



Journal Homepage: - [www.journalijar.com](http://www.journalijar.com)  
**INTERNATIONAL JOURNAL OF  
 ADVANCED RESEARCH (IJAR)**

Article DOI: 10.21474/IJAR01/5854  
 DOI URL: <http://dx.doi.org/10.21474/IJAR01/5854>



### RESEARCH ARTICLE

#### ROLES OF LARGE TUMOR SUPPRESSOR GENES IN BREAST CANCER.

Fang LIN<sup>1</sup> and Munankarmy AMIK<sup>2</sup>.

1. Professor, Department of Breast and Thyroid Surgery, Shanghai 10<sup>th</sup> People's Hospital, Tongji University School of Medicine, 301 Yanchang Road, Jing'an District, Shanghai 200072, P.R. China.
2. Resident, Department of Breast and Thyroid Surgery, Shanghai 10<sup>th</sup> People's Hospital, Tongji University School of Medicine, 301 Yanchang Road, Jing'an District, Shanghai 200072, P.R. China.

#### Manuscript Info

##### Manuscript History

Received: 13 September 2017  
 Final Accepted: 15 October 2017  
 Published: November 2017

##### Key words:-

Hippo pathway; breast cancer; larger tumor suppressor; tumorigenesis.

#### Abstract

The Hippo pathway is a tumor suppressor pathway that negatively regulates YAP and TAZ by regulating cell proliferation, cell growth and cell death. One of the core kinase of Hippo pathway, large tumor suppressor (LATS 1/2) are serine/threonine kinases which belongs to NDR family that are phosphorylated and activated by MST 1/2 and MOB 1/2 followed by phosphorylation of transcriptional co-activator YAP. Originally, isolated from *Drosophila* as tumor suppressor genes recently isolated from mice and human. Since a decade, there has been various studies in Hippo pathway but little is known about LATS expression in breast cancers. Hence, in this review we interpret the role of LATS in breast cancer and its significance as tumor suppressor gene.

Copy Right, IJAR, 2017.. All rights reserved.

#### Introduction

Cell development is essential form of every cell but we perceive little knowledge about the mechanism that controls the organ size[1][2]. For instance, when does a cell know to terminate cell division after it reaches its essential size? How does our body recognize when there is injury in the body and starts to regenerate? Since last two decades, much hard work had made progress to these mechanism to unveil cell proliferation, differentiation and its malignant transformation but still we know little knowledge about mechanism to control organ size[3].

Not only, the discovery of Hippo pathway play as a crucial regulator of organogenesis in *Drosophila* but also in terms of mammal development and tumorigenesis[4]. Hippo pathway consists of core kinase cascade known as Hpo (MST 1/2 orthologue of mammal), a member of Ste-20 protein kinase which phosphorylates Warts (LATS 1/2 orthologue of mammal). As a key regulator of this growth-inhibitory pathway, the large tumor suppressor (LATS)/Warts (Wts) gene encodes a Serine/Threonine protein kinase and somatic mutations in human LATS1 and LATS2 have been identified in primary tumors[5]. These protein kinases along with others help to regulate the normal phenomenon of cell differentiation, proliferation and apoptosis[6]. In a cell, phosphorylation of the transcriptional coactivators, Yes-associated protein (YAP) and WW domain containing transcription regulator 1 (TAZ) is controlled by LATS 1/2 which then, regulate cell proliferation, differentiation, and malignant transformation[7]. Hippo pathway has been deregulated in many types of cancer such as breast, lung, liver, colon, cervix, ovary and esophagus[8–13]. Regulation of gene expression take place in nucleus by transducing signal from cytoplasm to nucleus. Although there has been much understanding about the mechanism of signal transduction of core kinases, little is known in the other fields of Hippo pathway and lacks clear identification of mutational genes

**Corresponding Author:- Fang LIN.**

Address:- Department of Breast and Thyroid Surgery, Shanghai 10<sup>th</sup> People's Hospital, Tongji University School of Medicine, Shanghai 200072.

and cause of tumor progression. In this review, we relate the core kinase of Hippo pathway, LATS in terms of breast cancer cell.

### **The Hippo Pathway:-**

Hippo pathway was first discovered through multiple genetic screening in *Drosophila melanogaster* [14,15] (protein kinases explained are related to *D. melanogaster*). It is also known as Salvador – Warts – Hippo Pathway. With recent discoveries and valid hypothesis; Hippo pathway can trigger tumorigenesis in mice, mutation and alter gene expression and also core for control of organ growth as well as suppression of tumor. Hippo pathway has been linked with various critical biological processes and human diseases including organ growth control, stem cell function, tissue regeneration and tumor suppression [6]. Hippo pathway is constantly regulated in order to maintain the organ size and cellular proliferation during the development as well as homeostasis. Signaling tightly controls organ growth as well as tumor initiation and invasion. At first, animals with Hpo mutant eye discs produce adults with severely overgrown eyes and heads that are folded and darker than normal. The Hpo gene was thus named after its mutant adult head phenotype, which resembles that of the hippopotamus [16]. In *Drosophila*, lack of Hippo signaling in imaginal discs causes overgrowth because mutant cells proliferate faster than normal cell. And hence, they continue to proliferate beyond normal disc size and are resistant to the pro-apoptotic signal that would normally consume extra cells [16,17]. Thus, Hippo pathway confines cell proliferation and promotes cell apoptosis. Hippo pathway initially affects the number of cells produced and has only minor effects on tissue patterning.

With recent discoveries, several other genes have been identified and added in Hippo pathway, a complex signaling pathway with upstream and downstream regulators. In the center of the Hippo pathway lies two core kinases, the Ste20-like kinase - Hpo and NDR family kinases – Wts, these forms kinase cascade [16–18]. The Hpo and Wts kinases, along with their co-factors Salvador (Sav) and Mob as tumor suppressor (Mats) and Yorkie (Yki) transcriptional co-activator, forms the core kinases of Hippo pathway. In active state, Hpo together with co-factor protein kinase Sav facilitate to phosphorylate Wts and its cofactor Mats, which then activates Wts kinase activity, the pathway is now considered at an activated state. Activated Wts/Mats phosphorylates Yki (which is considered as a key event in the canonical Hippo pathway) at three different sites (S111, S168 and S250) [6,19,20] and hence deactivating the function of Yki. Though phosphorylation of Yki at different sites contribute to the regulation of Yki, phosphorylation at S168 plays crucial role which results to bind Yki to 14-3-3 phosphopeptide binding proteins, resulting to restrict location of Yki to nucleus thereby suppressing Yki transcriptional activity [19–21]. If Hippo signaling is not in active state, the major downstream effector of Hippo pathway, Yki binds to the transcription factor Scalloped (Sd) and Yki-Sd complex are translocated into the nucleus and bind to DNA via forming complexes with transcriptional enhancer factors TEAD 1-4. When Yki is activated, genes that promote organ growth such as cyclin E (promotes cell cycle progression) and diap1 (prevents cell apoptosis) is also activated [6]. Yki also bind with other transcription factors such as SMADs, TBX5, RUNX1/2 as well as P73 to regulate gene expression [22].

Multiple upstream regulators of Hippo pathway regulate Yki (YAP/TAP homolog of *D. melanogaster*) activity either by activating core kinases cascade to suppress function of Yki or by forming a complex system to restrict their nuclear access. These upstream regulators include: KIBRA-Ex-Mer complex, Scrib-Crumbs, GPCRs, Fat and Dachshous and Echinoid (orthologue of hemicentin 1 in mammals). Hence, downstream regulator, Yki acts as a cell growth promoter, whereas the upstream regulators, Hpo, Sav, Wts and Mats counteract the function of Yki. Most of the terms used in this review emphasizes in terms of mammals rather than *D. melanogaster*.

### **LATS as down regulator of transcriptional co-activator YAP:-**

With the recent articles so far, LATS 1/2 are only the two members of NDR kinase family in mammals that phosphorylates transcriptional co-activator YAP. The interaction of LATS 1 binds to both WW domains of YAP through its PPPY559 motif to inhibit the function of YAP [23]. YAP is known to be the substrate of LATS rather than NDR. To understand, how LATS acts as upstream regulator in Hippo YAP pathway, A. Hao et al. has identified the phosphorylation sites of LATS 1 in YAP. In mammals, YAP is phosphorylated in five different sites via phosphorylation of motif HXRXXS at S61A, S109A, S127A, S164A and S397A whereas, in *D. melanogaster* Yki is phosphorylated in two sites at S111 and S168 [23]. Phosphorylation at S168 is considered as an important factor because Yki binds to 14-3-3 phosphopeptide binding proteins, resulting to restrict location of Yki to nucleus [21]. Hence, phosphorylation of YAP by LATS 1 inhibits subcellular location to nucleus, leading to sequestration of YAP in the cytoplasm. Likewise, site of phosphorylation of YAP at S127 is also shown to be crucial in mammals and hence binding site for 14-3-3, this increases its interaction with cytoplasmic protein. Therefore,

LATS 1/2 regulate mammary cell proliferation and maturation through antagonism of YAP/TAZ. The down regulation of LATS kinases also alters P53, a tumor suppressor/regulator of homeostasis, to promote cell migration which thereby can lead to metastasis to other organs[24]. Mutation of tumor suppressor genes, loses its capabilities and acquire gain of function to enhance tumorigenesis and invasion.

#### **Expression of LATS in HER2 positive and TNBC:-**

The core kinases, LATS 1/2 of Hippo pathway also regulate with ataxia telangiectasia and Ras3-related protein ATR checkpoint kinase 1 (ATR-Chk1) and ataxia telangiectasia mutated ATM checkpoint kinase 2 (ATM-Chk2), which are the central nodes of DNA damage response (DDR) to minimize the expression of YAP[25]. Neither TAZ nor YAP, independently on whether they were considered in tumor cells or in neoplastic cells, showed a significant association with pCR (pathological complete response). In TNBC, both TAZ and YAP are closely related proteins, involved and possibly cooperate in metastatic dissemination. TAZ and YAP elicit a partial different array of oncogenic activities[26]. Tumors are mostly considered as positive or negative mostly on the basis of cellular localization and assessment of YAP/TAZ should be extended to the non-neoplastic compartment, in order to take into account the biological relevance of the Hippo pathway in CAFs (Cancer associated fibroblasts), TILs (tumor infiltrating lymphocytes) and endothelial cells[26]. Nuclear localization of YAP/TAZ is more prone in risk of recurrence. Hence, Hippo related micro environment needs vast studies in breast cancer in order to picture the significance of Hippo transducers in individual breast cancer subtypes.

#### **Regulation of Hippo pathway by interaction between LATS and KIBRA:-**

Recent studies show that KIBRA, regulates Hippo pathway by interacting with large tumor suppressor kinases. KIBRA associates with both large tumor suppressor kinases, LATS 1 and LATS 2 by interacting with endogenous protein, Merlin. It has been identified that WW domains in KIBRA are required to interact with LATS 2[27]. KIBRA binds with LATS 2 through its N-terminal 100 amino acids, which is the reason why deletion of first 100 amino acids from KIBRA terminates the interaction between KIBRA and LATS 2. KIBRA contains 2 WW domains in its N-terminal (amino acids 7-39 for WW1 and amino acids 54-86 for WW2). LATS 2 protein mutated in its PPxY motif partially lost interaction with KIBRA, further implying that another region besides the PPxY motif in LATS 2 is involved in associating with KIBRA[22]. It has been known that N-terminal amino acids of KIBRA, PPxY motif and LATS 1/2 are required for the interaction between KIBRA and LATS, to regulate the Hippo signaling pathway. KIBRA also promotes both large tumor suppressor kinases activity by stimulating phosphorylation on their hydrophobic sites[28]. KIBRA protein with its WW domain deleted is virtually unable to promote the phosphorylation of LATS 2, suggesting that the association with LATS 2 protein is required for KIBRA to activate the kinases. This interaction between WW domains of KIBRA and LATS is verified in human cell lines whereas deletion of any of WW domains in *D. melanogaster* has not yet been identified[27]. KIBRA significantly increases site of phosphorylation of YAP which suggests that KIBRA regulates YAP phosphorylation. KIBRA with its WW domains deleted was not able to promote YAP phosphorylation. But mutation of both WW domain alone could affect the ability of KIBRA to promote YAP phosphorylation. KIBRA also regulates LATS 1/2 and YAP phosphorylation independent of MST 1/2 kinases.

#### **Down regulation of LATS kinases serve as prognostic factor:-**

In a study of 30 breast tumors and 6 adjacent normal breast tissue (Y. Takahashi et al.) shows that hyper methylation of LATS kinases were seen in breast tumors whereas in normal breast tissue were not observed. Hence, correlating the LATS kinases level with clinico-pathologic parameter, decreased expression of LATS1 mRNA levels was clinically useful in prediction of survival. LATS level are a significant prognostic factor, independent of lymph node metastasis. Decrease in expression of LATS kinases level was significantly associated with a large tumor size[29]. Likewise, in terms of lung cancer, the relationship of YAP and LATS shows inverse proportional ie. when there is expression of nuclear YAP, cytoplasmic YAP didn't show any expression[30]. Also, regulation of YAP is associated with expression of other cell cycle markers. Not only, in breast, also in other organ such as lung, liver, blood vessels, esophagus and prostate shown similar results[5,31–35]. Therefore, loss of LATS kinase expression is highly associated with cell proliferation as well as organ size.

#### **Conclusion:-**

LATS, a tumor suppressor gene, is dedicated to promote phosphorylation of transcriptional co-activator YAP to restrict its nuclear location and hence deactivate its function. While, studies have been going through to restrict the nuclear location of YAP, we have only handful of knowledge regarding how LATS provoke as upstream regulator

in Hippo pathway. There has been evidence, not only LATS is phosphorylated by MST and MOB but also there are other co-factor genes that regulate the phosphorylation of LATS. Clearly, much more work needs to be done to understand the Hippo pathway as researchers have been discovering new regulating genes that could be crucial for development of mammary gland or its disease as well as in diagnosis and treatment for various types of cancer.

### References:-

1. Neto-Silva RM, de Beco S, Johnston LA. Evidence for a growth-stabilizing regulatory feedback mechanism between Myc and Yorkie, the drosophila homolog of Yap. *Dev Cell* 2010;19:507–20. doi:10.1016/j.devcel.2010.09.009.
2. Hayashi S, Yokoyama H, Tamura K. Roles of Hippo signaling pathway in size control of organ regeneration. *Dev Growth Differ* 2015;57:341–51. doi:10.1111/dgd.12212.
3. Yu FX, Zhao B, Guan KL. Hippo Pathway in Organ Size Control, Tissue Homeostasis, and Cancer. *Cell* 2015;163:811–28. doi:10.1016/j.cell.2015.10.044.
4. Harvey KF, Zhang X, Thomas DM. The Hippo pathway and human cancer. *Nat Rev Cancer* 2013;13:246–57. doi:10.1038/nrc3458.
5. Murakami H, Mizuno T, Taniguchi T, Fujii M, Ishiguro F, Fukui T, et al. LATS2 is a tumor suppressor gene of malignant mesothelioma. *Cancer Res* 2011;71:873–83. doi:10.1158/0008-5472.CAN-10-2164.
6. Huang J, Wu S, Barrera J, Matthews K, Pan D. The Hippo signaling pathway coordinately regulates cell proliferation and apoptosis by inactivating Yorkie, the Drosophila Homolog of YAP. *Cell* 2005;122:421–34. doi:10.1016/j.cell.2005.06.007.
7. Varelas X. The Hippo pathway effectors TAZ and YAP in development, homeostasis and disease. *Development* 2014;141:1614–26. doi:10.1242/dev.102376.
8. Cordenonsi M, Zanconato F, Azzolin L, Forcato M, Rosato A, Frasson C, et al. The Hippo transducer TAZ confers cancer stem cell-related traits on breast cancer cells. *Cell* 2011;147:759–72. doi:10.1016/j.cell.2011.09.048.
9. Zhou Z, Hao Y, Liu N, Raptis L, Tsao M-S, Yang X. TAZ is a novel oncogene in non-small cell lung cancer. *Oncogene* 2011;30:2181–6. doi:10.1038/onc.2010.606.
10. He C, Mao D, Hua G, Lv X, Chen X, Angeletti PC, et al. The Hippo/YAP pathway interacts with EGFR signaling and HPV oncoproteins to regulate cervical cancer progression. *EMBO Mol Med* 2015;7:1426–49. doi:10.15252/emmm.201404976.
11. Lam-Himlin DM, Daniels JA, Gayyed MF, Dong J, Maitra A, Pan D, et al. The Hippo Pathway in Human Upper Gastrointestinal Dysplasia and Carcinoma: A Novel Oncogenic Pathway. *Int J Gastrointest Cancer* 2007;37:103–9. doi:10.1007/s12029-007-0010-8.
12. Sohn BH, Shim J-J, Kim S-B, Jang KY, Kim SM, Kim JH, et al. Inactivation of Hippo Pathway Is Significantly Associated with Poor Prognosis in Hepatocellular Carcinoma. *Clin Cancer Res* 2016;22:1256–64. doi:10.1158/1078-0432.CCR-15-1447.
13. Wierzbicki PM, Rybarczyk A. The Hippo pathway in colorectal cancer. *Folia Histochem Cytobiol* 2015;53:105–19. doi:10.5603/FHC.a2015.0015.
14. Badouel C, Garg A, McNeill H. Herding Hippos: regulating growth in flies and man. *Curr Opin Cell Biol* 2009;21:837–43. doi:10.1016/j.ceb.2009.09.010.
15. Reddy BVVG, Irvine KD. The Fat and Warts signaling pathways: new insights into their regulation, mechanism and conservation. *Development* 2008;135:2827–38. doi:10.1242/dev.020974.
16. Udan RS, Kango-Singh M, Nolo R, Tao C, Halder G. Hippo promotes proliferation arrest and apoptosis in the Salvador/Warts pathway. *Nat Cell Biol* 2003;5:914–20. doi:10.1038/ncb1050.
17. Wu S, Huang J, Dong J, Pan D. hippo encodes a Ste-20 family protein kinase that restricts cell proliferation and promotes apoptosis in conjunction with salvador and warts. *Cell* 2003;114:445–56.
18. Xu T, Wang W, Zhang S, Stewart RA, Yu W. Identifying tumor suppressors in genetic mosaics: the Drosophila *lats* gene encodes a putative protein kinase. *Development* 1995;121:1053–63.
19. Dong J, Feldmann G, Huang J, Wu S, Zhang N, Comerford SA, et al. Elucidation of a universal size-control mechanism in Drosophila and mammals. *Cell* 2007;130:1120–33. doi:10.1016/j.cell.2007.07.019.
20. Zhao B, Wei X, Li W, Udan RS, Yang Q, Kim J, et al. Inactivation of YAP oncoprotein by the Hippo pathway is involved in cell contact inhibition and tissue growth control. *Genes Dev* 2007;21:2747–61. doi:10.1101/gad.1602907.
21. Oh H, Irvine KD. In vivo regulation of Yorkie phosphorylation and localization. *Development* 2008;135:1081–8. doi:10.1242/dev.015255.
22. Badouel C, Garg A, McNeill H. Herding Hippos: regulating growth in flies and man. *Curr Opin Cell Biol*

- 2009;21:837–43. doi:10.1016/j.ceb.2009.09.010.
23. Hao Y, Chun A, Cheung K, Rashidi B, Yang X. Tumor suppressor LATS1 is a negative regulator of oncogene YAP. *J Biol Chem* 2008;283:5496–509. doi:10.1074/jbc.M709037200.
  24. Furth N, Ben-Moshe NB, Pozniak Y, Porat Z, Geiger T, Domany E, et al. Down-regulation of LATS kinases alters p53 to promote cell migration. *Genes Dev* 2015;29:2325–30. doi:10.1101/gad.268185.115.
  25. Ercolani C, Di Benedetto A, Terrenato I, Pizzuti L, Di Lauro L, Sergi D, et al. Expression of phosphorylated Hippo pathway kinases (MST1/2 and LATS1/2) in HER2-positive and triple-negative breast cancer patients treated with neoadjuvant therapy. *Cancer Biol Ther* 2017;18:339–46. doi:10.1080/15384047.2017.1312230.
  26. Vici P, Mottollese M, Pizzuti L, Barba M, Sperati F, Terrenato I, et al. The Hippo transducer TAZ as a biomarker of pathological complete response in HER2-positive breast cancer patients treated with trastuzumab-based neoadjuvant therapy. *Oncotarget* 2014;5:9619–25. doi:10.18632/oncotarget.2449.
  27. Xiao L, Chen Y, Ji M, Dong J. KIBRA regulates hippo signaling activity via interactions with large tumor suppressor kinases. *J Biol Chem* 2011;286:7788–96. doi:10.1074/jbc.M110.173468.
  28. Xiao L, Chen Y, Ji M, Dong J. KIBRA regulates Hippo signaling activity via interactions with large tumor suppressor kinases. *J Biol Chem* 2011;286:7788–96. doi:10.1074/jbc.M110.173468.
  29. Takahashi Y, Miyoshi Y, Takahata C, Irahara N, Taguchi T, Tamaki Y, et al. Down-regulation of LATS1 and LATS2 mRNA expression by promoter hypermethylation and its association with biologically aggressive phenotype in human breast cancers. *Clin Cancer Res* 2005;11:1380–5. doi:10.1158/1078-0432.CCR-04-1773.
  30. Kim MH, Kim YK, Shin DH, Lee HJ, Shin N, Kim A, et al. Yes associated protein is a poor prognostic factor in well-differentiated lung adenocarcinoma. *Int J Clin Exp Pathol* 2015;8:15933–9.
  31. Strazisar M, Mlakar V, Glavac D. LATS2 tumour specific mutations and down-regulation of the gene in non-small cell carcinoma. *Lung Cancer* 2009;64:257–62. doi:10.1016/j.lungcan.2008.09.011.
  32. Luo SY, Sit K-Y, Sihoe ADL, Suen W-S, Au W-K, Tang X, et al. Aberrant large tumor suppressor 2 (LATS2) gene expression correlates with EGFR mutation and survival in lung adenocarcinomas. *Lung Cancer* 2014;85:282–92. doi:10.1016/j.lungcan.2014.05.025.
  33. Jiménez-Velasco A, Román-Gómez J, Agirre X, Barrios M, Navarro G, Vázquez I, et al. Downregulation of the large tumor suppressor 2 (LATS2/KPM) gene is associated with poor prognosis in acute lymphoblastic leukemia. *Leukemia* 2005;19:2347–50. doi:10.1038/sj.leu.2403974.
  34. Ishizaki K, Fujimoto J, Kumimoto H, Nishimoto Y, Shimada Y, Shinoda M, et al. Frequent polymorphic changes but rare tumor specific mutations of the LATS2 gene on 13q11-12 in esophageal squamous cell carcinoma. *Int J Oncol* 2002;21:1053–7.
  35. Xia J, Zeng M, Zhu H, Chen X, Weng Z, Li S. Emerging role of Hippo signalling pathway in bladder cancer. *J Cell Mol Med* 2017. doi:10.1111/jcmm.13293.