TRANSCRIPTOME ANALYSIS REVEALS ADAPTIVE RESPONSES TO HYPOXIA IN THE CEREBRAL CORTEX OF CHOLESTATIC MICE

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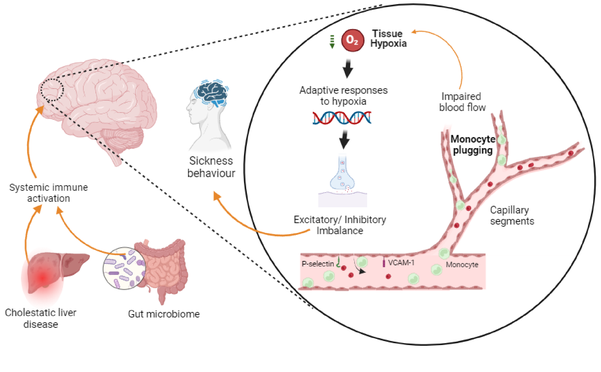
Introduction

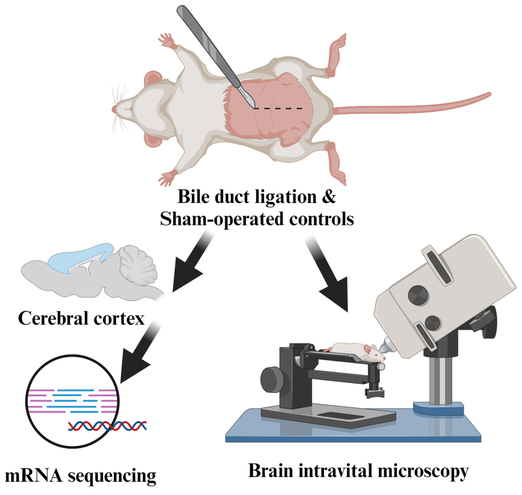
- Patients with cholestatic liver diseases (CLDs) commonly experience adverse disease-associated symptoms including altered mood, fatigue, cognitive dysfunction, decreased motivation, and social withdrawal *that significantly diminish their quality of life(QoL) and run a clinical course independent of liver disease severity*[1-3] (collectively termed sickness behaviors[3-6]).
- How CLD induces alterations in brain function, leading to sickness behaviors is poorly understood and represents a significant knowledge gap that impedes the development of new therapeutic approaches to treat them[7].
- Using near infrared spectroscopy (NIRS) we have shown frontal cerebral cortex (FC) hypoxia associated with significant dysregulation of cortical neurovascular coupling (mechanism linking transient neural activity to change in cerebral blood flow)[8, 9] in patients with the CLD primary biliary cholangitis (PBC)[10]. Findings that remain unexplained.
- Pathways driving hypoxia-related changes in brain function may play a role in driving CLD-associated sickness behavior development. We have previously identified marked accumulation of activated monocytes in cerebral blood [11].
- Therefore, we sued a mouse model of CLD to delineate whether cortical capillary plugging leads to brain hypoxia.

Hypothesis

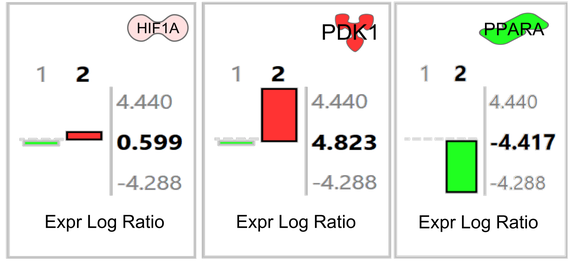
CLD's and associated gut dysbiosis impact systemic immunity and stimulate the migration of inflammatory monocytes from the peripheral circulation into brain cortical blood vessels resulting in capillary plugging and associated tissue hypoxia (Schematic shown below).



Methodology

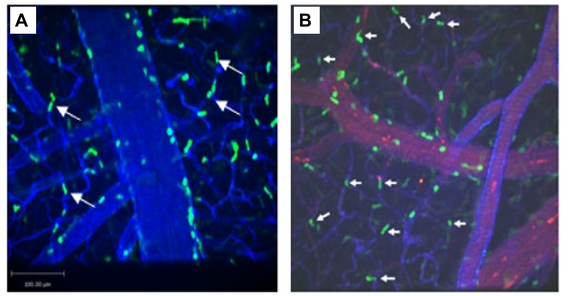


Results

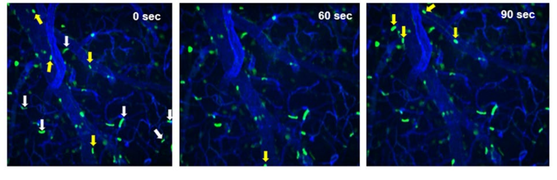


Upregulation of hypoxia-inducible factor HIF-1α and significant alterations of hypoxia-related genes in FC in BDL vs sham mice: RNAseq defined significantly differentially expressed genes (DEGs) in the FC in CLD vs control mice (n=6 mice/grp) and identified increased expression of hypoxia inducible factor1α gene (HIF1α;↑1.8-fold; p<0.013), and altered expression of a number of important hypoxia-linked genes - most importantly pyruvate dehydrogenase kinase 1 (PDK1; ↑124-fold; $p<1.42x10^{-5}$; key enzyme in shift to glycolysis in hypoxia), and peroxisome proliferator activated receptor alpha (PPARα; ↓83-fold; p<0.0062; a nuclear hormone-binding proteins have been implicated in lipid homeostasis).

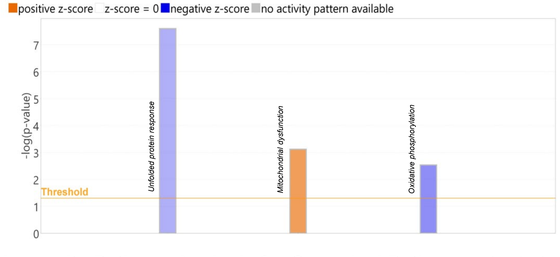
Results



Monocytes accumulate in cortical capillary branches of bile duct ligated (BDL) mice: Representative IVM images from frontal cortex (A) day 5 BDL, (B) day 10 BDL mice. Monocytes are green, capillary endothelium blue (APC anti-CD31) and platelets red (PE anti-CD49b). Note striking monocyte accumulation in small capillary branches in BDL mice (white arrows) compared to sham control where only a small number of capillary patrolling monocytes are seen (data not shown).



Monocytes in small cortical capillary branches in BDL mice are immobile: Representative IVM images from frontal cortex of a day 5 BDL mouse during a 90 sec. observation period. Monocytes are green (GFP+) and endothelium blue (APC anti-CD31). White arrows indicate stationary monocytes in small capillary and yellow arrows indicate monocytes (rolling/adhering along cerebral endothelium) in larger cortical capillaries.



Ingenuity Pathway Analysis for Canonical Pathways: Analysis of DEGs using IPA reveals significant dysregulation in several pathways classically implicated in the hypoxia response in FC of BDL vs sham mice (n=6/grp) , suggesting potential physiological relevance of the FC transcriptional fingerprint in CLD.

Conclusions

- Brain intravital microscopy revealed a marked accumulation of monocytes in the cortical capillaries of CLD that are immobile, suggesting they may contribute to impaired cerebral blood flow.
- RNAseq data defined ~3k significantly altered genes in the FC of BDL vs sham mice; a brain region highly relevant for sickness behavior development.
- BDL mice demonstrate gene expression changes in the FC suggesting tissue hypoxia – similar to findings in PBC patients.
- A therapeutic approach aimed at reducing leukocyte adhesion and capillary plugging could potentially reduce cortical hypoxia and reduce sickness behavior in patients.

Acknowledgements

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