Peptide-guided JC polyomavirus-like particles specifically target bladder cancer cells for gene therapy

Wei-Hong Lai, Chiung-Yao Fang, Ming-Chieh Chou, Mien-Chun Lin, Cheng-Huang Shen, Chun-Nun Chao, Yeong-Chin Jou, Deching Chang & Meilin Wang

Abstract: The ultimate goal of gene delivery vectors is to establish specific and effective treatments for human diseases. We previously demonstrated that human JC polyomavirus (JCPyV) virus-like particles (VLPs) can package and deliver exogenous DNA into susceptible cells for gene expression. For tissue-specific targeting in this study, JCPyV VLPs were conjugated with a specific peptide for bladder cancer (SPB) that specifically binds to bladder cancer cells. The suicide gene thymidine kinase was packaged and delivered by SPB-conjugated VLPs (VLP-SPBs). Expression of the suicide gene was detected only in human bladder cancer cells and not in lung cancer or neuroblastoma cells susceptible to JCPyV VLP infection in vitro and in vivo, demonstrating the target specificity of VLP-SPBs. The gene transduction efficiency of VLP-SPBs was approximately 100 times greater than that of VLPs without the conjugated peptide. JCPyV VLPs can be specifically guided to target particular cell types when tagged with a ligand molecule that binds to a cell surface marker, thereby improving gene therapy.

1. SPB binding specificity.

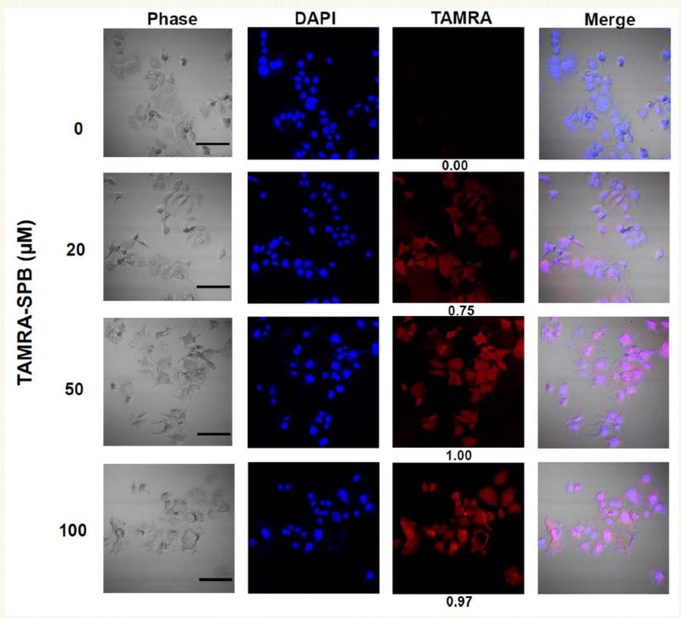

Figure 1. Binding of the bladder cancer-specific peptide (SPB) to HT-1376 bladder cancer cells. The SPB was conjugated with the fluorescent dye TAMRA to form a TAMRA-SPB conjugate, and the binding of TAMRA-SPB to bladder cancer cells was determined by detection of red fluorescence using a confocal microscope. Scale bar: 100 μm.

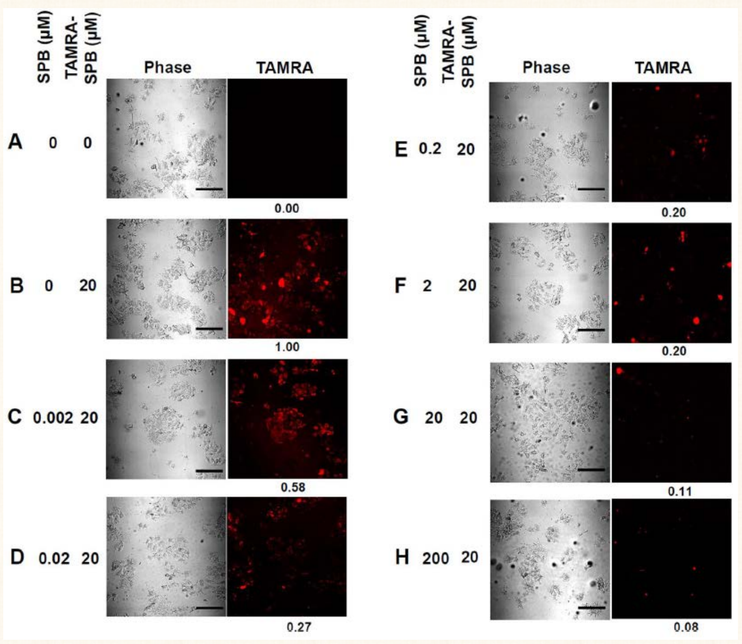

Figure 2. Binding specificity of the SPB to HT-1376 bladder cancer cells. The TAMRA-SPB conjugate was competed with the SPB at different concentrations, and binding was analyzed by detection of red fluorescence using a confocal microscope. The TAMRA-SPB conjugate was competed with the SPB at different concentrations, and binding was analyzed by detection of red fluorescence using a confocal microscope. (**A**) HT-1376 cells not treated with TAMRA-SPB or SPB were used as negative controls. (**B**) HT-1376 cells treated with 20 μM TAMRA-SPB were used as the positive control. HT-1376 cells were treated with 20 μM TAMRASPB, and the binding specificity was assessed by competing TAMRA-SPB with (**C**) 0.002 μM SPB, (**D**) 0.02 μM SPB, (**E**) 0.2 μM SPB, (**F**) 2 μM SPB, (**G**) 20 μM SPB, and (**H**) 200 μM SPB. The red fluorescence of TAMRA-SPB was visualized under a confocal microscope. Scale bar: 100 μm.

2. Binding specificity of JCPyV VLP-SPBs to bladder cancer cells.

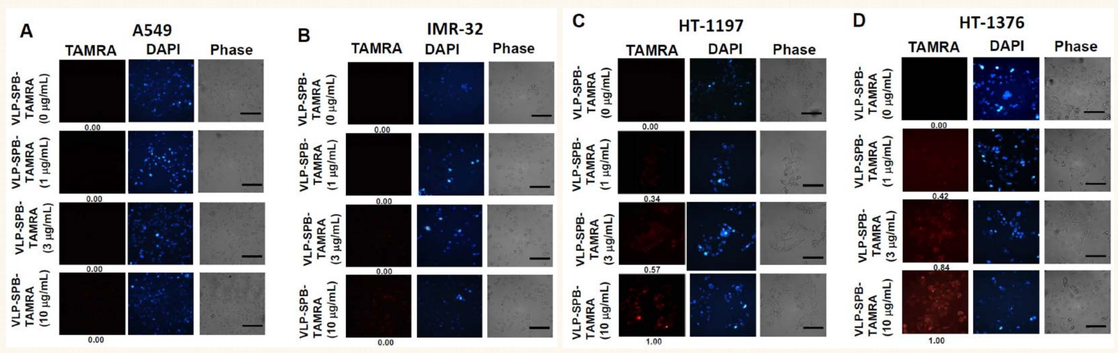

Figure 3. Determination of the binding specificity of JCPyV VLP-SPBs. JCPyV VLP-SPB-TAMRA at different concentrations was used to bind (**A**) A549 lung cancer cells, (**B**) IMR-32 neuroblastoma cells, (**C**) HT-1197 bladder cancer cells and (**D**) HT-1376 bladder cancer cells. The cells were observed under a fluorescent microscope. Scale bar: 100 μm.

3. Specific cytotoxicity of JCPyV VLP-SPBs to bladder cancer cells.

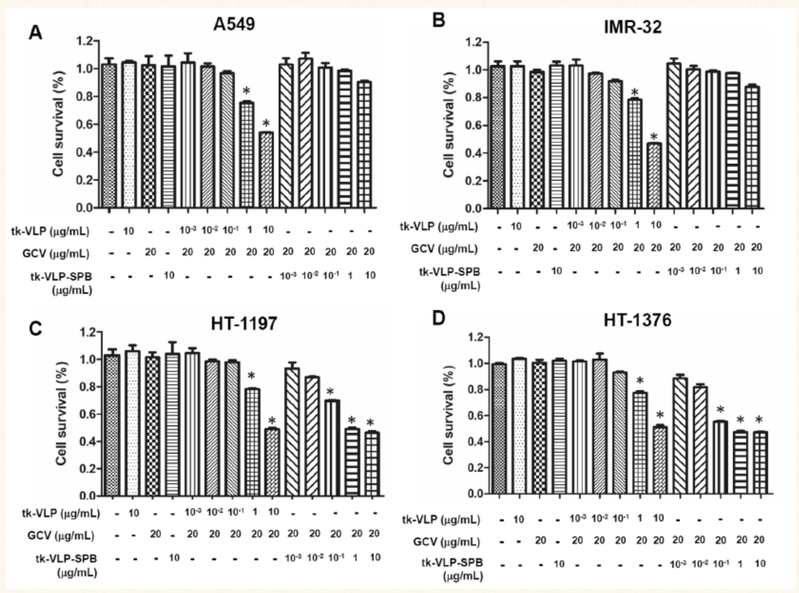

Figure 4. Determination of the specific cytotoxicity of tk-JCPyV VLP-SPBs. Specific cytotoxicity of tk-JCPyV VLP-SPBs to (**A**) A549 lung cancer cells, (**B**) IMR-32 neuroblastoma cells, (**C**) HT-1197 bladder cancer cells and (**D**) HT-1376 bladder cancer cells treated with different concentrations (0.001, 0.01, 0.1, 1, and 10 μg/mL) of tk-JCPyV VLPs or tk-JCPyV VLP-SPBs in the presence of 20 μg/mL ganciclovir (GCV). Cell viability was analyzed using CCK8 reagent. Experiments were triplicated. Kruskal–Wallis test was used to determine the statistics difference. . *: $P < 0.05$ was considered statistically significant and is indicated by asterisks (*).

4. Specific inhibition of bladder cancer cell growth by tk-JCPyV VLP-SPBs in a xenograft mouse model.

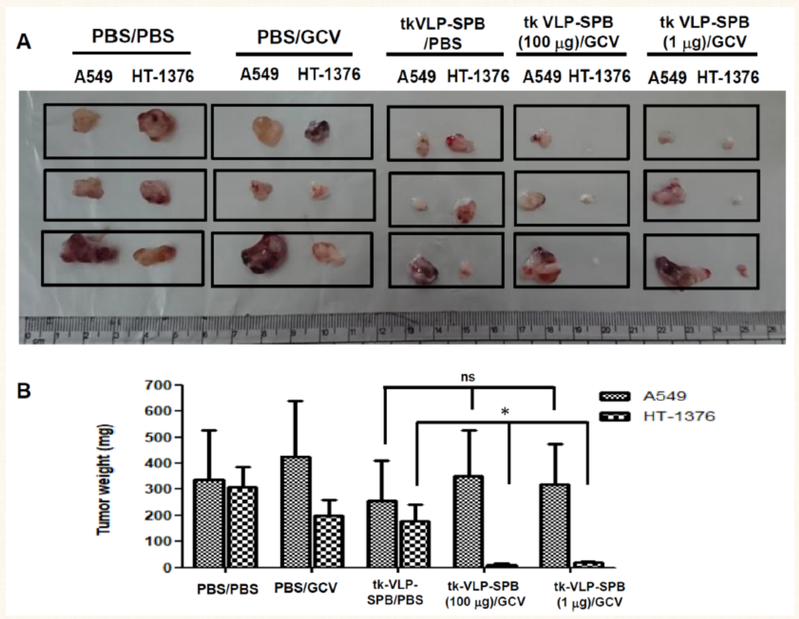

Figure 5. Specific inhibition of bladder cancer growth by tk-JCPyV VLP-SPBs in a xenograft mouse model. Mice were injected with A549 and HT-1376 cells in the left and right dorsal regions, respectively. The mice were divided into five groups and injected with PBS (PBS/PBS), GCV (PBS/GCV), tk-JCPyV VLP-SPB/PBS, tk-JCPyV VLP-SPB (100 μg)/GCV, or tk-JCPyV VLP-SPB (1 μg)/GCV. JCPyV VLP-SPB was injected into the tail vein, and GCV was administered by intraperitoneal injection. Injections were given once every three days for a total of 12 injections. Mice were anesthetized and euthanized with isoflurane, and the tumors were collected and weighed. (**A**) Photographs of tumors in the five groups. (**B**) Tumor weights in the five groups. *: $P < 0.05$ was considered statistically significant and is indicated by an asterisk (*). ns: not significant.

In summary, JCPyV VLPs can function as a gene therapy vector and can package a gene of interest driven by a tissue-specific promoter. The present results further demonstrated that conjugation with specific peptides allows the original tropism of JCPyV VLPs to be altered, thereby redirecting the encapsidated genes for expression in specific tissues and enhancing the gene transduction efficiency. Therefore, JCPyV VLPs could be designed as a flexible gene delivery vector targeted to different cell types for a variety of therapeutic purposes.