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）

1. Lee, T. Y., Wu, H. C*., Tseng, Y. L., Lin, C. T., "A novel peptide specifically binding to nasopharyngeal carcinoma for targeted drug delivery", *Cancer Research*(2004), volume 64, p.8002-p.8008.
2. Liu, I. J., Hsueh, P. R., Lin, C. T., Chiu, C. Y., Kao, C. L., Liao, M. Y., Wu, H. C.*, "Disease-specific B cell epitopes for serum antibodies from patients with severe acute respiratory syndrome (SARS) and serologic detection of SARS antibodies by epitope-based peptide antigens", *Journal of Infectious Diseases*(2004), volume 190, p.797-p.809.
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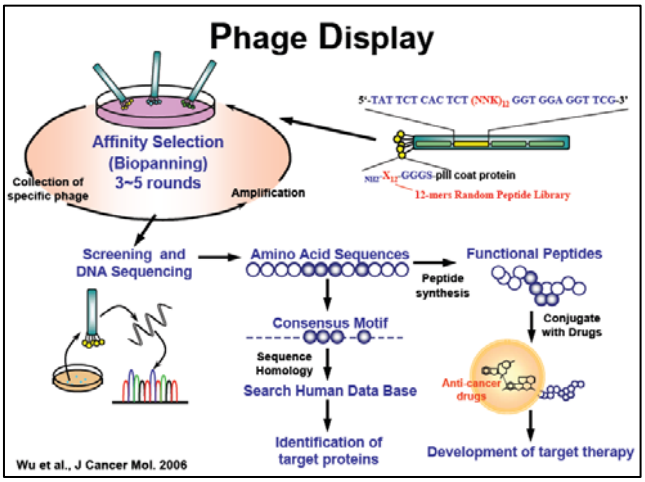
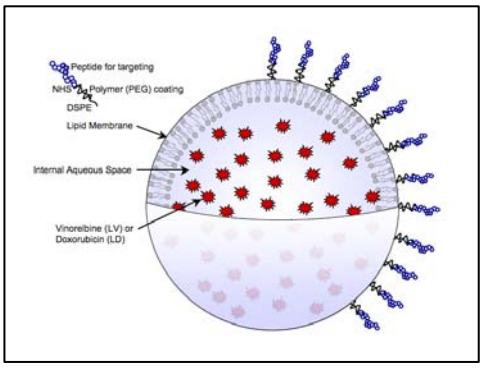
得獎著作簡介: （2000 字左右）

醫學界對於癌症的治療，至今仍然存在著很大的瓶頸，最主要的原因除了癌細胞會產生抗藥性之外，還有藥物不易達到壓力較高的腫瘤組織與腫瘤幹細胞等問題。為了避免抗癌藥物造成正常組織毒性而產生的副作用，常常只能給予次適當濃度的抗癌藥物，因此伴隨著抗藥性以及癌細胞轉移的發生，終究造成治療的失敗。為了解決這些問題，本實驗室發展出癌症配體標的療法(ligand-targeted therapy)。此法可將抗癌藥物大量累積在癌組織，有效地殺死癌細胞，並減低藥物的副作用，有希望發展成為新的癌症療法。

化學療法仍是現今治療癌症的主流，但這些藥物並不具腫瘤專一性，因而產生全身毒性。標的配體能自動導向癌細胞或腫瘤組織。當具辨識能力的標的配體與抗癌藥物連接後，便能將高劑量的化學藥物專一的帶至腫瘤組織。配體標的療法可以讓癌細胞暴露於局部高劑量的抗癌藥物中，進而被更有效的毒殺。於是，不完全的腫瘤毒殺效果所引起的癌症復發機會因而降低。再者，透過標的配體來提升藥物與腫瘤組織的親和力，將足夠的抗癌藥物傳送到腫瘤組織，便可克服癌症治療的一大障礙—腫瘤的高組織間質液壓(IFP)之問題。要發展配體標的療法，首先必須尋找有效的專一性配體。噬菌體顯現法，是一種將蛋白質或胜肽表現於噬菌體外套蛋白的技術。經由噬菌體所表現出來的隨機胜肽庫便可作為研究蛋白質間交互作用、篩選結合至受體或蛋白質的生物活性胜肽、尋找模擬特定疾病抗原，以及測定細胞或器官的專一性標的胜肽等。近年來，我們發展此技術以尋找表現於癌細胞以及腫瘤血管上的特殊受體(Cancer Research 2004 and 2007)。尋找腫瘤標的配體及研發標的治療策略如圖一所示。



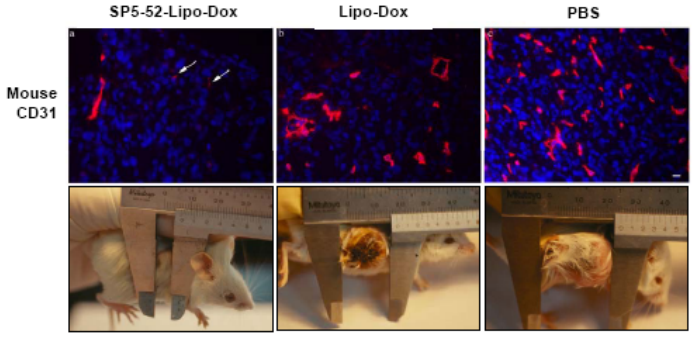
一旦我們尋獲癌細胞的標的配體後，便可以將此標的胜肽連接化學藥物，將抗癌藥物專一性地帶至腫瘤組織。許多針對抗癌藥物的奈米顆粒傳送系統已經進入了臨床試驗，並且有效的改善藥物動力學與藥理學而增進抗癌效能。微脂體是較先進的奈米藥物攜帶者形式，目前也使用於癌症臨床治療。因為微脂體具有下列特點：一、於循環系統中具長效性，二、具有累積於腫瘤內的能力，三、掌控藥物的釋放並能被癌細胞攝入，四、可以減少全身性的散佈、降低非專一性的毒性。藥物傳遞研究領域已經成功地製造出長效型的微脂體，且聚集於腫瘤組織並釋放包含於其中的藥物，此稱為被動擴散。被動式標的微脂體相較於小分子游離藥物(small molecular free drug)，能增加數倍藥物濃度於腫瘤組織內。因此，微脂體的藥物比一般的抗癌藥物具有更佳的療效。所以，我們選擇微脂體包裹抗癌藥物來連接標的胜肽，做成標的微脂體(targeting liposome)。我們做成的標的微脂體直徑範圍約 65-75 奈米，如圖二所示。然而，腫瘤的血管會有將近 100-600 奈米的破洞；而此裂縫使得微脂體得以從血管內進入腫瘤組織，卻不會經由血管進入無破洞的正常組織。標的微脂體藉著腫瘤標的配體專一性地將之攜帶至腫瘤處，大大地提高藥物的抗癌能力(Cancer Research 2004 and 2007; Mol Cancer Ther 2008)。



本實驗室採用 *in vitro* phage display 的方法，成功地找到可與鼻咽癌細胞專一性結合的標的胜肽，再運用此胜肽攜帶抗癌藥物進行標的治療。此治療方式在動物實驗證明有明顯的治療效果及減低副作用等優點(Cancer Research

2004)，已分別於 2006 年獲得台灣專利(Patent# I262192;專利期限:Jan. 01, 2006~Jun. 30, 2023)及 2007 年獲得美國專利(Patent# US 7,238,665;專利期限: Jul 03, 2007 ~ Mar 09, 2024)。Lancet Oncology (6: 6, 2005) 期刊也對此發現給予極高的評價，認為這些研究成果非常創新，未來對於癌症的治療及藥物運送，極有臨床潛力。

本實驗室也運用 *in vivo* phage display 的方法，找到數個可以與腫瘤組織或新生血管結合之標的胜肽，利用這些胜肽可將螢光物質及抗癌藥物專一性地帶至腫瘤組織及腫瘤的新生血管。此標的胜肽也被證明可與肺癌檢體中的血管專一性結合。本實驗室也更進一步證明帶有標的胜肽的抗癌藥物，具有極佳的療效且明顯降低藥物的副作用(Cancer Research 2007)，此研究成果已申請國際專利(US patent 2007; TW patent 2007; PCT patent 2008)。此專利已授權給生技公司進行前臨床測試。在我們的研究中，將我們所發現之標的胜肽做成標的微脂體，在動物模式實驗中，此配體標的療法明顯增進抗癌藥物對肺癌、腸癌、乳癌、肝癌、胰臟癌及口腔癌等癌症的治療效果，也降低了藥物的副作用(US patent 2007; TW patent 2007; PCT patent 2008)。此標的微脂體有效地抑制腫瘤血管的新生、明顯抑制腫瘤的生長及大幅提升其存活率(圖三；Cancer Research 2007)。此治療方式預期將會增進癌症病人的生活品質並有效的對抗癌症。



近年來，本實驗室也建立了從複雜的血清中，快速找出與疾病有關的抗原決定位(disease-specific epitopes)或結合配體。運用此策略已經研究出登革熱及嚴重急性呼吸道症候群(SARS)等感染病之抗原決定位。藉由數個抗原決定位證明 SARS 是由 SARS-CoV 感染，並發展出 SARS epitope-based 的偵測試劑。此研究成果已發表於重要的感染病研究期刊(Journal of Infectious Diseases 2004)，並於 2005 年通過專利審查(Patent# 225929;專利期限:Jan. 01, 2005~ May 08, 2023)。目前也研究出數個 spike protein 的抗原決定位，聯合這些抗原決定位當偵檢試劑，其靈敏度及專一性皆高達 95%(US Patent # 10/945,648)。這些抗原決定位對於 SARS 的致病機轉、偵檢試劑及疫苗研發提供重要資訊。

評審簡評：

吳漢忠博士實驗室近五年發展癌症標的治療法(ligand-target therapy)，此法採用 in vitro 或 in vivo phage display 的方法，找到可與鼻咽癌細胞專一性結合的 peptide ligand 或找到可以與肺癌腫瘤組織新生血管結合的 target peptide，利用這些 peptide 可將螢光物質及抗癌藥物專一性地帶至腫瘤組織及腫瘤的新生血管。此治療方式在動物實驗被証明有明顯的治療效果及降低藥物的副作用特性。這些研究成果具創新性，對癌症的治療及藥物的運送，極有潛力。已獲得台灣及美國專利，發表於重要的癌症研究期刊(Cancer Research 2004, 2007)。另外吳博士實驗室也建立由血清中，快速找出疾病有關的抗原決定區(disease-specific epitope)或 ligand。他運用此策略找出 SARS 感染症之 B-cell epitope。此結果已發表於感染病研究期刊(J. Infection Disease, 2004)，並獲得專利。這些抗原決定位對 SARS 的致病機轉、偵測或疫苗研發有助益。

2008 Academia Sinica Research Award for Junior Research Investigators

Name: Han-Chung Wu 	Education: Ph.D. Institute of Pathology, College of Medicine, National Taiwan University Employer(s)/Job Title(s): Institute of Cellular and Organismic Biology, Academia Sinica/ Associate Research Fellow (2005/09 ~ present) Graduate Institute of Oral Biology, College of Medicine, National Taiwan University/Adjunct Associate Professor (2005/02 ~ present) Graduate Institute of Oral Biology, College of Medicine, National Taiwan University/Assistant Professor (2001/08 ~ 2005/02)

Award publications：

1. Lee, T. Y., Wu, H. C*., Tseng, Y. L., Lin, C. T., "A novel peptide specifically binding to nasopharyngeal carcinoma for targeted drug delivery", *Cancer Research*(2004), volume 64, p.8002-p.8008.
2. Liu, I. J., Hsueh, P. R., Lin, C. T., Chiu, C. Y., Kao, C. L., Liao, M. Y., Wu, H. C.*, "Disease-specific B cell epitopes for serum antibodies from patients with severe acute respiratory syndrome (SARS) and serologic detection of SARS antibodies by epitope-based peptide antigens", *Journal of Infectious Diseases*(2004), volume 190, p.797-p.809.
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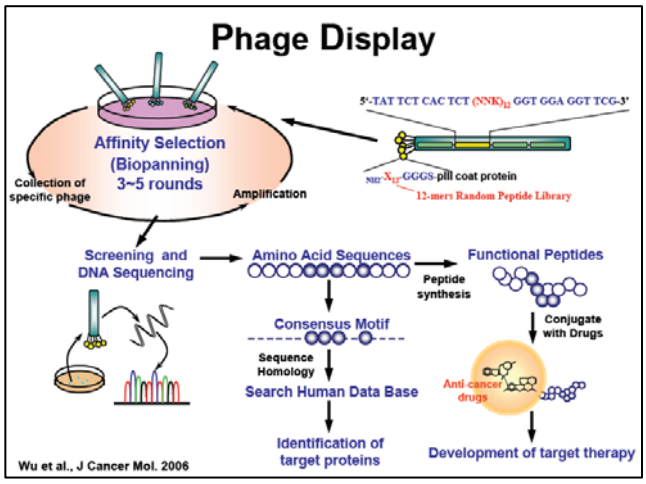
Summary of the Award publications（around 2000 words）：

Advances of medical science in the treatment of cancer have reached a bottle neck. The most important reasons for this are drug resistance, the difficulty of drug penetration in the high interstitial fluid pressure (IFP) of the solid tumors, and cancer stem cells. Trying to avoid the side effects that occur as a result of toxicities to normal tissues, we often give sub-optimal doses of anticancer chemotherapeutics, resulting in the eventual failure of therapy, which is often accompanied by the development of drug resistance and metastatic disease. Wishing to resolve these problems, our lab is

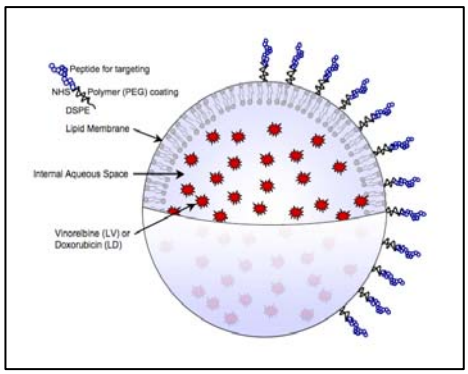
developing ligand-targeted therapy for cancer. This strategy increases the therapeutic efficacy of cancer treatment by increasing the drug accumulation in the tumor tissue which reduces the drugs' side effects on normal tissues. Ligand-targeted therapy makes possible tumor specificity and limited toxicity and shows promise in the development of novel therapies for cancer.

Traditional chemotherapy became one of the pillars for the treatment of cancer. These drugs are more prone systemically toxic normal cells because they are not tumor specific. Most cancer cells share many common features with the normal host cells from which they are derived. Therefore, high levels of selective toxicity cannot be achieved with anticancer chemotherapeutics because of the lack of unique molecular targets that would distinguish them from normal cells. When the recognized ligands link with cytotoxic reagents, they could bring sufficient chemical drugs to tumor mass. In this way, tumor cells can be exposed to abundant cytotoxic drugs and be killed. Ligand-targeted therapy may not only improve the therapeutic effectiveness of cancer treatment, it also allows avoiding the problem of toxicity to normal tissue. Accordingly, the phenomenon of incomplete tumor response, early disease relapse, and ultimately, the development of drug resistance stemming from suboptimal doses will be reduced. Furthermore, delivery of chemotherapeutic drugs to tumor tissue by affinity of targeting ligand may overcome an obstacle in cancer therapy caused by high tumor IFP and improve anticancer drug efficacy. Phage display, a selection technique in which a peptide or protein is expressed as a fusion with a coat protein of bacteriophage, results in a display of the fusion peptide or protein on the surface of the virion. Recently, we have developed phage display methods to identify the receptors expressed specifically on cancer cells and tumor vessels (Cancer Research 2004 and 2007). The strategy for identification of tumor-targeted ligand and development of ligand-targeted therapy is shown in Fig. 1.



Many nanopartical delivery systems for anticancer drugs have entered the clinic and have been shown to have improved anticancer effects because they can improve the pharmacokinetics and pharmacodynamics of their associated drug. Liposomes are the most advanced form of particulate drug carriers. The drug delivery research field has successfully constructed long circulating liposomes that accumulate in tumor

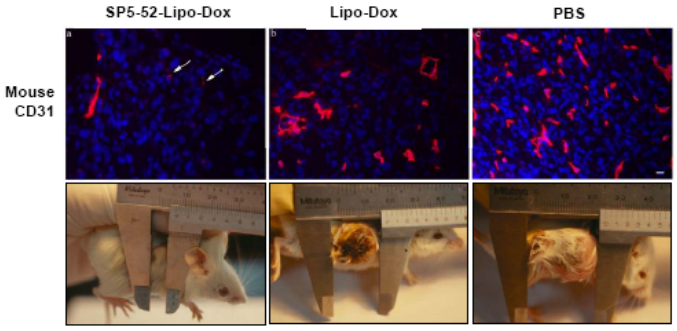
tissue where the entrapped drugs then have to leak out of the liposomes by “passive diffusion.” Passive targeting can result in increases in drug concentrations in solid tumors of several-fold relative to those obtained with free drugs. It is thought that the mechanism of action of the liposomal drugs is due to sustained release of drug from the liposomes and diffusion of the released drug throughout the tumor interstitial fluid, with subsequent uptake of the released drug by tumor cells. The range of diameters in targeting liposomes is approximately 65-75 nm (Fig. 2). However, because tumor vessels lack tight junctions between adjacent vasculature endothelial cells, the size of the gaps between the cells that line tumor blood vessels has been estimated to be 100-600 nm, which is large enough to allow the extravasation of most liposomes from the vessel into the tumor interstitial space through these pores. The targeting liposomes were found to have an enhanced anti-tumor effect and to decrease the side effect (Cancer Research 2004 and 2007; Molecular Cancer Therapeutics 2008).



Recently, we have developed methods of identifying the targeting ligand specifically bound to nasopharyngeal carcinoma (NPC) cells. In an effort to develop ligand-targeted therapy, we used peptide-linked liposomes that carried doxorubicin to treat SCID mice bearing human cancers. The targeting liposomes were found to have an enhanced anti-tumor effect and to have significant clinical potential in a targeted drug delivery system (Cancer Research 2004). This novel research was acclaimed by Lancet Oncology (6: 6, 2005) and patents were granted in 2006 and 2007 in Taiwan and the USA, respectively.

Using *in vivo* phage display, we have identified several ligands able to target tumor vessels of solid tumors. Targeting peptides recognized the neovasculature of multiple tumors in SCID mice, but did not target normal blood vessels. Using these peptide-functionalised liposomes for targeted therapy was found to have excellent therapeutic effects (Cancer Research 2007). Interestingly, the targeting liposome increased therapeutic efficacy to each of these six human cancers including human lung, colon, breast, liver, pancreatic and oral cancer xenografts (US patent 2007; Taiwan patent 2007; PCT patent 2008). This targeting liposome was also found to markedly inhibit the angiogenesis of tumors and have an enhanced anti-tumor effect (Fig. 3). The current study indicates that ligand-targeted therapy offers improved therapeutic effects over conventional anticancer drug therapy (Cancer Research 2007). Peptide-mediated liposomes that target tumor cells and vasculature are a new generation of chemotherapy delivery systems with superior pharmacokinetics,

controlled biodistribution, efficacy, and safety profiles. Academia Sinica has licensing-out these results to a biotechnology company for preclinical try.



We have setup a platform to quickly select disease-specific antigen mimics from serum samples of human patients. Using this technique, we identified several B-cell epitopes of SARS which corresponded to novel coronavirus (SARS-CoV) and developed a highly sensitive, specific and convenient serologic diagnostic reagent for SARS. Our results contributed to the development of diagnostic reagents and to our understanding of the pathogenesis of SARS (Journal of Infectious Diseases 2004). These results have led to a patent (Patent# 225929, 2005). For further identification of neutralizing epitopes, sixty-five immunopositive phage clones that bound specifically to other SARS patient serum samples were selected. These phage-borne peptides had five consensus motifs, including two which corresponded to the amino acid sequences reported for the Spike protein of SARS-CoV (US Patent # 10/945,648). By combining the immunopositive phage clones and synthetic peptide, we were able to obtain 95% diagnostic sensitivity and specificity for detecting infected serum from SARS patients (US Patent # 10/945,648).