Exosomes are phospholipid membrane-bound nanovesicles produced by living cells to mediate intercellular communication. Exosomes possess an innate ability to deliver macromolecular cargo across biological barriers and evade lysosomal degradation. For these reasons, exosomes have been investigated as potential drug nanocarriers. Exosomes show little evidence of natural tropism. Therefore, it would be beneficial to engineer them to display affinity ligands so that off-target delivery may be reduced. Current engineering methods include genetically engineering parent cells or may be reduced. Current engineering methods to display affinity ligands so that off-target delivery is designed bispecific antibodies that can direct delivery is modeled on engineered ICAM-expressing cells. Targeted exosomes incubated with wildtype ICAM-1 to the vascular endothelium due to its accessibility from the systemic circulation.

Methods

1. Exosome purification and ExTAb decoration

GFP-CD63 exosomes fluoresce green and AF647 labeled ExTAb fluoresce red; colocalization of green and red fluorescence indicate purified and decorated exosomes.

2. Biodistribution of exosomes with I-125 labeled antibodies

Radioactive exosomes were injected and blood was drawn at 5, 30, 60 minutes. Organs were harvested and weighed at 60 minutes. Counts were measured to determine %ID/g.

ExTAb Binding and Exosome Delivery

ExTAb binding affinity to targets was measured by ELISA. Dissociation constant for CD63, PECAM-1, ICAM-1 binding was 4.4 nM, 1.1 nM, and 2.1 nM respectively. Black curve shows specific binding and red curve shows nonspecific binding. In vitro exosome delivery was evaluated through flow cytometry. ICAM-1 targeted delivery was modeled on engineered ICAM-expressing cells. Targeted exosomes incubated with wildtype ICAM-1-non-expressing cells and untargeted exosomes incubated with ICAM-expressing cells showed very little change in GFP delivery compared to cells alone. Targeted exosomes incubated with ICAM-1 expressing cells had a significant shift in GFP fluorescence. ICAM-1 targeted delivery was modeled on mouse endothelial cells. Untargeted exosomes showed no difference in GFP fluorescence in the cells, but PECAM-1 targeted exosomes led to an increase in fluorescence. Decrease in shift could be due to overexpression of ICAM-1 in engineered cells.

In Vivo Biodistribution

In vivo biodistribution was evaluated through flow cytometry. Untargeted exosomes showed no difference in fluorescence in the cells, but PECAM-1 targeted exosomes led to an increase in fluorescence. ICAM-1 targeting causes highest uptake in kidneys while PECAM-1 targeted exosomes also show changes in uptake with targeting to the endothelium. ICAM-1 targeting causes highest uptake in kidneys while PECAM-1 targeting leads to highest uptake in heart.

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