Transactions of the Korean Nuclear Society Spring Meeting
Jeju, Korea, May 10-11, 2007

Gene Directed Enzyme Prodrug Therapy Using Rabbit Cytochrome P450 4B1 in Murine Colon Adenocarcinoma

Sung Joo Kim[a], Joo Hyun Kang[a], Tae Sup Lee[a], Kyeong Min Kim[a], Kwang Sun Woo[a], Wee Sup Chung[a], Gi Jeong Cheon[a,b], Chang Woon Choi[a, b], Sang Moo Lim[a, b]
[a]*Laboratory of Nuclear Medicine,* [b] *Department of Nuclear Medicine, Korea Institute of Radiological and Medical Sciences(KIRAMS), Seoul, Korea*

1. Introduction

The conventional cancer therapy is chemotherapy, surgical resection and/or radiotherapy. Chemotherapy using cytotoxic drug has some problems with lack of tumor selectivity resulting in toxicity to normal tissues. To enhance the tumor selectivity of cytotoxic drug, the application of suicidal gene therapy technology was designed. Suicidal gene therapy is based on the expression in tumor cells of a gene encoding an enzyme that converts a non-toxic prodrug into a cytotoxic product. Representative suicidal genes are Herpes simplex virus type 1 thymidine kinase (HSV1-tk) and cytosine deaminase (cd). Recently, a new prodrug-converting enzyme based on rabbit cytochrome P450 4B1 gene (cyp4B1) has been reported for therapy of experimental brain tumor. This enzyme activates the prodrugs such as 4-ipomeanol (4-IM) and 2-aminoanthracene (2-AA) to highly reactive furane epoxide and unsaturated dialdehyde intermediate, respectively. DNA alkylation seems to be the main mechanism of cytotoxicity of these activated drugs.

In this study, we isolated cyp4B1 cDNA from rabbit lung, transduced cyp4B1 expression vector into murine colon cancer cell, and then analyzed the cytotoxic properties of cyp4b1-activated 2-AA in cyp4B1 transduced cells to verify the cyp4B1 enzyme system for gene directed enzyme prodrug therapy.

2. Methods and Results

2.1. Cloning of rabbit CYP4B1 cDNA and construction of Cyp4B1 expression vector

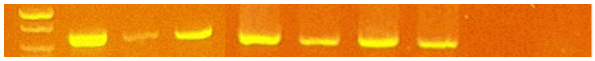
Total RNA was extracted from New Zealand white rabbit lung with the total RNA Isolation using TRI reagent (MRC, USA). By using the CapFishing™ Full-length cDNA Premix Kit (Seegene, USA), the full length cDNAs was synthesized from the total RNA of rabbit lung. Synthetic oligonucleotide primers (5′ primer, 5′-ATg CTC ggC TTC CTC TCC CgC CTg-3′; and 3′ primer, 5′-CTA CTT CTC AgC CTT ggg gCC CAG Agg-3′) were designed based on the reported rabbit CYP4B1 cDNA sequence (GenBank Accession Nos. AF332576). The coding region of rabbit cyp4B1 cDNA was amplified from the full-length cDNAs of rabbit lung using the CapFishing™ cDNA Isolation Kit (Seegene, USA). The resulting PCR product was gel purified and cloned into *E. coli* cloning vector pMD18-T. The recombinant plasmid was transformed into *E. coli* DH5α and the transformants were confirmed by DNA sequencing. To construct the cyp4B1-expressing vector in animal cells, pcDNA3.1/Hygro (Invitrogen, USA) vector was chosen and digested with BamHI and XbaI. Cyp4B1 gene was PCR amplified using primer set (Cyp4B1-S :ataggatccatgctcggcttcctctcc. Cyp4B1-AS : atatctagactacttctcagccttgggg) and digested with BamHI and XbaI. The purifed cyp4B1 product was ligated into pcDNA3.1/Hygro and the expression construct was named as pcDNA-cyp4B1.

2.2 Cell Line and Transfection

Murine BALB/c colon adenocarcinoma cell, CT-26 was grown as a monolayer in DMEM medium supplemented with 100,000 IU/L penicillin, 100 mg/L streptomycin, and 10% fetal bovine serum (FBS). Purified pcDNA-cyp4B1 vector was transfected into CT-26 using lipofectamine plus reagent (Invitrogen) according to the manufacturer's instructions. Stable transfectants were selected with 100 ug/ml of Hygromycin for 2 weeks and 10 individual clones were chosen.

2.3 Reverse Transcription-PCR Analysis for cyp4B1 expression

Total RNA was prepared from cell using total RNA extraction kit (Intron, Korea) according to the manufacturer's protocol. Purified RNA was used for template of one step RT-PCR kit (Qiagen, USA) with cyp4B1 specific primer set (Cyp4B1-S: cttccattacgacgtgctga, Cyp4B1-AS;tcatgcacatggtcaggtag). The samples were then subjected to 15 mins of denaturation at 94°C, 35 amplification cycles (30 secs at 94°C, 30 secs at 50°C, and 30secs at 72°C), and an additional 10 mins at 72°C. β-actin was amplified as a control using the same samples and same reaction condition with β-actin primers (Clontech, USA). The amplified products were analyzed by 2% EtBr–stained agarose gel electrophoresis. Cyp4B1 expressions were detected in cyp4B1 transduced cell lines but not in parental CT-26 cell as well as negative control (Neg) as shown in Figure.

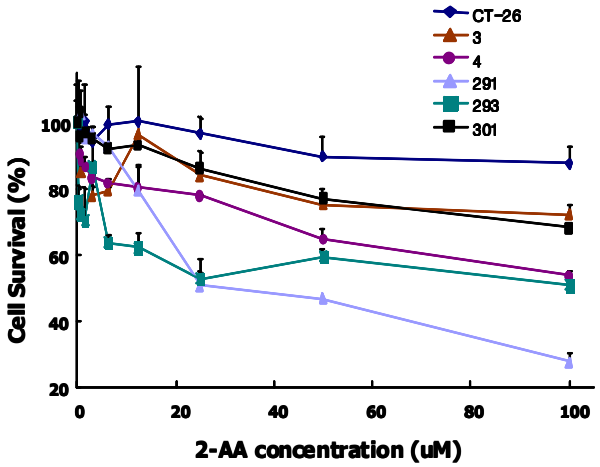


RT-PCR for cyp4B1 of cyp4B1 transduced clones (280, 281, 282, 291, 292, 301)

RT-PCR for beta-actin as control of cyp4B1 transduced clones (280, 281, 282, 291, 292, 301)

2.4 Functional evaluation of cyp4B1 expression with cytotoxicity

To assess the cyp4B1 expression in transduced cell, cyp4B1 mediated cytotoxicity treated with 2-AA was evaluated by MTT assay (Sigma, USA). The cells were seeded in a 96 well plate ($1X10^4$ cells/well), incubated at 37°C for overnight and treated with 2-AA at various concentrations ranging between 0 - 100 μM for 24 hrs or 48hrs. The cell viability after 2-AA treatment was measured with MTT assay according to the manufacturer's instructions. As shown in following figure, the cell survival (%) of cyp4B1 transduced clones (3, 4, 291, 293, 301) were markedly reduced after 48hrs of 2-AA treatment compared with that of parental cell, CT-26.



3. Conclusion

In conclusion, we constructed cyp4B1 expressing vector (pcDNA-cyp4B1) and observed the functionality of pcDNA-cyp4B1 by RT-PCR and cytotoxic activity treated with 2-AA in cyp4B1 transduced murine colon CT-26 cells. Cyp4B1-based prodrug gene therapy using 2-AA may have the potential to be used in colon adenocarcinoma management.

REFERENCES

1. JD Harris, AA Gutierrez, HC Hurst, K Sikora, and Lemoine NR, Gene therapy for cancer using tumour-specific prodrug activation. Gene ther Vol.1, p.170, 1994.
2. O. Greco, G.U. Dachs, :Gene directed Enzyme/Prodrug Therapy of Cancer: Historical Appraisal and Future Prospectives. J.Cell.Physiol. Vol.187, p.22, 2001.
3. S. Frank, S. Steffens, U. Fischer, A. Tlolko, N.G. Rainov and C.M. kramm, Differential cytotoxicity and bystander effect of the rabbit cytochrome P450 4B1 enzyme gene by two different prodrugs: Implications for pharmacogene therapy. Cancer Gene Therapy Vol.9, p.178, 2002.
4. P.B. Smith, H.F. Tiano, S. Nesnow , M.R. Boyd, R.M. Philpot and R. Langenbach. 4-Ipomeanol and 2-Aminoanthracene cytotoxicity in C3H/10$t_{1/2}$ cells expressing rabbit cytochrome P450 4B1, Biochemical Pharmacology Vol.50, p.1567,1995