Using vesicle lipid domains to enhance liposomal TRAIL

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Statement of Purpose: The spatial patterning of targeting proteins on liposome surfaces may offer a new strategy to control nanoparticle-cell interactions and enhance the efficacy of drug delivery. This idea is inspired by lipid rafts, phase segregated lipid regions present on cellular membranes, that play an important role in protein-protein interactions and downstream signaling processes. For example, TRAIL (Tumor necrosis factor-related apoptosis inducing ligand), a protein that induces apoptosis in cancer cells, is highly dependent on lipid raft formation for efficient signaling. Lipid phase segregation can be induced in synthetic vesicles, or liposomes, by mixing ternary compositions of saturated lipids, unsaturated lipids, and cholesterol. By controlling protein conjugation to saturated or unsaturated lipids, vesicles can localize proteins to specific domains, which has been demonstrated in giant unilamellar vesicles.[1] However, there has been limited exploration of vesicles with lipid domains for drug delivery applications. Furthermore, the effect of concentrating therapeutic proteins on vesicle lipid domains is unknown. In this work, we study the effects of lipid domains on liposomal TRAIL cytotoxicity. TRAIL has been shown to be more effective conjugated to liposomes, but the effects of mimicking TRAIL lipid rafts has not been explored.[2] Using Förster resonance energy transfer (FRET), we investigated how we could modulate the size of lipid domains in vesicles. We then explored the effects of concentrating liposomal TRAIL in lipid domains of different sizes on cytotoxicity. This work demonstrates the effect of mimicking native cell membrane environments on TRAIL interactions with cancer cells.

Methods: Small unilamellar vesicles of 100 nm were prepared comprised of 30 mol% cholesterol and varying amounts of 18:1 (Δ9) 1,2-dioleyoyl-sn-glycero-3-phosphocholine (DOPC) and 18:0 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) using thin-film hydration methods previously described.[3] FRET studies were conducted as described to confirm lipid segregation as a function of membrane composition.[3] For conjugating His-TRAIL, 1.0 mol% 18:1 1,2-dioleoyl-sn-glycero-3-[(N-(5-amino-1-carboxypentyl)iminodiacetic acid)succinyl] (nickel salt) (DGS-NTA(Ni)) and 1.0 mol% 18:0 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (DSPE-PEG2000) was added to vesicles. For TRAIL cytotoxicity studies, His-TRAIL (1 μg/mL, R&D Systems) was conjugated to vesicles containing DGS-NTA (5 mM) for 1 hour at 37°C, then dialyzed overnight with 100 kDa filters. TRAIL-vesicles were then incubated with Jurkat cells plated at 25,000 cells/well in a 96 well plate for 24 hours, and viability was detected using CellTiter-Glo (Promega).

Results: First, we wanted to determine if domains were present on 100 nm small unilamellar vesicles similar to those previously reported in giant unilamellar vesicles. To do this, we chose FRET dyes conjugated to one unsaturated lipid (NBD) and one saturated lipid (Rhod). In the absence of domains, the FRET pairs should be close in proximity, which leads to a lower FRET ratio. In the presence of domains, the dyes should segregate into different phases, leading to a higher FRET ratio. We observed an increase in FRET ratio as we increased the ratio of DSPC:DOPC composition, indicating the presence of lipid domains and domains of different sizes (Fig 1b). Next, we conjugated TRAIL to the unsaturated domains and looked at cytotoxicity of TRAIL against Jurkat cells. We saw that vesicles with domains exhibited better killing efficiency than vesicles without domains (Fig 1c). Furthermore, as we decreased the size of unsaturated domains, we saw an increase in TRAIL efficacy. Uniformly distributed TRAIL in DOPC vesicles without domains killed about 50% of cells at the highest concentration tested, while vesicles with the smallest domains killed 97% of the cells at the highest concentration.

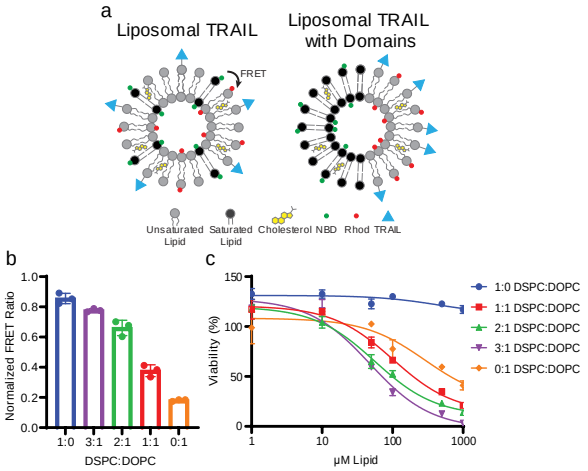

Figure 1: (a) Schematic of liposomal TRAIL with and without lipid domains. (b) FRET assay confirms the presence of lipid domains on 100 nm vesicles. (c) Cytotoxicity study demonstrates liposomal TRAIL with domains increases the efficacy of TRAIL cytotoxicity.

Conclusions: In summary, we demonstrated designed nanometer sized vesicles that exhibited lipid domains increases the efficacy of TRAIL cytotoxicity. Our approach demonstrates how mimicking cellular lipid rafts on vesicles could be a useful approach to enhance protein-protein interactions. In addition, our study provides a new strategy for enhancing liposomal drug delivery through utilizing lipid domains.

References: 1. (Momin, N. *Soft Matter* 2015, *11* (16), 3241–3250.) 2. (Yunker, P. J. *PNAS.* 2016, *113* (3), 608–613.) 3. (Peruzzi, J. A. *Angew. Chemie* 2019, *58* (51), 18683–18690.)