A Novel Mechanism Underlying Inflammatory Smooth Muscle Phenotype in Abdominal Aortic Aneurysm

Dunpeng Cai, Chenming Sun, Gui Zhang, Xingyi Que, Ken Fujise, Neal L Weintraub, Shi-You Chen

RATIONALE: Abdominal aortic aneurysm (AAA) is a permanent and localized dilatation of abdominal aorta with potentially fatal consequence of aortic rupture. No effective pharmacological approach has been identified to limit AAA progression and rupture. AAA is characterized by extensive aortic wall matrix degradation that contributes to arterial wall remodeling and eventual rupture, in which smooth muscle cell (SMC) phenotypic transition and MMPs (matrix metalloproteinases), especially MMP2 (matrix metalloproteinase-2) and MMP9, play critical roles.

OBJECTIVE: Our previous study showed that ADAR1 (adenosine deaminases acting on RNA 1) regulates SMC phenotype, which prompted us to study if ADAR1 is involved in AAA development.

METHODS AND RESULTS: We used Ang II (angiotensin II) infusion ApoE−/− mouse model combined with ADAR1 global and SMC-specific knockout to study the role of ADAR1 in AAA formation/dissection. Aortic transplantation was conducted to determine the importance of vascular cell ADAR1 in AAA development/dissection. Primary cultured SMC were used to study how ADAR1 regulates the inflammatory SMC phenotype and MMP production/activity. Patient specimens were obtained to investigate the relevance of ADAR1 expression to human AAA disease. ADAR1 was induced in abdominal aortic SMC in both mouse and human AAA tissues. Heterozygous knockout of ADAR1 diminished the Ang II–induced AAA/dissection in ApoE−/− mice. Mouse aortic transplantation showed that ADAR1 in vascular cells was essential for AAA formation. SMC-specific ADAR1 knockout reduced experimental AAA formation/dissection. Mechanistically, ADAR1 interacted with HuR (human antigen R) to increase the stability of MMP2 and MMP9 mRNA, leading to increased MMP levels and activities.

CONCLUSIONS: ADAR1 is novel regulator of AAA development/dissection, and thus may represent a potential new therapeutic target to hinder AAA growth and rupture.

GRAPHIC ABSTRACT: An online graphic abstract is available for this article.

Key Words: angiotensin II ♦ aortic aneurysm, abdominal ♦ interleukin ♦ matrix metalloproteinase ♦ phenotype
What Is Known?

- Abdominal aortic aneurysm (AAA) can have high morbidity and mortality associated with aorta rupture and dissection, especially in the elderly population. Pharmacological approaches to limit AAA progression and rupture have thus far proven ineffective.
- Vascular smooth muscle cell (SMC) phenotypic modulation, particularly the inflammatory SMCs play critical roles in AAA development.
- ADAR1 (adenosine deaminases acting on RNA 1) regulates RNA stability, mRNA splicing, microRNA processing, and RNA storage, etc. ADAR1 is upregulated in vascular pathologies such as atherosclerosis and neointimal SMC. ADAR1 promotes neointima formation following vascular injury.

What New Information Does This Article Contribute?

- ADAR1 is significantly upregulated in both mouse and human AAA lesions, especially in aortic SMCs, which contributes to the formation of the inflammatory SMC phenotype.
- ADAR1 deficiency either globally or specifically in SMCs diminished angiotensin II–induced AAA formation/dissection in ApoE−/− mice.
- ADAR1 promotes MMP (matrix metalloproteinase) 2 and MMP9 production/activities by interacting with human antigen R to increase MMP2 (matrix metalloproteinase-2) and MMP9 mRNA stability, leading to elastin degradation and AAA formation/dissection.

Nonstandard Abbreviations and Acronyms

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAA</td>
<td>abdominal aortic aneurysm</td>
</tr>
<tr>
<td>ACTA2</td>
<td>actin alpha 2, smooth muscle</td>
</tr>
<tr>
<td>ADAR1</td>
<td>adenosine deaminases acting on RNA 1</td>
</tr>
<tr>
<td>Ang II</td>
<td>angiotensin II</td>
</tr>
<tr>
<td>ECM</td>
<td>extracellular matrix</td>
</tr>
<tr>
<td>HuR</td>
<td>human antigen R</td>
</tr>
<tr>
<td>IL</td>
<td>interleukin</td>
</tr>
<tr>
<td>MMP</td>
<td>matrix metalloproteinase</td>
</tr>
<tr>
<td>MYH11</td>
<td>myosin heavy chain 11</td>
</tr>
<tr>
<td>VCAM-1</td>
<td>vascular cell adhesion molecule-1</td>
</tr>
</tbody>
</table>

AAA is a progressive vascular disease with >150,000 new cases and 10,000 deaths annually in the United States. Both open surgical and endovascular repair are associated with significant short- and long-term morbidity and mortality. Currently, there is no effective pharmacological treatment for AAA. Our previous study discovered that ADAR1 promotes SMC phenotypic modulation and artery remodeling following vascular injury. However, the goal of these studies was focused on SMC marker gene expression without analyzing the function of ADAR1 in the induction of inflammatory SMCs and aneurysm formation. In this study, by using global and SMC-specific deficient mice, aorta transplantation, and angiotensin II–induced AAA model, we show that ADAR1 is a novel essential regulator for the inflammatory SMC phenotype and AAA development. We further show that ADAR1 promotes SMC inflammation and AAA formation/dissection by stabilizing MMP2 and MMP9 mRNA and consequently increasing MMP production and activities, causing extracellular matrix degradation and weakening aorta wall. These findings provide key insights into the mechanism of AAA development and identify a potential new therapeutic target to hinder AAA growth and rupture.
AAA formation/dissection. Mechanistic studies identified a new role for ADAR1 to increase MMP2 and MMP9 mRNA stability by interacting with HuR (human antigen R), leading to increased MMP levels and activities.

METHODS
Data Availability
The detailed experimental materials and methods are available in the Data Supplement. The authors declare that the majority of supporting data are presented within this article and in the Data Supplement. The source data for the figures and the data that are not shown are available from the corresponding author upon reasonable request.

RESULTS
ADAR1 Expression Is Increased in Mouse and Human AAA Lesions
To test if ADAR1 is involved in the development of AAA, we used a well-established experimental aneurysm model that was generated by chronic infusion of Ang II (angiotensin II) into ApoE knockout (ApoE−/−) mice.10 Although immunostaining showed that ADAR1 was barely detectable in control aortas, consistent with our previous observation,15 it was significantly induced in AAA lesions (Figure 1A). Importantly, ADAR1 largely colocalized with ACTA2-positive cells in the media layer, indicating a potential role of SMC ADAR1 in AAA formation (Figure 1A and 1B). Both ADAR1 isoforms (p150 [150kd] and p110 [110kd]) were significantly upregulated in the AAA lesions as detected by Western blotting (Figure 1C and 1D). More significantly, ADAR1 was also upregulated in human AAA tissues and was largely colocalized with SMC (Figure 1E and 1F). Similar to the expression pattern in mouse AAA, both ADAR1 isoforms were significantly elevated in human AAA lesions (Figure 1G and 1H). These data suggest that ADAR1 may be involved in the AAA development in both mouse and human patients.

ADAR1 Deficiency Attenuates AAA Formation
To determine if ADAR1 is important for AAA development, we generated ADAR1 deficient mice in ApoE−/− background. Due to embryonic lethality of the homozygous ADAR1 knockout, ADAR1 heterozygous knockout mice (ADAR1−/−;ApoE−/−) were compared with ApoE−/− mice to determine the role of ADAR1 in AAA. Whereas ApoE−/− mice infused with Ang II for 28 days developed significant aortic dilation/dissection, ADAR1−/− mice were mostly protected against aneurysm formation (Figure 2A through 2E); blood pressure was similar in Ang II–infused ApoE−/− and ADAR1−/−;ApoE−/− mice. The incidence of AAA was reduced from 75% in ApoE−/− mice to 20% in ADAR1−/−;ApoE−/− mice. There was a 10% mortality rate in ApoE−/− mice infused with Ang II, but no mortality was observed in ADAR1−/−;ApoE−/− mice. To accurately measure the maximal aorta diameter in vivo, B mode ultrasound of abdominal aorta was performed (both longitudinally and transversely) in mice infused with saline or Ang II for 28 days. As shown in Figure 2B through 2D, Ang II infusion increased aortic diameter in ApoE−/− mice infused with Ang II, which was significantly blunted in the ADAR1−/− mice. The alterations of diameters were also confirmed by measuring the aortas and AAA sizes ex vivo (Figure 1A in the Data Supplement). These results demonstrated that ADAR1 plays an essential role in AAA development.

Medial degeneration and elastin degradation are hallmarks for AAA development, contributing to both aortic dilation and dissection. Hematoxylin and eosin staining showed that Ang II infusion caused marked aortic media degeneration/dissection and consequently intraluminal thrombus in ApoE−/− mice, but no statistical difference was observed in ADAR1−/−;ApoE−/− mice (Figure 2E). Consistently, elastin fragmentation, determined by Verhoeff elastic staining to quantify the elastin degradation index,17 was also markedly increased in ApoE−/− mice, but no statistical difference was observed the ADAR1−/− mice (Figure 2E and 2F).

ADAR1 Deficiency Reduces the Expression and Activities of MMP2 and MMP9 in AAA Lesions
MMP2 and MMP9 play critical roles in AAA formation/dissection and are largely responsible for elastin degradation. Consistent with previous studies,16,18,19 we observed a marked increase in both pro-MMP2 and pro-MMP9 and cleaved MMP2 and MMP9 protein levels in the abdominal aortas of Ang II–infused ApoE−/− mice. However, Ang II–infused ADAR1−/−;ApoE−/− mice exhibited no statistical difference in aortic MMP2 or MMP9 levels (Figure 3A through 3C). Because MMPs, especially MMP2, are expressed by phenotypically modulated SMC,20 we evaluated SMC phenotype in AAA tissues. As shown in Figure 3A, 3D, and 3E, in ApoE−/− mice, Ang II infusion led to increased expression of VCAM-1 (vascular cell adhesion molecule-1) but decreased expression of smooth muscle markers ACTA2 (actin alpha 2, smooth muscle) and calponin in the abdominal aorta, consistent with SMC phenotypic modulation. However, the expression of these markers was unaffected by Ang II infusion into ADAR1−/−;ApoE−/− mice (Figure 3A, 3D, and 3E), suggesting that ADAR1 deficiency prevented inflammatory SMC phenotypic modulation during AAA development. To examine MMP activity in AAA tissues, in situ zymography was performed. Ang II infusion resulted in significantly increased MMP activity in abdominal aortas of ApoE−/− mice as indicated by the green signal (Figure 3F), but no statistical difference was observed in ADAR1−/−;ApoE−/− mice (Figure 3F and 3G).
To evaluate the effect of ADAR1 deficiency on MMP2 and MMP9 activities individually, gelatin gel zymography was performed on aortic lysates. The activity of both MMP2 and MMP9 was significantly increased by Ang II in ApoE−/− mice, but no statistical difference was observed in ADAR1−/−;ApoE−/− mice (Figure 3H through 3J). These results suggest that ADAR1 deficiency modulated both MMP2 and MMP9 expression/activities in the context of AAA formation.

Vascular Cell ADAR1 Is Essential for AAA Formation

To test if ADAR1 expressed in vascular cells is essential for AAA formation, we generated a heterotopic transplantation model by grafting abdominal aorta of ApoE−/− or ADAR1−/−;ApoE−/− mice to recipient ApoE−/− mice (Figure 4A). The recipient aorta was ligated to detour the blood flow to the donor aorta as previously described. One week later, Ang II was infused for 28 days, and ultrasound was performed to measure the diameter of the donor aorta (Figure 4B). The diameter of transplanted ADAR1−/− aortas was significantly less than ApoE−/− mouse aortas (Figure 4C). Hematoxylin and eosin and Verhoeff elastic staining of the aorta sections showed significant dilation, media degeneration, and elastin fragmentation in transplanted ApoE−/− mouse aorta. However, these defects were attenuated in the transplanted ADAR1−/− aorta (Figure 4D and 4E). As a result, the elastin degradation index was significantly reduced in the transplanted ADAR1−/− aorta (Figure 4F). Moreover, the prevalence of AAA formation in the transplanted ADAR1−/− aorta was also significantly lower than the wild-type aorta (Figure 4G). These results indicate that vascular cell ADAR1 is vital for AAA formation in Ang II–infused ApoE−/− mice.
ADAR1 Deficiency in Smooth Muscle Ameliorates AAA Formation

Because ADAR1 has been shown to modulate SMC phenotype and is prominently expressed in medial SMCs in AAA tissues (Figure 1), we generated ADAR1 SMC-specific knockout mice by crossing ADAR1fl/fl mice with Mhy11-CreERT;ApoE−/− mice. Because homozygous ADAR1 deletion in SMC is lethal within 7 days after tamoxifen-induced Cre recombination (data not shown), ADAR1 SMC heterozygous knockout (ADAR1sm−/−) mice were employed. Whereas Mhy11-CreERT;ApoE−/− mice injected with tamoxifen and infused with Ang II for 28 days readily developed AAA with a 10% mortality rate, ADAR1sm−/− mice were resistant to aneurysm formation without any lethality (Figure 5A). The AAA incidence was reduced from 75% in ApoE−/− mice to 25% in ADAR1sm−/− mice. These findings were corroborated by ultrasound data, which confirmed a reduction in maximal aortic diameter in Ang II–infused ADAR1sm−/− mice as compared to control ApoE−/− mice (Figure 5B through 5D and Figure 1B in the Data Supplement). Histological
ADAR1 (adenosine deaminases acting on RNA 1) promoted MMP (matrix metalloproteinase)2 and MMP9 production and their activity in abdominal aortic aneurysm (AAA) lesions.

**Figure 3.** ADAR1 (adenosine deaminases acting on RNA 1) promoted MMP (matrix metalloproteinase)2 and MMP9 production and their activity in abdominal aortic aneurysm (AAA) lesions.

A–E, ApoE−/− and ADAR1−/− mice in ApoE−/− background were infused with saline or Ang II (angiotensin II; 1000 ng/[kg·min]) for 14 d. The protein levels of pre-MMP2 (matrix metalloproteinase-2) and active MMP2 (B), MMP9 (C), VCAM-1 (vascular cell adhesion molecule-1; D), ACTA (smooth muscle α-actin) 2, and calponin (E) were detected by Western blot and quantified by normalizing to GAPDH. *P=2.34×10−2 for pre-MMP2, 2.70×10−2 for cleaved-MMP2 (B), 1.87×10−2 for pre-MMP9, 2.87×10−2 for cleaved-MMP9 (C), 1.80×10−2 (D), 2.40×10−5 for ACTA2 (actin alpha 2, smooth muscle), 4.40×10−5 for calponin (E) vs wild-type (WT) with saline infusion, respectively (n=6); **P=3.31×10−2 for pre-MMP2, 2.40×10−2 for cleaved-MMP2 (B), 8.70×10−2 for pre-MMP9, 7.60×10−2 for cleaved-MMP9 (C), 2.10×10−2 (D), 3.20×10−5 for ACTA2, 4.20×10−5 for calponin (E) vs ApoE−/− mice with Ang II infusion, respectively (n=6).

F–G, In situ zymography of aorta showing increased MMP activity in Ang II–infused ApoE−/− mouse aorta, which was mitigated in ADAR1−/− aorta. Green fluorescence indicates MMP activities, which were quantified by normalizing to the fluorescent signal intensity in saline-treated mice. Nuclei were stained with DAPI. Scale bar=30 μm. *P=2.80×10−4 vs WT with saline infusion; **P=3.60×10−2 vs ApoE−/− mice with Ang II infusion, n=6. H–J, Aorta homogenates of ApoE−/− and ADAR1−/−; ApoE−/− mice infused with saline or Ang II for 14 d were prepared, and MMP2 and MMP9 activities were examined by gelatin zymography (H), measured by densitometry, and quantified by normalizing to the saline-infused group (I–J). *P=3.60×10−2 (I) and 2.90×10−2 (J) vs ApoE−/− mice with saline infusion, respectively (n=6); **P=3.30×10−2 (I) and 1.30×10−2 (J) vs ApoE−/− mice with Ang II infusion, respectively (n=6). All protein levels or activities were shown as fold inductions relative to the mean value of saline-infused ApoE−/− mouse group, which was set as 1 in each comparison. Kruskal-Wallis test with Dunn multiple comparisons tests were performed to determine statistical difference for all parts.
analyses showed that aortas from ADAR1sm−/− mice had significantly less elastin fragmentation as compared to ApoE−/− mice (Figure 5E and 5F). These results indicated that SMC ADAR1 plays an essential role in AAA formation in Ang II–infused ApoE−/− mice.

ADAR1 Promotes the Synthetic/Inflammatory SMC Phenotype and MMP Expression Independent of Its RNA Editing Function

SMC phenotypic modulation plays a role in the pathogenesis of AAA. The proinflammatory cytokine IL (interleukin)-1β is upregulated in AAA tissues and has been shown to induce synthetic/inflammatory SMC phenotype.23,24 Interestingly, in cultured SMC, IL-1β induced the expression of ADAR1, VCAM-1, MMP2, and MMP9 while downregulating the SMC markers MYH11 (myosin heavy chain 11), calponin, and ACTA2 (Figure 6A). Knockdown of ADAR1 using shRNA (short hairpin RNA) prevented IL-1β–induced downregulation of SMC marker proteins and blunted the upregulation of VCAM-1, MMP2, and MMP9 (Figure 6A through 6H). To determine if ADAR1 regulates activities of secreted MMPs from IL-1β–treated cells, supernatants of cultured SMC were collected, and MMP activity was detected by zymography. Consistent with the protein expression data, IL-1β increased the activity of secreted MMPs, which was prevented by knockdown of ADAR1 (Figure 6I). These results suggest that ADAR1 promotes the synthetic/inflammatory SMC phenotype and MMP production/activity.

Because ADAR1 is an RNA editing enzyme, we tested whether it participates in MMP2 and MMP9 pre-mRNA splicing. As shown in Figure 6J through 6L, both MMP2 and MMP9 pre-mRNAs were spliced normally in IL-1β–treated SMC. Knockdown of ADAR1 using specific shRNA reduced both the pre-mRNA and mature mRNAs (Figure 6J through 6L), suggesting that ADAR1 does not participate in MMP2 or MMP9 pre-mRNA splicing. Moreover, ADAR1 editing inhibitor 8-azaadenosine, which blocks ADAR1 editing function,25 also failed to alter...
Cai et al. 

IL-1β or ADAR1 regulation of MMP2, MMP9, or VCAM-1 mRNA expression (Figure II and IIIA through IIID in the Data Supplement). However, 8-azaadenosine was able to block IL-1β-induced or ADAR1-induced abnormal splicing of ACTA2 pre-mRNA (Figure II and IIIA and IIIE in the Data Supplement), consistent with our previous finding that ADAR1 regulates SMC marker genes through its RNA editing function. These results indicate that ADAR1 regulates MMP2/9 and inflammatory marker expression through an RNA editing-independent mechanism.

**ADAR1 Stabilizes MMP2 and MMP9 mRNA via Interacting With HuR**

Although ADAR1 did not affect MMP2 and MMP9 pre-mRNA splicing, knockdown of ADAR1 increased the mRNA degradation rates of MMP2 and MMP9 while their gene transcription was blocked by actinomycin D (Figure 7A through 7C), suggesting that ADAR1 promotes MMP2 and MMP9 mRNA stability. Because previous studies have shown that HuR, but not HuB, HuC, or HuD, stabilizes MMP2 and MMP9 mRNA by binding their 3′ UTRs (untranslated regions),26,27 we hypothesized that ADAR1 may interact with HuR to regulate MMP2 and MMP9 mRNA stability. To test this hypothesis, we first detected if ADAR1 interacts with HuR in SMC. Coimmunoprecipitation assay showed that ADAR1 physically interacted with HuR, and IL-1β treatment significantly enhanced the ADAR1-HuR interaction (Figure 7D and 7E). To test if ADAR1 cooperates with HuR to stabilize MMP2/9 transcripts, we knocked down ADAR1 in HuR-overexpressing SMC. As shown in Figure 7F through 7H, overexpression of HuR promoted MMP2 and MMP9 mRNA stability as evidenced by the reduced mRNA degradation rate.
Figure 6. ADAR1 (adenosine deaminases acting on RNA 1) regulated smooth muscle cell (SMC) phenotype and MMP (matrix metalloproteinase) level/activity independent of its editing function.

A–I. Mouse aortic SMCs were transduced with control (−) or shADAR1 (ADAR1 shRNA [short hairpin RNA]) adenoviral vector and then treated with 10 ng/mL of IL (interleukin)-1β for 24 h. Protein expressions of ADAR1, inflammatory cell marker VCAM-1 (vascular cell adhesion molecule-1), MMP2 (matrix metalloproteinase-2), MMP9, and SMC markers were detected by Western blot and quantified by normalizing to GAPDH, respectively. MMP activity was measured by in situ zymography (I). *P=1.45×10−2 for ADAR1 110 kD, 2.05×10−2 for ADAR1 150 kD (B), 2.08×10−3 for pre-MMP2, 1.45×10−3 for m-MMP2 (D), 1.85×10−3 for pre-MMP9, 2.35×10−3 for m-MMP9 (E), 2.11×10−2 (F), 1.41×10−2 (G), 8.41×10−3 (H), 1.85×10−3 (I) vs vehicle-treated cells (−), respectively; #P=1.98×10−2 for ADAR1 110 kD, 2.48×10−2 for ADAR1 150 kD (B), 3.11×10−2 (C), 2.53×10−2 for pre-MMP2, 1.83×10−2 for pre-MMP9, 2.53×10−2 for m-MMP9 (E), 4.05×10−2 (F), 2.05×10−2 (G), 1.35×10−2 (H), 2.53×10−2 (I) vs control adenovirus-transduced cells (−) with IL-1β treatment, respectively. n=6 for B–H; n=3 for I. J–L, Mouse aortic SMCs were transduced with control (−) or ADAR1 shRNA adenoviral vector (shADAR1) and then treated with vehicle (−) or 10 ng/mL of IL-1β for 24 h. The precursor (pre-) and mature (m)-mRNA levels of MMP2 and MMP9 were detected by real-time polymerase chain reaction (J). The pre-mRNA and m-mRNA levels of MMP2 (K) and MMP9 (L) were quantified by normalizing to GAPDH, respectively. *P=4.92×10−3 for pre-MMP2, 1.99×10−2 for m-MMP2 (K) and 4.92×10−3 for pre-MMP9, 9.22×10−3 for m-MMP9 (L) vs vehicle-treated cells (−); #P=5.98×10−3 for pre-MMP2, 3.40×10−2 for m-MMP2 (K) and 2.98×10−2 for pre-MMP9, 3.18×10−2 for m-MMP9 (L) vs control vector-transduced cells (−) with IL-1β treatment; n=6. All protein or mRNA levels were shown as fold induction relative to the mean value of vehicle (−) and control vector-transduced cells (−), which was set as 1 for each comparison. Kruskal-Wallis test with Dunn multiple comparisons tests was performed to determine statistical difference for all parts. MYH11 indicates myosin heavy chain 11.
Cai et al ADAR1 in Abdominal Aortic Aneurysm degradation rate. However, knockdown of ADAR1 diminished the ability of HuR in promoting MMP2 and MMP9 mRNA stability (Figure 7F through 7H). These results suggest that ADAR1 interacts with HuR to promote MMP2 and MMP9 mRNA stability, thus contributing to the phenotypic modulation of SMC and AAA formation.

**ADAR1 Mediates HuR Interaction With MMP2 and MMP9 mRNAs in AAA Lesions**

To test if ADAR1 is important for HuR binding to MMP2 and MMP9 transcripts in vivo, we performed RNA immunoprecipitation assay using lysates from mouse aortas and AAA tissues (Figure 8A). Significantly increased MMP2 and MMP9 mRNA binding with HuR was observed in abdominal aortas of Ang II–infused ApoE−/− mice. However, the binding was significantly blocked in Ang II–infused ADAR1−/−;ApoE−/− mice (Figure 8A through 8C), suggesting that ADAR1 is essential for HuR binding to MMP2/9 transcripts in AAA lesion. To determine if ADAR1 physically interacts with HuR in abdominal aorta during AAA formation, we performed Proximity Ligation Assay. Although ADAR1 barely interacts with HuR in saline-infused...
ApoE−/− mouse abdominal aorta, the interaction was significantly increased in Ang II–infused ApoE−/− mouse aortas, and attenuated in ADAR1 heterozygous mice (Figure 8D). More importantly, Proximity Ligation Assay assay showed that ADAR1–HuR interaction was also significantly increased in human AAA lesion, especially in the media layer as compared to the healthy aortas (Figure 8E), suggesting that ADAR1–HuR interaction could be also important for AAA development in human patients.
DISCUSSION

By using Ang II infusion of ApoE−/− mouse model, we found that ADAR1 is a novel factor essential for AAA formation/dissection. ADAR1 was induced in both mouse and human AAA lesions. Heterozygous knockout of ADAR1 significantly attenuated the aneurysm formation/dissection, as shown by the significantly decreased AAA prevalence and reduction of aortic dilation and elastin fragmentation as well as thrombus formation. Moreover, ADAR1 deficiency decreased the expression and activities of elastin degradation enzymes MMP2 and MMP9 in the abdominal aorta of Ang II–infused mice and restored SMC marker protein expression, indicating that ADAR1 promotes AAA formation by mediating SMC phenotypic modulation and facilitating the increased expression/activities of MMP2 and MMP9.

AAA formation/dissection is a complex process involving multiple molecular and cellular events including immune and vascular cell responses. Both immune and vascular cells play critical roles in AAA development and aorta dissection. ADAR1 in vascular cells appears to be required for the AAA formation because transplanting ADAR1−/− aorta to ApoE−/− mice significantly reduced the AAA formation and elastin degradation. These results are consistent with the predominant expression of ADAR1 in aortic media in both mouse and human aneurysm lesions. Indeed, heterozygous knockout of ADAR1 in SMC reduced AAA prevalence, attenuated aortic dilation, and decreased elastin degradation, demonstrating that SMC ADAR1 is essential for AAA formation/dissection. In addition to SMCs, ADAR1 has been shown to regulate immune cells, especially T-cell migration and function. It is likely that T-cell ADAR1 may also be important for AAA formation. However, because the majority of ADAR1-positive cells were SMC, ADAR1 is likely to promote aortic aneurysm/dissection in large part by regulating SMC phenotype. Future studies are necessary to determine if T-cell or macrophage ADAR1 is important for AAA formation/dissection.

ADAR1 is an RNA editing enzyme, and our previous studies have shown that ADAR1 disrupts SMC marker gene pre-mRNA splicing through its editing function, leading to reduction of their mature mRNAs. However, neither knockdown of ADAR1 nor the ADAR1 editing inhibitor affected the pre-mRNA splicing of MMP2 or MMP9, suggesting that ADAR1 editing function was not involved in the increased MMP2 and MMP9 expression/activities in aneurysm lesions. Rather, our data suggest that ADAR1 promotes MMP2 and MMP9 mRNA stability. Knockdown of ADAR1 accelerated MMP2 and MMP9 mRNA degradation and abolished HuR-mediated mRNA stability, indicating that ADAR1 is essential for HuR stabilization of MM2 and MMP9 mRNAs. Indeed, ADAR1 is required for HuR binding to MMP2 and MMP9 transcripts in mouse aortas during AAA development in vivo. These findings are also supported by the physical interaction between ADAR1 and HuR, which was promoted by IL-1β. More importantly, the physical interaction between ADAR1 and HuR was significantly increased in both mouse and human AAA lesions, suggesting that ADAR1-facilitated HuR binding to MMP2 and MMP9 mRNA could be an essential mechanism for AAA formation in human patients.

Because MMP9 is mainly expressed in macrophages during AAA development, the effects of ADAR1 on MMP9 production/activity could be attributed to both macrophages and SMC because ADAR1 is present in both the phenotypically modulated SMC and immune cells. In vitro evidence also showed that SMC express MMP9 with IL-1β treatment. However, MMP9 function in AAA development is controversial because MMP9 deficiency inhibits CaCl2-induced AAA but enhances Ang II–induced AAA. It appears that macrophage MMP9 plays a major role in CaCl2-induced AAA formation. Due to these discrepancies, we are unable to conclude how important the ADAR1 regulation of MMP9 is in AAA formation/dissection. Nevertheless, our data suggest that ADAR1, specifically SMC ADAR1, primarily regulates MMP2 production/activity to mediate AAA formation/dissection.

Taken together, our studies have identified a new role for ADAR1 in AAA formation/dissection. ADAR1 promotes inflammatory SMC phenotype and increases the expression/activities of MMP2 and MMP9. Mechanistically, ADAR1 interacts with HuR to promote MMP2 and MMP9 mRNA stability in inflammatory SMCs, leading to elastin degradation, aortic dilation/dissection, and aneurysm formation. Therefore, ADAR1 could be a novel potential target for hindering AAA development or rupture.

ARTICLE INFORMATION

Received April 14, 2021; revision received September 17, 2021; accepted September 22, 2021.

Affiliations

Departments of Surgery (D.C., X.Q., S.-Y.C.) and Department of Medical Pharmacology & Physiology (D.C., S.-Y.C.), University of Missouri School of Medicine, Columbia, Department of Physiology & Pharmacology, University of Georgia, Athens (C.S., G.Z., S.-Y.C.). Harborview Medical Center, Department of Medicine, University of Washington, Seattle (K.F.). Department of Medicine (N.L.W.) and Vascular Biology Center, Medical College of Georgia at Augusta University, Augusta, GA (N.L.W.).

Sources of Funding

This work was supported by grants from National Institutes of Health (HL117247, HL119053, HL135854, and HL147313).

Disclosures

None.

Supplemental Materials

Expanded Materials and Methods
Data Supplement Figures I–III
Data Supplement Table I
References 22–38
REFERENCES


