Expression of Genes Involved in Drosophila Wing Morphogenesis and Vein Patterning Are Altered by Spaceflight

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INTRODUCTION

Imaginal wing discs of Drosophila melanogaster (fruit fly) defined during embryogenesis ultimately result in mature wings of stereotyped (specific) vein patterning. Major regulators of wing disc development are the epidermal growth factor receptor (EGF), Notch, Hedgehog (Hh), Wingless (Wg), and Dpp signaling pathways. Highly stereotyped vascular patterning is also characteristic of tissues in other organisms flown in space such as the mouse retina and leaves of Arabidopsis thaliana. Genetic and other adaptations of vascular morphogenesis to space environmental factors have not yet been systematically quantified, despite widespread recognition of their critical importance for terrestrial and microgravity applications. Here we report changes in gene expression with space flight related to Drosophila wing morphogenesis and vein patterning. In addition, genetically modified phenotypes of increasingly abnormal ectopic vein patterning in the Drosophila wing were analyzed by NASA’s VESGen Generation Analysis (VESGEN) software. Our goal is to further develop insightful vascular mappings associated with bioinformatic dimensions of genetic or other molecular phenotypes for correlation with genetic and other molecular profiling relevant to NASA’s GeneLab and other Space Biology exploration initiatives.

METHODS

Gene Expression Analyses from Drosophila. Spaceflight-reared larvae and adult samples were collected, processed and analyzed as described previously by Marcu et al. Briefly, the Ga4-UAS transgenic line of Drosophila melanogaster that expresses two copies of eGFP under the control of the hemitector promoter was used in all experiments. RNA samples were processed and hybridized to Drosophila 2.0 Affymetrix® arrays. Six sets of larval arrays and 3 sets of adult arrays were used to provide repeats for statistical validation. The False Discovery Rate (FDR) criterion by Benjamini and Hochberg was applied to p-values.

VESGEN Mapping and Quantification. Binary vascular patterns extracted from grayscale images published by Johannes and Preiss of the Drosophila wing (Figure 1) were analyzed by automated user-interactive VESGEN software to generate parameters that include vessel diameter ($D_v$), fractal dimension ($D_f$) and densities of vessel area ($A_v$), length ($L_v$), number ($N_v$), and branch point ($B_p$) as described previously (Figures 2).

RESULTS

Microarray data from larval (Table 1) and adult flies (Table 2) returned from spaceflight significant changes in genes important for wing development and vein patterning compared to ground controls. For instance, the hedgehog pathway regulates the positioning of longitudinal veins such as L3 and L4. Expression of the gene Smoothered with hedgehog receptor activity was significantly down regulated (-0.8 fold; p-value < 0.000) in space returned adult flies. Similarly, expression of Rhomboid 7 (-0.7 fold; ectopic veins increased in number by $N_v$ from 1 in the wild-type to 18 in Class 5; for the ectopic vessels, $L_v$ increased from 0.0004 to 0.0095 px²) and Wingless (+0.8 fold; p-value < 0.000) were significantly down regulated in space-return adult flies compared to ground controls. Expression of Rhomboid and Aveugle is critical in EGF-regulated stereotypical patterning of veins. In the case of space-return larvae, expression of ash2 was significantly up-regulated (+0.6 fold; p-value < 0.000), suggesting possible changes in interven cell fate that determines interven patterning.

By confirming vascular parameters generated with VESGEN (Table 3, Figure 1), the eight stereotyped wing veins remained quite constant in genetically perturbed phenotypes compared to wildtype, including the most perturbed phenotype, Class 5. For example, $A_v$ for stereotyped Class 5 vessels is 1.03x and 1.15x that of the wildtype. In Class 5, only the stereotyped PCV is incomplete. However, ectopic veins increased in number by $N_v$ from 1 in the wild-type to 18 in Class 5; for the ectopic vessels, $L_v$ increased from 0.0004 to 0.0095 px²; $A_v$, $L_v$, and $N_v$ for ectopic vessels were 24x, 42x and 18x greater compared to wildtype.

CONCLUSIONS AND DISCUSSION

Major regulators of wing disc development include genes important for the epidermal growth factor receptor (EGF), Notch, Hedgehog (Hh), Wingless (Wg), and Dpp signaling pathways. Most of these genes also play a vital role in vein morphogenesis. We measured significant changes in expression for a number of such genes that include Smoothered, Rhomboid 7, Aveugle, and ash2. Altered wing venation of Drosophila resulting from a series of increasingly perturbed gene expression was successfully mapped by NASA’s VESGEN software to reveal that normal stereotypical vascular patterning was not significantly changed, despite the presence of increasingly abnormal ectopic vascularization. In the future, space-dependent changes in vascular patterning may be mapped by VESGEN to offer useful phenotypic read-out changes in genetic and other molecular signaling during Drosophila development and vascular adaptations of other important experimental model tissues such as Arabidopsis leaves and the rodent GI and retina (Figure 2).

REFERENCES


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