摘要

內皮細胞的方向性遷移對血管及其形態的發育扮演重要的角色。它受到許多引導因子嚴密的調控。先前，我們發現一個新穎蛋白: 凝血酶敏感蛋白區域包含 7A (Thrombospondin Type-1 Domain Containing 7A; THSD7A) 可能參與上述過程，但是其結構與功能的關聯機制尚未被深入了解。利用生物資訊分析的結果顯示，THSD7A 被預測為一個包含十一個凝血酶敏感蛋白重複區域 (TSR) 以及一個 RGD 序列的穿膜醣蛋白。這些特徵暗示 THSD7A 可能參與細胞的移動或細胞與胞外基質的交互作用。在我們先前的研究中指出，當斑馬魚體內 THSD7A 的表現減少時，會導致體節間血管 (ISV) 不正常的分歧與生長延遲。另一方面，我們觀察到在血管前端的內皮尖端細胞 (endothelial tip cell) 上生成過多的絲狀偽足 (filopodia)，其形成被視為調控遷移方向的關鍵因素。同時，我們也發現 THSD7A 的轉錄產物會表現在斑馬魚的神經系統中。基於上述的發現，我們假設 THSD7A 可能是一個從神經組織分泌出的血管引導蛋白，並且會在血管發育過程中扮演外源性負向調控內皮尖端細胞方向性遷移的因子。本篇論文研究的主要目的是利用活體外的分析方式找出 THSD7A 的功能性片段。首先，為了釐清人類 THSD7A 的轉譯後修飾與其同源異構體 (isoform)，我們藉由哺乳類細胞株表現其蛋白。我們亦利用桿狀病毒-昆蟲細胞表現系統成功建構THSD7A截切片段TF2之載體並表現蛋白。最後，我們利用穿透式細胞遷移試驗 (transwell migration assay) 與管柱生成試驗 (tube-like formation assay) 兩種與血管發育相關的活體外實驗，測試 TF2 的功能。結果顯示，THSD7A 是一個會被分泌到細胞外的醣蛋白。TF2-His 融合蛋白可以成功地藉由培養在 Express 5 培養基的 High5 昆蟲細胞表現並以適當的條件純化之。然而，我們發現 TF2 不會調控人類臍帶靜脈內皮細胞的遷移與管柱生成能力。這個結果暗示我們 THSD7A 的功能性片段可能不是座落在 TF2 上。

Abstract

Directed migration of endothelial cell (EC) plays a critical role in vascular growth and patterning processes. It is tightly regulated by various guidance cues and molecules. Previously, we identified a novel protein, Thrombospondin Type-1 Domain Containing 7A (THSD7A), which may involved in this process, but not much is known regarding its structural-functional mechanism. By bioinformatic analysis, THSD7A was predicted as a membrane glycoprotein which contains eleven thrombospondin-type-1 repeats (TSR) and one RGD motif. These characters imply THSD7A may be involved in cell migration and cell-to-ECM interactions. In our previous study, we found out that the down-regulation of zTHSD7A (THSD7A homolog in zebrafish) gene expression resulted in abnormal branching and stalled of intersegmental vessel (ISV). On the other hand, the endothelial tip cell at the leading front of growing vessel showed excessive filopodia formation which was thought to guide its direction. We also discovered that *Thsd7a* transcript was expressed in zebrafish nervous system. Based on these findings, we hypothesize that THSD7A may be a vessel guidance protein secreted by neural tissue and act as an exogenous negative regulator of the directed migration of endothelial tip cell during vascular growth. The specific aim of this thesis study is focusing on analyzing the functional domain of THSD7A *in vitro*. First, recombinant full-length human THSD7A was expressed by mammalian cells to reveal the isoforms of THSD7A and modification. Baculoviral vectors containing truncated fragment 2 (TF2) of THSD7A was then constructed and used to express the target protein region by insect cells system. The resulting TF2 were then tested with two *in vitro* angiogenic assays, which are the transwell migration assay and tube-like formation assay. These results demonstrated that there was a secreted form of THSD7A which was glycosylated. His-tagged TF2 (TF2-His) can indeed be expressed by High5 insect cells in Express 5 medium successfully, and an optimal purification procedure was established. However, we found TF2 has no activity on regulating the migration and tube-formation of HUVEC. This suggests that the functional domain of THSD7A may not locate within the TF2 region.