行政院國家科學委員會專題研究計畫　期中進度報告

剖析 RACI 有關犬腎上皮細胞形成囊泡及管腔之訊息傳導路徑(2/3)

計畫類別：個別型計畫
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執行單位：國立臺灣大學醫學院臨床醫學研究所

計畫主持人：周祖述

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處理方式：本計畫可公開查詢

中　華　民　國 93 年 5 月 15 日

In 1988, Dr. Ojakian reported an apical membrane protein, GP135 (also called GP135/170 due to the heavily glycosylated nature of the protein) could be identified by a monoclonal antibody generated against MDCK (Madin-Darby Canine Kidney) cells surface antigen. Previously, we had reported inhibition of small GTPase Rac1 activities blocked the apical lumen formation and reverse the apical polarized distribution of GP135 during the cyst formation of MDCK cells grown in collagen gel 3D culture system (Nat Cell Biol. 2001 3(9):831-8). To further characterize the role GP135 played in the process of Rac1 mediated apical pole formation, we set up to use the following scheme (Figure 1 and 2) to purify GP135 from MDCK cells lysate and do MS analysis followed by molecular cloning to obtain the cDNA of GP135.

The result shows GP135 belongs to the so called podocalyxin family, which is a member of the mucin superfamily (Figure 3). GP135 and the other podocalyxin like protein are protein with heavily glycosylation and undergo post-translational modification such as sialic acid addition which substantiate the function of podocalyxin as an anti-adhesive molecular the foot-processes of podocytes. To confirm, our clone is indeed the GP135 cDNA. We transfect the cDNA into HEK293 which normally do not express any protein identified by the original anti-GP135 monoclonal antibody generated by Dr. Ojakian. Indeed, the transfected HEK could be identified to express a high molecular weight protein recognized by the anti-GP135 monoclonal antibody, and the protein seemed to be localized at the membrane of the expressing HEK293 cells (Figure 4).

Our work in the next year is to create GP135 siRNA expressing stable clones of MDCK cells and to test them in the cyst assay so as to examine whether the function of GP135 is related to the apical formation and the cyst generation in epithelial cells (Figure 5).

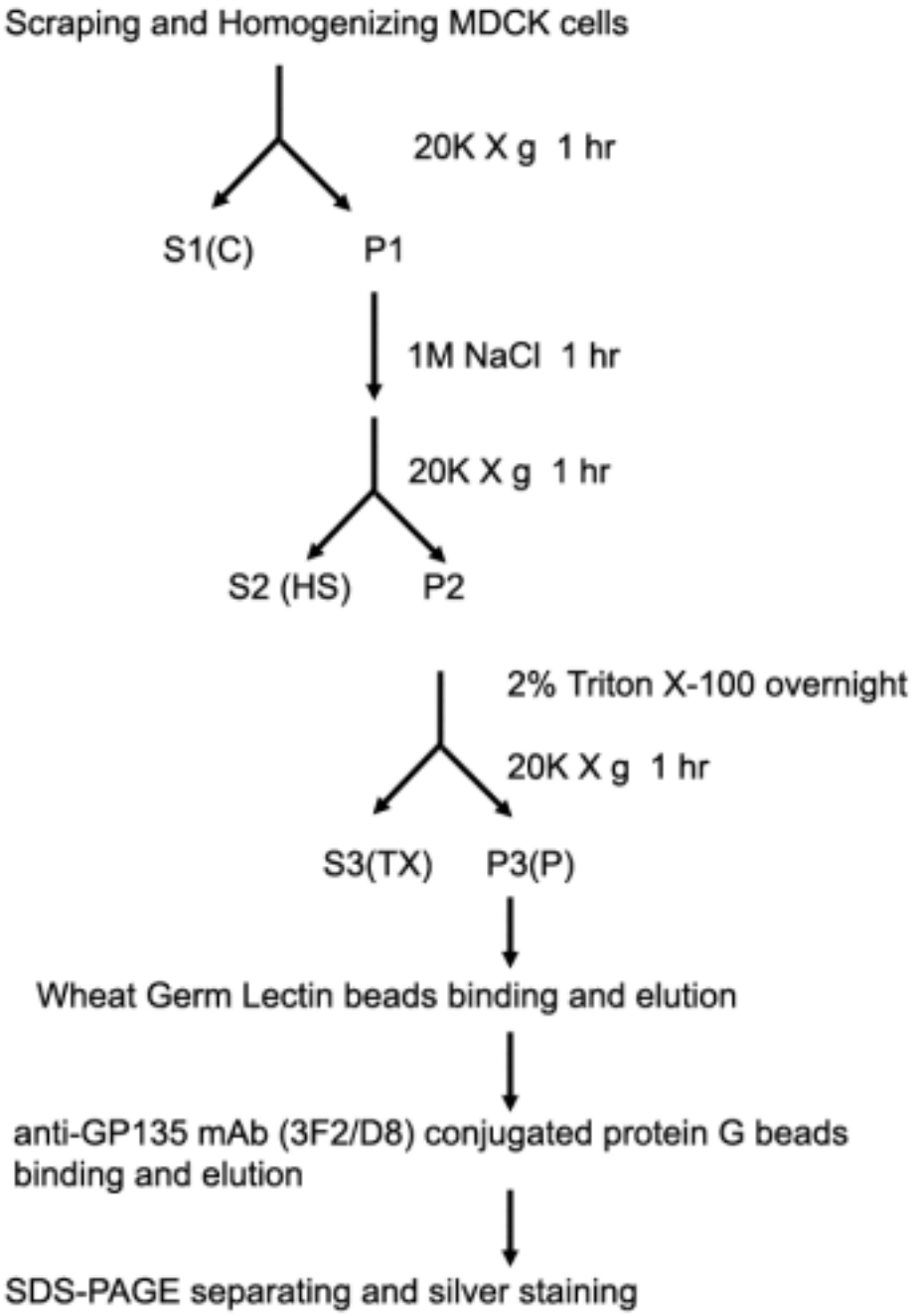


Figure 1. Purification Scheme of GP135

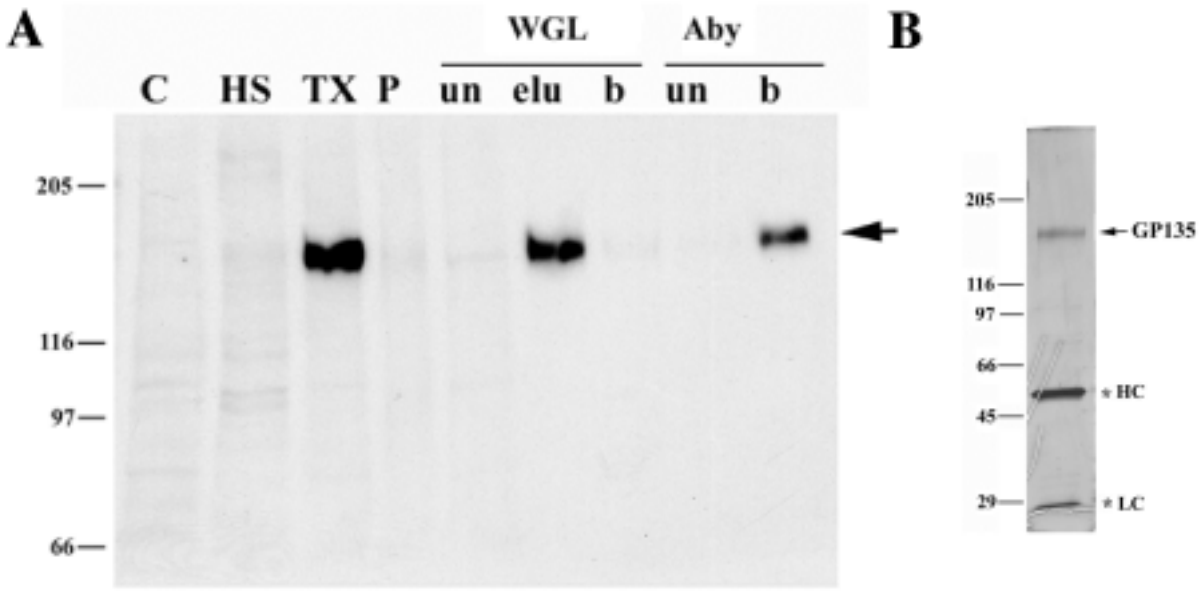


Figure 2 GP135 was purified using the protocol stated in figure 1.

The efficiency of purification was assayed by western blotting. Each sample was loaded in equal fraction and separated by 7.5% (GP135 and E-cadherin) or 12.5% (IkBa) SDS-PAGE and followed by western blotting with anti-GP135, anti-E cadherin polyclonal (E2), and anti-IkBa polyclonal antibodies.
C: cytosol, HS: high salt solublized, TX: triton solublized, P: triton insolublized,

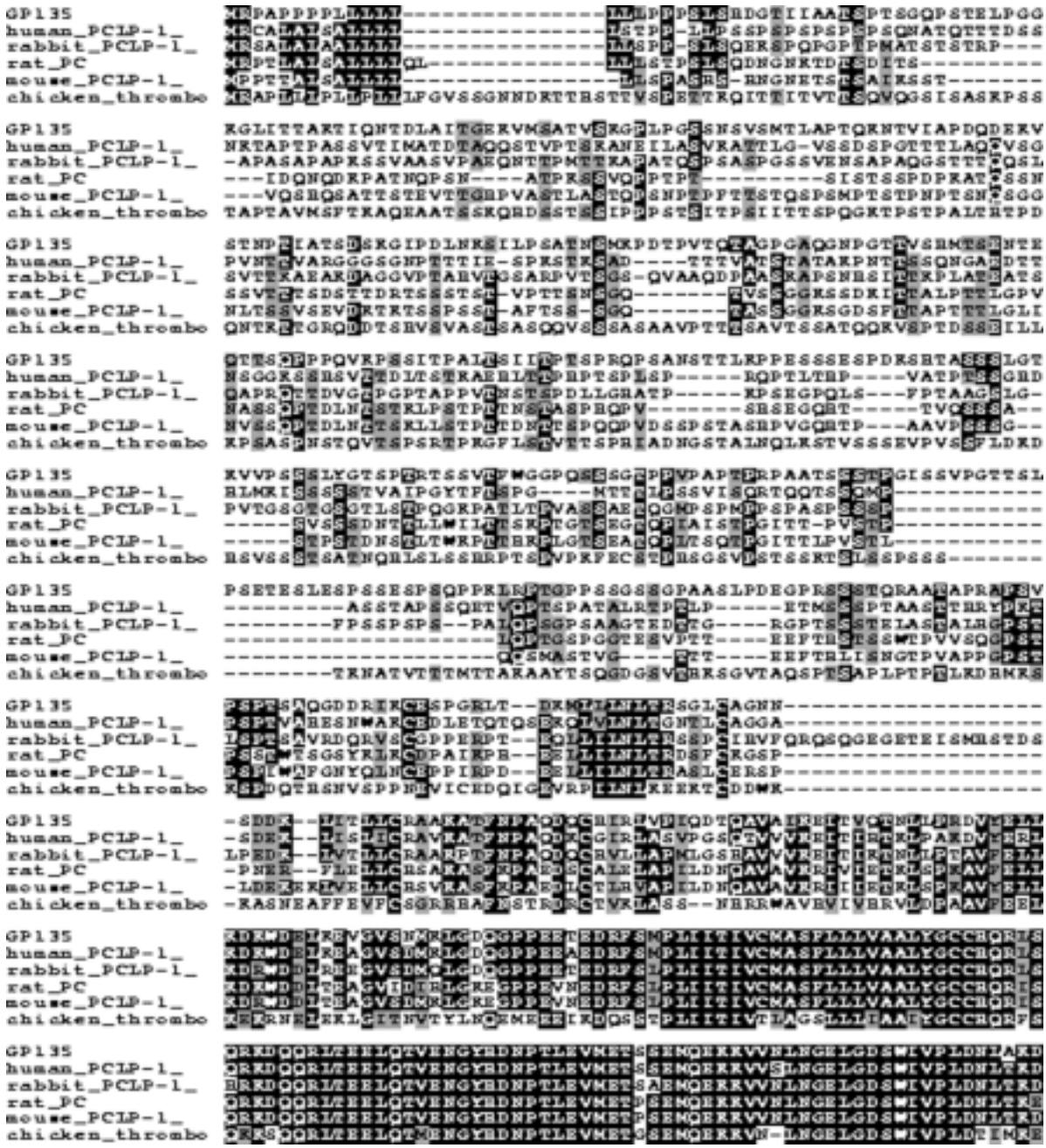
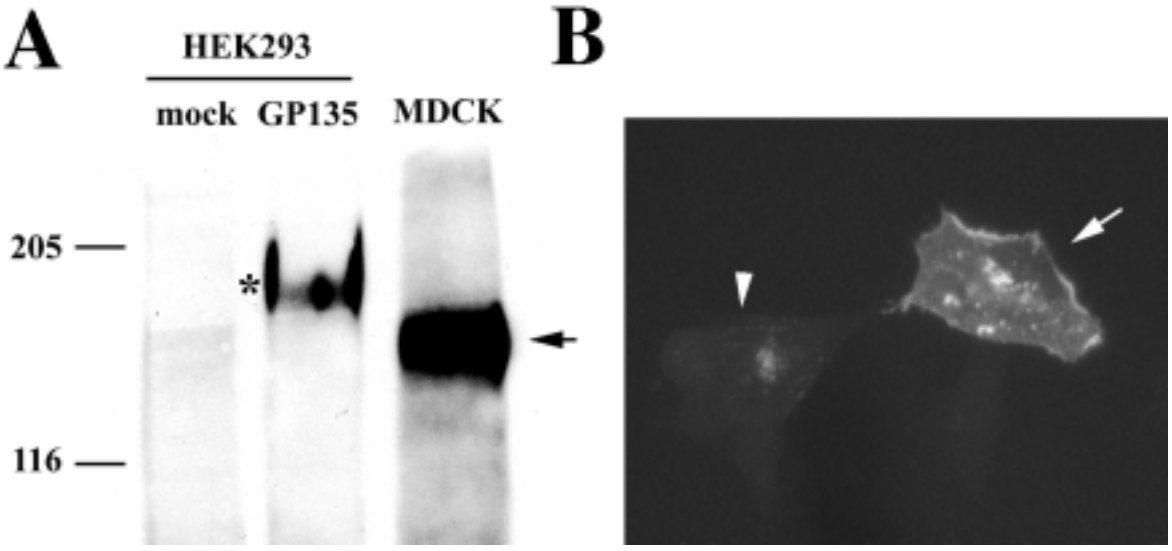
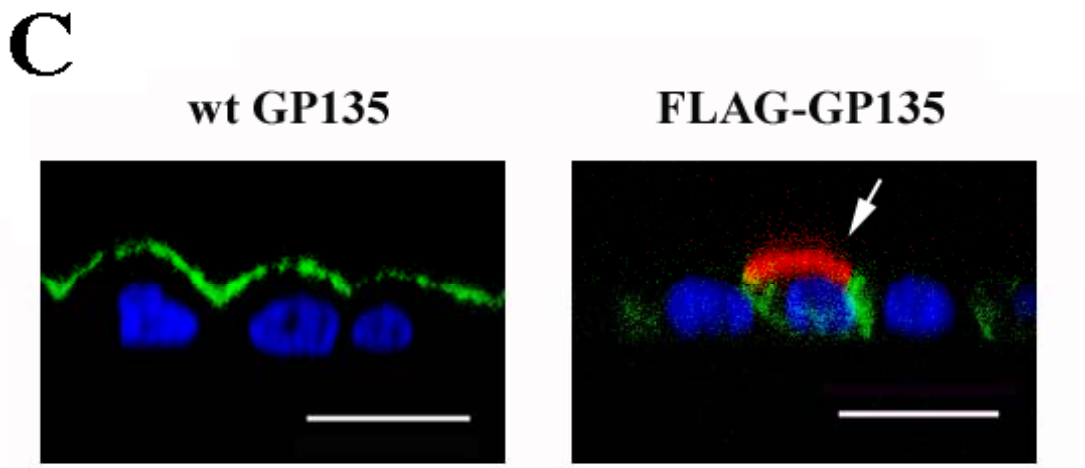


Figure 3. Amino acid sequence alignment of GP135 and 5 Podocalyxin family homologs.

Amino acid residues conserved among at least 4 species are highlighted. Black, amino acid


A
HEK293
mock
GP135
MDCK
205
116
*
B


C
wt GP135
FLAG-GP135

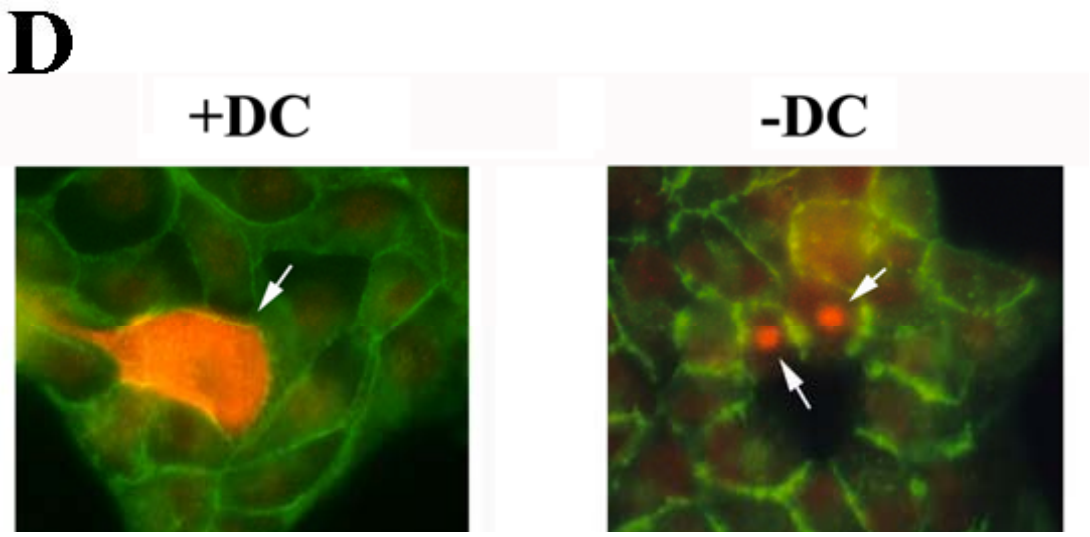

D
+DC
-DC

Figure 4. Expression of GP135 in HEK293 and MDCK cells.

(A) Lysates of HEK293 cells transiently transfected with pcDNA-GP135 or the mock vector were analyzed by western blotting with anti-GP135 antibody. A nearly 200 kDa band(*) was detected in the pcDNA-GP135 lane. This band was higher than the 170 kDa endogenous GP135 band (arrow) in MDCK total lysate.

(B) Immunofluorescence of HEK293 cells transient expressed GP135 with anti-GP135 antibody. The transfected cell (arrow) showed membrane staining pattern by comparing with the untransfected cell (arrowhead).

(C) FLAG-GP135 was expressed on MDCK cells apical domain.Left: wild type MDCK cells were stained GP135 (green) as control.Right: transfected cells were stained with polyclonal anti-FLAG (red) and monoclonal anti-E-cadherin (green) antibodies. A cell expressed FLAG-GP135 was indicated by arrow. Nuclei were visualized by Hoechst 33342 staining. The xz sections were collected by confocal microscopy. Bars, 20mm.

(D) MDCK mutant cells expressing constitutively active Rac1V12 under Tet-off inducible system were transiently expressed Flag-GP135 in the presence or absence of doxycycline (+/- DC). After 2 days of transfection and induction, cells were stained with polyclonal anti-FLAG (red) and monoclonal anti-E-cadherin (green) antibodies. Cells expressed FLAG-GP135 were indicated by arrows

Figure 5. Model of MDCK cyst formation in collagen gel and the role of Rac1 and VACS during this process