

Manuscript submitted to:

Volume 1, Issue 1, 20-33.

AIMS Genetics

DOI: 10.3934/genet.2014.1.20

Received date 28 October 2014, Accepted date 8 December 2014, Published date 14 December 2014

Review

Context-dependent interplay between Hippo and JNK pathway in *Drosophila*

Xianjue Ma *

Department of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA

* **Correspondence:** Email: XianjueMa@jhmi.edu; Tel: 410-502-3174.

Abstract: Both Hippo and JNK signaling have well-established roles in regulating many physiological processes, including cell proliferation, growth, survival, and migration. An increasing body of evidence shows that dysregulation of either Hippo or JNK pathway would lead to tumorigenesis. Recently, studies in *Drosophila* has coupled Hippo with JNK pathway in numerous ways ranging from tissue regeneration to growth control. In this review, I provide an overview of the current understanding of crosstalk between Hippo and JNK pathway in *Drosophila*, and discuss their context-dependent interactions in gut homeostasis, regeneration, cell competition and migration.

Keywords: Hippo; JNK; *Drosophila*; cell competition; ISC; migration

1. Introduction

1.1. The Hippo signaling pathway

The Hippo pathway was initially identified by genetic screens for growth regulating genes in *Drosophila melanogaster*. Recent studies have uncovered its function as a highly conserved tumor-suppressor pathway which plays essential roles in regulating multiple aspects of tumorigenesis, including cell growth, proliferation and survival [1,2,3]. The first identified four core components of Hippo pathway are the NDR family kinase Warts (Wts), the Ste20-like family kinase Hippo (Hpo), the WW-domain containing protein Salvador (Sav), and the adaptor protein Mob-as-tumor-suppressor (Mts) [4-12]. Hpo complexes with Sav to phosphorylate and activate Wts, which in turn, in conjunction with Mts, directly phosphorylates the transcriptional co-activator Yorkie (Yki), resulting in its cytoplasmic accumulation and activity inhibition [9,13,14]. Once the Hippo pathway is deactivated, Yki can translocate into nucleus to partner with different

DNA-binding transcription factors, including the TEAD/TEF family transcription factor Scalloped (Sd) [15,16,17], to activate the transcription of many cell growth and viability regulating genes, such as *cyclin E (cycE)*, *expanded (ex)* and *Drosophila inhibitor of apoptosis protein 1 (diap1)* [1,3]. In short, cells with reduced Hippo signal or increased Yki activity overgrowth [1,4-13]. So far, over 20 Hippo pathway regulators have been identified in *Drosophila* [18], and overwhelming evidence in mammals has further confirmed their conserved roles in regulating tumorigenesis and cancer development [2,19,20].

1.2. The JNK signaling pathway

The c-Jun N-terminal Kinase (JNK) pathway represents another key conserved eukaryotic signaling pathway. Initially identified as a stress-activated protein kinase (SAPK) [21], JNK can be activated by various stimuli ranging from UV irradiation to DNA damage, bacterial infection, and cytokines [22,23]. Upon stimulation, JNK will be activated by the mitogen-activated protein (MAP) phosphorylation kinase cascade composed of different members of JNK Kinase Kinases and JNK Kinases (JNKKs → JNKKs → JNKs) [23]. Then JNK translocates into the nucleus to phosphorylate and activate the prototypical transcription factors including AP-1 family members Jun and Fos [24]. In *Drosophila*, the JNK pathway is much simpler than in mammals, with only a single JNK (Basket, Bsk) and two JNKKs (Hemipterous, Hep and MKK4) [25]. In the past 18 years, increasing studies have revealed that JNK pathway is involved in a wide range of cellular processes including proliferation, cell competition, regeneration, survival and tumor progression [23,26,27]. Notably, unlike Hippo pathway, recent studies in *Drosophila* discovered that JNK activation can lead to tumor suppression or tumor progression depending on the context [28,29,30], which will be discussed in this review.

2. Crosstalk between Hippo and JNK signaling

2.1. Gut homeostasis and regeneration

Drosophila gut is under constant attack due to exposure to different pathogens and chemical stimuli during normal feeding. In order to maintain gut homeostasis, intestine stem cell (ISC) proliferation is required to ensure the replenishment of damaged cells [31,32], while disruption of this process would cause diseases including cancer [33]. *Drosophila* midgut epithelia is mainly composed of four cell types, namely ISC, enteroblast (EB) cells, absorptive enterocyte (EC) cells, and secretory enteroendocrine (ee) cells. The ISC division gives rise to a new ISC and enteroblast, the later further differentiates into either an absorptive enterocyte or a secretory enteroendocrine cell, based on the level of Notch signaling [34]. For the past decade, extensive studies using *Drosophila* midgut have uncovered a signaling network that maintains the homeostasis of gut epithelia, including Hippo and JNK pathway [35].

As a major stress sensor, JNK can be activated both in EC and ISC by bacterial infection or DNA damage to stimulate non-autonomous and autonomous ISC proliferation, respectively [36-40]. In the EC cells, JNK activation via Puc (a phosphatase which inhibits JNK activity) RNAi or Hep overexpression increased ISC mitosis [38] and resulted in nuclear Yki translocation and Hpo target genes expression [36,41], which could further trigger secretion of the Unpaired (Upd) cytokines to

drive non-autonomous ISC proliferation through JAK/STAT signaling pathway (Figure 1) [36,40,41]. Moreover, JNK activation in the progenitor cells of *Drosophila* midgut is sufficient to upregulate Wg expression, which in turn induces Myc-dependent ISC renewal (Figure 1) [42].

In addition, JNK and Hippo signaling have also been shown to regulate ISC proliferation in an autonomous manner [39,40,41,43]. Reducing JNK activity within ISCs significantly blocked *Ecc15* (*Erwinia carotovora carotovora* 15, Gram-negative bacteria) infection and paraquat-induced proliferation [37,44], whereas ectopic JNK activation by Hep or Rac1 (small GTPase acting upstream of JNK) overexpression promoted ISC self-renewal [39,45]. Similarly, silencing *yki* reduced excessive ISC proliferation induced by Gram-negative bacteria *Pseudomonas entomophila* (*Pe*) [41], while inactivation of Hippo signaling had an opposite effect [40,41]. Additionally, Ren and colleagues recently identified Myc as a crucial integrator of multiple signaling including Hippo and JAK/STAT in ISC regeneration (Figure 1) [46]. Importantly, unlike Myc, neither Yki nor JNK inhibition reduced ISC number under normal condition [36,43,44,46], highlighting their essential role as stress sensors in gut epithelia.

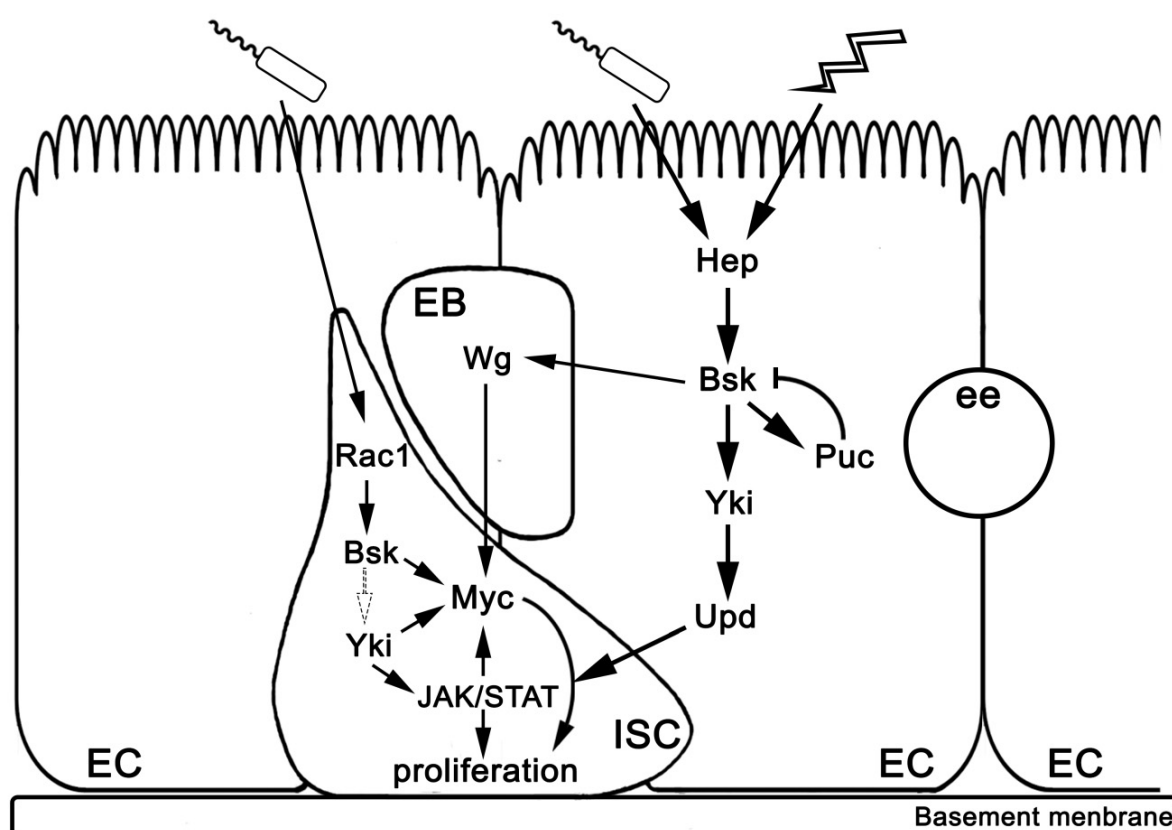


Figure 1. Crosstalk of JNK and Hippo signaling during *Drosophila* midgut regeneration. Upon damage or bacterial infection, JNK signaling acts autonomously in ECs and acts non-autonomously in ISC to stimulate regeneration through Hippo pathway inactivation. See text for details. Abbreviations: ISC, intestinal stem cell; EC, enterocyte; EB, enteroblast; ee, enteroendocrine cell; Wg, Wingless.

2.2. Regeneration in wing disc

JNK signaling activation has been considered as a universal hallmark of regenerating tissue in response to damage [47,48,49]. Besides, it is also shown that JNK plays critical roles in apoptosis-induced non-autonomous compensatory proliferation [50], which could accelerate the regeneration process. Two recent studies revealed that Hippo pathway also regulates regenerative growth in *Drosophila* wing disc. They found that Yki functions as a pivotal determinant for regeneration, as removing only one copy of *yki* could dramatically impair regenerating ability, while does not affect normal wing development [51,52]. Furthermore, activation of JNK by ectopic expression of Eiger (Egr, the *Drosophila* TNF homolog) or Hep^{CA} (a constitutive form of Hep) could induce Yki nuclear accumulation and target gene expression, both autonomously and non-autonomously [51,52]. In the former situation, JNK upregulates Wg secretion as well as Yki target gene expression, while it simultaneously triggers extensive cell death which may counteract the Yki induced growth advantage. In the latter scenario, JNK might propagate into neighboring cells [53] to induce non-autonomous compensatory proliferation via activation of Yki, Wg, Dpp, and Myc [18,50,51,54].

2.3. Cell competition

The phenomenon “cell competition” was first discovered in mosaic *Drosophila* proliferating wing epithelia [55], in which the faster growing cells (winner) would outcompete the slower growing cells (loser) via inducing apoptosis [56,57]. It is known that both JNK and Hippo pathway play essential roles in regulating cell competition [58,59], while not until recently, studies in fly have provided a link between cell competition and Hippo, JNK signaling [60-63].

2.3.1. Cell competition as tumor suppressor

The discovery of cell competition raised a key question: what is the physiological function of cell competition? Given the fact that around 85% of cancer arise from mutated epithelia cells (Cancerstats), cell competition has been rationally assumed as an intrinsic tumor suppressing mechanism removing “unwanted” cells to maintain normal tissue homeostasis [57]. Consistent with this hypothesis, mutant clones generated in *Drosophila* wing epithelium which lost the neoplastic tumor suppressor genes, including *lethal (2) giant larvae (l(2)gl)*, *discs large (dlg)* or *scribble (scrib)*, would be eliminated by JNK-dependent apoptosis or engulfment by neighboring wild-type cells (Figure 2A) [30,60,61,62,64-67]. Interestingly, Froldi et al found that *l(2)gl*^{-/-} clones express relative low levels of Myc compared to surrounding wild-type tissue [67]. Since Myc was recently identified as a direct target of Yki [68,69], and plays essential roles in cell competition and growth control [70,71], hence it is highly possible cell polarity disruption is related with Yki-Myc activation during cell competition (Figure 2A). Strikingly, it is also reported that in spite of facing cell competition, *scrib*^{-/-} clones can induce non-autonomous Yki activation in neighboring cells [51], which may further accelerate the elimination of *scrib*^{-/-} clones.

Several recent studies demonstrated that *scrib*^{-/-} clones indeed have a hyper-proliferate potential, which however was dramatically inhibited by JNK activity [28,62,64,72,73]. When *scrib*^{-/-} clones were protected from cell competition via expression of Bsk^{DN} (a dominant negative

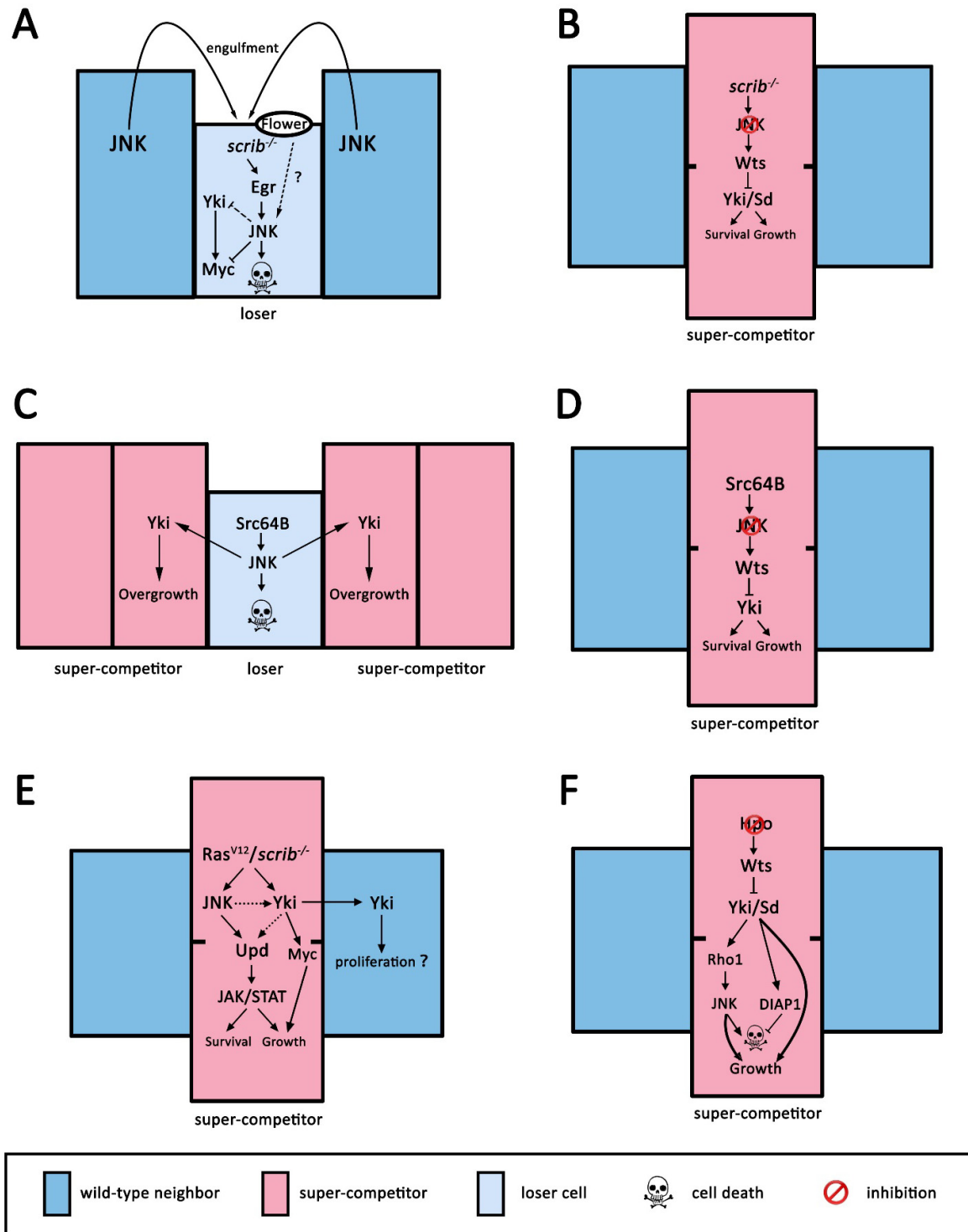


Figure 2. JNK and Hippo context-dependent interactions during cell competition. (A) *scrib*^{-/-} cells are eliminated by wild-type neighboring cells. (B) *scrib*^{-/-} cells with JNK inhibition induces Yki-mediated tumor growth. (C) Src64B overexpression clones causes non-autonomous overgrowth of surrounding tissue. (D) Blocking JNK signaling in Src-expressing clones releases inhibition of Yki activity and results in autonomous growth. (E) *Ras*^{V12} expression turn *scrib*^{-/-} cells into super-competitor by activating both Yki and JNK. (F) Hippo inactivation upregulates Rho1-JNK signaling to induce autonomous growth and super-competitor behavior.

form of *Drosophila* JNK, Bsk), they can ectopically proliferate and induce Yki target genes expression [62,72], in Sd/Yki-dependent manner [72]. Consistently, overexpression of Yki or Myc is sufficient to rescue *scrib*^{-/-} clones from being outcompeted [62]. Therefore, Yki level in *scrib*^{-/-} clones is an essential determinant of whether they would be outcompeted or became super-competitors when JNK signaling is dysregulated (Figure 2B).

Another intriguing question focusing on cell competition is how the loser cells were marked? A recent study shed lights on this puzzle [74]. The transmembrane protein Flower encodes three different isoforms, two of which are only detected in loser cells facing competition. Knocking down those two *flower* isoforms protected the losers from being outcompeted, and conversely, overexpression of them would lead to apoptosis and elimination. It would be worthwhile to further investigate whether Flower-induced competition also operates in a JNK- or Yki-dependent manner (Figure 2A).

Remarkably, it is also reported that unlike *scrib*, depletion of *l(2)gl* in eye epithelial alone is sufficient to suppress Hpo pathway activity, resulting in mild proliferation and Yki activation [75]. Similarly, blocking JNK via expression of Puc in *l(2)gl*^{-/-} cells did not produce tumor growth phenotype in wing epithelia, even though apoptosis was abolished [60].

2.3.2. Cell competition in promoting tumor progression

Interestingly, Enomoto and Igaki recently found that loser clones expressing oncoprotein Src64B could contribute to proliferation of surrounding winner cells, which also rely on both JNK and Hippo signaling [63]. Src64B overexpression clones have increased JNK activity, leading to their elimination by cell competition, while at the same time, JNK induces dramatic non-autonomous growth via Yki activation in neighboring cells (Figure 2C). Conversely, inhibiting JNK activity within Src64B overexpression clones not only blocked the non-autonomous overgrowth of surrounding tissue, but also results in autonomous proliferation and Yki hyperactivation (Figure 2D), highlighting anti-Yki effects of JNK activation in the presence of Src64B. However, the mechanism of how Src64B switches its functions from JNK-dependent pro-Yki to anti-Yki remains unknown. Hence, in addition to the tumor-suppressing role, cell competition could also act as a tumor-promoting factor, promoting JNK activation and subsequently Yki-mediated proliferation of neighboring cell. In accordance with this view, a recent study in *Drosophila* showed that cell competition could possess both tumor-suppressing and tumor-inducing roles depending on the population of loser cells [76].

2.3.3. Ras^{V12} hijacks *scrib*^{-/-} clones into super-competitors

Apart from the pro-apoptotic role discussed above, JNK pathway can promote oncogenic transformation and tumorigenesis as well [23,28,77,78]. In the *Drosophila* antennal-eye disc, expression of oncogenic Ras (*Ras*^{V12}) could convert loser *scrib*^{-/-} clones into super-competitors with massive overgrowth and proliferation [64,79]. Both JNK and Hippo pathways are essential for *scrib*^{-/-}/*Ras*^{V12} cooperation-induced growth, as reducing either JNK or Yki activity would be sufficient to block tumor growth (Figure 2E) [29,60,72,78]. By using eye epithelia as a model, Ohsawa and colleagues found JNK activation (via Egr overexpression) could cooperate with *Ras*^{V12} to autonomously activate Yki target genes expression, including *diap1* and *cycE*, indicating a

pro-Yki activity of JNK in the presence of *Ras*^{V12} [77]. Wu and colleagues investigated the cooperation between *scrib*^{-/-} and *Ras*^{V12} clones in both intra- and interclonal situations, and revealed a JNK-dependent upregulation of *upd*, which accelerated both tumor growth and metastasis [53]. In accordance with this genetic data, clones lacking JAK/STAT activity would be eliminated without JNK activation [80]. In consideration of the epistasis data obtained from Hippo and JAK/STAT signaling in *Drosophila* intestine homeostasis [40,41,43], it is highly possible that Yki may play essential roles in linking *scrib*^{-/-}/*Ras*^{V12} induced tumor growth and JAK/STAT activation (Figure 2E). Moreover, in addition to autonomous proliferation observed in *scrib*^{-/-}/*Ras*^{V12} clones, a non-autonomous effect on Hippo signaling was also seen in surrounding neighbors [62], probably owing to JNK propagation and subsequent compensatory proliferation [53].

In summary, the evidence outlined above indicates that in *Drosophila* JNK can have both pro- and anti-Yki function depending on the context, highlighting the importance for a further understanding of the regulatory crosstalk between Hippo and JNK in developing potential therapeutic treatments of different cancers.

2.3.4. Yki super-competitor property requires JNK activity

Missing core components of Hippo pathway or Yki overexpression are sufficient to turn clones into super-competitors via autonomous proliferation and induction of cell death in surrounding wild-type neighbors [60,68]. Apart from the involvement of Myc-mediated cell competition [68], our recent study provide a further mechanistic insight into the overgrowth properties caused by Hippo signaling loss (unpublished data, Figure 2F). By using *Drosophila* wing epithelia as a model, we found that impaired Hpo pathway activates JNK signaling *in vivo* and JNK activity is essential for Yki-induced growth and proliferation. Furthermore, we identified *rho1* as a direct transcriptional target of Yki/Sd complex. In agreement with previous studies [81,82], we found Rho1 overexpression promotes growth when cell death is compromised. Finally, we also demonstrate that the mammalian YAP is also able to activate *rho1* transcription and JNK activation in Sd-dependent manner when expressed in *Drosophila*, indicating a likely conserved role of Yap in regulating Rho1-JNK mediated growth in mammals (Figure 2F). Thus, our study reveals a novel means of crosstalk between Hippo and JNK signaling.

2.4. Ajuba links Hippo to JNK signaling

The direct evidence illuminating Hippo and JNK interaction came from a recent study by Sun and Irvine [83]. They found Hep^{CA} overexpression in the *Drosophila* wing epithelia can induce Yki nuclear accumulation and target gene expression, which were dramatically suppressed by activating Hippo pathway (Hpo or Wts overexpression). Their epistasis analysis further identified that Ajuba LIM protein (Jub), which has been shown to interact with Wts [84], functions as a bridge mediating Yorkie activation by JNK. Moreover, JNK can directly phosphorylate the mammalian Ajuba family protein LIMD1 and enhance its binding to LATS (Wts homolog in mammal), indicating a conserved role of JNK-Ajuba-Hippo signaling in mammalian cells.

2.5. Cell invasion and migration

Apart from regulating tumor growth and cell competition, JNK pathway also plays critical roles in cell invasion and migration [64,79,85]. For the past decade, *Drosophila* has been used extensively as an excellent model to investigate the mechanism of tumor invasion and metastasis [86,87,88]. Metastasis begins with the local invasion of tumor cells [89], and in *Drosophila* eye antennal discs, expression of *Ras*^{V12} can cooperate with mutants that simultaneously disrupt apical-basal cell polarity to induce invasive tumors into the ventral nerve cord (VNC) [64,79], while subsequent studies showed this invasion requires numerous JNK pathway components [78,85,90,91,92]. Despite the well documented role of Yap in promoting invasion [2,93], it is found that in *Drosophila* Yki activity is dispensable for invasive behavior of *scrib*^{-/-}/*Ras*^{V12} tumors, nevertheless the tumor size is significantly reduced [72]. Similarly, JNK activation specifically along the anterior/posterior boundary of wing disc is sufficient to induce cell invasion phenotype [85,94], while overexpression of Yki cannot [95], despite the dramatic upregulation of MMP1 (unpublished data), a protein required for basement membrane degradation and cell invasion [85,96]. Remarkably, Yuan and colleagues found that Yap actually can function as a negative regulator of invasion in breast cancer [97], they observed reduced Yap protein level in breast cancer sample and reducing Yap activity significantly increased cell migration and invasion. In accordance with this view, studies using *Drosophila* oocyte found that loss of Hippo activity (generating *hpo*^{-/-} or *wts*^{-/-} clones) markedly impeded border cell migration during oogenesis [98,99], phenocopying JNK loss-induced defects [100]. Given the complicated and context-dependent crosstalk between JNK and Hippo signaling (see above), it would be quite interesting to further investigate their corresponding roles in cell invasion and migration both in fly and mammal.

Recent studies in *Drosophila* also linked mechanical tension to Hippo and JNK signaling in tumor progression and invasive migration [101,102,103]. Cheerio (Cher/Filamin), an actin cross-linking protein, was found to be enriched in a JNK-dependent manner in invasive *scrib*^{-/-}/*Ras*^{V12} tumors [101]. Loss of *cher* not only dramatically impeded *scrib*^{-/-}/*Ras*^{V12} induced tumor growth and invasion, but also enabled larvae to form pupae [101], in contrast to *scrib*^{-/-}/*Ras*^{V12} tumor-bearing animals that mostly die as giant larvae [64,79]. In addition, it is also revealed that inhibition of *cher*, but not JNK signaling, suppressed Yki target gene elevation in the *scrib*^{-/-}/*Ras*^{V12} clones [101], however the mechanism of how Cher interacts with Hippo pathway remains unknown. In another study, Fernandez and colleagues showed that in the wing epithelia, actin-Capping Protein (CP) could antagonize oncoprotein Src64B induced overgrowth by restricting JNK signaling, and they also found Src64B upregulated Yki target genes expression via JNK, supporting a pro-Yki activity of JNK signaling [103]. Yet, whether and how JNK and Hippo signaling are coordinated in tumor invasion remains to be further addressed.

3. Conclusion

Both JNK and Hippo pathway regulate various essential biological processes including cell survival, growth and regeneration, and deregulation of either pathway is associated with cancer development in human [2,104]. For the past decade, thanks to the elegant genetic screen performed in *Drosophila*, a complex network composed of numerous novel components of Hippo and JNK pathway has been established, which shed lights on the treatment of related human cancers. JNK

signaling integrates with Hippo signaling in a context dependent manner (this review), and increasing studies show that both pathway possess components that are potential attractive drug targets for cancer treatment [105,106]. Thus, more comprehensive work is clearly needed to further address the regulatory mechanism between JNK and Hippo in diverse physiological functions *in vivo*. Moreover, given that studies have overwhelmingly proven that *Drosophila* can be an excellent drug screening model [107,108], it is promising to expect that the small fruit fly will make important contributions to the pharmaceutical intervention of Hippo and JNK related cancers.

Acknowledgements

I am grateful for Dr. Lei Xue for guidance and valuable advice, Dr. Tatsushi Igaki for personal communication, Dr. Yonggang Zheng for critical reading of the manuscript and helpful comments. I apologize to all the researchers whose work I have not cited due to space limitations.

Conflict of Interest

Author declares no conflicts of interest in this paper.

References

1. Pan D (2010) The hippo signaling pathway in development and cancer. *Dev Cell* 19: 491-505.
2. Harvey KF, Zhang X, Thomas DM (2013) The Hippo pathway and human cancer. *Nat Rev Cancer* 13: 246-257.
3. Pan D (2007) Hippo signaling in organ size control. *Genes Dev* 21: 886-897.
4. Xu T, Wang W, Zhang S, et al. (1995) Identifying tumor suppressors in genetic mosaics: the *Drosophila* *lats* gene encodes a putative protein kinase. *Development* 121: 1053-1063.
5. Justice RW, Zilian O, Woods DF, et al. (1995) The *Drosophila* tumor suppressor gene *warts* encodes a homolog of human myotonic dystrophy kinase and is required for the control of cell shape and proliferation. *Genes Dev* 9: 534-546.
6. Tapon N, Harvey KF, Bell DW, et al. (2002) *salvador* Promotes both cell cycle exit and apoptosis in *Drosophila* and is mutated in human cancer cell lines. *Cell* 110: 467-478.
7. Kango-Singh M, Nolo R, Tao C, et al. (2002) *Shar-pei* mediates cell proliferation arrest during imaginal disc growth in *Drosophila*. *Development* 129: 5719-5730.
8. Harvey KF, Pflieger CM, Hariharan IK (2003) The *Drosophila* *Mst* ortholog, *hippo*, restricts growth and cell proliferation and promotes apoptosis. *Cell* 114: 457-467.
9. Wu S, Huang J, Dong J, et al. (2003) *hippo* encodes a Ste-20 family protein kinase that restricts cell proliferation and promotes apoptosis in conjunction with *salvador* and *warts*. *Cell* 114: 445-456.
10. Udan RS, Kango-Singh M, Nolo R, et al. (2003) Hippo promotes proliferation arrest and apoptosis in the *Salvador/Warts* pathway. *Nat Cell Biol* 5: 914-920.
11. Pantalacci S, Tapon N, Leopold P (2003) The *Salvador* partner *Hippo* promotes apoptosis and cell-cycle exit in *Drosophila*. *Nat Cell Biol* 5: 921-927.
12. Lai ZC, Wei X, Shimizu T, et al. (2005) Control of cell proliferation and apoptosis by *mob* as tumor suppressor, *mats*. *Cell* 120: 675-685.

13. Huang J, Wu S, Barrera J, et al. (2005) The Hippo signaling pathway coordinately regulates cell proliferation and apoptosis by inactivating Yorkie, the Drosophila Homolog of YAP. *Cell* 122: 421-434.
14. Oh H, Irvine KD (2008) In vivo regulation of Yorkie phosphorylation and localization. *Development* 135: 1081-1088.
15. Wu S, Liu Y, Zheng Y, et al. (2008) The TEAD/TEF family protein Scalloped mediates transcriptional output of the Hippo growth-regulatory pathway. *Dev Cell* 14: 388-398.
16. Zhang L, Ren F, Zhang Q, et al. (2008) The TEAD/TEF family of transcription factor Scalloped mediates Hippo signaling in organ size control. *Dev Cell* 14: 377-387.
17. Goulev Y, Fauny JD, Gonzalez-Marti B, et al. (2008) SCALLOPED interacts with YORKIE, the nuclear effector of the hippo tumor-suppressor pathway in Drosophila. *Curr Biol* 18: 435-441.
18. Staley BK, Irvine KD (2012) Hippo signaling in Drosophila: recent advances and insights. *Dev Dyn* 241: 3-15.
19. Yu FX, Guan KL (2013) The Hippo pathway: regulators and regulations. *Genes Dev* 27: 355-371.
20. Mo JS, Park HW, Guan KL (2014) The Hippo signaling pathway in stem cell biology and cancer. *EMBO Rep* 15: 642-656.
21. Kyriakis JM, Avruch J (1990) pp54 microtubule-associated protein 2 kinase. A novel serine/threonine protein kinase regulated by phosphorylation and stimulated by poly-L-lysine. *J Biol Chem* 265: 17355-17363.
22. Davis RJ (2000) Signal transduction by the JNK group of MAP kinases. *Cell* 103: 239-252.
23. Weston CR, Davis RJ (2007) The JNK signal transduction pathway. *Curr Opin Cell Biol* 19: 142-149.
24. Angel P, Karin M (1991) The role of Jun, Fos and the AP-1 complex in cell-proliferation and transformation. *Biochim Biophys Acta* 1072: 129-157.
25. Igaki T (2009) Correcting developmental errors by apoptosis: lessons from Drosophila JNK signaling. *Apoptosis* 14: 1021-1028.
26. Chen F (2012) JNK-induced apoptosis, compensatory growth, and cancer stem cells. *Cancer Res* 72: 379-386.
27. Rios-Barrera LD, Riesgo-Escovar JR (2013) Regulating cell morphogenesis: the Drosophila Jun N-terminal kinase pathway. *Genesis* 51: 147-162.
28. Uhlirova M, Jasper H, Bohmann D (2005) Non-cell-autonomous induction of tissue overgrowth by JNK/Ras cooperation in a Drosophila tumor model. *Proc Natl Acad Sci U S A* 102: 13123-13128.
29. Cordero JB, Macagno JP, Stefanatos RK, et al. (2010) Oncogenic Ras diverts a host TNF tumor suppressor activity into tumor promoter. *Dev Cell* 18: 999-1011.
30. Igaki T, Pastor-Pareja JC, Aonuma H, et al. (2009) Intrinsic tumor suppression and epithelial maintenance by endocytic activation of Eiger/TNF signaling in Drosophila. *Dev Cell* 16: 458-465.
31. Kux K, Pitsouli C (2014) Tissue communication in regenerative inflammatory signaling: lessons from the fly gut. *Front Cell Infect Microbiol* 4: 49.
32. Amcheslavsky A, Jiang J, Ip YT (2009) Tissue damage-induced intestinal stem cell division in Drosophila. *Cell Stem Cell* 4: 49-61.
33. Apidianakis Y, Rahme LG (2011) Drosophila melanogaster as a model for human intestinal

- infection and pathology. *Dis Model Mech* 4: 21-30.
34. Ohlstein B, Spradling A (2007) Multipotent *Drosophila* intestinal stem cells specify daughter cell fates by differential notch signaling. *Science* 315: 988-992.
 35. Lucchetta EM, Ohlstein B (2012) The *Drosophila* midgut: a model for stem cell driven tissue regeneration. *Wiley Interdiscip Rev Dev Biol* 1: 781-788.
 36. Staley BK, Irvine KD (2010) Warts and Yorkie mediate intestinal regeneration by influencing stem cell proliferation. *Curr Biol* 20: 1580-1587.
 37. Buchon N, Broderick NA, Chakrabarti S, et al. (2009) Invasive and indigenous microbiota impact intestinal stem cell activity through multiple pathways in *Drosophila*. *Genes Dev* 23: 2333-2344.
 38. Jiang H, Patel PH, Kohlmaier A, et al. (2009) Cytokine/Jak/Stat signaling mediates regeneration and homeostasis in the *Drosophila* midgut. *Cell* 137: 1343-1355.
 39. Biteau B, Hochmuth CE, 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.
 40. Ren F, Wang B, Yue T, et al. (2010) Hippo signaling regulates *Drosophila* intestine stem cell proliferation through multiple pathways. *Proc Natl Acad Sci U S A* 107: 21064-21069.
 41. Shaw RL, Kohlmaier A, Polesello C, et al. (2010) The Hippo pathway regulates intestinal stem cell proliferation during *Drosophila* adult midgut regeneration. *Development* 137: 4147-4158.
 42. Cordero JB, Stefanatos RK, Scopelliti A, et al. (2012) Inducible progenitor-derived Wingless regulates adult midgut regeneration in *Drosophila*. *EMBO J* 31: 3901-3917.
 43. Karpowicz P, Perez J, Perrimon N (2010) The Hippo tumor suppressor pathway regulates intestinal stem cell regeneration. *Development* 137: 4135-4145.
 44. Biteau B, Jasper H (2011) EGF signaling regulates the proliferation of intestinal stem cells in *Drosophila*. *Development* 138: 1045-1055.
 45. Myant KB, Scopelliti A, Haque S, et al. (2013) Rac1 drives intestinal stem cell proliferation and regeneration. *Cell Cycle* 12: 2973-2977.
 46. Ren F, Shi Q, Chen Y, et al. (2013) *Drosophila* Myc integrates multiple signaling pathways to regulate intestinal stem cell proliferation during midgut regeneration. *Cell Res* 23: 1133-1146.
 47. Worley MI, Setiawan L, Hariharan IK (2012) Regeneration and transdetermination in *Drosophila* imaginal discs. *Annu Rev Genet* 46: 289-310.
 48. Bergantinos C, Corominas M, Serras F (2010) Cell death-induced regeneration in wing imaginal discs requires JNK signalling. *Development* 137: 1169-1179.
 49. Bosch M, Serras F, Martin-Blanco E, et al. (2005) JNK signaling pathway required for wound healing in regenerating *Drosophila* wing imaginal discs. *Dev Biol* 280: 73-86.
 50. Ryoo HD, Gorenc T, Steller H (2004) Apoptotic cells can induce compensatory cell proliferation through the JNK and the Wingless signaling pathways. *Dev Cell* 7: 491-501.
 51. Grusche FA, Degoutin JL, Richardson HE, et al. (2011) The Salvador/Warts/Hippo pathway controls regenerative tissue growth in *Drosophila melanogaster*. *Dev Biol* 350: 255-266.
 52. Sun G, Irvine KD (2011) Regulation of Hippo signaling by Jun kinase signaling during compensatory cell proliferation and regeneration, and in neoplastic tumors. *Dev Biol* 350: 139-151.
 53. Wu M, Pastor-Pareja JC, Xu T (2010) Interaction between Ras(V12) and scribbled clones induces tumour growth and invasion. *Nature* 463: 545-548.
 54. Smith-Bolton RK, Worley MI, Kanda H, et al. (2009) Regenerative growth in *Drosophila*

- imaginal discs is regulated by Wingless and Myc. *Dev Cell* 16: 797-809.
55. Morata G, Ripoll P (1975) Minutes: mutants of drosophila autonomously affecting cell division rate. *Dev Biol* 42: 211-221.
 56. Levayer R, Moreno E (2013) Mechanisms of cell competition: themes and variations. *J Cell Biol* 200: 689-698.
 57. Vincent JP, Fletcher AG, Baena-Lopez LA (2013) Mechanisms and mechanics of cell competition in epithelia. *Nat Rev Mol Cell Biol* 14: 581-591.
 58. Tyler DM, Li W, Zhuo N, et al. (2007) Genes affecting cell competition in Drosophila. *Genetics* 175: 643-657.
 59. Moreno E, Basler K, Morata G (2002) Cells compete for decapentaplegic survival factor to prevent apoptosis in Drosophila wing development. *Nature* 416: 755-759.
 60. Menendez J, Perez-Garijo A, Calleja M, et al. (2010) A tumor-suppressing mechanism in Drosophila involving cell competition and the Hippo pathway. *Proc Natl Acad Sci U S A* 107: 14651-14656.
 61. Grzeschik NA, Parsons LM, Richardson HE (2010) Lgl, the SWH pathway and tumorigenesis: It's a matter of context & competition! *Cell Cycle* 9: 3202-3212.
 62. Chen CL, Schroeder MC, Kango-Singh M, et al. (2012) Tumor suppression by cell competition through regulation of the Hippo pathway. *Proc Natl Acad Sci U S A* 109: 484-489.
 63. Enomoto M, Igaki T (2013) Src controls tumorigenesis via JNK-dependent regulation of the Hippo pathway in Drosophila. *EMBO Rep* 14: 65-72.
 64. Brumby AM, Richardson HE (2003) scribble mutants cooperate with oncogenic Ras or Notch to cause neoplastic overgrowth in Drosophila. *EMBO J* 22: 5769-5779.
 65. Tami Y, Bialucha CU, Tian AG, et al. (2010) Involvement of Lgl and Mahjong/VprBP in cell competition. *PLoS Biol* 8: e1000422.
 66. Ohsawa S, Sugimura K, Takino K, et al. (2011) Elimination of oncogenic neighbors by JNK-mediated engulfment in Drosophila. *Dev Cell* 20: 315-328.
 67. Froidi F, Ziosi M, Garoia F, et al. (2010) The lethal giant larvae tumour suppressor mutation requires dMyc oncoprotein to promote clonal malignancy. *BMC Biol* 8: 33.
 68. Ziosi M, Baena-Lopez LA, Grifoni D, et al. (2010) dMyc Functions Downstream of Yorkie to Promote the Supercompetitive Behavior of Hippo Pathway Mutant Cells. *PLoS Genet* 6: e1001140.
 69. Neto-Silva RM, de Beco S, Johnston LA (2010) Evidence for a Growth-Stabilizing Regulatory Feedback Mechanism between Myc and Yorkie, the Drosophila Homolog of Yap. *Dev Cell* 19: 507-520.
 70. Moreno E, Basler K (2004) dMyc transforms cells into super-competitors. *Cell* 117: 117-129.
 71. Grifoni D, Bellosta P (2014) Drosophila Myc: A master regulator of cellular performance. *Biochim Biophys Acta*.
 72. Doggett K, Grusche FA, Richardson HE, et al. (2011) Loss of the Drosophila cell polarity regulator Scribbled promotes epithelial tissue overgrowth and cooperation with oncogenic Ras-Raf through impaired Hippo pathway signaling. *BMC Dev Biol* 11: 57.
 73. Leong GR, Goulding KR, Amin N, et al. (2009) Scribble mutants promote aPKC and JNK-dependent epithelial neoplasia independently of Crumbs. *BMC Biol* 7: 62.
 74. Rhiner C, Lopez-Gay JM, Soldini D, et al. (2010) Flower forms an extracellular code that reveals the fitness of a cell to its neighbors in Drosophila. *Dev Cell* 18: 985-998.

75. Grzeschik NA, Parsons LM, Allott ML, et al. (2010) Lgl, aPKC, and Crumbs regulate the Salvador/Warts/Hippo pathway through two distinct mechanisms. *Curr Biol* 20: 573-581.
76. Ballesteros-Arias L, Saavedra V, Morata G (2014) Cell competition may function either as tumour-suppressing or as tumour-stimulating factor in *Drosophila*. *Oncogene* 33: 4377-4384.
77. Ohsawa S, Sato Y, Enomoto M, et al. (2012) Mitochondrial defect drives non-autonomous tumour progression through Hippo signalling in *Drosophila*. *Nature* 490: 547-551.
78. Igaki T, Pagliarini RA, Xu T (2006) Loss of cell polarity drives tumor growth and invasion through JNK activation in *Drosophila*. *Curr Biol* 16: 1139-1146.
79. Pagliarini RA, Xu T (2003) A genetic screen in *Drosophila* for metastatic behavior. *Science* 302: 1227-1231.
80. Rodrigues AB, Zoranovic T, Ayala-Camargo A, et al. (2012) Activated STAT regulates growth and induces competitive interactions independently of Myc, Yorkie, Wingless and ribosome biogenesis. *Development* 139: 4051-4061.
81. Brumby AM, Goulding KR, Schlosser T, et al. (2011) Identification of novel Ras-cooperating oncogenes in *Drosophila melanogaster*: a RhoGEF/Rho-family/JNK pathway is a central driver of tumorigenesis. *Genetics* 188: 105-125.
82. Khoo P, Allan K, Willoughby L, et al. (2013) In *Drosophila*, RhoGEF2 cooperates with activated Ras in tumorigenesis through a pathway involving Rho1-Rok-Myosin-II and JNK signalling. *Dis Model Mech* 6: 661-678.
83. Sun G, Irvine KD (2013) Ajuba family proteins link JNK to Hippo signaling. *Sci Signal* 6: ra81.
84. Das Thakur M, Feng Y, Jagannathan R, et al. (2010) Ajuba LIM proteins are negative regulators of the Hippo signaling pathway. *Curr Biol* 20: 657-662.
85. Uhlirva M, Bohmann D (2006) JNK- and Fos-regulated Mmp1 expression cooperates with Ras to induce invasive tumors in *Drosophila*. *EMBO J* 25: 5294-5304.
86. Miles WO, Dyson NJ, Walker JA (2011) Modeling tumor invasion and metastasis in *Drosophila*. *Dis Model Mech* 4: 753-761.
87. Willoughby LF, Schlosser T, Manning SA, et al. (2013) An in vivo large-scale chemical screening platform using *Drosophila* for anti-cancer drug discovery. *Dis Model Mech* 6: 521-529.
88. Pastor-Pareja JC, Xu T (2013) Dissecting Social Cell Biology and Tumors Using *Drosophila* Genetics. *Annu Rev Genet* 47: 51-74.
89. Friedl P, Alexander S (2011) Cancer invasion and the microenvironment: plasticity and reciprocity. *Cell* 147: 992-1009.
90. Ma X, Shao Y, Zheng H, et al. (2013) Src42A modulates tumor invasion and cell death via Ben/dUev1a-mediated JNK activation in *Drosophila*. *Cell Death Dis* 4: e864.
91. Ma X, Yang L, Yang Y, et al. (2013) dUev1a modulates TNF-JNK mediated tumor progression and cell death in *Drosophila*. *Dev Biol* 380: 211-221.
92. Ma X, Li W, Yu H, et al. (2014) Bendless modulates JNK-mediated cell death and migration in *Drosophila*. *Cell Death Differ* 21: 407-415.
93. Lamar JM, Stern P, Liu H, et al. (2012) The Hippo pathway target, YAP, promotes metastasis through its TEAD-interaction domain. *Proc Natl Acad Sci U S A* 109: E2441-2450.
94. Vidal M, Larson DE, Cagan RL (2006) Csk-deficient boundary cells are eliminated from normal *Drosophila* epithelia by exclusion, migration, and apoptosis. *Dev Cell* 10: 33-44.
95. Herranz H, Hong X, Cohen SM (2012) Mutual repression by bantam miRNA and Capicua links

- the EGFR/MAPK and Hippo pathways in growth control. *Curr Biol* 22: 651-657.
96. Srivastava A, Pastor-Pareja JC, Igaki T, et al. (2007) Basement membrane remodeling is essential for *Drosophila* disc eversion and tumor invasion. *Proc Natl Acad Sci U S A* 104: 2721-2726.
 97. Yuan M, Tomlinson V, Lara R, et al. (2008) Yes-associated protein (YAP) functions as a tumor suppressor in breast. *Cell Death Differ* 15: 1752-1759.
 98. Lucas EP, Khanal I, Gaspar P, et al. (2013) The Hippo pathway polarizes the actin cytoskeleton during collective migration of *Drosophila* border cells. *J Cell Biol* 201: 875-885.
 99. Lin TH, Yeh TH, Wang TW, et al. (2014) The Hippo Pathway Controls Border Cell Migration Through Distinct Mechanisms in Outer Border Cells and Polar Cells of the *Drosophila* Ovary. *Genetics* 198: 1087-1099.
 100. Llense F, Martin-Blanco E (2008) JNK signaling controls border cell cluster integrity and collective cell migration. *Curr Biol* 18: 538-544.
 101. Kulshammer E, Uhlirova M (2013) The actin cross-linker Filamin/Cheerio mediates tumor malignancy downstream of JNK signaling. *J Cell Sci* 126: 927-938.
 102. Rauskolb C, Sun S, Sun G, et al. (2014) Cytoskeletal tension inhibits Hippo signaling through an Ajuba-Warts complex. *Cell* 158: 143-156.
 103. Fernandez BG, Jezowska B, Janody F (2014) *Drosophila* actin-Capping Protein limits JNK activation by the Src proto-oncogene. *Oncogene* 33: 2027-2039.
 104. Wagner EF, Nebreda AR (2009) Signal integration by JNK and p38 MAPK pathways in cancer development. *Nat Rev Cancer* 9: 537-549.
 105. Park HW, Guan KL (2013) Regulation of the Hippo pathway and implications for anticancer drug development. *Trends Pharmacol Sci* 34: 581-589.
 106. Bubici C, Papa S (2014) JNK signalling in cancer: in need of new, smarter therapeutic targets. *Br J Pharmacol* 171: 24-37.
 107. Gladstone M, Su TT (2011) Chemical genetics and drug screening in *Drosophila* cancer models. *J Genet Genomics* 38: 497-504.
 108. Gonzalez C (2013) *Drosophila melanogaster*: a model and a tool to investigate malignancy and identify new therapeutics. *Nat Rev Cancer* 13: 172-183.

© 2014, Xianjue Ma, licensee AIMS Press. This is an open access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>)