Cell Reports
Article

Necrosis-Driven Systemic Immune Response Alters SAM Metabolism through the FOXO-GNMT Axis

Fumiaki Obata,[1,5] Erina Kuranaga,[2] Katsura Tomioka,[1] Ming Ming,[1] Asuka Takeishi,[1] Chun-Hong Chen,[3] Tomoyoshi Soga,[4] and Masayuki Miura[1,5,*]
[1]Department of Genetics, Graduate School of Pharmaceutical Sciences, The University of Tokyo, Bunkyo-ku, Tokyo 113-0033, Japan
[2]Laboratory for Histogenetic Dynamics, RIKEN CDB, Kobe 650-0047, Japan
[3]National Health Research Institutes, Zhunan, Miaoli County 35053, Taiwan
[4]Institute for Advanced Biosciences, Keio University, Tsuruoka, Yamagata 997-0052, Japan
[5]CREST, Japan Science and Technology Agency, Chiyoda-ku, Tokyo 102-0075, Japan
*Correspondence: miura@mol.f.u-tokyo.ac.jp
http://dx.doi.org/10.1016/j.celrep.2014.03.046

SUMMARY

Sterile inflammation triggered by endogenous factors is thought to contribute to the pathogenesis of acute and chronic inflammatory diseases. Here, we demonstrate that apoptosis-deficient mutants spontaneously develop a necrosis-driven systemic immune response in *Drosophila* and provide an in vivo model for studying the organismal response to sterile inflammation. Metabolomic analysis of hemolymph from apoptosis-deficient mutants revealed increased sarcosine and reduced S-adenosyl-methionine (SAM) levels due to glycine N-methyltransferase (Gnmt) upregulation. We showed that Gnmt was elevated in response to Toll activation induced by the local necrosis of wing epidermal cells. Necrosis-driven inflammatory conditions induced dFoxO hyperactivation, leading to an energy-wasting phenotype. Gnmt was cell-autonomously upregulated by dFoxO in the fat body as a possible rheostat for controlling energy loss, which functioned during fasting as well as inflammatory conditions. We propose that the dFoxO-Gnmt axis is essential for the maintenance of organismal SAM metabolism and energy homeostasis.

INTRODUCTION

Apoptosis is required for the proper removal of unneeded or damaged cells (Fuchs and Steller, 2011; Miura, 2011). When apoptosis is defective, the apoptosis-deficient cells either survive aberrantly or die by nonapoptotic cell death such as necrosis/necroptosis (Yuan and Kroemer, 2010; Kaczmarek et al., 2013). Necrotic cell death induces noninfectious "sterile" inflammation via the release of damage-associated molecular patterns (DAMPs). DAMP-mediated inflammation is involved in the pathogenesis of various diseases, including cancer, ischemia/reperfusion, neurodegeneration, atherosclerosis, and systemic inflammatory response syndrome (Martin et al., 2012; Rock et al., 2010; Zheng et al., 2011; Nagata et al., 2010; Grivennikov et al., 2010; Glass et al., 2010). Normally, tissue damage triggers an inflammatory response that is required for tissue repair; however, continuous or severe tissue damage can lead to hazardous inflammation. Organisms may have various responses, including an adaptive mechanism for the protection against severe inflammation, but this area has not been thoroughly investigated. To study such responses to sterile inflammation, we established a *Drosophila* model of a necrosis-driven systemic immune response using *dark* mutant flies, which exhibit defective wing apoptosis following eclosion.

Metabolism is tightly regulated by organisms under normal physiological states but exhibits flexibility in response to altered conditions. One of the most versatile metabolites, S-adenosyl-methionine (SAM), is a methyl-group donor required for the more than 200 known and putative methyltransferases encoded in the human genome (Lu and Mato, 2012; Petrossian and Clarke, 2011). In mammals, SAM is generated by methionine adenosyltransferase/SAM synthetase from ATP and methionine, and SAM levels are regulated primarily by glycine N-methyltransferase (Gnmt), which regulates SAM levels by consuming SAM to produce sarcosine in the liver (Luka et al., 2009). Either chronic depletion ($Mat1a^{-/-}$) or chronic elevation ($Gnmt^{-/-}$) of SAM results in spontaneous hepatocellular carcinoma and impaired liver regeneration (Martínez-Chantar et al., 2002, 2008; Chen et al., 2004; Varela-Rey et al., 2009), indicating that regulation of proper SAM levels is necessary for homeostasis. In yeast, SAM levels regulate proliferation and autophagy through the methylation of protein phosphatase 2A (PP2A) (Sutter et al., 2013). SAM's availability is also critical for lipogenesis in nematodes and HepG2 cells through the methylation of phosphoethanolamine and phosphatidyl-ethanolamine, respectively (Walker et al., 2011). Furthermore, a recent report indicates that the metformin-dependent lifespan increase in nematodes is mediated by altered folate and methionine/SAM metabolism in the gut microbiota (Cabreiro et al., 2013). These findings collectively demonstrate SAM's impact on cellular and organismal physiology. However, little is known about the mechanisms regulating the organismal SAM levels under physiological and pathological states. For example, the induced expression of nicotinamide N-methyltransferase (NNMT) in cancer cells was found to reduce SAM levels, thereby affecting histone and PP2A methylation (Ulanovskaya et al., 2013), yet the molecular mechanisms underlying NNMT induction remain to be elucidated. Administration of

retinoic acid in rats induced hepatic *Gnmt* expression by unknown mechanisms, resulting in reduced global DNA methylation (Ozias and Schalinske, 2003; Rowling et al., 2002). The reductions in SAM levels were observed in the brain and cerebrospinal fluid of Alzheimer's disease patients (Morrison et al., 1996; Linnebank et al., 2010), in the plasma of children with autism (James et al., 2004), and in the liver after partial hepatectomy (Huang et al., 1998) or in response to hepatic injury induced by ethanol (Halsted and Medici, 2012; Kharbanda, 2013) or carbon tetrachloride (Varela-Moreiras et al., 1995). SAM administration in these conditions impacts on the pathological consequences, implying that altered SAM levels are of medical importance. Nevertheless, molecular mechanisms underlying the regulation of SAM levels in vivo are not elucidated yet.

In this study, we revealed an energy-wasting phenotype in *Drosophila* models for a necrosis-driven systemic immune response. From the genetic studies with a combination to the metabolome analysis, we demonstrated that a FOXO-mediated system is activated that regulates SAM levels as a possible protective mechanism against energy loss under inflammatory conditions and starvation stress in vivo.

RESULTS

Defective Apoptosis of Wing Epidermal Cells Leads to a Necrosis-Driven Systemic Immune Response

Apoptosis is primarily executed by an evolutionarily conserved signaling process including caspases and their activator, apoptotic protease activating factor-1 (Apaf-1) (Green, 2005). In the hypomorphic *Drosophila Apaf-1* (*dark*/*dpf-1*/*HAC-1*) mutant $dark^{cd4}$, apoptosis is impaired during development, but a significant portion of the mutants survive to adulthood (Rodriguez et al., 1999). We noticed that the wings were gradually melanized within 5 days after eclosion in almost all of the $dark^{cd4}$ mutant flies examined (Figure 1A; Figure S1A). *Drosophila* wings are initially folded but expand within an hour after eclosion, followed by wing epidermal cell (WEC) apoptosis and elimination. Previous reports indicated that an inhibition of WEC apoptosis leads to incomplete cell elimination, resulting in the accumulation of cell remnants inside the wings and in wing melanization (Kimura et al., 2004; Link et al., 2007). Therefore, we assumed that apoptosis-deficient WECs eventually die by necrosis, triggering melanization. Indeed, we observed propidium iodide (PI)-positive necrotic cells in the wings of day 5 *dark* mutant flies (Figures 1B and S1B). We confirmed by Hoechst staining that these PI-positive particles were DNA of necrotic cells, and we also observed that some cells were still alive and were PI negative but Hoechst positive (Figures 1B and S1B). The wing melanization was completely rescued by introducing one copy of the genomic fragment containing *dark* ($dark^{BAC/+}$) (Figure 1C). Wing melanization was also observed when *dark* was knocked down by overexpressing short hairpin RNA for *dark* (*dark-sh*) by *WP-Gal4*, in which *Gal4* was expressed in WECs (Figures 1C, S1A, and S1C). The same phenotype was observed when *dronc* was knocked down by *WP-Gal4* or *dark* was knocked down by another wing driver, Bx^{MS1096}-*Gal4*, further confirming that WEC necrosis triggered wing melanization (Figure 1C).

Next, to investigate the global effects of WEC necrosis, we performed a microarray analysis on *dark* mutant flies. Many immune-related molecules, including antimicrobial peptides (AMPs), were upregulated dramatically (Tables S1 and S2). We hypothesized that the necrotic WECs triggered a severe inflammatory response. Notably, all of the flies with melanized wings developed an elevated immune response, as indicated by quantitative RT-PCR (qRT-PCR) analysis of the AMPs that were found to be highly elevated in the microarray analysis (Figures 1D and S1D). We assumed that the DAMPs produced by necrotic WECs gained access to the entire body through the circulating hemolymph, because *Drosophila* has an open circulatory system. To visualize which tissue(s) elicited the immune reaction, we checked the expression pattern of GFP reporters for two antimicrobial peptides, *Drosocin* and *Drosomycin*. Most of the fat body cells residing throughout the body were GFP positive, indicating the presence of systemic immune response driven by local WEC necrosis (Figures 1E–1G and S1E). These data collectively indicated that either the apoptosis-deficient mutants or the flies with specific-inhibition of WEC apoptosis could serve as an in vivo model for analyzing the organismal responses to a necrosis-driven systemic immune response.

Metabolic Profiling of the *dark* Mutant

Various metabolic intermediates have been identified as the missing links underlying biological processes that occur under both healthy and disease states. Indeed, several metabolites impact inflammation, including lipid mediators, azelaic acid monoester, and gut-bacterial-derived uracil or deoxycholic acid (von Moltke et al., 2012; Serhan et al., 2008; Matsubara et al., 2012; Lee et al., 2013; Yoshimoto et al., 2013). To identify the metabolite(s) and/or metabolic pathway(s) that could be affected by a necrosis-driven systemic immune response, we performed the metabolic profiling of the hemolymph, in which metabolites related to organismal homeostasis should be released. The direct analysis of metabolites in the hemolymph of adult *Drosophila* is straightforward but challenging because of its limited quantity. However, a recent development in metabolome analysis using capillary electrophoresis mass spectrometry (CE-MS) (Soga et al., 2003) enabled our analysis of the metabolic profiling of *dark* mutants. Among 90 metabolites identified, only three—gluconate, oxamate, and sarcosine—were elevated more than 2-fold in flies with two different *dark* mutant alleles compared to wild-type flies (Figure 2A; Table S3). Although the metabolic enzymes for gluconate and oxamate have not been identified in *Drosophila*, the metabolism of sarcosine is evolutionarily conserved between mammals and flies. Therefore, we focused on the sarcosine elevation in the *dark* mutant. We confirmed that sarcosine was elevated in flies with several different *dark* mutant alleles by analyzing whole-body homogenates using ultra-high-performance liquid chromatography with tandem mass spectrometry (UPLC-MS/MS) (Figure S2A).

Gnmt Is an Evolutionarily Conserved Regulator of SAM, and Its Expression Is Induced by Necrotic WECs

Sarcosine is synthesized by glycine N-methyltransferase (GNMT) and metabolized to glycine by sarcosine dehydrogenase (SARDH) in mammals, predominantly in the liver (Figure 2B). We