assays yielded results that were consistent with ELISA data and provided proof-of-principal for an inexpensive, portable assay (fig. 1). Transfections of normal cells produced negative results (not shown).

Preliminary in vivo experiments in an orthotopic model of transitional cell carcinoma show that reporters are detectable in the urine samples of tumor-bearing mice, but concentrations remain at background levels in treated mice that did not bear tumors.

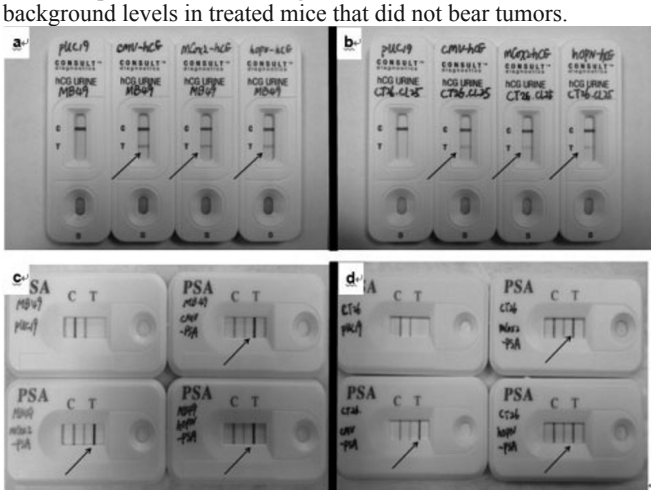

Figure 1. Lateral flow assays, indicating βhCG levels (panels a, b) and PSA levels (panels c, d) in the supernatants of bladder cancer (MB49 cells, panels, a, c) and colon cancer (CT26.CL25, panels b, d) following delivery of expression-targeted reporter plasmids under the control of pcmv, pcox2, or popn. The promoters used are indicated on each panel.

669. A Tumor Targeting Delivery System for Realizing siRNA as an Anti-Cancer Nanomedicine

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The field of siRNA as potent sequence-selective inhibitors of transcription has potential as anti-cancer therapeutics. However, until now low transfection efficiency, poor tissue penetration and non-specific immune stimulation by in vivo administered siRNAs have delayed their therapeutic application. Their potential as effective therapeutics hinges on the availability of a vehicle that can be systemically administered, safely and repeatedly, and will deliver the siRNA specifically and efficiently to the target tissue. A platform nanodelivery system has been developed comprising a self assembled, biodegradable, cationic liposomal nanoparticle, which bears targeting molecules that home to receptors, such as the transferrin receptor, on the surface of tumor cells. When systemically administered, this tumor-targeting nanocomplex can efficiently and selectively deliver molecular therapeutics to not only primary tumors, but also metastases and has the capability to cross the. We have successfully encapsulated and delivered plasmid DNA, miRNA/siRNA/AS ODN, MRI contrast agents, small molecules, and chemotherapeutic agents. Use of this nanocomplex for gene therapy has been shown to dramatically sensitize a number of human tumors in mouse models to radiotherapy and chemotherapy. This synergy has resulted in long term tumor elimination and life span prolongation in the animals. The nanocomplex carrying the wtp53 gene (SGT-53) is currently being evaluated in a number of human clinical trials where it has been shown to be well tolerated at therapeutic doses and has demonstrated anti-cancer activity both alone and in combination with standard chemotherapy.

This tumor-targeting nanoparticle delivery vehicle can also deliver siRNA to both primary and metastatic disease. We have also enhanced the efficiency of this complex by delivery of a modified hybrid (DNA-RNA) anti-HER-2 siRNA molecule. Scanning Probe Microscopy confirms that this modified complex maintains its nanoscale size. More importantly, we show that this nanoimmunolipsome-anti-HER-2 siRNA complex can sensitize human tumor cells to chemotherapeutics, silence the target gene and affect its downstream pathway components in vivo, and significantly inhibit tumor growth in a pancreatic cancer model. Thus, this complex has the potential to help translate the potent effects of siRNA into a clinically viable anticancer therapeutics.

670. P-Glycoprotein Knock-Down by Chitosan/MDR1-siRNA Nanoparticles Increases Sensitivity to Chemotherapeutics

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Purpose: Chemotherapy of Breast primary tumors can lead to the emergence of multidrug resistance (MDR) cancer cells. MDR cancer cells overexpress P-glycoprotein (P-gp) drug efflux pumps that desensitize them to chemotherapy. Although some chemical modulators aim to overcome multidrug resistant by interfering with the function of MDR1 gene, their toxicities limit clinical use. Consequently, small interfering RNA (siRNA) mediated sequence specific inhibition of the expression of MDR1 mRNA and resulting P-gp Knockdown may be effective in reversing MDR phenotype and increase the success of chemotherapy. Here we examine the ability of polymer/siRNA nanoparticles (NP) using the natural polysaccharide chitosan (CS) for efficient MDR1/siRNA cytosolic delivery and downregulation of MDR1 mRNA.

Methods: Nanoparticles were prepared by mixing siRNA specific to MDR1 with chitosans of specific molecular weight (MW), degree of deacetylation (DDA) and ratio of chitosan amine to RNA phosphate (N:P ratio). Physicochemical characterization and in vitro analysis in MCF-7 and MCF-7/MDR breast cancer cells by ESEM, DLS, SDS-PAGE, Western Blot and qRT-PCR, were performed in order to evaluate the potential of chitosan/siRNA based nanoparticles for P-gp knockdown in a breast cancer cell line.

Results : Chitosan/MDR1-siRNA nanoparticles size and ζ-potential varied from 80 to 150 nm and 20 to 30 mV respectively (Fig.1A). Chitosan/MDR1-siRNA nanoparticles were stable for at least 20 hours and were able to protect the RNA cargo when challenged with supra-physiological concentrations of nucleases. In vitro tests showed that nanoparticles uptake efficiency was ranged from 75 to 99% and that the MDR1 gene was silenced up to 60% (Fig.1B) with clear evidence of consequent P-gp knock-down (Fig.1C).

Conclusion: The delivery of MDR1/siRNA using chitosan based nanoparticles is a promising approach to reverse drug resistance and to increase breast cancer cells sensitivity to chemotherapeutics via MDR1 gene silencing.

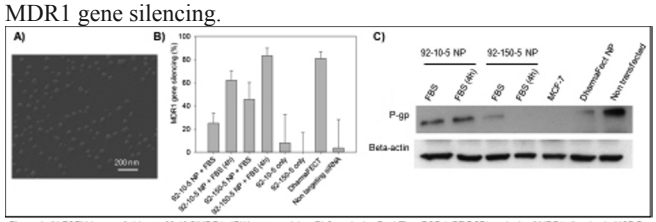

Figure 1: A) ESEM image of chitosan 92-10-5/MDR1-siRNA nanoparticles. B) Quantitative Real-Time PCR (qRT-PCR) analysis of MDR1 silencing in MCF-7 MDR cells transfected with chitosan /MDR1-siRNA nanoparticles with FBS or with FBS added 4hours post-transfection. C) Western blot analysis of P-gp expression levels in MCF-7 and MCF-7 MDR cells transfected with chitosan/MDR1-siRNA nanoparticles.