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# Experimental abdominal aortic aneurysm growth is inhibited by blocking the JAK2/STAT3 pathway

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ABSTRACT

*Background:* The JAK/STAT pathway is a vital transcription signaling pathway that regulates gene expression and cellular activity. Our recently published study highlighted the role of IL-17A in abdominal aortic aneurysm (AAA) formation and rupture. IL-17A has been proven to upregulate vascular endothelial growth factor (VEGF) expression in some diseases. However, no study has demonstrated the relationships among JAK2/STAT3, IL-17A and VEGF. Therefore, we hypothesized that IL-17A may up-regulate VEGF expression via the JAK2/STAT3 signaling pathway to amplify the inflammatory response, exacerbate neovascularization, and accelerate AAA progression.
*Methods:* To fully verify our hypothesis, two separate studies were performed: *i)* a study investigating the influence of JAK2/STAT3 on AAA formation and progression. *ii)* a study evaluating the relationship among IL-17A, JAK2/STAT3 and VEGF. Human tissues were collected from 7 AAA patients who underwent open surgery and 7 liver transplantation donors. All human aortic tissues were examined by histological and immunohistochemical staining, and Western blotting. Furthermore, mouse aortic tissues were also examined by histological and immunohistochemical staining and Western blotting, and the mouse aortic diameters were assessed by high-resolution Vevo 2100 microimaging system.
*Results:* Among human aortic tissues, JAK2/STAT3, IL-17A and VEGF expression levels were higher in AAA tissues than in control tissues. Group treated with WP1066 (a selective JAK2/STAT3 pathway inhibitor), IL-17A, and VEGF groups had AAA incidences of 25%, 40%, and 65%, respectively, while the control group had an incidence of 75%. Histopathological analysis revealed that the IL-17A- and VEGF-related inflammatory responses were attenuated by WP1066. Thus, blocking the JAK2/STAT3 pathway with WP1066 attenuated experimental AAA progression. In addition, in study *ii*, we found that IL-17A siRNA seemed to attenuate the expression of IL-17A and VEGF in vivo study; treatment with VEGF siRNA decreased the expression of VEGF, while IL-17A expression remained high. In an in vitro study, rhIL-17A treatment increased JAK2/STAT3 and VEGF expression in macrophages in a dose-dependent manner.
*Conclusion:* Blocking the JAK2/STAT3 pathway with WP1066 (a JAK2/STAT3 specific inhibitor) attenuates experimental AAA progression. During AAA progression, IL-17A may influence the expression of VEGF via the JAK2/STAT3 signaling pathway. This potential mechanism may suggest a novel strategy for nonsurgical AAA treatment.

## 1. Background

Abdominal aorta aneurysm (AAA) is a pivotal cause of death in elderly males, [1] and is characterized by an aortic diameter of at least 1.5 times the normal diameter or a maximum diameter >3 cm [2]. Surgical repair is the only efficient treatment for patients with large AAAs, which are defined as those with an aorta diameter ≥ 5.5 cm, but this option is not appropriate for patients with early or small AAAs. Small AAAs are defined as those with an aortic diameter >3 cm, but <5 cm based on aneurysm screening programs in the United States [3]. No specific medical therapies exist for early or small AAAs. Therefore, identifying a potential mechanism that attenuates AAA progression and that can become a novel strategy for nonsurgical AAA treatment is important.

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Multiple factors contribute to AAA formation and progression, such as male gender, dyslipidemia, smoking, hypertension, aging, and family history [4–6]. Inflammation and matrix degradation in the aneurysm microenvironment have been implicated in aneurysm wall weakening and are crucial for AAA formation and rupture. Matrix metalloproteinase (MMP) expression and vascular SMC apoptosis are regulated by inflammatory responses in the aneurysm microenvironment [7,8]. MMP family members, especially MMP-2 and MMP-9, are closely involved in neovascularization [9] and proteolytic extracellular matrix (ECM) degradation [10]. Previous studies have demonstrated that plasma MMP-2 and MMP-9 levels are elevated in AAAs [11–13]. The JAK/STAT pathway is a crucial signaling pathway in tumorigenesis, atherosclerosis, hypertension and diabetes [13–15]. Previous study demonstrated that incubating rat aortic vascular SMCs with cigarette smoke extract upregulates the JAK2/STAT3 pathway and its downstream targets MMP-2 and MMP-9 [16].

Previous studies highlighted the role of IL-17A [17] and VEGF [18–20] in AAA formation and rupture. IL-17A upregulates VEGF expression via the JAK2/STAT3 signaling pathway in some diseases, such as non-small-cell lung cancer [21], and in nucleus pulposus cells [22], but little is known about its role in AAA. Therefore, we preliminary investigated the relationship among JAK2/STAT3, IL-17A and VEGF and hypothesized that IL-17A may upregulate VEGF expression via the JAK2/STAT3 signaling pathway to amplify the inflammatory response, exacerbate neovascularization, and accelerate AAA progression.

## 2. Materials and methods

### 2.1. Human tissues analysis

A total of 7 abdominal aortic aneurysm patients who underwent open surgery between 2012 and 2017 were enrolled in this study. Normal specimens of abdominal aortic were collected from 7 liver transplantation donors between 2015 and 2017. Histological and immunohistochemical staining and Western blotting were performed on the human aorta tissues. All experiments were performed in accordance with the relevant guidelines and regulations of Huazhong University of Science and Technology and the Declaration of Helsinki. This study was approved by the Ethics Committee of Union Hospital and Huazhong University of Science and Technology, and written informed consent was obtained from each patient.

### 2.2. Mice and establishment of an aneurysm model

All protocols were approved by the Institutional Animal Care and Use Committee at Huazhong University of Science and Technology. Mice were housed in a specific pathogen-free environment on a 12 h light/dark cycle and maintained on normal laboratory chow and water. Sixteen-week-old male ApoE$^{-/-}$ mice weighing 25 to 30 g (purchased from HFK Biotechnology Co., Ltd., Beijing) were used to establish the aneurysm model.

The Ang II/ApoE$^{-/-}$ model was created as previously described [23]. We used subcutaneous osmotic pumps (Alzet Osmotic Pumps, model 2004, Durect Corporation) to administer Ang II (Sigma-Aldrich) at 1000 ng/kg/min for 28 days.

### 2.3. Transient transfection with siRNA

IL-17A-, VEGF-, and negative control siRNAs were purchased from Thermo Fisher (MA, USA). Transfection of macrophages was performed using Lipofectamine™ 2000 (Invitrogen) according to the manufacturer's instructions to verify the efficacy of the IL-17A- and VEGF-siRNAs.

### 2.4. Treatment

The mice received intraperitoneal (i.p.) injections of 3 μg/kg IL-17A siRNA (Thermo Fisher, MA, USA) or VEGF siRNA (Thermo Fisher, MA, USA). A pharmacological JAK2/STAT3 pathway inhibitor, WP1066 (Selleck Chemicals, Houston, TX), was also injected intraperitoneally into mice at 20 mg/kg in a total volume of 100 μl. All ApoE$^{-/-}$ mice ($n = 120$) were randomly divided into the following 6 groups after the subcutaneous osmotic pumps were implanted: the sham group (saline+0.5% dimethyl sulfoxide [DMSO] treatment, 0.5 ml, daily, i.p. injection), the control group (Ang II + 0.5% DMSO treatment, 0.5 ml, daily, i.p. injection), the WP1066 group (Ang II + WP1066, 0.1 ml, every other day, i.p. injection), the IL-17A group (Ang II+ IL-17A-siRNA, 0.5 ml, daily, i.p. injection), the VEGF group (Ang II+ VEGF-siRNA, 0.5 ml, daily, i.p. injection), and the siRNA control group (Ang II + negative control siRNA 0.5 ml, daily, i.p. injection). Aortic tissues were harvested after 28 days of treatment.

### 2.5. Assessment of aneurysm formation

We used a high-resolution Vevo 2100 microimaging system (Visualsonic) at 30 MHz to measure the aortic diameter in each group of mice on days 0, 7, 14, 21, and 28. The same experienced echocardiographer completed the mouse ultrasound measurements. A suprarenal aortic diameter increase of ≥50% or the occurrence of aortic dissection (AD) was considered aneurysmal in the mice. The survival ratios of all mouse groups were monitored daily.

### 2.6. Histological and immunohistochemical/immunofluorescent staining

The details are described in the supplementary material.

### 2.7. Western blotting

The details are described in the supplementary material.

### 2.8. Statistical analysis

Prism 5.0 (GraphPad Software, La Jolla, CA) was used for statistical analysis. The data are presented as the means ± SEMs, and a P value < .05 was considered to indicate statistically significant. $\chi 2$ test were used to analyze the incidence of AAA. Two-tailed Student's *t*-test (for parametric data) or Mann-Whitney test (for nonparametric data) were used to analyze differences between two groups. In addition, one-way analysis of variance (ANOVA) with the Bonferroni correction was used to compare multiple groups in the Ang II/ApoE model study.

## 3. Results

### 3.1. Inflammatory cell accumulation and JAK2 and STAT3 protein expression are increased in human AAA tissue

Human aortic samples were collected for analysis. EVG staining demonstrated that the structure of medial elastin was substantially damaged in the AAA group compared with the control group (Fig. 1, A-B and G). In addition, IL-17A was highly expressed in AAA patient aortic tissue specimens compared with control tissue specimens (Fig. 1, C and D). CD 31 staining revealed substantial neovascularization in the adventitia in the AAA group compared with the control group (Fig. 1, E-F and H). H&E staining revealed that the infiltration of inflammatory cells in the aortic wall in human AAA samples was more prominent than that in normal aorta samples (Supplemental Fig. 1, A-B). All parameter values were significantly increased in the AAA group, as determined by assessment of macrophage infiltration (Supplemental Fig. 1, C-D) and VEGF expression (Supplemental Fig. 1, E-F) in the adventitia

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in each aortic cross-section (ACS). In addition, JAK2 and STAT3 protein expression were substantially higher in the AAA group than in the control group, as determined by Western blot analysis (Fig. 1, I-J). Both IL-17A and VEGF were highly expressed in AAA patient aortic tissue specimens compared with control tissue specimens, as determined by Western blotting (Fig. 1, I-J).

### 3.2. The Ang II-induced EAAA mouse model imitate the pathology of human AAA

To fully investigate the roles of JAK2 and STAT3 in AAA formation and progression, we established an EAAA mouse model to imitate human AAA. A >50% increase in aortic diameter or aortic dissection (AD) occurrence is usually considered to indicate successful EAAA. We used the H&E and immunohistochemical (IL-17A, Mac2, CD31 and VEGF) staining to analyze the mouse aortic samples. All the parameters were substantially increased in the control group compared with the sham group (Fig. 2, C–F and H; Supplemental Fig. 2, A-F). EVG staining revealed that the EAAA mouse aortic structure was conspicuously disrupted (Fig. 2, A-B and G). Semi-quantitative histological analysis was used to count the number of CD31-positive cells (Fig. 2, H). In addition, JAK2 and STAT3 protein levels were notably higher in the control group than in the sham group (Fig. 2, I–J). All of these results demonstrated that the Ang II-induced EAAA mouse model can imitate the pathology of human AAA.

Inhibiting JAK2 and STAT3 Protein Expression Reduced the AAA Incidence and Increases the Survival Ratio in the Ang II/ ApoE Model.

The ultrasound results revealed visible changes in the harvested aortic tissue. Histological assessments and scaled digital photographs were comprehensively analyzed to characterize the EAAA, but the luminal diameter measurements taken by ultrasound were the most important parameters. Surprisingly, the increase in aortic diameter over the 28 days was significantly lower in the WP1066 group than in the control group (1.261 ± 0.09 mm versus 1.725 ± 0.102 mm, $P < .05$, Fig. 3, A, B, D). Treatment with WP1066 also reduced AAA incidence. In the WP1066 group, AAA incidence was substantially reduced from 75% (15 of 20 mice) to 25% (5 of 20 mice), and no AAA formation was found in the sham group (Fig. 3, C). Consistent with the reduction in aortic diameter and decreased incidence of AAA, the survival ratio was notably higher in the WP1066-treated group (95%; 1 died from AD on day 16) than in the control group (75%; 1 died from AD on each of days 4, 10, 12, 16 and 19) (Fig. 3, E).

### 3.3. WP1066 treatment exhibited protective effect in the Ang II/ApoE model

We performed a microscopic examination of aortic sections to identify the mechanisms contributing to the WP1066-induced AAA protective effect. Elastin fragmentation and neovascularization within the adventitia were substantially attenuated by WP1066 treatment, as demonstrated by EVG staining and CD31 immunohistochemistry (Fig. 4, A-C, G-I). Compared with the control group, the WP1066 group exhibited a significant advantage in the medial elastin fragmentation scoring ($P < .05$, Fig. 4, J). Consistent with the reduced medial elastin fragmentation scores, neovascularization was also reduced by WP1066 treatment ($P < .05$, Fig. 4, K). To assess inflammatory cell infiltration in aortic sections, we performed a histological analysis of $Mac2^+$ macrophages. Compared with the sham group, the control group exhibited significantly more $Mac2^+$ macrophages, whereas there were fewer $Mac2^+$ macrophages in the WP1066 group compared with the control group (Supplemental Fig. 3, D-F). H&E staining demonstrated that treatment with WP1066 decreased the expression of inflammatory cells. (Supplemental Fig. 3, A-C) Moreover, immunohistochemical staining of IL-17A (Fig. 4, D-F) and VEGF (Supplemental Fig. 3, G-I) also revealed a difference between the control and WP1066 groups. IL-17A and VEGF expression in the adventitia and adjoining tissue was substantially reduced in the WP1066 group compared with the control group. Additionally, immunofluorescence staining of IL-17A and

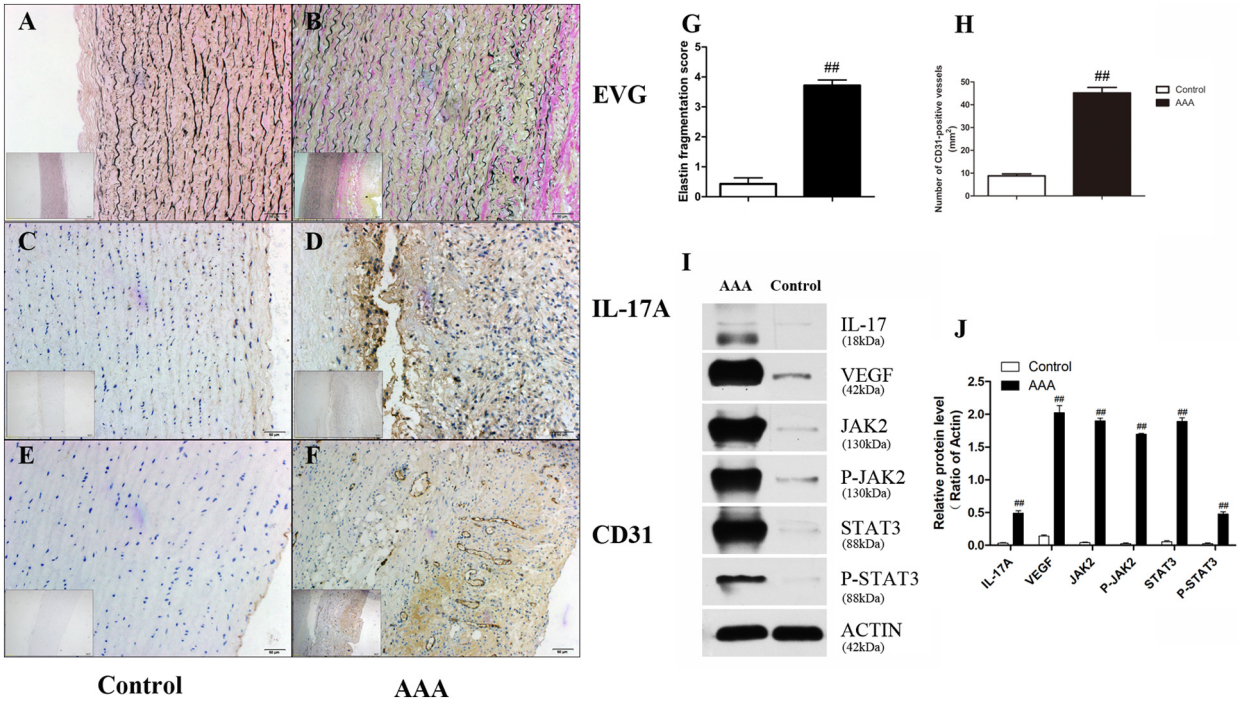


**Fig. 1.** Analysis of human aortic samples, the magnification of each histological images were ×200, bar = 50 μm. EVG staining shows degradation of the wavy structure of elastin in human AAA samples (B) compared with normal aorta (A). IL-17A expression was increased in the aortic wall of AAA (D) compared with that of normal aorta (C). CD31-positive microvessel density in the medial layer of AAA (F) was increased compared with that in normal aorta (E). Elastin fragmentation score was higher in AAA compared with the normal aorta (G). Number of CD31-positive vessels count was higher in AAA than normal aorta (H, n = 7). The protein expression (n = 4 per group) of JAK2, p-JAK2, STAT3, p-STAT3, IL-17A, and VEGF were increased in aortic tissue from patients compared with control specimens (I, J). Data are presented as the mean ± SEM, two-tailed Student's *t*-test was used to compare two groups. $^{\#}P < .05$ vs. Control, $^{\#\#}P < .01$ vs. Control.

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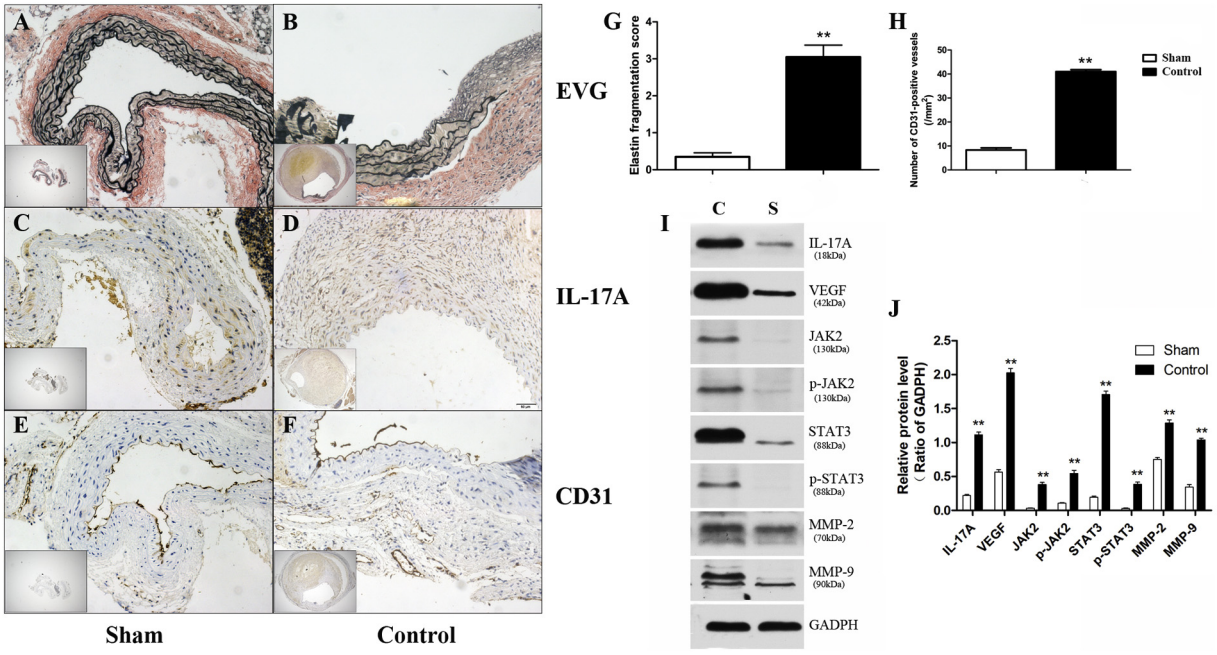


**Fig. 2.** Analysis of mouse aortic samples, the magnification of each histological images were ×200, bar = 50 μm. EVG staining shows degradation of the wavy structure of elastin in mouse AAA samples (B) compared with normal aorta (A). IL-17A expression was increased in the aortic wall of AAA (D) compared with that of normal aorta (C). CD31-positive microvessel density in the medial layer of AAA (F) was increased compared with that in normal aorta (E). Elastin fragmentation score was higher in AAA compared with the normal aorta (G). Number of CD31-positive vessels count was higher in AAA than normal aorta (H, n = 5). The protein expression (n = 4 per group) of JAK2, p-JAK2, STAT3, p-STAT3, IL-17A, and VEGF were increased in aortic tissue from mouse compared with control specimens (I, J). Data are presented as the mean ± SEM, two-tailed Student's *t*-test was used to compare two groups. *P < .05 vs. sham group, **P < .01 vs. sham group. C: Control, S: Sham.

VEGF demonstrated that treatment with WP1066 decreased the expression of inflammatory cytokines (Supplemental Fig. 4). Furthermore, JAK2, p-JAK2, STAT3, p-STAT3, MMP-2, MMP-9, IL-17A, and VEGF protein levels were determined by Western blot analysis. The detected protein levels were significantly higher in the control group than in the sham group. However, the level of these proteins

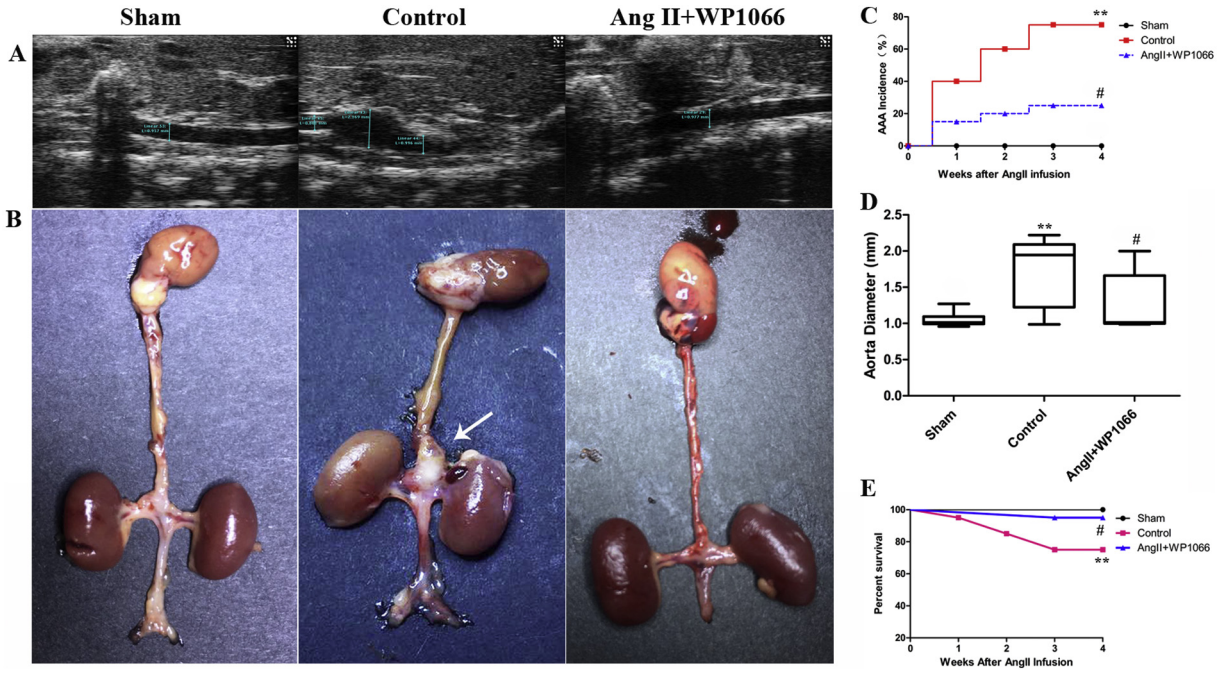


**Fig. 3.** Treated with WP1066 reduced abdominal aortic aneurysm incidence and increase the survival ratio in the Ang II/ApoE mice model. A, Typical ultrasound images of the abdominal aortic aneurysm from each group after 28 days infusion. B, Representative photographs showing the visible changes from each group (as indicated by the white arrowheads). C-E, Demonstrate the influence of each group in survival ratio (n = 20/group, 95% in WP1066 group, 75% in the control group), aorta diameter (WP1066 group vs control group, 1.261 ± 0.09 mm versus 1.725 ± 0.102 mm, P < .05), and AAA incidence (25% in WP1066 group, and 75% in the control group). Data are presented as the percentage or the mean ± SEM. The $\chi 2$ test, *t*-test, and one-way analysis of variance were used for data analysis. Compared with the sham group, **P < .01, n = 20/group; compared with the control group, #P < .05, n = 20 per group.

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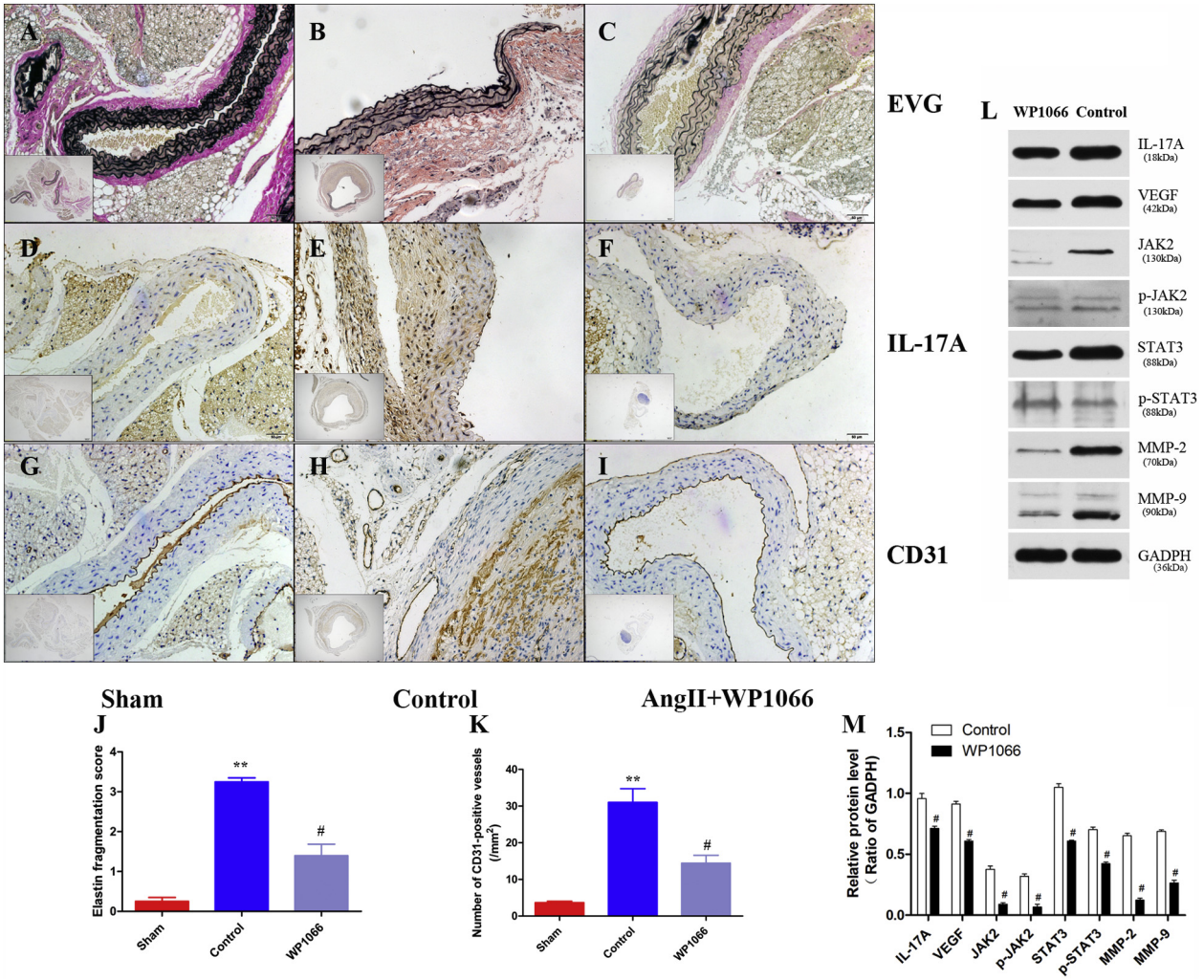


**Fig. 4.** Immunohistochemical staining of mouse aortic samples from each group, the magnification of each histological images were ×200, bar = 50 μm, n = 5 per group. A-C, EVG staining; D-F, IL-17A staining; G-I, CD31 stating. From the histological images, Control group shows amount of IL-17A infiltration, ruptured elastin lamellar architecture, and neovascularization. Treatment with WP1066 seems preserves the structure of the aortic wall and attenuates the infiltration of inflammatory cells and cytokines. J and K, representative the semiquantitative histological analysis (number of CD31-positive vessels count and media elastin destruction score). L and M, Western blot analysis of WP1066 group and control group (n = 4/group), WP1066 treatment decrease the protein expression of pro-inflammatory mediators (JAK2, p-JAK2, STAT3, p-STAT3, IL-17A, VEGF, MMP-2 and MMP-9) in the Ang II/ApoE model. Data are presented as the mean±SEM. Elastic destruction was analyzed with the Mann–Whitney test, and other data were analyzed with the 2-tailed Student's *t*-test. Compared with the sham group, $^{**}P < .01$; compared with the control group, $^{\#}P < .05$.

were substantially reduced in the WP1066 group. (Fig. 4, L and M) Those results suggest that WP1066 treatment exhibited protective effect in the Ang II/ApoE model.

### *3.4. IL-17A may influence VEGF expression via the JAK2/STAT3 signaling pathway*

We established two other Ang II/ApoE mouse model groups to investigate the relationship between IL-17A and VEGF. Surprisingly, AAA incidence and aortic diameter were lower in mice treated with IL-17A siRNA and VEGF siRNA compared with control mice (40%, 65% and 75%, respectively; 1.379 ± 0.105 mm, 1.610 ± 0.098 mm, and 1.725 ± 0.102 mm, respectively, Supplemental Fig. 5, A, B, D, E). In addition, treatment with IL-17A siRNA and VEGF siRNA increased the survival ratio from 75% (the control group value) to 90% (2 died from AD on days 9 and 18 in the IL-17A group, and 2 died from AD on days 16 and 21 in the VEGF group). (Supplemental Fig. 5, C) EVG staining (Supplemental Fig. 6, A-D, N) and CD31 (Supplemental Fig. 6, I-L, M) immunohistochemistry revealed significant improvement in the medial elastin fragmentation scores in the IL-17A and VEGF groups compared with the control group ($P < .01$, $P < .05$, respectively). In addition, only weak IL-17A (Supplemental Fig. 6, E-G) and VEGF (Supplemental Fig. 7, I-K) expression was found in the IL-17A group compared with the control group. Compared with the control group, the VEGF group did not exhibit reduced IL-17A expression (Supplemental Fig. 6, E, F and H) but did exhibit reduced VEGF expression (Supplemental Fig. 7, I, J and L) and neovascularization (Supplemental Fig. 6, I, J and L). Additionally, immunofluorescence staining revealed that treatment with IL-17A siRNA seemed to attenuate the expression of IL-17A and VEGF; treatment with VEGF siRNA only decreased the expression of VEGF, but IL-17A expression remained high (Supplemental Fig. 8). As shown above, we observed that WP1066 treatment not only reduced IL-17A and VEGF expression but also reduced the IL-17A and VEGF protein levels. Treatment with IL-17A siRNA attenuated IL-17A and VEGF protein levels and also reduced JAK2 and STAT3 protein expression (Supplemental Fig. 6, O and P). However, treatment with VEGF siRNA only reduced the protein level of VEGF but did not reduce IL-17A, JAK2, or STAT3 expression (Supplemental Fig. 6, O and P). In addition, in vitro, the protein levels of VEGF, JAK2, p-JAK2, STAT3, and p-STAT3 were altered in macrophages treated with different rhIL-17A concentrations (0 ng/ml, 10 ng/ml, 50 ng/ml, and 100 ng/ml), and the effects on these cytokines were dose-dependent (Supplemental Fig. 6, Q and R).

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Based on the above results, we hypothesize that IL-17A may influence VEGF expression via the JAK2/STAT3 signaling pathway.

## 4. Discussion

Abdominal aortic aneurysm is a permanent, complicated pathophysiological process and is the most prevalent vascular disease in older men, especially men older than 65 years [24,25]. Proteolysis, SMC apoptosis, chronic inflammatory reactions, neovascularization and other processes lead to AAA formation [6]. In AAA, the risk of death is associated with the diameter of the aneurysm, and surgery is the only effective treatment [26]. However, surgical treatment is not suitable for patients with small AAAs, and no specific drug treatment is available to prevent the progression of small AAAs [27]. Thus, finding a new, effective medical therapeutic strategy is necessary.

Compared with healthy individuals, patients with AAAs often have increased MMP-2 and MMP-9 levels in aortic tissue and serum [28]. MMP-2 and MMP-9 are associated with extracellular matrix and structural protein breakdown [11]. Thus, inhibiting the expression of MMP-2 and MMP-9 may affect the formation of AAA. Ghosh and his colleagues reported that the JAK/STAT pathway mediates MMP-2 and MMP-9 secretion in aortic vascular SMCs in vitro study [16]. Traditionally, the JAK/STAT signaling pathway plays important roles in cell proliferation, innate and adaptive immunity, tissue growth, protease expression and angiogenesis [29]. In addition, the JAK2/STAT3 pathway is reportedly involved in the pathogeneses of atherosclerosis, hypertension and diabetes [30]. Abundant evidence suggests that JAK/STAT is an ideal target for disease therapy [14,15]. In our previous work, results obtained with human aortic samples confirmed that JAK2 and STAT3 protein expression were significantly increased in the AAA tissues compared with the normal tissues. In our animal experiment, inhibition of JAK2 and STAT3 with a selective inhibitor, WP1066, decreased AAA formation, immune cell infiltration, inflammatory cytokine expression, and medial elastin scores. These results suggest that the JAK2/STAT3 signaling pathway plays a crucial role in AAA formation. The selective JAK2/STAT3 pathway inhibitor WP1066 was first introduced in 2007. Iwamaru and his colleagues [31] used WP1066 to inhibit the STAT3 pathway and induce apoptosis in malignant glioma cells. WP1066 has been demonstrated to alter proliferation/apoptotic mechanisms in vivo and ex vivo.

Inflammation plays a crucial role in AAA formation and progression. IL-17A has been proven to play an important role in the AAA inflammatory microenvironment. In a previous study, Wei [17] confirmed the role of IL-17A in the pathophysiological process of AAA. Moreover, IL-17A protein expression, medial elastin scores, and macrophage infiltration were decreased following treatment with IL-17A siRNA in our animal experiment. RNAi is commonly used to specifically inhibit gene expression and is a useful approach for gene function studies [32]. RNAi is faster and more efficient than traditional approaches (such as gene knockout) [33], and it may be a novel strategy for disease therapy [34]. IL-17A siRNA treatment clearly attenuated the incidence of mouse AAA in our experiment. VEGF is one of the most potent angiogenic and vascular permeability factors and plays essential roles in vascular formation. VEGF has received increasing attention because of its possible involvement in atherosclerosis through angiogenesis, endothelial regeneration, and inflammation in the vascular wall. Kaneko found that VEGF is overexpressed mainly in macrophages infiltrating the aortic wall and is associated with increased inflammatory cell infiltration and vasculogenesis in human AAA [18]. Our results with human aortic samples confirm the pathophysiological process of AAA. Treatment with VEGF siRNA not only attenuated AAA incidence but also decreased VEGF expression and neovascularization.

Based on the above results, we established the Ang II/ApoE mouse model to fully investigate the essential role of JAK2/STAT3 and preliminary investigated the potential relationship among JAK2/STAT3, IL-17A and VEGF in EAAA. The WP1066 group showed dramatically decreased in AAA formation in our study. The potential mechanism may involve the selective inhibitor of JAK2 and STAT3 by WP1066, because inhibition of this pathway affects multiple downstream molecules, thus enhancing the anti-aneurysm effect. For example, JAK2/STAT3 plays an important role in neovascularization by upregulating the expression of VEGF.

In addition, IL-17A siRNA treatment not only reduced IL-17A, VEGFA, MMP-2 and MMP-9 expression but also reduced JAK2 protein levels. These data suggest that IL-17A influences targets downstream of the JAK2 pathway in AAA, consistent with its effects in non-small-cell lung cancer [21]. Moreover, in vitro, treatment of macrophages with different rhIL-17A concentrations altered VEGF, JAK2, p-JAK2, STAT3, and p-STAT3 protein levels, and the effects on the expression of these cytokines were dose-dependent. These results suggest that IL-17A may influence VEGF expression via the JAK2/STAT3 pathway in AAA.

## 5. Conclusion

Blocking the JAK2/STAT3 pathway with WP1066 attenuates experimental AAA progression. During AAA progression, IL-17A may influence the expression of VEGF via the JAK2/STAT3 signaling pathway. This potential mechanism may suggest a novel strategy for nonsurgical AAA treatment.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijcard.2020.03.072.

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## Authors' contributions

J.X. and Z.W. designed and performed experiments, analyzed the data and wrote the manuscript. J.L. reviewed the manuscript. Y.S., X.C., W.C., H.Z. and C.Y. performed experiments.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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