01D09-3

HYPOXIA SENSIBILITY PLASMID VECTOR EXPRESSING BCL-2 SHORT HAIRPIN RNA SUPPRESSES THE GROWTH OF MOUSE RECTUM CARCINOMA
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RNA interference (RNAi) is a powerful therapeutic tool that leads to sequence-specific gene silencing in gene-related diseases such as cancers, neurodegenerative diseases and virus infections. In this expression vector, short hairpin RNA (shRNA) is derived from the U6 and H1 RNA polymerase III (Pol III) promoters and the cytomegalovirus (CMV) RNA polymerase II (Pol II) promoter. However Pol II promoter has a limited potential activity to express shRNA, it has a controlling effect on gene expression. In this study, we developed the plasmid vector, based on CMV promoter which expresses shRNA efficiently specifically in tumor. Tumor cell proliferation is occurred in reduced oxygen microenvironment and transcriptional mechanisms are enhanced by hypoxia inducible factor (HIF) and hypoxia responsive element (HRE). We constructed new synthetic promoter (HRE+CMV) by inserting HRE element into upstream of CMV promoter. Dual luciferase assay showed a high transcriptional activity in hypoxia conditions compared with CMV promoter in Colon-26 cells. Bcl-2, antiapoptotic oncogene and related to drug resistance, is overexpressed in tumor tissue. So, we designed bcl2 shRNA (shbcl2) expression vectors (HRE+CMV-shbcl2 and CMV-shbcl2). Real-time PCR and Western blot analysis revealed that HRE-CMV-shbcl-2 repressed bcl-2 mRNA and protein in hypoxia compared with CMV-shbcl2 in cultured cells. Furthermore, we confirmed that HRE+CMV-shbcl2 suppressed the growth of tumor proliferation in tumor-bearing mouse according to the reduction of bcl-2 expression compared with CMV-shbcl2. These results suggest that HRE+CMV promoter constructed in this study activates shRNA expression in hypoxia selectively in vitro and in vivo.

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DEVELOPMENT AND EVALUATION OF NOVEL NAPANALP FORMULATION OF PACLITAXEL
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Paclitaxel (PTX) is a powerful chemotherapeutic agent against wide range of cancers. Because of its poor water solubility, PTX is dissolved in the mixture of ethanol and Cremophor EL (1:1, v/v) for its clinical use. However, this formulation is associated with a number of pharmacological and pharmacokinetic concerns including the occurrence of serious hypersensitivity reactions in cancer patients. To overcome these problems, we examined o/w emulsion and liposome as a candidate of PTX nanocarrier. Emulsion was composed of egg phosphatidylcholine (EPC), Tween 80 and the mixture of triglycerides as surfactant, co-surfactant and oil component, respectively. Liposome was prepared with hydrogenated soybean phosphatidylcholine (HSPC) and cholesterol. To improve in-vivo behavior of these nanoparticles, PEGylation was also conducted. Moreover, to separately evaluate the in-vivo disposition characteristics of nanocarrier itself and the drug included, we used nanoparticles labeled with T-cholesterol hexadecylether (T-CHE) containing 14C-PTX. Although PEGylation significantly prolonged the blood circulation time of these nanoparticles after intravenous injection, AUC of PEG liposome was much larger than that of PEG emulsion. In addition, PEG liposome was found to be more stably incorporating PTX in the in-vivo situation while a rapid release of PTX from PEG emulsion was observed. On the other hand, in-vitro MTT assay indicated that cytotoxicity of PEG liposomal PTX was less potent than PTX formulated in PEG emulsion, reflecting the slower release of PTX from PEG liposome. These results suggest that PEG liposome would be a suitable nanocarrier for PTX. Now we are evaluating anti-tumor activity of PTX-loaded nanoparticles in a solid tumor-bearing mice model.