244f Profiling Membrane Protein Composition of the Brain Microvasculature Using Combinatorial Antibody Libraries

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Vertebrate brain blood vessels are impermeable to most materials and are therefore called the blood-brain barrier (BBB). As a result of this inherent impermeability, brain vascular endothelial cell membranes control the bidirectional passage of molecules and cells between the bloodstream and the brain. Thus this endothelial-based barrier is a bottleneck for the neuropharmaceutical industry, as 98% of brain drugs cannot cross the barrier to enter the brain. In addition during neurological disease conditions, the BBB is often compromised and the complement of membrane proteins is altered significantly. Thus, to better understand the BBB in health and disease, as well as to devise noninvasive drug transport systems, it is of utmost importance to profile the membrane proteome of the BBB. To this end, we have developed a combinatorial screening strategy that merges yeast antibody surface display technology with BBB endothelial cells. This panning method yields membrane protein-antibody pairs via yeast cell-endothelial cell conjugates without a priori knowledge of the membrane protein or single-chain antibody (scFv) identities. The method was first validated for mammalian cells presenting fluorescein as a model ligand. Specific yeast-mammalian cell conjugates required as few as 2000 fluorescein ligands per cell, and yeast displaying antifluorescein antibodies underwent 10^6-fold enrichment in just three rounds. The method was extended by using a yeast display library of human scFvs to probe the surface protein composition of BBB endothelial cells. After four rounds of selection, scFv clones that specifically bind to RBE4, a BBB endothelial cell line, were identified. A high throughput method was developed to subsequently determine the novelty of the RBE4-binding scFv clones and their relative capacity to mediate yeast-RBE4 cell interactions. Thus far, 11 unique scFv were found among the 120 characterized clones, and the antibody variable regions were derived from the VH3, VH6, and VL1 human germline families. Each of these clones was expressed in soluble form and scFv-mediated RBE4 cell internalization was investigated. The selected BBB-binding scFv clones can be used to clone the cognate RBE4 membrane antigens and could have downstream utility as drug targeting/delivery reagents.