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Photodynamic Therapy-Mediated Temporal Expression of Thymidine Kinase Genes Ligated to the Human Heat Shock Promotor: Preliminary *in vitro* Model Study of Enhanced Phototoxicity by PDT-Induced Gene Therapy

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PDT-mediated cyototoxicity basically depends on the penetrated light-dose into the tumor tissue. This limits the efficiency of PDT to the superficial tumor region typically less than 1 cm. The localized photochemical generation of reactive oxygen species, including singlet oxygen is known to increase expression of assortment of early response genes including heat shock protein. In order to increase PDT cytotoxicity in the treatment of solid tumor, it is desirable to combine PDT with other therapeutic effects. In this preliminary study we evaluated enhanced cytotoxicity from the PDT-mediated expression of thymidine kinase in a transfected tumor cell line. Two types of photosensitizers, a hematoporphyrin derivative(Photogem, Russia) and aluminium sulphonated phthalocyanine (Photosense, Russia) were used to evaluate the overexpression of hsp-70 in PDT-treated cell. Transient increase of hsp-70 was observed at 6-8 hrs later following irradiation in the photosense-treated cell whereas it was not observed in Photogem-treated cell. In the presence of ganciclovia, transfected cell showed a 17% increase in the cytotoxicity compared to the PDT only cell.

key words: PDT, heat shock protein-70 promoter, thymidine kinase, gene therapy

INTRODUCTION

PDT-mediated cytotoxicity relies on the localized photochemical generation of reactive oxygen species, including singlet oxygen. This leads to a rapid tumoricidal response mediated by both direct tumor cell toxicity and photodamage to the involved microvasculature. An assortment of early response genes including stress response genes has been shown to increase transiently following PDT-mediated oxidative stress. HSPs are activated by PDT at the level of transcription [1]. Transcriptional regulation of heat shock gene expression involves HSF (heat shock factor) binding to specific HSEs which was contained hsp promoter [2]. During cellular stress like oxidative stress, HSP-70 binds to denatured protein and allows monomeric HSF to trimerize and migrate to the nucleus where it then binds to HSE. HSP transcription is initiated upon phosphorlylation of the HSF trimer [2]. The hsp promoter has been used for over 10 years to selectively drive inducible expression of heterogous genes after hyperthermia and photodynamic therapy [3].

PDT-mediated cytotoxicity basically depends on the penetrated light dose into the tumor tissue which decays rapidly by scattering and absorption by tissue elements. In order to increase PDT cytotoxicity over the treatment of solid tumor, it is desirable to combine PDT with other therapeutic effects. In this preliminary study we evaluated enhanced cytotoxicity from the PDT-mediated expression of thymidine kinase genes ligated to the human heat shock promotor in a transfected tumor cell line.

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MATERIALS AND METHODS

Construction of an expression vector

The general construction scheme for the pLNH70-TK expression vector is shown in Figure 1. pGEM-7Zf(+) cloning vector was purchased from Promega. 1 kb fragment of herpes simplex virus thymidine kinase gene was derived from EcoRI digestion of pLTKSN [4]. pLNCZ vector was used for the template of the retrovirus vector. 0.4 kb fragment of the HSP 70 promoter was obtained from polymerase chain reaction of the human genomic DNA. The PCR primers were designed based on the sequences available from GenBank. The upstream primer (5'-CGCCATG-GAGACCAACACCCTTCC-3') corresponds to nucleotides 1-24. The downstream one (5'-AAGCTTCCGGACGCCGGAAACTCG-3') represents the reverse complement of nucleotides 387-407 and three extra nucleotides at 5' end (5'-AAG-3') to generate a HindIII restriction site. The amplified PCR fragment was cloned into pGEM-Teasy vector (Promega) before replacing CMV promoter of pLNC-TK.

Cell culture All cell lines, including 293 mGPHy, PG13 (packaging cell line characterized by expression of Gibbon ape leukemia virus envelope gene), and SNU 1041 (head and neck

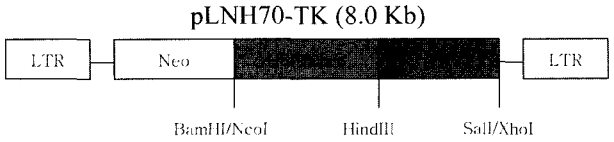

Figure 1. Construction scheme for the pLNH70-TK expression vector. 0.4 kb fragment of the hsp 70 promoter was derived from PCR of the human genomic DNA. 1 kb fragment of herpes simplex virus thymidine gene was derived from EcoRI digestion of pLTKSN. PLNCZ vector was used for the template of the retrovirus vector.

carcinoma cell, acquired from Seoul National Univ. in Korea) were grown in Dulbecco's Modified Eagle Medium (DMEM) with 4.5 g/L of glucose (Sigma D7777) supplemented with FCS (10%), penicillin (100 *u*/mL) and streptomycin (100 μg/mL). The cells were grown in a 37°C, 5% CO_2 incubator.

Construction of retrovirus vector-producing cells

To maximize virus infectivity, we used VSV-G (vesicular stomatitis virus G glycoprotein) vector system producing the progeny viruses encapsidated with VSV-G [5]. Production of the virus vectors with VSV-G envelopes was done by introductions of gene sequences of retrovirus vector and VSV-G to 293 mGPHy cells containing the *gag* and *pol* genes: Firstly, PG 13 packaging cells were transfected with pLNH70TK retrovirus vector plasmid to harvest LNH70TK retrovirus encapsidated with Gibbon ape leukemia virus envelope protein. Secondary, 293 mGPHy cells were infected with the medium taken from PG13 packaging cells to introduce the retrovirus vector sequences, then the cells were allowed two weeks for G418 (600 μg/mL) selection. Finally, to introduce *VSV-G* gene which is equivalent to the retrovirus *env* gene, the cells (293 mGPHy-LNH70TK) were undergone calcium phosphate transfection by adding 20 μg of plasmid pHCMV-G (obtained from Dr. Jane C. Burns) in 1 mL of calcium phosphate solution to the Neo^R (G418 resistant) 293 mGPHy-LNH70TK cells plated on the previous day (1×10^6 cells/100 mm dish). After 8 hours of incubation at 37°C with 5% CO_2 in air, calcium phosphate solution was aspirated to culture the cells with 10 mL of medium. Because VSV-G is cytotoxic, the medium containing retrovirus vectors was harvested after 48 hours of transfection.

Infection of Target Cells Infection of target cells was performed following our established protocol; 4 mL of a mixture of fresh non-selection medium, various amounts of virus-containing medium (filtered through 0.22 μm pore-size filter), and polybrene (5 μg/mL final concentration) were added to target cells which were plated on the previous day. Addition of selection medium (G418), X-gal staining or GFP assay of the cells was done on the next day after splitting.

RESULTS AND DISCUSSIONS

Hsp70-expression by PDT

RT-PCR was performed using the Acess RT-PCR system (Promega. Madison, USA). RNA (0.1 μg) was reverse transcribed and amplified for 25 cycles (1 min denaturation at 94°C, 1 min annealing at 61°C, and 1 min extension at 72°C). Primers HSP-70 (+ strand primer sequence : 5' cgacctgaacaagagcatcaa 3' and - strand primer sequence : 5' cttgtcgttggtgatggtgat 3') were added to reaction tubes for amplification. The resulting cDNA product (a 447 bp HSP-70 fragment) were size separated on a 1% agarose gel containing ethidium bromide and photographed under UV illumination. Two types of photosensitizers, a hematoporphyrin derivative(Photogem, Russia) and aluminium tetra-sulphonated phthalocyanine (Photosense, Russia) were used to evaluate the overexpression of hsp-70 in PDT-treated cell. Transient increase of hsp-70 was observed at 8 hours after irradiation with 25-100 mJ in the photosense-treated cell (Figure 2). In contrast it was not observed in photogem-treated cell. This contrast may be attributed to the differential subcellular distribution between two photosensitizers; hematoporphyrin derivative was known to localize in the plasma membrane and mitochondria, while more accumulation to lysosome was demonstrated by aluminium phthalocyanin [6]. The time for maximum induction of hsp-70 mRNA in the SNU cell was shifted to 6 hours when the irradiating light dose was increased to 200 mJ/cm^2. However increased photosensitizer, from 5 μg/mL to 10 μg/mL, did not give rise to any significant change in the hsp-70 induction.

PDT and cytotoxicity

Three light dose 25, 100, 200 mJ/cm^2 were irradiated with two different sensitizer concentration 5 μg/mL and 10 μg/mL. Cytotoxicity was evaluated by MTT assay in a number of time intervals after PDT treatment. In the presence of ganciclovia, transfected cell with TK-retroviral vector plasmid showed an increased cytotoxicity by 17% compared to the PDT only cell when measured at 8 hours or 10 days after PDT as shown in Figure 3.

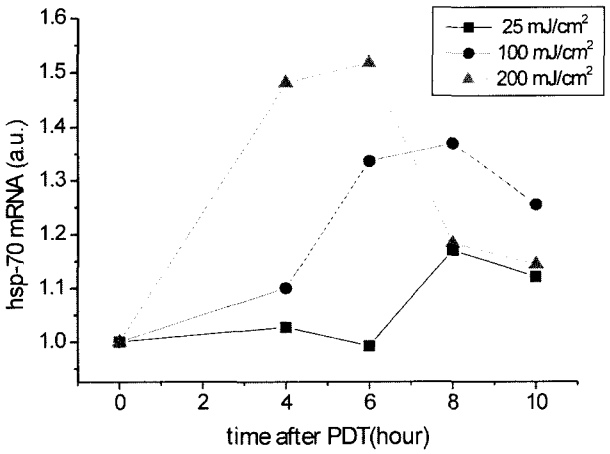

Figure 2. Temporal behavior of hsp-70 mRNA expression upon PDT treatment of the SNU cell with three different light doses. The time for maximum induction occurred at 6-8 hours after PDT.

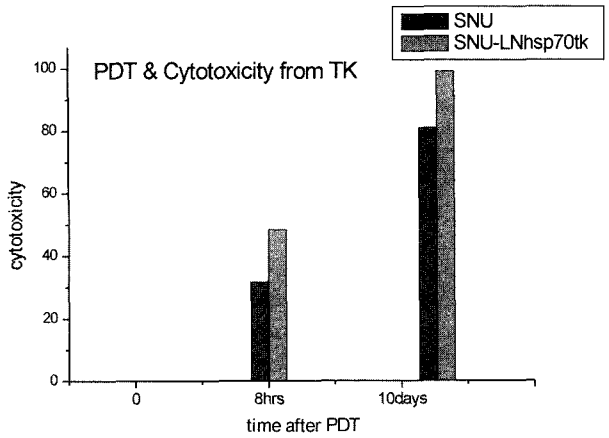

Figure 3. Comparison of photocytotoxicity in the transfected SNU cell with TK-retroviral vector plasmid and an usual SNU cell after PDT. MTT assay was evaluated in the presence of ganciclovia.

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