Dual Specific Suicide Gene Expression Plasmid Delivery Using Bio-Reducible Polymer for Gene Therapy of Liver Cancer

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ABSTRACT SUMMARY
For successful gene therapy of hepatocellular carcinoma, a hypoxia/hepatoma dual specific herpes simplex virus thymidine kinase (hsv-TK) gene expression plasmid was developed. pEpo-AFP-TK was constructed with the erythropoietin (Epo) enhancer and alpha-fetoprotein (AFP) promoter for hypoxia and hepatoma cell specific gene expression. MTT assay showed that, pEpo-AFP-TK transfected human hepatoma cell line (Huh7) had higher cell growth inhibition rate than pSV-TK or pAFP-TK. In addition, the pEpo-AFP-TK transfected Huh7 cells showed four times higher caspase 3/7 activity and over 80% of apoptosis positive cells ratio than controls under hypoxia.

INTRODUCTION
Hepatocellular carcinoma (HCC, also called hepatoma) is the most common type of a primary cancer of the liver. Currently, surgical resection and liver transplantation are considered as best treatment options. However, the narrow chance of treatments or recurrence of tumor after the surgical treatment brings less survival rate. For this reason, HCC remains one of the most difficult tumors to cure.¹

To overcome the limitation of current therapy methods, gene therapy proposed as a potential future treatment strategy. For the safe gene therapy, controllable therapeutic gene is one of the most important factors to avoid side-effects in normal tissues. The AFP is a major plasma protein, produced by the liver during fetal development. Although AFP gene is normally silent in adult liver, most hepatoma cells re-express a lot of AFP. Because of this feature, AFP is widely used as a hepatoma diagnosis marker.

Most of solid tumors including hepatoma have hypoxic regions and this characteristic suitable for physical targeting of gene regulation. In this study, a novel hypoxia/hepatoma dual specific TK gene expressed plasmid was delivered by bio-reducible non-toxic polymer, PAM-ABP, for successful hepatoma gene therapy.²

EXPERIMENTAL METHODS
Vector construction AFP promoter was amplified by PCR (<0.7kbp) and inserted into the p-SV-TK vector.³ The pEpo-AFP-TK was constructed by the insertion of the Epo enhancer fragment at the upstream of the AFP promoter of pAFP-TK.

Apoptosis assay Human hepatocarcinoma cell lines Huh7 was used. For the transfection, PAM-ABP/pAFP-TK or pEpo-AFP-TK complex was prepared at a 5/1 weight ratios. After transfection, cells were cultured in the presence of GCV (100µg/ml) under normoxia (20% oxygen) or hypoxia (1% oxygen) condition. After the 48hrs of incubation, caspase 3/7 assay and TUNEL assay were performed.

Cell growth inhibition assay Complexes were transfected into Huh7 cells with same condition of above method. At the end of the culture period, MTT assay was performed.

RESULTS AND DISCUSSION
Delivery of the herpes simplex virus thymidine kinase gene in combination with ganciclovir (hsv-TK/GCV) is a common strategy for cancer suicide gene therapy. Dual specific gene expression pEpo-AFP-TK was constructed with the Epo enhancer and AFP promoter for
hepatoma specific regulated gene expression (Figure.1).

To evaluate the cancer cell growth inhibition, pEpo-AFP-TK was transfected into Huh7 cells. pSV-TK and pAFP-TK were used as a control. Previously, bio-reducible gene delivery polymer, PAM-ABP was developed. PAM-ABP showed a higher gene delivery efficacy with low toxicity in various cell lines. For the efficient gene delivery, PAM-ABP was used as a gene delivery carrier. After the transfection, cell growth inhibition was measured by MTT assay. The result showed that cell viability in the pEpo-AFP-TK transfected cells was approximately 40% lower than under hypoxic condition. However, the pSV-TK or pAFP-TK transfected cells did not show higher cell growth inhibition efficiency compared with pEpo-AFP-TK under hypoxia (Figure. 2).

The anti-cancer effect by the pEpo-AFP-TK was measured by TUNEL assay and caspase3/7 assay. In Figure 3A, Epo-AFPL-TK transfected hepatoma cells showed that the over 80% TUNEL positive apoptotic cells rate compared with negative control. In addition, the caspase 3/7 assay results showed that pEpo-AFP-TK had approximately four times higher apoptosis mediated enzyme activity than pSV-TK under hypoxia (Figure3B). These results suggest that the combination of Epo enhancer and AFP promoter can significantly suppressed the hepatoma cell growth and induced the apoptosis by hypoxic hepatoma specific suicide gene expression.

Figure 3. Anti-cancer effect of pEpo-AFP-TK (A) TUNEL assay (B) Caspase3/7 activity

CONCLUSION
In this study, we developed hypoxia/hepatoma dual specific suicide gene expression plasmid. The in vitro transfection results suggest that hypoxia/hepatoma dual specific gene expression vector with the Epo enhancer and the AFP promoter may be useful for successful hepatoma specific gene therapy.

REFERENCES

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