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Extracellular matrix-modulated Heartless signaling in *Drosophila* blood progenitors regulates their differentiation via a Ras/ETS/FOG pathway and target of rapamycin function

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ABSTRACT

Maintenance of hematopoietic progenitors ensures a continuous supply of blood cells during the lifespan of an organism. Thus, understanding the molecular basis for progenitor maintenance is a continued focus of investigation. A large pool of undifferentiated blood progenitors are maintained in the *Drosophila* hematopoietic organ, the larval lymph gland, by a complex network of signaling pathways that are mediated by niche-, progenitor-, or differentiated hemocyte-derived signals. In this study we examined the function of the *Drosophila* fibroblast growth factor receptor (FGFR), Heartless, a critical regulator of early lymph gland progenitor specification in the late embryo, during larval lymph gland hematopoiesis. Activation of Heartless signaling in hemocyte progenitors by its two ligands, Pyramus and Thisbe, is both required and sufficient to induce progenitor differentiation and formation of the plasmatocyte-rich lymph gland cortical zone. We identify two transcriptional regulators that function downstream of Heartless signaling in lymph gland progenitors, the ETS protein, Pointed, and the Friend-of-GATA (FOG) protein, U-shaped, which are required for this Heartless-induced differentiation response. Furthermore, cross-talk of Heartless and target of rapamycin signaling in hemocyte progenitors is required for lamellocyte differentiation downstream of Thisbe-mediated Heartless activation. Finally, we identify the *Drosophila* heparan sulfate proteoglycan, Trol, as a critical negative regulator of Heartless ligand signaling in the lymph gland, demonstrating that sequestration of differentiation signals by the extracellular matrix is a unique mechanism employed in blood progenitor maintenance that is of potential relevance to many other stem cell niches.

Introduction

The maintenance of hematopoietic stem cells is a crucial process for the normal production of blood cells, but in addition, understanding its molecular basis could enhance the therapeutic benefits of this cell population. Studies of hematopoiesis and the regulation of hematopoietic progenitors have identified a variety of molecular mechanisms that regulate progenitor/stem cell maintenance (Arai and Suda, 2007; Seita and Weissman, 2010; Teitell and Mikkola, 2006). As in vertebrate systems, *Drosophila* hematopoiesis requires a population of multipotent progenitor cells that give rise to all differentiated hemocyte lineages (Mandal et al., 2007). Previous studies of *Drosophila* larval hematopoiesis have uncovered a complex network of signaling pathways that cooperate to regulate hemocyte progenitor maintenance. These include niche-derived Hedgehog signaling (Krzemien et al., 2007; Mandal et al., 2007), an adenosine deaminase growth factor A-mediated signal emanating from differentiated hemocytes (Mondal et al., 2011), and Wingless signaling, which autonomously regulates progenitor cell maintenance (Sinenko et al., 2009). In contrast, the signal(s) required for differentiation of *Drosophila* hemocyte progenitors during larval development remain largely unknown.

One wave of hematopoiesis in *Drosophila* occurs in the larval lymph gland (Lebestky et al., 2000; Tepass et al., 1994). Studies of the lymph gland have allowed genetic dissection of signaling networks that operate in a niche-, progenitor- or differentiated hemocyte-dependent manner to maintain blood homeostasis, owing to the ability to perform cell-type specific genetic manipulation with direct in vivo imaging of its effects on these distinct cell populations. In the lymph gland, multipotent blood progenitors termed prohemocytes express high levels of *Drosophila* (d)E-cadherin, or Shotgun, and are compactly arranged in a medial

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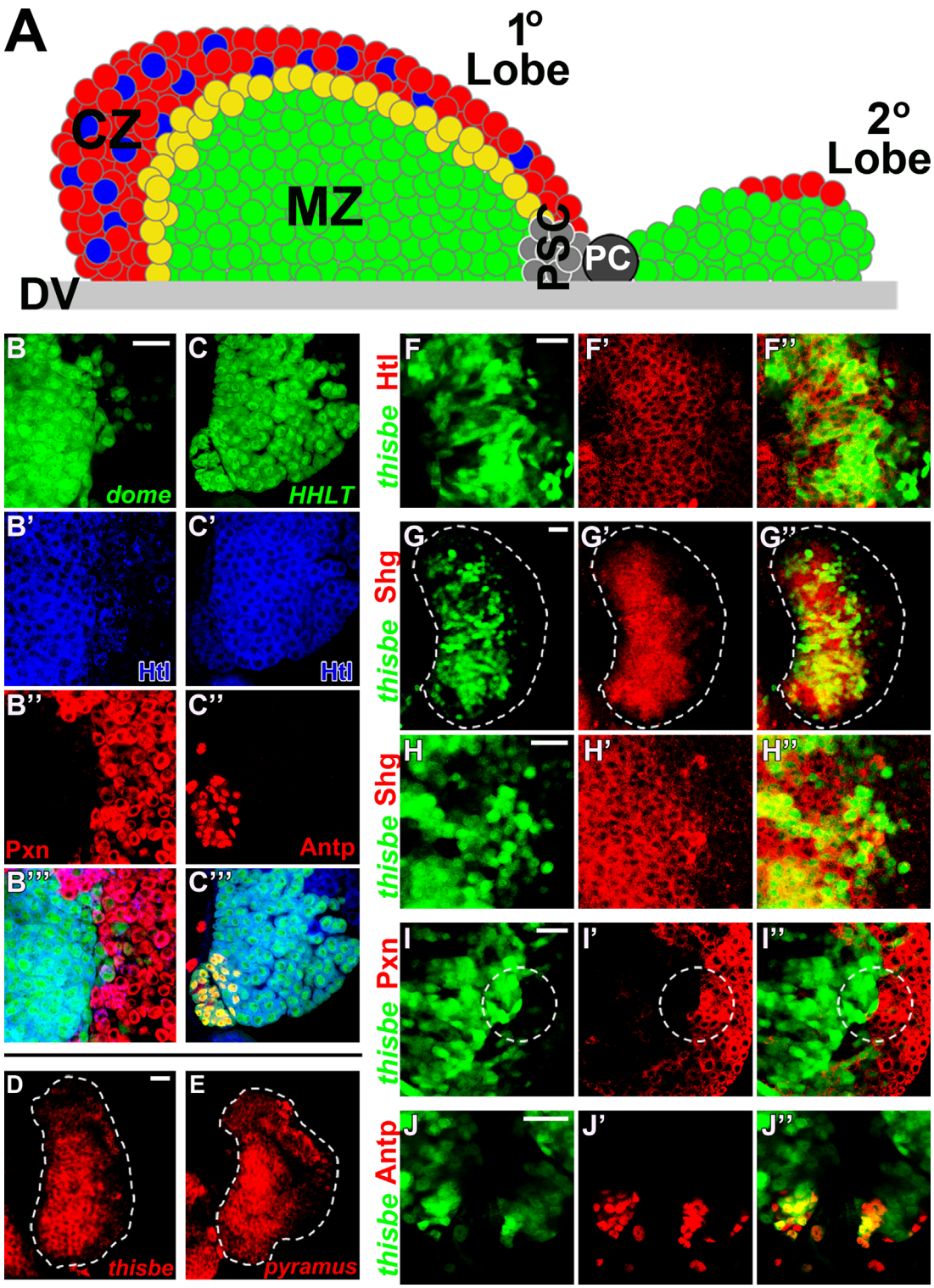

Fig. 1. *Expression patterns of Heartless and its ligands, Thisbe and Pyramus, in the lymph gland*. All panels represent wild-type lymph glands. In panels B–B''' *dome-Gal4* is used to drive *UAS-2xEGFP* (green) expression in MZ prohemocytes. In panels C–C''' *HHLT* (*hand-gal4, hml-gal4, UAS-2xEGFP, UAS-FLP; A5C-FRT-STOP-FRT-gal4*) is used to clonally express *UAS-2xEGFP* (green) in lymph gland hemocytes. In panels F–J'' *thisbe-Gal4* drives *UAS-2xEGFP* (green) expression in *thisbe*-expressing hemocytes. Peroxidasin (Pxn) expression is shown in red in panels B''–B''' and I'–I''. Antennapedia (Antp) expression is shown in red in panels C''–C''' and J–J''. Heartless (Htl) expression is shown in blue in panels B–C''' and in red in panels F–F''. The MZ marker, Shotgun (Shg), is shown in red in panels G–G''. *Thisbe* transcript expression is shown in red in panel D, and *pyramus* transcript expression is shown in red in panel E. (A) Schematic diagram of a wandering third instar lymph gland primary and secondary lobe. Differentiated plasmatocytes (red) and crystal cells (blue) localize to the peripheral cortical zone (CZ). Prohemocytes (green) are compactly arranged in the Medullary Zone (MZ). Posterior signaling center (PSC) cells (gray) localize to the posterior tip of the primary lobe. Secondary lobes mostly consist of undifferentiated progenitors (green) with few differentiated hemocytes (red). Pericardial cells (PC) intercalate between the primary and secondary lobes. DV, dorsal vessel. (B–B''') Heartless (Htl, blue) is strongly expressed in *dome*$^{+}$ hemocyte progenitors of the MZ (green), and is largely reduced in Pxn^{+} (red) differentiated hemocytes of the peripheral CZ. (C–C''') Heartless (blue) is detected in Antp^{+} cells of the PSC (red) at lower levels than adjacent hemocytes of the lymph gland. (D–E) Fluorescence in situ hybridization against *thisbe* (D) or *pyramus* (E) mRNA transcripts demonstrates highest expression in MZ hemocytes. (F–F'') *Thisbe* (green) expression overlaps (yellow) with Heartless (red) expression in medial lymph gland regions, although not all Heartless^{+} cells are *thisbe*$^{+}$. Thisbe > GFP is strongly expressed in a subset of MZ cells. (G–H'') *Thisbe* (green) expression overlaps (yellow) with Shotgun (Shg, red) in the MZ, confirming highest *thisbe* expression in MZ prohemocytes and reduced or negative *thisbe* expression in Shotgun-negative hemocytes of the peripheral CZ. (I–I'') High *thisbe* (green) expression rarely overlaps (yellow) with high Pxn expression, although *thisbe* expression often extends to the most medial Pxn^{+} hemocytes of the CZ. Co-expression of low levels of *thisbe* and low levels of Pxn are also observed in some scattered hemocytes. (J–J'') Most, but not all, Antp^{+} PSC cells express *thisbe* (green, overlap in yellow). Scale bars = 50 μm. Scale bar in A corresponds to A–B'''; scale bar in I corresponds to I–J.