Alteration of *Drp1* Expression in *Drosophila* Models of Parkinson Disease

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Abstract

Background: Parkinson Disease (PD) and other neurodegenerative diseases have a significant relationship with mitochondrial dysfunction. The substantial effect of mitochondrial dynamics in PD has led us to study the role of the gene encoding the mitochondrial fission protein *Drp1* in *Drosophila* models. *Drp1* is a member of the highly conserved, dynamin family of protein encoding genes. *Drp1* plays an essential role in the maintenance of the mitochondrial, peroxisomal, and endoplasmic reticulum (ER) dynamics, and has been found to regulate processes during homeostasis and cell survival.

Results: The directed expression of *Drp1* in *Drosophila melanogaster* neurons under the control of the *Ddc-Gal4* transgene decreases the lifespan and compromises climbing ability over time. The directed inhibition of *Drp1* produces a novel model of Parkinson Disease, as this causes little change in mean lifespan but a significant decrease in locomotor or climbing abilities. Interestingly, the loss of *park* dependent *Drosophila* model of PD is rescued by the directed inhibition of *Drp1*.

Conclusion: The compromised climbing abilities in flies with directed inhibition of *Drp1* has produced a new model for Parkinson Disease and can be used to further investigate the mechanism(s) underlying PD and other neurodegenerative diseases. Interestingly, the combined inhibition of both *Drp1* and *park* suppresses Parkinson Disease phenotypes and promotes survival.

Background

Mitochondria are critical organelles in the process of survival at the cellular level. Mitochondria accumulate damage over time to become dysfunctional and contribute to the process of ageing and eventually to the death of an organism [1]. Mitochondria are known to be the powerhouse of the cell, but its role goes beyond the production of ATP (oxidative phosphorylation); it is responsible for various aspects of energy homeostasis, oxidative stress, calcium handling, cell signalling, and cell survival [2]. The central role of mitochondria in energy regulation and signalling implies that its dysfunction would have devastating effects upon cellular functions. Such is especially true for the nervous system because subtle changes in signalling can have catastrophic consequences leading to neurodegenerative disease.

The shape and size of mitochondria are not fixed properties; mitochondrial morphology depends upon a number of factors, including stage of the cell cycle and cell type, and can change quite quickly in response to external stimuli or metabolic cues. Changes to the mitochondrial network seem to differentially influence a number of signalling pathways [3]. Therefore, mitochondria undergo fission and fusion frequently in order to change their structure in response to the specific requirements of the cell, under a wide range of circumstances. Mitochondrial fission allows segregation of damaged mitochondria components while the process of fusion facilitates the exchange of mitochondrial material vital to maintain homeostasis within the mitochondrial network. Mitochondrial fusion helps compromised mitochondria, with highly damaged DNA and protein, to actively exchange components with other more healthy mitochondria to decrease the severity of heteroplasmy, and help with functional

complementation [4, 5]. Mitochondrial fission allows for the segregation of irreversibly damaged portions of the mitochondrial network and subsequent degradation. Mitochondrial fission necessarily requires *dynammin-related protein 1 (Drp1)* and *FIS1* [6]. Nevertheless, an explicit understanding of the factors promoting fission and fusion remains limited.

The *Drp1* gene encodes a dynamin family GTPase protein comprised of a characteristic Dynamin like protein family domain (Dynamin and Mx protein domains), a dynamin central domain, and a dynamin GTPase effector domain. While predominantly located in the cytoplasm, a small fraction is located upon the cytoplasmic surface of the mitochondrial tubules. Overexpression of *Drp1* causes mitochondrial fragmentation, whereas inhibition results in the elongation of the mitochondrial network. *Drp1* protein function is regulated by post-translational modification via phosphorylation, where a well-documented phosphorylation site, S616, promotes fission through an increase in activity. In contrast, phosphorylation of another site, S637, acts to lessen fission through reduced activity [7, 8, 9]. *Drp1* polymerizes to form a spiral structure around the mitochondrial tubule, and then utilizes its GTPase activity to constrict the tubule and eventually cause fragmentation of the mitochondria [10, 11, 12 13]. Presumably, a low membrane potential promotes fission, while a high membrane potential obstructs fission [14]. Through its role in mitochondrial fission, *Drp1* controls mitochondrial morphology and function.

The role of *Drp1* protein is not limited to mitochondrial fission. In addition, it participates in peroxisomal fragmentation [15] and maintains the morphology and function of the Endoplasmic Reticulum (ER) [16, 17]. Also, Drp1 is required for a standard rate of cytochrome c release and caspase activation during programmed cell death [18]. The role of Drp1 in apoptosis is not clear, but the product of the *Bax* gene, a pro-apoptotic Bcl-2 family protein, has been found to co-localize with Drp1 at the site of mitochondrial fission [19]. Drp1 plays role in Bcl-2 regulated apoptosis, peroxisomal and ER fragmentation.

The Drp1 protein interacts with other proteins involved in a number of mitochondrial processes, such as the products of *Pink1 (PTEN-induced putative kinase 1)* and *park* [20]. Mutation of the *Pink1* and *park* genes are among the most prominent causes of early onset PD. The roles of *Pink1* and *park* are vital to ubiquitin-dependent mitophagy. The protein product of *park* is involved in the process of ubiquitylation that plays a crucial role in the proteasomal directed degradation of proteins, such as Drp1 [21]. Activated Akt can increase Drp1 phosphorylation and its localization to the mitochondria, to promote “mitofission” with an accompanying increase in the generation of reactive oxygen species [22]. *Drp1* knockouts increase nuclear translocation of transcription factor foxo and enhance the expression of its downstream targets [23]. Akt protein inhibits the nuclear translocation of foxo [24, 25] and regulates *Pink1/park* dependent mitophagy [26]. Foxo transcriptionally upregulates the *Pink1/park* pathway in mammals and *Pink1* appears to function upstream of foxo in *D. melanogaster* [27, 28]. Such a feedback mechanism implies a complex relationship between *Drp1* and *park*, *Akt1* and *foxo*.

Here, we propose that gain-of-function of *Drp1* results in a Parkinsonian-like phenotype. We use drosophila to model Parkinson disease because it is an excellent model system in which to study interactions between genes, including PD genes [29, 30]. The inhibition of *Drp1* in the dopaminergic

neurons of a mouse MPTP model of PD gives protection against mitochondrial translocation of p53 and the loss of dopaminergic neurons [31]. In our experiment, we used the *UAS-Gal4* system to direct and to inhibit the expression of the *Drp1* gene in *Drosophila*. The *GMR-GAL4* transgene directs expression in the developing eye [32] and the *Ddc-Gal4* transgene directs expression in dopaminergic and serotonergic neurons [33, 34]. Overexpression of *Drp1* has toxic effects; although, its inhibition slightly improves the lifespan, the climbing ability over time is compromised. In an established *park-RNAi* model of PD [35], we directed and inhibited the expression of the *Drp1* gene. The PD-like phenotypes of *Ddc-Gal4 park RNAi* were rescued by the expression of *Drp1-RNAi* transgenes. The strategy is to identify the basic mechanism in simple model organism and then further validate the finding in mammalian model organism.

Results

Drp1 is highly conserved between *Homo sapiens* and *Drosophila melanogaster*

The *D. melanogaster Drp1* protein sequence was sourced from NCBI protein and the conserved sequences were identified using NCBI CDD. NCBI protein Blast of *Drp1* protein of *D. melanogaster* (NP_608694.2) with the *H. sapiens*, identified dynamin-1-like protein (isoform 4) (NP_001265392.1), it is 65% identical with a bit score of 957. The multiple sequence alignment of the two proteins derived by Clustal Omega (Fig. 1A) shows a highly conserved dynamin-like protein family domain, a dynamin central domain, and a dynamin GTPase effector domain. Two well-documented phosphorylation sites are identified; S606 and S627 in *dynamin-1-like protein* isoform 4 of *H. sapiens*; and S616 and T637 in *Drp1* of *D. melanogaster*. A template-based modeling of *D. melanogaster Drp1* protein by use of a combination of empirically derived energy functions and physics-based simulated folding was produced using Phyre2. The modeled *D. melanogaster Drp1* protein (i) and the *H. sapiens* Dynamin-1 like protein (ii) from the NCBI database share an identical structure (Fig. 1B). The amino-terminus region of the *Drp1* protein is highly conserved and has a consensus LC3-interacting region (LIR) sequence for binding to the ATG8/LC3 protein as determined by the Eukaryotic Linear Motif (ELM) resource. As this protein is so highly conserved, it seems very likely that the functions are highly conserved.

The overexpression and inhibition of *Drp1* with *Ddc-Gal4*

The overexpression of *Drp1* by the *Ddc-Gal4* transgene results in a decreased lifespan of 56 days compared to the control of 68 days shown in Fig. 2A. Inhibition of *Drp1* by two distinct RNAi transgenes, via the *UAS-Drp1-RNAi1* and *UAS-Drp1-RNAi2* directed by the *Ddc-Gal4* transgene, results in lifespans of 70 and 72 days, respectively; very similar to the 68 days observed in the control, as determined by log-rank at a $P < 0.0001$ (Fig. 2A). While, the overexpression of *Drp1* by *Ddc-Gal4* does little to alter locomotion over time, the climbing abilities of flies expressing *Drp1-RNAi*'s are severely compromised as determined by 95% confidence interval in a nonlinear fitting of the climbing curve (Fig. 2B).

The inhibition of *Drp1* in the *Ddc-Gal4 UAS-park-RNAi* model of PD

The loss of function of *park* has led to the establishment of a number of *Drosophila* models of PD. The *Ddc-Gal4 park-RNAi UAS-lacZ* critical males have a median lifespan of 58 days. Overexpression of *Drp1* in the *Ddc-Gal4 park-RNAi* expressing flies results in a similar life span of 58 days which is not significantly different compared to the control. The two *UAS-Drp1-RNAi* transgenes, *UAS-Drp1-RNAi1* and *UAS-Drp1-RNAi2*, when expressed along with *Ddc-Gal4 park-RNAi*, results in a much-increased median life span of 84 and 76 days, respectively; determined by log-rank at a $P < 0.0001$ (Fig. 3A). The overexpression of *Drp1* by *Ddc-Gal4* along with *park-RNAi* slightly increases the climbing ability over time. However, the locomotor activity of the critical classes with the directed expression of the *Drp1-RNAi transgenes* are significantly increased (Fig. 3B) as determined by 95% confidence interval in a nonlinear fitting of the climbing curve.

Overexpression of Drp1 during development of the eye decreases ommatidia and bristle number

The inhibition and overexpression of *Drp1*, directed by the *GMR-Gal4* transgene in the developing eye of flies affects development. The expression of *UAS-Drp1* and *UAS-Drp1-RNAi1* in developing eye directed by *GMR-Gal4* transgene results in significantly higher mean number of ommatidia, 716.9 and 718.8, respectively compared to 703 for the *lacZ* control flies (Fig. 4B) as determined by unpaired T test with a P value of 0.0483 and 0.0484. The mean of interommatidial bristle produced through inhibition by the *UAS-Drp-RNAi1* and *UAS-Drp1-RNAi2* transgene was significantly higher at and 556.7 (P value = 0.0406) and 578.7 (P value = 0.0023) compare to 536 of control flies as determined by an unpaired T test. The mean number of interommatidial bristle for *UAS-Drp1* flies was 541.6 compared to 536 of control, which is not significant as determined by an unpaired T test (P value = 0.6128). The ommatidia area, compared to the control of 200 μm^2 per ommatidium, of the overexpression critical class was 213.7 μm^2 (P value = 0.0303) and the ommatidium area produced by the *UAS-Drp1-RNAi1* and *UAS-Drp1-RNAi2* transgenes were 225 μm^2 (0.0490) and 217 μm^2 (P value = 0.0011), which are all significantly different as determined by unpaired T test(Fig. 4D).

Discussion

The critical role played by the structure of the mitochondria in the function of the organelle suggests that the product of the *Drp1* gene acts as an essential component in the regulation of a number of sub-cellular processes. Excessive mitochondrial fragmentation is associated with dysfunctional metabolic diseases and a “hyper-fused” mitochondrial network serves to protect from metabolic insult and autophagy [36]. In the skeletal muscle of mice, *Drp1* overexpression causes the severe impairment of post-natal muscle growth as it results in attenuated protein syntheses and the downregulation of growth hormone pathways [37]. The high fat and high glucose conditions cause excessive oxidative stress and mitochondrial fragmentation mediated by the Drp1 protein [38, 39]. These phenotypes are similar to the overexpression of *DLP1/Drp1* and inhibition of the *MFN2* gene. In contrast, the levels of *MFN2* mRNA are increased by acute weight loss [40]. The effect of *Drp1* overexpression upon physiological processes is toxic.

The balance between mitochondrial fission and fusion is very delicate. For example, a newborn girl with microcephaly, along with other developmental defects, was shown to have defective mitochondrial and peroxisomal fission likely due to a dominant negative mutation in the *Drp1* homologue [41]. *Drp1* is essential for embryonic development in mice such that homozygous mutants or knockout *Drp1* mice die during embryogenesis [42]. The Drp1 protein assists in caspase-independent mitochondrial fission to amplify apoptosis [43]. The acute overexpression and inhibition of the *Drp1* gene has shown similar phenotypes [23], most likely due to an extreme disruption in mitochondrial morphology.

Increasing the expression of *Drp1* in the neurons of *Drosophila* under the *Ddc-Gal4* transgene seems to decrease longevity, and *Drp1* has been found to increase the ROS levels in mitochondria [44]. In our experiments, the lifespan of flies that overexpress *Drp1* was significantly decreased when compared to controls, which may be due to elevated oxidative stress beyond a threshold of a normal lifespan. The inhibition of *Drp1* in dopaminergic neurons through the directed expression of the *Drp1-RNAi*'s results in a slight increase in lifespan. *Drp1* overexpression and inhibition does significantly influence the number of ommatidia or interommatidial bristles, and significantly increase ommatidia area when expressed under the control of the GMR-Gal4 transgene.

The well-documented function of *Drp1* is the promotion of mitochondrial fission and the inhibition of mitochondrial fusion[45]. Mitochondria host a number of cellular processes, especially oxidative phosphorylation, and are thus under continuous cellular stress and require repair and replacement [46, 47, 48]. The lower tolerance of mitochondria to fission, compared to fusion, suggests that a continuous network of mitochondria can survive greater injury due to a lower rate of mitophagy and a slower rate of mitochondrial biogenesis, up-to an optimum level. The overexpression of *Drp1* results in the excessive fragmentation of the mitochondria such that its efficiency can be diminished to the point that functional complementation by fellow mitochondria is negligible. Mitochondria can become a distinct burden for the cell to upkeep instead of being an efficient "powerhouse of the cell". Clearly, the level of *Drp1* expression plays a key role in cell survival.

The *park* gene is crucial to the function of *Pink1*-dependent mitochondrial mitophagy. The loss of the park protein is a cause of great cellular stress as a major mitophagy mechanism is compromised to potentially result in the accumulation of non-functional mitochondria. Under normal circumstances, the park ubiquitin ligase recruits the Drp1 protein to mediate mitochondrial fragmentation during mitophagy [20, 49]. In mouse embryonic fibroblasts, loss of *park* does not have any effect upon the number of mitochondria [50]. Furthermore, the loss of *park* along with the loss of *Drp1* increases the number of mitochondria by threefold, which can be interpreted that *park* controls mitochondria fragmentation in a *Drp1* knockout background. Therefore, *park* can negatively regulate *Drp1*-independent mitochondrial division. The impact of the loss of *park* can be observed only when the majority of mitochondrial fission activity, mediated by *Drp1*, is absent.

In our experiments, the critical class flies that have the directed co-expression of *Drp1-RNAi* and *park-RNAi* inhibitory transgenes live longer than those that express *park-RNAi* and over-express *Drp1* under the *Ddc*-

Gal4 transgene. The inhibition of *Drp1* and *park* through directed RNA interference promotes survival. This could be due to the establishment of an altered mitochondrial network to enhance homeostasis and benefit cellular health.

Conclusion

The *Drp1* is not a major Parkinson disease gene, but it has been associated with cell death pathways in neurons. The overexpression of *Drp1* gene in neurons results in the reduced survival and an age-dependent decline in locomotor ability. The knockdown of *Drp1* in the *Ddc-Gal4*-transgenes of *Drosophila* results in an age-dependent loss in climbing ability, phenotypes that are strongly associated with neuronal degeneration and Parkinson disease. Thus, the compromised climbing abilities in flies with directed inhibition of *Drp1* have produced a new model of Parkinson Disease and can be used to investigate further the mechanisms underlying PD and other neurodegenerative diseases. The co-inhibition of the *parkin* with *Drp1* results in the rescue of the phenotypes observed, it is possible that *Drp1* and *parkin* participate in cellular pathways that promote cell death. Further studies are required to better understand the interaction between *parkin* and *Drp1* in these neurons. Overall our experiments are allowing us to contribute to our understanding of mitochondrial health and enhanced conditions of homeostasis.

Materials And Methods

Bioinformatic analysis

Protein sequences were obtained from the National Center of Biotechnology Information (NCBI) database (<https://www.ncbi.nlm.nih.gov/protein/>). The conserved domains were identified using NCBI Conserved Domain Database (<https://www.ncbi.nlm.nih.gov/cdd/>), and Eukaryotic Linear Motif (<http://elm.eu.org/>). Multiple sequence alignment was done using Clustal Omega (<https://www.ebi.ac.uk/Tools/msa/clustalo/>) to reveal the conservation of domains. The *Homo sapiens* Dynamin-1 like protein (DLP-1/Drp1) structure (PDB ID 4BEJ) was obtained from NCBI structure database (<https://www.ncbi.nlm.nih.gov/structure/>) and *Drosophila melanogaster Drp1* protein structure was developed using Phyre2 (<http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index>) modeling tool. The final models were edited with the PyMOL software (<https://pymol.org/2/>) to highlight the N-terminus, C-terminus and LIR regions.

Drosophila stocks and media

The *Ddc-Gal4* line was received from Dr. J. Hirsch of University of Virginia. The *GMR-Gal4¹²; UAS-lacZ⁴⁻¹⁻²; UAS-Drp1* (*y*[1] *w*[*]; *P*{*w*[+ *mC*] = FLAG-FIASH-HA-*Drp1*}3, *Ki*[1]); the *UAS-Drp1-RNAi1* (*y*[1] *v*[1]; *P*{*y*[+ *t*7.7] *v*[+ *t*1.8] = *TRiP.JF02762*}attP2)); and *UAS-Drp1-RNAi2* (*y*[1] *v*[1]; *P*{*y*[+ *t*7.7] *v*[+ *t*1.8] = *TRiP.HMC03230*}attP40) stocks were obtained from Bloomington Drosophila Stock Center at Indiana University, Bloomington, Indiana, USA. The *UAS-park-RNAi* line was obtained from Dr. B. Lu [51, 52]. The

Ddc-Gal4 park-RNAi line was produced through standard methods [53]. All flies were maintained on standard cornmeal/molasses/yeast/agar media treated with propionic acid and methylparaben to resist fungal growth. Stocks were maintained at room temperature ($22^{\circ} \pm 3^{\circ} \text{C}$), whereas crosses and experiments were kept at 25°C .

Survival assay

Female virgins of the *Ddc-Gal4* genotype were collected every 8 to 12 hours for several days. The confirmed virgin flies were then crossed with *UAS-lacZ*, *UAS-Drp1*, *UAS-Drp1-RNAi1* and *UAS-Drp1-RNAi2* males. Critical class male progeny were collected from mating's until approximately 250 flies of each genotype were obtained. To avoid over-crowding, the flies were maintained in cohorts of 25 or less per vial on standard media. Flies were scored every second day for viability and were transferred to new food every two to five days. Scoring continued until all flies had died [55]). Longevity data were analyzed using GraphPad Prism version 8 statistical software (*graphpad.com*), and survival curves were compared by Mantel-Cox test. Significance were determined at 95% confidence level ($P \leq 0.05$) with Bonferroni correction.

Locomotor analysis

Approximately 70 male flies of the critical class were collected within a 24-hour period and maintained as cohorts of 10 flies in each vial. Media was replenished twice a week. The climbing assay was performed as previously described according to a standard protocol [54, 55]. Briefly, every week 50 males were assayed, in groups of 10, for their ability to climb a glass tube divided into 5 levels of 2 cm each. The climbing index was calculated for each week using GraphPad prism version 8 statistical software. The climbing curve was fitted using non-linear regression and determined at a 95% confidence interval ($P \leq 0.05$).

Biometric analysis of the *Drosophila melanogaster* eye

Female virgins of the *GMR-Gal4* genotype were collected every 8 to 12 hours for several days. The confirmed virgins were then crossed with the males of *UAS-lacZ*, *UAS-Drp1*, *UAS-Drp1-RNAi1* and *UAS-Drp1-RNAi2* genotypes. Critical class male progeny were collected for each genotype. The collected flies were kept as cohorts of 10 flies or less upon fresh media and allowed to age for 3 to 4 days. The flies were prepared for scanning electron microscopy following the standard protocol [35]. Ommatidia and interommatidial bristle counts were performed on 10 or more flies of each genotype using National Institute of Health (NIH) ImageJ software. The ommatidium area was calculated by measuring the area of 5 distinct ommatidial "rosettes" per fly eye and then dividing by 7 to determine the mean area of each ommatidium; done on 10 eyes of each genotype. The Biometric analysis was performed using GraphPad Prism version 8 statistical software. Significance were determined at 95% confidence level ($P \leq 0.05$).

Abbreviations

Ddc
dopa decarboxylase
Drp1
dynamin related protein 1
GMR
glass multiple reporter
PD
Parkinson disease
Pink1
PTEN-induced putative kinase 1
RNAi
ribonucleic acid interference

Declarations

Ethics approval and consent to participate

The study was approved by the Animal Care Committee of the Memorial University of Newfoundland as a Category of Invasiveness Level A protocol. Consent was not applicable for the study.

Consent for publication

Not Applicable

Availability of data and materials

All data generated or analyzed during this study are included in the article.

Competing interests

The authors declare no competing interests.

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Author's contributions

Azra Hasan performed the statistical analysis of bioinformatic, survival, climbing and biometric data. Brian E Staveley devised and participated in the experimental design, supervision of the study and revision to the draft of manuscript.

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Azra Hasan & Brian E. Staveley

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Page 14/19

1 B (i)

B (ii)

Figure 2

B (i). The original Dynamin-1 like protein (DLP-1) structure of *H. sapiens* (NP_001265392.1) from the NCBI structure database. B (ii). The Phyre2 web portal for protein modelling, prediction and analysis mediated the development of a model of the Drp1 protein of *D. melanogaster* (NP_608694.2) from a 76% identical protein with a confidence of 100%. The N terminus is coloured in Magenta; C terminus is coloured in Red and a consensus ATG8 binding region at N terminus is coloured in orange.

2 A

B

Figure 3

Altered Drp1 expression under the control of Ddc-Gal4 influences the survival and climbing ability of flies. A. The GraphPad prism8 generated graph of the longevity assay for the expression of Drp1, Drp1 RNAi's under the control of Ddc-Gal4 transgene. The directed expression results in decreased median lifespan of 56 days compare to 68 days of control calculated by Log-rank Mantel Cox test, with Bonferroni correction. The inhibition of Drp1 under the control of Ddc-Gal4 transgene results in increased lifespan of 70 days with UAS-Drp1-RNAi1 and 72 days with UAS-Drp-RNAi2 compare to 68 days of control done by Log-rank Mantel Cox test, with Bonferroni correction. B. The GraphPad prism8 generated graph of the climbing abilities of flies with overexpression of Drp1, Drp1 RNAi's and control. The climbing ability of Drp1 overexpression and Drp1 RNAi's flies is significantly compromised compared to control as determined in nonlinear fitting of the climbing curve by 95% confidence interval.

3 A

B

Figure 4

Altered Drp1 expression when coupled with Ddc-Gal4 park-RNAi. A. The graph of longevity assay generated by GraphPad prism8 with altered Drp1 expression in Ddc-Gal4 park-RNAi expressing flies. The overexpression results in median lifespan of 58 days similar to 58 days of control (lacZ/Park-RNAi) determined by Log-rank Mantel-Cox test, with Bonferroni correction. The inhibition of Drp1 in neurons using Ddc-Gal4 transgene along with Park-RNAi results in increased lifespan of 84 days with UAS-Drp1-RNAi1 and lifespan of 76 days with UAS-Drp1-RNAi2 compare to 58 days of control done by Log-rank Mantel Cox test, with Bonferroni correction. B. The GraphPad prism8 generated graph of the climbing abilities of flies with overexpression of Drp1, Drp1 RNAi and control. The climbing abilities of and Drp1 RNAi flies has significantly increased compared to control as determined in nonlinear fitting of the climbing curve by 95% confidence interval.

Figure 5

The phenotypic effects of altered Drp1 expression in *D. melanogaster* eye. A. Scanning electron micrograph of the altered Drp1 expression under the control of GMR-Gal4 transgene. The genotypes are (a) GMR-Gal4/UAS-lacZ (Control); (b) GMR-Gal4/UAS-Drp1; (c) GMR-Gal4/UAS-Drp1-RNAi1 (d) GMR-Gal4/UAS-Drp1-RNAi2. B. The ommatidia number for control is 703, the inhibition and expression of Drp1 results in slight increase ommatidial count compared to control. C. The interommatidial bristle count for the control is 536 and for the expression and inhibition lines are slightly significant more for the two RNAi transgenes mean value of 556.7 and 578. D. The ommatidium area of the Drp1 expression transgene GMR-Gal4 UAS-Drp1 and the inhibition transgenes GMR-Gal4 UAS-Drp1-RNAi1 and GMR-Gal4 UAS-Drp1-RNAi2 are significantly higher than control.

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