Folate-functionalized Polymeric Micelle for Combinatorial Therapy to Overcome Drug Resistant Breast Cancer

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Statement of Purpose: Cancer continues to be one of the leading causes of death worldwide, with breast cancers accounting for nearly fourteen percent of all cancer related deaths in women.[1,2] Prognosis is intimately tied to surface protein expressions, often growth promoting elements such as Human Epidermal Factor Receptor-2 or drug-resistance inducing P-glycoprotein (P-gP) are overexpressed.[3,4] With breast cancers possessing high nucleic acid synthesis requirements, another marker often overexpressed is folate receptor alpha (FA +), a protein with a fortunately limited distribution elsewhere in the body.[5] This potential expression differential was exploited as a means to selectively target breast cancer by conjugating a folate (FA) moiety to the surface of PgP (Poly (lactic-co-glycolic acid)-graft-polyethylenimine) micelle for targeted delivery of siRNA and chemotherapeutic agents.

Methods: PgP was synthesized and characterized by ^{1}H-NMR and GPC previously in our 4D Lab.[6] To synthesize FA-PgP, FA was conjugated to the surface of PgP using Mal-PEG-SVA as a spacer. Following synthesis and purification, the structure of FA-PgP was characterized by ^{1}H-NMR. The feasibility of PgP and FA-PgP as a nucleic acid carrier was evaluated using the Monster Green Fluorescent Protein phMGFP Vector (pGFP (Promega), 2 µg/well) in MCF-7 (FA +) and MDA-MB-468 (FA -) cells in 10% serum-containing media. Transfections were performed by complexing pGFP with PgP, FA-PgP, or PgP/FA-PgP mixed micelle (1/2 w/w ratio) at various N/P ratios and subsequently applying the solutions to MCF-7 (FA +), MDA-MB-468 (FA -) (breast cancer) cells. In order to further characterize target specificity of FA-functionalized micelle, transfections were performed by complexing pGFP with FA-PgP and mixed micelles at N/P ratios of 25:1 in the presence of free FA, to act as a competitive inhibitor of FA mediated internalization pathway, in MCF-7 (FA +) and MDA-MB-468 (FA -) cells. Complexes of pGFP with polyethylenimine and folate functionalized polyethylenimine at N/P ratio of 5/1 were used as positive controls. At 48 hours post-transfection, cells were collected and transfection efficiency was assessed by flow cytometery and epiflourescent microphotography, while cytotoxicity was assessed by MTT assays.

Results: Folic acid was successfully conjugated to PgP and confirmed via ^{1}H-NMR. FA-PgP exhibited selectivity when comparing the transfection efficiencies against PgP in folate receptor alpha positive (MCF-7) and negative breast cancer cell lines (MDA-MB-468). Transfections with FA-PgP exhibited substantial decrease in efficiency compared to PgP in MDA-MB-468 (FA-) cells, with less reductive effect noted in MCF-7 (FA +) cells. A mixed micelle restored transfection in a manner proportional to PgP content (Fig 1). In the presence of free FA, transfection efficacies of both FA-PgP and mixed micelles were substantially impaired in MCF-7 (FA +) but not in MDA-MB-468 (FA -) cells (Fig 2), indicating free FA performed as competitive inhibitor against FA-PgP, providing additional evidence for pathway dependent sequestration.

Conclusions: Currently, we are evaluating FA-PgP or FA-PgP/PgP mixed micelle as a siRNA delivery carrier using siRNA targeting P-glycoprotein (P-gp siRNA) in MDA-MB-435 ADR (FA+). Future work includes utilizing combinatorial therapy of micelle/P-gp siRNAs with chemotherapeutics such as Doxorubicin or Paclitaxel to overcome drug resistance in breast cancers.

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References: 1. Sun et al, Nanoscale, 5:845-859 (2013) 2. Sayed et al, Ultrasonics, 53:979-91 (2013) 3. Hicks and Kulkarni, 129:263-273 (2008). 4. Thomas and Coley. Cancer Control. 10:159-165 (2003). 5. Meier et al, Radiology. 255:527-535 (2010). 6 Lee et al. Trans SFB p.917 (2010)

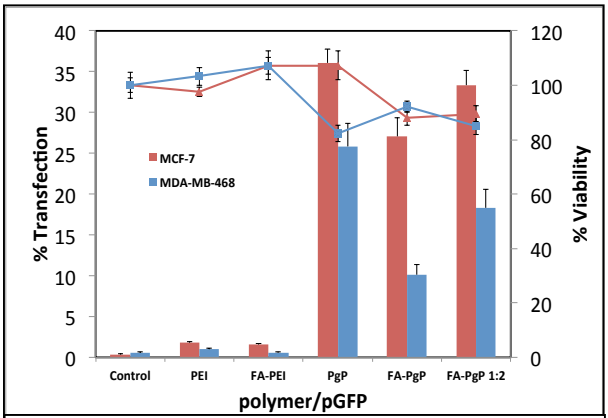

Figure 1: Transfection efficiency of various polyplexes in MCF 7 (FA+) and MBA-MB-468 (FA-) cells in 10% serum condition. Plasmid: pGFP, PEI or FA-PEI polyplexes: 5/1 N/P ratio; PgP, FA-PgP, FA-PgP 1:2 (FA-PgP/PgP (W/W)) : 25/1 N/P ratio

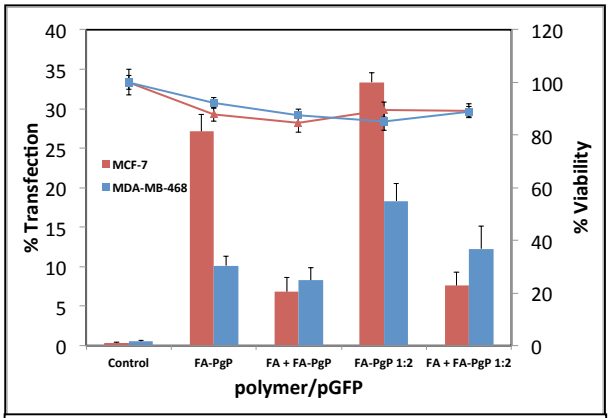

Figure 2: Transfection efficiency of various polyplexes in MCF 7 (FA+) and MBA-MB- 435 (FA-) (N/P). N/P ratio: 25/1, FA-PgP 1:2 = FA-PgP/PgP (w:w ratio), FA concentration in media:750 umol/L. (N=9) Data represents the mean ±SE.