Targeted delivery of therapeutic microRNA to HER2-positive breast cancer

Harada M1, Wang JH1, Forterre A1, Delcayre A2, Kanada M1,3, Contag CH1,3, Jeffrey SS4, Matin AC1

1 Microbiology & Immunology, Stanford University School of Medicine, 2 Exothera LLC, 3 Pediatrics, Radiology, Bioengineering, Stanford University School of Medicine, 4 Surgery, Stanford University School of Medicine

Extracellular vesicles (EVs), subpopulations of which include exosomes and microvesicles, are body's natural intracellular communicators that can transfer genetic and other biomolecular information. This led us to hypothesize that EVs could be used as a delivery tool for overcoming some current obstacles in gene therapy, including effective delivery and immunogenicity. Using exosome-display technology, our lab has previously reported success in engineering EVs capable of high level HER2 binding and functional delivery of therapeutic mRNA. Building upon this, we have developed a method to visualize EV targeting by the use of dual function EVs containing specific targeting ligands (anti-Her2 scFv) as well as reporter molecules for imaging.

MicroRNAs (miRNAs) are endogenous small non-coding RNAs which control diverse biological processes by regulating critical genes. Disease-specific miRNA dysregulation is common in almost all human malignancies and drug-resistance-related miRNAs have been identified. Thus, miRNA replacement therapy to restore the cell's normal function is an attractive and physiologically relevant approach for disease control.

In this study, EVs were isolated from transiently transfected HEK293FT cells by differential centrifugation followed by ultracentrifugation. Chimeric protein expression on EVs was determined by Western blot analysis and that of reporters was verified as follows: mCherry by fluorescent microscopy, and Gaussia luciferase by bioluminescent imaging in the presence of coelenterazine. ELISA assays using CD63 antibody revealed the capacity of specific binding of anti-HER2 scFv on EVs. Dual function EVs were internalized by HER2-overexpressing cells at 37°C, as revealed by fluorescent microscopy.

A DyLight Fluor-conjugated miRNA control mimic was co-transfected with anti-HER2 scFv to HEK293 FT cells to generate HER2-targeted EVs loaded with miRNAs. The resulting EVs successfully transferred miRNA to the HER2-overexpressing cell lines, BT474 and SKBR3. Analysis on the functional delivery and cellular localization are in progress to develop EVs as a therapeutic miRNA delivery tool. The success of this approach may provide an effective anti-cancer treatment.