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Drosophila as a starting point for developing therapeutics for the rare disease Duchenne Muscular Dystrophy

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Progress into developing therapeutics for rare diseases can be accelerated for those diseases that can be modeled in genetically tractable organisms. Here we comment on one disease, Duchenne Muscular Dystrophy (DMD), modeled in *Drosophila* that brought together disparate lines of research toward the goal of developing a therapeutic. Though the bioactive lipid sphingosine 1-phosphate (S1P) has been implicated in many anabolic processes in many cell types and tissues, including muscle, this work confirmed the therapeutic potential of assessing this pathway for DMD. Genetic dissection of sphingolipid metabolism showed the suppression of muscle structural and functional defects in flies. Moreover, improvement of muscle defects using known pharmacological agents that raise S1P levels in vivo highlight the potential of *Drosophila* as a drug-screening tool for DMD. We and others have extended S1P studies into the mouse model of DMD and have shown a partial amelioration of symptoms associated with DMD. Translation of this work to mammals makes the sphingolipid metabolism pathway a promising target for further drug development that may benefit the human condition.

Keywords: *Drosophila*, Duchenne Muscular Dystrophy, sphingosine 1-phosphate, THI, FTY720

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Commentary

Though there are many animal models for rare diseases[1] most of these are mammalian, which is apt as they are closely related to the human condition. However, a strong case can be made for using lower animals to model rare diseases. Rare disease phenotypes due to mutations in the homologous genes in lower animals such as *C. elegans* or *Drosophila melanogaster* would allow for the immense power of forward genetic screening to identify modifiers of these phenotypes (**Fig. 1A**). We have found that Duchenne Muscular Dystrophy (DMD), an X-linked age-dependent muscle wasting disease caused by the loss of the dystrophin gene could be modeled in *Drosophila*.[2] In addition to the notable muscle and neuronal defects, the reduction of Dystrophin in flies yielded another, visible phenotype, a defective posterior cross-vein in the wing.[2,3] Since visible phenotypes are more easily scored than functional phenotypes, i.e., movement phenotypes, we used the defective cross-vein to screen for modifiers of *Dystrophin* function.[4] The screens undertaken yielded not only modifiers of the cross-vein phenotype but also modifiers of muscle morphology.[5] We dissected the function of one strong suppressor of wing vein defect that also strongly suppressed the muscle phenotypes associated with the loss of dystrophin.[6] This suppressor, *wunen*, a human lipid phosphate phosphatase 3 homolog, revealed that altered sphingolipid metabolism can affect muscle wasting. In particular, the elevation of sphingosine 1-phosphate (S1P) can suppress dystrophic muscle degeneration. With the development of a sensitive myofibril integrity assay using immunohistochemical analysis and the use of the readily available activity monitors for insect movement we could rapidly assess both genetic and pharmacological candidates for muscle wasting suppression. It should be noted that the myofibril assay

is based on the loss of Projectin protein, a Titin homolog in *Drosophila* dystrophic mutants. Similarly, Titin has shown to extensively degrade in DMD patients,[7] further corroborating the similarity of the disease phenotypes between flies and humans. Hence analyzing Titin pattern in sarcomeres of dystrophic flies and mice may be a sensitive method for analyzing the beneficial effects of potential drugs.

Establishment and Use of Phenotypes Associated with the Genetic Disease Duchenne Muscular Dystrophy in *Drosophila*

It is known that S1P promotes anabolic processes in muscles by increasing muscle stem cell proliferation and muscle differentiation.[8,9] It was this knowledge that compelled us to clarify the role of S1P in suppressing *Drosophila* muscle wasting (**Fig. 1A**). With available mutants that altered sphingolipid metabolism, particularly, *UAS-lace*, the overexpression line that can elevate *lace* (serine palmitoyl CoA transferase) activity and the *Sply*, (S1P lyase) line, we were able to determine that alternate ways of increasing S1P promoted muscle wasting suppression. Moreover, with the reduction of *spinster*, a putative S1P transporter, we found that by presumably limiting S1P transport between cellular compartments and enriching S1P in the cytoplasmic compartment is sufficient to suppress dystrophic muscle wasting in flies. The information gleaned from these studies is an obvious starting point for future work in higher animals. Additionally, there are other modifiers from the initial screens[4] that suppress the cross-vein phenotype that have not been characterized as yet and their study may be just as fruitful as the study of *wunen* (**Fig. 1B**).

Elevation of S1P Promotes the Reduction of Pathological Symptoms Associated with DMD in the *mdx* Mouse

It would be interesting to see if genetic elevation of S1P in the dystrophic mouse model (*mdx*) would significantly suppress the phenotypes associated with DMD in mice. So far we[10] and others[11] have undertaken studies with a more therapeutic slant by analyzing small molecules that elevate S1P levels in *mdx* mice (**Fig. 1C**). More specifically, we have found that delivery of S1P itself into dystrophic muscle increases muscle progenitor cell proliferation; and have also found that systemic delivery of a putative S1P lyase inhibitor, 2-acetyl-4(5)-tetrahydroxybutyl imidazole (THI), dramatically reduces fibrosis and fat deposition in dystrophic mice as well as improves the function of acutely injured dystrophic muscle.[10] Furthermore, we have recently shown that THI can also promote a functional benefit to uninjured dystrophic muscle in a one month long treatment using young *mdx* mice.[10] It is also interesting to note that THI is a trace component of Caramel Color III, which is categorized by the FDA as GRAS (generally recognized as safe) making it an interesting candidate for possible use in humans. The outcomes with these small molecule delivery experiments establish that information gleaned from work in lower animals can powerfully translate to higher animals, which may then lead to clinical trials in humans.

Drosophila as a Tool for the Discovery of Small Molecule Therapeutics for DMD

As we have established muscle and activity phenotypes for dystrophic flies, we analyzed these to assess the efficacy of small molecules to suppress the dystrophic phenotypes. Using a candidate drug approach, we fed dystrophic flies solutions that contained THI, THI-oxime and FTY720. THI-oxime (or LX2931 from Lexicon Pharmaceuticals, Inc.) is a derivative of THI and is in clinical trials for treating rheumatoid arthritis[12] and FTY720 is an S1P agonist in addition to being an FDA approved drug used to treat Multiple Schlerosis (trade name Gilenya from Novartis, Inc.). Both compounds either elevate S1P or S1P signaling. We have found that all three compounds suppress muscle wasting when fed to dystrophic flies.[6] The THI result substantiates the similarity of muscular dystrophy in flies and mice, the results with the other two compounds are exciting and suggests that both compounds that are therapeutically used in humans may be effective in suppressing muscle wasting in DMD patients. Certainly it will be interesting to determine whether THI-oxime and FTY720 suppress muscle wasting in the *mdx* mouse, a prelude to future clinical trials. Importantly, these results have validated the possible use of *Drosophila* in a drug discovery screen for suppression of muscle wasting (**Fig. 1D**). A recent review summarized the efficacy of *Drosophila* as a human disease model organism useful for drug discovery.[13] With our work we have extended the list of human diseases to include the rare disease, Duchenne Muscular Dystrophy. There are many examples of drugs that are safe in mammalian cell culture ("hits" obtained in a screen) that translate to being toxic when tested in rodents. *Drosophila* may be extremely useful in obtaining immediately "higher quality hits" since the in vivo primary screen will address toxicity as well as suppression of phenotypes that are directly related to the disease.

In summary our work with THI, THI-oxime and FTY720 shows that the beneficial effects of a compound tested in dystrophic flies may translate to mammals. In the case of THI, this has been done by us and others.[10,11] It will be important now to extend this work to THI-oxime and FTY720. Likewise, new promising compounds identified in the fly can be immediately tested in well-established models of DMD, the *mdx* mouse and the more recently established *mdx* utrophin/+ mouse. Additionally promising drugs from an FDA approved library may provide valuable candidates for DMD drug therapeutics that have already been approved as medicine in humans and therefore this repurposing will shorten the time to the clinic compared with drugs that need to go through the FDA's Investigational New Drug (IND) procedures.

Furthermore, while these studies are addressing small molecules that might ameliorate the rare muscle disease, DMD, muscle degeneration is observed in other diseases and normal aging as well. Therefore, compounds that are identified should also be tested for other kinds of muscle wasting. Therefore, aside from being a therapeutic for muscle diseases, a small molecule that is also protective

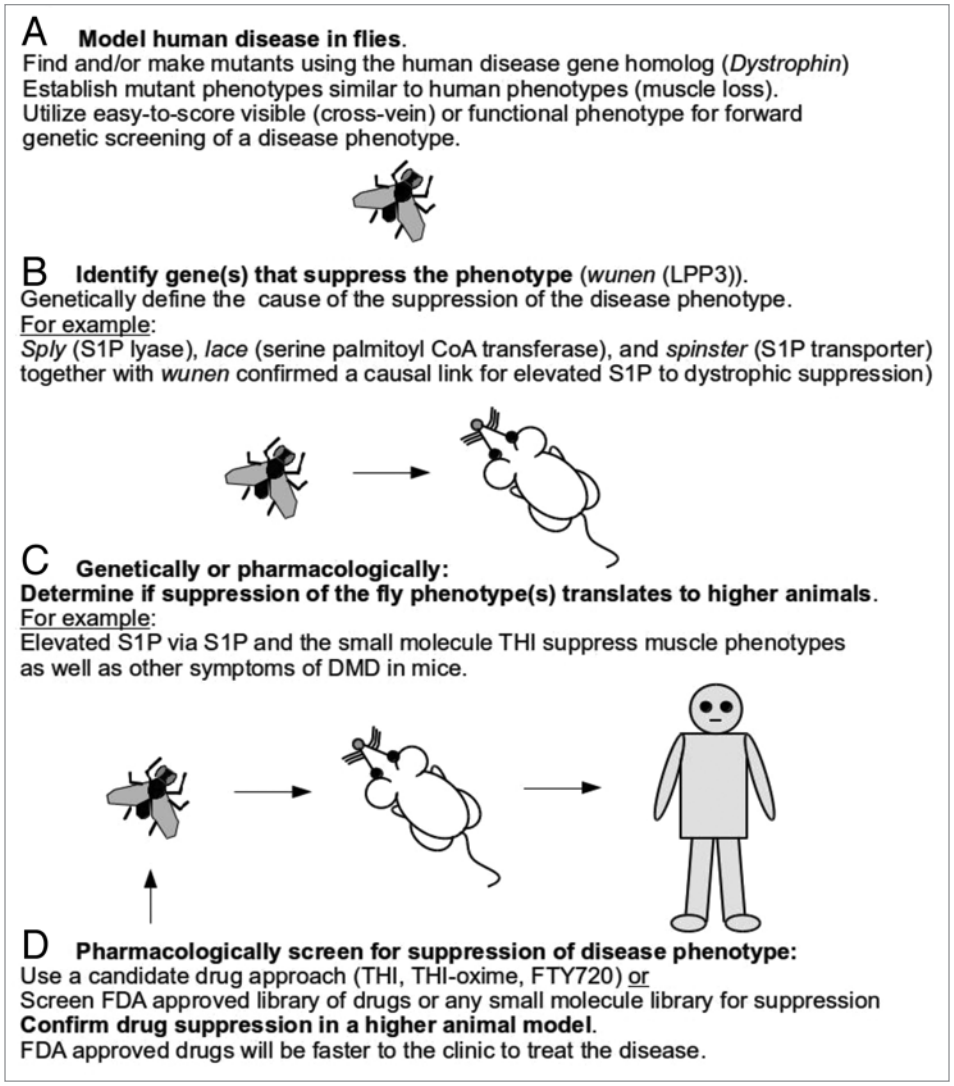

Figure 1. Strategy for the use of model organisms for rare disease study. (**A**) and (**B**) Establish phenotypes; screen easy-to-score phenotypes for modifier candidates of the disease process. Dissect identified pathways. DMD characterized by the loss of dystrophin protein is well modeled in *Drosophila*. Visually, the loss of Dystrophin can be scored in the posterior cross-vein of the fly wing, which allowed for easy screening of modifiers. Using muscle structural and functional phenotypes elevated S1P action was genetically confirmed to alleviate muscle wasting. (**C**) Extend studies to higher animals by genetic and/or pharmacological analysis. With DMD we found that using S1P and the S1P lyase inhibitor, THI partially ameliorated DMD pathology in *mdx* mice. (**D**) *Drosophila* may be used to screen drugs. A accelerate the process, complete FDA approved libraries of drugs can be screened in flies with the repurposing goal. Effective drugs may then be tested in mice where confirmation of efficacy may yield clinical trial candidates. Additionally, any small molecule library may be screened for muscle wasting suppression in flies.

or suppressive in normal age-dependent muscle degeneration—the dramatic loss of muscle strength that takes place in all of us above 20 years of age—would increase lifespan and be significantly beneficial to modern society.

Disclosure of Potential Conflicts of Interest

No potential conflict of interest was disclosed.

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