2008 年中央研究院「年輕學者研究著作獎」得獎人簡介

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得獎著作名稱：(請以申請時之格式填入)

A. Molecular Identification of Canine Podocalyxin Like Protein 1 As a Renal Tubulogenic Regulator. **Journal of the American Society of Nephrology**, 2005; 16: 1612-1622.

B. A bipartite signal regulates the faithful delivery of apical domain marker Podocalyxin/Gp135. **Molecular Biology of the Cell**, 2007 May;18(5):1710-1722

C. Pleomorphic extra-renal manifestation of the glomerular podocyte marker podocalyxin in tissues of normal beagle dogs. **Histochem Cell Biol** 2007 Apr;127(4):399-414

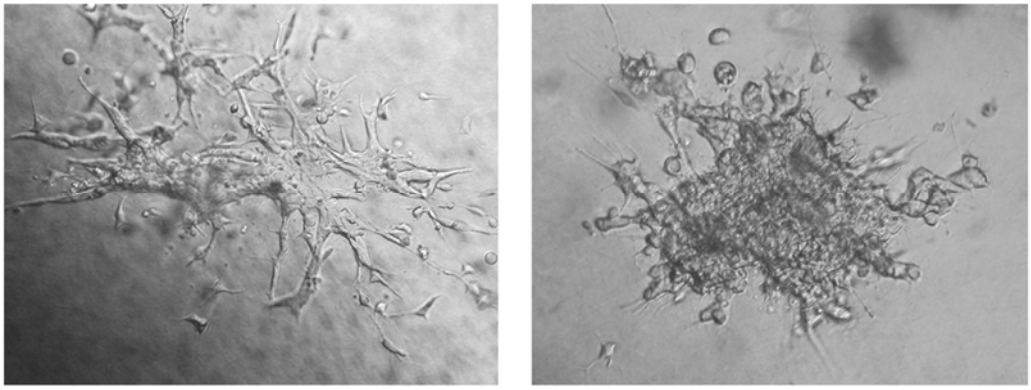
得獎著作簡介:

組成人體的器官分為實心或是具有管腔的器官。管腔器官包括呼吸道、腸胃道、和尿道等，其特徵為具有極性分化的上皮細胞內襯。這些上皮細胞內襯，不僅成為區隔生物體內外環境的障壁，同時也調控著重要物質以固定方向的輸送方式進出生物體。實心器官，雖然不具有特化的上皮細胞層，但穿梭其中的血管基本上是個連結不斷的管腔結構。在這些管腔結構內部也鋪滿了與管腔器官的內襯上皮細胞相類似的特化內皮細胞。上皮細胞與內皮細胞如何聚集形成具有管腔的組織，在細胞生物學中是個古老而尚未解決的問題。

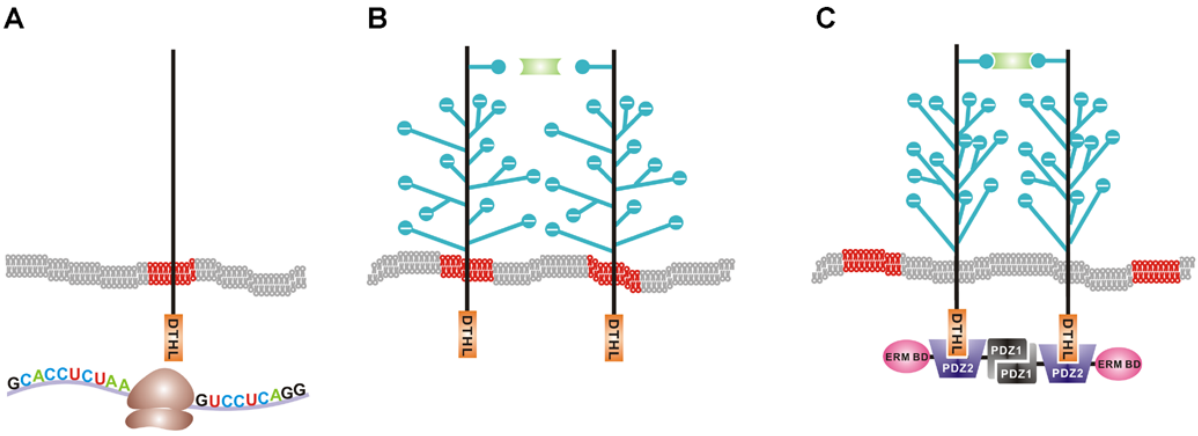
哺乳動物依賴腎臟進行血液廓清而得以生存，此一功能又倚賴腎臟內排列有條不紊的管腔結構。在每一個圍繞這些繁複管腔的腎臟上皮細胞中，各種膜蛋白包括孔道蛋白，運輸蛋白及幫浦蛋白會特化表現於頂部或底部細胞膜。此一高度極性分布的現象對於溶質以一定方向進出腎臟管腔極為重要。不論腎小管管腔新生或腎臟上皮細胞成熟極化的過程,皆可利用犬腎上皮細胞 MDCK 在實驗室裡忠實再現，因此 MDCK 一直做為研究腎臟發生學的良好典範。此外 GP135 被視為犬腎上皮細胞及老鼠腎小管的典型頂部膜蛋白已有數十年之久, 但我們實驗室的團隊最近確認 GP135 可調控肝細胞生長因子所誘導的管腔生成(圖一)。

經由詳細分析蛋白的運送過程，我們發現 Gp135 能否正確輸送，須經由細胞外的 O 型醣修飾區域與細胞內的 PDZ motif 兩段同等重要的訊號驅動。根據我們的觀察，EBP50 蛋白的 PDZ domain 藉由其 PDZ motif 反應，會在高基氏體與新生成的 Gp135 結合，並促成 Gp135 形成高單位複合體，以便正確地運送至頂部區域。無法與 EBP50 結合的 Gp135，或者是削減 EBP50 表現的細胞，會導致 Gp135 延遲離開脂筏，無法形成高單位複合體，並且被誤送至底部區域。根據這些發現，我們提出了一個模式，描繪出具有高負價電荷的膜蛋白，如何藉由細胞內合作蛋白的幫助，正確的組裝入運送至頂部區域的平台(圖二)。

經由這些研究所得到的訊息，不但可以幫助我們了解許多重要器官中管腔的形成機制，更可以解釋許多先天異常，如多囊性腎疾或膽管阻塞的致病機制。



圖一 Gp135 調控肝細胞生長因子所導引之管腔生成。包埋於膠原蛋白中的狗腎上皮細胞 MDCK，經由肝細胞生長因子的刺激會形成管腔; 此一現象經常被用以研究腎臟胚胎發育之模式。正常 MDCK 細胞在肝細胞生長因子刺激下，會生成典型的管腔(左);然而剔除 Gp135 的 MDCK 細胞無法生成管腔(右)。



圖二　在極化的上皮細胞中，Gp135 運送至頂部區域的步驟示意圖。(A) 原始的 Gp135 胜肽鍊，被轉譯並藉由訊號胜肽進入內質網之內腔 （ B ）Gp135 胜肽鍊從內質網被運送到高基氏體的過程中，同時開始進行 N 型與 O 型醣化及醣基末端唾液酸之修飾。雖然在內質網內腔推測有一凝集素，可以驅使 Gp135 形成複合體，但由於 Gp135 本身有唾液酸修飾造成高負電荷的狀態，會彼此排斥，因此無法順利的離開脂筏，形成高單位複合體。(C) 藉由 Gp135 尾端與 EBP50 的結合，與 EBP50 本身可以形成複合體的特性，Gp135 可以抵抗因為高負電荷所造成的排斥，順利的離開脂筏，並形成高單位複合體，順利運送至細胞頂部區域。

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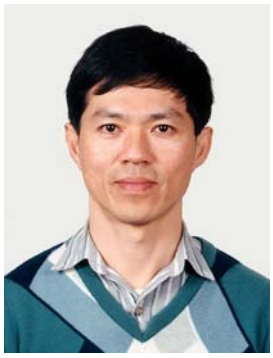
周祖述博士所提出的三篇代表作論文皆是針對 podocalyxin/Gp135 這一 mucin 蛋白的生物功能所做的研究探討。周博士的研究群是首先 clone 這一基因的團隊。本系列代表作的研究內容使用腎上皮細胞，將野株型 podocalyxin/Gp135 之 cDNA 送入細胞發現可促進肝生長因子(HGF)所誘導腎上皮細胞的小管形成，相對地使用 podocalyxin/Gp135 之 siRNA 送入細胞來 knockdown podocalyxin/Gp135 表現，則可抑制 HCF 所誘導腎上皮細胞的小管形成，這些証據指出 podocalyxin/Gp135 直接參與調控 HCF 所誘導腎小管形成。而 podocalyxin/Gp135 的這一作用需要其分子位於細胞膜外 O-glycosylation-rich 部位及其分子位於細胞內之 PDZ 部位來共同參與。周博士在 podocalyxin/Gp135 的生物功能研究具有很高的原創性，對腎小管形成機制的研究有很好的貢獻。

2008 Academia Sinica
Research Award for Junior Research Investigators

Name: Tzuu-Shuh Jou 	Education: National Taiwan University, M.D.（1978 ~1985） Stanford University, Ph.D.（1992 ~1998） Employer(s)/Job Title(s): Associate professor, National Taiwan University（2007~ ） Visiting Staff, National Taiwan University Hospital （1998~ ） National Taiwan University, assistant professor （1999~2007）

Award publications：

A. Molecular Identification of Canine Podocalyxin Like Protein 1 As a Renal Tubulogenic Regulator. **Journal of the American Society of Nephrology**, 2005; 16: 1612-1622.
B. A bipartite signal regulates the faithful delivery of apical domain marker Podocalyxin/Gp135. **Molecular Biology of the Cell**, 2007 May;18(5):1710-1722
C. Pleomorphic extra-renal manifestation of the glomerular podocyte marker podocalyxin in tissues of normal beagle dogs. **Histochem Cell Biol** 2007 Apr;127(4):399-414

Summary of the Award publications：

The human body is composed of many internal organs, which contain central lumen encircled by a polarized epithelial lining. This epithelial lining serves as the boundary between inner milieu and outer environment of the organism, while in the meantime plays a critical role in regulating selective transporting function across this barrier. Although essential in maintaining the homeostasis of the complicated chemical composition of human bodies, how the apical lumen is regulated still remains an enigma in cell biology.

The importance of lumen formation is best exemplified by kidney. Mammals depend on the kidney to perform blood filtering function in which orderly renal tubular structures are indispensable. Inside each of the renal epithelial cells lining these elaborate tubules, the localization of membrane proteins is highly polarized with various channel, transporter, and pumps selectively distributed at either the apical or basolateral domain. This polarized distribution pattern of membranous organization is pivotal for vectorial transport of solutes across epithelial barrier. Both the developmental regulation of renal tubulogenesis and the polarity formation of mature renal epithelia could be recapitulated using Madin-Darby canine kidney (MDCK) as a model. Although GP135 has served as the canonical hallmark of apical surface for decades, its molecular details and functions have remained obscure until we recently found GP135 is the glomerular sialomucin-podocalyxin and it could regulate HGF/SF (hepatocyte growth factor/scattering factor) induced tubulogenesis (Figure 1).

By detailed protein trafficking assays, we show that correct apical sorting of Gp135/podocalyxin depends on a bipartite signal composed of an extracellular O-glycosylation rich region and the intracellular PDZ domain binding motif. In accord with this observation, the PDZ domain protein, ezrin binding phosphoprotein 50 (EBP50), binds to newly synthesized Gp135 at Golgi apparatus, and facilitates oligomerization and sorting of Gp135 into clustering complex. Defective connection between Gp135 and EBP50 or EBP50 knock-down results in a delayed exit from the detergent resistant microdomain, failure of oligomerization, and basolateral missorting of Gp135. According to these findings, we propose a model depicting the way by which a highly negative charged transmembrane protein could be packed into an apical sorting platform with the aid of its cytoplasmic partner (Figure 2).

The information result from this study would help understand not only the mechanism governing the lumen formation in many vital organs, but also would help decipher the pathogenesis of many congenital anomalies such as polycystic kidney disease or biliary atresia.

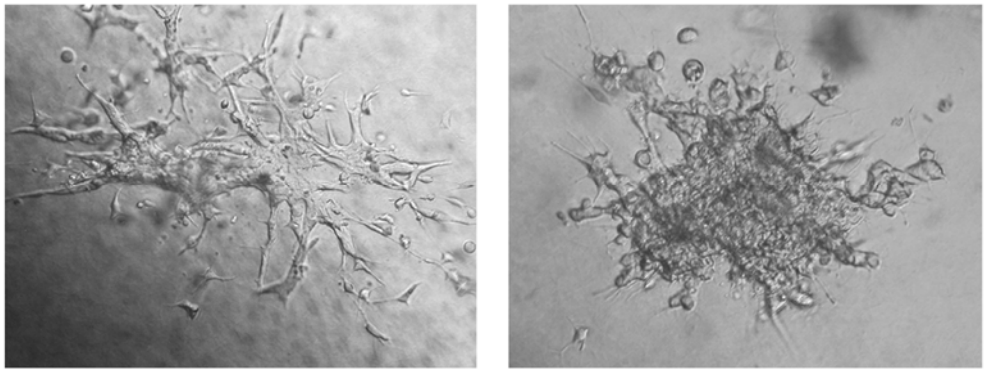


Figure 1. Gp135/podocalyxin regulates HGF/SF (hepatocyte growth factor/scattering factor) induced tubulogenesis. MDCK cells embedded in collagen were stimulated by HGF to recapitulate the morphogenesis of renal tubule formation. Control MDCK cells (left) developed branching tubular structures upon HGF addition, while Gp135/podocalyxin knock-down cells (right) failed to form the typical tubular structures.

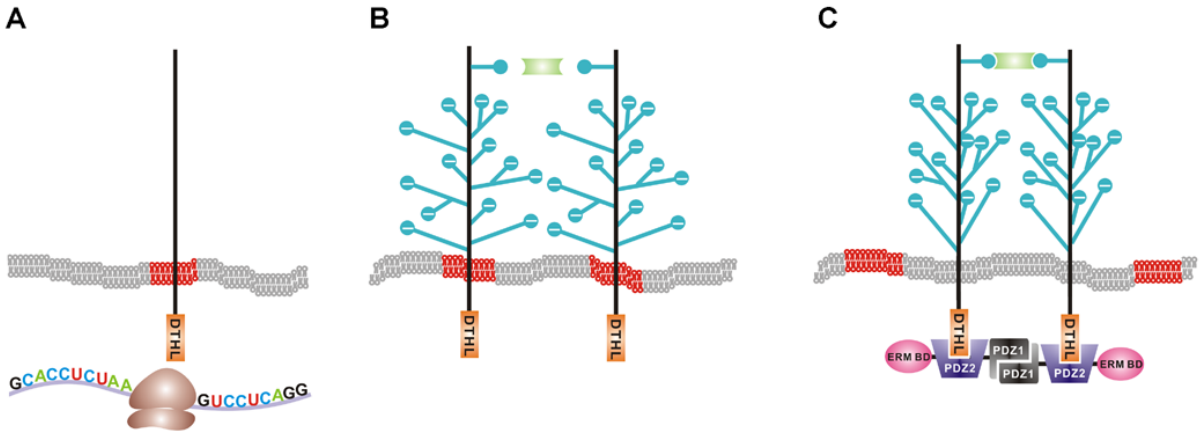


Figure 2. Multistep model for the apical sorting of podocalyxin/Gp135 in polarized epithelial cells. (A) As the nascent polypeptide of Gp135 is being released from the membrane-associated ribosome, the signal peptide of Gp135 has directed its entrance into the ER. (B) The polypeptide is undergoing N-linked glycosylation (not shown in the diagram) and O-linked glycosylation along its biosynthetic route through the ER/Golgi apparatus. Although the putative luminal lectin receptor can recognize the O-glycan as an apical sorting signal and cluster the nearby Gp135, the highly negative charged nature of the superimposed sialic acid modifications would impede the coalescence of the single lipid rafts (shown in red) into a functional apical sorting platform. (C) Binding of EBP50 to the cytoplasmic tail of Gp135 and the oligomerization of EBP50 would counteract the electric repulsive force by the sialic acid modifications incurred on the closely packed Gp135 and help clustering of multiple lipid rafts into a competent functional unit.