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RESEARCH LETTER



Sustained Activation of Endothelial YAP1 Causes Epithelioid Hemangioendothelioma

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Epithelioid hemangioendothelioma, first described in early 1980s,¹ is a rare malignant vascular neoplasm with significant morbidity and mortality.² Approximately 50% of epithelioid hemangioendotheliomas exhibit intravascular endothelial growth,³ yet their cellular origin, pathogenesis, and effective treatment remain undefined. Here, we identified stable nuclear expression of endothelial YAP1 (Yes1-associated transcriptional regulator) in pathological tissue samples from patients with epithelioid hemangioendothelioma. Mice expressing stable nuclear form of YAP1 in endothelial cells recapitulated the human intravascular epithelioid hemangioendothelioma phenotype. Sustained YAP1 activity induced mitosis and aberrant expression of lymphatic and epithelioid genes in blood endothelial cells. These results show sustained activation of endothelial YAP1 as a causal mechanism for intravascular epithelioid hemangioendothelioma.

See cover image

Patients with epithelioid hemangioendothelioma frequently exhibit signs and symptoms of deep vessel occlusion, like severe and sudden shortness of breath and swelling in the limbs.³ Indeed, half of the epithelioid hemangioendothelioma arise in preexisting blood vessels without affecting underlying structures.^{2,3} Recently, patients with epithelioid hemangioendothelioma revealed translocation t(11;X), which ablates regulatory sites for phosphorylation, cytoplasmic localization,

and proteosomal degradation within YAP1, generating a highly stable, nuclear, and constitutively active YAP1-TFE3 (transcription factor binding to IGHE enhancer 3) fusion protein.^{3,4} Transcriptional coactivator YAP1 functions as the principal effector of a highly conserved Hippo signaling. To limit cell proliferation and organ size, the core kinase of the Hippo pathway LATS1/2 (large tumor suppressor kinase 1/2) phosphorylates YAP1, which in turn promotes its cytosolic retention and proteosomal degradation.⁵

Despite the potential importance of dysregulated Hippo/YAP signaling in epithelioid hemangioendothelioma,⁴ it has remained unclear whether YAP1 activation alone is sufficient to drive the formation of epithelioid hemangioendothelioma in vivo. To investigate, we examined YAP1 expression in pathological tissues from patients diagnosed with epithelioid hemangioendothelioma. Morphological staining confirmed round and oval cells with abundant pale eosinophilic cytoplasm, cytoplasmic vacuolization, and inconspicuous nucleoli in large occlusive intravascular epithelioid hemangioendothelioma (Figure [A]). Within these lesions, we observed LYVE1 (lymphatic vessel endothelial hyaluronan receptor 1) and stable nuclear YAP1 expression in CDH5⁺ (cadherin 5) endothelial cells (Figure [A]). To determine endothelial cell-specific role of sustained YAP1 activity, we generated ≈3-month-old *YAP1^{5SA}; Cdh5^{CreERT2}* mice, in which tamoxifen activates sustained nuclear YAP1 expression⁵ in *Cdh5⁺* endothelial cells. At 3- to 6-week post-tamoxifen treatment, all endothelial YAP1 expressing male and female mice exhibited a hunched posture, reduced movement, wheezing, severe respiratory

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Nonstandard Abbreviations and Acronyms

CDH5	cadherin 5
LATS1/2	large tumor suppressor kinase 1/2
LYVE1	lymphatic vessel endothelial hyaluronan receptor 1
TFE3	transcription factor binding to IGDM enhancer 3
YAP1	Yes1-associated transcriptional regulator

distress, and sudden death consistent with deep vessel occlusion. Upon dissection and gross examination, we observed severe distention in the outflow tract and semilunar valve region (Figure [B]). Morphological staining of tamoxifen-treated *YAP1^{5SA}; Cdh5^{CreERT2}* hearts revealed large occlusive intravascular growth arising from endothelium of the pulmonary artery, the ascending aorta, the aortic arch, and the right atrium without affecting underlying structures, consistent with human phenotype (Figure [B]). These hearts exhibited aberrant growth of endocardial layer lining cardiac valves, mainly aortic and pulmonary valves, areas subjected to high shear stress, but not in liver, brain, and cardiac vasculature (Figure [B] and data not shown). These centripetal extending growth lesions were mainly composed of round and oval cells with pale eosinophilic cytoplasm and cytoplasmic vacuolization, embedded in a dense collagen stroma, consistent with human epithelioid hemangioendothelioma (Figure [B]). We observed similar large occlusive epithelioid hemangioendothelioma in tamoxifen-treated *YAP1^{5SA}; Cdh5^{CreERT2}* lung pulmonary arteries but not in areas subjected to low-flow shear stress, such as veins and lung capillaries (Figure [C]). Nuclear LacZ expression confirmed activation of *YAP1^{5SA}* allele in tamoxifen-treated mice and exhibited LacZ⁺ centripetal extending growth lesions arising from endothelium of the pulmonary artery and the aorta (Figure [C and E]).

To determine underlying transcriptional changes, we performed RNA-sequencing analyses of control and endothelial YAP1-expressing aortas derived from adult mice. Among 7888 differentially regulated transcripts, 52% were upregulated, while 48% were reduced in tamoxifen-treated *YAP1^{5SA}; Cdh5^{CreERT2}* compared with control. Gene set enrichment analyses revealed enrichment in YAP1 target genes and upregulation of the vast majority of mitotic cell cycle genes (Figure [D]). Expression of known epithelioid hemangioendothelioma markers, including endothelial cell surface receptors, was highly upregulated in endothelial YAP1-expressing arteries, consistent with aberrant endothelial morphology in human epithelioid hemangioendothelioma lesions (Figure [A, D, and E]). We observed highly mitotic *Cdh5*⁺

endothelial cells in epithelioid hemangioendothelioma lesions, suggesting cell autonomous role of YAP1 in endothelial cell proliferation (Figure [F] and data not shown). We observed similar gene expression changes in control and tamoxifen-treated *YAP1^{5SA}; Cdh5^{CreERT2}* lungs derived from adult mice (data not shown).

This study reveals the first causal link between sustained activation of endothelial YAP1 and intravascular epithelioid hemangioendothelioma. Fully penetrant and lethal phenotypes described in this study establish stabilization and activation of YAP1 as a somatic driver of epithelioid hemangioendothelioma. Our study also provides a critical animal model of human intravascular and cardiac epithelioid hemangioendothelioma. However, this model may not phenocopy all aspects of human YAP1-TFE3 fusion protein-associated epithelioid hemangioendothelioma, as TFE3 also regulates several cellular processes. Our findings support a model in which endothelial cells expressing stable and active YAP1 give rise to a highly mitotic form of intravascular epithelioid hemangioendothelioma in the aorta and the pulmonary artery, leading to severe deep vessel occlusion (Figure [B through F]).

ARTICLE INFORMATION

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Disclosures

None.

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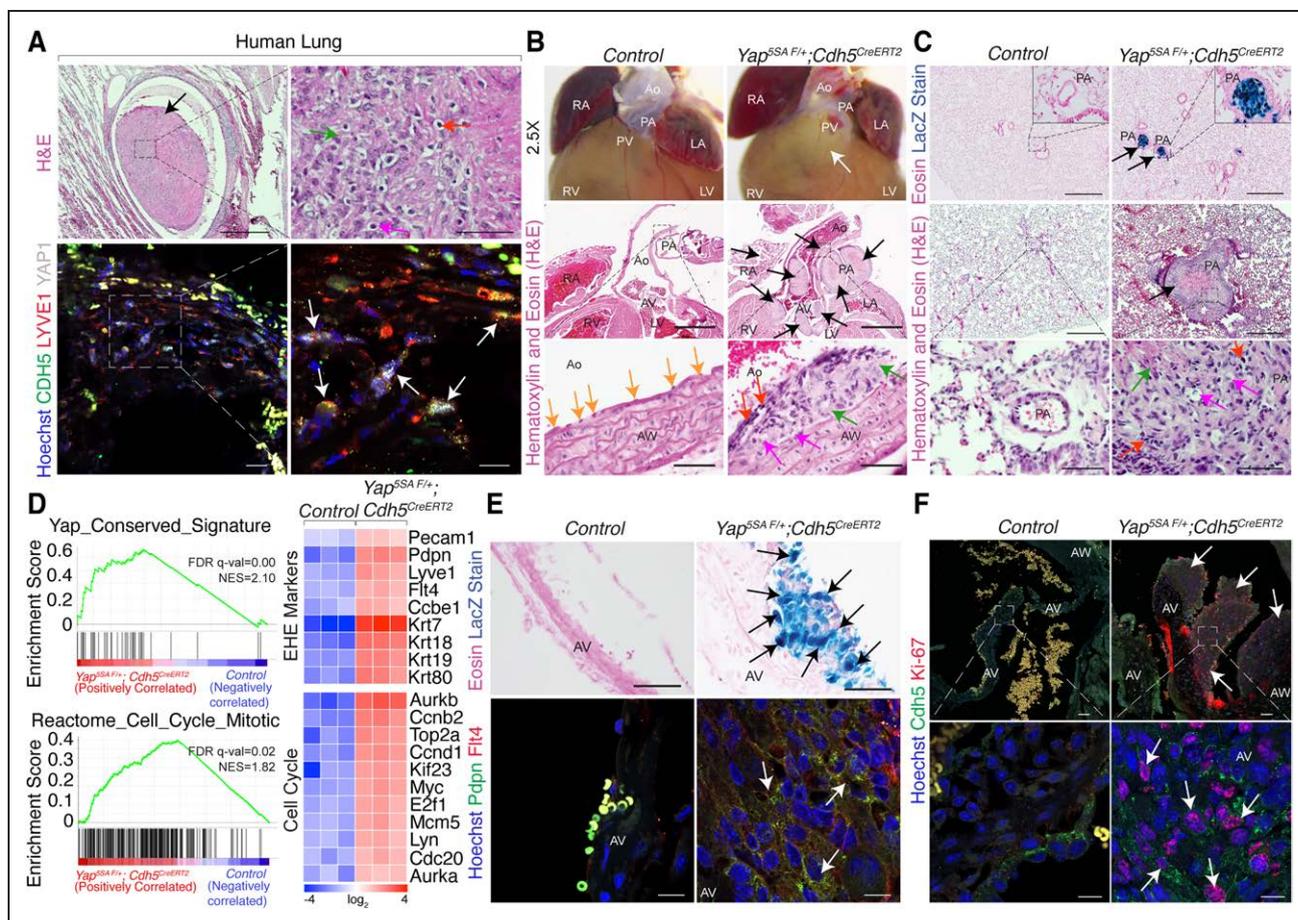


Figure. Stabilization and activation of endothelial YAP1 drives epithelioid hemangioendothelioma.

A, Analyses of human pathological tissues, previously diagnosed as epithelioid hemangioendothelioma. **Top**, Hematoxylin and eosin (H&E) stain shows large occlusive intravascular growth in artery within pathological lung tissue (black arrow), round and oval cells with cytoplasmic vacuolization (pink arrows), abundant pale eosinophilic cytoplasm (green arrows), and inconspicuous nucleoli (red arrows). **Bottom**, Coimmunofluorescent staining with Hoechst nuclear counterstain (blue) shows CDH5⁺ (cadherin 5; green)/LYVE1⁺ (lymphatic vessel endothelial hyaluronan receptor 1; red) endothelial cells with stable nuclear YAP1 (Yes1-associated transcriptional regulator) expression (white arrows). Scale bar, 500 μ m (**top left**), 50 μ m (**top right**), 20 μ m (**bottom left**), and 10 μ m (**bottom right**).

B, Control and tamoxifen-treated *YAP1^{5SA}; Cdh5^{CreERT2}* hearts from adult mice. **Top**, White arrow shows severe distention in the outflow tract and semilunar valve region. **Middle and bottom**, H&E stain shows large occlusive intravascular growth in artery (black arrows), round and oval cells with cytoplasmic vacuolization (pink arrows), abundant pale eosinophilic cytoplasm (green arrows), and inconspicuous nucleoli (red arrows; n=3). All experimental data verified in at least 3 independent experiments. Scale bar, 1000 μ m (**middle**) and 50 μ m (**bottom**).

C, Control and tamoxifen-treated *YAP1^{5SA}; Cdh5^{CreERT2}* lungs from adult mice. **Top**, Black arrows in eosin-stained sections show nuclear LacZ expressing (thus confirming *YAP1^{5SA}* allele expression) occlusive intravascular growth in pulmonary arteries. **Middle and bottom**, H&E stain shows large occlusive intravascular growth in pulmonary artery (black arrow), round and oval cells with cytoplasmic vacuolization (pink arrows), abundant pale eosinophilic cytoplasm (green arrows), and inconspicuous nucleoli (red arrows; n=3). All experimental data verified in at least 3 independent experiments. Scale bar, 500 μ m (**top and middle**) and 50 μ m (**bottom**).

D, Gene set enrichment analysis of differentially regulated, identified transcripts in tamoxifen-treated *YAP1^{5SA}; Cdh5^{CreERT2}* aortas (n=3). Heat map of top differentially regulated transcripts of epithelioid hemangioendothelioma markers and cell cycle categories (n=3). **E**, Control and tamoxifen-treated *YAP1^{5SA}; Cdh5^{CreERT2}* hearts from adult mice. Black arrows in eosin-stained sections show nuclear LacZ expressing (thus confirming *YAP1^{5SA}* allele expression) aberrant growth of endocardial layer lining aortic valve. Coimmunofluorescent staining with Hoechst nuclear counterstain (blue) shows Pdpn (podoplanin; green) and Flt4 (Fms related receptor tyrosine kinase 4; red) expressing endothelial cells with aberrant growth of endocardial layer lining aortic valve. Scale bar, 50 μ m (**top**) and 10 μ m (**bottom**).

F, Coimmunofluorescent staining with Hoechst nuclear counterstain (blue) shows highly mitotic Ki-67 (red) expressing and Cdh5⁺ (green) endothelial cells in tamoxifen-treated *YAP1^{5SA}; Cdh5^{CreERT2}* aortas and aortic valve. Scale bar, 20 μ m (**top**) and 10 μ m (**bottom**; n=3). AV indicates aortic valve; AW, aortic wall; EHE, epithelioid hemangioendothelioma; FDR, false discovery rate; LA, left atrium; LV, left ventricle; NES, normalized enrichment score; PA, pulmonary artery; PV, pulmonary valve; RA, right atrium; and RV, right ventricle.