The *Drosophila* TNF ortholog Eiger: Emerging physiological roles and evolution of the TNF system

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ABSTRACT

The TNF and TNFR superfamilies of proteins are conserved throughout evolution.

The first invertebrate orthologs of TNF and TNFR, Eiger and Wengen, were identified

in *Drosophila*, which enabled us to take advantage of its powerful genetics. Indeed,

genetic studies on Eiger in the last decade have discovered their signaling mechanisms

through activation of the JNK pathway and unveiled the role of Eiger-JNK signaling in

a variety of cellular and tissue processes such as cell death, cell proliferation, tissue

growth regulation, host defense, pain sensitization, and canalization. In this review,

we will describe the *in vivo* signaling of Eiger and its physiological roles in fly

development and homeostasis, and will discuss the evolution of the TNF/TNFR

systems.

1. Introduction

Many of the intra- and inter-cellular signaling pathways that regulate a variety of cellular processes such as cell proliferation, cell differentiation, cell survival, and cell death are highly conserved throughout evolution. Thus, accumulating studies using model organisms have made a great contribution to our knowledge of the basic principles as well as evolutionary variations of cellular signaling. *Drosophila* provides a powerful genetic model for studying the *in vivo* roles of the signaling pathways and their physiological regulations. *Drosophila* has the member of the tumor necrosis factor (TNF) Eiger and its receptor Wengen [1-3]. Genetic studies in *Drosophila* have identified evolutionarily conserved molecular functions of TNF family proteins and are now providing new dimensions toward understanding their physiological roles and evolution.

2. *Drosophila* TNF ortholog Eiger, a ligand for JNK signaling

The *Drosophila* ortholog of TNF was identified through an ectopic expression screen in *Drosophila* compound eye for genes that cause cell death, and was named

eiger (*eda-like cell death trigger*) [1]. *eiger* was also isolated independently by molecular approaches [2, 3]. The genomic database of *Drosophila* indicates that *eiger* is the sole member of the TNF superfamily in flies. *eiger* encodes a protein of 409 amino acids with a transmembrane (TM) domain and a C-terminal TNF homology domain, which shows highest homology with a TNF family member Ectodysplasia (EDA)-A2 (28%) [1]. Two isoforms of Eiger have been reported; one has 409 amino acids and the other isoform, Eiger-L, contains additional 6 amino acids (GESLLS) located outside the TNF homology domain [2, 3]. Both isoforms were shown to be expressed in flies [2]. Overexpression of Eiger in imaginal discs causes cell death by activation of the c-Jun N-terminal kinase (JNK) pathway through Misshapen (Msn, JNKKKK), dTAK1 (JNKKK), Hemipterous (Hep, JNKK), and Basket (Bsk, JNK) [1, 3]. As a receptor for Eiger, the fly TNF receptor (TNFR) homolog *wengen* was identified through a genetic screen for mutations that suppress Eiger-induced cell death [4]. *wengen* encodes a transmembrane protein with a TNFR homology domain in the extracellular region, which physically interacts with Eiger's TNF homology domain, and is required for Eiger-induced cell death [4]. Epitope-tagged expression of Wengen

in 293T cells showed that Wengen is a type I transmembrane protein, like most mammalian TNFR family members [2]. It was shown that overexpression of Eiger (Eiger-S) or Eiger-L together with Wengen in S2 cells induced cell death with the same extent [2].

Intriguingly, Wengen does not possess an intracellular motif called ‘death-domain’, which is commonly found in the TNFR superfamily members and is required for downstream activation of caspases. This suggests that there are other mechanism(s) for Wengen to transduce signals. Indeed, subsequent genetic studies have identified the *Drosophila* homolog of TAB2/3 (dTAB2), which links TNF receptor-associated factor (DTRAF1) to TAK1 [5], as well as DTRAF2 and the deubiquitinating enzyme dCYLD [6], as components of the Eiger-JNK signaling pathway. dCYLD, the fly homolog of the tumor suppressor mutated in familial cylindromatosis, facilitates Eiger signaling by deubiquitination of DTRAF2, which stabilizes the DTRAF2 protein and thereby triggers the JNK pathway. Further analyses for Eiger-induced signaling have shown that Bendless and dUev1a, which form an ubiquitin-conjugating E2 enzyme complex, act downstream of Eiger to elicit

both DTRAF2-mediated JNK-dependent cell death and NOPO (*Drosophila* ortholog of TRAF-interacting protein)-mediated caspase-dependent cell death [7-9] (Fig. 1).

TNF in mammals is synthesized as a membrane-anchored protein and is cleaved to generate a soluble ligand by a membrane-bound metalloproteinase called TNF-alpha converting enzyme (TACE). Although *Drosophila* has a gene encoding TACE homolog, it is unclear whether Eiger is processed by TACE to become a soluble protein *in vivo*. It has been shown that, when Eiger is expressed in *Drosophila* S2 cells, Eiger can be cleaved and secreted into the medium [2]. Furthermore, furin-like activity could cleave Eiger when expressed in 293T cells [10]. Interestingly, a recent study in *Drosophila* imaginal discs has shown that apoptotic cells induce additional apoptosis in the same tissue through Eiger-JNK signaling, a phenomenon named apoptosis-induced apoptosis (AiA) [11]. It was proposed that Eiger is produced in apoptotic cells and activates the JNK pathway in neighboring cells, thereby propagating the apoptotic JNK signaling in the tissue. This suggests that, under certain conditions, Eiger could be processed to become a soluble ligand and acts as a non-cell autonomous activator of JNK signaling. Such propagation of JNK activity within the imaginal disc

was also observed upon wounding [12]. However, it was shown that, when overexpressed in imaginal discs as clones, Eiger activates JNK signaling strictly in a cell-autonomous manner [13]. Overexpression of a modified Eiger, which lacks transmembrane domain but has additional hemagglutinin signal peptide at N-terminus (called ‘soluble Eiger’), in eye imaginal disc induced eye-ablation phenotype [10]. It is unclear whether this soluble Eiger can induce cell death through Wengen. Indeed, it was reported that physical interaction between soluble Eiger and Wengen was not observed [2]. Since Wengen has only a single cysteine-rich TNFR homology domain, affinity and/or specificity to the ligand could be low [2]. It is possible that soluble Eiger-induced JNK signaling is mediated through a receptor other than Wengen.

3. Metabolic control of Eiger signaling

To further dissect the downstream signaling pathway of Eiger-induced cell death, an unbiased genetic screen for dominant modifiers of Eiger-induced small-eye phenotype was conducted. A series of chromosomal deficiencies that cover more than 80% of the genome were screened and responsible genes for suppressor phenotypes

were identified using RNAi lines that target genes located within the chromosomal deletion. The screen resulted in the identification of a series of genes encoding energy production-related enzymes as suppressors of Eiger-induced cell death [14]. It was shown that metabolic enzymes act downstream of JNK to facilitate Eiger-induced cell death. Furthermore, as Eiger signaling is required for intrinsic tumor suppressor activity (see below), energy production-related enzymes were shown to play roles in such tumor suppression mechanisms. How does energy-production enzymes contribute to cell death? Clones of cells overexpressing Eiger in the eye disc were shown to be stained with dihydroethidium, which indicates that Eiger induces production of superoxide ($O_2^{\cdot-}$). In addition, a ROS-inducible *gstD* (glutathione S transferase D) promoter-GFP reporter, *gstD*-GFP, was induced when Eiger was ectopically expressed in the eye disc. Thus, Eiger can induce ROS production. Further genetic analyses indicated that energy production-related enzymes act upstream of ROS production, which could promote Eiger-induced cell death [14] (Fig. 1).

In mammals, some energy production-related enzymes are shown to act as tumor suppressors. For instance, succinate dehydrogenases (Sdh; SdhB, SdhC, and

SdhD), subunits of a TCA cycle enzyme, are classical tumor suppressors in pheochromocytoma or paraganglioma [15]. A specific isoform of pyruvate kinase, which is necessary for cellular metabolism to shift to aerobic glycolysis, works to promote tumorigenesis. Knockdown of pyruvate kinase M2 (PKM2) reduced cell proliferation rate and glucose metabolism [16]. In addition, pyruvate dehydrogenase (PDH) links the glycolytic pathway to the TCA cycle by transforming pyruvate to acetyl-CoA. The activity of PDH is suppressed in cancer cells, while reactivation of PDH induces cell death in a solid tumor cell line and xenografts [17]. In *Drosophila*, knockdown of PDH suppressed Eiger-induced cell death [14]. These observations suggest that mitochondrial energy production could act as a tumor suppressor probably through the generation of cytotoxic ROS production [18].

4. Physiological Roles of Eiger

Despite its potent ability to activate JNK signaling, the physiological roles of Eiger have been poorly understood until recently. Recent genetic studies have discovered that Eiger-JNK signaling plays crucial roles in a variety of cellular and tissue

processes such as tissue growth regulation (including both tumor suppression and tumor progression), host defense, pain sensitization, cell proliferation, and canalization.

4.1. Tumor-suppressing role of Eiger

Loss of apico-basal polarity is frequently associated with epithelial tumorigenesis. Indeed, *Drosophila* imaginal epithelium entirely mutant for evolutionarily conserved apico-basal polarity genes such as *scribble* (*scrib*) or *discs large* (*dlg*) results in tumorous overgrowth [19], suggesting an active role of polarity disruption in tumor progression. Interestingly, such polarity-deficient oncogenic mutant cells behave in a completely different way when surrounded by wild-type cells. Clones of cells mutant for *scrib* or *dlg* in otherwise wild-type tissue do not overgrow but are eliminated from the tissue by cell death [20, 21]. This suggests that normal epithelial tissue has an intrinsic tumor suppression mechanism that actively eliminates oncogenic polarity-deficient cells from the tissue. It has been shown that Eiger-JNK signaling plays crucial roles in this intrinsic tumor suppression (Fig. 2A). *scrib* mutant cells surrounded by wild-type cells elevate endocytosis, which causes translocation of

Eiger from the plasma membrane to early endosomes. Accumulation of Eiger in endosomes, in turn, triggers apoptotic JNK activation at the endocytic compartment [22] (Fig. 2B), where Eiger might meet adaptor or scaffold proteins that recruit downstream JNK signaling components. Similar endocytic activation of caspase by TNFR signaling has been reported in mammalian systems [23]. Intriguingly, most dying cells were observed at boundaries between *scrib* mutant clones and wild-type clones, suggesting an apoptotic cell-cell interaction between *scrib* mutant cells and wild-type cells. Indeed, Eiger-JNK signaling was also found to be specifically activated in wild-type cells nearby polarity-deficient clones. Furthermore, this JNK activation in wild-type cells promotes elimination of their neighboring mutant cells. Mechanistically, JNK activation in wild-type cells surrounding polarity-deficient cells induces expression of PVR, a PDGF/VEGF receptor homolog, which activates the engulfment pathway through actin cytoskeleton regulators ELMO-Mbc (a DOCK180 homolog) [13]. Polarity-deficient cells are thus actively eliminated by engulfment by neighboring wild-type cells (Fig. 2B). These results show that Eiger-JNK signaling in both polarity-deficient cells and surrounding wild-type cells are required for facilitating

cell elimination, and therefore dying cells were specifically observed at the boundaries between these two populations. It has been shown that this elimination of polarity-deficient clones is the result of ‘cell competition’ [13, 20, 22, 24], a struggle for existence among cells within the tissue [25]. While Eiger plays a central role in cell competition between *scrib* mutant cells and wild-type cells, it seems not to be involved in a classical *Minute* cell competition, a competition between wild-type cells and ribosomal protein mutant cells [13].

Systemic regulation of the elimination of polarity-deficient tumors has also been reported [26]. Eiger expression in imaginal disc tumors triggers activation of Toll signaling in the fat body, which suppresses imaginal tumor growth in a non-tissue autonomous manner. Eiger expression in hemocytes is required for triggering tumor cell death downstream of Toll activation in the fat body.

4.2. Tumor-promoting role of Eiger

Contrary to its tumor-suppressor function, Eiger can also function as a tumor-promoter in some contexts. In imaginal discs, clones of cells mutant for

apico-basal polarity genes such as *scrib* simultaneously expressing oncogenic Ras^{V12} (Ras^{V12}/*scrib*^{-/-} clones) result in tumorous overgrowth and metastatic behavior [20, 27]. JNK signaling activated in Ras^{V12}/*scrib*^{-/-} clones was shown to play an essential role in tumor growth, as blocking JNK in Ras^{V12}/*scrib*^{-/-} clones suppresses tumor growth and clonal activation of Ras and JNK signaling is sufficient to cause tumorous overgrowth [28]. Subsequently, it was shown that Eiger is the upstream trigger for this pro-tumor JNK signaling in Ras^{V12}/*scrib*^{-/-} clones [29] (Fig. 3A). Further genetic study has shown that co-activation of Ras and JNK signaling causes tumorous overgrowth through inactivation of the tumor-suppressor Hippo pathway [30]. Thus, Eiger-JNK signaling seems to switch its tumor-suppressor role to a tumor-promoting effect in the presence of oncogenic Ras through regulation of the Hippo pathway (Fig. 3B). Interestingly, it has also been shown that JNK activation in polarity-deficient clones is triggered by Eiger expressed in circulating hemocytes that are recruited to mutant clones [29].

4.3. Role of Eiger in host defense

Eiger is also shown to be involved in the process of host defense. It has

been shown that *eiger* expression is upregulated after exposure of *Drosophila* mbn-2 cells to LPS [31]. In addition, Eiger was shown to play a role in regulating the growth of extracellular pathogens, as *eiger* mutant flies show decreased phagocytosis that results in exclusion of extracellular pathogens [32]. On the other hand, *eiger* mutant flies show increased susceptibility to *Vibrio cholerae* infection [33]. *eiger* mutation does not affect the sensitivity to the intracellular pathogen *Salmonella typhimurium* but delays host death [34]. In addition, Eiger-JNK signaling was shown to be involved in crystal cell rapture that releases prophenoloxidase [35], a protein required for the process of host defense called melanization.

4.4 Role of Eiger in pain sensitization

Nociceptive (pain) sensitivity is crucial for a response to tissue damage to protect against injury. In *Drosophila* larvae, UV-induced tissue damage develops both thermal hyperalgesia (an exaggerated response to noxious thermal stimuli) and thermal allodynia (a responsiveness to subthreshold thermal stimuli that are not normally noxious). Knockdown of *wengen* in nociceptive sensory neurons abolished

development of UV-induced thermal allodynia. In addition, ectopic expression of Eiger in nociceptive sensory neurons was sufficient to induce allodynia even in the absence of UV irradiation. Thus, it was proposed that damaged epidermal cells produce Eiger, which is released from epidermal cells and binds to Wengen on nociceptive sensory neurons underlying the epidermal sheet and lowers the threshold of the behavioral response [36]. Hedgehog signaling was also shown to regulate nociceptive sensitization in the pathway parallel to Eiger/Wengen signaling[37].

4.5. Role of Eiger in glial proliferation

During *Drosophila* development, neuronal cell death occurs in the adult brain shortly after eclosion. Surrounding glial cells respond to this neuronal cell death to undergo cell division, as inhibition of neuronal cell death by the caspase inhibitor p35 suppresses surrounding glial proliferation. This glial cell division was attenuated in *eiger* mutant flies, whereas cell death soon after eclosion still occurs in mutant flies. Similarly, glial cells undergo cell division in response to brain injury in a manner dependent on Eiger [38]. Although the underlying mechanism is not known, these

results suggest that Eiger is involved in mitogenic cell-cell communication between apoptotic cells and surrounding viable cells.

4.6. Role of Eiger in canalization

The ‘constancy of the wild-type’ phenotype under varying developmental conditions, termed ‘canalization’, is achieved by buffering against stochastic fluctuations and environmental perturbations [39]. During dorsal patterning in *Drosophila* embryo, bone morphogenetic protein (BMP) signaling visualized by pMad staining shows a stable pattern centered on the dorsal midline [40]. Eiger expression is broadly observed in the dorsal region of stage 5 embryos and is restricted to the dorsal most cells at stage 6 [2, 40, 41]. Eiger expression depends on BMP signaling in the pregastrula embryo. Mean intensity of pMad staining is reduced to half in *eiger* mutant embryos or in embryos that lack maternal JNK activity. It was shown that Eiger acts in the positive feedback circuit to promote and refine BMP signaling. BMP signaling is antagonized by the BMP binding protein Crossveinless-2 (Cv-2). *zerknüllt* (*zen*) homeobox gene, a target of BMP signaling, regulates expression of both *eiger* and

cv-2. In embryos doubly mutant for *eiger* and *cv-2*, the variability of BMP signaling became very high, indicating that BMP signaling was ‘decanalized’. It has been reported that amnioserosa specification depends on BMP signaling and the variability of amnioserosa cell number is very low in wild-type embryos. This constancy of amnioserosa cell number is also decanalized in *eiger*, *cv-2* double mutant embryos. Decanalized status was not affected by higher (29 C) or lower (18 C) temperatures but was affected by maternal heterozygosity for *Medea*, an obligate BMP transcriptional cofactor. Thus, combined action of *eiger* and *cv-2* could canalize BMP signaling against genetic but not environmental fluctuations [40].

5. Invertebrate and ascidian TNF superfamilies

TNF ligand and receptor superfamilies have been identified throughout the animal kingdom based on the sequence similarities [42] (Fig. 4). Eiger is the first identified TNF superfamily ligand in invertebrates. Like Eiger in *Drosophila*, kuruma shrimp (*Marsupenaeus japonicus*) TNF ligand (MjTNF) is a type II transmembrane protein that shows 30.7% and 26.7% identities with Eiger and human EDA, respectively

[43]. The genome of purple sea urchin, *Stronglyocentrotus purpuratus*, has four TNF ligands and seven TNFR superfamilies [44]. Ascidian urochordate *Ciona intestinalis* has two TNF ligands and two TNFRs. Furthermore, sea anemone *N. vectensis* possesses one TNF ligand and two TNFRs [44]. While the genomes of ascidian, sea anemone and sea urchin all contain orthologs of EDA (CiEda in ascidian, NvTNF in sea anemone, Sp-Eda and Sp-Eda2 in sea urchin), they do not have orthologs of Fas, DR4, DR5, or TNFR1, and their ligands FasL, Trail, or TNF- α . EDA receptor (EDAR) ortholog was also found in sea urchin. Thus, EDA ligand/receptor members seem to be evolved early during metazoan evolution [44]. Orthologs of p75^{NTR}/NGFR and its ligand were also found in sea anemone, ascidian and sea urchin [44]. EDAR signaling is important for the development of epithelial appendages, while p75^{NTR}/NGFR is involved in neuronal survival [45-47]. Throughout the evolution of TNF superfamily members, EDAR and p75^{NTR}/NGFR subfamilies and their ligands including Eiger might have acquired roles in epithelial and neural development. Although *Drosophila* genome does not have p75^{NTR} homolog, a recent study on the X-ray structure of dimeric active Spätzle bound to the Toll ectodomain revealed that this ligand/receptor complex

resembled to NGF/p75^{NTR} complex rather than microbial ligands bound to Toll like receptor [48, 49]. In mammals, NGF induces oligodendrocyte cell death through p75^{NTR}/JNK signaling pathway [50, 51].

Apart from EDA and p75^{NTR} subfamilies, other TNF superfamilies were cloned from ascidian *Ciona savignyi* and *Ciona intestinalis* and named CsTL and CiTNF α , respectively. They show significant homologies with sea urchin *S. purpuratus* Sp-TL1A but not with *Drosophila* Eiger [52, 53]. CsTL and CiTNF α are ubiquitously expressed and have been shown to be upregulated in hemocytes after LPS injection, suggesting that these ligands play roles in inflammation and immune response.

A member of the TNF superfamily was also identified in molluscan disk abalone, which was named AbTNF- α . AbTNF- α is related to ascidian CsTL and is expressed in immune and non-immune tissues with a highest expression in gill. Another identified TNF superfamily member in molluscan disk abalone is AbFas ligand. AbFas ligand is constitutively expressed in hemocytes, gills, mantle, muscle, digestive tract, and digestive gland. Expressions of AbTNF- α and AbFas ligand are induced in

gill by pathogenic bacteria, viral haemorrhagic septicaemia virus, or LPS challenge [54, 55]. Expression patterns and the induction by immune stimulation suggest that AbTNF- α and AbFas ligand play roles in the immune system.

TNFR family signaling is mediated by binding of adapter molecules to the cytoplasmic domain of TNFR through its death-domain. Death-domain-containing adaptors such as FADD, TRADD, RAIDD/CRADD, and EDARADD bind homophilically to death-domain of receptors. *Drosophila* has two death-domain-containing adaptor proteins dFADD and dMyd88. Sea urchin has three death-domain-containing adaptors Sp-FADD, Sp-MyD88, and Sp-RAIDD/Cradd-19. Another adaptor protein family, TRAF family of proteins, binds to death-domain adaptor proteins as well as TNFRs directly [56]. Six TRAF proteins were found in mammals, three were found in *Drosophila* (dTRAF1, a TRAF4 ortholog; dTRAF3, a precursor for TRAF1, 2, 3 and 5; dTRAF2, a TRAF6 ortholog), and one was found in *C. elegans* [44, 57, 58]. Sea urchin has four TRAFs (Sp-TRAF1/2, Sp-TRAF3/5, Sp-TRAF4 and Sp-TRAF6) [44]. Thus, TRAF4 and TRAF6 orthologs seem to be evolved early during the metazoan evolution. In *Drosophila*, two TRAFs transduce

discrete signaling pathways. Overexpression of DTRAF1 causes JNK activation, while overexpression of DTRAF2 leads to activation of *Drosophila* NF- κ B proteins DIF and Relish [59]. Although Wengen can bind to both DTRAF1 and DRTAF2, only JNK signaling, but not the NF- κ B pathway, is activated by Eiger. DTRAF1 and DRTAF2 were both reported to be involved in Eiger-induced JNK activation [5, 6].

EDA binds to EDAR to form a trimeric ligands/receptor complex, and then the adaptor protein EDARADD is recruited. Subsequently, TAK1 is activated in the TRAF6/TAB2 complex, and TAK1 further activates the IKK complex. This leads to ubiquitination and degradation of I- κ B, resulting in activation of NF- κ B [60]. On the other hand, EDA also activates JNK [61]. Similar receptor complex was found in Eiger/Wengen signaling [5]. dTAK1 was shown to be activated in the complex of dTRAF1/dTAB2/Msn (Fig. 1). However, dTAK1's target is not the IKK complex but Hep, which activates downstream Bsk (JNK).

6. Evolution of the NF- κ B and JNK activation machineries through TNFR

In mammals, binding of TNF- α to TNFR1 activates membrane-bound signaling complex (complex I), which contains TRADD, TRAF2, cIAP1/2, and RIP1 [62]. Downstream of complex I, IKK complex is activated, which leads to degradation of I- κ B and subsequent activation of NF- κ B. TAK1 is also activated downstream of complex I and triggers p38 and JNK signaling [63]. Complex I endocytosis dissociates TRADD from the complex I, and then FADD is recruited to the complex (this new complex is called complex II). Caspase 8 is recruited to the complex II, thereby initiating the apoptotic pathway.

In *Drosophila*, binding of Eiger to Wengen activates JNK signaling through TAK1 but not NF- κ B. Is it possible that TNFR also mediates NF- κ B activation in *Drosophila*? *Drosophila* cytokine Spätzle/Toll signaling activates NF- κ B. Toll is a prototype of Toll-like receptor (TLR) but not a member of the TNFR superfamily. However, the Spätzle/Toll complex resembles to mammalian NGF/p75^{NTR} complex [48, 64] and p75^{NTR} is a member of the TNFR superfamily. Like TNFR1, binding of NGF to p75^{NTR} activates NF- κ B [65], JNK [66, 67], and caspase [68]. Thus, two different

TNFR-like signaling systems have been installed in *Drosophila*: Spätzle/Toll for NF- κ B activation and Eiger/Wengen for JNK activation (Fig. 5).

7. Amplification of inflammatory signaling in invertebrate

In mammals, upon bacterial infection or exposure of damage-associated molecular patterns (DAMPs), TLR directly senses the bacterial component or DAMPs and activates NF- κ B [69, 70]. After activation of NF- κ B, inflammatory cytokines including TNF- α are induced to amplify the inflammatory response. *Drosophila* has a different strategy to amplify the host inflammatory response against infection or DAMPs exposure through Toll. Proteolytic cascade of CLIP-domain serine protease [71] is utilized to activate proSpätzle after infection [72, 73] or DAMPs stimulation [74]. Spätzle prodomain normally masks the Spätzle's critical region required for binding to Toll, and the processing of the prodomain causes conformational change of Spätzle to be able to bind to Toll [75].

The hemolymph-clotting phenomenon is an efficient defense system found in the horseshoe crab *Limulus polyphemus* [76]. Studies using other horseshoe crab

Tachypleus tridentatus have shown that the proteolytic cascade of CLIP-domain serine protease is utilized for hemolymph coagulation upon LPS stimulation [77]. Coagulogen is processed by clotting enzyme and causes its conformational change to form coagulin gel. Structure of coagulogen exhibits a striking topological similarity to NGF and Spätzle [78, 79]. Thus, activation of neurotrophin-fold protein by the conformational changes through proteolytic cascade of CLIP-domain serine protease could be a prototypical signal amplification mechanism for host defense in invertebrate. In *Drosophila*, Spätzle/Toll, a TNFR-like signaling system, is activated upon necrotic cell death that could be commonly induced by tissue damage or under pathological conditions [80]. Toll activation leads to activation of dFoxO [81], which regulates glycine N-methyltransferase for the maintenance of organismal S-adenosyl-methionine metabolism and energy homeostasis [80]. This invertebrate amplification system for host defense could be widely utilized for sterile inflammation to maintain organismal homeostasis.

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Figure legends

Figure 1. Eiger-induced signaling that regulates a variety of cellular and tissue processes. See text for details.

Figure 2. Intrinsic tumor suppression by Eiger-JNK signaling. (A) Clones of cells mutant for *scrib* are eliminated from the imaginal epithelium in a manner dependent on Eiger-JNK signaling. (B) Polarity-deficient cells surrounded by wild-type cells cause endocytic activation of apoptotic Eiger-JNK signaling, while surrounding wild-type cells activate the engulfment pathway through Eiger-JNK signaling,

Figure 3. Eiger-JNK signaling switches its tumor-suppressor role to a tumor-promoting effect in the presence of oncogenic Ras. (A) Clones of cells mutant for *scrib* simultaneously expressing oncogenic Ras^{V12} result in tumor growth and invasion. (B) Ras signaling switches Eiger-JNK signaling from tumor-suppressor to tumor-promoting effect through regulation of the Hippo pathway.

Figure 4. Evolution of the TNF superfamily. The numbers of TNF superfamily ligands, receptors, and adaptor molecules associated with receptors are shown in each phylogenetic tree. The number in parenthesis indicate the number of receptors or adaptors with death-domains. The figure is adapted from [1, 3, 82, 83].

Figure 5. The Human and *Drosophila* TNF systems. In humans, EDAR and p75^{NTR} activate both NF- κ B and JNK signaling upon ligand binding. In *Drosophila*, EDA/EDAR ortholog Eiger/Wengen activates only JNK signaling. Spätzle/Toll, a structural homolog of NGF/p75^{NTR}, activates only NF- κ B signaling.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5