Molecular events regulating the pathogenesis of intracranial aneurysms: Special insight on hemodynamics and chronic inflammation

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Received: 2 July 2019 / Accepted: 10 September 2019
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Abstract Intracranial aneurysm (IA) can cause a lethal subarachnoid hemorrhage after rupture. Thereby, the correct understanding of the pathogenesis of the disease is essential to develop a novel therapeutic strategy to prevent progression. The accumulating evidence from simulation of hemodynamics targeting human cases has implied the role of hemodynamic force in IAs. In another point of view, experimental evidence mainly from animal studies has clarified the crucial role of macrophage-mediated long lasting-inflammation in the pathogenesis. However, how hemodynamic stress triggers such molecular events in arterial walls to develop IAs remains unclear. Recent experimental studies have revealed some of the potential machineries regulating hemodynamic stress-triggered IA formation. High walls shear stress activates endothelial cells and induces expression of MCP-1 at the earliest stage of IA formation. At adventitia, mechanical stretch induces MCP-1 expression in fibroblasts as well. MCP-1-mediated infiltration of macrophages into intracranial arterial walls thus occurs. In infiltrating macrophages, EP2 functions to exacerbate inflammation through formation of positive feedback loop, synergistic action with TNF- α and auto-amplification loop among macrophages. Given the nature of IAs as a vascular disease, further studies focused on hemodynamic force-mediated molecular events regulating the pathogenesis are necessary to understand the whole picture of the disease.

Keywords intracranial aneurysm, macrophage, chronic inflammation, wall shear stress, stretch

Introduction

Subarachnoid hemorrhage (SAH) has quite a poor prognosis and about a half of patients die despite of a modern technical advancement and an intensive care [1]. The major cause of SAH is rupture of an intracranial aneurysm (IA). IA is a regional bulging of intracranial arteries developed mainly at a bifurcation site of intracranial arteries and is histologically characterized by a disruption of the internal elastic lamina and degenerative changes in the media. The prevalence of IAs in a general public is high about 3~5% [2, 3] and thus many IAs are incidentally found before rupture through brain check or so. Given the devastating outcome of SAH once after the onset, the pre-emptive medicine targeting unruptured IAs to prevent progression and rupture is mandatory for social health [4]. However, today, treatment options to prevent rupture of IAs are limited to surgical interventions like the micro-neurosurgical clipping or the endovascular therapy like coiling. As these surgical interventions have a risk of complications including even death or severe morbidity in nature, the development of a novel therapeutic strategy based on the pathogenesis of IAs is demanded.

As reviewed and discussed in other articles in this special issue, the pathophysiology of IAs is greatly influenced by hemodynamic force loaded on arterial walls at bifurcation sites and thus many recognize IA as a hemodynamics-dependent disease. In addition, recent experimental findings have been accumulating evidence that macrophage-mediated chronic inflammation plays a crucial role in the pathogenesis of the disease [5–14]. In this process, some studies have implied the important role of hemodynamics to trigger inflammatory responses and to form inflammatory microenvironment leading to IA formation and progression [5, 11, 14–17]. In this mini-review article, I summarize some of the recently published articles and discuss the

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contribution of hemodynamic force to molecular events leading to IA formation.

Molecular events regulating IA formation and progression and the potential contribution of wall shear stress in this process

Through the accumulation of findings mainly obtained from computational fluid dynamic analyses of human cases with IAs and animal models of IAs, many believe that wall shear stress (WSS) influences IA formation and progression and rather WSS is considered as a trigger of the disease. These studies have consistently demonstrated the presence of high WSS at a prospective site of IA formation or neck portion of IAs [18–22]. On the contrary, the presence of excessive low WSS and concomitant turbulent flow in the dome of IAs and its correlation with the enlargement or rupture of lesions have also been demonstrated [22–26]. Therefore, machineries regulating IA formation and progression may be driven under quite a different flow-loading condition. However, intriguingly, in vitro experiment using a primary culture of endothelial cells has revealed that molecular events triggered by these different flow conditions are similar [12]. Both under the high WSS and the excessive low WSS condition, cultured endothelial cells produce adhesion molecules and chemoattractants for macrophages such as MCP-1 (Monocyte Chemoattractant Protein-1 also known as CCL2) [12]. In this process, concomitant presence of the turbulent flow further enhances expressions of adhesion molecules and chemoattractants. These observations are well consistent with the finding of MCP-1 expression in IA lesions, in which MCP-1 expression is sustained during the development of IAs [6, 12]. Here, MCP-1 is a major chemoattractant for macrophages. In the pathogenesis of IAs, MCP-1 plays a crucial role to trigger and maintain inflammatory responses in lesions which leads to IA formation and progression via recruiting macrophages [6, 10, 13]. As a genetic deletion of *Ccl2* (the gene encoding MCP-1) or an inhibition of MCP-1 by over-expression of its dominant negative form (7-ND) remarkably suppresses incidence or enlargement of IAs in an animal model [6, 13], MCP-1-mediated macrophage infiltration is presumably a critical step to initiate or progress the disease. In IA lesions, MCP-1 expression is induced in endothelial cells at the early stage of IA formation and its expression in endothelial cells is sustained during the progression of the disease even after the IA lesion is induced [6, 12]. Such a sustained expression of MCP-1 in endothelial cells enables a continuous macrophage infiltration in lesions, which may make macrophage-mediated inflammation being long-lasting. Because intracranial arteries lack vasa vasorum, trans-endothelial migration is presumably only way for macrophages to infiltrate into intracranial arterial walls to provide inflammatory microenvironment. In this point of view,

shear stress-induced expression of MCP-1 in endothelial cells plays a central role in the pathogenesis of IAs. Infiltrating macrophages in intracranial arterial walls produce various proteinases [8], leading to the degradation of extracellular matrix and facilitating the progression of the disease. Furthermore, macrophages produce cytokines like TNF- α and make inflammation being expanding and exacerbating [10, 27, 28]. In this step, machineries to amplify inflammation and to become chronic function in macrophages. In macrophages, prostaglandin E₂ (PGE₂) and its receptor subtype EP2 plays a crucial role as a mediator of chronicity of inflammatory responses in situ [5]. In mice deficient in EP2 specifically in macrophages, not only IA formation but also inflammatory responses such as NF- κ B activation in whole lesions are almost completely suppressed [5], confirming the crucial role of inflammatory responses evoked by macrophages. Furthermore, the selective EP2 antagonist, PF-04418948, remarkably suppresses further enlargement of pre-existing IAs induced in a rat model and inflammatory responses in lesions [5]. In vitro studies have provided the experimental evidence that EP2 signaling regulates chronicity of inflammation in multi steps and thus functions as a node of chronic inflammation. EP2 signaling cooperates with other cytokines present in inflammatory microenvironment in IA lesions like TNF- α and amplifies expression of pro-inflammatory genes induced by these cytokines in situ [5]. Formation of a transcription factor complex under synergistic stimulation of EP2 signaling and TNF- α signaling may be responsible for the amplification of pro-inflammatory gene expressions. Importantly, *PTGS2*, which encodes the inducible form of cyclooxygenase, an enzyme to produce PGE₂ named COX-2, is a gene whose expression is significantly amplified by EP2 signaling [5]. This means the formation of the positive feedback loop consisting of COX-2-PGE₂-EP2 cascade, making EP2-mediated inflammatory responses being long-lasting once triggered and amplified. EP2 signaling also activates a RNA-binding protein HuR to stabilize *CCL2* and thereby enhances protein expression of MCP-1 [5]. As MCP-1 secretion from macrophages recruits more macrophages themselves in lesions, under EP2 signaling the auto-amplification loop mediated by MCP-1 is formed among macrophages.

Combined above findings together suggest the following hypothesis (Figure 1); First, high WSS activates endothelial cells to express adhesion molecules and macrophages infiltrates into intracranial arterial walls across endothelial cell barrier. In addition to the sustenance of macrophage infiltration through activation of endothelial cells by the excessive low WSS and concomitant turbulent flow, macrophages infiltrated then form the auto-amplification loop under EP2 signaling and macrophage-mediated inflammation becomes long-lasting and amplified in situ. Through chronic inflammatory responses, cells in intracranial arterial walls are damaged and lost, resulting in the fragility of arterial walls.

Figure 1 Schematic drawing of events regulating intracranial aneurysm formation and progression. Macrophages infiltrates in intracranial arterial walls recruited by MCP-1 produced from endothelial cells and adventitial fibroblasts. Macrophages then form the auto-amplification loop to expand number of themselves. Also, in macrophages, EP2 signaling amplifies inflammatory responses via synergistically functioning with other cytokines or forming the positive feedback loop and functions as a node of chronic inflammation. Finally, by cytokines and proteinases produced by macrophages, degenerative changes of arterial walls are progressed. In these processes, two distinct hemodynamic forces, wall shear stress and mechanical stretch, trigger above events leading to the disease development and progression.

Molecular events regulating IA formation and the potential contribution of mechanical stretch

High WSS may become trigger of IA formation but fundamental question raises, why IA is formed at a specific bifurcation site although high WSS is basically loaded at every bifurcation sites. Recently published study provides us one explanation [14]. At the prospective site of IA formation in a rat model, a bifurcation site of intracranial arteries, transient outward bulging of arterial walls is observed in response to elevated systemic blood pressure to induce IAs at the earliest stage of IA formation [14]. In the adventitia of the prospective site of IA formation, fibroblasts present there activate when they are stretched by outward bulging and then express MCP-1. In in vitro study, mechanical stretch-loaded primary culture of adventitial fibroblasts consistently secretes MCP-1 and can recruit THP-1 cells, a human monocyte cell line, in a transwell system [14]. Given the expression of MCP-1 in endothelial cells and adventitial fibroblasts at the earliest stage of IA formation and the formation of auto-amplification loop among macrophages, two distinct hemodynamic forces, high WSS and mechanical stretch, drive macrophage infiltration into intracranial arteries and crucially contribute to provide inflammatory microenvironment which leads to IA formation and progression (Figure 1).

Acknowledgements We thank all of our staffs, technicians and secretaries for their dedicated efforts.

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