Targeted Delivery of Electromechanical Protein Prestin to the Brain Using Ultrasound

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Background, Motivation, and Objective:
Sonogenetics are promising for *in vivo* neuromodulation owing to ultrasound (US) can non-invasively stimulate cells in deep tissue. However, this system requires accurately transducing US-responsive proteins into target cells. Here, we introduce a non-invasively and non-viral approach to perform intracerebral gene delivery. This approach uses transient ultrasonic opening of the blood-brain barrier (BBB) to transduce neurons at specific site in the brain with engineered US sensing protein (prestin)-loaded microbubbles (prestin-MBs). The prestin is a transmembrane protein which exists in the US-sensing mammalian auditory system, functions as an electromechanical transducer. We further improved its US sensitivity by introducing specific amino acid substitution which frequently occurring in sonar species into mouse prestin. We demonstrated this concept in mice by using US with prestin-MBs to noninvasively modify and subsequently activate neurons within the substantia nigra (SN), showing that this enables spatiotemporal neuromodulation.

Statement of Contribution/Methods:
MBs composed of cationic phospholipid and C_3F_8 loaded mouse prestin plasmid via electrostatic interactions. The mean concentration and size of prestin-MBs were $(33.1 \pm 7.2) \times 10^9$/ mL and 0.9 ± 0.4 μm, respectively. SH-sy5y neuron-like cell and C57BL mice were used in this study. The gene transfection efficiency and BBB-opening region resulted from prestin-MBs with 1-MHz US (pressure = 0.1-0.5MPa, cycle = 50-10000, pulse repetition frequency (PRF): 0.5-5 Hz, sonication time = 60 s) were evaluated by yellow fluorescence protein and Evans blue staining, individually.

Results/Discussion:
The maximum prestin expression with highest cell viability occurred pressure of 0.5 MPa, cycle number of 5000, and PRF of 1 Hz. The cellular transfection rate by prestin-MBs with FUS could be achieved to 20 ± 2.5 %, which was 1.5 fold higher than commercial transfection agents (LT-1). *In vivo* data suggested that the optimal parameters for expressing transgene was 0.5 MPa, 48 h later, in US targeted region, while minimizing the erythrocyte extravasation. This technology provides a noninvasive and high spatial precision manner to delivery US-sensing gene into specific brain regions. Future works include long-term assessing the gene expression and achieving neuromodulation.