Identification of PSMA-Specific Peptides for Prostate Cancer Therapy

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Purpose
Prostate-specific membrane antigen (PSMA), a transmembrane protein with large extracellular domain, is an ideal target for prostate cancer targeted drug delivery because it is overexpressed on the surface of most prostate cancer cells, while its expression in normal cells and tissues is extremely low. In this study, our objective is to identify PSMA-specific peptides that can be potentially employed as ligands for targeted drug delivery in advanced prostate cancer therapy.

Methods
A combinatorial biopanning strategy was employed to identify PSMA specific peptide ligands in this study. Phage library was screened in vitro by biopanning against recombinant PSMA ECD and PSMA-positive LNCaP cells for four rounds. An additional round of in vivo phage biopanning was performed subsequently. Individual phage clones were randomly selected after biopanning, and ELISA was used to evaluate the affinity of each single phage clone to PSMA protein. By sequencing phage DNAs, the inserted peptide sequence of each phage clone was obtained. Binding affinity and specificity of the peptide candidates were examined by competitive inhibition and cellular uptake study. One of the peptide candidates shows the highest binding affinity and specificity. The peptide was selected and conjugated with a proapoptotic peptide D(KLAKLAK)2. This novel fusion peptide was incubated with LNCaP cells, and MTT assay was used to evaluate its cytotoxicity. Finally, biodistribution study was performed to exam whether this peptide can accumulate in prostate cancer xenografts.

Results
The S7 peptide which shows the high affinity to PSMA protein and PSMA positive cells is identified. FAM-labeled S7 peptide shows high uptake in PSMA-positive cells, while the uptake in PSMA-negative cells is negligible. Uptake of S7 peptide in LNCaP cells is reduced when the PSMA expression is knockdown by DHT treatment. The Kd value of S7 peptide to LNCaP and C4-2 cells are 8.22\(\mu\)M and 8.91\(\mu\)M, respectively. The S7-KLA fusion peptide specifically enter LNCaP cells and induce cell apoptosis with IC50 of 18.89\(\mu\)M. In the biodistribution study, compared to scrambled peptide, FAM-labeled S7 peptide shows significant higher uptake in C4-2 xenograft tumor and the tumor/muscle uptake ratio reaches 3:1.

Conclusion
By using phage display technology, we identified a group of peptides that can specifically bind to PSMA and PSMA-positive cells. Among all the candidates, the S7 peptide shows the highest binding affinity and specificity. All the results suggest that the S7 peptide holds a great promise as a targeting ligand for prostate cancer therapy.

Figure 1. Binding affinity of the S7 corresponding phages to PSMA-positive and negative cells.

Figure 2. Cellular uptake of FAM-labeled S7 peptide to DHT treated and non treated LNCaP cells.