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REVIEW

Turning back the clock: regression of abdominal aortic aneurysms via pharmacotherapy

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Abstract Abdominal aortic aneurysm (AAA) is a common disease that causes progressive expansion and rupture of the aorta with high mortality. There is a large and unmet need for nonsurgical treatment for AAA. Research has shown that an intricate network of inflammatory cells and interstitial cells contributes to the formation of AAA by producing pro-inflammatory mediators that activate enzymes to degrade the extracellular matrix (ECM) and impair ECM biosynthesis. Pharmacological agents such as statins and angiotensin-converting enzyme inhibitors may promote tissue stabilization in AAA by diminishing pro-inflammatory signaling and normalizing metabolism of the ECM. Our recent experiments in animal models demonstrate that inhibition of c-Jun N terminal kinase (JNK) inhibits multiple pathological processes and causes regression of established AAA. Thus, emerging evidence indicates that pharmacological intervention targeting pro-inflammatory signaling and abnormal ECM metabolism is a promising strategy for treatment of AAA.

Keywords Vascular disease · Signal transduction · Extracellular matrix · Inflammation · Therapy

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Introduction

Abdominal aortic aneurysm (AAA) is caused by a segmental weakening of the abdominal aortic walls, which leads to progressive aortic dilation. Although patients with AAA usually have no symptoms, it progresses with time, resulting in rupture of the diseased aorta. Aortic rupture frequently causes sudden death, with the mortality rate exceeding 50% even when the patient arrives at the hospital in time for surgical treatment. AAA poses a significant healthcare problem, affecting 6–9% of men over 65 years of age. In the United States, it is the tenth leading cause of

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death in men over 55 years of age [1]. Because AAA patients usually have no symptoms, the therapeutic goal is rupture prevention. The clinical strategy depends on the diameter of the aneurysm, which is the strongest predictor of rupture risk [2]. With aneurysms greater than 5.5 cm in diameter, the risk of rupture exceeds the risk for elective surgery. Thus, these large aneurysms are treated by surgical procedures which are currently the only established therapeutic option to prevent the aortic rupture. The diseased aorta can be replaced with an artificial graft by open surgery or by endovascular repair where an artificial graft is attached to a metal stent and inserted in the aorta through a catheter. In contrast, there is no effective therapy for small AAA. Graft replacement does not offer a survival advantage for small AAA [3, 4], and clinical trials to determine the effectiveness of endovascular repair for patients with small aneurysms are not yet complete [5]. Therefore, the standard practice for small aneurysms is "watchful waiting," in which periodical observations are made to assess AAA progression, until the risk of rupture reaches or exceeds the surgical risk. Thus, a nonsurgical therapy that slows progression of the disease would be a significant advance. An even greater advance would be a therapy that not only arrests disease progression but also induces healing and regression of the aneurysm.

Recently, we identified c-Jun N terminal kinase (JNK) as a key molecule in the pathogenesis of AAA [6]. JNK regulates various aspects of the molecular pathogenesis of AAA, promoting the destruction of extracellular matrix (ECM). Inhibition of JNK not only prevents the development of AAA in vivo but also causes regression of established AAA in animal models. Excellent textbooks and reviews of the AAA field are already available ([7, 8] among others). Rather than presenting a comprehensive review, this article summarizes current knowledge of the molecular pathogenesis of AAA as it relates to our study. Putative nonsurgical therapies for altering the pathology of AAA are described and discussed together with our recent findings.

Etiological considerations

Overview

Due to the silent nature of AAA, investigating when and how AAA develops is a challenge. Consequently, little is known about the initiation of AAA, but investigation has primarily consisted of case-control studies of genetic and environmental factors predisposing individuals to AAA. Although familial clustering of AAA has been reported and 15% of AAA patients has a family history of the disease [9], decades of studies reveal that AAA is most likely a polygenic disease under the influence of multiple environmental factors.

Genetic factors

As familial accumulation of AAA has been observed clinically, efforts have been made to identify genetic factors that predispose carriers to the development of AAA (reviewed in [10, 11]). Genome-wide screening in case-control studies has identified several genetic loci associated with AAA, including HLA class II, 19q13, and 4q31. The candidate gene approach has also revealed several genetic polymorphisms that predispose carriers to AAA development. These include polymorphisms of the genes for angiotensin-converting enzyme (ACE), matrix metalloproteinase (MMP)-9, plasminogen activator inhibitor-1 (PAI-1), and interleukin (IL)-10 among others. The reported odds ratios range from 1.34 (PAI-1) to 2.94 (MMP-9). These results have been difficult to replicate in some cases, as with other complex diseases, supporting the idea that AAA is multigenic and multifactorial.

Environmental factors

Smoking, age, gender, existence of coronary heart disease, hyperlipidemia, and hypertension all affect the development of AAA. Male gender is a strong risk factor, the effect of which may be mediated by multiple factors including genetic predisposition, hormonal environment, and anatomical factors such as relatively larger aortic diameter than female. Gender, as well as the remaining risk factors, is common to both AAA and atherosclerosis, and nearly all cases of AAA involve atherosclerotic changes. The causative relationship between atherosclerosis and AAA, however, is not firmly established. Indeed, diabetes mellitus, which is strongly linked to atherosclerosis, has repeatedly been shown to negatively correlate with AAA development and progression [9]. Thus, it seems that AAA and atherosclerosis are clinically distinct, although they share some common pathological features such as chronic inflammation and macrophage infiltration.

Chlamydia pneumoniae is a pathogen that is implicated in both AAA development and atherogenesis [9]. Its presumed role in AAA is based on the high prevalence of *C. pneumoniae* antibodies in the serum of AAA patients and on data showing greater progression of experimental AAA after *C. pneumoniae* infection. A clinical trial of macrolide roxithromycin for small AAA showed a beneficial effect of the antibiotic [12]. However, the pathogenetic role of *C. pneumoniae* is still elusive [12], in part, because not all AAA patients appear to be infected with *C. pneumoniae*, and infection of *C. pneumoniae* does not correlate with MMP production [9]. Thus, the effect of

macrolide may be attributable to its anti-inflammatory or antimicrobial activities [12, 13].

Etiology-oriented therapy

Because the etiology of AAA is largely unknown and probably multifactorial, development of etiology-oriented therapy has been difficult. Notable exceptions are certain monogenic diseases associated with AAA, including Marfan syndrome, Ehlers–Danlos syndrome type IV, and Loeys–Dietz syndrome. Although the genetic defects associated with these monogenic diseases are not yet correctable, a recent study demonstrated the therapeutic effectiveness of the angiotensin AT1 receptor antagonist that ameliorate overactive signaling of transforming growth factor-β (TGF-β) in a mouse model of Marfan syndrome [14]. Interestingly, it has been reported that ACE inhibitors retard the progression of experimental AAA [15] and may suppress aortic rupture in human AAA [16]. It remains to be seen whether AT1 signaling is involved in the etiology of AAA in general and AT1 antagonists are effective in treating human AAA; two reports have shown no beneficial effect of the AT1 antagonists on experimental or human AAA [15, 16].

Molecular pathogenesis of AAA

Overview

There has been extensive effort to unravel the molecular pathogenesis of AAA, understanding of which is requisite to the development of a nonsurgical therapy for the disease. Research has demonstrated the importance of chronic inflammation and degradation of ECM by various proteases to AAA. In addition, impairment of ECM biosynthesis is thought to play a role in the pathogenesis of AAA because AAA is accompanied by a progressive decrease in the number of vascular smooth muscle cells that normally synthesize ECM [17–21]. These pathological processes—inflammation, degradation of the ECM, and impairment of biosynthesis of the ECM—act in concert to promote the progressive destruction of the ECM. During chronic inflammation, inflammatory cells and interstitial cells secrete ECM-degrading proteases and pro-inflammatory cytokines; such cytokines further activate inflammatory signaling and may interfere with the normal biosynthesis of the ECM by interstitial cells. However, our knowledge of the molecular pathogenesis of AAA remains incomplete. For example, it is not known what triggers chronic inflammation and how it is maintained over a period of years or what interferes with the ordered biosynthesis of the ECM that should occur during tissue repair. In addition, the mechanisms for coordination of these events during AAA pathogenesis remain largely unknown.

Proteases

The mechanical strength of the aortic wall is maintained by the ECM, which is mainly composed of collagen and elastin fibers. The most prominent pathological feature of AAA is disruption of the ordered layers of the ECM, including the disappearance of elastic lamellae in the early stage of the disease. Disruption of elastin is sufficient for aneurysmal dilation of the aorta, and degradation of collagen is responsible for rupture [22, 23].

For these reasons, there has been a major effort to elucidate the mechanism for the degradation of the ECM, with a focus on elastolytic factors. This has led to the identification of various proteases in AAA tissue. Among them, the MMPs have drawn much attention, with MMP-9 and MMP-2 being the most extensively studied. A major breakthrough in AAA research was the finding that deletion of the genes for MMP-9 [24] and/or MMP-2 [25] completely protects mice from development of AAA. Subsequent to this finding, it was proposed that MMP inhibition is a potentially effective therapy for AAA.

Pro-inflammatory mediators

The chronic inflammation in AAA seems to be initiated and maintained by a complex interplay between innate and acquired immunity [26]. Infiltrating cellular components include macrophages, T cells, and B cells. Chronic inflammation is an essential component of AAA pathogenesis, as cytokine-stimulated macrophages are the major source of matrix-degrading enzymes such as MMP-9 and pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α.

The degradation products of elastin [27] or collagen [28] can initiate the inflammatory response, and cleavage of IL-8 by MMP-9 potentiates its ability to activate leukocytes [29]. These facts exemplify the intimate interplay between inflammatory signaling and MMP activities. However, these processes are experimentally separable: Mice deficient in MMP-9 and/or MMP-2 are protected from the development of AAA by infusion of elastase or $CaCl_2$ treatment of the aorta, but not protected from inflammatory responses [24, 25]. Doxycycline treatment inhibits the development of experimental AAA, but does not eliminate the inflammatory response [30], suggesting that the inflammatory response is maintained independently of MMP activities.

Various pro-inflammatory mediators are present in AAA tissue, including TNF-α, IL-1β, IL-6, and interferon (IFN)-γ [1]. In addition to these peptide mediators, lipid mediators

such as prostaglandin E2 [31–33] and leukotriene D4 (LTD4) [34] and the gaseous mediator nitric oxide [35, 36] have also been reported to play important roles in AAA. However, the precise roles of these inflammatory mediators and the relationships among them have not been elucidated and are likely to vary during the course of the disease.

These mediators affect not only inflammatory cells but also interstitial cells such as vascular smooth muscle cells, endothelial cells, and fibroblasts. For example, prostaglandin E2 inhibits the growth of vascular smooth muscle cells [32]. It was proposed that LTD4, a metabolic product of arachidonic acid 5-lipoxygenase (ALOX5), induces macrophage inflammatory protein-1α (MIP-1α) in macrophages and MIP-2 in endothelial cells, which in turn, recruit leukocytes into the aortic walls [34]. IFN-γ suppresses collagen I expression during tissue repair [37], and TNF-α suppresses the wound repair response of vascular smooth muscle cells [38] and decreases the expression of collagen type I and III in fibroblasts in a JNK-dependent manner [39]. Taken together, these findings suggest that inflammatory signaling suppresses normal ECM biosynthesis and tissue repair independent of ECM-degrading activities.

Impairment of ECM biosynthesis

In the healthy artery, interstitial cells such as vascular smooth muscle cells maintain the ordered structure of the ECM through active biosynthesis. Because vascular smooth muscle cells are depleted in human AAA, it has been hypothesized that impaired biosynthesis of the ECM plays a critical role in the pathogenesis of AAA [17–21].

Biosynthesis of collagen and elastin fibers, the major components of aortic ECM, is regulated at the level of expression as well as by post-translational modifications such as prolyl and lysyl hydroxylation and lysyl oxidation. These post-translational modifications are catalyzed by prolyl 4-hydroxylase (P4H), pro-collagen lysyl hydroxylase (PLOD), and lysyl oxidase (LOX). These ECM biosynthetic enzymes are essential for stable trimerization of collagen fibrils and cross-linking of collagen and elastin fibrils to form durable fibers. The critical role of ECM biosynthesis in maintaining the integrity of aortic walls is demonstrated by the finding that disruption of the LOX gene leads to aneurysm formation and aortic rupture [20]. In addition, it has been reported that expression of LOX is reduced in aneurysm-prone mice [18] and in experimental AAA [6, 17]. This may explain the ineffective maturation of the ECM in human AAA [19, 21]. A PLOD1 mutation in patients with Ehlers–Danlos syndrome causes a high risk of arterial rupture [40]. Taken together, these observations demonstrate the profound effect of impaired ECM biosynthesis on the integrity of arterial walls. Indeed, we found that adenoviral expression of exogenous LOX inhibits the development of experimental AAA in which endogenous LOX activity is suppressed [6].

Therapeutic targets in AAA

Overview

Based on their proposed roles in the pathogenesis of AAA, chronic inflammation, degradation of ECM by MMPs, and impaired biosynthesis of the ECM have been targeted with therapeutic interventions. These therapeutic strategies have proven effective to various extents in preventing the progression of experimental AAA, providing strong support for the working model of AAA pathogenesis.

Inhibition of MMP

MMP inhibition is the therapeutic strategy that has been most extensively explored in clinical trials involving AAA [41]. This is partly because of the prominent role of MMPs in AAA pathogenesis and partly because of the clinical availability of the MMP inhibitor doxycycline. Inhibition of MMP prevents the development of AAA in animal models; this has been demonstrated using both doxycycline [17, 30, 42] and another MMP inhibitor, BB-94 [43]. This underscores the critical role of MMPs in the pathogenesis of AAA. In addition, clinical trials of doxycycline show the feasibility of this approach and some favorable effects [44, 45], although these studies were designed to evaluate the safety of doxycycline and the therapeutic effect was not as clear as that demonstrated in animal models.

Another experimental approach for inhibiting MMP activity is the forced expression of tissue inhibitor of metalloproteinases (TIMP)-1 in smooth muscle cells and the seeding of these cells into an aortic xenograft. This has been shown to stabilize grafts and prevent rupture [46]. However, clinical application of the gene transfer approach awaits further technical advancements such as improved vector design and improved control of gene expression. The enduring nature of gene transfer may have advantages over conventional pharmacotherapy, as a one-time treatment may last for years. However, this may also be a disadvantage if adverse effects occur due to uncontrolled gene expression or faulty vector function.

Anti-inflammatory therapy

Experiments in animal models of AAA have demonstrated that immunosuppression with prednisone, cyclosporine [47], or rapamycin [48] prevents the development of experimental AAA induced by elastase infusion. However, general immunosuppression by steroids or cyclosporine

may not be sufficient to suppress the progression of AAA in humans [9], suggesting that more specific targets need to be identified.

Inhibition of the pro-inflammatory cytokine TNF-α [49] or the chemokine MCP-1 [50] suppresses the development of AAA in animal models. Interestingly, inhibition of IL-1β, another potent pro-inflammatory cytokine present in human AAA, is ineffective [49], suggesting that the various pro-inflammatory mediators play specialized roles in this context. More recently, it was shown that inhibition of NFκB, a critical transcription factor in cytokine signal transduction, by NFκB/Ets decoy oligonucleotide [51] or by a chemical inhibitor [52], prevents the development of experimental AAA.

The nonselective cyclooxygenase inhibitor indomethacin [31] and the selective cyclooxygenase-2 inhibitor celecoxib [33] have been reported to prevent AAA in animal models, demonstrating the critical roles of arachidonate metabolites in the development of AAA. A case-control study showed that AAA patients taking nonsteroidal anti-inflammatory drugs (NSAIDs) have aneurysms with a lower expansion rate than those in patients who do not take NSAIDs [32], suggesting that this class of drugs may be beneficial in this population. The gaseous mediator nitric oxide may be a therapeutic target, as it directly activates MMP-9 [53]. Furthermore, an inhibitor of inducible nitric oxide synthase (iNOS) prevents elastase-induced AAA development in animal models [36]. However, targeted deletion of the iNOS gene does not protect mice from elastase-induced AAA [54], suggesting that nitric oxide plays a context-specific role.

Other pharmacological interventions, including 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors (statins) [55–58] and ACE inhibitors [15, 16, 59], have been reported to prevent the progression of AAA possibly by suppressing inflammation. ACE inhibition is a promising therapy; a large-scale case-control study showed that treatment with ACE inhibitors is associated with reduced AAA rupture [16]. In contrast, AT1 receptor blockers do not show a beneficial effect with regard to AAA [15, 16], suggesting that the renin–angiotensin system plays a complex role in the pathogenesis of AAA. Statins, in addition to their lipid-lowering effect, suppress inflammatory signaling possibly by inhibiting the Rho family of small G-proteins. Case-control studies have demonstrated an association between use of statins and reduction in the levels of some MMPs [60] as well as a lower rate of expansion of AAA [58, 61]. ACE inhibitors and statins would merit larger systematic randomized clinical trials to confirm their benefits and to determine the optimal regimen for preventing the expansion and rupture of AAA.

MMP inhibition alone does not suppress infiltration of inflammatory cells [24, 25, 30]. Conversely, suppression of inflammatory signaling does not always reduce levels of MMP [31, 36]. Because pro-inflammatory mediators may promote the progression of AAA independent of MMPs, anti-inflammatory therapies may be a good strategy for treating AAA possibly in combination with MMP inhibition.

Stabilization of the ECM

ECM biosynthesis and stabilization have not been studied as extensively for AAA therapy as have MMP inhibitors and pro-inflammatory mediators. One reason for this is that although impairment of ECM biosynthesis has been demonstrated in experimental animals [17, 18] and is suspected in clinical settings [19, 21], the causal role of the impaired ECM biosynthesis in AAA pathogenesis is unclear. Other reasons include lack of knowledge regarding the molecular mechanisms underlying impaired ECM biosynthesis and the lack of reagents that specifically promote the biosynthesis of well-ordered and durable ECM. One proposed method for promoting ECM biosynthesis is seeding smooth muscle cells in an AAA model induced by xenografting a guinea pig aorta into a rat. Seeding of syngeneic rat vascular smooth muscle cells stabilizes aortic tissue [62], and this effect is enhanced by adenoviral expression of TGF-β1 [63]. These findings support the feasibility of therapy that increases ECM biosynthesis. This approach may be as effective as, and perhaps complementary to, inhibition of MMP. However, activation of the TGF-β pathway must be approached cautiously. Recent studies indicate that overactivation of TGF-β signaling in the aortic wall exacerbates inflammation and causes progression of AAA in humans [64, 65] and in an animal model of Marfan syndrome [14].

Regression of AAA via pharmacotherapy

Overview

Regression of AAA via pharmacotherapy, if clinically applicable, would offer a therapeutic option for patients with small aneurysms. Patients with larger aneurysms and a high risk of rupture might not immediately benefit from pharmacotherapy, as it would not instantaneously reduce rupture risk. However, pharmacotherapy could be used to treat patients with both high rupture risk and high surgical risk, for example, patients with AAA and multiple comorbidities such as pulmonary failure and renal failure.

It is becoming increasingly clear that the tissue degeneration observed in AAA is a consequence of an imbalance between tissue degradation and repair. However, active tissue repair is ongoing in aneurysms, as evidenced by increased expression of tropoelastin [21] and collagen [66].

It is thought that inhibition of MMP [17] or endovascular seeding of vascular smooth muscle cells [62] may sometimes cause regression of experimental AAA. Tissue repair in AAA also manifests clinically. Endovascular repair by insertion of stent-grafts often causes shrinkage of the aneurysmal aorta [67] and remission of the tissue degradation process [68], suggesting that regression of AAA is possible in certain situations.

As discussed above, the current view of AAA pathogenesis is that it is the result of a complex interplay among distinct pathological processes: chronic inflammation, ECM degradation, and impaired ECM biosynthesis. Each pathological process involves a network of signaling molecules and effector molecules whose levels of expression are differentially regulated [69–71]. In this regard, AAA progression is a highly ordered and regulated process. Although intervention in each process has proven effective in preventing the development or progression of experimental AAA, none of these therapeutic strategies reverses disease progression. This suggests that either AAA is a fundamentally irreversible destructive process or that we do not yet understand the key molecular mechanisms that prevent the healing of AAA. Alternatively, several simultaneous interventions may be required to induce efficient healing.

Although the mechanisms responsible for initiation of AAA are not known and may well be heterogeneous, the clinical course and pathology are known and fairly predictable. This suggests that various environmental and genetic stimuli may activate a final common pathway or "node" in the AAA signaling network that orchestrates chronic inflammation, ECM degradation, and impairment of ECM biosynthesis. Such a node, if it exists, would be an ideal therapeutic target for affecting multiple pathological processes and promoting healing of AAA.

Identification of JNK as a therapeutic target

In an attempt to identify a key therapeutic target for AAA, we assessed the phosphorylation status of signaling molecules in human AAA samples, including JNK, p38, extracellular signal-regulated kinase (ERK), signal transducer and activator of transcription (STAT) 2, STAT3, activating transcription factor (ATF) 2, inhibitor of κ light polypeptide gene enhancer in B cells (IκB)-α, Akt, glycogen synthase kinase (GSK) 3β, 70 kDa ribosomal protein S6 kinase (p70S6K), and 90 kDa ribosomal protein S6 kinase (p90RSK), all of which are regulated by reversible phosphorylation. Screening revealed increased phosphorylation of JNK, ERK, and STAT3 compared with a non-aneurysmal control. Of these proteins, JNK was a prime candidate because it is activated by stimuli that have been implicated in AAA pathogenesis: mechanical stress, oxidative stress, angiotensin II, TNF-α, IL-1β, IL-6, and IFN-γ. In addition, JNK induces MMP-9 in several cell lines [72–75]. A transcriptome analysis in vascular smooth muscle cells revealed that JNK upregulates pro-inflammatory molecules such as IL-1α, iNOS, ALOX5-activating protein (ALOX5AP), and the ECM-degrading MMP-9. At the same time, JNK downregulates TIMP-3, an endogenous inhibitor of MMPs, and critical ECM biosynthetic enzymes including PLOD1, P4HA1, and LOX. Therefore, JNK is an ideal therapeutic target because it coordinately regulates multiple pathological processes involved in tissue degradation, thus, serving as a node in the AAA signaling network (Fig. 1).

JNK inhibition by SP600125 in vivo completely prevented the development of AAA in response to abluminal application of $CaCl_2$ in mouse aorta (Fig. 2). Importantly, JNK inhibition strongly suppressed macrophage infiltration of the periaortic tissue, whereas doxycycline did not [30], suggesting that JNK is critically involved in the pro-inflammatory signaling process [76, 77]. In vivo JNK inhibition was also effective in treating established AAA induced by $CaCl_2$ treatment in wild-type mice and by continuous infusion of angiotensin II in ApoE knockout mice [78]. SP600125 caused significant regression of established AAA and normalized tissue architecture, indicating that JNK inhibition is a promising therapeutic modality.

Implications and future directions

Despite decades of effort, the exact etiology of AAA remains elusive and is likely to be heterogeneous. However,

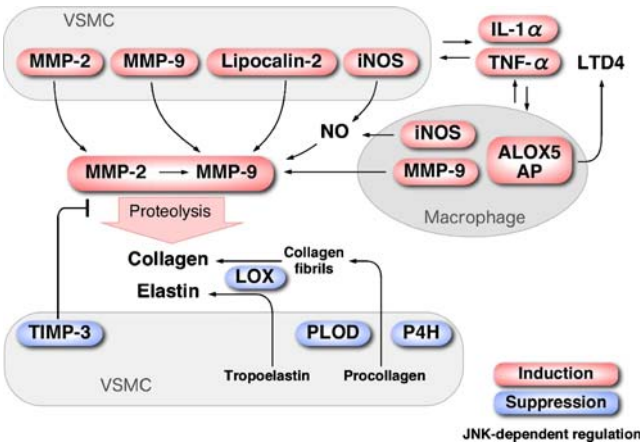

Fig. 1 JNK-regulated gene expression. JNK is activated in macrophages and in vascular smooth muscle cells (VSMCs) in AAA tissue. JNK induces the expression of MMPs and pro-inflammatory mediators, including TNF-α, IL-1α, and arachidonate 5-lipoxygenase-activating protein (ALOX5AP). Inducible nitric oxide synthase (*iNOS*) generates nitric oxide (*NO*), which activates MMP-9 by S-nitrosylation [53]. Lipocalin-2 binds to MMP-9 and maintains its activity [99]. In addition, JNK suppresses tissue inhibitor of metalloproteinase-3 (*TIMP*-3), an endogenous inhibitor of MMPs, and critical ECM biosynthetic enzymes, including prolyl 4-hydroxylase (*P4H*), pro-collagen lysyl hydroxylase (*PLOD*), and lysyl oxidase (*LOX*)

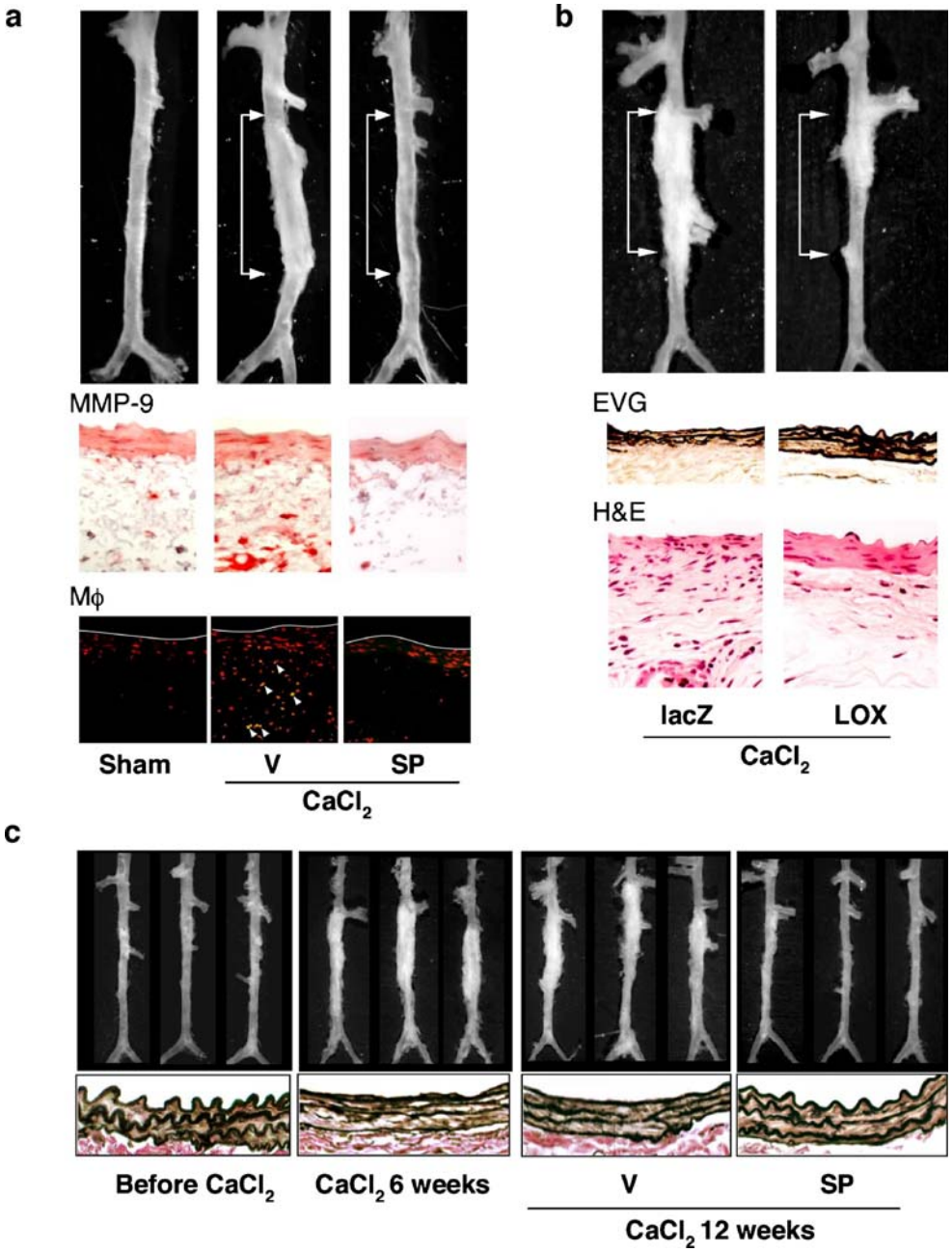

Fig. 2 JNK inhibition therapy for AAA. **a** $CaCl_2$ treatment of mouse aorta causes an increase in expression of MMP-9, infiltration of macrophages (Mϕ, *arrowheads*), and development of AAA after 10 weeks in mice treated with vehicle. Inhibition of JNK by SP600125 (*SP*) completely prevents development of AAA and suppresses MMP-9 and Mϕ infiltration. **b** Adenoviral gene transfer of LOX partially prevents the development of AAA. Compared to LacZ-transduced aorta (control), LOX-transduced aorta exhibit less disruption of elastic lamellae, as shown by elastica van Gieson (*EVG*) stain, and less cellular infiltration, as shown by hematoxylin–eosin (*H&E*) stain. **c** The mouse model of AAA was established 6 weeks after $CaCl_2$ treatment. After AAA was established, JNK inhibition was initiated via treatment with SP600125 (*SP*). After 6 weeks of SP600125 treatment, there was regression of AAA and repair of tissue architecture (modified from [6])

emerging evidence supports the notion that the vicious cycle of chronic inflammation and abnormal ECM metabolism is the final common pathway in the molecular pathogenesis of AAA. Central to this pathway is the signaling network, including JNK pathway, that coordinates inflammation and abnormal metabolism of the ECM. Pro-inflammatory mediators such as chemokines, cytokines, and angiotensin II cause infiltration and activation of inflammatory cells and increase in hemodynamic stress. This causes activation of the intracellular signaling network to dictate the destructive metabolism of the ECM and production of pro-inflammatory mediators. Abnormal ECM metabolism weakens the aortic wall, causing expansion of AAA and exposure of interstitial cells to higher mechanical and metabolic stress. This, in turn, exacerbates abnormal intracellular signaling. The central role of JNK in chronic inflammation and the abnormal metabolism of the ECM in AAA provides a framework for understanding the coordination of these pathological processes (Fig. 3) and an opportunity to develop a therapeutic intervention for reversing AAA.

A critical unanswered question is what triggers the abnormal activation of intracellular signaling and keeps it continuously active in human AAA. If the aforementioned vicious cycle maintains the chronic inflammation observed in AAA, what is the most effective point of intervention to

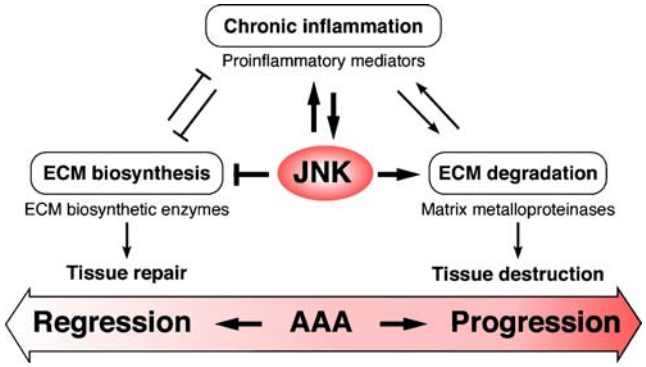

Fig. 3 The AAA signaling network. The current working hypothesis for the molecular pathogenesis of AAA is that JNK coordinates a pattern of gene expression that promotes tissue destruction and progression of AAA. Inhibition of JNK decreases chronic inflammation and degradation of the ECM and simultaneously allows recovery of ECM biosynthesis. Thus, JNK inhibition increases tissue repair and causes AAA to regress

end the cycle? Although our data support the JNK pathway as an important candidate, answering this question requires understanding of the complex network of inflammatory signaling in AAA and the relationship of the JNK pathway to other signaling pathways. For example, pro-inflammatory cytokines frequently activate both activator protein-1 (AP-1), the downstream target of the JNK pathway, and NFκB, and these transcription factors have been reported to synergistically activate downstream genes including MMP-9 [79] and pro-inflammatory cytokines [80, 81]. However, the JNK and NFκB pathways sometimes antagonize each other [82], exemplifying the complexity of the inflammatory signaling network. STAT3, another transcription factor activated in human AAA, may also cooperate with AP-1 in the IL-6-induced response to injury [83, 84] and in IL-17-induced expression of MMP-9 [85]. It remains to be seen whether intervention in these parallel signaling pathways promotes healing in AAA.

Concerning the molecular mechanisms by which JNK promotes the progression of AAA, pathological processes other than ECM metabolism may be involved. Numerous reports and our transcriptional profiling [6] indicate that JNK affects many cellular processes [76, 77], including cell proliferation, cell differentiation, metabolic pathways, cell migration [86], cell survival, and cell death [87, 88]. JNK may also be involved in the loss of vascular smooth muscle cells in the medial layer of the diseased aorta. Different JNK isoforms play different roles in various physiological [89, 90] and pathophysiological settings [91, 92], including AAA (KY and HA, unpublished observation). Thus, the isoform-specific role of JNK in AAA should be explored in detail. Isoform-specific inhibition of JNK may circumvent the possible adverse effects of systemic inhibition of all JNK isoforms.

With regard to reducing the side-effects of pharmacotherapy, local delivery of pharmacological agents should be considered. The efficacy of this approach was demonstrated in a recent study using doxycycline and a rat model of AAA [93]. The rapid advancement of drug-eluting stent technology and the growing prevalence of endovascular repair make stent-grafts an obvious choice for drug delivery. Regression of AAA by pharmacotherapy, if validated in humans, will improve graft fitting. JNK inhibition may also prevent thrombus formation, as recently demonstrated for coronary stent thrombosis [94]. Although the intramural thrombus may pose a barrier to the passive diffusion of pharmacological agents from an eluting stent-graft, the thrombus per se may be a therapeutic target [95, 96] because the mural thrombus may promote the progression and rupture of AAA [97] by actively producing proteases [98]. On the other hand, systemically administrated pharmacological agents may have better access to the adventitia, the site of inflammation in AAA, than agents released from inside the aorta. Further progress with drug delivery systems, including drug-eluting stent-grafts, and validation of their efficacy in vivo will advance the development of less invasive therapeutic strategies for AAA.

Conclusions

We are witnessing a rapid progress in understanding the molecular pathogenesis of AAA. This led to the proposal of promising pharmacotherapy with statins, ACE inhibitors, and JNK inhibitor among others. Accumulating knowledge obtained in the research of AAA may also create new avenues for improved therapeutic strategies not only for AAA but also for other diseases. Chronic inflammation and destructive remodeling of the ECM are widely observed in a variety of diseases, including rheumatoid arthritis, osteoarthritis, valvular heart disease, vulnerable atheromatous plaque, chronic obstructive pulmonary disease, and cancer. There may be a common abnormality of cellular signaling that positively regulates the destructive processes underlying these diseases and AAA. Then, how does the signaling network that is centered on the JNK pathway fit into the molecular pathogenesis of these diseases? How can therapeutic manipulation of the signaling pathways decrease the chronic inflammation and progressive tissue destruction observed in all of these pathologies? We are optimistic that the answers to these questions will lead to better future treatment of AAA and other diseases.

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