DREF, a Concertmaster for Hippo Pathway and JNK Pathway in *Drosophila*

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**Hippo Pathway and its Cross-talk with JNK Pathway**

Coordination of positive and negative regulation for cell proliferation is essential to achieve organ formation with proper size. During normal development and regeneration of organs such as after surgical excision, the organs reach at each proper size with cell proliferation and its arrest at proper timing. Studies, in the past decade, have defined a kinase cascade as a key signal transduction pathway to interpret this mechanism. The Hippo pathway was firstly identified in *Drosophila* as a tumor-suppressive signal cascade and plays a crucial role in controlling organ size [1-7]. Interestingly, its core kinases cassette is conserved evolutionally among metazoans, consisting of four proteins, Hippo (Hpo), Salvador (Sav), Mob (Mats) and Warts (Wts) in *Drosophila* [1-10] (Figure 1), and MST1/2, WW45, Mob1/2 and Lats1/2 in mammals, respectively [11-17]. The Ste20-like kinase, Wts is activated by phosphorylation [18], then the activated Hpo phosphorylates Wts, Sav, and Mats [7,19]. Sav binds to both Hpo and Wts to facilitate its reaction by serving as a scaffold [7]. Mats functions as a co-factor of Wts [10]. Then the activated Wts phosphorylates and inactivates the transcriptional co-activator Yorick (Yki) [20]. Consequently, the inactivated Yki is retained in cytoplasm, resulting in reduced transcription of its target genes such as cyclin E that promotes cell-proliferation and dIAP1 that inhibits cell death [20]. Loss-of-function mutations in hpo, wts, sav, or mats induce up-regulation of Yki activity, increase expression of cyclin E and dIAP1, and exhibit tumor-like overgrowth [21-26]. Similarly in mammals, MST1/2 binds to WW45 to phosphorylate and activate the complex of Mob1/2 and LAT51/2. In addition mammalian homologue of Yki, YAP and TAZ also function as transcriptional co-activators and promote cell proliferation [13,27-32].

Many of the Hpo pathway-related genes were identified by genetic screen in *Drosophila* [33]. Imaginal discs of *Drosophila* with hpo mutation exhibit severe tumor-like phenotypes that form dark and folded eye or head, resembling the hide of “hippopotamus” [6]. In particular, the interommatidial cells of eye disc with each mutation in *dIAP1* exhibit up-regulation of *Yki* with their function is at least partially redundant. They physically interact in severe tumor-like phenotype [34]. These observations suggest that the Hippo pathway activity (Figure 1). Mammalian homologues of Crb share conserved amino acids with the intracellular domain of *Drosophila* Crb, which is also reported to function in apical-basal polarity [40].

Moreover, Planar Cell Polarity (PCP) appears to be involved in the Hippo pathway function. Fat (Ft) is a large transmembrane cadherin-like protein involved in determination of PCP. Ft is reported to be the most upstream activator of the Hpo pathway [34,41-44]. Ft ligand, Dachsous (Ds) is another cadherin-like protein and Ft-interactant, Four-jointed (Fj) are also shown to be involved in the Hpo signaling. Ds and Fj are expressed in complementary gradient manner in imaginal discs [45,46]. Juxtaposition of cells that express different levels of Ft and Ds induces expression of the Hpo pathway target genes and cell proliferation in *Drosophila* wing discs. Moreover, uniform expression level of Ft and Ds inhibits cell proliferation [47,48]. Although the link between PCP and Hippo pathway appears to be complex, these findings suggest that the Hippo pathway activity is driven by partially redundant multiple inputs involving cell-cell contact and/or cell polarity (Figure 1).

One of the key mechanisms to control organ size is a so-called “contact inhibition”. In confluent cells, activation of Mer can be observed, which has been reported to require contact inhibition [49]. Also in mammals, cytoplasmic location and inactivation of YAP is induced by high cell density in the Hippo pathway-dependent manner. In addition, inhibition of YAP activity restores contact inhibition in human cells by disruption of WW45, a human homolog of Sav [50]. Moreover, in mammalian cells, the Hippo pathway components are required for contact inhibition of proliferation via cell contact through...
E-cadherin or α/β-catenin [51-54]. Taken together, these findings suggest that the Hippo pathway tightly links with cell contact inhibition, which induces proliferation arrest in order to control organ size.

Recent studies highlight another aspect of the Hippo pathway function. The Hippo pathway has been linked to regulation of organ regeneration. Yki activation in Intestinal Stem Cell (ISC) can be observed in response to damage and results in increasing of ISC proliferation in Drosophila [55]. Also in mice, YAP is related to intestine regeneration program after damage [56]. In Drosophila, intestine cells can be damaged by toxin or pathogens. In this damage-induced system, JNK and JAK/STAT pathways are known to be related to damage response and ISC proliferation. Damage signal is transmitted largely by JNK, and JAK/STAT induces ISC proliferation [57,58]. Currently, linkage between Yki and JNK, JAK/STAT pathways has been identified, in which JNK-dependent Yki activation in differentiated intestinal cells can be observed and induces expression of Upd, a JAK/STAT pathway ligand [59-61]. In Drosophila wing discs, cells that undergo apoptosis stimulate the nearby cells to proliferate. This phenomenon is called “compensatory cell proliferation” and is important to overcome tissue damage [62]. Also in this process, activation of Yki through the JNK pathway can be observed, and direct activation of JNK also induces Yki activation in surviving and nearby cells [63] (Figure 1).

Interaction between the Hippo pathway and JNK pathway is also shown in several other studies. In order to prevent diseases such as cancer, elimination of abnormal cells plays a central role in homeostatic mechanisms. Clones of cells mutant for the tumor suppressor gene scribble (scrib) are eliminated from Drosophila imaginal discs as “loser” by the mechanism called “cell competition” [64]. When all cells in imaginal discs are mutant for scrib, they induce hyperactivation of Yki that drives overgrowth into large neoplastic masses. However, this elimination of abnormal cells can be observed in imaginal discs containing both normal and scrib mutant cells [65-69]. Under these conditions, inhibition of Yki activation arises through JNK-dependent mechanisms in the scrib mutant cells to prevent overproliferation and induce apoptosis. These lines of striking evidence indicate that the Hippo pathway components play a crucial role in tumor suppression, and JNK tightly links with the Hippo pathway to control organ and tissue homeostasis (Figure 1).

DRE/DREF System Plays a Key Role in Transcriptional Regulation of Hippo Pathway- and JNK Pathway-Related Genes

Currently it is reported that Drosophila DRE (DNA Replication-Related Element) / DREF (DRE-Binding Factor) transcriptional regulatory system is essential for regulating the wts gene, a Hippo pathway core component and the basket (bsk) gene, a Drosophila JNK [70,71] (Figure 1). DRE/DREF system is known to closely relate to regulation of a number of cell proliferation-related genes [72]. However since many other genes have been identified as targets of the DRE/DREF system, it is now emerging that the DRE/DREF transcriptional regulatory system induces expression of genes that have a wide variety of functions [72]. Interestingly, Wts and Bsk are similar in function by which prevent inappropriate cell proliferation. Wts is a core component of the Hippo pathway, which functions as a tumor suppressor. And JNK serves a protective function for genome and promotes apoptosis just like p53, which is also known as a target of DRE/DREF [73]. In addition, as described above the Hippo pathway and the JNK pathway cooperate in tissue growth and regeneration. Thus DREF that regulates both wts and bsk genes appears to play a key role in coordination of these two signal transduction systems. In addition, genome database search revealed human DREF (hDREF)-binding consensus sequences in 5'-flanking region of the human lats1 and both jnk1 and 2 genes. Transcription of these genes may therefore also be regulated by the DRE/DREF system in human, as is the case of Drosophila. These findings suggest that DRE/ DREF system is a key regulator to achieve fine-tuning of tissue and organ growth and homeostasis in both Drosophila and human.

References

1. Justice RW, Zilian O, Woods DF, Noll M, Bryant PJ (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.

2. Xu T, Wang W, Zhang S, Stewart RA, Yu W (1995) Identifying tumor suppressors in genetic mosaics: the Drosophila lats gene encodes a putative protein kinase. Development 121: 1053-1063.

3. Tapon N, Harvey KF, Bell DW, Wahrer DC, Schiripo TA, 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.

4. Harvey KF, Pfieger CM, Hariharan IK (2003) The Drosophila Mst ortholog,
hippo, restricts growth and cell proliferation and promotes apoptosis. Cell 114: 457-467.
5. 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.
6. Udan RS, Kango-Singh M, Nolo R, Tao C, Halder G (2003) Hippo promotes proliferation arrest and apoptosis in the Salvador/Warts pathway. Nat Cell Biol 5: 914-920.
7. Wu S, Huang J, Dong J, Pan D (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.
8. Kango-Singh M, Nolo R, Tao C, Verstreken P, Hiesinger PR, et al. (2002) Sharpei mediates cell proliferation arrest during imaginal disc growth in Drosophila. Development 129: 5719-5730.
9. Jia J, Zhang W, Wang B, Trinko R, Jiang J (2003) The Drosophila Ste20 family kinase dMST functions as a tumor suppressor by restricting cell proliferation and promoting apoptosis. Genes Dev 17: 2514-2519.
10. Lai ZC, Wei X, Shimizu T, Ramos E, Rohraugh M, et al. (2005) Control of cell proliferation and apoptosis by mob as tumor suppressor, mats. Cell 120: 675-685.
11. Chan EH, Nouisaien M, Chalamalasetty RB, Schafer A, Nigg EA, et al. (2005) The Ste20-like kinase Mst2 activates the human large tumor suppressor kinase Lats1. Oncogene 24: 2076-2086.
12. Ling P, Lu TJ, Yuan CJ, Lai MD (2008) Biosignalling of mammalian Ste20-related kinases. Cell Signal 20: 1237-1247.
13. Lee JH, Kim TS, Yang TH, Koo BK, Oh SP, et al. (2008) A crucial role of WW45 in developing epithelial tissues in the mouse. EMBO J 27: 1231-1242.
14. Zhou D, Conrad C, Xia F, Park JS, Payer B, et al. (2009) Mst1 and Mst2 maintain hepatocyte quiescence and suppress hepatocarcinoma development through inactivation of the Yap1 oncogene. Cancer Cell 16: 425-438.
15. Hergovich A, Stegert MR, Schmitz D, Hemmings BA (2006) NDR kinases regulate essential cell processes from yeast to humans. Nat Rev Mol Cell Biol 7: 253-264.
16. Hergovich A, Cornils H, Hemmings BA (2008) Mammalian NDR protein kinases: from regulation to a role in centrosome duplication. Biochim Biophys Acta 1784: 3-15.
17. Hergovich A, Kohler RS, Schmitz D, Vichaikovski A, Cornils H, et al. (2009) The MST1 and MHOB1 tumor suppressors control human centrosome duplication by regulating NDR kinase phosphorylation. Curr Biol 19: 1692-1702.
18. Glaitschnig H, Rodan GA, Reszka AA (2002) Mapping of MST1 kinase sites of hMOB1 tumor suppressors control human centrosome duplication. Biochim Biophys Acta 1575: 275-283.
19. Wei X, Shimizu T, Lai ZC (2007) Mob as tumor suppressor is activated by Hippo kinase for growth inhibition in Drosophila. EMBO J 26: 1772-1781.
20. Huang J, Wu S, Barrera J, Matthews K, Pan D (2005) The Hippo signaling pathway coordinately regulates cell proliferation and apoptosis by inactivating Yorkie, the Drosophila Homolog of YAP. Cell 122: 421-434.
21. Reddy BV, Irvine KD (2008) The Fat and Warts signaling pathways: new insights into their regulation, mechanism and conservation. Development 135: 2827-2838.
22. Kango-Singh M, Singh A (2009) Regulation of organ size: insights from the Hippo signaling pathway. Curr Biol 16: 2101-2110.
23. Che J, Feng Y, Rauskolb C, Maltra S, Fehon R, et al. (2010) Delineation of a Fat tumor suppressor pathway. Nat Genet 38: 1142-1150.
24. Silva E, Tsatskis Y, Gardano L, Tapon N, McNeill H (2006) The tumor-suppressor gene fat controls tissue growth upstream of expanded in the hippo signaling pathway. Curr Biol 16: 2081-2089.
25. Tyler DM, Baker NE (2007) Expanded and fat regulate growth and differentiation in the Drosophila eye through multiple signaling pathways. Dev Biol 305: 187-201.
26. Clark HF, Brentrup D, Schneitz K, Bieber A, Goodman C, Noll M. (1995) Dachsous encodes a member of the cadherin superfamily that controls imaginal disc morphogenesis in Drosophila. Genes Dev 9: 1530-1542.
27. Villano JL, Katz FN (1995) Four-jointed is required for intermediate growth in the proximal-distal axis in Drosophila. Development 121: 2767-2777.
28. Rogulja D, Rauskolb C, Irvine KD (2008) Morphogen control of wing growth through the Fat signaling pathway. Dev Cell 15: 309-321.
29. Willecke M, Hara-ratoglu F, Sansores-Garcia L, Tao C, Halder G (2008) Boundaries of Dachsous Cadherin activity modulate the Hippo signaling pathway to induce cell proliferation. Proc Natl Acad Sci USA 105: 14987-14992.
30. Hergovich A (2011) MOB control: reviewing a conserved family of kinase regulators. Cell Signal 23: 1433. 1440.
31. Edgar BA (2006) From cell structure to transcription: Hippo forges a new path. Cell 124: 267-73.
32. Hamaratoglu F, Willecke M, Kango-Singh M, Nolo R, Hyun E, et al. (2006) The tumour-suppressor genes NF2/Merlin and Expanded act through Hippo signalling to regulate cell proliferation and apoptosis. Nat Cell Biol 8: 27-36.
33. McCartney BM, Kulkauskas RM, Leauness DR, Fehon RG (2000) The neurotrophin-like p25-homologue, Merlin, and the tumor suppressor expanded function together in Drosophila to regulate cell proliferation and differentiation. Development 127: 1315-1324.
34. Baumgartner R, Poembacher I, Buser N, Hafen E, Stocker H (2010) The WW domain protein Kibra acts upstream of Hippo in Drosophila. Dev Cell 18: 309-316.
35. Chen CL, Cajewski KM, Hamaratoglu F, Bossuyt W, Sansores-Garcia L, et al. (2010) The apical-basal cell polarity determinant Crumbs regulates Hippo signaling in Drosophila. Proc Natl Acad Sci USA 107: 15810-15815.
36. Robinson BS, Huang J, Hong Y, Moberg KH (2010) Crumbs regulates Salvador/Warts/Hippo signaling in Drosophila via the FERM-domain protein Expanded. Curr Biol 20: 582-590.
37. Ling C, Zheng Y, Yin F, Yu J, Huang J, et al. (2010) The apical transmembrane protein Crumbs functions as a tumor suppressor that regulates Hippo signaling by binding to Expanded. Proc Natl Acad Sci USA 107: 10532-10537.
38. Bazellieres E, Assemat E, Arsoanto JP, Le Bivic A, Massey-Harroche D (2009) Crumbs proteins in epithelial morphogenesis. Front Biosci 14: 2149-2169.
39. Bennett FC, Harvey KF (2006) Fat cadherin modulates organ size in Drosophila via the Salvador/Warts/Hippo signaling pathway. Curr Biol 16: 2101-210.1
40. Cho E, Feng Y, Rauskolb C, Maltra S, Fehon R, et al. (2006) Delineation of a Fat tumor suppressor pathway. Nat Genet 38: 1142-1150.
41. Silva E, Tsatskis Y, Gardano L, Tapon N, McNeill H (2006) The tumor-suppressor gene fat controls tissue growth upstream of expanded in the hippo signaling pathway. Curr Biol 16: 2081-2089.
42. Tyler DM, Baker NE (2007) Expanded and fat regulate growth and differentiation in the Drosophila eye through multiple signaling pathways. Dev Biol 305: 187-201.
43. Clark HF, Brentrup D, Schneitz K, Bieber A, Goodman C, Noll M. (1995) Dachsous encodes a member of the cadherin superfamily that controls imaginal disc morphogenesis in Drosophila. Genes Dev 9: 1530-1542.
44. Villano JL, Katz FN (1995) Four-jointed is required for intermediate growth in the proximal-distal axis in Drosophila. Development 121: 2767-2777.
45. Rogulja D, Rauskolb C, Irvine KD (2008) Morphogen control of wing growth through the Fat signaling pathway. Dev Cell 15: 309-321.
46. Willecke M, Hamaratoglu F, Sansores-Garcia L, Tao C, Halder G (2008) Boundaries of Dachsous Cadherin activity modulate the Hippo signaling pathway to induce cell proliferation. Proc Natl Acad Sci USA 105: 14987-14992.
47. Morrison H, Sherman LS, Legg J, Barine F, Isaacse C, et al. (2001) The NF2 tumor suppressor gene product,merlin, mediates contact inhibition of growth through interactions with CD44. Genes Dev 15: 921-927.
48. Zhao B, Wei X, Li W, Udan RS, Yang Q, et al. (2007) Inactivation of Yap oncoprotein by the Hippo pathway is involved in cell contact inhibition and tissue growth control. Genes Dev 21: 2747-2761.
49. Kim NG, Koh E, Chen X, Gumbiner BM (2011) E-cadherin mediates contact
inhibition of proliferation through Hippo signaling-pathway components. Proc Natl Acad Sci U S A 108: 11930-11935.
52. Silvis MR, Kreger BT, Lien WH, Klezovitch O, Rudakova GM, et al. (2011) α-catenin is a tumor suppressor that controls cell accumulation by regulating the localization and activity of the transcriptional coactivator Yap1. Sci Signal 4: ra33.
53. Schlegelmilch K, Mohseni M, Kirak O, Pruszak J, Rodriguez JR et al. (2011) Yap1 acts downstream of α-catenin to control epidermal proliferation. Cell 144: 782-795.
54. Robinson BS, Moberg KH (2011) Cell-cell junctions: α-catenin and E-cadherin help fence in Yap1. Curr Biol 21: R690-R692.
55. Staley BK, Irvine KD (2010) Warts and Yorkie mediate intestinal regeneration by influencing stem cell proliferation. Curr Biol 20: 1580-1587.
56. Cai J, Zhang N, Zheng Y, de Wilde RF, Mastra A, et al. (2010) The Hippo signaling pathway restricts the oncogenic potential of an intestinal regeneration program. Genes Dev 24: 2383-2388.
57. Beeke K, Lee WC, Michellli CA (2010) JAK/STAT signaling coordinates cell proliferation and multilignage differentiation in the Drosophila intestinal stem cell lineage. Dev Biol. 338: 28-37.
58. Jiang H, Patel PH, Kohlmaier A, Grenley MO, McEwen DG, et al. (2009) Cytokine/Jak/Stat signaling mediates regeneration and homeostasis in the Drosophila midgut. Cell 137: 1343-1355.
59. Karpowicz P, Perez J, Perrimon N (2010) The Hippo tumor suppressor pathway regulates intestinal stem cell regeneration. Development 137: 4135-4145.
60. Ren F, Wang B, Yue T, Yun EY, Ip YT, et al. (2010) Hippo signaling regulates Drosophila intestine stem cell proliferation through multiple pathways. Proc Natl Acad Sci USA 107: 21064-21069.
61. Shaw RL, Kohlmaier A, Polesello C, Veenken C, Edgar BA, et al. (2010) The Hippo pathway regulates intestinal stem cell proliferation during Drosophila adult midgut regeneration. Development 137: 4147-4158.
62. Fan Y, Bergmann A (2008) Apoptosis-induced compensatory proliferation. The Cell is dead. Long live the Cell! Trends Cell Biol 18: 467-473.
63. 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: 139-51.
64. Adachi-Yamada T, O’Connor MB (2004) Mechanisms for removal of developmentally abnormal cells: Cell competition and morphogenetic apoptosis. J Biochem 136: 13-17.
65. Brumby AM, Richardson HE (2003) scribble mutants cooperate with oncogenic Ras or Notch to cause neoplastic overgrowth in Drosophila. EMBO J. 22: 5769-5779.
66. Uhlbrova 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 USA 102: 13123-13128.
67. 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.
68. Igaki T, Pastor-Pareja JC, Aonuma M, Xu T (2009) Intrinsinc tumor suppression and epithelial maintenance by endocytic activation of Eiger/TNF signaling in Drosophila. Dev Cell 16: 458-465.
69. Bider D, Li M, Perrimon N (2000) Cooperative regulation of cell polarity and growth by Drosophila tumor suppressors. Science 289: 113-116.
70. Fujiwara S, Ida H, Yoshioka Y, Yoshida H, Yamaguchi M (2012) The warts gene as a novel target of the Drosophila DRE/DREF transcription pathway. Am J Cancer Res 2: 36-44.
71. Yoshioka Y, Ly LL, Yamaguchi M (2012) Transcription factor NF-Y is involved in differentiation of RT photoreceptor cell in Drosophila. Biol Open 1: 19-29.
72. Matsukage A, Hirose F, Yoo MA, Yamaguchi M (2008) The DRE/DREF transcriptional regulatory system: a master key for cell proliferation. Biochem Biophys Acta 1779: 81-89.
73. Tue NT, Thao DTP, Yamaguchi M (2010) Role of DREF in transcriptional regulation of the Drosophila p53 gene. Oncogene 29: 2060-2069.