• 計畫中文名稱	葡萄糖傳遞受體抑制劑應用於肝癌治療之分子機制探討		
• 計畫英文名稱	Studies on the Molecular Mechanisms of Glucose Transporter-Specific Inhibitors Applied on Liver Cancer Therapy		
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• 中文關鍵字	葡萄糖傳遞受體(glucose transporter 2; GLUT-2)抑制劑; 蜂膠; 抑癌作用; 細胞凋亡; 肝癌細胞		
• 英文關鍵字	Glucose transporter 2, GLUT-2, Phloretin, Anticancer, Apoptosis, Hepatomacells。		
• 中文摘要	台灣地區癌症的死亡率爲總死亡人數的第一位，其中肝癌患者佔了極重要 的比例。由於肝癌治療目前在臨床上尚無令人滿意的結果，因此本計劃期望利 用肝癌細胞對葡萄糖的營養需求，針對癌細胞表面之葡萄糖轉換受體 (Glucose-transporter, 簡稱 GLUT)，以專一性抑制劑進行分子標靶治療，期能達 到專一性抗癌目標。 先期結果說明: 本實驗室利用先進的雷射擷取顯微分離技術(Laser Capture Microdissection，簡稱 LCM)，將正常與腫瘤組織(圖 1A)加以分離並純化 cDNA 進行 real-time PCR 定量分析(圖 1B)。應用此技術目前已經完成十例肝癌檢體分 析，証實國人肝癌組織中二型葡萄糖轉運受體(簡稱 GLUT-2)的表現量較正常肝 組織高約 100 倍(圖 1C)。經搜尋文獻後發現 Phloretin(天然蘋果萃取成分，簡稱 爲 Ph)爲肝細胞中 GLUT-2 抑制劑，初步結果證實 Ph 可以誘發肝癌細胞凋亡作 用，而且血清中葡萄糖可干擾之(圖 2~3)。利用肝癌細胞培養(圖 4~5)或免疫缺 憾鼠活體腫瘤模式均證實 Ph 具有顯著的抗癌作用(圖 6,7)。我們假設 Ph 可抑制腫 瘤組織中 GLUT-2 受體，因此，將腫瘤老鼠進行正子掃瞄(micro PET)分析後證 實 Ph 的確可透過干擾 GLUT-2 受體對葡萄糖分子(18F-FDG)吸收達到抑癌的效 果(圖 7)。合併臨床抗癌藥物(Paclitaxel)可對 Ph 所誘發之肝癌細胞凋亡(圖 8~9) 及活體抑癌作用(圖 10)產生加成效果。 因此，本計畫利用肝癌細胞對葡萄糖代謝之需求，以細胞 GLUT-2 受體作爲 分子標靶，利用 Ph 作爲模式化合物，希望能探討其抑癌作用原理，建立癌症治 療之學理基礎，將來可以提供臨床醫師作爲一種創新的化學治療思考模式。本 計劃分三年擬完成下列計畫目標: 第一年計畫: 國人肝癌組織中葡萄糖轉換受體表現與致癌相關性之研究 目標一、利用 LCM 技術觀察肝癌組織中 GLUT-2 表現: 1.肝癌檢體收集: 目前已收集近五十例肝癌與正常組織(paired)樣本，利用 LCM 技術分離特定 型態之肝癌細胞，以 real-time PCR 技術分析國人肝癌組織中 GLUT 受體分型。 2.建立研究模式細胞株: 本計畫挑選二株基因表現迥異之肝癌細胞(HepG2 和 Hep3B)作		

	爲研究模式 細胞，理由如下: (a). Hep3B(HBsAg +/+)肝癌細胞可以表現 HBsAg 於癌細胞中 (Knowles et al., 1980)，反之 HepG2 則否(HBsAg -/-)，觀察 B 型肝炎病毒與 GLUT-2 在肝癌致癌訊息表達之相關性。(b).?了探討 p53 基因與 GLUT-2 之致癌 相關性，在 HepG2 爲 P53 基因 wild type，Hep3B 則爲 deleted(Bressac et al., 1990)。(c).過去文獻報導指出女性荷爾蒙，尤其是 estrogen 影響肝癌細胞增生與抑制細胞凋亡扮演極其重要的角色(Huang et al., 2006)。HepG2 可表現 ER(Ain et al., 1988)，Hep3B 則無法表現(Huang et al., 2006)。此二株細胞剛好可以用來探 討葡萄糖及其受體抑制劑所誘發抗癌訊息路徑下 ER 對抗癌作用或致癌作用扮 演何種角色。因此我們建立了 GLUT-2 基因 Adv-Tet-off 過度表現與 SiRNA 抑制 之肝癌模式細胞株 (詳見計畫書內文)。 3.組織微陣列晶片分析: 組織微陣列晶片技術可以同時用於評價數十例特定類型肝癌腫瘤樣本的 GLUT-2 表現，爲臨床預後提供判斷資料。 目標二、建立 GLUT-2 抑制(SiRNA)與過度表現(Tet-off)模式細胞株轉殖老鼠: 我們利用免疫缺陷(SCID)老鼠模式已建立肝癌腫瘤動物模式。本計劃將利 用這些模式進一步探討 Ph 之劑量、毒性乃至於最佳給藥模式。同時利用 GLUT-2 抑制(SiRNA)與過度表現(Tet-Off)細胞株進行活體腫瘤模式實驗，利用正子掃 描儀器(micro-PET)觀察活體內 GLUT-2 表現與癌細胞生長之關連性。動物實驗 之組織、檢體等將用來分析並建立 Ph 藥物作用的學理基礎，探討 Ph 在活體腫 瘤組織內的抗癌作用。 第二年計劃: GLUT-2 抑制劑抑癌作用之分子機制探討 目標一、探討肝癌細胞 GLUT-2 之基因表現與細胞週期調控: 1. 建立研究模式細胞株: 建立了 GLUT-2 之之 Adeno virus Si RNA 抑制(knock-down)及 Tet-Off 過度表 現之肝癌模式細胞株。 2.探討 GLUT-2 抑制劑如何抑制肝癌細胞週期變化: 本計劃希望探討 GLUT-2 受體抑制劑之抑癌作用，因此有必要先釐清血清中 葡萄糖的濃度是否影響 Ph 抑制肝癌細胞生長。我們初步研究成果證實 Ph 可誘 發肝癌細胞凋亡(~21.5%,圖 2C)，當血清中不含葡萄糖時，肝癌細胞對 Ph 的作 用變得更加敏感(~80%,圖 3A)，給予葡萄糖(>20 mM)可完全抑制並緩解 Ph 的作 用，而且原本細胞凋亡的現象變成 G2/M 細胞週期抑制(圖 3B)。Ph 如何抑制肝 癌細胞生長週期，必須在本計畫中釐清。利用 GLUT-2 抑制(SiRNA)與過度表現(Tet-Off)之模式細胞株，我們希望能夠釐清 Ph 所調控之肝癌細胞週期蛋白表現 在 GLUT-2 抑制或表現之細胞株有何差異。 3.觀察活體內腫瘤組織之細胞週期蛋白表現:
• 英文摘要	Malignant cells are known to have accelerated metabolism, high glucose requirements, and increased glucose uptake. Transport of glucose across the plasma membrane of mammalian cells is the first rate-limiting step for glucose metabolism and is mediated by facilitative glucose transporter (GLUT) proteins. Increased glucose transport in malignant cells has been associated with increased and deregulated expression of glucose transporter proteins, with over expression of GLUT in liver cancer cells, a characteristic feature. The eclectic-designed experiments in this study are hope to evaluate a novel therapeutic stratagem by using the glucose-transporter inhibitor which blocks the uptake of glucose in hepatocellular carcinoma cells. To test whether our hypothesis is available, ten cases of human hepatoma and normal tissues were separately dissected by Laser Capture Microdissection (LCM) and the expression level of type 2 GLUT (GLUT-2) in human normal and tumor tissues were determined by real-time PCR analysis. Our result revealed that the expression of GLUT-2 was higher (> ~100 folds) in tumor tissues than in normal part (Fig. 1). Animal studies also demonstrated that inhibition of glucose uptake could be detected in vivo by micro PET technique and the results shown that 18F-FDG absorption was nearly completely inhibited in the GLUT-2 inhibitor (Phloretin, Ph)-treated tumors (Fig. 6,7). Our preliminary studies indicated that GLUT can act as a 「molecular target」 for designing novel anticancer agents. Year-1 proposal: Characterization of the GLUT-2 phenotypes and its biological roles in human hepatocellular carcinoma tissues in Taiwan Aim-1: Quantitative analysis of GLUT-2 expression

levels by LCM/real time PCR technique in human hepatocellular carcinoma tissues: The LCM and real-time PCR analysis for GLUT-2 expression in human liver cancer tissues have been performed for more than 10 cases. Additional cases will be accumulated to more than 100 cases in which the clinical relevance of the GLUT-2 expression and its role in human liver cancer should be clearly investigated. Two hepatocellular carcinoma cell lines including HepG2 (HBsAg -/-) and Hep3B (HBsAg +/+) were selected to see the factors of hepatitis B virus infection and its role involved in GLUT-2-mediated cancer cell proliferation. The GLUT-2 knock-downed (by SiRNA) and over expressed (by tet-Off adenovirus) cell lines were also established in our laboratory to investigate their biological roles of GLUT-2 in cancer cell proliferation and its role in response to its specific inhibitors. Aim-2: Evaluations for the antitumor activities of GLUT-2 specific inhibitor in human liver cancer cell-xenografted tumor model: The human liver cancer cell-xenografted tumor model was established in the immuno-deficiency SCID mice (Fig. 6). Our results revealed that GLUT-2 specific inhibitor (Ph) have significant antitumor effects in the SCID mice through inhibition of the glucose uptake as evidenced by micro PET (18F-FDG) absorption analysis (Fig. 7). Such results implied that the GLUT-2 may have some significant role in human liver cancer growth in vivo. In this proposal, the GLUT-2 specific inhibitor was adapted as a molecular-targeting agent. However, the best protocols of drug administration is still not been established. The toxicity of the GLUT-2 inhibitors also needed to be carefully investigated. Year-2 proposal: Studies on the molecular mechanisms of GLUT-2 inhibitor-mediated antitumor effects Aim-1: Studies on the mechanisms of Ph-induced cell cycle regulation in human liver cancer 1. Establishment of the GLUT-2 knock-downed (SiRNA) and over expressed (tet-Off) cell lines: all these cell lines were established in our preliminary results (see the text of proposal). 2. In vitro studies of the cell cycle regulatory proteins treated by Ph in human liver cancer cells: Our preliminary results demonstrated that Hep G2 cells were more sensitive to Ph in the absence of glucose in culture medium (Fig. 3A). However, the Ph-induced apoptosis in HepG2 cells was completely attenuated when additional glucose added (Fig. 3B). G2/M cell cycle arrest instead of apoptosis was observed when the Ph-treated cells were rescued from high concentration of glucose (Fig. 3B). The G2/M phase cell cycle regulatory mechanisms will be investigated to study the Ph-induced cell growth arrest effects. The roles of GLUT-2 involved in cell growth signaling pathways will also be studied by using the GLUT-2 knock-downed and over expressed HepG2 cells. 3. In vivo studies of the cell cycle regulatory proteins in Ph-treated tumors: The GLUT-2 knock-downed and over-expressed HepG2-xenografted tumors dissected from the Ph-treated SCID mice were isolated for analysis of the cell cycle regulatory proteins. Such results will unveil the significance of GLUT-2-mediated cell survival signals and the in vivo mechanisms of GLUT-2 inhibitors-mediated antitumor effects. Aim-2: Studies on the mechanisms of Ph-induced cell apoptosis mechanisms in human liver cancer cells 1. Studies on the mechanisms of Ph-induced apoptosis in liver cancer cells: Our preliminary results revealed that GLUT-2 inhibitors could induce HepG2 cells apoptosis. However, glucose presence in the cultured medium protected the apoptosis inducing effects (Fig. 2~3). Such results revealed that GLUT-2 receptor may act as a molecular target to be applied in cancer chemotherapy. However, the molecular mechanisms of the GLUT-2 inhibitors-mediated apoptosis were still uncertain. In addition, how the glucose linked the survival signals through the GLUT-2 which eventually caused cell survival will also be illustrated. The GLUT-2 knock-downed and over-expressed cell lines may be used to definite the role of GLUT-2 in response to Ph-induced apoptosis. 2. In vivo observations of the apoptosis-signaling proteins in Ph-treated tumors: As described above, the GLUT-2 knock-downed and over-expressed liver cancer

	cell-xenografted tumors were isolated for analysis of the apoptosis regulatory proteins. Such results will unveil the significance of GLUT-2-mediated cell death signals and the in vivo mechanisms of GLUT-2 inhibitors-mediated antitumor effects. Year-3 proposal: Clinical applications of GLUT-2 inhibitors used for antitumor purpose in human liver cancer Aim 1: Evaluation of the antitumor efficacy by combine treatment of Ph with clinical used anticancer agents: Our results revealed that combine treated with lower concentration of Ph (50 μM) plus Paclitaxel (10 nM) significantly potentiate the apoptosis induction in HepG2 cells (Fig. 8). In vivo tumor models further support such observations (Fig. 10). Such results implied that combination of GLUT-2 inhibitors with clinical used anticancer agents may have significant benefits for liver cancer therapy. Our proposal need to be further studies for the administration protocols of combination therapy and improved the safety in an in vivo experimental model. Aim 2: Evaluation of the GLUT-2 inhibitors used in cancer chemopreventive purpose: Our preliminary studies revealed that Ph can be used as a candidate for anticancer agent through specific inhibition of the GLUT-2 function in human liver cancer. To our knowledge, the Ph has been isolated from apple juice and is exist around fruits and vegetables as a natural product. Such results promote us to ask whether the GLUT-2 or other Glu-1, 4 …. inhibitors occurred in natural products (such as Genestein exist in fruits) could be used as chemopreventive agents for cancer prevention. We demonstrated that combine treatment of two GLUT-2 inhibitors potentiate the apoptosis induction in HepG2 cells (Fig. 5). In the year-3 proposal, in vitro and in vivo experiment models must be set up to investigate whether combination of two other types of GLUT-2 inhibitors (such as Genestein, T-1095， Phloridzin，SB203580…) could potentiate the antitumor effects against the human liver cancer.