Project Closing Report

OTKA K-131484

I. The role of Headcase and its partners in the regulation of lymph gland progenitors

Aims of the work

In our previous work (OTKA PD-115534), we discovered that Headcase (Hdc) maintains blood cell progenitors in the niche of the larval hematopoietic organ, the lymph gland, by regulating the Hh and Dpp signalling pathways (Varga et al., 2019). In the framework of the current grant, we aimed to identify functional parts and potential interacting partners of Hdc, as well as to map the regulatory region of the gene.

Investigation of Hdc function and partners

For further studies, we generated three UAS-transgenes encoding HA tagged variants of the Hdc protein: one that contains the short isoform, one that contains the long isoform, and one that codes for the long isoform with the mutated internal STOP codon. The cloning was done in collaboration with Rita Sinka's research group (Department of Genetics, Faculty of Science and Informatics, University of Szeged). The transgenes were injected into embryos, and stocks carrying the transgenes were established. Immunostaining with the anti-HA antibody labeled a portion of larval plasmatocytes expressing *HA.hdc* with the *Hml-Gal4* driver in case of all three transgenic constructs. With Western blot using anti-HA antibody, we identified the short isoform of Hdc from larvae expressing the different versions of the *UAS-HA.hdc* transgene driven by the *actin-Gal4* driver, however, we could not detect the long Hdc isoform. However, we found that the short Hdc isoform is sufficient to rescue both the lethality and the hematopoietic phenotype of the mutant.

Structural studies of the Hdc protein

According to the LC-MS/MS results, it appeared that Hdc contains three potential phosphorilated sites, two serins and a threonin. We generated HA-tagged *hdc* transgenes carrying phospho-mimicking and phospho-dead mutations for each potential site one-by-one, and for all the three together. We found that the Headcase version containing the S2 phospho-mimicking site is significantly more effective in the rescue experiments than the other modified versions or the wild type transgene, which highlights the potential involvement of this serin in the function of the protein.

In silico investigation revealed that the Hdc protein contains extensive intrinsic disordered regions. Disordered proteins are particularly inclined to the generation of phase-separated cellular biocondensates, which underlie numerous specific biological activities, such as the formation of membraneless organelles. For the investigation of the phase separation capability of the Hdc protein, we started a collaboration with Ferenc Jankovics (Laboratory of *Drosophila* Germ Cell Differentiation, Institute of Genetics, HUN-REN BRC), and employed an *in vivo* optogenetic method. The assay is based on the light-dependent triggering of protein association, inducing phase transitions that lead to the formation of intracellular bodies. The full-length Hdc protein and two disordered Hdc fragments were tagged with Cry2, a light-sensitive protein known to self-associate upon exposure to blue light. The process of phase separation was tracked *in vivo* within the

cytoplasm of HEK cells using advanced microscopic techniques. Following photoactivation, we did not observe clustering of the tested Hdc protein constructs, suggesting that Hdc lacks the autonomous capacity to generate phase-separated condensates.

Role of Hdc in the regulation of the insulin/mTOR pathway

Since Hdc and its physical partner Unkempt (Unk) were described to contribute to the regulation of insulin/mTOR signalling in other tissues and organs, we were curious whether this pathway is involved in the hematopoietic phenotype of the mutation. We found that similarly to *hdc*, niche-specific silencing of *unk* resulted in lamellocyte differentiation (**Figure 1B,C**). In paralel, the activation of the insulin/mTOR pathway in the niche through the overexpression of a constitutively active form of the *PI3K* kinase or silencing the negative regulator *Pten* triggered lamellocyte differentiation (**Figure 1D,E**). In line with these findings, inactivation of the insulin/mTOR pathway by silencing *Akt*, the main kinase in the insulin pathway, or *raptor*, a component of the mTORC1 complex and a previously described physical interactor of Hdc in *hdc* silenced animals counteracted lamellocyte differentiation in the lymph gland and the appearance of these cells in the circulation (**Figure 1F,G**). Additionally, overexpression of *hdc* in *PtenRNAi* silenced larvae reduced the number of lamellocytes both in the lymph gland and in the circulation (**Figure 1H**). Collectively, these results suggest that Hdc is a negative regulator of the insulin/mTOR pathway in the niche, and in case *hdc* function is lost, continuous activation of the insulin/mTOR pathway induces lamellocyte differentiation in the lymph gland and lamellocytes are released into the circulation.

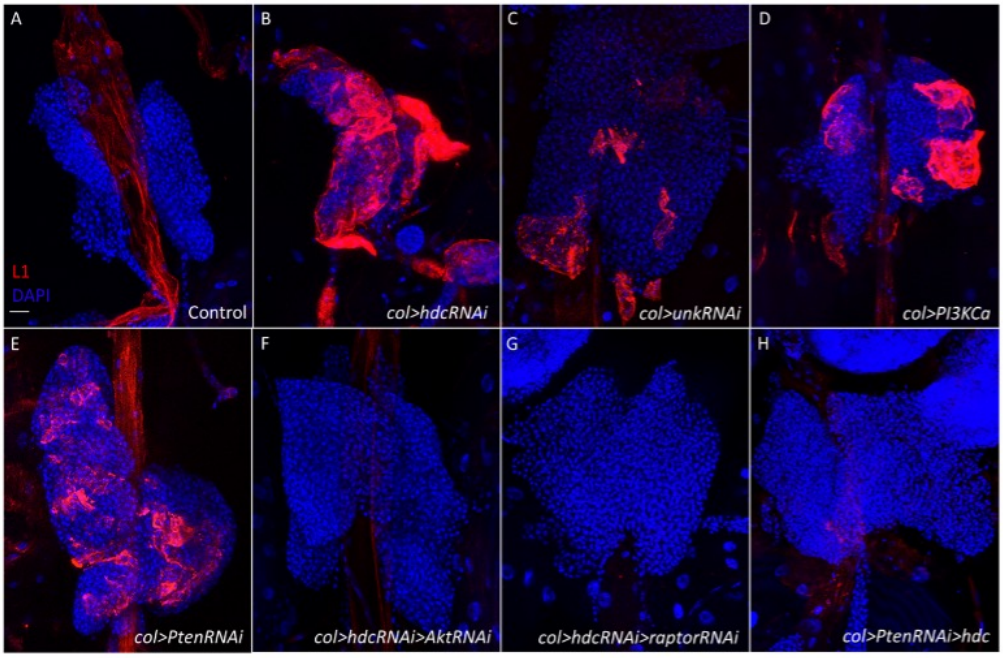

Figure 1. Hdc suppresses the insulin/mTOR pathway in the hematopoietic niche. (A-D) Lamellocytes (red) are absent from control lymph glands (A), while they are present when *hdc* is silenced in the niche (B), its partner *unk* is silenced (C), a constitutively active *Drosophila PI3K* is expressed (D), or the negative insulin pathway regulator *Pten* is silenced (E). (F-G) Lamellocyte differentiation in *hdc* silenced larvae is rescued when simultaneously either *Akt* (F) or *raptor* (G) is silenced. (H) Overexpression of *hdc* rescues lamellocyte differentiation in *Pten* silenced larvae. Nuclei are visualized by DAPI (blue). Scale bar: 20 μm.

The combined effect of hdc loss-of-function in the hematopoietic niche

Previously, it was shown that continuous activation of the insulin/mTOR pathway leads to an increase in the niche size. This was reinforced in our experiments, as we found that both overexpression of *PI3KCa* and the silencing of *Pten* resulted in significantly higher niche cell numbers as compared to the control. However, in case of *hdc* silencing, the number of niche cells remained comparable to that of the control, which underlines our original observation by Varga et al. (2019).

Since silencing *hdc* was shown to cause apoptosis in adult progenitor cells and other tissues, we asked whether the loss of niche cells could mask the effect of insulin/ mTOR activation in *hdc* silenced larvae. To test this possibility, we simultaneously expressed the apoptosis inhibitor *p35* while silencing *hdc* in the niche. Interestingly, we found that this increased the number of niche cells significantly, suggesting that our theory is correct. Moreover, we found that silencing *hdc* restored the number of niche cells in *PtenRNAi* silenced larvae, further confirming that *hdc* depletion causes cell death, which is the reason why we did not observe the expansion of niche cell numbers in *hdc* mutants. All of the above suggest that silencing *hdc* has a combined effect by increasing the size of the niche through the overactivation of the insulin/mTOR pathway and causing death of a proportion of niche cells. This all further supports the idea that Hdc negatively regulates the insulin/mTOR pathway in the niche.

Role of Hdc in the regulation of ROS levels in the niche

In the hematopoietic niche, continuous activation of the insulin/mTOR pathway was demonstrated to result in ROS accumulation serving as a non-cell autonomous signal that induces progenitor differentiation in the lymph gland. To investigate whether silencing *hdc* leads to higher ROS levels in the niche, we used the *gstD-GFP* reporter. Consistent with our expectation, a clear induction of the *gstD-GFP* reporter was observed in the niche of *hdc* silenced larvae as compared to control larvae (**Figure 2A-B'**). Furthermore, when *hdc* was silenced in the niche, we also detected an upregulation of the Forkhead box O (FoxO) reporter (*Thor-lacZ*), which was shown to become active upon ROS production in the niche, providing further evidence of oxidative stress (**Figure 2C-D'**).

Moreover, our experiments exploring genetic interactions revealed that overexpression of Catalase, an enzyme responsible for breaking down ROS, and overexpression of FoxO, a transcription factor that stimulates the expression of antioxidant enzymes suppressed lamellocyte differentiation in *hdc* silenced larvae, while silencing *foxo* enhanced the mutant phenotype (**Figure 2E-H**).

Taken together, these results suggest that metabolic dysfunction in the niche resulting from the activation of the insulin/mTOR pathway in *hdc* silenced larvae leads to cellular stress and ROS overproduction which prompts lamellocyte differentiation in a non-cell autonomous manner.

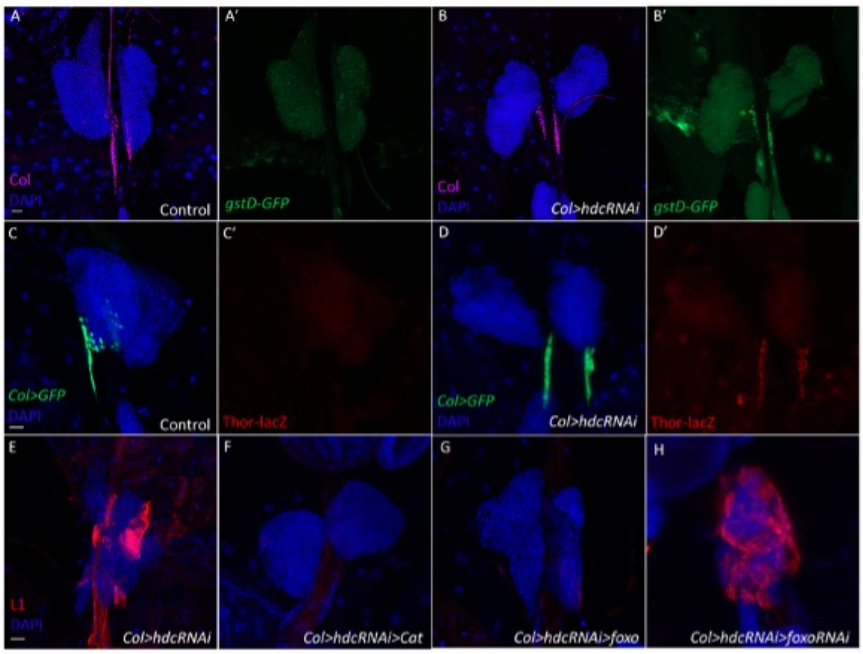

Figure 2. *hdc* silencing results in cellular stress in the niche.

(A-B') Silencing of *hdc* results in the activation of the *gstD-GFP* reporter in the niche in comparison to the control (blue: nuclei, green: ROS, magenta: Collier (niche-specific staining)). (C-D') Silencing *hdc* induces the transcription of *Thor* as detected by an anti-lacZ staining for *Thor-lacZ* reporter (blue: nuclei, green: niche, red: *Thor-LacZ*). (E-G) overexpression of *Cat* (F) and *foxo* (G) rescues lamellocyte differentiation in the lymph glands of *hdc* silenced larvae (E). (H) Silencing *foxo* enhances the lamellocyte differentiation phenotype associated with *hdc* silencing (blue: nuclei, red: lamellocytes). Scale bar: 20 μm.

Cell-autonomous function of Hdc in the hemocyte progenitors

Previously, we have demonstrated that *hdc* is expressed abundantly in the primary lobes of the lymph gland in second instar larvae, and its expression diminishes from the cortex with the formation of the cortical zone at the early third instar larval stage. By the end of the third instar larval stage, *hdc* expression disappears entirely from the primary lobes as most progenitors undergo differentiation and the lymph gland begins to disintegrate to release its content into the circulation (Varga et al., 2019).

Since the lymph gland of *hdc* loss-of-function larvae is reminiscent to immune-challenged lymph glands, we investigated whether *hdc* expression changes after immune induction with the parasitic wasp *Leptopilina boulardi*. To capture the moment that proceeds the disintegration of the primary lobes (which is estimated to occur 20h after parasitation), we examined lymph glands 16h after wasp infestation. Similarly to what was observed at the end of the larval stage, *hdc* expression was no longer detectable in the primary lobes of the lymph gland.

The temporal expression pattern of *hdc* during development and immune response suggests that in addition to its non-cell-autonomous regulatory function in the niche, Hdc is also cell-autonomously involved in preserving progenitor cells in an undifferentiated state. To investigate this possibility, we silenced *hdc* selectively in the medullary zone of the lymph gland. Consequently, we observed that lamellocytes differentiated in the lymph gland and appeared in the circulation of these larvae. Interestingly, lamellocyte differentiation was not observed when we silenced *hdc* with the intermediate zone-specific driver *CHIZ-Gal4*. Crystal cell counts of *hdc* silenced lymph glands were comparable to those of the control, indicating that *hdc* loss-of-function initiates the differentiation of progenitors into lamellocytes, but not into crystal cells.

Taken together, these results reveal a new cell-autonomous role for Hdc in core progenitors to suppress their premature differentiation into lamellocytes. Additionally, they shed light on the relevance of reduced *hdc* expression following wasp infestation, coinciding with the differentiation of progenitors into effector lamellocytes.

Hdc-regulated pathways in the medullary zone of the lymph gland

As silencing of *hdc* in the niche resulted in an accumulation of ROS, we sought to investigate whether knocking down *hdc* in the medullary zone would produce a comparable phenotype. The expression of the *gstD-GFP* reporter revealed elevated levels of ROS in *hdc* silenced lymph glands as compared to control samples (**Figure 3A,B**). In addition, we observed that the *hdc* phenotype was reversed by overexpression of Catalase and Foxo, also suggesting that ROS serve as a signal in the regulation of progenitor differentiation (**Figure 3 C,D**). We also noted that in the progenitors, silencing *unk*, a partner of Hdc in the insulin/mTOR pathway, resulted in lamellocyte differentiation (**Figure 3E**).

Although these results suggested that the insulin/mTOR pathway plays an important role in the maintenance of progenitors located in the medullary zone, our efforts to rescue the phenotype of *hdc* silenced larvae by knocking down components of the insulin/mTOR pathway (such as *Akt* and *raptor*) in the medullary zone proved unsuccessful (**Figure 3F,G**). However, inhibiting the EGFR and JUN pathways, through the expression of a dominant negative form of *EGFR* or *bsk*, respectively, resulted in the rescue of lamellocyte differentiation (**Figure 3H,I**). Similar results were obtained when the adhesion molecule E-cadherin was overexpressed in the medullary zone (**Figure 3J**). Since JNK and E-cad are regulated by ROS, these results provide additional evidence that ROS levels are involved in lamellocyte differentiation in case of *hdc* loss-of-function.

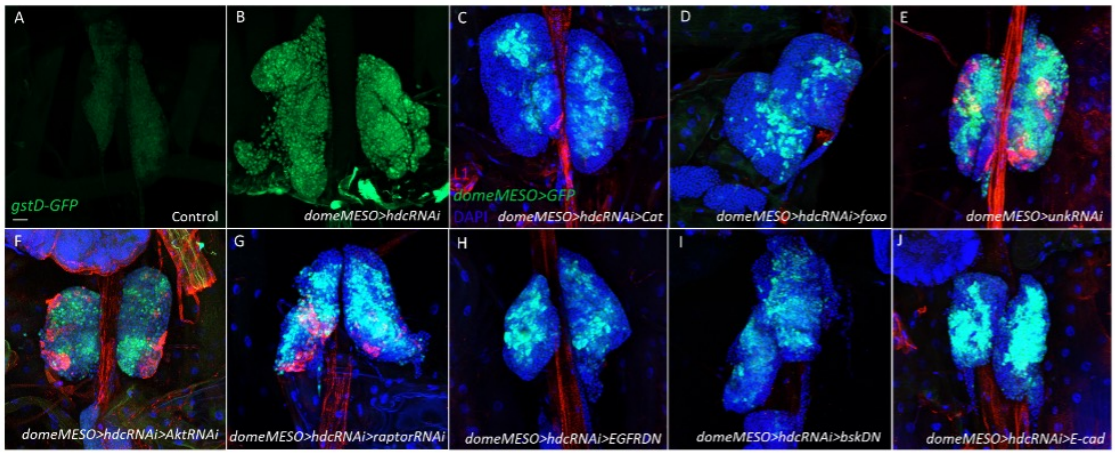

Figure 3. Role of Hdc in the medullary zone progenitors. (A-B) Silencing *hdc* in the medullary zone leads to higher levels of ROS as visualized by the *gstD-GFP* reporter (B) in comparison to the control (A). (C-D) Overexpression of *Cat* (C) or *foxo* (D) rescues lamellocyte differentiation in *hdc* silenced larvae. (E) Silencing *unkempt* results in similar phenotype to that of *hdc.* (F-I) Silencing *Akt* (F) *or raptor* (G) has no effect, while expression of a dominant negative version of *EGFR* (H), or *bsk* (I), or overexpression of *E-cad* (J) rescues *hdc* phenotype in the lymph gland (blue: nuclei, green: medullary zone, red: lamellocytes). Scale bar: 20 μm.

Screen for Hdc interacting partners

Using the tagged Hdc isoforms in LC-MS/MS experiments, we isolated a list of potential Hdc interacting partners, which were tested for genetic interaction with *hdc*. In our experiments, one of the best hits was Unkempt (Unk), a known Hdc partner. We found

that, similarly to *hdc*, niche-specific silencing of *unk* resulted in the differentiation of lamellocytes.

Among the top candidate partners was Calmodulin (Cam), a Calcium-binding messenger protein that interacts with various kinases and phosphatases. Silencing *Cam* rescued the lamellocyte phenotype in the circulation and partially in the lymph gland as well. The finding that Calmodulin plays a role in mTORC1 activation in HEK cells may serve as an explanation for our observation; *Cam* knock-down may rescue the *hdc* phenotype by inhibiting the mTOR pathway.

We started a collaboration with Ildikó Maruzs-Kristó and Péter Vilmos (Laboratory of *Drosophila* Nuclear Actin Research, Institute of Genetics, HUN-REN BRC) and used bimolecular fluorescence complementation (Split-YFP in S2 cells) to detect the interaction between Hdc and Cam. We also included three other possible interacting partners (Mlf, eRF3, Rps3) in the study. We got positive results in the interaction experiments between Hdc and Calmodulin, as well as in case of Hdc and eRF3. Next, we tested the interaction between Hdc and Calmodulin using Co-IP. However, in these experiments, we could not detect a physical interaction between the proteins, which suggests that the interaction may be indirect possibly mediated through other proteins of the mTOR complex.

As Sumo was also on the candidate list of Headcase interactors, we tested its genetic interaction with *hdc*. Silencing *Sumo* enhanced the *hdc* phenotype and we could also identify a Sumo binding site in the Hdc protein. These results suggest that either Hdc or one of its partners may be sumoylated.

As several candidate partners were ribosomal proteins, for further studies, we initiated a collaboration with Zoltán Villányi (Department of Biochemistry and Molecular Biology, University of Szeged). In our experiments, Hdc could be co-purified with ribosomes. Our results also indicated that Hdc containing phospho-mimicking serin and threonin sites (as described earlier) is associated stronger with ribosomes, while phospho-dead sites containing Hdc is rather associated to stress granules, suggesting that phosphorylation may change the cellular localisation of the protein. Our observation that Hdc is found at higher levels in ribosomal pellets when *raptor* is knocked down indicates that raptor is necessary for the release of Hdc from ribosomes.

Role of Mlf and its interacting partners in the regulation of the hematopoietic niche

One of the potential candidates we identified in our proteomic studies and genetic interaction screens was *Myeloid leukemia factor* (*Mlf*). This factor was especially interesting for us, as its human homologue was described in relation with leukemia, and silencing of *Mlf* in the lymph gland resulted in lamellocyte differentiation.

We found that while the effect of *Mlf* and *Hsc70-4* is specific to the lymph gland, it is not specific to a certain functional zone, since silencing them in all three zones of the lymph gland (the niche, medullary and cortical zones) lead to lamellocyte differentiation. In the niche, silencing *Mlf* and *Hsc70-4* lead to reduced niche size and in some cases total abolishment of the niche, which indicates that they have essential roles in preserving the identity of niche cells which in turn affects progenitor differentiation (**Figure 4B,C**). In the medullary zone, knocking down these two factors lead to enlargement and dispersal of the anterior lobes of the lymph gland. In the cortical zone, silencing *Mlf* and *Hsc70-4* lead to the dispersal of the lobes in most lymph glands, indicating that these proteins do not only suppress progenitor division and differentiation but also transdifferentiation of mature hemocytes into lamellocytes.

Silencing another Mlf partner, the transciption factor *Dref*, also resulted in lamellocyte differentiation, which was accompanied by disturbed niche cell localisation (**Figure 4D**). However, *Dref* silencing did not reduce the number of niche cells, which suggests that Dref function functions independently of Mlf and Hsc70-4. This is also underlined by the finding that *Dref* silencing in the medullary and cortical zones did not affect progenitor differentiation. At the moment, our aim is to unveil the signalling pathways through which Mlf, Hsc70-4, and Dref exert their effect in the lymph gland.

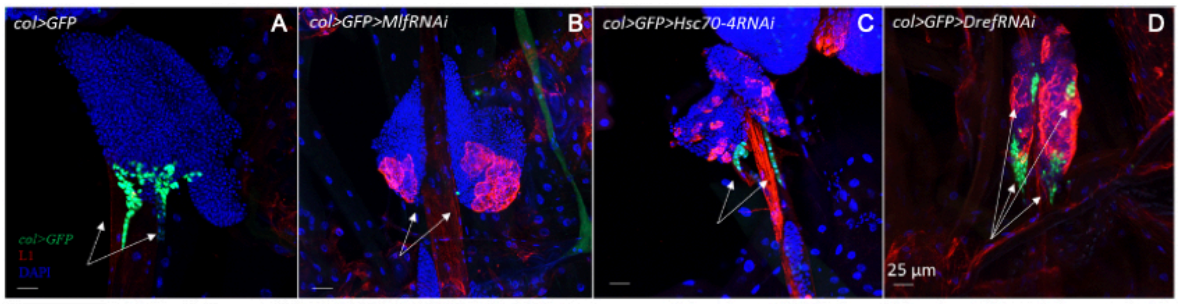

Figure 4. Role of Mlf and its partners in the hematopoietic niche. (A-C) Silencing of *Mlf* and its partners leads to lamellocyte differentiation (red). Silencing of *Mlf* (B) or *Hsc70-4* (C) in the niche leads to the reduction of niche cells as compared to the control (A). Silencing of the Mlf partner transcription factor *Dref* (D) leads to niche cell re-localisation to ectopic sites within the first lymph gland lobe (blue: nuclei, green: niche cells, red: lamellocytes). Scale bar: 25 µm.

Based on these results, Mlf, Hsc70-4 and Dref play an important role in maintaining niche identity, while the main function of Hdc is to enable signalling of the niche cells. We believe that these results give us new insight into the regulation of progenitor maintenance in the lymph gland, and opens a new possibility for further studies.

Investigation of the hdc regulatory region

When performing the rescue experiments with the short isoform of *hdc,* we found that the rescued flies showed a thorax closure phenotype, which we observed in the dead pupae of the hdc^{19} mutant, as well as in a low percentage of heterozygous adults. The phenotype was comparable to the effect of *dpp* silencing with the *pannier* (*pnr*) driver. This suggests that Hdc regulates Dpp signalling not only in the lymph gland, but also in the wing disc. In addition, we found that both silencing and overexpression of DPP in the *hdc* expression domain caused lethality, implying that DPP level is fine-tuned. Interestingly, we observed no thorax closure phenotype in the interallelic combination of the hdc^{19} driver and the $hdc^{\Delta 84}$ deletion when rescued with the *UAS-hdc.short* transgene. The explanation for these observations may be transvection, a phenomenon, in which the hdc^{19} driver traps the enhancer elements of the homologue chromosome. In the $hdc^{19}/hdc^{\Delta 84}$ combination, the deletion of the anchor region of the *hdc* gene may facilitate the homologue enhancers to act on the *hdc* promoter instead of the enhancer trap element, and thus contribute to the rescue of the phenotype.

To explore the regulatory region of the *hdc* gene, first we generated a transgene, which contains a *Gal4* gene together with a 2 kb long 5' sequence - a potential enhancer/ promoter - of the *hdc* gene. We also constructed transgenes, in which either *Gal4* or *GFP* were cloned together with a 4 kb long 5' sequence of *hdc*. The cloning was done in collaboration with Rita Sinka's research group (Department of Genetics, Faculty of Science and Informatics, University of Szeged). However, no GFP signal was detected in

either the *hdc-promoter-GFP* transgene carrying larvae or in the progeny of the *hdc-promoter-Gal4* crossed to *UAS-GFP* flies.

As we could not identify any of the *hdc* enhancer elements with the generated transgenes, we searched for the binding sites of the known regulator of *hdc*, *escargot* (*esg*) in the region of the *hdc* gene. We identified a single *esg* binding site in the first intron of the gene, and four *esg* binding sites in the 3' UTR of *hdc*. Based on these findings, we constructed a *Gal4* driver from the first intron of the gene, which contains an *esg* binding site. Unfortunately, we could not detect any relevant expression of this *Gal4* driver, which suggested that the enhancers of the gene are located at a longer distance from the promoter.

After being unsuccessful to find the regulatory elements of *hdc* with the previously generated constructs, we cloned eight segments systematically covering the 5' region and the first intron of the *hdc* gene with *Gal4*, and established stocks carrying the driver elements. None of these *Gal4* drivers was expressed in the lymph gland, however, two drivers were expressed in all imaginal discs, while other two were expressed in a specific pattern in imaginal discs.

II. Identification of markers and regulators of transdifferentiation

Aims of the work

In this part of the proposal, we aimed to identify factors that regulate the transdifferentiation of phagocytic plasmatocytes into capsule forming lamellocytes in our previously established *ex vivo* monitoring system. In these experiments, our goal was to reveal the relation of transcriptomic and morphological changes during the conversion, as well as to identify markers and regulators of transdifferentiation.

Characterization of larval blood cell composition

We optimised a single cell mass cytometry platform (SCMC) to analyse the blood cell composition of *Drosophila* larvae. We compared the antigene expression profile of single blood cells of naive wild type larvae with those of immune induced larvae and with tumorous mutants. We also analysed the blood cell composition of the amorphic *hdc* mutant, and revealed that although $hdc^{\Delta 84}$ larvae contain lamellocytes, their blood cell composition is vastly different from that of tumorous larvae (Balog et al., *Proteomics and Bioinformatics* 2021).

Revealing blood cell transdifferentiation routes

With the *ex vivo* cell culturing and differentiation method (developed in the framework of OTKA PD-115534), we mapped the potential transdifferentiation routes from plasmatocytes to lamellocytes. Our experiments revealed that upon immune induction, plasmatocytes are capable of differentiating into both type I and type II lamellocytes *ex vivo*. While type II lamellocytes are smaller cells than type I lamellocytes, they represent a differentiation end point; according to our regression plane analysis, type II lamellocytes do not differentiate into type I lamellocytes. The two lamellocyte types show distinct dynamics of differentiation; while type I lamellocytes appear in one early wave after immune induction, type II lamellocytes differentiate continuously during the investigated time

period. Massive transdifferentiation was observed only when hemocytes were isolated from the larvae 12 hours after immune induction, which indicates that *in vivo* signalling is important for the initialisation of the plasmatocyte-lamellocyte conversion. The work was done in collaboration with Péter Horváth's research group (Lendület Laboratory of Microscopic Image Analysis and Machine Learning, Institute of Biochemistry, HUN-REN BRC). This work served as a basis for a publication (Szkalisity et al., *Nature Communications* 2021).

For the better understanding of the transdifferentiation process, we repeated the time-lapse microscopy experiments in different immune conditions, such as wounding, wasp parasitation and in case of JAK dependent leukemia (hop^{Tum}). Although the morphology of type II lamellocytes was found to be very similar in all of the conditions, type I lamellocytes in tumorous mutants were often much larger (even exceeding 100 µm) than those differentiating after immune induction.

Transcriptome studies of transdifferentiating blood cells

We optimised our *ex vivo* transdifferentiation method to allow the isolation of individual hemocytes and performed transcriptome analysis on small pools of plasmatocytes and lamellocytes. To compare the gene expression profile of plasmatocytes with the two types of lamellocytes, after the analysis of cell fates with machine learning, we laser-dissected hemocytes from the membrane, and subdued the cells to transcriptome analysis (in collaboration with Lajos Pintér, Laboratory of Mutagenesis and Carcinogenesis Research, Institute of Genetics, HUN-REN BRC). We could underpin our first observation that type I and type II lamellocytes are distinct cell types with unique gene expression patterns. Interestingly, type II lamellocytes show a mixture of plasmatocyte and lamellocyte features. These cells are smaller than type I lamellocytes, and express the plasmatocyte-specific *eater-GFP* and the lamellocyte-specific *msn-Cherry* markers simultaneously. Although the lamellocyte specific *atilla* gene expression is lower in these cells, PPO3, which plays a role in the melanisation of the wasp egg is upregulated. *stg*, a mitotic marker also shows upregulation, which suggest that these cells, in contrast with type I lamellocytes, are capable of proliferation.

Based on the transcriptome data, we identified several genes that are differently expressed in plasmatocytes and lamellocytes. We used various bioinformatic tools to analyse the transcriptome data of previously isolated plasmatocytes and lamellocytes in collaboration with Balázs Vedelek (Laboratory of Actin Cytoskeleton Regulation, BRC). After counting the reads for each gene, we compared the plasmatocyte and lamellocyte samples. For differential expression, we used the DEapp software specifically developed for this aim. We detected changes in the transcript level of 118 genes. Gene ontology and pathway analysis of these genes yielded only limited results, however, we identified several interesting candidates (*atilla*, *Drip*, *CG8086* and *FoxP*) during our manual analysis.

Regulatory function of the identified genes

Based on our analysis, we selected candidate genes (*Drs*, *Mmp1*, *CG8086*, *Myo28B1*, *Drip*, *kis* and *rl*), which we tested systematically by silencing with the panhemocyte *He-Gal4* driver and the lamellocyte-specific *msn-Gal4* driver. The lamellocyte ratios following wounding and parasitation of the larvae were statistically evaluated. Although we got controversial results with pin induction, interestingly, silencing all of the studied genes with *msn-Gal4* resulted in a significantly reduced lamellocyte

number after wasp parasitation. We could not achieve the same effect with the *He-Gal4* driver, which may be due to the lower expression ensured by the driver element.

One of our candidates, *Drip* is an aquaporin channel, which presumably plays a role in the induction of morphological changes during transdifferentiation by transferring reactive oxygen species (ROS) into the intracellular space. We used an *in vivo* ROS reporter to elucidate the correlation of ROS elevation and *Drip* expression during transdifferentiation. We detected increased ROS levels in lamellocytes followed by the expression of the *Drip>RFP* in a portion of lamellocytes. Interestingly, silencing *Drip* in lamellocytes with the *msn-Gal4* driver in tumorous background (hop^{Tum}) resulted in a reduction of lamellocyte numbers.

For further studies, utilising CRISPR/Cas9, we inserted a T2A-Gal4 element in frame at the 3' end of the *atilla* gene. We generated an *atilla>mCD8::GFP; msnCherry* and an $atilla^{minos\text{-}GFP}$; *msn>mCD8::RFP* strain for obtaining Gal4 activity in the *atilla* (*atilla-Gal4*) and *msn* positive cells (*msn-Gal4*) independently. These lines will allow us the independent silencing of our candidate genes and monitoring their effect in the *msn* positive or *atilla* positive lamellocytes.

Exploring lamellocyte heterogeneity

We compared our data with single cell RNA sequencing datasets available from former transcriptomic studies, and we selected genes for *in vivo* and *ex vivo* expression analysis. In collaboration with Angela Giangrande's research group (IGMBC, Strasbourg, France), we attempted to generate CRIMIC reporters for three of the candidate genes, one of which (*Arc1*) was successful, however the reporter did not show specific expression in the hemocytes. The travel for the postdoc, Erika Gábor was supported by a Short Term EMBO Fellowship grant.

Besides *Arc1*, we used time-lapse microscopy to investigate the expression of *in vivo* markers for *Drs*, *Lsp1β*, *Antp*, *rl, ppn*, *Rac1*, *Atg8a*, *Snf4γ*, *Mmp1*, *Myo28B1* and *CG8086*. Of these genes, we found that *Myo28B1* and *CG8086* showed lamellocyte-specific expression after immune induction. The number of *Drs* and *Mmp1* expressing cells increased throughout the experiment after isolation of blood cells, which suggests that these genes may function during cellular stress response. The *ppn*, *Rac1*, *Atg8a, Snf4γ* genes were expressed in all hemocyte types both in induced and in uninduced larvae.

As these result indicated a heterogeneity of lamellocytes, we performed marker analysis at certain time-points following in parasitation, as well as in *ex vivo* time-lapse experiments, investigating whether our newly isolated markers were expressed in combination with the lamellocyte markers L1 and *msnCherry* after immune induction.

We tested the expression of 7 selected genes (*CG8086*, *Drip*, *kis*, *Myo28B1*, *Mmp1*, *Drs*, and *atilla*) in combination with the lamellocyte-specific *msnCherry* marker expression and L1 staining in naive larvae, as well as 16 h, 24 h, and 48 h following immune induction. We found a significant overlap of L1 and the examined markers. We detected a low percentage of hemocytes expressing either only *msnCherry* or only our gene-specific marker.

In *ex vivo* time-lapse experiments, we investigated marker expression 16 and 24 hours after wasp infestation in combination with *msnCherry.* At the 16 h time point following immune induction, the lymph gland is intact, while 24 h following immune induction, lamellocytes are released from the lymph gland as a contribution to the immune response. All the studied genes were found to be expressed in the circulation before lymph gland disruption.

In the time-lapse microscopic experiments, we observed that the *atilla*$^{minos\text{-}GFP}$, *Drip>GFP*, *CG8086>GFP*, *Myo28B1>GFP* were expressed exclusively in lamellocytes, while the *kis>GFP*, *Mmp1>GFP*, and *Drs-GFP* markers were expressed both in lamellocytes and plasmatocytes (**Figure 5**). The number of hemocytes expressing *Drip>GFP*, *kis>GFP* and *Drs-GFP* was stable in relation to *msnCherry* during the experiment, but while almost all of the lamellocytes expressed the *Drip*>GFP, only half of them were *kis>GFP* positive, and only a small portion of the cells were *Drs-GFP* positive. The ratio of *CG8086>GFP* and *Myo28B1>GFP* positive lamellocytes increased during the interval of the observation, as cells turned on these markers during lamellocyte differentiation. Interestingly, the ratio of *Mmp1>GFP* positive cells showed a higher increase in *ex vivo* compared to *in vivo* conditions, suggesting an upregulation of expression due to stress-response.

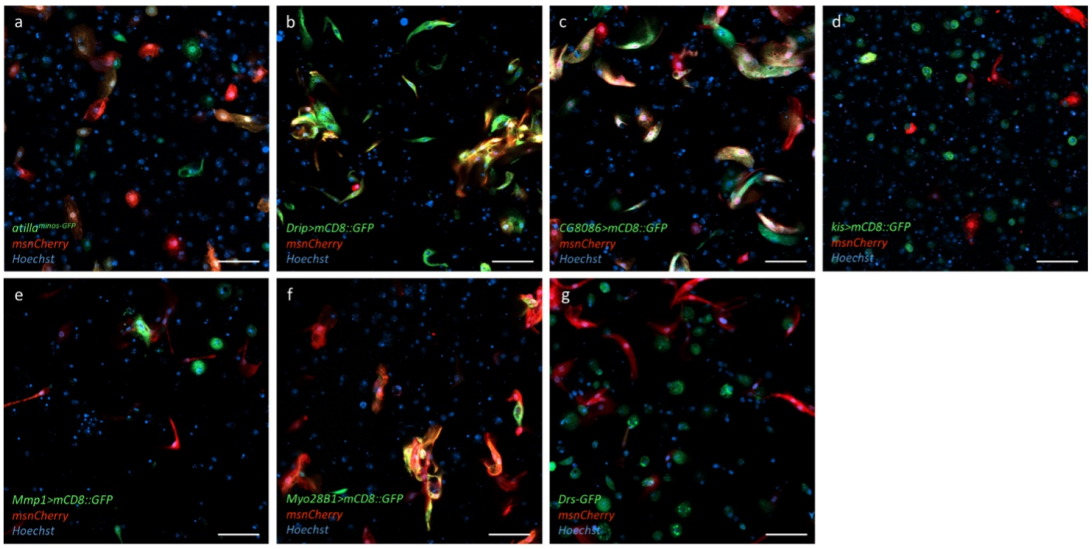

Figure 5. Expression of candidate genes in *ex vivo* hemocyte cultures. (a-g) The investigated markers (green) are expressed in hemocytes 24 hours following wasp infestation. Partial overlap of the markers were observed with the general lamellocyte marker *msnCherry* (red). Nuclei are stained with Hoechst (blue). Scale bars: 50 μm.

We detected the expression of the investigated markers in the lymph gland, which suggests that the expression of these genes are not exclusive to circulating hemocytes. We also observed the expression of all of the markers on the surface of the encapsulated parasite, indicating that the expression of these genes is not specific to a certain lamellocyte function.

Besides our candidate genes, we also examined the expression of reporters of the JAK/STAT (*10xSTAT-GFP*), Jun (*TRE-GFP*) and Toll (*Drs-GFP*) pathways at different time points after immune induction. We found that the JAK/STAT pathway is the most active 4-8h after immune induction, however, this activity is decreased after 24h. We could not detect *10xSTAT-GFP* expression in cells on the surface of the wasp egg, and the expression was not directly correlated with the *msnCherry* and L1 lamellocyte markers. The Jun pathway, however, was highly active in *msnCherry* positive lamellocytes both in the circulation and on the surface of the wasp egg. The expression of *Drs-GFP*, although not in direct correlation with *msnCherry*, was observed on the surface of the wasp egg.

Cluster analysis of the identified lamellocyte subsets

In collaboration with the Macrophage Polarisation Group, BRC, we reanalysed the single cell transcriptomic results of Tattikota et al. (2021). We defined the lamellocyte pool by the expression of *atilla*, and clustered lamellocytes into 8 sub-clusters. The pseudo-time analysis of transcriptome data defined that Cluster 4 as the pre-lamellocyte (progenitor) population. The top highly expressed genes of this cluster included the *Hml* and *NimC1* plasmatocyte markers, which confirms that these cells are the earliest lamellocytes in the process of transdifferentiation.

Based on the pseudo-time analysis Cluster 4 serves as a an ancestor of four independent branches: Cluster 5, Cluster 3 - Cluster 6, Cluster 0 - Cluster 1 - Cluster 2 and Cluster 7. Cells in Cluster 5 show both plasmatocyte and lamellocyte features; hemocytes of this cluster express several plasmatocyte markers (*Hml*, *Pxn*) at a high level, while also clearly showing *atilla* expression. Cells of this cluster are present in naive animals, but the size of the cluster increases 24 h after immune induction. Cluster 6 differentiates 24 hours after wasp parasitation through Cluster 3. The most dominant cluster 48 h after parasitisation is Cluster 2, which contains terminally differentiated classic lamellocytes. Based on their transcriptomic profile (expression of *atilla*, *msn, PPO3*, *Drip* and *shot*), these cells differentiate in two steps through Cluster 0 and Cluster 1. Cluster 7 which is sharply demarcated from the other clusters in the UMAP analysis (**Figure 6A**).

The expression of our candidate marker genes correlates with the cluster analysis: *Mmp1* serves as a marker for Cluster 4, *kis* is a marker for Cluster 5 and 6, *Drs* is a marker for Cluster 7, *Myo* is a marker for Cluster 0 and Cluster 1 (intermediates of Cluster 2), while *Drip* and *CG8086* are markers for Cluster 2 (**Figure 6B**). Following infestation, an increase in the size of Cluster 0, 1 and 2 were paralleled by the expression of *Drip>GFP, CG8086>GFP* and *Myo28B1>GFP*, which are the markers of these clusters. A lower number of cells belong to Cluster 7, but this cluster shows an increase following wasp infestation as well. The same was observed in the case of the *Drs-GFP* expressing cells. The ratio of *kis>GFP* expressing cells increased with lower dynamics compared to the *Drip>GFP, CG8086>GFP* and *Myo28B1>GFP*, similarly to the changes in the number of cells in Cluster 3, 6 and 5.

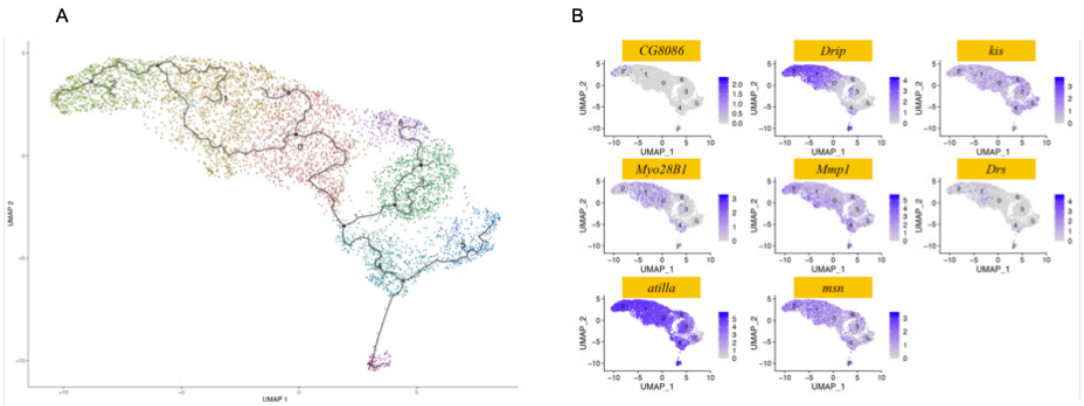

Figure 6. Cluster analysis of lamellocyte subsets. (A) Pseudo-time analysis of lamellocyte clusters reveal ontogenic pathways of lamellocyte differentiation. (B) Cluster-specific expression of the investigated transdifferentiation markers.

An ex vivo cell culturing system to study leukocyte plasticity

We set up a primary blood cell culturing system to study the details of transdifferentiation over a longer time period. Our experiments showed that during culturing, blood cells retained their capability to proliferate and their potential to transdifferentiate. The system also allowed for the maintenance and the observation of encapsulating lamellocytes on melanised wasp eggs. We found that the blood cell composition of leukemias are characteristic to the causing mutation, *e.g.* we found a very high lamellocyte rate in *hop*^{Tum} mutant larvae (**Figure 7**). By constructing a *UAS-blasticidin resistance* transgene, we set up a selection system for certain blood cell types, such as plasmatocytes, crystal cells, lamellocytes and the *crq-Gal4* expressing plasmatocyte subset. These experiments served as a basis for a publication (Kúthy-Sutus et al., *Cells* 2023).

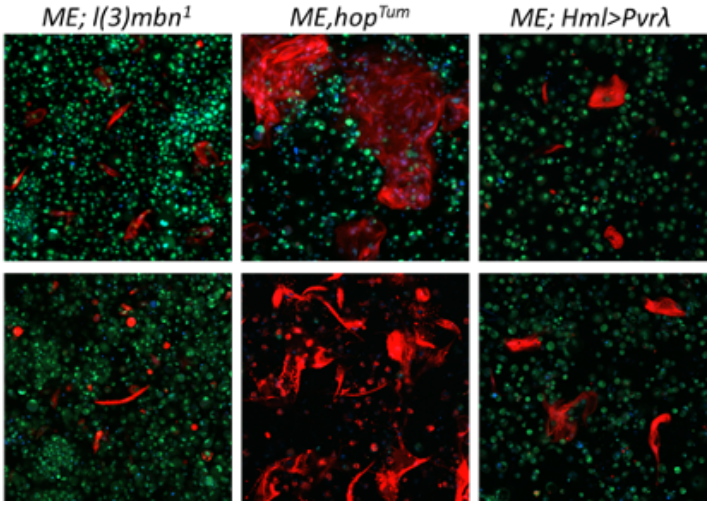

Figure 7. Differences in hemocyte composition and lamellocyte morphology in leukemia models. Lamellocyte (red) and plasmatocyte (green) ratio is significantly different in various leukemia models. Lamellocyte size and morphology also show huge variation in *ex vivo* cultures.

Continuation of the project

We aim to further analyse the regulation of lymph gland progenitor maintenance. For this, we plan to generate a null mutant for *Mlf* and perform the necessary rescue experiments. With genetic interaction experiments, we would like to identify the pathways via which Mlf and its partners exert their regulatory effect. We hope that revealing the exact function of these genes will help to better understand the regulatory processes that contribute to blood cell differentiation in hematopoietic compartments.

The newly revealed heterogeneity of lamellocytes and the results obtained in our *ex vivo* hemocyte differentiation system imply that certain lamellocyte clusters may be overrepresented in certain types of blood cell tumours. To further investigate the contribution of lamellocyte clusters to different leukemic conditions, we developed a method for sorting the lamellocytes and plasmatocytes with FACS based on their size and fluorescent marker expression. After sorting, hemocytes can be subdued to transcriptome analysis. In our preliminary study, we could detect a high expression of *Drip* in lamellocytes derived from wasp infested, *l(3)mbn*^{1} and *hop*^{Tum} larvae. In the future, we would like to compare the transcriptome profile of lamellocytes derived from wasp infested, and tumorous *l(3)mbn*^{1}, *hop*^{Tum} and *Hml>Bcr-Abl* larvae in collaboration with the Macrophage Polarization Group, Institute of Genetics, HUN-REN BRC.

Publications and activities related to the research

The results of this project were published in international scientific journals, presented as oral and poster presentations at national and international conferences (listed below). Our work also included science popularisation, teaching activities, university education and served as a basis for BSc, MSc and PhD theses (listed below) and a Short Term EMBO Fellowship grant contributing to international collaboration.

In the framework of the project, we published 6 scientific papers in international journals. One of these is not directly related to the project (Gábor et al., 2020), but we indicated it, since it involved a key experiment performed in *Drosophila* to identify a hemocyte marker. Two of the papers are reviews (Csordás et al., 2021 and Kharrat et al., 2022) summarising the latest results of scientific literature directly related to transdifferentiation in the circulation and to lymph gland progenitor maintenance.

There are 3 additional papers in preparation based on the work done in the framework of the grant: (1) on the role of Hdc in the regulation of the lymph gland progenitors via the insulin/mTOR pathway, (2) on the function of Mlf, Hsc70-4 and Dref in the lymph gland, and (3) on the transcriptomic, morphological and marker expression changes during transdifferentiation.

Articles

Gábor E, Cinege G, Csordás G, Rusvai M, Honti V, Kolics B, Török T, Williams MJ, Kurucz É, Andó I. 2020. Identification of reference markers for characterizing honey bee (*Apis mellifera*) hemocyte classes. DEV COMP IMMUNOL 109:103701. (IF: 3,192)

Csordás G, Gábor E, Honti V. 2021. There and back again: The mechanisms of differentiation and transdifferentiation in *Drosophila* blood cells. DEV BIOL 469:135-143. doi: 10.1016/j.ydbio.2020.10.006. (IF: 2,896)

Balog JÁ*, Honti V*, Kurucz É*, Kari B, Puskás LG, Andó I, Szebeni GJ. 2021. Immunoprofiling of *Drosophila* Hemocytes by Single-cell Mass Cytometry. GENOMICS PROTEOMICS BIOINFORMATICS S1672-0229(21)00055-3. doi: 10.1016/j.gpb.2020.06.022. (IF: 7,051)

Szkalisity A, Piccinini F, Beleon A, Balassa T, Varga IG, Migh E, Molnar C, Paavolainen L, Timonen S, Banerjee I, Ikonen E, Yamauchi Y, Ando I, Peltonen J, Pietiäinen V, Honti V, Horvath P. 2021. Regression plane concept for analysing continuous cellular processes with machine learning. NAT COMMUN 12(1): 2532. doi: 10.1038/s41467-021-22866-x. (IF: 14,919)

Kharrat B, Csordás G, Honti V. 2022. Peeling Back the Layers of Lymph Gland Structure and Regulation. INT J MOL SCI 23(14):7767. doi: 10.3390/ijms23147767. (IF: 6.208)

Kúthy-Sutus E, Kharrat B, Gábor E, Csordás G, Sinka R, Honti V. 2023. A Novel Method for Primary Blood Cell Culturing and Selection in *Drosophila melanogaster*. CELLS, 12(1), 24; https://doi.org/10.3390/cells12010024 (IF: 7.666)

Conference talks

2020. Magyar Immunológiai Társaság 49. Vándorgyűlése, online

Gábor Erika, Varga Gergely István, Géczi Aliz, Bayan Kharrat, Migh Ede, Beleon Attila, Horváth Péter, Széplaki Bence, Enyedi Márton, Pintér Lajos, Haracska Lajos, Honti Viktor: Vérsejtek transzdifferenciálódásának vizsgálata a mélytanulás módszerével *Drosophila melanogaster*-ben

2020. IV. Sejt-, Fejlődés- és Őssejt-biológusok éves találkozója, online

Gábor Erika, Varga Gergely István, Géczi Aliz, Bayan Kharrat, Migh Ede, Beleon Attila, Horváth Péter, Széplaki Bence, Enyedi Márton, Pintér Lajos, Haracska Lajos, Honti Viktor: Gépi tanulás alkalmazása a vérsejtek transzdifferenciálódásának vizsgálatára *Drosophila melanogaster*-ben

2021. IV. Móra Interdiszciplináris Szakkollégiumi Konferencia, online (SZTE Móra Ferenc Szakkollégium)

Géczi Aliz, Gábor Erika, Varga Gergely István, Bayan Kharat, Migh Ede, Beleon Attila, Horváth Péter, Széplaki Bence, Enyedi Márton, Pintér Lajos, Haracska Lajos, Honti Viktor: Vérsejt transzdifferenciálódás nyomon követése gépi tanulás segítségével *Drosophila melanogaster*ben

2021. Magyar Immunológiai Társaság 50. Vándorgyűlése, Kecskemét

Bayan Kharrat, Enikő Sutus, Erika Gábor, Rita Sinka, Ferenc Jankovics, Viktor Honti: Understanding the role of Headcase, the orthologue of HECA, in the *Drosophila* lymph gland

2021. Hungarian Molecular Life Sciences, Eger

Erika Gábor, Aliz Géczi, Ede Migh, Attila Beleon, Péter Horváth, Bence Széplaki, Márton Enyedi, Lajos Pintér, Lajos Haracska, Pierre Cattenoz, Angela Giangrande, Viktor Honti: Elucidating the regulation of hemocyte transdifferentiation with a complex deep learning based technology in *Drosophila melanogaster*

2021. 2nd Conference of the Visegrád Group Society for Developmental Biology, Szeged

Erika Gábor, Aliz Géczi, Ede Migh, Attila Beleon, Péter Horváth, Bence Széplaki, Márton Enyedi, Lajos Pintér, Lajos Haracska, Viktor Honti: Shedding light on the details of hemocyte transdifferentiation in *Drosophila melanogaster* with an artificial intelligence based technology

2021. A Magyar Tudomány Ünnepe, "Élettani és fejlődési folyamatok vizsgálata állatmodell rendszerekben" minikonferencia, Szeged

Géczi Aliz (Honti Viktor): Vérsejtek transzdifferenciálódásának vizsgálata ecetmuslicában

2022. *Drosophila* Blood Cell Biology Symposium, Strasbourg

Honti Viktor: *Drosophila* Blood Cell Differentiation Group

2022. Straub-napok, Szegedi Biológiai Kutatóközpont, Szeged

Honti Viktor: Ritka betegségek modellezése és *in vivo* gyógyszer panel szűrési kísérletek ecetmuslicában

2022. Magyar Immunológiai Társaság 51. Vándorgyűlése, Kecskemét

Gábor Erika, Perenyei-Gerecs Blanka, Bayan Kharrat, Sutus Enikő, Migh Ede, Beleon Attila, Horváth Péter, Takács Bertalan, Enyedi Márton, Pintér Lajos, Haracska Lajos, Vedelek Balázs, Honti Viktor: A vérsejtek transzdifferenciálódását szabályozó faktorok azonosítása és a differenciálódás útvonalainak feltérképezése *Drosophila melanogaster*ben

2022. "Genetikai Műhelyek Magyarországon" XXI. Minikonferencia, Szeged

Bayan Kharrat, Virág Nikolett, Sutus Enikő, Gábor Erika, Sinka Rita, Jankovics Ferenc, Honti Viktor: A *Drosophila* lárva nyirokszerve - a vérsejtképző őssejt niche modellje

2023. Hungarian Molecular Life Sciences, Eger

Bayan Kharrat, Nikolett Virág, Erika Gábor, Rita Sinka, Ferenc Jankovics, Viktor Honti: Identification of a novel network regulating lamellocyte fate in the lymph gland of *Drosophila melanogaster*

2023. Straub-napok, Szegedi Biológiai Kutatóközpont, Szeged

Erika Gábor, Blanka Perenyei-Gerecs, Bayan Kharrat, Ede Migh, Attila Beleon, Péter Horváth, Bence Széplaki, Márton Enyedi, Lajos Pintér, Lajos Haracska, Balázs Vedelek, Viktor Honti: Post-transcriptional analysis of *Drosophila* blood cells

2023. 27th European Drosophila Research Conference, Lyon

Bayan Kharrat, Nikolett Virág, Erika Gábor, Rita Sinka, Ferenc Jankovics, Viktor Honti: Dual role for the orthologue of HECA, Headcase, in blood cell progenitor maintenance in the *Drosophila* lymph gland

2023. SZBK 50. Jubileum, Szeged

Honti Viktor: Ellenanyagoktól a gépi tanulásig: a vérsejtképzés fejlődésbiológiai vizsgálata ecetmuslicában

Conference posters

2020. Magyar Immunológiai Társaság 49. Vándorgyűlése, online

Bayan Kharrat, Erika Gábor, Rita Sinka, Viktor Honti: Function and interacting partners of Headcase in *Drosophila melanogaster*

2020. IV. Sejt-, Fejlődés- és Őssejt-biológusok éves találkozója, online

Bayan Kharrat, Erika Gábor, Rita Sinka, Viktor Honti: Analysis of the function and interacting partners of Headcase in *Drosophila melanogaster*

2020. IV. Sejt-, Fejlődés- és Őssejt-biológusok éves találkozója, online

Géczi Aliz, Gábor Erika, Varga Gergely István, Bayan Kharrat, Migh Ede, Beleon Attila, Horváth Péter, Széplaki Bence, Enyedi Márton, Pintér Lajos, Haracska Lajos, Honti Viktor: Vérsejt transzdifferenciálódás nyomon követése gépi tanulás segítségével *Drosophila melanogasterben*

2021. Hungarian Molecular Life Sciences, Eger

Aliz Géczi, Erika Gábor, Bayan Kharrat, Ede Migh, Attila Beleon, Péter Horváth, Bence Széplaki, Márton Enyedi, Lajos Pintér, Lajos Haracska, Viktor Honti: A machine learning based approach to identify blood cell fates in *Drosophila melanogaster*

2021. Magyar Immunológiai Társaság 50. Vándorgyűlése, Kecskemét

Géczi Aliz, Gábor Erika, Varga Gergely István, Bayan Kharat, Migh Ede, Beleon Attila, Horváth Péter, Széplaki Bence, Enyedi Márton, Pintér Lajos, Haracska Lajos, Honti Viktor: Vérsejt transzdifferenciálódás nyomon követése gépi tanulás segítségével *Drosophila melanogaster*ben

2021. 2nd Conference of the Visegrád Group Society for Developmental Biology, Szeged

Aliz Géczi, Erika Gábor, Bayan Kharrat, Ede Migh, Attila Beleon, Péter Horváth, Bence Széplaki, Márton Enyedi, Lajos Pintér, Lajos Haracska, Viktor Honti: A machine learning based approach to identify blood cell fates in *Drosophila melanogaster* ***(poszterdíj)***

2021. Hungarian Molecular Life Sciences, Eger

Bayan Kharrat, Enikő Sutus, Erika Gábor, Nikolett Virág, Eszter Matiz, Rita Sinka, Ferenc Jankovics, Viktor Honti: Headcase as a player in stress response in the hematopoietic niche in *Drosophila melanogaster*

2021. 2nd Conference of the Visegrád Group Society for Developmental Biology, Szeged

Bayan Kharrat, Gergely István Varga, Ferenc Jankovics, Rita Sinka, Enikő Sutus, Erika Gábor, Viktor Honti: Role of Headcase in *Drosophila* hematopoiesis: what we know so far

2021. Hungarian Molecular Life Sciences, Eger

Enikő Sutus, Nikolett Virág, Ferenc Jankovics, Rita Sinka, Viktor Honti: Novel blood cell culturing method underlines leukocyte plasticity in *Drosophila melanogaster*

2021. Magyar Immunológiai Társaság 50. Vándorgyűlése, Kecskemét

Sutus Enikő, Virág Nikolett, Jankovics Ferenc, Sinka Rita, Honti Viktor: *Drosophila* primer vérsejt kultúrák differenciálódási potenciáljának vizsgálata

2021. 2nd Conference of the Visegrád Group Society for Developmental Biology, Szeged

Enikő Sutus, Nikolett Virág, Ferenc Jankovics, Rita Sinka, Viktor Honti: Differentiation potential analysis of *Drosophila* blood cells

2021. Magyar Immunológiai Társaság 50. Vándorgyűlése, Kecskemét

Gábor Erika, Géczi Aliz, Migh Ede, Beleon Attila, Horváth Péter, Széplaki Bence, Enyedi Márton, Pintér Lajos, Haracska Lajos, Honti Viktor: A vérsejtek transzdifferenciálódásának vizsgálata egy mesterséges intelligencián alapuló, komplex módszer segítségével *Drosophila melanogaster* modellszervezetben

2021 "Genetikai Műhelyek Magyarországon" XX. Minikonferencia, Szeged

Géczi Aliz, Gábor Erika, Bayan Kharrat, Migh Ede, Beleon Attila, Horváth Péter, Széplaki Bence, Enyedi Márton, Pintér Lajos, Haracska Lajos, Honti Viktor: Vérsejtek transzdifferenciálódását és vérsejt eredetű tumorok kialakulását szabályozó faktorok azonosítása gépi tanulás segítségével *Drosophila melanogaster*ben

2021 "Genetikai Műhelyek Magyarországon" XX. Minikonferencia, Szeged

Enikő Sutus, Nikolett Virág, Ferenc Jankovics, Rita Sinka, Viktor Honti: Differentiation potential analysis of *Drosophila* blood cells

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Bayan Kharrat, Gergely István Varga, Ferenc Jankovics, Rita Sinka, Enikő Sutus, Erika Gábor, Viktor Honti: Role of Headcase in *Drosophila* hematopoiesis: what we know so far

2022. Straub-napok, Szegedi Biológiai Kutatóközpont, Szeged

Erika Gábor, Aliz Géczi, Bayan Kharrat, Ede Migh, Attila Beleon, Péter Horváth, Bence Széplaki, Márton Enyedi, Lajos Pintér, Lajos Haracska, Balázs Vedelek and Viktor Honti: There and back again: the heterogeneity of *Drosophila* hemocytes

2022. Straub-napok, Szegedi Biológiai Kutatóközpont, Szeged

Enikő Sutus, Erika Gábor, Bayan Kharrat, Ferenc Jankovics, Rita Sinka and Viktor Honti: Studying *Drosophila* blood cell plasticity using primary blood cell cultures

2022. Straub-napok, Szegedi Biológiai Kutatóközpont, Szeged

Bayan Kharrat, Nikolett Virág and Viktor Honti: A new role for Myeloid leukemia factor (Mlf) in controlling lamellocyte fate in *Drosophila melanogaster*

2022. “Genetikai Műhelyek Magyarországon” XXI. Minikonferencia, Szeged

Gábor Erika, Géczi Aliz, Bayan Kharrat, Migh Ede, Beleon Attila, Horváth Péter, Takács Bertalan, Enyedi Márton, Pintér Lajos, Haracska Lajos, Vedelek Balázs, Honti Viktor: A vérsejtek transzdifferenciálódását szabályozó faktorok azonosítása és a differenciálódás útvonalainak feltérképezése *Drosophila melanogaster*ben

2022. “Genetikai Műhelyek Magyarországon” XXI. Minikonferencia, Szeged

Kúthy-Sutus Enikő, Gábor Erika, Kharrat Bayan, Faiyaz Ishrak, Honti Viktor: JAK2 indukált akut mieloid leukémia vizsgálata *Drosophila melanogaster*ben

2022. “Genetikai Műhelyek Magyarországon” XXI. Minikonferencia, Szeged

Virág Nikolett, Bayan Kharrat, Kúthy-Sutus Enikő, Gábor Erika, Sinka Rita, Jankovics Ferenc, Viktor Honti: Dual role for Headcase in hemocyte progenitor maintenance in the *Drosophila* lymph gland

2022. 51. Membrán-transzport Konferencia, Sümeg

Bayan Kharrat, Nikolett Virág, Viktor Honti: A new role for Myeloid Leukemia Factor (MLF) in controlling lamellocyte fate in *Drosophila melanogaster*

2022. 51. Membrán-transzport Konferencia, Sümeg

Sutus Enikő, Gábor Erika, Bayan Kharrat, Jankovics Ferenc, Sinka Rita, Honti Viktor: *Drosophila* vérsejtek plaszticitásának tanulmányozása primer vérsejtkultúrák segítségével

2022. 51. Membrán-transzport Konferencia, Sümeg

Gábor Erika, Géczi Aliz, Bayan Kharrat, Migh Ede, Beleon Attila, Horváth Péter, Széplaki Bence, Enyedi Márton, Pintér Lajos, Haracska Lajos, Vedelek Balázs, Honti Viktor: Oda és vissza: A *Drosophila* vérsejtek kalandos története ***(poszterdíj)***

2022. FEBS-IUBMB-ENABLE 1st International Molecular Biosciences PhD and Postdoc Conference, Seville, Spain

Bayan Kharrat, Nikolett Virág, Enikő Sutus, Erika Gábor, Rita Sinka, Ferenc Jankovics, Viktor Honti: Dual role for the orthologue of HECA, Headcase, in hemocyte progenitor maintenance in the *Drosophila* lymph gland

2023. Hungarian Molecular Life Sciences, Eger

Nikolett Virág, Bayan Kharrat, Erika Gábor, Rita Sinka, Ferenc Jankovics, Viktor Honti: A new cell-autonomous role for the HECA orthologue, Headcase, in blood cell progenitor maintenance in the *Drosophila* lymph gland

2023. Hungarian Molecular Life Sciences, Eger

Erika Gábor, Blanka Perenyei-Gerecs, Bayan Kharrat, Ede Migh, Attila Beleon, Péter Horváth, Bence Széplaki, Márton Enyedi, Lajos Pintér, Lajos Haracska, Balázs Vedelek, Viktor Honti: Characaterisation of new and old factors in the regulation of blood cell transdifferentiation in *Drosophila melanogaster*

2023. Straub-napok, Szegedi Biológiai Kutatóközpont, Szeged

Bayan Kharrat, Nikolett Virág, Viktor Honti: A novel function for Myeloid leukemia factor (Mlf) and two of its partners in maintaining blood cell progenitors in the larval lymph gland of *Drosophila melanogaster*

2023. Straub-napok, Szegedi Biológiai Kutatóközpont, Szeged

Nikolett Virág, Bayan Kharrat, Erika Gábor, Rita Sinka, Ferenc Jankovics, Viktor Honti: A new link between the orthologue of HECA, Headcase, and reactive oxygen species (ROS) in the *Drosophila* lymph gland

2023. "Genetikai Műhelyek Magyarországon" XXII. Minikonferencia, Szeged

Gábor Erika, Bayan Kharrat, Migh Ede, Beleon Attila, Horváth Péter, Takács Bertalan, Enyedi Márton, Pintér Lajos, Haracska Lajos, Vedelek Balázs, Honti Viktor: A vérsejtek heterogenitásának "poszt-transzkriptomikai" vizsgálata *Drosophila melanogaster*ben

2023. "Genetikai Műhelyek Magyarországon" XXII. Minikonferencia, Szeged

Bayan Kharrat, Nikolett Virág, Renáta Zagyva, Viktor Honti: Characterizing the role of Myeloid Leukemia Factor (MLF) and two of its partners in the larval lymph gland of *Drosophila melanogaster*

2023. FEBS-IUBMB-ENABLE 2nd International Molecular Biosciences PhD and Postdoc Conference, Cologne, Germany

Bayan Kharrat, Nikolett Virág, Erika Gábor, Rita Sinka, Ferenc Jankovics, Viktor Honti: The *Drosophila* lymph gland as a model for investigating hematopoietic stem cell (HSC) maintenance - Headcase as an example

2023. 27th European Drosophila Research Conference, Lyon

Bayan Kharrat, Nikolett Virág, Erika Gábor, Rita Sinka, Ferenc Jankovics, Viktor Honti: Dual role for the orthologue of HECA, Headcase, in blood cell progenitor maintenance in the *Drosophila* lymph gland

Science popularisation

- We organised programs for the “Kutatók éjszakája” event titled “Az ecetmuslica és az ember vére” every year.
- I took part in the “Science Snack” lecture series as a speaker.
- I had two demonstrations on research activity in the HUN-REN BRC in the framework of “Job Orientation Day” at Gábor Dénes secondary school, Szeged.
- We had a lab demonstration for secondary school students.
- I took part in a roundtable discussion at the Institute of Psychology, University of Szeged titled “A jövő embere”.

Supervision

The following students were involved in the work:

PhD- Bayan Kharrat (supervisor: Viktor Honti) - in progress

Benyhe-Kis Bernadett (supervisor: Zsolt Czimmerer and Viktor Honti) - in progress

MSc- Géczi Aliz (supervisor: Viktor Honti) - thesis defended

Ishrak Faiyaz (supervisor: Erika Gábor) - thesis defended

Lovász Dániel (supervisor: Viktor Honti) - in progress

BSc- Virág Nikolett (supervisor: Viktor Honti) - thesis defended (OTDK I. prize)

Perenyei-Gerecs Blanka (supervisor: Viktor Honti) - thesis defended

Zagyva Renáta (supervisor: Viktor Honti) - in progress

Simon Diána (supervisor: Erika Gábor) - in progress

Bihari Bence (supervisor: Erika Gábor) - in progress

Balogh Bálint Sándor - (supervisor: Viktor Honti) - in progress

Teaching activity, university courses

- I took part in the MSc course organised for English speaking students (University of Szeged) titled “Scientific Literature Sources” as a lecturer (“Blood of the Fly”).
- Every year, I organised the obligatory course at the Institute of Psychology, University of Szeged in titled “Etológia és magatartásgenetika”.
- I am an organiser of the course “Practical course on presenting scientific lectures“ (every semester) at the Institute of Genetics, HUN-REN BRC for PhD students. I also had a lecture in the framework of the course at the Institute of Genetics, HUN-REN BRC (“How (not) to write a scientific article”).
- As a lecturer, I took part in the courses titled “Molekuláris és sejtbiológia” and “Advanced Biochemistry, Biophysics, Molecular Cell Biology and Pharmacology” at the University of Szeged.