BASIC SCIENCE ARTICLE

The YAP signaling pathway promotes the progression of lymphatic malformations through the activation of lymphatic endothelial cells

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BACKGROUND: To investigate whether the YAP/TAZ (Yes-associated protein/transcriptional coactivator with PDZ binding motif) pathway contributes to the pathogenesis of lymphatic malformations (LMs).
METHODS: YAP, TAZ, CTGF (connective tissue growth factor), and Ki-67 were detected in LMs by immunohistochemistry. The colocalization of YAP and Ki-67 was analyzed by double immunofluorescence. Pearson's correlation and cluster analyses were performed to analyze the relationships between these proteins. Human dermal lymphatic endothelial cells (HDLECs) were used for mechanistic investigation. Rat models of LMs were established to investigate the role of the YAP pathway in LM development.
RESULTS: Compared with those in normal skin, the expression levels of YAP, TAZ, CTGF, and Ki-67 were significantly upregulated in lymphatic endothelial cells (LECs) of LMs. Interestingly, YAP and CTGF presented much higher expression levels in infected LMs. In experiments in vitro, lipopolysaccharide (LPS) enhanced the expression of YAP in a concentration- and time-dependent manner via the increased phosphorylation of Erk1/2 (extracellular signal-regulated kinase 1/2). Moreover, the proliferation, invasion, and tubule formation of HDLECs increased significantly in accordance with the activation of the YAP signaling pathway. Furthermore, LM rat models validated that LPS facilitated the development of LMs, which was dependent on the activation of YAP.
CONCLUSIONS: The data reveal that activation of the YAP signaling pathway in LECs may play a crucial role in the progression of LMs.

IMPACT:

- Compared with that in normal skin, the YAP signaling pathway was activated in LECs of LMs. Inhibiting the YAP signaling pathway attenuated the proliferation, invasion, and tubule formation of HDLECs. Additionally, the activation of the YAP signaling pathway could promote LM development in a rat model.
- Activation of the YAP signaling pathway in LECs may play a crucial role in the progression of LMs.
- The YAP signaling pathway was activated in LMs. Inhibition of the YAP signaling pathway could promote regression of the lesions.

INTRODUCTION

Lymphatic malformations (LMs) are congenital diseases that arise from the aberrant ontogeny of the lymphoid system during the embryonic period.[1] LMs mainly occur in places with abundant lymph, of which 45–52% occur in the head and neck regions. LMs are always present at birth and increase in size gradually with age.[2,3] Maxillofacial deformities and oral cavity dysfunctions caused by cervicofacial enlarged LM lesions cause patients enormous physical and psychological suffering and can be life threatening when the oropharynx is obstructed by swelling lesions.[4,5] The curative effect of LMs by surgery and sclerosing agent injection is different from person to person. Even worse, the

remedial effect is greatly reduced when LMs occur with local infection.[4] Nevertheless, the underlying mechanism is largely unknown.

Yes-associated protein (YAP) and its paralog TAZ (transcriptional coactivator with PDZ binding motif) are the major downstream effectors of the Hippo signaling pathway.[6] The activation of YAP/TAZ activates a number of target genes, including connective tissue growth factor (CTGF), which has been implicated in the progression of wound healing, inflammatory diseases, human fibrotic diseases, and tumor growth.[7,8] Recent studies have reported that the YAP signaling pathway is involved in embryogenesis, tissue regeneration, and homeostasis of somatic

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stem cells.[9] In addition, the overexpression of YAP has been found in tumors, inflammation, and cardiovascular system diseases.[6] Regarding lymphangiogenesis, YAP and TAZ play promoting roles in remodeling lymphatic plexus patterning and postnatal lymphatic valve maintenance by negatively regulating Prox1 expression.[10] Moreover, reports have proven that the expression of YAP is upregulated by infection and therefore contributes to nasal epithelial cell proliferation in chronic rhinosinusitis and activation of human vascular endothelial cells in sepsis-induced acute lung injury.[11,12] However, the accurate roles of the YAP pathway in LM development as well as the cellular physiology of lymphatic endothelial cells (LECs) in the presence or absence of infection are rarely reported.

In our present study, we found that the YAP signaling pathway was activated and had a close correlation with local infection in LMs. The activation of YAP contributed to the proliferation, invasion, and tubular formation of LECs in response to infection. Mechanistically, the potential role of the extracellular signal-regulated kinase 1/2 (Erk1/2) signaling pathway, one of the key signaling pathways in inflammation, was also investigated in these processes. Additionally, the role of YAP in the pathophysiologic development of LMs was further confirmed in LM rat models. Therefore, we propose a new pathogenesis mechanism of LMs, and targeting the YAP signaling pathway may be a promising therapeutic strategy for LMs.

METHODS

Clinical samples
Twenty LM samples and eight normal skin samples (SKs) were collected from the Hospital of Stomatology, Wuhan University. LM lesions (20) were collected from patients receiving surgery, but had not received any other therapies before surgery, and most patients (17/20) were under 12 years old. Relative information on the patients is shown in Supplementary Tables I and II. The research was approved by the review board of the Ethics Committee of the Hospital of Stomatology, Wuhan University, and all patients signed informed consent forms. All procedures were carried out in keeping with the National Institutes of Health guidelines with respect to the use of human specimens.

Immunohistochemistry, double-labeling immunofluorescence, and evaluation
According to our previous studies,[13–15] these experiments were carried out as described in the Supplement.

Cell culture and reagents
Human lymphatic endothelial cells (HDLECs, ScienCell, #2010) were cultured according to our previous study.[14] Lipopolysaccharide (LPS, Sigma, #L4391), the YAP inhibitor verteporfin (Cayman Chemical, #17334) and the Erk1/2 selected inhibitor U0126 (CST, #9903, 10 μM) were dissolved in phosphate-buffered saline (PBS) or dimethyl sulfoxide (DMSO) for further use. The cell medium was changed to 2% fetal bovine serum when the cells were treated with drugs.

Transfection and treatments
Knockdown of YAP was performed to explore its role in HDLEC biological behaviors, and a detailed procedure is included in the Supplement.

Western blot assay
Depending on our previous studies,[14] the procedures were as recorded in the Supplement.

Cell proliferation, migration, invasion, and tube formation assay
The procedure was performed as described in the Supplement.

LM models in rats
The LM models were established as in our previous studies.[14] Rats with lesions were randomly divided into four groups with different treatments (three times a week) for 8 weeks as follows: PBS, verteporfin (4 μg/mL), LPS (100 μg/mL), or LPS (100 μg/mL) + verteporfin (4 μg/mL). The specific process is described in the Supplement. Animal treatment and procedures were approved and supervised by the Ethics Committee for Animal Research, Wuhan University.

Statistical analysis
GraphPad Prism 6.0 (GraphPad Software Inc.) was used to perform Student's t test and Spearman's rank correlation test for statistical analysis. All data are presented as the mean values ± standard deviation (SD). $P < 0.05$ was considered statistically significant.

RESULTS

Upregulation of the YAP signaling pathway and its correlation with proliferation in LM samples
Podoplanin, a valid marker of lymphatic endothelia, is negatively expressed in the blood vasculature.[16] In this study, podoplanin-positive vessels were regarded as lymphatic vessels (Supplementary Fig. S1). To determine the activation of the YAP pathway in LMs, the expression levels of YAP, TAZ, and CTGF were detected in LMs and SKs. YAP/TAZ activation is demonstrated by its dephosphorylation and subsequent localization in the nucleus, as YAP/TAZ elicits its biological functions after translocation into the nucleus. Therefore, in the present study, positive YAP/TAZ staining in the nuclei of LECs was considered to indicate activated YAP/TAZ, and the statistical analysis only involved the activation of YAP/TAZ. The results showed that YAP, TAZ, and CTGF were strongly expressed in the LECs of LMs, but negative in SKs (Fig. 1a). Ki-67, a core marker of cellular proliferation, was also detected in LMs. In line with our previous studies,[14] the expression level of Ki-67 was significantly increased in LMs compared with SKs (Fig. 1a). More importantly, Student's t test further confirmed that the expression levels of YAP, TAZ, CTGF, and Ki-67 were significantly upregulated in LM samples compared with SKs (Fig. 1b). In addition, the results from the Spearman's rank test of the above detected proteins showed that the nuclear location of YAP was significantly correlated with CTGF and Ki-67 in LMs (Fig. 1c, d). Furthermore, the results from clustering analysis of the tested proteins showed that almost all the LM samples were clustered together and were obviously distinct from SKs (Fig. 1e). Additionally, we found a synchronous distribution for YAP and Ki-67 in the LECs of LMs, but not SKs with double-labeling immunofluorescence (Fig. 1f). Collectively, these results reveal that the YAP signaling pathway is active in LMs and has a close relationship with the proliferation of LECs in LMs.

YAP is related to local infection of LMs in experiments both in vitro and in vivo
Studies have well demonstrated that infection promotes the progression of human LMs.[14] Therefore, we next analyzed the expression of YAP, TAZ, and CTGF in infected and uninfected LMs, which were identified by their pathological and clinical findings. Interestingly, although no significant difference in TAZ staining between infected and uninfected LMs was observed, the quantitative analysis showed that the staining intensity of nuclear YAP and its downstream protein CTGF was remarkably elevated in infected LM samples compared with the uninfected group (Fig. 2a). LPS is a crucial product of Gram-negative microbiota that can promote the expression of inflammatory cytokines such as tumor necrosis factor and interleukins, thus leading to the pathogenesis and development of diseases.[17] In studies in vitro, we first found that LPS promoted HDLEC proliferation via YAP activation without

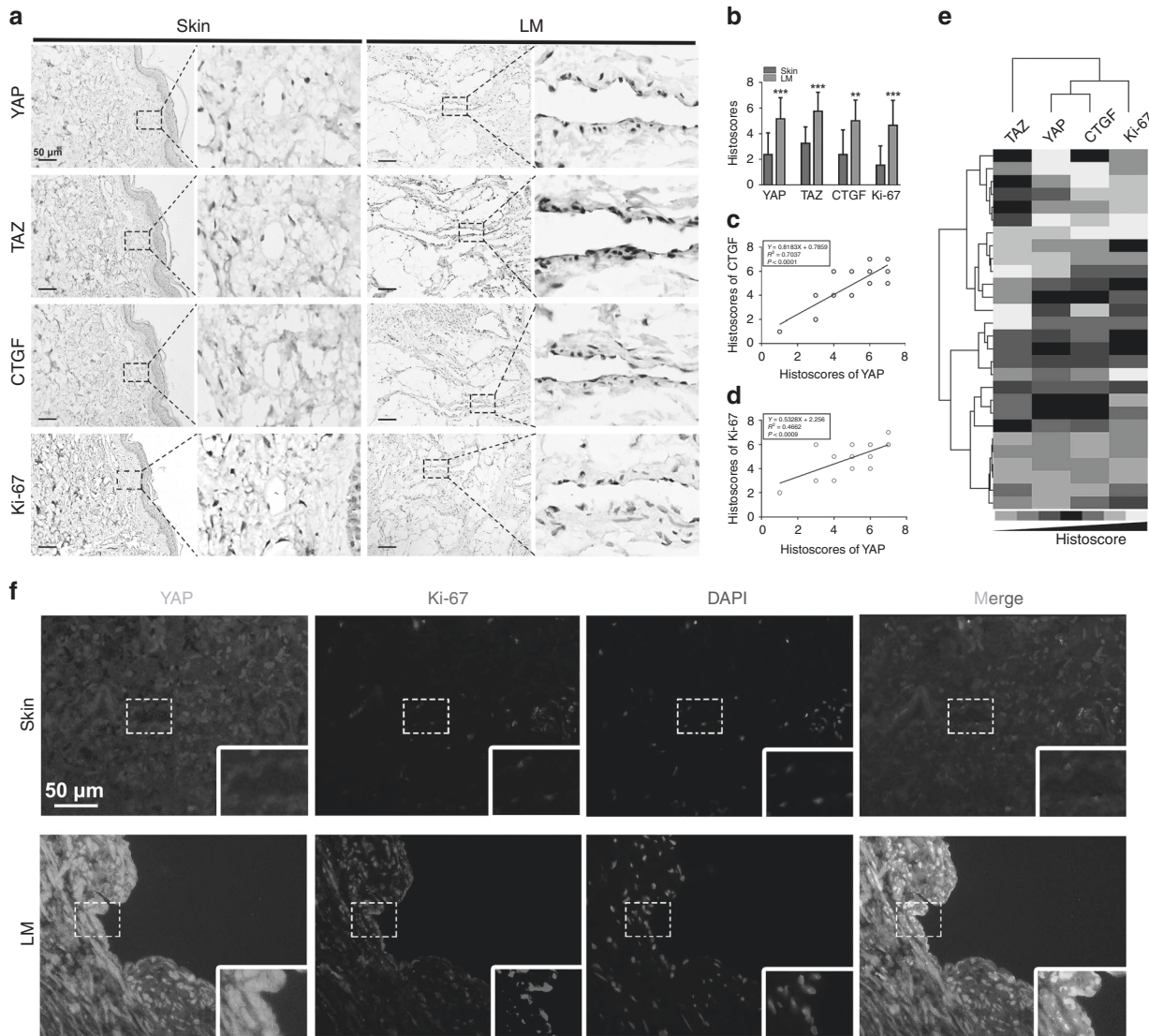The YAP signaling pathway promotes the progression of lymphatic...
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Fig. 1 Upregulation of the YAP signaling pathway and its correlation with proliferation in LM samples. a Representative immunohistochemical staining of YAP, TAZ, CTGF, and Ki-67 in normal skin (SK) and lymphatic malformation (LM) samples. **b** Quantitative analysis of the histoscores of the indicated markers in SK and LMs. The expression levels of YAP, TAZ, CTGF, and Ki-67 were much higher than those in SK. **c** Pearson's correlation analysis of YAP and CTGF in LM samples. **d** Pearson's correlation analysis of YAP and Ki-67 in LM samples. **e** Clustering analysis of the expression levels of YAP, TAZ, CTGF, and Ki-67 was performed, and the result was visualized with a heat map. **f** Double-labeling immunofluorescence analyses of YAP and Ki-67. Each point represents the mean ± SD. **$P < 0.01$; ***$P < 0.001$.

verteporfin (Fig. 2b, c). Subsequently, we detected the expression of YAP in HDLECs with or without LPS treatment. As expected, YAP and CTGF were massively potentiated by LPS in a dose- and time-dependent manner (Fig. 2d, e). Recent documents have indicated the vital function of Erk1/2 in the activation of the YAP signaling pathway.[18–20] Intriguingly, we also found that the activation of Erk1/2 in HDLECs was enhanced in response to LPS, which was parallel to the expression of YAP (Fig. 2d, e). In our studies, 10 µM U0126 successfully inhibited the phosphorylation of Erk1/2 (p-Erk1/2), but had no obvious effect on the expression of the total level of Erk1/2 (t-Erk1/2) in HDLECs (Fig. 2f). Interestingly, U0126 significantly attenuated LPS-induced YAP expression (Fig. 2f). Moreover, nuclear YAP localization was extremely enhanced in LPS-treated HDLECs, as demonstrated by cell immunofluorescence (Supplementary Fig. S2B). Taken together, these results suggest that YAP is very strongly associated with infection in LMs and that LPS promotes YAP expression in a dose- and time-dependent manner by activating the phosphorylation of Erk1/2 in LECs.

YAP is necessary for the migration, invasion, and tubular formation of HDLECs, even in infection conditions
To investigate the role of the YAP signaling pathway in HDLEC function, disruption of YAP by short hairpin RNA-YAP (shYAP)-mediated knockdown (Supplementary Fig. S2A) and pharmacological (verteporfin) methods was performed in studies in vitro. As shown in Fig. 3a–c, the migration, invasion, and tubular formation capacities of HDLECs decreased in a verteporfin dose-dependent manner and were significantly suppressed in the shYAP group (Fig. 3d–f). Considering the aforementioned findings elucidating LPS-enhanced expression and the essential role of YAP in HDLEC biological functions, we hypothesized that LPS promoted HDLEC biological behaviors via YAP activation. Therefore, LPS-treated HDLECs were incubated with verteporfin or shYAP. The final statistical results showed that LPS could remarkably drive the migration, invasion, and tubular formation of HDLECs, while inhibition of YAP (caused by verteporfin or shYAP) significantly attenuated the LPS-derived behavioral function in HDLECs (Fig. 3d–f). The above findings indicate that YAP is necessary for
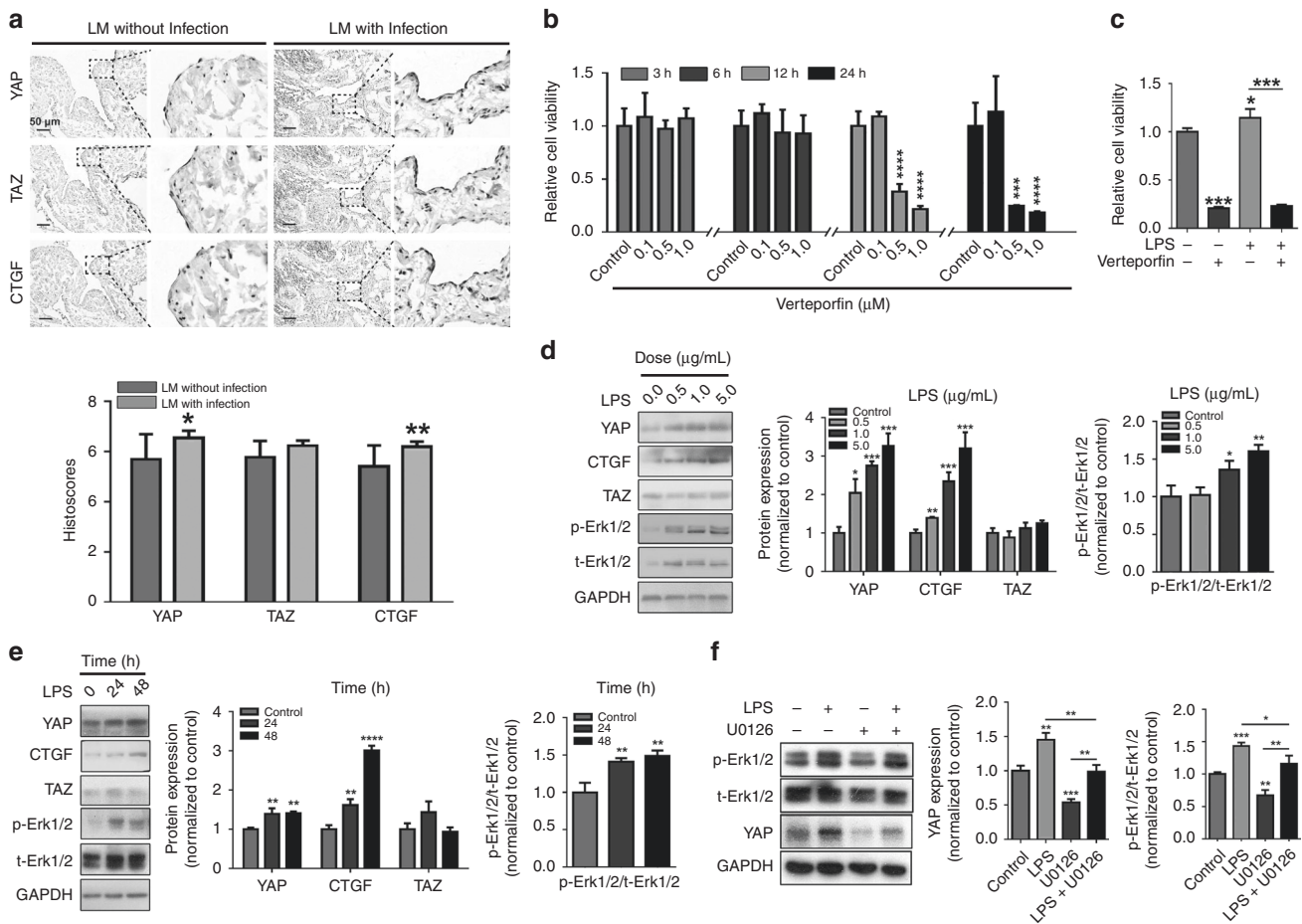
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Fig. 2 **YAP is related to local infection of LMs both in vitro and in vivo. a** Representative immunohistochemical images and analysis of YAP, TAZ, and CTGF in LMs with or without infection. **b** HDLECs were treated with different concentrations from 0.0 to 1.0 μM verteporfin for various times. Cell viability was measured at the same time by MTT assay. **c** Cell viability was detected using the MTT assay when the cells were treated with 0.5 μM verteporfin for 24 hours in the presence or absence of 5.0 μg/mL LPS stimulation. **d** Western blot of YAP, CTGF, TAZ, p-Erk1/2, and t-Erk1/2 in HDLECs after LPS challenge (0.0, 0.5, 1.0, and 5.0 μg/mL) for 24 h and quantitative analysis of YAP, CTGF, and TAZ with normalization to GAPDH and ratio of p-Erk/t-Erk in HDLECs with LPS treatment at a concentration gradient. **e** Western blot of YAP, CTGF, TAZ, p-Erk1/2, and t-Erk1/2 in HDLECs after 5.0 μg/mL LPS challenge (0, 24, and 48 h) and quantitative analysis of YAP, CTGF, and TAZ with normalization to GAPDH and the ratio of p-Erk/t-Erk in HDLECs with 5.0 μg/mL LPS treatment over a time period. **f** Western blot of p-Erk1/2, t-Erk1/2, YAP, and GAPDH in HDLECs treated with 5.0 μg/mL LPS for 24 h with or without 10 μM U0126 and quantitative analysis of YAP/GAPDH and p-Erk/t-Erk in HDLECs treated with LPS and/or U0126. Data are expressed as the mean ± SD. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$.

the migration, invasion, and tubular formation of HDLECs, and LPS was dependent on YAP activation to enhance these functions of HDLECs, which may be linked to pathological changes at the cellular level in fast-deteriorating LMs with infection.

Suppression of the YAP signaling pathway inhibits the formation of LM lesions in rat models
To explore the role of YAP in LM development, rat LM models were established with two injections (2 weeks apart) of Freund's incomplete adjuvant (FIA) subcutaneously into the neck as described in previous studies.[14] Rats with lesions were randomly divided into four groups with different local injection treatments (three times a week) for 8 weeks as follows: 200 μL PBS only as the control (FIA group), 4 μg/mL verteporfin in 200 μL PBS (FIA + verteporfin group), 100 μg/mL LPS in 200 μL PBS (FIA + LPS group), and 100 μg/mL LPS combined with 4 μg/mL verteporfin in 200 μL PBS (FIA + LPS + verteporfin group). The function of LPS was to simulate infected LM lesions.[14] In the present study, the lesions of the control group (FIA group) were much smaller than those of the FIA + LPS group (Fig. 4a–c), which is in line with previous studies.[14] Moreover, treatment with verteporfin

significantly suppressed the growth of LMs, even under LPS conditions (Fig. 4a–c). Immunohistochemical staining showed that Ki-67 was significantly decreased by verteporfin with or without LPS treatment (Fig. 4d). Verteporfin also reduced the level of VEGF-C (a specific regulator of lymphangiogenesis) in LECs of LMs with or without LPS treatment (Fig. 4e). On the other hand, during the administration of medication, the body weights of each group showed a trend of stable physiological growth with no significant difference among the groups (Supplementary Fig. S3A). In addition, hematoxylin and eosin staining of the harvested viscera showed no significant difference in inflammation changes and/or organ injury (Supplementary Fig. S3B). All of the above results showed that the suppression of YAP could effectively inhibit the development of FIA-induced LMs with high systematic biocompatibility, even under infection conditions.

DISCUSSION
LMs are characterized by enlarged and irregular lymphatic vessels and deteriorate with infection and trauma.[21,22] In the present study, we verified the abnormal activation of the YAP signaling

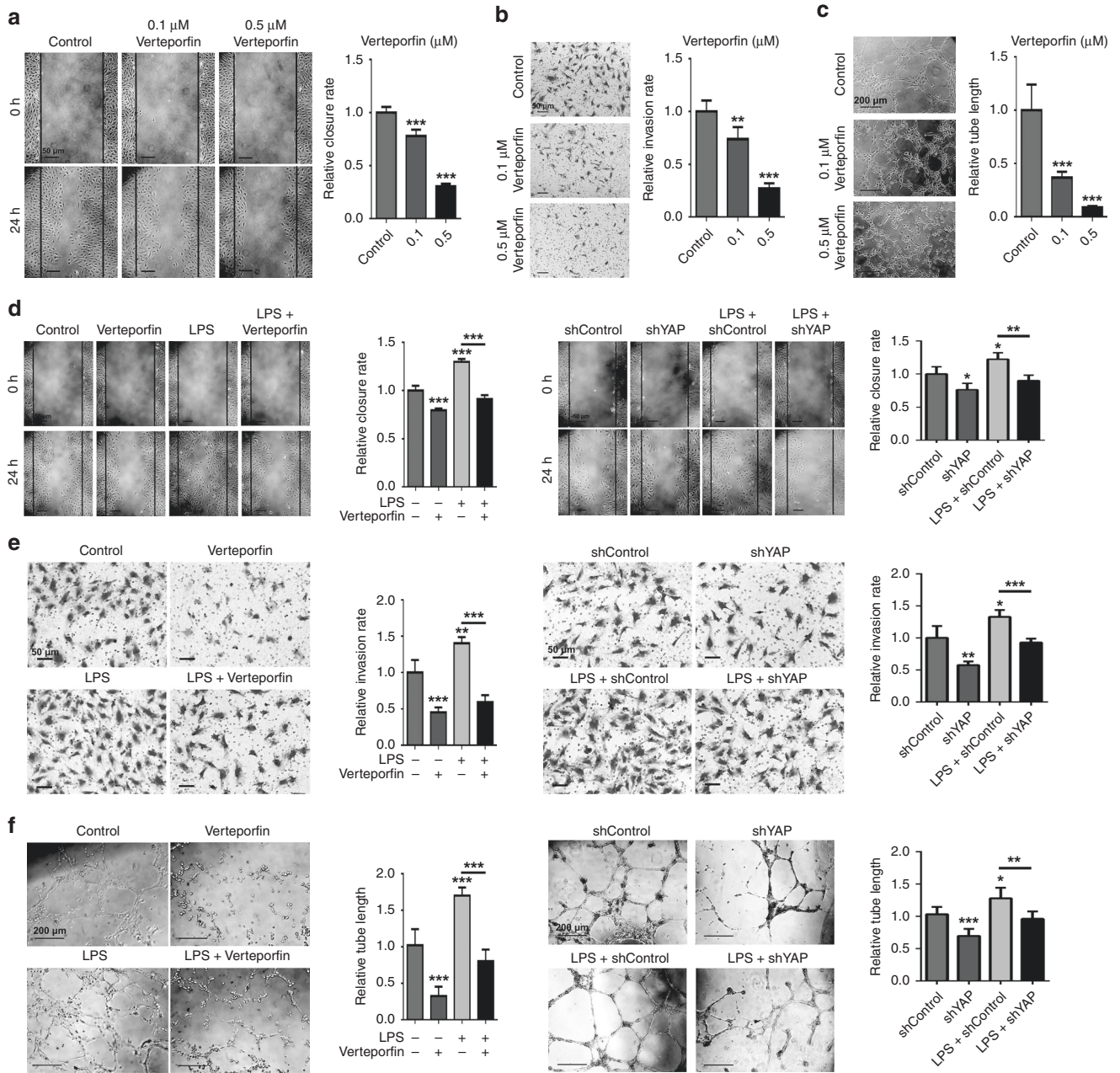The YAP signaling pathway promotes the progression of lymphatic...
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Fig. 3 YAP is necessary for the migration, invasion, and tubular formation of HDLECs, even under infection conditions. a Representative images of HDLEC migration determined by the wound healing assay after scratch treatment for 24 h and pretreatment with 0.1 or 0.5 μM verteporfin for 6 h and quantitative analyses of the relative gap closure rate. **b** Representative images of HDLECs migrating through Transwell inserts toward serum for 24 h with pretreatment with 0.1 or 0.5 μM verteporfin for 6 h and quantitative analyses of the relative invasion rate. **c** Representative images of HDLEC tubular formation capacity in a Matrigel tubular formation assay after the cells were seeded for 4 h with pretreatment with 0.1 or 0.5 μM verteporfin for 6 h and quantitative analyses of the perimeter of the formatted tube with relative tube length. **d** Representative images of wound healing assay after scratched treatment for 24 h in each group (control, 0.5 μM verteporfin, 5.0 μg/mL LPS, 5.0 μg/mL LPS + 0.5 μM verteporfin; shControl, shYAP, 5.0 μg/mL LPS + shControl, and 5.0 μg/mL LPS + shYAP) and quantitative analyses of the relative closure rates. **e** Representative images of HDLECs migrating through Transwell inserts in each group (control, 0.5 μM verteporfin, 5.0 μg/mL LPS, 5.0 μg/mL LPS + 0.5 μM verteporfin; shControl, shYAP, 5.0 μg/mL LPS + shControl, 5.0 μg/mL LPS + shYAP) and quantitative analyses of the relative invasion rates. **f** Representative images of HDLEC tubular formation capacity of each group (control, 0.5 μM verteporfin, 5.0 μg/mL LPS, 5.0 μg/mL LPS + 0.5 μM verteporfin; shControl, shYAP, 5.0 μg/mL LPS + shControl, and 5.0 μg/mL LPS + shYAP) in a Matrigel tubular formation assay after the cells were seeded for 4 h and quantitative analyses of the relative tube length. Each point represents the mean ± SD. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

pathway in the LECs of LMs, especially the link between hyperactivation of YAP and rapid progression of LMs with infection. Moreover, elevated activation of YAP was required for the proliferation, migration, invasion, and tubular formation of HDLECs. In addition, we found that the activation of p-Erk1/2 was necessary

for the elevated expression of YAP in response to infection. Finally, our in vivo study indicated that the inhibition of the YAP signaling pathway could suppress the development of LMs.

Recently, YAP has attracted increasing attention for its role in the development of pathological lymphatic vessels. Hyperactivated
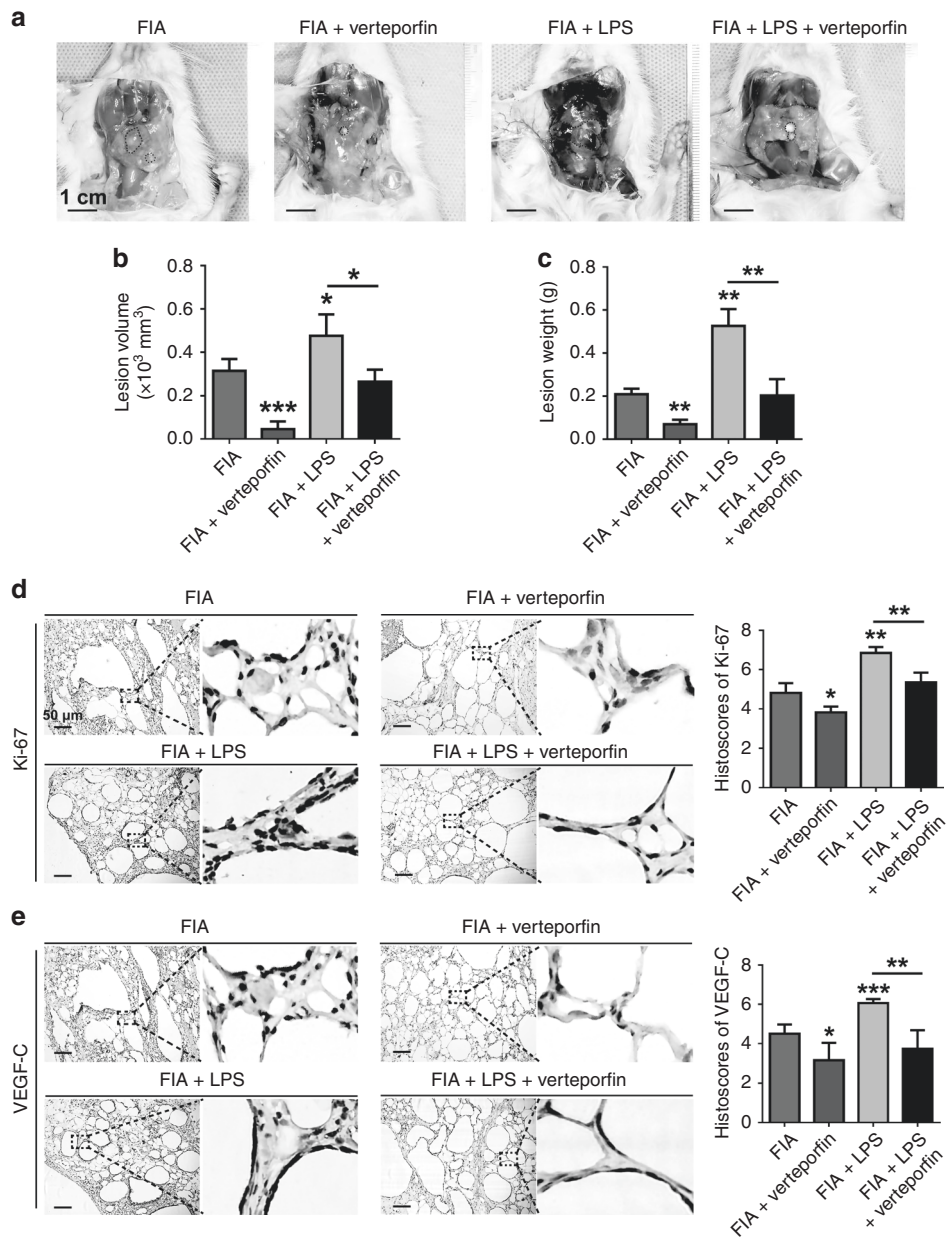
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Fig. 4 Suppression of the YAP signaling pathway inhibits the formation of LM lesions in rat models. a Representative images of LMs in rat models. **b** Lesion volumes in different groups. **c** Lesion weights in the four groups. **d** Representative images with partially enlarged details of immunohistochemical staining of Ki-67 in the lesions harvested from different groups and quantitative analysis of the histoscores. **e** Representative images with partially enlarged details of immunohistochemical staining of VEGF-C in the lesions harvested from different groups and quantitative analysis of histoscore. Each point represents the mean ± SD. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

YAP was significantly associated with TNM stage and lymphatic metastasis in various cancers, including gastric cancer and non-small-cell lung cancer.[9] Of interest to us, the YAP inhibitor verteporfin was applied not only in photodynamic therapy as a photosensitizer to reduce lymphangion-mediated tumor metastasis but also in immune rejection after corneal transplantation by promoting lymphatic regression.[23,24] Therefore, we hypothesized the potential role of the YAP signaling pathway in LM progression. In our study, we found that compared with those normal skin, the core markers in the YAP signaling pathway (YAP, TAZ, and CTGF) were significantly increased in the LECs of LMs. Amounting evidence has already indicated that the YAP signaling pathway plays a crucial role in vascular remodeling and promotes cardiovascular diseases, such as atherosclerosis, pulmonary arterial hypertension, and aortic aneurysms.[25] The deregulation of the

YAP/TAZ pathway might further enhance downstream target genes, such as *CTGF* and *CYR61*, to affect cellular physiology.[26] Elevated YAP alters different functions in vascular smooth muscle cells and endothelial cells, including cellular proliferation, apoptosis, and migration.[25] LECs exhibit high plasticity, as these cells can de-differentiate into endothelial cells with characteristics similar to blood endothelial cells or trans-differentiate into fibroblast-like cells.[27] Cho et al.[10] showed that YAP and TAZ played promoting roles in remodeling lymphatic plexus patterning as well as postnatal lymphatic valve maintenance, while genetic YAP/TAZ depletion inhibited the proliferation, metabolism, migration, and tubular formation of HDLECs. Our studies also showed that the inhibition of the YAP signaling pathway with verteporfin significantly prevented the proliferation, migration, invasion, and tubular formation of HDLECs in vitro. In addition, our

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data also confirmed that the expression levels of YAP and CTGF were positively correlated with the proliferation of LECs in LM samples. Thus, both existing studies and our present data suggest that the YAP signaling pathway may contribute to the development of LMs.

Infection is well known to significantly promote the progression of LMs.[14] We observed that YAP possessed a strong effect on chronic rhinosinusitis with nasal polyps as well as intestinal inflammation, which implicated a potential relationship between infection and the YAP signaling pathway.[11,28] Interestingly, the expression levels of YAP and CTGF were markedly increased in LECs of LMs with local infection. Moreover, in our in vitro experiments, YAP and CTGF expression was elevated in a time- and concentration-dependent manner under LPS stimulation. In LMs, we also found that the expression level of TAZ was obviously increased in the LECs of LMs compared with SKs. Nevertheless, the expression level of TAZ was similar in the LECs of LMs with or without infection. Although YAP and TAZ are considered to be quite redundant and similarly mediated by Hippo signaling, previous studies have also confirmed that they possess distinctions in structural, developmental and physiological aspects, which suggests that they are largely different in their regulatory mechanisms and downstream functions.[29,30] In human embryonic kidney cells, the activation of YAP has a stronger influence on cell spreading, proliferation, migration, and glucose uptake than the activation of TAZ.[29] Although the precise mechanism behind the different responses of YAP and TAZ to local infection remains to be investigated in LMs, our studies suggested that both YAP and TAZ influenced LM progression, while local infection might be mainly dependent on the subsequent activation of YAP, but not TAZ to accelerate the development of LMs.

To further uncover the mechanism by which local infection activates the YAP signaling pathway in LMs, we next examined the molecular regulatory network of the YAP signaling pathway. The Hippo cascade is considered a classical pathway in YAP administration through the phosphorylated regulatory serine-threonine kinase module comprising the large tumor suppressor 1 (LATS1) and LATS2 negatively regulating YAP by promoting phosphorylation.[31] Recently, increasing evidence on the identification of more regulatory signaling pathways has shown that the regulation of YAP is considerably complex. Mammalian target of rapamycin could promote YAP degradation through autophagy.[32] Moreover, reverse-regulated proteins in Hippo signaling, such as G-protein-coupled receptor, wingless/int, and epidermal growth factor (EGF) signaling, trigger inhibition of the kinases LATS1 and LATS2, which in turn dephosphorylate and activate YAP by inducing translocation into the nucleus.[33–35] As one of the vital effectors of EGF signaling, Erk1/2 inhibition results in the downregulation of YAP, while its mechanism in regulating YAP expression in LMs is not clear.[20] In our current study, p-Erk1/2 was found to be upregulated synchronously with the elevated expression of YAP caused by LPS stimulation. Then, we applied the highly selective Erk1/2 phosphorylation blocker U0126 to suppress the phosphorylation of Erk1/2, and the results showed that the LPS-enhanced YAP expression was attenuated by Erk1/2 inhibition. These data are supported by several reports, which showed that p-Erk1/2 regulated YAP activation in various mammalian cells, including cementoblasts, hepatocellular carcinoma cells, and gastric cancer cells.[36–38] The deregulation of the YAP/TAZ pathway might further enhance downstream target genes, such as CTGF, to affect cellular physiology.[39] Correspondingly, we also found that the activation of the YAP signaling pathway is required for LPS-enhanced cellular proliferation, migration, invasion, and tubule formation in HDLECs, which further confirmed the pivotal role of the YAP signaling pathway in inflected LMs.

For clinical significance, we investigated the influence of the inhibition of YAP on the growth of LM lesions in rat models.

Consistent with our hypothesis, the growth of FIA-induced LMs in rats was obviously inhibited by verteporfin administration, followed by reduced proliferation and pro-lymphangiogenesis in LECs with high systematic biocompatibility. Clinically, in most cases, local infections could significantly aggravate the progression of LMs. Interestingly, verteporfin administration also significantly attenuated the growth of FIA-induced LMs under LPS stimulation. Encouragingly, verteporfin has already been used as a clinical drug with minimal side effects for the treatment of age-related macular degeneration.[40] Although further studies are still needed to elucidate the clinical significance, these findings suggest that suppression of the YAP signaling pathway may be a feasible therapeutic target for LMs.

In summary, we reported that the activation of the YAP signaling pathway played a pivotal role in the progression of LMs. LPS facilitated YAP expression by promoting the phosphorylation of Erk1/2. The activation of YAP was required for the proliferation, migration, invasion, and tubular formation of HDLECs, even under LPS stimulation. These biological activities were effectively suppressed by verteporfin both in vitro and in vivo, which implies that targeting the YAP signaling pathway in LMs may be a promising approach for the treatment of LMs.

AUTHOR CONTRIBUTIONS
W.Z. and H.J. performed the immunohistochemical staining, cell experiments, and animal experiments and wrote the manuscript; Y.Z. and K.H. collected clinical data, performed immunohistochemical staining, and analyzed the data; J.Z. collected clinical data and helped design the study; Y.C. designed the study, analyzed the data, revised the manuscript, and is the guarantor of the work; and X.Z. and D.M. performed the cell transfection experiments and revised the paper.

ADDITIONAL INFORMATION
The online version of this article (https://doi.org/10.1038/s41390-020-0863-0) contains supplementary material, which is available to authorized users.

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