Microglial metabolism

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Complex roles for mitochondrial complexes in microglia

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Metabolism impacts various cellular types, and microglia are no exception. Two recent studies in *Nature Metabolism* demonstrate that impairing the mitochondrial respiratory chain, via deficiencies in complex I or complex III, affects microglia in a highly context-dependent manner.

Cells generate energy in the form of ATP using two major metabolic routes – glycolysis and oxidative phosphorylation (OXPHOS). OXPHOS, which occurs in the mitochondrion, is also known as mitochondrial respiration[1]. It involves a series of metabolic processes through which electrons are transferred to acceptor molecules by a chain of protein complexes (from complex I to complex IV), collectively known as the electron transport chain. This process generates an electrochemical proton gradient that ultimately leads to the synthesis of ATP via the ATP synthase (complex V). This electron transport within the electron transport chain, which can happen both in the forward and reverse direction, plays crucial roles in maintaining the balance between energy production and reactive oxygen species (ROS) generation, being essential for cell survival, proliferation and function[2].

Decades of research have shown how OXPHOS shapes cellular metabolism and function, highlighting its contribution to cellular homeostasis, extending well beyond ATP synthesis[1]. While mitochondrial respiration is generally considered crucial for cellular integrity, there are specific contexts where it is not required for cell survival[3], indicating a repurposing of mitochondria to drive diverse cellular metabolic states and needs. As early as 1923, Otto Warburg observed that cancer cells tend to prefer glycolysis over mitochondrial respiration for fast ATP production, even in the presence of oxygen[4]. While it is now established that mitochondrial respiration in cancer cells varies remarkably with disease stage – and can even be dispensable – much less is known about its role in physiology. Furthermore, the specific role of individual mitochondrial complexes in controlling cellular functions in complex, healthy and diseased microenvironments remains poorly understood.

Microglia, the resident innate immune cells of the central nervous system, are emerging as highly metabolically flexible cells that play key roles in various physiological functions, from brain maturation to maintenance of homeostasis, as well as being critical players in infections and diseases[5]. Recent evidence shows that microglia in the developing brain require both glycolysis and OXPHOS to perform efficient phagocytosis[6]. Microglia can rapidly adapt to sudden changes in metabolite availability and sustain their energetic requirements[7]. In the diseased brain (for example, in 5XFAD mice, an Alzheimer's disease (AD) mouse model), these cells progressively enter a general metabolic breakdown associated with a loss of phagocytic capacity, which can be partially rescued by reprogramming their metabolism towards glycolysis[8]. The genetic ablation of the glycolytic enzyme Hexokinase-2 (*Hk2*), which is upregulated in reactive microglia, leads to reduced glycolytic and bioenergetic flow, inducing aberrant inflammatory responses upon lipopolysaccharide injection and in a model of stroke[9], and promoting phagocytosis and amyloid clearance in a model of AD, possibly via increasing lipid metabolism[10].

This collective evidence supports a strong correlation between metabolism and microglial phenotype and function.

In this issue of *Nature Metabolism*, two studies identify a role for mitochondrial respiration in microglia by mechanistically addressing the function of the core subunits of the mitochondrial complex I and complex III[11,12] (Fig. 1).

Mora-Romero and colleagues deleted the core subunit of the mitochondrial complex I NADH–ubiquinone oxidoreductase core subunit S2 (*Ndufs2*) using the *Cx3cr1-cre* mouse line, creating a mouse model deficient for complex I activity in microglia (MGcCI). MGcCI microglia exhibited drastically reduced mitochondrial basal and maximal respiration, increased glycolysis, reduced ATP production and accumulated NADH. Transcriptomic analysis of MGcCI microglia from 1-month-old (juvenile) and 3-month-old (adult) mice revealed enrichment in genes associated with glycolysis, OXPHOS, the pentose phosphate pathway and the one-carbon folate pathway, indicating extensive metabolic rewiring, as seen in lipopolysaccharide-treated monocyte-derived cells. Additionally, both juvenile and adult MGcCI microglia showed the emergence of the disease-associated microglia (DAM) signature and partial repression of the homeostatic signature. Time-dependent changes were observed in mTORC and TNF signalling pathways, downregulated in juvenile and enriched in adult MGcCI microglia.

The authors then assessed microglial phagocytic capacity, both under physiological conditions and after administering sub-epileptogenic doses of kainate to induce hippocampal neuronal apoptosis in vivo. Adult MGcCI microglia exhibited altered morphology and weakened housekeeping phagocytosis. Interestingly, both juvenile and adult MGcCI mice had increased astrocytic reactivity, especially in cortical areas, and an enrichment of inflammatory genes characteristic of reactive astrocytes in neurodegeneration, including TNF signalling via NFkB and IFN-α-related responses.

Microglia depletion through the colony stimulating factor 1 receptor (CSF1r) inhibitor PLX3397 significantly reduced reactive astrocytes in the cortex, unlike the *Lyz2*$^{Cre/+}$; *Ndufs2*$^{flox/flox}$ (LYcCI) mouse model, where CI function is lost in myeloid lineages but not in microglia. Impairment in microglial mitochondrial respiratory chain had no notable impact on synapses in juvenile mice but led to a reduction in presynaptic markers VGLUT and VGAT in adults. Surprisingly, adult MGcCI mice experienced sudden weight loss and died between 3 and 4 months of age, with the exact cause of this early mortality remaining unclear. Whether the observed cellular and behavioural changes are

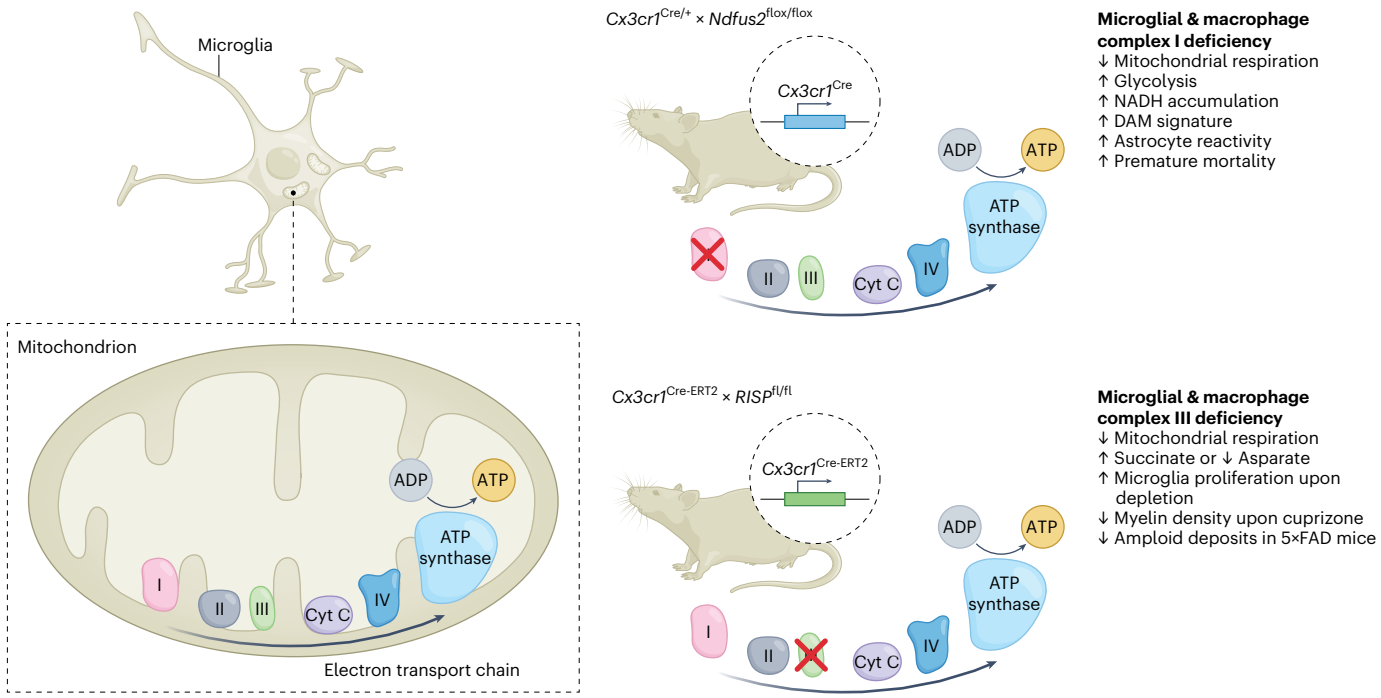

Fig. 1 | Widespread consequences of complex I and complex III deficiency in microglia. The deletion of *Ndfus2* and *Uqcrfs1* (RISP) in microglia and macrophages within mouse models, resulting in deficiencies in complex I and complex III, carries extensive implications that emphasize the essential role of microglial OXPHOS in brain function.

due to intrinsic microglial roles or dysfunctional microglia–astroglia coupling leading to synapse loss and increased astrocytic reactivity, culminating in premature death, is yet to be established.

The different transcriptomic and functional profiles of MGcCI at juvenile and adult stages may reflect the exhaustion of dynamic compensatory adaptations to respiratory chain impairment, in combination with the inherent changes of microglial activation states at different ages. These data suggest that mitochondrial complex I is essential for maintaining proper bioenergetics and function in microglia during development, where forward electron transport is primarily used for ATP production. Conversely, recent evidence indicates that in adult mice with chronic neuroinflammation, time-specific genetic and pharmacological perturbation of mitochondrial complex I activity in microglia may have a beneficial role by reducing the production of ROS generated through complex I via reverse electron transfer[13]. This delicate balance between the physiological and pathological roles of mitochondrial complexes is an exciting area for future research.

Stoolman and colleagues instead conditionally deleted *Uqcrfs1*, a gene encoding the Rieske iron-sulfur protein (RISP) subunit of mitochondrial complex III subunit in the microglia of adult mice using the inducible *Cx3cr1*$^{Cre\text{-}Ert2}$ and the *Ai14lsl* tomato reporter. Using novel RISP-cKO mice (*CX3CR1*$^{Cre\text{-}Ert2\text{-}yfp/wt}$ × *RISP*$^{flox/flox}$ × Ai14^{lsl}), the authors assessed the requirement of mitochondrial respiratory chain for microglial survival, proliferation and response after demyelinating injury, as well as in 5xFAD models of neurodegeneration. As with complex I (ref. 11), complex III deficiency in microglia also resulted in reduced mitochondrial respiration, although it is unclear whether this was accompanied by a metabolic reprogramming towards glycolysis.

RISP-cKO led to the accumulation of succinate and reduction in aspartate, which is known to be central for cell proliferation[14]. Paradoxically, however, RISP-cKO microglia displayed an increased proliferative capacity, at least upon depletion with PLX3397. Bulk RNA-seq of microglia from one-year-old mice indicated an enrichment in the DAM signature and a decrease in the expression of TNF-α responsive genes in RISP-cKO mice. Phenotypically, these mice did not exhibit any deficits, showing normal motor and spatial memory function. However, RISP-cKO mice displayed reduced myelin density, as quantified by MRI, during the recovery phase following cuprizone-induced demyelination. Transcriptomic analysis of microglia isolated two weeks into the remyelination phase revealed that, unlike controls, RISP-cKO cells failed to upregulate genes associated with TNF-α-responsive NF-kB activation, known to be necessary for remyelination[15]. The authors further investigated the potential consequences of RISP cKO in 5xFAD mice. Through histological assessment, they observed a decrease in amyloid burden and enhanced plaque coverage by microglia in the hippocampus. However, these changes did not correlate with any improvement in cognitive performance. While these initial findings are intriguing and warrant further investigations, the underlying mechanisms of complex III-driven regulation of microglia responses to myelin damage and regeneration, and AD-like pathology in vivo remain largely unclear.

These two complementary approaches to inactivate the respiratory chain via different complexes underscore the significant roles of microglial OXPHOS. These roles appear to be closely dependent on the age of the mice and the specific context in which they are assessed, highlighting the remarkable heterogeneity and versatility of microglia in both physiological and pathological settings. However, it is

important to emphasize that the significant differences in experimental design and timing of gene inactivation in the two studies make direct comparisons between complex I and complex III deletion challenging. In Mora-Romero et al.[11], complex I is constitutively removed from microglia (and potentially other cell types), resulting in substantial effects. Conversely, in Stoolman et al.[12] microglial complex III deletion is induced only in adulthood. This distinction likely accounts for some of the observed differences: complex I loss leads to early mortality, while conditional complex III loss results in only mild phenotypes under unchallenged conditions.

Following the groundbreaking discovery that, in a mouse model of multiple sclerosis, disease-associated microglia undergo reverse electron transport where mitochondria produce ROS instead of ATP, inhibiting RET was found to reduce oxidative damage and neurodegeneration in the spinal cord and prompted a shift in microglia back to homeostasis[13]. The works by Mora-Romero et al.[11] and Stoolman et al.[12] provide further insights and contexts to appreciate the complex roles of mitochondria in microglial biology. Although these studies offer compelling evidence linking OXPHOS to microglial function, they also raise additional questions about the underlying cellular and molecular mechanisms. They suggest potential impacts on other core mitochondrial functions and signalling properties that contribute to homeostasis and the resolution of inflammation in response to both intrinsic and extrinsic cues. Notably, whether the inactivation of complex I and complex III also impacts on ROS production[13], or more generally whether complexes of the respiratory chain play moonlight functions in microglia, beyond their canonical enzymatic roles is an intriguing possibility that remains to be investigated.

Overall, these findings strengthen the evidence that metabolic reprogramming is crucial for microglial functions in both the healthy and diseased brain. They also suggest that targeting mitochondrial metabolism in microglia holds the promise of inspiring a new set of precise therapeutic interventions for acute and chronic central nervous system disorders.

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References

1. Zhu, J. & Thompson, C. B. *Nat. Rev. Mol. Cell Biol.* **20**, 436–450 (2019).
2. Lennicke, C. & Cochemé, H. M. *Mol. Cell* **81**, 3691–3707 (2021).
3. Li, A. M. & Ye, J. *Annu. Rev. Cancer Biol.* **8**, 35–58 (2024).
4. Thompson, C. B. et al. *Nat. Metab.* **5**, 1840–1843 (2023).
5. Paolicelli, R. C. et al. *Neuron* **110**, 3458–3483 (2022).
6. He, D. et al. *Immunity* **55**, 159–173.e9 (2022).
7. Bernier, L. P. et al. *Nat. Commun.* **11**, 1559 (2020).
8. Baik, S. H. et al. *Cell Metab.* **30**, 493–507.e6 (2019).
9. Hu, Y. et al. *Nat. Metab.* **4**, 1756–1774 (2022).
10. Leng, L. et al. *Nat. Metab.* **4**, 1287–1305 (2022).
11. Mora-Romero, B. et al. *Nat. Metab.* https://doi.org/10.1038/s42255-024-01081-0 (2024).
12. Stoolman, J. S. et al. *Nat. Metab.* https://doi.org/10.1038/s42255-024-01080-1 (2024).
13. Peruzzotti-Jametti, L. et al. *Nature* **628**, 195–203 (2024).
14. Sullivan, L. B. et al. *Cell* **162**, 552–563 (2015).
15. Arnett, H. A. et al. *Nat. Neurosci.* **4**, 1116–1122 (2001).

Competing interests

S.P. is founder, CSO and shareholder (>5%) of CITC Ltd.