A Drosophila model of combined D-2- and L-2-hydroxyglutaric aciduria reveals a mechanism linking mitochondrial citrate export with oncometabolite accumulation

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ABSTRACT

The enantiomers of 2-hydroxyglutarate (2HG) are potent regulators of metabolism, chromatin modifications and cell fate decisions. Although these compounds are associated with tumor metabolism and commonly referred to as oncometabolites, both D- and L-2HG are also synthesized by healthy cells and likely serve endogenous functions. The metabolic mechanisms that control 2HG metabolism in vivo are poorly understood. One clue towards how cells regulate 2HG levels has emerged from an inborn error of metabolism known as combined D- and L-2HG aciduria (D-/L-2HGA), which results in elevated D- and L-2HG accumulation. Because this disorder is caused by mutations in the mitochondrial citrate transporter (CIC), citrate must somehow govern 2HG metabolism in healthy cells. The mechanism linking citrate and 2HG, however, remains unknown. Here, we use the fruit fly Drosophila melanogaster to elucidate a metabolic link between citrate transport and L-2HG accumulation. Our study reveals that the Drosophila gene scheggia (sea), which encodes the fly CIC homolog, dampens glycolytic flux and restricts L-2HG accumulation. Moreover, we find that sea mutants accumulate excess L-2HG owing to elevated lactate production, which inhibits L-2HG degradation by interfering with L-2HG dehydrogenase activity. This unexpected result demonstrates that citrate indirectly regulates L-2HG stability and reveals a feedback mechanism that coordinates L-2HG metabolism with glycolysis and the tricarboxylic acid cycle. Finally, our study also suggests a potential strategy for preventing L-2HG accumulation in human patients with CIC deficiency.

This article has an associated First Person interview with the first author of the paper.

KEY WORDS: 2-Hydroxylutarate, Oncometabolite, L2HGDH, Scheggia, SLC25A1

INTRODUCTION

Although the enantiomers of 2-hydroxyglutarate (2HG) have emerged as potent oncometabolites capable of influencing a wide range of cellular processes, both D- and L-2HG are also produced by healthy tissues (Ye et al., 2018). Mammalian cells produce D-2HG as a result of γ-hydroxybutyrate metabolism and phosphoglycerate dehydrogenase activity (Fan et al., 2015; Struys et al., 2005b), whereas L-2HG is generated by malate dehydrogenase and lactate dehydrogenase A in response to hypoxia, acidic cellular conditions and decreased electron transport chain activity (Intlekofer et al., 2015, 2017; Oldham et al., 2015; Reinecke et al., 2012; Nadtochyi et al., 2016; Mullen et al., 2011; Teng et al., 2016). Furthermore, yeast and Drosophila generate D- and L-2HG, respectively, under standard growth conditions (Becker-Kettern et al., 2016; Li et al., 2017). Overall, these results suggest that D- and L-2HG serve endogenous biological functions and emphasize the need to understand how 2HG metabolism is controlled in vivo.

Despite the fact that D- and L-2HG can dramatically influence the cellular physiology of healthy tissues, most 2HG studies focus on the role of these compounds in cancer cell lines and, as a result, the molecular mechanisms that regulate endogenous 2HG accumulation remain poorly understood. In fact, most of our current understanding about endogenous D- and L-2HG metabolism stems from a class of rare human diseases that are collectively known as the 2HG acidurias (2HGAs) (Kranendijk et al., 2012). For example, patients with L2HGA accumulate L-2HG owing to loss-of-function mutations in the FAD-dependent enzyme L-2HG dehydrogenase (L2HGDH) (Rzem et al., 2004), which converts L-2HG to 2-oxoglutarate (2OG). Similarly, D2HGA type I results from the absence of D-2HG dehydrogenase (D2HGDH) activity and an inability to degrade D-2HG (Struys et al., 2005a). Overall, these studies illustrate how the 2HGA disorders provide essential clues for understanding endogenous 2HG metabolism.

In addition to the disorders associated with a single 2HG enantiomer, a small subset of 2HGA patients exhibit elevated levels of both D- and L-2HG. This rare disease, which is known as combined D-/L-2HGA, results in severe neurological and muscular defects, developmental delays and childhood lethality (Muntau et al., 2000). Considering that this disorder is caused by loss-of-function mutations in the mitochondrial citrate carrier (CIC; encoded by SLC25A1) (Nota et al., 2013; Palmieri, 2013), citrate transport must somehow govern 2HG accumulation. However, the mechanism linking these metabolites is unknown.

We recently discovered that Drosophila larvae accumulate high concentrations of L-2HG during normal larval growth (Li et al., 2017). Moreover, we determined that flies, like mammals, rely on lactate dehydrogenase (dLDH; FBgn0001258) to synthesize L-2HG from the tricarboxylic acid (TCA) cycle intermediate 2OG (Li et al., 2017). These findings demonstrate that fundamental aspects of L-2HG metabolism are conserved between flies and humans, and suggest that studies in Drosophila will be essential for understanding how L-2HG accumulation is controlled in vivo. Here, we exploit the fly model to examine the metabolic link...
between citrate and L-2HG. By studying a hypomorphic mutation in the Drosophila gene scheggia (sea; FBgn0037912), which encodes the fly SLC25A1 homolog (Carrià et al., 2008; Morciano et al., 2009), we demonstrate that loss of mitochondrial citrate efflux results in elevated glucose catabolism, increased lactate production and enhanced L-2HG accumulation. The elevated L-2HG levels observed in sea mutants, however, are not the result of excess synthesis, but rather are caused by decreased degradation. Moreover, our studies indicate that sea mutants accumulate excess L-2HG as a result of increased lactate synthesis, which inhibits the enzyme that degrades L-2HG, dL2HGDH (FBgn0032729) (Li et al., 2017). Overall, our findings present a metabolic feedback loop by which L-2HG levels are controlled by the combined outputs of glycolysis and the TCA cycle, and suggest that a similar mechanism could be active in mammals.

RESULTS

sea mutant larvae accumulate excess L-2HG

To determine whether the Drosophila homolog of SLC25A1 influences 2HG accumulation, we used gas chromatography-mass spectrometry (GC-MS) to quantify both D- and L-2HG in sea mutant larvae (sea^{Δ24}/Df), which exhibit a significant reduction in mitochondrial CIC activity (Morciano et al., 2009). When compared with a genetically matched control strain (sea^{Δ24}/Df), both D- and L-2HG were significantly elevated in sea mutants (Fig. 1A,B), with L-2HG representing the majority of the 2HG pool. Although these observations differ from patients with combined D-/L-2HGA, in which D-2HG is the more abundant enantiomer (Muntau et al., 2000), the metabolic profile of sea^{Δ24}/Df mutants clearly indicates that the inverse relationship between CIC activity and L-2HG accumulation is present in flies.

Glycolysis and the TCA cycle are disrupted in sea mutants

Combined D-/L-2HGA patients not only exhibit increased 2HG levels and decreased citrate accumulation, but also possess elevated levels of lactate, 2OG, succinate, fumarate and malate (Nota et al., 2013; Prasun et al., 2015). To determine whether sea mutants display similar metabolic defects, we used GC-MS-based metabolomics to quantify the relative abundance of metabolites in glycolysis and the TCA cycle. Multivariate analysis of the resulting data sets revealed that sea^{Δ24}/Df mutant larvae exhibit a distinct metabolic profile when compared with either sea^{Δ24}/Df or w^{1118}/Df controls (Fig. 1C). Targeted analysis of these data revealed that sea^{Δ24}/Df mutants and combined D-/L-2HGA patients display similar metabolic phenotypes, including decreased citrate levels and elevated concentrations of pyruvate, lactate, fumarate and malate (Fig. 1D). Similar metabolic changes were observed when the sea^{Δ24} mutation was analyzed in a second genetic background (trans to a second deficiency that also uncovers the sea locus; Fig. S1). Moreover, the sea^{Δ24}/Df metabolic phenotypes were rescued by ubiquitous expression of a sea complementary DNA (cDNA) from a UAS-sea transgene, indicating that the metabolic profile displayed by sea^{Δ24}/Df mutants specifically results from the loss of CIC activity (Fig. 1E,F).

Glycolytic flux is elevated in sea mutants

Considering that Drosophila larvae primarily synthesise pyruvate, lactate and L-2HG from glucose (Li et al., 2017;
Tennessen et al., 2011), our data suggested that the sea\textsuperscript{Δ24/Df} mutants accumulate excess L-2HG owing to increased glycolytic flux. We tested this hypothesis by feeding \textsuperscript{13}C\textsubscript{6}-glucose to both sea\textsuperscript{Δ24/Df} mutants and sea\textsuperscript{pro/Df} controls, and selectively monitoring \textsuperscript{13}C incorporation into pyruvate, lactate, citrate and 2HG. When compared with the control strain, sea\textsuperscript{Δ24/Df} larvae exhibited a 60% increase in the rate of lactate (m+3) synthesis, a modest increase in the accumulation rate of labeled pyruvate (m+3) and 2HG (m+2), and a slight, but significant, decrease in the rate of m+2 citrate synthesis (Fig. 2A). These observations confirm that glycolytic flux is elevated in sea mutants, and are consistent with a recent study that observed enhanced glucose consumption and increased lactate production in CIC-deficient human cells (Jiang et al., 2017).

To determine whether increased glycolytic flux is necessary for elevated L-2HG accumulation, we used a Phosphofructokinase (Pfk) RNAi transgene (UAS-Pfk-RNAi; FBgn003071) to attenuate glycolysis in both control and mutant larvae. Ubiquitous expression of this construct in a wild-type background reduced Pfk mRNA levels by 80%, and induced a similar reduction in pyruvate, lactate and 2HG levels (Fig. S2A,B). Similarly, Pfk-RNAi expression in a sea\textsuperscript{Δ24/Df} mutant background induced an 80% decrease in Pfk mRNA levels, a 75% decrease in 2HG and a ~50% decrease in pyruvate and lactate (Fig. 2B,C). Overall, these results support a model in which Drosophila CIC activity restricts L-2HG accumulation by inhibiting glucose catabolism.

In order to further explore the mechanism linking citrate with glycolysis and L-2HG, we used RNA sequencing (RNA-seq) to determine whether Drosophila CIC activity influences the expression of glycolytic enzymes (Table S1). This analysis revealed that only two of the 25 Drosophila genes that encode glycolytic enzymes exhibited a >1.5-fold increase in sea\textsuperscript{Δ24/Df} mutants, and no gene in this 25-gene subset exhibited a greater than twofold increase (Fig. 3A; Table S2). Quantitative reverse transcription PCR (qRT-PCR) analysis also confirmed that Pfk, dLdh and dL2HGDH mRNA levels were comparable between sea\textsuperscript{Δ24/Df} mutants and sea\textsuperscript{pro/Df} controls (Fig. 3B). Moreover, we observed no changes in either Malate dehydrogenase I or 2 gene expression (FBgn0262782; FBgn0262559; Table S1), both of which have been implicated in mammalian L-2HG synthesis (Ye et al., 2018). Overall, these results demonstrate that Drosophila CIC activity does not significantly influence the transcription of key genes directly associated with L-2HG metabolism and glycolysis.

Because our gene expression studies demonstrated that Drosophila CIC activity controls glycolysis at post-transcriptional levels, we next examined the possibility that changes in intracellular citrate distribution directly influence glycolysis and L-2HG metabolism. Considering that citrate inhibits glycolytic flux in mammals (Kemp and Gunasekera, 2002), and that cytosolic citrate is significantly depleted in sea mutants (Morciano et al., 2009), we tested whether exogenous citrate treatment could rescue the sea mutant phenotypes. Indeed, sea\textsuperscript{Δ24/Df} mutants fed a citrate-supplemented diet for 24 h not only accumulated excess citrate but also exhibited a significant decrease in pyruvate, lactate and 2HG (Fig. 3C). These results indicate that citrate restricts glycolytic flux in Drosophila larvae and suggest that sea mutants accumulate excess L-2HG in response to decreased cytosolic citrate levels. Moreover, our observations support the findings of a recent human case study, in which a patient with combined D-/L-2HGA exhibited decreased urinary 2HG levels and reduced cardiac symptoms following citrate treatment (Muhlhause et al., 2014).

**sea mutants accumulate excess L-2HG owing to decreased degradation**

The correlation between excess L-2HG accumulation and increased glycolytic flux does not necessarily indicate that sea mutants synthesize more L-2HG. Rather, elevated L-2HG levels could result from increased synthesis, decreased degradation or a combination of both processes. In this regard, we previously demonstrated that L-2HG synthesis and degradation are both regulated by lactate metabolism. Not only does Drosophila LDH directly synthesize L-2HG from 2OG, but lactate also stabilizes the larval L-2HG pool by inhibiting dL2HGDH activity (Li et al., 2017). Consistent with these earlier observations, we found a highly positive correlation between lactate and 2HG levels in both sea\textsuperscript{Δ24/Df} mutants and sea\textsuperscript{pro/Df} controls (r=0.973, P<0.01; Fig. 4A), suggesting that sea mutants, similar to wild-type larvae, coordinate L-2HG accumulation with lactate production. We tested this possibility by measuring 2HG levels in sea\textsuperscript{Δ24/Df} mutants that expressed a UAS-dLdh-RNAi transgene. Relative to the control strains, dLdh-RNAi depleted dLdh mRNA levels in sea mutants by 80% and induced a ~60% reduction in both the lactate and 2HG pools (Fig. 4B; Fig. S3), demonstrating that dLDH influences L-2HG levels in mutant larvae.

The manner by which sea mutants accumulate L-2HG suggests two possible mechanisms: (1) sea mutants rely on dLDH to synthesize L-2HG at a higher rate than control larvae; (2) elevated lactate levels inhibit dL2HGDH activity and interfere with L-2HG

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**Fig. 2. sea mutants exhibit elevated levels of glycolytic flux.** (A) The relative metabolic flux rates from \textsuperscript{13}C\textsubscript{6}-glucose into pyruvate (pyr), lactate (lac), 2HG and citrate (cit). n=4. (B) Relative Pfk mRNA levels in sea\textsuperscript{Δ24/Df} mutant larvae that ubiquitously express a UAS-Pfk-RNAi transgene. n=3 samples containing 15 mid-L3 larvae collected from independent mating bottles. (C) Pfk-RNAi reduces pyruvate, lactate, and 2HG levels in sea mutant larvae. n=6 samples containing 15 mid-L3 larvae collected from independent mating bottles. All data are shown as means ± s.e.m. *P<0.05, **P<0.01, ***P<0.001. Data were analyzed using a two-tailed Student’s t-test with Bonferroni correction for multiple comparisons. Data are representative of at least two independent experiments.
degradation. We distinguished between these possibilities by measuring 2HG abundance in dL2HGDH<sup>12/14</sup>, sea<sup>Δ24</sup>/Df double mutants, which are able to synthesize, but not degrade, L-2HG. If loss of Drosophila CIC activity induces excess L-2HG synthesis, then dL2HGDH<sup>12/14</sup>, sea<sup>Δ24</sup>/Df double mutants should accumulate more L-2HG than the dL2HGDH<sup>12/14</sup> single mutant. In contrast, if sea mutants accumulate L-2HG owing to decreased degradation, then L-2HG levels will be similar in both genetic backgrounds. Our GC-MS analysis supports the latter model, as 2HG levels in sea<sup>Δ24</sup>/Df mutants accumulate L-2HG owing to decreased degradation, more L-2HG than the sea<sup>prec</sup>/Df double mutants exhibited increased levels of lactate and pyruvate, as well as decreased citrate levels (Fig. 4C). In contrast, if loss of dL2HGDH activity is inhibited by acidic pH (Nadtochiy et al., 2016), activity is inhibited by acidic pH (Nadtochiy et al., 2016),

**DISCUSSION**

L-2HG accumulation is coordinately regulated by glycolysis and the TCA cycle

The CIC plays a central role in cellular metabolism by controlling the amount of citrate that exits the TCA cycle and enters the cytosol. This function serves many purposes in cellular physiology, such as providing substrate for fatty acid synthesis, controlling histone acetylation and regulating cellular redox balance (Palmieri, 2013; Dolce et al., 2014; Morciano et al., 2009). In addition, cytosolic citrate serves to inhibit glycolytic flux, whether by inhibiting PFK protein in mammals (Kemp and Gunasekera, 2002), or by a yet to be determined mechanism in insects. This feedback mechanism fine-tunes central carbon metabolism by serving as a signal to slow glycolysis during times of sufficient energy production (Fig. 5). Our findings suggest that the role of citrate as a negative regulator of glycolysis represents the primary mechanism that induces L-2HG accumulation in sea mutants.

Based on our study and previous observations in human cell culture (Jiang et al., 2017), CIC deficiency induces elevated glycolytic flux and decreased citrate production (Fig. 5). As observed in Drosophila, human patients and CIC-deficient cells, these metabolic disruptions result in enhanced lactate synthesis, which, at least in the fly, inhibits dL2HGDH and stabilizes the L-2HG pool (Li et al., 2017; Jiang et al., 2017; Nota et al., 2013; Prasun et al., 2015). Such a model would also explain why citrate treatment could reduce 2HG levels in a patient with combined D-/L-2HGA (Mühlhausen et al., 2014), as partial restoration of cytosolic citrate levels would inhibit PFK enzyme activity and reduce glycolytic flux (Kemp and Gunasekera, 2002). Although it is tempting to speculate that citrate also inhibits Drosophila PFK, we would note that insect PFK homologs, when isolated from adult muscle tissue, are not inhibited by citrate (Nunes et al., 2016). Regardless of the mechanism, our findings demonstrate that citrate governs glycolytic flux in Drosophila larvae.

Our findings also highlight the role of dL2HGDH in regulating L-2HG accumulation. Although dLDH synthesizes most of the larval L-2HG pool, the kinetics of this reaction are poor and the only reasonable explanation for how flies accumulate such high L-2HG levels rests upon our observations that dL2HGDH activity is sensitive to lactate (Li et al., 2017). Because larval metabolism is highly glycolytic, aerobic lactate production stabilizes the L-2HG pool and allows for dramatic accumulation of this metabolite. Moreover, the positive correlation between L-2HG and lactate levels rests upon our observations that dL2HGDH activity is sensitive to lactate (Li et al., 2017). Because larval metabolism is highly glycolytic, aerobic lactate production stabilizes the L-2HG pool and allows for dramatic accumulation of this metabolite. Moreover, the positive correlation between L-2HG and lactate.
accumulating minimal amounts of D-2HG, suggesting that the metabolic enzymes driving D-2HG accumulation in humans have either diverged in flies such that they no longer synthesize this molecule, or that D-2HG is only produced under specific cellular conditions. When considered in this context, the lack of Drosophila D-2HG production highlights the importance of L-2HG in cellular metabolism, as the mechanisms that control L-2HG accumulation are conserved across phyla. Moreover, these observations reinforce the notion that Drosophila genetics provides a powerful tool for dissecting the metabolic mechanisms that underlie L-2HG metabolism.

Finally, the sea mutant phenotypes raise important questions regarding the subcellular regulation of L-2HG metabolism. LDH-dependent production of L-2HG likely requires export of 2OG from the mitochondria into the cytosol. L2GDH, however, is a mitochondrial enzyme (Rzem et al., 2004), thus raising the question of how L-2HG is transported across the mitochondrial membrane. Moreover, our results indicate that lactate is a key regulator of L2GDH. Although the exact nature of this feedback mechanism remains to be elucidated, including how changes in CIC activity influence the activity, abundance and localization of the enzymes involved in lactate and L-2HG metabolism, our findings hint at the possibility that lactate is either synthesized in, or transported into, the mitochondria. Such a metabolic mechanism would be of significant interest considering recent excitement surrounding the proposed mitochondrial lactate shuttle (Brooks, 2018). Overall, these basic questions illustrate how little is known about the endogenous L-2HG metabolism and highlight the need for further study of this compound in model systems.

The many faceted roles of 2HG in disease and metabolism

Combined D-/L-2HGA is associated with severe neurometabolic symptoms, developmental delays and childhood lethality (Muntau et al., 2000). Similarly, sea null mutations induce an early larval lethal phenotype, while the hypomorphic sea allele used in this study results in pupal lethality (Morciano et al., 2009). In addition, all examined sea mutant strains exhibit chromosomal abnormalities that include chromosome breaks and decreased histone acetylation (Morciano et al., 2009). Although these phenotypic parallels between flies and humans might suggest that 2HG accumulation is driving the sea mutant phenotypes, our ongoing studies indicate that larval development is largely resistant to elevated 2HG levels (Li et al., 2017). In fact, the amount of L-2HG in wild-type larvae is comparable to levels observed in L-2HGA and combined D-/L-2HGA patients (Muntau et al., 2000; Li et al., 2017; Barth et al., 1992), indicating that L-2HG, which is the primary 2HG enantiomer present in sea mutants, is not toxic to the development of flies maintained under standard culture conditions. Moreover, dL2GDH mutant strains, which accumulate L-2HG to levels comparable with those seen in sea mutants, are completely viable and can be maintained as homozygous stocks (Li et al., 2017). These observations suggest that the sea mutant phenotypes potentially result from changes in cytosolic acetyl-CoA availability and altered redox balance. Although this result differs from humans, in whom the accumulation of L-2HG causes severe neurometabolic symptoms (Barth et al., 1992; Ye et al., 2018), it does not diminish the importance of studying Drosophila L-2HG metabolism because the metabolic mechanisms underlying L-2HG accumulation are conserved between flies and humans. Moreover, the ability of Drosophila to withstand high levels of L-2HG accumulation establishes the fly as an important model for studying combined D-/L-2HGA, as it provides a system in which to identify disease
phenotypes that are caused by loss of CIC activity, but are not dependent on 2HG accumulation. Finally, our observations raise the question of why humans are sensitive to elevated L-2HG levels whereas flies are resistant. These organism-specific differences in L-2HG toxicity suggest that future comparisons of L-2HG metabolism in insects and mammals hold potential to identify factors that render cells sensitive to the pathological effects of this oncometabolite.

MATERIALS AND METHODS

Drosophila husbandry and genetics

Fly stocks were maintained on standard Bloomington Drosophila Stock Center (BDSC) media. Larvae were raised on molasses agar plates with yeast paste spread on the surface. All BDSC stocks used in this study are listed in Table S3. The sea^{Δ24} mutants and the precise excision control strain (sea^{rev}); previously noted as Rev^{Δ24}) were kindly provided by Dr Giovanni Cenci (Morciano et al., 2009). All experiments used a trans-heterozygous combination of sea^{Δ24} and a molecularly defined deficiency. All controls consisted of a trans-heterozygous combination of sea^{rev} and the same deficiency. The UAS-sea strain was generated by injecting the Drosophila Genomics Resource Center (DGRC) plasmid UFO06122 into BDSC stock 8621. dL2HGDH mutant strains used were as previously described (Li et al., 2017).

Sample collection

Trans-heterozygous larvae of the appropriate genotypes (sea^{Δ24}/DF and sea^{rev}/DF) were separated from siblings that harbored a TM3, P[Dfd-GMR-nvYFP]3, Sb[1] balancer chromosome based on the absence of YFP expression. For each experiment, individual samples were collected from independent mating bottles as described (Li and Tennessen, 2018). Each sample contained 15 middle-third-instar (mid-L3) larvae of mixed sex from independent mating vials. All GC-MS experiments were repeated a

Metabolomics and metabolic flux analysis

Samples for GC-MS analysis were processed as previously described (Li and Tennessen, 2018). For each experiment, six individual samples were collected from independent mating vials. All GC-MS experiments were repeated a minimum of two times. Metabolite extraction, derivatization and GC-MS analysis were conducted as described previously (Li et al., 2017; Li and Tennessen, 2018). Spectral data preprocessing was performed using MetAlign software (Lommen, 2009). Data were normalized to both the sample mass and the amount of lactate produced by glycolysis. In contrast, mutations in sea result in decreased CIC activity, decreased cytosolic citrate levels and increased glycolytic flux. As a result, sea mutants synthesize excess lactate, which interferes with L2HGDH activity and promotes L-2HG accumulation. CIC, mitochondrial citrate carrier; LDH, lactate dehydrogenase; 2-LHG, L-2-hydroxyglutarate; TCA, tricarboxylic acid; 2OG, 2-oxoglutarate.

qRT-PCR

Total RNAs were extracted using Trizol reagent (Thermo Fisher Scientific). cDNA was made using the Maxima First Strand cDNA Synthesis Kit (Thermo Fisher Scientific), and quantitative PCR was performed using FastStart Essential DNA Green Master Kit (Roche Diagnostics) in a LightCycler 96 instrument (Roche Diagnostics). The primers for rp49 (also known as RpL32) and dLdh were the same as reported previously (Li et al., 2017). Additional primer sequences are described in Table S4.

Acknowledgements

We thank Jonathan Karty, Angela Hansen, Doug Rusch and Chris Hemmerich for technical assistance; and Dr Giovanni Cenci (University of Rome) for strains and helpful advice.


Table S1. RNA-seq analysis of sea[24]/Df mutants compared to sea[prec]/Df controls. For each sample, RNA was extracted from 15 mid-L3 larvae of the appropriate genotypes and transcript abundance was measured using RNA-seq.

Click here to Download Table S1

Table S2. The expression of genes encoding glycolytic enzymes were compared between sea[24]/Df mutants and sea[prec]/Df controls. Data derived from Supplemental Table S1.

Click here to Download Table S2
Table S3. Bloomington Drosophila Stock Center (BDSC) strains used for genetic analysis.

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Table S4. Oligos used for qRT-PCR analysis.

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Figure S1. Metabolic defects induced by the sea$^{24}$ mutation were confirmed using a different deficiency strain $Df(3R)BSC469$ ($Df$). Data are shown as mean ± SEM. $n = 6$, *$P < 0.05$, **$P < 0.01$. 
Figure S2. Changed metabolic profiles caused by Pfk knockdown. (A) Expression of dsRNA for RNAi of Pfk (Pfki) down-regulates the transcriptional levels of Pfk significantly. Data shown as mean ± SEM, n = 3, ***P < 0.001. (B) Changed metabolites induced by Pfk knockdown. Data shown as mean ± SEM, n = 6, **P < 0.01, ***P < 0.001.
**Figure S3.** Relative dLdh mRNA levels in sea^{Δ24}/Df mutant larvae that ubiquitously express a UAS-dLdh-RNAi transgene. Data are shown as mean ± SEM, n = 3, ***P < 0.001.
How would you explain the main findings of your paper to non-scientific family and friends?

Human mutations in a gene encoding the protein mitochondrial citrate carrier (CIC) cause a rare inborn error of metabolism known as combined D-/L-2-hydroxyglutaric aciduria. A key feature of this disease is elevated levels of another protein, 2-hydroxyglutarate (2-HG), which are associated with severe neurological defects and developmental delay. In our study, we demonstrated that, in fruit flies, mutations in this gene induce metabolic defects that are similar to those observed in human patients. Moreover, we found that CIC regulates L-2-HG stability by governing the production of lactate, hinting at a potential treatment for patients with this disease.

What are the potential implications of these results for your field of research?

Prior to our work, the mechanism by which the loss of CIC function results in 2-HG accumulation was unknown. Our study reveals a previously unknown metabolic mechanism that explains why human CIC mutations result in elevated L-2-HG accumulation and provides a potential treatment strategy for combined D-/L-2HG aciduria.

What are the main advantages and drawbacks of the model system you have used as it relates to the disease you are investigating?

"[T]he combination of a vast range of available genetic tools with modern metabonomic techniques [...] can help to dissect the mechanisms underlying metabolic diseases in further detail."

There are many advantages to using the Drosophila model system. For my study, I think the most important one is the combination of a vast range of available genetic tools with modern metabonomic techniques, which can help to dissect the mechanisms underlying metabolic diseases in further detail. The drawback of using a Drosophila model to study D-/L-2-HG aciduria is that, unlike humans, the fly can withstand high levels of 2-HG without severe neurological defects. Regardless, the metabolic regulation of 2-HG accumulation is conserved between humans and flies.

Describe what you think is the most significant challenge impacting your research at this time and how will this be addressed over the next 10 years?

A significant challenge impacting our research is to study the metabolism of 2-HG in a tissue-specific manner at high resolution, because fruit flies are relatively small. With the development of modern mass spectrometry imaging techniques, this challenge will probably be addressed over the next 10 years.

What changes do you think could improve the professional lives of early-career scientists?

I think, for early-career scientists, persistence in research is very important. In addition, communication skills, hard work, and efficient time management are also essential.

What’s next for you?

I will continue to use the genetic power of Drosophila models and metabolomics to study gene functions in the regulation of metabolic homeostasis.

Reference