英文摘要

A novel neural protein, thrombospondin type I domain containing 7A (THSD7A), is found to be expressed in neural tube and affect endothelial cell migration during developmental stage of zebrafish. To further study the effect THSD7A involved in endothelial cell migration, we focus on the post-translational modification and the underlying mechanisms. We constructed the full-length *THSD7A* with a FLAG-tag and overexpressed it in human embryonic kidney 293T (HEK293T) cells. We found that THSD7A is a membrane associated N-glycoprotein and can release a soluble THSD7A into the cultured medium. After harvested the soluble THSD7A in cultured medium and performed angiogenic assays, we found that soluble THSD7A promotes human umbilical vein endothelial cell (HUVEC) migration and tube formation. HUVEC sprouts in collagen matrix were increased in the presence of soluble THSD7A. The number of branching points of new vessels in zebrafish subintestinal vessel (SIV) was also increased after soluble THSD7A injection. Interestingly, the filopodia formation and the distribution patterns of vinculin and phosphorylated focal adhesion kinase (FAK) were affected in the presence of soluble THSD7A. These results implied that soluble THSD7A involved in the focal adhesion assembly. The phosphorylation level of FAK in HUVEC was also increased by addition of soluble

THSD7A. Taken together, THSD7A was demonstrated to be a membrane-associated N-glycoprotein that can be released a soluble form from cell surface. Soluble THSD7A promotes endothelial cell migration and tube formation during angiogenesis via a FAK-involved manner and may be a novel neuroangiogenic factor.

中文摘要

Thrombospondin type I domain containing 7A (THSD7A)，一個新穎蛋白質，被發現可大量表現於神經系統且影響內皮細胞的遷移及血管形成。在這篇研究中，我們研究其轉譯後修飾作用以及引發血管新生的下游機制，以探討 THSD7A 在血管新生過程中如何調控血管生長。我們以 Western blot 分析 Full-length *THSD7A*-transfected human embryonic kidney 293T cells (HEK293T) 發現 THSD7A 是一個 membrane associated N-glycoprotein；且在 cultured medium 中，發現一個 THSD7A 的 soluble form。我們收集了 soluble THSD7A 並進行 angiogenic assays 發現 soluble THSD7A 能促進 human umbilical vein endothelial cell (HUVEC) 的移動、管柱生成和新芽生成。而在斑馬魚動物實驗中，發現 soluble THSD7A 能夠有效的增加 subintestinal vessel (SIV) 新生血管的分支數目，形成不正常的血管網路。有趣的是，我們同時也觀察到有 soluble THSD7A 存在時，HUVEC 會有較多的 filopodia。vinculin 以及 phosphorylated focal adhesion kinase (FAK) 在 HUVEC 中的分佈同樣也受到 soluble THSD7A 的影響，表示 soluble THSD7A 與 focal adhesion assembly 有關。HUVEC 中 FAK 的磷酸化程度同樣也被 soluble THSD7A 調控，暗示 soluble THSD7A 可能影響細胞骨架的重組。綜合以上實驗結果，我們驗證了 THSD7A，一個 membrane associated N-glycoprotein，會釋放 soluble form 至細胞外。Soluble THSD7A 能

在血管新生的過程中，經由 FAK-dependent mechanism 促進內皮細胞移動，而可能扮演一個新穎的神經血管作用因子。