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14. ABSTRACT

The proposed research is a proof-of-concept study that focuses on testing a new cancer targeting strategy that aims at enhancing nanodelivery of drugs to osteopontin (OPN) that are often overexpressed in advanced prostate cancer cells and their microenvironment. To evaluate this strategy, lipid-based nanocarrier that targets OPN (i.e. OPN-LN) was developed, characterized and compared with non-targeting LN. This OPN-LN was used as the key platform to study the OPN targeting strategy. In the reporting period, OPN-LN was prepared by conjugation of OPN antibody onto the surface of lipid nanocarriers using SATA as the conjugation reagent. Our data show that the OPN-LN have good dispersion stability (no noticeable aggregation at 37 ^{o}C in 2 days) and regular morphology. When compared with non-targeting LN, OPN-LN were more efficiently taken up by PC-3M prostate cancer cells which were shown to be OPN expressing as indicated by Western blotting analysis. No significant increase in non-specific toxicity was observed after OPN antibody conjugation. In brief, OPN targeting apparently can improve the nanodelivery to OPN expressing prostate cancer cells. The impact on anticancer efficacy will be evaluated in the next reporting period.

15. SUBJECT TERMS

Osteopontin, prostate cancer, targeted delivery, nanomedicine

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1. **INTRODUCTION:**

The proposed research is a proof-of-concept study that focuses on testing a new cancer targeting strategy that aims at enhancing nanodelivery of drugs to the glycoproteins (e.g. osteopontin, OPN) that are often overexpressed in advanced prostate cancer cells and their microenvironment. The purpose is to establish this novel delivery strategy for effective and safe prostate cancer therapy. To evaluate this strategy, in the first stage, it was proposed that a nanocarrier that targets OPN will be developed and characterized, and this nanocarrier will be used for determining the feasibility of the proposed OPN-targeting strategy (i.e. Aim #1 – To determine the feasibility of OPN-targeted strategy for enhancing nanomedicine delivery to OPN-rich targets). In the second stage, this OPN-targeting nanocarrier will be loaded with an anticancer drug and the in vitro therapeutic activities against prostate cancer cells with TIC behaviors will be studied (i.e. Aim #2 – To study the therapeutic effects of OPN-targeted delivery on metastatic prostate cancer).

2. **KEYWORDS:**

Osteopontin, prostate cancer, targeted delivery, nanomedicine

3. **ACCOMPLISHMENTS:**

- **What were the major goals of the project?**

Specific Aim 1: To determine the feasibility of OPN-targeting strategy for enhancing nanomedicine delivery to OPN-rich targets.

- Objective 1: Prepare and characterize OPN-targeting carrier (month 1-6) (completed in 12 months)
- Objective 2: Evaluate the effect of decorating a nanocarrier with OPN-targeting moieties on OPN-binding (month 1-6) (completed in 12 months)
- Objective 3: Evaluate the effect of OPN-targeting on nanomedicine delivery to cell culture (month 4-9) (completed in 12 months)

Specific Aim 2 To study the therapeutic effects of OPN-targeted delivery on metastatic prostate cancer

- objective 1: Preparation of prostaspheres (month 1-6) (not completed)

- objective 2: Evaluate the therapeutic effects of OPN-targeting system carrying a hedgehog pathway inhibitor (month 3-12) (not completed)

- **What was accomplished under these goals?**

Major activities and specific objectives: In this year, we focused on developing OPN-targeting carriers and using the most stable carrier as the platform for evaluation of the feasibility of OPN-targeting strategy to enhance nanomedicine delivery. The goal is to complete the works described under Specific Aim 1 (i.e. To determine the feasibility of OPN (osteopontin)-targeted strategy for enhancing nanomedicine delivery to OPN-rich targets). To achieve this goal, our group has followed the SOW and performed the subtasks under three specific objectives, including

- objective 1: Prepare and characterize OPN-targeting carrier
- objective 2: Evaluate the effect of decorating a nanocarrier with OPN-targeting moieties on OPN-binding
- objective 3: Evaluate the effect of OPN-targeting on nanomedicine delivery to cell culture

Significant results: The more significant results are summarized as below

a. Preparation of Osteopontin(OPN)-targeting carrier (under Objective 1). Lipid nanoparticles were prepared using a blend of biocompatible lipids and phospholipids such as triglyceride, triolein, DSPC (1,2-distearoyl-sn-glycero-3-phosphocholine), and/or PEG-DSPE (Polyethylene glycol-distearoyl-glycero-phosphoethanolamine). These nanoparticles were conjugated with OPN-antibody using SATA as conjugation agent. The OPN-targeting lipid nanoparticles (OPN-LN) showed no visible aggregation and precipitation at 37 ^{o}C in 72 hours. OPN-LN were further characterized and the results are shown as below.

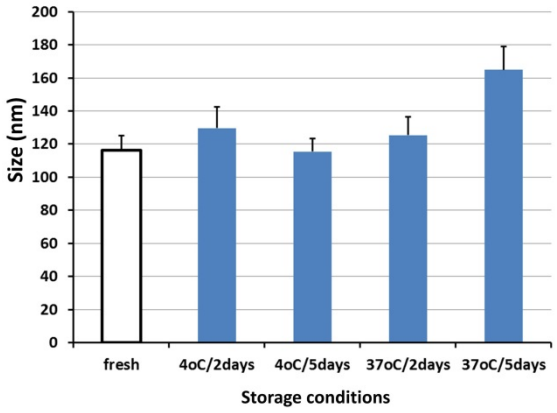
b. Dynamic light scattering: Size, PDI, zeta potential (under Objective 1). The average size, polydispersity (PDI) of size and zeta potentials of LN before and after conjugation with OPN-antibody are measured using dynamic light scattering technique (Zetasizer, Malvern, UK) and shown below. In addition, in preparation of the works under Specific Aim 2 we also attempted to encapsulate a hedgehog pathway inhibitor cyclopamine (CP), and the data are as follows:

	Average diameter (nm)	PDI	Zeta potential (mV)
Blank LN, no OPN-	149.4	0.196	-20.0

antibody			
Blank LN, +OPN-antibody coating	154.3	0.174	-18.2
LN-loading CP, no OPN-antibody	161.7	0.181	-28.7
LN-loading CP, +OPN-antibody	169.8	0.198	-25.9

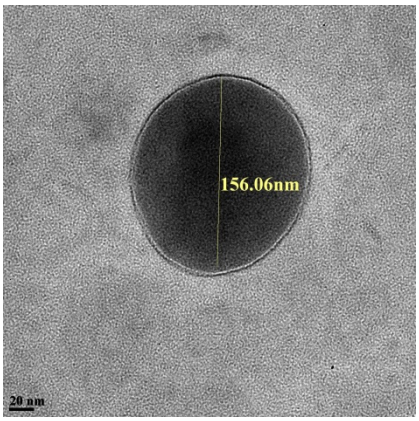
The data indicate that conjugation of OPN-antibody onto LN and encapsulation of CP drug did not significantly increase the average size of the nanocarriers (some modest increases were observed but not statistically significant). The PDI value reflects the distribution of nanocarrier size. PDI values <0.3 in all samples suggest that the conjugation also did not lead to the formation of some excessively large particulate matters. The modestly negative zeta potentials as shown in the table help to maintain the dispersed state of OPN-LN. In general, OPN-antibody conjugation and loading of drug do not have any detrimental impact on the size/charge aspect of the lipid nanocarriers.

c. Dispersion stability study (under Objective 1). To rule out the possibility OPN-LN forming aggregates that may affect their interaction with the target cells, their size at different conditions (4 ^{o}C or 37 ^{o}C, up to 5 days) was monitored using dynamic light scattering. The results are summarized as below:



It is shown that OPN-LN remained similar in size even at 37 ^{o}C for 2 days. Some increase in size suggesting modest aggregation occurred after 5 days of incubation. In general, the OPN-LN can be considered fairly resistant to aggregation and this should not be a major factor affecting the cell-nanocarrier interaction.

d. Electron microscopy (EM) (under Objective 1): To double-check the size data from dynamic light scattering and learn the morphology of OPN-LN, transmission electron microscopy imaging was performed. A representative image is shown as follows:



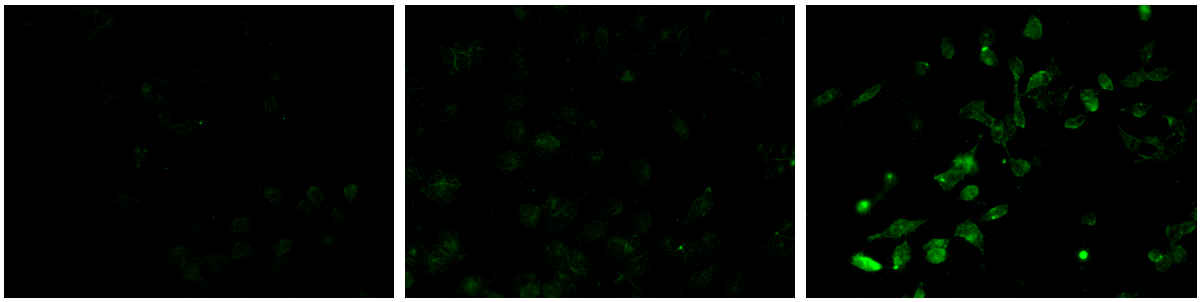
OPN-LN was shown to be spherical and regular in morphology. The size is consistent with the dynamic light scattering measurement.

e. OPN-antibody conjugation (under Objective 1). OPN-LN was prepared with different antibody to LN ratios. ELISA was performed to evaluate the amount of unconjugated, free antibody and the result obtained was used to determine the efficiency of antibody conjugation (amount of antibody conjugated x 100%/ amount of antibody added). The result shows a conjugation efficiency of the nanoformulation at over 30%.

f. Confirmation of OPN expression in prostate cancer cells (Objective 2): To determine the OPN expression levels in different human prostate cancer cell lines, Western blotting analysis was performed using anti-human OPN mouse IgG as primary antibody and goat anti-mouse IgG-HRP as secondary antibody.

OPN was expressed in all four prostate cancer cells tested (From left to right: PC-3M, Du145, LNCap, PC3). The strongest OPN expression was observed in PC-3M. PC-3M is a metastatic subline of PC3 prostate epithelial cancer cells. Because of its high expression it was chosen as the primary cell line for subsequent *in vitro* studies.

g. Evaluate interaction of OPN-LN and OPN-expressing prostate cancer cells (Objective 2): To study the correlation between OPN-antibody conjugation and interaction with the OPN expressing prostate cancer cells, fluorescent microscope imaging was performed. PC-3M cells were used and LN were labeled with FITC-conjugated DSPC-PEG. Representative images are shown below (from left to right: non-targeting LN, OPN-LN with low density of OPN antibody, OPN-LN with high density of OPN antibody).



The cellular uptake of LN noticeably improved with surface decoration OPN-antibodies on the LN. This demonstrates the feasibility of using OPN-targeting for improved nanoparticle-cell interaction.

h. Evaluate baseline cell toxicity of OPN-LN (Objective 3): Sometimes active targeting can render a nanocarrier inherently more toxic to cells (even without drug). This may not necessarily be beneficial as the toxicity derived from the nanocarrier device itself is often less disease specific and may affect the non-target cells. We performed MTT viability assay using prostate cells with low OPN expression level (PC3). There is no significant difference between the viabilities of PC3 cells treated with OPN-LN, non-targeting LN and vehicle control (all without any drug encapsulated). The data indicate that OPN-antibody conjugation did not affect the baseline toxicity of the nanocarrier.

Goals to be completed in coming reporting period: While Specific aim #1 has almost been completed, because the main technician who handled the cancer cell lines has found another position during this period, several objectives listed under Specific aim #2 (*i.e*. To study the therapeutic effects of OPN-targeted delivery on metastatic prostate cancer) remain unfinished. To sum up, these include "preparation of prostaspheres" and "evaluate the therapeutic effects of OPN-targeting system carrying a hedgehog pathway inhibitor" as listed in the SOW. A no-cost extension for one year has been approved and the studies under these objectives will be conducted in this period.

- **What opportunities for training and professional development has the project provided?**
  Nothing to report

- **How were the results disseminated to communities of interest?**
  Nothing to report

- **What do you plan to do during the next reporting period to accomplish the goals?**

We will focus on completing the two subtasks under Specific Aim 2. We have started cultivating prostaspheres and more time is needed to confirm their stability. We will try to encapsulate hedgehog pathway inhibitors such as CP and GDC-0449 in OPN-LN. Some preliminary studies about their encapsulation (as shown in "Significant results" described above) have been completed but detailed ones such as their drug encapsulation efficiencies still need to be determined. The drug loaded OPN-LN will be evaluated in the prostaspheres to learn their efficacy.

4. **IMPACT:** *Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

- **What was the impact on the development of the principal discipline(s) of the project?**

Nothing to report

- **What was the impact on other disciplines?**

Nothing to report

- **What was the impact on technology transfer?**

Nothing to report

- **What was the impact on society beyond science and technology?**

Nothing to report

5. **CHANGES/PROBLEMS:**

- **Changes in approach and reasons for change**

No significant changes in the approach.

- **Actual or anticipated problems or delays and actions or plans to resolve them**

The main technician has found another job during this period, causing delay in many experiments especially the ones under Specific Aim #2. We have applied for a no-cost extension for one additional year and just got the approval. The PI will perform the remaining studies during this period.

- **Changes that had a significant impact on expenditures**

Nothing to report

- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

Nothing to report

- **Significant changes in use or care of human subjects**

Nothing to report

- **Significant changes in use or care of vertebrate animals**

Nothing to report

- **Significant changes in use of biohazards and/or select agents**

Nothing to report

6. **PRODUCTS:**

Publications, conference papers, and presentations

Nothing to report

7. **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**

- **What individuals have worked on the project?**

Name:	Ho-Lun Wong
Project Role:	PI
Researcher Identifier ORCID ID:	0000-0002-0349-7708
Nearest person month worked:	12
Contribution to Project:	He designed the project, performed nanocarrier preparation and characterization, conducted some in vitro works, and is mainly responsible for data analysis and manuscript writing
Funding Support:	NIH 1R01CA168917
Name:	Jan Romano
Project Role:	Part time technician
Researcher Identifier (e.g. ORCID ID):	Not applicable
Nearest person month worked:	7
Contribution to Project:	Ms Romano has assisted Dr. Wong in nanoparticle preparation and characterization, and was responsible for cancer cell culture and also assisted in some in vitro experiments
Funding Support:	None

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

Nothing to report

- **What other organizations were involved as partners?**

Nothing to report

8. **SPECIAL REPORTING REQUIREMENTS**

Nothing to report

9. **APPENDICES:** Nothing to report