A New Paradigm to Identify Brain-Targeting Ligands

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Being able to selectively deliver therapies to the brain will greatly enhance the treatment of disorders such as Alzheimer's disease by increasing therapy efficiency and decreasing detrimental side-effects.

Current brain delivery strategies employ ligands which bind targets highly expressed on the surface of the brain vasculature. However, such strategies have inherent brain-specificity limitations as the targets are not exclusively expressed on the brain vasculature, leading to increased off-target therapy accumulation in peripheral organs. Hence, alternative approaches to identify targeting ligands are required to achieve truly brain-selective therapy delivery.

We are developing a novel strategy which identifies ligands based not on their increased *binding* to the brain vasculature, but rather on their increased *retention* on the brain vasculature (scheme 1). By exploiting the particularly low rate of endocytosis of brain endothelial cells (EC) (a key aspect of the physiology of the brain vasculature[1]), we should be able to bind ligands to protein targets expressed in the vasculature of both peripheral organs and the brain, but which are endocytically removed from the former while being retained on the surface of the latter. Such ligands selectively retained on the brain vasculature would in turn serve as 'artificial targets' to increase the delivery of therapies specifically to the brain.

We have established the proof of this principle[2] by employing a biotin-conjugated ligand binding the endothelial protein PECAM-1 to deliver avidin-functionalized nanoparticles. We have shown *in vivo* that if the nanoparticles are injected shortly after administration of the anti-PECAM1 ligand, there is strongest targeting to the lung, brain, heart and pancreas, reflecting the expression levels of PECAM1 in these organs. However, as the time-interval increases, targeting to peripheral organs steadily decreases, while targeting to the brain remains constant, reflecting the differential removal of the anti-PECAM1 ligand from the various vasculature surfaces. Such differential removal leads to nanoparticle targeting solely to the brain at long time-intervals (fig. 1), indicating successful generation of artificial targets selectively on the brain vasculature.

Using phage-display technology, we have developed an *in vitro* assay based on this novel paradigm to identify candidate peptides which initially bind to liver, lung and brain EC, but are retained specifically on the surface of brain EC. We have shown that the most promising candidate peptide derived from this screening allows delivery of model proteins into brain endothelial cells.

Figures

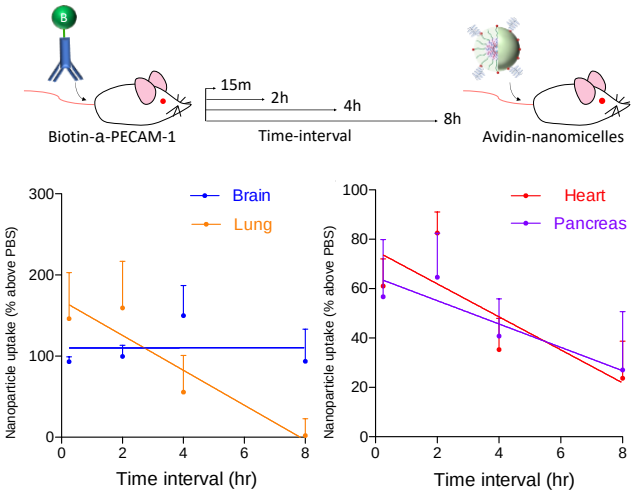

Figure 1. Brain targeting based on selective retention of ligands on the brain vasculature. Avidin-functionalized nanoparticles are targeted to the lung, brain, heart and pancreas when administered shortly after injection of a biotinylated α-PECAM1 ligand. Targeting to peripheral organs decreases with time due to removal of the α-PECAM1 ligand from the vasculature surface, leading to specific brain targeting at long time-intervals.

References

1. Ben-Zvi et al., *Nature*, 2014; 509 (7501): 507-11.
2. Gonzalez-Carter et al, *PNAS*, 2020; 117 (32): 19141-50

Scheme 1. A new paradigm to identify brain-targeting ligands.