Characterization of disease-related phenotypes in a *Drosophila* model of Mucopolysaccharidosis type I

De Filippis C.\(^1,2\), Napoli B.\(^3\), Rigon L.\(^1\), Guarato G.\(^4\), Tomanin R.\(^1,2\) and Orso G.\(^4\)

\(^1\) Laboratory of Diagnosis and Therapy of Lysosomal Disorders, Department of Women’s and Children’s Health, University of Padova, Via Giustiniani 3, 35128 Padova, Italy; \(^2\) Fondazione Istituto di Ricerca Pediatrica “Città della Speranza”, Corso Stati Uniti 4, 35127 Padova, Italy; \(^3\) Scientific Institute, IRCCS Eugenio Medea, Laboratory of Molecular Biology, Via Don Luigi Monza 20, 23842 Bosio Parini, Lecco, Italy; \(^4\) Department of Pharmaceutical and Pharmaceutical Sciences, University of Padova, Via Marzolo 5, 35131 Padova, Italy

**Introduction**

Mucopolysaccharidosis type I (MPS I) is a metabolic, rare, autosomal recessive disease, caused by mutations in the gene encoding for the lysosomal enzyme α-L-iduronidase (IDUA), that lead to a lack of enzymatic activity [1]. IDUA is responsible for the degradation of the glycosaminoglycans (GAGs) heparan- and dermatan-sulphate, which therefore accumulate undegraded in lysosomes, leading to a progressive multi-organ dysfunction, including the central nervous system in the severe forms [2]. Although clinically well characterized, still little is known about the mechanisms underlying the pathology. Therefore, we developed a *Drosophila melanogaster* model for MPS I, which displays disease-related phenotypes, to better characterize the pathogenesis of the disease and to search for biomarkers, that can be useful to address new therapeutic strategies.

**Advantages of the use of *Drosophila melanogaster***

- Easy to handle and short life cycle
- Availability of transgenic fluorescent lines for *in vivo* studies
- Tissue-specific gene expression
- Chance of pharmacological screening
- Studies of the transport of drugs across the emato-encephalic barrier

**Materials and methods**

- The model was generated using the RNAi approach, which allows the down-regulation of the *D-ida* gene using the UAS-Gal4 system
- Real-time qPCR, IDUA activity analysis and GAGs analysis were performed on RNA and proteins extracted from 3rd instar larvae
- Confocal images were recorded on fixed samples of 3rd instar larvae stained with antibody anti-Ref(2)p
- Live imaging of Lysotracker assay was performed on fresh dissected 3rd instar larvae

**Figure 1.** Schematic representation of UAS-Gal4 system. The ubiquitous down-regulation of *D-ida* with the ubiquitous driver Tubulin-Gal4, leads to a reduction of the gene expression up to 61% and to a complete lethality at pupal stage.
Figure 2. A reduction of IDUA activity to one third compared to controls (A), although causing a complete lethality at pupal stage, doesn’t lead to a significative accumulation of GAGs (B) in 3rd instar larvae where D-idua was ubiquitously down-regulated (driver Tubulin-Gal4). N=60/group. Down-regulation of D-idua in (C) neurons (driver Elav-Gal4) and (D) glial cells (driver Repo-Gal4) leads to a mild, progressive impairment in the climbing performance of adult flies, which could be due to both neurological or muscular impairment. N=100/group. All data are expressed as mean ± SEM.

Figure 3. Increased number and size of lysosomes in brain (A) and muscles (B) of 3rd instar larvae where D-idua was ubiquitously down-regulated, resembles the well known phenotype already seen in vitro on patient’s fibroblasts and in the mouse model. (C) Reduction of the percentage of acidified lysosomes in muscles of 3rd instar larvae, where D-idua was ubiquitously down-regulated. N=10. Data are presented as mean ± SEM.
Figure 4. (A) Schematic representation of crossings performed. (B) Representative confocal images of 3rd instar larvae muscles where D-idua was ubiquitously down-regulated, and relative quantification of (C) lysosomes (Lamp1), autophagosome (Atg8a and Ref(2)p) and auto-lysosomes, and (D) % of autophagosomes-lysosomes fused. In (C), asterisks indicate a statistically significant difference from Ctrl and hash marks indicate a statistically significant difference from RNAi. The increase of the autophagic flux followed by a block of the autophagosomes-lysosomes fusion is ameliorated in starvation conditions, where we can observe a reduction of the Lamp1-positive particles and an increased percentage of autophagosomes fused with lysosomes, similar to the controls. Arrows indicate autophagosomes. STD indicates standard conditions; STV indicates a starvation of 4h. N=10/group. Data are expressed as mean ± SEM.
Figure 5. (A) Schematic representation of crossings performed. (B) Representative confocal images of 3rd instar larvae muscles where D-idua was ubiquitously down-regulated and relative quantification of (C) autophagosomes (green+red and Ref(2)p), and auto-lysosomes (red without green), and (D) % of mature auto-lysosomes. In (C), asterisks indicate a statistically significant difference from Ctrl; hash marks indicate a statistically significant difference from RNAi and crosses indicate a statistically significant difference from Ctrl STV. Also in this case, we can observe an improvement of the autophagic flux in starvation conditions, indicated both by the decrease of the Ref(2)p marker, which is correctly degraded after the fusion of the autophagosomes with the lysosomes, and by the increase of the percentage of correct mature autolysosomes. Arrows indicate autophagosomes. STD indicates standard conditions; STV indicates a starvation of 4h. N=10/group. Data are expressed as mean ± SEM.
Figure 6. qPCR of genes involved in (A) the glycolytic pathway and (B) the lipid synthesis pathway in 3rd instar larvae where D-idua was ubiquitously down-regulated. Concerning the glycolysis, we can observe an upregulation of the expression of the genes Tpi (Triose phosphate isomerase), Pfk (Phosphofructokinase) and Ldh (Lactate dehydrogenase), that return almost at the control levels in starvation conditions. The same consideration can be done for the Acc (Acetil-CoA carboxylase) gene for the lipid synthesis pathway. STV indicates a starvation of 4h. N=20/group. Data are expressed as mean ± SEM.

Conclusions and future perspectives

The D. melanogaster model for MPS I that we developed represents a promising tool for the study of the disease:

• IDUA have a fundamental role in the fruit fly development, since, when it is ubiquitously down-regulated, it causes lethality at pupal stage.

• The resulting reduced enzyme activity leads to an increase of the number and size of lysosomes, a phenotype which correlates with what already observed in patient’s fibroblasts and in the mouse model [3,4]; however, although increased in number and size, lysosomes probably don’t have a proper function, since they are not correctly acidified.

• The alteration of the autophagic pathway can be partially rescued by starvation, which is a method to induce autophagy. The activation of autophagy can represent a strategy of the cell to compensate the lack of activity of the lysosomes, and to remove undegraded molecules which accumulate inside the cell [5]; therefore the use of drugs that induce autophagy could be a therapeutic strategy in order to improve the outcome of the pathology, at least from a molecular point of view. In this case, this model could be very useful, because it permits a rapid screening of a lot of molecules in a very short time.

• The metabolic alterations observed in the glycolytic and lipid genesis pathways are also related to the alteration of autophagy, since the cell can use the products of these pathways to generate the autophagic particles. Moreover, the activation of these metabolic pathways could be a strategy used by the cell to produce some molecules that cannot obtain from the degradation activity of the lysosomes, which don’t work properly [6].

• Further investigation about the mild climbing impairment will be done to assess a potential neurological involvement.

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Laura Rigon
Rosella Tomanin
Giulia Guarato
Genny Orso
Barbara Napoli
Corresponding author: concetta.defilippis93@gmail.com

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