Flow-dependent YAP/TAZ activities regulate endothelial phenotypes and atherosclerosis

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The focal nature of atherosclerotic lesions suggests an important role of local hemodynamic environment. Recent studies have demonstrated significant roles of Yes-associated protein (YAP) and transcriptional coactivator with PDZ-binding motif (TAZ) in mediating mechanotransduction and vascular homeostasis. The objective of this study is to investigate the functional role of YAP/TAZ in the flow regulation of atheroprone endothelial phenotypes and the consequential development of atherosclerotic lesions. We found that exposure of cultured endothelial cells (ECs) to the atheroprone disturbed flow resulted in YAP/TAZ activation and translocation into EC nucleus to up-regulate the target genes, including cysteine-rich angiogenic inducer 61 (CYR61), connective tissue growth factor (CTGF), and ankyrin repeat domain 1 (ANKRD1). In contrast, the atheroprotective laminar flow suppressed YAP/TAZ activities. En face analysis of mouse arteries demonstrated an increased nuclear localization of YAP/TAZ and elevated levels of the target genes in the endothelium in atheroprone areas compared with athero-protective areas. YAP/TAZ knockdown significantly attenuated the disturbed flow induction of EC proliferative and proinflammatory phenotypes, whereas overexpression of constitutively active YAP was sufficient to promote EC proliferation and inflammation. In addition, treatment with statin, an antiatherosclerotic drug, inhibited YAP/TAZ activities to diminish the disturbed flow-induced proliferation and inflammation. In vivo blockade of YAP/TAZ translation by morpholino oligos significantly reduced endothelial inflammation and the size of atherosclerotic lesions. Our results demonstrate a critical role of the activation of YAP/TAZ by disturbed flow in promoting atheroprone phenotypes and atherosclerotic lesion development. Therefore, inhibition of YAP/TAZ activation is a promising athero-protective therapeutic strategy.

atherogenesis | disturbed flow | endothelial cells | mechanotransduction

Atherosclerosis, the most common cause of acute myocardial infarction and ischemic stroke, results from endothelial dysregulation in the arterial wall (1, 2). The hemodynamic forces acting on the arterial wall play a crucial role in determining the function and phenotype of endothelial cells (ECs) (3, 4). In the straight part of the arterial tree, the blood flow pattern is laminar with a significant forward direction (laminar shear stress, LS), and the resulting gene expression and structural/functional consequences in ECs are athero-protective, i.e., antiproliferation and antiinflammation. In contrast, the local flow patterns at branch points and aortic arch are disturbed, having oscillations with little forward component (oscillatory shear stress, OS), and the EC phenotypes and gene expression are atheroprone, i.e., proliferation and proinflammation (3, 4). Thus, the different mechanisms of mechanotransduction in response to flow patterns with or without a clear direction lead to the distinct EC phenotypes in different regions of the arterial tree. Multiple pathways mediating the flow-regulated functional outcomes (athero-protective vs. atheroprone) have been established (3), but there is a relative lack of information on the downstream functional effectors.

Yes-associated protein (YAP) and transcriptional coactivator with PDZ-binding motif (TAZ) are emerging as master signaling regulators in organ growth control and tumor suppression (5–7).

YAP and TAZ (YAP/TAZ) activities are canonically modulated by the Hippo pathway kinase large tumor suppressor (LATS)-dependent phosphorylation. The dysregulation of Hippo pathway and hyperactivation of YAP/TAZ has been reported in many types of human cancers (6–8). YAP/TAZ are retained in the cytoplasm and inactive in gene transcription when phosphorylated; in contrast, the dephosphorylated form of YAP/TAZ translocates into the nucleus to promote the target gene transcription via interactions with the transcription factor partners (e.g., TEA domain transcription factors, TEADs) (9). Many biochemical factors such as cytokines (e.g., IL-6), growth factors (e.g., EGF), and drugs (e.g., statins) have been shown to regulate YAP/TAZ activities and functional outcomes (10–13). Recent studies have reported that YAP/TAZ serve as major mechanotransducers responding to extracellular biophysical cues, such as cell geometry and matrix stiffness, in modulating stem cell fate (14). YAP has been shown to mediate vascular remodeling in the progression of carotid stenosis (15). Two of the YAP/TAZ transcriptional targets, cysteine-rich angiogenic inducer 61 (CYR61) and connective tissue growth factor (CTGF), are highly expressed in atherosclerotic or injured, but not in normal, human arteries (16, 17). However, it remains to be determined whether YAP/TAZ mediate a mechanosensitive pathway that plays a significant role in the hemodynamic regulation of vascular homeostasis in health and disease.

In this study, we provide evidence that atherogenic OS, but not athero-protective LS, resulted in YAP/TAZ phosphorylation and nuclear localization in ECs both in vitro and in vivo. Therefore, we hypothesized that OS signals through YAP/TAZ to induce

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Significance

Yes-associated protein (YAP)/transcriptional coactivator with PDZ-binding motif (TAZ) are emerging as key regulators of cell fate in response to biochemical and biophysical cues, but their role in modulating vascular homeostasis in health and disease is not clearly understood. In studying the flow regulation of YAP/TAZ activities in vascular endothelial cells, we find that disturbed flow (without a clear direction), but not laminar flow (with a clear direction), activates YAP/TAZ to promote the atheroprone phenotypes (proliferation and inflammation). We also demonstrate that inhibition of YAP/TAZ attenuates the atheroprone phenotypes and lesion progression in atherosclerotic mice. These findings indicate that YAP/TAZ serve as important mechanotransducers in the disturbed flow-induced atherogenesis and provide insights to the development of therapeutic strategy for cardiovascular diseases.

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the proproliferative and proinflammatory responses of ECs and the consequential atherogenesis. Our results have validated this hypothesis by demonstrating that knocking down YAP/TAZ repressed the OS induction of EC proliferation and inflammation; in contrast, overexpression of YAP/TAZ led to the expression of these atheroprone EC phenotypes. In addition, we found that statin, a cholesterol-lowering drug, enhanced athero-protection in ECs through YAP/TAZ inactivation. These findings provided a rationale for exploring the regulatory role of YAP/TAZ in the atherosclerotic mouse model. Our in vivo studies demonstrated that systemic inhibition of YAP/TAZ attenuated the development of atherosclerotic lesions induced by the partial carotid ligation (PL) procedure in apolipoprotein E-deficient (ApoE$^{-/-}$) mice, suggesting that YAP/TAZ activation in ECs plays a causative role in the initiation and progression of atherosclerosis. Thus, our findings have established the role of YAP/TAZ activation in mediating the atherogenesis induced by disturbed flow and provided mechanistic and therapeutic insights for atherosclerosis.

Results

To assess the role of YAP/TAZ in regulating vascular homeostasis and diseases, we examined their expression and localization in normal and atherosclerotic arteries in the ApoE$^{-/-}$ mice. Immunofluorescence staining of the normal carotid artery showed that YAP/TAZ-positive cells were present in abundance in the endothelium in tunica intima, but few in the media layer (Fig. S1A). In contrast, in the atherosclerotic carotid artery and the aortic arch, YAP/TAZ staining was prominent in both the endothelium and the media layer, and the intimal hyperplasia plaque (Fig. S1 B and C). These results demonstrate that, although YAP/TAZ are enriched in ECs under normal physiological conditions, they are aberrantly elevated in atherosclerotic vessels, suggesting that YAP/TAZ may serve as important regulators of EC functions and that dysregulation of YAP/TAZ may contribute to the development of atherosclerosis. Therefore, we further investigated the mechanisms by which vascular YAP/TAZ are regulated and their functional role in modulating vascular phenotypes.

Differential Regulation of YAP/TAZ Activities Under Different Flow Patterns in Vitro and in Vivo. EC phenotypes and functions are modulated by the flow patterns generated by local hemodynamic forces. The activation of YAP/TAZ in ECs is determined by the hypophosphorylation of YAP at the Ser127 residue, increases of YAP/TAZ and their target gene expression, and nuclear localization of YAP/TAZ. To investigate whether the flow pattern plays a role in modulating YAP/TAZ activities in ECs, we performed in vitro shear stress experiments (OS vs. LS) by using the parallel flow chamber and in vivo flow perturbation experiments using the PL model in ApoE$^{-/-}$ mice (18, 19). Human umbilical vein ECs (HUVECs) were subjected to OS at 0.5 ± 4 dyn/cm^2 or LS at 12 dyn/cm^2 for 12 and 24 h, and the levels of YAP phosphorylation and YAP/TAZ expression were examined by Western blot analysis. Our results demonstrated that OS, but not LS, led to a significant decrease of YAP phosphorylation and a marked increase of YAP expression. We found that 24-h OS elevated TAZ expression, in comparison with the LS condition (Fig. 1A). The hypophosphorylation of YAP and induction of YAP/TAZ under OS were confirmed in human aortic ECs (HAECs) in vitro (Fig. S2A). These findings suggest that OS results in YAP/TAZ activation in both HUVECs and HAECs. The OS activation of YAP/TAZ was further demonstrated by the nuclear staining of YAP/TAZ that was detected by an antibody recognizing both YAP and TAZ, whereas LS caused cytoplasmic retention of the inactive YAP/TAZ (Fig. 1B). Quantitative analysis of the intensity of YAP/TAZ staining in the nuclear and cytoplasmic regions confirmed the nuclear localization of YAP/TAZ under OS, but not under LS. To elucidate the flow regulation of YAP/TAZ activation in vivo, we harvested the left common carotid arteries (LCA) from ApoE$^{-/-}$ mice subjected
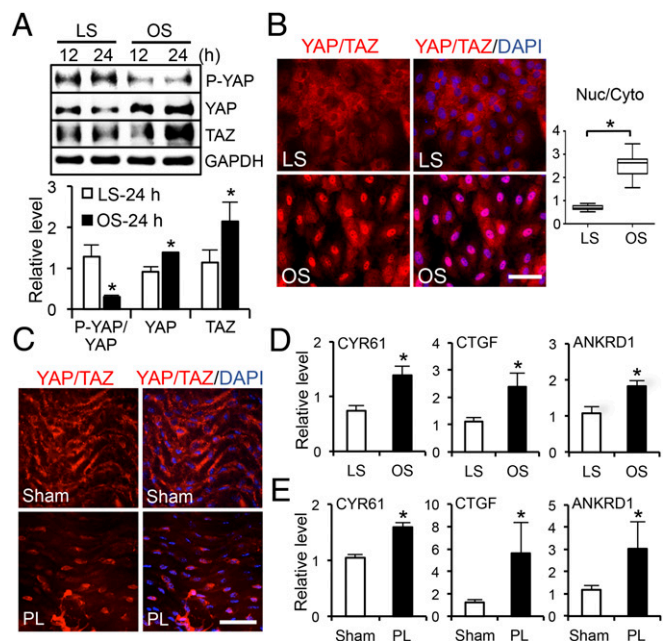

Fig. 1. Disturbed flow induces activation and nuclear localization of YAP/TAZ. (A) Western blot analysis of P-YAP, YAP, TAZ, and GAPDH in ECs subjected to LS or OS for the indicated time. The graphic data are mean ± SD of band intensity normalized to its internal control and LS 12-h group, $n = 3$. *$P < 0.05$ vs. LS-24 h. (B and C) Immunofluorescence staining of YAP/TAZ in ECs subjected to LS or OS (B) and in the endothelium of PL- or sham-operated carotid arteries (C). (Scale bars: 100 μm.) $n = 4$. The graphic data in B are the YAP/TAZ intensity ratio (nuclear/cytoplasmic) from cells randomly selected from three independent experiments. *$P < 0.05$ vs. LS. (D and E) qRT-PCR analysis of YAP/TAZ target genes expression in ECs subjected to OS or LS (D) and in PL or sham groups (E) (mean ± SD, $n = 3$, *$P < 0.05$ vs. LS or sham).

to PL and sham operation, where the endothelium had experienced atheroprone disturbed flow and athero-protective laminar flow, respectively. En face staining showed YAP/TAZ accumulation in the nuclei of ECs in the PL group, but a cytoplasmic and diffused distribution in ECs in the sham group (Fig. 1C). Quantitative RT-PCR (qRT-PCR) analysis revealed that the expression levels of YAP/TAZ target genes, including CYR61, CTGF, and ankyrin repeat domain 1 (ANKRD1), were significantly higher in ECs under OS than LS (Fig. 1D and Fig. S2B). In agreement with our in vitro results, the levels of YAP/TAZ target genes were higher in the PL than the sham group in our in vivo study (Fig. 1E). In addition, YAP/TAZ activation and expression were found to be higher in the disturbed flow region of the inner curvature of aortic arch (AA) than the laminar flow region of the straight segment of thoracic aorta (TA) (Fig. S2 C and D). Consistent with this finding, the expression of the YAP/TAZ target genes were also elevated in AA, in comparison with those in TA (Fig. S2E).

Disturbed Flow Promotes EC Proliferation Through YAP/TAZ Activation. In light of such differential regulations of YAP/TAZ phosphorylation, localization, and target gene expression by flow patterns found both in vitro and in vivo, we further studied the functional role of YAP/TAZ in modulating EC phenotypes. YAP/TAZ are known to promote cell growth in many tissues. To determine the role of flow regulation of YAP/TAZ activities in modulating endothelial proliferation, we performed loss-of-function experiments in ECs by using silencing RNAs against YAP/TAZ (siYT) and gain-of-function experiments using plasmids overexpressing constitutively active FLAG-5SA-YAP mutant (5SA-YAP) and wild-type HA-TAZ (TAZ) to mimic the OS activation of YAP/TAZ. The results demonstrated that knocking down YAP/TAZ significantly inhibited
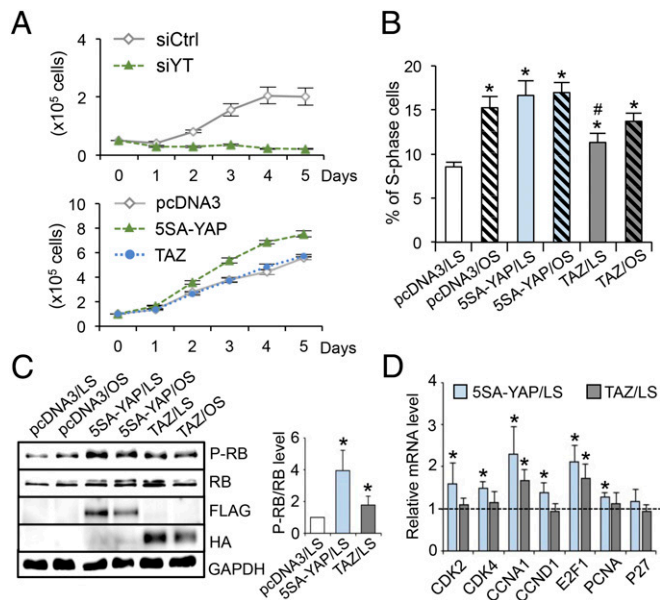
Fig. 2. YAP/TAZ regulate the EC growth and cell cycle progression. (A) EC growth curves with YAP/TAZ knockdown (Upper) and overexpression (Lower). ECs were transfected with silencing RNAs against YAP/TAZ (siYT) or scramble control (siCtrl) and plasmids expressing FLAG-5SA-YAP (5SA-YAP), HA-TAZ (TAZ), or pcDNA3, and then seeded onto six-well plates. The number of ECs were counted daily for 5 d. $n = 3$. (B–D) ECs were transfected with 5SA-YAP, TAZ, or pcDNA3 plasmids, followed by OS and LS experiments for the analysis of cell cycle (B), retinoblastoma (RB) phosphorylation (C), and cell cycle regulatory genes expression (D). (B–D) Mean ± SD, $n = 3$, *$P < 0.05$ vs. pcDNA3/LS and #$P < 0.05$ vs. 5SA-YAP/LS.

EC growth (Fig. 2 A, Top), and that overexpression of 5SA-YAP, but not TAZ, markedly increased EC growth (Fig. 2 A, Bottom). In accordance with our previous report (18), exposure of ECs to long-term LS led to a reduction of cell numbers in the S phase and growth arrest in the G_0/G_1 phase (Fig. 2B). Flow cytometry analysis showed that overexpression of 5SA-YAP mitigated the LS-induced cell cycle arrest, with the percentage of S-phase cells increased from $8.54 \pm 1.13\%$ to $16.64 \pm 3.23\%$; overexpression of TAZ under the same transfection condition had a lesser effect as the percentage of S-phase cells increased to only $11.31 \pm 1.99\%$ (Fig. 2B). To identify the molecules that mediate the YAP regulation of cell cycle progression, we examined the phosphorylation level of retinoblastoma protein (RB) and expression levels of cell cycle regulatory genes in G_1-to-S transition (i.e., CDK2/4, CCNA1, CCND1, E2F1, p27, and PCNA). In agreement with the flow cytometry analysis, overexpression of 5SA-YAP exerted a stronger effect than TAZ in promoting the hyperphosphorylation of RB under both LS and OS conditions (Fig. 2C) and expressions of cell cycle regulatory genes (Fig. 2D). These results suggest that LS inactivates YAP/TAZ to exert the antiproliferative effect on ECs; in contrast, OS results in YAP/TAZ activation to program ECs to a proliferative phenotype. The inactivation of YAP/TAZ by LS and the resulting cell cycle suppression would contribute to the lower EC turnover rate and the quiescent EC phenotype in athero-protective regions of the arterial tree in comparison with the atheroprone regions.

Disturbed Flow Promotes Inflammation Through YAP/TAZ Activation. We investigated whether YAP/TAZ mediates the proinflammatory responses in ECs under disturbed flow. The effects of YAP/TAZ knockdown or overexpression on their target gene expressions were examined by qRT-PCR analysis (Fig. S3 A–C). Application of OS, but not LS, up-regulated the levels of VCAM1 and ICAM1 in EC lysates (Fig. 3A and Fig. S3D) and on EC surfaces (Fig. 3B), and the OS-induction of adhesion molecules was accompanied by an increase

in the number of THP1 monocytes attached to ECs (Fig. 3C). YAP/TAZ knockdown decreased the OS-induced proinflammatory responses, as evidenced by the reductions of EC adhesion molecule expression and monocyte attachment (Fig. 3 A–C), whereas overexpression of 5SA-YAP and TAZ increased the proinflammatory responses under static condition (Fig. 3 D and E). Moreover, YAP/TAZ knockdown attenuated the VCAM1 expressions in ECs induced by inflammatory cytokines (TNFα and IL1β) (Fig. S3E). A previous report identified β2 integrin (ITGB2) as one of the potential targets induced by YAP/TAZ activation (9). Because of the unique role of ITGB2 in the chemosensory adhesion machinery of bloodborne cells, we further examined the role of YAP/TAZ in monocytes. YAP/TAZ knockdown blunted the up-regulation of β2 integrin and ICAM1 induced by TNFα in THP1 cells and reduced their adherence to the activated ECs (Fig. 3 F–H and Fig. S4A) without altering the plasticity of THP1 monocyte-to-macrophage differentiation (Fig. S4 B and C). Taken together, these results support our hypothesis that the YAP/TAZ activation by disturbed flow and cytokines enhance monocyte–EC interaction and contribute, at least partially, to atherogenesis.

Statin Exerts Athero-Protective Effects Through Inactivation of YAP/TAZ. Statin, a commonly used antiatherosclerotic drug, has been shown to repress YAP activity and prevent YAP-mediated transcription in tumor growth (12, 13). Similar to the LS, statin treatment exerts
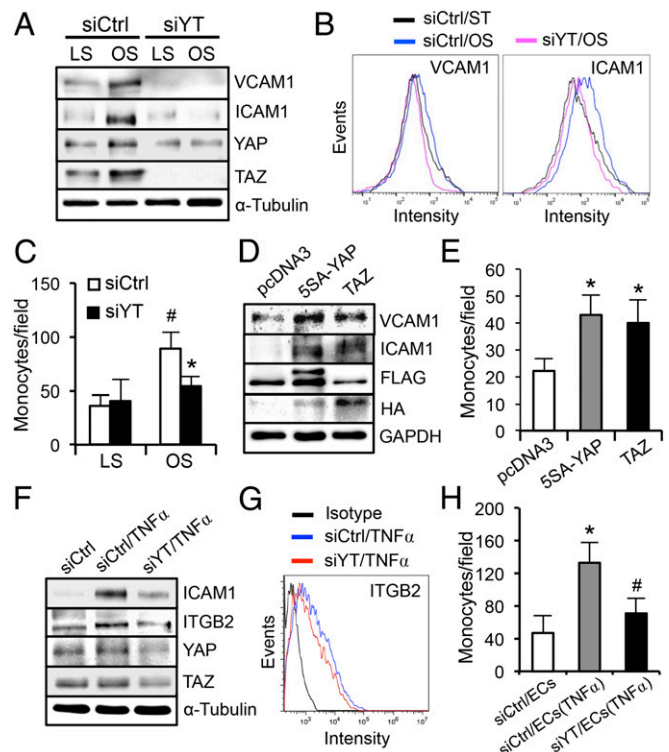

Fig. 3. YAP/TAZ activation promotes the disturbed flow-induced proinflammatory responses. (A–C) ECs were transfected with siYT or siCtrl, subjected to 24-h LS or OS, and followed by Western blot analysis of VCAM1, ICAM1, and YAP/TAZ (A), flow-cytometric analysis of VCAM1 and ICAM1 surface expression (B), and THP1 monocyte adhesion assay (C), *$P < 0.05$ vs. siCtrl/OS and #$P < 0.05$ vs. siCtrl/LS. (D and E) ECs were transfected with 5SA-YAP, TAZ, or pcDNA3 plasmids and subjected to the Western blot analysis of VCAM1 and ICAM1 (D) and monocyte adhesion assay (E). *$P < 0.05$ vs. pcDNA3. (F–H) THP1 monocytes were transfected with siYT or siCtrl, treated with tumor necrosis factor-α (TNFα, 10 ng/mL) for 6 h, and then subjected to Western blot analysis of ICAM1 and ITGB2 (F), flow cytometric analysis of ITGB2 surface expression (G), and adhesion assay to the TNFα-activated ECs (H). *$P < 0.05$ vs. siCtrl/EC and #$P < 0.05$ vs. siCtrl/EC(TNFα).

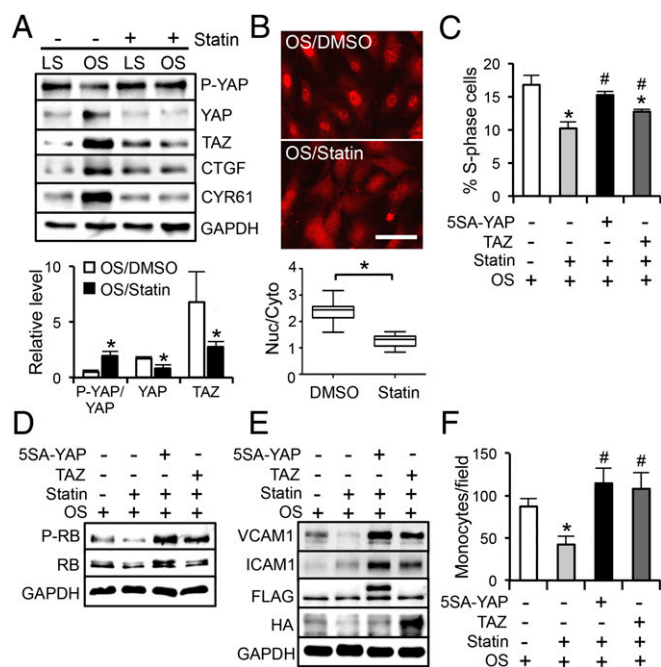CELL BIOLOGY

Fig. 4. Statin inhibits YAP/TAZ to exert antiproliferative and antiinflammatory effects on ECs. (*A*) Western blot analysis of P-YAP, YAP, TAZ, CTGF, and CYR61 in ECs subjected to LS or OS in the presence of 1 μM simvastatin (statin) or DMSO control. *P < 0.05 vs. OS/DMSO. (*B*) Staining of YAP/TAZ localization in ECs subjected to 24-h OS in the presence of statin or DMSO. (Scale bar: 100 μm.) The graphic data are the nuclear/cytoplasmic ratio of YAP/TAZ intensity from cells randomly selected from three independent experiments. *P < 0.05 vs. OS/DMSO. (*C* and *D*) ECs transfected with 5SA-YAP, TAZ, or pcDNA3 were subjected to OS in the presence of statin or DMSO, followed by the analyses of cell cycle (*C*) and RB phosphorylation (*D*). (*E* and *F*) The transfected ECs were subjected to OS in the presence of statin or DMSO, followed by the analysis of VCAM1, ICAM1, and GAPDH (*E*) and monocyte adhesion assay (*F*). (*C* and *F*) Mean ± SD, n = 3, *P < 0.05 vs. OS/DMSO/pcDNA3 and #P < 0.05 vs. OS/statin/pcDNA3.

athero-protective effects on vascular ECs (20). We further investigated whether YAP/TAZ mediate the statin-modulated athero-protective effects. Our results demonstrated that exposure of ECs to OS in the presence of 1 μM simvastatin (statin), but not the solvent control (dimethyl sulfoxide, DMSO), resulted in hyperphosphorylation of YAP, reductions of YAP/TAZ and their target genes (Fig. 4*A*), and a decrease of nuclear YAP/TAZ (Fig. 4*B*), suggesting that statin treatment inactivates YAP/TAZ and may lead to inhibitory effects on EC proliferation and inflammation. Indeed, reconstitution of YAP/TAZ activities by overexpression of 5SA-YAP or TAZ attenuated the statin suppression of EC cell cycle progression (Fig. 4 *C* and *D*). Statin treatment also decreased the VCAM1 expression and the monocyte attachment to ECs induced by OS. Introduction of 5SA-YAP and TAZ reversed these inhibitory effects exerted by statin (Fig. 4 *E* and *F*). In agreement with the in vitro findings, our in vivo results demonstrated that daily statin administration (15 mg/kg, 3 d) resulted in YAP inactivation and reduction, but did not alter TAZ expression, in the AA inner curvature and the TA straight segment ([Fig. S5*A*](#)). En face staining of the PL-operated LCAs showed YAP/TAZ accumulation in the nuclei of ECs from the vehicle control group, but a cytoplasmic and diffused YAP/TAZ distribution pattern in ECs from the statin-treated group ([Fig. S5*B*](#)). Taken together, our results suggest that the pleiotropic effects of statin on the suppression of the atheroprone EC phenotypes are mediated, at least in part, through YAP/TAZ inactivation.

YAP/TAZ Inhibition Attenuates Endothelial Activation and Atherosclerotic Lesion Development in PL-Operated LCAs. We next investigated the role of disturbed flow-induced YAP/TAZ activation on the

development of atherosclerotic lesions. The PL-induced flow disturbance in LCAs of ApoE$^{-/-}$ mice results in severe carotid atherosclerosis in 4 wk (19). To perturb the disturbed flow activation of YAP/TAZ in vivo, we designed the morpholino (MO)-based oligos to inhibit YAP/TAZ translation ([*SI Materials and Methods*](#)). ApoE$^{-/-}$ mice fed the Paigen diet were given MO-YAP/TAZ (MO-YT) or MO-Control (MO-Ctrl) at a dose of 10 nmol (~5 mg/kg) through tail-vein injections 72 h before and immediately after the PL procedure; the oligos were given twice a week after the operation for up to 4 wk (Fig. 5*A*). The expression levels of YAP/TAZ and endothelial inflammation were examined 1 wk after operation. The results showed that administration of MO-YT, but not MO-Ctrl, significantly diminished YAP/TAZ protein levels in the arteries (Fig. 5*B*). Functionally, this reduction of YAP/TAZ decreased the PL-induced adhesion molecule expression in the intima (Fig. 5*C*) and the consequential leukocyte attachment (Fig. 5*D*), as indicated by the immunofluorescence staining analysis of VCAM1 and CD45, respectively. After 4 wk of MO-oligos injections, the levels of cholesterol, HDL, and LDL in the serum of ApoE$^{-/-}$ mice fed the Paigen diet were comparable between the MO-Ctrl and MO-YT groups, but significantly higher than the control littermates that were fed a regular chow ([Table S1](#)). These results demonstrate that the YAP/TAZ modulation of vascular functions is not mediated through the blood lipids. Importantly, in vivo blockade of YAP/TAZ translation significantly reduced the overall size of atherosclerotic plaques in the PL-operated LCAs (Fig. 5*E* and [Fig. S6](#)). Histological analysis of the frozen sections and quantification of luminal lesion size confirmed that YAP/TAZ inhibition by MO-YT strongly attenuated the disturbed flow-induced carotid atherosclerosis (from 175,820 to 30,964 μm^2) (Fig. 5*F*). These results demonstrate that the YAP/TAZ activation induced by disturbed flow synergizes with atherogenic factors, such as hypercholesterolemia, to promote the atheroprone phenotypes and atherosclerosis development.

Discussion

The detailed mechanisms for atheroprone EC phenotypes, such as dysregulated turnover and maladaptive inflammation, induced by the disturbed flow patterns at the arterial branches and curvature, remain to be elucidated (3, 4). Recent reports demonstrated that, in addition to their responses to chemical stimuli, YAP/TAZ serve as important transducers relaying mechanical signals to modulate gene expression profiles and functional consequences through their transcription factor partners (14, 21, 22). Our current findings elucidate the mechanotransducing role of YAP/TAZ in hemodynamic regulation of endothelial homeostasis and diseases. As summarized in Fig. 6, we have revealed a mechanism that YAP/TAZ are critical regulators in mediating the disturbed flow-induced atheroprone EC phenotypes and the consequential development of atherosclerosis. This conclusion is supported by the in vitro and in vivo findings that (*i*) the disturbed flow, but not steady laminar flow, induces YAP/TAZ activation, as indicated by hypophosphorylation of YAP, nuclear localization of YAP/TAZ, and up-regulations of YAP/TAZ and their target genes; (*ii*) the disturbed flow activation of YAP/TAZ promotes the cell cycle progression, proinflammatory gene expression, and monocyte adhesion; (*iii*) the statin suppression of endothelial proliferation and inflammation is mediated by inhibition of YAP/TAZ activities; (*iv*) the inhibition of YAP/TAZ attenuates the PL-induced endothelial inflammation and the consequential progression of carotid atherosclerosis in ApoE$^{-/-}$ mice.

The regulation of YAP/TAZ activities, as indicated by the phosphorylation status of YAP and the resulting cytoplasmic/nuclear localization, are controlled canonically by LATS kinases (7). Recent reports have shown that mechanical stimuli modulate YAP/TAZ activities via a Rho-GTPases/actomyosin-dependent, but LATS-independent, manner (14, 22). In our hands, depletion of LATS1 was not sufficient to reduce the LS-induced YAP phosphorylation in ECs ([Fig. S7*A*](#)); instead, modulations of Rho-GTPase
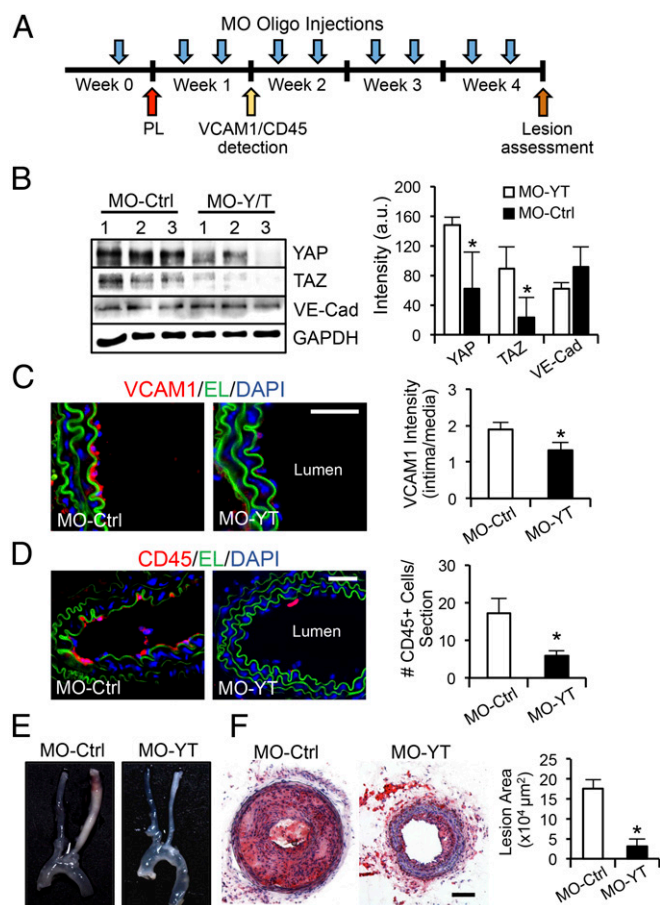
Fig. 5. YAP/TAZ play important roles in atherogenesis. (*A*) Experimental design of morpholino oligo treatments (MO-Ctrl vs. MO-YT, 10 nmol) in ApoE$^{-/-}$ mice fed the Paigen diet. The oligos were i.v. injected into animals 72 h before the PL, immediately after the PL, and twice a week afterward. (*B*) One-week post-PL, thoracic aortas were harvested for the analysis of YAP, TAZ, VE-Cadherin, and GAPDH expression. $n = 3$. (*C* and *D*) The sections of LCAs were examined by the immunofluorescence staining of VCAM1 (red) (*C*) and CD45 (red) (*D*). Elastic lamina (EL, green) and nuclear staining (DAPI, blue) are shown to indicate vessel structure. The VCAM1 intensity ratio (intima/media) and the number of CD45-positive cells attached to intima layer were quantified. $n = 4$. (*E* and *F*) Four-week post-PL, the arterial tissues were isolated to examine the atherosclerotic lesions (*E*), and the LCAs were sectioned for immunohistochemistry analysis (Oil Red O and Hematoxylin) and quantification of lesion area. $n = 6$ (*F*). (*B*–*D* and *F*): mean ± SD, *$P < 0.05$ vs. MO-Ctrl. (Scale bars: 100 μm.)

activities with an active RhoA (V14-Rho) and a Rho inhibitor (C3 exoenzyme) strongly regulated YAP/TAZ activities (Fig. S7*B*), suggesting the flow regulation of YAP/TAZ activities does not solely depend on LATS kinases but also through the modulation of Rho-GTPase activities. In addition to the Hippo and Rho-GTPase pathways, other known mechanosensors, such as G protein-coupled receptors and integrins (23, 24), and mechanotransduction pathways, including AMP-activated protein kinase, and VE-cadherin–mediated phosphatidylinositol 3-kinase (PI3K)/AKT signaling (25, 26), have been shown to modulate YAP/TAZ activities through phosphorylation. It would be worthwhile to further delineate the Hippo signaling cascade and the cross-talk between the flow-sensitive pathways that modulate YAP/TAZ activities. YAP/TAZ–TEADs complex has been shown to induce expressions of its target genes controlling cell growth and cell cycle progression (9, 27). It is likely that the OS-activated YAP/TAZ act through TEADs to promote EC proliferation. Further investigation will be required to dissect the transcriptional regulation of YAP/TAZ-target gene

expressions and the functional network in ECs in response to different flow patterns.

CYR61 and CTGF, the best known targets of YAP/TAZ, have been associated with the chemosensory adhesion and migration of leukocytes (16, 17). Our in vitro and in vivo data demonstrate that disturbed flow patterns increased the expressions of CYR61 and CTGF in ECs, in comparison with steady laminar flow (Fig. 1 *D* and *E*). CYR61 and CTGF have also been found to be highly expressed in atherosclerotic arteries (16, 17). In our studies, we have found that, just as CYR61 and CTGF, YAP/TAZ are highly expressed transmurally in the atherosclerotic arteries, but their expression is only restricted to the intima layer at the lesion-free regions (Fig. S1). Furthermore, perturbations of YAP/TAZ by the silencing RNAs and the plasmids overexpressing YAP resulted in down- and up-regulations of CYR61 and CTGF, respectively (Fig. S3 *A* and *B*). Taken together, our findings indicate that the dysregulation of YAP/TAZ induced by disturbed flow and other atherogenic factors are responsible for the CYR61 and CTGF overexpressions and, hence, play a proatherogenic role in promoting the inflammatory-fibrotic responses in atherosclerosis.

Dysregulation of YAP/TAZ causes aberrant cell growth, leading to a series of developmental disorders and cancers (5, 7). In the vascular system, smooth muscle-specific deletions of YAP result in profound cardiac defects and vascular abnormalities (28). The inductions of YAP expression and activation following arterial injury enhance neointima formation and the subsequent stenosis as a result of the unrestrained smooth muscle cell proliferation (15). We demonstrate that perturbations of YAP/TAZ in ECs resulted in significant changes in EC growth, indicating that YAP/TAZ are essential for proliferation and cell cycle progression of ECs. The OS activation of YAP/TAZ induced strong proinflammatory responses in ECs (Fig. 3), and the loss of YAP/TAZ attenuated the induction of VCAM1 expression by the inflammatory cytokines (Fig. S3*E*); these findings indicate that YAP/TAZ activation exerts a proinflammatory effect in response to the elevation of cytokines at the atherosclerotic lesions, as well as the atheroprone flow conditions. However, the inactivation of YAP/TAZ by atheroprotective LS counteracted the effects resulted from these risk factors associated with atherosclerosis. Our findings establish that YAP/TAZ activities modulate endothelial proliferation and inflammation, with the disturbed flow activation of YAP/TAZ playing a causative role in mediating the atheroprone phenotypes.

In addition to the blockade of cholesterol synthesis, statin exerts the beneficial pleiotropic effects on vascular cells, including the
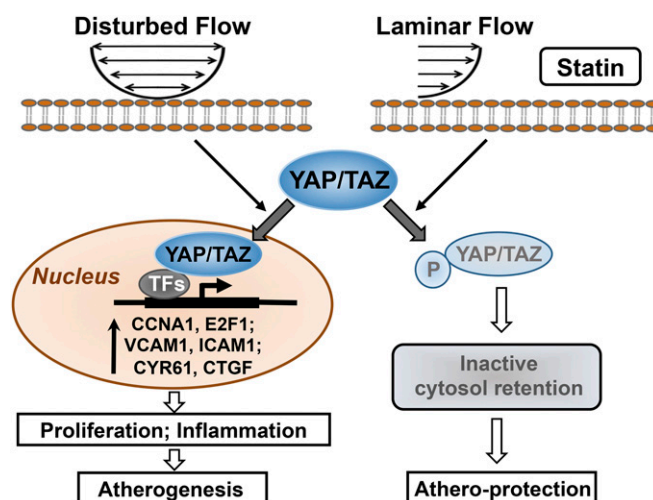

Fig. 6. Schematic diagram of YAP/TAZ signaling and their modulation of EC phenotypes in response to disturbed flow, laminar flow, and statin.

improvement of endothelial function and attenuation of vascular inflammation (20). Our findings provide a mechanistic explanation for such pleiotropic effects of statin on vascular cells. We found that statin repressed the OS-induced EC proliferation and inflammation, and that statin caused the reduction of YAP/TAZ activities and cytoplasmic retention of YAP/TAZ both in vitro and in vivo, similar to the findings in cancer cells (12, 13). Most importantly, reconstitution of the YAP/TAZ activities blocked the statin suppressions of EC proliferation and inflammation (Fig. 4). These findings demonstrate that inactivation of YAP/TAZ plays a critical role in the athero-protective effects of statin on ECs. It has been reported that statin therapy also inhibits the activities of proatherogenic Rho-GTPase/kinase (ROCK) pathway and improves the outcome in patients with atherosclerosis (29). One may speculate that the beneficial effects of statin inhibition of Rho/ROCK on atherosclerosis are attributable to YAP/TAZ inactivation in vascular cells. In this regard, YAP/TAZ inhibition may serve as a potential antiatherosclerotic strategy with better specificity and fewer off-target effects.

The proatherogenic role of YAP/TAZ activation in the pathogenesis and progression of atherosclerosis was further established by our in vivo loss-of-function studies. Morpholino-based antisense oligomers have been developed and tested in a range of disease models, including that for coronary artery restenosis after angioplasty, through translational inhibition (30). Using the rodent model to induce carotid atherosclerosis by disturbed flow patterns, we showed that the i.v. injections of morpholino oligos against

YAP/TAZ reduced the PL-induced atheroprone EC phenotypes within the 1-wk treatment period, and that prolonged inhibition of YAP/TAZ (4 wk) attenuated the atherosclerotic lesion formation (Fig. 5). These findings provide the preclinical evidence that YAP/TAZ are potential therapeutic targets for atherosclerosis.

In conclusion, using the in vitro cell perfusion system and the in vivo PL-induced atherosclerotic mouse model, we have demonstrated that YAP/TAZ serve as critical mechanotransducers in modulating endothelial proliferation and inflammation in response to local hemodynamics, and that YAP/TAZ activation mediates the disturbed flow-induced atheroprone phenotypes and atherosclerotic lesion formation. Our findings provide strong evidence for the atherogenic role of YAP/TAZ activation and insights for the development of therapeutic intervention for atherosclerosis.

Materials and Methods

The sources of reagents and antibodies, and the methods for cell culture, flow channel experiments, transient transfection, qRT-PCR, Western blot, flow cytometric analysis of cell cycle, monocyte adhesion assay, animal experiments, histological characterization of the arterial tissues, quantification of atherosclerotic lesions, and statistical analysis are described in SI Materials and Methods. The primer sets used in this study are listed in Table S2. All animal experiments performed in this study were in accordance with NIH guidelines and approved by the Animal Care and Use Committee of the University of California at San Diego. The details are provided in SI Materials and Methods.

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