Engineering exosomes towards therapeutics

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Introduction: Therapeutic Potential of Exosomes

Exosomes are extracellular nanovesicles that are naturally secreted by most of the cells in our body for transporting and communicating between tissue and organs. Their unique features and therapeutic potential are summarized in the table below.

Table 1: Therapeutic potential of exosomes.

Traits	Potential Therapeutic Benefit
Small size (~30-100 nm)	Easy transportation throughout the body
Lipid bilayer	Protection of biological cargo
Naturally secreted	No immune response
Ability to cross the blood brain barrier	CNS drug delivery

Approach: Surface Engineering

To harness the therapeutic potential of exosomes, we engineer the surface of the exosomes using a tetraspanin CD63 scaffold or a vesicular stomatitis virus G glycoprotein scaffold.

Tetraspanin CD63

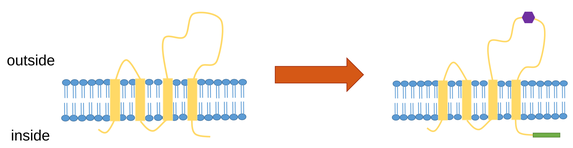

Figure1: Tetraspanin CD63 scaffold unmodified (left) and modified (right).

Benefits of using tetraspanin CD63:

- Natural molecule on exosome surface
- "M" morphology allows variety of options
  - Multiple intracellular and extracellular domains

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Vesicular Stomatitis Virus G Glycoprotein (VSVG)

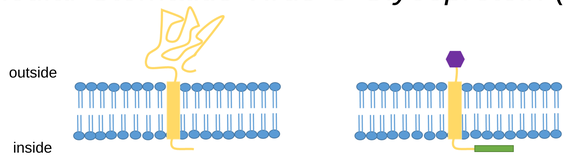


Figure 2: VSVG scaffold unmodified (left) and modified (right).

Benefits of using VSVG:

- Enhanced production and uptake of exosomes
- Simple structure and membrane configuration

Methods

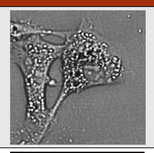
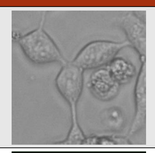
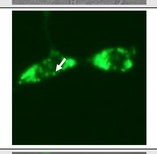
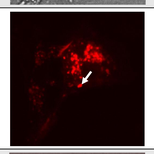
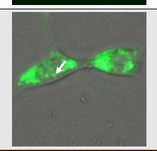
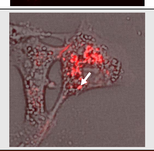
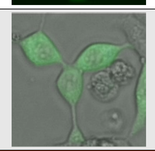
Create Design
- Plasmid design
- 3rd party production of plasmid

Produce the Design
- Transfection of plasmid into mammalian cells
  - Use lipofectamine transfection reagent

Evaluation of Production
- Fluorescent live cell monitoring
  - Searching for localization into exosomes

Results

Table 2: Exosome localization of CD63-GFP and VSVG-RFP in mammalian cells.

	Tetraspanin CD63-GFP	VSVG-RFP	Cytosolic GFP
Phase			
Fluorescent			
Overlay			
	HEK 293	Hep G2	HEK 293

Discussion

Our research has confirmed that tetraspanin CD63 and VSVG are potential scaffolds for the surface engineering of exosomes, displayed by the successful localization of the modified protein into exosomes. Further modifications of these scaffolds could have many applications including therapy, delivery of biologically active cargo, and specifically targeted therapy (Table 2).

Table 3: Potential applications of engineered exosomes.

Application	Proposed Modification
Visualizing exosome biogenesis	Fusion with red/green fluorescent protein (RFP/GFP)
Drug delivery	Loading of drug inside of exosome
Targeted Therapy	Cell-specific targeting molecule on outside surface of exosome

This research can be further advanced through creating designs for multiple purposes such as a TNF-receptor treatment for rheumatoid arthritis and targeted drug delivery for cancers. Our researches focus on exosome-based therapeutics, and our immediate goal is to build up a general strategy for exosome surface engineering using mammalian cells.

References

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