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TITLE: Identification of a Protein for Prostate-Specific Infection

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14. ABSTRACT In this proposal, we will identify and clone a protein that can be used to generate infection-specific gene therapy vector. We expect that using this protein to modify various gene therapy vectors, we can specifically deliver cytotoxic genes into prostate cancer cells using systemic treatment, and eventually eradicate metastatic prostate cancer cells in patients. During the second year, we continue screening our cDNA library that we prepared during the first funding year. We have screened approximately 8,000 more colonies. So that the total colonies with the first year we have done are 9,200. We found that peptides from two genes can efficiently increase lentiviral vectors to infect LNCaP prostate cells. So, in the coming year, we will insert the sequences that encode these two peptides to modify gene therapy vectors and to generate tissue specific vectors with higher efficacy in gene delivery.					
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Introduction:

Using gene therapy vectors to eradicate prostate cells is extensively studied. It is expected that if we can use a target-specific gene therapy to deliver cytotoxic genes into metastatic prostate cancer cells in patients, we can eradicate these malignant cells and cure the patients. To find an approach to generate infection-specific vectors is critical for target-specific cancer cell eradication. Because viral vectors infect cells by the binding of their surface proteins to their receptors on the target cells, it is expected that infection-specific vectors can be generated by modifying their surface proteins with proteins that can specifically bind to target cells. In our Preliminary Studies, we found that the lentiviral vector (an HIV-based retroviral vector) generated from a cell line derived from human oral tissue (HOT) can specifically infect LNCaP prostate cancer cells. Virus generated from other cell lines has much lower infectivity to LNCaP cells (200 to 1200-fold lower). These results strongly suggest that the HOT cells express a very specific membrane protein that can be picked by lentiviral vectors to modify their envelope. With such protein on its envelope, the viral vectors are able to specifically infect LNCaP cells.

If this is the case, we expect that a protein for generating infection-specific vector can be obtained by screen the cDNA library of the HOT cell line.

Body:

The approved SOW is listed below.

- Task 1. To prepare cDNA library in eukaryotic gene expression vectors (months 1-8).
- Task 2. To perform first-round screening to identify the cDNA clone groups that contain the cDNA clones encoding the protein responsible for tissue-specific infection. (months 9-20)
- Task 3. To perform the second round of screening to identify the individual clones that contain the cDNA encoding the protein responsible for tissue-specific infection (months 21-24)
- Task 4. To sequence the identified gene (months 25-26)
- Task 5. To use sequence analysis to characterize the identified gene (months 27-28)
- Task 6. To generate deletions of the identified gene and to use them to confirm the functional domains of the identified gene (months 31-36)

To follow the schedule, we should complete Tasks 1-4. We actually achieved our goal with some modifications as described in the following sections.

Progress of our research:

During the second year, we have screened more cDNA colonies. In our original proposal, we plan to screen 6,000 to 12,000 colonies. However, combined with the numbers of colonies, we have screened 9,200 colonies and we have not identified the colony that can specifically increase the transduction efficiency by lentiviral vectors. So we changed our strategy by using particular peptides to test the help viral vector gene delivery and we found that two peptides demonstrated significant effects in enhancing lentiviral vector gene delivery.

We make progress following our SOW.

1. Screening cDNA clones isolated from HOT cells

Following our research plan, we co-transfected the CG cells with cDNA clones isolated from the HOT cells and a lentiviral vector that carries the GFP gene. Lentiviral vector released from the CG cells were used to infect LNCaP cells. Because our previous studies demonstrated that viral vectors produced from CG cell line have very low infectivity, we expect that by adding a gene that can increase the binding between viral vector and the target cells, and such increased infection can be detected by counting GFP positive cells in the infected LNCaP cell culture. However, although we have screened 9,200 colonies, we have not identified any cDNA clone that could significantly increase the infection of LNCaP cells.

2. Two peptides significantly increasing LNCaP infection by lentiviral vectors

Based on the results described, we speculated that the lentiviral vectors may need two or even more proteins on their surface to interact with LNCaP cells. Our previous approach may work when only one protein is required for LNCaP cell infection. One approach is to use peptides derived from an identified gene that is able to increase lentiviral vector infection, to increase lentiviral vector infection. We have found CLDN-7 protein increase lentiviral vector infection (reference 1). To test that whether this gene is able to increase infection of LNCaP, we added a peptide from this gene into the infection process of LNCaP. Our results demonstrated that a peptide with 15 amino acid residues (YDSVLALSAALQATR) significantly increased LNCaP infection (Fig. 1). Using similar approaches, we have identified that another peptide with sequence of RGCICRCIGRGCICRCIG can also significantly increase LNCaP infection (Fig. 1)

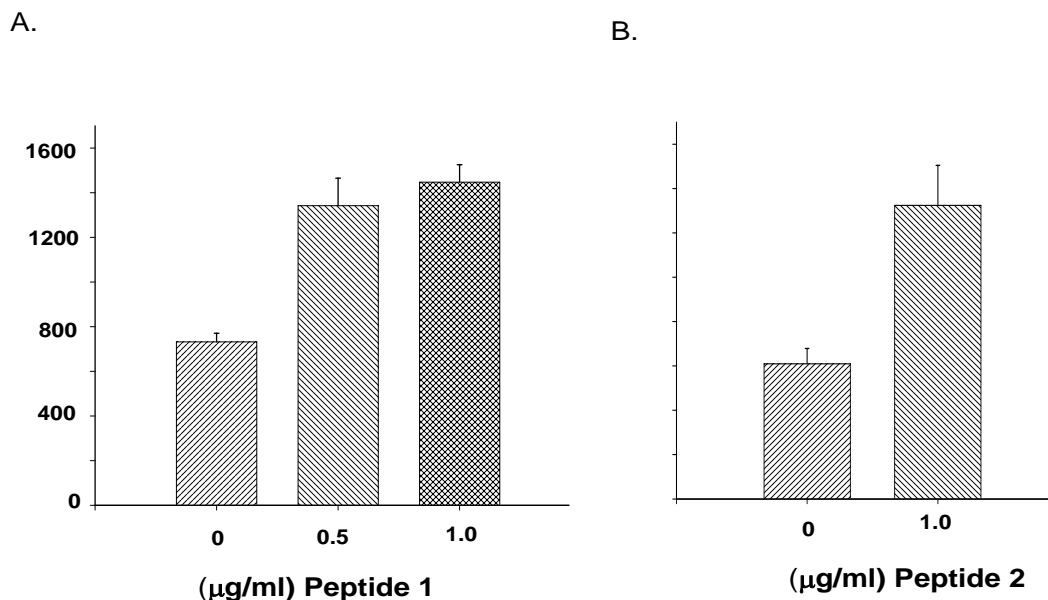


Fig. 1. Two peptides significantly increase LNCaP cell infection. By adding a peptide with sequence of RGCICRCIGRGCICRCIG (panel A) or YDSVLALSAALQATR (panel B) into LNCaP cell culture medium during gene delivery by using lentiviral vectors, infection of LNCaP cells can be significantly increased.

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Key Research accomplishments:

Two peptides that can significantly increase lentiviral infection of LNCaP cells have been identified.

Reportable Outcomes:

A manuscript titled “Regulation of PSA expression by a claudin-7-binding protein, JAM-A” has been submitted.

Conclusions:

We have extensively screened a cDNA library containing approximately 10^5 cDNA clones and have found that the cDNA clones isolated from HOTA cells do not significantly enhance lentiviral infection of LNCaP cells. The results suggest that the tissue-specific infection of LNCaP cells by lentiviral vector is involved in two or more surface proteins. Based on such fact, we changed our strategy. We have identified two peptides that are able to significantly increase the infection of LNCaP cells. We are now using these two peptides to modify lentiviral surface proteins. We expected that by modifying lentiviral vector surface proteins, such as gp120, using these two peptides, we should be able to generate a prostate-tissue specific vector.

Reference:

1. Zheng JY, Xie Y, Campbell R, Chen H, Arasteh A, Chen IS, James Berenson J, and Pang S. Involvement of claudin-7 in gp120-independent HIV infection. *Retrovirology*, 2005, 2:79