• 計畫中文名稱	斑馬魚 Syndecan-3 基因在胚胎型態發育過程中功能之分析		
• 計畫英文名稱	The Role of Syndecan-3 Morphogenesis and Development in Zebrafish		
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• 研究人員	周志銘		
• 中文關鍵字	--		
• 英文關鍵字	--		
• 中文摘要	Syndecans 爲 HSPGs (Heparan-sulfate proteoglycans) 家族成員之一，而 syndecans 家族主要是由四種第一型穿膜蛋白所組成，此家族成員廣泛的分佈在哺乳類細胞的表面 且參與許多的生理過程，同時也扮演許多生化反應中受質-受體（ligand-receptor）結合 反應中協同受體 (co-receptor) 的角色。 Syndecan-3 (N-syndecan) 爲哺乳類 proteoglycans 家族四個成員之一，在哺乳類的 相關研究中顯示此基因主要表現在神經系統，在胚胎發育神經系統形成的過程中可能參 與細胞附著 (cell adhesion)、神經樹突生長的引導 (neurite guidance) 和細胞的轉移 (cell migration) ，同時也有研究指出此基因會和海馬迴 (hippocampus) 中突觸的多變性 (synaptic plasticity) 調節有關，而且會與許多生長因子及細胞外的間質蛋白結合，扮演 重要的生物功能調節。雖然 syndecan-3 在哺乳類的研究中發現主要參與在胚胎發育過 程腦部的發育和骨骼肌的分化和發育，但是在其他脊椎動物胚胎發育過程中其扮演的角 色仍不清楚。肝素結合蛋白神經促進因子（Heparin binding neurotrophic factor / Pleiotrophin；HBNF/PTN)，這類蛋白不論在細胞的生長或胚胎發育過程中神經系統生物 的形成過程，皆具有相當重要的功能。在體外培養的神經細胞研究中發現改變 syndecan-3 蛋白外肝鹽結合多糖類或利用 syndecan-3 蛋白的多元抗體加入培養的神經 細胞內都可抑制由肝素結合蛋白神經促進因子 (HBNF/PTN-induced neurite outgrowth) 所促進的神經纖維生長，在這些研究中推測 syndecan-3 可能扮演的角色爲肝素結合蛋 白神經促進因子 (HBNF/PTN) 的接受體，而 HBNF 如何促進神經纖維生長的相關研究 還不是很明確，其促進神經纖維生長的分子機轉有待進一步的研究。 本研究主要將探討 syndecan-3 (zSyn3) 基因在斑馬魚胚胎發育早期可能參與腦 部、身體形態和肌肉組織發生的過程以及對於肝素結合蛋白神經促進因子 (HBNF) 在 神經生長調節的分子機轉。先前本實驗室的研究 (Chang et al.,2004)，利用具神經組織專 一性表現的啓動子(HuC promoter) 來調控綠色螢光蛋白 (green fluorescence protein, GFP)，標定斑馬魚胚胎發育過程中的神經組織，成功的建立肝素結合蛋白神經促進因子 (HBNF) 所誘導促進斑馬魚神經纖維生		

	長的活體分析系統。本計畫擬先探討 syndecan-3 基因對於斑馬魚胚胎發育過程早期的影響並利用實驗室已建立的斑馬魚神經纖維生長 的活體分析系統，進一步探討 syndecan-3 對於肝素結合蛋白神經促進因子 (HBNF) 在 神經生長調節的分子機轉同時將利用 α-actin：EGFP 的斑馬魚轉殖株，探討 syndecan-3 基因表現對於肌肉發育的影響。主要研究的目標如下：目標一、分析 syndecan-3 基因 表現與斑馬魚胚胎發育早期之關係。目標二、將研究 syndecan-3 基因在胚胎發育時期 腦部發育、身體的形成和肌肉組織發育中扮演的重要功能。目標三、探討 HBNF 所引 發的神經生長是否必需有 syndecan-3 基因參與。若完成此三目標將有助於瞭解 syndecan-3 在斑馬魚胚胎發育過程中參與腦部發育及其與身體型態發育過程的重要性 以及在 HBNF/PTN 所促進的神經生長過程中扮演的角色。
• 英文摘要	The syndecans are a family of heparan-sulfate proteoglycans (HSPGs), which are involved in many physiological processes and are co-receptors for a variety of ligand-receptor interaction. Syndecan-3 (N-syndecan) is one of the four mammalian syndecans and it is mainly expressed in the nervous system, especially during development. Syndecan-3 has one transmembrane domain, a short cytoplasmic tail of 34 amino acids, and an extracellular domain that carries heparan sulfate chains. Syndecans have been suggested to function as co-receptors with other signaling receptors, such as FGF receptors and integrins. It has been suggested to function in cell adhesion, neurite guidance, and cell migration during development of the nervous system. Syndecan-3 has also been implicated in the regulation of synaptic plasticity in the hippocampus. Syndecan-3 palys an important role in regulation of skeletal muscle differentiation and development. However, the roles of syndecan3 in the embryogenesis of other vertebrate species remain to be elucidated. Heparin binding neurotrophic factor/Pleiotrophin (HBNF/PTN) was first isolated as a heparin-binding protein that was eluted from a heparin affinity column with high salt concentration. Such a high affinity-binding property suggests that heparin or heparin-type carbohydrates may play important roles in the biological function of HBNF/PTN. Both the heparan sulfate side chains of syndecan-3 and polyclonal anti-syndecan-3 inhibit HBNF/PTN-induced neurite outgrowth in the cultured neurons. Syndecan-3 is interest due to its high expression in nerve and its potential to serve as FGF receptor co-receptor that mediates a variety of biological responses. In our previous study, we established an in vivo neurite outgrowth assay in zebrafish embryos that provided a direct observation of HBNF-induced neurite outgrowth from GFP-labeled neurons during zebrafish development. Using this assay, the effects of manipulating syndecan-3 gene activity can be monitored. Recently study was found that knocking down syndecan-3 in zebrafish may interfere formation of the posterior body. Syndecan-3 regulates the formation of the posterior body in zebrafish are largely unknown. Whether the syndecan-3 interfere HBNF induced neurite outgrowth, need further analysis. In this project, we propose to apply an in vivo neurite outgrowth assay in zebrafish embryos to define the causal role of syndecan-3 in the HBNF promotion of neurite outgrowth and use α-actin:EGFP transgenic zebrafish to analysis syndecan-3 function during zebrafish development. Specific Aim 1 will analysis the role of syndecan-3 gene during early development stage. In the experiment of loss-of-function, morpholino oligonucleotide (MO) technology will be applied. Through injection of zSyn-3 MO to knockdown syndecan-3 gene expression during developmental stage will be investigated. Specific aim 2 will determine the essential roles of syndecan-3 in brain development, the morphogenesis of the posterior body and skeletal muscle development in zebrafish. The hypothesis to be tested syndecan-3 whether coordinated Wnt, Bmp or Fgf signaling for formation of the brain and body morphogenesis. Specific aim 3 will examine whether syndecan-3 is essential for HBNF promotion of neurite outgrowth. Through coinjection of zSyn-3 MO and HBNF/HuC-GFP, the effect of knockdown syndecan-3 expression on HBNF-induce of neurite outgrowth will be investigated and provided an in vivo assay system. Completion of these three specific aims define whether syndecan-3 play causal role in HBNF/PTN promotion of neurite outgrowth and whether coordinated Wnt, Bmp or Fgf provide synergistic

	effects in brain development and the morphogenesis of the posterior body in zebrafish.