Título Identification and Functional Analysis of Healing Regulators in Drosophila

Tipo de Producto Publicación Científica

Autores Álvarez-Fernández, Carmen; Tamirisa, Srividya; Prada, Federico; Chernomoretz, Ariel; Podhajcer, Osvaldo; Blanco, Enrique and Martín-Blanco, Enrique

Código del Proyecto y Título del Proyecto

A15T02. Utilización del modelo de Drosophila melanogaster para evaluar el efecto en la ingesta del glutamato monosódico (GMS) y la transcriptómica de la cicatrización de heridas

Responsable del Proyecto

Prada, Federico

Línea

Biociencias

Área Temática

ABI

Fecha

Febrero, 2015

INTEC
Instituto de Tecnología

UADE FUNDACIÓN UADE

PLOS | GENETICS

RESEARCH ARTICLE

Identification and Functional Analysis of Healing Regulators in *Drosophila*

Carmen Álvarez-Fernández[1‡¤a], Srividya Tamirisa[1‡], Federico Prada[2¤b], Ariel Chernomoretz[3], Osvaldo Podhajcer[2], Enrique Blanco[4], Enrique Martín-Blanco[1]*

1 Instituto de Biología Molecular de Barcelona, Consejo Superior de Investigaciones Científicas. Parc Cientific de Barcelona, Barcelona, Spain, **2** Terapia Molecular y Celular, Fundación Instituto Leloir, Buenos Aires, Argentina, **3** Departamento de Física, Universidad de Buenos Aires, Buenos Aires, Argentina, **4** Departament de Genètica and Institut de Biomedicina (IBUB), Universitat de Barcelona, Barcelona, Spain

¤a Current address: Laboratory of Oncological Hematology and Transplantation, Institute of Biomedical Research, IIB Sant Pau, Barcelona, Spain and Department of Hematology, Hospital Santa Creu i Sant Pau, Barcelona, Spain
¤b Current address: Departamento de Biotecnología y Tecnología Alimentaria, UADE, Lima, Buenos Aires, Argentina
‡ These authors equally contributed to this work.
* embbmc@ibmb.csic.es

CrossMark
click for updates

OPEN ACCESS

Citation: Álvarez-Fernández C, Tamirisa S, Prada F, Chernomoretz A, Podhajcer O, Blanco E, et al. (2015) Identification and Functional Analysis of Healing Regulators in *Drosophila*. PLoS Genet 11(2): e1004965. doi:10.1371/journal.pgen.1004965

Editor: Michael Joseph Galko, Univ of Texas MD Anderson Cancer Center, UNITED STATES

Received: June 19, 2014

Accepted: December 20, 2014

Published: February 3, 2015

Copyright: © 2015 Álvarez-Fernández et al. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: All relevant data are within the paper and its Supporting Information files except the original microarrays, which have been deposited in the NCBI-GEO (accession number: GSE62863).

Funding: CAF and FP were supported by the EU FP6 STREP project WOUND and ST held a Spanish FPU PhD studentship. Research in the EMB laboratory is funded by grants of the EU (FP6 STREP project WOUND), the Spanish Ministry of Economy and Competitivity (DGI and CONSOLIDER grants) and the Generalitat de Catalunya (SGR). The funders had no role in study design, data collection and

Abstract

Wound healing is an essential homeostatic mechanism that maintains the epithelial barrier integrity after tissue damage. Although we know the overall steps in wound healing, many of the underlying molecular mechanisms remain unclear. Genetically amenable systems, such as wound healing in *Drosophila* imaginal discs, do not model all aspects of the repair process. However, they do allow the less understood aspects of the healing response to be explored, e.g., which signal(s) are responsible for initiating tissue remodeling? How is sealing of the epithelia achieved? Or, what inhibitory cues cancel the healing machinery upon completion? Answering these and other questions first requires the identification and functional analysis of wound specific genes. A variety of different microarray analyses of murine and humans have identified characteristic profiles of gene expression at the wound site, however, very few functional studies in healing regulation have been carried out. We developed an experimentally controlled method that is healing-permissive and that allows live imaging and biochemical analysis of cultured imaginal discs. We performed comparative genome-wide profiling between *Drosophila* imaginal cells actively involved in healing versus their non-engaged siblings. Sets of potential wound-specific genes were subsequently identified. Importantly, besides identifying and categorizing new genes, we functionally tested many of their gene products by genetic interference and overexpression in healing assays. This non-saturated analysis defines a relevant set of genes whose changes in expression level are functionally significant for proper tissue repair. Amongst these we identified the TCP1 chaperonin complex as a key regulator of the actin cytoskeleton essential for the wound healing response. There is promise that our newly identified wound-healing genes will guide future work in the more complex mammalian wound healing response.

analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

Author Summary

Two major challenges in our understanding of epithelial repair and regeneration is the identification of the signals triggered after injury and the characterization of mechanisms initiated during tissue repair. From a clinical perspective, a key question that remains unanswered is "Why do some wounds fail to heal?" Considering the low genetic redundancy of *Drosophila* and its high degree of conservation of fundamental functions, the analysis of wound closure in imaginal discs, whose features are comparable to other post-injury events, seems to be a good model. To proceed to genomic studies, we developed a healing-permissive *in vitro* culture system for discs. Employing this method and microarray analysis, we aimed to identify relevant genes that are involved in healing. We compared cells that were actively involved in healing to those not involved, and identified a set of upregulated or downregulated genes. They were annotated, clustered by expression profiles, chromosomal locations, and presumptive functions. Most importantly, we functionally tested them in a healing assay. This led to the selection of a group of genes whose changes in expression level and functionality are significant for proper tissue repair. Data obtained from these analyses must facilitate the targeting of these genes in gene therapy or pharmacological studies in mammals.

Introduction

Damage to an organism initiates a cascade of events that includes inflammation and the formation and remodeling of new tissue. Multiple studies have revealed significant similarities between how tissues are rebuilt during repair episodes and how they are built during development [1]. Thus, when considering epithelial repair, clear parallels exist at the structural level, as well as in signaling and the control of gene expression with the embryonic dorsal closure or the fusion of imaginal discs in *Drosophila*, ventral enclosure in *C. elegans* or eyelid closure in vertebrates [1–5]. Remarkably, co-assembly of actin cables and filopodial protrusions are instrumental in all these processes, with the majority being dependent on signaling by the JNK cascade [3, 6–9].

In invertebrates, the immediate wound healing response involves the formation of a temporary plug that encapsulate invading microbes, along with the activation of melanization and cross-linking enzymes [10]. In *Drosophila* larvae the outer part of the plug forms a scab within the first few hours of being wounded. The surrounding epidermal cells then orient themselves towards the wound and spread to reestablish a continuous epithelium. In larval epithelia wounds, the inactivation of the JNK pathway inhibits epidermal spreading and reepithelialization [11]. Remarkably, healing of incisional wounds in *Drosophila* adults also proceeds through a JNK dependent, lamellipodial-directed, epidermal cell spreading and scab formation [12]. In contrast, and similar to vertebrate early embryos, *Drosophila* embryos wounded by laser beams accumulate an actomyosin cable at the leading edge and display dynamic filopodial protrusions. The same is observed when imaginal discs are subjected to mechanical or genetic injuries [3, 13, 14]. The actin cable and the filopodial protrusions appear to participate in efficient sealing. The cable pulls the wound margins like a purse-string and assembles in response to different signaling events, including the activity of the JNK pathway [3, 13].

While we begin to understand some of the mechanisms of wound healing, many aspects remain unanswered. Genetically amenable systems, such as the healing of *Drosophila* imaginal discs, do not model all aspects of the repair process, such as inflammation or connective tissue contraction and fibrosis. However, they do allow the less understood aspects of the healing response to be explored; e.g. which cues regulate the migratory and proliferative machinery of

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4315591/