Regulation of a serine protease homolog by the JNK pathway during thoracic development of Drosophila melanogaster

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A R T I C L E  I N F O

Article info
Received 16 August 2014
Revised 24 January 2015
Accepted 27 January 2015

Keywords:
Drosophila
JNK pathway
Thorax closure
Serine protease homolog
Imaginal disc
Scarface

A B S T R A C T

The importance of the Jun N-terminal Kinase (JNK) pathway during normal development and tumor invasion has been well documented in Drosophila. Here, this pathway plays important roles in epithelial morphogenesis, wound healing, apoptosis, immunity and regulation of lifespan. However, which downstream molecules facilitate these effects is not very well elucidated. In this study, data are presented on a serine protease homolog (SPH), scarface. These data show that scarface is under regulatory control of the JNK pathway and that this pathway is both necessary and sufficient for its expression within the context of thoracic development. Consequently, down-regulation of scarface results in a thoracic-cleft phenotype that phenocopies the JNK pathway defect. A possible role of scarface during thoracic development in Drosophila is discussed.

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1. Introduction

Development of Drosophila thorax has been an excellent model that has aided in our understanding of the morphogenetic processes involved in formation of an organism [1,2]. Several of the processes involved in thoracic development, like epithelial mesenchymal transition, migration, tissue invasion and extracellular matrix remodeling are also processes co-opted by cells that become cancerous and metastatic [3,4]. For this reason an understanding of gene functions, their regulation and processes they control during metastatic [3,4]. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
Serine proteases (SP) are catalytically active enzymes that perform a host of functions in animals ranging from food digestion to immunity to development to blood coagulation [10,11]. Serine protease homologs (SPHs) on the other hand, while being similar to serine proteases, have a catalytic triad substitution rendering them catalytically compromised [12,13]. The exact function of these SPH serine proteases, have a catalytic triad substitution rendering them SPH homologs (SPHs) on the other hand, while being similar to form a host of functions in animals ranging from food digestion to...
peripodial membrane cells play important roles during thorax development and are regions where the JNK pathway is also active [1].

3.3. JNK pathway is necessary and sufficient for scarface expression in the wing disc

The finding that Scarface protein is expressed in the peripodial membrane, and the peripodial stalk taken in combination with the known roles of JNK pathway in these tissues, suggested that scarface could be regulated by the JNK pathway. To test the necessity of JNK pathway for Scarface expression, we utilized a strong mutation in the D-JNKK encoded by the gene hemipterous (hepR75) [21]. Wing discs derived from larvae hemizygous for hepR75 and also carrying the scarface protein trap exhibited diminished Scarface expression as judged by reduced GFP levels in the peripodial stalk and peripodial membrane (Fig. 3, arrow compared to Fig. 2 B, B', E). This demonstrated that JNK pathway is necessary for Scarface expression.

We next tested to see if JNK pathway was sufficient for the expression of Scarface. To test this we up-regulated this pathway
using an upstream component of the JNK pathway called dTAK1 [16] and the UAS-Gal4 system [19]. Because JNK pathway activation results in larval lethality, we controlled the temporal expression of dTAK1 by placing it under the control of Gal80ts as previously described [4]. Induction of dTAK1 expression was achieved by shifting the larvae of the genotype Ptc-gal4, UAS-RFP,
3.4. scarface knockdown mimics JNK pathway mutant phenotype

Having established that Scarface is expressed in the peripodial stalk and peripodial membrane (JNK pathway is active here) cells under the regulatory control of JNK signaling, we reasoned that Scarface could be one of the effectors of JNK signaling within the context of thoracic development. If this were the case then Scarface knockdown in the developing thorax should result in phenotypes reminiscent of JNK pathway defect (thoracic cleft) [1,2,5]. To knockdown scarface in the developing thorax a UAS-scarface RNAi line was overexpressed in the domain of Pannier-Gal4 (Pnr-Gal4) expression and Apterous-Gal4 (Ap-Gal4) expression in combination with UAS-Dcr2 (for RNAi effect enhancement). The Pnr-Gal4 drives expression of a UAS transgene in the notum area of the wing disc destined to form the dorsal medio-lateral region of the adult thorax as well as thoracic bristles [22]. Apterous-Gal4, on the other hand expresses in the dorsal compartment of the wing disc which encompasses the region destined to form the future thorax [22] scarface knockdown using the Ap-Gal4 driver resulted in loss of bristles from the medio-lateral region of the thorax and the thorax displayed a mild thoracic cleft (arrow in Fig. 5B). scarface knockdown using the Pnr-Gal4 driver resulted in a much stronger thoracic cleft and loss of bristles from the medio-lateral region of the thorax (arrow in Fig. 5D). The thoracic cleft phenotype is reminiscent of JNK pathway defect and has been recovered for mutations in various components of the JNK pathway [5]. The regulation of scarface by the JNK pathway during thoracic development combined with the thoracic cleft phenotype generated when scarface is knocked down, suggests that part of the function of JNK during thoracic development is effected through SPH scarface.

3.5. Possible function of scarface during Drosophila thorax development

JNK pathway plays a central role in thorax development and mediates this in at least two ways. First, it regulates the MMPs which in turn regulate the BM degradation, a step critical for the process of disc eversion [4]. Second, JNK signaling along with DPP also regulates the cytoskeletal dynamics and adhesion, properties important for the proper movement of epithelial sheets [6]. A third role of JNK pathway in thoracic development could be the regulation of BM integrity mediated by the SPH scarface during thoracic closure. While this needs to be unequivocally demonstrated during thoracic closure, evidence pointing to a similar function of JNK pathway in embryonic dorsal closure mediated by scarface already exists. It has been demonstrated by Sorrosal et al. (2010), that scarface is under regulatory control of the JNK pathway and is required for proper Laminin localization within the BM during embryonic dorsal closure [18].

While JNK regulation of scarface and its involvement in thoracic closure has been established in this study, what cellular role does scarface play during thoracic development in general and thoracic closure in particular (considering that it is not a catalytically active protein) is open to speculation. It has been suggested that SPHs have evolved into “catalytically dead regulatory molecules” and

\[ w; \text{Ptc-Gal4,UAS-srcRFP},sf^{\text{plus}/+}; \text{UAS-dTAK1/Tubulin-Gal80}^{\text{ts}} \]
may exert their regulatory effects by sequestering substrates for serine proteases in a context dependent manner or by stabilizing cellular structures. For example, mutations in a SPH in Drosophila encoded by the gene masquerade (mas) \[23\] results in muscle attachment defects suggesting that this protein has a stabilizing effect on muscle-cell and matrix interaction. It is possible that, just as scarface stabilizes the integrity of BM by ensuring proper localization of Laminin during embryonic development\[18\], it may be performing a similar role during thoracic closure also. Future experiments may provide evidence confirming this idea.

During metamorphosis, the larval wing imaginal discs undergo a process of disc eversion where the imaginal discs are everted out of the larval body cavity. A hallmark of this disc eversion process is the degradation of BM mediated by the actions of MMPs under regulatory control of the JNK pathway \[4\]. However, once the disc eversion has occurred, the wing disc epithelial cells spread and move towards each other by crawling over the larval epidermis \[3,6\]. For spreading and movement to occur, modulation of the cell-matrix interaction with the underlying larval cells would be required. That this cell-matrix interaction modulation in thoracic closure could be brought about by SPH Scarface is evidenced by the thoracic cleft phenotype generated as a result of scarface knockdown. In the absence of this protein, (through RNAi knockdown) the cell-matrix interaction maybe destabilized, resulting in impeded and delayed movement of the two discs towards each other and generation of the thoracic cleft phenotype.

As mentioned earlier, experiments performed to study the function of Scarface in embryonic development have demonstrated that Scarface is indeed required for BM integrity through proper localization of Laminin \[18\] in the BM \[24\]. It has also been demonstrated in this study and others that scarface is under regulatory control of the JNK pathway \[17,18\]. Given that BM integrity is compromised during tumor metastasis and because JNK pathway is up-regulated in migrating tumors \[4,8,25\], it is attractive to suggest that SPH Scarface may play a role in tumor metastasis by regulating BM integrity. Future experiments will help us better understand the role of this SPH in tumor metastasis using a Drosophila model \[26,27\].

**Author contributions**

AS conceptualized, designed, performed the experiments, analyzed data and wrote the paper. QD helped with dissection, fixation and mounting of discs presented in Supplementary Fig. 1.

**Acknowledgements**

Many thanks are due to Dr. Tian Xu for his generous help with the continuation of this project in his laboratory and for his support. Thanks are also due to Dr. Richard Mann for the Scarface protein trap line. The BDSC and VDRC are acknowledged for various reagents used in this study. Part of this work was supported by an Anna Fuller Fund Postdoctoral Fellowship to AS at Yale University. Research in my laboratory at WKU is supported by the WKU Department of Biology startup funds, WKU Research Foundation RCAP-I grant # 11-8032 and by a KBRIN-AREA grant funded through a parent grant from the National Institute of General Medical Sciences of the National Institutes of Health under award number 5P20GM103436-13.

**Appendix A. Supplementary data**

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.fob.2015.01.008.
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