Characterizing the dual role of Headcase in hemocyte progenitor maintenance in *Drosophila melanogaster*

Ph.D. Dissertation

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Introduction

Due to the significant level of homology of regulatory pathways, the lymph gland, the multi-lobed hematopoietic organ of the *Drosophila* larva serves as an efficient model of mammalian hematopoietic niches. In the primary lobes of the lymph gland, one can distinguish blood cell (hemocyte) progenitors in the medullary zone (MZ), differentiated hemocytes in the cortical zone (CZ), and a hematopoietic niche called the posterior signaling centre (PSC) that signals to the progenitors to control their differentiation.

In the CZ of the lymph gland and in the circulation, two types of mature hemocytes can be found: phagocytic plasmatocytes and melanizing crystal cells. Following immune induction by wasp infestation, wounding, or tumor formation, a third type of hemocytes, the lamellocytes, appear in the lymph gland and circulation, and encapsulate larger sized invaders, such as the parasitic wasp egg. Since these cells are not present in uninduced conditions, their presence in naive animals indicates a dysfunction in hemocyte progenitor maintenance mechanisms in the larva, similarly to what is observed in

mutants of the orthologue of the human tumor suppressor HECA, *headcase* (*hdc, heca*).

Hdc is an imaginal cell factor involved in multiple developmental processes, such as imaginal disc morphogenesis, maintenance of the testicular and intestinal stem cells, and the regulation of tracheal branching. In the hematopoietic system, *hdc* is expressed exclusively in the lymph gland. Our colleagues have found previously that the loss of *hdc* function leads to the differentiation of lamellocytes without immune induction, which suggested that Hdc is involved in the maintenance of the hematopoietic niche. This phenotype was rescued by overactivating the Hh or Dpp signaling in the PSC, indicating that Hdc functions upstream to these pathways in the niche. However, the mechanism behind lamellocyte differentiation in these larvae remained unknown, since intriguingly, knocking down *hdc* did not affect niche size or identity. Moreover, the role of Hdc in the other domains of the lymph gland (the MZ and the CZ) was not studied previously. The interaction with its two previously described partners, Unk and Raptor, in regard to hematopoiesis was also not investigated, and we did not

have information on potential Hdc interactors in the lymph gland. Since the human homologue of Hdc, HECA, behaves as a tumor suppressor in several tumor models, characterizing the mechanism of Hdc function in the lymph gland would be beneficial not only in the field of *Drosophila* hematopoiesis but it may be crucial for human tumorigenesis studies as well.

Aims and objectives

Given that the underlying mechanism of how Hdc maintains progenitor state of hemocytes is not yet understood, we aimed to investigate the regulatory role of Hdc in the *Drosophila* lymph gland.

Specific aims:

- As literature data suggested a connection between Hdc and the insulin/mTOR pathway, we aimed to investigate whether the hematopoietic phenotype of *hdc* is mediated by insulin/mTOR signaling.
- Previously it was shown that *hdc*, while compromising PSC function, does not affect the number or identity of PSC cells. Since the insulin/mTOR was shown to play

a role in PSC size determination, we aimed to understand why the PSC size remains unaltered in *hdc* larvae.

- Former data suggested that *hdc* expression is downregulated during larval development, possibly to allow for progenitor differentiation at later stages. We asked whether *hdc* expression is also repressed following immune induction with parasitic wasp, thereby contributing to the differentiation of progenitors to lamellocytes during immune response.
- We wanted to explore the possibility of a cell-autonomous role of Hdc in the regulation of lymph gland hemocyte progenitors, as well as to characterize the signaling pathways required for Hdc function in these cells.
- Since only two partners of Hdc (Unk and Raptor) were described previously, we set out to isolate novel interacting partners of Hdc in the lymph gland.

Applied methods

3.1. Classical *Drosophila* genetics

3.2. Immunostaining, imaging, and processing of lymph gland samples

3.3. Immunostaining, imaging, and counting of circulating hemocytes

3.4. Immune induction by *Leptopilina boulardi* parasitic wasp

3.5. Cloning of HA tagged Hdc isoforms, and testing their functionality by rescue experiments

3.6. Screening for novel interactors of Hdc using LC/MS-MS

3.7. Validating the candidate Hdc interactors with genetic interactions, Split-YFP and Co-IP

3.8. Statistical analysis of data

Results and conclusion

Despite the differences at the structural level between the *Drosophila* lymph gland and mammalian hematopoietic compartments, the signaling pathways responsible for controlling hemocyte progenitor maintenance are remarkably similar to those found in mammals. Studies aiming to better understand hemocyte differentiation within the lymph gland are highly facilitated by transgenic tools that allow for context-specific manipulation of signaling pathways and analyzing the resulting phenotype. One of the aims of the work presented in this thesis was to further investigate the previously reported non-cell-autonomous function of the imaginal cell factor, Hdc, in the hematopoietic niche. Although it was documented that PSC-specific silencing of *hdc* leads to the differentiation of lamellocytes that are typically not present in naive larvae, the precise mechanism underlying the phenotype was not previously understood. Here, through extensive genetic interaction experiments, we have demonstrated that Hdc exerts this function by negatively regulating the insulin/mTOR pathway in the niche. When *hdc* is depleted

in the niche, the overactivation of this pathway triggers reactive oxygen species (ROS) accumulation, which promotes lamellocyte differentiation in the lymph gland, and their consequent appearance in the hemolymph. This is supported by our observations that scavenging ROS from the niche alleviates the phenotype, while interfering with the stress protective machinery, boosts lamellocyte differentiation.

Although overactivation of insulin/mTOR signaling was described to result in elevated PSC cell number, we did not observe this phenotype in *hdc* larvae. We showed that *hdc* depletion causes cell death in the niche independently from the insulin/mTOR pathway, thereby concealing mTOR overactivation and resulting in a niche size indistinguishable from the control.

Moreover, we have described a novel cell-autonomous role for Hdc in suppressing progenitor differentiation in the MZ. We found that even though the insulin/mTOR pathway is not involved in the *hdc* phenotype in the MZ, similarly to the niche, knocking down *hdc* in MZ progenitors leads to ROS elevation, which affects cell-cell

adhesion and induces the activity of the EGFR and JNK pathways leading to premature progenitor loss.

Besides understanding Hdc function in the lymph gland, we aimed to isolate new Hdc interacting partners. We used HA-tagged Hdc transgenic protein in LC-MS/MS to search for possible interactors. We identified a genetic interaction and an indirect physical interaction between Hdc and Calmodulin (Cam), a calcium binding protein that was previously described to be required for the activity of the insulin/mTOR pathway in human cell lines, which suggests that Cam may play a similar role in the hematopoiesis in *Drosophila*. Moreover, through our genetic interaction screens, we have identified a novel role for 4 genes (*eIF5B*, *CCT1*, *eRF3*, and *Hsc70-4*) in suppressing lamellocyte fate in the niche. This opens new ways for further research into the function of each of these genes in blood cell differentiation in *Drosophila*.

The important role of Hdc in progenitor maintenance in the lymph gland is further corroborated by our observations that *hdc* expression decreases in the primary lobes of the lymph gland in response to immune induction by parasitic wasp, and at the end of larval stages as

progenitors prepare to differentiate. Considering this and previous data demonstrating that *hdc* expression is lost in the imaginal cells as they start to differentiate into adult tissues, we conclude that Hdc may play a general suppressive role in the maintenance of progenitors and imaginal cells of the larva. Given that the function of the top LC-MS/MS candidate interactors of Hdc is to enable basic cellular tasks, such as translation, and protein folding, it is likely that Hdc acts as a chaperon, which facilitates proper cellular function. This is also underlined by our finding that *hdc* loss-of-function leads to cellular stress, resulting in various consequences, such as triggering precursor cell differentiation.

We hope that our findings help to shed light on the regulation of blood cell development in *Drosophila*, as well as parallel mechanisms implicated in HSC differentiation and hematopoietic disorders in humans.

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Publication List (MTMT ID: 10084192)

Number of scientific publications: **2**

Total impact factor: **11.6**

Conference abstracts: **1**

Journal articles used for attaining the PhD degree

Kúthy-Sutus, E.*, Kharrat, B.*, Gábor, E., Csordás, G., Sinka, R., & Honti, V. (2022). A Novel Method for Primary Blood Cell Culturing and Selection in *Drosophila melanogaster*. *Cells*, *12*(1), 24. doi: 10.3390/cells12010024. (*: shared first author). **(I.F: 6.0 (2022))**

Kharrat, B., Csordás, G., & Honti, V. (2022). Peeling back the layers of lymph gland structure and regulation. *International Journal of Molecular Sciences*, *23*(14), 7767. doi: 10.3390/ijms23147767. **(I.F: 5.6 (2022))**

Conference talks

Dual role for the orthologue of HECA, Headcase, in blood cell progenitor maintenance in the *Drosophila* lymph gland

Bayan Kharrat, Nikolett Virág, Erika Gábor, Rita Sinka, Ferenc Jankovics, Viktor Honti

The 27th European *Drosophila* Research Conference 2023 (Lyon, France)

The *Drosophila* lymph gland as a model for investigating hematopoietic stem cell (HSC) maintenance - Headcase as an example (flash talk)

Bayan Kharrat, Nikolett Virág, Erika Gábor, Rita Sinka, Ferenc Jankovics, Viktor Honti

The 2nd FEBS-IUBMB-ENABLE Conference 2023 (Cologne, Germany)

Identification of a novel network regulating lamellocyte fate in the lymph gland of *Drosophila melanogaster*

Bayan Kharrat, Nikolett Virág, Erika Gábor, Rita Sinka, Ferenc Jankovics, Viktor Honti

Hungarian Molecular Life Science Conference 2023 (Eger, Hungary)

Understanding the role of Headcase, the orthologue of HECA, in the *Drosophila* lymph gland

Bayan Kharrat, Enikő Sutus, Erika Gábor, Rita Sinka, Ferenc Jankovics, Viktor Honti.

50th Annual Meeting of The Hungarian Society for Immunology 2021 (Kecskemét, Hungary)

Role of Headcase in *Drosophila* hematopoiesis: what we know so far (flash talk)

Bayan Kharrat, Gergely István Varga, Ferenc Jankovics, Rita Sinka, Enikő Sutus, Erika Gábor, Viktor Honti

The 2nd Conference of the Visegrád Group Society for Developmental Biology 2021 (Szeged, Hungary)

Declaration

I declare as the supervisor and the corresponding author of the below listed publications, that the contribution of Bayan Kharrat was significant in these publications and that the doctoral process is based on these publications. The results reported in the PhD dissertation were not used to acquire any PhD degree in the past and will not be used in the future either.

Kúthy-Sutus, E.*, Kharrat, B.*, Gábor, E., Csordás, G., Sinka, R., & Honti, V. (2022). A Novel Method for Primary Blood Cell Culturing and Selection in *Drosophila melanogaster*. *Cells*, *12*(1), 24. doi: 10.3390/cells12010024. (*: shared first author). **(I.F: 6.0 (2022))**

Kharrat, B., Csordás, G., & Honti, V. (2022). Peeling back the layers of lymph gland structure and regulation. *International Journal of Molecular Sciences*, *23*(14), 7767. doi: 10.3390/ijms23147767. **(I.F: 5.6 (2022))**

Szeged, 02.04.2024

Viktor Honti, Ph.D.