Functional polyesters enable selective small RNA delivery to orthotopic lung tumor cells over normal cells

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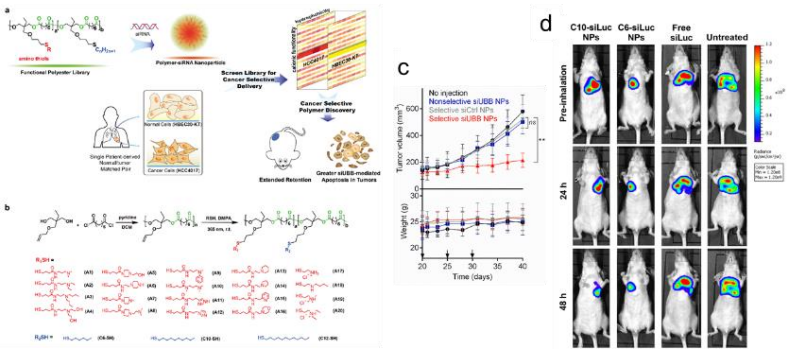
Statement of Purpose: Conventional chemotherapeutics nonselectively kill all rapidly dividing cells, which produces numerous side effects. To address this challenge, we report the discovery of functional polyesters that are capable of delivering small RNA drugs selectively to lung cancer cells and not to normal lung cells. Selective polyplex nanoparticles (NPs) were identified by high-throughput library screening on a unique pair of matched cancer/normal cell lines obtained from a single patient.[1] Selective NPs promoted rapid endocytosis into HCC4017 cancer cells, but were arrested at the membrane of HBEC30-KT normal cells during the initial transfection period. When injected into tumor xenografts in mice, cancer selective NPs were retained in tumors for over one week, while nonselective NPs were cleared within hours. This translated to improved siRNA-mediated cancer cell apoptosis and significant suppression of tumor growth. Selective NPs were also able to mediate gene silencing in xenograft and orthotopic tumors via intravenous injection or aerosol inhalation, respectively. Notably, we found that by altering the administration route from i.v. to aerosol, the NPs could avoid liver accumulation and instead be specifically localized only in the lungs. This resulted in significant gene silencing in the HCC1299 and A549 orthotopic lung tumors.[2] Due to the ability to deliver small RNAs to non-liver targets, this approach provides a privileged route for gene silencing in the lungs. Overall, this work highlights that different cells respond differentially to the same drug carrier, an important factor that should be considered in the design and evaluation of all nanoparticle carriers. Because no targeting ligands are required, these functional polyester NPs provide a new approach for selective drug delivery to tumor cells that may improve efficacy and reduce adverse side effects of cancer therapies.

Methods: Polymer synthesis. A scalable (100+ gram) synthetic strategy based on the polymerization of trimethylolpropane allyl ether (TPAE) with diacid chlorides (poly(TPAE-co-AC)s) was employed.[3] >800 structurally unique polyesters were prepared by thiol-ene modification of base polymers with different molecular weights.[1] **Tumor models.** For the orthotopic tumor model, 3 million HCC1299-Luc or A549-Luc cells (25 µL, 50% Matrigel) were orthotopically implanted to the left lung of the nude mouse to develop single module orthopotic lung tumor. Strong luciferase signal was detected by bioluminescence imaging in 2-3 weeks after surgical implantation.

Results: Cancer selective NPs were identified by utilizing a matched tumor/normal cell line pair (HCC4017/HBEC30-KT) derived from the normal and cancerous lung tissues of a single patient (**Fig. 1a-b**). Selective and Nonselective siUBB nanoparticles were injected into HCC4017 xenograft tumors to compare selective delivery potential *in vivo*. Only Selective Nps reduced tumor burden (**Fig. 1c**). Aerosolized NPs can deliver siRNA *in vivo* to surgically implanted orthotopic lung tumors. The decrease of luminance intensity in the lung indicates the siLuc-mediated luciferase knockdown in orthotopic A549 tumors after inhalation of siLuc NPs (1 mg/kg siLuc, 400 µL) (**Fig. 1d**).



Conclusions: Ideal cancer therapeutics accurately hit tumors and avoid side effects on healthy cells. We utilized a patient-derived pair of matched cancer/normal cell lines to discover selective nanoparticles that could deliver a cytotoxic siRNA to kill cancer cells and not normal cells. The finding that cells respond differently to the same nanoparticle has profound implications for gene therapy because cell type specificity of drug carriers *in vivo* could alter clinical patient outcomes. Our data suggest that selectivity is an underappreciated reality that should be carefully considered when evaluating drug carriers. The combination of well-defined molecular targets and nanoparticle delivery to targeted cells are likely both required to improve cancer drug accuracy in the clinic.

References: (1) Yan, Y.; Liu, L.; Xiong, H.; Miller, J. B.; Zhou, K.; Kos, P.; Huffman, K. E.; Elkassih, S.; Norman, J. W.; Carstens, R.; Kim, J.; Minna, J. D.; Siegwart, D. J. Functional polyesters enable selective siRNA delivery to lung cancer over matched normal cells. *Proc. Natl. Acad. Sci. U.S.A.* **2016**, *113*, E5702-E5710.
(2) Yan, Y.; Zhou, K.; Xiong, H.; Miller, J. B.; Motea, E. A.; Boothman, D. A.; Liu, L.; Siegwart, D. J. Aerosol delivery of stabilized polyester-siRNA nanoparticles to silence gene expression in orthotopic lung tumors. **2016**, Submitted, under external review.
(3) Yan, Y.; Siegwart, D. J. Scalable synthesis and derivation of functional polyesters bearing ene and epoxide side chains. *Polym. Chem.* **2014**, *5*, 1362-1371.