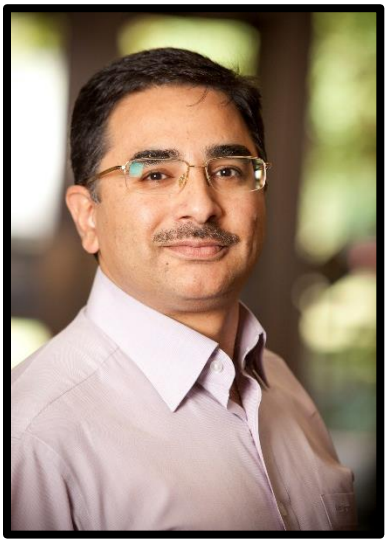
THE GRADUATE COLLEGE OF THE
UNIVERSITY OF OKLAHOMA HEALTH SCIENCES CENTER

ANNOUNCES THE FINAL EXAMINATION OF

Hooman Yari

FOR THE DEFENSE OF THE DOCTOR OF PHILOSOPHY DEGREE
GRADUATE COLLEGE
Graduate Pharmaceutical Sciences



Monday, November 4, 2019 | 10:00 am |
College of Pharmacy, Room 339

Prostate Specific Membrane Antigen-Targeted Theranostic Liposomes for Active Targeting of Prostate

COMMITTEE IN CHARGE: Vibhudutta Awasthi, PhD, Chair; Nathan Shankar, PhD; Michael A. Ihnat, PhD; Alamdar Hussain, PhD;. Rheal A. Towner, PhD

ABSTRACT: Prostate specific membrane antigen (PSMA) is a marker for diagnosis of prostate cancer and can be used to help in the delivery of therapeutics/diagnostics to advanced and metastasized prostate cancer tumors. Here we report on the use of theranostic liposome-based delivery systems for specific delivery of therapeutics/diagnostics to PSMA-expressing ($PSMA^+$) cells. These liposome-based preparations were formulated by surface-modification of theranostic liposomes with lipopolymer compounds that bind to the PSMA protein. The lipopolymer P^3 is comprised of a glutamate-urea-lysin (Glu-urea-Lys)-based PSMA-binding motif conjugated to a palmitate residue by using a poly (ethylene glycol)$_{2000}$ chain as the linker. The lipopolymer DP^2 consists of the same (Glu-urea-Lys)-based PSMA-binding motif and the poly(ethylene glycol)$_{2000}$ chain, but it includes a distearoylphosphatidylcholine (DSPE) residue at its hydrophobic tail. These liposomes were radiolabeled with ^{99m}Tc radionuclide and loaded with doxorubicin to study their specificity towards $PSMA^+$ prostate cancer cells. We confirmed the differential expression of PSMA in LNCaP and PC cells by immunoblotting and flow cytometry. We found that the uptake of ^{99m}Tc-labeled P^3-liposomes by LNCaP cells was >3-fold higher than ^{99m}Tc-labeled Plain-liposomes. We also

determined the amount of doxorubicin delivered to LNCaP cells by these liposomes, and found that the highest amount of doxorubicin was delivered by DP^2-liposome, followed by P^3-liposome, and both were significantly greater than Plain-liposome ($p < 0.05$). Cell-based cytotoxicity assay results showed that doxorubicin-loaded P^3-liposomes were significantly more toxic to LNCaP cells ($p < 0.05$), but not to PSMA-negative PC3 cells. Compared to doxorubicin-loaded Plain-liposomes, the IC_{50} value of doxorubicin-loaded P^3-liposomes was reduced by ~5-fold in LNCaP cells.

We investigated the capability of DP^2-liposomes for delivering their content to LNCaP cells in the presence of blood plasma. We found that the amount of doxorubicin delivered by DP^2-liposomes to LNCaP cells was significantly decreased in the presence of plasma ($p = 0.002$). Bovine serum albumin (BSA) showed a similar effect and decreased the amount of doxorubicin delivered by DP^2-liposomes to LNCaP cells ($p < 0.05$), which could not be regained by pre-incubation of BSA with disodium glucarate at 100 x excessive molar ratio. Binding of ^{99m}Tc-radiolabeled DP^2-liposomes to PSMA was measured in a cell-free assay, and further revealed that the binding of DP^2-liposomes to PSMA was significantly decreased in the presence of plasma or BSA ($p < 0.0001$).

These results together suggest that surface functionalization of liposomes with small PSMA-binding (Glu-urea-Lys)-based molecules provides a viable platform for the specific delivery of theranostics to $PSMA^+$ prostate cancer. However, exposure of liposomes to blood and its components especially serum albumin impairs the interaction of targeted liposomes with $PSMA^+$ cells, possibly due to the adsorption of proteins on the surface of the liposomes. This phenomenon may negatively affect efficiency and specificity of the delivery system *in vivo*. Further studies are required to pinpoint the mechanism of the inhibitory effect of plasma and serum albumin on the interaction of targeted liposomes with $PSMA^+$ cells.