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) venation patterning. Major regulators of wing disc development are the epidural 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 patterning 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 wing venation in the *Drosophila* wing, were analyzed by NASA’s VESGEN GenExpression Analysis (VESGEN) software. Our goal is to further develop insightful vascular mappings associated with bioinformatic dimensions of genetic or other molecular phenomena for 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 Gal4-UAS transgenic line of *Drosophila melanogaster* that expresses two copies of eGFP under the control of the hemolymph 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 image-interactive VESGEN software to generate parameters that include vessel diameter (*D*), fractal dimension (*Df*) and densities of vessel area (*A*), length (*L*), number (*N*), and branch point (*B*) as described previously (Figure 2).

RESULTS

Microarray data from larvae (Table 1) and adult flies (Table 2) returned from space measured 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 Smoothened with hedgehog receptor activity was significantly down regulated (-0.8 fold; *p*-value=0.00) in space-retarded adult flies. Similarly, expression of Rhomboid 7 (-0.7 fold; *p*-value=0.00) and Aveugle (-0.8 fold; *p*-value=0.00) were significantly down regulated in space-retarded adult flies compared to ground controls. Expression of Rhomboid and Aveugle is critical in EGF-regulated stereotyped patterning of veins. In the case of space-retarded larval expression, Aveugle was significantly up-regulated (+0.6 fold; *p*-value=0.00), suggesting possible changes in vein cell fate that determines vein 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* and *L* for stereotyped Class 5 vessels are 1.03× and 1.15× that of the wildtype. In Class 5, only the stereotyped PCV is incomplete. However, ectopic veins increased in number by *N* from 1 in the wildtype to 18 in Class 5; for the ectopic vessels, *L* increased from 0.0004 to 0.0095 px-px *A*, and *N* for ectopic vessels are 24, 42× and 18× 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 wing vein morphogenesis. We measured significant changes in expression for a number of such genes that include Smoothened, 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-outs of 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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