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Autophagy promotes aortic adventitial fibrosis via the IL-6/Jak1 signaling pathway in Takayasu's arteritis

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ABSTRACT

Background: Autophagy is a ubiquitous and evolutionarily conserved self-rescue process. Studies have shown that autophagy is involved in the pathogenesis of multiple diseases; however, whether autophagy is associated with the pathogenesis of Takayasu's arteritis (TA), a large vessel idiopathic inflammatory disease characterized by vascular fibrosis, remains unclear. Moreover, although IL-6 is believed to be a direct target for TA treatment, anti-IL-6 treatment could not block TA-associated fibrosis in some cases, which impairs the aortic function of patients and can result in death. Thus, identify the mechanisms associated with TA is extremely important. Based on the relationship between autophagy and IL-6, we investigated the role of autophagy in the vascular fibrosis of TA induced by IL-6.

Methods: Autophagy proteins (LC3 and Atg3), IL-6, and markers of fibrosis (collagen 1 and α-SMA) were detected in tissues with TA lesions via immunochemistry, immunofluorescence, and Western blot, respectively. Different stages of autophagy were analyzed by the specific inhibitors, 3-methyladenosine (early stage), hydroxychloroquine sulfate (late stage), and bafilomycin A1 (late stage). Autophagosomes were detected using electron microscopy and a viral-vector transfection assay. The fibrosis profiles induced by IL-6-dependent autophagy was assessed with an ELISA.

Results: The expression of autophagy, IL-6, and fibrosis markers were elevated and correlated with each other in the adventitia tissues of TA patients. Furthermore, exogenous IL-6/IL-6Rα could significantly increase autophagy and fibrosis *in vitro*. An autophagy inhibitor was found to significantly block both autophagy and fibrosis induced by IL-6. Finally, IL-6 was found to significantly promote autophagy-induced fibrosis through the activation of the Jak1 pathway.

Conclusions: IL-6-induced autophagy plays an important role in vascular fibrosis of TA. Targeting autophagy pathways might represent a novel therapeutic option for the treatment of TA.

1. Introduction

It has been well-established that autophagy is a ubiquitous and evolutionarily conserved self-rescue process required to maintain hemostasis when cells are exposed to environmental stress [1,2]; however, whether autophagy is associated with the pathogenesis of Takayasu's arteritis (TA) remains unclear.

TA is a large vessel idiopathic inflammatory disease characterized by vascular fibrosis [3]. Our previous studies have found that the elevated level of IL-6 in the local tissue can promote aortic adventitial fibrosis, leading to vessel thickening and remodeling, subsequently influencing patient outcome and quality-of-life [4,5]. Although clinically, anti-inflammatory therapies (e.g., DMARDs) appear to effectively block the progression of TA [6,7], some reports have shown that such therapy could not reduce vascular fibrosis during the long-term follow-up [6–10]. Therefore, there is an urgent need to elucidate the potential novel mechanisms associated with inflammation-induced adventitial fibrosis, to improve patient treatment.

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Recently, multiple cytokines have been shown to regulate autophagy, among which IL-6 was found to have several functions associated with autophagy [11]. For example, IL-6 could promote autophagy in the human neuroblastoma cell line, SHSY5Y [12], but inhibit autophagy in the human histiocytic lymphoma cell line, U937, and bronchial epithelial cell line, N20 [13,14]. However, whether autophagy is associated with IL-6-induced aortic fibrosis of TA remains unknown.

In the present study, we found that IL-6-induced autophagy plays an extremely important role in the vascular fibrosis of TA through systemic analysis, which indicated that targeting the autophagy pathway might represent a novel effective therapeutic option for TA treatment.

2. Materials and methods

2.1. Patients

Patients diagnosed with TA according to the American College of Rheumatology criteria 1990 for Takayasu arteritis were enrolled in this study from the Zhongshan Hospital of Fudan University in 2017 [15] (Supplementary Table 1). Pathological specimens were obtained from our hospital for further detection and analysis. Moreover, normal specimens, including aortic arteries from heart transplantations or other aortic operations were used as controls. Written informed consent was obtained from all of the subjects prior to enrollment. The study protocol was reviewed and approved by the Ethics Committee of Zhongshan Hospital of Fudan University (**Approval No. B2016–168)**, and the investigation conformed to the declaration of Helsinki.

2.2. Pathological staining of fibrosis, autophagy, and inflammation

To detect the extent of fibrosis, vessel specimens embedded in paraffin were dewaxed and subjected to Masson and Sirius-Red staining according to the manufacturers' instructions. The procedures used to perform immunohistochemistry (IHC) and immunofluorescence (IF) were as previously described [5]. Antibodies specific to LC3 (Abcam, Cambridge, UK) and P62 (Abcam) were used to confirm the level of autophagy in the two groups using IHC. Mouse anti-human IL-6 (Abcam), rabbit anti-human α-SMA (Abcam), and sheep anti-human Atg3 (R&D systems, Minnesota, USA) were used to explore their relationship with Donkey anti-mouse-Alexa Flour 594 (Jackson ImmunoResearch, PA, USA), Donkey anti-rabbit-Alexa Flour 488 (Jackson ImmunoResearch), and Donkey anti-sheep-Alexa Flour 647 (Abcam) by laser confocal microscopy (Leica, Wetzlar, German). The areas positive in the IHC pictures and integrated optical density (IOD) from the IF photos were evaluated by three independent investigators using Image J software.

2.3. Cell culture and treatment

Primary human aortic adventitial fibroblasts (HAAFs) (Sciencecell Research Laboratories, Carlsbad, CA, USA) were cultured as previously described [5]. At passage six, the cells were gently washed three times with complete culture medium (Sciencecell) and stimulated with IL-6 and IL-6Rα (R&D systems, Minnesota, USA) at the indicated time and concentrations. Autophagy inducers, rapamycin (CST, Boston, USA) and brefeldin A (medchemexpress, MCE, NJ, USA), were incubated with the fibroblasts for 12 h as positive controls [16]. Autophagy inhibitors, 3-methyladenine (3-MA), hydroxychloroquine sulfate (HCQ) and bafilomycin A1 (baf A1), together with the signaling pathway inhibitors, Itacitinib (all from MCE) and tofacitinib (Pfizer, NY, USA), were used to pre-treat the cells for 1 h, followed by IL-6/IL-6Rα (abbreviated as IL6) intervention with corresponding reagents.

2.4. Transmission electron microscopy (TEM)

The cells were centrifuged and fixed in 2.5% glutaraldehyde containing 0.1 M sodium cacodylate. After fixing in 1% osmic acid, the samples were dehydrated in graded acetone, embedded in Epon812, and cut into 50 nm ultra-thin sections. The images were acquired with a Tecnai sprite biotwin at 120 KV (FEI, OR, USA) after staining the sections with uranyl acetate and lead citrate at the Yuyi Test Center (Shanghai, China). The initial autophagic vacuole (AVi) and degradative autophagic vacuole (AVd) was judged and counted at the scale of 500 nm by three independent researchers in a blinded manner as described previously [13].

2.5. mCherry-GFP-LC3 adenovirus transfection

The cells were transfected with mCherry-GFP-LC3 adenoviral vectors (Hanbio, Shanghai, China) and cultured on glass coverslips according to the manufacturers' instructions. Following the addition of IL6 and baf A1, the cells were observed with confocal microscopy (Leica). According to a previous study [17], mCherry appears red and the GFP appears green when used to label cellular components. In an autophagy assay, because the green fluorescence of GFP is quenched in the lysosomes, the autophagosome (not binding with the lysosomes) is labeled as yellow puncta while the autolysosome (binding with the lysosomes) was labeled as the red-only puncta under a confocal microscope. Based on the different color of the autophagosomes and autolysosomes, an increase in autophagy flux can be detected using an mCherry-GFP-LC3 adenoviral vector. When both colors are elevated in cells, the level of autophagy is increased. Conversely, when only elevated autophagosomes are observed, it indicates that the autophagy flux was blocked before binding to the lysosomes. In the present study, the autophagy profile was determined by counting the yellow (autophagosome) and red-only (autolysosome) puncta, respectively, in 20 cells by three independent researchers using Adobe Photoshop (Adobe, CA, USA).

2.6. ELISA

Cells were treated with IL6 and inhibitors for the indicated time periods. Cell culture medium supernatants were collected to measure the concentration of fibrosis associated protein fibronectin, Collagen 1α, Collagen 3, and TGF-β1 with an ELISA (R&D systems), according to the manufacturer's instructions.

2.7. Western blot (WB)

A WB was performed as previously described [5]. Specific antibodies against fibronectin, collagen 1, collagen 3, CTGF, α-SMA, TGF-β, P62, and LC3B, and β-actin were purchased from Abcam. A phospho-Jak family antibody sampler kit was obtained from CST. Image J software was used to detect the band intensities with Tanon LAS-3000 detection systems (Tanon, Shanghai, China) and the super ECL detection reagent (Yeasen, Shanghai, China). The expression of the target protein was normalized to the level of β-actin and the normal control.

2.8. Statistical analysis

The data was expressed as the mean ± standard deviation (SD). A Pearson's correlation analysis was used to explore the relationship between variables. GraphPad software was used to construct figures and analyze the differences between two groups with a *t*-test or between two more groups with a one-way ANOVA together with multiple comparisons, if necessary. The sample size of each group was three unless indicated otherwise. With a two-tailed test, a *P* value less than 0.05 was statistically significant.

Please see the Supplementary Methods for additional methods.

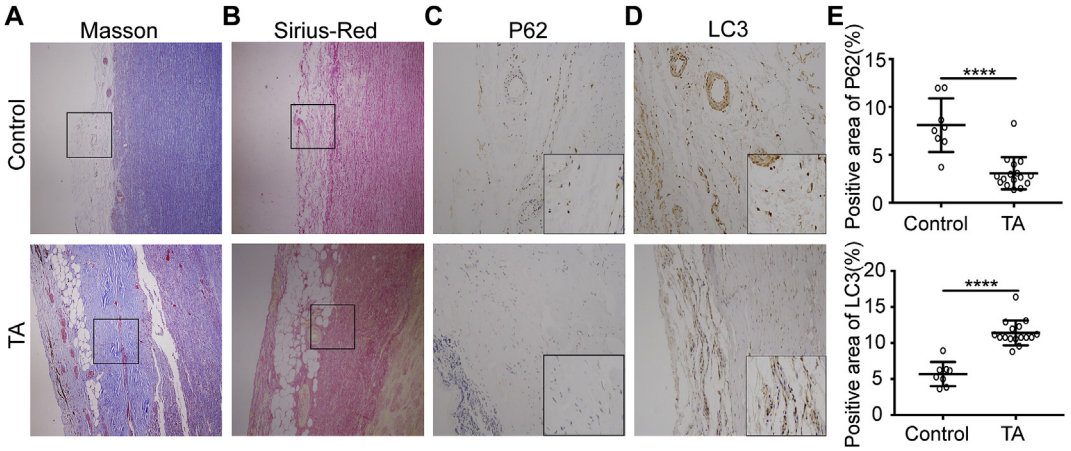

Fig. 1. Autophagy is present and elevated in the adventitia with fibrosis in TA patients. (A) The fibrotic conditions of the outer membrane of the aorta in TA and the control with Masson Staining. In the adventitia, the intensity of the blue staining indicates the level of collagen (magnification: × 40). (B) Sirius-Red staining of aortic adventitia fibrosis in TA and the control. In the adventitia, the intensity of the red staining indicates the level of extracellular matrix accumulation (magnification: × 40). (C) Immunohistochemistry showing the expression of autophagy substrate P62 in the outer membrane of TA and normal aorta (magnification: × 100/ × 400). (D) Immunohistochemistry showing the expression of the autophagy-associated protein, LC3B, in the adventitia of TA and normal aorta (magnification: × 100/ × 400). (E) The scoring of P62 and LC3 in C and D. $^{****}P < 0.0001$. (n = 24). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3. Results

3.1. Autophagy was increased in the tissues from TA patients

A total of 16 TA (TA group) and 8 control aortic specimens (Control group) were used for the analysis. The corresponding demographic information is summarized in Supplementary Table 1.

The Masson (Fig. 1A) and Sirius-Red staining (Fig. 1B) show that the extent of fibrosis in the adventitia of TA was much more severe compared to that of the control. Moreover, IHC staining revealed that autophagy protein, LC3, was significantly increased ($P < 0.001$) whereas the autophagy substrate, P62, was significantly decreased in TA (Fig. 1C–E). These results indicate that autophagy was increased in the fibrotic regions of TA adventitia.

3.2. Elevated levels of IL-6 were associated with autophagy in the tissues from TA patients

Since we found that IL-6 was associated with vascular fibrosis in TA [5], we evaluated the level of IL-6, α-SMA, and autophagy catalytic enzymes Atg3 in TA patients. The results revealed that the expression of these proteins was much higher in the outer membrane of TA than that of the control, and extended analysis revealed that they were significantly correlated ($P < 0.05$) (Fig. 2A–D). The colocation analysis showed that the fibroblasts were surrounded by IL-6, with elevated α-SMA and Atg3 in IF (Fig. 2E). These results suggest that IL-6, autophagy, and fibrosis are closely connected in the vascular fibrosis of TA.

3.3. IL-6 promoted the autophagy of HAAFs in an in vitro assay

Next, we added exogenous IL-6/IL-6α into the HAAF culture and explored the relationship between IL-6, autophagy, and fibrosis and the associated regulating mechanisms *in vitro*.

The results showed that compared with the normal (normal control, NC) and positive controls, IL-6 significantly increased the level of autophagy-related protein, LC3-II, and gene profile expression (e.g., *Atg3, P62, LC3B, Atg4a, Atg12,* and *beclin-1*) at 8 h (Fig. 3A and C, Supplementary Fig. 1A). In contrast, the substrate, P62, decreased moderately when IL-6 was added (Fig. 3B), which was confirmed following stimulation with different concentrations of IL-6 at 8 h (Fig. 3D). These data imply that IL-6 might enhance autophagy in HAAFs.

3.4. IL-6-induced autophagy prior to the fusion of autophagosomes and lysosomes

Given that transmission electron microscopy (TEM) could be used to observe the autophagy status, the early-stage autophagy autophagosomes (AVi) could be identified by a double membrane with the engulfed cytosolic contents. Late-stage autophagy autolysosomes (AVd) could be identified by the partially degraded contents due to autophagosome and lysosome fusion [17]. We demonstrated that IL-6 promoted autophagy with TEM by using inhibitors to block autophagy at different stages in the HAAFs (Supplementary Fig. 1B–D and K). The results revealed that compared with the NC, IL-6 increased the number of AVi and AVd with TEM (Fig. 4A and B). The inhibitor, baf A1, which can block the fusion between autophagosomes and lysosomes, significantly increased the boundary of AVi morphologically. The number of AVi were also significantly increased when IL-6 was added to baf A1, without a significant change in AVd (Fig. 4A and B).

Adenovirus transfection (mCherry-GFP-LC3) can be used to monitor the autophagy status [17]. In our study, we treated the HAAF with IL-6 and found that there were an increased number of autophagosomes (yellow puncta) and autolysosomes (red-only puncta) in the cells. In the presence of baf A1, IL-6 significantly increased the number of autophagosomes without a significant change in autolysosomes (Fig. 4C and D).

The WB results showed that the inhibitors, baf A1 and HCQ (which block the degradation of autophagosomes), could further increase the level of LC3-II with IL-6 at 8 h, while 3-MA (which blocks the formation of autophagosomes) could mitigate the accumulation of LC3-II caused by IL-6 (Fig. 4E–G). In contrast to the WB results, the three inhibitors demonstrated a similar ability to significantly decrease the gene expression of autophagy (Supplementary Fig. 1E–G). Taken together, these results demonstrate that IL-6 promotes autophagy prior to the maturation step of autophagy.

3.5. Blocking autophagy alleviated the IL-6-induced fibrosis in vivo

To observe the level of fibrosis induced by autophagy, the baseline levels of fibrosis-related protein and gene expression was detected. In WB and PCR, treatment with IL-6 increased the expression of fibrosis-related proteins (e.g., fibronectin and collagen 1) at 4 h, 8 h, and 12 h, with the levels peaking at 8 h (Supplementary Fig. 2A and 2B). A dose-

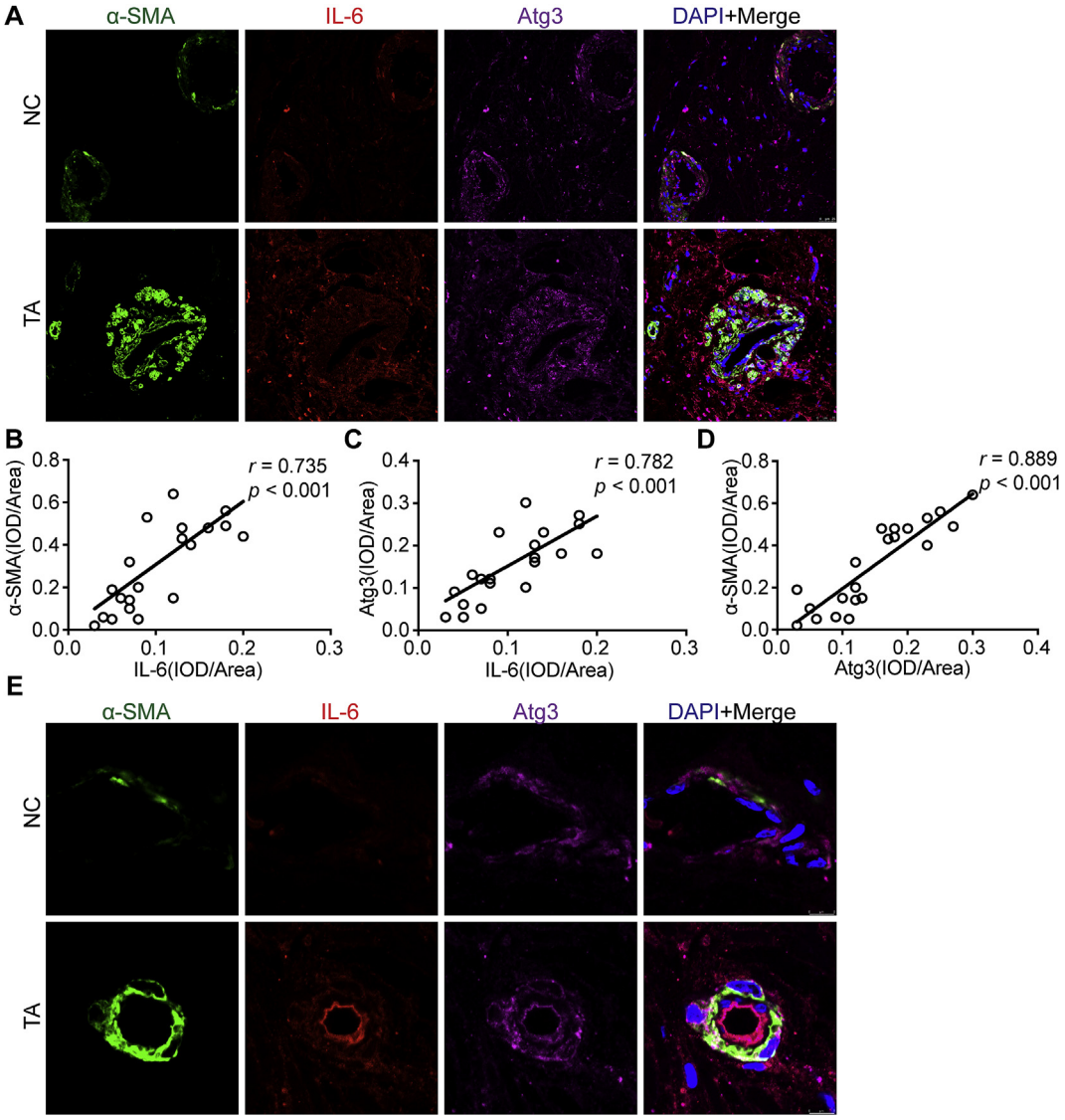

Fig. 2. The colocalization and correlation analysis of Atg3, α-SMA, and IL-6 in the adventitia in TA and the control. (A–D) Correlation analysis of Atg3, α-SMA, and IL-6 in the adventitia in TA and the control (magnification: × 400). (E) Colocation analysis of Atg3, α-SMA, and IL-6 in the adventitia of TA and the control (magnification: × 2000). (n = 24).

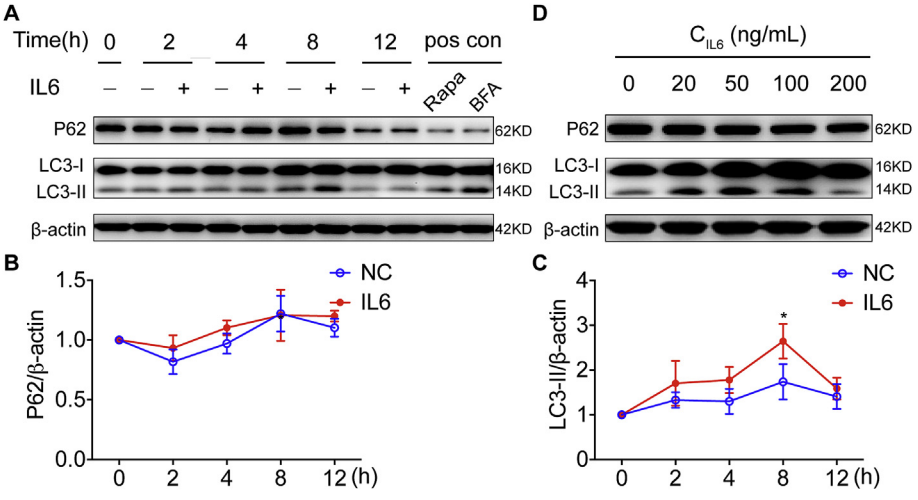

Fig. 3. IL6 influences the expression of autophagy-associated proteins in HAAF. (A) The expression level of autophagy-related proteins P62 and LC3-II changes with the stimulation time. Treatment of stimulated HAAFs with the mTOR inhibitor, Rapa (rapamycin), and endoplasmic reticulum stress inducer, BFA (brefeldin A), for 12 h were used as positive controls (pos con). (B and C) The level of P62 and LC3-II normalized to the β-actin at the corresponding stimulation time points. (D) The effect of different concentrations of IL6 on P62 and LC3 at 8 h of stimulation. $^{*}P < 0.05$. (n = 3).

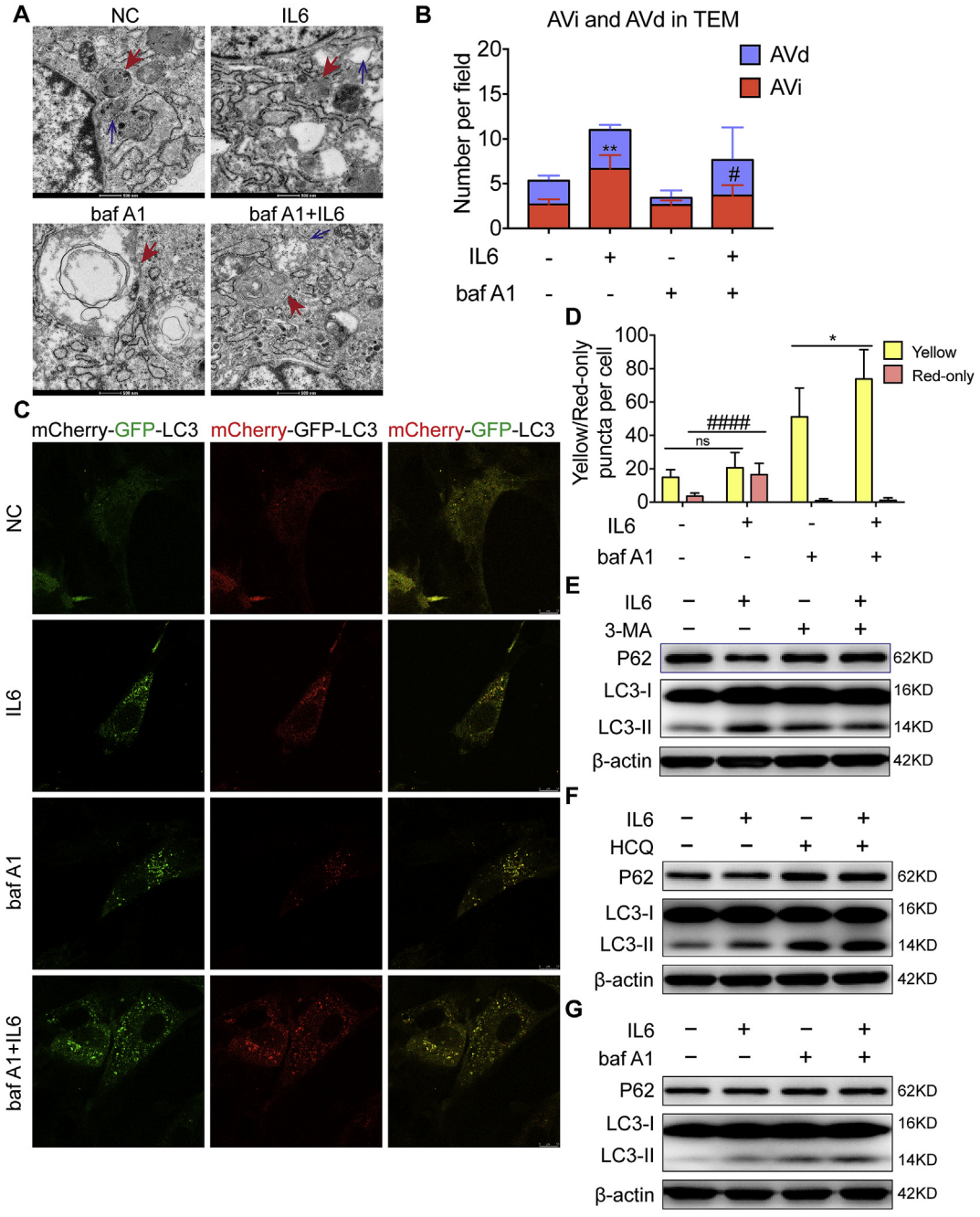

Fig. 4. IL-6 promotes autophagy in HAAF. (A and B) The autophagosomes changed with the addition of IL-6 and autophagy inhibitor, bafilomycin A1 (10 nM) at 8 h. The larger red arrow points to the initial autophagic vacuole (AVi), while the smaller blue arrow refers to the degradative autophagic vacuole (AVd). *IL-6 vs NC in AVi; # IL6 vs IL6 + baf A1 in AVi. (C and D) Detection of autophagosomes (yellow puncta) and autolysosomes (red puncta) in the presence of IL-6 and baf A1 (10 nM) by transfection with mCherry-GFP-LC3 adenovirus at 8 h *baf A1 vs baf A1 + IL-6 in the yellow puncta; #NC vs IL-6 in the red-only puncta. (E–G) The influence of autophagy inhibitors 3-methyladenosine (3-MA) (50 μM) hydroxychloroquine sulfate (HCQ) (10 nM) and baf A1 (10 nM) on IL-6-induced autophagy at 8 h. See Supplemental Fig. 1H–J for the statistical results. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{\#}P < 0.05$; $^{\#\#\#\#}P < 0.0001$; (n = 3). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

dependent pro-fibrosis effect was also observed at 8 h (Supplementary Fig. 2C).

Moreover, we observed that IL-6 could augment the expression of fibrosis protein profiles, including fibronectin, collagen 1, α-SMA, and TGF-β, which could be partially blocked by three autophagic inhibitors, 3-MA, baf A1, and HCQ (Fig. 5A–D). Similar results were found using quantitative PCR (Supplementary Fig. 2D–G). Since most of these proteins are secretory proteins, the level of fibrosis proteins in the culture supernatants was also examined. Compared with the NC, IL-6 significantly increased the level of fibronectin ($P = 0.04$), collagen 1α ($P = 0.003$), collagen 3 ($P = 0.04$), and TGF-β1 ($P = 0.04$), among which the effects on fibronectin and collagen 1α secretion could be reversed by baf A1 (Fig. 5E–H). Collectively, these results indicate that blocking autophagy can mitigate the fibrosis induced by IL-6.

3.6. Fibrosis promoted by IL-6/autophagy was dependent on Jak1 activation

To investigate the signaling pathways involved in IL-6-induced autophagy, the Jak family was screened. Jak1 was significantly activated

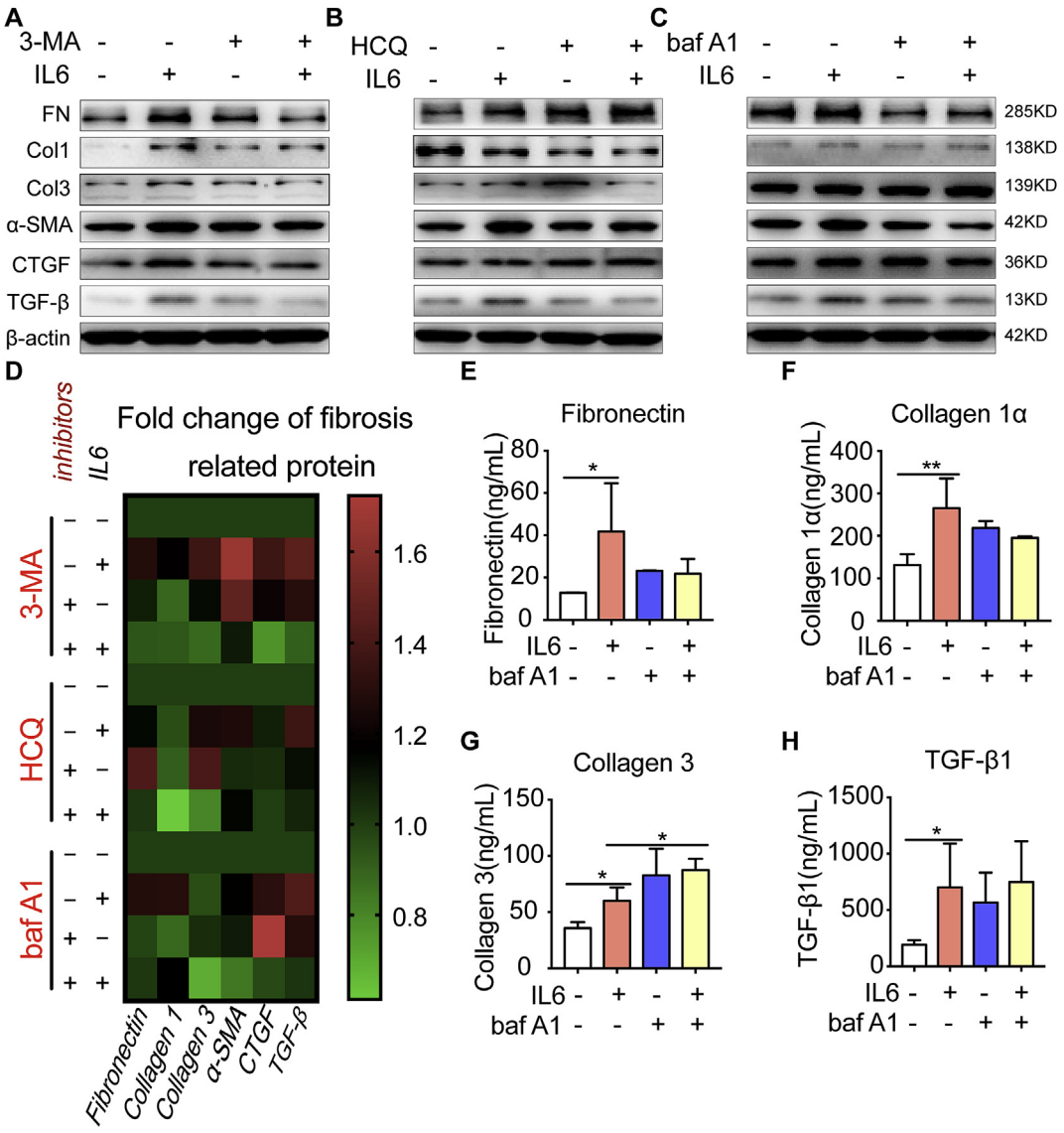

Fig. 5. Fibrosis elevated by IL-6 could be abrogated by autophagy inhibitors. (A–C) The influence of autophagy inhibitors 3-MA, HCQ, and baf A1 on IL-6-induced fibrosis. (D) The statistics for A–C shown by the *mean* (heat map). (E–H) The concentration of fibronectin, collagen 1α, collagen 3, and TGF-β1 in the cell culture supernatants. $^{*}P < 0.05$; $^{**}P < 0.01$; (n = 3).

when HAAFs were exposed to IL-6 (Fig. 6A, Supplementary Fig. 3A). To inhibit the activity of Jak1, the Jak1 inhibitors, Itacitinib and tofacitinib, were used. Following exposure to the inhibitors, the upregulated levels of LC3-II, as well as the elevated number of autophagosomes and autolysosomes induced by IL-6 were significantly reduced (Fig. 6B–E, Supplementary Fig. 3B–E). Moreover, the fibrosis-related proteins, fibronectin and collagen 1α, induced by IL-6 were significantly reduced in both the cell lysates and culture supernatants (Fig. 6F–P). Finally, the alleviated phosphorylation of stat3 and the augmented bcl-2 was observed in the cell lysates as well in the downstream (Supplementary Fig. 3F – H). These results suggest that IL-6/autophagy promotes vascular fibrosis in TA through Jak1/stat3 activation (see Fig. 7).

4. Discussion

Clinically, it is often found that in some patients who have received anti-inflammatory therapy, vascular fibrosis development could not be inhibited, which results in the dysfunction of the aorta and related organs [6,7]. This reveals that vascular fibrosis is an extremely complex process. Autophagy is a well-established self-rescue process, but it has now been found that abnormal autophagy is involved in multiple diseases [18]. In the present study, we found that autophagy and IL-6 were severely elevated in the fibrotic aortic specimens from TA patients, and further analysis showed that they were collocated in the lesioned tissues. This implies that autophagy is related to elevated levels of IL-6 and fibrosis in TA patients.

We previously found that increased levels of IL-6 could promote aortic adventitial fibrosis, further resulting in vessel thickening and remodeling [4,5]. In the study of autophagy, IL-6 has been found to play a double-edged sword, promoting or inhibiting autophagy in different cell lines [12–14]. Thus, we sought to determine whether elevated IL-6 in the tissue promotes or inhibits autophagy.

To address this question, we performed an *in vitro* analysis. We found that exogenous IL-6/IL-6Rα could induce autophagy, including increased mature autophagosomes and autophagy-related genes and proteins. It has been reported that IL-6 could promote autophagy during the late stage (maturation step) in the SHSY5Y cell line [12]. We found that IL-6 enhanced autophagy at an early stage with inhibitors 3-MA, HCQ, and baf A1, respectively.

It has been found that the promotion of autophagy during the early

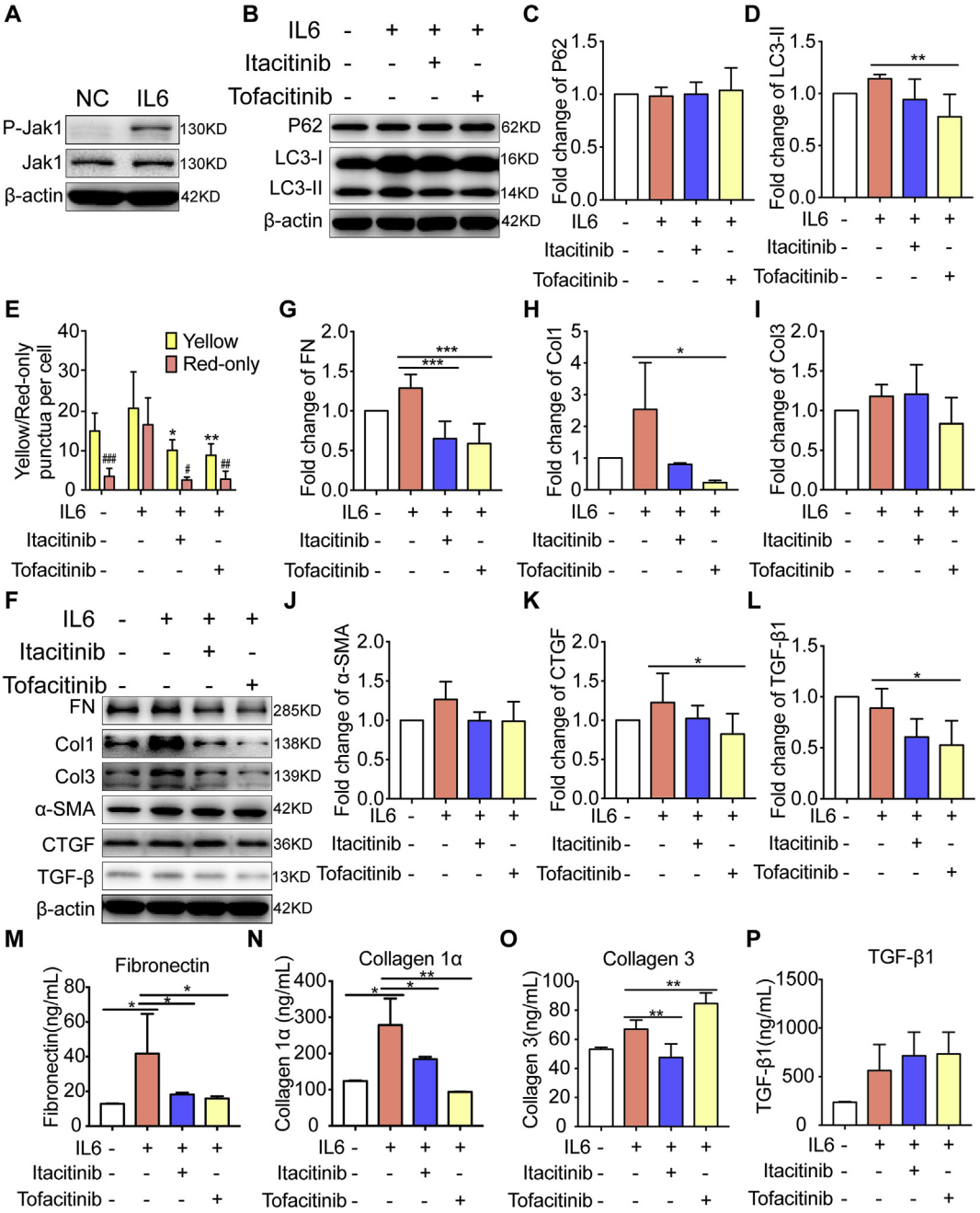

Fig. 6. Jak1 activation was involved in autophagy-induced fibrosis mediated by IL-6. (A) The influence of IL-6 on the phosphorylation of Jak1. (B–D) Change in P62 and LC3 when Itacitinib and tofacitinib were added in IL-6-induced autophagy. (E) The changes in the yellow and red-only puncta when Itacitinib and tofacitinib were added in IL-6-induced autophagy. *IL-6 vs inhibitors + IL-6 in yellow puncta; # NC and inhibitors + IL-6 vs IL-6 in red-only puncta. (F–L) Fibrosis-related protein changes with the addition of Jak1 inhibitors. (M–P) Changes in fibrosis-related proteins in the cell culture supernatants in the presence of Jak1 inhibitors. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{\#}P < 0.05$; $^{\#\#}P < 0.01$; $^{\#\#\#}P < 0.001$ (n = 3). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

and late stages differs with the pathogenesis of disease. The former (early stage) is an initiation factor which is very important in the development of disease, while the latter (late stage) is an enhancer in the process of disease [19]. In the present study, we found that IL-6 promoted autophagy at the early stage, indicating that IL-6 is critical in the autophagy-related pathogenesis of disease.

Given that fibrosis is an end-stage pathogenesis of TA, we further explored the role of IL-6-induced autophagy in the development of fibrosis. The results showed that the interruption of autophagy could significantly abrogate fibrosis induced by IL-6.

Studies have found that autophagy can both promote or inhibit fibrosis [20]. For example, autophagy could increase mouse kidney interstitial and liver fibrosis [21,22]. However, in mouse mesangial cells, enhanced autophagy was found to inhibit fibrosis through the degradation of collagen 1 [23]. In the present study, it was found that in TA, autophagy promoted fibrosis via IL-6, indicating that targeting both autophagy and IL-6 could effectively alleviate vascular fibrosis clinically.

It has been well-established that HCQ is widely used for the treatment of rheumatological diseases. As an alkaline molecule, HCQ can increase the lysosomal pH and prevent lysosomal degradation [24,25]. In autophagy flux, the autophagosome and the lysosomes fuse in order

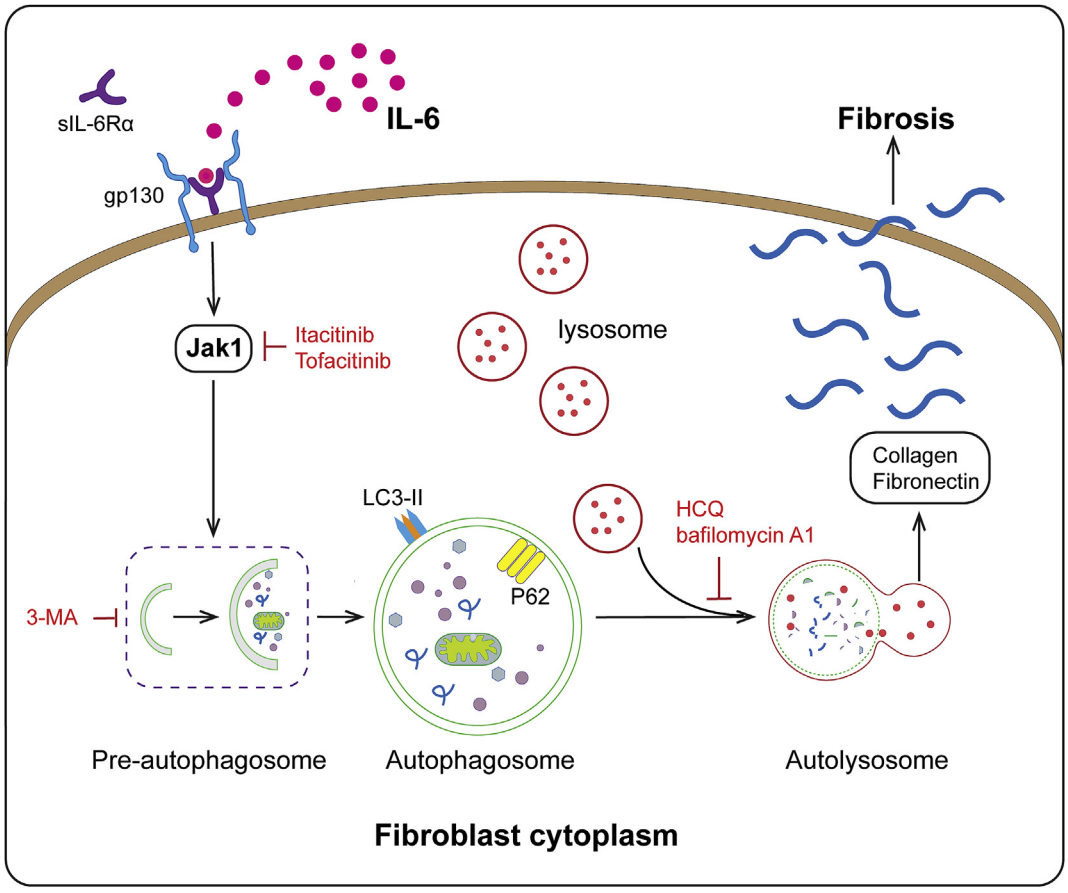

Fig. 7. Research summary. In HAAF, IL-6 could activate the Jak1 signaling pathway to facilitate autophagy in the presence of soluble IL-6Rα(sIL-6Rα), which could be interrupted by the Jak1 inhibitors, Itacitinib and tofacitinib. 3-MA was found to inhibit the initiation of the beclin-1 complex (or VPS34 complex) while HCQ and baf A1 could alter the pH to prevent the degradation of autolysosomes in the process of autophagy. This increased autophagy could subsequently promote fibrosis and influence the vascular remodeling.

to degrade their contents during the maturation step [26]. In an analysis of autophagy, it was demonstrated that HCQ could block the autophagy flux during the maturation step [12], indicating that it acts on the late stage of autophagy in relation to the development of fibrosis. In this study, we found that HCQ could effectively alleviate IL-6/autophagy-induced vascular fibrosis, indicating that this is a popular pathway in inflammatory fibrosis. Accordingly, in a clinical setting, HCQ has been found to mediate both structural and functional protection against vascular lesions in patients with chronic kidney disease and systemic arteritis [27,28]. Therefore, the potential mechanisms might also be autophagy-dependent.

We have previously found that IL-6 promoted fibrosis through activation of the Jak2/stat3 pathway in TA patients [5]. Similarly, in adult ventricular myocytes, IL-6 was found to activate the Jak2/Stat3 signaling pathway [29]; however, in U937 cells, IL-6 could regulate autophagy through the Jak1/Stat3/bcl2/BECN1 pathway [13]. In the present study, we found that IL-6 initiated autophagy through Jak1/stat3 activation, indicating that IL-6-induced fibrosis is dependent on the combination of Jak1 and Jak2. Correspondingly, two pathways should be inhibited simultaneously to obtain the optimal anti-fibrotic effect in TA patients.

Tofacitinib is used to treat patients with rheumatoid arthritis [30]. Moreover, mechanistic studies have shown that tofacitinib could inhibit Jaks in a dose-dependent manner [31]. In this study, we found that tofacitinib could inhibit autophagy, which was identified by IL-6/Jak1 activation. Therefore, in combination with the results of the Jak1 inhibitor, Itacitinib, we speculate that tofacitinib selectively inhibits the Jak1 signaling pathway in IL-6-induced autophagy.

To date, tofacitinib has been found to alleviate vascular stiffness in rheumatoid arthritis [30] without significantly elevating the incidence of cardiovascular disease [32,33]; however, this agent has not been used to treat TA to date. In the present study, we found that tofacitinib could effectively block IL-6/autophagy-induced vascular fibrosis, which indicates that tofacitinib could be used to treat TA clinically in the future.

In conclusion, our findings suggest that autophagy promoted by IL-6/Jak1 augments the vascular fibrosis of TA. Inhibition of the autophagy pathway could prevent vascular remodeling in TA.

Conflicts of interest

None.

Acknowledgement

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jaut.2019.01.010.

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References

[1] N. Mizushima, Autophagy: process and function, Genes Dev. 21 (2007) 2861–2873 https://doi.org/10.1101/gad.1599207.

[2] B. Levine, V. Deretic, Unveiling the roles of autophagy in innate and adaptive immunity, Nat. Rev. Immunol. 7 (2007) 767–777 https://doi.org/10.1038/nri2161.

[3] S.L. Johnston, R.J. Lock, M.M. Gompels, Takayasu arteritis: a review, J. Clin. Pathol. 55 (2002) 481–486.

[4] X. Kong, Y. Sun, L. Ma, H. Chen, L. Wei, W. Wu, et al., The critical role of IL-6 in the pathogenesis of Takayasu arteritis, Clin. Exp. Rheumatol. 34 (2016) S21–S27.

[5] X. Kong, L. Ma, Z. Ji, Z. Dong, Z. Zhang, J. Hou, et al., Pro-fibrotic effect of IL-6 via aortic adventitial fibroblasts indicates IL-6 as a treatment target in Takayasu arteritis, Clin. Exp. Rheumatol. 36 (2018) 62–72.

[6] X.F. Kong, X.J. Zhang, P. Lv, X.M. Cui, L.L. Ma, H.Y. Chen, et al., Treatment of Takayasu arteritis with the IL-6R antibody tocilizumab vs. cyclophosphamide, Int. J. Cardiol. 266 (2018) 222–228 https://doi.org/10.1016/j.ijcard.2017.12.066.

[7] A. Mekinian, M. Resche-Rigon, C. Comarmond, A. Soriano, J. Constans, L. Alric, et al., Efficacy of tocilizumab in Takayasu arteritis: multicenter retrospective study of 46 patients, J. Autoimmun. 91 (2018) 55–60 https://doi.org/10.1016/j.jaut.2018.04.002.

[8] Y. Sun, L. Ma, Z. Ji, Z. Zhang, H. Chen, H. Liu, et al., Value of whole-body contrast-enhanced magnetic resonance angiography with vessel wall imaging in quantitative assessment of disease activity and follow-up examination in Takayasu's arteritis, Clin. Rheumatol. 35 (2016) 685–693 https://doi.org/10.1007/s10067-015-2885-2.

[9] J. Loricera, R. Blanco, S. Castaneda, A. Humbria, N. Ortego-Centeno, J. Narvaez, et al., Tocilizumab in refractory aortitis: study on 16 patients and literature review, Clin. Exp. Rheumatol. 32 (2014) S79–S89.

[10] M. Bredemeier, C.M. Rocha, M.V. Barbosa, E.H. Pitrez, One-year clinical and radiological evolution of a patient with refractory Takayasu's arteritis under treatment with tocilizumab, Clin. Exp. Rheumatol. 30 (2012) S98–S100.

[11] J. Harris, Autophagy and cytokines, Cytokine 56 (2011) 140–144 https://doi.org/10.1016/j.cyto.2011.08.022.

[12] X.Z. Li, C.Y. Sui, Q. Chen, X.P. Chen, H. Zhang, X.P. Zhou, Promotion of autophagy at the maturation step by IL-6 is associated with the sustained mitogen-activated protein kinase/extracellular signal-regulated kinase activity, Mol. Cell. Biochem. 380 (2013) 219–227 https://doi.org/10.1007/s11010-013-1676-9.

[13] B.B. Qin, Z. Zhou, J.Q. He, C.L. Yan, S.P. Ding, IL-6 inhibits starvation-induced autophagy via the STAT3/bcl-2 signaling pathway, Sci Rep-Uk 5 (2015), https://doi.org/ARTN-15701-10.1038/srep15701.

[14] Y. Qi, M. Zhang, H. Li, J.A. Frank, L. Dai, H. Liu, et al., Autophagy inhibition by sustained overproduction of IL6 contributes to arsenic carcinogenesis, Cancer Res. 74 (2014) 3740–3752 https://doi.org/10.1158/0008-5472.CAN-13-3182.

[15] W.P. Arend, B.A. Michel, D.A. Bloch, G.G. Hunder, L.H. Calabrese, S.M. Edworthy, et al., The American-College-of-Rheumatology 1990 criteria for the classification of Takayasu arteritis, Arthritis Rheum. 33 (1990) 1129–1134.

[16] W.X. Ding, H.M. Ni, W. Gao, Y.F. Hou, M.A. Melan, X. Chen, et al., Differential effects of endoplasmic reticulum stress-induced autophagy on cell survival, J. Biol. Chem. 282 (2007) 4702–4710 https://doi.org/10.1074/jbc.M609267200.

[17] D.J. Klionsky, K. Abdelmohsen, A. Abe, M.J. Abedin, H. Abeliovich, A.A. Arozena, et al., Guidelines for the use and interpretation of assays for monitoring autophagy (3rd edition), Autophagy 12 (2016) 1–222 https://doi.org/10.1080/15548627.2015.1100356.

[18] J.S. Rockel, M. Kapoor, Autophagy: controlling cell fate in rheumatic diseases, Nat. Rev. Rheumatol. 13 (2017) 193 https://doi.org/10.1038/nrrheum.2017.17.

[19] L.G. Friedman, Y.H. Qureshi, W.H. Yu, Promoting autophagic clearance: viable therapeutic targets in Alzheimer's disease, Neurotherapeutics 12 (2015) 94–108 https://doi.org/10.1007/s13311-014-0320-z.

[20] D. Del Principe, P. Lista, W. Malorni, A.M. Giammarioli, Fibroblast autophagy in fibrotic disorders, J. Pathol. 229 (2013) 208–220 https://doi.org/10.1002/path.4115.

[21] M.J. Livingston, H.F. Ding, S. Huang, J.A. Hill, X.M. Yin, Z. Dong, Persistent activation of autophagy in kidney tubular cells promotes renal interstitial fibrosis during unilateral ureteral obstruction, Autophagy 12 (2016) 976–998 https://doi.org/10.1080/15548627.2016.1166317.

[22] K.M. Kim, C.Y. Han, J.Y. Kim, S.S. Cho, Y.S. Kim, J.H. Koo, et al., G alpha(12) overexpression induced by miR-16 dysregulation contributes to liver fibrosis by promoting autophagy in hepatic stellate cells, J. Hepatol. 68 (2018) 493–504 https://doi.org/10.1016/j.jhep.2017.10.011.

[23] S.I. Kim, H.J. Na, Y. Ding, Z.B. Wang, S.J. Lee, M.E. Choi, Autophagy promotes intracellular degradation of type I collagen induced by transforming growth factor (TGF)-beta 1, J. Biol. Chem. 287 (2012) 11677–11688 https://doi.org/10.1074/jbc.M111.308460.

[24] S. Lombard-Platlet, P. Bertolino, H. Deng, D. Gerlier, C. Rabourdin-Combe, Inhibition by chloroquine of the class II major histocompatibility complex-restricted presentation of endogenous antigens varies according to the cellular origin of the antigen-presenting cells, the nature of the T-cell epitope, and the responding T cell, Immunology 80 (1993) 566–573.

[25] C. Munz, Autophagy beyond intracellular MHC class II antigen presentation, Trends Immunol. 37 (2016) 755–763 https://doi.org/10.1016/j.it.2016.08.017.

[26] S.R. Yoshii, N. Mizushima, Monitoring and measuring autophagy, Int. J. Mol. Sci. 18 (2017), https://doi.org/10.3390/ijms18091865.

[27] A.M. Shukla, C. Bose, O.K. Karaduta, E.O. Apostolov, G.P. Kaushal, T. Fahmi, et al., Impact of hydroxychloroquine on atherosclerosis and vascular stiffness in the presence of chronic kidney disease, PLoS One 10 (2015) e0139226 https://doi.org/10.1371/journal.pone.0139226.

[28] A. Casian, S.R. Sangle, D.P. D'Cruz, New use for an old treatment: hydroxychloroquine as a potential treatment for systemic vasculitis, Autoimmun. Rev. 17 (2018) 660–664 https://doi.org/10.1016/j.autrev.2018.01.016.

[29] X. Yu, R.H. Kennedy, S.J. Liu, JAK2/STAT3, not ERK1/2, mediates interleukin-6-induced activation of inducible nitric-oxide synthase and decrease in contractility of adult ventricular myocytes, J. Biol. Chem. 278 (2003) 16304–16309 https://doi.org/10.1074/jbc.M212321200.

[30] A.K. Kume K, S. Yamada, T. Kanazawa, K. Hatta, Tofacitinib Monotherapy Improves Arterial Stiffness in Conventional Dmards Active Rheumatoid Arthritis Patients. A Cohort Study, Arthritis & rheumatology, Hoboken, NJ), 2017, p. 69 abstract.

[31] J.A. Hodge, T.T. Kawabata, S. Krishnaswami, J.D. Clark, J.B. Telliez, M.E. Dowty, et al., The mechanism of action of tofacitinib - an oral Janus kinase inhibitor for the treatment of rheumatoid arthritis, Clin. Exp. Rheumatol. 34 (2016) 318–328.

[32] J.J. Wu, B.E. Strober, P.R. Hansen, O. Ahlehoff, A. Egeberg, A.A. Qureshi, et al., Effects of tofacitinib on cardiovascular risk factors and cardiovascular outcomes based on phase III and long-term extension data in patients with plaque psoriasis, J. Am. Acad. Dermatol. 75 (2016) 897–905 https://doi.org/10.1016/j.jaad.2016.06.012.

[33] J.R. Curtis, H. Schulze-Koops, L. Takiya, C.A. Mebus, K.K. Terry, P. Biswas, et al., Efficacy and safety of tofacitinib in older and younger patients with rheumatoid arthritis, Clin. Exp. Rheumatol. 35 (2017) 390–400.