Headcase is a hematopoietic regulator in *Drosophila melanogaster*

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Ph. D. thesis

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Introduction

There are numerous similarities between the hematopoiesis of human and the fruit fly, *Drosophila melanogaster*. Human blood cell differentiation starts in the early embryo in multiple waves (pimitive- and definitive hematopoiesis) and takes place in different hematopoietic organs, which serve as compartments. Similarly, in the fruit fly, primitive (embryonic) and definitive (larval) hematopoiesis and compartmentalization of hemocytes can be distinguished. The main location of human postembryonic hematopoiesis is the hematopoietic stem cell niche, in which the hematopoietic stem cell generates the different blood cell types by unequal divisions. Hematopoietic stem cells also exist in *Drosophila*. These cells are also located in a hematopoietic niche, the lymph gland. Besides sharing common anatomical and functional features, the *Drosophila* and the human immune system utilize homologues genes for the defense of the organism. Therefore *Drosophila melanogaster* proved to be an excellent model to study innate immunity.

During my Ph. D. work, I investigated the genetic regulation of the larval hematopoiesis of the fruit fly. Three effector cell types were identified in *Drosophila* larva: the phagocytic plasmatocyte, the melanizing crystal cell and the large, flattened lamellocyte, which differentiates only upon immune induction to form a multi-layered, melanized capsule around dangerous particles. The hemocytes are located in three hematopoietic compartments: the circulation, the sessile hematopoietic tissue and the lymph gland.

Most of the knowledge available on the regulation of blood cell differentiation comes from studies of the lymph gland. The primary lobe of this paired-lobed organ has three functional zones: the cortical zone, which contains differentiated hemocytes, the medullary zone consisting of blood cell progenitors and the posterior signaling centre (PSC), which is a niche that maintains the undifferentiated state of prohemocytes. In the primary lobe, aligned

cooperation of several signal transduction pathways is responsible for the regulation of effector differentiation and progenitor maintenance. The hematopoietic processes in the other two compartments are less understood.

During my work, I studied the possible hematopoietic function of Headcase (Hdc), the *Drosophila* homologue of a human tumor suppressor, HECA. Hdc is a cytoplasmic protein with disordered structure, which was described as a repressor of numerous differentiation processes. It can act both in a cell-autonomous and in a non-cell-autonomous manner. We observed that Hdc is expressed in the lymph gland, however the cells leaving the organ upon immune induction and differentiating into lamellocytes lose their *hdc* expression. This observation suggested that Hdc may play a role in the regulation of hemocyte differentiation.

Aims

Since *hdc* is expressed in the lymph gland, but its activity turns off during effector hemocyte differentiation upon immune induction, we were curious about its potential role in the regulation of the maturation of blood cells. Moreover, new knowledge on the function of Hdc can be beneficial not only in *Drosophila* hematopoiesis, but also in connection with other developmental processes and in the field of human tumor biology. To shed light on the role of Hdc in hematopoiesis we planned to:

1. generate a *hdc-Gal4* driver to investigate the expression pattern of the gene in the live animal,
2. monitor the expression of *hdc* in the hematopoietic compartments during larval development,
3. isolate and characterize an amorphic *hdc* allele,
4. study the effect of the *hdc* mutation on the hemocyte differentiation,
5. identify the focus of the *hdc* mutation,
6. carry out a genetic interaction screen to isolate the possible cooperators of Hdc.

Methods

1. Preparation of hemocyte samples
2. Dissection of lymph gland and imaginal discs
3. Application of indirect immunofluorescence
4. Fluorescent microscopy
5. Confocal microscopy
6. *In vivo* confocal video-microscopy
7. Immune induction
8. X-gal staining
9. P element conversion
10. Polymerase chain reaction
11. Generation of recombinant lines
12. Isolation of P element insertions
13. Mutagenesis with P element remobilization
14. Genetic interaction screen

Summary of the results

1. To follow *hdc* expression *in vivo,* we carried out a P-element conversion screen, and generated the *hdc^{19}-Gal4* transgenic driver, which was tested with the *UAS-GFP* reporter. The pattern of the driver followed precisely the expression of *hdc;* it was active in the imaginal tissues and in the lymph gland. The exact genomic position of the insertion was determined with DNA sequencing, which revealed that the exchange of the P-elements was virtually precise. We found that, similarly to the hypomorphic *hdc* alleles, the newly generated *hdc^{19}-Gal4* insertion caused pupal lethality. With the overexpression and the silencing of the factor, we verified that the lethality was provoked by the P-element and not by second-site mutations, which might have appeared during the conversion process. Based on these results, we concluded that *hdc^{19}-Gal4* is a hypomorphic allele of *hdc*.

2. By the usage of the new driver, we observed that during larval development the initial extensive expression of *hdc* in the primary lobes of the lymph gland decreased gradually by the end of the larval stage. Moreover, while we noticed significant *hdc^{19}-Gal4* expression in the hematopoietic niche of second instar larvae, wandering larvae completely lacked *hdc* activity in the PSC. These results strengthened our hypothesis on the possible role of Hdc in the development of the lymph gland and on the differentiation of hemocytes within the organ.

3. In a P-element remobilization mutagenesis screen, we isolated a deficiency (*hdc$^{\Delta 84}$*) of almost 2 kilobases, which overlapped with the complete 5' untranslated region and the first exon of *hdc*.

4. We observed spontaneous lamellocyte differentiation in the larvae homozygous for *hdc$^{\Delta 84}$* and the previously generated *hdc^{43}* null alleles, as well as in *hdc^{19}-Gal4* larvae.

Accordingly, we concluded that Hdc plays a role in the regulation of hematopoiesis as a repressor of the lamellocyte fate.

5. In order to find the focus of *hdc*, we used several drivers with different expression patterns and a *hdc*-specific RNA interference construct to silence the factor in the distinct functional compartments of the lymph gland. On the basis of the hematopoietic phenotype of the different silencing combinations, we determined the focus of *hdc* in the PSC. We found that Hdc is required in the hematopoietic niche to impede the differentiation of lymph gland prohemocytes. Furthermore, when we silenced the factor in the entire *Dot* hemocyte lineage, we detected both autonomous and non-autonomous lamellocyte differentiation.

6. We assumed that Hdc represses lamellocyte differentiation through the interaction with the signal transduction pathways in the lymph gland. Accordingly, we investigated the genetic interaction of *hdc* with the elements of distinct signaling cascades, which were described to affect the differentiation of lamellocytes. Our results implied that Hdc cooperates with the Dpp, the Hh and the JAK/STAT pathways.

7. On the basis of the results presented in this study, we set up our model on the function of Hdc in the hematopoietic niche of the lymph gland and on the cooperation of the factor with the different signaling cascades in the regulation of lamellocyte differentiation.

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List of publications

Publications supporting the dissertation:

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