Targeted Delivery of micro-RNA by Ephrin-A1 Conjugated Nanoliposomal Particles (NLP) for Malignant pleural Mesothelioma

Hung-Yen Lee Ph.D[1, 3], Kamal A. Mohammed Ph.D [1, 2] , Fredric Kaye, MD[4], Parvesh Sharma, PhD[5], Brij M. Moudgil, PhD[5], and Najmunnisa Nasreen Ph.D[1, 2]

[1]Division of Pulmonary, Critical Care & Sleep Medicine, Department of Medicine, [2]NF/SGVHS, Malcom Randall VA Medical Center, University of Florida, [3]Biomaterials Center, Department of Materials Sciences and Engineering, University of Florida, [4]Hematology and oncology, and [5]Particle Engineering Research Center, Material Science & Engineering, University of Florida, Gainesville, FL.

Statement of Purpose: MicroRNA (miRNA) via complementary binding to mRNA silences gene expression. However, an effective carrier is needed for miRNA delivery due to the high vulnerability of miRNA and low uptake by cancer cells when administered *in vivo*. EphA2 receptor tyrosine kinase is overly expressed in lung cancer cells. Ephrin-A1, the ligand of EphA2 receptor, specifically binds to the EphA2 receptor on cancer cell membrane and inhibits tumor proliferation and migration.[1-3] In this study, to enhance the delivery of miRNA, miRNA were encapsulated in the PEG-modified nanoliposomal DOTAP/Cholesterol particles and Ephrin-A1 was conjugated on the surface of particles for targeted delivery. In this study we used Ephrin-A1 conjugated NLP as a delivery vehicle of miRNA for intra-pleural therapy (IPT) as a potential strategy to treat localized tumors of MPM. W**e** hypothesized that treatment of MPM tumor cells by Ephrin-A1 conjugated NLP attenuates tumor growth via induction of let-7 miRNA or miRNA-302b and repression of EphA2 in an orthotopic mouse model of MPM. In addition, the combination therapy of miRNA and Ephrin-A1 was expected to show improved treatment effectiveness.

Methods: The nanoliposomal particles were prepared by hydrating the dry film of lipid mixture and extruded through filters. The miRNA were entrapped in the nanoliposomes and the Ephrin-A1 protein were coupled with the cyanuric chloride-activated PEGylated lipid, 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[cyanur(polyethylene glycol)-2000] (Fig.1), on the liposome surface. The miRNA/ephrin-A1/liposome complexes were examined *in vitro* and *in vivo* in an orthotopic mouse model of MPM.

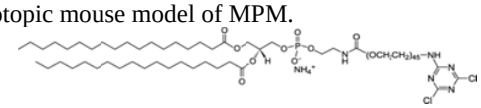
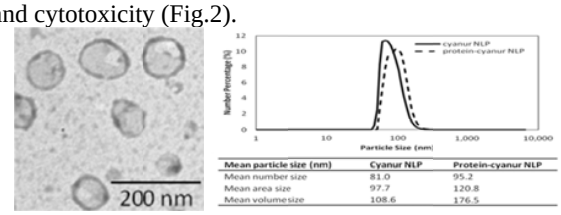


Figure 1. DSPE-PEG(2000)-cyanur (Avanti Lipids, Inc.).

Results: Nanoliposomal particles in a diameter of 100 nm were prepared and characterized for their stability, uptake and cytotoxicity (Fig.2).



Mean particle size (nm)	Cyanur NLP	Protein-cyanur NLP
Mean number size	81.0	95.2
Mean area size	97.7	120.8
Mean volume size	108.6	176.5

Figure 1. TEM image and particle size distribution of NLP and protein conjugated NLP.

The encapsulation efficiency of miRNA in the nanoliposomes was higher than 95%. The conjugation efficiency of Ephrin-A1 on NLP reached up to 40% when Ephrin-A2 and NLP were mixed in the weight ratio of 1:1. The PEG-modified nanoliposomes showed low toxicity, high cellular uptake and improved transfection efficiency for the lung cancer cells. This miRNA/Ephrin-A1/liposome complex showed inhibition on cell invasion in the wound healing assay (Fig.3).

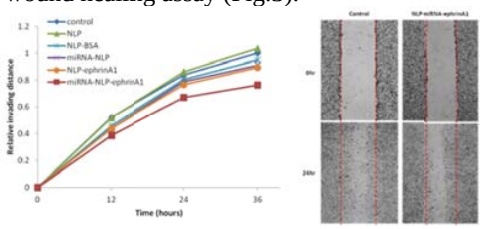


Figure 3. The invading distance from the edge of wound were measured over time and shown in relative distance to the control sample.

In tumor growth assay, these liposome complexes significantly reduced the tumor size and number on 3-D matrigel and showed higher effectiveness when compared to the NLP complex alone (Fig.4a). Intra-pleural delivery of NLP conjugated EphrinA1 and microRNA-302b significantly reduced the tumor burden in mouse model of MPM (Fig.4b).

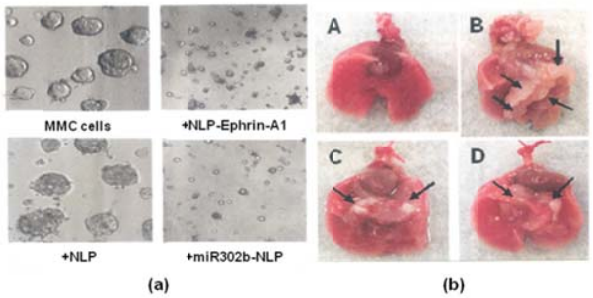


Figure 4. (a) Ephrin-A1 conjugated and miR-302b encapsulated NLPs inhibited MPM tumor growth. (b) Intrapleural delivery of Ephrin-A1 conjugated and miR-302b encapsulated NLPs inhibited MPM tumor growth in vivo. A: control mice lung without tumor; B: mice received tumor alone; C: mice received tumor and treated with NLP-Ephrin-A1; D: mice received tumor and treated with miR302b-NLP. Arrows point to tumor sites.

Conclusions: The targeted delivery of EphrinA1 and miR-302b through nanoliposomal particles suppressed the tumor growth. Thus the engineered Ephrin-A1 conjugated NLP could be a potential therapeutic strategy for in vivo delivery of small RNA molecules to treat the patients with localized tumors such as MPM.

References: [1.] Nasreen N. Cancer. 2006; 15;107(10).
[2.] Nasreen N. Cancer Lett. 2007;258(2):215-22.
[3.] Khodayari N. Cancer Gene Ther. 2012;18(11):806-16.