



Loss of MPC1 reprograms retinal metabolism to impair visual function

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Glucose metabolism in vertebrate retinas is dominated by aerobic glycolysis (the “Warburg Effect”), which allows only a small fraction of glucose-derived pyruvate to enter mitochondria. Here, we report evidence that the small fraction of pyruvate in photoreceptors that does get oxidized by their mitochondria is required for visual function, photoreceptor structure and viability, normal neuron–glial interaction, and homeostasis of retinal metabolism. The mitochondrial pyruvate carrier (MPC) links glycolysis and mitochondrial metabolism. Retina-specific deletion of MPC1 results in progressive retinal degeneration and decline of visual function in both rod and cone photoreceptors. Using targeted-metabolomics and ¹³C tracers, we found that MPC1 is required for cytosolic reducing power maintenance, glutamine/glutamate metabolism, and flexibility in fuel utilization.

MPC | retinal degeneration | mitochondrial metabolism | pyruvate | glutamine

Vertebrate retinas favor aerobic glycolysis, also called the “Warburg effect.” Retinas convert 80–96% of glucose into lactate rather than fully oxidizing it to CO₂ in their mitochondria (1, 2). The Warburg effect is critical to maintaining the integrity of the photoreceptor outer segment (OS) and promoting glucose transport from retinal pigment epithelium (RPE) (3–5). Still, retinas are abundant with mitochondria and demonstrate active oxidative phosphorylation (6). Dysfunctional mitochondrial metabolism is emerging as an important cause for retinal diseases (7–9). It remains to be elucidated the importance of mitochondrial glucose oxidation in visual function and retinal energy metabolism *in vivo*.

Pyruvate is the key intermediate linking glycolysis and mitochondrial metabolism. Glucose-derived pyruvate is mostly reduced into lactate in the retina through lactate dehydrogenase (LDH). This reaction converts NADH into NAD⁺ to allow NAD⁺ recycling for glycolysis. Lactate could also be exported to fuel glial cells and RPE cells (5, 10). A small fraction of pyruvate from glycolysis enters mitochondria to be oxidized to acetyl-CoA through the pyruvate dehydrogenase (PDH) complex. The recently identified mitochondrial pyruvate carrier (MPC) transports pyruvate into the mitochondrial matrix (11, 12). MPC expression is associated with the Warburg effect and cell proliferation. In mammals, MPC has two subunits, MPC1 and MPC2, which form a multimeric complex of uncertain stoichiometry (11). Both subunits are required for the stability of the complex; loss of one subunit results in loss of the other (13–15). Deletion or inhibition of MPC increases proliferation of stem cells and cancers, while MPC overexpression increases pyruvate oxidation and suppresses cell growth (16, 17). In multiple cancers, MPC1 is deleted or underexpressed (16). Global knockout of either the MPC1 or the MPC2 subunit leads to embryonic

lethality, which can be rescued by a ketogenic diet (15, 18). Liver-specific knockout of MPC impairs gluconeogenesis and whole-body glucose homeostasis (13, 14). Inhibition of MPC in *ex vivo* retinal culture or in primary cortical neuron culture switches the preferred substrate for the mitochondrial TCA cycle from glucose to glutamine (19, 20).

In this study, we have generated a retina-specific knockout of MPC1 to block mitochondrial pyruvate import specifically in the retina. We studied the impact of the retina-specific loss of MPC1 on visual function, retinal viability, and retinal energy metabolism. Our results demonstrate that mitochondrial pyruvate transport in the retina is essential for maintaining the integrity of photoreceptors, regulating substrate utilization, and for neurotransmitter synthesis.

Results

Generation of Mice Lacking MPC1 in the Retina. To generate retina-specific knockout of MPC1 mice, we crossed homozygous MPC1^{flox/flox} mice with homozygous Six3 Cre transgene mice (Fig. 1A). The Six3 Cre mice express Cre recombinases in the developing neural retina at embryonic day 10 (21). The heterozygous MPC1^{flox/WT}-Six3-Cre mice were bred with heterozygous MPC1^{flox/WT} to generate MPC1^{flox/flox}-Six3-Cre (MPC KO) and their littermate controls MPC1^{flox/flox} (FL) or Six3 Cre (Cre).

Significance

Mitochondrial pyruvate carrier (MPC) is responsible for transporting pyruvate from glycolysis into mitochondria for oxidative phosphorylation. In the vertebrate retinas, the majority of glucose is metabolized into lactate by glycolysis rather than being oxidized in the mitochondria. Here, we show that although a small percentage of glucose is transported through MPC, deletion of MPC impairs visual function, decreases the viability of photoreceptors, disrupts photoreceptor ultrastructure, and causes glial activation. We find that MPC is required for maintaining the retinal metabolism in amino acids, neurotransmitters, and ketone bodies.

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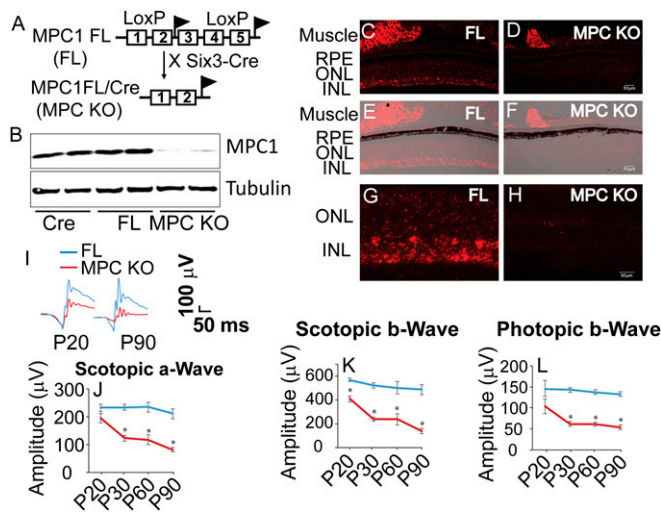


Fig. 1. Loss of MPC1 in retina impairs visual function. (A) Schematic for generation of MPC1 knockout mice in the retina. (B–H) MPC KO retina have deficiency in MPC1 expression. (B) Immunoblot of MPC1 or β -tubulin in retinas ($n = 4$). (C–H) In situ hybridization of MPC1 ($n = 3$). (I–L) MPC KO causes progressive decline in visual function. Representative ERG traces at P20 and P90 at -12 dB (I). ERGs were recorded at -12 dB at different postnatal days (J–L). Mean \pm SE; $n = 6$; * $P < 0.05$ vs. MPC KO littermates (ANOVA).

MPC KO and their littermate controls did not differ in size and gross eye morphology. To examine whether MPC1 is deleted, we probed immunoblots of retinal protein extracts with an MPC1 antibody and found that MPC1 is almost completely eliminated in the MPC KO retina (Fig. 1B) while expression of β -tubulin (the loading control) remained constant. An MPC1 antibody suitable for immunofluorescence is not available currently. We tested commercially available MPC1 antibodies from Sigma, Abcam, and Invitrogen and found none could be validated. Therefore, to localize MPC1, we detected MPC1 mRNA expression by in situ hybridization on retina frozen sections. Muscle expresses MPC1, thus we included a piece of ocular muscle as a positive control (14). MPC1 mRNA is highly expressed in muscle and retinas in FL littermates, but MPC1 mRNA expression was eliminated in MPC KO retinas (Fig. 1C–H), confirming that MPC1 is deleted specifically in the retina.

MPC KO Mice Have Impaired Visual Function. To investigate visual function, we recorded electroretinograms (ERG) for both dark-adapted rod responses (scotopic a-wave and b-wave) and light-adapted cone responses (photopic b-wave) between postnatal day 20 (P20) and P90. Scotopic a-wave responses in MPC KO were normal at P20 but progressively declined at P30, P60, and P90 (Fig. 1I and J and SI Appendix, Fig. S1A and B). However, scotopic b-wave responses were decreased significantly at P20 and then declined similarly to the a-wave (Fig. 1K). Additionally, the percent reduction of the b-wave was \sim twofold more than the a-wave, suggesting that neurotransmitters in the photoreceptors might be deficient or that there is a detrimental effect in the secondary neurons in the retina. The cone response was also impaired at P30 and it remained constant until P90. At P90, both rod and cone ERGs in MPC KO mice were less than 50% of their littermate controls at all light intensities (SI Appendix, Fig. S1B–D), indicating that MPC1 is essential to maintain visual function.

Loss of MPC1 Causes Retinal Degeneration. Photoreceptor degeneration is a common cause for visual impairment. To investigate whether deletion of MPC1 causes retinal degeneration, we quantified the thickness of retinal layers by both H&E

staining of eye sections and OCT. Six different positions covering both dorsal and ventral regions in the sections of both FL and KO were analyzed by ImageJ for thickness and number of nuclei (SI Appendix, Fig. S2). Since Six3 Cre expresses in the retina at embryonic day 10 and most of the retinal cells are matured 2 wk after birth (22), we tested eyes earlier at P8 and P14 to investigate a possible retinal developmental defect. In the P8 retinas, we found no difference in retinal morphology between MPC KO and littermate controls (SI Appendix, Fig. S3). In the P14 retinas, the thickness of all layers was normal except slight decrease of OS length close to the optic nerve, suggesting that photoreceptor OS development may be slightly impaired. From P20, OS was shortened but other layers were normal in the MPC KO retina (Fig. 2A–C). The OS length and number of nuclei in the outer nuclear layer (ONL) progressively decreased after P20 (Fig. 2D–J). OCT of P90 mice confirmed that loss of MPC1 causes photoreceptor degeneration (Fig. 2J). Other layers like the inner plexiform layer (IPL) also were smaller after P30 (Fig. 2K), suggesting MPC1 is important for the viability of inner retinal cells.

MPC-Deficient Retinas Have Distinctive Ultrastructural Defects. Because MPC KO mice possess significantly shorter rod photoreceptor OS, we investigated photoreceptor ultrastructure by transmission electron microscopy (TEM). In the plastic sections, apart from the shortened rod OS, photoreceptors in the P90 MPC KO retinas were largely normal. Their relationship to the RPE of MPC KO eyes were similar to FL controls; both MPC KO and FL retinas had apical processes extending from the adjacent RPE layer, and the OSs were well-organized with stacked disk membranes. Inner segment ellipsoids containing properly organized mitochondria were evident, as were structurally normal connecting cilia, anchored in the distal ellipsoid by a basal body (SI Appendix, Fig. S4).

However, in the IS-OS junction layer, MPC KO retinas displayed a distinctive and reproducible defect, which was never observed in FL controls (Fig. 3B–H). To our knowledge, this ultrastructural defect has not been described in the published literature, and represents a unique retinal phenotype. The distal portion of the IS and the proximal region of the OS were each

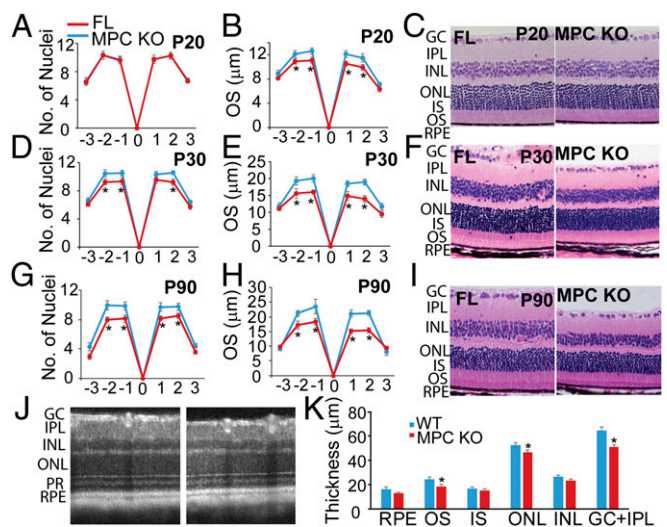


Fig. 2. Loss of MPC1 results in progressive retinal degeneration. (A–I) Representative H&E staining images at -2 position to the optic nerve head (0 position) and quantification of retinal layer thickness at six different locations from eyes at P20, P30, and P90. * $P < 0.05$ (ANOVA), $n = 40$ from four different animals for each age. (J and K) Representative OCT image and OCT quantifications of retinal thickness at all layers. * $P < 0.05$ (t test), $n = 6$.

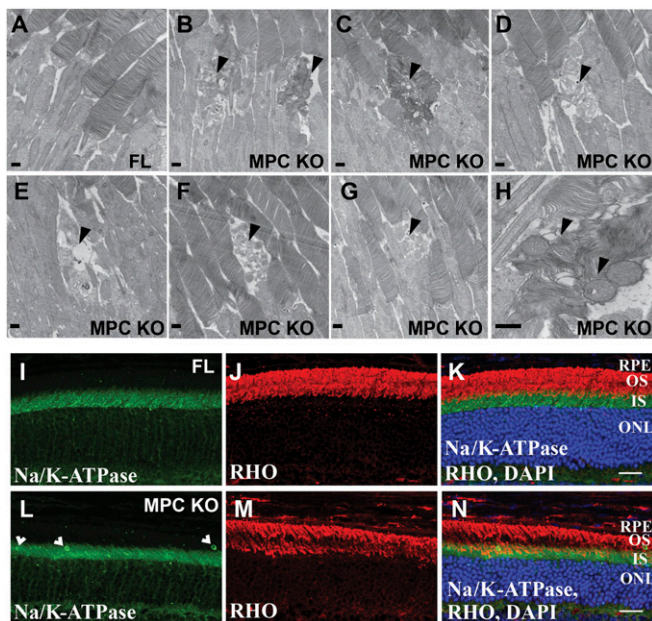


Fig. 3. Loss of MPC1 disrupts the integrity of IS-OS junction layer. (A–H) TEM images of FL and MPC KO retinas in the IS-OS junction layer. The disrupted areas were marked by black arrowheads. $n = 4$. (I–N) Immunostaining showed abnormal vacuoles marked by white arrowheads in the IS and IS-OS junction in the MPC KO retinas. RHO, rhodopsin. (Scale bars: A–H, 500 nm; I–N, 20 μm .) $n = 3$.

involved. Higher-magnification views demonstrated that disruptions in normal cell structure might be caused by a shed portion of an ellipsoid region including isolated swollen mitochondria, adjacent to packets of disorganized OS disk membrane (Fig. 3H). To estimate the frequency of shed ellipsoid regions, the number of such features observed was normalized to the number of cell bodies present in a continuous ultrathin section. These defects were observed at a frequency of roughly 4% relative to the total rod photoreceptor population. To further examine these defects, we stained IS membrane with Na/K ATPase (23, 24) and OS with rhodopsin. MPC KO retinas formed small vacuoles in the IS and IS-OS junction (Fig. 3I–N) but not in the FL retinas, supporting the structural damage in the IS.

MPC KO Alters the Retinal Metabolic Profile. Next, we used targeted steady-state metabolomics to investigate how loss of MPC1 influences energy metabolism. We used LC MS/MS to target 93 metabolites covering major pathways in the metabolism of glucose, amino acids, lipids, and nucleotides (SI Appendix, Table S1). Pyruvate was the most increased metabolite in the MPC KO retina at P20 in both volcano plot and t test (Fig. 4A and B), confirming that MPC1 is deleted in the retina and blocks mitochondrial pyruvate oxidation. Consistent with our previous analysis of the effects of an MPC inhibitor ex vivo (19), aspartate accumulated, while glutamate and glutamine were diminished, in the MPC KO retina. Additionally, 3-hydroxybutyrate (3-HB), CoA, and glutathione (GSH) were diminished by ~50% or more in the MPC KO retina (Fig. 4A and B). Since RPE can be an important source of 3-HB by oxidizing phagocytosed outer segments (25), we also quantified metabolites in the RPE/choroid. We did not find differences in 3-HB, pyruvate, or other metabolites in the RPE/choroid between MPC KO and their littermate controls (Fig. 4C), indicating that the metabolic changes are restricted to the retina. We hypothesized that loss of MPC1 blocks the entry of pyruvate into mitochondria and depletes α -ketoglutarate, a precursor for synthesis of glutamate, glutamine,

and GSH (Fig. 4D). To compensate for the mitochondrial energy deficit, the MPC KO retina may increase consumption of 3-HB to provide acetyl-CoA for the TCA cycle (Fig. 4D).

MPC KO Impairs Retinal Glucose Metabolism. To test the hypothesis in Fig. 4D that glucose-derived pyruvate is critical for retinal amino acid metabolism, we incubated freshly isolated retinas at P20 with uniformly ^{13}C -labeled glucose ($\text{U-}^{13}\text{C}$ glucose) for 1 h, and we used GC MS to quantify the labeled metabolites in glycolysis, mitochondrial TCA cycle, and amino acid metabolism. The labeled carbons from each six-carbon molecule of labeled glucose (M6) that is metabolized through glycolysis can be found in the three-carbon (M3) 3-phosphoglycerate (3PG), in the M3 amino acid serine (Ser), in the M3 phosphoenolpyruvate (PEP), or in the M3 molecule pyruvate (Pyr). M3 pyruvate can enter mitochondria through MPC1 to be oxidized to acetyl-CoA along with the loss of one carbon as CO_2 (Fig. 5A). Therefore, the intermediates within the first round of the TCA cycle include two labeled carbons from ^{13}C glucose (M2). Consistent with our expectation that loss of MPC1 would block transport of pyruvate into mitochondria for oxidation, M3 pyruvate increased ~3.5 fold in the MPC KO (Fig. 5F), similar to the effect MPC1 loss has on total pyruvate (Fig. 4B), but the glucose consumption remained unchanged (SI Appendix, Fig. S5). Interestingly, MPC deficiency increased the labeled serine and glycine, but not 3PG, PEP, and lactate. It has been reported that pyruvate can be transaminated into alanine to enter the TCA cycle when MPC1 is deleted in the liver (14). However, in retina the labeled alanine was not increased but decreased in the MPC KO. In the mitochondrial intermediates, MPC KO decreased all of the labeled intermediates except M2 aspartate (Asp). M2 glutamine (Gln) decreased ~5 \times while M2 Asp increased ~3 \times .

The amount of pyruvate increases while lactate remains constant, so the retinal lactate/pyruvate ratio decreases in MPC KO retinas (Fig. 5Q). It is surprising that lactate does not increase, as pyruvate and lactate are interconverted through LDH. Because lactate and pyruvate can easily be exported into the medium, we

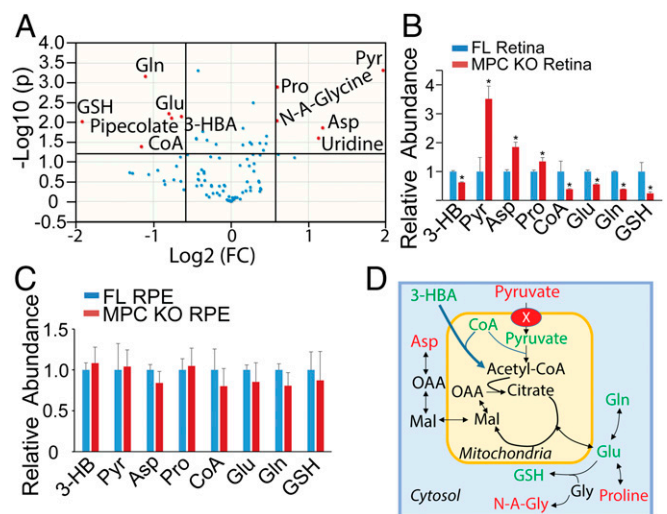


Fig. 4. Loss of MPC1 alters the metabolic profile in the retina. (A) Mouse retinas at P20 were analyzed by LC MS/MS for metabolomics. Volcano plot ($P < 0.05$ and fold change > 1.5 -fold were significant). y axis is the \log_{10} of P value, and x axis is the \log_2 of fold change. Significantly changed metabolites in mouse retina (B) and RPE/choroid (C). * $P < 0.05$ vs. FL (t test), $n = 6$. Relative abundance is the ion intensity relative to FL. (D) A proposed model for the metabolic change. Knockout of MPC1 accumulates cytosolic Pyr and aspartate (Asp) and depletes 3-HBA, glutamate (Glu), and glutamine (Gln). Mal, malate; Pro, proline.

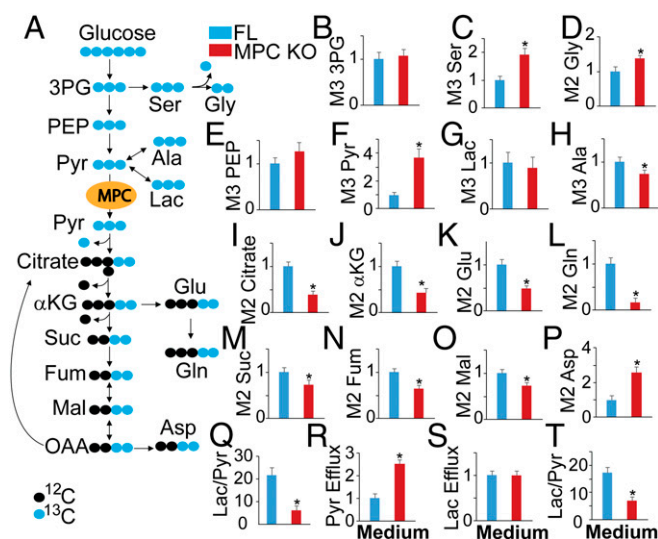


Fig. 5. MPC1 knockout blocks the entry of ^{13}C -glucose-derived pyruvate into mitochondria. (A) A schematic of ^{13}C -glucose labeling. U- ^{13}C glucose was metabolized into M3 3PG, serine (Ser), PEP, Pyr, alanine (Ala), and lactate (Lac). Pyr enters the mitochondria through MPC and oxidized through TCA cycle. (B–P) Mouse retinas at P20 were incubated with 5 mM U- ^{13}C glucose for 1 h. The y axis was ^{13}C abundance relative to FL. (Q) The MPC knockout reduced the Lac/Pyr ratio. (R–T) The efflux of Pyr into the medium was increased but the Lac remained unchanged. * $P < 0.05$ vs. FL (t test), $n = 6$. Fum, fumarate; Suc, succinate.

examined the medium metabolites and found a parallel increase of pyruvate and normal lactate (Fig. 5 R–T). Taken together, MPC KO blocks glucose oxidation in the mitochondria, accumulates pyruvate and aspartate, and reduces mitochondrial intermediates, especially glutamine.

MPC Deficiency Enhances Consumption of Ketone Bodies. The amount of 3-HB, a ketone body, is substantially diminished in MPC KO retinas (Fig. 4 A and B). We hypothesized that loss of MPC1 could cause a retina to oxidize more ketone bodies in its mitochondria. To test this hypothesis, we incubated retinas with 2,4- ^{13}C 3-HB in the presence of 5 mM unlabeled glucose and analyzed the labeled metabolites by GC MS. ^{13}C 3-HB first must be oxidized by NAD^+ to form acetoacetate to supply acetyl-CoA for the TCA cycle (SI Appendix, Fig. S6). After 1 h incubation, about 20–50% of mitochondrial intermediates were replaced with ^{13}C in the WT retinas (Fig. 6A), indicating that retinas can efficiently utilize ketone bodies as a fuel source. As expected, MPC deficiency significantly increases the percentage of labeled fraction (enrichment of ^{13}C metabolite in total pool) for all of the mitochondrial intermediates (Fig. 6A). Since enrichment can be influenced by pool size, we determined the relative amounts of labeled intermediates (abundance of ^{13}C metabolites) (Fig. 6B). The amounts of labeled α -ketoglutarate (αKG), fumarate, malate, proline, and aspartate are significantly enhanced in MPC KO retinas, while glutamine still is lower than in littermate controls. To confirm whether MPC KO retinas consume more ^{13}C 3-HB, we quantified medium metabolites and found that MPC-KO retinas consumed more ^{13}C 3-HB than FL retinas (Fig. 6C). However, pyruvate still accumulated (produced from the 5 mM unlabeled glucose) as it did with ^{13}C glucose alone (SI Appendix, Fig. S6). Lactate was unchanged and the lactate/pyruvate ratio was substantially diminished in MPC KO retinas. Ketone body is generated by fatty acids, which can also be oxidized directly in the mitochondria transported through acylcarnitines. We found most of acylcarnitine are decreased in the MPC KO retinas (SI Appendix, Fig. S7), suggesting that fatty

oxidation is enhanced in the mitochondria. Overall, these results demonstrate that loss of MPC1 enhances the use of ketone bodies to rescue the deficiency in TCA cycle intermediates. However, ketone bodies do not rescue the pyruvate accumulation and deficiency in glutamine.

MPC-Deficient Retinas Accumulate Aspartate at the Expense of Glutamine. Glutamine can be a source of carbons for mitochondrial intermediates once it has been converted to glutamate and then to αKG . Glutamine also is a precursor for proline, gamma-aminobutyric acid (GABA) and 5-oxo-proline. To investigate the effect of MPC deficiency on glutamine metabolism, we incubated retinas with uniformly labeled ^{13}C glutamine. In the first round of the TCA cycle, M5 glutamine loses a carbon through αKG dehydrogenase to generate the M4 intermediates of the TCA cycle (Fig. 6D). M4 aspartate accumulates about fivefold and M4 citrate is depleted by half in MPC KO retinas compared with controls. Aspartate accumulates because there is not enough acetyl-CoA to convert oxaloacetate to citrate (Fig. 6E). In normal mitochondria carbons that enter from M5 glutamine in the first cycle continue into subsequent cycles where they ultimately are fully oxidized to CO_2 . Intermediates in the second round of the cycle appear as M3 or M2 (Fig. 6D). These isotopomers are scarce in MPC-deficient retinas, indicating an overall slower rate of glutamine oxidation. M2 aspartate accumulates to higher levels in MPC-deficient retinas because without pyruvate, a bottleneck occurs at citrate synthesis. (Fig. 6F). Overall, glutamine oxidation appears to be slowed because of the limited supply of acetyl CoA required to catalyze complete oxidation of the carbons from glutamine. Consistent with this, we found that consumption of ^{13}C glutamine in the medium by MPC-deficient retinas is diminished (SI Appendix, Fig. S8).

Surprisingly, carbons from ^{13}C glutamine appear in both M1 and M3 pyruvate in MPC-deficient retinas (SI Appendix, Fig. S9 A–C). M3 pyruvate could be made by malic enzyme converting M4 malate into pyruvate. The deficiency of mitochondrial pyruvate may stimulate this pathway to generate pyruvate inside mitochondria.

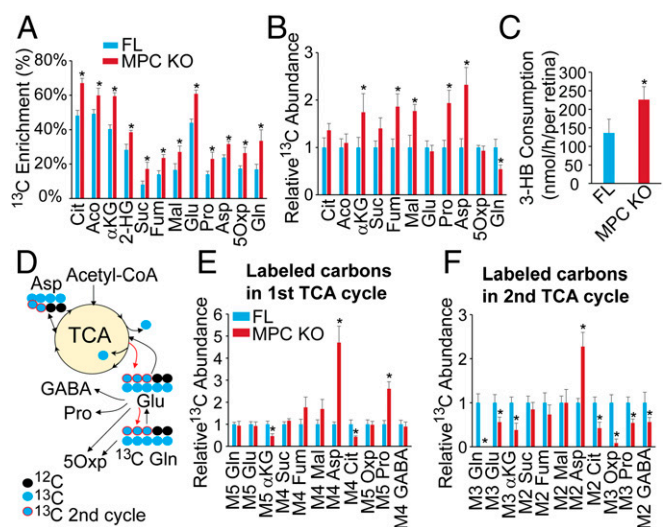


Fig. 6. The knockout of MPC1 enhances ketone body oxidation and accumulates Asp from Gln. (A–C) Mouse retinas were incubated with ^{13}C 5 mM 3-HB and glucose for 1 h. ^{13}C enrichment is the percentage of each ^{13}C -labeled isotopomer out of the total of all isotopomers. ^{13}C abundance represents the absolute abundance of each ^{13}C -labeled isotopomer. 3-HB increased mitochondrial intermediates except Gln. (D–F) Mouse retinas were incubated with 2 mM ^{13}C Gln and glucose for 1 h. Incorporation of ^{13}C from Gln was blocked at the step of citrate synthesis and accumulated as ^{13}C Asp. * $P < 0.05$ vs. FL (t test), $n = 6$. Aco, aconitate; Cit, citrate; 5Oxo, 5-oxoproline.

Indeed, M6 citrate (two carbons from M3 pyruvate plus four carbons from oxaloacetate) and M3 citrate (one carbon from M1 pyruvate and two carbons from the second cycle intermediates) accumulate to higher levels in MPC KO retinas (*SI Appendix, Fig. S9 C and E*). However, this alternative pathway for pyruvate production accounts for only ~1% of total pyruvate (*SI Appendix, Fig. S9D*).

Consistent with our previous finding (1), glutamine increases the lactate/pyruvate ratio (*SI Appendix, Fig. S9F*) and it increases 3-HB more than threefold in WT retinas. We found that the decrease of 3-HB that occurs in MPC retinas can be prevented by supplementation with glutamine (*SI Appendix, Fig. S9G*). These results confirm that MPC-deficient retinas can use glutamine, but their inability to efficiently synthesize glutamine still causes an overall reduction of glutamine content.

MPC Deficiency Causes Mitochondrial Energy Deficit. To test mitochondrial function, we measured oxygen consumption in isolated mitochondria and levels of total ATP and NAD/NADH. As expected, the oxygen consumption rate was severely impaired in MPC KO retinal mitochondria (*SI Appendix, Fig. S10A*). The levels of ATP and NADH did not change at P20 retinas, most probably due to the compensational use of ketone bodies and fatty acid. However, the levels of ATP and NADH were decreased in MPC KO retinas at P30 (*SI Appendix, Fig. S10 B–D*), indicating that mitochondrial dysfunction is critical for the retinal degeneration.

MPC Deficiency Activates Müller Glial Cells. Glutamine is synthesized by glutamine synthetase (GS), which is localized specifically to Müller glial cells. Müller glia are hypersensitive to changes in the retinal environment. To examine whether Müller glial cells were activated by MPC deficiency, we immunostained retinal sections with GS and glial fibrillary acidic protein (GFAP, a marker of glial activation). In FL retinas, GS-stained Müller glial cells were well organized, and the minimal GFAP expression was restricted to Müller cell end feet, where the GFAP signal overlapped with GS (*SI Appendix, Fig. S11 A–C*). However, in MPC-deficient retinas, GS staining was more irregular. GFAP was up-regulated, which reached out to all layers where Müller glia are present including the IPL, inner nuclear layer (INL), and ONL (*SI Appendix, Fig. S11 D–F*). The Müller glia in the MPC KO retinas were disorganized (*SI Appendix, Fig. S11 D–F*), indicating activation and damage. To quantify the protein expression, we tested the whole retinal protein extracts with immunoblots. Consistently, the expression of GS was down-regulated while GFAP was up-regulated in the MPC KO retinas (*SI Appendix, Fig. S11 G and H*).

Discussion

Retina metabolism is dominated by aerobic glycolysis (the Warburg effect) with a small fraction of the pyruvate produced by glycolysis being oxidized in mitochondria. In this study, we report that mitochondrial pyruvate transport is required for normal retinal function and photoreceptor viability. MPC controls how a retina uses metabolic fuels. It influences retinal cytosolic reducing power, glutaminolysis, ketone body oxidation, and glutamine synthesis, which are critical for photoreceptor function and integrity.

Mitochondrial Pyruvate Transport in Visual Function and Retinal Viability. Visual function declines progressively in retinas without MPC. The scotopic b-wave is affected early and severely. The b-wave reflects glutamatergic transmission between photoreceptors and biopolar cells. The depletion of glutamine/glutamate and accumulation of aspartate may be the major contributor to the reduced b-wave response. Intraocular injection of a glutamine synthetase inhibitor in rats caused a similar suppression of the scotopic b-wave response (26). In isolated rat retinas, exogenous glutamine or glutamate is required to maintain b-wave

responses and inhibition of glutamate transporters causes rapid loss of the b-wave (27). The monocarboxylate transporter inhibitor, α -cyano-4-hydroxycinnamate (4-CIN), potently inhibits MPC (28). Intravitreal injection 4-CIN suppresses the b-wave response, lowers glutamate, and raises aspartate (29), similar to the changes that occur in MPC KO mice.

Shortening of outer segments occurs as early as P14 in MPC KO retinas. The daily turnover of vertebrate outer segments creates a high demand for synthesis from lipids and amino acids. Pyruvate oxidation through the TCA cycle can be an important source for de novo lipid synthesis and nonessential amino acid synthesis. We found that blocking import of pyruvate into mitochondria leads to oxidation of ketone bodies that are derived from lipids. We speculate that the compensatory increase of ketone body and glutamine oxidation may not be fast enough to meet the high demand for energy and biosynthesis, because glutamine/glutamate, glycine, and glutathione are significantly depleted in the MPC KO retina (*Fig. 4 A and B*). Consistent with this hypothesis, mutation of the gene encoding human isocitrate dehydrogenase (IDH), a key enzyme in the TCA cycle, exclusively causes retinal degeneration (7). Loss of PDH or loss of citrate synthase causes light-induced photoreceptor degeneration in *Drosophila* (30) and pyruvate administration can protect mouse retinas from light damage (31). Furthermore, glial activation in response to diminished glutamine may aggravate retinal degeneration. GS expression is slightly up-regulated in MPC KO retinas, apparently by compensation, but its activity is substantially impaired based on our ^{13}C labeling results with either ^{13}C glucose or ^{13}C glutamine. GS is a potent neuroprotectant and inhibition of GS activity can lead to retinal cell death (32).

MPC Influences Pyruvate Oxidation and Lactate/Pyruvate Ratio. Like cancer cells, photoreceptors express high levels of the M2 isoform of pyruvate kinase (PKM2) and the LDH isoform A (LDHA) (3, 10, 33–35). These isoforms are generally associated with aerobic glycolysis in cancer cells and other proliferating cell types, whereas MPC expression is negatively correlated with aerobic glycolysis in cancer cells (16). Consistent with this observation, MPC expression is much lower in the photoreceptor layer than in ocular muscle and in the inner retinal layers (*Fig. 1C*). Knockout of MPC1 inhibits pyruvate oxidation. It also causes accumulation of serine and glycine (*Fig. 5*). Increased de novo serine synthesis could enhance phospholipid synthesis, but this effect may be counteracted in the MPC KO retinas by enhanced oxidation of fatty acids.

There is no increase in lactate production from both the retina and the RPE with MPC deficiency although pyruvate accumulates to a level substantially higher than normal (*Fig. 5*). Similarly, there was no increase in lactate in retinas cultured *ex vivo* with an MPC inhibitor (19). We showed previously that a high cytosolic lactate/pyruvate ratio in normal retinas can drive malate aspartate shuttle activity, which diverts α -ketoglutarate away from being oxidized by α -ketoglutarate dehydrogenase (1). In MPC-deficient retinas, it is the accumulation of oxaloacetate and aspartate that drives malate aspartate shuttle activity. Because two carbons from pyruvate are needed to make citrate, oxaloacetate accumulates in MPC KO retinas. The oxaloacetate is converted by aminotransferase activity into aspartate (*SI Appendix, Fig. S12*), which is transported to the cytoplasm. There it is converted back to oxaloacetate, which forms malate while oxidizing NADH to NAD⁺. The malate exchanges with α -KG from the matrix, thereby diverting the α -KG from oxidation. The increased amount of NAD⁺ in the cytoplasm contributes to the decreased lactate/pyruvate ratio in MPC-deficient retinas. NAD⁺ also can oxidize 3-HB to acetoacetate to provide some matrix acetyl-CoA. The metabolic flexibility to use glutamine and lipids to compensate for MPC loss has been reported in other MPC-deficient cells

(14, 36). This may partially explain why retinal degeneration in MPC-deficient retinas is not very severe. However, despite this adaptive metabolic pathway, the balance of retinal neurotransmitter and the integrity of photoreceptors still cannot be maintained without MPC1. A ketogenic diet can rescue gestation of global MPC1-deficient embryos, but the pups die within minutes after birth (15).

MPC Influences Neuron–Glial Interaction. Photoreceptor neurons, Müller glial cells, and RPE cells have a symbiotic metabolic relationship. Disruption of these metabolic relationships can cause photoreceptor degeneration or gliosis (37). The up-regulated GFAP and disorganized Müller glial cells in MPC-deficient retinas suggest that MPC is critical for neuron–glial interaction.

Disruption of glutamine metabolism is likely to be a key factor in glial dysfunction. Glutamine is synthesized in glial cells and transported to photoreceptors where it is converted to glutamate to be used as a neurotransmitter or as fuel (*SI Appendix, Fig. S12*). We have reported evidence that Müller cells use lactate and aspartate from photoreceptors to synthesize glutamine (10). Why does MPC deficiency deplete glutamine? Müller cells lack the mitochondrial aspartate–glutamate carrier (AGC) (10) that

diverts oxaloacetate away from the TCA cycle. This gives Müller cell mitochondria the ability to use aspartate to make glutamine (10) by adding carbons from acetyl-CoA to oxaloacetate to make citrate. That citrate then is oxidized to α -KG for glutamate and glutamine synthesis. However, when MPC is deficient, acetyl-CoA from pyruvate is unavailable, thereby blocking this pathway for synthesis of glutamate and glutamine in Müller glia.

Materials and Methods

All of the reagents, animals, and key resources were detailed in the key resources form (*SI Appendix*). Mouse experiments were performed in accordance with the National Institutes of Health guidelines and the protocol approved by the Institutional Animal Care and Use Committee of West Virginia University. MPC1 RNA in situ hybridization, metabolite analysis by mass spectrometry, EM, immunohistochemistry, and other methods were performed as reported (38, 39) and described in details in *SI Appendix*.

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