計畫編號：BM02-00
計畫名稱：微生物致病機轉之探討
計畫主持人：陳培哲
計畫摘要(中)：

在這項計畫裡，我們的主題將研究微生物的致病機轉(pathogenesis)。其機轉將透過使用不同的病原體和甚至不同的代理系統、從不同的層次加以探討。從微生物的黏著和擴散的層次來看，我們將針對會侵入人體肝或者眼睛的獨特的 Klebsiella 系統加以研究。在這個題目裡有許多新發展，但是有更多需要被研究的議題。在感染之後微生物的基因表現的層次來看，我們將研究與性激素路徑有相互作用的人類 papilloma 病毒。對病毒細胞相互作用的層次來看，我們將應用 HIV 系統探索 SMN-Vpr 相互作用並且檢驗一種瞄準 SMN 作為 HIV 的治療的新可能性。對微生物的清除層次來看，這是慢性病毒感染的一個非常難的問題，特別為慢性 B 型肝炎病毒感染。最近我們發現透過干擾素合併 ribavirin 治療雙重慢性 C 型和 B 型肝炎患者能清除大約 10~20%的 B 型肝炎。我們將進一步探討其背後機制。最後，微生物的致病機轉可能非常錯綜複雜。就 HBV X 蛋白質而論，這蛋白質在宿主基因互動，抑制 proteasome 活性，活化訊息路徑以及和肝細胞癌發生等各方面都有影響。不過，在哺乳動物的細胞裡進行遺傳學研究相當困難。我們將探索一個使遺傳學的分析更加容易的系統，Drosophila 系統。我們將表現 HBV X 基因在 Drosophila 的具體組織內並且觀察其臨床表徵(phenotype)。之後我們將使用蒼蠅遺傳學發現在 Drosophila 裡影響 HBx phenotypes 的宿主基因。希望以這種新方法，我們能鑑定演化上涉及 HBx 蛋白質功能的宿主因子。最終，我們的主題研究能夠發現微生物的致病機轉並且在未來治療方面提供重要的訊息。

計畫摘要(英)：

In this program project, we will focus upon the theme of microbial pathogenesis. The mechanisms will be addressed from different levels by using different pathogens and even surrogate systems. For microbial adhesion and invasiveness, we will study this in the Klebsiella system which has been unique for its capacity to bind and to invade human liver or eyes. There are new progresses in this topic but much more needed to be studied. For microbial gene expression after infection, we will adopt

the human papilloma virus which displays an interesting interaction with sex hormone pathways. For virus-cell interaction, we will employ the HIV system to explore the SMN-Vpr interaction and examine a new possibility of targeting SMN as an anti-HIV therapy. For the microbial clearance, this is a very difficult issue for chronic viral infection, especially for chronic hepatitis B virus infection. Fortunately, recently we found that co-infection by hepatitis C virus and treatment with interferon can clear HBV in about 20-25% of the hepatitis B infected patients. It thus suggested an interesting mechanism for HBV clearance, probably via virus interference and by interferon therapy. Finally, the pathogenesis of microbials can be very complicated in the mammalian hosts or cells. In the case of HBV X protein, this pleotropic protein has been implicated in host gene activation, proteasome inhibition, activation for signaling pathways and in liver carcinogenesis. However, as the genetic study in mammalian cells is difficult to conduct and so far has not yet been done. We will like to explore a novel system which makes genetic analysis much more easier, the drosophila system. It is possible to express HBV X gene in specific tissues of Drosophila and to drive certain phenotype. Later we can apply fly genetics to discover host genes influencing HBx phenotypes in Drosophila. Hopefully, by this new approach, we can identify the evolutionally-conserved host factors involved in HBx protein functions. In the long run, we can study and assay the human counterparts in details. These projects are mostly novel and original to explore important pathogenesis of infectious agents and will provide innovative information to this field.

計畫編號：BM02-01
計畫名稱：社區性肝膿瘍克雷伯氏肺炎桿菌與腸細胞之黏著和入侵
計畫主持人：王錦堂
計畫摘要(中)：

克雷伯氏肺炎桿菌（Klebsiella pneumoniae）引起的社區性化膿性肝膿瘍（community-acquired pyogenic liver abscess）已是全球重要的新興感染症，位於 K1 莢膜多醣體生合成區的 magA 基因已知和細菌高黏性、血清與細胞吞噬抗性與對老鼠的毒性有關，因此莢膜血清型 (尤其是 K1 與 K2) 在致病上扮演非常重要的角色，但克雷伯氏肺

炎桿菌引起的化膿性肝膿瘍之詳細致病過程仍未完全了解。克雷伯氏肺炎桿菌普遍存在腸胃道中，由我們分離健康自願者糞便中克雷伯氏肺炎桿菌菌株的初步結果，約有 12%菌株屬於莢膜血清型 K1，而約 5%菌株是莢膜血清型 K2，以聚合酶連鎖反應分析數段侵襲性相關的基因片段，發現血清型 K1 與 K2 之腸道寄生株的基因體型和血清型 K1 與 K2 之肝膿瘍分離株相似，且其對老鼠之致病力也相同。先前學者對其他腸內菌的研究已經證實腸道中的細菌可以穿過腸道上皮細胞的屏障，進而引起全身的敗血症。因此綜合我們的初步結果和細菌轉位的理論基礎，我們提出一個假設” 腸道中的克雷伯氏肺炎桿菌可能可以穿過腸道上皮細胞的屏障到血液中再由門脈流至肝臟，引起化膿性肝膿瘍和菌血症” 。因此本計畫希望可以 (1) 比較引起化膿性肝膿瘍菌株，非侵襲性菌株與腸道寄生株對腸道細胞的黏著與侵入能力 (2) 找出負責與腸道細胞黏著和侵入的基因 (3) 進一步分析負責與腸道細胞黏著和侵入的基因的功能 (4) 與細菌黏著後腸道細胞之細胞生物與分子生物學變化。最後希望這些結果可以幫助我們了解克雷伯氏肺炎桿菌與腸道上皮細胞的交互作用及細菌如何造成感染，並作為發現阻斷黏著與侵入的治療方法基礎。

計畫摘要(英)：

Klebsiella pneumoniae causing community-acquired pyogenic liver abscess (PLA) has been an emerging infectious disease worldwide. The magA/ K1 capsular polysaccharide (cps) biosynthesis region was responsible for mucoviscosity, resistance to serum and phagocytosis and virulence to mice. The capsular genotype/ serotype (especially K1 and K2) is believed to play a vital role in the pathogenesis, but the details of pathogenesis of PLA caused by K. pneumoniae remained unclear. The reservoir for K. pneumoniae is the gastrointestinal tract. In our preliminary study, the ~12% K. pneumoniae strains isolated from stool collected from health volunteers belonged to K1 capsular serotype and ~5% strains isolated from stool were serotype K2. The genotype and virulence to mice of these K1 and K2 colonization strains were similar with those of the K1 and K2 PLA strains. Previous studies have believed that enteric microbes could pass through the intestinal epithelial barrier and result in systemic sepsis. Based on our preliminary result and the hypothesis of bacterial translocation, we proposed a hypothesis that “K.

pneumoniae colonized in the intestine might pass through the intestinal epithelial barrier, get access to liver, and result in PLA and bacteremia”. This project plans to: (1) compare the adhesion and invasion abilities to the intestinal epithelial cells among colonization, noninvasive and PLA strains. (2) identify the genes responsible for the adhesion and invasion with the intestinal epithelial cells. (3) characterize the function of putative adhesin(s) and invasin(s) and their interaction with intestinal epithelial cells (4) study the cellular and molecular biological changes of enterocytes during infection. These results will help us to understand the interaction of K. pneumoniae and intestinal epithelia and how the bacteria get access to infection and provide the novel candidates for blocking the adherence and invasion.

計畫編號：BM02-02
計畫名稱：研究人類乳突瘤病毒基因調控的機轉
計畫主持人：陳小梨
計畫摘要(中)：

HPV 所導致的子宮頸癌中，HPV-16 是經常被發現的一株病毒，並可表達三個會導致轉型的致癌基因—E6、E7 及 E5。在 HPV 引起的子宮頸癌中，賀爾蒙也被視為導致癌症的原因之一，然而目前對於賀爾蒙如何調控 HPV 基因表現的研究極為稀少。先前研究中，我們選殖出了一個全新的基因並命名為 NRIP(nuclear receptor interaction protein, NRIP)，且證明 NRIP 的功能乃是作為與賀爾蒙相關核接受體—糖皮質激素受器(GR)及 androgen receptor(AR)的輔活化因子，增強 GR 及 AR 對於 mouse mammary virus tumor promoter(MMTV)啟動子的轉錄活性(Tsai et al., 2005; Chen et al., 2007)。我們初期研究結果認為 GR 可以活化 HPV 基因表現，而 NRIP 則可增進 GR 活化 HPV 基因表現的作用。在我們初步的結果中，如果以被 HPV-16 感染的細胞株 SiHa 及 CaSki 為對象，將 NRIP 基因 knock down 掉，會導致病毒 E6、E7 mRNA 的表現量降低，且會抑制細胞生長。因此我們假設 NRIP 及 GR 可以調控 HPV 的基因表現，本計畫主要探討 GR 及 NRIP 對 HPV 造成子宮頸癌的調控基轉。

另外；在 HPV-16 的啟動子上有三個 AP-1 的結合區，因此 c-jun 可加以活化 HPV-16 的基因表現。而 HPV-16 的 E5 蛋白質乃是屬於膜蛋白，並且具有使細胞轉型的能力，我們最新發表的文章中也已證明

E5 可以導致 c-Jun 蛋白質的表現並且將其磷酸化，且 c-jun 是 HPV 的轉錄因子之一，因此 E5 與 HPV 基因表現的相關性乃值得加以研究。此外，我們亦已證明生長因子受體(growth factor receptor)--ErbB4 會活化 c-jun 基因表現且促進其磷酸化，HPV-16 的 E5 蛋白可與 ErbB4 結合。若同時表現 ErbB4 及 HPV-16 E5 蛋白時，E5 會抵消由 ErbB4 所引起的 c-Jun 蛋白質表現及磷酸化現象(Chen et al., 2007)。更重要一點，我們初期結果發現 ErbB4 是-醣蛋白並找到一個胺基酸是醣化的重要胺基酸，因此 ErbB4 及其醣化 ErbB4 在 HPV 基因調控中所可能扮演的角色將相當有趣且值得探討。

在此計畫中，我們擬定四個研究主題：

(1) 瞭解 GR 如何調控 HPV 基因表現。
(2) 瞭解 NRIP 如何增強 GR 調控的基因表現及 NRIP 如何自我調控。
(3) 闡明核受體、ErbB4 醣化及病毒蛋白 E5 在 HPV 引起的子宮頸癌中所分別扮演的角色及如何相互影響。
(4) 研究新的治療癌症製劑 siNRIP 造成細胞死亡的機轉。

計畫摘要(英)：

Human papillomavirus type 16 (HPV-16) is the most frequent viruses in HPV-caused cancers; and encodes three transforming oncogenes E6, E7 and E5. Steroid hormones are identified to act as cofactors with HPVs in the etiology of cervical cancer. However the regulation mechanism of HPV gene expression by hormone receptor is rare. Previously we have identified a novel gene-NRIP and characterized NRIP role as a hormone-dependent coactivator of glucorticoid receptor (GR) (Tsai et al., 2005). Previously, the entire regulatory region of HPV-16 genome has been identified at least three glucocorticoid hormone receptor response elements (GREs). Our preliminary results show GR can activate HPV gene expression (Figure 1 and 2); and NRIP can increase GR-driven HPV gene expression (Figure 2A). When knock-down NRIP gene in HPV-16 containing SiHa and CasKi cells, it reduces HPV-16 E6 and E7 gene expression resulting the inhibition of cell growth (Figure 4 and 5). Therefore, the regulatory mechanisms of GR and NRIP to HPV gene expression will be investigated. Moreover, except three GRE sites, there are three AP1 binding sites in HPV-16 promoter, and c-jun is reported to activate HPV gene expression. HPV-16 E5 is a membrane and

oncogenic protein. Our recent published paper shows E5-inducing c-Jun expression and phosphorylation (Figure 6). Hence, the mechanisms of E5-regulating HPV gene will be investigated. Additionally, we also demonstrated that growth factor receptor-ErbB4 can activate c-jun gene expression and phosphorylation (Figure 6). HPV-16 E5 can bind to ErbB4, however when co-expressing E5 and ErbB4, E5 can abrogate ErbB4-induced c-Jun protein expression and phosphorylation (Figure 6, Chen et al., 2007). Most intriguingly, our preliminary results found that ErbB4 is a glycosylated protein and identified which amino acid 358 responsible for its glycosylation (Figure 7 and 8). Therefore, the mechanisms of ErbB4 and its glycosylation form involving in HPV gene regulation via c-Jun will be investigated. In this study, there are four major aims to be approached:

(1) To understand the mechanisms of GR-regulating HPV gene expression.
(2) To understand the mechanisms of NIRP-enhancing GR-mediated HPV gene expression.
(3) To illustrate the cross talk between GR (nuclear receptor), ErbB4 and its glycosylation status (growth factor receptor), 16E5 (viral oncoprotein) in HPV-causing cervical cancer.
(4) To identify a novel therapeutic agent siNRIP for cervical cancer and investigate the apoptosis mechanism.

計畫編號：BM02-04
計畫名稱：雙重感染慢性 B 型和 C 型肝炎患者經治療後 B 型肝炎表面抗原消失的機制探討
計畫主持人：劉俊人
計畫摘要(中)：

在大多數國家，有慢性病毒的肝炎的病患通常只有單一 C 型病毒(HCV)或者Ｂ型肝炎病毒(HBV)感染。但是在 HBV 或 HCV 感染盛行國家例如台灣，雙重 HBV 和 HCV 感染病患不在少數。而且，有雙重 HBV 和 HCV 感染病患比單一 C 型病毒(HCV)或者Ｂ型肝炎病毒(HBV)感染病患有更嚴重的肝疾病和肝細胞癌(HCC)發生的危險，因此應該被更積極治療。台灣一向是全球肝病療法的指標，對於各種肝炎之治療皆有相當成效，唯獨罹患慢性 B、C 型肝炎雙重感染者一

直令醫界束手無策。有感於全球有百萬慢性 B、C 型肝炎雙重感染病患亟待治療，我們在長達兩年的大型臨床試驗中，使用長效型干擾素(pegylated interferon)合併雷巴威林（ribavirin）療法，針對三百餘名患者進行追蹤評估。本研究共收案 321 人，其中有 161 位慢性 B、C 型肝炎雙重感染與 160 位單純慢性 C 型肝炎感染的病患接受治療，結果發現高達 75%可以達到 C 型肝炎持續性病毒反應的治療目標。使我們驚奇的是其中 10%雙重 HBV 和 HCV 感染患者經治療後血清 HBsAg 轉變成陰性，這是一般慢性 HBV 患者經抗病毒治療後的最終、最理想治療標的。相對地，自然病程或者治療後引起的 HBsAg 消失比率，在慢性 HBV 單一感染病患裡每年只有大約 0-3%。因此值得進一步探討慢性 B、C 型肝炎雙重感染患者經治療後 HBsAg 消失的相關機制。

以前的報告指明 HBsAg 消失與收錄患者時的年齡、肝炎的嚴重度、男性、和肝硬變的存在等因素有相關。病毒因子，例如 HBV 基因型 B 或者有急性 HCV 重覆感染，也已經被證實與 HBsAg 的消失有關。不過，幾個關鍵的問題仍有待釐清。首先，過去研究指出雙重慢性 C 型和 B 型肝炎患者，HCV 和 HBV 複製是交互式互相影響的，並且通常 HCV 在體外試管內會抑制 HBV 複製。有關於這相互作用，是否 HCV 和 HBV 在活體內能感染相同的肝細胞，目前仍然是未知的。若 HCV 和 HBV 在活體內能感染相同的肝細胞，那麼在 HCV RNA 被清除之後，HBV 會怎樣表現也未知。其次，HBV 能以隱藏的感染型態(OHB)存在。OHB 意指 HBsAg 陰性，但是在血清或者肝臟裡可以檢測得到 HBV DNA。OHB 的存在取決於 HBsAg 和 HBV DNA 分析方法的敏感度，造成 OHB 的原因則包括患者自然感染中復原，病毒表面基因突變而未被偵測，或者 HBsAg 濃度太低而無法被偵測到。經治療發生的 HBsAg 消失到底是代表病患的確從 HBV 感染中痊癒、或者有 OHB 之虞，也須進一步澄清。再者，是否任何 HBV 專一性或非專一性免疫反應會影響 HBsAg 和 HBV DNA 的消失仍留待被驗証。最後，任何Ｂ型肝炎病毒因子能否影響 HBsAg 消失也有待證實。

為了釐清這些問題，我們假設 C 型肝炎病毒因子能在雙重感染 C 型和 B 型肝炎的病患影響 HBsAg 的消失。若 HBV 和 HCV 可以感染相同的 hepatocyte，則 HCV 結構或者功能蛋白質可能直接影響 HBV 的複製和 HBsAg 的表現。另一方面，如果 HCV 和 HBV 不能感染相

同的 hepatocyte，則 HCV 可能藉由宿主免疫反應間接影響 HBV DNA 的複製。利用我們此一治療族群，我們希望(1)驗証是否 HCV 和 HBV 能感染相同的 hepatocyte，(2)研究是否 HCV 蛋白質能影響 HBV 的複製和 HBsAg 的表現，(3)研究隱藏型 HBV 感染的可能性，(4)研究 HBV 專一性或非專一性免疫反應在 HBsAg 消失上的影響，以及(5)研究 HBsAg 基因變異，基因型和其他 HBV 因子對 HBsAg 消失的影響。

關於慢性 HBV 感染，HBsAg 消失是一個關鍵的事件，通常代表著肝炎活性降低和長期良好的預後。雖然如此，自然病程或者經治療引起的 HBsAg 消失仍不常見。而且，HBsAg 消失的機制基本上仍未知。我們意外發現大約 10%雙重感染病患在治療之後失去 HBsAg。如果我們能在這群病患裡釐清 HBsAg 消失的機制，在短時期內，我們也許能驗証 HCV 和 HBV 感染之間的相互作用，我們能釐清 HBsAg 消失意指 HBV 感染痊癒或者只反映出隱藏型 HBV 感染的存在。同時，如果隱藏型 HBV 感染存在，我們的研究結果也可以澄清在肝臟裡面 HBV 持續存在或者消失過程中病毒和免疫因子所扮演的角色。最後，我們能為將來的治療提供研發方向，使病患減少罹患 HBV 相關肝疾。

計畫摘要(英)：

In most countries, patients with chronic viral hepatitis usually have single hepatitis C virus (HCV) or hepatitis B virus (HBV) infection. However, in areas where HBV infection is endemic such as Southeast Asia, Far East and southern Europe, subjects infected with both hepatitis C and B are substantial. We have treated hepatitis C and B dually infected patients in a pilot study by using standard IFN in combination with ribavirin for 6 months. We found that a sustained HCV virologic clearance rate (SVR) in hepatitis C and B dually infected patients could be achieved to an extent comparable to that in simple hepatitis C. After a follow-up of >2 years, HCV RNA remained undetectable in 89% of patients with sustained clearance of serum HCV RNA 6 months post-treatment. To our surprise, 21% of them lost serum HBsAg, which is the ultimate endpoint of antiviral therapy for chronic HBV infection. A recent nationwide multicenter clinical trial in Taiwan using peg-interferon alfa plus ribavirin demonstrated that the HCV SVR can be further increased to be around 75%, and consistently the HBsAg clearance could

be obtained in 10% of the treated patients. In contrast, the spontaneous or treatment-induced HBsAg clearance rate is only around 0-3% annually in patients with chronic HBV infection alone. Thus it is interesting to clarify the mechanisms associated with the clearance of HBsAg in this treatment cohort with dual hepatitis B and C.

Previous observations suggest that the probability of HBsAg seroclearance correlated positively with age at entry, sustained remission of hepatitis, marginally significantly with male gender, and the presence of liver cirrhosis. Viral factors such as HBV genotype B or superinfection with acute HCV infection have also been shown to correlate with the clearance of HBsAg. However, several critical issues remain to be resolved in patients with dual chronic hepatitis C and B. First, it has been shown that HCV and HBV replication is reciprocally interactive, and usually HCV suppresses HBV replication in vitro. Regarding this interaction, whether HCV and HBV can infect the same hepatocyte in vivo is still unknown. How HBV behaves after the clearance of HCV RNA is also unknown. Second, HBV can exist in the form of occult HBV infection (OHB) infection, defined as negative for HBsAg but positive for HBV DNA in serum or liver. One of the critical questions regarding HBsAg loss after treatment is whether the patient is indeed recovered from HBV infection or has OHB. Third, whether any HBV-specific or non-specific immune responses influence the clearance of HBsAg and HBV DNA remains to be clarified. Fourth, whether any hepatitis B viral factor can influence the clearance of HBsAg in dually infected patients is not studied well.

To address these issues, we hypothesize that hepatitis C viral factors can influence the clearance of HBsAg in patients dually infected with hepatitis C and B. HBV and HCV might infect the same hepatocyte, then HCV structure or functional proteins may directly influence the replication of HBV and the expression of HBsAg. Alternatively, if HCV and HBV do not infect the same hepatocyte, then HCV may influence the replication of HBV DNA indirectly by host immune responses. Taking advantage of the treatment cohort, we aim (1) to clarify whether HCV and HBV can infect the same hepatocyte, (2) to study HBV and HCV

interactions in vitro by transfection assay, (3) to study the possibility of occult HBV infection, (4) to study the influence of HBV-specific and non-specific host immune systems in the circulation or liver compartments on the clearance of HBsAg, and (5) to study the influence of HBsAg gene mutant, genotype and other HBV factors on the loss of HBsAg. HBsAg loss is a critical event and usually indicates remission of hepatitis activity and a favorable long-term outcome. If we can clarify the underlying mechanisms of HBsAg loss in this group of patients, we may provide directions for future treatment development.

計畫編號：BM02-05
計畫名稱：利用果蠅染色體缺失篩選以及 RNA 干擾篩選尋找 B 型肝炎病毒蛋白 X 之修飾基因
計畫主持人：陳培哲
計畫摘要(中)：
計畫摘要(中)：

肝癌為台灣地區主要癌症死亡原因之一。肝癌的發生與 B 型肝炎病毒慢性感染有密切相關。雖然流行病學證據顯示 B 型肝炎病毒的感染會導致肝癌的發生，但是詳細的分子機制並不清楚。HBx 是一種 B 型肝炎病毒製造的調節蛋白，即使 B 型肝炎病毒嵌入宿主染色體後也可以製造出 HBx 蛋白。HBx 除了影響病毒複製之外，許多研究指出 HBx 也參與肝癌的發生。HBx 基因轉殖在特定品系的小鼠肝臟細胞會促使肝腫瘤生成,因此推測 HBx 確實會促進腫瘤的形成。雖然目前研究已知 HBx 可調節基因的轉錄作用、基因毒性的壓力反應、蛋白質的降解作用、細胞凋亡和許多訊息傳遞路徑，但 HBx 促進肝癌腫瘤生成的分子機制仍不明瞭。長久以來一直懷疑腫瘤生成的過程中 HBx 可能需要其他分子協同作用。至於是那些分子和 HBx 共同參與肝癌形成則有待進一步研究。為了探究 HBx 所調節之新的細胞功能以及與 HBx 協同作用的分子，我們計劃利用果蠅染色體缺失篩選尋找調節 HBx 蛋白功能的修飾基因，也就是可能會和 HBx 協同作用促使肝癌發生的分子。我們使用於果蠅眼睛發育時，表現 HBx 蛋白時會顯現出 rough eye 的表現型作為篩選的依據，對超過涵蓋 95%果蠅基因體的染色體缺失果蠅株進行 dose-sensitive 的遺傳篩選。經過第一次染色體缺失的篩選後發現包含修飾基因的染色體基因座後，將進一步利用不同部分覆蓋此段修飾基因座之染色體缺失的果蠅株以

縮小修飾基因座的範圍。一旦將修飾基因座縮小到適當長度後，我們可以利用修飾基因座的範圍內每個基因的 RNA 干擾的轉植果蠅篩選出可改變 HBx 活性的修飾基因。此外會利用現有突變株確認找到的修飾基因，或利用高解析染色體刪除的技術取得涵蓋修飾基因的微小缺失染色體進行確認。此種篩選的方式有涵蓋幾乎所有果蠅的基因的優勢，而且每個基因可得以被一視同仁地篩選過沒有偏頗。因此我們期盼發現一些新的 HBx 修飾基因，幫助我們了解 HBx 調節何種細胞功能以及 HBx 如何調控這些功能。我們可以利用果蠅篩選結果對照其哺乳類動物的異物種同源基因，進一步地在小鼠肝癌動物模式及臨床病人驗體中進行研究 HBx 與其修飾基因如何協同作用導致肝癌形成。總結，我們提出利用果蠅 rough eye 的表現型去進行全面性的果蠅基因篩選。預期這些研究結果應該可以進一步了解慢性 B 型肝炎導致肝癌的機轉。

計畫摘要(英)：

Heptocellular carcinoma (HCC) is one of the leading cancer related mortalities in Taiwan. The development of HCC is tightly associated with chronic HBV infection. Although compelling epidemiologic evidence suggests that HBV infection leads to the development of HCC, the underlying molecular mechanism is not entirely clear. HBx protein is a regulatory protein encoded by both replicating and integrated subviral genomes. In additional to its essential role for viral replication, several lines of evidence implicates that HBx plays a role in the development of HCC. Transgenic mice expressing HBx in hepatocytes develop liver tumor, suggesting that HBx indeed directly promotes tumor formation. Although HBx has been reported to regulate gene transcription, genotoxic stress response, protein degradation, apoptosis, and several signaling pathways, the molecular mechanisms by which HBx contributes to HCC carcinogenesis remain illusive. It has been speculated that there are missing links between HBx and carcinogenesis. To explore novel cellular functions regulated by HBx, we propose a non-biased screen looking for functional modifiers of HBx protein by using fly genetics. We propose a dose-sensitive genetic screen against a set of chromosome deficient fly stocks that cover about 95% of fly genome using a rough eye phenotype generated by overexpressing HBx during eye development. The

chromosome locus harboring a modifier (an enhancer or a suppressor) identified from first run of deficiency screen will be further narrowed down by different deficient stocks in which chromosome deletions partially overlap with the chromosome deletion of the original deficiency stock. Once the modifier locus has been narrowed down to a reasonably short region, we can identify the candidate gene that modify HBx activity by screening the RNAi transgenic flies of the genes encoded by the locus that modifies the rough eye in HBx transgenic fly. The identity of the modifier will be then independently confirmed by available p-element mutants of the gene. Alternatively the modifier gene can be confirmed by a recently available high resolution chromosome deletion technique which allows us to generate a small deletion of the desired locus by FRT site directed chromosome recombination. Because the design of the screen is close to saturation (the deficiency chromosome cover about 95% of the genome) and each gene is equally represented in deficiency stocks, we expect to discover some novel HBx modifiers which will be very informative to our understanding what cellular functions may be regulated by HBx and how HBx may regulate them. Provided that the fundamental cellular functions are often conserved between mammals and insects, the genetic interaction between HBx and the mammal ortholog of the identified modifier can be immediately tested in the perspective of the identified cellular function. Furthermore, the implication of the identified modifier and cellular function in HCC carcinogenesis especially pertaining to its genetic interaction with HBx can be studied in animal models and clinical specimens. In summery, we propose to use Drosophila rough eye phenotype to conduct a non-biased, saturate genetic screen, which is not yet possible in mice, looking for fundamental pathways that are regulated by HBx. The results should provide new insights to HCC carcinogenesis.