Suicidal gene therapy with rabbit cytochrome P450 4B1/4-ipomeanol, 2-aminoanthracene system in glioma cell

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1. Introduction

Suicidal gene therapy is based on the transduction of tumor cells with "suicide" genes encoding for prodrug-activating enzymes that render target cells susceptible to prodrug treatment [1]. Suicidal gene therapy results in the death of tumor with the expression of gene encoding enzyme that converts non-toxic prodrug into cytotoxic product. Cytochrome P450 4B1 (CYP4B1) activates 4-ipomeanol (4-ipo) and 2-aminoanthracene (2-AA) to cytotoxic furane epoxide and unsaturated dialdehyde intermediate. In this study, therapeutic effects of suicidal gene therapy with rabbit CYP4B1/4-ipo or CYP4B1/2-AA system.

2. Methods and Results

2.1 Establishment of C6-fLuc-CYP4B1 (C6-CL)

Rat glioma cell line C6 was purchased from the American Type Culture Collection (ATCC). One day prior to infection with lentiviral vector, C6 cells were seeded in 6-well plate at a density of 5×10^5 cells/well. Lentiviral vector infections were exposed 6 h by using polybrene. Stable cell line was selected by treatment of blasticidin(5 ㎍/㎖) for 1week. Then, high fLuc activity selected well for experiment (C6-L). pcDNA3.1/Hygro vector expressing cytochrome P450 4B1 under CMV promoter was transfected into C6-L cells and Hygromycin (500 ㎍/㎖) selection (C6-CL)

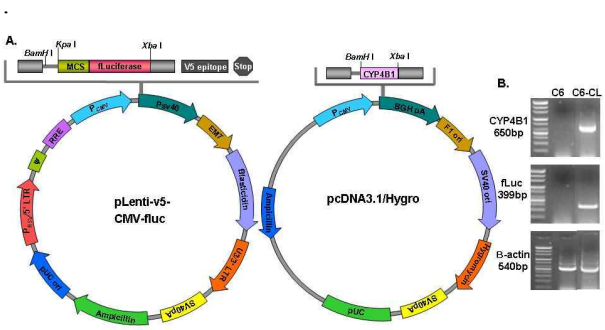

Fig 1. A. Lentiviral vector and CYP4B1 expressed vector construct B. To determine the gene expression of CYP4B1 and fLuc, C6-CL cells were analyzed with RT-PCR.

2.2 Prodrug induced cytotoxicity

C6-CL cells were seeded into 96-well plates in triplicate at a density of 5×10^3 cells/well. After 24 h, cell culture media containing various concentrations of 4-ipo or 2-AA ranging from 0 to 1 mM or 0 to 0.1 mM were added and incubated for 96h or 72h. The number of surviving cells was determined by MTT assay kit. The color development was measured and analyzed by ELISA reader with dual filter (595 nm and 655 nm). C6-CL cells were seeded into 6-well plates in triplicate at a density of 1×10^5 cells/well. After 24 h, cell culture media with various concentrations of 4-ipo or 2-AA ranging from 0 to 1 mM or 0 to 0.1 mM were added and incubated for 96h or 72h. The number of surviving cells was determined by cell count. Trypan-blue solution was mixed with cells, adding 20 ㎕ of trypan-blue solution to 20 ㎕ of cell suspension. The suspension was loaded into a C-Chip and scored with a microscope. MTT assay and trypan-blue dye exclusion showed that IC_{50} of C6-CL was 0.3 mM and < 0.01 mM after 4-ipo or 2-AA treatment at 96hrs or 72hrs exposure, respectively. Cell survivals of C6-CL were more rapidly reduced after treatment of 4-ipo or 2-AA than those of C6-L cells (Fig 2).

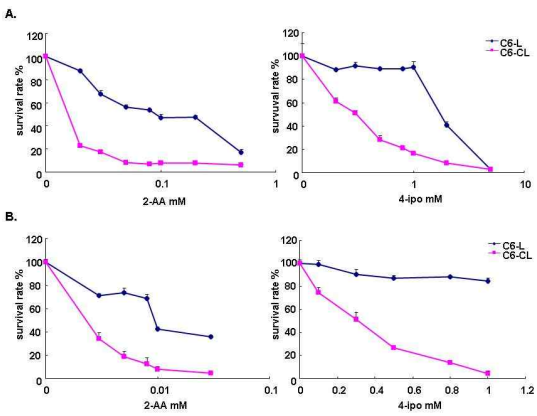

Fig 2. A. MTT assay. B. Trypan-blue dye exclusion assay. The survival rates of C6-CL cell was more rapidly decreased with increasing 4-ipo or 2-AA dose than of C6-L.

2. 3 Luciferase activity assay

C6-CL cells seeded into 24-well plates in duplication at the concentration of 5×10^4 cells/well. After 24h incubation, cell culture media containing various concentrations of 4-ipo ranging from 0 to 1 mM were added and incubated for 96 h. A luciferase assay kit

used for the evaluation of luciferase activities by using luminometer. The dose-dependent reduction in luciferase activity in C6-CL cells was observed treated with 4-ipo (Fig 3).

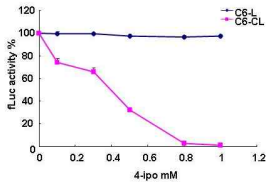

Fig 3. Luciferase assay. Dose-dependent reduced luciferase activity in C6-CL cells.

2.4 Cellular uptake of [^{3}H]-ipomeanol

C6-CL cells were seeded in 6-well plates in triplicate at a density of 5×10^5 cells/well 1 day prior to a test. The cells were incubated for 24 h, and incubated with [^{3}H] ipomeanol, to a concentration of 1μCi/well for an additional 30, 60, 180, 360, 720 and 1,440 min. After incubation, the supernatants were removed and rinsed with cold PBS at once, the adherent cells were stripped with 0.2% SDS. Cells were harvested in plastic counter tube and 10 ㎖ of scintillation cocktail was added. The CYP4B1 expression level was determined by beta counting of [^{3}H]-ipomeanol for 1 min. As shown in Fig 4, [^{3}H]-ipomeanol uptake of C6-CL was higher than C6-L according to increasing incubation time.

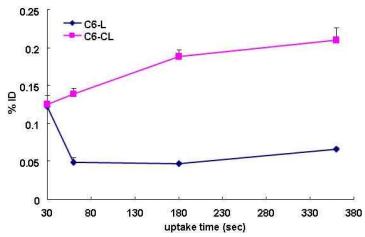

Fig 4. C6-CL cells showed higher [^{3}H]-ipomeanol uptake than C6-L cells at all time points.

3. Conclusions

The use of rabbit CYP4B1 as a prodrug-activating enzyme for gene therapy of malignant glioma has been reported recently [2]. We demonstrated that the CYP4B1/4-ipo or 2-AA for prodrug-activating system effectively induce cytotoxicity of glioma cells at low prodrug concentration. To further evaluate the potential of CYP4B1/4-ipo or 2-AA for prodrug-activating gene therapy of glioma, its therapeutic efficiency monitored in vivo as well as in vitro by bioluminescence, when fLuc gene was used as a bioluminescence reporter gene. .

REFERENCES

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