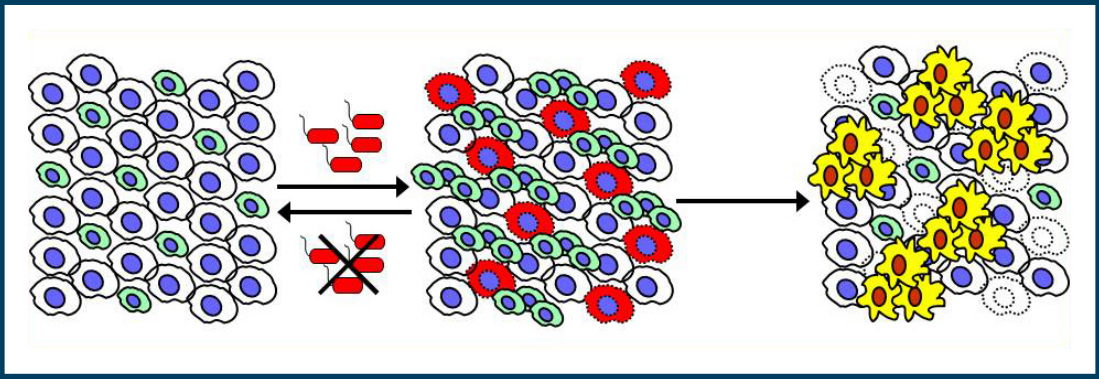
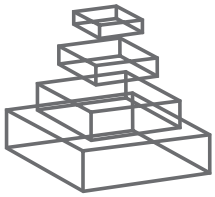
frontiers
RESEARCH TOPICS



MODEL ORGANISMS IN INFLAMMATION AND CANCER

Topic Editors
Yiorgos Apidianakis and Dominique Ferrandon

frontiers in
CELLULAR AND INFECTION MICROBIOLOGY



frontiers

ISSN 1664-8714
ISBN 978-2-88919-370-7
DOI 10.3389/978-2-88919-370-7

ABOUT FRONTIERS

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

FRONTIERS JOURNAL SERIES

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing.
All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

DEDICATION TO QUALITY

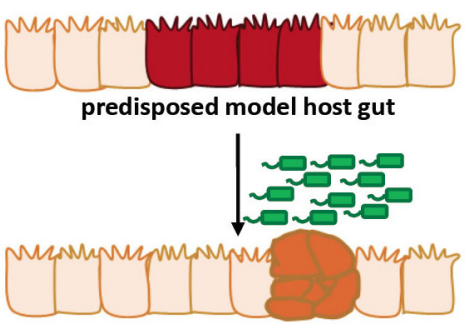
Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view.
By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

WHAT ARE FRONTIERS RESEARCH TOPICS?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area!
Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: researchtopics@frontiersin.org

MODEL ORGANISMS IN INFLAMMATION AND CANCER

Topic Editors:
Yiorgos Apidianakis, University of Cyprus, Cyprus
Dominique Ferrandon, Centre National de la Recherche Scientifique, France



Microbes mediate inflammation and facilitate tumor formation in genetically (and otherwise) predisposed hosts. The phenomenon is evident in humans and can be effectively studied using both invertebrate (e.g. Drosophila) and mammalian model hosts.

A link between inflammation and cancer was initially made by Rudolf Virchow back in the 19th century. Nowadays many cancers are considered dependent on inflammatory responses to microbial and damaged-self stimuli and both arms of immunity, innate and adaptive, are playing a role in promoting cancer. Moreover, besides environmental factors, opportunistic pathogens contribute to inflammation and cancer. Nevertheless, microbial influence on chronic disease is sometimes difficult to discern, especially in the context of polymicrobial communities, such as those found in the digestive tract. In this light, model organisms provide important insights into immune and growth signals that promote cancer, and suggest therapies that will selectively target potentially harmful microbes or modulate host responses. A number of review and opinion articles in this series address novel aspects and paradigms of the interactions between the microbiota and the host in relation to inflammation and cancer.

Table of Contents

frontiers in
CELLULAR AND INFECTION MICROBIOLOGY

EDITORIAL
published: 24 September 2014
doi: 10.3389/fcimb.2014.00134

Modeling hologenome imbalances in inflammation and cancer

Yiorgos Apidianakis[1]* and Dominique Ferrandon[2]

[1] *Department of Biological Sciences, University of Cyprus, Nicosia, Cyprus*
[2] *UPR9022 CNRS, University of Strasbourg Institute for Advanced Study, Strasbourg, France*
**Correspondence: apidiana@ucy.ac.cy*

Edited and reviewed by:
Yousef Abu Kwaik, University of Louisville School of Medicine, USA

Keywords: *Drosophila*, human, mouse, innate immunity, microbiota, hologenome, diet, aging

Genetics play a pivotal role in cancer. This is best exemplified in sporadic intestinal cancer development, which usually starts with mutations in APC then in Ras, p53 and TGFβ (Sears and Garrett, 2014). Nevertheless, intestinal bacteria, diet and lifestyle contribute significantly to mucosal inflammation and cancer (Anand et al., 2008; Kostic et al., 2014; Sears and Garrett, 2014). An effective approach to study the aforementioned factors may be to analyze them combinatorially. In this regard, intestinal dysbiosis is a useful concept to describe harmful changes in the constitution of the microbiota. Another imbalance occurs during inflammatory bowel disease due to an excessive immune response to the intestinal microbiota, which in turn may lead to dysbiosis and perpetuate inflammation. Suspected factors, such as immune system mutations or tissue-damaging microbial strains, may not suffice to promote inflammation and cancer in the absence of co-founding factors that create or sustain an imbalance. Thus, a broad unifying concept may describe disease as dysfunctional interactions among environmental factors, such as diet and lifestyle, microbiota composition, and the genetic of the host. Moreover, aging affects the onset of inflammation and cancer, the host microbiota and the occurrence of sporadic mutations. Accordingly, the host genetic background and that of the microbiome, define the intestinal hologenome, which is influenced by age and the environment toward homeostasis or disease. Thus, intestinal disease may ensue when the intestinal hologenome is imbalanced, that is, when a genetically predisposed or old host interacts with its dysbiotic microbiota in an inadequate or harmful dietary or lifestyle-shaped environment.

The review and opinion articles accompanying this editorial describe key aspects of modeling the hologenome with an emphasis on intestinal infection, inflammation and cancer. One major issue discussed is the adaptation of Koch's postulates in order to assess causation between the human opportunistic pathogen *Pseudomonas aeruginosa* and intestinal disease in patients with cancer (Markou and Apidianakis, 2014). While *Enterobacteriaceae* are suspected contributors to intestinal inflammation and cancer, *P. aeruginosa* exemplifies the opportunistic nature of many bacterial species toward colonization and disease. The suggested guidelines therefore provide a simple framework within which clinical associations, experimental data, and improved outcomes upon treatment against suspected bacteria need to be taken into account in order to prove causation.

Experimental data can be obtained with the various mouse models of intestinal inflammation and cancer described comprehensively by Gkouskou et al. (2014). This review article describes the contributing role of microbiota as a whole, as well as that of specific bacterial species in exacerbating the disease. Interestingly, *Enterobacteriaceae* and *Bacteroides* species contribute to disease progression in various mouse models. In addition, intestinal dysbiosis is influenced by diet, antibiotics, and an immune genetic background conducive to exacerbated adaptive and diminished innate immune response. The authors highlight the potential of targeting the dysbiosis-inflammation-tumorigenesis axis for the development of novel therapeutic strategies for IBD and colorectal cancer.

Whereas studies on bacteria dominate the literature on the role of dysbiosis in inflammation and cancer, viruses were historically the first microbes to be linked to cancer. A modern approach to this issue is described by Iacovides and colleagues who suggest that the interplay between cancer and cell stemness can be influenced by oncogenic viruses (Iacovides et al., 2013). These viruses interfere with signaling pathways that are traditionally associated with self-renewal and lineage-commitment. Thus virus-associated cancers can serve as models to understand the link between viral infection, cancer, and stemness.

Innate immune and stress responses lie at the intersection of apoptosis and cell proliferation during inflammation and cancer. In this regard the simple model organism *Caenorhabditis elegans* has provided valuable insights into the tight regulation of apoptosis during development, infection, and DNA damage (Arvanitis et al., 2013). These findings have been taken a few steps further with the use of *Drosophila* models of infection and cancer, as reviewed by Bangi (2013). This review illustrates the key role of stress, innate immunity, and inflammatory signaling pathways in promoting intestinal stem cell proliferation and tumorigenesis. Prominent among these pathways is the c-Jun-N-terminal kinase (JNK) cascade, which in an oncogenic background can be diverted from tissue damage- or infection-mediated apoptosis to tumor cell proliferation and invasion (Apidianakis et al., 2009; Cordero et al., 2010; Bangi et al., 2012). Ligoxygakis and colleagues contribute a thorough review on *Drosophila* hemocytes, describing the multifaceted roles of these innate immunity cells in development, immunosurveillance, and tumorigenesis (Wang et al., 2014). Kim and Lee explain the multiple roles of *Drosophila*

Duox, an NADPH oxidase, the homologs of which mediate bacterial killing via oxygen radicals in mammalian mucosae and phagocytes (Kim and Lee, 2014). The authors provide insights into the role of *Duox* in gut immunity, homeostasis of the intestinal epithelium, and stem cell proliferation. Complementarily, Ayyaz and Jasper put in perspective aging and three responses of *Drosophila* to intestinal microbes, namely, *Duox*, the Immune deficiency NF-κB pathway, and the renewal of intestinal enterocytes (Ayyaz and Jasper, 2013). These two reviews provide a comprehensive analysis of intestinal dysbiosis and accompanying intestinal cell renewal, which is a homeostatic arm of the intestinal host defense induced either by pathogenic or seemingly innocuous bacteria, and showcase the usefulness of *Drosophila* as a model for the study of intestinal immunity, inflammation, and disease.

Regenerative and tumor-promoting cytokines in *Drosophila* and mammals may not necessarily emanate from tissue infiltrating blood cells (Panayidou and Apidianakis, 2013; Gkouskou et al., 2014). The review by Kux and Pitsouli highlights that regeneration signals are not confined to the *Drosophila* intestinal epithelium (Kux and Pitsouli, 2014). Neighboring tissues, such as muscles, trachea and potentially the neural system communicate with intestinal epithelial cells, and thus might contribute to the intestinal stem cell niche. Accordingly, regenerative or tumor-promoting inflammatory signaling may be controlled not only by tumors and their microenvironment, but also by remote organs. Taking a far-reaching perspective, Droujinine and Perrimon provide an educated guess on the tissues that may systemically provide inflammatory and other inter-organ signals either locally or systemically (Droujinine and Perrimon, 2013). The authors foresee the existence of a vast inter-organ communication network (ICN) of peptides, proteins, and metabolites that act in-between organs to coordinate cellular processes, either under homeostatic or stress conditions. A unique strength of the *Drosophila* model is that biochemical studies can be combined to *in vivo* genome-wide organ-specific genetic screens to identify ICN components.

ACKNOWLEDGMENT

Yiorgos Apidianakis is supported by Fondation Sante and Marie Curie CIG-303586.

REFERENCES

Anand, P., Kunnumakara, A. B., Sundaram, C., Harikumar, K. B., Tharakan, S. T., Lai, O. S., et al. (2008). Cancer is a preventable disease that requires major lifestyle changes. *Pharm. Res.* 25, 2097–2116. doi: 10.1007/s11095-008-9661-9

Apidianakis, Y., Pitsouli, C., Perrimon, N., and Rahme, L. (2009). Synergy between bacterial infection and genetic predisposition in intestinal dysplasia. *Proc. Natl. Acad. Sci. U.S.A.* 106, 20883–20888. doi: 10.1073/pnas.0911797106

Arvanitis, M., Li, D. D., Lee, K., and Mylonakis, E. (2013). Apoptosis in *C. elegans*: lessons for cancer and immunity. *Front. Cell. Infect. Microbiol.* 3:67. doi: 10.3389/fcimb.2013.00067

Ayyaz, A., and Jasper, H. (2013). Intestinal inflammation and stem cell homeostasis in aging *Drosophila melanogaster*. *Front. Cell. Infect. Microbiol.* 3:98. doi: 10.3389/fcimb.2013.00098

Bangi, E. (2013). Drosophila at the intersection of infection, inflammation, and cancer. *Front. Cell. Infect. Microbiol.* 3:103. doi: 10.3389/fcimb.2013.00103

Bangi, E., Pitsouli, C., Rahme, L. G., Cagan, R., and Apidianakis, Y. (2012). Immune response to bacteria induces dissemination of Ras-activated Drosophila hindgut cells. *EMBO Rep.* 13, 569–576. doi: 10.1038/embor.2012.44

Cordero, J. B., Macagno, J. P., Stefanatos, R. K., Strathdee, K. E., Cagan, R. L., and Vidal, M. (2010). Oncogenic Ras diverts a host TNF tumor suppressor activity into tumor promoter. *Dev. Cell* 18, 999–1011. doi: 10.1016/j.devcel.2010.05.014

Droujinine, I. A., and Perrimon, N. (2013). Defining the interorgan communication network: systemic coordination of organismal cellular processes under homeostasis and localized stress. *Front. Cell. Infect. Microbiol.* 3:82. doi: 10.3389/fcimb.2013.00082

Gkouskou, K. K., Deligianni, C., Tsatsanis, C., and Eliopoulos, A. G. (2014). The gut microbiota in mouse models of inflammatory bowel disease. *Front. Cell. Infect. Microbiol.* 4:28. doi: 10.3389/fcimb.2014.00028

Iacovides, D., Michael, S., Achilleos, C., and Strati, K. (2013). Shared mechanisms in stemness and carcinogenesis: lessons from oncogenic viruses. *Front. Cell. Infect. Microbiol.* 3:66. doi: 10.3389/fcimb.2013.00066

Kim, S. H., and Lee, W. J. (2014). Role of DUOX in gut inflammation: lessons from Drosophila model of gut-microbiota interactions. *Front. Cell. Infect. Microbiol.* 3:116. doi: 10.3389/fcimb.2013.00116

Kostic, A. D., Xavier, R. J., and Gevers, D. (2014). The microbiome in inflammatory bowel disease: current status and the future ahead. *Gastroenterology* 146, 1489–1499. doi: 10.1053/j.gastro.2014.02.009

Kux, K., and Pitsouli, C. (2014). Tissue communication in regenerative inflammatory signaling: lessons from the fly gut. *Front. Cell. Infect. Microbiol.* 4:49. doi: 10.3389/fcimb.2014.00049

Markou, P., and Apidianakis, Y. (2014). Pathogenesis of intestinal *Pseudomonas aeruginosa* infection in patients with cancer. *Front. Cell. Infect. Microbiol.* 3:115. doi: 10.3389/fcimb.2013.00115

Panayidou, S., and Apidianakis, Y. (2013). Regenerative inflammation: lessons from Drosophila intestinal epithelium in health and disease. *Pathogens* 2, 209–231. doi: 10.3390/pathogens2020209

Sears, C. L., and Garrett, W. S. (2014). Microbes, microbiota, and colon cancer. *Cell Host Microbe* 15, 317–328. doi: 10.1016/j.chom.2014.02.007

Wang, L., Kounatidis, I., and Ligoxygakis, P. (2014). Drosophila as a model to study the role of blood cells in inflammation, innate immunity and cancer. *Front. Cell. Infect. Microbiol.* 3:113. doi: 10.3389/fcimb.2013.00113

Conflict of Interest Statement: The authors declare that the study was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 03 September 2014; accepted: 05 September 2014; published online: 24 September 2014.

*Citation: Apidianakis Y and Ferrandon D (2014) Modeling hologenome imbalances in inflammation and cancer. Front. Cell. Infect. Microbiol. **4**:134. doi: 10.3389/fcimb.2014.00134*

This article was submitted to the journal Frontiers in Cellular and Infection Microbiology.

frontiers in
CELLULAR AND INFECTION MICROBIOLOGY

OPINION ARTICLE
published: 07 January 2014
doi: 10.3389/fcimb.2013.00115

Pathogenesis of intestinal *Pseudomonas aeruginosa* infection in patients with cancer

Panayiota Markou and Yiorgos Apidianakis*

Department of Biological Sciences, University of Cyprus, Nicosia, Cyprus
**Correspondence: apidiana@ucy.ac.cy*

Edited by:
Dominique Ferrandon, Centre National de la Recherche Scientifique, France

Reviewed by:
John C. Alverdy, University of Chicago, USA

Keywords: inflammation, tumour, *Drosophila*, human, gut, epithelial damage, regeneration

In 1882, Robert Koch suggested four postulates that establish causation between an infectious agent and a particular disease: (1) the infectious organism must be found in abundance in all diseased, but not in healthy, organisms, (2) the infectious organism must be isolated from the diseased host and grown in culture, (3) the disease must be reproduced when the cultured organism is introduced into a healthy organism and (4) the same organism must be reisolated from the experimentally diseased host (Tabrah, 2011; Breitschwerdt et al., 2013). In **Figure 1** we suggest an adaptation to the original postulates of Koch to be used as a framework to assess the causation between intestinal *Pseudomonas aeruginosa* and intestinal disease in patients with cancer. In the following sections we describe the prevalence of *P. aeruginosa* in cancer and the immunosuppressive and stress-inducing conditions of cancer that facilitate the growth, dissemination and virulence of intestinal *P. aeruginosa*. In addition, we describe work showing that *P. aeruginosa* promotes intestinal epithelium cancer-related phenotypes when introduced in tumor prone model hosts.

CANCER AND OTHER IMMUNOSUPPRESSIVE CONDITIONS PROMOTE THE PREVALENCE OF *P. aeruginosa*

Bacteremia is a major cause of life-threatening complications in patients with cancer, especially those who receive anticancer chemotherapy. Cancer patients are more vulnerable to invasive infection, due to ulcerative lesions in mucosal surfaces and immune suppression secondary to chemotherapy (Safdar and Armstrong, 2001). Many studies associate bloodstream infections in cancer patients with Gram-negative bacteria (Oliveira et al., 2007; Bos et al., 2013; Montassier et al., 2013).

P. aeruginosa is a Gram-negative opportunistic bacterium that causes various infections. Common community-acquired infections with *P. aeruginosa* are skin and soft tissue infections, ulcerative keratitis and otitis externa, while hospital-acquired infections include bloodstream infections, pneumonias and urinary tract infections (Driscoll et al., 2007). Infections may be associated with a high rate of morbidity and mortality in immunocompromised hosts, such as those suffering from chemotherapy-induced neutropenia, patients with cystic fibrosis or severe burns and individuals who receive intensive care (Driscoll et al., 2007; Kerr and Snelling, 2009; Worth and Slavin, 2009; Stuart et al., 2010; Rafla and Tredget, 2011).

P. aeruginosa intestinal carriage increases from ∼3% in normal people to ∼20% in hospitalized patients (Stoodley and Thom, 1970). In a case-control study the intestinal colonization by *P. aeruginosa* in cancer patients was 10% before and 31% after hospitalization (Andremont et al., 1989). Studies conducted in oncology–hematology units, found an overall intestinal carriage of *P. aeruginosa* between 11.7 and 37% (Thuong et al., 2003). In another case-control study *P. aeruginosa* intestinal colonization was identified in 17% of controls and 60% of blood cancer patients (Vuotto et al., 2013). These epidemiology data suggest that intestinal colonization by *P. aeruginosa* is prominent among hospitalized cancer patients (Andremont et al., 1989; Vuotto et al., 2013).

The intestinal carriage of *P. aeruginosa* is likely a consequence of the opportunistic nature of this species. Most *P. aeruginosa* infections appear secondary to a breach in host defences. In addition to compromised host immunity, intestinal microbiota play a major role in intestinal defence to infection (Levison, 1973). Thus systemic exposure to antibiotics, which alters intestinal microbiota by reducing the abundance of certain microbes creates the opportunity for intestinal growth of *P. aeruginosa* and other pathogenic bacteria (Hentges et al., 1985).

INTESTINAL *P. aeruginosa* AS A SOURCE OF SYSTEMIC AND REMOTE INFECTIONS

The translocation of endogenous intestinal *P. aeruginosa* extraluminally is an important pathogenic phenomenon and a cause of systemic infections, especially in neutropenic patients with hematological malignancies (Okuda et al., 2010). During the translocation process, bacteria and their products cross the intestinal barrier by traveling between or through the cells of the intestinal epithelium, causing infection and massive inflammation (Alexander et al., 1990; Papoff et al., 2012). Lung infections caused by *P. aeruginosa* are frequent in patients and can occur by direct contamination of the lungs by gastrointestinal flora or through hematogenous spread from the intestine to the lungs. Sepsis and mortality in immunocompromised patients are the results of the presence of highly virulent strains of *P. aeruginosa* within the intestinal tract and the pathogen's ability to adhere to the

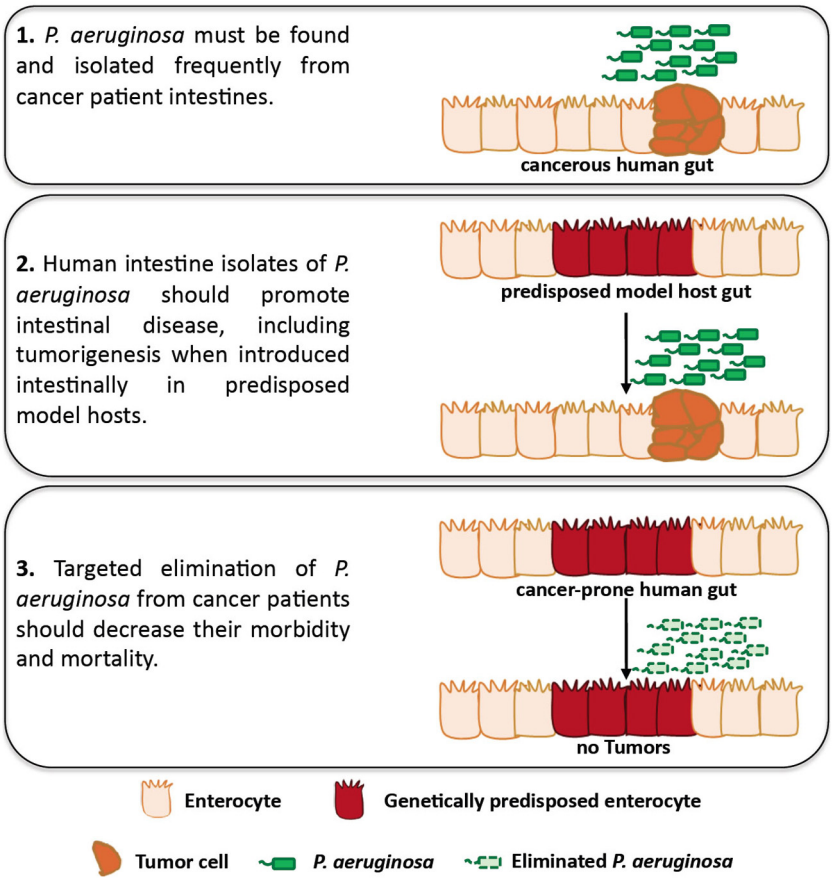


FIGURE 1 | An adaptation of Koch's postulates assessing causation between intestinal *P. aeruginosa* and disease in patients with cancer. Studies using *Drosophila* and mammalian hosts may assess the role of *P. aeruginosa* in facilitating intestinal disease, including intestinal *P. aeruginosa* growth, dissemination, virulence and tumorigenesis, in predisposed hosts. In addition, clinical studies can be designed to assess the presence of *P. aeruginosa* in cancer patients and its role in intestinal disease, including tumorigenesis for which clinical data are lacking.

intestinal epithelial barrier (Marshall et al., 1993; Alverdy et al., 2000; Osmon et al., 2004; Zaborina et al., 2006; Okuda et al., 2010).

P. aeruginosa uses different virulence factors that can damage epithelial cells, such as enzymes (proteases and elastases), toxins, adhesins, flagella and protein secretion systems (Sundin et al., 2004). The T3SS enables the injection of at least four effector proteins (ExoS, ExoT, ExoU, and ExoY) into the host cell. ExoS injected into the host epithelial cell migrates to the membrane where it binds to the mammalian factor FXYD3, expressed specifically in the colon and stomach (Okuda et al., 2010). Thus, ExoS may assist the penetration of *P. aeruginosa* through the intestinal epithelial barrier, impairing the defence function of tight junctions against bacterial penetration (Okuda et al., 2010). Moreover, gut inflammation and apoptosis–which can be initiated by the *Pseudomonas* quinolone signal (PQS)–lead to tight junction disruption and an increase of epithelial barrier permeability (Alverdy et al., 2005; An, 2008). Similarly, *P. aeruginosa* lectin PA-I, which is associated with adhesion to epithelial cell layer, is produced after intestinal ischemia and secreted into the intestinal lumen, causing tight junction interruption, epithelial barrier dysfunction and increase of its permeability (Seal et al., 2011).

INTESTINAL *P. aeruginosa* EXHIBITS ENHANCED VIRULENCE UPON STRESS, SURGERY, TRAUMA, AND MAYBE CANCER

Cohort studies show that infections are more frequent, severe and lethal among surgical patients (Craven et al., 1988; Sax et al., 2011). Surgical injury can shift the dynamics of the host-pathogen interaction leading to phenotype transformation or phase variation that develops as microbes adapt and respond to novel environments, causing morbidity and mortality (Babrowski et al., 2013). *P. aeruginosa* escalates its virulence and promotes systemic inflammation during various conditions of host stress (Seal et al., 2011). In patients colonized by *P. aeruginosa*, the prolonged surgical injury releases stress-related host factors that can trigger the otherwise dormant colonizers, making them invasive and lethal (Babrowski et al., 2013). Defence mechanisms such as degradative proteases and lipases, exopolysaccharide capsule and outer membrane-derived vesicles (OMVs), which serve as a secretion mechanism for virulence factors,

help the pathogen to survive in the host environment (Macdonald and Kuehn, 2013). OMVs are induced in response to physiological stressors and secreted during infection serving multiple roles in bacterial pathogenesis (Macdonald and Kuehn, 2013). In surgically stressed hosts interferon-gamma, endogenous opioids and the hypoxic end-products adenosine and inosine are released into the intestinal lumen where they bind bacteria and activate the expression of PA-I lectin and other virulence factors of *P. aeruginosa*. The PA-I lectin alters the tight junction permeability of the intestinal epithelium to exotoxin A, leading to lethal gut derived sepsis (Long et al., 2008). Moreover, local intestinal depletion of extracellular phosphate (hypophosphatemia), which occurs after surgical injury, can activate virulent pathways due to bacterial sensing of low phosphate, shifting the phenotype of *P. aeruginosa* to that of a lethal strain (Long et al., 2008). Because interferon-gamma, opioids and hypoxia are part of the host response and the therapeutic regiments administered to cancer patients (Dunn et al., 2005; Vaupel and Mayer, 2007), the conditions that accompany cancer may also provide the signals for *P. aeruginosa* virulence induction.

CAN *P. aeruginosa* SIMILARLY TO OTHER GASTROINTESTINAL BACTERIA FACILITATE CANCER?

Bacteria may initiate oncogenesis because they can induce inflammation and produce cell damaging toxins that facilitate tumorigenesis (Collins et al., 2011; Tjalsma et al., 2012). The characteristic single polar flagella and type 4 pili of *P. aeruginosa* function as initiators of inflammation and adhesins, respectively (Gellatly and Hancock, 2013). *P. aeruginosa* induces Toll-like receptors to activate cytokines, chemokines and COX-2 and recruit cells of the innate and adaptive immune system (Hussain et al., 2003; Holt et al., 2008; de Lima et al., 2012). Epithelial adherence is a property of various bacteria associated with gastrointestinal disease and cancer, such as *Bacteroides fragilis, Streptococcus bovis, Escherichia coli* and *Helicobacter pylori* (Toprak et al., 2006; Selgrad et al., 2008; Abdulamir et al., 2011; Arthur et al., 2012). Cell wall antigens of *S. bovis* induce overexpression of COX-2 and NF-κB *in vitro*, which promote cellular proliferation and angiogenesis (Tafe and Ruoff, 2007; Abdulamir et al., 2009).

E-cadherin, a cell adhesion molecule serves as an antagonist of invasion and metastasis and is found mutated in human carcinomas (Cavallaro and Christofori, 2004; Berx and van Roy, 2009). *B. fragilis* secreted factor BFT cleaves E-cadherin, which is usually bound inside the plasma membrane to β-catenin. The cleavage releases catenin in the cytosol leading to the transcription of the oncogene *c-myc* (Hardy et al., 2000). Similarly, *P. aeruginosa* secreted factor LasI can disrupt adherens junctions and reduce the expression and distribution of E-cadherin and β-catenin in the cell membrane, resulting in changes in cell junction associations and enhanced paracellular permeability (Vikström et al., 2009).

Interestingly, intestinal innate immune responses and stem cells may drive tumor initiation, maintenance and metastasis (Schwitalla et al., 2013). Cancer development is assisted by apoptotic programmed cell death in the tumor microenvironment (Evan and Littlewood, 1998; Lowe et al., 2004; Adams and Cory, 2007) and *P. aeruginosa* uses many virulence factors that induce epithelial cell apoptosis. Intestinal infection with *P. aeruginosa* in *Drosophila* activates the c-Jun N-terminal kinase (JNK) pathway, which causes apoptosis of enterocytes and leads to proliferation of intestinal stem cells (Apidianakis et al., 2009). Importantly, genetic predisposition via an oncogenic form of Ras1/K-Ras oncogene, can synergize with inflammatory signals to induce stem cell-originating tumors characterized by alterations in cell polarity and tissue architecture. Moreover, sustained intestinal infection with *P. aeruginosa* in *Drosophila* induces the Imd/NF-κB pathway, which synergizes with the oncogene $Ras1^{V12}$ to activate the JNK pathway. This leads to invasion and dissemination of oncogenic hindgut cells to distant sites (Bangi et al., 2012; Christofi and Apidianakis, 2013).

CONCLUSIONS

P. aeruginosa is a common colonizer of the human intestine upon hospitalization, immunosuppression, antibiotic treatment, surgery, severe trauma and other conditions that cancer patients may face. Not only is *P. aeruginosa* carriage increased in the aforementioned conditions, but also bacteria become more virulent and damaging to the intestinal epithelium upon surgery, injury, and severe stress. Moreover, human isolates of *P. aeruginosa* can induce intestinal pathology and cancer-related epithelial phenotypes in genetically predisposed model hosts. Thus, *P. aeruginosa* appears to have the opportunity and the ability to promote intestinal disease in predisposed hosts, although further proof on the ability of this bacterium to promote tumorigenesis in mammalian models of infection is needed. The lack of epidemiological data linking *P. aeruginosa* to intestinal disease and potentially tumorigenesis in cancer patients may reflect the lack of clinical studies assessing bacterial growth and virulence in relation to cancer recurrence. Because the titter, distribution and virulence of *P. aeruginosa* in the intestine may be very dynamic (Tjalsma et al., 2012), future studies should be designed to repeatedly assess intestinal *P. aeruginosa* abundance and virulence in cancer patients versus healthy individuals. Clinical samples can be assessed for the presence of *P. aeruginosa* via classical microbiology, and next-generation sequencing may offer the chance to assess *P. aeruginosa* transcriptome during infection. Importantly, definite proof of causation of *P. aeruginosa* in morbidity and mortality of cancer patients can only be achieved if targeted elimination of *P. aeruginosa* from these patients improves the outcome of their disease. In **Figure 1** we illustrate a roadmap to specifically assess the role of *P. aeruginosa* in intestinal disease and tumorigenesis. It is conceivable that similar principles can be used to assess causality between intestinal disease and many other opportunistic pathogens harbored by the human gut.

REFERENCES

Abdulamir, A. S., Hafidh, R. R., and Abu Bakar, F. (2011). The association of streptococcus bovis/gallolyticus with colorectal tumors: the nature and the underlying mechanisms of its etiological role. *J. Exp. Clin. Cancer Res.* 30, 11. doi: 10.1186/1756-9966-30-11

Abdulamir, A. S., Hafidh, R. R., Mahdi, L. K., Al-jeboori, T., and Abubaker, F. (2009). Investigation into the controversial association of streptococcus gallolyticus with colorectal

cancer and adenoma. *BMC Cancer* 9:403. doi: 10.1186/1471-2407-9-403

Adams, J. M., and Cory, S. (2007). The bcl-2 apoptotic switch in cancer development and therapy. *Oncogene* 26, 1324–1337. doi: 10.1038/sj.onc.1210220

Alexander, J. W., Boyce, S. T., Babcock, G. F., Gianotti, L., Peck, M. D., Dunn, D. L., et al. (1990). The process of microbial translocation. *Ann. Surg.* 212, 496–510. discussion: 511–512. doi: 10.1097/00000658-199010000-00012

Alverdy, J., Holbrook, C., Rocha, F., Seiden, L., Wu, R. L., Musch, M., et al. (2000). Gut-derived sepsis occurs when the right pathogen with the right virulence genes meets the right host: evidence for *in vivo* virulence expression in *Pseudomonas aeruginosa*. *Ann. Surg.* 232, 480–489. doi: 10.1097/00000658-200010000-00003

Alverdy, J., Zaborina, O., and Wu, L. (2005). The impact of stress and nutrition on bacterial-host interactions at the intestinal epithelial surface. *Curr. Opin. Clin. Nutr. Metab. Care* 8, 205–209. doi: 10.1097/00075197-200503000-00016

An, G. (2008). Introduction of an agent-based multi-scale modular architecture for dynamic knowledge representation of acute inflammation. *Theor. Biol. Med. Model.* 5, 11. doi: 10.1186/1742-4682-5-11

Andremont, A., Marang, B., Tancrede, C., Baume, D., and Hill, C. (1989). Antibiotic treatment and intestinal colonization by *Pseudomonas aeruginosa* in cancer patients. *Antimicrob. Agents Chemother.* 33, 1400–1402. doi: 10.1128/AAC.33.8.1400

Apidianakis, Y., Pitsouli, C., Perrimon, N., and Rahme, L. (2009). Synergy between bacterial infection and genetic predisposition in intestinal dysplasia. *Proc. Natl. Acad. Sci. U.S.A.* 106, 20883–20888. doi: 10.1073/pnas.0911797106

Arthur, J. C., Perez-Chanona, E., Mühlbauer, M., Tomkovich, S., Uronis, J. M., Fan, T. J., et al. (2012). Intestinal inflammation targets cancer-inducing activity of the microbiota. *Science* 338, 120–123. doi: 10.1126/science.1224820

Babrowski, T., Romanowski, K., Fink, D., Kim, M., Gopalakrishnan, V., Zaborina, O., et al. (2013). The intestinal environment of surgical injury transforms *Pseudomonas aeruginosa* into a discrete hypervirulent morphotype capable of causing lethal peritonitis. *Surgery* 153, 36–43. doi: 10.1016/j.surg.2012.06.022

Bangi, E., Pitsouli, C., Rahme, L. G., Cagan, R., and Apidianakis, Y. (2012). Immune response to bacteria induces dissemination of ras-activated drosophila hindgut cells. *EMBO Rep.* 13, 569–576. doi: 10.1038/embor.2012.44

Berx, G., and van Roy, F. (2009). Involvement of members of the cadherin superfamily in cancer. *Cold Spring Harb. Perspect. Biol.* 1, a003129. doi: 10.1101/cshperspect.a003129

Bos, M. M., Smeets, L. S., Dumay, I., and de Jonge, E. (2013). Bloodstream infections in patients with or without cancer in a large community hospital. *Infection* 41, 949–988. doi: 10.1007/s15010-013-0468-1

Breitschwerdt, E. B., Linder, K. L., Day, M. J., Maggi, R. G., Chomel, B. B., and Kempf, V. A. (2013). Koch's postulates and the pathogenesis of comparative infectious disease causation associated with bartonella species. *J. Comp. Pathol.* 148, 115–125. doi: 10.1016/j.jcpa.2012.12.003

Cavallaro, U., and Christofori, G. (2004). Cell adhesion and signalling by cadherins and ig-CAMs in cancer. *Nat. Rev. Cancer* 4, 118–132. doi: 10.1038/nrc1276

Christofi, T., and Apidianakis, Y. (2013). Ras-oncogenic drosophila hindgut but not midgut cells use an inflammation-like program to disseminate to distant sites. *Gut Microbes* 4, 54–59. doi: 10.4161/gmic.22429

Collins, D., Hogan, A. M., and Winter, D. C. (2011). Microbial and viral pathogens in colorectal cancer. *Lancet Oncol.* 12, 504–512. doi: 10.1016/S1470-2045(10)70186-8

Craven, D. E., Kunches, L. M., Lichtenberg, D. A., Kollisch, N. R., Barry, M. A., Heeren, T. C., et al. (1988). Nosocomial infection and fatality in medical and surgical intensive care unit patients. *Arch. Intern. Med.* 148, 1161–1168. doi: 10.1001/archinte.1988.00380050165024

de Lima, C. D. M., Calegari-Silva, T. C., Pereira, R. M. S., de Oliveira Lima Santos, S. A., Lopes, U. G., Plotkowski, M. C. M., et al. (2012). ExoU activates NF-κB and increases IL-8/KC secretion during *Pseudomonas aeruginosa* infection. *PLoS ONE* 7:e41772. doi: 10.1371/journal.pone.0041772

Driscoll, J. A., Brody, S. L., and Kollef, M. H. (2007). The epidemiology, pathogenesis and treatment of *Pseudomonas aeruginosa* infections. *Drugs* 67, 351–368. doi: 10.2165/00003495-200767030-00003

Dunn, G. P., Ikeda, H., Bruce, A. T., Koebel, C., Uppaluri, R., Bui, J., et al. (2005). Interferon-gamma and cancer immunoediting. *Immunol. Res.* 32, 231–245. doi: 10.1385/IR:32:1-3:231

Evan, G., and Littlewood, T. (1998). A matter of life and cell death. *Science* 281, 1317–1322. doi: 10.1126/science.281.5381.1317

Gellatly, S. L., and Hancock, R. E. (2013). *Pseudomonas aeruginosa*: new insights into pathogenesis and host defenses. *Pathog. Dis.* 67, 159–173. doi: 10.1111/2049-632X.12033

Hardy, R. G., Meltzer, S. J., and Jankowski, J. A. (2000). ABC of colorectal cancer. Molecular basis for risk factors. *BMJ* 321, 886–889. doi: 10.1136/bmj.321.7265.886

Hentges, D. J., Stein, A. J., Casey, S. W., and Que, J. U. (1985). Protective role of intestinal flora against infection with *Pseudomonas aeruginosa* in mice: influence of antibiotics on colonization resistance. *Infect. Immun.* 47, 118–122.

Holt, P. G., Strickland, D. H., Wikstrom, M. E., and Jahnsen, F. L. (2008). Regulation of immunological homeostasis in the respiratory tract. *Nat. Rev. Immunol.* 8, 142–152. doi: 10.1038/nri2236

Hussain, S. P., Hofseth, L. J., and Harris, C. C. (2003). Radical causes of cancer. *Nat. Rev. Cancer* 3, 276–285. doi: 10.1038/nrc1046

Kerr, K. G., and Snelling, A. M. (2009). *Pseudomonas aeruginosa*: a formidable and ever-present adversary. *J. Hosp. Infect.* 73, 338–344. doi: 10.1016/j.jhin.2009.04.020

Levison, M. E. (1973). Effect of colon flora and short-chain fatty acids on growth *in vitro* of *Pseudomonas aeruginosa* and enterobacteriaceae. *Infect. Immun.* 8, 30–35.

Long, J., Zaborina, O., Holbrook, C., Zaborin, A., and Alverdy, J. (2008). Depletion of intestinal phosphate after operative injury activates the virulence of *P. aeruginosa* causing lethal gut-derived sepsis. *Surgery* 144, 189–197. doi: 10.1016/j.surg.2008.03.045

Lowe, S. W., Cepero, E., and Evan, G. (2004). Intrinsic tumour suppression. *Nature* 432, 307–315. doi: 10.1038/nature03098

Macdonald, I. A., and Kuehn, M. J. (2013). Stress-induced outer membrane vesicle production by *Pseudomonas aeruginosa*. *J. Bacteriol.* 195, 2971–2981. doi: 10.1128/JB.02267-12

Marshall, J. C., Christou, N. V., and Meakins, J. L. (1993). The gastrointestinal tract. The "undrained abscess" of multiple organ failure. *Ann. Surg.* 218, 111–119. doi: 10.1097/00000658-199308000-00001

Montassier, E., Batard, E., Gastinne, T., Potel, G., and de La Cochetiere, M. F. (2013). Recent changes in bacteremia in patients with cancer: a systematic review of epidemiology and antibiotic resistance. *Eur. J. Clin. Microbiol. Infect. Dis.* 32, 841–850. doi: 10.1007/s10096-013-1819-7

Okuda, J., Hayashi, N., Okamoto, M., Sawada, S., Minagawa, S., Yano, Y., et al. (2010). Translocation of *Pseudomonas aeruginosa* from the intestinal tract is mediated by the binding of ExoS to an na, K-ATPase regulator, FXYD3. *Infect. Immun.* 78, 4511–4522. doi: 10.1128/IAI.00428-10

Oliveira, A. L., de Souza, M., Carvalho-Dias, V. M., Ruiz, M. A., Silla, L., Tanaka, P. Y., et al. (2007). Epidemiology of bacteremia and factors associated with multi-drug-resistant gram-negative bacteremia in hematopoietic stem cell transplant recipients. *Bone Marrow Transplant.* 39, 775–781. doi: 10.1038/sj.bmt.1705677

Osmon, S., Ward, S., Fraser, V. J., and Kollef, M. H. (2004). Hospital mortality for patients with bacteremia due to staphylococcus aureus or *Pseudomonas aeruginosa*. *Chest* 125, 607–616. doi: 10.1378/chest.125.2.607

Papoff, P., Ceccarelli, G., d'Ettorre, G., Cerasaro, C., Caresta, E., Midulla, F., et al. (2012). Gut microbial translocation in critically ill children and effects of supplementation with pre- and pro biotics. *Int. J. Microbiol.* 2012, 151393. doi: 10.1155/2012/151393

Rafla, K., and Tredget, E. E. (2011). Infection control in the burn unit. *Burns* 37, 5–15. doi: 10.1016/j.burns.2009.06.198

Safdar, A., and Armstrong, D. (2001). Infectious morbidity in critically ill patients with cancer. *Crit. Care Clin.* 17, 531–570, vii–viii. doi: 10.1016/S0749-0704(05)70198-6

Sax, H., Uckay, I., Balmelli, C., Bernasconi, E., Boubaker, K., Muhlemann, K., et al. (2011). Overall burden of healthcare-associated infections among surgical patients. Results of a national study. *Ann. Surg.* 253, 365–370. doi: 10.1097/SLA.0b013e318202fda9

Schwitalla, S., Fingerle, A. A., Cammareri, P., Nebelsiek, T., Göktuna, S. I., Ziegler, P. K., et al. (2013). Intestinal tumorigenesis initiated by dedifferentiation and acquisition of stem-cell-like properties. *Cell* 152, 25–38. doi: 10.1016/j.cell.2012.12.012

Seal, J. B., Alverdy, J. C., Zaborina, O., and An, G. (2011). Agent-based dynamic knowledge representation of *Pseudomonas aeruginosa* virulence activation in the stressed gut: towards characterizing host-pathogen interactions in gut-derived sepsis. *Theor. Biol. Med. Model.* 8, 33. doi: 10.1186/1742-4682-8-33

Selgrad, M., Malfertheiner, P., Fini, L., Goel, A., Boland, C. R., and Ricciardiello, L. (2008). The role of viral and bacterial pathogens in gastrointestinal cancer. *J. Cell. Physiol.* 216, 378–388. doi: 10.1002/jcp.21427

Stoodley, B. J., and Thom, B. T. (1970). Observations on the intestinal carriage of *Pseudomonas aeruginosa*. *J. Med. Microbiol.* 3, 367–375. doi: 10.1099/00222615-3-3-367

Stuart, B., Lin, J. H., and Mogayzel, P. J. Jr. (2010). Early eradication of *Pseudomonas aeruginosa* in patients with cystic fibrosis. *Paediatr. Respir. Rev.* 11, 177–184. doi: 10.1016/j.prrv.2010.05.003

Sundin, C., Thelaus, J., Broms, J. E., and Forsberg, A. (2004). Polarisation of type III translocation by *Pseudomonas aeruginosa* requires PcrG, PcrV and PopN. *Microb. Pathog.* 37, 313–322. doi: 10.1016/j.micpath.2004.10.005

Tabrah, F. L. (2011). Koch's postulates, carnivorous cows, and tuberculosis today. *Hawaii Med. J.* 70, 144–148.

Tafe, L. J., and Ruoff, K. L. (2007). *Streptococcus bovis:* answers and questions. *Clin. Microbiol. Newsl.* 29, 49–55. doi: 10.1016/j.clinmicnews.2007.03.004

Thuong, M., Arvaniti, K., Ruimy, R., de la Salmoniere, P., Scanvic-Hameg, A., Lucet, J. C., et al. (2003). Epidemiology of *Pseudomonas aeruginosa* and risk factors for carriage acquisition in an intensive care unit. *J. Hosp. Infect.* 53, 274–282. doi: 10.1053/jhin.2002.1370

Tjalsma, H., Boleij, A., Marchesi, J. R., and Dutilh, B. E. (2012). A bacterial driver – passenger model for colorectal cancer: beyond the usual suspects. *Nat. Rev. Microbiol.* 10, 575–582. doi: 10.1038/nrmicro2819

Toprak, N. U., Yagci, A., Gulluoglu, B. M., Akin, M. L., Demirkalem, P., Celenk, T., et al. (2006). A possible role of bacteroides fragilis enterotoxin in the aetiology of colorectal cancer. *Clin. Microbiol. Infect.* 12, 782–786. doi: 10.1111/j.1469-0691.2006.01494.x

Vaupel, P., and Mayer, A. (2007). Hypoxia in cancer: significance and impact on clinical outcome. *Cancer Metastasis Rev.* 26, 225–239. doi: 10.1007/s10555-007-9055-1

Vikström, E., Bui, L., Konradsson, P., and Magnusson, K. E. (2009). The junctional integrity of epithelial cells is modulated by *Pseudomonas aeruginosa* quorum sensing molecule through phosphorylation-dependent mechanisms. *Exp. Cell Res.* 315, 313–326. doi: 10.1016/j.yexcr.2008.10.044

Vuotto, F., Berthon, C., Lemaitre, N., Duhamel, A., Balkaran, S., Le Ray, E., et al. (2013). Risk factors, clinical features, and outcome of *Pseudomonas aeruginosa* bacteremia in patients with hematologic malignancies: a case-control study. *Am. J. Infect. Control* 41, 527–530. doi: 10.1016/j.ajic.2012.07.012

Worth, L. J., and Slavin, M. A. (2009). Bloodstream infections in haematology: risks and new challenges for prevention. *Blood Rev.* 23, 113–122. doi: 10.1016/j.blre.2008.10.003

Zaborina, O., Kohler, J. E., Wang, Y., Bethel, C., Shevchenko, O., Wu, L., et al. (2006). Identification of multi-drug resistant *Pseudomonas aeruginosa* clinical isolates that are highly disruptive to the intestinal epithelial barrier. *Ann. Clin. Microbiol. Antimicrob.* 5, 14. doi: 10.1186/1476-0711-5-14

Received: 26 September 2013; accepted: 22 December 2013; published online: 07 January 2014.

Citation: Markou P and Apidianakis Y (2014) Pathogenesis of intestinal Pseudomonas aeruginosa infection in patients with cancer. Front. Cell. Infect. Microbiol. ***3****:115. doi: 10.3389/fcimb.2013.00115*

This article was submitted to the journal Frontiers in Cellular and Infection Microbiology.

frontiers in
CELLULAR AND INFECTION MICROBIOLOGY

REVIEW ARTICLE
published: 28 February 2014
doi: 10.3389/fcimb.2014.00028

The gut microbiota in mouse models of inflammatory bowel disease

Kalliopi K. Gkouskou[1,2†], Chrysoula Deligianni[3†], Christos Tsatsanis[3] and Aristides G. Eliopoulos[1,2,4*]

[1] Molecular and Cellular Biology Laboratory, Division of Basic Sciences, University of Crete Medical School, Heraklion, Greece
[2] Laboratory of Translational Medicine and Experimental Therapeutics, University of Crete Medical School, Heraklion, Greece
[3] Department of Clinical Chemistry, University of Crete Medical School, Heraklion, Greece
[4] Laboratory of Cancer Biology, Institute of Molecular Biology and Biotechnology–FORTH, Heraklion, Greece

Edited by:
Yiorgos Apidianakis, University of Cyprus, Cyprus

Reviewed by:
Triantafyllos Chavakis, Technische Univerasität Dresden, Germany
Christos Polytarchou, University of California Los Angeles, USA

****Correspondence:***
Aristides G. Eliopoulos, University of Crete Medical School, GR-71003 Heraklion, Crete, Greece
e-mail: eliopag@med.uoc.gr
† These authors have contributed equally to this work.

The intestine and the intestinal immune system have evolved through a symbiotic homeostasis under which a highly diverse microbial flora is maintained in the gastrointestinal tract while pathogenic bacteria are recognized and eliminated. Disruption of the balance between the immune system and the gut microbiota results in the development of multiple pathologies in humans. Inflammatory bowel diseases (IBD) have been associated with alterations in the composition of intestinal flora but whether these changes are causal or result of inflammation is still under dispute. Various chemical and genetic models of IBD have been developed and utilized to elucidate the complex relationship between intestinal epithelium, immune system and the gut microbiota. In this review we describe some of the most commonly used mouse models of colitis and Crohn's disease (CD) and summarize the current knowledge of how changes in microbiota composition may affect intestinal disease pathogenesis. The pursuit of gut-microbiota interactions will no doubt continue to provide invaluable insight into the complex biology of IBD.

Keywords: microbiota, colitis, mouse models, IBD, Crohn's disease

INTRODUCTION

The lower gastrointestinal tract of healthy adult humans contains more than 100 trillion bacteria (Ley et al., 2008), termed the gut "microbiota," which are involved in complex interactions with host mucosal epithelial and immune cells and shape fundamental physiological processes such as digestion, energy homeostasis, and development of gut-associated lymphoid tissues (Bakhtiar et al., 2013). Surface antigens and metabolic end-products of gut microbiota modulate the activation of resident immune cells and the production of cytokines which protect against potential pathogens (Cario, 2013). However, this homeostatic relationship is perturbed in inflammatory bowel diseases (IBD), a group of chronic relapsing and remitting disorders of the gastrointestinal tract manifesting as Crohn's disease (CD) or ulcerative colitis (UC). UC usually affects only the rectum and shows continuous inflammation, whereas CD may occur anywhere along the gastrointestinal tract and is characterized by discontinuous lesions in the intestinal wall.

One of the most important and devastating complications of the long-standing inflammation in IBD is colorectal cancer development. The first case of UC-associated carcinoma of the intestine was reported by Crohn and Rosenberg (1925), and CD was connected to cancer in 1945 (Warren and Sommers, 1948). Subsequent studies confirmed that patients with IBD, especially UC, have increased risk for developing colorectal cancer and this risk increases further with the severity of inflammation (reviewed in Danese and Mantovani, 2010; Rubin et al., 2012).

The realization of the intimate relationship between the microbiota and intestinal homeostasis has spurred large collaborative efforts aiming to identify and characterize the microorganisms which associate with health and disease in humans. The European MetaHIT [Metagenomics of the Human Intestinal Tract, (Qin et al., 2010)] project and the Human Microbiome Project [HMP, (Peterson et al., 2009)] explore multi-"omic" data to define the role of human microbiome in health and disease along with the development of a reference set of microbial genome sequences. However, experimental model systems such as the mouse and Drosophila continue to provide critical insight into how host-microbiota homeostasis is established, maintained or perturbed (Kostic et al., 2013).

Herein, we review the phenotypic, cellular, and molecular characteristics of some of the most widely-used mouse models of experimental IBD and colitis-associated cancer (CAC) and the impact of microbiota on these pathologies (**Figure 1**).

CHEMICAL AND GENETIC MOUSE MODELS OF INFLAMMATORY BOWEL DISEASE AND COLITIS-ASSOCIATED COLON CANCER

DEXTRAN SODIUM SULFATE-INDUCED COLITIS

An established model of IBD employs the chemical Dextran Sodium Sulfate (DSS). DSS administered to the drinking water in repeated cycles triggers a state of chronic intestinal inflammation by binding to medium-chain-length fatty acids present in the mouse colon, inducing disruption of colonic epithelial barrier (Laroui et al., 2012). The ensuing tissue damage

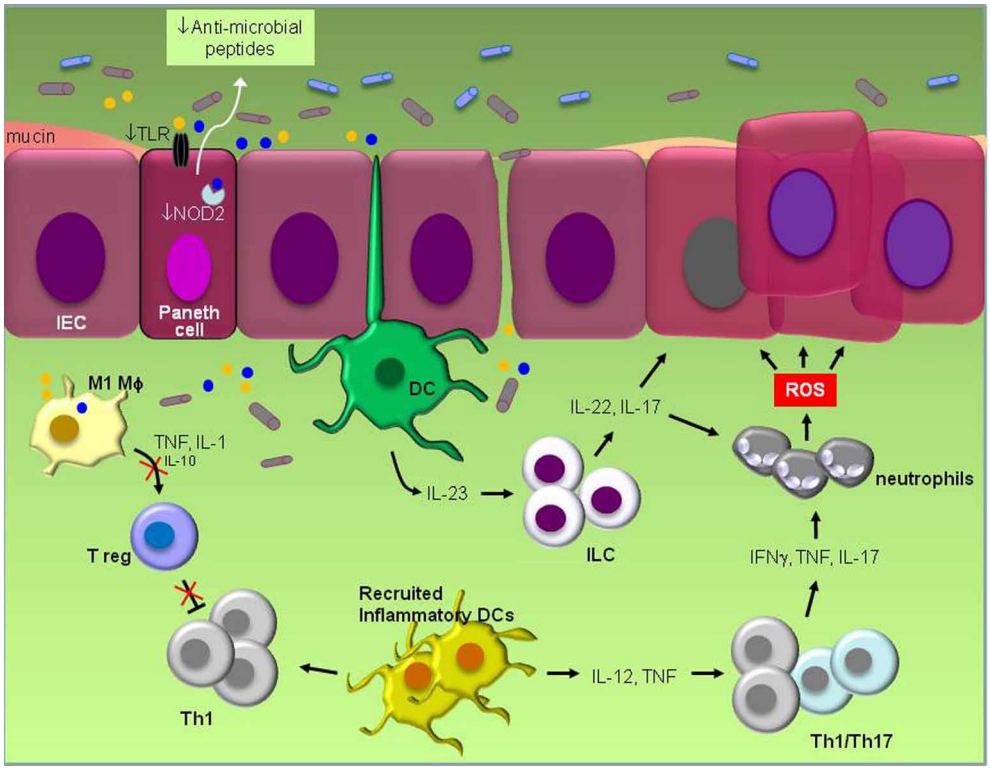


FIGURE 1 | Schematic representation of known pathogenic events in experimental IBD. Defective TLR and NOD signaling in Paneth epithelial cells leads to reduced "sensing" of bacterial products (yellow and blue circles) and reduced production of anti-microbial peptides. The ensuing disruption of microbiota balance which may also be influenced by the frequent use of antibiotics and/or diet stimulates inflammation that is largely orchestrated by resident dendritic cells (DCs). Their activation by products of pathogenic bacteria induces IL-23 which in turn engages innate lymphoid cells (ILC) to produce IL-22 and IL-17. Inflammation also results in the recruitment of inflammatory DCs which secrete IL-12 and TNF and increase IFNγ, TNF and IL-17-producing Th1/Th17 cells. Cytokines secreted by ILCs and Th1/Th17 cells promote both the recruitment of neutrophils that produce DNA-damaging reactive oxygen species (ROS) and the survival of intestinal epithelial cells (IEC) by the engagement of STAT3 signal transduction, eventually leading to malignant transformation. Suppression of regulatory T cell (T_{reg}) activity by pro-inflammatory M1 macrophages which secrete high TNF and IL-1 but low IL-10 levels unleashes inflammation and allows macrophages to produce oxidative products and mutagens which are believed to contribute to carcinogenesis. Reduced production of mucus by Goblet cells impacts on microbial composition and gastrointestinal barrier function.

allows exposure of innate immune cells to commensal bacteria accompanied by a robust Th1-type immune response to eliminate infiltrating pathogens and promote tissue healing. Multiple cell types participate in the pathogenesis of DSS-induced colitis including gut epithelial cells, $CD4^+$ and $CD8^+$ T lymphocytes, regulatory T cells, neutrophils and macrophages, resembling the pathogenic events in human colitis. Mucosal macrophages may prime the local inflammatory response through both phagocytosis of DSS and activation by bacteria products. The contribution of macrophage polarization phenotype to the development of CAC has been described using this model including the demonstration that Akt2 deficient mice are partly protected from DSS-induced colitis because of a macrophage phenotype shift from M1 to M2 in the colonic mucosa (Arranz et al., 2012).

Chronic inflammation induced by prolonged administration of DSS results in malignancy only in a small proportion of animals (Okayasu et al., 1990, 1996) but adenocarcinoma development readily occurs upon intraperitoneal injection of the mutagen azoxymethane (AOM) followed by repeated DSS cycles (reviewed in Wirtz et al., 2007; Chen and Huang, 2009). AOM is metabolized *in vivo* to methylazoxymethanol (MAM) by cytochrome P450 (Sohn et al., 2001). MAM and its derivatives are direct DNA mutagens although tumor formation requires additional cellular and molecular events associated with chronic inflammatory imbalance. Indeed, the degree of inflammation correlates with the development of dysplasia in minor lesion aberrant crypt foci and is linked to the nuclear translocation of β-catenin (Cooper et al., 2000). Impairment of indoleamine 2,3 dioxygenase-1 (IDO-1) activity, a molecule which catabolizes tryptophan in the kynurenine pathway and is expressed in inflamed and neoplastic intestinal epithelial cells, reduces nuclear β-catenin and cell proliferation (Thaker et al., 2013). Inflammatory cytokines such as TNF, IL-6, and IL-1α which have been implicated in human IBD and IBD-associated colorectal carcinogenesis, also largely dictate the outcome of AOM/DSS-induced pathology (Becker et al., 2004; Van Hauwermeiren et al., 2013; Bersudsky et al., in press). Interestingly, mice deficient in myeloid translocation gene related-1 (MTGR1) are resistant to AOM/DSS-induced CAC despite the preservation of an active inflammatory infiltrate. Tumor resistance in these animals arises

from increased malignant cell death and impaired wound-healing (Barrett et al., 2011), suggesting that in addition to the severity of inflammation, AOM/DSS-induced carcinogenesis depends on apoptosis and wound-healing regulatory pathways.

Mutations in p53 are abundant in both sporadic and IBD-associated colorectal cancer in humans, suggesting a pivotal role for this tumor suppressor in intestinal disease pathogenesis. However, whereas p53 mutations are late genetic events in sporadic CRC, they are observed in inflamed colonic tissue well before neoplastic lesions become detectable (Hussain et al., 2000). Thus, p53 mutations probably have an initiating role in human IBD-associated cancer. In the mouse colon, AOM/DSS-induced pathology is largely amplified by either mutations or loss of WT p53. Knock-in mice carrying a germline mutated p53 allele encoding p53R172H, the mouse equivalent of the human hot spot mutant p53R175H (Lang et al., 2004), develop adenocarcinomas even in the absence of AOM treatment (Cooks et al., 2013). The accelerated tumorigenesis in these animals results from a combination of amplified and prolonged inflammation and augmented capacity of mutated p53-containing epithelial cells to evade apoptosis. P53-deficient or $p53^{+/-}$ mice also develop multiple tumors upon exposure to DSS without the requirement of AOM administration (Fujii et al., 2004; Chang et al., 2007). Therefore, AOM/DSS induces a state of chronic intestinal inflammation which progresses to cancer with molecular, histopathological and phenotypic characteristics that resemble the human disease.

Another carcinogen used in combination with DSS is 1, 2-dimethylhydrazine (DMH). DMH is metabolized in liver and its derivatives induce the production of diazonium by gut epithelial cells. The aforementioned metabolite exerts mutagenic effects through oxidative stress and methylation events (Hamiza et al., 2012).

TNBS-INDUCED INFLAMMATORY BOWEL DISEASE

Intrarectal administration of the contact sensitizing allergen 2,4,6-trinitrobenzenesulfonic acid (TNBS) initiates acute T cell-mediated, IL-12 driven intestinal inflammation (Scheiffele and Fuss, 2002; Neurath and Finotto, 2009). Ethanol is required to disrupt the mucosal barrier, whereas TNBS is proposed to haptenize microbiota or colonic autologous proteins rendering them immunogenic. The overall phenotypic and histopathological features of TNBS-induced colitis mostly resemble those characterizing CD. Recently, the TNBS model was used for the identification of $rVEGF164_b$, a VEGF-A isoform, as an inhibitory molecule of angiogenesis in IBD (Cromer et al., 2013). Thus, TNBS is considered as a suitable model to study both gut inflammation and the mechanism involved in colonic healing in IBD. Using this model we have recently described the efficacy of antisense oligonucleotides targeting CD40, a TNF family receptor that triggers Th1 and innate immune responses upon stimulation by its ligand, in treating early stage and established colitis (Arranz et al., 2013).

ADENOMATOUS POLYPOSIS COLI MUTATION-INDUCED ADENOMA MODEL

Mutations in the Adenomatous polyposis coli (APC) gene in humans are critically involved in familial adenomatous polyposis (FAP) and represent an early genetic aberration in sporadic colorectal cancer (Liang et al., 2013). The multiple intestinal neoplasia (Min) mouse, one of the first genetic models used to study intestinal cancer in rodents, bears a point mutation in the Apc gene ($Apc^{min/+}$) and develops numerous adenomas. Exposure of $Apc^{min/+}$ mice to DSS alone mimics CAC and results in accelerated tumorigenesis (Tanaka et al., 2006). In addition to inflammation, $Apc^{min/+}$-induced carcinogenesis can be influenced by oxidative stress. Thus, Cheung et al. (2012) showed that ablation of nuclear factor-erythroid 2 related factor 2 (Nrf2) attenuates anti-oxidative stress pathways and increases proliferation in the intestinal crypts leading to enhanced intestinal carcinogenesis in $Apc^{min/+}$ mice. This observation is pertinent to the role of gut microbiome in disease pathogenesis, identifying microbial metabolites as modulators of carcinogenesis in part through induction of chronic oxidative stress (Arthur et al., 2012).

IKK-γ (NEMO) DEFICIENCY IN INTESTINAL EPITHELIAL CELLS

Intestinal epithelial-cell-specific inhibition of NF-κB through conditional ablation of NEMO/IKKγ, the regulatory subunit of the IKK signaling complex essential for NF-κB activation, spontaneously causes severe chronic intestinal inflammation in mice (Nenci et al., 2007). Histological examination of colon sections from these animals revealed extensive apoptosis of colonic epithelial cells leading to disruption of epithelial integrity and translocation of bacteria from the lumen into the mucosa. Notably, these mice exhibit reduced expression of defensin-3, an antimicrobial peptide primarily produced by specialized intestinal epithelial cells, called Paneth. Low defensin copy number has been reported to correlate with predisposition to IBD in humans (Wehkamp et al., 2006) and unpublished data from our laboratory suggest that defensin expression is higher in the proximal compared to distal colon reflecting their differential susceptibility to DSS-induced pathology (Gkouskou and Eliopoulos, in preparation).

INTERLEUKIN-10 (IL-10)-DEPENDENT INFLAMMATORY BOWEL DISEASE

Genome-wide association studies have identified SNPs flanking the IL-10 gene to be linked to UC (Franke et al., 2008). IL-10-deficient mice exhibit intolerance to their intestinal microbiota, have altered responses to inflammatory or autoimmune stimuli and develop spontaneous enterocolitis and adenocarcinoma (Sturlan et al., 2001). A similar intestinal phenotype was observed in mice with a T cell specific IL-10 deficiency, underscoring the importance of T cell derived IL-10 and IL-10-dependent regulatory T-cells in the regulation of mucosal T cell responses and disease pathogenesis (Erdman et al., 2003).

T CELL ADOPTIVE TRANSFER MODEL

Initially developed by the group of Fiona Powrie (Powrie et al., 1994), mouse $CD4^+$ T lymphocytes which express high CD45RB ($CD4^+CD45RB^{Hi}$) can be adoptively transferred into immunodeficient SCID or $RAG1/2^{-/-}$ mice, where they traffic to the intestine and induce gut inflammation. Recipient mice repopulated with $CD4^+CD45RB^{Lo}$ T cells or total $CD4^+$ T lymphocytes do not develop colitis, despite their ability to colonize the host gut. This phenomenon is attributed to the presence of $CD25^+$ $FoxP3^+$ regulatory T cells within the $CD4^+CD45RB^{Lo}$

population (Read et al., 2000) and adoptive transfer of $CD4^+CD25^-$ T cells has thus been proposed as the most suitable T cell transfer model of enterocolitis (Kjellev et al., 2006). IL-10 appears to have an important role in the pathogenesis of the disease in this model as SCID mice administered both $CD4^+CD45RB^{Hi}$ and regulatory T cells together with anti-IL-10 receptor antibodies develop colitis (Kjellev et al., 2006).

THE GUT MICROBIOTA IN MOUSE MODELS OF IBD

Several lines of evidence support a role for the microbiota in experimental colitis. Early studies reported a significant increase in members of *Bacteroidaceae* and *Clostridium* spp. families, in particular *Bacteroides distasonis* and *Clostridium ramosum*, in the intestines of mice exposed to DSS (Okayasu et al., 1990) (**Table 1**). Subsequent reports showed elevated 16S rRNA gene copy numbers of the mucin-degrading Gram-negative bacterium *Akkermansia muciniphila* and of *Enterobacteriaceae* to correlate with disease activity in mice administered DSS (Hakansson et al., 2014). A breakthrough in appreciating the major impact of gut microbiota on disease pathogenesis came by the observations that treatment with antibiotics or germ-free breeding of various mouse models of IBD is associated with significantly less severe bowel inflammation and carcinogenesis. Thus, Dove and colleagues showed that $Apc^{Min/+}$ mice housed in germ-free environment display more than 50% reduction in tumor development compared to the same animals housed in standard specific pathogen-free (SPF) conditions (Dove et al., 1997). IL-10 deficient mice were also found to be resistant to spontaneous colitis when kept in germ-free environment (Sellon et al., 1998).

Analysis of different classes of antibiotics indicated differential and localized roles of bacteria species in the establishment and perpetuation of colitis in IL-$10^{-/-}$ mice after colonization with SPF bacteria. Ciprofloxacin was found to be most effective in caecal inflammation by controlling aerobic organisms, including *E. coli* and *E. faecalis*, whereas metronidazole was preferentially active in the colon and selectively decreased anaerobic bacteria and *Bacteroides* spp. (Hoentjen et al., 2003). Interestingly, whereas induction of colitis in IL-$10^{-/-}$ mice born under SPF conditions and in mice exposed to DSS is prevented by ciprofloxacin and metronidazole respectively, these antibiotics have minimal effect after the onset of colitis (Hans et al., 2000; Madsen et al., 2000). In contrast, vancomycin-imipenem reduces total luminal bacteria, eliminates specific aerobic and anaerobic organisms and effectively treats established disease (Hoentjen et al., 2003). These results suggest that some intestinal bacteria species may orchestrate the initiation of inflammation whereas other subsets may have a role in perpetuating colitis (Rath et al., 2001). In line with this notion, colonic polyps developed in $Apc\Delta^{468}$/IL-$10^{-/-}$ mice are significantly enriched in two genera of the phylum *Bacteroidetes*, namely *Bacteroides* and *Porphyromonas* as compared with uninvolved tissue (Dennis et al., 2013) (**Table 1**). The interplay between oncogenes and microbiota species in the development of gut pathologies is also highlighted by studies in Drosophila which have demonstrated that the human pathogen *Pseudomonas aeruginosa* synergizes with the RasV12 oncogene to induce intestinal dysplasia and metastasis-like phenotype (Apidianakis et al., 2009; Bangi et al., 2012).

Further evidence supporting the significance of microbes in colitis development has been provided by studies describing a communicable form of colitis induced by deficiency of T-bet in cells of the innate immune system. T-bet is a transcription factor with a pivotal role in the development of Th1 cells and in the regulation of adaptive and innate immune responses. Loss of T-bet in mice lacking B and T cells ($T\text{-}bet^{-/-}/RAG\text{-}1^{-/-}$) results in spontaneous colitis which is transmissible to wild-type animals (which express T-bet) upon cross-fostering or co-housing (Garrett et al., 2007).

Nutrition plays an important role in the establishment of microbial flora which in turn affects metabolism of several macro- and micronutrients. For example, a high *Firmicutes* to *Bacteroidetes* ratio and low microbial diversity is indicative of a high-calorie diet and obesity in humans (Ley et al., 2006). A telling example of how genetics, microbiota and the immune system may interact to promote chronic gut inflammation is highlighted by a recent study by Devkota et al. (2012) which demonstrated that the ingestion of saturated fat by IL-$10^{-/-}$ mice induces a more severe form of chronic colitis compared to the disease that normally develops in these animals. This diet was

Table 1 | Microorganisms reported to associate with IBD in the mouse.

Type of disease or model	Microorganisms	Final effect	References
DSS colitis	*Bacteroides distasonis, Clostridium ramosum, Akkermansia muciniphila, Enterobacteriaceae*	Increased numbers correlate with acute and chronic ulcerative colitis	Okayasu et al., 1990; Hakansson et al., 2014
Colitis in IL-10 deficient mice	*Enterobacteriaceae* and adherent-invasive *E. coli*	Increased numbers correlate with inflammation (*Enterobacteriaceae)* and cancer *(E. coli)*	Arthur et al., 2012; Yang et al., 2013b
Colitis in $Apc\Delta^{468}$/IL-$10^{-/-}$ mice	*Bacteroides* and *Porphyromonas* genera	Increased numbers correlate with inflammation and colon polyposis	Dennis et al., 2013
TNBS colitis	*Enterobacteriaceae, Bacteroides*	Increased numbers correlate with inflammation	Ettreiki et al., 2012

Differences in intestinal microbiota composition due to different housing conditions have been reported (Yang et al., 2013b).

shown to stimulate the formation of taurocholine-conjugated bile acids leading to intestinal dysbiosis characterized by the overgrowth of the rare sulfate-reducing pathogenic bacteria *Bilophilia wadsworthia* (Devkota et al., 2012). The modulation of microbiota species and density has also highlighted the important role of bacteria in gut homeostasis and disease. Thus, administration of VSL#3 probiotics, a mixture of *Lactobacillus, Bifidobacterium* and *Streptococcus salivarious* strains, has shown to confer beneficial effects on various mouse models of colitis and in humans suffering from IBD (Isaacs and Herfarth, 2008). Intriguingly, VSL#3 does not reduce colitis-associated colon cancer in the mouse (Arthur et al., 2013).

Direct evidence for the role of pathogenic bacteria in IBD has been provided by studies using the aerobic bacterium *Helicobacter hepaticus.* Immunodeficient $RAG^{-/-}$ mice infected with *H. hepaticus* and treated with AOM develop invasive colon carcinoma after 3–5 months, at the sites of highest inflammation in the colon and cecum (Fox et al., 2011). This model has also assisted in the identification of a genetic interval on the telomeric part of mouse chromosome 3 designated *Hiccs* (*Helicobacter hepaticus*-induced colitis and associated cancer susceptibility), which harbors 8 genes and 5 micro RNAs and confers protection against *H. hepaticus*-induced chronic colitis and inflammation-driven colon cancer (Boulard et al., 2012).

What are the mechanisms by which bacteria dysbiosis triggers inflammatory bowel disease? Several studies have highlighted a prominent role for TLR and NOD family members as key sensors of and responders to microbe-associated molecular patterns. The effects of *Nod2* mutations are of particular interest because they have been implicated in human IBD and *Nod2* knockout mice have diminished ability to prevent intestinal colonization of pathogenic bacteria (Petnicki-Ocwieja et al., 2009; Couturier-Maillard et al., 2013). Impaired TLR and NOD function in Paneth epithelial cells affects their capacity to produce antimicrobial factors which kill pathogenic bacteria, resulting in a shift in the composition of gut microbiota (**Figure 1**). Frequent use of antibiotics or personal habits, including diet may also influence this shift. The concomitant release of ATP, other metabolic products and DNA by microbia (Atarashi et al., 2008; Hall et al., 2008) may lead to increased production of IL-23 by resident monocytes, such as dendritic cells, which in turn stimulates innate lymphoid cells to secrete IL-17, IL-22, and IFNγ (Buonocore et al., 2010). IL-17 is of particular relevance to colitis as it is linked to reduced regulatory T cell (T_{reg}) activity. Resident T_{reg} produce IL-10 which inhibits Th1 cells and monocyte effector functions associated with inflammation. Suppression of T_{reg} activity thereby unleashes inflammation, leading to a switch in the differentiation program of $Ly6C^{hi}$ monocytes from anti-inflammatory M2 macrophages to inflammatory dendritic cells and M1 macrophages in the colon (Rivollier et al., 2012) which produce a plethora of pro-inflammatory cytokines, oxidative products and mutagens such as *trans*-4-hydroxy-2-nonenal (4-HNE) (Yang et al., 2013a). Reactive oxygen species (ROS) generated by recruited neutrophils may also cause DNA damage in epithelial cells.

The production by pathogenic bacteria of secondary bile acids that have carcinogenic effects is believed to contribute to the dysbiosis-inflammation-tumorigenesis axis (Sommer and Backhed, 2013). Additional host genetic factors may influence the cross-talk between microbiota and IBD. For example, production of mucus by Goblet cells, especially mucin 2 (MUC2), has a significant impact on microbial composition and gastrointestinal barrier function. Altered MUC2 expression and/or glycosylation leads to accompanying intestinal pathologies, including IBD and colon cancer (Yang et al., 2008).

CONCLUSIONS AND FUTURE DIRECTIONS

In the intestine, the symbiotic relationship between the host and the microbiota influences nutrition, metabolism, immune system functions, development and normal physiology, as well as susceptibility to IBD and CAC. Accumulating experimental, epidemiological, and clinical evidence highlights the potential of targeting the dysbiosis-inflammation-tumorigenesis axis for the development of new therapeutic strategies for IBD and colorectal cancer. Much of the current knowledge of the regulation of this axis comes from studies exploring the effects of particular pathogenic bacteria using chemical or genetic models of CAC. High-throughput human microbiome studies confirm that the genetic make-up, environmental factors and personal habits influence the bacteria communities among individuals; however, further studies are warranted to fully appreciate how a particular microbiota is established and orchestrates the immune responses toward the development of colitis and CAC. The establishment of "humanized" gnotobiotic mice, animals that carry only human-derived gut microbes (Turnbaugh et al., 2009) is expected to improve human disease modeling and provide further insight into how environmental factors, including diet, may influence the microbiota and shape gut physiology and disease pathogenesis. Similarly, it would be important to assess changes in the gut flora during aging and evaluate their impact on IBD susceptibility. In line with this notion, recent studies in Drosophila show that immunosenescence associated with aging results in dysbiosis and triggers an inflammatory response which promotes intestinal stem cell over-proliferation and dysplasia (Guo et al., 2014). Further studies are also needed to determine whether changes in particular microbiota species induced by inflammation may impact on progression to cancer.

Future research could also lead to the development of beneficial (probiotic) microbes or inhibitors of specific microbes and/or their products which "normalize" the intestinal flora and can improve human health. As the current repertoire of probiotics is limited, further studies to explore the potential of fecal microbiota transplantation (FMT) therapy, the infusion of fecal bacteria from a healthy individual into a recipient patient, for the treatment of intestinal disorders are warranted. FMT has demonstrated tremendous efficacy in treating refractory *Clostridium difficile* infection, and there are case reports of successful management of UC using FMT in humans (Lemon et al., 2012). A more focused approach requires the identification of species or bacterial products and metabolites which normalize the inflamed gut mucosa. In this regard, the isolation of 17 human clostridia species and the discovery of microbial-derived short-chain fatty acids that can stimulate the expansion of T_{reg} cells in mice (Atarashi et al.,

2013; Smith et al., 2013) opens up new therapeutic options for the treatment of IBD.

The microbiome plays an important role in immunity and energy metabolism and will thus be important to determine if the microbial gut ecology may also impact on non-gastrointestinal diseases, including obesity, cancer and neurological disorders.

ACKNOWLEDGMENTS

This work was supported by the European Commission FP7 programmes INFLA-CARE (EC contract number 223151) to Aristides G. Eliopoulos and "Translational Potential" (TransPOT; EC contract number 285948) to Aristides G. Eliopoulos and Christos Tsatsanis. Aristides G. Eliopoulos also acknowledges co-funding of this review by the General Secretariat of Research and Technology of Greece through the Operational Program Competitiveness and Entrepreneurship (OPC II), NSRF 2007-2013, action "SYNERGASIA 2011," Project THERA-CAN (contract number 11ΣΥΝ_1_485).

REFERENCES

Apidianakis, Y., Pitsouli, C., Perrimon, N., and Rahme, L. (2009). Synergy between bacterial infection and genetic predisposition in intestinal dysplasia. *Proc. Natl. Acad. Sci. U.S.A.* 106, 20883–20888. doi: 10.1073/pnas.0911797106

Arranz, A., Doxaki, C., Vergadi, E., Martinez de la Torre, Y., Vaporidi, K., Lagoudaki, E. D., et al. (2012). Akt1 and Akt2 protein kinases differentially contribute to macrophage polarization. *Proc. Natl. Acad. Sci. U.S.A.* 109, 9517–9522. doi: 10.1073/pnas.1119038109

Arranz, A., Reinsch, C., Papadakis, K. A., Dieckmann, A., Rauchhaus, U., Androulidaki, A., et al. (2013). Treatment of experimental murine colitis with CD40 antisense oligonucleotides delivered in amphoteric liposomes. *J. Control. Release* 165, 163–172. doi: 10.1016/j.jconrel.2012.11.008

Arthur, J. C., Gharaibeh, R. Z., Uronis, J. M., Perez-Chanona, E., Sha, W., Tomkovich, S., et al. (2013). VSL#3 probiotic modifies mucosal microbial composition but does not reduce colitis-associated colorectal cancer. *Sci. Rep.* 3, 2868. doi: 10.1038/srep02868

Arthur, J. C., Perez-Chanona, E., Muhlbauer, M., Tomkovich, S., Uronis, J. M., Fan, T. J., et al. (2012). Intestinal inflammation targets cancer-inducing activity of the microbiota. *Science* 338, 120–123. doi: 10.1126/science.1224820

Atarashi, K., Nishimura, J., Shima, T., Umesaki, Y., Yamamoto, M., Onoue, M., et al. (2008). ATP drives lamina propria T(H)17 cell differentiation. *Nature* 455, 808–812. doi: 10.1038/nature07240

Atarashi, K., Tanoue, T., Oshima, K., Suda, W., Nagano, Y., Nishikawa, H., et al. (2013). Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota. *Nature* 500, 232–236. doi: 10.1038/nature12331

Bakhtiar, S. M., LeBlanc, J. G., Salvucci, E., Ali, A., Martin, R., Langella, P., et al. (2013). Implications of the human microbiome in inflammatory bowel diseases. *FEMS Microbiol. Lett.* 342, 10–17. doi: 10.1111/1574-6968.12111

Bangi, E., Pitsouli, C., Rahme, L. G., Cagan, R., and Apidianakis, Y. (2012). Immune response to bacteria induces dissemination of Ras-activated Drosophila hindgut cells. *EMBO Rep.* 13, 569–576. doi: 10.1038/embor.2012.44

Barrett, C. W., Fingleton, B., Williams, A., Ning, W., Fischer, M. A., Washington, M. K., et al. (2011). MTGR1 is required for tumorigenesis in the murine AOM/DSS colitis-associated carcinoma model. *Cancer Res.* 71, 1302–1312. doi: 10.1158/0008-5472.CAN-10-3317

Becker, C., Fantini, M. C., Schramm, C., Lehr, H. A., Wirtz, S., Nikolaev, A., et al. (2004). TGF-beta suppresses tumor progression in colon cancer by inhibition of IL-6 trans-signaling. *Immunity* 21, 491–501. doi: 10.1016/j.immuni.2004.07.020

Bersudsky, M., Luski, L., Fishman, D., White, R. M., Ziv-Sokolovskaya, N., Dotan, S., et al. (in press). Non-redundant properties of IL-1alpha and IL-1beta during acute colon inflammation in mice. *Gut* doi: 10.1136/gutjnl-2012-303329

Boulard, O., Kirchberger, S., Royston, D. J., Maloy, K. J., and Powrie, F. M. (2012). Identification of a genetic locus controlling bacteria-driven colitis and associated cancer through effects on innate inflammation. *J. Exp. Med.* 209, 1309–1324. doi: 10.1084/jem.20120239

Buonocore, S., Ahern, P. P., Uhlig, H. H., Ivanov, I. I., Littman, D. R., Maloy, K. J., et al. (2010). Innate lymphoid cells drive interleukin-23-dependent innate intestinal pathology. *Nature* 464, 1371–1375. doi: 10.1038/nature08949

Cario, E. (2013). Microbiota and innate immunity in intestinal inflammation and neoplasia. *Curr. Opin. Gastroenterol.* 29, 85–91. doi: 10.1097/MOG.0b013e32835a670e

Chang, W. C., Coudry, R. A., Clapper, M. L., Zhang, X., Williams, K. L., Spittle, C. S., et al. (2007). Loss of p53 enhances the induction of colitis-associated neoplasia by dextran sulfate sodium. *Carcinogenesis* 28, 2375–2381. doi: 10.1093/carcin/bgm134

Chen, J., and Huang, X. F. (2009). The signal pathways in azoxymethane-induced colon cancer and preventive implications. *Cancer Biol. Ther.* 8, 1313–1317. doi: 10.4161/cbt.8.14.8983

Cheung, K. L., Lee, J. H., Khor, T. O., Wu, T. Y., Li, G. X., Chan, J., et al. (2012). Nrf2 knockout enhances intestinal tumorigenesis in Apc(min/+) mice due to attenuation of anti-oxidative stress pathway while potentiates inflammation. *Mol. Carcinog.* 53, 77–84. doi: 10.1002/mc.21950

Cooks, T., Pateras, I. S., Tarcic, O., Solomon, H., Schetter, A. J., Wilder, S., et al. (2013). Mutant p53 prolongs NF-kappaB activation and promotes chronic inflammation and inflammation-associated colorectal cancer. *Cancer Cell* 23, 634–646. doi: 10.1016/j.ccr.2013.03.022

Cooper, H. S., Murthy, S., Kido, K., Yoshitake, H., and Flanigan, A. (2000). Dysplasia and cancer in the dextran sulfate sodium mouse colitis model. Relevance to colitis-associated neoplasia in the human: a study of histopathology, B-catenin and p53 expression and the role of inflammation. *Carcinogenesis* 21, 757–768. doi: 10.1093/carcin/21.4.757

Couturier-Maillard, A., Secher, T., Rehman, A., Normand, S., De Arcangelis, A., Haesler, R., et al. (2013). NOD2-mediated dysbiosis predisposes mice to transmissible colitis and colorectal cancer. *J. Clin. Invest.* 123, 700–711. doi: 10.1172/JCI62236

Crohn, B., and Rosenberg, H. (1925). The sigmoidoscopic picture of chronic ulcerative colitis (non-specific). *Am. J. Med. Sci.* 170, 220–228. doi: 10.1097/00000441-192508010-00006

Cromer, W. E., Ganta, C. V., Patel, M., Traylor, J., Kevil, C. G., Alexander, J. S., et al. (2013). VEGF-A isoform modulation in an preclinical TNBS model of ulcerative colitis: protective effects of a VEGF164b therapy. *J. Transl. Med.* 11, 207. doi: 10.1186/1479-5876-11-207

Danese, S., and Mantovani, A. (2010). Inflammatory bowel disease and intestinal cancer: a paradigm of the Yin-Yang interplay between inflammation and cancer. *Oncogene* 29, 3313–3323. doi: 10.1038/onc.2010.109

Dennis, K. L., Wang, Y., Blatner, N. R., Wang, S., Saadalla, A., Trudeau, E., et al. (2013). Adenomatous polyps are driven by microbe-instigated focal inflammation and are controlled by IL-10-producing T cells. *Cancer Res.* 73, 5905–5913. doi: 10.1158/0008-5472.CAN-13-1511

Devkota, S., Wang, Y., Musch, M. W., Leone, V., Fehlner-Peach, H., Nadimpalli, A., et al. (2012). Dietary-fat-induced taurocholic acid promotes pathobiont expansion and colitis in Il10-/- mice. *Nature* 487, 104–108. doi: 10.1038/nature11225

Dove, W. F., Clipson, L., Gould, K. A., Luongo, C., Marshall, D. J., Moser, A. R., et al. (1997). Intestinal neoplasia in the ApcMin mouse: independence from the microbial and natural killer (beige locus) status. *Cancer Res.* 57, 812–884.

Erdman, S. E., Rao, V. P., Poutahidis, T., Ihrig, M. M., Ge, Z., Feng, Y., et al. (2003). CD4(+)CD25(+) regulatory lymphocytes require interleukin 10 to interrupt colon carcinogenesis in mice. *Cancer Res.* 63, 6042–6050.

Ettreiki, C., Gadonna-Widehem, P., Mangin, I., Coeffier, M., Delayre-Orthez, C., and Anton, P. M. (2012). Juvenile ferric iron prevents microbiota dysbiosis and colitis in adult rodents. *World J. Gastroenterol.* 18, 2619–2629. doi: 10.3748/wjg.v18.i21.2619

Fox, J. G., Ge, Z., Whary, M. T., Erdman, S. E., and Horwitz, B. H. (2011). *Helicobacter hepaticus* infection in mice: models for understanding lower bowel inflammation and cancer. *Mucosal Immunol.* 4, 22–30. doi: 10.1038/mi.2010.61

Franke, A., Balschun, T., Karlsen, T. H., Sventoraityte, J., Nikolaus, S., Mayr, G., et al. (2008). Sequence variants in IL10, ARPC2 and multiple other loci contribute to ulcerative colitis susceptibility. *Nat. Genet.* 40, 1319–1323. doi: 10.1038/ng.221

Fujii, S., Fujimori, T., Kawamata, H., Takeda, J., Kitajima, K., Omotehara, F., et al. (2004). Development of colonic neoplasia in p53 deficient mice with experimental colitis induced by dextran sulphate sodium. *Gut* 53, 710–716. doi: 10.1136/gut.2003.028779

Garrett, W. S., Lord, G. M., Punit, S., Lugo-Villarino, G., Mazmanian, S. K., Ito, S., et al. (2007). Communicable ulcerative colitis induced by T-bet deficiency in the innate immune system. *Cell* 131, 33–45. doi: 10.1016/j.cell.2007.08.017

Guo, L., Karpac, J., Tran, S. L., and Jasper, H. (2014). PGRP-SC2 promotes gut immune homeostasis to limit commensal dysbiosis and extend lifespan. *Cell* 156, 109–122. doi: 10.1016/j.cell.2013.12.018

Hakansson, A., Tormo-Badia, N., Baridi, A., Xu, J., Molin, G., Hagslatt, M. L., et al. (2014). Immunological alteration and changes of gut microbiota after dextran sulfate sodium (DSS) administration in mice. *Clin. Exp. Med*. doi: 10.1007/s10238-013-0270-5. [Epub ahead of print].

Hall, J. A., Bouladoux, N., Sun, C. M., Wohlfert, E. A., Blank, R. B., Zhu, Q., et al. (2008). Commensal DNA limits regulatory T cell conversion and is a natural adjuvant of intestinal immune responses. *Immunity* 29, 637–649. doi: 10.1016/j.immuni.2008.08.009

Hamiza, O. O., Rehman, M. U., Tahir, M., Khan, R., Khan, A. Q., Lateef, A., et al. (2012). Amelioration of 1,2 Dimethylhydrazine (DMH) induced colon oxidative stress, inflammation and tumor promotion response by tannic acid in Wistar rats. *Asian Pac. J. Cancer Prev.* 13, 4393–4402. doi: 10.7314/APJCP.2012.13.9.4393

Hans, W., Scholmerich, J., Gross, V., and Falk, W. (2000). The role of the resident intestinal flora in acute and chronic dextran sulfate sodium-induced colitis in mice. *Eur. J. Gastroenterol. Hepatol.* 12, 267–273. doi: 10.1097/00042737-200012030-00002

Hoentjen, F., Harmsen, H. J., Braat, H., Torrice, C. D., Mann, B. A., Sartor, R. B., et al. (2003). Antibiotics with a selective aerobic or anaerobic spectrum have different therapeutic activities in various regions of the colon in interleukin 10 gene deficient mice. *Gut* 52, 1721–1727. doi: 10.1136/gut.52.12.1721

Hussain, S. P., Amstad, P., Raja, K., Ambs, S., Nagashima, M., Bennett, W. P., et al. (2000). Increased p53 mutation load in noncancerous colon tissue from ulcerative colitis: a cancer-prone chronic inflammatory disease. *Cancer Res.* 60, 3333–3337.

Isaacs, K., and Herfarth, H. (2008). Role of probiotic therapy in IBD. *Inflamm. Bowel Dis.* 14, 1597–1605. doi: 10.1002/ibd.20465

Kjellev, S., Lundsgaard, D., Poulsen, S. S., and Markholst, H. (2006). Reconstitution of Scid mice with CD4+CD25- T cells leads to rapid colitis: an improved model for pharmacologic testing. *Int. Immunopharmacol.* 6, 1341–1354. doi: 10.1016/j.intimp.2006.04.017

Kostic, A. D., Howitt, M. R., and Garrett, W. S. (2013). Exploring host-microbiota interactions in animal models and humans. *Genes Dev.* 27, 701–718. doi: 10.1101/gad.212522.112

Lang, G. A., Iwakuma, T., Suh, Y. A., Liu, G., Rao, V. A., Parant, J. M., et al. (2004). Gain of function of a p53 hot spot mutation in a mouse model of Li-Fraumeni syndrome. *Cell* 119, 861–872. doi: 10.1016/j.cell.2004.11.006

Laroui, H., Ingersoll, S. A., Liu, H. C., Baker, M. T., Ayyadurai, S., Charania, M. A., et al. (2012). Dextran sodium sulfate (DSS) induces colitis in mice by forming nano-lipocomplexes with medium-chain-length fatty acids in the colon. *PLoS ONE* 7:e32084. doi: 10.1371/journal.pone.0032084

Lemon, K. P., Armitage, G. C., Relman, D. A., and Fischbach, M. A. (2012). Microbiota-targeted therapies: an ecological perspective. *Sci. Transl. Med.* 4, 137rv5. doi: 10.1126/scitranslmed.3004183

Ley, R. E., Hamady, M., Lozupone, C., Turnbaugh, P. J., Ramey, R. R., Bircher, J. S., et al. (2008). Evolution of mammals and their gut microbes. *Science* 320, 1647–1651. doi: 10.1126/science.1155725

Ley, R. E., Turnbaugh, P. J., Klein, S., and Gordon, J. I. (2006). Microbial ecology: human gut microbes associated with obesity. *Nature* 444, 1022–1023. doi: 10.1038/4441022a

Liang, J., Lin, C., Hu, F., Wang, F., Zhu, L., Yao, X., et al. (2013). APC polymorphisms and the risk of colorectal neoplasia: a HuGE review and meta-analysis. *Am. J. Epidemiol.* 177, 1169–1179. doi: 10.1093/aje/kws382

Madsen, K. L., Doyle, J. S., Tavernini, M. M., Jewell, L. D., Rennie, R. P., and Fedorak, R. N. (2000). Antibiotic therapy attenuates colitis in interleukin 10 gene-deficient mice. *Gastroenterology* 118, 1094–1105. doi: 10.1016/S0016-5085(00)70362-3

Nenci, A., Becker, C., Wullaert, A., Gareus, R., van Loo, G., Danese, S., et al. (2007). Epithelial NEMO links innate immunity to chronic intestinal inflammation. *Nature* 446, 557–561. doi: 10.1038/nature05698

Neurath, M. F., and Finotto, S. (2009). Translating inflammatory bowel disease research into clinical medicine. *Immunity* 31, 357–361. doi: 10.1016/j.immuni.2009.08.016

Okayasu, I., Hatakeyama, S., Yamada, M., Ohkusa, T., Inagaki, Y., and Nakaya, R. (1990). A novel method in the induction of reliable experimental acute and chronic ulcerative colitis in mice. *Gastroenterology* 98, 694–702.

Okayasu, I., Ohkusa, T., Kajiura, K., Kanno, J., and Sakamoto, S. (1996). Promotion of colorectal neoplasia in experimental murine ulcerative colitis. *Gut* 39, 87–92. doi: 10.1136/gut.39.1.87

Peterson, J., Garges, S., Giovanni, M., McInnes, P., Wang, L., Schloss, J. A., et al. (2009). The NIH Human Microbiome Project. *Genome Res.* 19, 2317–2323. doi: 10.1101/gr.096651.109

Petnicki-Ocwieja, T., Hrncir, T., Liu, Y. J., Biswas, A., Hudcovic, T., Tlaskalova-Hogenova, H., et al. (2009). Nod2 is required for the regulation of commensal microbiota in the intestine. *Proc. Natl. Acad. Sci. U.S.A.* 106, 15813–15818. doi: 10.1073/pnas.0907722106

Powrie, F., Leach, M. W., Mauze, S., Menon, S., Caddle, L. B., and Coffman, R. L. (1994). Inhibition of Th1 responses prevents inflammatory bowel disease in scid mice reconstituted with CD45RBhi CD4+ T cells. *Immunity* 1, 553–562. doi: 10.1016/1074-7613(94)90045-0

Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K. S., Manichanh, C., et al. (2010). A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* 464, 59–65. doi: 10.1038/nature08821

Rath, H. C., Schultz, M., Freitag, R., Dieleman, L. A., Li, F., Linde, H. J., et al. (2001). Different subsets of enteric bacteria induce and perpetuate experimental colitis in rats and mice. *Infect. Immun.* 69, 2277–2285. doi: 10.1128/IAI.69.4.2277-2285.2001

Read, S., Malmstrom, V., and Powrie, F. (2000). Cytotoxic T lymphocyte-associated antigen 4 plays an essential role in the function of CD25(+)CD4(+) regulatory cells that control intestinal inflammation. *J. Exp. Med.* 192, 295–302. doi: 10.1084/jem.192.2.295

Rivollier, A., He, J., Kole, A., Valatas, V., and Kelsall, B. L. (2012). Inflammation switches the differentiation program of Ly6Chi monocytes from antiinflammatory macrophages to inflammatory dendritic cells in the colon. *J. Exp. Med.* 209, 139–155. doi: 10.1084/jem.20101387

Rubin, D. C., Shaker, A., and Levin, M. S. (2012). Chronic intestinal inflammation: inflammatory bowel disease and colitis-associated colon cancer. *Front. Immunol.* 3:107. doi: 10.3389/fimmu.2012.00107

Scheiffele, F., and Fuss, I. J. (2002). Induction of TNBS colitis in mice. *Curr. Protoco. Immunol.* Chapter 15, Unit 15.19. doi: 10.1002/0471142735.im1519s49

Sellon, R. K., Tonkonogy, S., Schultz, M., Dieleman, L. A., Grenther, W., Balish, E., et al. (1998). Resident enteric bacteria are necessary for development of spontaneous colitis and immune system activation in interleukin-10-deficient mice. *Infect. Immun.* 66, 5224–5231.

Smith, P. M., Howitt, M. R., Panikov, N., Michaud, M., Gallini, C. A., Bohlooly, Y. M., et al. (2013). The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. *Science* 341, 569–573. doi: 10.1126/science.1241165

Sohn, O. S., Fiala, E. S., Requeijo, S. P., Weisburger, J. H., and Gonzalez, F. J. (2001). Differential effects of CYP2E1 status on the metabolic activation of the colon carcinogens azoxymethane and methylazoxymethanol. *Cancer Res.* 61, 8435–8440.

Sommer, F., and Backhed, F. (2013). The gut microbiota–masters of host development and physiology. *Nat. Rev. Microbiol.* 11, 227–238. doi: 10.1038/nrmicro2974

Sturlan, S., Oberhuber, G., Beinhauer, B. G., Tichy, B., Kappel, S., Wang, J., et al. (2001). Interleukin-10-deficient mice and inflammatory bowel disease associated cancer development. *Carcinogenesis* 22, 665–671. doi: 10.1093/carcin/22.4.665

Tanaka, T., Kohno, H., Suzuki, R., Hata, K., Sugie, S., Niho, N., et al. (2006). Dextran sodium sulfate strongly promotes colorectal carcinogenesis in Apc(Min/+) mice: inflammatory stimuli by dextran sodium sulfate results in development of multiple colonic neoplasms. *Int. J. Cancer* 118, 25–34. doi: 10.1002/ijc.21282

Thaker, A. I., Rao, M. S., Bishnupuri, K. S., Kerr, T. A., Foster, L., Marinshaw, J. M., et al. (2013). IDO1 metabolites activate beta-catenin signaling to promote cancer cell proliferation and colon tumorigenesis in mice. *Gastroenterology* 145, 416–425.e1–e4. doi: 10.1053/j.gastro.2013.05.002

Turnbaugh, P. J., Ridaura, V. K., Faith, J. J., Rey, F. E., Knight, R., and Gordon, J. I. (2009). The effect of diet on the human gut microbiome: a metagenomic analysis in humanized gnotobiotic mice. *Sci. Transl. Med.* 1, 6ra14. doi: 10.1126/scitranslmed.3000322

Van Hauwermeiren, F., Armaka, M., Karagianni, N., Kranidioti, K., Vandenbroucke, R. E., Loges, S., et al. (2013). Safe TNF-based antitumor therapy following p55TNFR reduction in intestinal epithelium. *J. Clin. Invest.* 123, 2590–2603. doi: 10.1172/JCI65624

Warren, S., and Sommers, S. C. (1948). Cicatrizing enteritis as a pathologic entity; analysis of 120 cases. *Am. J. Pathol.* 24, 475–501.

Wehkamp, J., Chu, H., Shen, B., Feathers, R. W., Kays, R. J., Lee, S. K., et al. (2006). Paneth cell antimicrobial peptides: topographical distribution and quantification in human gastrointestinal tissues. *FEBS Lett.* 580, 5344–5350. doi: 10.1016/j.febslet.2006.08.083

Wirtz, S., Neufert, C., Weigmann, B., and Neurath, M. F. (2007). Chemically induced mouse models of intestinal inflammation. *Nat. Protoc.* 2, 541–546. doi: 10.1038/nprot.2007.41

Yang, I., Eibach, D., Kops, F., Brenneke, B., Woltemate, S., Schulze, J., et al. (2013b). Intestinal microbiota composition of interleukin-10 deficient C57BL/6J mice and susceptibility to *Helicobacter hepaticus*-induced colitis. *PLoS ONE* 8:e70783. doi: 10.1371/journal.pone.0070783

Yang, K., Popova, N. V., Yang, W. C., Lozonschi, I., Tadesse, S., Kent, S., et al. (2008). Interaction of Muc2 and Apc on Wnt signaling and in intestinal tumorigenesis: potential role of chronic inflammation. *Cancer Res.* 68, 7313–7322. doi: 10.1158/0008-5472.CAN-08-0598

Yang, Y., Wang, X., Huycke, T., Moore, D. R., Lightfoot, S. A., and Huycke, M. M. (2013a). Colon macrophages polarized by commensal bacteria cause colitis and cancer through the Bystander effect. *Transl. Oncol.* 6, 596–606.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 09 January 2014; accepted: 14 February 2014; published online: 28 February 2014.

*Citation: Gkouskou KK, Deligianni C, Tsatsanis C and Eliopoulos AG (2014) The gut microbiota in mouse models of inflammatory bowel disease. Front. Cell. Infect. Microbiol. **4**:28. doi: 10.3389/fcimb.2014.00028*

This article was submitted to the journal Frontiers in Cellular and Infection Microbiology.

frontiers in
CELLULAR AND INFECTION MICROBIOLOGY

MINI REVIEW ARTICLE
published: 25 December 2013
doi: 10.3389/fcimb.2013.00066

Shared mechanisms in stemness and carcinogenesis: lessons from oncogenic viruses

Demetris Iacovides, Stella Michael†, Charis Achilleos† and Katerina Strati*

Department of Biological Sciences, University of Cyprus, Nicosia, Cyprus

Edited by:
Dominique Ferrandon, Centre National de la Recherche Scientifique, France

Reviewed by:
Jean-Marie Peloponese, Centre National de la Recherche Scientifique, France
Dohun Pyeon, University of Colorado School of Medicine, USA

***Correspondence:**
Katerina Strati, Department of Biological Sciences, University of Cyprus, 1 University Avenue, 2109 Nicosia, Cyprus
e-mail: strati@ucy.ac.cy
† These authors have contributed equally to this work.

A rise in technologies for epigenetic reprogramming of cells to pluripotency, highlights the potential of understanding and manipulating cellular plasticity in unprecedented ways. Increasing evidence points to shared mechanisms between cellular reprogramming and the carcinogenic process, with the emerging possibility to harness these parallels in future therapeutics. In this review, we present a synopsis of recent work from oncogenic viruses which contributes to this body of knowledge, establishing a nexus between infection, cancer, and stemness.

Keywords: cancer, stemness, reprogramming, HBV, HCV, HPV, EBV, KSHV

INTRODUCTION

Long-standing observations have noted a number of parallels between the homeostasis of cancer cells and that of stem cells. A complicated picture includes the involvement of tissue stem cells as the cells-of-origin for some cancers, a stem cell compartment thought to maintain most tumors [commonly known as cancer stem cells (CSCs)], as well as more recent concepts of differentiated cells being reprogrammed back to pluripotency during the carcinogenic process (Lapouge et al., 2011; Friedmann-Morvinski et al., 2012). Several publications have shown that classic tumor suppressors such as p53 and pRb have emerging roles in the regulation of stemness (Conklin and Sage, 2009; Bonizzi et al., 2012). In addition to that, genes generally known for their key roles in stem cell biology, for example Nanog, appear to be deregulated in a number of cancers (Zhang et al., 2012; Lu et al., 2013). In the cutting edge field of reprogramming cells to pluripotency, key players in tumor suppression have been implicated in crucial roadblocks to the reprogramming process. While there is still a lot to be understood, it has been proposed that understanding the complicated relationship between stemness and cancer may hold the key to more successful future therapies; for example targeting cancer stem cells may reduce the possibility of future cancer recurrence.

Virally-induced cancers, thought to account for about 20% of the global cancer incidence, have long been studied to enable better understanding of the clinical manifestation of the disease as well as for their value as models of carcinogenesis overall (Farrell, 2002). Such cancers are attributed mainly to Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), Human Papilloma Virus (HPV), Epstein-Barr Virus (EBV), Kaposi's Sarcoma-associated Herpes virus (KSHV), Human T-cell Leukemia Virus-1 (HTLV-1), and more recently, Merkel Cell Polyoma Virus (MCPyV) (Samanta et al., 2003; Bonilla Guerrero and Roberts, 2005; Bajaj et al., 2007; Schiffman et al., 2007; Saha et al., 2010; Jeong et al., 2012; Amber et al., 2013; Cook et al., 2013). These viruses encode proteins shown to impinge on various cellular processes including cell cycle regulation, apoptosis, cell signaling, transcriptional regulation, and epigenetic regulation, resulting in carcinogenesis (Saha et al., 2010). We present here evidence which implicates oncogenic viruses in the regulation of pluripotency at various levels. We argue that virus-associated cancers can serve as models to understand the general link between cancer and stemness, as well as the distinct role that infection plays in these cases. It should be noted that other types of infectious agents, most notably the leprosy bacterium and Helicobacter pylori, have also been shown to modulate stemness-associated processes and pathways in host cells, raising the possibility that strategies involving the manipulation of cellular stemness may serve as evolutionary advantages to pathogens (Fujii et al., 2012; Wegner, 2013). Here, we review the available evidence for regulation of stemness by oncogenic viruses with particular emphasis on results coming from *in vivo* model systems. We also propose key questions that remain to be addressed.

INTERACTION OF ONCOGENIC VIRUSES WITH TISSUE STEM CELLS

Tissue stem cells and committed tissue progenitor cells destined for terminal differentiation are target cells of several oncogenic viruses. While no known oncogenic virus displays exclusive tropism for such specific cell populations, infection of either a stem or progenitor population may provide the opportunity of a longer-lived cellular reservoir for viral replication. In addition, infection of these cells might in some cases enable viruses to evade the immune system, since tissue progenitor/stem cells might be

immune privileged (Di Trapani et al., 2013), even though this notion is still controversial (Tseng et al., 2010).

Gammaherpesviruses, including KSHV and its murine cousin MHV68 and EBV infect primarily resting mature B cells. However, these cells are short-lived and non-proliferating, which points to the possibility that herpesviruses may also be able to infect a progenitor, stem cell-like population of B cells, which normally gives rise to mature B cells, in order to ensure continuous viral genome propagation and viral latency maintenance. Indeed, there is some evidence that both human and murine gammaherpesviruses infect hematopoetic progenitor cells. KSHV has been detected in immature hematopoetic cells in the bone marrow of transplant recipients (Luppi et al., 2000; Lapouge et al., 2011) and in hematopoietic progenitor cells in Kaposi's sarcoma patients (Henry et al., 1999; Friedmann-Morvinski et al., 2012), whereas MHV68 was detected in immature splenic B cells in the mouse (Marques et al., 2003; Collins et al., 2009). Moreover, KSHV-infected human hematopoietic progenitor stem cells gave rise to KSHV-infected mature human B-cells and monocytes when transplanted in NOD/SCID mice (Wu et al., 2006). Coleman et al. examined developing B cell infection by MHV68, a model for gammaherpesviruses, in a fully immunocompetent mouse host. They showed that this virus establishes long-term latency in immature B cells in the bone marrow as well as in transitional B cells in the spleen (Coleman et al., 2010). Since these self-renewing stem cell populations of developing B cells give rise to mature resting B cells, the authors speculate that infection of these cell populations by herpesviruses might play a key role in the maintenance of lifelong infection in the host.

Even though the direct involvement of Human Cytomegalovirus (HCMV) in tumor initiation is still not well-documented, a variety of malignancies have been associated with HCMV infections and persistence but the association is more widely accepted for malignant gliomas (Harkins et al., 2002; Samanta et al., 2003; Soderberg-Naucler, 2006; Michaelis et al., 2009). In normal brain tissue, HCMV appears to primarily target cells in the subventricular zone (SVZ) of the brain (Perlman and Argyle, 1992; Fritschy et al., 1996; Odeberg et al., 2007), which is the source of local stem cells and progenitor cells within this organ (Seri et al., 2006). Differentiation of neural precursors into mature neurons seems to reduce susceptibility to HCMV infection (Lokensgard et al., 1999; Cheeran et al., 2005) and activation of PDGFR alpha (essential to the self-renewal potential of neural stem cells) (Kofman et al., 2011) by HCMV is necessary for successful infection (Soroceanu et al., 2008). These results further support the possibility that the primary cell reservoir for HCMV, at least in the brain, is the stem cell compartment (Dziurzynski et al., 2012), and that infection of HCMV of this cell population might be a way for the virus to successfully establish lifelong latency in the host.

HPVs are strongly associated with a number of malignancies, most notably cervical carcinoma (CC). Several studies have proposed the existence of multiple HPV target cells within the host epithelium. There is increasing support for the hypothesis that stem cells of the transformation zone (TZ) of the cervical epithelium are the primary site of persistent HPV infection (Lopez et al., 2012). Given the anatomical observation that a lot of cervical cancers are derived from the TZ, a connection between infection of tissue stem cells and eventual carcinogenesis has been proposed. The long latency period between infection with HPV and development of cervical dysplasias supports the hypothesis that these cells can be targets of HPV infection and serve as a vehicle for long-term established viral latency in the cervix. Using laser capture microdissection in a rabbit oral papillomavirus (ROPV) model system, Maglennon et al. (2011) showed that ROPV indeed persists in a latent state, even after immune-mediated regression of induced papillomas, and that the site of latency is a subset of basal epithelial cells which the authors propose are the epithelial stem cells. It should be noted that expression of papillomavirus genes in stem cells has been shown to modulate their behavior *in vivo* and may be associated with ensuing carcinogenesis. In a study using mice transgenic for the HPV16 oncogenes our group showed that expression of viral oncogenes in label-retaining epithelial stem cells caused aberrant mobilization (Michael et al., 2013). In a related study, using animals expressing the entire HPV16 viral genome in all basal cells of stratified epithelia, skin cancers were shown to derive from tissue stem cells (da Silva-Diz et al., 2013).

VIRUSES GIVING RISE TO CANCER STEM CELLS

CSCs are cells within a tumor that possess stem cell properties, namely the ability to self-renew and give rise to progeny destined for differentiation to regenerate tumor cell diversity. Though genetic changes or oncogenic infection of an undifferentiated cell is usually thought to give rise to tumor initiating cells, tumors have been shown to originate from differentiated cells as well (Friedmann-Morvinski et al., 2012). It has been suggested that cellular reprogramming mediated by oncogenic viruses may promote the formation of tumor initiating cells or CSCs. The term "tumor initiating cells," strictly referring to the initial cells from which a tumorigenic transformation occurs, is used interchangeably in most cases, describing the ability of CSCs to fully regenerate, or "reinitiate" the tumor.

Several reports have implicated oncogenic viruses in the generation of CSCs. Arzumanyan et al. recently showed that HBV might induce initiation of hepatocellular carcinomas (HCC) by activating cellular factors that promote stemness (Arzumanyan et al., 2011). HBV encoded X antigen (HBVx), important in the viral life cycle as well as carcinogenesis, was shown to activate stemness associated factors Oct-4, Nanog, Klf4, beta catenin, and EpCAM *in vitro*. In addition, this protein was shown to induce cell migration, sphere formation, and growth in soft agar, all phenotypic characteristics of CSCs. These results were confirmed in liver biopsies obtained from HCC patients, since the above stemness associated markers were observed in the majority of HBV associated HCCs (Arzumanyan et al., 2011). Interestingly, microarray data from HBV-associated HCC showed that miR-181, recently found to contribute to tumorigenesis (Agami, 2010), was over-expressed in hematopoietic stem cells (HSCs) and CSCs, and was also found to be upregulated in HBx-expressing cells and HBx-positive liver biopsies (Arzumanyan et al., 2011) suggesting that this micro-RNA might be involved in stemness or CSCs induction and maintenance in HBV-associated HCCs.

The HCV has also been implicated in induction of CSCs. Machida et al. isolated tumor initiating stem-like cells from transgenic mice expressing HCV core, as well as from patients with HCC, and showed that the Tlr4-Nanog pathway was upregulated in these cells and was necessary for their tumorigenic properties (Machida et al., 2009, 2012). Nanog, a stem/progenitor cell marker was further shown to be upregulated through activation of the TLR4 pathway by NS5A, a non-structural protein encoded by HCV (Machida et al., 2012). Furthermore, a study by Ali et al. showed that infection of cultured hepatic cells with an HCV subgenomic replicon resulted in acquisition of CSC characteristics, including expression of Lgr5, c-myc, and DCAMKL-1 (Ali et al., 2011). A DCAMKL-1 enriched cell population was subsequently shown to form tumors with expression of proteins associated with metastatic potential in athymic nude mice. Importantly, removing the HCV replicon from these cells dramatically reduced expression of the stem cell-associated markers. The results correlated well with analysis of liver biopsies from HCV-infected patients, further highlighting the possibility that HCV promotes a CSC-like phenotype *in vivo*.

Several studies have suggested the possibility that EBV might exert its tumorigenic properties at least in part by giving rise to CSCs within the infected tissue. In an important study, Kong et al. investigated the role of EBV LMP2A protein in CSC modulation in nasopharyngeal carcinoma (NPC) cells, and showed that expression of this protein induced cell invasion and epithelial-mesenchymal transformation (EMT) (Kong et al., 2010). Overexpression of LMP2A was found to enrich stem cell like cells within the NPC tumor cell population, and increased the number of cells that were capable of re-establishing tumors in nude mice (Kong et al., 2010). These results were subsequently confirmed in NPC patient biopsies, further suggesting that a possible mechanism of tumorigenesis in EBV-infected tissues is the modulation of the tissue stem cell compartment and the induction of tumor initiating cancer stem cells. A subsequent study showed that, similar to LMP2A, EBV encoded LMP1 latent membrane protein also stimulated EMT, induced a CSC/CPC-like phenotype and enhanced the self-renewal potential in nasopharyngeal epithelial cell lines, further supporting EBV involvement in modulation of cellular plasticity and induction of CSC cellular phenotypes (Kondo et al., 2011). This notion is also highlighted by a more recent study (Lun et al., 2012), which showed up-regulation of multiple stem cell markers in an EBV-positive NPC cell line with increased tumorigenic potential and high resistance to chemotherapy. Finally, a recent study by Port et al. demonstrated that NPC is frequently associated with deregulation of the Hedgehog (HH) pathway, a pathway that is associated with stem cell maintenance. In an *in vitro* model of NPC, the authors showed that EBV activates the HH pathway through induction of the SHH ligand, which leads to increased expression of stemness-associated genes and induction of stem cell phenotypes in these cells (Port et al., 2013).

The long length of papillomavirus infection usually preceding malignant pathologies has been proposed to relate to latency of viral infection in tissue stem cells. Infected tissue stem cells may serve as tumor initiating or CSC in HPV-induced CCs. In support of this hypothesis, a study showed that the invasive and metastatic potential of cervical squamous cell carcinoma (CSCC) was correlated with cancer stem cell-associated genes, and supported the idea that high-risk HPV might induce CSC phenotypes in the TZ of the cervical epithelium (Liu et al., 2010). In addition, expression of HPV E6 and E7 viral oncogenes was shown to induce epigenetic reprogramming in human keratinocytes, through modulation of chromatin structure and global methylation/acetylation events involving cellular factors that have significant role in tumorigenesis and stemness. For example, Hyland et al. showed that E6/E7-expressing primary human foreskin keratinocytes have elevated levels of the EZH2 methyltransferase and the KDM6A demethylase, which results in a reduction of global H3K27 trimethylation and upregulation of downstream targeted HOX genes (Hyland et al., 2011). Reduction in trimethylation of H3K27 associated with elevated EZH2 was also demonstrated in high-grade squamous cervical intraepithelial lesions. In a related study, McLaughlin et al. demonstrated that repressive H3K27 trimethylation was reduced in HPV-positive cervical lesions, and that this was a result of E7-mediated induction of KDM6A and KDM6B demethylases, which subsequently lead to significantly higher expression of homeobox genes (McLaughlin-Drubin et al., 2011). These findings support the possibility that HPV-induced epigenetic reprogramming is important in viral oncogenesis, and further highlight the commonalities between stemness and carcinogenesis, at least in the context of the oncogenic virus life cycle. Further research is needed to fully understand whether HPV-associated cancers are related to cellular reprogramming of infected tissue stem cells or more differentiated cells. The impact of such reprogramming on the viral life cycle also remains unknown.

PATHWAYS TARGETED BY ONCOGENIC VIRUSES ARE ASSOCIATED WITH STEMNESS

A number of reports have shown that classic tumor suppressors and their pathways, notably p53 and pRb, which are long known to be targets of oncogenic viruses (Felsani et al., 2006; Levine, 2009), have important roles in modulation of stemness.

The p53/ARF pathway is a well-established stemness repressor and cells in which this pathway is inactivated can be more efficiently reprogrammed to pluripotency (Hanna et al., 2009; Hong et al., 2009; Kawamura et al., 2009; Li et al., 2009; Marion et al., 2009; Utikal et al., 2009). p53 was also recently found to induce miR-34a and miR-145, which negatively regulate stemness-associated factors (Xu et al., 2009; Jain et al., 2012). More recently, two separate reports further highlighted the importance of p53 in stem cell biology. Chiche et al. showed that somatic loss of p53 resulted in higher numbers of stem/progenitor cells in mammary epithelium (Chiche et al., 2013). Sato et al. reported that p53 activation promoted proteosome-dependent degradation of Nanog and differentiation of glioma stem cells (Sato et al., 2013). It is therefore possible that p53 inactivation, a common strategy of oncogenic viruses, may contribute positively to the viral life cycle in a way additional to the proposed viral escape of apoptosis of infected cells.

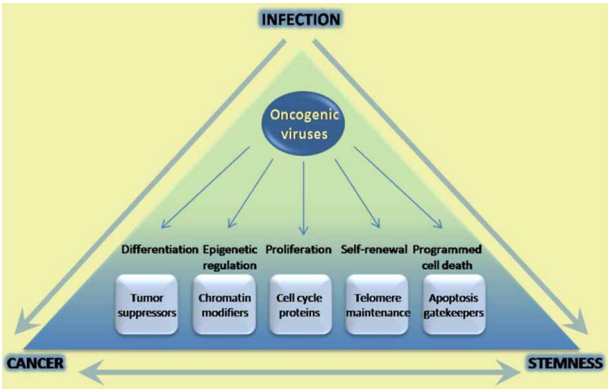


FIGURE 1 | Infection with oncogenic viruses highlights parallels in cancer and stem cell biology. Oncogenic viruses modulate a variety of cellular pathways with parallel roles in the carcinogenic process and stem cell homeostasis. The parallels between these two processes have been extensively documented, and increasingly well-understood in terms of being able to reprogram cell state. However little has been done in the way of uncovering potential roles of these pathways in infection success. Increasing understanding of common pathways modulated may yield better tools to prevent and treat infection, as well as ensuing carcinogenesis.

The retinoblastoma tumor suppressor (pRb) is another major target of oncogenic viruses, since inhibition of Rb liberates the E2F transcription factor, which stimulates entry of the cell into the cell cycle, thus favoring viral replication. Increasing evidence has implicated this pathway in stemness modulation, initially in plants (Ebel et al., 2004; Wildwater et al., 2005) and subsequently in animals (Liu et al., 2009). Accumulating evidence reinforces the role of pRb in stem cell homeostasis (Conklin and Sage, 2009). The pRb pathway was shown to have a critical role as a roadblock in the reprogramming of human fibroblasts to iPSCs, as well as cell fate determination, as elegantly shown by Calo et al. (2010). Conceivably then, like p53 inactivation, the inactivation of pRb could promote cellular plasticity and stemness, which in turn would confer an ideal niche for virus persistence and latency.

There is mounting evidence supporting the recently suggested notion that tumor suppressor pathways, traditionally key targets of oncogenic viruses, might play a significant role in cellular plasticity and modulation of stemness. Even cellular factors activated by genetic events in virally-induced cancers such as c-myc in Burkitt's lymphoma, have well-described involvement in cancer as well as stemness (Dang, 2012; Buganim et al., 2013). Therefore, it is also not surprising that factors traditionally involved in stemness and cellular plasticity are increasingly being identified as targets of oncogenic viruses. Indeed, HCV, HBV, and EBV have been shown to regulate a number of pluripotency and stem cell-associated factors (Ruf et al., 1999; Machida et al., 2009; Ali et al., 2011; Lun et al., 2012). In addition, telomerase activation and telomere maintenance are important in both cancer and stemness, and it is therefore not surprising that oncogenic viruses evolved to regulate these processes. Most, if not all, tumor viruses, including the oncogenic retrovirus HTLV-1, induce transcriptional activation of telomerase (Kuhlmann et al., 2007; Bellon and Nicot, 2008), and EBV and HPV are also known to regulate telomerase post-transcriptionally.

DISCUSSION

Oncogenic viruses cause cancer after long-term infection of their natural niche. These viruses interfere with signaling pathways that are important in a number of major cellular processes including cell proliferation and cell division, apoptosis, and cell differentiation. Accumulating evidence suggests that oncogenic viruses may also manipulate cellular stemness in various ways. Stem cells or progenitor cells are targets of infection and normal cell homeostasis is disrupted as a result. Moreover, pathways that are traditionally associated with self-renewal and lineage-commitment have been shown to be transcriptionally regulated by viral oncoproteins. Regulation of such pathways, and of oncogenic pathways now understood to play key roles in stemness, may lead to cellular reprogramming. Whether regulation of stemness is necessary for ensuing carcinogenesis, or whether it has any impact on the viral life cycle, has not been conclusively addressed. However, it is conceivable that infection of tissue stem cells might positively affect the viral life cycle, especially in terms of establishing a successful chronic infection (**Figure 1**). It should also be noted that regulation of innate immunity and inflammation, also known to be linked to carcinogenesis, is now beginning to be linked to stemness as well (e.g., TLR4-Nanog, TLR3) (Machida et al., 2009; Lee et al., 2012). Additional studies are necessary in order to fully investigate this notion, especially in the context of *in vivo* infection models. As we continue to explore the parallels between cellular stemness and the carcinogenic process, oncogenic viruses continue to serve as excellent paradigms with plenty to teach.

REFERENCES

Agami, R. (2010). microRNAs, RNA binding proteins and cancer. *Eur. J. Clin. Invest.* 40, 370–374. doi: 10.1111/j.1365-2362.2010.02279.x

Ali, N., Allam, H., May, R., Sureban, S. M., Bronze, M. S., Bader, T., et al. (2011). Hepatitis C virus-induced cancer stem cell-like signatures in cell culture and murine tumor xenografts. *J. Virol.* 85, 12292–12303. doi: 10.1128/JVI.05920-11

Amber, K., McLeod, M. P., and Nouri, K. (2013). The merkel cell polyomavirus and its involvement in merkel cell carcinoma. *Dermatol. Surg.* 39, 232–238. doi: 10.1111/dsu.12079

Arzumanyan, A., Friedman, T., Ng, I. O., Clayton, M. M., Lian, Z., and Feitelson, M. A. (2011). Does the hepatitis B antigen HBx promote the appearance of liver cancer stem cells? *Cancer Res.* 71, 3701–3708. doi: 10.1158/0008-5472.CAN-10-3951

Bajaj, B. G., Murakami, M., and Robertson, E. S. (2007). Molecular biology of EBV in relationship to AIDS-associated oncogenesis. *Cancer Treat. Res.* 133, 141–162. doi: 10.1007/978-0-387-46816-7_5

Bellon, M., and Nicot, C. (2008). Regulation of telomerase and telomeres: human tumor viruses take control. *J. Natl. Cancer Inst.* 100, 98–108. doi: 10.1093/jnci/djm269

Bonilla Guerrero, R., and Roberts, L. R. (2005). The role of hepatitis B virus integrations in the pathogenesis of human hepatocellular carcinoma. *J. Hepatol.* 42, 760–777. doi: 10.1016/j.jhep.2005.02.005

Bonizzi, G., Cicalese, A., Insinga, A., and Pelicci, P. G. (2012). The emerging role of p53 in stem cells. *Trends Mol. Med.* 18, 6–12. doi: 10.1016/j.molmed.2011.08.002

Buganim, Y., Faddah, D. A., and Jaenisch, R. (2013). Mechanisms and models of somatic cell reprogramming. *Nat. Rev. Genet.* 14, 427–439. doi: 10.1038/nrg3473

Calo, E., Quintero-Estades, J. A., Danielian, P. S., Nedelcu, S., Berman, S. D., and Lees, J. A. (2010). Rb regulates fate choice and lineage commitment *in vivo*. *Nature* 466, 1110–1114. doi: 10.1038/nature09264

Cheeran, M. C., Hu, S., Ni, H. T., Sheng, W., Palmquist, J. M., Peterson, P. K., et al. (2005). Neural precursor cell susceptibility to human cytomegalovirus diverges along glial or neuronal differentiation pathways. *J. Neurosci. Res.* 82, 839–850. doi: 10.1002/jnr.20682

Chiche, A., Moumen, M., Petit, V., Jonkers, J., Medina, D., Deugnier, M. A., et al. (2013). Somatic loss of P53 leads to Stem/Progenitor cell amplification in both mammary epithelial compartments, basal and luminal. *Stem Cells* 9, 1857–1867. doi: 10.1002/stem.1429

Coleman, C. B., Nealy, M. S., and Tibbetts, S. A. (2010). Immature and transitional B cells are latency reservoirs for a gammaherpesvirus. *J. Virol.* 84, 13045–13052. doi: 10.1128/JVI.01455-10

Collins, C. M., Boss, J. M., and Speck, S. H. (2009). Identification of infected B-cell populations by using a recombinant murine gammaherpesvirus 68 expressing a fluorescent protein. *J. Virol.* 83, 6484–6493. doi: 10.1128/JVI.00297-09

Conklin, J. F., and Sage, J. (2009). Keeping an eye on retinoblastoma control of human embryonic stem cells. *J. Cell. Biochem.* 108, 1023–1030. doi: 10.1002/jcb.22342

Cook, L. B., Elemans, M., Rowan, A. G., and Asquith, B. (2013). HTLV-1: persistence and pathogenesis. *Virology* 435, 131–140. doi: 10.1016/j.virol.2012.09.028

Dang, C. V. (2012). MYC on the path to cancer. *Cell* 149, 22–35. doi: 10.1016/j.cell.2012.03.003

da Silva-Diz, V., Sole-Sanchez, S., Valdes-Gutierrez, A., Urpi, M., Riba-Artes, D., Penin, R. M., et al. (2013). Progeny of Lgr5-expressing hair follicle stem cell contributes to papillomavirus-induced tumor development in epidermis. *Oncogene* 32, 3732–3743. doi: 10.1038/onc.2012.375

Di Trapani, M., Bassi, G., Ricciardi, M., Fontana, E., Bifari, F., Pacelli, L., et al. (2013). Comparative study of immune regulatory properties of stem cells derived from different tissues. *Stem Cells Dev.* 22, 2990–3002. doi: 10.1089/scd.2013.0204

Dziurzynski, K., Chang, S. M., Heimberger, A. B., Kalejta, R. F., McGregor Dallas, S. R., Smit, M., et al. (2012). Consensus on the role of human cytomegalovirus in glioblastoma. *Neuro Oncol.* 14, 246–255. doi: 10.1093/neuonc/nor227

Ebel, C., Mariconti, L., and Gruissem, W. (2004). Plant retinoblastoma homologues control nuclear proliferation in the female gametophyte. *Nature* 429, 776–780. doi: 10.1038/nature02637

Farrell, P. J. (2002). Tumour viruses–could they be an achilles' heel of cancer? *Eur. J. Cancer* 38, 1815–1816. doi: 10.1016/S0959-8049(02)00241-1

Felsani, A., Mileo, A. M., and Paggi, M. G. (2006). Retinoblastoma family proteins as key targets of the small DNA virus oncoproteins. *Oncogene* 25, 5277–5285. doi: 10.1038/sj.onc.1209621

Friedmann-Morvinski, D., Bushong, E. A., Ke, E., Soda, Y., Marumoto, T., Singer, O., et al. (2012). Dedifferentiation of neurons and astrocytes by oncogenes can induce gliomas in mice. *Science* 338, 1080–1084. doi: 10.1126/science.1226929

Fritschy, J. M., Brandner, S., Aguzzi, A., Koedood, M., Luscher, B., and Mitchell, P. J. (1996). Brain cell type specificity and gliosis-induced activation of the human cytomegalovirus immediate-early promoter in transgenic mice. *J. Neurosci.* 16, 2275–2282.

Fujii, Y., Yoshihashi, K., Suzuki, H., Tsutsumi, S., Mutoh, H., Maeda, S., et al. (2012). CDX1 confers intestinal phenotype on gastric epithelial cells via induction of stemness-associated reprogramming factors SALL4 and KLF5. *Proc. Natl. Acad. Sci. U.S.A.* 109, 20584–20589. doi: 10.1073/pnas.1208651109

Hanna, J., Saha, K., Pando, B., van Zon, J., Lengner, C. J., Creyghton, M. P., et al. (2009). Direct cell reprogramming is a stochastic process amenable to acceleration. *Nature* 462, 595–601. doi: 10.1038/nature08592

Harkins, L., Volk, A. L., Samanta, M., Mikolaenko, I., Britt, W. J., Bland, K. I., et al. (2002). Specific localisation of human cytomegalovirus nucleic acids and proteins in human colorectal cancer. *Lancet* 360, 1557–1563. doi: 10.1016/S0140-6736(02)11524-8

Henry, M., Uthman, A., Geusau, A., Rieger, A., Furci, L., Lazzarin, A., et al. (1999). Infection of circulating CD34+ cells by HHV-8 in patients with kaposi's sarcoma. *J. Invest. Dermatol.* 113, 613–616. doi: 10.1046/j.1523-1747.1999.00733.x

Hong, H., Takahashi, K., Ichisaka, T., Aoi, T., Kanagawa, O., Nakagawa, M., et al. (2009). Suppression of induced pluripotent stem cell generation by the p53-p21 pathway. *Nature*. 460, 1132–1135. doi: 10.1038/nature08235

Hyland, P. L., McDade, S. S., McCloskey, R., Dickson, G. J., Arthur, K., McCance, D. J., et al. (2011). Evidence for alteration of EZH2, BMI1, and KDM6A and epigenetic reprogramming in human papillomavirus type 16 E6/E7-expressing keratinocytes. *J. Virol.* 85, 10999–11006. doi: 10.1128/JVI.00160-11

Jain, A. K., Allton, K., Iacovino, M., Mahen, E., Milczarek, R. J., Zwaka, T. P., et al. (2012). p53 regulates cell cycle and microRNAs to promote differentiation of human embryonic stem cells. *PLoS Biol.* 10:e1001268. doi: 10.1371/journal.pbio.1001268

Jeong, S. W., Jang, J. Y., and Chung, R. T. (2012). Hepatitis C virus and hepatocarcinogenesis. *Clin. Mol. Hepatol.* 18, 347–356. doi: 10.3350/cmh.2012.18.4.347

Kawamura, T., Suzuki, J., Wang, Y. V., Menendez, S., Morera, L. B., Raya, A., et al. (2009). Linking the p53 tumour suppressor pathway to somatic cell reprogramming. *Nature* 460, 1140–1144. doi: 10.1038/nature08311

Kofman, A., Marcinkiewicz, L., Dupart, E., Lyshchev, A., Martynov, B., Ryndin, A., et al. (2011). The roles of viruses in brain tumor initiation and oncomodulation. *J. Neurooncol.* 105, 451–466. doi: 10.1007/s11060-011-0658-6

Kondo, S., Wakisaka, N., Muramatsu, M., Zen, Y., Endo, K., Murono, S., et al. (2011). Epstein-barr virus latent membrane protein 1 induces cancer stem/progenitor-like cells in nasopharyngeal epithelial cell lines. *J. Virol.* 85, 11255–11264. doi: 10.1128/JVI.00188-11

Kong, Q. L., Hu, L. J., Cao, J. Y., Huang, Y. J., Xu, L. H., Liang, Y., et al. (2010). Epstein-barr virus-encoded LMP2A induces an epithelial-mesenchymal transition and increases the number of side population stem-like cancer cells in nasopharyngeal carcinoma. *PLoS Pathog.* 6:e1000940. doi: 10.1371/journal.ppat.1000940

Kuhlmann, A. S., Villaudy, J., Gazzolo, L., Castellazzi, M., Mesnard, J. M., and Duc Dodon, M. (2007). HTLV-1 HBZ cooperates with JunD to enhance transcription of the human telomerase reverse transcriptase gene (hTERT). *Retrovirology* 4:92. doi: 10.1186/1742-4690-4-92

Lapouge, G., Youssef, K. K., Vokaer, B., Achouri, Y., Michaux, C., Sotiropoulou, P. A., et al. (2011). Identifying the cellular origin of squamous skin tumors. *Proc. Natl. Acad. Sci. U.S.A.* 108, 7431–7436. doi: 10.1073/pnas.1012720108

Lee, J., Sayed, N., Hunter, A., Au, K. F., Wong, W. H., Mocarski, E. S., et al. (2012). Activation of innate immunity is required for efficient nuclear reprogramming. *Cell* 151, 547–558. doi: 10.1016/j.cell.2012.09.034

Levine, A. J. (2009). The common mechanisms of transformation by the small DNA tumor viruses: the inactivation of tumor suppressor gene products: P53. *Virology* 384, 285–293. doi: 10.1016/j.virol.2008.09.034

Li, H., Collado, M., Villasante, A., Strati, K., Ortega, S., Canamero, M., et al. (2009). The Ink4/Arf locus is a barrier for iPS cell reprogramming. *Nature* 460, 1136–1139. doi: 10.1038/nature08290

Liu, W. K., Jiang, X. Y., and Zhang, Z. X. (2010). Expression of PSCA, PIWIL1 and TBX2 and its correlation with HPV16 infection in formalin-fixed, paraffin-embedded cervical squamous cell carcinoma specimens. *Arch. Virol.* 155, 657–663. doi: 10.1007/s00705-010-0635-y

Liu, Y., Clem, B., Zuba-Surma, E. K., El-Naggar, S., Telang, S., Jenson, A. B., et al. (2009). Mouse fibroblasts lacking RB1 function form spheres and undergo reprogramming to a cancer stem cell phenotype. *Cell Stem Cell* 4, 336–347. doi: 10.1016/j.stem.2009.02.015

Lokensgard, J. R., Cheeran, M. C., Gekker, G., Hu, S., Chao, C. C., and Peterson, P. K. (1999). Human cytomegalovirus replication and modulation of apoptosis in astrocytes. *J. Hum. Virol.* 2, 91–101.

Lopez, J., Ruiz, G., Organista-Nava, J., Gariglio, P., and Garcia-Carranca, A. (2012). Human papillomavirus infections and cancer stem cells of tumors from the uterine cervix. *Open Virol. J.* 6, 232–240. doi: 10.2174/1874357901206010232

Lu, X., Mazur, S. J., Lin, T., Appella, E., and Xu, Y. (2013). The pluripotency factor nanog promotes breast cancer tumorigenesis and metastasis. *Oncogene.* doi: 10.1038/onc.2013.209. [Epub ahead of print].

Lun, S. W., Cheung, S. T., Cheung, P. F., To, K. F., Woo, J. K., Choy, K. W., et al. (2012). CD44+ cancer stem-like cells in EBV-associated nasopharyngeal carcinoma. *PLoS ONE* 7:e52426. doi: 10.1371/journal.pone.0052426

Luppi, M., Barozzi, P., Schulz, T. F., Setti, G., Staskus, K., Trovato, R., et al. (2000). Bone marrow failure associated with human herpesvirus 8 infection after transplantation. *N. Engl. J. Med.* 343, 1378–1385. doi: 10.1056/NEJM200011093431905

Machida, K., Chen, C. L., Liu, J. C., Kashiwabara, C., Feldman, D., French, S. W., et al. (2012). Cancer stem cells generated by alcohol, diabetes, and hepatitis C virus. *J. Gastroenterol. Hepatol.* 27(Suppl. 2), 19–22. doi: 10.1111/j.1440-1746.2011.07010.x

Machida, K., Tsukamoto, H., Mkrtchyan, H., Duan, L., Dynnyk, A., Liu, H. M., et al. (2009). Toll-like receptor 4 mediates synergism between alcohol and HCV

in hepatic oncogenesis involving stem cell marker nanog. *Proc. Natl. Acad. Sci. U.S.A.* 106, 1548–1553. doi: 10.1073/pnas.0807390106

Maglennon, G. A., McIntosh, P., and Doorbar, J. (2011). Persistence of viral DNA in the epithelial basal layer suggests a model for papillomavirus latency following immune regression. *Virology* 414, 153–163. doi: 10.1016/j.virol.2011.03.019

Marion, R. M., Strati, K., Li, H., Murga, M., Blanco, R., Ortega, S., et al. (2009). A p53-mediated DNA damage response limits reprogramming to ensure iPS cell genomic integrity. *Nature* 460, 1149–1153. doi: 10.1038/nature08287

Marques, S., Efstathiou, S., Smith, K. G., Haury, M., and Simas, J. P. (2003). Selective gene expression of latent murine gammaherpesvirus 68 in B lymphocytes. *J. Virol.* 77, 7308–7318. doi: 10.1128/JVI.77.13.7308-7318.2003

McLaughlin-Drubin, M. E., Crum, C. P., and Münger, K. (2011). Human papillomavirus E7 oncoprotein induces KDM6A and KDM6B histone demethylase expression and causes epigenetic reprogramming. *Proc. Natl. Acad. Sci. U.S.A.* 108, 2130–2135. doi: 10.1073/pnas.1009933108

Michael, S., Lambert, P. F., and Strati, K. (2013). The HPV16 oncogenes cause aberrant stem cell mobilization. *Virology* 443, 210–225. doi: 10.1016/j.virol.2013.04.008

Michaelis, M., Doerr, H. W., Cinatl, J. (2009). The story of human cytomegalovirus and cancer: increasing evidence and open questions. *Neoplasia* 11, 1–9. doi: 10.1593/neo.81178

Odeberg, J., Wolmer, N., Falci, S., Westgren, M., Sundtrom, E., Seiger, A., et al. (2007). Late human cytomegalovirus (HCMV) proteins inhibit differentiation of human neural precursor cells into astrocytes. *J. Neurosci. Res.* 85, 583–593. doi: 10.1002/jnr.21144

Perlman, J. M., and Argyle, C. (1992). Lethal cytomegalovirus infection in preterm infants: clinical, radiological, and neuropathological findings. *Ann. Neurol.* 31, 64–68. doi: 10.1002/ana.410310112

Port, R. J., Pinheiro-Maia, S., Hu, C., Arrand, J. R., Wei, W., Young, L. S., et al. (2013). Epstein-barr virus induction of the hedgehog signalling pathway imposes a stem cell phenotype on human epithelial cells. *J. Pathol.* 231, 367–377. doi: 10.1002/path.4245

Ruf, I. K., Rhyne, P. W., Yang, H., Borza, C. M., Hutt-Fletcher, L. M., Cleveland, J. L., et al. (1999). Epstein-barr virus regulates c-MYC, apoptosis, and tumorigenicity in burkitt lymphoma. *Mol. Cell. Biol.* 19, 1651–1660.

Saha, A., Kaul, R., Murakami, M., and Robertson, E. S. (2010). Tumor viruses and cancer biology: modulating signaling pathways for therapeutic intervention. *Cancer Biol. Ther.* 10, 961–978. doi: 10.4161/cbt.10.10.13923

Samanta, M., Harkins, L., Klemm, K., Britt, W. J., and Cobbs, C. S. (2003). High prevalence of human cytomegalovirus in prostatic intraepithelial neoplasia and prostatic carcinoma. *J. Urol.* 170, 998–1002. doi: 10.1097/01.ju.0000080263.46164.97

Sato, A., Okada, M., Shibuya, K., Watanabe, E., Seino, S., Suzuki, K., et al. (2013). Resveratrol promotes proteasome-dependent degradation of nanog via p53 activation and induces differentiation of glioma stem cells. *Stem Cell Res.* 11, 601–610. doi: 10.1016/j.scr.2013.04.004

Schiffman, M., Castle, P. E., Jeronimo, J., Rodriguez, A. C., and Wacholder, S. (2007). Human papillomavirus and cervical cancer. *Lancet* 370, 890–907. doi: 10.1016/S0140-6736(07)61416-0

Seri, B., Herrera, D. G., Gritti, A., Ferron, S., Collado, L., Vescovi, A., et al. (2006). Composition and organization of the SCZ: a large germinal layer containing neural stem cells in the adult mammalian brain. *Cereb. Cortex* 16(Suppl. 1), i103–i111. doi: 10.1093/cercor/bhk027

Soderberg-Naucler, C. (2006). Does cytomegalovirus play a causative role in the development of various inflammatory diseases and cancer? *J. Intern. Med.* 259, 219–246. doi: 10.1111/j.1365-2796.2006.01618.x

Soroceanu, L., Akhavan, A., and Cobbs, C. S. (2008). Platelet-derived growth factor-alpha receptor activation is required for human cytomegalovirus infection. *Nature* 455, 391–395. doi: 10.1038/nature07209

Tseng, H. C., Arasteh, A., Paranjpe, A., Teruel, A., Yang, W., Behel, A., et al. (2010). Increased lysis of stem cells but not their differentiated cells by natural killer cells; de-differentiation or reprogramming activates NK cells. *PLoS ONE* 5:e11590. doi: 10.1371/journal.pone.0011590

Utikal, J., Polo, J. M., Stadtfeld, M., Maherali, N., Kulalert, W., Walsh, R. M., et al. (2009). Immortalization eliminates a roadblock during cellular reprogramming into iPS cells. *Nature* 460, 1145–1148. doi: 10.1038/nature08285

Wegner, M. (2013). Mighty bugs: leprosy bacteria turn schwann cells into stem cells. *Cell* 152(1–2), 15–6. doi: 10.1016/j.cell.2013.01.001

Wildwater, M., Campilho, A., Perez-Perez, J. M., Heidstra, R., Blilou, I., Korthout, H., et al. (2005). The RETINOBLASTOMA-RELATED gene regulates stem cell maintenance in arabidopsis roots. *Cell* 123, 1337–1349. doi: 10.1016/j.cell.2005.09.042

Wu, W., Vieira, J., Fiore, N., Banerjee, P., Sieburg, M., Rochford, R., et al. (2006). KSHV/HHV-8 infection of human hematopoietic progenitor (CD34+) cells: Persistence of infection during hematopoiesis *in vitro* and *in vivo*. *Blood* 108, 141–151. doi: 10.1182/blood-2005-04-1697

Xu, N., Papagiannakopoulos, T., Pan, G., Thomson, J. A., and Kosik, K. S. (2009). MicroRNA-145 regulates OCT4, SOX2, and KLF4 and represses pluripotency in human embryonic stem cells. *Cell* 137, 647–658. doi: 10.1016/j.cell.2009.02.038

Zhang, J., Espinoza, L. A., Kinders, R. J., Lawrence, S. M., Pfister, T. D., Zhou, M., et al. (2012). NANOG modulates stemness in human colorectal cancer. *Oncogene* 32, 4397–4405. doi: 10.1038/onc.2012.461

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 01 August 2013; paper pending published: 05 September 2013; accepted: 03 December 2013; published online: 25 December 2013.

*Citation: Iacovides D, Michael S, Achilleos C and Strati K (2013) Shared mechanisms in stemness and carcinogenesis: lessons from oncogenic viruses. Front. Cell. Infect. Microbiol. **3**:66. doi: 10.3389/fcimb.2013.00066*

This article was submitted to the journal Frontiers in Cellular and Infection Microbiology.

frontiers in
CELLULAR AND INFECTION MICROBIOLOGY

OPINION ARTICLE
published: 18 October 2013
doi: 10.3389/fcimb.2013.00067

Apoptosis in *C. elegans*: lessons for cancer and immunity

Marios Arvanitis[1†], De-Dong Li[1,2†], Kiho Lee[1] and Eleftherios Mylonakis[1]*

[1] *Department of Medicine, Division of Infectious Diseases, Rhode Island Hospital, Warren Alpert Medical School of Brown University, Providence, RI, USA*
[2] *School of Pharmacy, Second Military Medical University, Shanghai, China*
**Correspondence: emylonakis@lifespan.org*
[†] *These authors have contributed equally to this work.*

Edited by:
Yiorgos Apidianakis, University of Cyprus, Cyprus

Reviewed by:
Yiorgos Apidianakis, University of Cyprus, Cyprus

Keywords: *Caenorhabditis elegans*, apoptosis, cancer, immunity, cell death

INTRODUCTION

Since 1974, when Sydney Brenner first introduced *Caenorhabditis elegans* in the scientific community as a model organism (Brenner, 1974), the nematode has been extensively used as a model system to study cellular biology. One of the most intriguing fields of molecular biology that have been investigated using this model host and that led to the 2002 Nobel Prize in Physiology or Medicine, is programmed cell death. Apoptosis is an evolutionarily conserved method employed by multicellular organisms to maintain tissue homeostasis during development and differentiation (Kuranaga, 2011), but can also serve as a way to prevent growth of cells mutated due to DNA damage (Bailly and Gartner, 2013).

THE MAIN APOPTOTIC PATHWAY

In *C. elegans*, apoptosis is a normal component of growth. During development 1090 somatic cells are generated for each hermaphrodite, of which 131 invariantly undergo apoptosis (Sulston and Horvitz, 1977; Sulston et al., 1983). Interestingly, the main effectors of apoptosis in the worm are conserved in mammalian organisms. Indeed, the main apoptotic pathway in the nematode starts with the activation of EGL-1 in the cells that are destined to die. EGL-1 is a BH3 only protein which, when activated, binds to and inhibits CED-9, the only BCL-2-like protein in *C. elegans*, and thus negates its inhibiting effect on CED-4. CED-4 is the analog of mammalian APAF-1 and it serves as an activator of CED-3, a caspase, which then leads to cell death (Gartner et al., 2008).

Under-expression or mutation of effectors within the apoptotic pathway that are conserved in this model host has been known to lead to uncontrolled cellular proliferation and cancer in mammalian organisms. Indeed, *bcl-2* mutations are found in a wide range of human cancers (Ciardiello and Tortora, 2002). In support of this notion, a recent article reported an alternative mechanism of programmed cell-death activation on the nematode that involves inactivation of CED-9 by DRE-1. DRE-1 is the worm analog of the human protein FBXO10, which is known to be mutated or expressed at low levels in human diffuse large B-cell lymphomas (Chiorazzi et al., 2013).

Meanwhile, apoptosis seems to play an active role in *C. elegans* innate immunity as was shown by three pivotal studies. In the first one, Aballay et al. found that *Salmonella typhimurium* colonization of the *C. elegans* intestine leads to an increased level of cell death in the worm, dependent on the well-characterized EGL-1/CED-9/CED-4/CED-3 pathway (Aballay and Ausubel, 2001). In an ensuing article, the same researchers proved that *Salmonella*-induced apoptosis requires the *C. elegans* homolog of the mammalian p38 mitogen-activated protein kinase (MAPK) encoded by the *pmk-1* gene, a well-characterized and conserved innate immune effector (Aballay et al., 2003). Inactivation of *pmk-1* by RNAi blocked *Salmonella*-elicited *C. elegans* apoptosis, and epistasis analysis showed that CED-9 lies downstream of PMK-1 (Aballay et al., 2003). These results suggest that the apoptosis and immune response pathways are linked at some point to ensure the survival of the multicellular organism. A potential explanation for this link would be that apoptosis might be triggered by the host immune system to serve a protective role against the infectious process by eliminating infected cells thus hindering the dissemination of the invading pathogen. Interestingly, a similar relationship between immunity and apoptosis was recently shown in *Drosophila melanogaster* flies (Apidianakis et al., 2009). Specifically, the researchers investigated a *Pseudomonas aeruginosa* gut infection model in flies and found that the infection activated the c-Jun-N-terminal Kinase (JNK) pathway which in turn promoted apoptosis of infected enterocytes. Further, this phenomenon led to a subsequent over-proliferation of intestinal stem cells as a compensatory mechanism, thus suggesting a close interaction between immunity and pathways that control cell fate. In another article, researchers found that DAPK-1, the *C. elegans* ortholog of the tumor suppressor death-associated protein kinase, which is a known regulator of apoptosis and autophagy, decreases innate immune responses to barrier damage, thus protecting the worms from inflammation due to uncontrolled over activation of their immune system (Tong et al., 2009). This finding reveals a unique interplay between apoptosis, inflammation and cancer, suggesting that, throughout evolution, programmed cell death has acquired the role of protecting the organism from a wide variety of environmental insults.

APOPTOSIS AND DNA DAMAGE RESPONSES

The apoptotic pathway is directly linked to DNA damage control. To prevent growth of transformed cells, cell-cycle control proteins inhibit mitotic progression and promote apoptosis in response to DNA damage signals. These proteins are known as tumor suppression proteins and perhaps the most widely acknowledged

among them is p53. Indeed, mutations in p53 have been found invariably in almost all different types of human cancer (Goh et al., 2011). The only p53-like protein in *C. elegans* is encoded by *cep-1* that is required for DNA-damage and UV-induced apoptosis. In the nematode, p53 is activated in response to DNA damage response signals and it induces *egl-1* and *ced-13* that encodes another BH3 only protein that serves in parallel to EGL-1 to promote apoptotic pathway initiation in such cases (Bailly and Gartner, 2013).

Interestingly, p53 is highly regulated in all organisms. In *C. elegans*, the main regulatory protein of p53 seems to be ATR, a serine/threonine protein kinase that recognizes single-stranded DNA generated by the recession of double-strand DNA breaks (Bailly and Gartner, 2013). However, recent studies have shown that there are other proteins that can regulate p53 either in parallel or together with ATR. For example, deletion of the gene encoding histone demethylase JMJD2, the human homologs of which are amplified in cases of cancer, slows DNA replication, blocks progression to S phase, and promotes ATR/p53-dependent apoptosis in the nematodes (Black et al., 2010). Further, a pivotal article elucidated the connection between Hypoxia-Inducible Factor 1 (HIF-1), a protein that is found to be upregulated in solid tumors and is associated with cancer prognosis, and apoptosis. The researchers used *C. elegans* to show that HIF-1 upregulates TYR-2, a member of the tyrosinase family in sensory neurons, which is then secreted and acts on the germline to antagonize *cep-1* dependent apoptosis (Sendoel et al., 2010). This observation not only identifies a potential adjunctive therapeutic target for tumors carrying the increased *hif-1* phenotype, but also shows that inhibition of apoptosis can sometimes be a non-autonomous cell response in multicellular organisms.

Importantly, an interesting interplay between pathways that are related to aging, cancer, and apoptosis was suggested by Perrin et al. (2013). The researchers investigated the interactions between DAF-2 (an insulin/IGF-1 homolog associated with aging), CEP-1, and AKT-1 (a protein that belongs to the Protein Kinase B/AKT family of protein kinases that are implicated in a wide range of human cancers). While AKT-1 inhibits CEP-1 and thus decreases DNA damage-induced apoptosis, DAF-2 antagonizes these effects and promotes apoptosis by parallel pathways through inhibition of AKT-2 and activation of *Ras* signaling. Therefore, the insulin/IGF receptors could serve as potential targets in AKT-dependent cancers.

Finally, other significant and recently identified effectors of the DNA damage response mechanism are the microRNAs (miRNAs), which have been shown to have an altered expression in tumor tissues and are implicated in the regulation of cellular response to radiation-induced DNA damage. Importantly, in a recent article, researchers investigated the role of miR-34, a conserved type of miRNA, on *C. elegans* and found that miR-34 has a differential effect in apoptotic vs. non-apoptotic cell death after radiation. In fact, miR-34 was shown to protect cells from non-apoptotic death while serving a role in promoting apoptosis (Kato et al., 2009). The significance of this finding becomes evident when one considers that the main response to radiation in some types of cancer is non-apoptotic death. Therefore, compounds that are able to lower miR-34 levels in these malignant cells could serve as important adjuncts to radiotherapy.

APOPTOTIC CORPSE CLEARANCE

The final piece of the apoptotic machinery is the engulfment and degradation of the apoptotic corpse which is induced by certain signals expressed on the membrane of the dying cells. Notably, the most well-recognized of these signals, a protein known as phosphatidylserine, has been proven to serve a substantial role in protecting mammalian organisms from lung inflammatory disorders (Savill et al., 2003), thus establishing the role of this pathway as a method to prevent inappropriate immune activation due to accumulating dead cell remnants. In *C. elegans*, the engulfment is mediated by two pathways that include multiple proteins like CED-1, CED-2, CED-5, CED-6, CED-7, CED-12, and both converge at CED-10 (a Rac family GTPase) (Kinchen et al., 2005), while the degradation is mediated by signals involving the RAB-5 protein and ending with lysosomal degradation of the corpse (Conradt and Xue, 2005). Contributing to the well-appreciated notion that disturbance of the corpse degradation pathway can be related to various autoimmune disorders (Nagata et al., 2010), Haskin et al. described a molecular link between CED-1 and innate immunity in *C. elegans* (Haskins et al., 2008). They found that in *C. elegans*, CED-1 upregulates a family of genes encoding proteins with prion-like glutamine/asparagine (Q/N)-rich domains, known to be activated by ER stress and thought to aid in the unfolded protein response (Urano et al., 2002), thus rendering *ced-1* mutant worms immunocompromised and very susceptible to *Salmonella enterica* infection. These findings indicate that *ced-1* is required for the transcriptional activation of an unfolded protein response pathway essential for proper response to invading pathogens (Lamitina and Cherry, 2008). Despite the fact that the investigators suggested that the function of CED-1 in innate immunity is not dependent upon its function in apoptotic corpse engulfment, the importance of this observation cannot be overlooked as it implies that at least some of the effectors of the engulfment pathway can in fact have multiple roles, functioning to protect the organism against noxious stimuli resulting either from within the cell (in case of abnormally folded proteins) or from its surrounding environment (in the case of apoptotic corpse clearance).

On the other hand, the interaction between dying cell removal and cancer is less clear. In a recent article, researchers showed that *sli-1*, the homolog of the mammalian proto-oncogene *c-Cbl*, is able to inhibit engulfment of the dying cells through a previously unidentified pathway (Anderson et al., 2012). More importantly though, in another study, Suzuki et al. showed that XK-family proteins promote phosphatidylserine exposure on the membrane of dying cells in response to apoptotic signals and found that XK-Related Protein 8, a member of the XK family, is epigenetically repressed in some types of human cancer cells (Suzuki et al., 2013). Both of these findings suggest a mechanistic association between autoimmunity and cancer in which apoptotic corpse degradation seems to have a central role.

CONCLUSIONS

It is evident that research based on *C. elegans* has provided us with a wide variety of previously unrecognized interactions

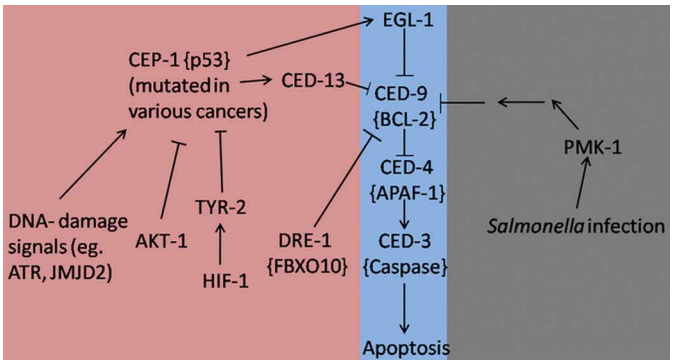


FIGURE 1 | Apoptosis signaling pathways in *C. elegans* during development (Purple), stress/infection (Gray), and hyperproliferation (Pink). Lines with arrowheads represent activation while lines with barheads represent deactivation. Mammalian homologs are shown in brackets when applicable.

between programmed cell death and pathways that contribute to immunity or lead to cancer (**Figure 1**). It is now widely accepted that the apoptotic machinery serves a much wider role in multicellular organisms than what was previously acknowledged. It seems that this pathway is necessary to maintain tissue homeostasis not only under normal development but especially under conditions that are associated with cellular stress. In fact, apoptosis should be considered as the last physiologic safeguard in response to environmental insults. When all other repair mechanisms fail, programmed cell death is activated as a failsafe mechanism to sacrifice the affected cells for the greater good of the organism. Keeping this simple principle in mind, it is easy to deduce the interactions between apoptosis, cancer, and immunity. More specifically, infectious processes are well-recognized environmental insults, therefore programmed cell death can aid in preventing the dissemination of the pathogen especially when it comes to intracellular microbes. Similarly, in the case of cancer-inducing insults, like ionizing radiation, the apoptotic machinery is triggered in an effort to kill the malignant cells and protect the host from their uncontrolled proliferation that could be detrimental to its well-being. On the other hand, uncontrolled activation of programmed cell death can negatively impact the organism. Therefore, finding a way to specifically induce the apoptotic pathway in the affected cells could provide us with a powerful weapon in our fight against human diseases, like cancer and infectious processes.

REFERENCES

Aballay, A., and Ausubel, F. M. (2001). Programmed cell death mediated by ced-3 and ced-4 protects *Caenorhabditis elegans* from Salmonella typhimurium-mediated killing. *Proc. Natl. Acad. Sci. U.S.A.* 98, 2735–2739. doi: 10.1073/pnas.041613098

Aballay, A., Drenkard, E., Hilbun, L. R., and Ausubel, F. M. (2003). *Caenorhabditis elegans* innate immune response triggered by *Salmonella enterica* requires intact LPS and is mediated by a MAPK signaling pathway. *Curr. Biol.* 13, 47–52. doi: 10.1016/S0960-9822(02)01396-9

Anderson, C., Zhou, S., Sawin, E., Horvitz, H. R., and Hurwitz, M. E. (2012). SLI-1 Cbl inhibits the engulfment of apoptotic cells in *C. elegans* through a ligase-independent function. *PLoS Genet.* 8:e1003115. doi: 10.1371/journal.pgen.1003115

Apidianakis, Y., Pitsouli, C., Perrimon, N., and Rahme, L. (2009). Synergy between bacterial infection and genetic predisposition in intestinal dysplasia. *Proc. Natl. Acad. Sci. U.S.A.* 106, 20883–20888. doi: 10.1073/pnas.0911797106

Bailly, A., and Gartner, A. (2013). Germ cell apoptosis and DNA damage responses. *Adv. Exp. Med. Biol.* 757, 249–276. doi: 10.1007/978-1-4614-4015-4_9

Black, J. C., Allen, A., Van Rechem, C., Forbes, E., Longworth, M., Tschop, K., et al. (2010). Conserved antagonism between JMJD2A/KDM4A and HP1gamma during cell cycle progression. *Mol. Cell* 40, 736–748. doi: 10.1016/j.molcel.2010.11.008

Brenner, S. (1974). The genetics of *Caenorhabditis elegans*. *Genetics* 77, 71–94.

Chiorazzi, M., Rui, L., Yang, Y., Ceribelli, M., Tishbi, N., Maurer, C. W., et al. (2013). Related F-box proteins control cell death in *Caenorhabditis elegans* and human lymphoma. *Proc. Natl. Acad. Sci. U.S.A.* 110, 3943–3948. doi: 10.1073/pnas.1217271110

Ciardiello, F., and Tortora, G. (2002). Inhibition of bcl-2 as cancer therapy. *Ann. Oncol.* 13, 501–502. doi: 10.1093/annonc/mdf191

Conradt, B., and Xue, D. (2005). "Programmed cell death," in *WormBook*, ed The *C. elegans* Research Community (WormBook). doi: 10.1895/wormbook.1.32.1. Available online at: http://www.wormbook.org

Gartner, A., Boag, P. R., and Blackwell, T. K. (2008). "Germline survival and apoptosis," in *WormBook*, ed The *C. elegans* Research Community (WormBook). doi: 10.1895/wormbook.1.145.1. Available online at: http://www.wormbook.org

Goh, A. M., Coffill, C. R., and Lane, D. P. (2011). The role of mutant p53 in human cancer. *J. Pathol.* 223, 116–126. doi: 10.1002/path.2784

Haskins, K. A., Russell, J. F., Gaddis, N., Dressman, H. K., and Aballay, A. (2008). Unfolded protein response genes regulated by CED-1 are required for *Caenorhabditis elegans* innate immunity. *Dev. Cell* 15, 87–97. doi: 10.1016/j.devcel.2008.05.006

Kato, M., Paranjape, T., Muller, R. U., Nallur, S., Gillespie, E., Keane, K., et al. (2009). The mir-34 microRNA is required for the DNA damage response *in vivo* in *C. elegans* and *in vitro* in human breast cancer cells. *Oncogene* 28, 2419–2424. doi: 10.1038/onc.2009.106

Kinchen, J. M., Cabello, J., Klingele, D., Wong, K., Feichtinger, R., Schnabel, H., et al. (2005). Two pathways converge at CED-10 to mediate actin rearrangement and corpse removal in *C. elegans*. *Nature* 434, 93–99. doi: 10.1038/nature03263

Kuranaga, E. (2011). Caspase signaling in animal development. *Dev. Growth Differ.* 53, 137–148. doi: 10.1111/j.1440-169X.2010.01237.x

Lamitina, T., and Cherry, S. (2008). Dangerous liaisons: the apoptotic engulfment receptor CED-1 links innate immunity to the unfolded protein response. *Dev. Cell* 15, 3–4. doi: 10.1016/j.devcel.2008.06.007

Nagata, S., Hanayama, R., and Kawane, K. (2010). Autoimmunity and the clearance of dead cells. *Cell* 140, 619–630. doi: 10.1016/j.cell.2010.02.014

Perrin, A. J., Gunda, M., Yu, B., Yen, K., Ito, S., Forster, S., et al. (2013). Noncanonical control of *C. elegans* germline apoptosis by the insulin/IGF-1 and Ras/MAPK signaling pathways. *Cell Death Differ.* 20, 97–107. doi: 10.1038/cdd.2012.101

Savill, J., Gregory, C., and Haslett, C. (2003). Cell biology. Eat me or die. *Science* 302, 1516–1517. doi: 10.1126/science.1092533

Sendoel, A., Kohler, I., Fellmann, C., Lowe, S. W., and Hengartner, M. O. (2010). HIF-1 antagonizes p53-mediated apoptosis through a secreted neuronal tyrosinase. *Nature* 465, 577–583. doi: 10.1038/nature09141

Sulston, J. E., and Horvitz, H. R. (1977). Post-embryonic cell lineages of the nematode, *Caenorhabditis elegans*. *Dev. Biol.* 56, 110–156. doi: 10.1016/0012-1606(77)90158-0

Sulston, J. E., Schierenberg, E., White, J. G., and Thomson, J. N. (1983). The embryonic cell lineage of the nematode *Caenorhabditis elegans*.

Dev. Biol. 100, 64–119. doi: 10.1016/0012-1606(83)90201-4

Suzuki, J., Denning, D. P., Imanishi, E., Horvitz, H. R., and Nagata, S. (2013). Xk-related protein 8 and CED-8 promote phosphatidylserine exposure in apoptotic cells. *Science* 341, 403–406. doi: 10.1126/science.1236758

Tong, A., Lynn, G., Ngo, V., Wong, D., Moseley, S. L., Ewbank, J. J., et al. (2009). Negative regulation of *Caenorhabditis elegans* epidermal damage responses by death-associated protein kinase. *Proc. Natl. Acad. Sci. U.S.A.* 106, 1457–1461. doi: 10.1073/pnas.0809339106

Urano, F., Calfon, M., Yoneda, T., Yun, C., Kiraly, M., Clark, S. G., et al. (2002). A survival pathway for *Caenorhabditis elegans* with a blocked unfolded protein response. *J. Cell Biol.* 158, 639–646. doi: 10.1083/jcb.200203086

Received: 15 August 2013; accepted: 03 October 2013; published online: 18 October 2013.

*Citation: Arvanitis M, Li D-D, Lee K and Mylonakis E (2013) Apoptosis in C. elegans: lessons for cancer and immunity. Front. Cell. Infect. Microbiol. **3**:67. doi: 10.3389/fcimb.2013.00067*

This article was submitted to the journal Frontiers in Cellular and Infection Microbiology.

frontiers in
CELLULAR AND INFECTION MICROBIOLOGY

MINI REVIEW ARTICLE
published: 19 December 2013
doi: 10.3389/fcimb.2013.00103

Drosophila at the intersection of infection, inflammation, and cancer

Erdem Bangi*

Department of Developmental and Regenerative Biology, Icahn School of Medicine at Mount Sinai, New York, NY, USA

Edited by:
Yiorgos Apidianakis, University of Cyprus, Cyprus

Reviewed by:
Terry Kwok, Monash University, Australia
Sung Ouk Kim, University of Western Ontario, Canada

****Correspondence:***
Erdem Bangi, Department of Developmental and Regenerative Biology, Icahn School of Medicine at Mount Sinai, One Gustave L. Levy Place, Annenberg 25-40 New York, NY 10029, USA
e-mail: erdem.bangi@mssm.edu

Recent studies show that both cellular and humoral aspects of innate immunity play important roles during tumor progression. These interactions have traditionally been explored in vertebrate model systems. In recent years, Drosophila has emerged as a genetically tractable model system for studying key aspects of tumorigenesis including proliferation, invasion, and metastasis. The absence of adaptive immunity in Drosophila provides a unique opportunity to study the interactions between innate immune system and cancer in different genetic contexts. In this review, I discuss recent advances made by using Drosophila models of cancer to study the role of innate immune pathways Toll/Imd, JNK, and JAK-STAT, microbial infection and inflammation during tumor progression.

Keywords: Drosophila, innate immune response, inflammation, infection, cancer

The interaction between the tumor and the immune system is a complex, multi-step process in which both innate and adaptive branches of the immune system participate (Finn, 2012). The outcome of this antitumor response is variable and unpredictable; it can be tumor suppressive or tumor promoting depending on the immunogenicity and genetic composition of the tumor and the strength of patient's immune response (Finn, 2012). Several recent studies report that at least some aspects of the relationship between the immune system and cancer are also conserved in flies (Pastor-Pareja et al., 2008; Apidianakis et al., 2009; Cordero et al., 2010; Bangi et al., 2012): Drosophila immune system also recognizes and responds to tumors and this response can be tumor promoting or tumor suppressive depending on the genetic composition of the tumor. Here, I briefly summarize studies that use Drosophila to explore the role of the innate immune system during tumor progression.

INFLAMMATION, TUMOR ASSOCIATED HEMOCYTES (TAHs), AND INVASION

The first potential link between cancer and inflammation was proposed 2000 years ago, when the Roman physician Galenos suggested that cancers evolved from inflammatory lesions (Trinchieri, 2012). The first experimental evidence supporting this remarkable observation would not emerge until 1863, when German scientist and physician Rudolf Virchow observed that leukocytes were associated with neoplastic tissues, re-establishing this forgotten link between cancer and inflammation (Balkwill and Mantovani, 2001). Now, it is a well-established fact that inflammation impacts every aspect of of tumor development and progression (Trinchieri, 2012).

An early step in the anti-tumor response is the recruitment of macrophages and other blood cells mediating the innate immune response to the tumor site (Finn, 2012). These cells phagocytose tumor cells and secrete inflammatory cytokines to both maintain the innate immune response and promote and support activation of the adaptive immune response (Finn, 2012). While the Drosophila immune system shows evidence of some primed responses (Kvell et al., 2007), flies lack adaptive immunity as we know it in mammals. However, both the cellular and humoral aspects of the innate immune response and the pathways that mediate them are highly conserved (Hoffmann et al., 1999).

The cellular arm of the Drosophila immune response consists of circulating blood cells called hemocytes. There are three morphologically distinct types of hemocytes in Drosophila that share a common developmental and evolutionary origin with mammalian blood cells (Hartenstein, 2006). Plasmatocytes are the most common hemocyte type in Drosophila, comprising more than 95% of all hemocytes. Plasmatocytes resemble mammalian phagocytes and like macrophages, they are recruited to sites of infections or wounds to phagocytose apoptotic cells, invading microbes, and other foreign bodies (Tepass et al., 1994; Franc et al., 1999; Elrod-Erickson et al., 2000). Like their mammalian counterparts, Drosophila hemocytes are also recruited to epithelial tumors (Pastor-Pareja et al., 2008). Epithelial tumors are often established in Drosophila by generating patches of epithelial cells (clones) mutant for apical/basal polarity genes such as *scrib* (*scr*), *lethal giant larvae* (*lgl*), or *discs large* (*dlg*) while also expressing the oncogenic form of Drosophila *dRas1* (e.g., $scrib^{-/-}$ $dRas1^{V12}$) (Pastor-Pareja et al., 2008; Gonzalez, 2013). Cells mutant for apical/basal polarity genes alone are quickly eliminated from the epithelium by apoptosis in a JNK dependent manner (Rudrapatna et al., 2012). However, co-expressing $dRas1^{V12}$ in these polarity-defective cells leads to invasive tumors as JNK pathway activation in these tumors promotes MMP expression, basement membrane degradation and invasion instead of apoptosis (Brumby and Richardson, 2003; Pagliarini and Xu, 2003).

Using a $scrib^{-/-}$ $dRas1^{V12}$tumor model, Pastor-Pareja and collegues showed that hemocytes infiltrate epithelial tumors

in Drosophila (Pastor-Pareja et al., 2008). Tumor bearing animals also show increased numbers of circulating hemocytes and enlarged lymph glands as a result of increased hemocyte proliferation. Interestingly, this anti-tumor response is remarkably similar to the immune response to experimentally induced aseptic wounds, consistent with the idea that tumors are like wounds that never heal (Dvorak, 1986).

The mechanism by which hemocytes are recruited to tumors is not clear. However, Tumor Associated Hemocytes (TAHs) are preferentially found in the regions of the tumor where the basement membrane is disrupted (Pastor-Pareja et al., 2008). Basement membrane disruption in the absence of tumors by overexpression of MMP2 is sufficient to induce hemocyte recruitment but not proliferation, indicating that basement membrane break-down is only one of the signals mediating this immune response.

Local activation of JNK signaling in the tumor cells is critical for the maintenance of the anti-tumor response (Pastor-Pareja et al., 2008). JNK signaling promotes the secretion of JAK-STAT activating cytokines (Upd ligands) from the tumor; this initiates a positive feedback loop that activates *upd* expression in hemocytes and the fat body (also the site of antimicrobial peptide expression and release in response to infection). The increased JAK-STAT pathway activity in the hemocytes is required to induce hemocyte proliferation in response to tumors.

Tumor Necrosis Factor (TNF) signaling is another critical component of the inflammatory response activated in response to microbial infection, tissue damage and malignant cells (Waters et al., 2013a). While both tumor suppressive and tumor promoting roles for this pathway have been well established, the molecular mechanisms mediating these different responses are less clear (Waters et al., 2013b). Drosophila has a highly conserved but simplified TNF pathway with a single TNF ligand called Eiger (Egr) (Igaki et al., 2002; Moreno et al., 2002). Removal of $scrib^{-/-}$ or $lgl^{-/-}$ cells from the epithelium also requires TNF/Eiger indicating a conserved role for TNF signaling as a tumor suppressor pathway in Drosophila within these genetic contexts (Igaki et al., 2009; Cordero et al., 2010).

Cordero and collegues showed that hemocyte attachment and infiltration of tumors provoke tumor cells to induce high levels of *egr* expression in TAHs (Cordero et al., 2010). By transfusing hemocytes into tumor bearing larvae with *egr* mutant or wild-type hemocytes, Cordero and collegues show that *egr* expression in TAHs is required to induce JNK signaling and MMP expression in tumor cells and that these defects can be partially rescued by transfusing animals with $egr^{+/+}$ hemocytes. Most importantly, removing *egr* from TAH's has drastically different consequences on tumors with different genotypes: $scrib^{-/-}$ tumors cannot be eliminated from the tissue without $egr^{+/+}$ hemocytes, indicating a tumor suppressive role for TAHs and TNF signaling in this genetic context (**Figures 1A,C**). In contrast, Egr signaling from the TAHs is essential for $scrib^{-/-}$ $dRas1^{V12}$ cells to become invasive tumors (**Figures 1B,C**) indicating a tumor promoting role for this pathway in this genetic context.

Activation of JNK signaling and induction of MMP1 expression are a part of the normal immune response to facilitate delamination of abnormal cells from the epithelium and promote further infiltration of the wound or infection by hemocytes. As both JNK and TNF pathways are strong inducers of cell death, these MMP expressing cells are normally quickly eliminated by apoptosis to ensure tissue integrity. However, these studies suggest that if these JNK/MMP1 positive tumor cells persist long enough in the tissue, for instance as a result of additional mutations that prevent apoptosis, they can further promote degradation of the basement membrane and infiltration by additional TAHs. This in turn leads to a positive feedback loop that increases the number of JNK/MMP positive cells within the tumor and thereby its metastatic potential (**Figures 1A–C**).

Aspects of bacterial infection can also be studied by directly expressing pathogen-derived proteins in host tissues. For instance, Drosophila models of *H. pylori* infection have been generated by expressing the *H. pylori* virulence factor CagA in Drosophila tissues (Botham et al., 2008; Wandler and Guillemin, 2012). Certain virulent strains of *H. pylori* possess a secretion system that allows them to directly inject the CagA protein into gastric epithelial cells and can promote the development of gastric carcinoma in a small percentage of infected individuals (Peek and Blaser, 2002; Hatakeyama, 2008; Wroblewski et al., 2010). Wandler and Guillemin showed that CagA expression in discrete domains in the Drosophila wing disc epithelium leads to the activation of apoptosis in a subset of CagA expressing cells in a JNK signaling dependent fashion (Wandler and Guillemin, 2012). Interestingly, loss of *egr* function in the whole animal increased the number of apoptotic CagA expressing cells, but not when *egr* was only reduced in CagA expressing cells. This suggests a non-cell-autonomous role for Egr in apoptotic cell clearance. The authors propose a model whereby loss of Egr from the neighboring wildtype epithelial cells mediate elimination of apoptotic CagA expressing cells from the epithelium. CagA expression also synergized with oncogenic Ras to facilitate JNK mediated tumor progression and invasion, however, the role of Egr in this context has not been investigated. Furthermore, potential roles for the core immune signaling pathways and the cellular immune response in this process remain unexplored possibilities. It will be interesting to see if hemocytes also associate with tumors in this paradigm and whether similar pro-tumor and anti-tumor roles for Egr/TNF signaling can be elucidated.

TOLL/Imd SIGNALING, MICROBIAL INFECTION, AND CANCER

Recognition or pathogen and damage associated molecular patterns by the immune system is a key component of mounting an effective host defense. In Drosophila, this innate immune response is mediated by two pathways: Recognition of Gram positive bacteria and fungi depends on secreted factors that regulate the processing and activation of the Toll receptor ligand Spatzle (Spz) (Lemaitre et al., 1996). Subsequent activation of the Toll pathway leads to the expression and secretion of antimicrobial peptides (AMPs) mediated by NFκB related molecules Dorsal and Dif (Valanne et al., 2011). On the other hand, Gram negative bacteria are recognized by pattern recognition receptors called PGRPs, ultimately leading to activation of another NFκB related molecule called Relish as well as JNK pathway in

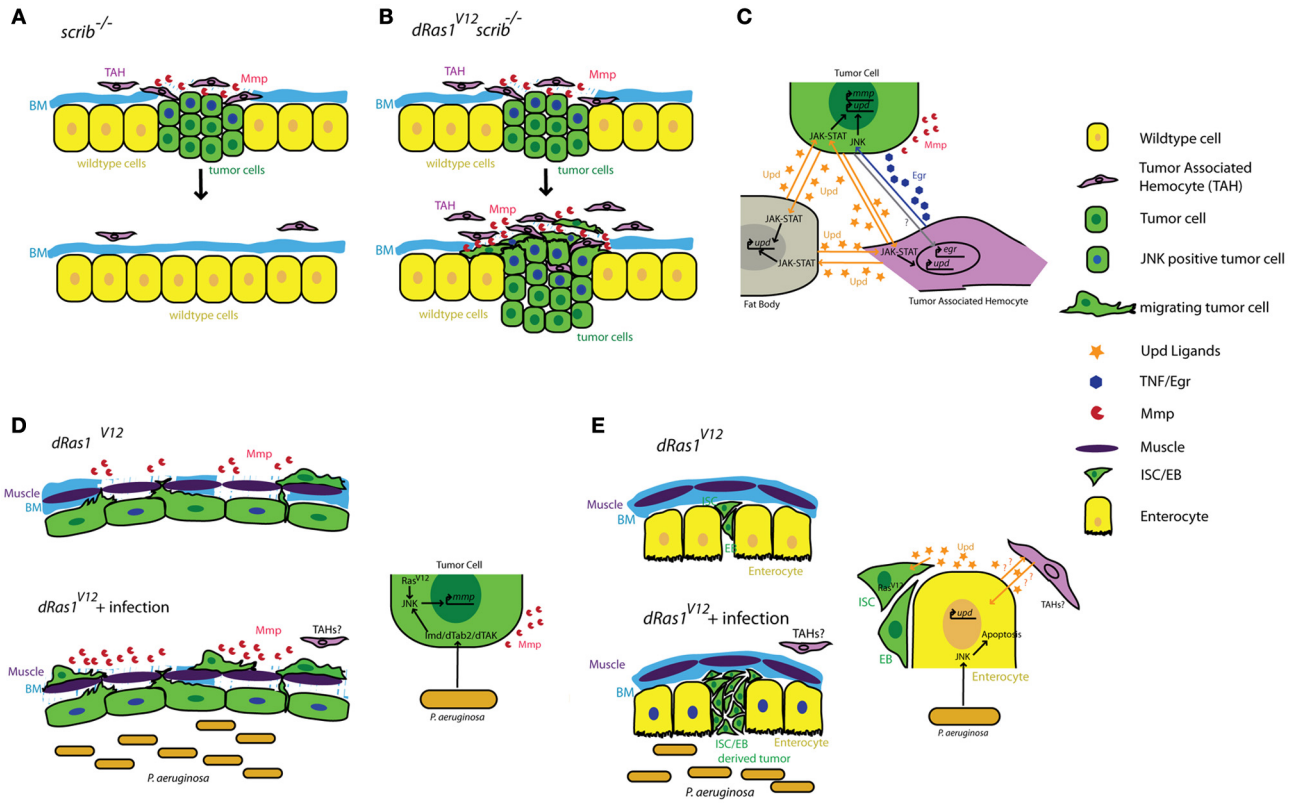


FIGURE 1 | Summary of tumor cell-immune system interactions in Drosophila. **(A)** Clones of cells defective for cell polarity genes such as *scrib* (green) generated in the context of a wild type tissue (yellow) are rapidly eliminated from the epithelium with the help of tumor associated hemocytes (TAHs, purple). **(B)** This antitumor immune response is subverted in *scrib*$^{-/-}$ *dRas1*^{V12} cells, leading to the establishment of an invasive tumor. **(C)** Reciprocal interactions between tumor cells (green) TAHs (purple), and fat body (gray) result in both local and systemic release of inflammatory cytokines in a positive feedback loop. **(D)** Microbial infection synergizes with *dRas1*^{V12} to induce activation of JNK signaling (blue nuclei) and Mmp expression (red) in transformed hindgut epithelial cells, leading to migration and dissemination. **(E)** Microbial infection induces apoptosis of differentiated enterocytes (yellow), and synergizes with with *dRas1*^{V12} to induce hyperplastic ISC/EB-like tumors. BM, Basement Membrane; Mmp, Matrix Metalloprotease; ISC, Intestinal Stem Cell; EB, Enteroblast.

an Imd dependent fashion (Choe et al., 2002; Ramet et al., 2002; Kallio et al., 2005). In mammals, Toll Related Receptor (TLR) signaling is activated by direct binding of pathogen associated molecules, leading to NFκB-mediated induction of AMP expression (Takeuchi and Akira, 2010). In addition, pathogen associated peptidoglycan fragments are recognized by NOD-like Receptors (NLRs), which leads to activation of NFκB and JNK pathways (Lavelle et al., 2010). Even though there are some differences in the activation mechanisms of these pathways, most of the downstream pathway components and their roles are highly conserved between mammals and Drosophila (see reference 30 for an in-depth comparative analysis).

Stimulation of innate immune responses by microbial components can also modulate migratory potential of epithelial cells (Wang et al., 2003; Merrell et al., 2006) and recent identification of functionally active TLRs in several tumor cell lines point to important roles for TLR signaling in epithelial tumor progression and metastasis (Huang et al., 2005, 2008; Kelly et al., 2006; Rakoff-Nahoum and Medzhitov, 2009). In recent years several groups took advantage of the high degree of conservation of core immune signaling pathways in Drosophila to explore the relationship between innate immune responses and tumor progression.

The gastrointestinal tract is a prominent component of both mammalian and Drosophila immune systems. The intestinal epithelium expresses several TLRs and studies both in murine models and in Drosophila reveal that intestinal epithelial cells respond to microbial infection by secreting AMPs, a Toll/Imd/TLR signaling mediated process (O'Neil et al., 1999; Apidianakis et al., 2005). Interestingly, chronic activation of the immune response is thought to facilitate intestinal tumorigenesis in genetically predisposed individuals (Pasparakis, 2008; Secher et al., 2010), again suggesting a pro-tumorigenic role for Toll/Imd/TLR signaling in the intestine. We found that acute activation of the Imd pathway in response to microbial infection interacts with pre-existing oncogenic mutations to promote tumorigenesis in a *dRas1*^{V12} induced model of colon cancer in Drosophila (Bangi et al., 2012). When targeted to the hindgut epithelium—the functional equivalent of the mammalian colon—*dRas1*^{V12} activates JNK signaling and MMP expression in a subset of the hindgut epithelial cells. These transformed cells eventually migrate out of the epithelium to colonize

distant sites within the animal. While JNK/MMP positive cells do not migrate themselves, both JNK signaling and MMP expression is necessary for the dissemination phenotype. Microbial infection of these animals using a previously established infection paradigm by oral feeding of the Gram negative bacterium *Pseudomonas aeruginosa* (Apidianakis and Rahme, 2009, 2011) leads to a significant enhancement of $dRas1^{V12}$ induced dissemination in an Imd dependent fashion. Microbial infection in this case increases the metastatic potential of the tumor by increasing the number of JNK/MMP1 positive cells, thereby further compromising the integrity of the tissue and facilitating the migration of $dRas1^{V12}$ transformed cells (**Figure 1D**).

By contrast in the midgut, microbial infection synergizes with $dRas1^{V12}$ to induce intestinal hyperplasia but not invasion or dissemination; in this model, $dRas1^{V12}$ was targeted to intestinal stem cells (ISCs) and undifferentiated enteroblasts (EBs), the immediate progeny of ISCs (Apidianakis and Rahme, 2009; Pitsouli et al., 2009) (**Figure 1E**). Hyperplasia is driven by bacteria-induced death of differentiated midgut cells. Curiously, JNK induced secretion of JAK-STAT inducing cytokines (Upd-1, -2, -3) by the dying midgut cells is known to be a key mediator of tissue regeneration (Jiang et al., 2009), reminiscent of the positive feedback loop created between TAHs and tumor cells in the imaginal disc tumor models discussed above (Pastor-Pareja et al., 2008). Adult hemocytes have been reported to respond to microbial infection by phagocytosing invading pathogens in multiple infection paradigms (Elrod-Erickson et al., 2000; Kocks et al., 2005; Nehme et al., 2007). However, there is no evidence that they infiltrate the adult gut as part of the immune response and whether they contribute to hyperplasia and dissemination phenotypes in these intestinal cancer models have not been investigated.

ANTIVIRAL IMMUNITY AND CANCER

In addition to bacterial and fungal infection paradigms, several Drosophila models of viral infection also exist; these include models that use natural viruses that infect Drosophila as well as several viruses that cause disease in humans and those that directly express various viral proteins in Drosophila tissues (Bier and Guichard, 2012; Merkling and van Rij, 2013). The major immune defense against viral infection in insects is the RNA interference pathway, however, several recent reports indicate possible roles for the evolutionarily conserved core immune signaling pathways Toll, Imd, and JAK-STAT in antiviral immunity (Dostert et al., 2005; Zambon et al., 2005; Costa et al., 2009). It would be interesting to combine these viral infection models with available Drosophila cancer models to explore interactions between viral infection, antiviral immunity and cancer.

DROSOPHILA OFFERS NEW TOOLS TO EXPLORE LINKS BETWEEN IMMUNOLOGY AND CANCER

The presence of an antitumor immune response in Drosophila opens up new avenues of research in the field of tumor immunology. The absence of an adaptive immune response precludes modeling certain aspects of immune response. However, signaling pathways that mediate the interactions between tumor cells and the innate immune system (JNK, JAK-STAT, TNF, Toll/Imd/TLR) as well as the way these pathways interact with each other are highly conserved in flies.

The sophisticated genetic tools available in Drosophila can be used for genetic dissection of conserved aspects of the anti-tumor immune response. For instance, multiple independent targeted and inducible expression systems are available in Drosophila (del Valle Rodriguez et al., 2012), making it possible to separately label and genetically manipulate tumor cells and cells of the immune system. An increasing number of genetically complex tumor models are being reported in Drosophila (Gonzalez, 2013). For instance, 30 multigenic models of colon cancer in the adult Drosophila gut have recently been generated and characterized in our laboratory (Bangi et. al., in review). These models allow us to explore the mechanisms by which the innate immune system reacts to tumors with different genetic compositions.

Lastly, Drosophila is emerging as a useful platform for cancer drug discovery: flies provide a high degree of conservation of cancer relevant pathways as well as appropriate sensitivity to compounds targeting these pathways (Bangi et al., 2011; Gonzalez, 2013). Compound screens in Drosophila using organismal lethality or other complex phenotypic read outs of cancer are revealing new anti-cancer agents with promising activity in mammalian models (Dar et al., 2012). With these tools, Drosophila can be useful both as a genetic model system for tumor immunology but also as a drug discovery platform to screen for compounds that target the immune system and its interactions with tumor cells.

ACKNOWLEDGMENTS

I would like to thank Ross Cagan for his feedback on the manuscript. Erdem Bangi is supported by grants R01-CA109730 and R01-EY011495.

REFERENCES

Apidianakis, Y., Mindrinos, M. N., Xiao, W., Lau, G. W., Baldini, R. L., Davis, R. W., et al. (2005). Profiling early infection responses: pseudomonas aeruginosa eludes host defenses by suppressing antimicrobial peptide gene expression. *Proc. Natl. Acad. Sci. U.S.A.* 102, 2573–2578. doi: 10.1073/pnas.0409588102

Apidianakis, Y., Pitsouli, C., Perrimon, N., and Rahme, L. (2009). Synergy between bacterial infection and genetic predisposition in intestinal dysplasia. *Proc. Natl. Acad. Sci. U.S.A.* 106, 20883–20888. doi: 10.1073/pnas.0911797106

Apidianakis, Y., and Rahme, L. G. (2009). Drosophila melanogaster as a model host for studying Pseudomonas aeruginosa infection. *Nat. Protoc.* 4, 1285–1294. doi: 10.1038/nprot.2009.124

Apidianakis, Y., and Rahme, L. G. (2011). Drosophila melanogaster as a model for human intestinal infection and pathology. *Dis. Model. Mech.* 4, 21–30. doi: 10.1242/dmm.003970

Balkwill, F., and Mantovani, A. (2001). Inflammation and cancer: back to Virchow? *Lancet* 357, 539–545. doi: 10.1016/S0140-6736(00)04046-0

Bangi, E., Garza, D., and Hild, M. (2011). *In vivo* analysis of compound activity and mechanism of action using epistasis in Drosophila. *J. Chem. Biol.* 4, 55–68. doi: 10.1007/s12154-010-0051-5

Bangi, E., Pitsouli, C., Rahme, L. G., Cagan, R., and Apidianakis, Y. (2012). Immune response to bacteria induces dissemination of Ras-activated Drosophila hindgut cells. *EMBO Rep.* 13, 569–576. doi: 10.1038/embor.2012.44

Bier, E., and Guichard, A. (2012). Deconstructing host-pathogen interactions in Drosophila. *Dis. Model. Mech.* 5, 48–61. doi: 10.1242/dmm.000406

Botham, C. M., Wandler, A. M., and Guillemin, K. (2008). A transgenic Drosophila model demonstrates that the Helicobacter pylori CagA protein functions as a eukaryotic Gab adaptor. *PLoS Pathog.* 4:e1000064. doi: 10.1371/journal.ppat.1000064

Brumby, A. M., and Richardson, H. E. (2003). Scribble mutants cooperate with oncogenic Ras or Notch to cause neoplastic overgrowth in Drosophila. *EMBO J.* 22, 5769–5779. doi: 10.1093/emboj/cdg548

Choe, K. M., Werner, T., Stoven, S., Hultmark, D., and Anderson, K. V. (2002). Requirement for a peptidoglycan recognition protein (PGRP) in Relish

activation and antibacterial immune responses in Drosophila. *Science* 296, 359–362. doi: 10.1126/science.1070216

Cordero, J. B., Macagno, J. P., Stefanatos, R. K., Strathdee, K. E., Cagan, R. L., and Vidal, M. (2010). Oncogenic ras diverts a host TNF Tumor suppressor activity into Tumor promoter. *Dev. Cell* 18, 999–1011. doi: 10.1016/j.devcel.2010.05.014

Costa, A., Jan, E., Sarnow, P., and Schneider, D. (2009). The Imd pathway is involved in antiviral immune responses in Drosophila. *PLoS ONE* 4:e7436. doi: 10.1371/journal.pone.0007436

Dar, A. C., Das, T. K., Shokat, K. M., and Cagan, R. L. (2012). Chemical genetic discovery of targets and anti-targets for cancer polypharmacology. *Nature* 486, 80–84. doi: 10.1038/nature11127

del Valle Rodriguez, A., Didiano, D., and Desplan, C. (2012). Power tools for gene expression and clonal analysis in Drosophila. *Nat. Methods* 9, 47–55. doi: 10.1038/nmeth.1800

Dostert, C., Jouanguy, E., Irving, P., Troxler, L., Galiana-Arnoux, D., Hetru, C., et al. (2005). The Jak-STAT signaling pathway is required but not sufficient for the antiviral response of drosophila. *Nat. Immunol.* 6, 946–953. doi: 10.1038/ni1237

Dvorak, H. F. (1986). Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing. *N.Engl. J. Med.* 315, 1650–1659. doi: 10.1056/NEJM198612253152606

Elrod-Erickson, M., Mishra, S., and Schneider, D. (2000). Interactions between the cellular and humoral immune responses in Drosophila. *Curr. Biol.* 10, 781–784. doi: 10.1016/S0960-9822(00)00569-8

Finn, O. J. (2012). Immuno-oncology: understanding the function and dysfunction of the immune system in cancer. *Ann. Oncol.* 23 (Suppl. 8), viii6–viii 9. doi: 10.1093/annonc/mds256

Franc, N. C., Heitzler, P., Ezekowitz, R. A., and White, K. (1999). Requirement for croquemort in phagocytosis of apoptotic cells in Drosophila. *Science* 284, 1991–1994. doi: 10.1126/science.284.5422.1991

Gonzalez, C. (2013). Drosophila melanogaster: a model and a tool to investigate malignancy and identify new therapeutics. *Nat. Rev. Cancer* 13, 172–183. doi: 10.1038/nrc3461

Hartenstein, V. (2006). Blood cells and blood cell development in the animal kingdom. *Annu. Rev. Cell Dev. Biol.* 22, 677–712. doi: 10.1146/annurev.cellbio.22.010605.093317

Hatakeyama, M. (2008). SagA of CagA in Helicobacter pylori pathogenesis. *Curr. Opin. Microbiol.* 11, 30–37. doi: 10.1016/j.mib.2007.12.003

Hoffmann, J. A., Kafatos, F. C., Janeway, C. A., and Ezekowitz, R. A. (1999). Phylogenetic perspectives in innate immunity. *Science* 284, 1313–1318. doi: 10.1126/science.284.5418.1313

Huang, B., Zhao, J., Li, H., He, K. L., Chen, Y., Chen, S. H., et al. (2005). Toll-like receptors on tumor cells facilitate evasion of immune surveillance. *Cancer Res.* 65, 5009–5014. doi: 10.1158/0008-5472.CAN-05-0784

Huang, B., Zhao, J., Unkeless, J. C., Feng, Z. H., and Xiong, H. (2008) TLR signaling by tumor and immune cells: a double-edged sword. *Oncogene* 27, 218–224. doi: 10.1038/sj.onc.1210904

Igaki, T., Kanda, H., Yamamoto-Goto, Y., Kanuka, H., Kuranaga, E., Aigaki, T., et al. (2002). Eiger, a TNF superfamily ligand that triggers the Drosophila JNK pathway. *EMBO J.* 21, 3009–3018. doi: 10.1093/emboj/cdf306

Igaki, T., Pastor-Pareja, J. C., Aonuma, H., Miura, M., and Xu, T. (2009). Intrinsic tumor suppression and epithelial maintenance by endocytic activation of Eiger/TNF signaling in Drosophila. *Dev. Cell* 16, 458–465. doi: 10.1016/j.devcel.2009.01.002

Jiang, H., Patel, P. H., Kohlmaier, A., Grenley, M. O., McEwen, D. G., and Edgar, B. A. (2009). Cytokine/Jak/Stat signaling mediates regeneration and homeostasis in the Drosophila Midgut. *Cell* 137, 1343–1355. doi: 10.1016/j.cell.2009.05.014

Kallio, J., Leinonen, A., Ulvila, J., Valanne, S., Ezekowitz, R. A., and Ramet, M. (2005). Functional analysis of immune response genes in Drosophila identifies JNK pathway as a regulator of antimicrobial peptide gene expression in S2 cells. *Microbes Infect.* 7, 811–819. doi: 10.1016/j.micinf.2005.03.014

Kelly, M. G., Alvero, A. B., Chen, R., Silasi, D. A., Abrahams, V. M., Chan, S., et al. (2006). TLR-4 signaling promotes tumor growth and paclitaxel chemoresistance in ovarian cancer. *Cancer Res.* 66, 3859–3868. doi: 10.1158/0008-5472.CAN-05-3948

Kocks, C., Cho, J. H., Nehme, N., Ulvila, J., Pearson, A. M., Meister, M., et al. (2005). Eater, a transmembrane protein mediating phagocytosis of bacterial pathogens in Drosophila. *Cell* 123, 335–346. doi: 10.1016/j.cell.2005.08.034

Kvell, K., Cooper, E. L., Engelmann, P., Bovari, J., and Nemeth, P. (2007). Blurring borders: innate immunity with adaptive features. *Clin. Dev. Immunol.* 2007, 83671. doi: 10.1155/2007/83671

Lavelle, E. C., Murphy, C., O'Neill, L. A., and Creagh, E. M. (2010). The role of TLRs, NLRs, and RLRs in mucosal innate immunity and homeostasis. *Mucosal Immunol.* 3, 17–28. doi: 10.1038/mi.2009.124

Lemaitre, B., Nicolas, E., Michaut, L., Reichhart, J. M., and Hoffmann, J. A. (1996). The dorsoventral regulatory gene cassette spatzle/Toll/cactus controls the potent antifungal response in Drosophila adults. *Cell* 86, 973–983. doi: 10.1016/S0092-8674(00)80172-5

Merkling, S. H., and van Rij, R. P. (2013). Beyond RNAi: antiviral defense strategies in Drosophila and mosquito. *J. Insect. Physiol.* 59, 159–170. doi: 10.1016/j.jinsphys.2012.07.004

Merrell, M. A., Ilvesaro, J. M., Lehtonen, N., Sorsa, T., Gehrs, B., Rosenthal, E., et al. (2006). Toll-like receptor 9 agonists promote cellular invasion by increasing matrix metalloproteinase activity. *Mol. Cancer Res.* 4, 437–447. doi: 10.1158/1541-7786.MCR-06-0007

Moreno, E., Yan, M., and Basler, K. (2002). Evolution of TNF signaling mechanisms: JNK-dependent apoptosis triggered by Eiger, the Drosophila homolog of the TNF superfamily. *Curr. Biol.* 12, 1263–1268. doi: 10.1016/S0960-9822(02)00954-5

Nehme, N. T., Liegeois, S., Kele, B., Giammarinaro, P., Pradel, E., Hoffmann, J. A., et al. (2007). A model of bacterial intestinal infections in Drosophila melanogaster. *PLoS Pathog.* 3:e173. doi: 10.1371/journal.ppat.0030173

O'Neil, D. A., Porter, E. M., Elewaut, D., Anderson, G. M., Eckmann, L., Ganz, T., et al. (1999). Expression and regulation of the human beta-defensins hBD-1 and hBD-2 in intestinal epithelium. *J. Immunol.* 163, 6718–6724.

Pagliarini, R. A., and Xu, T. (2003). A genetic screen in Drosophila for metastatic behavior. *Science* 302, 1227–1231. doi: 10.1126/science.1088474

Pasparakis, M. (2008). IKK/NF-kappaB signaling in intestinal epithelial cells controls immune homeostasis in the gut. *Mucosal Immunol.* 1 (Suppl. 1), S54–S57. doi: 10.1038/mi.2008.53

Pastor-Pareja, J. C., Wu, M., and Xu, T. (2008). An innate immune response of blood cells to tumors and tissue damage in Drosophila. *Dis. Model. Mech.* 1, 144–154. discussion: 53. doi: 10.1242/dmm.000950

Peek, R. M. Jr., and Blaser, M. J. (2002). Helicobacter pylori and gastrointestinal tract adenocarcinomas. *Nat. Rev. Cancer* 2, 28–37. doi: 10.1038/nrc703

Pitsouli, C., Apidianakis, Y., and Perrimon, N. (2009). Homeostasis in infected epithelia: stem cells take the lead. *Cell Host Microbe* 6, 301–307. doi: 10.1016/j.chom.2009.10.001

Rakoff-Nahoum, S., and Medzhitov, R. (2009). Toll-like receptors and cancer. *Nat. Rev. Cancer* 9, 57–63. doi: 10.1038/nrc2541

Ramet, M., Manfruelli, P., Pearson, A., Mathey-Prevot, B., and Ezekowitz, R. A. (2002). Functional genomic analysis of phagocytosis and identification of a Drosophila receptor for E. coli. *Nature* 416, 644–648. doi: 10.1038/nature735

Rudrapatna, V. A., Cagan, R. L., and Das, T. K. (2012). Drosophila cancer models. *Dev. Dyn.* 241, 107–118. doi: 10.1002/dvdy.22771

Secher, T., Gaillot, O., Ryffel, B., and Chamaillard, M. (2010). Remote control of intestinal tumorigenesis by innate immunity. *Cancer Res.* 70, 1749–1752. doi: 10.1158/0008-5472.CAN-09-3401

Takeuchi, O., and Akira, S. (2010). Pattern recognition receptors and inflammation. *Cell* 140, 805–820. doi: 10.1016/j.cell.2010.01.022

Tepass, U., Fessler, L. I., Aziz, A., and Hartenstein, V. (1994). Embryonic origin of hemocytes and their relationship to cell death in Drosophila. *Development* 120, 1829–1837.

Trinchieri, G. (2012). Cancer and inflammation: an old intuition with rapidly evolving new concepts. *Annu. Rev. Immunol.* 30, 677–706. doi: 10.1146/annurev-immunol-020711-075008

Valanne, S., Wang, J. H., and Ramet, M. (2011). The Drosophila Toll signaling pathway. *J. Immunol.* 186, 649–656. doi: 10.4049/jimmunol.1002302

Wandler, A. M., and Guillemin, K. (2012). Transgenic expression of the Helicobacter pylori virulence factor CagA promotes apoptosis or tumorigenesis through JNK activation in Drosophila. *PLoS Pathog.* 8:e1002939. doi: 10.1371/journal.ppat.1002939

Wang, J. H., Manning, B. J., Wu, Q. D., Blankson, S., Bouchier-Hayes, D., Redmond, H. P., et al. (2003). Endotoxin/lipopolysaccharide activates NF-kappa B and enhances tumor cell adhesion and invasion through a beta 1 integrin-dependent mechanism. *J. Immunol.* 170, 795–804.

Waters, J. P., Pober, J. S., and Bradley, J. R. (2013a). Tumour necrosis factor in infectious disease. *J. Pathol.* 230, 132–147. doi: 10.1002/path.4187

Waters, J. P., Pober, J. S., and Bradley, J. R. (2013b). Tumour necrosis factor and cancer. *J. Pathol*. 230, 241–248. doi: 10.1002/path.4188

Wroblewski, L. E., Peek, R. M. Jr., and Wilson, K. T. (2010). Helicobacter pylori and gastric cancer: factors that modulate disease risk. *Clin. Microbiol. Rev*. 23, 713–739. doi: 10.1128/CMR.00011-10

Zambon, R. A., Nandakumar, M., Vakharia, V. N., and Wu, L. P. (2005). The Toll pathway is important for an antiviral response in Drosophila. *Proc. Natl. Acad. Sci. U.S.A*. 102, 7257–7262. doi: 10.1073/pnas.0409181102

Conflict of Interest Statement: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 05 September 2013; accepted: 06 December 2013; published online: 19 December 2013.

Citation: Bangi E (2013) Drosophila at the intersection of infection, inflammation, and cancer. Front. Cell. Infect. Microbiol. ***3****:103. doi: 10.3389/fcimb.2013.00103*

This article was submitted to the journal Frontiers in Cellular and Infection Microbiology.

frontiers in
CELLULAR AND INFECTION MICROBIOLOGY

REVIEW ARTICLE
published: 09 January 2014
doi: 10.3389/fcimb.2013.00113

Drosophila as a model to study the role of blood cells in inflammation, innate immunity and cancer

Lihui Wang , Ilias Kounatidis and Petros Ligoxygakis*

Laboratory of Genes and Development, Department of Biochemistry, University of Oxford, Oxford, UK

Edited by:
Yiorgos Apidianakis, University of Cyprus, Cyprus

Reviewed by:
Sung O. Kim, University of Western Ontario, Canada
Valerio Iebba, 'Sapienza' University of Rome, Italy

***Correspondence:**
Petros Ligoxygakis, Laboratory of Genes and Development, Department of Biochemistry, University of Oxford, New Biochemistry Building, South Parks Road, Oxford OX1 3QU, UK
e-mail: petros.ligoxygakis@bioch.ox.ac.uk

Drosophila has a primitive yet effective blood system with three types of haemocytes which function throughout different developmental stages and environmental stimuli. Haemocytes play essential roles in tissue modeling during embryogenesis and morphogenesis, and also in innate immunity. The open circulatory system of *Drosophila* makes haemocytes ideal signal mediators to cells and tissues in response to events such as infection and wounding. The application of recently developed and sophisticated genetic tools to the relatively simple genome of *Drosophila* has made the fly a popular system for modeling human tumorigensis and metastasis. *Drosophila* is now used for screening and investigation of genes implicated in human leukemia and also in modeling development of solid tumors. This second line of research offers promising opportunities to determine the seemingly conflicting roles of blood cells in tumor progression and invasion. This review provides an overview of the signaling pathways conserved in *Drosophila* during haematopoiesis, haemostasis, innate immunity, wound healing and inflammation. We also review the most recent progress in the use of *Drosophila* as a cancer research model with an emphasis on the roles haemocytes can play in various cancer models and in the links between inflammation and cancer.

Keywords: haemocytes, haematopoiesis, plasmatocyte, macrophage, innate immunity, tumor, inflammation

INTRODUCTION

Drosophila has undoubtedly been a powerful model organism for the study of nearly all essential and fundamental biological processes. What we have learned from the fruit fly has expanded our knowledge in life science at an unprecedented speed. This is in particular due to the recent availability of the complete annotated genome, a versatile array of genomic modifying techniques and powerful life imaging tools. Cellular and molecular mechanisms underlying many basic biological processes have been discovered to be highly conserved between *Drosophila* and mammals. For example, the Notch, Hedgehog (Hg) and Wingless (Wnt) pathways first identified in *Drosophila* embryogenesis and the Runt and Hippo signaling pathways conserved in the *Drosophila* haematopoiesis and tissue growth are also implicated in the progression of various human cancers (Geissler and Zach, 2012; Harvey et al., 2013). Indeed the past decade has witnessed a rapidly emerging trend for *Drosophila* to be used in modeling human tumor growth, progression, invasion and metastasis and as a test-bed for therapeutic discovery (reviews in Harris, 2005; Crozatier and Vincent, 2011; Miles et al., 2011; Hsu, 2012; Gonzalez, 2013).

Most forms of human cancers progress step by step from mutations in the oncogene, the tumor suppressor gene and signaling molecules and can eventually kill the host by spreading uncontrollable immortal growth of mutant malignant tissues into different organs. On the route to spread and invade, cancer cells can influence their microenvironment via the interaction with the infiltrated blood cells, gradually disabling the host immunosurvellience and finally breaking the stromal barrier to become invasive and metastatic (Dunn et al., 2004). It is at the metastatic stage that many lives would be claimed. Therefore the outcome from the tug of war in the tumor microenvironment between malignant cancerous cells that undergo constant somatic mutations and surrounding blood cells plays a vital role in the prevention and intervention of tumorigenesis. In addition, chronic inflammation has been well-documented as contributing to and promoting the initiation and progression of various cancers (Coussens and Werb, 2002; Mantovani et al., 2008; Aggarwal et al., 2009). It is now generally accepted that an inflammatory microenvironment is necessary for tumor progression and metastasis (Wu and Zhou, 2009; Grivennikov et al., 2010). Macrophages in particular have been reported to facilitate many aspects of this process in different cancers and also to intervene in the anti-cancer therapies (De Palma and Lewis, 2013; Lee et al., 2013b). Apart from the role of macrophages in cancer development, they have been for many years subjected to extensive research as the key player in inflammatory responses which accompany infection,

Abbreviations: AMP, antimicrobial peptide; AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; Bsk, Basket; Chn, Charlatan; CML, chronic myeloid leukemia; CNS, central nerve system; Col, Collier; Dif, Dorsal related immunity; Dom, Domeless; ECM, extracellular matrix proteins; EGF, epidermal growth factor; FOG, Friend of GATA; GADD45, growth arrest and DNA damage-inducible gene 45; Gcm, Glial-cells-missing; Hg, Hedgehog; Hml, Hemolectin; HSC, haematopoietic stem cells; JNK, Jun N-terminal kinase; LG, lymph gland; Lgl, lethal giant larvae; Lz, Lozenge; MARCM, mosaic analysis with a repressible cell marker system; PDGF, platelet-derived growth factor; PI3K, phosphatidyl-inositol 3-kinase; PPAE, Prophenoloxidase activating enzyme; PPO, Prophenoloxidase; PSC, posterior signaling center; ROS, reactive oxygen species; Ser, Serrate; Smo, Smoothened; Srp, Serpent; TF, Transglutaminase; Upd, Unpaired; Ush, U-shaped; VEGF, vascular endothelial growth factor; Wnt, Wingless.

tissue damage and wound healing (Mantovani et al., 2013; Novak and Koh, 2013). Therefore inflammation, immunity and cancer are inter-linked and any imbalance can result in serious health issues. Blood cells such as macrophages appear to be the link and have a crucial role in influencing and maintaining the equilibrium between protection (immunity and inflammation) and regeneration/tissue homeostasis (where cancer can be considered a malignant proliferative and invasive tissue). Animal models such as mice have revealed invaluable insights into the multi-step interaction of mammalian innate immunity with associated inflammatory responses in defining the cancer microenvironment. These innate immune responses can include the complement pathways (Ricklin and Lambris, 2013), pro-inflammatory cytokine and chemokine production (Sethi et al., 2012; Candido and Hagemann, 2013). However, the multi-layered interaction in the context of a generally slow progression of the human cancer has created fragmentary and controversial results in the mouse model and thus inevitably slows down our progress to understand the disease. *Drosophila*, on the contrary, as a simply-formed and genetically tractable multi-cellular organism, has been used to dissect processes of development (tissue homeostasis) and innate immunity with such precision that the time is now ripe for us to look into the active dialogs between these fundamental processes in the context of mammalian inflammation and cancer. *Drosophila* has a primitive open blood circulation system with only three types of blood cells or haemocytes circulating in the haemolymph during a fly's life span. The majority of the circulating haemocytes in the haemolymph are macrophage-like cells that engulf and degrade apoptotic cells and invading pathogens. Haemocytes perform vital roles through their contribution both to cellular and humoral immune responses in the fly. In combination with the currently well-developed *Drosophila* tumor models, the roles of haemocytes in tumor regression and/or progression can be explored and important clues can be obtained to understand further the inflammatory responses in relation to tumors; the focus can be directed more at the molecular and cellular level by use of sophisticated genetic manipulation and live imaging tools. Tian Xu and colleagues have done pioneering work in this direction by using a *Drosophila* tumor model to investigate the role of haemocytes in the tumor growth control during a systemic inflammatory response (Pastor-Pareja et al., 2008). In this review, we first give a brief overview of *Drosophila* haematopoiesis. This is followed by a discussion of the roles of haemocytes in *Drosophila* at different developmental stages. Next we consider how haemocytes function in tissue injury and wound healing. *Drosophila* leukemia model and interaction between immunity and tumorigenesis are also discussed. Finally, perspectives for possible future research opportunities in the interplay of inflammation, immunity and cancer revolving around the blood cells are discussed.

Drosophila HAEMATOPOIESIS

The blood system of *Drosophila* is rather primitive compared to the great complexity in vertebrates. The fruit fly does not have a vascular network to separate the blood cells from other tissues and organs and its internal organs are bathed in haemolymph. Meanwhile vertebrates have many different types of blood cells. Each type has evolved to perform specialized functions during million years of evolution. On the contrary only three major types of blood cells, collectively termed haemocytes, have been identified in the fruit fly and none of them has acquired the capability to undergo DNA rearrangement and somatic hypermutation to generate a vast repertoire for immunological memory in the B- and T-lymphocytes. Therefore *Drosophila* relies on a very simple system to fulfil basically all the roles that vertebrate blood cells can play. However there is extensive conservation in the molecular mechanisms of haematopoiesis in both *Drosophila* and mammals.

As in vertebrates, *Drosophila* haematopoiesis takes place in two phases: primitive haematopoiesis and definitive haematopoiesis (for more detailed review see Evans et al., 2003; Crozatier and Meister, 2007; Krzemien et al., 2010). Briefly, the site for primitive haematopoiesis resides in the precephalic mesoderm which gives rise to the early wave of haemocyte generation in the embryo (**Figure 1A**). At the end of embryogenesis, a specialized organ termed Lymph Gland (LG) originating from the lateral mesoderm starts to appear along the dorsal vessel and becomes fully mature during the first half of larval development (**Figure 1B**). Definite haematopoiesis initiates in the LG (**Figures 1B**, **2B**) and generates terminally differentiated haemocytes at the onset of metamorphosis; the cells are released during the pupal stage with disintegration of the LG. After the disappearance of LG, no haematopoiesis will occur in the pupa or in the adult fly. Haemocytes persisting through the whole life stages of the fly

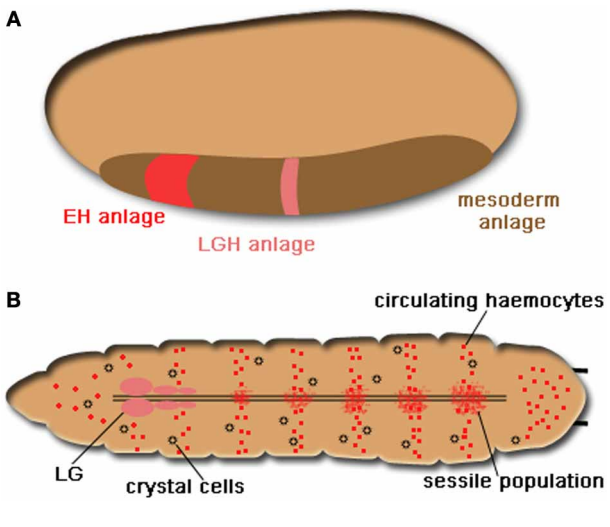


FIGURE 1 | *Drosophila* haematopoiesis. (A) The embryonic haematopoiesis. Two waves of haemocyte generation take place in *Drosophila*. During embryogenesis, haemocytes originate from the precephalic mesoderm as indicated in red in the region of mesoderm anlage (shown in brown), The pink region denotes the embryonic origin that will give rise to lymph gland haemocytes (LGH) in the larva. **(B)** Larval haematopoiesis. The LG (in pink) composes of the primary and secondary lobes and is located in the anterior end of the larva along the dorsal aorta. The sessile haemocyte population distributes diffusely along the segmental borders of the larva and consists of functional differentiated haemocytes and a few prohaemocytes with an embryonic origin (shown in the same red color). Until the end of the third star, circulating haemocytes including plasmatocytes and crystal cells (small black circles) are derived from the embryonic haemocytes.

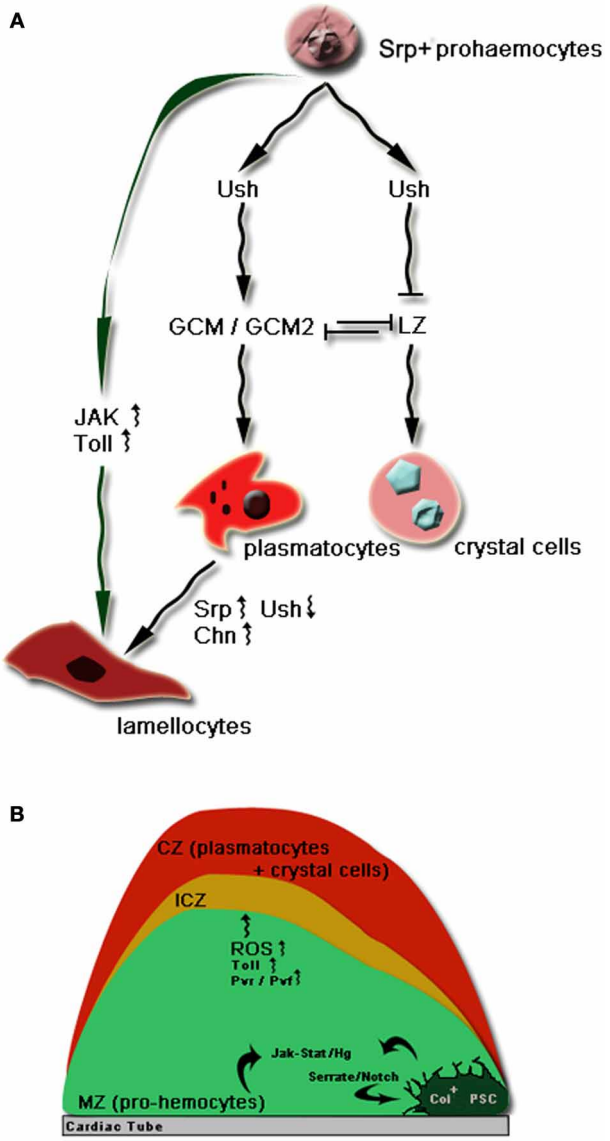


FIGURE 2 | (A) The major transcriptional network in the lineage commitment of *Drosophila* prohaemocytes (Lebestky et al., 2000; Evans et al., 2003; Waltzer et al., 2010). Prohaemocytes express the early GATA transcription factor, Serpent (Srp) for haematopoietic development (Lebestky et al., 2000). The expression of Srp defines the haemocyte anlage from embryogenesis. Later on the Srp expressing prohaemocytes turn on the transcription of the *Drosophila* friend of GATA, U-shaped (Ush). Ush together with Srp activates the expression of another transcription factor Gilial cell missing (GCM) and its isoform GCM2. Activation of GCM/GCM2 turns on the expression of plasmocyte cell markers and thus commits prohaemocytes into plasmatocyte specification (Fossett et al., 2001). In a small population of prohaemocytes, the expression of a *Drosophila* Runx family member of transcription factors, Lozenge (Lz) antagonizes Ush and also inhibits the expression of Gcm/Gcm2. Cells expressing Lz adopt the crystal cell fate (Bataillé et al., 2005). Lamellocytes do not appear in normal circumstances but can be induced rapidly from the LG or sessile haemocytes by parasitic infection in the larva. Most recent lineage tracing studies have pointed out a direct differentiation of lamellocytes from plasmatocytes by the upregulation of Srp and Charlatan (Chn) and downregulation of Ush to suppress the plasmatocyte

(Continued)

FIGURE 2 | Continued
transcriptional profile (Stofanko et al., 2010). However direct differentiation from the LG prohaemocytes may also contribute to the total population of lamellocytes in particular by the over-activation of JAK/STAT and Toll signaling. **(B)** Compartments of the primary lobes of the LG and key signaling pathways in larval haemocyte specification (Krzemień et al., 2007). The primary lobes are the major sites for the larval haemocyte differentiation. The primary lobe can be divided into three major compartments: the Cortical Zone (CZ), the Medullary Zone (MZ) and the Posterior Signaling Center (PSC) (Jung et al., 2005). There is also a region that contains a population of intermediate haemocytes from undifferentiated prohaemocytes. This region is sometimes termed Intermediate Cortical Zone (ICZ). The stem cell-like fate of prohaemocytes is regulated by communication between the PSC cells and MZ via filopodia of the PSC cells. Activation of JAK/STAT and Hg signaling by the PSC cells maintains the undifferentiated status of prohaemocytes (Krzemień et al., 2007; Mandal et al., 2007). Meanwhile the transcription factor Collier (Col) expression defines PSC cell identity and is controlled by the Serrate/Notch signaling (Lebestky et al., 2003). Toll signaling is also important in the survival and proliferation of prohaemocytes while increased ROS level and Pvr/Pvf signaling can also contribute to the differentiation of prohaemocytes to plasmatocytes (Qiu et al., 1998; Brückner et al., 2004).

therefore have either embryonic or larval lineage (Holz et al., 2003).

LG forms at the end of embryogenesis with a single pair of lobes called the anterior or primary lobes. Larval haematopoiesis takes place primarily in the primary lobes to temporally and spatially regulate the haemocyte differentiation. The primary lobes can be physically divided into three compartments (**Figure 2B**): the cortical and the medullary zones and the Posterior Signaling Center (PSC) (Jung et al., 2005). PSC is essential in controlling haemostasis in healthy larvae by a direct cell-cell communication via filopodia, thin cytoplasmic extensions (Krzemień et al., 2007; Mandal et al., 2007). This cellular contact provides a platform for the interplay of a network of key signaling pathways required for normal larval haematopoiesis (**Figure 2A**).

Based on different morphological features three major types of haemocytes can be identified throughout the life cycle of the fruit fly, namely, plasmatocytes, crystal cells and lamellocytes (**Figure 2A**) (Lanot et al., 2001; Hartenstein, 2006). Plasmatocytes are the dominating haemocyte population during all *Drosophila* developmental stages. They are macrophage-like cells that are primarily responsible for the removal of apoptotic debris, phagocytosis of invading microbes and repair of damaged tissues. Crystal cells are larger in size than plasmatocytes and are named from the paracystalline inclusions in the cytoplasm. The crystal cell inclusions are believed to contain large quantities of components involved in a process called melanisation involving a cascade of serine proteases leading to melanin synthesis (Jiravanichpaisal et al., 2006). Melanin is important to prevent haemolymph loss in wound sites, immobilize microbial pathogens and facilitate wound healing (see below). In addition, its free radical oxidative by-product can directly kill microorganisms. Although they constitute only a small proportion of 5% of the total population of *Drosophila* haemocytes in the embryo and larvae, crystal cells are major executioners in *Drosophila* innate immunity. Lamellocytes are physically distinctive from both plasmatocytes and crystal cells. They are flat and adhesive and are the largest haemocytes

observed in *Drosophila* (Lanot et al., 2001). They do not appear in the embryo or in healthy larvae but can be induced quickly to differentiate from the LG or sessile population along the border of larval segments to engulf foreign particles larger than those that can be phagocytosized by plasmatocytes, such as the parasitoid eggs (Sorrentino et al., 2002; Lee et al., 2009). This process is termed encapsulation. Lamellocytes can also launch a melanisation cascade to kill the parasitic invaders with the aid of crystal cells and thus are essential in the *Drosophila* immunity against parasite infection (Krzemien et al., 2010).

The key players including primarily transcription factors and the corresponding molecular mechanisms are briefly summarized and illustrated in **Figure 2**.

HAEMOCYTES IN IMMUNITY

In the wild, *Drosophila* feeds on rotting fruits and lives in a microorganism-enriched environment so fruit flies constantly face the danger of physical injury and gastrointestinal infection. The selection pressure from the hostile environment must be one of the driving forces for *Drosophila* to develop multilayered defense responses so that it can survive and propagate. Not surprisingly the cellular and molecular mechanisms in the various facets of *Drosophila* innate immunity have been phylogenetically conserved. *Drosophila* can mount an array of cellular and humoral responses when challenged by pathogens such as bacteria, fungi, viruses and parasites. The cellular responses include direct engulfment of small objects such as the bacteria, as in phagocytosis, and encapsulation of larger objects such as parasitoid eggs. The humoral responses take place primarily in the haemolymph and three major events can occur during an immune challenge depending on the nature of the invading pathogen: (1) direct killing by Antimicrobial peptide (AMP) released into haemolymph from rapid de novo synthesis in the haemocyte and fat body; (2) direct killing by Hydrogen Peroxide (H_2O_2) or Nitric Oxide (NO) agents produced during melanisation; and (3) immobilization of opportunistic pathogens by blood coagulation at an open wound (for more in depth reviews see Lemaitre and Hoffmann, 2007; Kounatidis and Ligoxygakis, 2012). In this review, we focus on the roles of haemocytes in the *Drosophila* host defense and the key signaling pathways in orchestrating the different strategies deployed against a wide range of pathogens.

Molecular basis of haemocyte migration and motility in embryos

Drosophila haemocytes respond to numerous signals during development or following injury and/or infection (Wood and Jacinto, 2007). These signals can include migrating cues during embryogenesis, inflammatory and stress chemoattractants from the injury site and pathogen invasion in the haemolymph or in the tissue. During development, embryonic plasmatocytes are highly motile cells and they migrate from the precephalic mesoderm around stage 10 of the embryogenesis to start to disperse the entire embryo (Tepass et al., 1994). Haemocytes migrate along several invariant main routes throughout the embryo: toward the tail, along the ventral nerve cord, along the dorsal vessel and the gut primordium. The cell migration is guided by Pvr/Pvf signaling. The receptor tyrokinase Pvr is the homolog of the vertebrate Platelet-derived growth factor (PDGF) and Vascular endothelial growth factor (VEGF) receptors. The Pvr has three ligands: PDGF- and VEGF-related factor (Pvf)-1, Pvf-2 and Pvf-3. Haemocytes express Pvr and are attracted by Pvf2 and Pvf3 expressed in different tissues of the embryo. For example, the nerve cord expresses Pvf2 and Pvf3 spatially and temporally in its different compartments to attract haemocytes to move along the Central Nerve System (CNS) (Cho et al., 2002; Wood et al., 2006). The impressively fixed migrating patterns for haemocytes to populate the entire embryo from anterior to posterior and from dosal to ventral rely on sustained motility and cell polarity and highly organized cell shape to enable smooth and rapid movement along the tissue surface. By use of live confocal microscopy, plasmatocytes were discovered to move with large, polarized and actin-rich filopodia and lamellopodia during the embryonic migration (Wood et al., 2006). Toward the end of embryogenesis, these cytoplasmic protrusions become highly dynamic and continually extend and retract to survey the surrounding microenvironment. The Rho family small GTPase Rac, Rac1 and Rac2, function redundantly to control the lamellopodia formation and thus the successful dispersal of haemocytes in the embryo (Paladi and Tepass, 2004). A *Drosophila* PDZ guanine-nucleotide exchange factor (PDZ-GEF) Dizzy was also identified to be required for the embryonic haemocyte migration (Huelsmann et al., 2006). In the absence of Dizzy, the cytoplasmic protrusions are reduced in size and thus slow down the migration rate of the cells. Overexpression of Dizzy in haemocytes generates cells with abnormally extended protrusions. Dizzy is believed to act upstream of the Ras superfamily member of small GTPase Rap1 to regulate integrin dependent adhesion of haemocytes to the epithelia and to maintain their cellular "microspikes" throughout migration (Huelsmann et al., 2006). Siekhaus et al. discovered that during embryonic haemocyte migration *Drosophila* haemocytes invade an epithelial barrier as they move into the tail despite an open blood system (Siekhaus et al., 2010). A mutant of RhoL, another *Drosophila* GTPase homolog specifically expressed in haemocytes blocks this epithelia invasion but not other aspects of guided migration. RhoL interferes with Rap1mediated integrin adhesion by moving Rap1 away from a concentration in the cytoplasm to the leading edge during invasive migration. RhoL therefore functions as a regulator for integrin adhesion and Rap1 localization during the invasion. Inhibition of integrin-based adhesion is necessary to regulate the cadherin interactions that allow plasmatocytes to transmigrate from the head region, through the epithelium, to the posterior of the embryo. These findings revealed a striking similarity of the stepwise migratory process during *Drosophila* development with vertebrate immune cell transmigration during inflammation.

Apart from moving along fixed developmental migratory patterns, embryonic plasmatocytes respond to the epithelia wound by migrating rapidly to the site of injury. This process also shares many physiological relevancies with the vertebrate inflammation. Similar to the developmental migration, this deployment to the injury site also requires Rac-mediated lamellopodia formation (Stramer et al., 2005). Stramer and co-workers showed that Rho signaling is necessary for haemocytes to retract from sites of cell

matrix and disengage from cell-cell contact. During the migration to the wound, CDC42 is required to maintain the plasmatocyte polarity. In contrast to the developmental dispersal of haemocytes in response to Pvr/Pvf cues, the chemotaxic signals from the injury site activate a different mechanism to mobilize plasmatocytes. This *Drosophila* inflammation induced cell migration depends on a phosphatidyl-inositol 3-kinase (PI3K) signaling which is also used by the mammalian neurophils in response to chemotaxic cues (Stramer et al., 2005). Therefore, it can be concluded that actin protrusion formation controlled by the Rac signaling in the cell motility is essential for plasmatocyte migration in these two different processes. Ena is another player identified recently to regulate the actin protrusions in the embryonic haemocyte (Tucker et al., 2011). Ena is the *Drosophila* homolog of Mena, member of the evolutionarily conserved Ena/VASP family of actin cytoskeletal regulators. Mena promotes metastasis and invasive motility of breast cancer cells *in vivo*. Tucker et al. found that Ena stimulates lamellipodial dynamics and positively regulates the number and length of filopodia. Overexpression of Ena in the haemocyte results in dramatic increase in the migration rate. One of the phenotypes can be also observed from overexpression of Mena in the mammalian fibroblast.

Postembryonic haemocyte migration

In the larva, overexpression of Rac in the haemocyte disrupts the sessile haemocytes population and causes a large migration of haemocytes into the circulation. Sessile haemocyte activation and mobilization require the Jun N-terminal kinase (JNK) Basket (Bsk) and Rac1. Bsk is also found to regulate the turnover of focal adhesions in the circulating haemocyte in the larva (Williams et al., 2006). These findings suggest that the Rho and JNK signaling are conserved, underlining their roles in the formation of cytoplasmic protrusions and actin focal adhesions for proper plasmatocyte mobility and migration to support their cellular roles in development and immunity. Two very recent studies on the postembryonic haemocyte migration have shed more light on the cellular dynamics and molecular basis of this process. An *ex vivo* culturing system using the primary larval or pre-pupal haemocytes has been developed to allow a real time analysis and manipulation to examine the roles of cytoskeleton dynamics in plasmatocyte migration (Sampson and Williams, 2012). From this system, it was found that larval circulating haemocytes are less motile than the pre-pupal haemocytes and thus unable to migrate. The extending and retracting rates of the protrusions appear dormant while the prepupal haemocytes have normal dynamic protrusions potentially required in the morphogenesis. The same study also reinforced the role of Rho family members: Rac1 and Rac2 and CDC42 to sustain the size of the filopodia and lamellopodia in the prepual haemocytes. Absence of these genes caused a static phenotype of pre-pual haemocytes similar to what was observed in haemocytes from third instar larvae. Though an *in vivo* assay still awaits to confirm these findings, the importance of Rho signaling in the actin cytoskeleton shaping has been strengthened in the 'walking' of the haemocyte along the extracellular matrix.

Another *in vivo* study based on MARCM (Mosaic Analysis with a Repressible Cell Marker System) investigated the integrin adhesion activation and maturation in the migration of the sessile haemocyte population in the late larval stage into pupal stage (Moreira et al., 2013). The *Drosophila* βPS integrin myosperoid and integrin containing adhesion regulators such as Rhea and Fermitin were found to be required in pupal haemocyte migration.

Cellular immunity mediated by plasmatocytes

Plasmatocytes represent around 90% of the total circulating haemocyte population in all developmental stages of *Drosophila*. They are professional macrophages which function as sentinels to maintain cell and tissue homeostasis and to recognize pathogen entry for subsequent immune reactions.

In the embryo, mature plasmatocytes function primarily as scavengers to remove apoptotic cell debris during embryogenesis. The clearance of apoptotic cell debris is dependent on scavenger receptors: CD36 homolog Croquemort (Franc et al., 1996), Draper (Manaka et al., 2004) and NimC4/Simu (Kurant et al., 2008). In the larval stage, recognition and rapid engulfment of invading microbes such as bacteria rely on the cell surface receptors Eater (Kocks et al., 2005) and NimC1 (Kurucz et al., 2007) and in the adult fly on Draper also (Cuttell et al., 2008). Loss of function of mutants in those receptors results in functional deficiency of phagocytosis of Gram-positive bacteria such as *Staphylococcus. aureus* and Gram-negative bacteria such as *Escherichia coli.* Eater, NimC1, NimC4 and Draper together with CED in *C. elegans* belong to a large protein family conserved across the metazoan animal kingdom including other insects such as *Anopheles* and humans (Kurucz et al., 2007). Epidermal Growth Factor (EGF)-like repeats are abundantly found in the extracellular domains of these bacterial phagocytosis receptors and there is evidence to show the direct interaction and binding of the EGF-like repeats with bacteria in Eater (Kocks et al., 2005) and Draper (Hashimoto et al., 2009). These interesting findings suggest that proteins with GF-like repeats may play an evolutionary conserved role in phagocytosis in the entire animal kingdom (**Table 1**).

Another receptor that binds directly microorganisms and participates in phagocytosis is Dscam, which is a member of the Ig superfamily with an essential function in neuron interconnection. By alternative splicing as many as 18,000 isoforms can be theoretically generated in the haemocyte and fat body (Watson et al., 2005). Haemocyte-specific Dscam silencing reduces the phagocytic uptake of bacteria. The existence of a potential extensive repertoire of thousands of Ig-domain-containing proteins in the recognition of a variety of pathogens in *Drosophila* and other invertebrates has opened up a new avenue in exploring the possibility of an "adaptive" immunity across animal plyla that have been considered as having only innate responses (Watson et al., 2005; Schmucker and Chen, 2009).

Haemocyte-mediated humoral response

In response to pathogens that manage to gain entry into the haemocoel *Drosophila* can mount a robust systemic immune humoral response. The hallmark of this response is the rapid synthesis of a broad spectrum of AMPs against bacteria and fungi both in the haemocyte and in the fat body. AMPs are secreted into the haemolymph and directly kill the microbes at an

Table 1 | Key genes involved in innate immunity, inflammatory responses and wound healing in *Drosophila* haemocytes.

Blood cells	Innate immune response	Key genes
Plasmatocytes	Phagocytosis / AMP production	Croquemort (Crq) Draper NimC4/Sim Eater NimC1 PGPR-LC/PGRP-LE Dscam
	Blood coagulation	Hemolectin (hml) Transglutaminase (TF)
	Inflammation / Wound healing	Eiger Psidin Spatzle
	Cytokine-like	Hayan UPd3
	Cytoskeleton modulation	Rac1/Rac2 Dizzy Zir RhoL Rap1 Cdc42 Ena
Crystal cells	Wound healing / Melanisation	Srp7 DoxA1 CG8193
Lamellocytes	Parasite infection	DoxA3 Charlatan

optimal concentration. Although the fat body—the *Drosophila* equivalent of mammalian liver—is the prominent site of AMP synthesis, plasmatocytes play important roles in triggering the AMP production as haemocyte ablation can abolish the AMP expression in larval fat body (Shia et al., 2009). The roles plasmatocytes can play in the systemic immune response can be achieved by signaling between the site of infection and the fat body or by degradation of invading pathogens. To date, a haemocyte-released cytokine, Unpaired-3 (Upd-3) has been proposed to activate the JAK/STAT pathway in response to septic injury by binding to the fat body Domeless (Dom) receptor (Agaisse et al., 2003). Nevertheless the precise role of this pathway and its overall contribution to the host defense remains to be established. Spätzle is another cytokine secreted by haemocytes, processed by a serine protease cascade in the haemolymph and required for the Toll signaling pathway controlled AMP synthesis in the fat body (Shia et al., 2009). The Toll signaling has been well characterized in the Dorsal-Ventral patterning during embryogenesis and AMP production against mainly fungi, Gram positive bacteria, *Pseudomonas aeruginosa* (a Gram negative bacterium) and also stress/danger signals (reviews in Valanne et al., 2011; Kounatidis and Ligoxygakis, 2012). The core Toll signaling event is the degradation of *Drosophila* NFκB Inhibitor homolog Cactus followed by the activation and translocation of the *Drosophila* NFκB transcription factors Dorsal or Dorsal related immunity factor (Dif) into the nucleus. Dorsal and Dif are homologs of mammalian p50 and p65. Apart from these secreted cytokines, a cytoplasmic lysosomal protein called Psidin has been found to be the link between the haemocyte phagocytosis and AMP activation in the fat body in the larval immune response (Brennan et al., 2007). Psidin is required both for the phagocytic degradation of internalized bacteria and for the induction of one of the AMPs, Defensin, in the fat body. This interesting finding suggests a likely "antigen" presentation mechanism of the haemoctye to the fat body for the activation of AMP. Contrary to these findings, plasmatocyte ablation does not affect the antimicrobial responses upon systemic infection in the adult fly (Charroux and Royet, 2009; Defaye et al., 2009). This might suggest that tissue specific humoral responses, such as local expression of AMP and cytokines independent of haemocytes in the gut or in the trachea, play dominant roles in the adult immunity against pathogens.

AMP production in haemocytes

AMP production plays a vital role throughout the life cycle of *Drosophila*. Many tissues that have direct contact with the microorganisms such as the trachea, the gut and malpighian tubules have the capability to synthesize AMP and kill the microbes locally and efficiently. For microbes that manage to gain entry into the circulation via an open wound or the digestive or reproductive tracts, the fly can mount a systemic humoral response to produce large amounts of AMP from mainly the fat body into the haemolymph. Although haemocytes are not the major organ in the fly for systemic AMP production, the signaling pathways in control of AMP synthesis are activated in haemocytes like in the other tissues during a concerted immune response, in particular in the embryonic haemocytes (reviewed by Lemaitre and Hoffmann, 2007; Kounatidis and Ligoxygakis, 2012).

Melanisation

Melanisation in arthropods is generally believed to play an important and central role in arthropod defense reactions such as wound healing, encapsulation, microbe immobilization and the production of toxic intermediates that are speculated to kill invading microorganisms (Cerenius and Söderhäll, 2004). As described briefly above, crystal cells are the major haemocytes responsible for the melanisation reaction in the larva. Melanisation can be immediately induced at the site of cuticular injury or on the surface of parasites invading the haemocoel. It involves formation of black pigmentations resulting from *de novo* synthesis and deposition of melanin. Prophenoloxidase (PPO) is the enzyme required in melanisation to catalyze the oxidation of mono- and di-phenols to ortho-quinones, which polymerize into melanin. PPO in normal physical conditions is enzymatically inert. A serine protease known as prophenoloxidase activating enzyme (PPAE) acts upstream to cleave and turn PPO into active phenoloxidase (PO). Like PPO, PPAE also exists as an inactive zymogen and it is processed by a tightly regulated serine protease cascade in a step wise way into enzymatically functional form leading to the final melanin formation. As in other invertebrates, a recent *in vitro* study on the *Drosophila* PPOs suggested

a direct binding of PPOs to bacteria and fungi which might play a role to initiate their activation (Yang et al., 2013). The *Drosophila* genome encodes three PPOs: DoxA1, DoxA3 and CG8193 (Irving et al., 2005). Crystal cells express DoxA1 and CG8193 while lamellocytes express exclusively DoxA3, a strong indication that Dox3 participates in the encapsulation that accompanies melanisation. Melanisation is diminished in the *domino* mutant that lacks haemocytes (Braun et al., 1998) and in the *Black cells* (Bc) mutant with aberrant crystal cells (Rizki et al., 1980; Corbo and Levine, 1996) and the Lz knockout, which is devoid of crystal cells (Peeples et al., 1969). One serine protease Sp7 has been reported to be involved in PPO activation and expressed also in the crystal cells (Castillejo-López and Häcker, 2005). In the absence of crystal cells in the adult fly, melanisation perhaps relies on the activation of proteolytic cascades in the haemolyph including PPAE, PPO and serine proteases. The cascades are tightly regulated by serine protease inhibitors in the haemolymph such as Serpin27 A to restrict the reaction to the site of injury and to prevent the spread of systemic melanisation (De Gregorio et al., 2002; Ligoxygakis et al., 2002). Two serine proteases MP1 and MP2 are reported to activate the cascade in response to different microbial changes (Tang et al., 2006). This pathogen-specific activation of melanisation can be attributed to PGLP-LC and PGRP-LE expressed both by haemocytes and the fat body (Takehana et al., 2004; Schmidt et al., 2008). However the connection between other types of pathogen receptors (for example, Gram-positive bacteria and fungi etc.) has not been linked to melanisation triggering. To date only one PPAE has been identified in the melanisation cascade of the adult fly (Leclerc et al., 2006).

Encapsulation

Lamellocytes are the major executioner of encapsulation during parasite infection in the *Drosophila* larva. Encapsulation involves three key steps with coordinated actions from both plasmatocytes and lamellocytes. Firstly circulating plasmatocytes sense and recognize the entry of parasitoid eggs in the haemocoel and attach to the egg chorion. Secondly a massive proliferation and differentiation of sessile compartments and of haemocytes in the LG to lamellocytes is induced via unknown signaling molecules within a few hours to appear in the circulation where the lamellocytes form a multi-layered capsule around the eggs. Eventually the lamellocytes, like the crystal cells, release their cellular content such as PPO to activate the melanisation process and kill the parasites, possibly by the cytotoxic by products from the localized melanisation reaction (Nappi et al., 1995). To date genes that have been reported to play a role in encapsulation process are involved primarily in cell-cell interaction such as αPS4/βPSintegrins (myospheroid) (Irving et al., 2005; Wertheim et al., 2005) and in cytoskeleton remodeling for motility and migration, such as RhoGTPase protein family member Rac1(Williams et al., 2006), Rac2 and CDC42 (Williams et al., 2005). Rac2 and CDC42 are activated by the *Drosophila* homolog of Rho guanine nucleotide exchange factor (RhoGEF), Zir (Sampson and Williams, 2012; Sampson et al., 2012). Rac1 and Rac2 function in a non-redundant manner. Interaction between Rac1and myospheroid has recently been reported to be required in the directed localization of β-integrin on the cell surface of lamellocytes in response to parasitoid eggs (Xavier and Williams, 2011). Rac1 requires the JNK pathway component Bsk to regulate the formation of actin- and focal adhesion kinase (FAK)-rich placodes in haemocyte migration and both are required for the proper encapsulation of wasp eggs (Williams et al., 2006). A recent screen to target genes involved in the cell adhesion and shape change not only strengthened the previous findings but also discovered more conserved components in these cellular processes that participate in the encapsulation reaction, for example the extracellular matrix proteins (ECM) (Howell et al., 2012). Loss of function of ECM components results in failure to encapsulate. In correlation with the previous discovery on the encapsulation of mechanically damaged self-tissue, it is plausible that exposure of ECM by foreign particle intrusion or deposition of ECM on the eggs can be the initiative signal in encapsulation (Rizki and Rizki, 1980; Howell et al., 2012). Genome-wide analysis of the transcriptional profiles in haemocytes after parasitoid infection has offered many interesting and promising candidate genes that are differentially regulated in the encapsulation reaction (Wertheim et al., 2005). This study also reinforced the importance of the Toll and JAK/STAT signaling pathways in the differentiation and proliferation of lamellocytes in the LG (Sorrentino et al., 2004). In addition, the haemocyte-specific transmembrane protein Hemese has been reported to play a modulatory role to keep lamellocyte proliferation in check from overacting during parasitoid egg infection (Kurucz et al., 2003). Despite these findings, the molecular nature of the signals sent from plasmatocytes for lamellocyte differentiation in the LG remains elusive. It has been proposed that a signal delivered to the PSC initiates lamellocyte differentiation as the PSC-restricted expression of Collier (Col), the *Drosophila* homolog of human Early B cell factor, is required upon parasite invasion (Crozatier et al., 2004).

Blood coagulation

Haemocytes have essential roles in blood coagulation, not only to maintain haemostasis but also to defend against pathogens. It has been found that Hemolectin (Hml) expressed mainly by plasmatocytes is required in blood coagulation. Blood coagulation led by plasmatocytes is independent of both melanin production and phenoloxidase activity, which is also part of the wound healing process (Goto et al., 2003). By proteomics and pull out analysis, important components of the blood clot have been isolated and subjected to detailed genetic and cellular investigations (Karlsson et al., 2004; Scherfer et al., 2004). Among these the best-characterized clotting factors are *Drosophila* Transglutaminase (TF) and Fondue. *Drosophila* TF is the only mammalian blood coagulation factor homolog (Factor XIIIa) found in the fly and uses Fondue as its substrate to form the blood clot. Unlike *hemolectin* (*hml*) mutants shown to affect only coagulation (Goto et al., 2003), ubiquitous silencing of *fondue* also results in cuticle defects in the pupa as well as in the clot forming in larvae (Scherfer et al., 2006). Hml is expressed mainly by plasmatocytes and contains domains found in coagulation factors (Goto et al., 2001, 2003). It is suggested that TF/Fondue acts more actively in cross-linking of fibers formed by Hml, reacting promptly to bleeding and injury (Scherfer et al., 2006; Lindgren et al.,

2008). Interestingly TF is most likely expressed in haemocytes (Johansson et al., 2005) while Fondue is expressed in the fat body under control of the Toll signaling pathway (Scherfer et al., 2006). This strongly suggests that cellular and humoral factors are required in blood coagulation with contributions from both haemocytes and the fat body. Therefore, the lack of a signal sequence in TF gene (like PPO, another enzyme expressed in haemocytes) suggests that its release from haemocytes may be a key step in the initiation of coagulation. Most recently Wang et al. proposed a conserved innate immune mechanism based on TF's ability to use a potential microbial surface substrate to sequestrate and immobilize bacteria to the clot formed in blood coagulation (Wang et al., 2010).This interesting piece of work provides direct evidence for the blood coagulation factor to directly bind to microbes in the process of blood clot formation.

THE *Drosophila* "INFLAMMATORY RESPONSE"

All organisms have developed various mechanisms to maintain structural and physiological integrity in response both to external injury and to internal disruption. Wound healing, tissue repair and regeneration are essential processes for multi-cellular organisms to survive and proliferate against constant environmental or physical assaults. Therefore it is highly feasible to hypothesize that wound healing is an ancient process that evolved before the divergence of insects and mammals and this view can be supported by evidence from extensive research conducted in various model organisms on the pathways underlying this basic process.

Wound healing depends on complex molecular and cellular networks involving different types of cells and tissues. This complexity increases with the basic mechanisms varying in a tissue-specific, developmental stage dependent, and damage related manner. For example, in fruit fly embryos wound healing occurs rapidly via actin cable assembly and filopodial extension by cells at the wound margin, and proceeds without blood clot formation (Kiehart et al., 2000; Wood et al., 2002). Again, despite the substantial structural differences between *Drosophila* and mammalian epidermis, embryonic wound healing in mammalian embryos appears to be similar to that in *Drosophila*. It is also a rapid process involving actin cable formation without apparent haemostatic or inflammatory response (Martin and Lewis, 1992).

Haemocytes in embryonic wound healing

Although blood clots are not formed and required during the embryonic wound healing, microarray analysis comparing the transcriptional profile of wild type and haemocyte-absent embryos still revealed interesting haemocyte signature genes involved specifically in wound healing. These include phospholipase A2 conserved also in the mammalian inflammatory response (Stramer et al., 2008). From this study, a *Drosophila* ortholog of a novel mouse inflammatory-responsive gene Growth Arrest and DNA Damage-inducible gene 45 (GADD45) (Takekawa and Saito, 1998) was found to be induced in the damaged epidermal cells. This finding reinforces the idea that inflammatory responses are ancient processes for organisms to respond to danger signals. JNK signaling has also been reported to be essential in the epithelial wound healing in the embryo (Rämet et al., 2002; Wood et al., 2002). The earliest signal that triggers the haemocyte attraction to a wound in embryos has been recently identified as the calcium wave from the damaged epithelial cells following immediate laser wounding. Blocking this calcium flash inhibits H_2O_2 synthesis which relies on the activation of an NAPDH oxidase, DUOX (Razzell et al., 2013). In response to H_2O_2 that transiently outcompetes developmental migrating cues, haemocytes are quickly recruited to the injury site in the embryo (Moreira et al., 2010). The establishment of calcium flux-induced H_2O_2 production in the inflammatory response associated with wound healing from *Drosophila* will certainly give more insights into the signaling events taking place in wound induced inflammation in mammals.

Haemocytes in tissue injury and wound repair in the larva

The mammalian epithelial tissue can summon a set of humoral and cellular reactions lasting from days to months in response to tissue damage until the damage cap is properly closed and the injured cells or tissues are removed and replaced. The reactions typically include the rapid formation of a blood clot at the injury site and recruitment of inflammatory blood cells followed by spreading of the damaged epithelium across the wound gap to restore tissue integrity (Of and Healing, 1999; Singer and Clark, 1999). Likewise, *Drosophila* larval wound healing shares many similarities to postembryonic wound healing in mammals. By developing an aseptic puncture wounding in the third instar larva in combination with *in vivo* life imaging, Galko and Krasnow (2004) have established a system to study the process of *Drosophila* postembryonic wound healing. They have characterized the wound healing process by three key stages: (1) primary clot formation during blood coagulation: larvae bleed following the puncture wounding and the primary clot forms in the wound gap; (2) scab and syncytium formation: the primary clot is further cross-linked and hardened by melanisation to form a scab while epidermal cells surrounding the primary clot migrate toward it and then fuse to form a syncytium; and (3) central syncytium formation: more epidermal cells are attracted to the initial synticium and a larger central syncytium forms. JNK pathway is activated in the epidermal cells of the syncytium in a gradient manner to emanate signals for the epidermal cells to move along or through the wound clot to rebuild a continuous epithelium with its basal lamina and apical cuticle lining. Crystal cells are the haemocyte required in the formation of the scab. The scab stabilizes the wound site, establishes a physical barrier to the external microbes, prevents the over-activation of JNK pathway which can result in chronic wounding and provides a scaffold for re-epithelialisation. Scab forming and wound closure are controlled by independent genetic and signaling pathways as re-epithelialisation can still be activated at the wound gap though it never heals in the absence of a scab (Galko and Krasnow, 2004). Presumably, multiple signals must be produced to spatiotemporally co-ordinate this dynamic flow of cellular events involving crystal cells, epidermal cells and also plasmatocytes to remove cell debris for tissue remodeling in addition to phagocytosis of invading microbial pathogens from the open wound. For example: the signals from damaged sites to initiate blood coagulation as discussed in the previous section are still to be identified. What also remains unknown at present is the signal to attract crystal cells to the primary clot, the signals during the formation of a scab (perhaps from crystal cells or plasmacytes)

to negatively modulate the JNK activity and the signal to recruit plasmatocytes to the wound site. These signals may be able to behave like mammalian chemokines or cytokines and possess distinct characteristics in terms of the range and the different signaling pathways they can activate. In addition, there could also be mitogenic signals from apoptotic cells to stimulate cell migration and regeneration (Bergmann and Steller, 2010). Although the identity of these invertebrate inflammatory signaling molecules remains largely unknown, in combination with studies on other arthropods the interconnection between inflammatory responses and wound healing seems to be phylogenetically conserved (Theopold et al., 2004; Eleftherianos and Revenis, 2011). Recent studies have begun to reveal the molecular identities of some of these signals. A blood borne Pvf1 ligand has been found to be expressed by epidermal cells at the wounding edge and to function in an autocrine manner to activate the motility of epidermal cells in wound closure (Wu et al., 2009). In a study by mutant screening using crystal cell rupture and melanisation as the readout, Bidla et al. reported that the rapid rupture of crystal cells and subsequent local melanisation in the clot at injury depended on the JNK pathway and on Eiger, the *Drosophila* homolog of tumor necrosis factor (Bidla et al., 2007).The most interesting finding is that endogenous signals such as Eiger released from crystal cells and plasmatocytes undergoing apoptosis followed by secondary necrosis can function independently of microbial elicitors in triggering the PPO activation, which can support the idea that endogenously induced 'death signals' initiate inflammatory and repair responses. Nevertheless, the molecular nature of the signal to initiate JNK pathway by the mechanical stress for the crystal cells to rupture still remains to be discovered to date.

In a study to reveal the genetic and molecular networks in control of systemic wound response after physical wounding in *Drosophila* larvae and adult flies, Nam et al. reported that a redox signal released from proPPO activation via the blood borne serine protease Hayan is required for the downstream activation of JNK signaling to protect remote internal tissues from systemic wound response induced by local physical trauma (Nam et al., 2012). Forced expression of Hayan in the haemocyte, but not in other tissues, rescued the wound induced mortality in the *hayan* loss of function mutant suggesting that a redox dependent mechanism communicates between circulating haemocytes (most probably plasmatocytes) and the remote internal tissues in a process similar to systemic inflammation in mammals.

The cellular and genetic basis of wound healing has recently been studied in detail by using a *Drosophila* embryo laser wounding model and it is revealed not surprisingly that a coordinated process exists involving myosin, E-cadherin, Echinoid, the plasma membrane, microtubules and the CDC42 small GTPase which respond dynamically during wound repair (Abreu-Blanco et al., 2011, 2012). The wound healing mechanism in *Drosophila* larvae was also explored by the development of a targeted large scale *in vivo* RNAi screen in the larval epidermis. Likewise, in the embryo, components in the JNK pathway and genes involved in the remodeling of actin cytoskeleton also actively participate in the larval wound healing (Lesch et al., 2010). Key genes in the haemocyte mediated innate immunity and in the *Drosophila* inflammatory response are summarized in **Table 1**. For recent reviews on the topic of wound healing, (see Belacortu and Paricio, 2011; Ríos-Barrera and Riesgo-Escovar, 2013).

Haemocytes in infection induced inflammation

The last decade has witnessed a rapid growth of research in gut immunity in *Drosophila* (reviews in Royet, 2011; Kounatidis and Ligoxygakis, 2012). In 2009 a genome-wide RNAi screen revealed a large numbers of genes in both haemocytes and the fat body to be regulated following intestinal *Serratia macescens* infection (Cronin et al., 2009). By ontology enrichment analysis, this study found a strong enrichment of genes in haemocytes implicated in processes including phagocytosis, responses to external stimuli and vesicle trafficking. The critical role of JAK/STAT signaling in the gut immunity was reinforced to function through regulation of intestinal stem cell proliferation which controls the gut epithelial cell haemostasis. Though potential signaling pathways were not the focus of this genome-wide analysis, the large number of genes to be either upregulated or downregulated in haemocytes suggests important modulatory roles that haemocytes can play in organ to organ communication in response to internal infection or inflammation. Recently, Juang and colleagues found that intestinal ROS signal triggers a systemic AMP expression in the fat body following oral feeding of Ecc15 (*Erwinia carotovaro* subsp.) to larvae. The ROS stress in the gut induces NO expression and transduces the signal to haemocytes by a NO dependent pathway (Wu et al., 2012). NO-dependent signaling mediated by haemocytes has been also observed following gastrointestinal infection by *Candida albicans* (Glittenberg et al., 2011). In the absence of haemocytes, the AMP production was greatly reduced but not completely abolished in the fat body. Though ROS has long been recognized to be involved in the initiation of inflammatory bowel diseases in humans (Rezaie et al., 2007), this recent finding from *Drosophila* can offer further insight into the potential role of macrophages in triggering a systemic inflammation during inflammatory bowel diseases. In addition, gut-associated macrophage-like cells were also found in the larval gut and their number was regulated by the PI3K signaling pathway (Zaidman-Rémy et al., 2012). This situation closely resembles the mouse colitis model (for review see Lin and Hackam, 2011). Taken together, research in *Drosophila* has revealed many similarities in the important signaling roles macrophage-like blood cells can fulfil during either local tissue-specific or systemic inflammation following internal infection. On the other hand, more recently, Panayidou and Apidianakis have given a comprehensive review on using *Drosophila* as the model to study interlinking mechanisms underlying intestinal cell proliferation, differentiation and maintenance during bacterial infection and intestinal stress. The authors therefore proposed a regenerative inflammation phenomenon independent of haemocytes conserved between *Drosophila* and mammals in cancer progression (Panayidou and Apidianakis, 2013).

CANCER AND IMMUNITY IN *Drosophila*

Drosophila as the model to study haematopoiesis and its associated leukemia

In adult mammals such as humans and mice, bone marrow is the organ that houses haematopoietic stem cells (HSC) which

give rise to both the myeloid and lymphoid lineage. Mammalian HSC possesses the ability to self-renew and the pluripotency to differentiate into a great variety of blood cells in response to signals from its microenvironment, which has been termed the HSC niche (review in Wang and Wagers, 2011). The HSC niche has been a subject for vigorous research since the concept has been fully accepted (Wang and Wagers, 2011; Lensch, 2012). The dynamic communication between HSC and its niche has been shown to be fundamental in the control and regulation of haematopoietic process in vertebrates. Any dysfunction in the genetic and cellular mechanism underlying the HSC and niche interaction can result in blood borne cancers such as AML (Oh and Humphries, 2012). However, the structural and cellular complexity of the bone marrow niche has hindered the progress of fully understanding the basic genetic and molecular events fundamental to the haematopoietic process and their application in potential human diseases. The *Drosophila* larval PSC as discussed previously functions as a primitive niche to instruct the different fate that the prohaemocyte would adopt or to help the prohamemocyte to maintain its stem cell status (Crozatier and Meister, 2007). The LG primary lobes represent a very simplified HSC and niche model compared to its mammalian counterpart (Mandal et al., 2004). Although there are obvious restraints such as the limited number of differentiated blood cell types and the complete absence of lymphocytes, this simplicity can further our understanding of the basic signaling and cellular communication mechanisms involved in particular between the HSC and its microenvironment (review in Crozatier and Vincent, 2011).

Signaling pathways in Drosophila haematopoiesis and tumorigenesis

Research in *Drosophila* haematopoiesis has revealed a number of pathways in control of prohaemocyte proliferation and differentiation. Overexpression of the *Drosophila* JAK gene hop^{Tum-1} causes proliferation of prohaemocytes and leads to melanotic tumor formation in the LG (Harrison et al., 1995). This discovery preceded the demonstration that mutated constitutive activation of JAK/STAT signaling could result in human leukemia (Lacronique et al., 1997). Compared to the vertebrate system, the *Drosophila* JAK/STAT pathway is much simplified and shows nearly complete absence of genetic redundancy. The *Drosophila* genome encodes only three upstream ligands of JAK/STAT pathway Unpaired (Upd1-3) while the mammalian JAK/STAT can be activated by a large group of cytokines and growth factors. Dom is the only transmembrane receptor upstream of one JAK kinase (Hop) and the one STAT transcription factor (STAT92E). Therefore the misexpression of a dominant-active form of STAT92E can also promote tumorigenesis in the eye of the *Drosophila* adult flies and melanotic tumor formation in the larva (Ekas et al., 2010). A systematic genome-wide RNAi screening for genes required for JAK/STAT pathway activity in cultured *Drosophila* haemocyte-like cells also identified interacting genes that can function as suppressors of leukemia-like blood cell tumors in humans (Müller et al., 2005). Increasing evidence from clinical research in human AML has pinpointed a role for JAK/STAT signaling pathway to be implicated in AML pathogenesis. In particular an activating mutation on the human JAK2 has been discovered to be responsible for various forms of AML (Lee et al., 2013a; Vainchenker and Constantinescu, 2013).

Apart from the JAK/STAT signaling pathway, the Notch, Hg, Wnt, and JNK pathways have all been identified as regulators of prohaemocyte fate (Mandal et al., 2007; Owusu-Ansah and Banerjee, 2009; Sinenko et al., 2009). Hg signaling in the PSC has been identified to maintain the undifferentiated fate of prohaemocytes in the larval medullary zone of the primary lobes in the LG (Mandal et al., 2007). In a mouse B cell lymphoma model, Hg signaling from the stromal cells was also shown to provide an important survival signal for B- and plasma-cell malignancies *in vitro* and *in vivo* (Dierks et al., 2007).The Hg signaling pathway was also discovered to be required in the maintenance of cancer stem cells of chronic myeloid leukemia (CML) (Zhao et al., 2009). Loss of Smoothened (Smo) the downstream transmembrane G protein coupled receptor in the JAK/STAT pathway causes depletion of CML stem cells whereas constitutively active Smo augments CML stem cell number and accelerates the disease. These studies are reminiscent of Hg signaling implicated in the communication between PSC niche and the prohaemocyte in *Drosophila* haematopoiesis.

Notch signaling pathway has been well conserved in vertebrate haematopoiesis, in particular in lymphoid cell commitment (Radtke et al., 2005; Tanigaki and Honjo, 2007). In *Drosophila* LG, the Serrate-mediated Notch signaling from the PSC is required to maintain normal levels of Col transcription and thus PSC cell identity (Krzemień et al., 2007). In addition, a non-canonical and ligand-independent activation of Notch signaling has also been reported to determine crystal cell fate in the LG. The *Drosophila* ortholog of mammalian hypoxia-inducible factor–a (HIF-a), Sima, activates full length Notch receptor under conditions of normal oxygen availability and commits the prohemocyte to the crystal cell lineage (Mukherjee et al., 2011). In fact, the first human Notch was originally identified from human Acute lymphoblastic leukemia (ALL) and since then Notch signaling has been discovered to be involved in many forms of human leukemia (review in Pancewicz and Nicot, 2011).

Wnt signaling has been shown to promote proliferation of prohaemocytes and prevent differentiation at the same time by controlling the PSC niche (Sinenko et al., 2009). In addition, Wnt signaling also positively regulates the proliferation and maintenance of PSC cells while inhibition of Wnt signaling results in fewer PSC cells than observed in control flies. Likewise in vertebrates, Wnt signaling has been reported to play important roles in the HSC homeostasis and maintenance of its microenvironment for self renewal (for the most recent review see Seke Etet et al., 2013) and thus has been implicated in many forms of haematologic malignancy such as AML (Gandillet et al., 2011) and CML (Nagao et al., 2011). To elucidate the controversial role of reactive oxygen species (ROS) in the haematopoietic system, work done by Owusu-Ansah and Banerjee set out to make use of the *Drosophila* haematopoietic model to study levels of ROS in the *in vivo* proliferation and differentiation of prohaemocytes. It was found that increased levels of ROS promote the differentiation of prohaemocytes while inhibition of the ROS level delays the differentiation of prohaemocytes into mature haemocytes. Interestingly, through a downstream signaling pathway that involves JNK and FoxO

activation as well as Polycomb downregulation, increasing the haematopoietic progenitor ROS beyond their basal level triggers premature differentiation of prohaemocytes into all three mature haemocytes found in *Drosophila*. Therefore a moderately high level of ROS can be the developmental signal for the population of haemocyte progenitor to commit to lineage differentiation (Owusu-Ansah and Banerjee, 2009) (**Figure 2B**). In mammals, higher ROS level was also observed during the common myeloid progenitor differentiation in response to oxidative stress (Tothova et al., 2007). A recent study by Dragojlovic-Munther and Martinez-Agosto demonstrated that the tumor suppressors TSC and PTEN also have important roles in controlling blood progenitor proliferation through a common TOR- and 4EBP-dependent pathway in the LG. Loss of function of *Tsc2* or *Pten* in prohaemocytes increases TOR signaling and causes overgrowth of the LG by haemocyte hyper-proliferation accompanied by a higher level of ROS. This study illustrates further how TSC and PTEN influence TOR function in response to physical stress such as starvation, hypoxia or increased ROS level during infection (Dragojlovic-Munther and Martinez-Agosto, 2012). Interestingly, the PTEN/mTOR signaling pathway has indeed been the therapeutic target in the treatment of human leukemia (Martelli et al., 2011).

Collectively these studies demonstrate the strength of *Drosophila* as an excellent model to study the HSC and its microenvironment interaction and shed light on the potential for therapeutic prevention of various hematological malignancies by dissecting its underlining genetic and cellular mechanisms.

Drosophila human leukemia model

Apart from the elucidation of basic molecular signaling pathways in the HSC and its niche interaction, *Drosophila* can also be used directly to model human leukemia. In addition to overactivation of JAK/STAT signaling, human AMLs can result from the chromosomal translocation of the transcription factor AML1, a RUNX domain protein, to form a protein fusion product with ETO (Hatlen et al., 2012). Targeted expression of human AML1-ETO fusion transcription factor in the haemocyte lineage cells by using the UAS/GAL4 system caused human leukaemic-like phenotypes such as hyper-proliferation of the circulating haemocytes resulting from the expansion of prohaemocytes in the LG (Sinenko et al., 2010). The successful establishment of the AML1-ETO leukemia model in *Drosophila* allowed a rapid tissue-specific genetic screening to identify suppressors for the hyperproliferation phenotype. The authors thus were able to show that ROS is a signaling factor promoting maintenance of normal as well as aberrant haemocyte precursors which suggested the importance of antioxidant enzymes and their regulators as targets for further study in the context of leukemia (Sinenko et al., 2010). In another independent genetic screening for modifiers in the AML1-ETO *Drosophila* model, Osman et al. identified calpainB as required for AML1-ETO-induced blood cell disorders in *Drosophila* by using an *in vivo* RNAi-based screen for suppressors of AML1-ETO. Remarkably, calpain was also found to interact with AML1-ETO in the human leukemic blood cell line Kasumi-1 (Osman et al., 2009). Therefore these studies have paved new avenues in our understanding of the pathogenesis of haematopoietic associated leukemia by developing and recapitulating the fundamental features of the disease in *Drosophila* and will contribute significantly to more precise and effective AML therapy.

Drosophila and human solid tumor

During the past two decades, the completion of the *Drosophila* genome and the development of advanced genome editing tools have given unprecedented stimulus and fast expansion on the use of *Drosophila* as a model for cancer. In particular, *Drosophila* has been instrumental for the discovery of three fundamental mechanisms involved in tumor progression and metastasis: (1) the role of Hippo signaling pathway in control of cell growth and survival together with Scrib/Dlg/Lgl signaling pathway in cell polarity to regulate organ sizes; (reviews in Enomoto and Igaki, 2011; Martin-Belmonte and Perez-Moreno, 2012; Harvey et al., 2013); (2) the *in situ* cell competition mechanism for morphogens during the formation of epithelium where slow growing cells are outcompeted and removed (review in Levayer and Moreno, 2013); and (3) apoptosis induced mitogenic signals in compensatory proliferation to replace surrounding damaged tissues (review in Fan and Bergmann, 2008). All these pathways underline the basic cellular communication, shaping and tissue organization which if disrupted can lead to tumorigenesis and facilitate tumor metastasis.

Despite a very short life span, cancer research to investigate directly the tumor progression and invasion in *Drosophila* can be dated back to nearly a century ago. It was observed that *Drosophila* can naturally develop hereditary tumors; carcinogens such as X-rays have been used to discover mutants with abnormal growth (Bridges and Brehme, 1944; Salomon and Jackson, 2008). The first fly strain carrying a mutation in a tumor suppressor gene called *lethal giant larvae* (*lgl*) was isolated 70 years ago. However only recently has Lgl been identified as a component of a signaling circuit in the regulation of apico-basal polarity in epithelial cells: Scrib/Dlg/Lgl (Humbert et al., 2008). Loss of function of the genes in this pathway can lead to the formation of neoplasma in the brain and imaginal disks in the developing larva. Based on the neoplastic phenotype observed in the scrib signaling mutants, Gateff was one of the pioneers who carried out more screenings to isolate recessive lethal mutations that could promote neoplastic overgrowth in the brain, imaginal disks or haematopoietic organ (Gateff, 1978). A tissue transplantation technique was developed from Gateff's study to assess the malignancy and invasive capacity of the neoplastic clones arising from various tissues. This technique involves the implantation of cancerous cell clones into the abdomen of wild type adult flies. Flies implanted with malignant cell clones usually die within 2 weeks and histological examination usually observes a massive invasion of cancerous cells into various tissues in the adult fly. This method resembles the tail veil intravenous injection of tumor cell lines into the mouse to model cancer in small rodents. This pioneering work fully established the potential for *Drosophila* to be used as a whole organism model to recapitulate key stages in cancer pathogenesis. The *in vivo* lac-Z reporter gene expression system can be utilized to quantify the proliferation rate and metastatic index of donor tumors in a tissue specific context in the normal wild type host (Beaucher et al., 2007). By using mutant clones of labeled cells such as GFP within

a specific tissue, the invasive and metastatic behavior of cancer cells can be observed *in situ* by *in vivo* live imaging (Brumby and Richardson, 2003; Pagliarini and Xu, 2003). The recent development of MARCM system allows the establishment of large homozygous mutant cells clones within a normal or heterozygous tissue. A similar system is the FLP/FRT site directed recombination that can also generate genetic mosaics in the targeted organ or tissue. The mutant cells usually overexpress a UAS-tagged transgene fused with a reporter gene like GFP to allow imaging. These systems have been used by two independent groups to identify genes in cooperation with known oncogenes to induce tumor growth and invasion in the context of normal tissue (Brumby and Richardson, 2003; Pagliarini and Xu, 2003). By overexpressing an activated Ras (Ras^{v12}) in the *Drosophila* eye imaginal disk and screening for the entire *Drosophila* genome, Pagliarini and Xu indentified Scrib as the promoting factor for the metastatic transformation of the otherwise benign tumor caused by overexpression of Ras oncogene alone (Pagliarini and Xu, 2003). Similar work by using the *Drosophila* eye as an *in vivo* "test tube" to investigate genetic interactions during tumor progression has proved to be extremely fruitful in dissecting the Scrib tumor suppressor signaling pathway and Hippo pathway and human oncogenes and tumor suppressors such as Ras and PTEN. Critical reviews of the most recent progress in the use of *Drosophila* in cancer modeling and therapeutic potentials can be found elsewhere (Miles et al., 2011; Stefanatos and Vidal, 2011; Gonzalez, 2013; Tipping and Perrimon, 2013).

Innate immunity in Drosophila tumor progression and tumor invasion model

Like other aspects of tumor progression that can be modeled and studied comparatively in *Drosophila*, the interplay of innate immunity mediated inflammation and tumor growth and invasion can be investigated accordingly. Of note is the establishment of *Drosophila* intestinal tumor model to study the molecular and cellular mechanisms linking inflammation and cancer pathogenesis (reviewed in Christofi and Apidianakis, 2013). The *Drosophila* cytokines Upd1-3 have been reported to activate the JAK/STAT signaling in promoting intestinal stem cell proliferation upon enteric infection or JNK-mediated stress response and thus the innate immunity plays essential roles in gut tissue homeostasis (Jiang et al., 2009). Moreover, overexpression of *Drosophila* Ras oncogene in association with bacterial infection can result in the formation of intestinal dysplasia (Apidianakis et al., 2009). More recently it is revealed that both the activation of Ras and bacterial induced IMD signaling can activate JNK pathway, which culminates in the up-regulation of matrix metalloproteinase 1 and thus cell invasion and migration (Bangi et al., 2012). Two studies have also emerged to explore the roles of haemocytes in the tumor progression and both relied on induced $Ras^{v12}Scrib^{-/-}$ model in the *Drosophila* eye disks, which has been used to offer complementary views of systemic innate immunity to tumor growth and invasion. Under the condition of established tumor, the *Drosophila* haemocyte could be recruited to the tumor site and tumor associated haemocytes are the major source of *Drosphila* TNFα, Eiger, to promote the growth and invasion of the tumor cells into other tissues (Cordero et al., 2010), while a TNF-dependent mechanism in *Drosophila* eliminates cells deficient for the polarity tumor suppressors Scrib or Dlg to maintain the tissue homeostasis and keep any malignant growth in check (Brumby and Richardson, 2003). In an earlier study by Tian Xu and co-workers, the same eye imaginal disk derived $Ras^{v12}Scrib^{-/-}$ tumor was found to induce a systemic proliferation of haemocytes via the JNK-JAK/STAT signaling cross-talk conserved also in response to tissue injury. The disrupted tumor basal membrane recruited

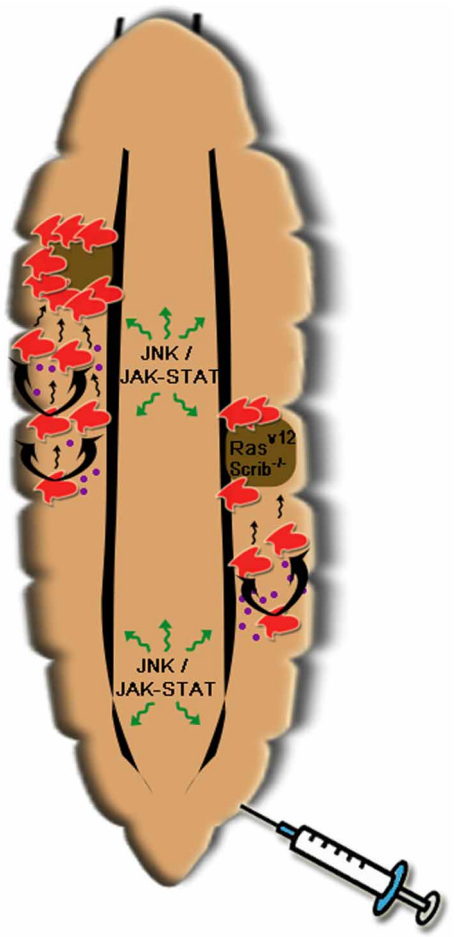


FIGURE 3 | A simple illustration of roles of haemocytes in the *Drosophila* tumor model. Both tumor ($Ras^{v12}Scrib^{-/-}$) and wounding (as illustrated by a needle) can trigger proliferation of circulating haemocytes in the larva. This is mediated by cytokines (most probably Upd 1–3 shown in purple dots) induced by JNK signaling in response to wounding or the invasive tumor itself. In responses to the cytokines, JAK/STAT signaling in haemocytes or the fat body (not shown) can be activated and thus can further amplify the cytokine expression to promote haemocyte proliferation. Meanwhile disruption of basal membrane in the tumor attracts the adherent of haemocytes and limits the tumor growth perhaps by the synthesis of *Drosophila* TNFα, Eiger. This cartoon delineates that a step wise proliferation of haemocytes induced by wounding can be used to counter tumor growth. Modeling of these two processes (tumor growth and inflammation) in the *Drosophila* larva points out the potential beneficial effect that systemic inflammation can exert on tumorigenesis and will open more avenues for the basic research on innate immunity in particular macrophages' role in human tumor (see text for more detail).

circulating haemocytes in a manner reminiscence of wound healing (Pastor-Pareja et al., 2008). This study also modeled for the first time in *Drosophila* the anti-tumor effect of a systemic inflammation response induced by mechanical injury and provided critical insight into the cross-talk between the different signaling pathways in regulating the multiple step progression of tumor.

PERSPECTIVE

Cancer has a complex biology. As a rationalization, Hanahan and Weinberg identified six major successive changes in human tumor development (Hanahan and Weinberg, 2011). The increasing awareness of this daunting complexity has turned more scientists to develop *Drosophila* as the complementary genetic tool to dissect each of the hallmark processes and to offer fundamental insights into the underlying genetic and cellular basis of the disease. In future, the *Drosophila* model can continue to be used as the reductionist system to investigate more of the cross-talks between these fundamental cancer biological processes. For example, the contrasting roles of immunity to control tumor growth or to be hijacked by the tumor can be modeled in the fly in a temporal and spatial manner. The unique advantage of *Drosophila* to be used as a whole organism has started to show promising potential in cancer drug screening and testing recently (Dar et al., 2012). This advantage can be fully explored by modeling simultaneously or sequentially physiological processes such as wound or infection induced innate immune/inflammatory response in the progression of cancer (**Figure 3**). The study of the role of haemocytes in response to wounding and invasive tumors will shed some light to the fundamentals of macrophages in immunity, inflammation and tumor microenvironment in human cancer.

ACKNOWLEDGMENTS

We would like to thank all members of our laboratory for discussions. Our work is funded by the European Research Council and the Biological and Biotechnological Sciences Research Council (UK).

REFERENCES

Abreu-Blanco, M. T., Verboon, J. M., Liu, R., Watts, J. J., and Parkhurst, S. M. (2012). *Drosophila* embryos close epithelial wounds using a combination of cellular protrusions and an actomyosin purse string. *J. Cell. Sci.* 125(Pt 24), 5984–5997. doi: 10.1242/jcs.109066

Abreu-Blanco, M. T., Verboon, J. M., and Parkhurst, S. M. (2011). Cell wound repair in *Drosophila* occurs through three distinct phases of membrane and cytoskeletal remodeling. *J. Cell. Biol.* 193, 455–464. doi: 10.1083/jcb.201011018

Agaisse, H., Petersen, U. M., Boutros, M., Mathey-Prevot, B., and Perrimon, N. (2003). Signaling role of hemocytes in *Drosophila* JAK/STAT-dependent response to septic injury. *Dev. Cell.* 5, 441–450. doi: 10.1016/S1534-5807(03)00244-2

Aggarwal, B. B., Vijayalekshmi, R. V, and Sung, B. (2009). Targeting inflammatory pathways for prevention and therapy of cancer: short-term friend, long-term foe. *Clin. Cancer Res.* 15, 425–430. doi: 10.1158/1078-0432

Apidianakis, Y., Pitsouli, C., Perrimon, N., and Rahme, L. (2009). Synergy between bacterial infection and genetic predisposition in intestinal dysplasia. *Proc. Natl. Acad. Sci. U.S.A.* 106, 20883–20888. doi: 10.1073/pnas.0911797106.

Bangi, E., Pitsouli, C., Rahme, L. G., Cagan, R., and Apidianakis, Y. (2012). Immune response to bacteria induces dissemination of Ras-activated *Drosophila* hindgut cells. *EMBO Rep.* 13, 569–576. doi: 10.1038/embor.2012.44

Bataillé, L., Augé, B., Ferjoux, G., Haenlin, M., and Waltzer, L. (2005). Resolving embryonic blood cell fate choice in *Drosophila*: interplay of GCM and RUNX factors. *Development* 132, 4635–4644. doi: 10.1242/dev.02034

Beaucher, M., Goodliffe, J., Hersperger, E., Trunova, S., Frydman, H., and Shearn, A. (2007). *Drosophila* brain tumor metastases express both neuronal and glial cell type markers. *Dev. Biol.* 301, 287–297. doi: 10.1016/j.ydbio.2006.09.019

Belacortu, Y., and Paricio, N. (2011). *Drosophila* as a model of wound healing and tissue regeneration in vertebrates. *Dev. Dyn.* 240, 2379–2404. doi: 10.1002/dvdy.22753

Bergmann, A., and Steller, H. (2010). Apoptosis, stem cells, and tissue regeneration. *Sci. Signal.* 3, re8. doi: 10.1126/scisignal.3145re8

Bidla, G., Dushay, M. S., and Theopold, U. (2007). Crystal cell rupture after injury in *Drosophila* requires the JNK pathway, small GTPases and the TNF homolog Eiger. *J. Cell. Sci.* 120(Pt 7), 1209–1215. doi: 10.1242/jcs.03420

Braun, A., Hoffmann, J. A., and Meister, M. (1998). Analysis of the *Drosophila* host defense in domino mutant larvae, which are devoid of hemocytes. *Proc. Natl. Acad. Sci. U.S.A.* 95, 14337–14342. doi: 10.1073/pnas.95.24.14337

Brennan, C. A., Delaney, J. R., Schneider, D. S., and Anderson, K. V. (2007). Psidin is required in *Drosophila* blood cells for both phagocytic degradation and immune activation of the fat body. *Curr. Biol.* 17, 67–72. doi: 10.1016/j.cub.2006.11.026

Bridges, C. B., and Brehme, K. S. (1944). *The Mutants of Drosophila Melanogaster. Publs Carnegie Instn*, 552: vii + 257pp.

Brückner, K., Kockel, L., Duchek, P., Luque, C. M., Rørth, P., and Perrimon, N. (2004). The PDGF/VEGF receptor controls blood cell survival in *Drosophila*. *Dev. Cell* 1, 73–84. doi: 10.1016/j.devcel.2004.06.007

Brumby, A. M., and Richardson, H. E. (2003). scribble mutants cooperate with oncogenic Ras or Notch to cause neoplastic overgrowth in *Drosophila*. *EMBO J.* 22, 5769–5779. doi: 10.1093/emboj/cdg548

Candido, J., and Hagemann, T. (2013). Cancer-related inflammation. *J. Clin. Immunol.* 33, S79–S84. doi: 10.1007/s10875-012-9847-0

Castillejo-López, C., and Häcker, U. (2005). The serine protease Sp7 is expressed in blood cells and regulates the melanization reaction in *Drosophila*. *Biochem. Biophys. Res. Commun* 338, 1075–1082. doi: 10.1016/j.bbrc.2005.10.042

Cerenius, L., and Söderhäll, K. (2004). The prophenoloxidase-activating system in invertebrates. *Immunol. Rev.* 198, 116–126. doi: 10.1111/j.0105-2896.2004.00116.x

Charroux, B., and Royet, J. (2009). Elimination of plasmatocytes by targeted apoptosis reveals their role in multiple aspects of the *Drosophila* immune response. *Proc. Natl. Acad. Sci. U.S.A.* 106, 9797–9802. doi: 10.1073/pnas.0903971106

Cho, N. K., Keyes, L., Johnson, E., Heller, J., Ryner, L., Karim, F., et al. (2002). Developmental control of blood cell migration by the *Drosophila* VEGF pathway. *Cell* 108, 865–876. doi: 10.1016/S0092-8674(02)00676-1

Christofi, T., and Apidianakis, Y. (2013). Ras-oncogenic *Drosophila* hindgut but not midgut cells use an inflammation-like program to disseminate to distant sites. *Gut. Microbes* 4, 54–59. doi: 10.4161/gmic.22429

Corbo, J. C., and Levine, M. (1996). Characterization of an immunodeficiency mutant in *Drosophila*. *Mech. Dev.* 55, 211–220. doi: 10.1016/0925-4773(96)00506-0

Cordero, J. B., Macagno, J. P., Stefanatos, R. K., Strathdee, K. E., Cagan, R. L., and Vidal, M. (2010). Oncogenic Ras diverts a host TNF tumor suppressor activity into tumor promoter. *Dev. Cell.* 18, 999–1011. doi: 10.1016/j.devcel.2010.05.014

Coussens, L. M., and Werb, Z. (2002). Inflammation and cancer. *Nature* 420, 860–867. doi: 10.1038/nature01322

Cronin, S. J. F., Nehme, N. T., Limmer, S., Liegeois, S., Pospisilik, J. A., Schramek, D., et al. (2009). Genome-wide RNAi screen identifies genes involved in intestinal pathogenic bacterial infection. *Science* 325, 340–343. doi: 10.1126/science.1173164

Crozatier, M., and Meister, M. (2007). *Drosophila* haematopoiesis. *Cell. Microbiol.* 9, 1117–1126. doi: 10.1111/j.1462-5822.2007.00930.x

Crozatier, M., Ubeda, J.-M., Vincent, A., and Meister, M. (2004). Cellular immune response to parasitization in *Drosophila* requires the EBF ortholog collier. *PLoS Biol.* 2:E196. doi: 10.1371/journal.pbio.0020196

Crozatier, M., and Vincent, A. (2011). *Drosophila*: a model for studying genetic and molecular aspects of haematopoiesis and associated leukaemias. *Dis. Model Mech.* 4, 439–445. doi: 10.1242/dmm.007351

Cuttell, L., Vaughan, A., Silva, E., Escaron, C. J., Lavine, M., Van Goethem, E., et al. (2008). Undertaker, a *Drosophila* Junctophilin, links

Draper-mediated phagocytosis and calcium homeostasis. *Cell* 135, 524–534. doi: 10.1016/j.cell.2008.08.033

Dar, A. C., Das, T. K., Shokat, K. M., and Cagan, R. L. (2012). Chemical genetic discovery of targets and anti-targets for cancer polypharmacology. *Nature* 486, 80–84. doi: 10.1038/nature11127

De Gregorio, E., Han, S.-J., Lee, W.-J., Baek, M.-J., Osaki, T., Kawabata, S., et al. (2002). An immune-responsive Serpin regulates the melanization cascade in *Drosophila*. *Dev. Cell.* 3, 581–592. doi: 10.1016/S1534-5807(02)00267-8

De Palma, M., and Lewis, C. E. (2013). Macrophage regulation of tumor responses to anticancer therapies. *Cancer Cell* 23, 277–286. doi: 10.1016/j.ccr.2013.02.013

Defaye, A., Evans, I., Crozatier, M., Wood, W., Lemaitre, B., and Leulier, F. (2009). Genetic ablation of *Drosophila* phagocytes reveals their contribution to both development and resistance to bacterial infection. *J. Innate Immun.* 1, 322–334. doi: 10.1159/000210264

Dierks, C., Grbic, J., Zirlik, K., Beigi, R., Englund, N. P., Guo, G. R., et al. (2007). Essential role of stromally induced hedgehog signaling in B-cell malignancies. *Nat. Med.* 13, 944–951. doi: 10.1038/nm1614

Dragojlovic-Munther, M., and Martinez-Agosto, J. A. (2012). Multifaceted roles of PTEN and TSC orchestrate growth and differentiation of *Drosophila* blood progenitors. *Development* 139, 3752–3763. doi: 10.1242/dev.074203

Dunn, G. P., Old, L. J., and Schreiber, R. D. (2004). The immunobiology of cancer immunosurveillance and immunoediting. *Immunity* 21, 137–148. doi: 10.1016/j.immuni.2004.07.017

Ekas, L. A, Cardozo, T. J., Flaherty, M. S., McMillan, E. A, Gonsalves, F. C., and Bach, E. A. (2010). Characterization of a dominant-active STAT that promotes tumorigenesis in *Drosophila*. *Dev. Biol.* 344, 621–636. doi: 10.1016/j.ydbio.2010.05.497

Eleftherianos, I., and Revenis, C. (2011). Role and importance of phenoloxidase in insect hemostasis. *J. Innate Immun.* 3, 28–33. doi: 10.1159/000321931

Enomoto, M., and Igaki, T. (2011). Deciphering tumor-suppressor signaling in flies: genetic link between Scribble/Dlg/Lgl and the Hippo pathways. *J. Genet. Genomics* 38, 461–470. doi: 10.1016/j.jgg.2011.09.005

Evans, C. J., Hartenstein, V., and Banerjee, U. (2003). Thicker than blood: conserved mechanisms in *Drosophila* and vertebrate hematopoiesis. *Dev. Cell.* 5, 673–690. doi: 10.1016/S1534-5807(03)00335-6

Fan, Y., and Bergmann, A. (2008). Apoptosis-induced compensatory proliferation. The Cell is dead. Long live the Cell! *Trends Cell. Biol.* 18, 467–473. doi: 10.1016/j.tcb.2008.08.001

Fossett, N., Tevosian, S. G., Gajewski, K., Zhang, Q., Orkin, S. H., and Schulz, R. A. (2001). The Friend of GATA proteins U-shaped, FOG-1, and FOG-2 function as negative regulators of blood, heart, and eye development in *Drosophila*. *Proc. Natl. Acad. Sci. U.S.A.* 98, 7342–7347. doi: 10.1073/pnas.131215798

Franc, N. C., Dimarcq, J. L., Lagueux, M., Hoffmann, J., and Ezekowitz, R. A. (1996). Croquemort, a novel *Drosophila* hemocyte/macrophage receptor that recognizes apoptotic cells. *Immunity* 4, 431–443. doi: 10.1016/S1074-7613(00)80410-0

Galko, M. J., and Krasnow, M. A. (2004). Cellular and genetic analysis of wound healing in *Drosophila* larvae. *PLoS Biol.* 2:E239. doi: 10.1371/journal.pbio.0020239

Gandillet, A, Park, S., Lassailly, F., Griessinger, E., Vargaftig, J., Filby, A., et al. (2011). Heterogeneous sensitivity of human acute myeloid leukemia to β-catenin down-modulation. *Leukemia* 25, 770–780. doi: 10.1038/leu.2011.17

Gateff, E. (1978). Malignant neoplasms of genetic origin in *Drosophila melanogaster*. *Science* 200, 1448–1459. doi: 10.1126/science.96525

Geissler, K., and Zach, O. (2012). Pathways involved in *Drosophila* and human cancer development: the Notch, Hedgehog, Wingless, Runt, and Trithorax pathway. *Ann. Hematol.* 91, 645–669. doi: 10.1007/s00277-012-1435-0

Glittenberg, M. T., Kounatidis, I., Christensen, D., Kostov, M., Kimber, S., Roberts, I., et al. (2011). Pathogen and host factors are needed to provoke a systemic host response to gastrointestinal infection of *Drosophila* larvae by *Candida albicans*. *Dis. Model Mech.* 4, 515–525. doi: 10.1242/dmm.006627

Gonzalez, C. (2013). *Drosophila melanogaster*: a model and a tool to investigate malignancy and identify new therapeutics. *Nat. Rev. Cancer* 13, 172–183. doi: 10.1038/nrc3461

Goto, A., Kadowaki, T., and Kitagawa, Y. (2003). Drosophila hemolectin gene is expressed in embryonic and larval hemocytes and its knock down causes bleeding defects. *Dev. Biol.* 264, 582–591. doi: 10.1016/j.ydbio.2003.06.001

Goto, A., Kumagai, T., Kumagai, C., Hirose, J., Narita, H., Mori, H., et al. (2001). A *Drosophila* haemocyte-specific protein, hemolectin, similar to human von Willebrand factor. *Biochem. J.* 359, 99–108. doi: 10.1042/0264-6021:3590099

Grivennikov, S. I., Greten, F. R., and Karin, M. (2010). Immunity, inflammation, and cancer. *Cell* 140, 883–899. doi: 10.1016/j.cell.2010.01.025

Hanahan, D., and Weinberg, R. A. (2011). Hallmarks of cancer: the next generation. *Cell* 144, 646–674. doi: 10.1016/j.cell.2011.02.013

Harris, H. (2005). A long view of fashions in cancer research. *Bioessays* 27, 833–838. doi: 10.1002/bies.20263

Harrison, D. A., Binari, R., Nahreini, T. S., Gilman, M., and Perrimon, N. (1995). Activation of a *Drosophila* Janus kinase (JAK) causes hematopoietic neoplasia and developmental defects. *EMBO J.* 14, 2857–2865.

Hartenstein, V. (2006). Blood cells and blood cell development in the animal kingdom. *Annu. Rev. Cell. Dev. Biol.* 22, 677–712. doi: 10.1146/annurev.cellbio.22.010605.093317

Harvey, K. F., Zhang, X., and Thomas, D. M. (2013). The Hippo pathway and human cancer. *Nat. Rev. Cancer* 13, 246–257. doi: 10.1038/nrc3458

Hashimoto, Y., Tabuchi, Y., Sakurai, K., Kutsuna, M., Kurokawa, K., Awasaki, T., et al. (2009). Identification of lipoteichoic acid as a ligand for draper in the phagocytosis of Staphylococcus aureus by *Drosophila* hemocytes. *J. Immunol.* 183, 7451–7460. doi: 10.4049/jimmunol.0901032

Hatlen, M. A., Wang, L., and Nimer, S. D. (2012). AML1-ETO driven acute leukemia: insights into pathogenesis and potential therapeutic approaches. *Front. Med.* 6:3. doi: 10.1007/s11684-012-0206-6

Holz, A., Bossinger, B., Strasser, T., Janning, W., and Klapper, R. (2003). The two origins of hemocytes in *Drosophila*. *Development* 130, 4955–4962. doi: 10.1242/dev.00702

Howell, L., Sampson, C. J., Xavier, M. J., Bolukbasi, E., Heck, M. M. S., and Williams, M. J. (2012). A directed miniscreen for genes involved in the *Drosophila* anti-parasitoid immune response. *Immunogenetics* 64, 155–161. doi: 10.1007/s00251-011-0571-3

Hsu, T. (2012). Complex cellular functions of the von Hippel-Lindau tumor suppressor gene: insights from model organisms. *Oncogene* 31, 2247–2257. doi: 10.1038/onc.2011.442

Huelsmann, S., Hepper, C., Marchese, D., Knöll, C., and Reuter, R. (2006). The PDZ-GEF dizzy regulates cell shape of migrating macrophages via Rap1 and integrins in the *Drosophila* embryo. *Development* 133, 2915–2924. doi: 10.1242/dev.02449

Humbert, P. O., Grzeschik, N. A., Brumby, A. M., Galea, R., Elsum, I., and Richardson, H. E. (2008). Control of tumourigenesis by the Scribble/Dlg/Lgl polarity module. *Oncogene* 27, 6888–6907. doi: 10.1038/onc.2008.341

Irving, P., Ubeda, J. M., Doucet, D., Troxler, L., Lagueux, M., Zachary, D., et al. (2005). New insights into *Drosophila* larval haemocyte functions through genome-wide analysis. *Cell. Microbiol.* 7, 335–350. doi: 10.1111/j.1462-5822.2004.00462.x

Jiang, H., Patel, P. H., Kohlmaier, A., Grenley, M. O., McEwen, D. G., and Edgar, B. A. (2009). Cytokine/Jak/Stat signaling mediates regeneration and homeostasis in the *Drosophila* midgut. *Cell* 137, 1343–1355. doi: 10.1016/j.cell.2009.05.014

Jiravanichpaisal, P., Lee, B. L., and Söderhäll, K. (2006). Cell-mediated immunity in arthropods: hematopoiesis, coagulation, melanization and opsonization. *Immunobiology* 211, 213–236. doi: 10.1016/j.imbio.2005.10.015

Johansson, K. C., Metzendorf, C., and Söderhäll, K. (2005). Microarray analysis of immune challenged *Drosophila* hemocytes. *Exp. Cell. Res.* 305, 145–155. doi: 10.1016/j.yexcr.2004.12.018

Jung, S. H., Evans, C. J., Uemura, C., and Banerjee, U. (2005). The *Drosophila* lymph gland as a developmental model of hematopoiesis. *Development* 132, 2521–2533. doi: 10.1242/dev.01837

Karlsson, C., Korayem, A. M., Scherfer, C., Loseva, O., Dushay, M. S., and Theopold, U. (2004). Proteomic analysis of the *Drosophila* larval hemolymph clot. *J. Biol. Chem.* 279, 52033–52041. doi: 10.1074/jbc.M408220200

Kiehart, D. P., Galbraith, C. G., Edwards, K. A., Rickoll, W. L., and Montague, R. A. (2000). Multiple forces contribute to cell sheet morphogenesis for dorsal closure in *Drosophila*. *J. Cell. Biol.* 149, 471–490. doi: 10.1083/jcb.149.2.471

Kocks, C., Cho, J. H., Nehme, N., Ulvila, J., Pearson, A. M., Meister, M., et al. (2005). Eater, a transmembrane protein mediating phagocytosis of bacterial pathogens in *Drosophila*. *Cell* 123, 335–346. doi: 10.1016/j.cell.2005.08.034

Kounatidis, I., and Ligoxygakis, P. (2012). *Drosophila* as a model system to unravel the layers of innate immunity to infection. *Open Biol.* 2, 120075. doi: 10.1098/rsob.120075

Krzemien, J., Crozatier, M., and Vincent, A. (2010). Ontogeny of the *Drosophila* larval hematopoietic organ, hemocyte homeostasis and the dedicated cellular immune response to parasitism. *Int. J. Dev. Biol.* 54, 1117–1125. doi: 10.1387/ijdb.093053jk

Krzemień, J., Dubois, L., Makki, R., Meister, M., Vincent, A., and Crozatier, M. (2007). Control of blood cell homeostasis in *Drosophila* larvae by the posterior signalling centre. *Nature* 446, 325–328. doi: 10.1038/nature05650

Kurant, E., Axelrod, S., Leaman, D., and Gaul, U. (2008). Six-microns-under acts upstream of Draper in the glial phagocytosis of apoptotic neurons. *Cell* 133, 498–509. doi: 10.1016/j.cell.2008.02.052

Kurucz, E., Márkus, R., Zsámboki, J., Folkl-Medzihradszky, K., Darula, Z., Vilmos, P., et al. (2007). Nimrod, a putative phagocytosis receptor with EGF repeats in *Drosophila* plasmatocytes. *Curr. Biol.* 17, 649–654. doi: 10.1016/j.cub.2007.02.041

Kurucz, E., Zettervall, C., Sinka, R., Vilmos, P., Pivarcsi, A., Ekengren, S., et al. (2003). Hemese, a hemocyte-specific transmembrane protein, affects the cellular immune response in Drosophila. Proc. Natl. Acad. Sci. U.S.A. 100, 2622–2627. doi: 10.1073/pnas.0436940100

Lacronique, V., Boureux, A., Valle, V. D., Poirel, H., Quang, C. T., Mauchauffé, M., et al. (1997). A TEL-JAK2 fusion protein with constitutive kinase activity in human leukemia. *Science* 278, 1309–1312. doi: 10.1126/science.278.5341.1309

Lanot, R., Zachary, D., Holder, F., and Meister, M. (2001). Postembryonic hematopoiesis in *Drosophila*. *Dev. Biol.* 230, 243–257. doi: 10.1006/dbio.2000.0123

Lebestky, T., Chang, T., Hartenstein, V., and Banerjee, U. (2000). Specification of *Drosophila* hematopoietic lineage by conserved transcription factors. *Science* 288, 146–149. doi: 10.1126/science.288.5463.146

Lebestky, T., Jung, S. H., and Banerjee, U. (2003). A Serrate-expressing signaling center controls *Drosophila* hematopoiesis. *Genes Dev*. 17, 348–353. doi: 10.1101/gad.1052803

Leclerc, V., Pelte, N., El Chamy, L., Martinelli, C., Ligoxygakis, P., Hoffmann, J. A., et al. (2006). Prophenoloxidase activation is not required for survival to microbial infections in *Drosophila*. *EMBO Rep.* 7, 231–235. doi: 10.1038/sj.embor.7400592

Lee, H. J., Daver, N., Kantarjian, H. M., Verstovsek, S., and Ravandi, F. (2013a). The role of JAK pathway dysregulation in the pathogenesis and treatment of acute myeloid leukemia. *Clin. Cancer Res.* 19, 327–335. doi: 10.1158/1078-0432.CCR-12-2087

Lee, H. W., Choi, H. J., Ha, S. J., Lee, K. T., and Kwon, Y. G. (2013b). Recruitment of monocytes/macrophages in different tumor microenvironments. *Biochim. Biophys. Acta* 1835, 170–179. doi: 10.1016/j.bbcan.2012.12.007

Lee, M. J., Kalamarz, M. E., Paddibhatla, I., Small, C., Rajwani, R., and Govind, S. (2009). Virulence factors and strategies of Leptopilina spp.: selective responses in *Drosophila* hosts. *Adv. Parasitol.* 70, 123–145. doi: 10.1016/S0065-308X(09)70005-3

Lemaitre, B., and Hoffmann, J. (2007). The host defense of *Drosophila melanogaster*. *Annu. Rev. Immunol.* 25, 697–743. doi: 10.1146/annurev.immunol.25.022106.141615

Lensch, M. W. (2012). An evolving model of hematopoietic stem cell functional identity. *Stem Cell Rev.* 8, 551–560. doi: 10.1007/s12015-012-9347-x

Lesch, C., Jo, J., Wu, Y., Fish, G. S., and Galko, M. J. (2010). A targeted UAS-RNAi screen in *Drosophila* larvae identifies wound closure genes regulating distinct cellular processes. *Genetics* 186, 943–957. doi: 10.1534/genetics.110.121822

Levayer, R., and Moreno, E. (2013). Mechanisms of cell competition: themes and variations. *J. Cell. Biol.* 200, 689–698. doi: 10.1083/jcb.201301051

Ligoxygakis, P., Pelte, N., Ji, C., Leclerc, V., Duvic, B., Belvin, M., et al. (2002). A serpin mutant links Toll activation to melanization in the host defence of *Drosophila*. *EMBO J.* 21, 6330–6337. doi: 10.1093/emboj/cdf661

Lin, J., and Hackam, D. J. (2011). Worms, flies and four-legged friends: the applicability of biological models to the understanding of intestinal inflammatory diseases. *Dis. Model. Mech.* 4, 447–456. doi: 10.1242/dmm.007252

Lindgren, M., Riazi, R., Lesch, C., Wilhelmsson, C., Theopold, U., and Dushay, M. S. (2008). Fondue and transglutaminase in the *Drosophila* larval clot. *J. Insect Physiol.* 54, 586–592. doi: 10.1016/j.jinsphys.2007.12.008

Manaka, J., Kuraishi, T., Shiratsuchi, A., Nakai, Y., Higashida, H., Henson, P., et al. (2004). Draper-mediated and phosphatidylserine-independent phagocytosis of apoptotic cells by *Drosophila* hemocytes/macrophages. *J. Biol. Chem.* 279, 48466–48476. doi: 10.1074/jbc.M408597200

Mandal, L., Banerjee, U., and Hartenstein, V. (2004). Evidence for a fruit fly hemangioblast and similarities between lymph-gland hematopoiesis in fruit fly and mammal aorta-gonadal-mesonephros mesoderm. *Nat. Genet.* 36, 1019–1023. doi: 10.1038/ng1404

Mandal, L., Martinez-Agosto, J. A., Evans, C. J., Hartenstein, V., and Banerjee, U. (2007). A Hedgehog- and Antennapedia-dependent niche maintains *Drosophila* haematopoietic precursors. *Nature* 446, 320–324. doi: 10.1038/nature05585

Mantovani, A., Allavena, P., Sica, A., and Balkwill, F. (2008). Cancer-related inflammation. *Nature* 454, 436–444. doi: 10.1038/nature07205

Mantovani, A., Biswas, S. K., Galdiero, M. R., Sica, A., and Locati, M. (2013). Macrophage plasticity and polarization in tissue repair and remodelling. *J. Pathol.* 229, 176–185. doi: 10.1002/path.4133

Martelli, A. M., Evangelisti, C., Chappell, W., Abrams, S. L., Bäsecke, J., Stivala, F., et al. (2011). Targeting the translational apparatus to improve leukemia therapy: roles of the PI3K/PTEN/Akt/mTOR pathway. *Leukemia* 25, 1064–1079. doi: 10.1038/leu.2011.46

Martin, P. and Lewis, J (1992). Actin cables and epidermal movement in embryonic wound healing. *Nature* 360, 179–183. doi: 10.1038/360179a0

Martin-Belmonte, F., and Perez-Moreno, M. (2012). Epithelial cell polarity, stem cells and cancer. *Nat. Rev. Cancer* 12, 23–38. doi: 10.1038/nrc3169

Miles, W. O., Dyson, N. J., and Walker, J. A. (2011). Modeling tumor invasion and metastasis in *Drosophila*. *Dis. Model Mech.* 4, 753–761. doi: 10.1242/dmm.006908

Moreira, C. G. A, Jacinto, A., and Prag, S. (2013). *Drosophila* integrin adhesion complexes are essential for hemocyte migration *in vivo*. *Biol. Open* 2, 795–801. doi: 10.1242/bio.20134564

Moreira, S., Stramer, B., Evans, I., Wood, W., and Martin, P. (2010). Prioritization of competing damage and developmental signals by migrating macrophages in the *Drosophila* embryo. *Curr. Biol.* 20, 464–470. doi: 10.1016/j.cub.2010.01.047

Mukherjee, T., Kim, W. S., Mandal, L., and Banerjee, U. (2011). Interaction between Notch and Hif-alpha in development and survival of *Drosophila* blood cells. *Science* 332, 1210–1213. doi: 10.1126/science.1199643

Müller, P., Kuttenkeuler, D., Gesellchen, V., Zeidler, M. P., and Boutros, M. (2005). Identification of JAK/STAT signalling components by genome-wide RNA interference. *Nature* 436, 871–875. doi: 10.1038/nature03869

Nagao, R., Ashihara, E., Kimura, S., Strovel, J. W., Yao, H., Takeuchi, M., et al. (2011). Growth inhibition of imatinib-resistant CML cells with the T315I mutation and hypoxia-adaptation by AV65–a novel Wnt/β-catenin signaling inhibitor. *Cancer Lett.* 312, 91–100. doi: 10.1016/j.canlet.2011.08.002

Nam, H. J., Jang, I. H., You, H., Lee, K. A., and Lee, W. J. (2012). Genetic evidence of a redox-dependent systemic wound response via Hayan protease-phenoloxidase system in *Drosophila*. *EMBO J.* 31, 1253–1265. doi: 10.1038/emboj.2011.476

Nappi, A. J., Vass, E., Frey, F. and Carton, Y. (1995). Superoxide anion generation in *Drosophila* during melanotic encapsulation of parasites. *Eur. J. Cell. Biol.* 68, 450–456.

Novak, M. L., and Koh, T. J. (2013). Macrophage phenotypes during tissue repair. *J. Leukoc. Biol.* 93, 875–881. doi: 10.1189/jlb.1012512

Of, B., and Healing, W. (1999). Cutaneous wound healing. *N. Engl. J. Med.* 738–746.

Oh, I. H., and Humphries, R. K. (2012). Concise review: multidimensional regulation of the hematopoietic stem cell state. *Stem Cells* 30, 82–88. doi: 10.1002/stem.776

Osman, D., Gobert, V., Ponthan, F., Heidenreich, O., Haenlin, M., and Waltzer, L. (2009). A *Drosophila* model identifies calpains as modulators of the human leukemogenic fusion protein AML1-ETO. *Proc. Natl. Acad. Sci. U.S.A.* 106, 12043–12048. doi: 10.1073/pnas.0902449106

Owusu-Ansah, E., and Banerjee, U. (2009). Reactive oxygen species prime *Drosophila* haematopoietic progenitors for differentiation. *Nature* 461, 537–541. doi: 10.1038/nature08313

Pagliarini, R. A., and Xu, T. (2003). A genetic screen in *Drosophila* for metastatic behavior. *Science* 302, 1227–1231. doi: 10.1126/science.1088474

Paladi, M., and Tepass, U. (2004). Function of Rho GTPases in embryonic blood cell migration in *Drosophila*. *J. Cell. Sci.* 117(Pt 26), 6313–6326. doi: 10.1242/jcs.01552

Panayidou, S., and Apidianakis, Y. (2013). Regenerative inflammation: lessons from *Drosophila* intestinal epithelium in health and disease *Pathogens* 2, 209–231. doi: 10.3390/pathogen2020209

Pancewicz, J., and Nicot, C. (2011). Current views on the role of Notch signaling and the pathogenesis of human leukemia. *BMC Cancer* 11:502. doi: 10.1186/1471-2407-11-502

Pastor-Pareja, J. C., Wu, M., and Xu, T. (2008). An innate immune response of blood cells to tumors and tissue damage in *Drosophila*. *Dis. Model Mech.* 1, 144–154. doi: 10.1242/dmm.000950

Peeples, E. E., Geisler, A., Whitcraft, C. J., and Oliver, C. (1969). Comparative studies of phenol oxidase activity during pupal development of three lozenge mutants (lzs, lz, lzk) of *Drosophila melanogaster*. *Genetics* 62, 161–170.

Qiu, P., Pan, P. C., and Govind, S. (1998). A role for the *Drosophila* Toll/Cactus pathway in larval hematopoiesis. *Development* 125, 1909–1920.

Radtke, F., Wilson, A., and MacDonald, H. R. (2005). Notch signaling in hematopoiesis and lymphopoiesis: lessons from *Drosophila*. *BioEssays* 27, 1117–1128. doi: 10.1002/bies.20315

Rämet, M., Lanot, R., Zachary, D., and Manfruelli, P. (2002). JNK signaling pathway is required for efficient wound healing in *Drosophila*. *Dev. Biol.* 241, 145–156. doi: 10.1006/dbio.2001.0502

Razzell, W., Evans, I. R., Martin, P., and Wood, W. (2013). Calcium flashes orchestrate the wound inflammatory response through DUOX activation and hydrogen peroxide release. *Curr. Biol.* 23, 424–429. doi: 10.1016/j.cub.2013.01.058

Rezaie, A., Parker, R. D., and Abdollahi, M. (2007). Oxidative stress and pathogenesis of inflammatory bowel disease: an epiphenomenon or the cause? *Dig. Dis. Sci.* 52, 2015–2021. doi: 10.1007/s10620-006-9622-2

Ricklin, D., and Lambris, J. D. (2013). Complement in immune and inflammatory disorders: pathophysiological mechanisms. *J. Immunol.* 190, 3831–3838. doi: 10.4049/jimmunol.1203487

Ríos-Barrera, L. D., and Riesgo-Escovar, J. R. (2013). Regulating cell morphogenesis: the *Drosophila* Jun N-terminal kinase pathway. *Genesis* 51, 147–162. doi: 10.1002/dvg.22354

Rizki, R. M., and Rizki, T. M. (1980). Hemocyte responses to implanted tissues in *Drosophila melanogaster* larvae. *Rouxs Arch. Dev. Biol.* 213, 207–213.

Rizki, T. M., Rizki, R. M., and Grell, E. H. (1980). A mutant affecting the crystal cells in *Drosophila melanogaster*. *Rouxs Arch. Dev. Biol.* 181, 91–99.

Royet, J. (2011). Epithelial homeostasis and the underlying molecular mechanisms in the gut of the insect model *Drosophila melanogaster*. *Cell. Mol. Life Sci.* 68, 3651–3660. doi: 10.1007/s00018-011-0828-x

Salomon, R. N., and Jackson, F. R. (2008). Tumors of testis and midgut in aging flies. *Fly* 2, 265–268.

Sampson, C. J., Valanne, S., Fauvarque, M.-O., Hultmark, D., Rämet, M., and Williams, M. J. (2012). The RhoGEF Zizimin-related acts in the *Drosophila* cellular immune response via the Rho GTPases Rac2 and Cdc42. *Dev. Comp. Immunol.* 38, 160–168. doi: 10.1016/j.dci.2012.05.004

Sampson, C. J., and Williams, M. J. (2012). Real-time analysis of *Drosophila* post-embryonic haemocyte behaviour. *PLoS ONE* 7:e28783. doi: 10.1371/journal.pone.0028783

Scherfer, C., Karlsson, C., Loseva, O., Bidla, G., Goto, A., Havemann, J., et al. (2004). Isolation and characterization of hemolymph clotting factors in *Drosophila melanogaster* by a pullout method. *Curr. Biol.* 14, 625–629. doi: 10.1016/j.cub.2004.03.030

Scherfer, C., Qazi, M. R., Takahashi, K., Ueda, R., Dushay, M. S., Theopold, U., et al. (2006). The Toll immune-regulated *Drosophila* protein Fondue is involved in hemolymph clotting and puparium formation. *Dev. Biol.* 295, 156–163. doi: 10.1016/j.ydbio.2006.03.019

Schmidt, R. L., Trejo, T. R., Plummer, T. B., Platt, J. L., and Tang, A. H. (2008). Infection-induced proteolysis of PGRP-LC controls the IMD activation and melanization cascades in *Drosophila*. *FASEB J.* 22, 918–929. doi: 10.1096/fj.06-7907com

Schmucker, D., and Chen, B. (2009). Dscam and DSCAM: complex genes in simple animals, complex animals yet simple genes. *Genes Dev.* 23, 147–156. doi: 10.1101/gad.1752909

Seke Etet, P. F., Vecchio, L., Bogne Kamga, P., Nchiwan Nukenine, E., Krampera, M., and Nwabo Kamdje, A. H. (2013). Normal hematopoiesis and hematologic malignancies: role of canonical Wnt signaling pathway and stromal microenvironment. *Biochim. Biophys. Acta.* 1835, 1–10. doi: 10.1016/j.bbcan.2012.08.002

Sethi, G., Shanmugam, M. K., Ramachandran, L., Kumar, A. P., and Tergaonkar, V. (2012). Multifaceted link between cancer and inflammation. *Biosci. Rep.* 32, 1–15. doi: 10.1042/BSR20100136

Shia, A. K. H., Glittenberg, M., Thompson, G., Weber, A. N., Reichhart, J. M., and Ligoxygakis, P. (2009). Toll-dependent antimicrobial responses in *Drosophila* larval fat body require Spätzle secreted by haemocytes. *J. Cell. Sci.* 122(Pt 24), 4505–4515. doi: 10.1242/jcs.049155

Siekhaus, D., Haesemeyer, M., Moffitt, O., and Lehmann, R. (2010). RhoL controls invasion and Rap1 localization during immune cell transmigration in *Drosophila*. *Nat. Cell. Biol.* 12, 605–610. doi: 10.1038/ncb2063

Sinenko, S. A, Hung, T., Moroz, T., Tran, Q. M., Sidhu, S., Cheney, M. D., et al. (2010). Genetic manipulation of AML1-ETO-induced expansion of hematopoietic precursors in a *Drosophila* model. *Blood* 116, 4612–4620. doi: 10.1182/blood-2010-03-276998

Sinenko, S. A., Mandal, L., Martinez-Agosto, J. A., and Banerjee, U. (2009). Dual role of wingless signaling in stem-like hematopoietic precursor maintenance in *Drosophila*. *Dev. Cell.* 16, 756–763. doi: 10.1016/j.devcel.2009.03.003

Singer, A. J., and Clark, R. A. (1999). Cutaneous wound healing. *N. Engl. J. Med.* 341, 738–746. doi: 10.1197/j.aem.2006.03.189

Sorrentino, R. P., Carton, Y., and Govind, S. (2002). Cellular immune response to parasite infection in the *Drosophila* lymph gland is developmentally regulated. *Dev. Biol.* 243, 65–80. doi: 10.1006/dbio.2001.0542

Sorrentino, R. P., Melk, J. P., and Govind, S. (2004). Genetic analysis of contributions of dorsal group and JAK-Stat92E pathway genes to larval hemocyte concentration and the egg encapsulation response in *Drosophila*. *Genetics* 166, 1343–1356. doi: 10.1534/genetics.166.3.1343

Stefanatos, R. K. A, and Vidal, M. (2011). Tumor invasion and metastasis in *Drosophila*: a bold past, a bright future. *J. Genet. Genomics* 38, 431–438. doi: 10.1016/j.jgg.2011.09.004

Stofanko, M., Kwon, S. Y., and Badenhorst, P. (2010). Lineage tracing of lamellocytes demonstrates *Drosophila* macrophage plasticity. *PLoS ONE* 5:e14051. doi: 10.1371/journal.pone.0014051

Stramer, B., Winfield, M., Shaw, T., Millard, T. H., Woolner, S., and Martin, P. (2008). Gene induction following wounding of wild-type versus macrophage-deficient *Drosophila* embryos. *EMBO Rep.* 9, 465–471. doi: 10.1038/embor.2008.34

Stramer, B., Wood, W., Galko, M. J., Redd, M. J., Jacinto, A., Parkhurst, S. M., et al. (2005). Live imaging of wound inflammation in *Drosophila* embryos reveals key roles for small GTPases during *in vivo* cell migration. *J. Cell. Biol.* 168, 567–573. doi: 10.1083/jcb.200405120

Takehana, A., Yano, T., Mita, S., Kotani, A., Oshima, Y., and Kurata, S. (2004). Peptidoglycan recognition protein (PGRP)-LE and PGRP-LC act synergistically in *Drosophila* immunity. *EMBO J.* 23, 4690–4700. doi: 10.1038/sj.emboj.7600466

Takekawa, M., and Saito, H. (1998). A family of stress-inducible GADD45-like proteins mediate activation of the stress-responsive MTK1/MEKK4 MAPKKK. *Cell* 95, 521–30. doi: 10.1016/S0092-8674(00)81619-0

Tang, H., Kambris, Z., Lemaitre, B., and Hashimoto, C. (2006). Two proteases defining a melanization cascade in the immune system of *Drosophila*. *J. Biol. Chem.* 281, 28097–28104. doi: 10.1074/jbc.M601642200

Tanigaki, K., and Honjo, T. (2007). Regulation of lymphocyte development by Notch signaling. *Nat. Immunol.* 8, 451–456. doi: 10.1038/ni1453

Tepass, U., Fessler, L. I., Aziz, a, and Hartenstein, V. (1994). Embryonic origin of hemocytes and their relationship to cell death in *Drosophila*. *Development* 120, 1829–1837.

Theopold, U., Schmidt, O., Söderhäll, K., and Dushay, M. S. (2004). Coagulation in arthropods: defence, wound closure and healing. *Trends Immunol.* 25, 289–294. doi: 10.1016/j.it.2004.03.004

Tipping, M., and Perrimon, N. (2013). *Drosophila* as a model for context-dependent tumorigenesis. *J. Cell. Physiol.* 1–20. doi: 10.1002/jcp.24427

Tothova, Z., Kollipara, R., Huntly, B. J., Lee, B. H., Castrillon, D. H., Cullen, D. E., et al. (2007). FoxOs are critical mediators of hematopoietic stem cell resistance to physiologic oxidative stress. *Cell* 128, 325–339. doi: 10.1016/j.cell.2007.01.003

Tucker, P. K., Evans, I. R., and Wood, W. (2011). Ena drives invasive macrophage migration in *Drosophila* embryos. *Dis. Model Mech.* 4, 126–134. doi: 10.1242/dmm.005694

Vainchenker, W., and Constantinescu, S. N. (2013). JAK/STAT signaling in hematological malignancies. *Oncogene* 32, 2601–2613. doi: 10.1038/onc.2012.347

Valanne, S., Wang, J. H., and Rämet, M. (2011). The *Drosophila* Toll signaling pathway. *J. Immunol.* 186, 649–656. doi: 10.4049/jimmunol.1002302

Waltzer, L., Gobert, V., Osman, D., and Haenlin, M. (2010). Transcription factor interplay during *Drosophila* haematopoiesis. *Int. J. Dev. Biol.* 54, 1107–1115. doi: 10.1387/ijdb.093054lw

Wang, L. D., and Wagers, A. J. (2011). Dynamic niches in the origination and differentiation of haematopoietic stem cells. *Nat. Rev. Mol. Cell. Biol.* 12, 643–655. doi: 10.1038/nrm3184

Wang, Z., Wilhelmsson, C., Hyrsl, P., Loof, T. G., Dobes, P., Klupp, M., et al. (2010). Pathogen entrapment by transglutaminase–a conserved early innate immune mechanism. *PLoS Pathog.* 6:e1000763. doi: 10.1371/journal.ppat.1000763

Watson, F. L., Püttmann-Holgado, R., Thomas, F., Lamar, D. L., Hughes, M., Kondo, M., et al. (2005). Extensive diversity of Ig-superfamily proteins in the immune system of insects. *Science* 309, 1874–1878. doi: 10.1126/science.1116887

Wertheim, B., Kraaijeveld, A. R., Schuster, E., Blanc, E., Hopkins, M., Pletcher, S. D., et al. (2005). Genome-wide gene expression in response to parasitoid attack in *Drosophila*. *Genome Biol.* 6, R94. doi: 10.1186/gb-2005-6-11-r94

Williams, M. J., Ando, I., and Hultmark, D. (2005). *Drosophila melanogaster* Rac2 is necessary for a proper cellular immune response. *Genes Cells* 10, 813–823. doi: 10.1111/j.1365-2443.2005.00883.x

Williams, M. J., Wiklund, M.-L., Wikman, S., and Hultmark, D. (2006). Rac1 signalling in *the Drosophila* larval cellular immune response. *J. Cell. Sci*. 119(Pt 10), 2015–2024. doi: 10.1242/jcs.02920

Wood, W., Faria, C., and Jacinto, A. (2006). Distinct mechanisms regulate hemocyte chemotaxis during development and wound healing in *Drosophila melanogaster*. *J. Cell. Biol.* 173, 405–416. doi: 10.1083/jcb.200508161

Wood, W., and Jacinto, A. (2007). *Drosophila melanogaster* embryonic haemocytes: masters of multitasking. *Nat. Rev. Mol. Cell. Biol.* 8, 542–551. doi: 10.1038/nrm2202

Wood, W., Jacinto, A., Grose, R., Woolner, S., Gale, J., Wilson, C., et al. (2002). Wound healing recapitulates morphogenesis in *Drosophila* embryos. *Nat. Cell. Biol.* 4, 907–912. doi: 10.1038/ncb875

Wu, S. C., Liao, C. W., Pan, R. L., and Juang, J. L. (2012). Infection-induced intestinal oxidative stress triggers organ-to-organ immunological communication in *Drosophila*. *Cell Host Microbe* 11, 410–417. doi: 10.1016/j.chom.2012.03.004

Wu, Y., Brock, A. R., Wang, Y., Fujitani, K., Ueda, R., and Galko, M. J. (2009). A blood-borne PDGF/VEGF-like ligand initiates wound-induced epidermal cell migration in *Drosophila* larvae. *Curr. Biol.* 19, 1473–1477. doi: 10.1016/j.cub.2009.07.019

Wu, Y., and Zhou, B. P. (2009). Inflammation: a driving force speeds cancer metastasis. *Cell Cycle* 8, 3267–3273. doi: 10.4161/cc.8.20.9699

Xavier, M. J., and Williams, M. J. (2011). The Rho-family GTPase Rac1 regulates integrin localization in *Drosophila* immunosurveillance cells. *PLoS ONE* 6:e19504. doi: 10.1371/journal.pone.0019504

Yang, B., Lu, A., Peng, Q., Ling, Q. Z., and Ling, E. (2013). Activity of fusion prophenoloxidase-GFP and its potential applications for innate immunity study. *PLoS ONE* 8:e64106. doi: 10.1371/journal.pone.0064106

Zaidman-Rémy, A., Regan, J. C., Brandão, A. S., and Jacinto, A. (2012). The *Drosophila* larva as a tool to study gut-associated macrophages: PI3K regulates a discrete hemocyte population at the proventriculus. *Dev. Comp. Immunol*. 36, 638–647. doi: 10.1016/j.dci.2011.10.013

Zhao, C., Chen, A., Jamieson, C. H., Fereshteh, M., Abrahamsson, A., and Blum, J. (2009). Hedgehog signalling is essential for maintenance of cancer stem cells in myeloid leukaemia. *Nature* 458, 776–779. doi: 10.1038/nature07737

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 01 October 2013; accepted: 21 December 2013; published online: 09 January 2014.

*Citation: Wang L, Kounatidis I and Ligoxygakis P (2014) Drosophila as a model to study the role of blood cells in inflammation, innate immunity and cancer. Front. Cell. Infect. Microbiol. **3**:113. doi: 10.3389/fcimb.2013.00113*

This article was submitted to the journal Frontiers in Cellular and Infection Microbiology.

frontiers in
CELLULAR AND INFECTION MICROBIOLOGY

REVIEW ARTICLE
published: 10 January 2014
doi: 10.3389/fcimb.2013.00116

Role of DUOX in gut inflammation: lessons from *Drosophila* model of gut-microbiota interactions

Sung-Hee Kim[1,2] and Won-Jae Lee[1,2]*

[1] School of Biological Science and Institute of Molecular Biology and Genetics, Seoul National University, Seoul, South Korea
[2] National Creative Research Initiative Center for Symbiosystem, Seoul National University, Seoul, South Korea

Edited by:
Dominique Ferrandon, Centre National de la Recherche Scientifique, France

Reviewed by:
Heinrich Jasper, Buck Institute for Research on Aging, USA
Nicolas Buchon, Cornell University, USA

***Correspondence:**
Won-Jae Lee, Seoul National University, Seoul 151-742, South Korea
e-mail: lwj@snu.ac.kr

It is well-known that certain bacterial species can colonize the gut epithelium and induce inflammation in the mucosa, whereas other species are either benign or beneficial to the host. Deregulation of the gut-microbe interactions may lead to a pathogenic condition in the host, such as chronic inflammation, tissue injuries, and even cancer. However, our current understanding of the molecular mechanisms that underlie gut-microbe homeostasis and pathogenesis remains limited. Recent studies have used *Drosophila* as a genetic model to provide novel insights into the causes and consequences of bacterial-induced colitis in the intestinal mucosa. The present review discusses the interactions that occur between gut-associated bacteria and host gut immunity, particularly the bacterial-induced intestinal dual oxidase (DUOX) system. Several lines of evidence showed that the bacterial-modulated DUOX system is involved in microbial clearance, intestinal epithelial cell renewal (ECR), redox-dependent modulation of signaling pathways, cross-linking of biomolecules, and discrimination between symbionts and pathogens. Further genetic studies on the *Drosophila* DUOX system and on gut-associated bacteria with a distinct ability to activate DUOX may provide critical information related to the homeostatic inflammation as well as etiology of chronic inflammatory diseases, which will enhance our understanding on the mucosal inflammatory diseases frequently observed in the microbe-contacting epithelia of humans.

Keywords: dual oxidase, gut immunity, epithelial cell renewal, gut microbiota, uracil, reactive oxygen species, gut-microbe interactions

INTRODUCTION

Bacteria heavily colonize multiple sites in our body. These sites include various mucosal epithelia such as the respiratory, gastrointestinal, and urogenital tracts. It is now evident that commensal community members form an ecosystem in these sites and that this microbial ecosystem impacts diverse ranges of the host physiology (Turnbaugh et al., 2006; Ryu et al., 2008; Garrett et al., 2010; Shin et al., 2011; Storelli et al., 2011). In particular, in the intestine of human beings, approximately one hundred trillion bacterial cells can be found (Gill et al., 2006; Qin et al., 2010). Because any eukaryotic organ readily responds to bacteria by mounting acute inflammation, one of the most important questions is how host mucosal epithelia that are in continuous contact with a diverse range of bacteria manage such microbial burdens. Recent studies in different animal models demonstrated the reciprocal interactions between gut microbiota and the host innate immunity, where the host immunity controls the community of gut-contacting bacteria that in turn modulates the host immunity (Artis, 2008; Ryu et al., 2008; Round and Mazmanian, 2009; Cerf-Bensussan and Gaboriau-Routhiau, 2010; Littman and Pamer, 2011; Maslowski and Mackay, 2011; Hooper et al., 2012). The balanced interactions between the host immunity and the gut-associated bacteria are of central importance to achieve host-microbe symbiosis. However, it is clear that dysregulation of this relationship may cause chronic inflammation and/or metabolic disorders via bacterial stimulation of the host immune system (Turnbaugh et al., 2006; Wen et al., 2008; Garrett et al., 2010; Vijay-Kumar et al., 2010). Several animal model systems are introduced to dissect the molecular relationship between gut microbiota and gut inflammation (Koropatnick et al., 2004; Bates et al., 2007; Cani et al., 2008; Mazmanian et al., 2008; Ryu et al., 2008; Fraune et al., 2009; Kanther and Rawls, 2010). Although striking advances were made in recent years by taking advantage of technical innovations such as pyro-sequencing and omics technologies, the exact molecular mechanism of gut-microbiota interactions is only partly understood. This is probably due to the complexity of the host immune signaling pathways and also that of commensal community. *Drosophila*, a classical model for developmental biology and innate immunity, is now being introduced in the field of gut-microbiota interactions (Corby-Harris et al., 2007; Cox and Gilmore, 2007; Dietzl et al., 2007; Ren et al., 2007; Drysdale, 2008; Ryu et al., 2008; Apidianakis and Rahme, 2011; Chandler et al., 2011; Shin et al., 2011; Storelli et al., 2011; Wong et al., 2011; Broderick and Lemaitre, 2012; Charroux and Royet, 2012). Its elegant genetic tool box, simple commensal community, well-established knowledge on innate immune system, and easy to generate gnotobiotic animals make it possible to provide a novel insight on the dynamic dialog between bacterial and host cells. Genetic evidence demonstrated that reactive oxygen species (ROS), produced by dual oxidase (DUOX), a member

of the intestinal nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, are involved in diverse aspects of gut-microbe interactions, such as microbial clearance, intestinal epithelial cell renewal (ECR), redox-dependent modulation of signaling pathways, cross-linking of biomolecules, and discrimination between symbionts and pathogens. In the current review, recent advances on the regulation of DUOX in *Drosophila* gut as well as its role on the gut cell homeostasis and gut inflammation are discussed.

GUT-INTERACTING BACTERIA IN *Drosophila*

Due to its open anatomical structure, gut epithelia are in constant contact with diverse ranges of microbial cells. These include resident "autochthonous" bacteria and also transiently passing "allochthonous" bacteria derived from the environment (Dillon and Dillon, 2004; Ley et al., 2008). In *Drosophila*, it is important to note that it is still unclear whether these resident autochthonous bacteria reside inside gut (i.e., stable colonization for a long time period) or transiently colonize gut (i.e., colonization for a short time period, but still longer persistence time when compared to that of transiently passing bacteria). Autochthonous symbionts (e.g., *Commensalibacter intestini*, *Acetobacter pomorum*, and *Lactobacillus plantarum*) constitute an important portion of resident bacteria that are believed to be beneficial to the host physiology (Ryu et al., 2008; Shin et al., 2011; Storelli et al., 2011). For example, *A. pomorum* and *L. plantarum* are known to enhance host development by stimulating important host signaling pathways such as insulin signaling and Tor signaling (Shin et al., 2011; Storelli et al., 2011). However, it is important to note that not all resident bacteria are symbiotic. For instance, *Gluconobacter morbifer* is considered a pathobiont, i.e., the resident bacterial species that is normally benign within a host, but can be conditionally pathogenic when commensal community is deregulated (Ryu et al., 2008). It has been shown that the pathobiont *G. morbifer* becomes pathogenic when the number of this bacterium exceeds a certain threshold following deregulation of gut immunity. In addition to these resident bacteria, the gut is also in contact with several other non-resident allochthonous bacteria that are introduced by the environment. *Erwinia carotovora* is a naturally occurring *Drosophila*-associated bacterium derived from the environment (Buchon et al., 2009b). *E. carotovora* is considered as an opportunistic pathogen because this bacterium does not harm the normal host but it can turn pathogenic when the host immune system is impaired (Ha et al., 2005a, 2009a,b). Among the allochthonous bacteria, certain species such as *Pseudomonas entomophila* and *Serratia marcescens*, are life threatening and thus classified as entomopathogens that are able to kill the host upon gut infection (Vodovar et al., 2005; Nehme et al., 2007). Therefore, it is evident that the host must draw maximum benefits from symbionts while antagonizing potentially pathogenic effects from pathogens and pathobionts, thereby achieving gut-microbiota homeostasis.

GUT IMMUNITY IN *Drosophila*

Due to the fact that the intestine harbors large amounts of bacterial cells, one of the most important questions is to understand the interactions between the host immunity and bacteria. Genetic analyses in *Drosophila* demonstrated that the gut epithelia are able to mount two distinct immune pathways: the immune deficiency (IMD) pathway that controls antimicrobial peptide (AMP) production, and the DUOX pathway that controls microbicidal ROS production (Lemaitre and Hoffmann, 2007; Bae et al., 2010; Royet et al., 2011; Buchon et al., 2013; Lee and Brey, 2013). As a plethora of excellent reviews on the IMD pathway, a *Drosophila* homolog of the mammalian NF-κB pathway can be found in several journals (Lemaitre and Hoffmann, 2007; Ganesan et al., 2010; Royet et al., 2011), the details on this pathway will not be described here. Several studies utilizing the IMD pathway mutant flies generated four interesting observations. First, the IMD pathway mutant flies are fairly resistant to gut infection, indicating that the IMD pathway is dispensable for the host resistance against gut infection in most cases (Ha et al., 2005a,b, 2009a,b). Second, chronic activation of the IMD pathway provokes modification of the gut commensal community, leading to the overgrowth of the opportunistic pathobionts (Ryu et al., 2008). Third, the IMD pathway mutant flies harbor higher amounts of gut microbiota (Buchon et al., 2009a). The second and third points indicate that the IMD pathway regulates the commensal community structure in a quantitative and qualitative manner. Finally, some bacteria that can subvert DUOX-dependent ROS are regulated by IMD-dependent AMPs, indicating that the IMD pathway likely plays a complementary role to the DUOX system, at least under certain circumstances (Ryu et al., 2010). In contrast to the IMD pathway mutant animals, animals with a reduced DUOX activity are highly susceptible to gut infection, indicating that DUOX-dependent ROS generation plays a major role in the control of gut-associated bacteria (Ha et al., 2005a; Bae et al., 2010). The DUOX system, particularly the diverse roles of DUOX in gut physiology, will be explored in further details.

DUOX, A MEMBER OF THE NADPH OXIDASE FAMILY

The role of ROS in the innate immune system was best illustrated by an oxidative burst in phagocytes (Babior, 2004). In this system, $gp91^{Phox}$, a NADPH oxidase (now called NOX2), is responsible for the production of the superoxide anion (Segal, 2005). An analysis of the human genome sequence revealed several homologs of $gp91^{Phox}$, now referred to as the NOX and DUOX family enzymes (Lambeth, 2004; Leto and Geiszt, 2006; Sumimoto, 2008). At present, five NOXs and two DUOXs have been identified in humans (Lambeth, 2004; Leto and Geiszt, 2006; Sumimoto, 2008), only one NOX and one DUOX homolog were observed in *Drosophila* (Donko et al., 2005; Ha et al., 2005a; Bae et al., 2010). These enzymes are found to be expressed in various non-phagocytic cells, including mucosal epithelial cells, suggesting novel physiological roles of ROS in diverse ranges of cells and tissues other than the phagocytes (Geiszt et al., 2003; El Hassani et al., 2005; Ha et al., 2005a; Allaoui et al., 2009; Fischer, 2009). Synthesis of the thyroid hormone in the thyroid gland is catalyzed by thyroperoxidase that requires the presence of H_2O_2, which is generated via the oxidation of NADPH by an NADPH oxidase in the thyroid (Dupuy et al., 1999; De Deken et al., 2000). DUOX was originally identified as a thyroid NADPH oxidase; however, it was later found to be expressed in the mucosal epithelia of the respiratory and gastrointestinal tracts (Geiszt et al., 2003; El Hassani et al., 2005). The DUOX gene is highly conserved

amongst various organisms, from *Caenorhabditis elegans* to mammals (Edens et al., 2001; Ha et al., 2005a; Kawahara and Lambeth, 2007; Flores et al., 2010). The DUOX gene in the *Drosophila* genome is situated in the cytogenetic location 23B2-23B3, on the left arm of chromosome 2. The general structural organization of DUOX was well-conserved in all the studied organisms, and is presented in the **Figure 1**. The enzyme includes an extracellular peroxidase homology domain, a trans-membrane domain, a calcium-modulated EF hand domain, and a NADPH oxidase domain. Although the role of DUOX in the midgut has been most intensively studied, *DUOX* expression level in the midgut is found to be modest. High *DUOX* expression is observed in different organs in larvae (e.g., trachea, hindgut, and central nervous system) and adult (e.g., ovary, spermatheca, crop, and head) (see high-throughput expression data, such as FlyAtlas Anatomy Microarray analysis, in Flybase), suggesting distinct biological roles of DUOX in different organs.

THE ROLE OF DUOX IN THE OXIDANT-DEPENDENT ANTIMICROBIAL RESPONSE IN EPITHELIA

Following the identification of DUOX1/2 expression in the mammalian mucosal epithelia, several lines of evidence demonstrated that DUOX is a source of non-phagocytic ROS in the epithelial cells of the respiratory and gastrointestinal tracts (Geiszt et al., 2003; El Hassani et al., 2005). Because these cells function as a barrier that is in contact with microorganisms, it is believed that DUOX-dependent ROS may act as a microbicide, similar to phagocytic ROS. In this system, DUOX produces extracellular H_2O_2 that is used for the oxidative conversion of SCN^- to hypothiocyanate ($OSCN^-$) by the enzymatic action of lactoperoxidase in the mucosal fluids (Leto and Geiszt, 2006; van der Vliet, 2008; Fischer, 2009) (**Figure 1**). Because hypothiocyanate can kill the bacteria, this DUOX-lactoperoxidase system is believed to provide a robust antimicrobial defense network in mammalian epithelial cells (Forteza et al., 2005; Boots et al., 2009; Gattas et al., 2009). However, because all of these observations in the mammalian system were made in *in vitro* cultured primary cells/tissues or cell lines, the precise *in vivo* role of DUOX in the host antimicrobial defense in an organism remains to be elucidated in mammals. The most direct evidence on the *in vivo* role of DUOX was first provided in a *Drosophila* gut infection model system (Ha et al., 2005a). As mentioned earlier, in contrast to the essential role of AMP-based immunity when microorganisms enter the body (i.e., systemic infection), AMP-based immunity plays only a minor role when microorganisms are introduced in the gut by oral ingestion (i.e., gut infection). For example, AMP-deficient mutant animals are apparently healthy following a gut infection, suggesting the existence of other immune systems that can regulate the bacteria in the gut epithelia (Ha et al., 2005a,b). It was demonstrated that DUOX-knockdown (KD) flies are highly susceptible to gut infections by various microorganisms. Tissue-specific KD experiments showed that the DUOX activity in the gut epithelia is responsible for host resistance to gut infection (Ha et al., 2009b). Additional biochemical studies showed that DUOX is the source of infection-induced ROS in *Drosophila* gut (Buchon et al., 2009a; Ha et al., 2009a,b). Later, the importance of DUOX in gut immunity was also demonstrated in the *C. elegans* and zebrafish model systems (Flores et al., 2010; Hoeven et al., 2011). Although DUOX-mutant mice are available, they exhibit pleiotropic phenotypes such as dwarfism, which makes it difficult to unambiguously conclude the role of DUOX in this animal model (Johnson et al., 2007). Further analysis using conditional knockout animal models will be necessary to validate the *in vivo* role of DUOX in mucosal immunity.

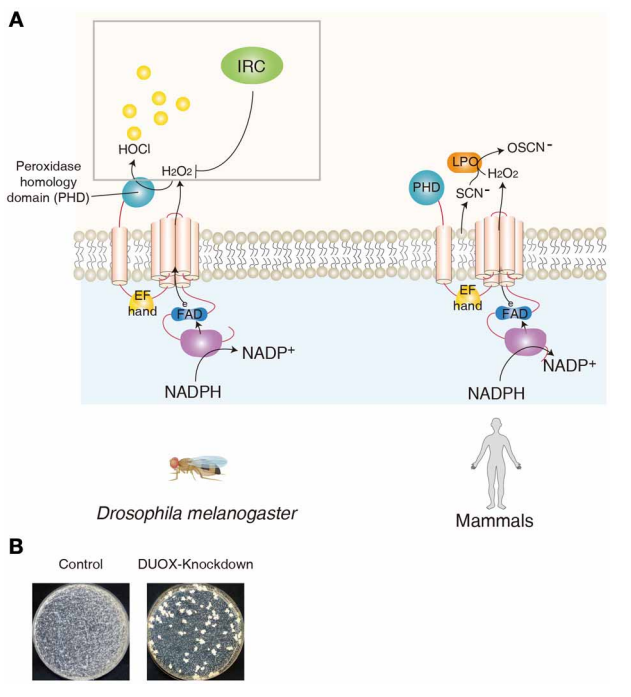


FIGURE 1 | DUOX as a mucosal antimicrobial system in *Drosophila* and human. (A) Similar domains of DUOX enzymes between *Drosophila* and human are shown. In *Drosophila*, peroxidase homology domain of DUOX converts H_2O_2 into HOCl in the presence of chloride. DUOX-dependent H_2O_2 molecules are eliminated by immune-regulated catalase (IRC) activity. In human, DUOX-dependent H_2O_2 is used for the oxidative conversion of SCN^- to $OSCN^-$ by the enzymatic action of lactoperoxidase in the mucosal fluids. **(B)** Modification of gut commensal community members in flies carrying reduced DUOX activity. Midgut of control flies and that of DUOX-knockdown flies are dissected and the homogenates of midguts are spread on Mannitol agar plate. Representative images are shown.

How does *Drosophila* DUOX antagonize bacterial growth *in vivo*? It has been suggested that the NADPH oxidase domain of DUOX produces H_2O_2 in the gut lumen, and a peroxidase homology domain, the second domain of DUOX, converts H_2O_2 into HOCl in the presence of chloride (Ha et al., 2005a) (**Figure 1**). In support of this notion, the recombinant peroxidase homology domain can kill the bacteria only in the presence of both H_2O_2 and chloride (Ha et al., 2005a).

In the absence of gut infection, the metazoan gut harbors significant amounts of bacterial cells under conventional conditions (Ley et al., 2008; Lee and Lee, 2013). This commensal community structure (both in terms of bacterial diversity and density) is known to be actively shaped by the host immunity (Artis, 2008; Pedron and Sansonetti, 2008; Ryu et al., 2008; Round and Mazmanian, 2009; Cerf-Bensussan and Gaboriau-Routhiau,

2010; Littman and Pamer, 2011; Maslowski and Mackay, 2011; Hooper et al., 2012; Lee and Lee, 2013). It has been shown that a regulated level of IMD pathway potential is essential for a normal commensal community structure (Ryu et al., 2008). As the DUOX system is the primary host immune system that provides a robust antimicrobial response in the microbe-laden epithelia in metazoans, it is expected that the loss-of-DUOX activity would result in dysregulation of the commensal community (Ha et al., 2009a). On examination of the gut microbiota of DUOX-KD flies cultured in a growth plate, it is consistently observed that the gut commensal community of DUOX-KD flies is highly modified, as evidenced by the presence of higher bacterial cell number, different shapes of bacterial colonies, and the presence of fungi (Ha et al., 2009a) (**Figure 1**). This indicates that the absence of a major defense system leads to a severe dysregulation of the gut-associated microbiota. Given that DUOX-KD flies under conventional (CV) conditions had a short life span that could be completely rescued under germ-free (GF) condition (Ha et al., 2009a), and that the monoassociation of DUXO-KD flies with each of the resident symbiotic bacteria did not affect their survival rate, the dysregulated commensal community may be the direct cause of mortality. However, opportunistic pathogens and/or pathobionts responsible for the lethality of conventional DUOX-KD flies remain to be elucidated.

Unlike AMPs specific to prokaryotic cells, microbicidal ROS are also cytotoxic to eukaryotic host cells. Therefore, ROS production must be tightly regulated to avoid excess oxidative stress. It was found that flies lacking secretory immune-regulated catalase (IRC) showed high lethality against gut infection due to oxidative stress (Ha et al., 2005b) (**Figure 1**). As IRC possesses a H_2O_2-scavenging activity, this observation indicates that infection-induced ROS are dynamically removed by IRC. Therefore, it is likely that DUOX-dependent ROS generation and IRC-dependent ROS removal modulate redox-dependent innate immunity to antagonize pathogen growth, while protecting host cells from an excess immune response (Ha et al., 2005a,b).

MICROBIAL LIGANDS FOR DUOX ACTIVATION

The identification of the DUOX system in the gut epithelia raises an important question of how a host senses different bacteria to induce DUOX activation. In *Drosophila*, meso-diaminopimelic acid-type peptidoglycan (PG) primarily released from Gram-negative bacteria acts as an agonist for the IMD activation in the gut (Leulier et al., 2003; Royet et al., 2011). However, PG was unable to induce a DUOX-dependent ROS generation, indicating that ligands other than PG (non-PG ligands) are derived from the bacteria to induce DUOX activation (Ha et al., 2009a,b; Bae et al., 2010). Because most microorganisms, including yeast and Gram-positive bacteria, can also activate the DUOX system, these non-PG ligands are believed to commonly exist in diverse microorganisms. In contrast to the robust DUOX activation following gut epithelial contact with allochthonous bacteria, most symbiotic autochthonous bacteria do not cause DUOX activation (Lee et al., 2013). This observation suggests that non-PG ligands may acts as pathogen-specific ligands that may be absent and/or reduced in symbionts, allowing a distinction between allochthonous and autochthonous bacteria. It has recently been found that this non-PG ligand is indeed secreted from allochthonous bacteria but not from the autochthonous bacteria (Lee et al., 2013). Chemical analyses of this non-PG ligand have revealed that it is a uracil nucleobase. Synthetic uracil is found to be very capable of stimulating DUOX activation (range approximately 100 pM–100 nM) whereas other nucleobases are inefficient ligands under similar concentrations. Furthermore, uracil is unable to activate the IMD pathway, indicating that uracil-based immunity is distinct to PG-based immunity (Lee et al., 2013). This uracil-based immune system is unique because PG-based immune systems fail to distinguish between pathogens and symbionts because both bacteria have a similar capacity to induced the PG-dependent IMD pathway (Lee et al., 2013). All of these observations suggest that the gut epithelia selectively mount DUOX activation by sensing pathogen-derived uracil. Mutant pathogens with reduced uracil secretion (e.g., uracil auxotrophic *E. carotovora* strain) could avoid DUOX activation with this being lethal to the host, whereas the wild type *E. carotovora* strain would not harm the normal host (Lee et al., 2013) (**Figure 2**). These observations demonstrate that the recognition of pathogen-derived uracil is essential for the control of opportunistic pathogens such as *E. carotovora* and host survival. These observations also raise the interesting possibility that a reduction of uracil secretion may be employed as a virulence mechanism for the pathogen to avoid host immunity (**Figure 2**). It would be interesting to see whether host-killing *Drosophila* pathogens use this strategy to avoid the host DUOX system.

As uracil can be found in any living cells including symbiotic or pathogenic bacteria, it is presently unclear why symbiotic bacteria do not secret uracil whereas pathogens do so. The mechanism of uracil secretion from the bacteria is presently unknown. The secretion of uracil in the case of *E. coli* is only observed when growth conditions are unfavorable, e.g., in response to entry into the stationary phase or to a perturbation of balanced growth conditions (Rinas et al., 1995). This observation indicates that uracil release is controlled by the bacterial cells depending on the environmental conditions. It is unclear why bacteria release uracil under unfavorable condition. One interesting possibility is that it may act as a bacterial survival signal to overcome the stringent conditions. For example, *Pseudomonas aeruginosa* can respond to exogenous uracil by reprogramming the bacterial gene expressions involved in virulence, quorum sensing, and biofilm formation (Ueda et al., 2009). Therefore, one can speculate that uracil release is a normal bacterial response to resist stressful conditions; this is beneficial for the survival of bacterial cells. In this context, it is possible that gut environments are stressful conditions for most environment-derived opportunistic pathogens which initiate uracil release *in situ* to promote their survival. However, this survival strategy is potentially dangerous to the host cells. Therefore, host may have evolved to sense the bacterial status from uracil presence, subsequently antagonizing pathogens before they mount their survival strategy. Another interesting point is that, as uracil can be also found in any eukaryotic cells, it may act as a danger signal released from damaged host cells. In this case, it is possible that host could mount innate immunity by sensing uracil released from host cells damaged by pathogens (e.g., by intracellular pathogens). Further detailed

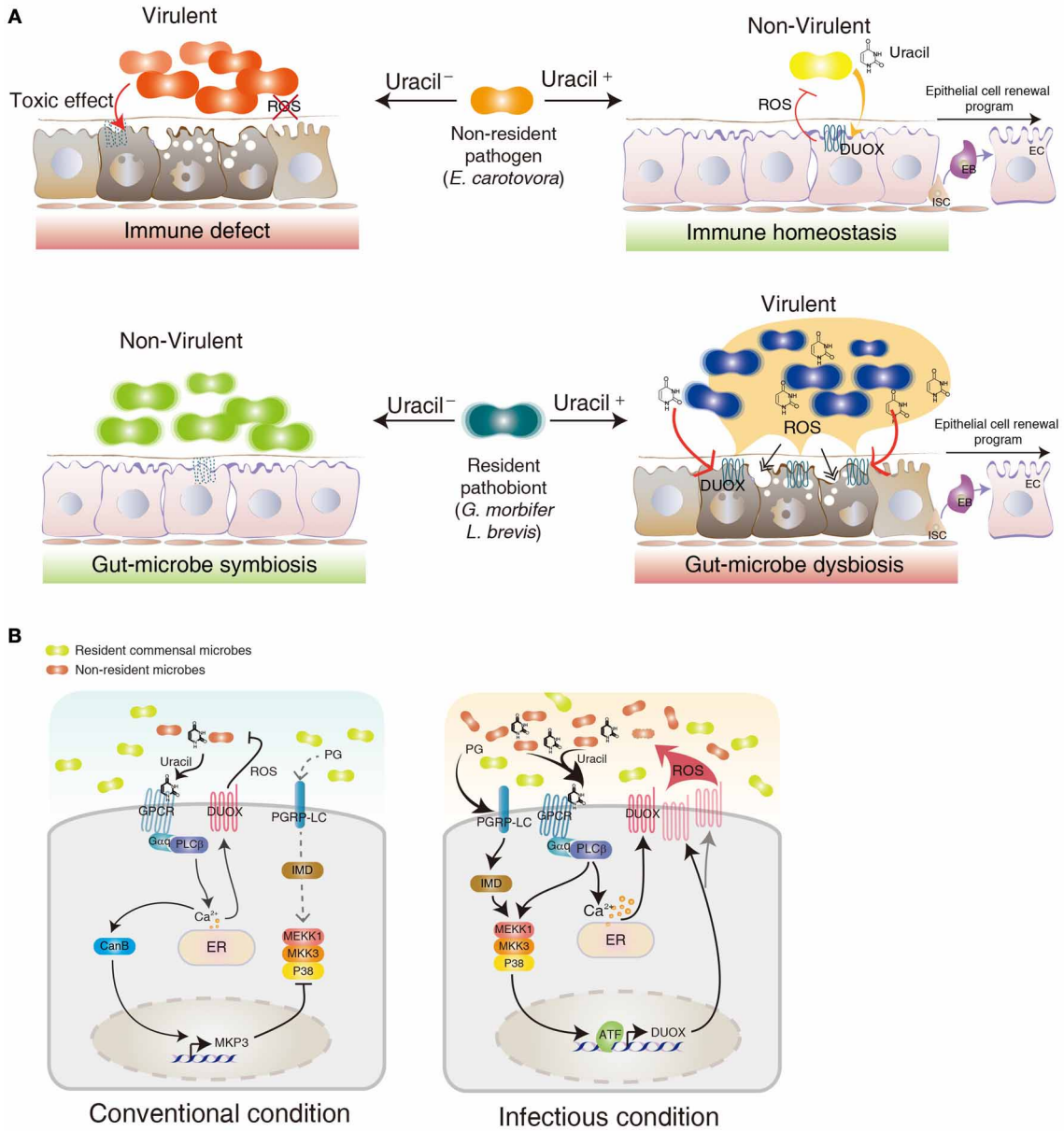


FIGURE 2 | Role of DUOX in gut-microbe interactions. (A) Different gut physiologies depending different uracil-releasing states (Uracil$^-$ and Uracil$^+$ for uracil non-releasing and releasing state, respectively) and different gut-colonizing ability (resident vs. non-resident) of each bacterium in a *Drosophila* gut environment. **(B)** DUOX regulatory mechanism in conventional and infectious conditions. See text for more details.

investigations of all these interesting possibilities will be needed to better understand the complex interactions between host immunity and different gut-associated autochthonous/allochthonous bacteria.

Monoassociation of GF animals with each type of commensal bacteria revealed that most symbiotic autochthonous bacteria do not elicit a DUOX activation probably due to the absence of uracil release (Lee et al., 2013; Valanne and Ramet, 2013). This observation indicates that symbiotic autochthonous bacteria may have evolved to adapt to the gut environment by avoiding DUOX activation possibly by modifying the pathway of uracil secretion. However, some resident bacteria, such as *G. morbifer* and *L. brevis*, do induce a chronic DUOX activation, suggesting that these gut-dwelling pathobionts may chronically release the uracil that is responsible for the chronic DUOX activation (Lee et al., 2013) (**Figure 2**). Chronic DUOX activation results in gut cell apoptosis and early host death, which is reminiscent of the phenotypes found in chronic inflammatory diseases. The reduction of uracil release by generating URA$^-$ mutant pathobionts is sufficient to prevent all the disease phenotypes, with a resulting bacterial phenotypic shift from pathobionts to symbionts (Lee et al., 2013) (**Figure 2**). These observations demonstrate that

uracil release from gut-dwelling bacteria can act as a virulence factor of the opportunistic pathobionts. It is presently unknown why pathobionts are generally benign within a normal commensal community but become pathogenic under certain conditions. If uracil excretion can be controlled by the bacteria in a context-dependent manner, one intriguing possibility is that pathobionts can become pathogenic when they initiate their uracil secretion pathway under certain dysregulated gut environments (**Figure 2**). Future studies on the mechanism of the uracil secretion pathway and its differential regulation between the symbiont and pathobionts will be needed to better understand the physiological characteristics of pathobionts and symbionts.

Interestingly, uracil can also stimulate DUOX activation in *C. elegans* as well as in human bronchial and intestinal epithelial cells (Lee et al., 2013). It would be interesting to investigate whether the uracil-mediated DUOX activation mechanism is involved in the etiology and pathogenesis of mammalian epithelial inflammatory diseases that arise from abnormal mucosa-microbe interactions.

THE DUOX REGULATORY MECHANISM

Gut epithelial cells are in continuous contact with basal amounts of bacterial ligands such as PG and uracil (Lee and Lee, 2013). As chronic and/or overactivation of the DUOX system may lead to a deleterious effect on host cells, DUOX activation must be tightly regulated to avoid oxidative damages while preserving intact microbicidal activity (Ha et al., 2009b; Lee and Lee, 2013). At present, genetic analyses have revealed that two signaling pathways are controlling DUOX-dependent ROS generation (Ha et al., 2009b). The DUOX-activity pathway composed of PLCβ-calcium signaling is responsible for the induction of DUOX enzymatic activity whereas the DUOX-expression pathway composed of the MEKK1-MKK3-p38 MAPK-ATF2 transcription factor is responsible for the induction of DUOX gene expression (Ha et al., 2009b) (**Figure 2**).

It is known that these two pathways are differentially activated depending on the local microbial burdens. By comparing the GF animals (devoid of any bacterial cells) and CV animals (having normal symbiotic microflora as well as some environment-derived microorganisms) it was found that CV animals consistently showed higher basal ROS levels than those found in GF animals or GF animals monoassociated with symbiotic commensal bacteria (Lee et al., 2013). This observation indicates that gut-associated microflora other than symbionts found in the CV environment stimulates basal levels of DUOX activity. Basal levels of DUOX are known to be required for the routine control of gut-introduced microorganisms such as dietary yeast, *Saccharomyces cerevisiae* (Ha et al., 2009b). In this condition, basal PLCβ activity induces low calcium mobilization to maintain the basal DUOX activation because the DUOX enzyme is dependent on calcium concentration (**Figure 2**). When gut epithelia are further subjected to gut infection, the PLCβ-calcium signaling becomes maximally activated to induce full DUOX activity (Ha et al., 2009b) (**Figure 2**). It is important to note that this PLCβ-calcium signaling is activated by uracil but not by PG, indicating that the IMD pathway and the DUOX pathway are distinct (Lee et al., 2013). As a variety of microbial cells can induce DUOX activation, it is likely that uracil is released from many microbial cells in the gut. Under infectious conditions, the DUOX-expression pathway becomes activated by two different bacterial ligands, uracil, and PG (Ha et al., 2009b; Lee et al., 2013) (**Figure 2**). Uracil activates MEKK1-MKK3-p38 in a PLCβ-dependent manner possibly by PKC activation, whereas PG activates MEKK1-MKK3-p38 in a PGRP-LC and IMD-dependent manner (**Figure 2**). It should be noted that MEKK1 mutant animals having an intact DUOX-activity pathway but impaired DUOX-expression pathway survive normally under CV conditions (Ha et al., 2009b). They are, however, highly susceptible to gut infections. These observations indicate that the DUOX-activity pathway alone is required and sufficient for the control of routine microbial burdens whereas both DUOX-activity and the DUOX-expression pathway are required for the control of high microbial burdens.

It is important to note that the basal DUOX-activity pathway is required for the inhibition of the DUOX-expression pathway under CV conditions (Ha et al., 2009a,b; Bae et al., 2010) (**Figure 2**). For example, PLCβ mutant flies showed constitutive p38 MAPK activation and DUOX gene overexpression under CV conditions but not GF conditions (Ha et al., 2009a). It has been shown that basal PLCβ-calcium signaling induces calcium-dependent calcineurin B and MAPK phosphatase 3 (MKP3) gene expression (Ha et al., 2009b) (**Figure 2**). MKP3 negatively regulates p38 phosphorylation. As the calcineurin inhibitor FK506 abolished MKP3 gene expression, Calcineurin B acts as an upstream component of MKP3 (Ha et al., 2009b). MKP3-KD flies having a high DUOX-expression pathway activation exhibited a short life span under CV conditions due to oxidative stress, indicating that the negative regulation of the DUOX-expression pathway by the DUOX-activity pathway is required to avoid excess oxidative stress under routine gut-microbe interactions (Ha et al., 2009b; Bae et al., 2010).

DUOX IN GUT INTEGRITY

In addition to its direct microbicidal actions, other interesting aspects of DUOX are also documented (**Figure 3**). In *Anopheles gambiae*, DUOX is known to be involved in gut permeability by forming a dityrosine network of the peritrophic membrane, a non-cellular semi-permeable layer of chitin polymers covering the midgut epithelia (Kumar et al., 2010). In this system, DUOX-dependent H_2O_2 acts as a substrate of secreted heme peroxidase that catalyzes protein cross-linking in the mucin layer. In an *Anopheles* with reduced DUOX expression, gut permeability increases due to the reduction of dityrosine cross-linking of the peritrophic membranes (Kumar et al., 2010). It was shown that DUOX activity mediates cross-linking between macromolecules, e.g., between collagen and other proteins, via di- and tri-tyrosine linkage, for the formation of the cuticular extracellular matrix in *Caenorhabditis elegans* (Edens et al., 2001). In the sea urchin eggs, DUOX-dependent H_2O_2 is shown to be essential for the oxidative cross-linking of the fertilization envelop (Wong et al., 2004). Similarly, *Drosophila* DUOX was found to be involved in the stabilization of the adult wing, possibly by tyrosine cross-linking (Anh et al., 2011). Therefore, bacterial-induced DUOX activity may regulate the formation of a physical barrier such as the peritrophic membrane that provides a buffered zone between commensal

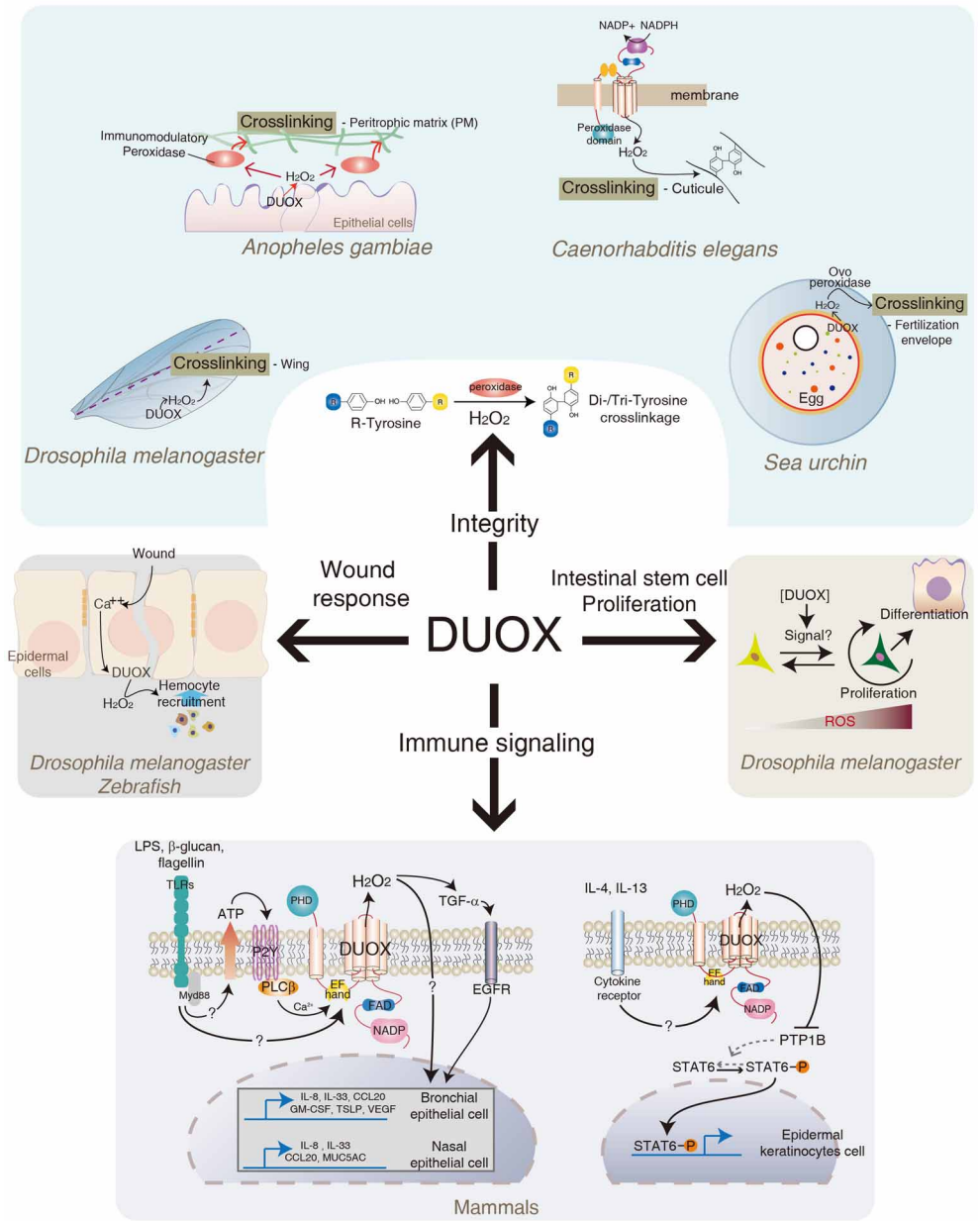


FIGURE 3 | Role of DUOX in diverse biological activities. In addition to its original function in redox-dependent antimicrobial defense in mucosa described in **Figures 1**, **2**. DUOX system is also involved in cross-linking of biomolecules, intestinal epithelial cell renewal, redox-dependent modulation of signaling pathways, and wound healing in different metazoans. See text for more details.

bacteria and enterocytes. In this regard, it is interesting to note that DUOX-KD flies under CV condition showed spontaneous IMD pathway activation when the flies became old (Lee and Lee, Unpublished observation), which was abolished in GF DUOX-KD flies. These results suggest that increased peritrophic membrane permeability and/or increased bacterial burden observed in DUOX-KD flies are responsible for spontaneous IMD pathway activation. Further studies will be needed to elucidate the exact cause of spontaneous IMD pathway activation in aged DUOX-KD flies. In mammals, DUOX is known to be involved in the expression of MUC5AC mucin, one of the major components of airway mucus, in the airway epithelia in response to different stimuli (Shao and Nadel, 2005). In this case, DUOX-dependent H_2O_2 acts as a second messenger to modulate signaling pathways, leading to MUC5AC expression, although the exact mechanisms remain to be elucidated. In the *Drosophila* genome, 17 mucins and 19 mucin-related proteins are identified (Syed et al., 2008). It would be interesting to see whether DUOX activity also mediates the expression of these mucins in the midgut epithelia.

DUOX IN INTESTINAL STEM CELL ACTIVATION

The process of gut infection introduces a high density of bacterial cells into the gut lumen, which inevitably damages the epithelial cells lining the intestinal tract. These damaged cells need to be replaced by newly emerged cells to maintain gut cell homeostasis. It was recently shown that bacterial infection induces an ECR program that is responsible for replenishing the damaged cells (Amcheslavsky et al., 2009; Buchon et al., 2009a,b; Chatterjee and Ip, 2009; Cronin et al., 2009; Jiang et al., 2009). This ECR program includes intestinal stem cell (ISC) proliferation and differentiation. Although the ECR program controls the normal turn-over rate of gut epithelial cells, the infection process accelerates the ECR program due to the massive gut cell loss (Buchon et al., 2009a,b, 2010; Chatterjee and Ip, 2009; Jiang et al., 2009). Upon gut infection, each ISC produces one daughter cell that retains the fate of its parent cell, and one postmitotic enteroblast that in turn differentiates into either an enterocyte or an enteroendocrine cell (Micchelli and Perrimon, 2006; Ohlstein and Spradling, 2006, 2007). Several signaling pathways such as growth factor signaling and JAK-STAT signaling pathways are known to be involved in the ECR program (Buchon et al., 2009b, 2010; Cronin et al., 2009; Jiang and Edgar, 2009; Jiang et al., 2009; Xu et al., 2011; Zhou et al., 2013). Interestingly, flies with reduced DUOX activity fail to mount a normal ECR program following gut infection, as evidence by reduced ISC proliferation and differentiation (Buchon et al., 2009a). Based on this result, it has been proposed that DUOX-dependent ROS molecule is one of major inducers to initiate the ECR program. Given that ingestion of tissue damaging agents such as sodium dodecyl sulfate or paraquat could initiate ECR, it is speculated that the increase in the ECR program is not a direct effect of ROS but rather an effect of the ROS-induced host cell damage (Buchon et al., 2009a). Alternatively, DUOX-dependent ROS molecule may act as a direct signaling molecule to initiate ECR program. It is routinely observed that the DUOX-KD flies exhibited a higher gut cell apoptosis index in a CV condition when compared to that observed in control flies; i.e., more than 2% in 13-day-old DUOX-KD flies vs. less than 0.2% in control flies of the same age (unpublished observation). Despite the high gut cell apoptosis index, these DUOX-KD flies demonstrated a reduced rate of ECR program, raising an alternative possibility in that a certain level of ROS acts as a critical signal to initiate the ECR program. In agreement with this notion, recent evidences showed that ISCs in *Drosophila* are under redox-control and that reduced ROS level favors stemness whereas elevated ROS level initiates the differentiation program (Biteau et al., 2008; Buchon et al., 2009a; Lee, 2009; Owusu-Ansah and Banerjee, 2009; Hochmuth et al., 2011; Jasper and Bohmann, 2013) (**Figure 2**). It has been proposed that different ROS levels modulate the specificity and intensity of the signal response as well as the adhesive properties of stem cells within a niche. Interestingly, *L. plantarum*, but not other bacterial species, was recently shown to induce NOX-dependent ROS to modulate ECR program in *Drosophila* (Jones et al., 2013). In the study of interactions between gut and a specific bacterium, it is important to note that bacterial micro-diversity within the same species even with 100% identical 16S rRNA was reported in many bacteria (Jaspers and Overmann, 2004). Distinct physiology, such as phenotypic and genomic diversity, among different strains of the same species, *L. plantarum*, was also reported (Siezen et al., 2010). For example, a recent report showed that a *L. plantarum* IBDML1 strain is unable to promote *Drosophila* larval growth whereas a *L. plantarum* strain WJL strain can promote larval development under the same experimental conditions (Storelli et al., 2011), indicating that the physiological characteristics of microorganisms should be studied in a strain level, but not in a species level. Therefore, it is possible that each bacterial strain may differentially influence ECR program by activating distinct enzymes (i.e., NOX or DUOX) with different mode of enzyme activation in terms of intensity and duration. This important issue can be clarified by clearly establishing the ROS-inducing mode of each bacterial strain and the molecular mechanisms by which ROS modulate intracellular signaling pathways involved in ISC proliferation and differentiation. The ingestion of uracil is sufficient to induce all aspects of the ECR program such as ISC proliferation and differentiation as well as JAK-STAT activation (Lee et al., 2013). Thus, the uracil-induced ECR program will provide a unique opportunity to dissect the molecular mechanism by which DUOX modulates ISC regulation.

DUOX IN SIGNAL TRANSDUCTION

Although H_2O_2 is a well-known cytotoxic molecule that can damage the host, it became evident that the physiological concentration of H_2O_2 is essential for the relay of many important intracellular signaling pathways (Sauer and Wartenberg, 2005; Rhee, 2006; Stone and Yang, 2006). In this regard, it is interesting to note that DUOX is found to be activated following ligand-dependent stimulation of TLRs in mammals (**Figure 3**). For example, interactions between the microbial components and TLRs, such as flagellin/TLR5, LPS/TLR4, and β-1,3-glucan/TLR2, are shown to induce DUOX activation in human airway epithelial cells (Koff et al., 2008; Joo et al., 2012; Ryu et al., 2013) (**Figure 3**). However, the mechanism by which TLR stimulation leads to DUOX activation is less clear. Co-immunoprecipitation experiments showed that DUOX is physically associated, directly or indirectly, with at least some members of the TLR family, such as TLR2 and TLR5 (Joo et al., 2012; Ryu et al., 2013). One possibility is that this TLR stimulation following ligand binding may induce structural changes of TLR, which somehow contributes to the DUOX activation state. Alternatively, TLR stimulation induces DUOX activation by intracellular calcium mobilization. For example, upon TLR stimulation, cells release ATP that induces PLCβ-dependent calcium mobilization via purinergic receptor activation (Boots et al., 2009) (**Figure 3**). As calcium mobilization can directly modulate the DUOX enzyme activity via its EF-hand domains, it can be speculated that bacterial ligands capable of inducing calcium, directly or indirectly, could induce calcium-dependent DUOX activation and H_2O_2 production. Importantly, the absence of DUOX-dependent H_2O_2 production abolished the expression of TLR-downstream target genes in epithelial cells, such as IL8 and Mucin 5AC, and CCL20 chemokines, highlighting the importance of DUOX-dependent H_2O_2 in TLRs signaling pathways (Koff et al., 2008; Joo et al., 2012; Ryu et al., 2013). It is presently unclear how DUOX-dependent H_2O_2 contributes to the expression of inflammatory

genes in epithelial cells. One possible mechanism is that DUOX-dependent H_2O_2 somehow converts the latent form of TNF-α converting enzyme (TACE) to its active form, which in turn cleaves the proform of TGF-α to its active form (Koff et al., 2008). The active form of TGF-α in turn induces EGFR signaling activation to induce inflammatory gene expression such as IL8. However, other H_2O_2-dependent and ligand-independent EGFR activations are also described (Boots et al., 2009). In this system, DUOX-dependent H_2O_2 activates Src kinase, which in turn activates EGFR in a ligand-independent manner. In *Drosophila* and zebrafish, DUOX-dependent H_2O_2 production in response to tissue injury is shown to be critical to attract hemocyte recruitment and wound repair gene expression (Niethammer et al., 2009; Moreira et al., 2010) (**Figure 3**). Epithelial injury in *Drosophila* embryo induces DUOX-dependent ROS generation that is in turn required for the induction of ERK-dependent wound repair genes such as *dopa decarboxylase* and *tyrosine hydrolase* (Juarez et al., 2011; Razzell et al., 2013). How does H_2O_2 modulate such diverse signaling pathways? It is well-known that H_2O_2 can modify protein structure and function by the oxidation of some amino acid residues such as cysteine (Stadtman and Levine, 2003). Several redox-regulated signaling molecules have been documented (Veal et al., 2007). These include transcription factors (e.g., c-Jun/c-Fos, Nrf-2/Keap-1), several kinases (JNK, MEKK1, I-κB kinase, Src tyrosine kinase), and phosphatase (e.g., PTEN and PTP). Indeed, it has been shown that the Th2 cytokines, IL4 and IL13, induce DUOX-dependent ROS generation in normal human epidermal keratinocytes, and that DUOX-dependent ROS induces oxidative inactivation of the catalytic cysteine 215 of the protein tyrosine phosphatase 1B (Hirakawa et al., 2011). Inactivation of protein tyrosine phosphatase 1B acts as a positive feedback loop that prolongs the duration of IL4- and IL13-induced STAT6 phosphorylation (**Figure 3**). Given that DUOX activation acts genetic upstream of JAK-STAT activation during ISC differentiation in *Drosophila*, it would be interesting to examine whether a similar mechanism operates in the ECR program in *Drosophila* gut epithelia. In sum, all the relevant evidences suggest that the ligand-dependent generation of physiological concentration of DUOX-dependent H_2O_2 likely plays a critical role in the initiation and amplification of diverse signaling pathways, including inflammatory and wound repair signaling. The identification of target redox-regulated signaling molecules controlled by DUOX-dependent H_2O_2 will clearly elucidate the exact molecular mechanism of DUOX-mediated signaling pathways.

CONCLUSION

Signal-dependent ROS productions are now considered to play a pivotal role in a diverse range of host physiology. Genetic studies using the *Drosophila* model system unambiguously demonstrated the *in vivo* role of mucosal DUOX on bacterial control (Ha et al., 2005a). Strikingly, its unique mode of activation by bacteria-derived uracil makes it possible to distinguish between bacteria that release uracil and bacteria that cannot (Lee et al., 2013). Considering that the uracil-releasing ability and gut-colonizing ability of each bacterium determines the total amount and duration of uracil released *in situ*, respectively, these two bacterial characteristics are the factors controlling the intensity of DUOX activity *in vivo*. Insufficient DUOX activation by allochthonous bacteria may result in an infectious condition, whereas long-term DUOX activation by autochthonous bacteria may lead to chronic inflammation (Lee et al., 2013). In this regard, it is important to investigate the bacterial mechanism of uracil release and its regulation in different bacteria. This information may provide a novel insight on the molecular mechanisms of gut-microbe symbiosis and gut-microbe pathogenesis. It is also exciting to observe diverse DUOX functions in the mucosal epithelia. In addition to its antimicrobial response, it becomes evident that DUOX plays a central role in gut permeability and modulation of signal transductions involved in immune gene expression, wound healing, and stem cell regulation. Biochemical analyses on the identification of redox-controlled signaling molecules will provide a clearer picture on the mechanism of DUOX-modulated signaling pathways. One issue however remains; the host receptors responsible for DUOX activation. Analysis on the DUOX-activating signaling pathway revealed that G-protein coupled receptors (GPCRs) are involved in the recognition of bacterial ligands or other stimuli to initiate DUOX activation (Ha et al., 2009a; Lee et al., 2013). Approximately 300 GPCRs have been identified in the *Drosophila* genome (Brody and Cravchik, 2000; Hewes and Taghert, 2001). Preliminary genetic screening revealed that multiple GPCRs seem to be involved in the DUOX activation during gut-microbe interactions. The identification and characterization of these GPCRs and their respective ligands will provide a better understanding of the mechanism of how gut epithelia sense environmental ligands for DUOX activation, and of how each GPCR contributes to DUOX-modulated gut physiology.

ACKNOWLEDGMENTS

This study was supported by the National Creative Research Initiative Program (Grant no. 2006-0050687 to Won-Jae Lee) and the Basic Science Research Program (NRF-2013R1A1A2013250 to Sung-Hee Kim) from the National Research Foundation of the Ministry of Science, ICT, and Future Planning of Korea.

REFERENCES

Allaoui, A., Botteaux, A., Dumont, J. E., Hoste, C., and De Deken, X. (2009). Dual oxidases and hydrogen peroxide in a complex dialogue between host mucosae and bacteria. *Trends Mol. Med.* 15, 571–579. doi: 10.1016/j.molmed.2009.10.003

Amcheslavsky, A., Jiang, J., and Ip, Y. T. (2009). Tissue damage-induced intestinal stem cell division in Drosophila. *Cell Stem Cell* 4, 49–61. doi: 10.1016/j.stem.2008.10.016

Anh, N. T., Nishitani, M., Harada, S., Yamaguchi, M., and Kamei, K. (2011). Essential role of Duox in stabilization of Drosophila wing. *J. Biol. Chem.* 286, 33244–33251. doi: 10.1074/jbc.M111.263178

Apidianakis, Y., and Rahme, L. G. (2011). *Drosophila melanogaster* as a model for human intestinal infection and pathology. *Dis. Model. Mech.* 4, 21–30. doi: 10.1242/dmm.003970

Artis, D. (2008). Epithelial-cell recognition of commensal bacteria and maintenance of immune homeostasis in the gut. *Nat. Rev. Immunol.* 8, 411–420. doi: 10.1038/nri2316

Babior, B. M. (2004). NADPH oxidase. *Curr. Opin. Immunol.* 16, 42–47. doi: 10.1016/j.coi.2003.12.001

Bae, Y. S., Choi, M. K., and Lee, W. J. (2010). Dual oxidase in mucosal immunity and host-microbe homeostasis. *Trends Immunol.* 31, 278–287. doi: 10.1016/j.it.2010.05.003

Bates, J. M., Akerlund, J., Mittge, E., and Guillemin, K. (2007). Intestinal alkaline phosphatase detoxifies lipopolysaccharide and prevents inflammation in

zebrafish in response to the gut microbiota. *Cell Host Microbe* 2, 371–382. doi: 10.1016/j.chom.2007.10.010

Biteau, B., Hochmuth, C. E., and Jasper, H. (2008). JNK activity in somatic stem cells causes loss of tissue homeostasis in the aging Drosophila gut. *Cell Stem Cell* 3, 442–455. doi: 10.1016/j.stem.2008.07.024

Boots, A. W., Hristova, M., Kasahara, D. I., Haenen, G. R., Bast, A., and van der Vliet, A. (2009). ATP-mediated activation of the NADPH oxidase DUOX1 mediates airway epithelial responses to bacterial stimuli. *J. Biol. Chem.* 284, 17858–17867. doi: 10.1074/jbc.M809761200

Broderick, N. A., and Lemaitre, B. (2012). Gut-associated microbes of *Drosophila melanogaster*. *Gut Microbes* 3, 307–321. doi: 10.4161/gmic.19896

Brody, T., and Cravchik, A. (2000). *Drosophila melanogaster* G protein-coupled receptors. *J. Cell Biol.* 150, F83–F88. doi: 10.1083/jcb.150.2.F83

Buchon, N., Broderick, N. A., Chakrabarti, S., and Lemaitre, B. (2009a). Invasive and indigenous microbiota impact intestinal stem cell activity through multiple pathways in Drosophila. *Genes Dev.* 23, 2333–2344. doi: 10.1101/gad.1827009

Buchon, N., Broderick, N. A., Poidevin, M., Pradervand, S., and Lemaitre, B. (2009b). Drosophila intestinal response to bacterial infection: activation of host defense and stem cell proliferation. *Cell Host Microbe* 5, 200–211. doi: 10.1016/j.chom.2009.01.003

Buchon, N., Broderick, N. A., Kuraishi, T., and Lemaitre, B. (2010). Drosophila EGFR pathway coordinates stem cell proliferation and gut remodeling following infection. *BMC Biol.* 8:152. doi: 10.1186/1741-7007-8-152

Buchon, N., Broderick, N. A., and Lemaitre, B. (2013). Gut homeostasis in a microbial world: insights from *Drosophila melanogaster*. *Nat. Rev. Microbiol.* 11, 615–626. doi: 10.1038/nrmicro3074

Cani, P. D., Bibiloni, R., Knauf, C., Neyrinck, A. M., Neyrinck, A. M., Delzenne, N. M., et al. (2008). Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. *Diabetes* 57, 1470–1481. doi: 10.2337/db07-1403

Cerf-Bensussan, N., and Gaboriau-Routhiau, V. (2010). The immune system and the gut microbiota: friends or foes? *Nat. Rev. Immunol.* 10, 735–744. doi: 10.1038/nri2850

Chandler, J. A., Lang, J. M., Bhatnagar, S., Eisen, J. A., and Kopp, A. (2011). Bacterial communities of diverse Drosophila species: ecological context of a host-microbe model system. *PLoS Genet.* 7:e1002272. doi: 10.1371/journal.pgen.1002272

Charroux, B., and Royet, J. (2012). Gut-microbiota interactions in non-mammals: what can we learn from Drosophila? *Semin. Immunol.* 24, 17–24. doi: 10.1016/j.smim.2011.11.003

Chatterjee, M., and Ip, Y. T. (2009). Pathogenic stimulation of intestinal stem cell response in Drosophila. *J. Cell. Physiol.* 220, 664–671. doi: 10.1002/jcp.21808

Corby-Harris, V., Pontaroli, A. C., Shimkets, L. J., Bennetzen, J. L., Habel, K. E., and Promislow, D. E. (2007). Geographical distribution and diversity of bacteria associated with natural populations of *Drosophila melanogaster*. *Appl. Environ. Microbiol.* 73, 3470–3479. doi: 10.1128/AEM.02120-06

Cox, C. R., and Gilmore, M. S. (2007). Native microbial colonization of *Drosophila melanogaster* and its use as a model of *Enterococcus faecalis* pathogenesis. *Infect. Immun.* 75, 1565–1576. doi: 10.1128/IAI.01496-06

Cronin, S. J., Nehme, N. T., Limmer, S., Liegeois, S., Pospisilik, J. A., Schramek, D., et al. (2009). Genome-wide RNAi screen identifies genes involved in intestinal pathogenic bacterial infection. *Science* 325, 340–343. doi: 10.1126/science.1173164

De Deken, X., Wang, D., Many, M. C., Costagliola, S., Libert, F., Vassart, G., et al. (2000). Cloning of two human thyroid cDNAs encoding new members of the NADPH oxidase family. *J. Biol. Chem.* 275, 23227–23233. doi: 10.1074/jbc.M000916200

Dietzl, G., Chen, D., Schnorrer, F., Su, K. C., Barinova, Y., Fellner, M., et al. (2007). A genome-wide transgenic RNAi library for conditional gene inactivation in Drosophila. *Nature* 448, 151–156. doi: 10.1038/nature05954

Dillon, R. J., and Dillon, V. M. (2004). The gut bacteria of insects: nonpathogenic interactions. *Annu. Rev. Entomol.* 49, 71–92. doi: 10.1146/annurev.ento.49.061802.123416

Donko, A., Peterfi, Z., Sum, A., Leto, T., and Geiszt, M. (2005). Dual oxidases. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 360, 2301–2308. doi: 10.1098/rstb.2005.1767

Drysdale, R. (2008). FlyBase: a database for the Drosophila research community. *Methods Mol. Biol.* 420, 45–59. doi: 10.1007/978-1-59745-583-1_3

Dupuy, C., Ohayon, R., Valent, A., Noel-Hudson, M. S., Deme, D., and Virion, A. (1999). Purification of a novel flavoprotein involved in the thyroid NADPH oxidase. Cloning of the porcine and human cdnas. *J. Biol. Chem.* 274, 37265–37269. doi: 10.1074/jbc.274.52.37265

Edens, W. A., Sharling, L., Cheng, G., Shapira, R., Kinkade, J. M., Lee, T., et al. (2001). Tyrosine cross-linking of extracellular matrix is catalyzed by Duox, a multidomain oxidase/peroxidase with homology to the phagocyte oxidase subunit gp91phox. *J. Cell Biol.* 154, 879–891. doi: 10.1083/jcb.200103132

El Hassani, R. A., Benfares, N., Caillou, B., Talbot, M., Sabourin, J. C., Belotte, V., et al. (2005). Dual oxidase2 is expressed all along the digestive tract. *Am. J. Physiol. Gastrointest. Liver Physiol.* 288, G933–G942. doi: 10.1152/ajpgi.00198.2004

Fischer, H. (2009). Mechanisms and function of DUOX in epithelia of the lung. *Antioxid. Redox Signal.* 11, 2453–2465. doi: 10.1089/ars.2009.2558

Flores, M. V., Crawford, K. C., Pullin, L. M., Hall, C. J., Crosier, K. E., and Crosier, P. S. (2010). Dual oxidase in the intestinal epithelium of zebrafish larvae has anti-bacterial properties. *Biochem. Biophys. Res. Commun.* 400, 164–168. doi: 10.1016/j.bbrc.2010.08.037

Forteza, R., Salathe, M., Miot, F., Forteza, R., and Conner, G. E. (2005). Regulated hydrogen peroxide production by Duox in human airway epithelial cells. *Am. J. Respir. Cell Mol. Biol.* 32, 462–469. doi: 10.1165/rcmb.2004-0302OC

Fraune, S., Abe, Y., and Bosch, T. C. (2009). Disturbing epithelial homeostasis in the metazoan Hydra leads to drastic changes in associated microbiota. *Environ. Microbiol.* 11, 2361–2369. doi: 10.1111/j.1462-2920.2009.01963.x

Ganesan, S., Aggarwal, K., Paquette, N., and Silverman, N. (2010). NF-kappaB/Rel proteins and the humoral immune responses of *Drosophila melanogaster*. *Curr. Top. Microbiol. Immunol.* 349, 25–60. doi: 10.1007/82_2010_107

Garrett, W. S., Gallini, C. A., Yatsunenko, T., Michaud, M., DuBois, A., Delaney, M. L., et al. (2010). Enterobacteriaceae act in concert with the gut microbiota to induce spontaneous and maternally transmitted colitis. *Cell Host Microbe* 8, 292–300. doi: 10.1016/j.chom.2010.08.004

Gattas, M. V., Forteza, R., Fragoso, M. A., Fregien, N., Salas, P., Salathe, M., et al. (2009). Oxidative epithelial host defense is regulated by infectious and inflammatory stimuli. *Free Radic. Biol. Med.* 47, 1450–1458. doi: 10.1016/j.freeradbiomed.2009.08.017

Geiszt, M., Witta, J., Baffi, J., Lekstrom, K., and Leto, T. L. (2003). Dual oxidases represent novel hydrogen peroxide sources supporting mucosal surface host defense. *FASEB J.* 17, 1502–1504. doi: 10.1096/fj.02-1104fje

Gill, S. R., Pop, M., DeBoy, R. T., Eckburg, P. B., Turnbaugh, P. J., Samuel, B. S., et al. (2006). Metagenomic analysis of the human distal gut microbiome. *Science* 312, 1355–1359. doi: 10.1126/science.1124234

Ha, E. M., Lee, K. A., Park, S. H., Kim, S. H., Nam, H. J., Lee, H. Y., et al. (2009a). Regulation of DUOX by the Galphaq-phospholipase Cbeta-Ca2+ pathway in Drosophila gut immunity. *Dev. Cell* 16, 386–397. doi: 10.1016/j.devcel.2008.12.015

Ha, E. M., Lee, K. A., Seo, Y. Y., Kim, S. H., Lim, J. H., Oh, B. H., et al. (2009b). Coordination of multiple dual oxidase-regulatory pathways in responses to commensal and infectious microbes in drosophila gut. *Nat. Immunol.* 10, 949–957. doi: 10.1038/ni.1765

Ha, E. M., Oh, C. T., Bae, Y. S., and Lee, W. J. (2005a). A direct role for dual oxidase in Drosophila gut immunity. *Science* 310, 847–850. doi: 10.1126/science.1117311

Ha, E. M., Oh, C. T., Ryu, J. H., Bae, Y. S., Kang, S. W., Jang, I. H., et al. (2005b). An antioxidant system required for host protection against gut infection in Drosophila. *Dev. Cell* 8, 125–132. doi: 10.1016/j.devcel.2004.11.007

Hewes, R. S., and Taghert, P. H. (2001). Neuropeptides and neuropeptide receptors in the *Drosophila melanogaster* genome. *Genome Res.* 11, 1126–1142. doi: 10.1101/gr.169901

Hirakawa, S., Saito, R., Ohara, H., Okuyama, R., and Aiba, S. (2011). Dual oxidase 1 induced by Th2 cytokines promotes STAT6 phosphorylation via oxidative inactivation of protein tyrosine phosphatase 1B in human epidermal keratinocytes. *J. Immunol.* 186, 4762–4770. doi: 10.4049/jimmunol.1000 791

Hochmuth, C. E., Biteau, B., Bohmann, D., and Jasper, H. (2011). Redox regulation by Keap1 and Nrf2 controls intestinal stem cell proliferation in Drosophila. *Cell Stem Cell* 8, 188–199. doi: 10.1016/j.stem.2010.12.006

Hoeven, R., McCallum, K. C., Cruz, M. R., and Garsin, D. A. (2011). Ce-Duox1/BLI-3 generated reactive oxygen species trigger protective SKN-1 activity via p38 MAPK signaling during infection in *C. elegans*. *PLoS Pathog.* 7:e1002453. doi: 10.1371/journal.ppat.1002453

Hooper, L. V., Littman, D. R., and Macpherson, A. J. (2012). Interactions between the microbiota and the immune system. *Science* 336, 1268–1273. doi: 10.1126/science.1223490

Jasper, H., and Bohmann, D. (2013). Redox regulation of stem cell function. *Oxid. Stress Redox Regul.* 343–357. doi: 10.1007/978-94-007-5787-5_12

Jaspers, E., and Overmann, J. (2004). Ecological significance of microdiversity: identical 16S rRNA gene sequences can be found in bacteria with highly divergent genomes and ecophysiologies. *Appl. Environ. Microbiol.* 70, 4831–4839. doi: 10.1128/AEM.70.8.4831-4839.2004

Jiang, H., and Edgar, B. A. (2009). EGFR signaling regulates the proliferation of Drosophila adult midgut progenitors. *Development* 136, 483–493. doi: 10.1242/dev.026955

Jiang, H., Patel, P. H., Kohlmaier, A., Grenley, M. O., McEwen, D. G., and Edgar, B. A. (2009). Cytokine/Jak/Stat signaling mediates regeneration and homeostasis in the Drosophila midgut. *Cell* 137, 1343–1355. doi: 10.1016/j.cell.2009.05.014

Johnson, K. R., Marden, C. C., Ward-Bailey, P., Gagnon, L. H., Bronson, R. T., and Donahue, L. R. (2007). Congenital hypothyroidism, dwarfism, and hearing impairment caused by a missense mutation in the mouse dual oxidase 2 gene, Duox2. *Mol. Endocrinol.* 21, 1593–1602. doi: 10.1210/me.2007-0085

Jones, R. M., Luo, L., Ardita, C. S., Richardson, A. N., Kwon, Y. M., Mercante, J. W., et al. (2013). Symbiotic lactobacilli stimulate gut epithelial proliferation via Nox-mediated generation of reactive oxygen species. *EMBO J.* 32, 3017–3028. doi: 10.1038/emboj.2013.224

Joo, J. H., Ryu, J. H., Kim, C. H., Kim, H. J., Suh, M. S., Kim, J. O., et al. (2012). Dual oxidase 2 is essential for the toll-like receptor 5-mediated inflammatory response in airway mucosa. *Antioxid. Redox Signal.* 16, 57–70. doi: 10.1089/ars.2011.3898

Juarez, M. T., Patterson, R. A., Sandoval-Guillen, E., and McGinnis, W. (2011). Duox, Flotillin-2, and Src42A are required to activate or delimit the spread of the transcriptional response to epidermal wounds in Drosophila. *PLoS Genet.* 7:e1002424. doi: 10.1371/journal.pgen.1002424

Kanther, M., and Rawls, J. F. (2010). Host-microbe interactions in the developing zebrafish. *Curr. Opin. Immunol.* 22, 10–19. doi: 10.1016/j.coi.2010.01.006

Kawahara, T., and Lambeth, J. D. (2007). Molecular evolution of Phox-related regulatory subunits for NADPH oxidase enzymes. *BMC Evol. Biol.* 7:178. doi: 10.1186/1471-2148-7-178

Koff, J. L., Shao, M. X., Ueki, I. F., and Nadel, J. A. (2008). Multiple TLRs activate EGFR via a signaling cascade to produce innate immune responses in airway epithelium. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 294, L1068–L1075. doi: 10.1152/ajplung.00025.2008

Koropatnick, T. A., Engle, J. T., Apicella, M. A., Stabb, E. V., Goldman, W. E., and McFall-Ngai, M. J. (2004). Microbial factor-mediated development in a host-bacterial mutualism. *Science* 306, 1186–1188. doi: 10.1126/science.1102218

Kumar, S., Molina-Cruz, A., Gupta, L., Rodrigues, J., and Barillas-Mury, C. (2010). A peroxidase/dual oxidase system modulates midgut epithelial immunity in *Anopheles gambiae*. *Science* 327, 1644–1648. doi: 10.1126/science.1184008

Lambeth, J. D. (2004). NOX enzymes and the biology of reactive oxygen. *Nat. Rev. Immunol.* 4, 181–189. doi: 10.1038/nri1312

Lee, K. A., Kim, S. H., Kim, E. K., Ha, E. M., You, H., Kim, B., et al. (2013). Bacterial-derived uracil as a modulator of mucosal immunity and gut-microbe homeostasis in Drosophila. *Cell* 153, 797–811. doi: 10.1016/j.cell.2013.04.009

Lee, K. A., and Lee, W. J. (2013). Drosophila as a model for intestinal dysbiosis and chronic inflammatory diseases. *Dev. Comp. Immunol.* 42, 102–110. doi: 10.1016/j.dci.2013.05.005

Lee, W. J. (2009). Bacterial-modulated host immunity and stem cell activation for gut homeostasis. *Genes Dev.* 23, 2260–2265. doi: 10.1101/gad.1858709

Lee, W. J., and Brey, P. T. (2013). How microbiomes influence metazoan development: insights from history and Drosophila modeling of gut-microbe interactions. *Annu. Rev. Cell Dev. Biol.* 29, 571–592. doi: 10.1146/annurev-cellbio-101512-122333

Lemaitre, B., and Hoffmann, J. (2007). The host defense of *Drosophila melanogaster*. *Annu. Rev. Immunol.* 25, 697–743. doi: 10.1146/annurev.immunol.25.022106.141615

Leto, T. L., and Geiszt, M. (2006). Role of Nox family NADPH oxidases in host defense. *Antioxid. Redox Signal.* 8, 1549–1561. doi: 10.1089/ars.2006.8.1549

Leulier, F., Parquet, C., Pili-Floury, S., Ryu, J. H., Caroff, M., Lee, W. J., et al. (2003). The Drosophila immune system detects bacteria through specific peptidoglycan recognition. *Nat. Immunol.* 4, 478–484. doi: 10.1038/ni922

Ley, R. E., Lozupone, C. A., Hamady, M., Knight, R., and Gordon, J. I. (2008). Worlds within worlds: evolution of the vertebrate gut microbiota. *Nat. Rev. Microbiol.* 6, 776–788. doi: 10.1038/nrmicro1978

Littman, D. R., and Pamer, E. G. (2011). Role of the commensal microbiota in normal and pathogenic host immune responses. *Cell Host Microbe* 10, 311–323. doi: 10.1016/j.chom.2011.10.004

Maslowski, K. M., and Mackay, C. R. (2011). Diet, gut microbiota and immune responses. *Nat. Immunol.* 12, 5–9. doi: 10.1038/ni0111-5

Mazmanian, S. K., Round, J. L., and Kasper, D. L. (2008). A microbial symbiosis factor prevents intestinal inflammatory disease. *Nature* 453, 620–625. doi: 10.1038/nature07008

Micchelli, C. A., and Perrimon, N. (2006). Evidence that stem cells reside in the adult Drosophila midgut epithelium. *Nature* 439, 475–479. doi: 10.1038/nature04371

Moreira, S., Stramer, B., Evans, I., Wood, W., and Martin, P. (2010). Prioritization of competing damage and developmental signals by migrating macrophages in the Drosophila embryo. *Curr. Biol.* 20, 464–470. doi: 10.1016/j.cub.2010.01.047

Nehme, N. T., Liegeois, S., Kele, B., Giammarinaro, P., Pradel, E., Hoffmann, J. A., et al. (2007). A model of bacterial intestinal infections in *Drosophila melanogaster*. *PLoS Pathog.* 3:e173. doi: 10.1371/journal.ppat.0030173

Niethammer, P., Grabher, C., Look, A. T., and Mitchison, T. J. (2009). A tissue-scale gradient of hydrogen peroxide mediates rapid wound detection in zebrafish. *Nature* 459, 996–999. doi: 10.1038/nature08119

Ohlstein, B., and Spradling, A. (2006). The adult Drosophila posterior midgut is maintained by pluripotent stem cells. *Nature* 439, 470–474. doi: 10.1038/nature04333

Ohlstein, B., and Spradling, A. (2007). Multipotent Drosophila intestinal stem cells specify daughter cell fates by differential notch signaling. *Science* 315, 988–992. doi: 10.1126/science.1136606

Owusu-Ansah, E., and Banerjee, U. (2009). Reactive oxygen species prime Drosophila haematopoietic progenitors for differentiation. *Nature* 461, 537–541. doi: 10.1038/nature08313

Pedron, T., and Sansonetti, P. (2008). Commensals, bacterial pathogens and intestinal inflammation: an intriguing menage a trois. *Cell Host Microbe* 3, 344–347. doi: 10.1016/j.chom.2008.05.010

Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K. S., Manichanh, C., et al. (2010). A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* 464, 59–65. doi: 10.1038/nature08821

Razzell, W., Evans, I. R., Martin, P., and Wood, W. (2013). Calcium flashes orchestrate the wound inflammatory response through DUOX activation and hydrogen peroxide release. *Curr. Biol.* 23, 424–429. doi: 10.1016/j.cub.2013.01.058

Ren, C., Webster, P., Finkel, S. E., and Tower, J. (2007). Increased internal and external bacterial load during Drosophila aging without life-span trade-off. *Cell Metab.* 6, 144–152. doi: 10.1016/j.cmet.2007.06.006

Rhee, S. G. (2006). Cell signaling. H2O2, a necessary evil for cell signaling. *Science* 312, 1882–1883. doi: 10.1126/science.1130481

Rinas, U., Hellmuth, K., Kang, R., Seeger, A., and Schlieker, H. (1995). Entry of *Escherichia coli* into stationary phase is indicated by endogenous and exogenous accumulation of nucleobases. *Appl. Environ. Microbiol.* 61, 4147–4151.

Round, J. L., and Mazmanian, S. K. (2009). The gut microbiota shapes intestinal immune responses during health and disease. *Nat. Rev. Immunol.* 9, 313–323. doi: 10.1038/nri2515

Royet, J., Gupta, D., and Dziarski, R. (2011). Peptidoglycan recognition proteins: modulators of the microbiome and inflammation. *Nat. Rev. Immunol.* 11, 837–851. doi: 10.1038/nri3089

Ryu, J. H., Ha, E. M., and Lee, W. J. (2010). Innate immunity and gut-microbe mutualism in Drosophila. *Dev. Comp. Immunol.* 34, 369–376. doi: 10.1016/j.dci.2009.11.010

Ryu, J. H., Kim, S. H., Lee, H. Y., Bai, J. Y., Nam, Y. D., Bae, J. W., et al. (2008). Innate immune homeostasis by the homeobox gene caudal and commensal-gut mutualism in Drosophila. *Science* 319, 777–782. doi: 10.1126/science.1149357

Ryu, J. H., Yoo, J. Y., Kim, M. J., Hwang, S. G., Ahn, K. C., Ryu, J. C., et al. (2013). Distinct TLR-mediated pathways regulate house dust mite-induced allergic disease in the upper and lower airways. *J. Allergy Clin. Immunol.* 131, 549–561. doi: 10.1016/j.jaci.2012.07.050

Sauer, H., and Wartenberg, M. (2005). Reactive oxygen species as signaling molecules in cardiovascular differentiation of embryonic stem cells and tumor-induced angiogenesis. *Antioxid. Redox Signal.* 7, 1423–1434. doi: 10.1089/ars.2005.7.1423

Segal, A. W. (2005). How neutrophils kill microbes. *Annu. Rev. Immunol.* 23, 197–223. doi: 10.1146/annurev.immunol.23.021704.115653

Shao, M. X., and Nadel, J. A. (2005). Dual oxidase 1-dependent MUC5AC mucin expression in cultured human airway epithelial cells. *Proc. Natl. Acad. Sci. U.S.A.* 102, 767–772. doi: 10.1073/pnas.0408932102

Shin, S. C., Kim, S. H., You, H., Kim, B., Kim, A. C., Lee, K. A., et al. (2011). Drosophila microbiome modulates host developmental and metabolic homeostasis via insulin signaling. *Science* 334, 670–674. doi: 10.1126/science.1212782

Siezen, R. J., Tzeneva, V. A., Castioni, A., Wels, M., Phan, H. T., Rademaker, J. L., et al. (2010). Phenotypic and genomic diversity of *Lactobacillus plantarum* strains isolated from various environmental niches. *Environ. Microbiol.* 12, 758–773. doi: 10.1111/j.1462-2920.2009.02119.x

Stadtman, E. R., and Levine, R. L. (2003). Free radical-mediated oxidation of free amino acids and amino acid residues in proteins. *Amino Acids* 25, 207–218. doi: 10.1007/s00726-003-0011-2

Stone, J. R., and Yang, S. (2006). Hydrogen peroxide: a signaling messenger. *Antioxid. Redox Signal.* 8, 243–270. doi: 10.1089/ars.2006.8.243

Storelli, G., Defaye, A., Erkosar, B., Hols, P., Royet, J., and Leulier, F. (2011). *Lactobacillus plantarum* promotes Drosophila systemic growth by modulating hormonal signals through TOR-dependent nutrient sensing. *Cell Metab.* 14, 403–414. doi: 10.1016/j.cmet.2011.07.012

Sumimoto, H. (2008). Structure, regulation and evolution of Nox-family NADPH oxidases that produce reactive oxygen species. *FEBS J.* 275, 3249–3277. doi: 10.1111/j.1742-4658.2008.06488.x

Syed, Z. A., Hard, T., Uv, A., and van Dijk-Hard, I. F. (2008). A potential role for Drosophila mucins in development and physiology. *PLoS ONE* 3:e3041. doi: 10.1371/journal.pone.0003041

Turnbaugh, P. J., Ley, R. E., Mahowald, M. A., Magrini, V., Mardis, E. R., and Gordon, J. I. (2006). An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* 444, 1027–1031. doi: 10.1038/nature05414

Ueda, A., Attila, C., Whiteley, M., and Wood, T. K. (2009). Uracil influences quorum sensing and biofilm formation in *Pseudomonas aeruginosa* and fluorouracil is an antagonist. *Microb. Biotechnol.* 2, 62–74. doi: 10.1111/j.1751-7915.2008.00060.x

Valanne, S., and Ramet, M. (2013). Uracil debases pathogenic but not commensal bacteria. *Cell Host Microbe* 13, 505–506. doi: 10.1016/j.chom.2013.04.013

van der Vliet, A. (2008). NADPH oxidases in lung biology and pathology: host defense enzymes, and more. *Free Radic. Biol. Med.* 44, 938–955. doi: 10.1016/j.freeradbiomed.2007.11.016

Veal, E. A., Day, A. M., and Morgan, B. A. (2007). Hydrogen peroxide sensing and signaling. *Mol. Cell* 26, 1–14. doi: 10.1016/j.molcel.2007.03.016

Vijay-Kumar, M., Aitken, J. D., Carvalho, F. A., Cullender, T. C., Mwangi, S., Srinivasan, S., et al. (2010). Metabolic syndrome and altered gut microbiota in mice lacking Toll-like receptor 5. *Science* 328, 228–231. doi: 10.1126/science.1179721

Vodovar, N., Vinals, M., Liehl, P., Basset, A., Degrouard, J., Spellman, P., et al. (2005). Drosophila host defense after oral infection by an entomopathogenic Pseudomonas species. *Proc. Natl. Acad. Sci. U.S.A.* 102, 11414–11419. doi: 10.1073/pnas.0502240102

Wen, L., Ley, R. E., Volchkov, P. Y., Stranges, P. B., Avanesyan, L., Stonebraker, A. C., et al. (2008). Innate immunity and intestinal microbiota in the development of Type 1 diabetes. *Nature* 455, 1109–1113. doi: 10.1038/nature07336

Wong, C. N., Ng, P., and Douglas, A. E. (2011). Low-diversity bacterial community in the gut of the fruitfly *Drosophila melanogaster*. *Environ. Microbiol.* 13, 1889–1900. doi: 10.1111/j.1462-2920.2011.02511.x

Wong, J. L., Creton, R., and Wessel, G. M. (2004). The oxidative burst at fertilization is dependent upon activation of the dual oxidase Udx1. *Dev. Cell* 7, 801–814. doi: 10.1016/j.devcel.2004.10.014

Xu, N., Wang, S. Q., Tan, D., Gao, Y., Lin, G., and Xi, R. (2011). EGFR, Wingless and JAK/STAT signaling cooperatively maintain Drosophila intestinal stem cells. *Dev. Biol.* 354, 31–43. doi: 10.1016/j.ydbio.2011.03.018

Zhou, F., Rasmussen, A., Lee, S., and Agaisse, H. (2013). The UPD3 cytokine couples environmental challenge and intestinal stem cell division through modulation of JAK/STAT signaling in the stem cell microenvironment. *Dev. Biol.* 373, 383–393. doi: 10.1016/j.ydbio.2012.10.023

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 30 September 2013; paper pending published: 27 November 2013; accepted: 23 December 2013; published online: 10 January 2014.

*Citation: Kim S-H and Lee W-J (2014) Role of DUOX in gut inflammation: lessons from Drosophila model of gut-microbiota interactions. Front. Cell. Infect. Microbiol. **3**:116. doi: 10.3389/fcimb.2013.00116*

This article was submitted to the journal Frontiers in Cellular and Infection Microbiology.

frontiers in
CELLULAR AND INFECTION MICROBIOLOGY

REVIEW ARTICLE
published: 16 December 2013
doi: 10.3389/fcimb.2013.00098

Intestinal inflammation and stem cell homeostasis in aging *Drosophila melanogaster*

Arshad Ayyaz and Heinrich Jasper*

Buck Institute for Research on Aging, Novato, CA, USA

Edited by:
Dominique Ferrandon, Centre National de la Recherche Scientifique, France

Reviewed by:
Bruno Lemaitre, Ecole Polytechnique Fédérale de Lausanne, Switzerland
Won-Jae Lee, Seoul National University, South Korea

***Correspondence:**
Heinrich Jasper, Buck Institute for Research on Aging, 8001 Redwood Boulevard, Novato, CA 94945-1400, USA
e-mail: hjasper@buckinstitute.org

As a barrier epithelium, the intestinal epithelium has to coordinate physiological functions like digestion and nutrient resorption with the control of commensal bacteria and the prevention of pathogenic infections. It can therefore mount powerful innate immune and inflammatory responses, while, at the same time, maintaining tissue homeostasis through regenerative processes. How these different functions are coordinated remains unclear, and further insight is required to understand the age-related loss of homeostasis in this system, as well as the etiology of inflammatory and proliferative diseases of the gut. Recent work in *Drosophila melanogaster* has provided important new insight into the regulation of regenerative activity, innate immune homeostasis, commensal control, as well as age-related dysfunction in the intestine. Interestingly, many of the identified processes and mechanisms mirror similar homeostatic processes in the vertebrate intestine. This review summarized the current understanding of how innate immune responses, changes in commensal bacteria, and other challenges influence regenerative activity in the aging intestinal epithelium of flies and draws parallels to similar processes in mammals.

Keywords: stem cell, tissue homeostasis, aging, dysbiosis, dysplasia

INTRODUCTION

As a major barrier epithelium, the intestinal epithelium is the first line of defense against pathogenic microorganisms, while at the same time managing the beneficial interaction between commensal bacteria and the host. Accordingly, it mounts highly coordinated and regulated stress and immune responses to govern these interactions. Dysfunction in these signaling mechanisms can cause intestinal dysbiosis and chronic inflammation, and these pathologies can in turn negatively influence epithelial homeostasis, causing dysplasias and cancers (Gonda et al., 2009; Uronis et al., 2009; Kaser et al., 2010; Niwa et al., 2010; Clemente et al., 2012; Kostic et al., 2012). Deeper insight into the interaction between the intestinal epithelium, the commensal microbiota, and stress and innate immune signaling in epithelial cells is thus paramount to developing rational therapies and preventive strategies for these diseases. Such insight is further expected to significantly contribute to our understanding of changes in tissue homeostasis in the aging organism.

Elderly individuals are more susceptible to infectious diseases, including inflammatory disorders (Clemente et al., 2012), colorectal cancer (Patel et al., 2009), metabolic imbalance (Roberts and Rosenberg, 2006) and gastrointestinal infections (Duncan and Flint, 2013). Interestingly, various other age-related physiological complications, for instance obesity (Kallus and Brandt, 2012), insulin resistance (De Bandt et al., 2011), and general frailty (Claesson et al., 2012) have been associated with changes in the intestinal microbiota, suggesting that age-related changes in epithelial/commensal interactions impact not only inflammatory diseases of the gut, but potentially overall health and lifespan.

Recent advances in sequencing techniques that allow "metagenomic" strategies have revolutionized the study of microbiota associated with the human intestine (Qin et al., 2010; Kamada et al., 2013; Koeth et al., 2013; Stecher et al., 2013). An average human gut harbors as many as 10^{14} bacterial cells belonging to 400–1000 different species. Composition of this microbiota is highly variable among individuals and changes along the lifespan of individuals (Biagi et al., 2010; Claesson et al., 2011, 2012; Lozupone et al., 2012; Schloissnig et al., 2013). At the same time, the composition of the microbiota is remarkably stable in the short term (Power et al., 2013), suggesting that a tightly controlled immune response maintains a diverse array of "commensals" while simultaneously eliminating hazardous microbes in healthy intestines.

Age-related changes in microbiota composition are thus likely a consequence of changes in the ability of the intestinal epithelium to properly control the type and number of microorganisms colonizing the gut. These changes are in turn expected to be caused by deregulation of epithelial signaling events and by a breakdown of epithelial homeostasis that occur due to common age-associated cellular changes. Broadly, the aging process is characterized by the loss of proteostasis, accumulation of DNA damage, increased oxidative stress, metabolic imbalances and deregulated stress signaling (Paaby and Schmidt, 2008; Karpac and Jasper, 2009; Kenyon, 2010). While the progression toward age-related dysfunction in the intestinal epithelium remains unclear, it can be anticipated that the damage to epithelial cells resulting from such general age-associated molecular changes is likely to affect epithelial interactions with commensal microbial communities. At the same time, these changes also cause increased vulnerability to pathobionts in older guts (Biagi et al., 2012; Schloissnig et al., 2013). The resulting chronic stimulation of immune and inflammatory responses is further likely to promote

tissue dysfunction by impacting regenerative and homeostatic processes.

The interactions between microbiota, stress and immune signaling in epithelial cells, as well as regenerative processes in the epithelium, represent a complex and wide-ranging field of study, and simple animal models are needed to provide fundamental insight into these interactions. The availability of powerful genetic tools for *D. melanogaster*, as well as its short lifespan and the relative simplicity of its intestine, but also the presence of complex epithelial interactions with commensals and of regenerative processes that resemble similar processes in mammals, have recently elevated the fly to a model organism of choice in this context. A large number of studies have already provided important mechanistic insight into epithelial/commensal interactions, as well as age-related changes in these interactions and their consequences for epithelial homeostasis (Buchon et al., 2009a, 2013a; Apidianakis and Rahme, 2011; Hochmuth et al., 2011; Karpac et al., 2011; Rera et al., 2011; Lee and Brey, 2013).

In the wild, *D. melanogaster* feeds on rotten fruits and vegetables. Such feeding behavior exposes them to repeated interactions with a variety of microbes. Distinct mechanisms have therefore evolved in fruit flies that enable them to maintain intestinal tissue homeostasis and survive in a microbe-rich environment (Ferrandon, 2013). In older flies, however, a widespread growth of intestinal microbial populations is associated with hyperplasia and misdifferentiation of intestinal stem cells (ISCs) and their progeny, leading to loss of tissue homeostasis (Buchon et al., 2009a; Biteau et al., 2010). Interestingly, over-expression of stress-protective genes in ISCs is sufficient to rescue this age-related loss of homeostasis and to increase *Drosophila* lifespan (Biteau et al., 2010; Rera et al., 2011, 2012). This finding supports the notion that managing the loss of intestinal homeostasis is critical for health and lifespan in metazoans, and highlights the usefulness of flies as models for inflammatory diseases of the gut. In this review we will summarize the current understanding of the interaction between innate immune responses, commensal microbiota, and proliferative homeostasis in the aging intestinal epithelium in *D. melanogaster*.

THE *Drosophila* INTESTINE

The gastrointestinal tract in *Drosophila* can be subdivided into the crop, foregut, midgut and hindgut (**Figure 1**). The crop is a food storage organ which is attached to the distal end of the foregut, via a thin tube. The midgut can further be divided into anterior, middle and posterior regions. The anterior midgut (AM) encompasses the proventriculus, and opens into the acidic middle midgut (MM; also called copper cell region). The posterior midgut, in turn, extends from the MM to a fusion point where it is connected to the hindgut and to malpighian tubules (Buchon et al., 2013b; Marianes and Spradling, 2013).

The *Drosophila* intestinal epithelium is a monolayer composed of three types of cells; the polyploid enterocytes (EC) form the majority of the midgut cell population, followed by hormone secreting enteroendocrine (EE) cells and the proliferating ISCs. ECs are absorptive cells but also secrete digestive enzymes in some parts of the gut, and play a central role in mounting innate immune responses to infection and in managing the commensal population. Proteases, lipases (such as LipA), carbohydratases, catalytic peptidoglycan recognition proteins (PGRPs) and lysozymes are among the digestive enzymes secreted by midgut cells (Sieber and Thummel, 2012; Lemaitre and Miguel-Aliaga, 2013). The MM, in turn, contains acid secreting copper cells, most likely to aid digestion.

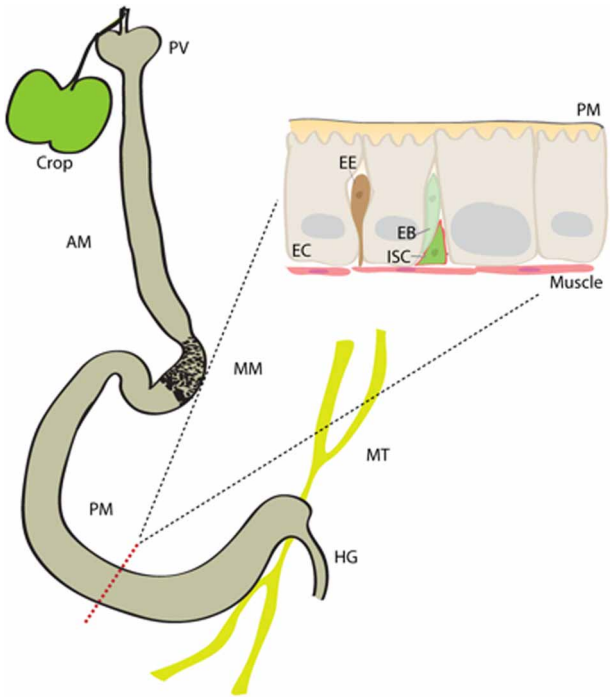


FIGURE 1 | The *Drosophila intestine*. The midgut in *Drosophila* is subdivided into anterior midgut (AM), middle midgut (MM) and posterior midgut (PM) regions. It contains single population of mitotically active intestinal stem cells (ISCs), which spread throughout from anterior to posterior regions. An ISC asymmetrically divides to generate an intermediate enteroblast (EB), which eventually differentiates either into an enterocyte (EC) or enteroendocrine (EE) cell. proventriculous (PV), hindgut (HG), Malpighian tubules (MT), peritrophic matrix (PM).

Regenerative processes in the intestinal epithelium differ along the gastrointestinal tract, and are influenced by local signals in each compartment (Buchon et al., 2013b; Guo et al., in press; Li et al., 2013; Marianes and Spradling, 2013). Interestingly, this compartmentalization seems to decline in the aging intestine, causing widespread deregulation of stem cell activity (Buchon et al., 2013b).

Regeneration of the posterior midgut epithelium is best understood so far. ISCs in this area can mount rapid and widespread regenerative responses to damage. During this renewal, ISCs divide asymmetrically to produce a population of non-differentiated progenitors calles enteroblasts (EBs) (Micchelli and Perrimon, 2006; Ohlstein and Spradling, 2006). EBs are not mitotically active, and differentiate into either an EC or an EE cell, depending on differential Notch signaling activity (Ohlstein and Spradling, 2007; Biteau et al., 2011a; Perdigoto et al., 2011; Cordero and Sansom, 2012; Kapuria et al., 2012). ISCs are also known to divide symmetrically to expand their own

population (O'brien et al., 2011; Goulas et al., 2012). The ISCs are located close to the basal membrane (BM) of the epithelium and are in close proximity to the surrounding circular visceral muscle. The BM and visceral muscle, but also EBs and ECs, influence ISC proliferative activity and maintenance (Bardin et al., 2010; Biteau and Jasper, 2011; Xu et al., 2011; Cordero et al., 2012a; Goulas et al., 2012; Zhou et al., 2013).

CONTROL OF ISC PROLIFERATION

The proliferative activity of ISCs is very plastic. While low levels of homeostatic proliferation are generally observed in young, healthy guts, strong regenerative activity is observed in response to insults that damage the epithelium. EGF, Insulin/IGF (IIS), and p38MAPK signaling pathways are essential for ISC proliferation (Park et al., 2009; Biteau et al., 2010, 2011a; Biteau and Jasper, 2011). Constitutive activation of EGF receptor (EGFR) or insulin receptor (InR) increases the rate of ISC proliferation, indicating that RTK signaling can modulate ISC activity in accordance with the metabolic status of the animal (Biteau and Jasper, 2011; Karpac et al., 2011; O'brien et al., 2011; Xu et al., 2011). Long-term stem cell maintenance is further ensured by mechanisms that prevent activation of Target of Rapamycin (TOR) signaling (Amcheslavsky et al., 2011; Kapuria et al., 2012; Quan et al., 2013), and by muscle—derived Wingless (Sackton et al., 2007; Lin et al., 2008; Takashima et al., 2008; Cordero and Sansom, 2012; Cordero et al., 2012b).

While the signaling pathways listed above are required for homeostatic proliferation and maintenance of ISCs, various stress signaling pathways have been identified that govern induction of ISC proliferation when the intestinal epithelium is exposed to a stress or is injured. Stressors that trigger ISC proliferation include oxidative stress (Biteau et al., 2008; Choi et al., 2008; Buchon et al., 2009a), bacterial infection (Apidianakis et al., 2009; Buchon et al., 2009b; Cronin et al., 2009; Jiang et al., 2009; Guo et al., 2013), DNA damage (Amcheslavsky et al., 2011; Guo et al., 2013), aging (Biteau et al., 2008, 2010, 2011b; Choi et al., 2008; Buchon et al., 2009a; Karpac et al., 2009; Hochmuth et al., 2011), and factors that cause apoptosis and damage to ECs (Jiang et al., 2009; Amcheslavsky et al., 2011). Jun-N-terminal Kinase (JNK) (Biteau et al., 2008; Choi et al., 2008), JAK/Stat signaling (Buchon et al., 2009a; Cronin et al., 2009; Jiang et al., 2009) and the Hippo/Yorkie pathway (Karpowicz et al., 2010; Ren et al., 2010; Shaw et al., 2010; Staley and Irvine, 2010) are all critical for stress-induced ISC proliferation [reviewed in Biteau et al. (2011a)]. The integration of these inductive signals with signaling pathways that play a permissive role for proliferation, as well as the exact cellular interactions during a regenerative response, are only beginning to be understood. Following bacterial infection or an injury, interleukin-6-like cytokines of the Unpaired (Upd) family, especially Upd 2 and 3 are induced in and secreted by damaged and dying ECs (Jiang et al., 2009; Osman et al., 2012; Zhou et al., 2013). Upds activate JAK/Stat signaling, either in ISCs directly (Buchon et al., 2009a; Cronin et al., 2009; Jiang et al., 2009), or in visceral muscle, where it induces the EGF-like ligand Vein, which in turn stimulates ISC proliferation (Jiang and Edgar, 2009; Buchon et al., 2010; Biteau and Jasper, 2011; Xu et al., 2011; Zhou et al., 2013).

The JNK pathway also plays a dual role in stimulating ISC proliferation: JNK is activated by reactive oxygen species (ROS) in both ECs and ISCs. Its activation in ISCs induces their proliferation by phosphorylating the AP-1 transcription factor Fos (Biteau et al., 2008; Biteau and Jasper, 2011; Hochmuth et al., 2011). Interestingly, Fos is phosphorylated both by JNK and EGFR pathways, and thus integrates growth factor and stress signals to induce ISC proliferation (Ciapponi et al., 2001; Biteau and Jasper, 2011). JNK activation in ECs, on the other hand, can stimulate Upd induction and induce ISC proliferation, but does not seem to be required for the regenerative response to a challenge (Jiang et al., 2009) nor for survival of the host upon pathogenic infection (Buchon et al., 2009a). Forced activation of JNK in ECs induces Upd expression by promoting Yorkie nuclear localization (Karpowicz et al., 2010; Ren et al., 2010; Shaw et al., 2010; Staley and Irvine, 2010).

The need for epithelial renewal after pathogenic infections suggest that to maintain homeostasis, signaling mechanisms that control innate immune and inflammatory responses and signaling pathways that regulate ISC proliferation have to be highly coordinated. Recent years have seen tremendous progress in our understanding of the cellular and molecular mechanisms governing this coordination (Buchon et al., 2013a).

INTESTINAL IMMUNITY

The *Drosophila* intestine contains physical and chemical barriers to prevent microbial infections [reviewed in Ferrandon (2013)]. As a first barrier, a peritrophic matrix, consisting of chitin and glycoproteins covers the intestinal epithelium, preventing direct contact of microbes and other lumen contents with epithelial cells. The peritrophic matrix is secreted by the proventriculus with possible contributions by ECs (Hegedus et al., 2009). Loss of peritrophic matrix components renders flies susceptible to infections, highlighting the importance of the peritrophic matrix as a physical barrier against bacteria (Kuraishi et al., 2011).

A second line of defense is the secretion of antimicrobial peptides (AMPs) by ECs in the intestinal epithelium (Tzou et al., 2000; Liehl et al., 2006; Ryu et al., 2006; Nehme et al., 2007; Buchon et al., 2009b). Invading bacteria are recognized by their peptidoglycans (PGN; structural components of the bacterial cell wall) (Zaidman-Remy et al., 2006). PGNs bind to PGRP-LC and -LE resulting in activation of the IMD/Relish (but not the Toll) pathway (Bosco-Drayon et al., 2012; Neyen et al., 2012), which in turn induces AMP transcription [immune signaling in *D. melanogaster* is comprehensively reviewed in Ferrandon et al. (2007); Lemaitre and Hoffmann (2007); Ha et al. (2009a); Davis and Engstrom (2012); Buchon et al. (2013a); Lee and Brey (2013)]. Relish belongs to the family of highly conserved Nuclear Factor-κB (NF-κB) transcription factors, and is a required component of the IMD pathway, which is related to the mammalian tumor necrosis factor receptor (TNFR) pathway (Hoffmann, 2003). NF-κB and TNFR pathways are critical for epithelial immunity in mammals (Xavier and Podolsky, 2007; Meylan et al., 2011; De Jong et al., 2012): NF-κB activation in epithelial cells modulates immune responses to environmental challenges and microbial infections (Pasparakis, 2012), and cytokines and chemokines secreted by epithelial cells act on immune and

non-immune cells to modulate the cellular immune response. Chronic activation of NF-κB and of the TNFR pathway in epithelial cells results in the development of intestinal inflammation (Meylan et al., 2011; Wullaert et al., 2011; De Jong et al., 2012).

In flies, the IMD/Rel pathway is kept inactive in normal, homeostatic, conditions by a variety of negative regulators, including Caudal (Ryu et al., 2008), PGRPs of the SC, LB and LF class (Zaidman-Remy et al., 2006; Maillet et al., 2008; Paredes et al., 2011), USP36 (Thevenon et al., 2009) and PIRK (Lhocine et al., 2008) (**Figure 2**). These regulators are of particular importance in the maintenance of not only the commensal population, but also of proliferative homeostasis in the intestinal epithelium: loss of the homeobox transcription factor Caudal, for example, leads to a shift in commensal populations in the fly intestine, eliminating beneficial bacterial species and allowing outbreaks of pathogenic species. At the same time, stress signaling is ectopically activated, and stem cell proliferation is strongly induced, resulting in dysplasia-like phenotypes (Ryu et al., 2008; Buchon et al., 2009a; Biteau et al., 2010). These conditions are reminiscent of the dysplasia and inflammation observed in aging flies, where microbial expansion is associated with hyperactivation of the IMD, JAK/Stat and JNK signaling pathways, and with epithelial dysplasia (Buchon et al., 2009a).

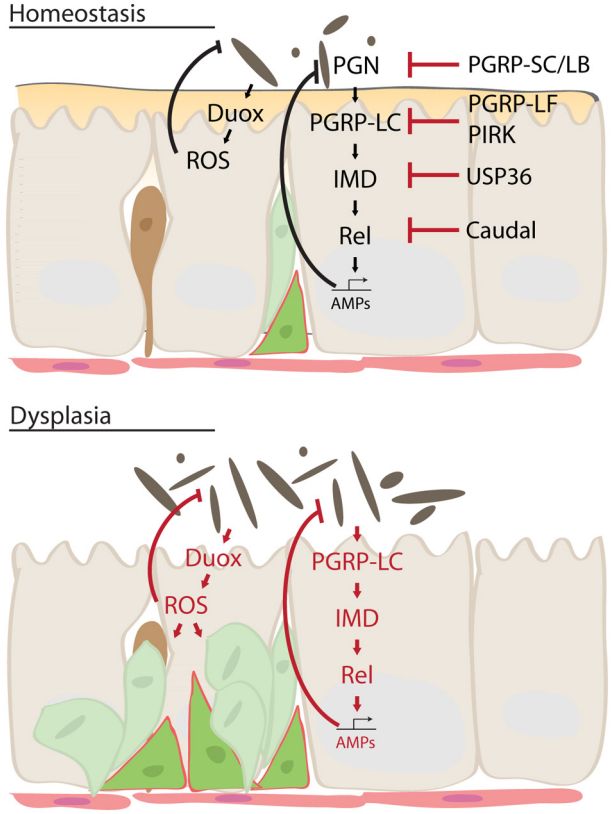


FIGURE 2 | Mechanism of intestinal dysplasia. Under normal homeostatic conditions, activity of Immune deficiency (IMD) pathway is tightly regulated by multiple factors. This ensures moderate innate immune response sufficient to keep intestinal microbes in check while preventing excessive immune activation. In an aging intestine, however, loss of these regulatory mechanisms leads to chronic inflammation and dysbiosis, which results in dysplasia and disruption of tissue homeostasis. Dual oxidase (Duox), reactive oxygen species (ROS), peptidoglycan (PGRP), Relish (Rel), poor Imd response upon knock-in (PIRK), antimicrobial peptides (AMP).

The third part of the intestinal immune response against microbes is the production of ROS by ECs. ROS are produced by the transmembrane protein dual oxidase (DUOX), a member of the NADPH oxidase family, which is transcriptionally induced in ECs and activated in response to a bacterial challenge (Ha et al., 2005; Ryu et al., 2010). Under homeostatic conditions, ROS are produced at moderate levels in response to the interaction of the epithelium with resident autochthonous bacterial species. During infection with transient allochthonous bacteria, however, production of ROS is increased by two mechanisms: an unknown G-Protein Coupled Receptor (GPCR) activates Phospholipase C-β (PLCβ) and triggers inositol-1,4,5-triphosphate (IP3)-induced Ca2+ release. Ca2+ is bound by EF-hands in DUOX, stimulating its activity (Ha et al., 2009a). A second mechanism involves activation of p38 MAPKinase, which transcriptionally induces Duox (Ha et al., 2009a,b). Young flies are believed to protect themselves from the cytotoxic effects of ROS by secreting an extracellular immune-related catalase (IRC), which neutralizes ROS (Ha et al., 2005). However, excessive ROS are generated and accumulate in the intestine of aged flies, presumably as a consequence of constant stimulation by immune resistant intestinal microbes. This excessive ROS accumulation is a likely cause of the age-related loss of epithelial homeostasis (Buchon et al., 2009a; Hochmuth et al., 2011).

INTESTINAL MICROBIOTA

A young and healthy *Drosophila* intestine contains a relatively simple microbiota comprising about 5–20 microbial species. Major constituents of these commensals are beneficial microbes, such as *Acetobacter pomorum* and *Lactobacillus plantarum* which promote growth and development in flies when reared on a restricted diet (Ryu et al., 2008; Chandler et al., 2011; Shin et al., 2011; Storelli et al., 2011; Wong et al., 2011). These microbes do not activate the intestinal immune system, allowing colonization of the gut. Resident pathobionts or invading potential pathogens, on the other hand, are readily recognized. One example of such a pathobiont, *Gluconobacter morbifer,* constitutes only a minor proportion of the healthy intestinal community. Under favorable conditions, however, it can take over the gut, causing gut pathologies and lethality of the host (Ryu et al., 2008). Moreover, many negative regulators have also been identified that prevent chronic activation of the IMD pathway induced by indigenous microbes (Lhocine et al., 2008; Paredes et al., 2011; Bosco-Drayon et al., 2012).

Until recently, it was not known how the *Drosophila* immune system differentiates between friends and foes. Recent elegant work by the Lee lab shows, however, that pathogenic bacteria, in contrast to beneficial symbionts, are constantly secreting Uracil, which is recognized by the *Drosophila* immune system. Uracil is recognized by an unknown GPCR, which activates the PLCb/IP3/Ca/Duox pathway discussed above to produce ROS. Many opportunistic pathogens such as *Vibrio fluvialis, Klebsiella pneumonia, Erwinia carotovora carotovora, Shigella sonnei, Pseudomonas aeruginosa* and *Serratia marcescens*, but not symbionts like *A. pomorum*, *L. plantarum* and *Commensalibacter intestini*, secrete significant quantities of Uracil (Lee et al., 2013).

Mono-association of flies with *G. morbifer* leads to chronic inflammation, induces apoptosis and shortens *Drosophila* lifespan, while these effects were not observed in germ free control flies or in flies mono-associated with a mutant *G. morbifer* lacking Uracil secretion. Uracil-producing pathobionts may also contribute significantly to the age-related increase in epithelial Duox-mediated ROS production, and thus to the age-associated dysfunction in epithelial homeostasis. It is clear that understanding causes and consequences of age-related changes in the commensal microbiome is an important task for future studies.

INFLAMMATION REGENERATION CROSSTALK

The Duox-induced innate immune response has thus important implications for the etiology of the dysfunction of the intestinal epithelium observed in aging flies. When flies are raised on a conventional diet, internal and external microbial populations expand with age (Ren et al., 2007; Guo et al., in press) and this expansion correlates with the age-associated accumulation of ROS in the gut (Buchon et al., 2009a). The increasing concentration of ROS stimulates ISC proliferation directly by activating JNK or inhibiting the Nrf2 homolog CncC (Biteau et al., 2008; Hochmuth et al., 2011), or indirectly by damaging ECs and stimulating Upd expression (Jiang et al., 2009). Although increased ISC activity is essential for regeneration in young epithelia in response to an insult, excessive ISC proliferation in aging animals results in the accumulation of mis-differentiated cells and the loss of tissue homeostasis, and is thus deleterious to animal health (Biteau et al., 2008, 2010; Hochmuth et al., 2011). Accordingly, overexpressing antioxidants or other stress-protective factors within ISCs not only rescues this age-related dysplasia, but also extends lifespan in *Drosophila* (Biteau et al., 2010; Hochmuth et al., 2011; Rera et al., 2011). These observations further support the notion that age-associated changes in the intestinal microbiota play a critical role in the development of age-related pathologies of the intestine, a concept that further studies in the fly should be able to test.

Why does the commensal microbiota expand in aging animals? It is unclear whether malfunctioning of the host immune response causes commensal populations to overgrow, or if expansion of immune-resistant intestinal commensals is the initiating event that causes the described aged-related intestinal pathology. Deregulation of innate immune signaling in aging epithelia can be observed, and may be brought about by age-related activation of stress signaling, in particular of the JNK signaling pathway (Buchon et al., 2009a; Karpac et al., 2009, 2013). The interaction between the IMD pathway and JNK is multifactorial and complex: JNK-mediated activation of the transcription factor Foxo can induce Rel expression in larvae (Karpac et al., 2011). In larval fatbodies, activation of TAK1 by infection not only promotes Relish nuclear localization, but also activates Hemipterous (JNKK), which phosphorylates and activates Basket (JNK) (Silverman et al., 2003; Park et al., 2004; Kallio et al., 2005). Another IMD pathway component, DREDD, may also directly activate JNK upon immune stimulation (Guntermann and Foley, 2011). Furthermore, JNK and Foxo have been shown to induce AMP transcription, in part independently of Relish (Delaney et al., 2006; Becker et al., 2010). Recent work in larvae and adults highlights the need to study these interactions in a spatially- and temporally-resolved manner in order to characterize the complex interactions between innate immune responses and stress and inflammatory signals *in vivo* (Karpac et al., 2011, 2013). Interestingly, a recent study from our lab identified age-related activation of Foxo in ECs as a driving force in the disruption of innate immune homeostasis, resulting in immune senescence. Foxo inhibits the expression of PGRP-SC2, resulting in chronic, excessive activation of Relish, and impairing the ability of the intestine to clear bacteria (Guo et al., in press).

While germ-free conditions can rescue age-related dysplasia (Buchon et al., 2009a), and pharmacological inhibition of the NFκB signaling pathway can reportedly extend *Drosophila* lifespan (Moskalev and Shaposhnikov, 2011), the evidence for a role of intestinal microbiota in influencing fly longevity remains controversial (Brummel et al., 2004; Ren et al., 2007). It is likely that rearing flies under sterile conditions results in the removal of not only deleterious species, but also of beneficial commensals, and experiments assessing fly lifespan under germ-free conditions may thus result in variable outcomes. However, moderate down-regulation of ISC proliferation has been shown to not only rescue the age-related intestinal disruption but also to extend lifespan (Biteau et al., 2010; Hochmuth et al., 2011). Conditions that can keep the commensal bacterial population in check, promoting innate immune homeostasis and proliferative homeostasis in the intestinal epithelium, are thus expected to be beneficial for the animal's health. Accordingly, we find that managing the commensal population by preventing the age-related loss of PGRP-SC2 expression is sufficient to limit age-related dysplasia and extend lifespan (Guo et al., in press).

CONCLUSION

To maintain intestinal homeostasis, a highly selective immune response has to ensure that pathogenic microorganisms are eliminated, while commensals can thrive. Moreover, the inflammatory response triggered by pathogens and commensals alike has to be carefully contained to prevent excessive stem cell activation and dysplasias. It may not be surprising that these carefully balanced responses are misregulated in aging animals, making the host more susceptible to invading microbes, and promoting inflammatory and dysplastic conditions. A better understanding of the molecular parameters driving these age-related changes, however, promises to provide insight into avenues for therapeutic intervention that may not only be applied to inflammatory diseases and cancers of the gut, but potentially to allay tissue dysfunction in the normally aging human intestine.

ACKNOWLEDGMENTS

This work was supported by the National Institute on Aging (NIH RO1 AG02812).

REFERENCES

Amcheslavsky, A., Ito, N., Jiang, J., and Ip, Y. T. (2011). Tuberous sclerosis complex and Myc coordinate the growth and division of Drosophila intestinal stem cells. *J. Cell Biol.* 193, 695–710. doi: 10.1083/jcb.201103018

Apidianakis, Y., Pitsouli, C., Perrimon, N., and Rahme, L. (2009). Synergy between bacterial infection and genetic predisposition in intestinal dysplasia. *Proc. Natl. Acad. Sci. U.S.A.* 106, 20883–20888. doi: 10.1073/pnas.0911797106

Apidianakis, Y., and Rahme, L. G. (2011). *Drosophila melanogaster* as a model for human intestinal infection and pathology. *Dis. Model. Mech.* 4, 21–30. doi: 10.1242/dmm.003970

Bardin, A. J., Perdigoto, C. N., Southall, T. D., Brand, A. H., and Schweisguth, F. (2010). Transcriptional control of stem cell maintenance in the Drosophila intestine. *Development* 137, 705–714. doi: 10.1242/dev.039404

Becker, T., Loch, G., Beyer, M., Zinke, I., Aschenbrenner, A. C., Carrera, P., et al. (2010). FOXO-dependent regulation of innate immune homeostasis. *Nature* 463, 369–373. doi: 10.1038/nature08698

Biagi, E., Candela, M., Fairweather-Tait, S., Franceschi, C., and Brigidi, P. (2012). Aging of the human metaorganism: the microbial counterpart. *Age (Dordr.)* 34, 247–267. doi: 10.1007/s11357-011-9217-5

Biagi, E., Nylund, L., Candela, M., Ostan, R., Bucci, L., Pini, E., et al. (2010). Through ageing, and beyond: gut microbiota and inflammatory status in seniors and centenarians. *PLoS ONE* 5:e10667. doi: 10.1371/journal.pone.0010667

Biteau, B., Hochmuth, C. E., and Jasper, H. (2008). JNK activity in somatic stem cells causes loss of tissue homeostasis in the aging Drosophila gut. *Cell Stem Cell* 3, 442–455. doi: 10.1016/j.stem.2008.07.024

Biteau, B., Hochmuth, C. E., and Jasper, H. (2011a). Maintaining tissue homeostasis: dynamic control of somatic stem cell activity. *Cell Stem Cell* 9, 402–411. doi: 10.1016/j.stem.2011.10.004

Biteau, B., Karpac, J., Hwangbo, D., and Jasper, H. (2011b). Regulation of Drosophila lifespan by JNK signaling. *Exp. Gerontol.* 46, 349–354. doi: 10.1016/j.exger.2010.11.003

Biteau, B., and Jasper, H. (2011). EGF signaling regulates the proliferation of intestinal stem cells in Drosophila. *Development* 138, 1045–1055. doi: 10.1242/dev.056671

Biteau, B., Karpac, J., Supoyo, S., Degennaro, M., Lehmann, R., and Jasper, H. (2010). Lifespan extension by preserving proliferative homeostasis in Drosophila. *PLoS Genet.* 6:e1001159. doi: 10.1371/journal.pgen.1001159

Bosco-Drayon, V., Poidevin, M., Boneca, I. G., Narbonne-Reveau, K., Royet, J., and Charroux, B. (2012). Peptidoglycan sensing by the receptor PGRP-LE in the Drosophila gut induces immune responses to infectious bacteria and tolerance to microbiota. *Cell Host Microbe* 12, 153–165. doi: 10.1016/j.chom.2012.06.002

Brummel, T., Ching, A., Seroude, L., Simon, A. F., and Benzer, S. (2004). Drosophila lifespan enhancement by exogenous bacteria. *Proc. Natl. Acad. Sci. U.S.A.* 101, 12974–12979. doi: 10.1073/pnas.0405207101

Buchon, N., Broderick, N. A., Chakrabarti, S., and Lemaitre, B. (2009a). Invasive and indigenous microbiota impact intestinal stem cell activity through multiple pathways in Drosophila. *Genes Dev.* 23, 2333–2344. doi: 10.1101/gad.1827009

Buchon, N., Broderick, N. A., Poidevin, M., Pradervand, S., and Lemaitre, B. (2009b). Drosophila intestinal response to bacterial infection: activation of host defense and stem cell proliferation. *Cell Host Microbe* 5, 200–211. doi: 10.1016/j.chom.2009.01.003

Buchon, N., Broderick, N. A., Kuraishi, T., and Lemaitre, B. (2010). Drosophila EGFR pathway coordinates stem cell proliferation and gut remodeling following infection. *BMC Biol.* 8:152. doi: 10.1186/1741-7007-8-152

Buchon, N., Broderick, N. A., and Lemaitre, B. (2013a). Gut homeostasis in a microbial world: insights from *Drosophila melanogaster*. *Nat. Rev. Microbiol.* 11, 615–626. doi: 10.1038/nrmicro3074

Buchon, N., Osman, D., David, F. P. A., Fang, H. Y., Boquete, J.-P., Deplancke, B., et al. (2013b). Morphological and molecular characterization of adult midgut compartmentalization in Drosophila. *Cell Rep.* 3, 1725–1738. doi: 10.1016/j.celrep.2013.04.001

Chandler, J. A., Lang, J. M., Bhatnagar, S., Eisen, J. A., and Kopp, A. (2011). Bacterial communities of diverse Drosophila species: ecological context of a host-microbe model system. *PLoS Genet.* 7:e1002272. doi: 10.1371/journal.pgen.1002272

Choi, N.-H., Kim, J.-G., Yang, D.-J., Kim, Y.-S., and Yoo, M.-A. (2008). Age-related changes in Drosophila midgut are associated with PVF2, a PDGF/VEGF-like growth factor. *Aging Cell* 7, 318–334. doi: 10.1111/j.1474-9726.2008.00380.x

Ciapponi, L., Jackson, D. B., Mlodzik, M., and Bohmann, D. (2001). Drosophila Fos mediates ERK and JNK signals via distinct phosphorylation sites. *Genes Dev.* 15, 1540–1553. doi: 10.1101/gad.886301

Claesson, M. J., Cusack, S., O'sullivan, O., Greene-Diniz, R., De Weerd, H., Flannery, E., et al. (2011). Composition, variability, and temporal stability of the intestinal microbiota of the elderly. *Proc. Natl. Acad. Sci. U.S.A.* 108(Suppl.) 4586–4591. doi: 10.1073/pnas.1000097107

Claesson, M. J., Jeffery, I. B., Conde, S., Power, S. E., O'connor, E. M., Cusack, S., et al. (2012). Gut microbiota composition correlates with diet and health in the elderly. *Nature* 488, 178–184. doi: 10.1038/nature11319

Clemente, J. C., Ursell, L. K., Parfrey, L. W., and Knight, R. (2012). The impact of the gut microbiota on human health: an integrative view. *Cell* 148, 1258–1270. doi: 10.1016/j.cell.2012.01.035

Cordero, J. B., and Sansom, O. J. (2012). Wnt signalling and its role in stem cell-driven intestinal regeneration and hyperplasia. *Acta Physiol. (Oxf.)* 204, 137–143. doi: 10.1111/j.1748-1716.2011.02288.x

Cordero, J. B., Stefanatos, R. K., Myant, K., Vidal, M., and Sansom, O. J. (2012a). Non-autonomous crosstalk between the Jak/Stat and Egfr pathways mediates Apc1-driven intestinal stem cell hyperplasia in the Drosophila adult midgut. *Development* 139, 4524–4535. doi: 10.1242/dev.078261

Cordero, J. B., Stefanatos, R. K., Scopelliti, A., Vidal, M., and Sansom, O. J. (2012b). Inducible progenitor-derived Wingless regulates adult midgut regeneration in Drosophila. *EMBO J.* 31, 3901–3917. doi: 10.1038/emboj.2012.248

Cronin, S. J. F., Nehme, N. T., Limmer, S., Liegeois, S., Pospisilik, J. A., Schramek, D., et al. (2009). Genome-wide RNAi screen identifies genes involved in intestinal pathogenic bacterial infection. *Science* 325, 340–343. doi: 10.1126/science.1173164

Davis, M. M., and Engstrom, Y. (2012). Immune response in the barrier epithelia: lessons from the fruit fly *Drosophila melanogaster*. *J. Innate Immun.* 4, 273–283. doi: 10.1159/000332947

De Bandt, J.-P., Waligora-Dupriet, A.-J., and Butel, M.-J. (2011). Intestinal microbiota in inflammation and insulin resistance: relevance to humans. *Curr. Opin. Clin. Nutr. Metab. Care* 14, 334–340. doi: 10.1097/MCO.0b013e328347924a

De Jong, H. K., Parry, C. M., Van Der Poll, T., and Wiersinga, W. J. (2012). Host-pathogen interaction in invasive Salmonellosis. *PLoS Pathog.* 8:e1002933. doi: 10.1371/journal.ppat.1002933

Delaney, J. R., Stöven, S., Uvell, H., Anderson, K. V., Engström, Y., and Mlodzik, M. (2006). Cooperative control of Drosophila immune responses by the JNK and NF-kappaB signaling pathways. *EMBO J.* 25, 3068–3077. doi: 10.1038/sj.emboj.7601182

Duncan, S. H., and Flint, H. J. (2013). Probiotics and prebiotics and health in ageing populations. *Maturitas* 75, 44–50. doi: 10.1016/j.maturitas.2013.02.004

Ferrandon, D. (2013). The complementary facets of epithelial host defenses in the genetic model organism *Drosophila melanogaster*: from resistance to resilience. *Curr. Opin. Immunol.* 25, 59–70. doi: 10.1016/j.coi.2012.11.008

Ferrandon, D., Imler, J.-L., Hetru, C., and Hoffmann, J. A. (2007). The Drosophila systemic immune response: sensing and signalling during bacterial and fungal infections. *Nat. Rev. Immunol.* 7, 862–874. doi: 10.1038/nri2194

Gonda, T. A., Tu, S., and Wang, T. C. (2009). Chronic inflammation, the tumor microenvironment and carcinogenesis. *Cell cycle* 8, 2005–2013. doi: 10.4161/cc.8.13.8985

Goulas, S., Conder, R., and Knoblich, J. A. (2012). The Par complex and integrins direct asymmetric cell division in adult intestinal stem cells. *Cell Stem Cell* 11, 529–540. doi: 10.1016/j.stem.2012.06.017

Guntermann, S., and Foley, E. (2011). The protein Dredd is an essential component of the c-Jun N-terminal kinase pathway in the Drosophila immune response. *J. Biol. Chem.* 286, 30284–30294. doi: 10.1074/jbc.M111.220285

Guo, L., Karpac, J., Tran, S. L., and Jasper, H. (in press). Lifespan extension by promoting immune homeostasis and limiting commensal dysbiosis in the Drosophila intestine. *Cell.*

Guo, Z., Driver, I., and Ohlstein, B. (2013). Injury-induced BMP signaling negatively regulates Drosophila midgut homeostasis. *J. Cell Biol.* 201, 945–961. doi: 10.1083/jcb.201302049

Ha, E. M., Oh, C. T., Bae, Y. S., and Lee, W. J. (2005). A direct role for dual oxidase in Drosophila gut immunity. *Science* 310, 847–850. doi: 10.1126/science.1117311

Ha, E. M., Lee, K. A., Park, S. H., Kim, S. H., Nam, H. J., Lee, H. Y., et al. (2009a). Regulation of DUOX by the Galphaq-phospholipase Cbeta-Ca2+ pathway in Drosophila gut immunity. *Dev. Cell* 16, 386–397. doi: 10.1016/j.devcel.2008.12.015

Ha, E. M., Lee, K. A., Seo, Y. Y., Kim, S. H., Lim, J. H., Oh, B. H., et al. (2009b). Coordination of multiple dual oxidase-regulatory pathways in responses to commensal and infectious microbes in drosophila gut. *Nat. Immunol.* 10, 949–957. doi: 10.1038/ni.1765

Hegedus, D., Erlandson, M., Gillott, C., and Toprak, U. (2009). New insights into peritrophic matrix synthesis, architecture, and function. *Annu. Rev. Entomol.* 54, 285–302. doi: 10.1146/annurev.ento.54.110807.090559

Hochmuth, C. E., Biteau, B., Bohmann, D., and Jasper, H. (2011). Redox regulation by Keap1 and Nrf2 controls intestinal stem cell proliferation in Drosophila. *Cell Stem Cell* 8, 188–199. doi: 10.1016/j.stem.2010.12.006

Hoffmann, J. A. (2003). The immune response of Drosophila. *Nature* 426, 33–38. doi: 10.1038/nature02021

Jiang, H., and Edgar, B. A. (2009). EGFR signaling regulates the proliferation of Drosophila adult midgut progenitors. *Development* 136, 483–493. doi: 10.1242/dev.026955

Jiang, H., Patel, P. H., Kohlmaier, A., Grenley, M. O., Mcewen, D. G., and Edgar, B. A. (2009). Cytokine/Jak/Stat signaling mediates regeneration and homeostasis in the Drosophila midgut. *Cell* 137, 1343–1355. doi: 10.1016/j.cell.2009.05.014

Kallio, J., Leinonen, A., Ulvila, J., Valanne, S., Ezekowitz, R. A., and Rämet, M. (2005). Functional analysis of immune response genes in Drosophila identifies JNK pathway as a regulator of antimicrobial peptide gene expression in S2 cells. *Microbes Infect.* 7, 811–819. doi: 10.1016/j.micinf.2005.03.014

Kallus, S. J., and Brandt, L. J. (2012). The intestinal microbiota and obesity. *J. Clin. Gastroenterol.* 46, 16–24. doi: 10.1097/MCG.0b013e31823711fd

Kamada, N., Seo, S.-U., Chen, G. Y., and Núñez, G. (2013). Role of the gut microbiota in immunity and inflammatory disease. *Nat. Rev. Immunol.* 13, 321–335. doi: 10.1038/nri3430

Kapuria, S., Karpac, J., Biteau, B., Hwangbo, D., and Jasper, H. (2012). Notch-mediated suppression of TSC2 expression regulates cell differentiation in the Drosophila intestinal stem cell lineage. *PLoS Genet.* 8:e1003045. doi: 10.1371/journal.pgen.1003045

Karpac, J., Biteau, B., and Jasper, H. (2013). Misregulation of an adaptive metabolic response contributes to the age-related disruption of lipid homeostasis in Drosophila. *Cell Rep.* 4, 1250–1261. doi: 10.1016/j.celrep.2013.08.004

Karpac, J., Hull-Thompson, J., Falleur, M., and Jasper, H. (2009). JNK signaling in insulin-producing cells is required for adaptive responses to stress in Drosophila. *Aging Cell* 8, 288–295. doi: 10.1111/j.1474-9726.2009.00476.x

Karpac, J., and Jasper, H. (2009). Insulin and JNK: optimizing metabolic homeostasis and lifespan. *Trends Endocrinol. Metab.* 20, 100–106. doi: 10.1016/j.tem.2008.11.004

Karpac, J., Younger, A., and Jasper, H. (2011). Dynamic coordination of innate immune signaling and insulin signaling regulates systemic responses to localized DNA damage. *Dev. Cell* 20, 841–854. doi: 10.1016/j.devcel.2011.05.011

Karpowicz, P., Perez, J., and Perrimon, N. (2010). The Hippo tumor suppressor pathway regulates intestinal stem cell regeneration. *Development* 137, 4135–4145. doi: 10.1242/dev.060483

Kaser, A., Zeissig, S., and Blumberg, R. S. (2010). Inflammatory bowel disease. *Annu. Rev. Immunol.* 28, 573–621. doi: 10.1146/annurev-immunol-030409-101225

Kenyon, C. J. (2010). The genetics of ageing. *Nature* 464, 504–512. doi: 10.1038/nature08980

Koeth, R. A., Wang, Z., Levison, B. S., Buffa, J. A., Org, E., Sheehy, B. T., et al. (2013). Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis. *Nat. Med.* 19, 576–585. doi: 10.1038/nm.3145

Kostic, A. D., Gevers, D., Pedamallu, C. S., Michaud, M., Duke, F., Earl, A. M., et al. (2012). Genomic analysis identifies association of Fusobacterium with colorectal carcinoma. *Genome Res.* 22, 292–298. doi: 10.1101/gr.126573.111

Kuraishi, T., Binggeli, O., Opota, O., Buchon, N., and Lemaitre, B. (2011). Genetic evidence for a protective role of the peritrophic matrix against intestinal bacterial infection in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. U.S.A.* 108, 15966–15971. doi: 10.1073/pnas.1105994108

Lee, K.-A., Kim, S.-H., Kim, E.-K., Ha, E.-M., You, H., Kim, B., et al. (2013). Bacterial-derived uracil as a modulator of mucosal immunity and gut-microbe homeostasis in Drosophila. *Cell* 153, 797–811. doi: 10.1016/j.cell.2013.04.009

Lee, W.-J., and Brey, P. T. (2013). How microbiomes influence metazoan development: insights from history and Drosophila modeling of gut-microbe interactions. *Annu. Rev. Cell Dev. Boil.* 29, 571–592. doi: 10.1146/annurev-cellbio-101512-122333

Lemaitre, B., and Hoffmann, J. (2007). The host defense of *Drosophila melanogaster*. *Annu. Rev. Immunol.* 25, 697–743. doi: 10.1146/annurev.immunol.25.022106.141615

Lemaitre, B., and Miguel-Aliaga, I. (2013). The digestive tract of *Drosophila melanogaster*. *Annu. Rev. Genet.* 47, 377–404. doi: 10.1146/annurev-genet-111212-133343

Lhocine, N., Ribeiro, P. S., Buchon, N., Wepf, A., Wilson, R., Tenev, T., et al. (2008). PIMS modulates immune tolerance by negatively regulating Drosophila innate immune signaling. *Cell Host Microbe* 4, 147–158. doi: 10.1016/j.chom.2008.07.004

Li, H., Qi, Y., and Jasper, H. (2013). Dpp signaling determines regional stem cell identity in the regenerating adult Drosophila gastrointestinal tract. *Cell Rep.* 4, 10–18. doi: 10.1016/j.celrep.2013.05.040

Liehl, P., Blight, M., Vodovar, N., Boccard, F., and Lemaitre, B. (2006). Prevalence of local immune response against oral infection in a Drosophila/Pseudomonas infection model. *PLoS Pathog.* 2:e56. doi: 10.1371/journal.ppat.0020056

Lin, G., Xu, N., and Xi, R. (2008). Paracrine wingless signalling controls self-renewal of Drosophila intestinal stem cells. *Nature* 455, 1119–1123. doi: 10.1038/nature07329

Lozupone, C. A., Stombaugh, J. I., Gordon, J. I., Jansson, J. K., and Knight, R. (2012). Diversity, stability and resilience of the human gut microbiota. *Nature* 489, 220–230. doi: 10.1038/nature11550

Maillet, F., Bischoff, V., Vignal, C., Hoffmann, J., and Royet, J. (2008). The Drosophila peptidoglycan recognition protein PGRP-LF blocks PGRP-LC and IMD/JNK pathway activation. *Cell Host Microbe* 3, 293–303. doi: 10.1016/j.chom.2008.04.002

Marianes, A., and Spradling, A. C. (2013). Physiological and stem cell compartmentalization within the Drosophila midgut. *eLife* 2:e00886. doi: 10.7554/eLife.00886

Meylan, F., Richard, A. C., and Siegel, R. M. (2011). TL1A and DR3, a TNF family ligand-receptor pair that promotes lymphocyte costimulation, mucosal hyperplasia, and autoimmune inflammation. *Immunol. Rev.* 244, 188–196. doi: 10.1111/j.1600-065X.2011.01068.x

Micchelli, C. A., and Perrimon, N. (2006). Evidence that stem cells reside in the adult Drosophila midgut epithelium. *Nature* 439, 475–479. doi: 10.1038/nature04371

Moskalev, A., and Shaposhnikov, M. (2011). Pharmacological inhibition of NF-κB prolongs lifespan of *Drosophila melanogaster*. *Aging* 3, 391–394.

Nehme, N. T., Liégeois, S., Kele, B., Giammarinaro, P., Pradel, E., Hoffmann, J. A., et al. (2007). A model of bacterial intestinal infections in *Drosophila melanogaster*. *PLoS Pathog.* 3:e173. doi: 10.1371/journal.ppat.0030173

Neyen, C., Poidevin, M., Roussel, A., and Lemaitre, B. (2012). Tissue- and ligand-specific sensing of gram-negative infection in drosophila by PGRP-LC isoforms and PGRP-LE. *J. Immunol.* 189, 1886–1897. doi: 10.4049/jimmunol.1201022

Niwa, T., Tsukamoto, T., Toyoda, T., Mori, A., Tanaka, H., Maekita, T., et al. (2010). Inflammatory processes triggered by *Helicobacter pylori* infection cause aberrant DNA methylation in gastric epithelial cells. *Cancer Res.* 70, 1430–1440. doi: 10.1158/0008-5472.CAN-09-2755

O'brien, L. E., Soliman, S. S., Li, X., and Bilder, D. (2011). Altered modes of stem cell division drive adaptive intestinal growth. *Cell* 147, 603–614. doi: 10.1016/j.cell.2011.08.048

Ohlstein, B., and Spradling, A. (2006). The adult Drosophila posterior midgut is maintained by pluripotent stem cells. *Nature* 439, 470–474. doi: 10.1038/nature04333

Ohlstein, B., and Spradling, A. (2007). Multipotent Drosophila intestinal stem cells specify daughter cell fates by differential notch signaling. *Science* 315, 988–992. doi: 10.1126/science.1136606

Osman, D., Buchon, N., Chakrabarti, S., Huang, Y. T., Su, W. C., Poidevin, M., et al. (2012). Autocrine and paracrine unpaired signaling regulate intestinal stem cell maintenance and division. *J. Cell Sci.* 125, 5944–5949. doi: 10.1242/jcs.113100

Paaby, A. B., and Schmidt, P. S. (2008). Functional significance of allelic variation at methuselah, an aging gene in Drosophila. *PLoS ONE* 3:e1987. doi: 10.1371/journal.pone.0001987

Paredes, J. C., Welchman, D. P., Poidevin, M., and Lemaitre, B. (2011). Negative regulation by amidase PGRPs shapes the Drosophila antibacterial response and protects the fly from innocuous infection. *Immunity* 35, 770–779. doi: 10.1016/j.immuni.2011.09.018

Park, J. M., Brady, H., Ruocco, M. G., Sun, H., Williams, D., Lee, S. J., et al. (2004). Targeting of TAK1 by the NF-kappa B protein Relish regulates the JNK-mediated immune response in Drosophila. *Genes Dev.* 18, 584–594. doi: 10.1101/gad.1168104

Park, J.-S., Kim, Y.-S., and Yoo, M.-A. (2009). The role of p38b MAPK in age-related modulation of intestinal stem cell proliferation and differentiation in Drosophila. *Aging* 1, 637–651.

Pasparakis, M. (2012). Role of NF-κB in epithelial biology. *Immunol. Rev.* 246, 346–358. doi: 10.1111/j.1600-065X.2012.01109.x

Patel, B. B., Yu, Y., Du, J., Levi, E., Phillip, P. A., and Majumdar, A. P. N. (2009). Age-related increase in colorectal cancer stem cells in macroscopically normal mucosa of patients with adenomas: a risk factor for colon cancer. *Biochem. Biophys. Res. Commun.* 378, 344–347. doi: 10.1016/j.bbrc.2008.10.179

Perdigoto, C. N., Schweisguth, F., and Bardin, A. J. (2011). Distinct levels of Notch activity for commitment and terminal differentiation of stem cells in the adult fly intestine. *Development* 138, 4585–4595. doi: 10.1242/dev.065292

Power, S. E., O'toole, P. W., Stanton, C., Ross, R. P., and Fitzgerald, G. F. (2013). Intestinal microbiota, diet and health. *Br. J. Nutr.* 1–16. doi: 10.1017/S0007114513002560

Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K. S., Manichanh, C., et al. (2010). A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* 464, 59–65. doi: 10.1038/nature08821

Quan, Z., Sun, P., Lin, G., and Xi, R. (2013). TSC1/2 regulates intestinal stem cell maintenance and lineage differentiation through Rheb-TORC1-S6K but independently of nutritional status or Notch regulation. *J. Cell Sci.* 126, 3884–3892. doi: 10.1242/jcs.125294

Ren, C., Webster, P., Finkel, S. E., and Tower, J. (2007). Increased internal and external bacterial load during Drosophila aging without life-span trade-off. *Cell Metab.* 6, 144–152. doi: 10.1016/j.cmet.2007.06.006

Ren, F., Wang, B., Yue, T., Yun, E.-Y., Ip, Y. T., and Jiang, J. (2010). Hippo signaling regulates Drosophila intestine stem cell proliferation through multiple pathways. *Proc. Natl. Acad. Sci. U.S.A.* 107, 21064–21069. doi: 10.1073/pnas.10127 59107

Rera, M., Bahadorani, S., Cho, J., Koehler, C. L., Ulgherait, M., Hur, J. H., et al. (2011). Modulation of longevity and tissue homeostasis by the Drosophila PGC-1 homolog. *Cell Metab.* 14, 623–634. doi: 10.1016/j.cmet.2011.09.013

Rera, M., Clark, R. I., and Walker, D. W. (2012). Intestinal barrier dysfunction links metabolic and inflammatory markers of aging to death in Drosophila. *Proc. Natl. Acad. Sci. U.S.A.* 109, 21528–21533. doi: 10.1073/pnas.1215849110

Roberts, S. B., and Rosenberg, I. (2006). Nutrition and aging: changes in the regulation of energy metabolism with aging. *Physiol. Rev.* 86, 651–667. doi: 10.1152/physrev.00019.2005

Ryu, J. H., Ha, E. M., and Lee, W. J. (2010). Innate immunity and gut-microbe mutualism in Drosophila. *Dev. Comp. Immunol.* 34, 369–376. doi: 10.1016/j.dci. 2009.11.010

Ryu, J.-H., Ha, E.-M., Oh, C.-T., Seol, J.-H., Brey, P. T., Jin, I., et al. (2006). An essential complementary role of NF-kappaB pathway to microbicidal oxidants in Drosophila gut immunity. *EMBO J.* 25, 3693–3701. doi: 10.1038/sj.emboj. 7601233

Ryu, J. H., Kim, S. H., Lee, H. Y., Bai, J. Y., Nam, Y. D., Bae, J. W., et al. (2008). Innate immune homeostasis by the homeobox gene caudal and commensal-gut mutualism in Drosophila. *Science* 319, 777–782. doi: 10.1126/science.1149357

Sackton, T. B., Lazzaro, B. P., Schlenke, T. A., Evans, J. D., Hultmark, D., and Clark, A. G. (2007). Dynamic evolution of the innate immune system in Drosophila. *Nat. Genet.* 39, 1461–1468. doi: 10.1038/ng.2007.60

Schloissnig, S., Arumugam, M., Sunagawa, S., Mitreva, M., Tap, J., Zhu, A., et al. (2013). Genomic variation landscape of the human gut microbiome. *Nature* 493, 45–50. doi: 10.1038/nature11711

Shaw, R. L., Kohlmaier, A., Polesello, C., Veelken, C., Edgar, B. A., and Tapon, N. (2010). The Hippo pathway regulates intestinal stem cell proliferation during Drosophila adult midgut regeneration. *Development* 137, 4147–4158. doi: 10.1242/dev.052506

Shin, S. C., Kim, S.-H., You, H., Kim, B., Kim, A. C., Lee, K.-A., et al. (2011). Drosophila microbiome modulates host developmental and metabolic homeostasis via insulin signaling. *Science* 334, 670–674. doi: 10.1126/science.1212782

Sieber, M. H., and Thummel, C. S. (2012). Coordination of triacylglycerol and cholesterol homeostasis by DHR96 and the Drosophila LipA homolog magro. *Cell Metab.* 15, 122–127. doi: 10.1016/j.cmet.2011.11.011

Silverman, N., Zhou, R., Erlich, R. L., Hunter, M., Bernstein, E., Schneider, D., et al. (2003). Immune activation of NF-kappaB and JNK requires Drosophila TAK1. *J. Biol. Chem.* 278, 48928–48934. doi: 10.1074/jbc.M304802200

Staley, B. K., and Irvine, K. D. (2010). Warts and Yorkie mediate intestinal regeneration by influencing stem cell proliferation. *Curr. Biol.* 20, 1580–1587. doi: 10.1016/j.cub.2010.07.041

Stecher, B., Maier, L., and Hardt, W.-D. (2013). 'Blooming' in the gut: how dysbiosis might contribute to pathogen evolution. *Nat. Rev. Microbiol.* 11, 277–284. doi: 10.1038/nrmicro2989

Storelli, G., Defaye, A., Erkosar, B., Hols, P., Royet, J., and Leulier, F. (2011). Lactobacillus plantarum promotes Drosophila systemic growth by modulating hormonal signals through TOR-dependent nutrient sensing. *Cell Metab.* 14, 403–414. doi: 10.1016/j.cmet.2011.07.012

Takashima, S., Mkrtchyan, M., Younossi-Hartenstein, A., Merriam, J. R., and Hartenstein, V. (2008). The behaviour of Drosophila adult hindgut stem cells is controlled by Wnt and Hh signalling. *Nature* 454, 651–655. doi: 10.1038/nature07156

Thevenon, D., Engel, E., Avet-Rochex, A., Gottar, M., Bergeret, E., Tricoire, H., et al. (2009). The Drosophila ubiquitin-specific protease dUSP36/Scny targets IMD to prevent constitutive immune signaling. *Cell Host Microbe* 6, 309–320. doi: 10.1016/j.chom.2009.09.007

Tzou, P., Ohresser, S., Ferrandon, D., Capovilla, M., Reichhart, J. M., Lemaitre, B., et al. (2000). Tissue-specific inducible expression of antimicrobial peptide genes in Drosophila surface epithelia. *Immunity* 13, 737–748. doi: 10.1016/S1074-7613(00)00072-8

Uronis, J. M., Muhlbauer, M., Herfarth, H. H., Rubinas, T. C., Jones, G. S., and Jobin, C. (2009). Modulation of the intestinal microbiota alters colitis-associated colorectal cancer susceptibility. *PLoS ONE* 4:e6026. doi: 10.1371/journal.pone.0006026

Wong, C. N., Ng, P., and Douglas, A. E. (2011). Low-diversity bacterial community in the gut of the fruitfly *Drosophila melanogaster*. *Environ. Microbiol.* 13, 1889–1900. doi: 10.1111/j.1462-2920.2011.02511.x

Wullaert, A., Bonnet, M. C., and Pasparakis, M. (2011). NF-κB in the regulation of epithelial homeostasis and inflammation. *Cell Res.* 21, 146–158. doi: 10.1038/cr.2010.175

Xavier, R. J., and Podolsky, D. K. (2007). Unravelling the pathogenesis of inflammatory bowel disease. *Nature* 448, 427–434. doi: 10.1038/nature06005

Xu, N., Wang, S. Q., Tan, D., Gao, Y., Lin, G., and Xi, R. (2011). EGFR, Wingless and JAK/STAT signaling cooperatively maintain Drosophila intestinal stem cells. *Dev. Biol.* 354, 31–43. doi: 10.1016/j.ydbio.2011.03.018

Zaidman-Remy, A., Herve, M., Poidevin, M., Pili-Floury, S., Kim, M. S., Blanot, D., et al. (2006). The Drosophila amidase PGRP-LB modulates the immune response to bacterial infection. *Immunity* 24, 463–473. doi: 10.1016/j.immuni. 2006.02.012

Zhou, F., Rasmussen, A., Lee, S., and Agaisse, H. (2013). The UPD3 cytokine couples environmental challenge and intestinal stem cell division through modulation of JAK/STAT signaling in the stem cell microenvironment. *Dev. Biol.* 373, 383–393. doi: 10.1016/j.ydbio.2012.10.023

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 15 October 2013; paper pending published: 04 November 2013; accepted: 27 November 2013; published online: 16 December 2013.

*Citation: Ayyaz A and Jasper H (2013) Intestinal inflammation and stem cell homeostasis in aging Drosophila melanogaster. Front. Cell. Infect. Microbiol. **3**:98. doi: 10.3389/fcimb.2013.00098*

This article was submitted to the journal Frontiers in Cellular and Infection Microbiology.

frontiers in
CELLULAR AND INFECTION MICROBIOLOGY

MINI REVIEW ARTICLE
published: 24 April 2014
doi: 10.3389/fcimb.2014.00049

Tissue communication in regenerative inflammatory signaling: lessons from the fly gut

Kristina Kux and Chrysoula Pitsouli*

Department of Biological Sciences, University of Cyprus, Nicosia, Cyprus

Edited by:
Dominique Ferrandon, Centre National de la Recherche Scientifique, France

Reviewed by:
Huaqi Jiang, The University of Texas Southwestern Medical Center, USA
Masayuki Miura, The University of Tokyo, Japan

***Correspondence:**
Chrysoula Pitsouli, Department of Biological Sciences, University of Cyprus, PO Box 20537, Nicosia 1678, Cyprus
e-mail: pitsouli@ucy.ac.cy

The intestine, as a barrier epithelium, serves in the first line of defense against invading pathogens and damaging agents that enter the body via food ingestion. Maintenance of intestinal homeostasis is therefore key to organismal health. To maintain homeostasis, intestinal stem cells (ISCs) continuously replace lost or damaged intestinal epithelial cells in organisms ranging from *Drosophila* to humans. Interestingly, intestinal damage upon ingestion of chemicals or pathogenic bacteria leads to an inflammatory response in the *Drosophila* intestine, which promotes regeneration and predisposes to tumorigenesis. This regenerative inflammatory signaling culminates in proliferation and differentiation of ISCs that replenish the damaged intestinal cells and is regulated by the interplay of conserved cell-cell communication pathways, such as the JNK, JAK/STAT, Wnt/Wingless, Notch, InR, PVR, EGFR, and Hippo. These pathways are induced by signals emanating not only from the damaged intestinal epithelial cells, but also from neighboring tissues associated with the intestinal epithelium, such as the muscles and the trachea, or distant tissues, such as the wounded epidermis and the brain. Here we review tissue communication during homeostasis and regenerative inflammatory signaling in *Drosophila* focusing on the signals that emanate from non-intestinal epithelial tissues to ensure intestinal integrity.

Keywords: *Drosophila*, homeostasis, intestine, stem cells, signaling pathways, regenerative inflammatory signaling, tissue communication

THE *DROSOPHILA* INTESTINE

Due to its functional, structural and cellular similarity to the human intestine, the *Drosophila* intestine has evolved to an excellent model for studying signaling events that control intestinal homeostasis, which, when deregulated, can cause disease (Pitsouli et al., 2009; Apidianakis and Rahme, 2011; Jiang and Edgar, 2011; Jiang et al., 2011).

The adult *Drosophila* intestinal tract is anatomically and functionally separated in three main domains. The foregut, which comprises the esophagus, the crop and the cardia, is followed by the equivalent of the human small intestine, the midgut, and the equivalent of the colon, the hindgut (Demerec, 1950). The intestinal mono-layered tube is ensheathed along its length by circular and longitudinal visceral muscles (VMs) that ensure mixing and grinding, and forward-pushing of the food, respectively (Bayliss and Starling, 1899). The epithelium is covered toward the lumen by the peritrophic membrane (PM), which functions as a structural barrier and contains chitin and glycoproteins (Kuraishi et al., 2011). Between the VM and the intestinal epithelium lies the extracellular matrix-rich basement membrane (BM) (Ohlstein and Spradling, 2006). An extensively ramified network of intestinal trachea responsible for oxygen transport is closely associated with the VMs and reaches the epithelium (Li et al., 2013b). Furthermore, neuronal innervations attach to the esophagus and the cardia, as well as the midgut-hindgut boundary and the rectum, whereas most of the midgut is devoid of innervations (Cognigni et al., 2011) (**Figure 1**).

The *Drosophila* midgut has recently emerged as a favorite model of intestinal homeostasis. The midgut cells align basally on the BM and are apically separated from the intestinal content by the PM. Four different cell types constitute the midgut epithelium: the differentiated enterocytes (ECs) and enteroendocrine cells (EEs), with absorptive and secretory properties, respectively, the transient enteroblasts (EBs), and the self-renewing intestinal stem cells (ISCs). The ISCs are evenly distributed in the epithelium, reside basally close to the BM and replenish lost cells continuously (Micchelli and Perrimon, 2006; Ohlstein and Spradling, 2006).

HOMEOSTASIS AND REGENERATION IN THE *DROSOPHILA* MIDGUT

The *Drosophila* midgut is continuously damaged during feeding, as well as by chemicals and pathogens, and needs to be constantly renewed. Homeostatic renewal is ensured via ISC division and differentiation. The ISC division is usually asymmetric and produces two types of daughters: one ISC and one progenitor cell, the EB. The EB does not divide further, but differentiates directly into either an EC or an EE (Micchelli and Perrimon, 2006; Ohlstein and Spradling, 2006, 2007; Jiang and Edgar, 2011).

Intestinal homeostasis is coordinated by the combined action of conserved signaling pathways. In addition to Notch that controls ISC commitment and differentiation depending on its levels (Micchelli and Perrimon, 2006; Ohlstein and Spradling, 2007; Perdigoto et al., 2011), the Wnt/Wg pathway is an important

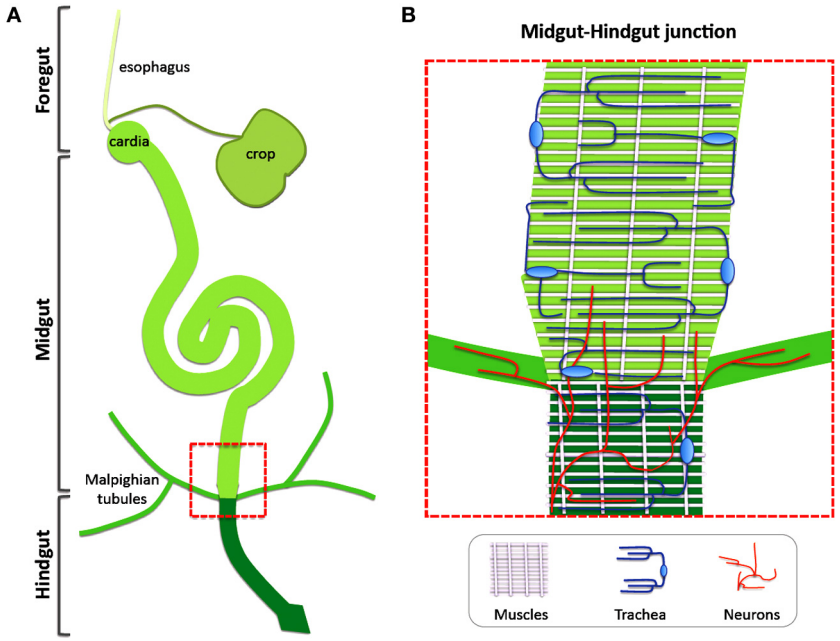


FIGURE 1 | Non-intestinal epithelial tissues are closely connected with the *Drosophila* gut. (A) The intestinal tract of the *Drosophila* adult is separated in three main domains: the foregut, the midgut and the hindgut. **(B)** Visceral muscles, intestinal trachea and neurons are integral parts of the intestinal tract. A zoom-up of the boxed area in **(A)** Corresponding to the midgut-hindgut junction is shown to demonstrate the different tissues. Circular and longitudinal visceral muscles ensheath the intestine, tracheal cells generate a vast gas-transporting tubular network around the muscles and neuronal innervations are present at the hindgut-midgut boundary and the malpighian tubules to regulate intestinal physiology.

regulator of ISC maintenance and proliferation (Lin et al., 2008; Lee et al., 2009), the Epidermal Growth Factor Receptor (EGFR) and the Target-of-rapamycin (Tor) pathways regulate basal levels of ISC proliferation, the latter in response to nutrition (Amcheslavsky et al., 2011; Biteau and Jasper, 2011; Jiang et al., 2011; Xu et al., 2011) and the Platelet Derived Growth Factor Receptor (PDGFR)/Vascular Endothelial Growth Factor Receptor (VEGFR) pathway, known as PVR in flies, acts in an autocrine manner to control ISC differentiation (Bond and Foley, 2012).

Strikingly, the *Drosophila* midgut exhibits the remarkable ability to regenerate after damage. Ingestion of chemicals, like bleomycin or paraquat, and enteric infection with *Pseudomonas species or Erwinia carotovora carotovora 15* (*Ecc15*) activate a process of regenerative inflammatory signaling, whereby damaged cells produce inflammatory signals that trigger regenerative pathways to replace lost cells and maintain tissue integrity (Panayidou and Apidianakis, 2013). EC damage results in JNK signaling activation, release of IL6-related cytokines (called Unpaired1–3 in flies), induction of EGFs in the intestinal epithelium, as well as the VM, and secretion of Wg from EBs (Biteau et al., 2008; Apidianakis et al., 2009; Buchon et al., 2009a,b; Jiang and Edgar, 2009; Biteau and Jasper, 2011; Jiang et al., 2011). These in turn activate the JAK/STAT, EGFR/Ras/MAPK, and Wg/Wnt cascades in the ISCs to promote proliferation (Jiang and Edgar, 2009; Jiang et al., 2011; Cordero et al., 2012a,b). The EGFR/Ras/MAPK pathway plays a key role in the proliferative response and it is required for both JNK and JAK/STAT-induced ISC proliferation (Buchon et al., 2010; Jiang et al., 2011). In addition, the Hippo pathway acts as a stress sensor in the intestine and responds to changes in epithelial integrity (Karpowicz et al., 2010; Ren et al., 2010; Shaw et al., 2010; Staley and Irvine, 2012). The PVR pathway mediates the response to oxidative stress and aging (Choi et al., 2008) and the injury-induced BMP/Dpp pathway negatively regulates ISC proliferation during the reversion of regeneration-to-maintenance (Guo et al., 2013).

Interestingly, the source of the regeneration signals is not confined to the intestinal epithelium. Accumulating evidence suggests that neighboring tissues, such as the muscle, the trachea and potentially the neurons communicate with the intestinal epithelial cells, and thus might function as part of the ISC niche (**Figure 2**). In the following sections, we review the recent literature on the local and systemic signals emanating from non-intestinal epithelial tissues that ensure intestinal homeostasis during basal tissue maintenance and regenerative inflammatory signaling in *Drosophila.*

THE VISCERAL MUSCLE: A SOURCE OF Wg, EGFs, UPDs AND INSULIN-LIKE PEPTIDES

Wnt/Wg SIGNALING

The first report of inter-organ communication between the adult intestinal epithelium and neighboring tissue, which serves as a functional "ISC niche," came from a study investigating the role of Wnt/Wg signaling in gut homeostasis (Lin et al., 2008). The authors observed *wg* gene expression in the VM and Wg protein accumulation between the VM and the BM suggesting that VM-secreted Wg reaches the ISCs through the BM. Loss of *wg*

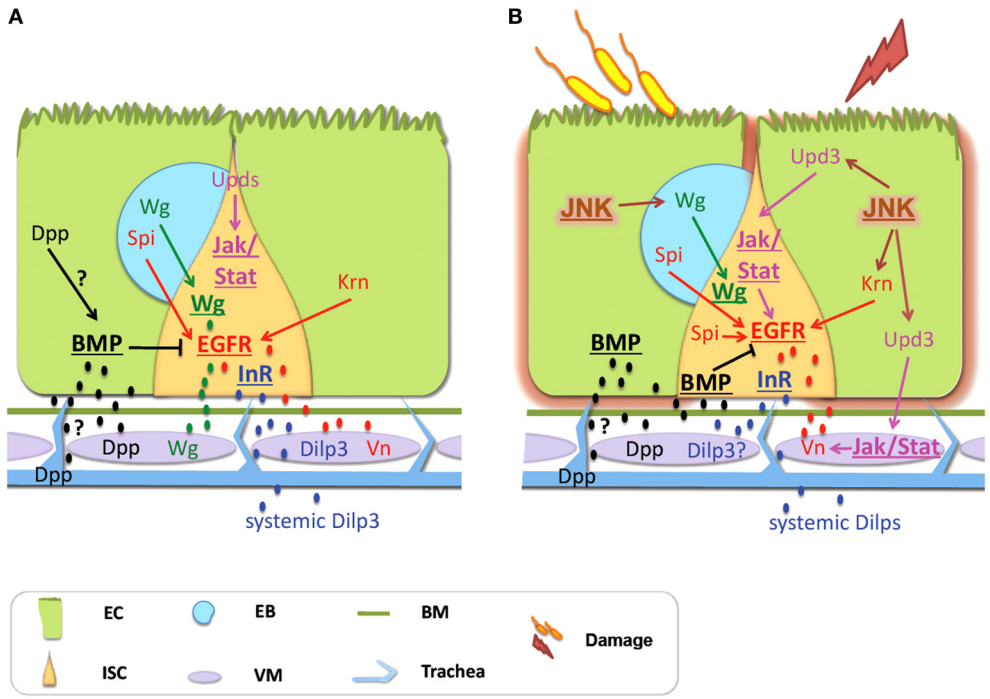


FIGURE 2 | Signals derived from non-intestinal epithelial tissues control intestinal homeostasis in *Drosophila*. (A) Physiological Homeostasis: Basal proliferation is controlled via the EGFR and the Wg pathways, whereas JAK/STAT controls differentiation. Vn secreted by the VM, as well as Krn and Spi coming from the EC and the EB, respectively, activate the EGFR pathway. Wg coming from the VM and the EB activates the Wg pathway. Dilp3 secreted from the VM and systemic Dilps activate InR signaling in response to nutrition. Dpp secreted by the VM and possibly by the ECs and the trachea activates the BMP pathway, which inhibits EGFR. Differentiation is regulated by JAK/STAT with the Upd cytokines coming mainly from the ECs. **(B)** Regenerative inflammatory signaling: Enteric infection and ingestion of chemicals induce intestinal damage that promotes regeneration via compensatory ISC proliferation. The EGFR, the Wg and the JAK/STAT pathways control ISC proliferation. JNK signaling is a stress sensor induced in the ECs. It activates EGFR signaling in ISCs and induces Wg in the EBs that activates Wg signaling in the ISCs. The EGFR ligands come from the VM (secreted Vn), the ECs (Krn) and the EBs (Spi). The JNK and JAK/STAT induce proliferation by activating EGFR signaling. Upd3 derived from damaged ECs induces the JAK/STAT pathway in the ISCs and the VM. In the VM, Vn is induced by JAK/STAT activity. BMP signaling is required for the shift from regeneration to basal maintenance; it inhibits EGFR signaling. The BMP ligand Dpp comes from the VM. Additionally, Dpp is expressed in the trachea but this source seems dispensable. Although the InR promotes proliferation, the source and identity of its ligands remain unclear. Signaling pathways are shown in bold and underlined. Abbreviations: EC, enterocyte; ISC, intestinal stem cell; EB, enteroblast; Vn, Vein; Spi, Spitz; Krn, Keren; Wg, Wingless.

significantly reduced the ISC number, whereas ISC clones lacking the Wg receptors or core components of the pathway contained fewer ISCs, suggesting that paracrine VM-produced Wg induces the pathway in the ISCs to promote their self-renewal (Lin et al., 2008). Furthermore, careful analysis of *Adenomatous polyposis coli* (*Apc*) mutant clones, which activate Wnt/Wg signaling, has uncovered a proliferative, not a self-renewal, role of Wg in the ISCs (Lee et al., 2009). Nevertheless, the proliferative effect of Wnt/Wg signaling is mild and was later shown that Wnt/Wg, EGFR and JAK/STAT cooperatively regulate homeostatic ISC proliferation and maintenance (Xu et al., 2011).

Interestingly, a recent report showed that Wg coming from the VM and additional Wg from the epithelium act in concert to regulate ISC maintenance and self-renewal in unchallenged flies (Cordero et al., 2012b). Strikingly, intestinal damage triggered by ingestion of Dextrane Sulfate Sodium (DSS) or *Pseudomonas entomophila* caused Wg upregulation exclusively in the EBs and not the VM. Elegant tissue-specific *wg* inactivation experiments (in the VM and the epithelium), instead of the temperature sensitive *wg* mutation that broadly removes *wg* (Lin et al., 2008), showed that EB-secreted Wg signals to neighboring ISCs to activate downstream pathway components and ISC proliferation (Cordero et al., 2012b).

EGFR/RAS/MAPK AND JAK/STAT SIGNALING

The EGFR pathway was initially shown to regulate development of the midgut epithelium by controlling the proliferation of the adult midgut progenitors (AMPs) (Jiang and Edgar, 2009). Several independent studies subsequently established its key role in ISC proliferation during homeostasis and regeneration (Buchon et al., 2010; Biteau and Jasper, 2011; Jiang et al., 2011; Xu et al., 2011). The three EGFs, Vein (Vn), Spitz (Spi) and Keren (Krn) trigger the EGFR pathway activity in the adult intestine. Vn is expressed in the VM (Buchon et al., 2010; Biteau and Jasper, 2011; Jiang et al., 2011; Xu et al., 2011), whereas Spi and Krn are expressed in the midgut epithelium. Overexpression of *vn*, *spi* or *krn* in the VM, ISCs/EBs or ECs is sufficient to induce ISC proliferation (Buchon et al., 2010; Jiang et al., 2011; Xu et al., 2011). Nevertheless, there are conflicting reports regarding the necessity of each of the three EGFs in ISC proliferation. Although Jiang

et al. (2011) report that neither VM-specific *vn* RNAi nor ISC/EB-specific *spi* RNAi produce an effect, other groups report effects on proliferation (Buchon et al., 2010; Biteau and Jasper, 2011; Xu et al., 2011) and long-term ISC maintenance (Xu et al., 2011) in VM-specific *vn* RNAi. Clearly, the EGFR ligands function redundantly in the *Drosophila* intestine: removing them in combinations produces stronger effects and overexpression of one can rescue loss of another, i.e., overexpression of secreted *spi* in the VM can rescue *vn* RNAi (Xu et al., 2011).

The EGFR ligand redundancy is also observed in stressed or damaged intestines. For example, in response to enteric infection with *Ecc15 vn* is strongly induced in the VM and VM-specific *vn* knockdown impairs ISC proliferation (Buchon et al., 2009b, 2010; Zhou et al., 2013), albeit not fully indicating the redundant function of VM *vn* with other EGFs (Zhou et al., 2013). Indeed, impaired proliferation was also observed by loss of *spi* or *krn* in progenitor cells (Buchon et al., 2010). In addition, VM *vn* is necessary for the ISC regenerative response to damage with paraquat or bleomycin (Biteau and Jasper, 2011). Furthermore, *Pseudomonas entomophila* oral infection leads to induction of *vn* in the VM, *spi* in ISCs/EBs and *krn* in ECs, but only the simultaneous knockdown of *krn* with *spi* or with *vn* impairs stress-induced proliferation underscoring redundancy in EGF function (Jiang et al., 2011).

Both the EGFR and the JAK/STAT pathways are activated during regenerative inflammatory signaling and emerging evidence suggests their interplay at the level of ligand induction. Earlier studies agree that JAK/STAT primarily acts autonomously in the ISCs to regulate their proliferation and differentiation in response to damage (Buchon et al., 2009a,b; Cronin et al., 2009; Jiang and Edgar, 2009). Closer examination of the cell-type specific expression and function of the JAK/STAT ligands has shown that the Upds are induced in distinct cell types: *upd1* is expressed in ISCs/EBs (Osman et al., 2012) and possibly the longitudinal VM (Lin et al., 2010) and it is moderately induced upon bacterial ingestion (Jiang and Edgar, 2009; Osman et al., 2012), *upd2* is probably produced by both progenitors and ECs and exhibits an additive effect to *upd3* in epithelial regeneration upon *Ecc15* infection (Osman et al., 2012), and *upd3* is expressed in ECs and it is strongly induced upon infection (Jiang and Edgar, 2009; Osman et al., 2012; Zhou et al., 2013). Intriguingly, recent evidence suggests that JAK/STAT exhibits a non-autonomous effect on ISC proliferation in response to damage via the activation of EGFs in the VM and the EBs. Specifically, the Upd3-activated JAK/STAT signaling induces *vn* in the VM (Buchon et al., 2010; Jiang et al., 2011; Zhou et al., 2013) and *spi* in the EBs (Zhou et al., 2013). The release of Upd3 from damaged ECs and EBs leads to strong induction of STAT92E activity in ISCs/EBs (Buchon et al., 2009b; Jiang and Edgar, 2009; Zhou et al., 2013) and the VM (Buchon et al., 2010; Zhou et al., 2013) and STAT activation in the VM is sufficient to induce *vn* (Jiang et al., 2011), whereas loss of JAK/STAT activity from the VM leads to loss of VM *vn* and reduces ISC proliferation (Buchon et al., 2010). Interestingly, upon infection with *Pseudomonas entomophila* JAK/STAT activity is dispensable for the induction of *vn* in the VM suggesting that additional signals might be involved in its induction (Jiang et al., 2011).

INSULIN SIGNALING

The insulin pathway promotes ISC proliferation and differentiation during feeding, aging and regeneration (Amcheslavsky et al., 2009; Biteau et al., 2011; Choi et al., 2011) in *Drosophila*. Nevertheless, the source of the Insulin Receptor (InR) ligands that control these processes remains largely unknown. Two of the eight *Drosophila* insulin-like peptides, *Dilp3* and *Dilp7*, are expressed in the intestine: *Dilp7* is expressed in intestinal neurons and regulates intestinal physiology (Cognigni et al., 2011), whereas *Dilp3* is expressed in foregut and midgut muscles (Veenstra et al., 2008). Interestingly, VM-derived Dilp3, supplemented by systemic Dilps, acts directly on the ISCs via the *Drosophila* InR to promote their proliferation and regulates adaptive midgut growth during food intake via both asymmetric and symmetric ISC divisions (O'Brien et al., 2011). Although the inactivation of brain neurons producing systemic Dilps partially inhibits DSS- and bleomycin-induced midgut regeneration (Amcheslavsky et al., 2009), it remains to be tested if intestinal Dilps are also involved.

THE INTESTINAL TRACHEA: A SOURCE OF Dpp?

Oxygenation of the adult *Drosophila* intestine is achieved via a highly ramified tracheal network overlaying the musculature. The importance of the trachea for intestinal development was highlighted in the silkworm, *Manduca sexta*, where the tracheal and intestinal epithelia grow co-ordinately during metamorphosis (Nardi et al., 2011). In *Drosophila*, tracheal cells project fine extensions through the VM of the adult intestine, which closely contact the intestinal epithelium to allow gas exchange (Li et al., 2013b).

A role of BMP/Dpp signaling in *Drosophila* intestinal homeostasis was first described during larval development, when Dpp is required to keep AMPs undifferentiated (Mathur et al., 2010). Recently, the first study investigating the role of BMP/Dpp signaling in *Drosophila* adult intestinal homeostasis (Li et al., 2013b) showed that loss of BMP/Dpp signaling from the ECs results in ISC proliferation mediated via the ectopic activation of EGFs (*spi* in the ISCs, EBs, ECs, and the VM; and *vn* in the VM). Interestingly, expression of the Dpp ligand is found in tracheal cells and trachea-specific *dpp* RNAi knockdown leads to reduced BMP/Dpp activity in the intestinal epithelium concurrent with increased ISC proliferation suggesting that trachea-derived Dpp is necessary for midgut homeostasis by counteracting stress factors and protecting ECs from apoptosis (Li et al., 2013b).

Interestingly, two additional studies investigating the role of Dpp in intestinal maintenance and regeneration arrived to different conclusions. Guo et al. (2013) report regional differences in *dpp* expression: strong *dpp* in the circular VM of the middle midgut, highly variable *dpp* in the circular VM of the anterior and posterior midgut, and *dpp* expression in the intestinal trachea of unchallenged flies, whereas Li et al. (2013a) report regional graded *dpp* expression in ECs of the middle midgut, but not in the VM or the trachea. Nevertheless, both studies agree that paracrine Dpp acts on ISCs of the middle midgut (the source of the ligand may be both the VM and the ECs) and is necessary and sufficient for the differentiation of specialized midgut ECs, the copper cells (Guo et al., 2013; Li et al., 2013a). Furthermore, intestinal inflammation caused by bleomycin or paraquat induces

dpp strongly along the midgut in the VM and trachea and leads to BMP/Dpp signaling activation in most ECs and ISCs (Guo et al., 2013). Using highly VM-specific drivers to knockdown *dpp*, Guo et al. (2013) observed strong reduction of BMP/Dpp activity in the midgut suggesting that VM-derived *dpp* is required to induce and maintain the BMP/Dpp signaling. Intriguingly, inactivating *dpp* by RNAi in the VM, but not in the trachea, impaired BMP/Dpp activity in ISCs and led to their proliferation, whereas the proliferative effect observed by depleting downstream components of the BMP/Dpp pathway in ECs (Li et al., 2013b) could not be reproduced (Guo et al., 2013). Since Li et al. (2013b) aged the flies significantly to assess the effects of trachea-specific Dpp knockdown, and the aging intestine exhibits increased intestinal regeneration (Biteau et al., 2008), the age of the flies might have contributed to the observed discrepancies. Furthermore, differences in the genetic background, the diet and the intestinal microbiota of the flies maintained in different laboratories could have also contributed.

EPIDERMAL INJURY, DISTANT FROM THE INTESTINE, INDUCES INTESTINAL REGENERATION

Intriguing recent findings by Takeishi et al. (2013) indicate that aseptic trauma of the adult epidermis induces a systemic wound response that causes renewal of the intestinal epithelium necessary for survival. Specifically, wounding induces ROS in the ECs, followed by caspase-dependent EC apoptosis, which leads to *upd3* activation, ISC proliferation and intestinal regeneration. If caspase activity is blocked in the ECs, regeneration is inhibited and the flies succumb to the trauma leading the authors to suggest that EC apoptosis is essential to counteract a lethal factor present in the hemolymph of wounded flies. Although the nature of the lethal factor remains unknown, it seems that the intestinal response to epidermal injury acts in parallel to ROS-mediated neuronal JNK activation that protects the organism from trauma (Nam et al., 2012).

CONCLUSIONS-PERSPECTIVES

Although the role of the intestinal nervous system in regenerative inflammatory signaling remains unclear, accumulating evidence in *Drosophila* suggests a key function of the intestinal neurons in physiology. Parallel to systemic signals, gut-specific innervations regulate food intake, fluid and ion balance, as well as physiological intestinal responses triggered by diet or internal metabolic changes (Cognigni et al., 2011). Strikingly, nutrient- and oxygen-responsive neurons, through insulin- and VIP-like peptides, regulate the growth and plasticity of the intestinal tracheal system (Linneweber et al., 2014). Therefore, the nervous system, the trachea and the intestine are intimately connected to maintain physiological homeostasis. Since infection and tumorigenesis affect gut physiology and excretion in *Drosophila* (Apidianakis et al., 2009), it will be interesting to test if the intestinal neurons are implicated in regeneration.

An emerging theme in intestinal regeneration of both *Drosophila* and mammals is the interplay of different signaling pathways that coordinate ISC activity during physiological and regenerative homeostasis. Strikingly, regulatory signals exchanged between the epithelium and surrounding tissues control intestinal maintenance. In *Drosophila*, homeostasis, physiology and regenerative inflammatory signaling are regulated by signals secreted from the intestinal VM (Wnt/Wg, IL6/Upds, EGFs, insulin-like peptides, TGF-beta/Dpp), the trachea (TGF-beta/Dpp) and the neurons (insulin-like peptides, neuropeptides). In mammals epithelial-mesenchymal interactions involving Hh, PDGF, and BMP signaling drive the modeling of the epithelium (Crosnier et al., 2006). Paneth cells, which constitute part of the intestinal niche, express essential regulatory signals, like EGF, TGF-a, Wnt3 or Delta-like-4, which directly control ISC proliferation (Sato et al., 2009, 2011), and stromal cells secrete IL6 (Rigby et al., 2007; Grivennikov et al., 2009; Jiang and Edgar, 2012). In addition, the intestinal subepithelial myofibroblasts, which ensheath the intestinal epithelial cells and closely contact the enteric neurons, express IL23, Wnts and VEGF during inflammation (Andoh et al., 2007), the gut immune cells communicate with intestinal neurons during inflammation (Buhner and Schemann, 2012) to often cause changes in their morphology and density that relate to pathophysiology of the disease, i.e., pain (Demir et al., 2013). Finally, the intestinal blood vessels change their morphology in response to inflammatory signals (Cromer et al., 2011). These observations further underscore the signaling homologies between *Drosophila* and mammals in intestinal homeostasis and regenerative inflammation. Clearly, studies in the *Drosophila* intestinal system will broaden our understanding of tissue communication in mammalian homeostasis.

ACKNOWLEDGMENTS

Our work is funded by FP7-PEOPLE-Marie Curie CIG 303727 and the Fondation Santé.

REFERENCES

Amcheslavsky, A., Ito, N., Jiang, J., and Ip, Y. T. (2011). Tuberous sclerosis complex and Myc coordinate the growth and division of *Drosophila* intestinal stem cells. *J. Cell Biol.* 695–710. doi: 10.1083/jcb.201103018

Amcheslavsky, A., Jiang, J., and Ip, Y. T. (2009). Tissue damage-induced intestinal stem cell division in *Drosophila*. *Cell Stem Cell* 4. 49–61. doi: 10.1016/j.stem.2008.10.016

Andoh, A., Bamba, S., Brittan, M., Fujiyama, Y., and Wright, N. A. (2007). Role of intestinal subepithelial myofibroblasts in inflammation and regenerative response in the gut. *Pharmacol. Ther.* 114, 94–106. doi: 10.1016/j.pharmthera.2006.12.004

Apidianakis, Y., Pitsouli, C., Perrimon, N., and Rahme, L. (2009). Synergy between bacterial infection and genetic predisposition in intestinal dysplasia. *Proc. Natl. Acad. Sci. U.S.A.* 106, 20883–20888. doi: 10.1073/pnas.0911797106

Apidianakis, Y., and Rahme, L. G. (2011). *Drosophila melanogaster* as a model for human intestinal infection and pathology. *Dis. Model Mech.* 4, 21–30. doi: 10.1242/dmm.003970

Bayliss, W. M., and Starling, E. H. (1899). The movements and innervation of the small intestine. *J. Physiol.* 24, 99–143.

Biteau, B., Hochmuth, C. E., and Jasper, H. (2008). JNK activity in somatic stem cells causes loss of tissue homeostasis in the aging *Drosophila* gut. *Cell Stem Cell* 3, 442–455. doi: 10.1016/j.stem.2008.07.024

Biteau, B., Hochmuth, C. E., and Jasper, H. (2011). Maintaining tissue homeostasis: dynamic control of somatic stem cell activity. *Cell Stem Cell* 9, 402–411. doi: 10.1016/j.stem.2011.10.004

Biteau, B., and Jasper, H. (2011). EGF signaling regulates the proliferation of intestinal stem cells in *Drosophila*. *Development* 138, 1045–1055. doi: 10.1242/dev.056671

Bond, D., and Foley, E. (2012). Autocrine platelet-derived growth factor-vascular endothelial growth factor receptor-related (Pvr) pathway activity controls

intestinal stem cell proliferation in the adult *Drosophila* midgut. *J. Biol. Chem.* 287, 27359–27370. doi: 10.1074/jbc.M112.378018

Buchon, N., Broderick, N. A., Chakrabarti, S., and Lemaitre, B. (2009a). Invasive and indigenous microbiota impact intestinal stem cell activity through multiple pathways in *Drosophila*. *Genes Dev.* 23, 2333–2344. doi: 10.1101/gad.1827009

Buchon, N., Broderick, N. A., Kuraishi, T., and Lemaitre, B. (2010). *Drosophila* EGFR pathway coordinates stem cell proliferation and gut remodeling following infection. *BMC Biol.* 8:152. doi: 10.1186/1741-7007-8-152

Buchon, N., Broderick, N. A., Poidevin, M., Pradervand, S., and Lemaitre, B. (2009b). *Drosophila* intestinal response to bacterial infection: activation of host defense and stem cell proliferation. *Cell Host Microbe* 5, 200–211. doi: 10.1016/j.chom.2009.01.003

Buhner, S., and Schemann, M. (2012). Mast cell-nerve axis with a focus on the human gut. *Biochim. Biophys. Acta* 1822, 85–92. doi: 10.1016/j.bbadis.2011.06.004

Choi, N. H., Kim, J. G., Yang, D. J., Kim, Y. S., and Yoo, M. A. (2008). Age-related changes in *Drosophila* midgut are associated with PVF2, a PDGF/VEGF-like growth factor. *Aging Cell* 7, 318–334. doi: 10.1111/j.1474-9726.2008.00380.x

Choi, N. H., Lucchetta, E., and Ohlstein, B. (2011). Nonautonomous regulation of *Drosophila* midgut stem cell proliferation by the insulin-signaling pathway. *Proc. Natl. Acad. Sci. U.S.A.* 108, 18702–18707. doi: 10.1073/pnas.1109348108

Cognigni, P., Bailey, A. P., and Miguel-Aliaga, I. (2011). Enteric neurons and systemic signals couple nutritional and reproductive status with intestinal homeostasis. *Cell Metab.* 13, 92–104. doi: 10.1016/j.cmet.2010.12.010

Cordero, J. B., Stefanatos, R. K., Myant, K., Vidal, M., and Sansom, O. J. (2012a). Non-autonomous crosstalk between the Jak/Stat and Egfr pathways mediates Apc1-driven intestinal stem cell hyperplasia in the *Drosophila* adult midgut. *Development* 139, 4524–4535. doi: 10.1242/dev.078261

Cordero, J. B., Stefanatos, R. K., Scopelliti, A., Vidal, M., and Sansom, O. J. (2012b). Inducible progenitor-derived Wingless regulates adult midgut regeneration in *Drosophila*. *EMBO J.* 31, 3901–3917. doi: 10.1038/emboj.2012.248

Cromer, W. E., Mathis, J. M., Granger, D. N., Chaitanya, G. V., and Alexander, J. S. (2011). Role of the endothelium in inflammatory bowel diseases. *World J. Gastroenterol.* 17, 578–593. doi: 10.3748/wjg.v17.i5.578

Cronin, S. J., Nehme, N. T., Limmer, S., Liegeois, S., Pospisilik, J. A., Schramek, D., et al. (2009). Genome-wide RNAi screen identifies genes involved in intestinal pathogenic bacterial infection. *Science* 325, 340–343. doi: 10.1126/science.1173164

Crosnier, C., Stamataki, D., and Lewis, J. (2006). Organizing cell renewal in the intestine: stem cells, signals and combinatorial control. *Nat. Rev. Genet.* 7, 349–359. doi: 10.1038/nrg1840

Demerec, M. (1950). *Biology of Drosophila*. New York, NY: Wiley & Sons, Inc., CSHL Press.

Demir, I. E., Schafer, K. H., Tieftrunk, E., Friess, H., and Ceyhan, G. O. (2013). Neural plasticity in the gastrointestinal tract: chronic inflammation, neurotrophic signals, and hypersensitivity. *Acta Neuropathol.* 125, 491–509. doi: 10.1007/s00401-013-1099-4

Grivennikov, S., Karin, E., Terzic, J., Mucida, D., Yu, G. Y., Vallabhapurapu, S., et al. (2009). IL-6 and Stat3 are required for survival of intestinal epithelial cells and development of colitis-associated cancer. *Cancer Cell* 15, 103–113. doi: 10.1016/j.ccr.2009.01.001

Guo, Z., Driver, I., and Ohlstein, B. (2013). Injury-induced BMP signaling negatively regulates *Drosophila* midgut homeostasis. *J. Cell Biol.* 201, 945–961. doi: 10.1083/jcb.201302049

Jiang, H., and Edgar, B. A. (2009). EGFR signaling regulates the proliferation of *Drosophila* adult midgut progenitors. *Development* 136, 483–493. doi: 10.1242/dev.026955

Jiang, H., and Edgar, B. A. (2011). Intestinal stem cells in the adult *Drosophila* midgut. *Exp. Cell Res.* 317, 2780–2788. doi: 10.1016/j.yexcr.2011.07.020

Jiang, H., and Edgar, B. A. (2012). Intestinal stem cell function in *Drosophila* and mice. *Curr. Opin. Genet. Dev.* 22, 354–360. doi: 10.1016/j.gde.2012.04.002

Jiang, H., Grenley, M. O., Bravo, M. J., Blumhagen, R. Z., and Edgar, B. A. (2011). EGFR/Ras/MAPK signaling mediates adult midgut epithelial homeostasis and regeneration in *Drosophila*. *Cell Stem Cell* 8, 84–95. doi: 10.1016/j.stem.2010.11.026

Karpowicz, P., Perez, J., and Perrimon, N. (2010). The Hippo tumor suppressor pathway regulates intestinal stem cell regeneration. *Development* 137, 4135–4145. doi: 10.1242/dev.060483

Kuraishi, T., Binggeli, O., Opota, O., Buchon, N., and Lemaitre, B. (2011). Genetic evidence for a protective role of the peritrophic matrix against intestinal bacterial infection in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. U.S.A.* 108, 15966–15971. doi: 10.1073/pnas.1105994108

Lee, W. C., Beebe, K., Sudmeier, L., and Micchelli, C. A. (2009). *Adenomatous polyposis coli* regulates *Drosophila* intestinal stem cell proliferation. *Development* 136, 2255–2264. doi: 10.1242/dev.035196

Li, H., Qi, Y., and Jasper, H. (2013a). Dpp signaling determines regional stem cell identity in the regenerating adult *Drosophila* gastrointestinal tract *Cell Rep.* 4, 10–18. doi: 10.1016/j.celrep.2013.05.040

Li, Z., Zhang, Y., Han, L., Shi, L., and Lin, X. (2013b). Trachea-derived *dpp* controls adult midgut homeostasis in *Drosophila*. *Dev. Cell* 24, 133–143. doi: 10.1016/j.devcel.2012.12.010

Lin, G., Xu, N., and Xi, R. (2008). Paracrine Wingless signalling controls self-renewal of *Drosophila* intestinal stem cells. *Nature* 455, 1119–1123. doi: 10.1038/nature07329

Lin, G., Xu, N., and Xi, R. (2010). Paracrine *unpaired* signaling through the JAK/STAT pathway controls self-renewal and lineage differentiation of *Drosophila* intestinal stem cells. *J. Mol. Cell Biol.* 2, 37–49. doi: 10.1093/jmcb/mjp028

Linneweber, G. A., Jacobson, J., Busch, K. E., Hudry, B., Christov, C. P., Dormann, D., et al. (2014). Neuronal control of metabolism through nutrient-dependent modulation of tracheal branching. *Cell* 156, 69–83. doi: 10.1016/j.cell.2013.12.008

Mathur, D., Bost, A., Driver, I., and Ohlstein, B. (2010). A transient niche regulates the specification of *Drosophila* intestinal stem cells. *Science* 327, 210–213. doi: 10.1126/science.1181958

Micchelli, C. A., and Perrimon, N. (2006), Evidence that stem cells reside in the adult *Drosophila* midgut epithelium. *Nature* 439, 475–479. doi: 10.1038/nature04371

Nam, H. J., Jang, I. H., You, H., Lee, K. A., and Lee, W. J. (2012). Genetic evidence of a redox-dependent systemic wound response via hayan protease-phenoloxidase system in *Drosophila*. *EMBO J.* 31, 1253–1265. doi: 10.1038/emboj.2011.476

Nardi, J. B., Bee, C. M., Miller, L. A., Mathur, D., and Ohlstein, B. (2011). Cell renewal in adjoining intestinal and tracheal epithelia of *Manduca*. *J. Insect Physiol.* 57, 487–493. doi: 10.1016/j.jinsphys.2011.01.008

O'Brien, L. E., Soliman, S. S., Li, X., and Bilder, D. (2011). Altered modes of stem cell division drive adaptive intestinal growth. *Cell* 147, 603–614. doi: 10.1016/j.cell.2011.08.048

Ohlstein, B., and Spradling, A. (2006). The adult *Drosophila* posterior midgut is maintained by pluripotent stem cells. *Nature* 439, 470–474. doi: 10.1038/nature04333

Ohlstein, B., and Spradling, A. (2007). Multipotent *Drosophila* intestinal stem cells specify daughter cell fates by differential Notch signaling. *Science* 315, 988–992. doi: 10.1126/science.1136606

Osman, D., Buchon, N., Chakrabarti, S., Huang, Y. T., Su, W. C., Poidevin, M., et al. (2012). Autocrine and paracrine unpaired signaling regulate intestinal stem cell maintenance and division. *J. Cell Sci.* 125(Pt 24), 5944–5949. doi: 10.1242/jcs.113100

Panayidou, S., and Apidianakis, Y. (2013). Regenerative Inflammation: lessons from *Drosophila* intestinal epithelium in health and disease. *Pathogens* 2, 209–231. doi: 10.3390/pathogens2020209

Perdigoto, C. N., Schweisguth, F., and Bardin, A. (2011). Distinct levels of Notch activity for commitment and terminal differentiation of stem cells in the adult fly intestine. *Development* 138, 4585–4595. doi: 10.1242/dev.065292

Pitsouli, C., Apidianakis, Y., and Perrimon, N. (2009). Homeostasis in infected epithelia: stem cells take the lead. *Cell Host Microbe* 6, 301–307. doi: 10.1016/j.chom.2009.10.001

Ren, F., Wang, B., Yue, T., Yun, E. Y., Ip, Y. T., and Jiang, J. (2010). Hippo signaling regulates *Drosophila* intestine stem cell proliferation through multiple pathways. *Proc. Natl. Acad. Sci. U.S.A.* 107, 21064–21069. doi: 10.1073/pnas.1012759107

Rigby, R. J., Simmons, J. G., Greenhalgh, C. J., Alexander, W. S., and Lund, P. K. (2007). Suppressor of cytokine signaling 3 (SOCS3) limits damage-induced crypt hyper-proliferation and inflammation-associated tumorigenesis in the colon. *Oncogene* 26, 4833–4841. doi: 10.1038/sj.onc.1210286

Sato, T., van Es, J. H., Snippert, H. J., Stange, D. E., Vries, R. G., van den Born, M., et al. (2011). Paneth cells constitute the niche for Lgr5 stem cells in intestinal crypts. *Nature* 469, 415–418. doi: 10.1038/nature09637

Sato, T., Vries, R. G., Snippert, H. J., van de Wetering, M., Barker, N., Stange, D. E., et al. (2009). Single Lgr5 stem cells build crypt-villus structures *in vitro* without a mesenchymal niche. *Nature* 459, 262–265. doi: 10.1038/nature07935

Shaw, R. L., Kohlmaier, A., Polesello, C., Veelken, C., Edgar, B. A., and Tapon, N. (2010). The Hippo pathway regulates intestinal stem cell proliferation during *Drosophila* adult midgut regeneration. *Development* 137, 4147–4158. doi: 10.1242/dev.052506

Staley, B. K., and Irvine, K. D. (2012). Hippo signaling in *Drosophila*: recent advances and insights. *Dev. Dyn.* 241, 3–15. doi: 10.1002/dvdy.22723

Takeishi, A., Kuranga, E., Tonoki, A., Misaki, K., Yonemura, S., Kanuka, H., et al. (2013). Homeostatic epithelial renewal in the gut is required for dampening a fatal systemic wound response in *Drosophila*. *Cell Rep.* 3, 919–930. doi: 10.1016/j.celrep.2013.02.022

Veenstra, J. A., Agricola, H. J., and Sellami, A. (2008). Regulatory peptides in fruit fly midgut. *Cell Tissue Res.* 334, 499–516. doi: 10.1007/s00441-008-0708-3

Xu, N., Wang, S. Q., Tan, D., Gao, Y., Lin, G., and Xi, R. (2011). EGFR, Wingless and JAK/STAT signaling cooperatively maintain *Drosophila* intestinal stem cells. *Dev. Biol.* 354, 31–43. doi: 10.1016/j.ydbio.2011.03.018

Zhou, F., Rasmussen, A., Lee, S., and Agaisse, H. (2013). The UPD3 cytokine couples environmental challenge and intestinal stem cell division through modulation of JAK/STAT signaling in the stem cell microenvironment. *Dev. Biol.* 373, 383–393. doi: 10.1016/j.ydbio.2012.10.023

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 30 October 2013; accepted: 04 April 2014; published online: 24 April 2014.
Citation: Kux K and Pitsouli C (2014) Tissue communication in regenerative inflammatory signaling: lessons from the fly gut. Front. Cell. Infect. Microbiol. ***4****:49. doi: 10.3389/fcimb.2014.00049*
This article was submitted to the journal Frontiers in Cellular and Infection Microbiology.

frontiers in
CELLULAR AND INFECTION MICROBIOLOGY

OPINION ARTICLE
published: 19 November 2013
doi: 10.3389/fcimb.2013.00082

Defining the interorgan communication network: systemic coordination of organismal cellular processes under homeostasis and localized stress

Ilia A. Droujinine[1]* and Norbert Perrimon[1,2]*

[1] *Department of Genetics, Harvard Medical School, Boston, MA, USA*
[2] *Howard Hughes Medical Institute, Boston, MA, USA*
**Correspondence: perrimon@receptor.med.harvard.edu; droujinine@g.harvard.edu*

Edited by:
Yiorgos Apidianakis, University of Cyprus, Cyprus

Reviewed by:
Chrysoula Pitsouli, University of Cyprus, Cyprus

Keywords: interorgan communication network, non-cell autonomous signaling, systemic disease, organismal homeostasis, systemic stress response

Following the acquisition of multicellularity, organisms with increasing levels of specialized cells, tissues, and organs emerged during evolution. To coordinate specialized organs, long-distance interorgan communication systems appeared. The central nervous system evolved to regulate many organ behaviors, using hormones or neurons. In addition, organs developed systems to directly communicate their states to one another. This is illustrated by the lack of nervous systems in plants and simple animals like sponges, which can perform complex systemic functions (Lough and Lucas, 2006; Srivastava et al., 2010).

Developmental or homeostatic events within cells or tissues have been extensively studied. For example, maintenance of the integrity of the *Drosophila* gut involves stem cell proliferation and differentiation, partially driven by local JAK/STAT, EGF, MAPK, and Wnt signaling (Panayidou and Apidianakis, 2013). Recently, it has become clear that individual organs themselves are also able to communicate their states. However, the nature of the interorgan signaling mechanisms remains largely a mystery.

Here, we review the emerging data supporting the existence of a vast interorgan communication network (ICN). The ICN is the network of peptides, proteins, and metabolites that act between organs to coordinate essential and specialized cellular processes under homeostasis and stress (**Figure 1**). We propose that studies in *Drosophila*, where, unlike in mammals, biochemical studies can be combined with genome-wide *in vivo* tissue-specific genetic screens, are poised to identify many ICN components. Characterization of the ICN will further understanding of systemic diseases such as cancer-associated muscle cachexia.

FUNCTION OF THE ICN: SYSTEMIC INTEGRATION OF HOMEOSTASIS

A limited number of studies in mammals, *C. elegans*, and *Drosophila* showed that perturbed tissues affect organismal growth and metabolism via largely unknown signals. The *Drosophila* fat-body (liver and adipose functional equivalent) responds to dietary signals by releasing factors affecting insulin secretion, growth, and metabolism (Britton and Edgar, 1998; Colombani et al., 2003; Géminard et al., 2009). For instance, in response to high dietary fat and sugar, the fat-body-derived leptin-like factor Unpaired-2 systemically controls release of insulin from insulin-producing cells in the brain (Rajan and Perrimon, 2012). Further, unknown nutrition-dependent signals control intestinal, neural, and germline stem cell division through local or systemic insulin signaling (LaFever and Drummond-Barbosa, 2005; Chell and Brand, 2010; O'Brien et al., 2011; Sousa-Nunes et al., 2011). Also, localized organ growth perturbations delay systemic development via inhibition of insulin signaling (DiAngelo et al., 2009), and insulin (Karpac et al., 2011) and ecdysteroid synthesis, partially through insulin-like Dilp8 (Colombani et al., 2012; Garelli et al., 2012).

In mammals, leptin is secreted by adipose tissue with nutritional surplus, controlling the neuroendocrine system (Zhang et al., 1994; Ahima et al., 1996). Also, exercise and muscle overexpression of PGC1-α increases the production of the secreted factor Irisin, a fragment of the transmembrane protein FNDC5, which stimulates metabolism and fat browning (Böstrom et al., 2012). Moreover, exercising muscle secretes interleukin-6 (Steensberg et al., 2000), possibly regulating systemic glucose and lipid metabolism by acting on muscle, liver, fat, intestinal L-cells, and pancreatic alpha-cells (Febbraio et al., 2004; Petersen et al., 2005; Ellingsgaard et al., 2011; Pedersen, 2011; Pedersen and Febbraio, 2012). Interestingly, liver or muscle autophagy controls whole-body glucose and fatty-acid metabolism, partially through FGF-21 (Kim et al., 2013). Finally, a number of gut-derived hormones including gastrin, ghrelin, cholecystokinin, glucagon-like peptide-1, and others affect insulin secretion, systemic fatty-acid metabolism, and feeding (Drucker, 2007). Strikingly, metabolic control is conserved, as leptin can rescue *Drosophila* Unpaired-2 deficiency, and both function through similar neuronal circuits (Vong et al., 2011; Rajan and Perrimon, 2012).

Intracellular pathways induce factors which regulate aging, stress resistance, and distal cellular functions. In *C. elegans*, germ-line absence extends life-span (Arantes-Oliveira et al., 2002) and causes systemic proteasomal activity increase, via unknown signals (Vilchez et al., 2012). In addition, tissue-specific induction of mitochondrial (Durieux et al., 2011), cytoplasmic (van Oosten-Hawle et al., 2013),

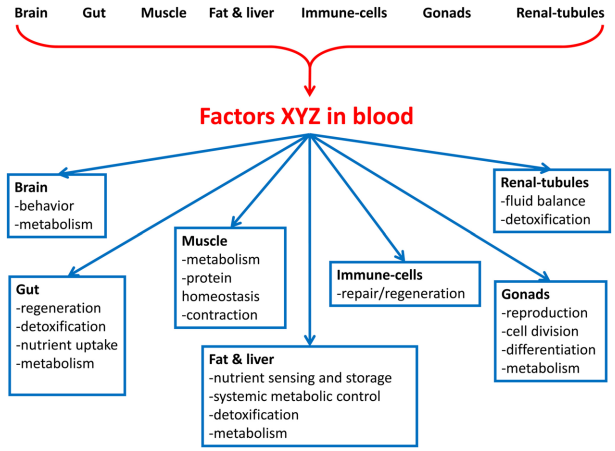


FIGURE 1 | Overview of the interorgan communication network (ICN). The ICN is the network of peptides, proteins, and metabolites that act between organs to coordinate organismal cellular processes under homeostasis and stress. Organs in the body secrete factors that act to influence the physiology of cells in distal organs. Processes that may be connected between organs include aging, protein homeostasis, nutrient uptake, metabolism, cell division, cell movement, detoxification, organelle biogenesis, and secretion of local and systemic signals. The signals may be nutrients, wastes, toxins, metabolites, nucleic acids, proteins, and peptides.

and endoplasmic reticulum (ER; Taylor and Dillin, 2013) unfolded protein responses result in their systemic propagation, via poorly characterized factors. Neurotransmitter signaling partially mediates ER stress (Taylor and Dillin, 2013), but not heat-shock response propagation (van Oosten-Hawle et al., 2013). Moreover, systemic signaling to the brain causes behavioral avoidance of the stress-inducer (Melo and Ruvkun, 2012).

In *Drosophila*, gut, muscles, and fat-body are essential in stress resistance and aging. Gut infection or oxidative stress induces fat-body anti-microbial peptide secretion via unknown mechanisms (Foley and O'Farrell, 2003; Wu et al., 2012). Fat-body overexpression of FOXO transcription factor increases lifespan (Giannakou et al., 2004). Moreover, adult muscle-specific overexpression of FOXO prevents aging of other organs by decreasing accumulation of protein aggregates and increasing autophagy (Demontis and Perrimon, 2010). In addition, activation of muscle TOR or p38-MAPK signaling controls systemic aging and stress resistance (Vrailas-Mortimer et al., 2011). Also, muscle fatty-acid metabolism is essential for lifespan-increasing effects of dietary restriction (Katewa et al., 2012). Moreover, maintenance of gut homeostasis by stem-cell expression of PGC-1 or FOXO targets improves lifespan and metabolic homeostasis (Biteau et al., 2010; Rera et al., 2011).

Also, exposure of old mice to young blood results in restoration of muscle and liver regeneration, suggesting that systemic factors control aging (Conboy et al., 2005). For example, GDF-11 is a BMP ligand which slows myocardial aging through unknown mechanisms (Loffredo et al., 2013). Interestingly, TGF-β has been implicated in regulating reactive oxygen species production in the aorta, endothelial structure, blood-pressure, and cardiomyocyte function (Buday et al., 2010).

Systemic factors also control cell proliferation and tissue regeneration. In *Drosophila*, distal wounds control gut proliferative homeostasis via unknown mechanisms (Takeishi et al., 2013). Moreover, insulin regulates intestinal stem-cell proliferation (Amcheslavsky et al., 2009; Choi et al., 2011). In mammals, muscle from dystrophin-mutant mice may remotely alter wound healing (Straino et al., 2004). Also, liver-secreted betatrophin controls pancreatic beta-cell proliferation (Yi et al., 2013).

Unknown factors may also be controlled by reproduction. In insects, mating and fertilization induces numerous uncharacterized transcriptional changes in multiple organs (Rogers et al., 2008; Avila et al., 2011). In *Drosophila* females, mating increases mating receptivity, feeding, and egg-laying; changes movement; and decreases lifespan (Fowler and Partridge, 1988; Barnes et al., 2008; Avila et al., 2011). Some changes are associated with transfer of male accessory gland peptides (e.g., sex peptide) to females (Wigby and Chapman, 2005; Carvalho et al., 2006). Conversely, systemic factors may control reproduction. For instance, in *Drosophila*, insulin controls female germline stem cell proliferation (LaFever and Drummond-Barbosa, 2005). In *C. elegans*, oocyte and germline maintenance during aging is regulated by TGF-β and insulin via unknown relay signals (Luo et al., 2010).

In addition, systemic factors may regulate offspring fitness. In mice, paternal diet influences offspring metabolism (Carone et al., 2010; Ng et al., 2010). Moreover, the injury of fathers' and grandfathers' livers increases the regenerative capacity of their offspring's livers (Zeybel et al., 2012). Similarly, in *Drosophila*, tissue-specific stress causes heritable developmental alterations (Stern et al., 2012).

Finally, because alterations in its composition influence systemic physiology (e.g., metabolism; Claus et al., 2008), the microbiome is part of the ICN. For instance, obesity-induced changes in gut microbiome increase systemic deoxycholic acid that acts as a liver DNA-damaging and cancer-promoting agent (Yoshimoto et al., 2013).

In conclusion, there is growing evidence that many organismal functions mediate various aspects of interorgan communication through secreted factors. Understanding the roles of these factors, and how their activities are integrated to the organism's functions is the next big challenge. Further, as systematic screens have not been performed for such factors, it is likely that many additional ones remain to be identified.

STRUCTURE OF THE ICN

Gene-expression analyses of organs have shown the existence of organ-to-organ coexpression networks that change in disease and aging, suggesting of unexplored interorgan processes and common responses of tissues to systemic factors (Keller et al., 2008; Dobrin et al., 2009; Huang et al., 2011). These analyses

revealed that at least 40% of the interorgan features are not in single-tissue networks, and that the highly connected genes in the interorgan networks are poorly connected in the single-tissue networks (Dobrin et al., 2009).

What are the factors/nodes that connect the organs/hubs in the ICN? At their simplest and most evolutionary ancient form, signals may be nutrients, wastes, toxins, or metabolites. For instance, liver-produced beta-hydroxybutyrate inhibits histone deacetylases (Shimazu et al., 2013). Communication may also be in the form of circulating nucleic acids (e.g., miRNAs; Mitchell et al., 2008). Finally, proteins and peptides may be classical developmental regulators or novel. Intriguingly, "intracellular" proteins can be secreted outside the cell, as an isoform containing a signal sequence (e.g., PTEN-long; Hopkins et al., 2013), or through non-classical secretion (e.g., aP2; Cao et al., 2013)

An important feature that differentiates local tissue and developmental networks from the ICN, is the large distance over which signaling acts, meaning that concentration and specificity of the factors could be lower. To remedy this, a dense network of closely acting factors could exist, such that one factor acts on a neighboring tissue, which secretes a relay signal. Alternatively, signals may be carried along "molecular tracks" to their destination. These may be blood vessels or tissue regions containing "guidance factors"—putative weak affinity receptors to common structural features to groups of secreted factors. In addition, binding proteins (Mantovani et al., 2001) or proteases may be secreted to modulate local or systemic signaling. For example, *Drosophila* insulin-binding proteins ImpL2 (Honegger et al., 2008) or secreted decoy of insulin (Okamoto et al., 2013) bind to and inhibit insulin, locally or systemically. The mammalian ImpL2 homologs, insulin-like growth factor (IGF) binding proteins transport and regulate IGFs (Hwa et al., 1999; Honegger et al., 2008).

Factors may also be modified with fatty-acids, cholesterol, or glycans, regulating their stability, transport (Nusse, 2003; Linder and Deschenes, 2007; Moremen et al., 2012), and interaction with abundant and stable components including apolipoproteins (Panáková et al., 2005). These molecules can then deliver factors to target organs. For example, Hedgehog can be lipidated, interact with apoliproteins, and act distally (Palm et al., 2013). Finally, signaling can occur extracellularly through protease cascades (e.g., *Drosophila* spatzle-Toll; Morisato and Anderson, 1994) or phosphorylation (Yalak and Vogel, 2012).

ICNs IN HUMAN BIOLOGY AND DISEASE

Elucidation of the ICN will be valuable for disease biology. Many disorders begin locally, and ultimately involve the entire organism by affecting behavior, cell recruitment, metabolism, proliferation, and activation (McCance and Huether, 2002). For example, muscle defects are associated with alterations in wound healing (Straino et al., 2004), regeneration, hepatocyte proliferation (Conboy et al., 2005), dyslipidemia, hypertension, type 2 diabetes, cardiovascular diseases, cancer, Alzheimer's and Parkinson's diseases (Pedersen, 2011). Moreover, cachexia, wound-healing, and hematopoiesis defects occur in cancer (Devereux et al., 1979; Egeblad et al., 2010).

Also, organ failure patients who receive organ function replacement therapy eventually succumb to disease, with systemic defects. For instance, kidney failure patients receiving kidney function replacement hemodialysis suffer from malnutrition and lung defects (McCance and Huether, 2002; Doi et al., 2011; White et al., 2011). This suggests that organs have essential functions beyond their "classic" roles, for example, by regulating distal organs through secreted factors. Importantly, blood-borne signals mediate critical systemic homeostatic adjustments from local perturbations, illustrated by control of systemic physiology by electrical cycling of paralyzed muscles in spinal-cord injured tetraplegic humans (Kjaer et al., 1996; Pedersen, 2011).

CONCLUSIONS

Great strides are being made toward understanding intracellular and tissue homeostasis. The next step is to understand the structure, function, and components of the ICN. The main questions are the nature of the interorgan communication factors and their roles in maintaining whole-organism homeostasis. Also, how does the ICN change during development, aging, and disease? The current transcriptomic, proteomic, metabolomic, and genome-wide tissue-specific genetic manipulation technologies will allow answering these questions. Importantly, systematic *in vivo* identification of systemic factors is impractical in mammals. Thus, the ICN may be constructed for *Drosophila*, for which all of the above tools are available, and applied to mammals. Thus, "organ-sensing" RNAi screens can now be done, where genes are inactivated by tissue-specific RNAi, and function of another organ is assessed. Within the next decade, we expect a surge of interest to define the structure and function of the ICN.

ACKNOWLEDGMENTS

Ilia A. Droujinine is in part supported by NSERC PGS-D. We thank Akhila Rajan and Edward Owusu-Ansah for their insightful comments on the manuscript.

REFERENCES

Ahima, R. S., Prabakaran, D., Mantzoros, C., Qu, D., Lowell, B., Maratos-Flier, E., et al. (1996). Role of leptin in the neuroendocrine response to fasting. *Nature* 382, 250–252. doi: 10.1038/382250a0

Amcheslavsky, A., Jiang, J., and Ip, Y. T. (2009). Tissue damage-induced intestinal stem cell division in *Drosophila*. *Cell Stem Cell* 4, 49–61. doi: 10.1016/j.stem.2008.10.016

Arantes-Oliveira, N., Apfeld, J., Dillin, A., and Kenyon, C. (2002). Regulation of life-span by germ-line stem cells in *Caenorhabditis elegans*. *Science* 295, 502–505. doi: 10.1126/science.1065768

Avila, F. W., Sirot, L. K., LaFlamme, B. A., Rubinstein, C. D., and Wolfner, M. F. (2011). Insect seminal fluid proteins: identification and function. *Annu. Rev. Entomol.* 56, 21–40. doi: 10.1146/annurev-ento-120709-144823

Barnes, A. I., Wigby, S., Boone, J. M., Partridge, L., and Chapman, T. (2008). Feeding, fecundity and lifespan in female *Drosophila melanogaster*. *Proc. Biol. Sci.* 275, 1675–1683. doi: 10.1098/rspb.2008.0139

Biteau, B., Karpac, J., Supoyo, S., DeGennaro, M., Lehmann, R., and Jasper, H. (2010). Lifespan extension by preserving proliferative homeostasis in *Drosophila*. *PLoS Genet.* 6:e1001159. doi:10.1371/journal.pgen.1001159

Böstrom, P., Wu, J., Jedrychowski, M. P., Korde, A., Ye, L., Lo, J. C., et al. (2012). A PGC1-α-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. *Nature* 481, 463–468. doi: 10.1038/nature10777

Britton, J. S., and Edgar, B. A. (1998). Environmental control of the cell cycle in *Drosophila*: nutrition activates mitotic and endoreplicative cells by distinct mechanisms. *Development* 125, 2149–2158.

Buday, A., Orsy, P., Godó, M., Mózes, M., Kökény, G., Lacza, Z., et al. (2010). Elevated systemic TGF-beta impairs aortic vasomotor function through activation of NADPH oxidase-driven superoxide production and leads to hypertension, myocardial remodeling, and increased plaque formation in apoE(-/-) mice. *Am. J. Physiol. Heart Circ. Physiol.* 299, H386–H389. doi: 10.1152/ajpheart.01042.2009

Cao, H., Sekiya, M., Ertunc, M. E., Burak, M. F., Mayers, J. R., White, A., et al. (2013). Adipocyte lipid chaperone AP2 is a secreted adipokine regulating hepatic glucose production. *Cell Metab.* 17, 768–778. doi: 10.1016/j.cmet.2013.04.012

Carone, B. R., Fauquier, L., Habib, N., Shea, J. M., Hart, C. E., Li, R., et al. (2010). Paternally induced transgenerational environmental reprogramming of metabolic gene expression in mammals. *Cell* 143, 1084–1096. doi: 10.1016/j.cell.2010.12.008

Carvalho, G. B., Kapahi, P., Anderson, D. J., and Benzer, S. (2006). Allocrine modulation of feeding behavior by the sex peptide of *Drosophila*. *Curr. Biol.* 16, 692–696. doi: 10.1016/j.cub.2006.02.064

Chell, J. M., and Brand, A. H. (2010). Nutrition-responsive glia control exit of neural stem cells from quiescence. *Cell* 143, 1161–1173. doi: 10.1016/j.cell.2010.12.007

Choi, N. H., Lucchetta, E., and Ohlstein, B. (2011). Nonautonomous regulation of *Drosophila* midgut stem cell proliferation by the insulin-signaling pathway. *Proc. Natl. Acad. Sci. U.S.A.* 108, 18702–18707. doi: 10.1073/pnas.1109348108

Claus, S. P., Tsang, T. M., Wang, Y., Cloarec, O., Skordi, E., Martin, F. -P., et al. (2008). Systemic multicompartmental effects of the gut microbiome on mouse metabolic phenotypes. *Mol. Syst. Biol.* 4, 219. doi: 10.1038/msb.2008.56

Colombani, J., Andersen, D. S., and Léopold, P. (2012). Secreted peptide Dilp8 coordinates *Drosophila* tissue growth with developmental timing. *Science* 336, 582–585. doi: 10.1126/science.1216689

Colombani, J., Raisin, S., Pantalacci, S., Radimerski, T., Montagne, J., and Léopold, P. (2003). A nutrient sensor mechanism controls *Drosophila* growth. *Cell* 114, 739–749. doi: 10.1016/S0092-8674(03)00713-X

Conboy, I. M., Conboy, M. J., Wagers, A. J., Girma, E. R., Weissman, I. L., and Rando, T. A. (2005). Rejuvenation of aged progenitor cells by exposure to a young systemic environment. *Nature* 433, 760–764. doi: 10.1038/nature03260

Demontis, F., and Perrimon, N. (2010). FOXO/4E-BP signaling in *Drosophila* muscles regulates organism-wide proteostasis during aging. *Cell* 143, 813–825. doi: 10.1016/j.cell.2010.10.007

Devereux, D. F., Thistlethwaite, P. A., Thibault, L. E., and Brennan, M. F. (1979). Effects of tumor bearing and protein depletion on wound breaking strength in the rat. *J. Surg. Res.* 27, 233–238. doi: 10.1016/0022-4804(79)90135-5

DiAngelo, J. R., Bland, M. L., Bambina, S., Cherry, S., and Birnbaum, M. J. (2009). The immune response attenuates growth and nutrient storage in *Drosophila* by reducing insulin signaling. *Proc. Natl. Acad. Sci. U.S.A.* 106, 20853–20858. doi: 10.1073/pnas.0906749106

Dobrin, R., Zhu, J., Molony, C., Argman, C., Parrish, M. L., Carlson, S., et al. (2009). Multi-tissue coexpression networks reveal unexpected subnetworks associated with disease. *Genome Biol.* 10, R55. doi: 10.1186/gb-2009–2010-5-r55

Doi, K., Ishizu, T., Fujita, T., and Noiri, E. (2011). Lung injury following acute kidney injury: kidney-lung crosstalk. *Clin. Exp. Nephrol.* 15, 464–470. doi: 10.1007/s10157-011-0459-4

Drucker, D. J. (2007). The role of gut hormones in glucose homeostasis. *J. Clin. Invest.* 117, 24–32. doi: 10.1172/JCI30076

Durieux, J., Wolff, S., and Dillin, A. (2011). The cell-non-autonomous nature of electron transport chain-mediated longevity. *Cell* 144, 79–91. doi: 10.1016/j.cell.2010.12.016

Egeblad, M., Nakasone, E. S., and Werb, Z. (2010). Tumors as organs: complex tissues that interface with the entire organism. *Dev. Cell* 18, 884–901. doi: 10.1016/j.devcel.2010.05.012

Ellingsgaard, H., Hauselmann, I., Schuler, B., Habib, A. M., Baggio, L. L., Meier, D. T., et al. (2011). Interleukin-6 enhances insulin secretion by increasing glucagon-like peptide-1 secretion from L cells and alpha cells. *Nat. Med.* 17, 1481–1489. doi: 10.1038/nm.2513

Febbraio, M. A., Hiscock, N., Sacchetti, M., Fischer, C. P., and Pedersen, B. K. (2004). Interleukin-6 is a novel factor mediating glucose homeostasis during skeletal muscle contraction. *Diabetes* 53, 1643–1648. doi: 10.2337/diabetes.53.7.1643

Foley, E., and O'Farrell, P. H. (2003). Nitric oxide contributes to induction of innate immune responses to gram-negative bacteria in *Drosophila*. *Genes Dev.* 17, 115–125. doi: 10.1101/gad.1018503

Fowler, K., and Partridge., L. (1988). A cost of mating in female fruitflies. *Nature* 338, 760–761. doi: 10.1038/338760a0

Garelli, A., Gontijo, A. M., Miguela, V., Caparros, E., and Dominguez, M. (2012). Imaginal discs secrete insulin-like peptide 8 to mediate plasticity of growth and metabolism. *Science* 336, 579–582. doi: 10.1126/science.1216735

Géminard, C., Rulifson, E. J., and Léopold, P. (2009). Remote control of insulin secretion by fat cells in *Drosophila*. *Cell Metab.* 10, 199–207. doi: 10.1016/j.cmet.2009.08.002

Giannakou, M. E., Goss, M., Jünger, M. A., Hafen, E., Leevers, S. J., and Partridge, L. (2004). Long-lived *Drosophila* with overexpressed dFOXO in adult fat body. *Science* 305, 361. doi: 10.1126/science.1098219

Honegger, B., Galic, M., Köhler, K., Wittwer, F., Brogiolo, W., Hafen, E., et al. (2008). Imp-L2, a putative homolog of vertebrate IGF-binding protein 7, counteracts insulin signaling in Drosophila and is essential for starvation resistance. *J. Biol.* 7, 10. doi: 10.1186/jbiol72

Hopkins, B. D., Fine, B., Steinbach, N., Dendy, M., Rapp, Z., Shaw, J., et al. (2013). A secreted PTEN phosphatase that enters cells to alter signaling and survival. *Science* 341, 399–402. doi: 10.1126/science.1234907

Huang, T., Zhang, J., Xie, L., Dong, X., Zhang, L., Cai, Y. D., et al. (2011). Crosstissue coexpression network of aging. *OMICS* 15, 665–671. doi: 10.1089/omi.2011.0034

Hwa, V., Oh, Y., and Rosenfeld, R. G. (1999). The insulin-like growth factor-binding protein (IGFBP) superfamily. *Endocr. Rev.* 20, 761–787. doi: 10.1210/er.20.6.761

Karpac, J., Younger, A., and Jasper, H. (2011). Dynamic coordination of innate immune signaling and insulin signaling regulates systemic responses to localized DNA damage. *Dev. Cell* 20, 841–854. doi: 10.1016/j.devcel.2011.05.011

Katewa, S. D., Demontis, F., Kolipinski, M., Hubbard, A., Gill, M. S., Perrimon, N., et al. (2012). Intramyocellular fatty-acid metabolism plays a critical role in mediating responses to dietary restriction in *Drosophila melanogaster*. *Cell Metab.* 16, 97–103. doi: 10.1016/j.cmet.2012.06.005

Keller, M. P., Choi, Y. J., Wang, P., Davis, D. B., Rabaglia, M. E., Oler, A. T., et al. (2008). A gene expression network model of type 2 diabetes links cell cycle regulation in islets with diabetes susceptibility. *Genome Res.* 18, 706–716. doi: 10.1101/gr.074914.107

Kim, K. H., Jeong, Y. T., Oh, H., Kim, S. H., Cho, J. M., Kim, Y. N., et al. (2013). Autophagy deficiency leads to protection from obesity and insulin resistance by inducing Fgf21 as a mitokine. *Nat. Med.* 19, 83–92. doi: 10.1038/nm.3014

Kjaer, M., Pollack, S. F., Mohr, T., Weiss, H., Gleim, G. M., Bach, F. W., et al. (1996). Regulation of glucose turnover and hormonal responses during electrical cycling in tetraplegic humans. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 271, R191–R199.

LaFever, L., and Drummond-Barbosa, D. (2005). Direct control of germline stem cell division and cyst growth by neural insulin in *Drosophila*. *Science* 309, 1071–1073. doi: 10.1126/science.1111410

Linder, M. E., and Deschenes, R. J. (2007). Palmitoylation: policing protein stability and traffic. *Nat. Rev. Mol. Cell Biol.* 8, 74–84. doi: 10.1038/nrm2084

Loffredo, F. S., Steinhauser, M. L., Jay, S. M., Gannon, J., Pancoast, J. R., Yalamanchi, P. et al. (2013). Growth differentiation factor 11 is a circulating factor that reverses age-related cardiac hypertrophy. *Cell* 153, 828–839. doi: 10.1016/j.cell.2013.04.015

Lough, T. J., and Lucas, W. J. (2006). Integrative plant biology: role of phloem long-distance macromolecular trafficking. *Annu. Rev. Plant Biol.* 57, 203–232. doi: 10.1146/annurev.arplant.56.032604.144145

Luo, S., Kleemann, G. A., Ashraf, J. M., Shaw, W. M., and Murphy, C. T. (2010). TGF-β and insulin signaling regulate reproductive aging via oocyte and germline quality maintenance. *Cell* 143, 299–312. doi: 10.1016/j.cell.2010.09.013

Mantovani, A., Locati, M., Vecchi, A., Sozzani, S., and Allavena, P. (2001). Decoy receptors: a strategy to regulate inflammatory cytokines and chemokines. *Trends Immunol.* 22, 328–336. doi: 10.1016/S1471-4906(01)01941-X

McCance, K. L., and Huether, S. E. (2002). *Pathophysiology: The Biologic Basis for Disease in Adults and Children, 4th Edn.* St. Louis, MO: Mosby.

Melo, J. A., and Ruvkun, G. (2012). Inactivation of conserved *C. elegans* genes engages pathogen- and xenobiotic-associated defences. *Cell* 149, 452–466. doi: 10.1016/j.cell.2012.02.050

Mitchell, P. S., Parkin, R. K., Kroh, E. M., Fritz, B. R., Wyman, S. K., Pogosova-Agadjanyan, E. L.,

et al. (2008). Circulating microRNAs as stable blood-based markers for cancer detection. *Proc. Natl. Acad. Sci. U.S.A.* 105, 10513–10518. doi: 10.1073/pnas.0804549105

Moremen, K. W., Tiemeyer, M., and Nairn, A. V. (2012). Vertebrate protein glycosylation: diversity, synthesis and function. *Nat. Rev. Mol. Cell Biol.* 13, 448–463. doi: 10.1038/nrm3383

Morisato, D., and Anderson, K. V. (1994). The spätzle gene encodes a component of the extracellular signaling pathway establishing the dorsal-ventral pattern of the *Drosophila* embryo. *Cell* 76, 677–688. doi: 10.1016/0092-8674(94)90507-X

Ng, S. F., Lin, R. C., Laybutt, D. R., Barres, R., Owens, J. A., and Morris, M. J. (2010). Chronic high-fat diet in fathers programs β-cell dysfunction in female rat offspring. *Nature* 467, 963–966. doi: 10.1038/nature09491

Nusse, R. (2003). Wnts and Hedgehogs: lipid-modified proteins and similarities in signaling mechanisms at the cell surface. *Development* 130, 5297–5305. doi: 10.1242/dev.00821

O'Brien, L. E., Soliman, S. S., Li, X., and Bilder, D. (2011). Altered modes of stem cell division drive adaptive intestinal growth. *Cell* 147, 603–614. doi: 10.1016/j.cell.2011.08.048

Okamoto, N., Nakamori, R., Murai, T., Yamauchi, Y., Masuda, A., and Nishimura, T. (2013). A secreted decoy of InR antagonizes insulin/IGF signaling to restrict body growth in Drosophila. *Genes Dev.* 27, 87–97. doi: 10.1101/gad.204479.112

Palm, W., Swierczynska, M. M., Kumari, V., Ehrhart-Bornstein, M., Bornstein, S. R., and Eaton, S. (2013). Secretion and signaling activities of lipoprotein-associated hedgehog and non-sterol-modified hedgehog in flies and mammals. *PLoS Biol.* 11:e1001505. doi: 10.1371/journal.pbio.1001505

Panáková, D., Sprong, H., Marois, E., Thiele, C., and Eaton, S. (2005). Lipoprotein particles are required for Hedgehog and Wingless signalling. *Nature* 435, 58–65. doi:10.1038/nature03504

Panayidou, S., and Apidianakis, Y. (2013). Regenerative inflammation: lessons from *Drosophila* intersinal epithelium in health and disease. *Pathogens* 2, 209–231. doi: 10.3390/pathogens2020209

Pedersen, B. K. (2011). Muscles and their myokines. *J. Exp. Biol.* 214, 337–346. doi: 10.1242/jeb.048074

Pedersen, B. K., and Febbraio, M. A. (2012). Muscles, exercise, and obesity: skeletal muscle as a secretory organ. *Nat. Rev. Endocrinol.* 8, 457–465. doi: 10.1038/nrendo.2012.49

Petersen, E. W., Carey, A. L., Sacchetti, M., Steinberg, G. R., Macaulay, S. L., Febbraio, M. A., et al. (2005). Acute IL-6 treatment increases fatty acid turnover in elderly humans *in vivo* and in tissue culture *in vitro*. *Am. J. Physiol. Endocrinol. Metab.* 288, E155–E162. doi: 10.1152/ajpendo.00257.2004

Rajan, A., and Perrimon, N. (2012). *Drosophila* cytokine unpaired 2 regulates physiological homeostasis by remotely controlling insulin secretion. *Cell* 151, 123–137. doi: 10.1016/j.cell.2012.08.019

Rera, M., Bahadorani, S., Cho, J., Koehler, C. L., Ulgherait, M., Hur, J. H., et al. (2011). Modulation of longevity and tissue homeostasis by the *Drosophila* PGC-1 homolog. *Cell Metab.* 14, 623–634. doi: 10.1016/j.cmet.2011.09.013

Rogers, D. W., Whitten, M. M., Thailayil, J., Soichot, J., Levashina, E. A., Catteruccia, F. (2008). Molecular and cellular components of the mating machinery in *Anopheles gambiae* females. *Proc. Natl. Acad. Sci. U.S.A.* 105, 19390–19395. doi: 10.1073/pnas.0809723105

Shimazu, T., Hirschey, M. D., Newman, J., He, W., Shirakawa, K., Le Moan, N., et al. (2013). Suppression of oxidative stress by β-hydroxybutyrate, an endogenous histone deacetylase inhibitor. *Science* 339, 211–214. doi: 10.1126/science.1227166

Sousa-Nunes, R., Yee, L. L., and Gould, A. P. (2011). Fat cells reactivate quiescent neuroblasts via TOR and glial insulin relays in *Drosophila*. *Nature* 471, 508–512. doi: 10.1038/nature09867

Srivastava, M., Simakov, O., Chapman, J., Fahey, B., Gauthier, M. E. A., Mitros, T., et al. (2010). The *Amphimedon queenslandica* genome and the evolution of animal complexity. *Nature* 466, 720–726. doi: 10.1038/nature09201

Steensberg, A., van Hall, G., Osada, T., Sacchetti, M., Saltin, B., and Klarlund Pedersen, B. (2000). Production of interleukin-6 in contracting human skeletal muscles can account for the exercise-induced increase in plasma interleukin-6. *J. Physiol.* 529, 237–242. doi: 10.1111/j.1469-7793.2000.00237.x

Stern, S., Fridmann-Sirkis, Y., Braun, E., and Soen, Y. (2012). Epigenetically heritable alteration of fly development in response to toxic challenge. *Cell Rep.* 1, 528–542. doi: 10.1016/j.celrep.2012.03.012

Straino, S., Germani, A., Di Carlo, A., Porcelli, D., De Mori, R., Mangoni, A., et al. (2004). Enhanced arteriogenesis and wound repair in dystrophin-deficient mdx mice. *Circulation* 110, 3341–3348. doi: 10.1161/01.CIR.0000147776.50787.74

Takeishi, A., Kuranaga, E., Tonoki, A., Misaki, K., Yonemura, S., Kanuka, H., et al. (2013). Homeostatic epithelial renewal in the gut is required for dampening a fatal systemic wound response in *Drosophila*. *Cell Rep.* 3, 919–930. doi: 10.1016/j.celrep.2013.02.022

Taylor, R. C., and Dillin, A. (2013). XBP-1 is a cell-nonautonomous regulator of stress resistance and longevity. *Cell* 153, 1435–1447. doi: 10.1016/j.cell.2013.05.042

van Oosten-Hawle, P., Porter, R. S., and Morimoto, R. I. (2013). Regulation of organismal proteostasis by transcellular chaperone signaling. *Cell* 153, 1366–1378. doi: 10.1016/j.cell.2013.05.015

Vilchez, D., Morantte, I., Liu, Z., Douglas, P. M., Merkwirth, C., Rodrigues, A. P. C., et al. (2012). RPN-6 determines *C. elegans* longevity under proteotoxic stress conditions. *Nature* 489, 263–268. doi: 10.1038/nature11315

Vong, L., Ye, C., Yang, Z., Choi, B., Chua, S. Jr., and Lowell, B. B. (2011). Leptin action of GABAergic neurons prevents obesity and reduces inhibitory tone to POMC neurons. *Neuron* 71, 142–154. doi: 10.1016/j.neuron.2011.05.028

Vrailas-Mortimer, A., del Rivero, T., Mukherjee, S., Nag, S., Gaitanidis, A., Kadas, D., et al. (2011). A muscle-specific p38 MAPK/Mef2/MnSOD pathway regulates stress, motor function, and life span in *Drosophila*. *Dev. Cell* 21, 783–785. doi: 10.1016/j.devcel.2011.09.002

White, L. E., Chaudhary, R., Moore, L. J., Moore, F. A., and Hassoun, H. T. (2011). Surgical sepsis and organ crosstalk: the role of the kidney. *J. Surg. Res.* 167, 306–315. doi: 10.1016/j.jss.2010.11.923

Wigby, S., and Chapman, T. (2005). Sex peptide causes mating costs in female *Drosophila melanogaster*. *Curr. Biol.* 15, 316–321. doi: 10.1016/j.cub.2005.01.051

Wu, S. C., Liao, C. W., Pan, R. L., and Juang, J. L. (2012). Infection-induced intestinal oxidative stress triggers organ-to-organ immunological communication in Drosophila. *Cell Host Microbe* 11, 410–417. doi: 10.1016/j.chom.2012.03.004

Yalak, G., and Vogel, V. (2012). Extracellular phosphorylation and phosphorylated proteins: not just curiosities but physiologically important. *Sci. Signal.* 5, re7. doi: 10.1126/scisignal.2003273

Yi, P., Park, J. S., and Melton, D. A. (2013). Betatrophin: a hormone that controls pancreatic β cell proliferation. *Cell* 153, 747–758. doi: 10.1016/j.cell.2013.04.008

Yoshimoto, S., Loo, T. M., Atarashi, K., Kanda, H., Sato, S., Oyadomari, S., et al. (2013). Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome. *Nature* 499, 97–101. doi: 10.1038/nature12347

Zeybel, M., Hardy, T., Wong, Y. K., Mathers, J. C., Fox, C. R., Gackowska, A., et al. (2012). Multigenerational epigenetic adaptation of the hepatic wound-healing response. *Nat. Med.* 18, 1369–1377. doi: 10.1038/nm.2893

Zhang, Y., Proenca, P., Maffei, M., Barone, M., Leopold, L., and Friedman, J. M. (1994). Positional cloning of the mouse obese gene and its human homologue. *Nature* 372, 425–432. doi: 10.1038/372425a0

Received: 20 August 2013; accepted: 30 October 2013; published online: 19 November 2013.

*Citation: Droujinine IA and Perrimon N (2013) Defining the interorgan communication network: systemic coordination of organismal cellular processes under homeostasis and localized stress. Front. Cell. Infect. Microbiol. **3**:82. doi: 10.3389/fcimb.2013.00082*

This article was submitted to the journal Frontiers in Cellular and Infection Microbiology.