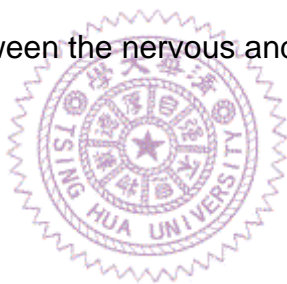# Abstract

Angiogenesis is a highly organized process under the control of guidance cues that direct endothelial cell (EC) migration, proliferation and differentiation. Recently, many molecules that were initially described as regulators of neural guidance were subsequently shown to also direct EC migration during angiogenesis. Here we report a novel protein, Thrombospondin-type Ι Domain-containing Protein 7A (THSD7A), which is required for EC migration and involved in the vascular patterning during development.

Identified by SAGE (serial analysis of gene expression) database mining and immunohistochemistry, THSD7A is highly expressed in human placenta vasculatures. To determine the function of THSD7A, we altered endogenous THSD7A expression in human umbilical vein endothelial cells (HUVECs) for subsequent angiogenesis assays. Our data indicated that downregulation of THSD7A in HUVECs enhanced cell migration and promoted tube formation, while overexpression of a THSD7A carboxyl-terminal fragment inhibited HUVEC migration and disrupted tube formation. Immunohistological analysis revealed that THSD7A was expressed at the leading edge of migrating HUVECs, and it co-localized with $\alpha_V\beta_3$ integrin and paxillin. This distribution was dispersed from focal adhesions after disruption of the actin cytoskeleton, suggesting the involvement of THSD7A in $\alpha_V\beta_3$ integrin and paxillin that mediates cytoskeletal reorganization during directed EC migration.

To characterize THSD7A in vivo, we performed whole-mount *in-situ* hybridization to reveal the spatiotemporal expression of *THSD7A* orthologue during zebrafish embryonic development, by which we detected zebrafish

thsd7a transcripts in the central nervous system. Notably, this expression exhibited a unique pattern along the ventral edge of neural tube, correlating with the growth path of angiogenic intersegmental vessels (ISVs). Antisense oligonucleotide-mediated gene knockdown of Thsd7a caused a lateral deviation of angiogenic ECs below the *thsd7a*-expressing sites, resulting in aberrant ISV patterning.

Collectively, our study revealed that THSD7A mediates angiogenic EC migration via cytoskeletal reorganization, and that zebrafish Thsd7a is a neural protein required for ISV angiogenesis during development. Future analysis on this novel protein shall provide a new perspective on the underlying mechanisms of directed EC migration, and shed light on the complex communication network between the nervous and vascular systems.

Abstract in Chinese 中文摘要

血管新生是一個高度組織化的過程，包括血管內皮細胞的遷移，複製與分化，並受各類誘導分子調控。近年來，許多參與神經系統發育的分子，亦被發現擁有調控血管內皮細胞遷移的能力，並參與血管新生的過程。本論文研究一新穎蛋白質，Thrombospondin-type I domain-containing protein 7A (THSD7A)。從實驗結果發現，此新穎蛋白質 THSD7A 可調控血管內皮細胞的遷移與形成管狀構造的能力，並參與神經與血管交互作用的發育過程。

由基因表現系列分析（serial analysis of gene expression）資料庫與組織染色結果中發現，THSD7A 高度表現於人類胎盤血管壁組織與臍帶靜脈內皮細胞（Human Umbilical Vein Endothelial Cell；HUVEC）。為了進一步了解其功能，我在人類臍帶靜脈內皮細胞內改變 THSD7A 的表現，並觀察此對血管新生的影響。實驗數據顯示，抑制 THSD7A 可提高 HUVEC 遷移的能力，並促進其管狀結構的形成。另一方面，當超量表現 THSD7A 3' 端保守片段於 HUVEC 中時，可抑制其遷移與形成管狀結構的能力。我們進一步利用免疫染色分析，定位出 THSD7A 表現分佈於遷移中細胞的最前端，並與細胞骨架分子$\alpha_V\beta_3$ integrin 和 paxillin 共位。而當細胞骨架被破壞時，亦可改變 THSD7A 於細胞內的分佈。此結果指出，THSD7A 可能透過$\alpha_V\beta_3$ integrin 和 paxillin 參與細胞骨架的組織重組，而進一步調控血管內皮細胞的遷移。

為了研究 THSD7A 在活體內的特性，我們利用全胚胎原位雜交法（whole mount *in-situ* hybridization)偵測其於斑馬魚發育過程中的表現時間與位置。不同於人類胎盤血管系統的表現，斑馬魚 *thsd7a* 高量表達於發育中的中央神經系統，並沿神經管腹側形成一特殊的表現式樣。此特殊的表現位置正座落於斑馬魚體節血管新生至神經系統的路徑上。利用嗎啉反義核苷酸介導的基因剔除技術抑制 Thsd7a 的蛋白質表達，可造成遷移中的內皮細胞偏軌，破壞體節血管新生的典型樣式。

綜合本論文數據結果，我們發現 THSD7A 可透過細胞骨架重組以調控血管內皮細胞的遷移，並於斑馬魚發育中，參與神經與血管的交互作用。本研究結果發表一新穎蛋白質以探討血管新生的分子機轉，並有助於釐清神經與血管交互作用的複雜機制。