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Anti-HER2 scFv-Directed Extracellular Vesicle-Mediated mRNA-Based Gene Delivery Inhibits Growth of HER2-Positive Human Breast Tumor Xenografts by Prodrug Activation

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Introduction: Lack of specific targeting/insufficient genetic material delivery have hampered gene directed enzyme prodrug (GDEPT) therapies. We have developed "EXO-DEPT" exosomes that specifically target $HER2^+$ breast cancer, delivering a gene (as mRNA) that encodes our improved bacterial enzyme, HChrR6 which activates the prodrugs CNOB and CB1954. Drug generated by CNOB is MCHB, which is fluorescent. Exosomes being 'nature's own antigen delivery system' are superior to other delivery vehicles, e.g., viruses and nanoparticles. mRNA is superior to DNA for gene delivery: It is directly translated upon delivery to the cytosol and eliminates the danger of insertional mutagenesis.

Methods: To direct the exosomes specifically to $HER2^+$ cells/tumors, the chimeric protein, EVHB, was constructed composed of high-affinity anti-HER2 scFv antibody (ML39) and lactadherin C1-C2 domains, capable of tethering to exosome surface. Transfection of HEK293 cells with a new plasmid, generated mRNA-loaded exosomes ("plasmid exosomes"); their incubation with EVHB generated plasmid-EXO-DEPT exosomes. CNOB to MCHB conversion was measured from MCHB fluorescence, cell viability by MTT assay. To load exosomes with a plasmid-free method, *in vitro* transcribed (IVT) HChrR6 mRNA was used ("IVT-exosomes") because CB1954 has already been in clinical trials, this prodrug was also used.

Results: Plasmid-EXO-DEPT treatment specifically enabled $HER2^+$ BT474 cells to convert CNOB into MCHB (actinomycin D–independent), showing successful mRNA transfer. These EXO-DEPT+CNOB treatment caused complete growth arrest of orthotopic BT474 xenografts in mice. IVT-EXO-DEPTs proved more efficient; some two-log fewer number along with CB1954 resulted in complete inhibition of the tumor growth. Hematological analyses and pathological examination of several organs showed no signs of toxicity.

Conclusion: This is the first time that functional exogenous mRNA was delivered by exosomes. The EXO-DEPT prodrug regimen successfully treats $HER2^+$ tumors in mice without offsite toxicity. Use of IVT-EXO-DEPTs eliminates the danger of introduction of potentially harmful plasmid genetic material during the treatment. Since the ML39 anti-HER2 scFv can be replaced by other similar targeting ligands, the approach is generic and can treat any cancer/disease in which a receptor is over-expressed. Since exosomes can cross the blood brain barrier, this regimen can be adapted to teat brain metastasized cancer/brain diseases.

Biography:

Dr. Matin has been a full professor at Stanford University for several years and is affiliated with several programs, including the Stanford Cancer Research Institute. He has contributed to many areas of biological research, including discovery of new drugs and therapeutic enzymes and their improvement as well as specific targeting of these to cancer. He did his Ph. D. at UCLA, spent some years in the Netherlands (State University of Groningen), where he directed a research group, before joining Stanford. He is recipient of numerous awards and honors.