. Furthermore, the sIA wall often shows a gradient from almost intact to degenerated tissue in the neck-to-fundus direction.[27] Thus, differential factors between unruptured and ruptured sIA walls are indicated to be more subtle than thought, most likely even being different wall areas within a single sIA.

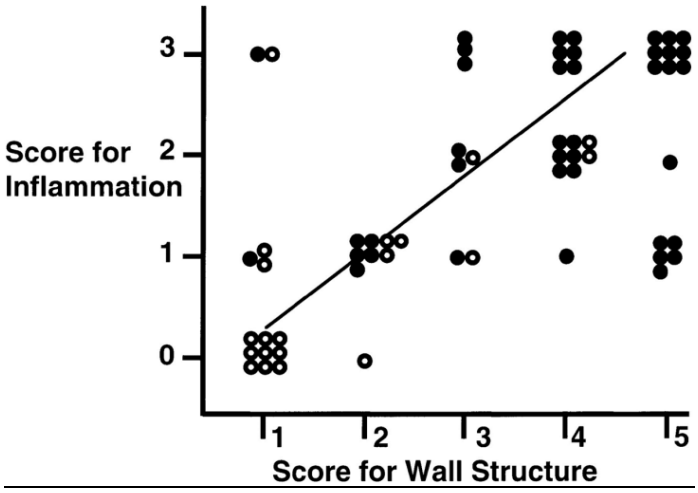


Figure 5. Significant correlation between structural integrity and degree of inflammatory cell infiltration in human sIA walls. Open circles: unruptured sIAs, closed circles: ruptured sIAs. Scores from 0 (normal) to 3 (densely populated) or 5 (complete disruption) of inflammatory cell invasion and wall integrity respectively, based on IHC and scanning electron microscopy. Figure adapted from Kataoka *et al.*, 1999. [F6]

Aneurysm growth and mural cell death

The geometry of sIAs is not stable and grows over time. It is thought that sIAs grow through different mechanisms. Size and shape of sIAs can alter by distention as a result of hemodynamic pressure exerted by the bloodflow, by proliferation of mural cells or a combination of the two.[6] Although sIA geometry was not reported to be directly correlated with wall degeneration, size and geometry are associated with sIA rupture.[16] In a recent study, an association has been made between sIA geometry and the profile of signaling pathways involving apoptosis, cell proliferation and stress signaling in ruptured and unruptured sIAs.[85] MAP kinases are thought to induce smooth muscle cell proliferation in vascular walls as a response to various stimuli such as cytokines, growth factors and hemodynamic and stretch stress, thus possibly leading to sIA growth.[86] Phosphorylation and levels of MAP kinases c-p54 Jun N-terminal kinase (JNK) and p38 were positively correlated with sIA size and negatively with undulation index, which is associated with sIA rupture.[87] A down-regulation of anti-apoptotic Bad protein was observed in ruptured sIAs compared to unruptured sIAs, as well as a down-regulation of phosphorylated m-TOR, indicating decreased cell survival and proliferation in ruptured sIAs.[85, 86]

As loss of mural cells is associated with sIA rupture, it is important to know how mural cells die before it can be elucidated why they are lost. In a recent study, the degree of apoptosis in ruptured

and unruptured sIAs was assessed by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining. Ruptured sIA walls were associated with apoptosis in several studies which was also confirmed by electron microscopy.[88,89,77] Although, this association was not significant in every study. Cleaved caspase-9 activity, a marker of intrinsic apoptosis, has been found to be significantly up-regulated in ruptured walls, whereas no activity of cleaved caspase 3 and 8 was detected.[88] In several studies assessing cell death in the sIA wall, the degree of apoptosis, as assessed with TUNEL staining, is relatively low compared to the degree of mural cell loss. Furthermore, in histological studies, fibrin-like debris was frequently observed in areas with few mural cells.[6] This resembled fibrinoid necrosis which is a consequence of vascular damage caused by the immune response.[90] These findings indicate a significant role for necrosis in sIA wall deterioration. However, it should be kept in mind that apoptotic and necrotic cells and debris could be cleared from the sIA wall by phagocytic cells, thus biasing these observations.

Oxidative stress and molecular mediators of inflammation

Among others, thrombosis in the sIA wall, which is associated with sIA rupture, is a significant source of oxidative stress.[27] Increased expression of hemeoxygenase-1, a marker for oxidative stress, was associated with sIA wall degeneration and rupture.[6] Oxidative stress damages cell membranes, oxidizes intra- and extracellular lipids and triggers apoptosis by activating caspase-9. Oxidized lipids can trigger both apoptosis and necrosis due to their cytotoxic effects.[91] These oxidized lipids are found both in both mural cells and in the ECM in sIA walls.[75] These areas strongly represent atherosclerotic lesions, where macrophages become foam cells after internalizing lipids and act as antigen presenting cells and trigger an immune response against the oxidized lipids.[92] Patients with unruptured and ruptured sIAs have been shown to exhibit differences in humoral immune response against oxidized lipid epitopes.[77]

Activation of the complement system, assessed by staining for the membrane attack complex (MAC), the end-product of complement activation, has been associated with ruptured sIAs and inflammatory cell infiltration.[76] Based on (immune) electron microscopy, cell death was observed in MAC positive areas of the sIA wall.[77] However, it is not clear how complement activation can lead to vessel wall degeneration and sIA rupture. MACs can cause cell death by apoptosis and necrosis induction.[76] However, MACs are not often observed on cell surface in sIA walls, but in ECM debris in hypocellular regions. These observations suggest complement activation as a reaction to necrosis rather than the cause of it.[77] An interesting alternative hypothesis is that sub-lytic MAC concentrations may have protective effects against apoptosis in the sIA wall, thus explaining the

rarity of co-localization of MAC+ and TUNEL+ cells.[77] It has previously been demonstrated that sub-lytic MAC concentrations inhibit apoptosis in VSMCs by regulation of apoptotic pathways and growth factor production.[93]

Little is known about the role of inflammatory cytokines in sIA rupture. Up to now, TNF-α is the only cytokine of which expression was found to be up-regulated in ruptured human sIAs.[66] As inflammatory cell infiltration is correlated with sIA rupture it is very likely that expression of other cytokines such as IL-1β is also up-regulated in ruptured sIA walls. However, up to now this remains a speculation.[30]

The role of inflammation in sIA rupture has recently been indirectly demonstrated by Hasan *et al*. In a retrospective case-control study a 60% reduction in sIA rupture by daily intake of aspirin has been reported.[94] Although the mechanism behind this protective effect is not clear yet, it is speculated that it is mediated in part by aspirin inhibition of COX-2 and mPGES-1. Their expression is up-regulated in walls of sIAs compared to STA control tissue and greater in walls of ruptured sIAs compared to unruptured sIAs.[95] This pathway could provide a promising target for drugs against sIA rupture.

Observations from gene expression studies

As mentioned before, currently, only 5 genome-wide microarray gene expression studies have been published with a total of 60 sIA tissues analyzed.[78] Besides using different gene expression analysis platforms, the study designs differ in experimental design. In some studies both unruptured and ruptured sIA tissues were compared to healthy control tissue, thus addressing the etiology of sIAs. Other studies, which will be discussed here, elaborated or specifically compared ruptured with unruptured sIA tissue, thereby aiming to identify factors leading to sIA rupture.

In 2009, Shi *et al*. have reported an indication for differential gene expression patterns between ruptured and unruptured sIAs in their study of global gene expression patterns of the sIA wall without further specification of the involved genes.[96] A study by Krischek *et al.* in 2008 yielded only two genes that are significantly, differentially expressed between ruptured and unruptured sIAs. These were the genes encoding for an unspecified nucleoprotein and ribosomal protein L22, the core protein for the large ribosomal subunit.[97] In the two largest microarray studies, conducted by Marchese *et al*. in 2010 and Kurki *et al.* in 2011, a significant up-regulation of genes corresponding to vascular remodeling, extracellular matrix degradation, cell death, oxidative stress and leukocyte migration, was observed in ruptured sIAs.[28, 98] Specifically, ruptured sIA walls exhibited significant up-

regulation of matrix MMP-2 and 9, pro-apoptotic genes such as Fas, Bid and Bax, TLR signaling, NF-κB, hypoxia inducible factor-1A (HIF-1A), iNOS and ETS transcription factor binding sites. TIMP-3 and anti-apoptotic genes, such as Bcl-X(L) and Bcl-2 were down-regulated. Surprisingly, the study of Pera *et al*. from 2010 reported that although genes associated with the inflammatory response are up-regulated in sIA tissue compared to healthy control tissue, they are down-regulated in ruptured sIAs compared to unruptured.[99] Except the results from Pera *et al.*, the genomic studies are in agreement with the processes as observed in the histopathological and experimental studies as described above. However, it is hard to determine whether the differences in gene expression are the cause of sIA rupture or a reaction to it.[28] Another limitation of these gene expression studies is the difficulty in pinpointing the cell type responsible for the differences in gene expression profiles, since the sIA wall comprises a mixture of cell types.[28] Lastly, it is difficult to compare micro-array studies with each other. Due to the use of different micro-array platforms, genes with altered expression in one study might not come out in another platform. Also, the statistical power is limited due to the small amount of tissues used in each study.[78]

DISCUSSION

In this literature study, currently available data concerning the cellular and molecular mechanisms of sIA formation and rupture has been reviewed. The findings are summarized in Figure 6: Hemodynamic stress, in particular altered WSS, causes endothelial dysfunction. Through the release of growth factors and alterations in NO homeostasis, activated endothelial cells induce inflammatory responses and remodeling in the vessel wall. Based on data from observational and experimental studies, the two main roles in the pathophysiology of sIA formation and eventual rupture appear to be reserved for inflammation and vascular remodeling. Inflammatory pathways, macrophage infiltration and humoral components such as pro-inflammatory cytokines and complement are up-regulated in sIA tissue. Increased expression and activity of MMPs and altered growth factor signaling contribute to vascular remodeling in sIA tissue. The interplay between inflammation and remodeling in the sIA wall leads to morphologic changes including disruption and degeneration of the endothelium and IEL, mural cell death, but also proliferation and re-organization of the ECM. Subsequently, the sIA grows and changes geometry, which in turn further facilitates hemodynamic stress. It is hypothesized that unruptured sIAs represent adaptive changes in the vascular wall to hemodynamic stress and subsequent inflammation and remodeling, while maladaptive changes result in sIAs prone to rupture. Ruptured sIAs are reported to have higher degrees of inflammation, apoptosis, ECM breakdown and oxidative stress compared to unruptured sIAs. This was evident on cellular, molecular and gene expression level. Although significant efforts have been made, the reasons why some sIA undergo maladaptive changes and rupture remain unresolved due to the poor understanding of the interplay between the various, complex, cellular and molecular mechanisms of sIA pathogenesis. The main questions to be answered in order to decipher sIA rupture consider the extent in which unruptured and ruptured sIAs share pathogeneses and the causality of the observed pathological mechanisms. If these questions could be answered, the pathogenesis of sIA rupture could be elucidated. However, the currently available data fails to answer these questions. Therefore, a hypothesis for sIA rupture based on the observed pathological processes is proposed here.

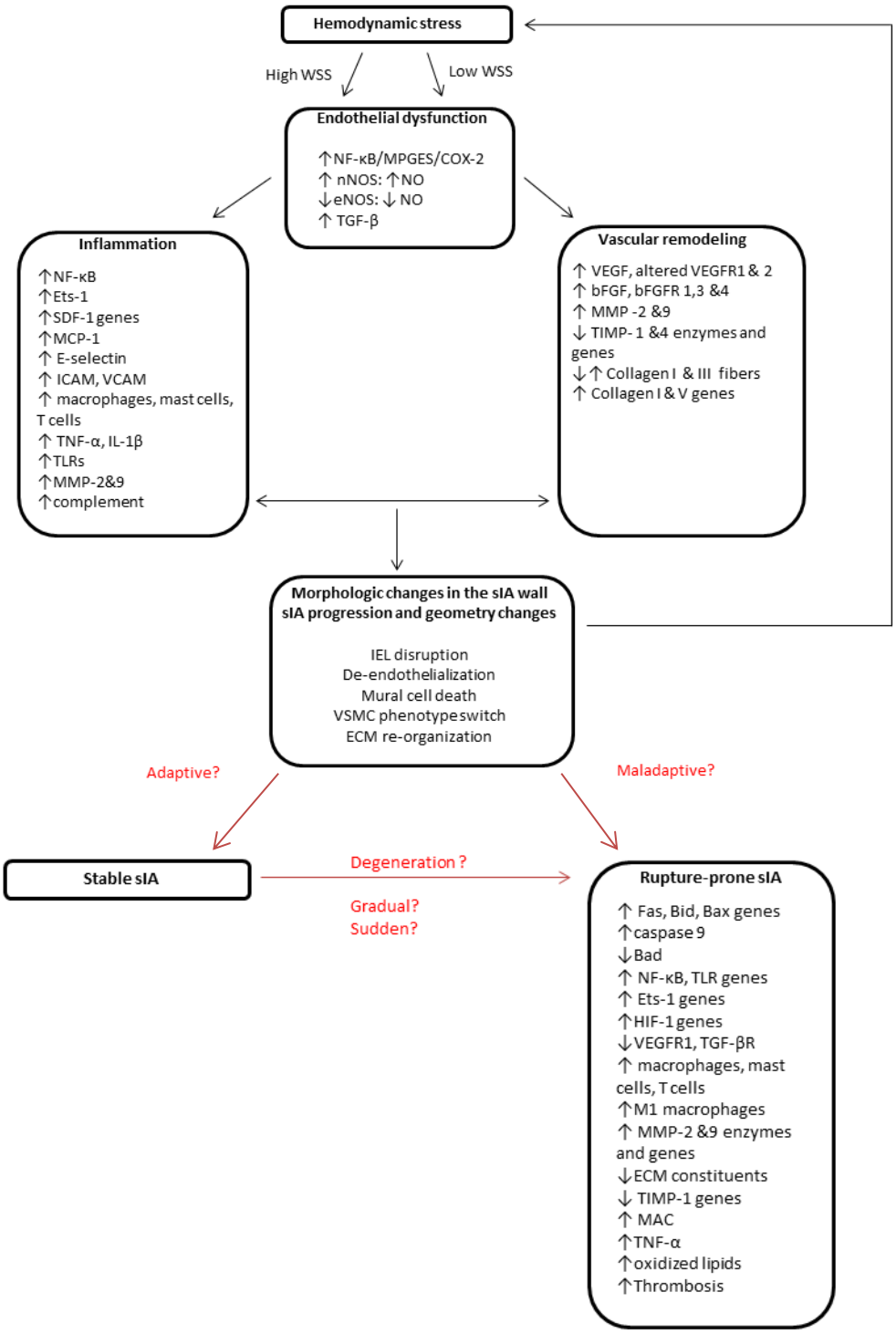


Figure 6. Flow chart of the pathophysiologic processes and molecular mediators of sIA formation and eventual rupture. Endothelial dysfunction caused by altered WSS induces inflammatory processes and vascular remodeling, which in turn influence each other and lead to sIA progression. If unruptured and ruptured sIAs have distinct pathogeneses or if stable sIAs degenerate and eventually rupture is yet unknown (in red).

The balance of vascular remodeling and inflammation: hypothesis for sIA rupture pathogenesis

The interplay between vascular remodeling and inflammation is complex and partially unknown. On one hand they are both involved in maintenance and repair mechanisms in response to tissue injury induced by hemodynamic stress, while on the other hand they are themselves possible sources of tissue injury.[38] Shifts in this balance of repair and injury can be caused by increased hemodynamic stress caused by the altering sIA geometry, possibly as well as external factors, causing a loop reinforcing inflammation and vascular remodeling.[27] As the balance of inflammation and remodeling shifts from repair to injury-inducing, mural cell death increases as well as ECM degradation, thereby weakening the tensile strength of the sIA wall and leading to rupture.[27, 38] It is hypothesized that if inflammation and vascular remodeling work as a repair mechanism, the wall of unruptured sIAs can remain intact for many years. However, Frösen *et al*. have shown that a range of phenotypes, ranging from thickened, ECM-rich to hypocellular and fragile wall areas exist in a single sIA.[27] This, together with the activity of MAP kinases, indicates an ongoing remodeling process in sIAs.[38] However, the question is whether sIAs prone to rupture are distinct from the origin of their pathogenesis from sIAs that never rupture. Alternatively, a stable sIA could undergo degenerative changes gradually and rupture, or a shift in inflammation and remodeling balance towards rupture could suddenly occur (Figure 6). It should be kept in mind that a combination of these theories is possible and that eventual rupture is dependent on the microenvironment of the individual sIA. Recently a Japanese case study reported a patient presenting two sIAs, one ruptured and the other unruptured.[100] Although cases like these are very rare, they could provide insight into the roles of the micro-environment of individual sIAs in their potential rupture. Methodically it is very difficult to identify when and if rupture will occur as it cannot be stated for certain that sIAs exist which will never rupture given an infinite amount of time. Thus, comparing unruptured and ruptured sIAs could possibly be ineffective.

Causality of pathological mechanisms

An important step towards elucidating the mechanisms of sIA rupture is clarifying the causality of the thus far observed pathogenic processes in the sIA wall. However, as many of the described mechanisms are interrelated, this could prove to be difficult. For example, sIA growth by cell proliferation or distention by blood flow is influenced by the biologic processes in the wall, while these processes themselves are influenced by sIA geometry and growth.[6] Regarding the inflammatory response, it is not clear yet whether inflammation is a cause or reaction to degeneration and rupture of the sIA wall. In both histological[26, 27] studies and gene expression

studies[28], different times from rupture to resection of the sIA wall did not seem to significantly correlate to of histological inflammation and aneurysm wall fragility, leukocyte density and differential gene expression. This suggests their presence in the sIA wall prior to rupture. Furthermore, activation of the complement system in the sIA wall seems to be chronic, as significant accumulation of complement factor C3d has been detected, which occurs in chronic inflammatory states.[75] However, current data does not indicate loss of mural cells induced by inflammatory cells. Macrophages induce apoptosis through TNF-α, among other mechanisms.[101] Studies have not been able to detect caspase-8 activation, the mediator of TNF-α induced apoptosis, despite an up-regulation of TNF-α in ruptured sIA walls. [66,6] Besides the interrelation of the observed pathological mechanisms, the methodological issues mentioned above make it very difficult to assess causality.

Implications for future research

Much more knowledge must be gained on all levels involved in sIA pathogenesis and rupture. As hemodynamic stress most likely lies at the basis of sIA formation, and has been shown to differ between ruptured and unruptured sIAs, it is a prime candidate as a starting point to decipher the origins of sIA rupture. Further development of CFD simulations of patient-derived sIA characteristics and also post-mortem tissues of healthy patients could provide insight into the altering hemodynamic stresses on sIA formation and subsequent rupture.[34] As the balance of inflammation and vascular remodeling responses seems to be a key player in sIA formation and rupture, it could be interesting to investigate the roles of factors influencing this balance more extensively. This feasibility is demonstrated by Hasan *et al*. with their preliminary results of the lowered prevalence of sIA formation in humans by daily intake of aspirin.[94] Also, as reported by Aoki *et al*., the use of anti-hypertensive drug Imidapril, which acts as an MMP-9 inhibitor, reduces sIA size in rats.[69] As these both are easily obtainable, orally administered drugs, their therapeutic potential could prove to be very valuable and deserves further investigation. Lastly, results from published gene-expression studies have thus far only been validated by retrospective analysis with histopathological studies.[78] It could prove interesting to use the altered gene expression profile as a starting point for a detailed, functional analysis of the found targets in human sIA walls and animal models. Ultimately, it would be of highest significance to deduce biomarkers for sIA rupture from observations regarding their pathogenesis, aside from sIA geometry which is currently used.[17]

Conclusions

Despite the significant efforts that have been made, the underlying cellular and molecular mechanisms of sIA formation and rupture still remain poorly understood. A growing body of evidence supports the theory that hemodynamic stress causes endothelial dysfunction which subsequently induces inflammation and remodeling in the vascular wall, thus leading to sIA formation. The same processes seem to be up-regulated on cellular, molecular and gene expression level in ruptured compared to unruptured sIAs. However, due to methodological issues it is not possible to address if ruptured and unruptured sIAs at least partially share a pathogenesis or if they are distinct pathological processes. Therefore, the question as to why some sIAs rupture and other do not, may remain unresolved for a long time.

APPENDIX

Limitations of human studies and applications of animal models

As mentioned before, human sIA material only provides end-point data as it is obtained after (preventive) micro-surgical procedures or post-mortem. This limits the use of human tissue samples. The stresses on the sIA during surgical procedure can directly increase the chance of rupture and indirectly activate pathways predisposing the sIA for rupture. Also, as patients undergoing these surgeries are predisposed to sIA formation and/or rupture and possibly other pathologies by the previously mentioned risk factors. Therefore, control samples from unaffected arteries may not provide perfect control conditions and obviously samples of walls of intracranial arteries from healthy subjects cannot be extracted. Additionally, many of the human sIA studies are based on samples obtained from Japanese and Finnish sIA and SAH patients. This could provide a biased view on sIA formation and causes of rupture as the Finnish and Japanese population have been shown to have a significantly higher risk of sIA.[21] To overcome these limitations and to systematically study underlying mechanisms of sIA formation, animal models have proven to be useful.[25] However, a fundamental problem in animal models is, as mentioned before, the low incidence of spontaneous rupture. The only reported incidence of rupture in an animal model was a rat-model of hypertension-induced IA formation with a rupture rate of only 3% in 3 months after sIA induction.[25] Although this closely resembles human sIA rupture rates of 3.2% in the general population[9], it is unfeasible for experimental conditions. Additionally, as shown by Kadrivel *et al*., rabbit models of IA can survive for at least 5 years without rupture.[81] In 2009, Nuki *et al*. have reported the induction of sIAs in mice by injection of elastase and continuous infusion of angiotensin II. These induced IAs appeared to rupture spontaneously, however the exact incidence was not reported.[102] This major limitation has to be looked further into as the development of an animal model exhibiting spontaneous sIA rupture would be of highest value in studying sIA rupture in a systematic, temporally-focused manner. Another limitation of animal studies are the differences in severity of IA phenotypes between the different models, making it difficult to interpret and compare the results. sIAs induced by elastase injections tend to be larger and macroscopically apparent compared to IAs induced by MCP-1 knock-out and macrophage depletion, which also show more subtle changes in histology.[53, 102] However, the fundamental concern regarding the lack of spontaneous rupture in experimentally-induced sIAs is a major pitfall in the translation to human sIAs, also for the future assessment of potential drugs against sIA formation, progression and rupture.

SUMMARY IN LAYMAN'S TERMS

Saccular intracranial aneurysms (sIAs) are berry-like outpouchings of arteries in the brain. When and if a sIA ruptures, bleeding in the compartments surrounding the brain occurs, resulting in death or severe disabilities. Therapies against sIA rupture are equally risky and current methods to predict if a sIA will rupture are unreliable. Therefore, it is pivotal to asses why some sIAs rupture and other do not, based on their bio-molecular mechanisms. However, these are still poorly understood and highly intricate. A growing body of evidence suggests that changes in blood flow through the brain arteries initiate dysfunction of the arterial cells. This results in higher inflammation and detrimental changes in the structure of the arterial wall, leading to sIA formation. These mechanisms have been found to be active in higher degrees in cells from ruptured sIAs compared to cells from unruptured sIAs, suggesting their role in sIA rupture. However, due to methodological issues, it is difficult to interpret the causality of these processes and thereby the extent in which unruptured and ruptured sIAs differ from each other.

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