The Role of PI3K/PTEN in resident brain myeloid cells during homeostasis and EAE

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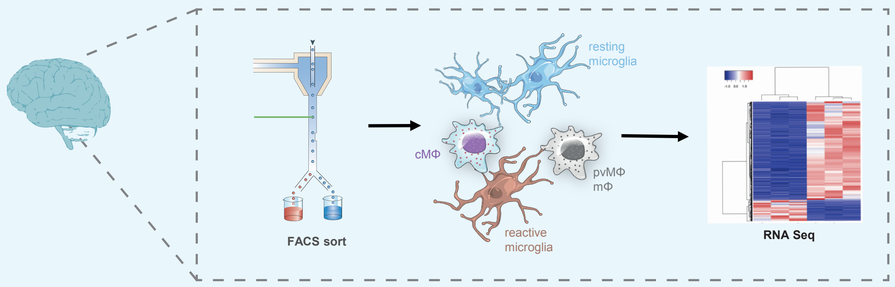
Background and Central Hypothesis

Central nervous system associated macrophage subsets comprising microglia, choroid plexus, meningeal and perivascular macrophages represent crucial immune effector cells that play important roles during health and various neuroinflammatory, neurodegenerative and neurodevelopmental disorders. In multiple sclerosis, a chronic inflammatory autoimmune disease of the CNS, microglia and CNS resident macrophages have been described to contribute to detrimental but also to neuroprotective processes, yet their exact roles are poorly defined und understudied. Therefore, studying the unique signatures and dissecting distinct functions of these resident brain myeloid subsets is of paramount importance. Cellular metabolism and metabolic reprogramming critically dictates myeloid effector functions. The PI3K pathway, a central sensor of environmental cues links immune signals and metabolic fluxes. The main aim of this poject is to generate important knowledge about the unique transcriptional and metabolic signatures of microglia and CNS associated macrophages during health and disease. As cellular metabolism can dictate myeloid cell functions, I hypothesize that PI3K may be critically involved in the metabolic reprogramming of CNS macrophages and aim to shed more light on the complex connection of myeloid cell metabolism and central effector functions in the context of autoimmunedriven CNS inflammation.

Characterization of CNS macrophage populations

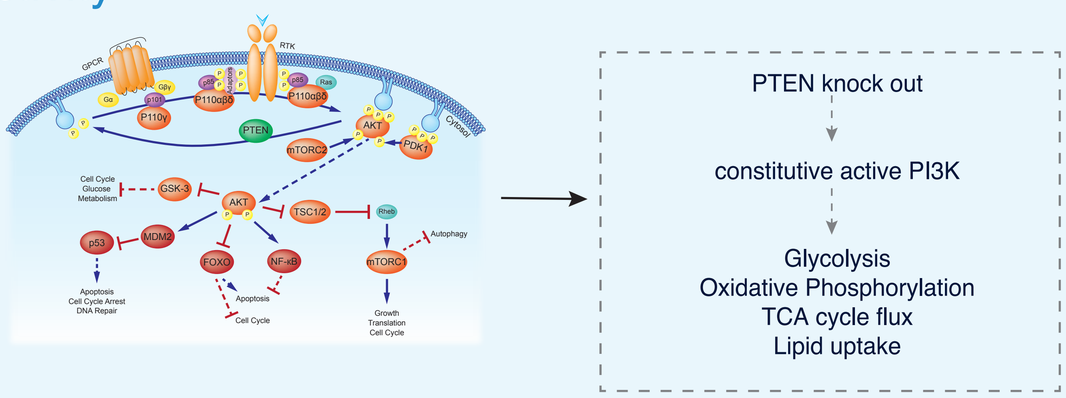


1. What are the unique transcriptional signatures of CNS myeloid cells, microglia, choroid plexus macrophages and other brain macrophages (meningeal and perivascular), during homeostasis?

2. What are the global transcriptional changes in CNS myeloid cells during autoimmune-driven neuroinflammation?

3. What are the transcriptional differences between reactive and resting resident brain myeloid cells at disease peak?

Metabolic profiles of CNS macrophages and PI3K activity



PTEN knock out

↓

constitutive active PI3K

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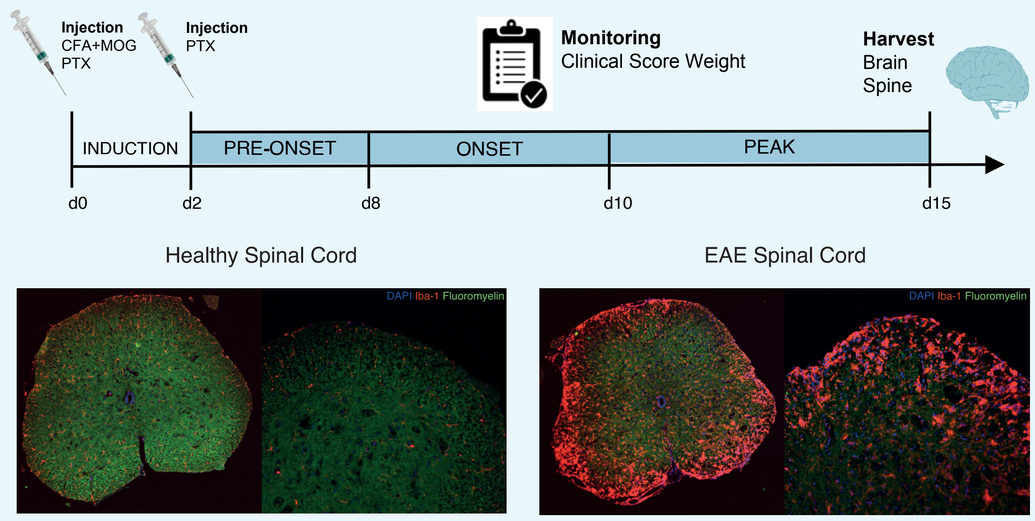
Glycolysis
Oxidative Phosphorylation
TCA cycle flux
Lipid uptake

1. What is the basal metabolic state of CNS-associated macrophage populations?

2. How do metabolic profiles of myeloid cells change upon neuroinflammation? Are profiles different between resident CNS macrophages and infiltrating macrophages?

3. What is the PI3K activity in CNS macrophages and what are the changes upon neuroinflammation. Is PI3K critically involved in immunometabolic changes of resident vs. infiltrating myeloid cells?

Consequences of perturbed myeloid PI3K activity



1. How does PTEN deficiency skew cellular metabolism in microglia and CNS macrophages *in vivo*?

2. What are the *in vivo* consequences of sustained PI3K activity in CNS associated macrophages? By using different genetic Cre-recombinase targeting approaches PTEN deficiency will be differentially induced in the different macrophage populations and contribution to EAE pathogenesis will be assessed.

3. How do PI3K driven metabolic changes in CNS macrophages influence imflammatory properties of CNS macrophages (cytokine production, T cell stimulation)?

4. Does PTEN deficiency lead to the enrichment/depletion of specific metabolites in macrophages or in the CNS microenvironment?

Research Methods

To assess unique transcriptional profiles of the different CNS macrophage populations, cells will be isolated, FACS sorted based on a previously established gating strategy and RNA sequencing will be conducted. For the characterization of CNS macrophages during autoimmune-driven neuroinflammation, the mouse model of MS, experimental autoimmune encephalomyelitis (EAE) will be used.

Metabolic signatures of CNS associated macrophages will be assessed by different approaches which comprise extracellular flux analysis, *in vivo* and *in vitro* lipid and glucose uptake assays and targeted metabolomics.

To determine *in vivo* consequences of skewed myeloid PI3K activity during neuroinflammation, EAE will be conducted in different knock out, reporter and fate mapping mouse strains. Furthermore, clinical score, weight loss, cytokine profile, T cell profiles and histological changes will be assessed. Reactivity state and activity of PI3K signaling of CNS macrophages will be determined by thorough flow cytometric analysis.

Outlook

Overall, I expect these approaches will shed significant light on the signatures of CNS macrophages during health and disease and may reveal perturbations in particular metabolic pathways that could be relevant to the reactivity of these cell types during disease.