In vitro evaluation of herpes simplex virus type 1 thymidine kinase reporter system in dynamic studies of transcriptional gene regulation.

鄧文炳
Hsieh CH;Liu RS;Wang HE;Hwang JJ;Deng WP;Chen JC;Chen FD

Abstract

The herpes simplex virus type 1 thymidine kinase (HSV1-TK) reporter system is being used to directly and indirectly monitor therapeutic gene expression, immune cell trafficking and protein-protein interactions in various living animals. However, the issues of HSV1-TK enzyme stability in living cells and whether this reporter system is optimal for dynamic studies of gene expression events in genetic imaging have not be addressed. The purpose of the present study was to evaluate the application of this reporter system in dynamic studies of transcriptional gene regulation. To achieve this purpose, we established two tetracycline-inducible murine sarcoma cell lines, tetracycline-turn-off HSV1-tk-expressing cell line (NG4TL4/tet-off-HSV1-tk) and tetracycline-turn-off Luc-expressing cell line (NG4TL4/tet-off-Luc), to create an artificially regulated gene expression model in vitro. The dynamic transcriptional events mediating a series of doxycycline (Dox) inductions were monitored by HSV1-TK or by the firefly luciferase reporter gene using HSV1-TK enzyme activity assay and luciferase assay, respectively. The results of dynamic gene expression studies showed that the luciferase gene is an optimal reporter gene for monitoring short-timescale, dynamic transcriptional events mediating a series of Dox inductions, whereas the HSV1-tk is not optimal to achieve this purpose. Furthermore, the enzyme half-life of HSV1-TK in NG4TL4 cells is about 35 h after cycloheximide-induced protein inhibition. On the other hand, the results of an efflux assay of [(131)I] FIAU and [(3)H] GCV revealed that the molecular probe phosphorylated by HSV1-TK can be trapped long term within HSV1-TK stably transformed cells. Therefore, a long half-life radionuclide is not suitable for dynamic gene expression studies. Based on these results, we suggest that the HSV1-TK reporter system is not optimal for monitoring short-timescale dynamic processes such as kinetic gene expression controlled by inducible promoters or a less stable protein with a more rapid turnover due to the limitations of the half-life of the HSV1-TK enzyme and the cellular retention time of their phosphorylated molecular probes.