Combinatorial Therapy of siMDR1 and Doxorubicin to Mediate Drug Resistance in Breast Cancer Models

[1]David Oglesby, [2]Wendy Cornett, and [1]Jeoung Soo Lee
[1]Drug Design, Development, and Delivery laboratory, Bioengineering, Clemson University, Clemson, SC, USA
[2]Surgery Department, Greenville Health System, Greenville, SC, USA

Statement of Purpose: Despite declines in breast cancer mortality due to recent advances in early detection and treatment, highly metastatic forms of breast cancer remain particularly challenging to effectively treat. Current treatments include surgical options, often limited due to challenges of localization, and hormone therapy, which is ineffective in roughly 15%, of cases where neither estrogen, progesterone, nor HER2 are overexpressed [1,2]. Chemotherapy with cytotoxic agents typically requires some convalescence time between treatments – allowing rapidly growing cancer types to develop resistance. Pump-dependent resistance is particularly difficult to treat as it functions through overexpression of P-glycoprotein, an efflux pump which can produce resistance to a range of chemotherapeutics [3]. Our lab has previously developed a micellar, cationic copolymer poly(lactide-co-glycolide)-g-polyethylenimine (PgP) and demonstrated its capacity as a vehicle for intracellular delivery of nucleic acid [4]. Here, we examine the capacity of PgP as a vehicle for co-delivery of Doxorubicin (DOX) and siMDR1 to drug resistant triple-negative cancer cells in order to concurrently treat cells and silence the target gene responsible for their resistance *in vitro*.

Methods: PgP micelles were designed and synthesized to carry hydrophobic drugs in the PLGA core and negatively charged nucleic acids through electrostatic interactions with positively charged amine groups in the corona as previously described [4]. Doxorubicin loading in PgP was assessed absorption at 520 nm by UV/Vis spectrometer. The stability of PgP/siMDR1 was assessed through heparin competition assays using 2% agarose gel electrophoresis. Knockdown efficiency of PgP/siMDR1 was evaluated by RT-PCR in mRNA level and western blot in protein level at 48 hours post-transfection with PgP/siMDR1 at N/P ratios of 30/1, 45/1, and 60/1 in MDA-MB-435 ADR cells in 10% serum-containing media. Cytotoxicity of DXR-HCl combination with PgP/MDR1 polyplex was determined by MTT assay performed after 48 hour incubation with PgP/siMDR1 complexed at N/P of 45:1 followed by incubation in media containing DOX-HCl (13.5 μM). PgP only, PgP/siNT(non-targeting siRNA), and media only were used as controls. Cytotoxicity of DXR loaded PgP (DXR-PgP, 1mg/mL PgP) compared to DOX-HCl and PgP alone was also measured by MTT assay.

Results: PgP was determined to be an effective carrier of DOX, showing approximately 42% loading efficiency limited by solubility of DOX in methanol. Cytotoxicity studies comparing DOX-HCl, PgP alone, and DOX/PgP showed cell viability at a 2.5 μM dose of 99%, 98%, and 90%, respectively in MDA-MB-435 ADR cells. We also found that PgP can effectively bind siRNA and can successfully deliver RNA to cells in serum conditions, and can also be consistently loaded. PgP/siMDR1 polyplexes were shown to mediate knockdown of P-glycoprotein as well as a significant knockdown of MDR1 mRNA up to 63% (**Fig. 2**) in MDA-MB-435 ADR cells compared to an untreated control group. Cytotoxicity studies via MTT assay showed that cell viability of PgP/siRNA was 91%, 76%, and 70% for complexes prepared at N/P ratios of 30/1, 45/1, and 60/1, respectively.

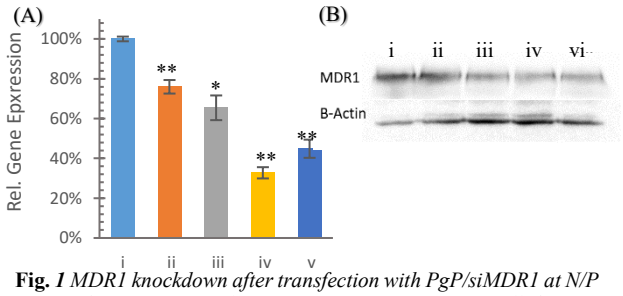

Fig. *1* *MDR1 knockdown after transfection with PgP/siMDR1 at N/P ratios of 30:1, 45:1, and 60:1 in MDA-MB-435 ADR cells* by *RT-PCR (A) and Western blot (B) . i) control, ii) 30:1,iii) 45:1, iv) 60:1 and v) LF 3000, respectively. *:P< 0.001 and **:P<0.0001 compared to control*

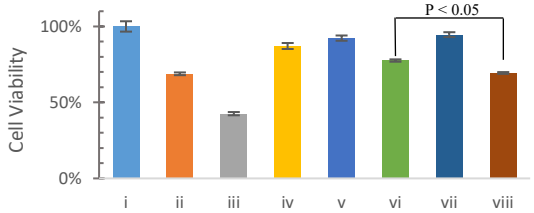

Fig. 2 Cell Viability after sequential treatment *of MDA-MB-435 ADR cells with PgP/siMDR1 N/P 45:1 and DOX-HCl (13.5* μM*), assessed by MTT assay. i) control, ii) DOX-HCl, iii) Dox-PgP, iv) PgP only, v) PgP/siNT, vi) PgP/siNT+DOX, vii) PgP/siMDR1, and viii) PgP/siMDR1+DOX, respectively. As indicated, cell viability of PgP/siMDR1+DOX was significantly lower compared to PgP/siNT+DOX (P<0.05)*

Conclusions: Sequential treatment of PgP/siMDR1 and Doxorubicin-HCl showed a synergistic effect over DOX-HCl treatment alone and was significant when compared to sequential treatment with PgP/siNT. PgP/siMDR1 polyplexes mediated significant knockdown of P-glycoprotein in MDA-MB-435 ADR cells *in vitro*. These results demonstrate the potential of PgP as a vector for RNAi therapy for mediation of pump-dependent drug resistance. Future studies will examine efficacy of the complexed and loaded particle *in vivo*, and functionality of the particle when conjugated with potential targeting moieties.

Acknowledgements: This research was supported by NIGMS of the National Institutes of Health under grant number 5P20GM103444-07

References:

[1] Cleator, S *et al.* (2007) *Lancet Oncol* **8**(3): 235-44
[2] Dent, R. *et al*. (2007) *Clin Cancer Res* **13**(15):4429-34
[3]Creixell, M. *et al.* (2012) *Nano Today* **7**(4)367-379.
[4] Gwak, S.J., *et al*. (2016) *Acta Biomater.* 35:98-108.