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Up regulation of isoleucyl-tRNA synthetase promotes vascular smooth muscle cells dysfunction via p38 MAPK/PI3K signaling pathways

Bowen Li[a,b,c], Zhiwei Wang[a,b,*], Ruoshi Chen[a,b,c], Junmou Hong[a,b,c], Qi Wu[a,b,c], Junxia Hu[a,b,c], Zhipeng Hu[a,b], Min Zhang[a,b]

[a] Department of Cardiovascular Surgery, Renmin Hospital of Wuhan University, 238# Jiefang Road, Wuhan 430000, Hubei Province, People's Republic of China
[b] Cardiovascular Surgery Laboratory, Renmin Hospital of Wuhan University, 9# Zhangzhidong Road, Wuhan 430000, Hubei Province, People's Republic of China
[c] Central Laboratory, Renmin Hospital of Wuhan University, 9# Zhangzhidong Road, Wuhan 430000, Hubei Province, People's Republic of China

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ABSTRACT

The pathogenesis of abdominal aortic aneurysm remains unclear. The aim of the present study was to establish whether isoleucyl-tRNA synthetase (Iars) regulates the differentiation and apoptosis of vascular smooth muscle cells (VSMCs) during the development of abdominal aortic aneurysm (AAA). In addition, the contribution of various signaling pathways towards this process was ascertained. The study demonstrated that the expression of Iars, p-p38, osteopontin (OPN) and Bcl-2-associated X protein (Bax) clearly increased, while levels of p-PI3K and smooth muscle 22 alpha (SM22α) decreased significantly in AAA tissues. Inhibition of Iars significantly reduced the incidence of angiotensin II (AngII)-induced AAA in mice, coincident with decreased activity of the p38 MAPK pathway and increased PI3K pathway activity. AngII-induced phenotypic switching and apoptosis of VSMCs decreased following the inhibition of Iars in vitro. Upregulation of the IARS gene induced phenotypic switching and apoptosis in VSMCs in addition to increased p38 MAPK pathway activation and reduced PI3K pathway activation. Following pretreatment with an activator of the PI3K pathway, expression of Iars and the phenotypic markers of VSMCs were not affected, while apoptosis of VSMCs decreased. Similarly, inhibition of the p38 MAPK pathway in VSMCs did not affect the expression of Iars or the degree of cell apoptosis, but reduced phenotypic switching was observed. Conclusively, upregulation of Iars regulates the phenotypic switching and apoptosis of VSMCs. Targeting Iars may be a promising strategy to prevent abdominal aortic aneurysm.

1. Introduction

Abdominal aortic aneurysm (AAA) is a permanent, localized dilation of the aorta, occurring in nearly 9% of adults older than 65 years of age in the United States, leading to approximately 15,000 annual deaths following rupture [1,2]. Despite decades of research into AAA, there remains a paucity of fundamental understanding of the mechanisms that cause it or factors that block its progression. Thus, a critical step in the development of novel pharmacologic therapies for AAA is elucidation of the molecular basis for the disease.

Many researchers have established that vascular smooth muscle cells (VSMCs) play an important role in the development of arterial diseases because their function is to remodel the vascular wall. Studies have shown that there was phenotypic switching and apoptosis of VSMCs during the formation of aortic aneurysm [3–6]. Previously, we established that the p38 mitogen-activated protein kinase (MAPK) pathway is involved in the process of phenotypic switching in VSMCs [5]. However, the mechanisms responsible for modulation of the p38 MAPK pathway and apoptosis of VSMCs remains unclear.

The classic function of aminoacyl-tRNA synthetases (AARSs) is synthesis of proteins which specifically recognize the side chains and tRNAs of amino acids, catalyzing the binding of specific amino acids to specific tRNAs. This ensures that the genetic information carried by individual mRNA strands is accurate, reflected in the amino acid sequence of that protein [7]. Recently, a number of AARSs have been recognized as having involvement in other functions in addition to

Abbreviations: AAA, abdominal aortic aneurysm; Iars, isoleucyl-tRNA synthetase; VSMC, vascular smooth muscle cell; MAPK, mitogen-activated protein kinase; PI3K, phosphoinositide 3-kinase; AngII, angiotensin II; OPN, osteopontin; Bax, Bcl-2-associated X protein; M, mupirocin; DA, 1,3-dicaffeoylquinic acid; IHC, Immunohistochemistry; WB, Western blotting; PCR, polymerase chain reaction

* Corresponding author at: Department of Cardiovascular Surgery, Renmin Hospital of Wuhan University, 238# Jiefang Road, Wuhan 430000, Hubei Province, People's Republic of China.
E-mail address: wangzhiwei@whu.edu.cn (Z. Wang).

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protein synthesis, such as immunity, angiogenesis, transcriptional regulation and apoptosis [8,9]. Isoleucyl-tRNA synthetase (Iars), a member of the amino acid tRNA transport synthetase family which is encoded by the IARS gene [10], has been reported to be responsible for hypertrophic cardiomyopathy and inhibit angiogenesis [11,12]. Our search and analysis of the GEO database (GLP10787) found that mRNA expression of IARS in the aorta of patients with abdominal aortic aneurysm (AAA) was significantly greater than that of non-AAA patients (Supplementary Fig. 1). An imbalance in aortic medial damage and repair is an important characteristic of aortic disease. Whether Iars can affect the occurrence of AAA by inhibiting the repair process of the aortic wall has not been reported. In a preliminary experiment we confirmed that the protein expression of Iars was greater in tissues suffering AAA.

Herein, we wished to clarify whether Iars influenced the development of AAA by regulation of the survival and differentiation of VSMCs, and the signaling pathways involved in these processes.

2. Materials and methods

2.1. Immunohistochemistry (IHC)

All experimental protocols regarding human materials were conducted according to the Declaration of Helsinki and were approved by the ethical committee of Renmin Hospital of Wuhan University (WDRY2015-K021). Pathological aortic tissues were obtained from patients who had undergone abdominal aortic aneurysmectomy and artificial blood vessel replacement. The normal aorta were derived from organ donors who had no family history of aortic diseases and had no previous history of cardiovascular diseases and long-term medication. The baseline characteristics of the AAA patients and Aortic donors are presented in Table 1. IHC was conducted as previously described [5]. The prepared slides were incubated with primary antibody overnight at 4 °C, for detailed information on the use of primary antibodies, see Supplementary Table 1. The stained slides were then incubated with secondary antibody for 1 h at 37 °C and then developed with diaminobenzidine. Immunohistochemical analysis was conducted as previously described [13].

2.2. Western blotting (WB)

Aortic tissues were rinsed twice with PBS and proteins extracted using ice-cold lysis buffer containing a 1% protease inhibitor (Sigma, USA), as previously described [5]. Western blot assays were carried out as previously described. Primary antibodies used in our work were as follows: anti-Iars (Clone: ab229643; Abcam, dilution: 1:4000), anti-PI3K (Clone: ab189403; Abcam, dilution: 1:500), anti-phospho-PI3K (Clone: ab182651; Abcam, dilution:1:700), anti-p38 (Clone:D13E1; Cell Signaling Technology, dilution: 1:1000), anti-phospho-p38 (Clone:28B10; Cell Signaling Technology, dilution: 1:1500), anti-Bax (Clone: ab32503; Abcam, dilution: 1:4000), anti-SM22α (Clone: ab14106; Abcam, dilution: 1:1000), anti-osteopontin (OPN, Clone: ab91655; Abcam, dilution: 1:1000) and anti-α-tubulin (Clone:ab7291; Abcam, dilution: 1:2000).

2.3. RNA extraction and quantitative real-time PCR (RT-PCR)

Total RNA was extracted from cells/aortic tissues using RNAiso Plus reagent (Takara, Japan) and used as a template from which complementary DNA was reverse transcribed using a First Strand complementary DNA Synthesis Kit (Takara, Japan). RT-PCR was conducted as previously described [5]. The primers used were as follows: IARS-F:5-ACCACCTTCACTCACA-3, IARS-R:5-TCGTTTTTCTCGTACC-3; SM22a-F:5- AGTGCAGTCCAAAATCGAGAAG-3, SM22a-R:5- CTTGCTC AGAATCACGCCAT-3; OPN-F:5- CTCCATTGACTCGAACGACTC-3, OPN-R:5- CAGGTCTGCGAAACTTCTTAGAT-3; Bax-F:5- CCCGAGAGGTCTTT TTCCGAG-3, Bax-R:5- CCAGCCCATGATGGTTCTGAT-3; GAPDH-F:5-GGAGCGAGATCCCTCCAAAAT-3, GAPDH-R:5- GGCTGTTGTCATACTT CTCATGG-3.

Table 1
Characteristics of the AAA patients and aortic donors.

	Abdominal aortic aneurysm group	Aortic donors group	*p*-Value
Age (year)	50.24 ± 6.58	46.36 ± 5.74	0.177
Male/female (n)	8/2	7/3	1.000
BMI	24.53 ± 6.66	24.37 ± 6.18	0.956
Drinking history (n)	7	7	1.000
Smoking history (n)	9	8	1.000
Drug use history (n)	0	0	1.000
LVEF(%)	58.13 ± 5.003	57.96 ± 6.042	0.946
Type of AAA (n)	10	0	< 0.0001***
Suprarenal AAA	0	0	
Subrenal AAA	10	0	
Comorbidities (n)	10	0	
Hypertensive disease	10	0	< 0.0001***
Diabetes mellitus	2	0	0.474
Heart disease	0	0	
COPD	1	0	1.000
Renal insufficiency	0	0	

LVEF = left ventricle ejection fraction; COPD = chronic obstructive pulmonary disease.
*** $p < 0.0001$ vs. AAA.

2.4. Animals and in vivo experiment

48 eight-week-old male C57/BL6 mice (20 ± 0.54 g) were used in this study, all mice were randomly divided into 4 groups (12 per group). All experiments were conducted in accordance with the guidelines for the management and use of laboratory animals of China and were approved by the Institutional Ethics Committee on Animal Use (WDRY2015-K021). According to a previously-published protocol, 0.5 ml/L calcium chloride ($CaCl_2$) was used to infiltrate the abdominal aorta for 15 min, in the 5th week after the $CaCl_2$ treatment, 1 μg/kg/min AngII was injected into the mice by a subcutaneous pump for one week. And the mice were dissected after 6 weeks to observe the formation of AAA [14] (Ca + A group). To investigate the effect of the expression of Iars on AAA, 2% Mupirocin (Clone: S4297, Selleck), a Iars inhibitor, was administered by smearing on the aorta of both $CaCl_2$ intervention (M + Ca + A group) and no $CaCl_2$ + AngII(M group) animals.

2.5. VSMCs culture and treatments with AngII

Human aortic smooth muscle cells (HASMCs) were purchased from ATCC (PCS-100-012). HASMCs were then incubated in DMEM containing 10% FBS, 1% mycillin, 2 mM glutamine, 50 mg/mL gentamycin and 50 mg/mL amphotericin-B at 37 °C in an atmosphere containing 5% CO_2. The cells treated with 0.1 μM AngII for 12 h as previously reported [5]. To study the effect of inhibiting Iars, cells were pretreated with 10 μM Mupirocin and then either treated after 30 min with AngII (M + AngII) or untreated (M).

2.6. Lentivirus transfection and intervention in signaling pathways

A recombinant lentivirus was constructed using a three-plasmid co-transfection system in human embryonic kidney 293T cells to convey the expression of Iars and puromycin resistance, as previously described [15]. Transfection efficiency was determined using western blotting.

To study the role of the PI3K signaling pathway, cells were pre-treated with 10 μM 1,3-dicaffeoylquinic acid (Clone: HY-N1412; MCE) (DA), an activator of the PI3K pathway, 30 min prior to lentivirus treatment (DA + Iars). To investigate the role of the p38 MAPK pathway, the p38 MAPK inhibitor SB203580 (Clone: HY-10256; MCE) (S) was added 30 min prior to upregulation of Iars (S + Iars).

2.7. Detection of VSMC apoptosis

Annexin V-FITC/PI apoptosis kit (Clone: 556547, BD) was used to detect VSMC apoptosis according to the protocols. Annexin V-FITC added followed by propidium iodide (PI) and mixed uniformly then incubated for 5–15 min at room temperature in the dark. The negative control comprised normal cells without Annexin V-FITC or PI. The cells with the strongest apoptosis effect were denoted as positive controls 1 and 2, plus Annexin V-FITC alone and PI alone, respectively.

2.8. Statistical analyses

Data were presented as mean ± standard deviation. Paired and/or unpaired Student *t*-tests were used to evaluate the statistical significance of differences between the means of two groups, while analysis of variance (ANOVA) was performed to determine significance across multiple groups. A p-value < 0.05 was considered statistically significant. GraphPad Prism 6.0 was used for statistical analyses. All graphs were fitted with Sigma Plot Version 10.0 software.

3. Results

3.1. Apoptosis and phenotypic switching of VSMCs in AAA tissues is accompanied by increased Iars expression

Using IHC and WB, it was evident that the expression of Iars and Bax was significantly greater in the pathological tissues than the normal group (Fig. 1A–D, I,J). In addition, expression of p-p38 and the synthetic phenotypic marker of VSMCs, OPN was substantially greater in the AAA tissues (Fig. 1G–J,K,L), while expression of the contractile phenotypic marker, SM22α was lower (Fig. 1E,F,I,J). These results indicate that apoptosis and phenotypic switching were observed in the pathological specimens and that Iars had involvement in this process.

3.2. PI3K pathway activity was inhibited in AAA

To understand the response of the PI3K pathway in the pathogenesis of AAA, we quantified PI3K and p-PI3K expression within the medial layer of normal and pathological aorta samples using WB. The results demonstrate that p-PI3K levels decreased in the AAA tissues (Fig. 1K,L), suggesting that PI3K pathway inhibition occurred in AAA pathogenesis.

3.3. Inhibition of Iars attenuated $CaCl_2$ + AngII-induced AAA in mice

To investigate the expression of Iars during the formation of an AAA, a $CaCl_2$ + AngII-induced (Ca + A group) model of AAA was established (Fig. 2A). Of 12 mice, 9 formed an AAA, and 3 of them developed AAA rupture. To determine the role of Iars during AAA formation, mice were treated with 2% Mupirocin during induction of AAA by $CaCl_2$ + AngII (M + Ca + A group). Inhibition of Iars by Mupirocin was also performed in non-induced mice (M group). The formation rate of AAA in the M + Ca + A group was 16.7% (2 of 12), a considerably lower rate than that of the Ca + A group (Fig. 2B). The use of Mupirocin alone did not affect the formation of AAA (Fig. 2B). In addition, the largest aortic diameter of the mice in group Ca + A was significantly larger than the other three groups, while the aortic thickness of this group was thinner than others (Fig. 2C–E). The expression of Iars, p-p38, OPN and Bax was higher in the AAA mice (Fig. 2F–l, 3A,B,G,H), while SM22a and p-PI3K were lower (Fig. 2J,K, 3E,F). Meanwhile, the

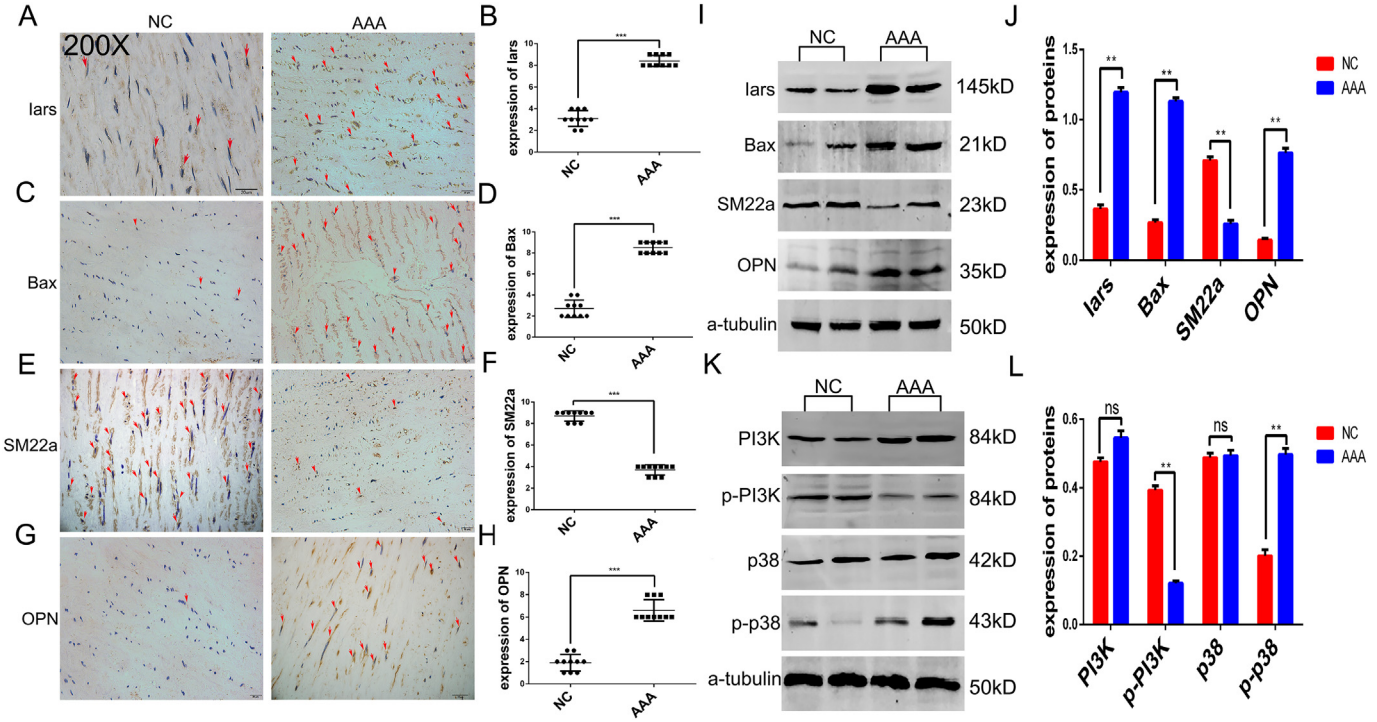

Fig. 1. Expression of Iars, and apoptosis and phenotypic switching in VSMCs increased in the aneurysm specimens. (A,B,I) IHC and WB demonstrated that the expression of Iars, (C,D,I) an apoptosis marker (Bax) and (G,H,I) a synthetic phenotypic marker of VSMCs (OPN) increased significantly in the AAA tissues (AAA, $n = 10$), (E,F,I) while expression of the contractile phenotypic marker of VSMCs (SM22α) declined. (K,L) Expression of p-PI3K decreased in AAA tissues, while p-p38 was upregulated. No significant difference in total protein expression of these two indicators was observed in the negative control (NC, $n = 10$) or AAA tissues. *t*-test, $^{**}p < 0.0001$ vs. NC.

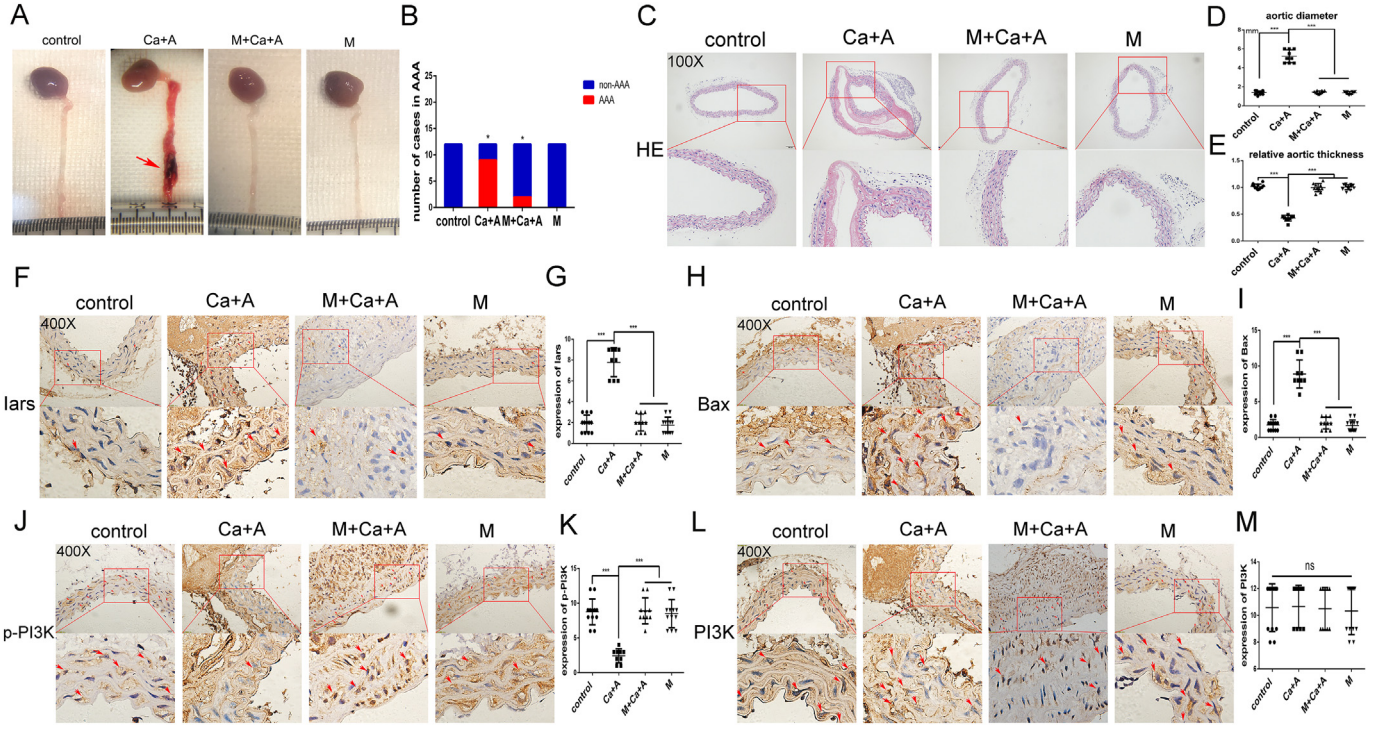

Fig. 2. Inhibition of Iars reduced $CaCl_2$ + AngII-induced formation of AAA, apoptosis and phenotypic switching of VSMCs. (A–C) After application of Mupirocin, the inhibitor of Iars, $CaCl_2$ + AngII-induced formation of AAA decreased significantly (M + Ca + A), whereas use of the inhibitor alone (M) did not change injury to the aorta. (D,E) The largest aortic diameter of the mice in group Ca + A was significantly larger than the other three groups, while the aortic thickness of this group was thinner than others. (F,G) Expression of Iars significantly increased in AAA aortas. (J–M) Inhibition of Iars in vivo increased activity of the PI3K pathway and (H,I) decreased the expression of Bax. $n = 12$, one-way ANOVA test, $^{***}p < 0.0001$ vs. Ca + A.

expression of phenotypic markers of VSMCs and Bax, p38 MAPK pathway activity and PI3K pathway activity were not affected by the inhibitor (Figs. 2F–M, 3A–H). The data above suggested that upregulation of Iars plays a pivotal role in AAA formation, with inhibition of Iars attenuating the disease.

3.4. Inhibition of Iars in vitro reduced AngII-induced phenotypic switching and apoptosis of VSMCs

Our previous research established that AngII induced phenotypic switching of VSMCs through the p38 MAPK pathway [5] (Fig. 4A–D). In the present study we observed that AngII also induced apoptosis (Fig. 4A, E, F) while also reducing activity of the PI3K pathway (Fig. 4C,D). After inhibition of Iars by Mupirocin (M + AngII), AngII was unable to induce phenotypic switching (Fig. 4A,B), modify signaling pathway activity (Fig. 4C,D) or apoptosis of VSMCs (Fig. 3A,E,F). These results suggest that Iars mediates AngII-induced phenotypic switching and the apoptosis of VSMCs.

3.5. Upregulation of IARS-induced apoptosis and phenotypic switching of VSMCs via the PI3K and p38 MAPK pathways

To ascertain whether Iars could trigger downstream phenotypic switching and apoptosis of VSMCs, HASMCs were treated in vitro with

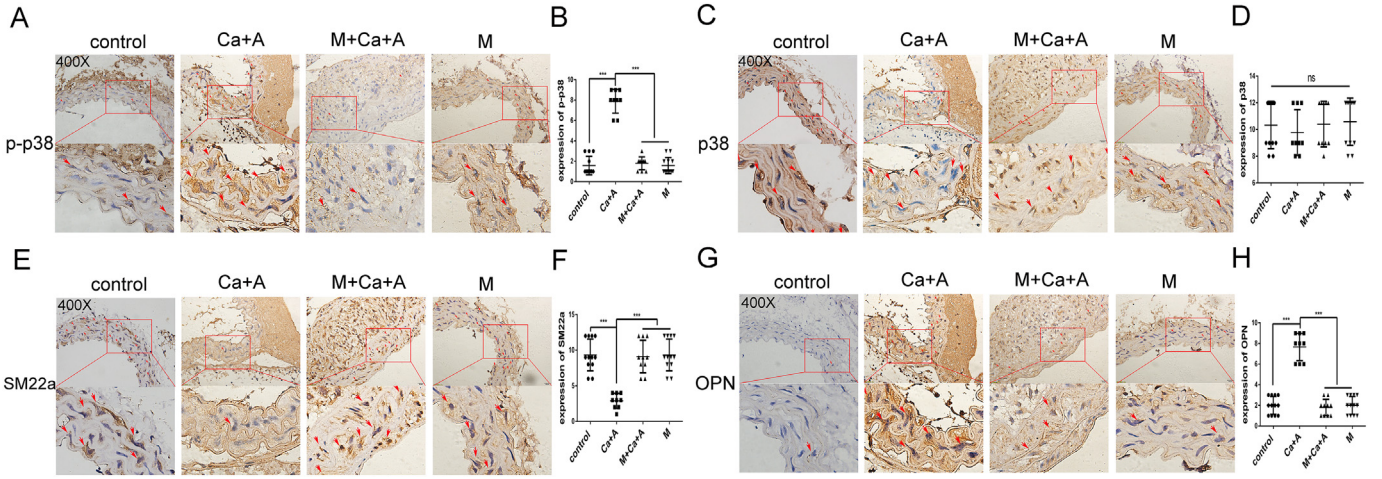

Fig. 3. (A-D) The Iars inhibitor decreased p38 MAPK pathway activity and (G,H) reduced OPN expression, while (E,F) maintain the contraction phenotype of VSMCs. $n = 12$, one-way ANOVA test, $^{***}p < 0.0001$ vs. Ca + A.

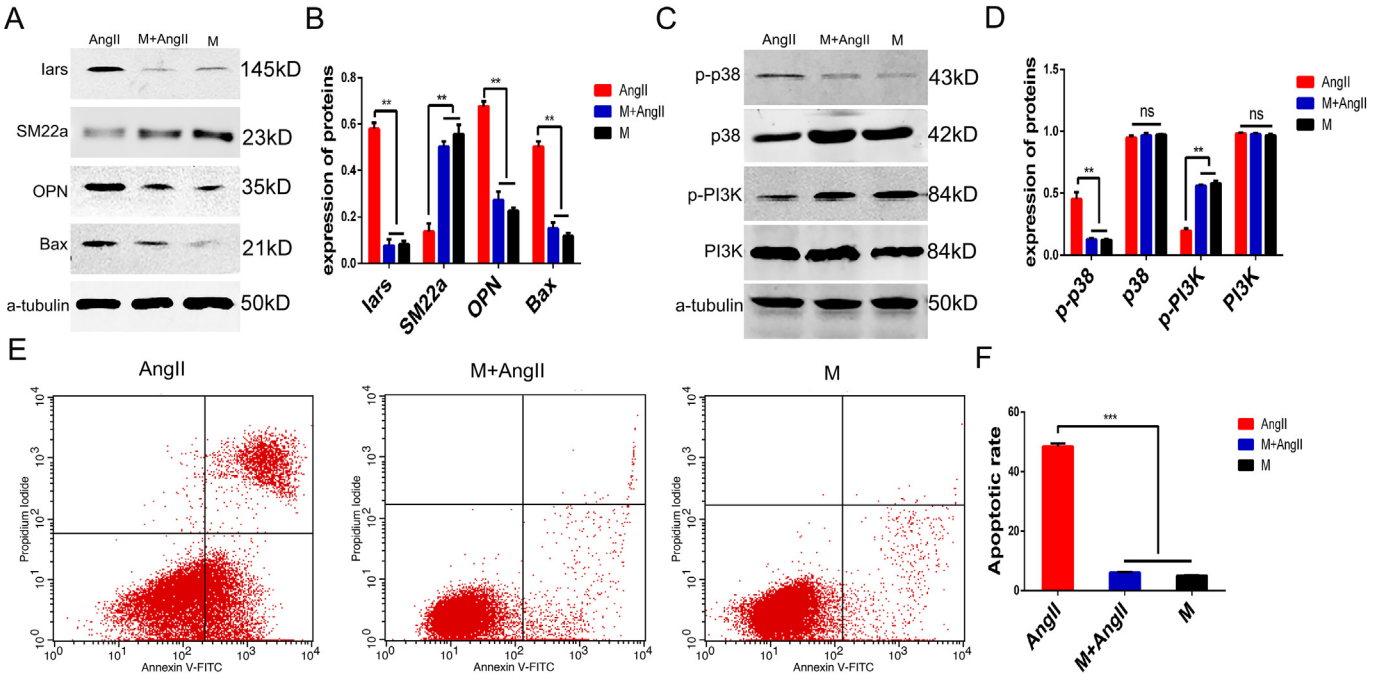

Fig. 4. Inhibition of Iars reduced Ang II-induced phenotypic switching and apoptosis of VSMCs. (A,B) Ang II induced the increase of OPN and Bax, while the expression of SM22a reduced, inhibition of Iars in vitro blocked the induction of Ang II (M + Ang II). (C,D) The inhibitor blocked AngII-induced phosphorylation of p38 while maintaining phosphorylation of PI3K. (E,F) The intervention of AngII caused a large number of apoptosis of VSMCs, while the inhibition of Iars significantly decreased the apoptosis rate of cells. The use of inhibitor alone (M) did not affect VSMCs function or apoptosis or changes in related pathways. $n = 3$, two-way ANOVA test, $^{***}p < 0.0001$ vs. AngII.

lentivirus. Thereafter, samples were analyzed using WB with antibodies against p-PI3K, Bax, p-p38, SM22α and OPN. Meanwhile, mRNAs of SM22α, OPN and Bax were detected by RT-PCR. The expression of Bax, p-p38 and OPN were significantly higher than those of the control, while p-PI3K and SM22a levels decreased (Fig. 5A–E). Flow cytometry demonstrated that the number of apoptotic VSMCs increased after Iars upregulation (Fig. 5J,K).

To verify the impact of the PI3K pathway on VSMCs after the upregulation of Iars, HASMCs were pretreated with a specific activator (DA) of the PI3K pathway immediately before the addition of lentivirus. WB data indicated that the PI3K pathway was activated, repressing the expression of Bax (Fig. 5F,G). And that activation of PI3K decreased VSMCs apoptosis. Otherwise, the PI3K activator did not affect Iars expression or the phenotypic markers of VSMCs and p38 pathway activity (Fig. 5F,G).

Previously, we determined that the p38 MAPK pathway regulates the phenotypic switching of VSMCs [5]. In this study, we established that inhibition of the p38 MAPK pathway blocked Iars-induced phenotypic switching of VSMCs (Fig. 5H,I), while VSMCs apoptosis and the expression of p-PI3K, Bax and Iars was unaffected (Fig. 5H–K). Collectively, these data suggest that Iars regulates the apoptosis of VSMCs via the PI3K pathway and phenotypic switching in cells via the P38 MAPK pathway.

More detailed results can be found in the attached data.

4. Discussion

Abdominal aortic aneurysm is a disease that is seriously life-threatening. However, the cause of the disease has not yet been fully elucidated. Therefore, the study of possible mechanisms of pathogenesis is of great importance for future prevention, diagnosis and treatment of the disease. Studies have shown that aortic medial degeneration is the principal pathological feature of aortic aneurysm [16,17]. In this study we discovered a possible mechanism underlying AAA. Isoleucyt-tRNA synthetase can induce apoptosis and phenotypic switching in VSMCs during AAA formation via the PI3K and p38 MAPK signaling pathways, respectively.

Cells respond to changes in their microenvironment by modifying many cellular programs, including cell survival, proliferation, differentiation, metabolism, interactions with other cells and numerous homeostatic loops. VSMCs switch their phenotype, from contractile to synthetic, in response to these microenvironmental cues in a process known as phenotypic switching. The release of various stress factors such as Ang II, oxidative stress and inflammatory factors during the pathogenesis of aortic aneurysm is significantly elevated, leading to VSMC dysfunction [18,19]. We have demonstrated that the expression of OPN and Bax was considerably greater, and SM22α lower, in the medial layer of specimens taken from tissues exhibiting AAA than did nonpathological controls, thereby suggesting that phenotypic switching and apoptosis had taken place. Our finding is consistent with previous research [20,21] that reported phenotypic switching and apoptosis of VSMCs was observed in aortic aneurysm tissues, but the precise mechanisms of which were unclear.

It has been reported that in addition to regulating protein biosynthesis, Iars also performs an important role in the regulation of immunity, transcription, angiogenesis [22,23]. However, to the best of our knowledge no studies have examined the differentiation and apoptosis of VSMCs induced by AARS, and nothing is known about its modulation by Iars. In addition to demonstrating phenotypic switching and apoptosis in VSMCs in AAA specimens, we also found that the expression of Iars increased significantly in such tissues, which led us to speculate that Iars performs an important role in the pathogenesis of AAA. To test this hypothesis, Iars was inhibited in one group of mice while AAA formation was induced with $CaCl_2$ plus AngII to ascertain the role of Iars on the development of AAA. Unexpectedly, inhibition of Iars significantly reduced the formation of AAA, and reduced phenotypic switching and apoptosis in VSMCs. Additionally, we also observed corresponding changes in the activation status of the PI3K and p38 MAPK pathways. These results indicate that Iars, and the PI3K and p38 MAPK pathways are involved in the formation of AAAs but their precise

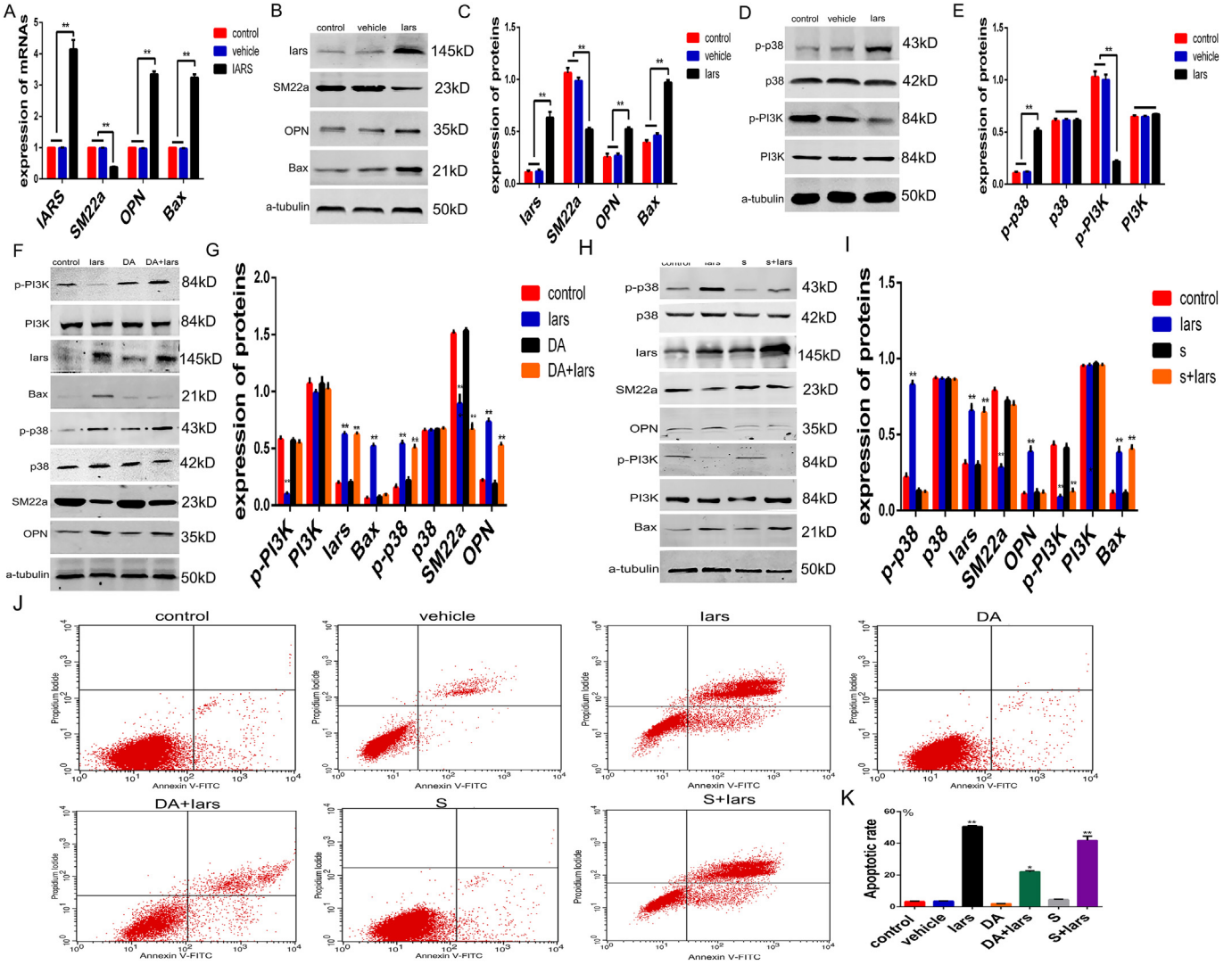

Fig. 5. Up-regulation of IARS induced apoptosis and phenotypic switching in VSMCs via the PI3K and p38 MAPK pathways, respectively. (A–C) Upregulation of Iars (Iars) significantly increased the expression of OPN and Bax and decreased the expression of SM22a in vitro. (D,E) Iars increased the activity of the p38 MAPK pathway, but decreased the activity of the PI3K pathway. (F,G,J,K) Activation of PI3K attenuated Iars-induced Bax expression in VSMCs (DA + Iars), without affecting phenotypic markers or the p38 MAPK pathway. (H,I,J,K) Similarly, a p38 MAPK inhibitor (SB203580) only decreased the phenotypic switching of VSMCs (S + Iars) and did not influence apoptosis or the PI3K pathway. Using either the activator (DA) or inhibitor (S) alone did not affect the cells or pathways. $n = 3$, two-way ANOVA test, $^{***}p < 0.0001$ vs. Iars.

roles and mechanisms of action still require elucidation.

In our previous study, we demonstrated that the p38 MAPK pathway mediates AngII-induced phenotypic switching of VSMCs [5]. In the present study, we observed that AngII additionally induced the expression of Iars. When the IARS gene in HASMCs was overexpressed, it was subsequently observed by Western blot and RT-PCR analysis that the expression of OPN tended to be elevated, while SM22α exhibited significant downregulation, thereby suggesting that Iars induced the phenotypic switching of VSMCs. However, while the p38 MAPK pathway was inhibited, upregulation of Iars did not induce the phenotypic switching of VSMCs, but VSMCs apoptosis still occurred, and inhibition of the p38 MAPK pathway did not affect the expression of Iars. These data suggest that the p38 MAPK pathway mediates Iars-induced phenotypic switching of VSMCs, but not their apoptosis.

Apoptosis is a spontaneous process of programmed cell death that occurs both physiologically and pathologically, operating under tight regulation [24,25]. The phosphoinositide 3-kinase (PI3K) signaling pathway is critical to many cellular functions including the regulation of cell growth, proliferation and apoptosis [26,27]. The principal pathological manifestation of aortic medial layer degeneration is increased apoptosis and dysfunction of VSMCs. We demonstrated that PI3K pathway activity in the medial layer of AAA tissues declined, suggesting that the PI3K pathway is highly likely to perform a role in this process, consistent with observations made by Zhu and Liu [28,29]. However, the mechanisms for suppressing the activity of the PI3K pathway during the pathogenesis of AAA have not yet been reported. In the present study, we demonstrated that Iars affects the activity of the PI3K pathway, both in vivo and in vitro. The change we observed only affected cell apoptosis and not the expression of Iars or VSMC phenotypic markers. These observations suggest that the PI3K pathway mediates Iars-induced apoptosis in VSMCs, rather than phenotypic switching.

We acknowledge that our investigation is limited by the nature of the study's experimental design. Accordingly, it is not possible from our data to determine whether inhibition of Iars affected the function of other organs while reducing the incidence of AAA. Moreover, since Mupirocin is ineffective through both oral administration and injection, it is currently only possible to suppress Iars by direct application to the aorta. Experimentation with a special aortic Iars-knockdown animal model may be critical in verifying our findings and help identify a new

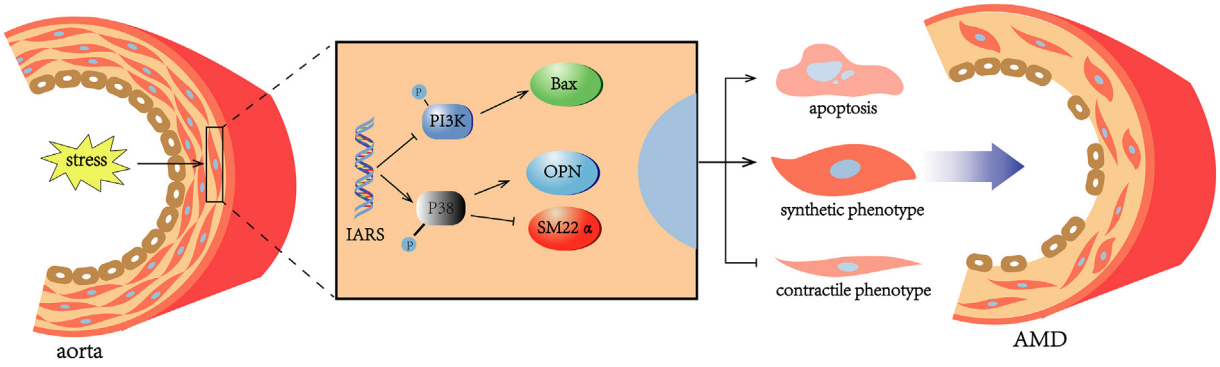

Fig. 6. Various stresses caused up-regulation of IARS expression in aortic VSMCs, which in turn increased phenotypic switching and apoptosis of cells by increasing p38 MAPK pathway activity and decreasing PI3K pathway activity, respectively, resulting in loose aortic wall structure and AMD development.

method of inhibiting Iars in the future.

In the present study, we have shown that upregulation of Iars promotes phenotypic switching and apoptosis in VSMCs during AAA formation via the p38 MAPK and PI3K pathways, respectively (Fig. 6). Our data may offer insight into a previously unknown mechanism of degeneration of the aortic medial layer. Our study suggests that Iars may be an attractive target for strategies aiming to prevent the formation of AAA.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lfs.2019.03.052.

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Disclosure of conflict of interest

The authors report no competing interest in the article.

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