連續性造影技術監測第一型泡疹病毒胸腺嘧啶激酶;基因轉導的老鼠肉瘤細胞在活體內之標的轉移

Serial in vivo imaging of the targeted migration of HSV1-tk gene transduced murine sarcoma

中文摘要

以非侵入性造影追蹤活體內的基因表現，可說是一種有效監測基因治療的方法。第一型泡疹病毒胸腺嘧啶激酶基因(HSV1-tk)被廣泛使用在基因表現之造影，原因是它不但可當作報導基因(reporter gene)；同時也可當作具有治療性的”自殺基因”(suicide gene)，應用在癌症的基因治療上。許多放射性標幟的核甘類似物(nucleoside analogue)可當作 HSV1-thymidine kinase 的受質探針，藉由被磷酸化而滯留在細胞內。運用核子分子造影技術，除了可依據 HSV1-tk 基因轉導之細胞累積受質探針的程度來反應 HSV1-tk 基因表現之外，也能以受質探針為指標來追蹤細胞在活體內的位置及移動。

本研究的主要目的是使用非侵入性造影方式，監測具轉移性(metast-atic)的老鼠肉瘤細胞在活體內的位置、移動及存活，來建立一個穩定的肺部腫瘤模式，以應用在癌症基因治療的研究上。我們先確定所使用的 HSV1-tk 基因轉導肉瘤細胞株 YD-SML-TK，在體外可以選擇性地累積放射性標幟的類核甘藥物[131I]FIAU，並且在攝取[131I]FIAU 後仍然可維持其存活。藉由讓 YD-SML-TK 細胞在體外及體內攝取[131I]FIAU 的方式，我們使用 SPECT 進行平面伽瑪攝影(planar gamma camera imaging)來追蹤細胞在 FVB/N 小鼠體內的轉移。結果顯示，攝取[131I]FIAU 後的 YD-SML-TK 仍保有其轉移至肺部的能力。在 RT-PCR 的分析上，也顯示出 YD-SML-TK 細胞在肺部形成腫瘤後還繼續進行 HSV1-tk 基因的表現。此外，每天從腹腔給予前驅藥物 GCV 治療的老鼠，從平面伽瑪攝影的影像可看出，肺部腫瘤在第 7 天有明顯的消退。

英文摘要

Noninvasive imaging of gene expression is a useful method for monitoring gene therapy in vivo. Herpes simplex virus type 1 thymidine kinase gene (HSV1-tk) is widely used as a reporter gene for imaging gene expression as well as a therapeutic “suicide gene” for cancer gene therapy. Many radiolabed nucleoside analogues are specific substrate of HSV1-TK and, upon entering the cell, can be phosphorylated by HSV-1-thymidine kinase, resulting in the intracellular retention of the radiolabeled products. Thus, accumulation of the phosporylated nucleotides would reflect HSV1-tk gene expression and can be determined by nuclear molecular imaging technologies for localizing and tracking these cells in the living subjects.

In this research I have taken the experimental approach of noninvasive imaging to monitor the location, migration, and survival of metastatic murine sarcoma in living mice for the establishment of a stable lung tumor model of cancer gene therapy. First I

demonstrated HSV1-tk transduced YD-SML-TK sarcoma cells incubated with [131I]FIAU in vitro could selectively accumlate this radiolabeled substrate with minimal effect on their viability. Also, YD-SML-TK cells labeled with [131I]FIAU in vitro or in vivo could be tracked in FVB/N mice by serial planar gamma camera imaging. Future more, radiolabed YD-SML-TK cells still retained their capability of metastasis to the lungs. Expression of the HSV-tk gene in YD-SML-TK cells-induced lung tumor could be demonstrated by RT-PCR analysis. Planar gamma camera images showed regression of YD-SML-TK lung tumor at day 7 of consecutive daily treatment with the prodrug ganciclovir.