建立反轉錄病毒感染小鼠初級 **T** 細胞的基因轉殖系統

Establishment of Retrovirus Transduction in Murine Primary T Lymphocytes

中文摘要

反轉錄病毒，目前普遍被應用作爲哺乳類動物細胞基因轉殖的工具。與一般轉染（transfection）不同的是，反轉錄病毒所攜帶的基因直接嵌入標的細胞的染色體中表達，而非短暫存在於細胞中。本篇論文主要建立反轉錄病毒感染小鼠初級 T 細胞（primary T cell）的基因轉殖系統。第一階段，生產高效價（high titer）之反轉錄病毒。我們利用 PhoenixE 包裝細胞株(package cell line)提供病毒結構 gag/pol、env，再以 pBMN-EGFP 或 pGC-YFP 兩個攜帶病毒包裝訊息（y）並會表達螢光蛋白的病毒載體 DNA，經轉染法生產病毒。將所生產之病毒感染 3T3 細胞後，以流式細胞儀測定感染率（即細胞表達螢光蛋白之比率），再依其感染率計算病毒效價。實驗結果顯示，轉染時放入 Chloroquine 可提高病毒產量，而以離心方式感染 3T3 則可提高病毒對 3T3 的感染率。此外，病毒冷凍儲存 4 個月內效價並未下降。仍可穩定地製造出效價達到 1x106 unit/ml 的病毒。

第二階段，以初級 T 細胞爲感染對象，分別使用三種 T 細胞進行實驗。一. 小鼠脾臟細胞經過 PMA&ionomycin 活化後，以反轉錄病毒感染，可得到 22%的細胞表達黃色螢光蛋白。二. 我們利用過去實驗室所建立小鼠腸道表皮間隙 gdT 細胞的活化系統，以抗 gd 型 T 細胞受器抗體活化小鼠腸道表皮間隙 gdT 細胞，不同活化時間點給予病毒感染，以流式細胞儀偵測黃色螢光蛋白質，發現在活化的同時（0 小時）感染細胞，可以得到最佳的感染率爲 10%。三. 來自脾臟及淋巴結的 CD8 細胞，經過抗 b 型 T 細胞受器抗體和抗 CD28 抗體活化，24 小時後，以反轉錄病毒感染，得到 15%感染率。

未來，將會利用已建立之反轉錄病毒傳導系統，把有興趣的基因放入小鼠腸道表皮間隙 gdT 細胞和 CD8 細胞，研究這些基因對於細胞活化及存活的影響。

英文摘要

Retroviral transduction is a technique for introducing genes of interest into mammalian cells. The genes introduced this way can integrate into host chromosome and express stably. Moreover, retrovirus can transduce primary cells that are difficult to receive genes delivered by conventional methods. My project has aimed to establish retroviral transduction of murine primary lymphocytes. The first part of my work was to produce high titer recombinant retrovirus. PhoenixE cell line carrying plasmids encoding retroviral core(gag)/polymerase(pol) or envelope(env) genes was used to produce recombinant virus after transfecting viral vector which contains retroviral packaging signal(y) and the gene of insterest. Two viral vectors,

pBMN-EGFP and pGC-YFP, were used to transfect the packaging cell line. I determined the optimal amounts of plasmid DNA and chloroquine for CaPO4 transfection. Then, I tested conditions for spin infection. Finally, I could produce the recombinant retrovirus with a titer around 1x106 unit/ml.

The second part of my work was using the retrovirus to transduce murine lymphocytes. Three types of murine lymphocytes were used. The first cell type was total spleen cells activated by PMA&ionophore. Twenty-two percent of activated spleenocytes were transduced as determined by YFP expression. The second cell type was TCRgd+ intestinal intraepithelial lymphocytes (gdiIEL) activated by anti-TCRgd Ab. Ten percent of gd-iIEL expressing YFP during primary activation were transduced. The third cell type was spleen and lymph node CD8 cells activated with immobilized anti-TCRb Ab&anti-CD28 Ab. After retrovirus transduction, fifteen percent of activated CD8 cells expressed YFP.

The established experimental conditions will allow us to introduce gene of interest into gdiIEL and CD8 cells to study gene function.