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Regression of Abdominal Aortic Aneurysms by Pharmacological Inhibition of c-Jun N-terminal Kinase

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Abstract Abdominal aortic aneurysm (AAA) is characterized by chronic inflammation and proteolytic degradation of the extracellular matrix (ECM). Recently, we identified c-Jun N-terminal kinase (JNK) as a key molecule in the pathogenesis of AAA. Human AAA tissue showed a high level of active JNK. In *in vitro* studies, JNK not only activated the expression of ECM-degrading enzymes, but also suppressed the expression of enzymes for ECM biosynthesis. Finally, pharmacological inhibition of JNK not only prevented the development of AAA but also caused the regression of established AAA in mouse models. Thus, pharmacological inhibition of JNK may provide a novel therapeutic option for treatment of human AAA.

Key words: abdominal aortic aneurysm, c-Jun N-terminal kinase, pharmacological therapy

Introduction

Abdominal aortic aneurysm (AAA) is a common disease that, when surgical treatment is inapplicable, results in rupture of the aorta with high mortality. Although a pharmacologic therapy for AAA is eagerly awaited, the degradation of the extracellular matrix (ECM) and the destruction of the aortic walls in AAA have been considered irreversible processes. We found, for the first time, the regression of AAA by a pharmacological therapy, and demonstrated our findings in a paper entitled "Regression of abdominal aortic aneurysm by inhibition of c-Jun N-terminal kinase," published in *Nature Medicine* in 2005.[1] Here, we summarize the paper, which received the Sojinkai award in 2007.

Activation of JNK in human AAA tissue

We observed a significant increase in the activity of c-Jun N-terminal kinase (JNK), a stress-activated protein kinase, in human AAA walls, as well as its linear correlation with matrix metalloproteinase (MMP)-9 expression. Other mitogen-activated protein kinases, namely ERK and p38, did not show such a correlation. Active JNK colocalized with MMP-9 in both macrophages and vascular smooth muscle cells (VSMCs) in human AAA.

Role of JNK in ECM metabolism

To obtain insight into the role of JNK in AAA, we screened for JNK-dependent genes in VSMCs using a DNA microarray. We

adopted the gain-of-function and loss-of-function strategies for the JNK pathway in the transcriptional profiling in rat VSMCs to identify genes regulated by JNK. According to our analysis, JNK upregulates the positive regulators of ECM-degrading MMP-9, including inducible nitric oxide synthase, TrkC, interleukin-1alpha, lipocalin-2 and MMP-9 itself. In fact, we found that SP600125, a specific JNK inhibitor, significantly suppressed the secretion of MMP-9 and MMP-2 and prevented collagen degradation in human AAA walls in *ex vivo* culture. Simultaneously, JNK downregulates the crucial enzymes for ECM biosynthesis, such as lysyl hydroxylase (PLOD, essential for the stability of collagen fibers), prolyl 4-hydroxylase (P4H, the rate-limiting enzyme for collagen biosynthesis) and lysyl oxidase (LOX, responsible for the cross-linking of collagen and elastin fibers).

Therefore, JNK is an ideal therapeutic target for AAA because it promotes ECM degradation and concurrently suppresses ECM biosynthesis, thus dictating the ECM metabolism toward degradation in the pathogenesis of AAA.

Pharmacologic treatment of AAA in mice

These findings led us to hypothesize that JNK is essential for development of AAA *in vivo*. To test this hypothesis, we treated a calcium-induced AAA model in mice with SP600125. Pharmacologic inhibition of JNK by SP600125 suppressed the development of experimental AAA, with reduced cellular infiltration, reduced activities of JNK and MMP-9 and preserved architecture of the aortic wall. These data demonstrate the central role of JNK in the development of AAA *in vivo*.

We next investigated the role of ECM biosynthesis in AAA development. During development of the calcium-induced AAA model, the local activity of LOX, a critical enzyme for stabilization of collagen and elastin fibers, decreased below the basal level while the serum level of MMP-9 was elevated. These findings suggested that the ECM metabolism shifts to degradation during AAA development. To enhance ECM biosynthesis, we performed local gene delivery of LOX using an adenoviral vector. Local overexpression of LOX attenuated the development of AAA and preserved aortic tissue architecture. These data suggest that impaired ECM biosynthesis contributes to the pathogenesis of AAA.

Because we found that JNK not only activates the degradation pathway, but also suppresses the biosynthetic pathway of ECM in AAA, we hypothesized that JNK inhibition might cause regression of AAA, which requires tissue repair including active ECM biosynthesis (Fig. 1). We found that inhibition of JNK by SP600125 caused a significant reduction in aneurysm diameters in established calcium-induced AAA. Surprisingly, SP600125 was effective in normalizing tissue architecture. Furthermore, serial echographic studies demonstrated that treatment with SP600125 significantly reduced the aneurysm diameter in another AAA model developed in ApoE-null mice by continuous angiotensin II infusion. These data indicated that pharmacologic treatment with the JNK inhibitor caused healing of aneurysmal tissue and regression of established AAA.

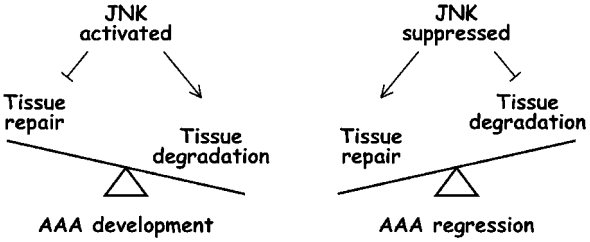

Fig. 1 The role of JNK in development (left panel) and regression (right panel) of AAA. JNK regulates the balance between tissue repair and degradation.

Conclusions

Our findings indicate that JNK activation causes abnormal ECM metabolism in the pathogenesis of AAA and that inhibition of JNK not only prevents ECM destruction but also restores its biosynthesis. Thus, JNK may represent a novel therapeutic target for AAA. This demonstration of regression of established AAA by a pharmacological therapy has changed our view on therapeutic

strategies for AAA.[2)3)]

Acknowledgments

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