

#	Session	Name	Affiliation	Poster Title
18	Wednesday/Thursday	Nielsen, Morten	University of Aarhus	Sortilin regulates blood-brain barrier integrity
19	Wednesday/Thursday	Nirwane, Abhijit	University of South Florida, Morsani School of Medicine	Capillary pericytes differentiate into microglia and macrophages in ischemic brain
20	Wednesday/Thursday	O'Brown, Natasha	Harvard Medical School	Identification of a novel neuronal signal that induces blood-brain barrier development
21	Wednesday/Thursday	O'Connor, Claire	Smurfit Institute of Genetics, Trinity College Dublin	AAV-mediated regulation of Claudin-5 at the blood brain barrier as a gene therapy based approach for neurological disorders
22	Wednesday/Thursday	Oh, Juhee	University of Minnesota	Murine CNS and bone marrow distribution of the aurora A kinase inhibitor alisertib: pharmacokinetics and exposure at the sites of efficacy and toxicity
23	Wednesday/Thursday	Pareja, Javier	University of Bern, Theodor Kocher Institute	Brain endothelial antigen presentation detains CD8+ T cells at the blood-brain barrier leading to its breakdown
24	Wednesday/Thursday	Porkolab, Gergo	Biological Research Centre, Szeged	cARLA: a small molecular cocktail for robust induction of blood-brain barrier properties
25	Wednesday/Thursday	Power, Liam	Tufts University School of Graduate Biomedical Science, Tufts School of Medicine	Development of a Chronic In Vitro Model of the Blood Brain Barrier based on 3D silk scaffolds
26	Wednesday/Thursday	Pyun, Jae	Monash University	Copper bis(thiosemicarbazone) complex modulates P-glycoprotein expression and function at the blood-brain barrier
27	Wednesday/Thursday	Qin, Zhenpeng	University of Texas at Dallas	Reversibly Modulating the Blood-Brain Barrier by Laser Stimulation of Molecular-Targeted Nanoparticles
28	Wednesday/Thursday	Riviere-Cazaux, Cecile	Mayo Clinic	Glioma reconnaissance using intraoperative microdialysis for extracellular tumor metabolomics
29	Wednesday/Thursday	Rodgers, Louis	University of Kentucky	Repurposing FDA-approved PI3K/Akt Inhibitors to Improve Brain Uptake of Anticancer Drugs in Glioblastoma Resection Models
30	Wednesday/Thursday	Salian, Vrishali	University of Minnesota, Department of Pharmaceutics	Impact of cerebrovascular inflammation positive feedback loop in Alzheimer's disease progression

CARLA: a small molecular cocktail for robust induction of blood-brain barrier properties

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Background

Problem I: species differences at the BBB → translational gap
→ human BBB culture models are needed

Problem II: human stem cell-derived BBB models with a definitive endothelial identity have an incomplete BBB phenotype, especially regarding barrier tightness

Solution: targeting developmentally relevant signaling pathways at the BBB

Hypothesis: activation of Wnt/β-catenin and inhibition of TGF-β signaling converge and interact with the cAMP/PKA pathway in brain ECs

Approach: combination of small molecules (cheap, easy to implement)

the best combination in our screen:

CARLA

Methods

Cells:

- endothelial cells derived from human umbilical cord blood stem cells (Cecchi et al., 2014; PMID: 24936790)
- in monoculture (for the screening) or
- in co-culture with brain pericytes (in all other experiments)

Screening of compounds:

- real time, impedance-based assay (E-plate 96, RTCA SP, Agilent)
- normalized cell index correlates with barrier tightness and adherence

Validation of barrier tightness:

- transendothelial electrical resistance (TEER) measurements (EVOM6 voltohmmeter with STX-2 electrodes)
- solute permeability (1 h permeability on an orbital shaker after 48 h treatment, Horiba Fluorolog 3 spectrofluorometer, P_{app} : apparent permeability coefficient)

RNA-sequencing:

- massive analysis of cDNA ends (MAC-seq)
- DESeq2 for differential expression analysis
- gProfiler for pathway analysis

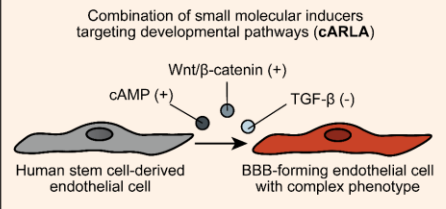
Changes at the protein level:

- immunocytochemistry and confocal microscopy (Leica SP5)
- image analysis in FIJI

Functional tests:

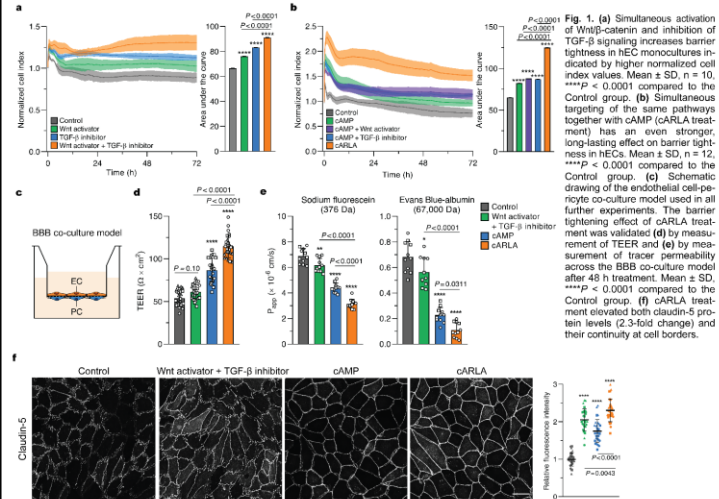
- rhodamine 123 bidirectional (A-B, B-A) transport assay (1 h on an orbital shaker, Horiba Fluorolog 3 spectrofluorometer)
- zeta potential measurement by dynamic light scattering (Malvern Zetasizer Nano)

Summary

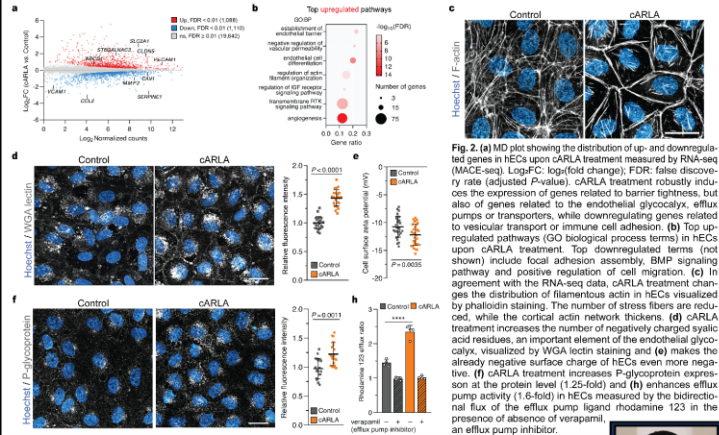


Results

I. CARLA treatment robustly and synergistically enhances barrier tightness



II. CARLA treatment not only improves barrier tightness, but induces a complex BBB phenotype



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