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Liposome-aided metabolic engineering of tumor surface immunogenicity

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ABSTRACT

Approaches to increase tumor immunogenicity are of therapeutic potentials. We herein reported the use of liposomes for covalent incorporation of neoantigen on tumor surfaces with DNP-conjugated sialic acid (^{DNP}Sia). Relative to free ^{DNP}Sia, sugar-encapsulated biotinylated liposomes (^{DNP}Sia@LP@biotin) enables effective cell surface expression of ^{DNP}Sia on biotin receptor (BR)-expressing cells over BR-free cells in vitro, and on tumor cell surfaces with high tumor-to-normal tissue contrast in a mice model. These findings suggest the potentials of targetable liposomes for modulating tumor surface immunity via metabolic oligosaccharide engineering.

Cancer cells often evade immune system surveillance, necessitating effective approaches to redirect the immune system against tumors, such as cytotoxic T cells genetically engineered with chimeric antigen receptors[1–3] or ligand–antigen adaptor molecules targeting cell surface receptors.[4–10] Previously, we reported the covalent incorporation of "non-self" immunogen to the glycocalyx of tumor cells using a dinitrophenyltaed sialic acid,[11] which is located at the exterior of cell surface to recruit antibodies. Albeit markedly suppressed melanoma metastasis in mice, the intravenously injected sialic acid analog preferentially accumulates in tumor foci and yet is obviously present in healthy tissues such as heart.[11] As such, it is of practical interest to enhance in vivo expression of immunogens on tumor cell surfaces with high tumor-to-healthy tissue contrast.

Liposomes accumulate in tumor tissues via the enhanced permeability and retention (EPR) effect[12–14] and have been widely used to encapsulate water-soluble drugs for drug delivery.[15,16] In addition folate-targeted liposomes have been exploited for investigations on tumor-associated sialoglycoproteins.[17,18] As such, we envisioned that ^{DNP}Sia-encapsulating liposomes with surface displaying ligands cognate to tumor receptors would be of use to facilitate selective and enhanced metabolic incorporation of ^{DNP}Sia into tumor surfaces (Scheme 1).

Biotin has been used to ferry diverse functional molecules into tumors by targeting cell surface biotin receptor (BR).[19,20] Based on these findings, we first assessed the use of biotin-displaying liposomes to target BR-expressing tumor cells. As a proof of concept, we first prepared 5-carboxyfluorescein (CF) doped nanoscaled liposomes (CF@LP@biotin), via the film dispersion method,[21] using DPPC, cholesterol, DSPE-PEG2000, and DSPE-PEG2000-Biotin (60:40:4:1 M ratio) dispersed in aqueous solution of CF. In parallel, Biotin–free liposomes was prepared as the control (CF@LP). CF@LP@biotin was incubated with BR-expressing cell lines including HeLa cells, A549 and MCF-7 or BR-free NIH3T3 cells[19,22,23] for 15 min at 37 °C. Bright fluorescence was identified on cell surface of BR-expressing cells whereas no signals were identified on NIH3T3 cells (Fig. 1A). In contrast, dim signals were observed on HeLa, A549 and MCF-7 cells incubated with CF@LP devoid of biotin (Fig. 1B). Next, HeLa cells were cultivated with CF@LP@biotin for varied periods of time. Confocal microscopy revealed bright fluorescence inside cells after 6 h incubation (Fig. S6), indicative of efficient uptake of these biotinylated liposomes by cells. Collectively, these results validated the use of biotinylated liposomes for selective targeting of BR-expressing cells.

With the demonstrated selective uptake of CF@LP@biotin of by BR^+ cells, we synthesized ^{DNP}Sia bearing a 3, 5-dinitrobenoyl group at C-9 via amidation of 9-amino-9-deoxy sialic acid (Fig. 2). Compared to the previous DNP-Sia diad bridged by an amino linker,[11] ^{DNP}Sia features an amide linker and could be prepared in a much higher yield (Supporting information). We then prepared ^{DNP}Sia-encapsulated liposomes (^{DNP}Sia@LP@biotin) with diameters of at 195 nm (Fig. S1). Reversed-phase HPLC and UV absorption spectrum analysis showed that the molar ratios of ^{DNP}Sia to lipid were approximately 1:2 (Figs. S4–S5),

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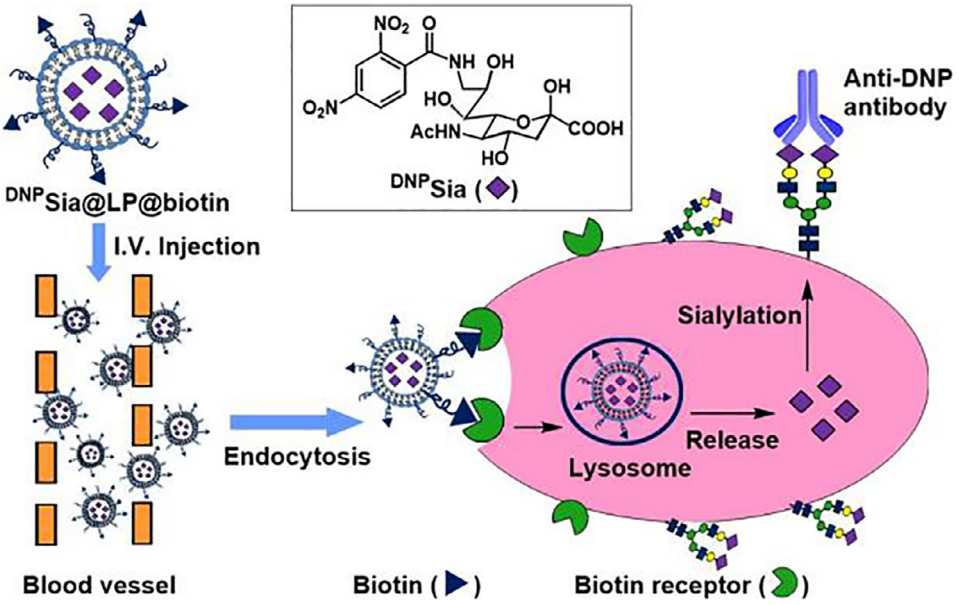

Scheme 1. Schematic for selective tumor surface engineering with liposome-aided delivery of ^{DNP}Sia.

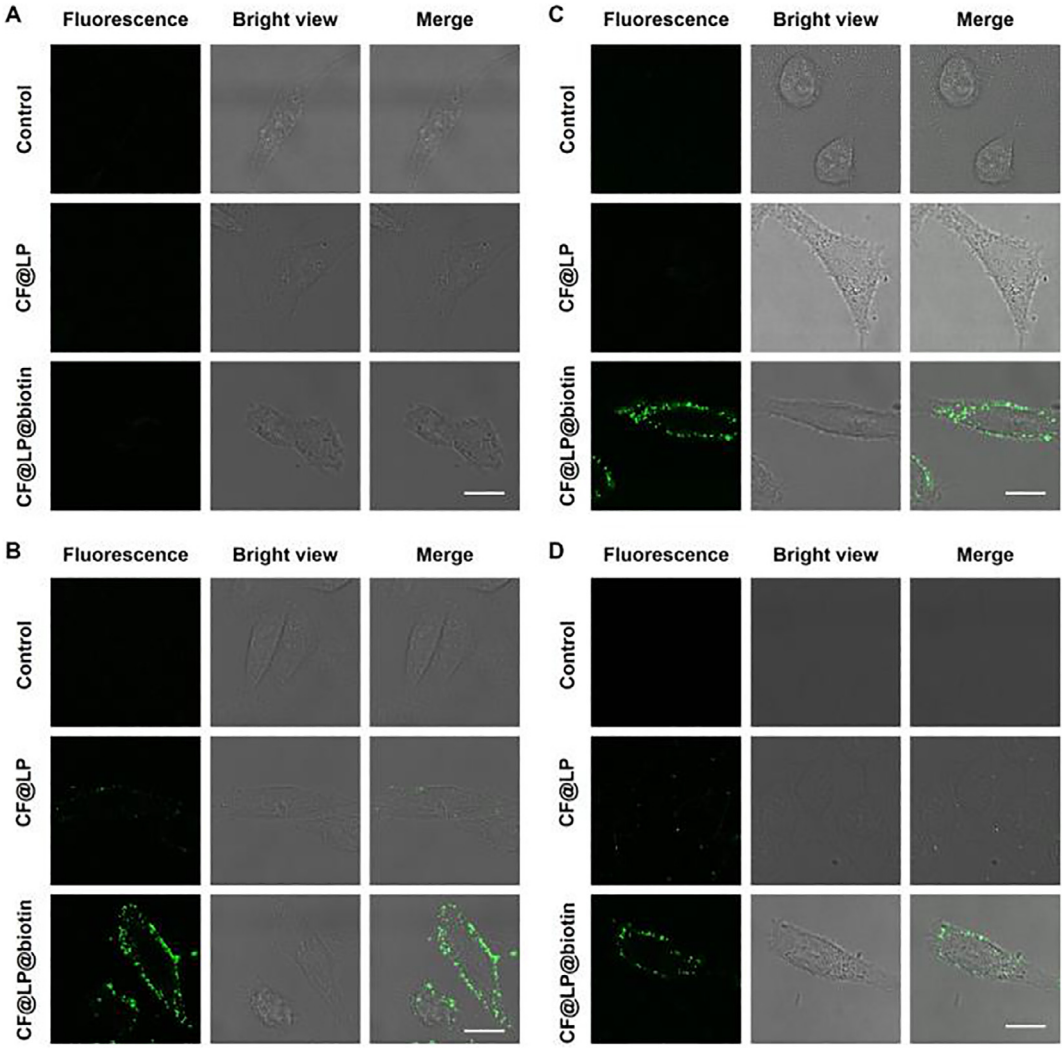

Fig. 1. Preferential binding of CF@LP@biotin with BR^+ cells. NIH3T3 (A), HeLa (B), A549 (C) and MCF-7 (D) cells were treated with CF@LP@biotin or biotin-free CF@LP for 15 min before confocal microscopy analysis. Scale bars: 25 μm.