In vitro developments of early diagnosis by radioactive isotope labeled targeting peptides

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摘要

肺癌主要分成小細胞肺癌(small cell lung cancer)與非小細胞肺癌(non-small cell lung cancer)兩類。非小細胞肺癌可再分為肺腺癌、鱗狀上皮癌與大細胞肺癌。小細胞肺癌約佔肺癌病例的 15%，肺腺癌佔的比例最高，約 50-55%。治療肺癌目前以化學藥物治療同時併用放射治療，治療療程大約 4-6 個月，而化學藥物治療的缺點是正常細胞的損傷，造成病患的免疫力下降，還會增加感染機會。肺癌的初期症狀不明顯，約有三分之一的病患被發現時已是肺癌第三期，治療難度高、存活率低。因此，肺癌之早期檢測與標靶治療非常重要。為了解決上述缺點，具有辨認癌細胞之高度專一性的材料是治療與檢測的關鍵。

目前沈正煌醫師的研究團隊已利用 phage display 的方式，篩檢出兩條具有高度專一性的標靶胜肽，分別辨認非小細胞肺癌與小細胞肺癌。計畫目標是利用此標靶胜肽與現行抗癌藥物合成出胜肽藥物複合體(peptide-drug conjugate)，以及委託國衛院與核研所於標靶胜肽標幟放射性同位素（I-125、Ga-68、In-111），以作為癌症之早期檢測與放射性治療。我的任務是找出這兩條標靶胜肽分別在非小細胞肺癌細胞(A549 與 H520)與小細胞肺癌(H146)所標靶的對象。

我們先將標靶胜肽標記螢光，利用螢光影像辨認標靶胜肽於細胞株中所處的位胞器。之後再將該胞器之蛋白質從細胞中分離出來。同時，利用大腸桿菌大量表達連接螢光蛋白之標靶胜肽，進行 pull-down assay 以捕捉標靶胜肽所結合之蛋白質，再經質譜定序，辨認該蛋白質之身份。實驗室同仁所合成出的胜肽藥物複合體是否仍可辨認標靶對象，有待檢測。因應同位素標幟，標靶胜肽修飾得先做修飾。碘同位素可利用 tyrosine 標幟，但這兩條標靶胜肽都沒有 tyrosine，額外鍵結 tyrosine 後的辨識效果仍待測試。過渡金屬同位素的標幟可透過螯合劑，鍵結螯合劑後的辨識效果也有待測試。我期待這些工作進行順利，成果有助於肺癌治療與早期檢測。

Abstract

Lung cancer is broadly categorized into two groups: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). NSCLC can be further divided into adenocarcinoma, squamous cell carcinoma, and large cell carcinoma. SCLC represents around 15% of lung cancer cases, with adenocarcinoma being the most prevalent subtype, accounting for approximately 50-55% of cases. Typically, lung cancer treatment combines chemotherapy and radiation therapy over a span of 4-6 months. However, chemotherapy has drawbacks such as harming healthy cells, weakening the patient's immune system, and elevating infection risks. Early-stage lung cancer symptoms tend to be subtle, resulting in around one-third of patients receiving a late-stage diagnosis. This poses treatment challenges and lowers survival rates, underscoring the importance of early detection and targeted therapy in managing lung cancer. To contest these issues, materials with exceptional specificity in identifying cancer cells are crucial for both treatment and detection.

Dr. Cheng-Huang Shen's research team has recently discovered two extremely precise targeting peptides through phage display. These peptides effectively differentiate between NSCLC and SCLC. Our collaborative project aims to synthesize peptide-drug conjugates (PDCs) by integrating these targeting peptides with established anticancer drugs. Furthermore, we intend to collaborate with the National Health Research Institutes and the Institute of Nuclear Energy Research to tag the targeting peptides with radioactive isotopes (^{125}I, ^{68}Ga, ^{111}In) to enhance early cancer detection and facilitate radiotherapy.

My task is to identify the targets of these two targeting peptides in NSCLC cell lines (A549 and H520) and SCLC (H146). The target peptides will be labeled with fluorescence for subcellular location determination and protein isolation. Additionally, we will express peptides with fluorescent proteins in *Escherichia coli* for protein capture via pull-down assays and mass spectrometry identification. Further verification is needed for the synthesized peptide-drug conjugates target recognition. For isotope labeling, modifications are required as the peptides lack tyrosine. We will test the efficacy of additional tyrosine linkage for iodine isotopes and assess recognition after chelator binding for transition metal isotopes. Successful completion of these tasks will contribute to the advancement of lung cancer treatment and early detection.