Prostate Cancer Specific Adenoviral Vectors to Increase the Therapeutic Index of Targeted Radiotherapy

Final Report

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Introduction

The goal of this proposal was to construct and evaluate adenoviral vectors encoding for the human somatostatin receptor subtype 2 (SSTr2) in the context of human prostate cancer. It was hypothesized that these vectors can specifically increase SSTr2 expression in prostate cancer, which can then be targeted with the somatostatin analog, ^{64}Cu-TETA-octreotide, for therapeutic purposes. A major limitation of adenoviral vectors as gene delivery vehicles are that they lack selectivity for infecting target tissue (tumor). To address this issue, adenoviral vectors were constructed that contain SSTr2 driven by the cyclooxygenase-2 (COX-2) promoter. It has been shown that prostate cancers overexpress COX-2. Thus construction of an adenoviral vector that contains a COX-2 promoter should limit SSTr2 expression to COX-2 positive tissues. The AdCOX-2SSTR2 virus was constructed and evaluated in prostate cancer cells and in mice bearing prostate cancer xenografts.

Materials and Methods

Construction of Adenoviral Vectors

AdCMVSSTr2 was produced and titered against 293 human transformed embryonic kidney cells as previously described. The AdCOX-2SSTr2 was constructed through homologous recombination in *Escherichia coli* using the AdEasy system. The COX-2 promoter (-883/+59, SacI-HindIII fragment) was derived from phPES2 (provided by Drs. Inoue and Tanabe at National Cardiovascular Center Research Institute, Japan) and placed in front of SSTr2 for selective expression. Though three major control regions of the COX-2 promoter (binding sites of NF-kB, NFIL-6 and CRE) exist within 300 base pairs from transcription initiation site, the longer control regions were used to achieve as much fidelity as possible. The viruses were propagated in the adenovirus packaging cell line, 293, and purified by double CsCl density gradient centrifugation, followed by dialysis against phosphate-buffered saline with 10% glycerol. The vectors were titrated by plaque assay and stored at –80°C until usage.

Quantitation of SSTr2 expression after AdCOX-2SSTr2 infection.

DU-145 or PC-3 cells were infected with AdCMVSSTr2 or AdCOX-2SSTr2 at 100 plaque forming units (pfu) per cell. The cells were seeded such that they were ~80% confluent at the time they were infected and then harvested 2 days later for the membrane preparation. The membranes were prepared by rinsing the cells once with PBS, scraping them from the bottom of the flasks into PBS and centrifuging into a pellet. The pellets were then suspended in cold lysis buffer (10 mM Tris-Cl, pH 7.2, 2 mM EDTA, 2 mM $MgCl_2$, 0.5 mM PMSF) for 15 min, vortexed, centrifuged and the supernatant collected. This was repeated 4 times. The supernatants were centrifuged at 28,000 x g for 30 min at 4°C. After centrifugation, the pellet was resuspended in 0.25 ml buffer (20 mM glyglycine, 1 mM $MgCl_2$, 250 mM sucrose) and the

protein concentration determined. Aliquots were frozen at -80°C. For the binding assay, 25 μg of the membrane preparation was washed twice and resuspended in 0.2 ml binding buffer (10 mM HEPES, pH 7.4, 1 mM EDTA, 5 mM $MgCl_2$, 10 μg/ml leupeptin, 10 μg/ml pepstatin, 0.5 μg/ml aprotinin, 200 μg/ml bacitracin, and 0.1% BSA). Various concentrations (0.5 –100 nM) of ^{64}Cu-TETA-octreotide were added to the wells in triplicate in the presence or absence of Tyr^1-somatostatin as an inhibitor such that the final volume in each well was 110 μl. The plate was incubated at room temperature for 30 min with shaking, and then the wells were rinsed twice with buffer and the filters dried and removed. Samples were counted on an automated well-type gamma counter and the data was analyzed using the one-site binding equation in the GraphPad Prism Software to generate maximum binding capacities (B_{max}).

***In vivo* expression of SSTr2 after AdCOX-2 infection.**

SCID mice were implanted s.c. with 1 x 10^7 DU-145 or PC-3 cells mixed 1:1 with Matrigel in a final volume of 200 μl each rear flank. Three weeks later the tumors were either injected with vehicle, or injected directly with 3 x 10^8 pfu of AdCMVSSTr2 or AdCOX-2SSTr2. Two days after adenoviral injection, the mice were injected i.v. with ^{64}Cu-TETA-octreotide, and the mice were sacrificed 1 h later. The blood, lung, liver, spleen, kidney, muscle, bone, pancreas, and tumors were harvested, weighed, and counted in a gamma counter. Samples were corrected for radioactive decay to calculate the percent injected dose per gram (% ID/g) of tissue by comparison with a standard representing the injected dose.

Results

AdCOX-2SSTr2 was constructed and validated by RT-PCR. Infection of DU-145 cells or PC-3 cells with AdCMVSSTr2 at 100 pfu/cell resulted in SSTr2 expression of 9,485 ± 1,689 fmol/mg of protein and 3,540 ± 1,090 fmol/mg, respectively. Infection of the COX-2 positive DU-145 cells with AdCOX-2SSTr2 resulted in SSTr2 expression of 7,150 ± 2,518 fmol/mg compared to 1,966 ± 82 fmol/mg for the PC-3 cells. This represents 75% of expression of AdCMVSSTr2 for DU-145 cells and 56% of AdCMVSSTr2 for PC-3 cells.

Biodistribution of the animals bearing DU-145 tumors injected with vehicle, AdCMVSSTr2, and AdCOX-2SSTr2 showed uptake of 1.4 ± 0.9, 3.5 ± 2.2, and 1.8 ± 0.8 % injected dose per gram of tumor (% ID/g), respectively. While biodistribution of the animals bearing PC-3 tumors injected with vehicle, AdCMVSSTr2, and AdCOX-2SSTr2 showed uptake of 0.9 ± 0.2, 2.7 ± 0.7, and 1.6 ± 0.2 % ID/g, respectively. There were no significant differences between groups for the DU-145 tumors, while all groups were significantly different from the other for the PC-3 tumors ($P < 0.003$).

Discussion

These studies show that AdCOX-2SSTr2 was evaluated in DU-145 and PC-3 cells and showed that SSTr2 expression was 75% and 56% of AdCMVSSTr2, respectively. However, the DU-145 *in vivo* studies showed that there was no significant uptake between the groups with regard to tumor uptake of ^{64}Cu-TETA-octreotide. For the PC-3 tumors, it was shown that infection with AdCMVSSTr2 and AdCOX-2SSTr2 resulted in uptake of ^{64}Cu-TETA-octreotide that was significantly greater than control tumors. However, this uptake was rather unimpressive and thus precluded the use of this system in therapeutic studies. Therefore, the remaining studies focused

on developing SSTr2 as a reporter gene for imaging of gene transfer. These studies are summarized briefly below using A-427 cells as a model system.

Characterization of SSTr2 in stable cell lines.

Most radiolabeled somatostatin analog analyses have been performed in rat pancreatic cells that express low levels of somatostatin receptor subtype 2 (SSTr2). A human cell line that overexpresses human somatostatin receptor subtype 2 would be beneficial for the evaluation of radiolabeled somatostatin analogs. The human non-small cell lung cancer line A-427 was stably transfected with a hemagglutinin (HA)-tagged human SSTr2. Expression of the receptor was evaluated *in vitro* using flow cytometry, competitive binding analysis, internalization assays, and quantitative polymerase chain reaction. The receptor expression was also validated in an *in vivo* mouse model in biodistribution and micro positron emission tomography (microPET) imaging studies using the somatostatin analog octreotide (OC), which was linked to the ^{64}Cu chelator 1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid (TETA), or ^{64}Cu-TETA-OC. Stable clones were isolated, and four clones (#2, #4, #5, and #7) were expanded for further examination. *In vitro* assays showed that clone #4 had no expression of SSTr2, while the others had various levels in the order of #7 > #2 > #5. Biodistribution studies with ^{64}Cu-TETA-OC showed the same rank order with tumor uptake of the clones ranging from 0.8 to 6.5% ID/g. MicroPET imaging confirmed good uptake of ^{64}Cu-TETA-OC in clone #7 and background uptake in clone #4. These studies show that the overexpression of human SSTr2 in a human cell line is a valid and effective means for analyzing radiolabeled somatostatin analogs both *in vitro* and *in vivo* and that the overexpression can be easily monitored using microPET imaging with ^{64}Cu-TETA-OC.

Evaluation of a technetium-94m somatostatin analog for imaging of gene transfer.

Gene therapy trials would benefit greatly from the use of non-invasive imaging to determine the location, magnitude, and time course of gene transfer. The somatostatin receptor subtype 2 (SSTr2) has previously been used as a reporter probe for gamma camera imaging of gene transfer in animal models. Positron-emission tomography (PET) has greater sensitivity than gamma camera imaging and therefore would have an advantage for imaging of SSTr2 gene transfer. An adenovirus (AdHASSTr2) encoding *sstr2* that contains an *N*-terminal hemagglutinin (HA) epitope was used for evaluating SSTR2 gene transfer. The somatostatin analog, Demotate 1 (Tyr^3-octreotate conjugated with the 1,4,8,11-tetraazaundecane chelator) was used for chelation of the positron-emitter, ^{94m}Tc ($t_{1/2}$ = 52 min), and targeting to SSTr2. Gene transfer was evaluated *in vitro* in A-427 non-small cell lung cancer cells after infection with AdHASSTr2 using ^{94m}Tc-Demotate 1 binding and internalization assays. *In vivo* biodistribution and microPET studies were conducted in mice bearing A-427 xenografts directly injected with AdHASSTr2 to determine the tumor localization of ^{94m}Tc-Demotate 1. ^{94m}Tc-Demotate 1 bound with high affinity and was rapidly internalized into AdHASSTr2-infected A-427 cells. Biodistribution studies showed uptake of ^{94m}Tc-Demotate 1 in tumors infected with AdHASSTr2 (4.0% ID/g at 2 h) and background uptake in tumors infected with a control adenovirus (0.8% ID/g at 2 h). The uptake of ^{94m}Tc-Demotate 1 in AdHASSTr2 infected tumors was greater than the uptake in all other tissues except for the kidneys and the SSTr2 positive pancreas. MicroPET imaging showed similar results with clear uptake of ^{94m}Tc-Demotate 1 in AdHASSTr2 infected tumors, background uptake in control tumors and clearance through the kidneys. These studies show that the positron-emitting somatostatin analog, ^{94m}Tc-Demotate 1, could be used to

determine SSTr2 gene transfer via microPET imaging which will improve the sensitivity of the SSTr2 reporter gene system.