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Review

Review

Hippo pathway in mammary gland development and breast cancer

Peiguo Shi[1,2], Jing Feng[3,*], and Ceshi Chen[1,*]

[1]Key Laboratory of Animal Models and Human Disease Mechanisms of the Chinese Academy of Sciences and Yunnan Province, Chinese Academy of Sciences, Kunming Institute of Zoology, Kunming 650223, China, [2]University of Chinese Academy of Sciences, Beijing 100049, China, and [3]Department of laboratory medicine & Central Laboratory, South Medical University Affiliated Fengxian Hospital, Shanghai 201499, China

*Correspondence address. Tel: +86-871-65181944; Fax: +86-871-65181945; E-mail: chenc@mail.kiz.ac.cn (C.C.)/Tel: +86-21-57412836; Fax: +86-21-57412830; E-mail: fengjing8801530@163.com (J.F.)

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Abstract

Accumulated evidence suggests that the Hippo signaling pathway plays crucial roles in mammary gland development and breast cancer. Key components of the Hippo pathway regulate breast epithelial cell proliferation, migration, invasion, and stemness. Additionally, the Hippo pathway regulates breast tumor growth, metastasis, and drug resistance. It is expected that the Hippo pathway will provide novel therapeutic targets for breast cancer. This review will discuss and summarize the roles of several core components of the Hippo pathway in mammary gland development and breast cancer.

Key words: Hippo pathway, YAP, TAZ, mammary gland development, breast cancer

Introduction

The Hippo pathway was discovered ~20 years ago in *Drosophila melanogaster* [1,2]. The Hippo pathway is largely conserved in mammals; however, it becomes more complex in mammals than in *Drosophila* [3–7]. The Hippo pathway is regulated by various upstream signals, such as cell–cell contact [8,9], extracellular matrix [10], and cell stress [11]. In addition, Hippo pathway is regulated by G-protein-coupled receptors (GPCRs) [12] and PI3K [13,14]. When Hippo pathway is activated, phosphorylated mammalian sterile 20-like kinase 1/2 (Mst1/2) interact with Sav1 (also known as WW45) to form a complex [4]. The activated Mst1/2 complex directly phosphorylates the large tumor suppressor 1 and 2 (LATS1/2) [7] and MOBKL1A/B (also known as MOB1) that forms another kinase complex with LATS1/2 [5] (**Fig. 1**). Phosphorylated and activated LATS1/2 phosphorylates transcription coactivators YAP and TAZ at S127 and S89, respectively [15–18], leading to the YAP/TAZ cytoplasm retention by 14-3-3 or degradation [17–19]. Unphosphorylated YAP and TAZ translocate into the nucleus to interact with transcription factors, including TEAD1–4 [20–22], Smads [23], p73 [24], and so on. The transcription complexes regulate expression of a number of downstream target genes, for example, *CTGF* [22] and *Cyr61* [25], by which the Hippo pathway modulates various cellular behaviors [26,27].

The Hippo pathway is believed to be a pivotal pathway that controls organ size in *Drosophila* and mammals by coordination of cell proliferation and survival [15,28–40] (**Fig. 1**). Furthermore, the Hippo pathway plays an important role in stem cells of several organs, including liver, skin, intestine, heart, and so on [33,41–46]. Additionally, the Hippo pathway also determines the self-renewal and differentiation of embryonic stem cell [47,48], mesenchymal stem cell [49], induced pluripotent stem cell [47,50], and cancer stem cell (CSC) [51,52]. The Hippo pathway regulates embryonic development and organ homeostasis. The aberration of the pathway causes different diseases, such as cancer [35,52–61], cardiovascular diseases [62], and neurodegenerative diseases [63]. In this review, we focus on several core components of the Hippo pathway in mammary gland development and breast cancer.

The Hippo Pathway Regulates Mammary Gland Development

Mammary gland development can be divided into embryonic and postnatal development stages. Postnatal mammary gland development is further divided into puberty, pregnancy, lactation, and involution periods. There is only a rudimentary ductal structure invasion into

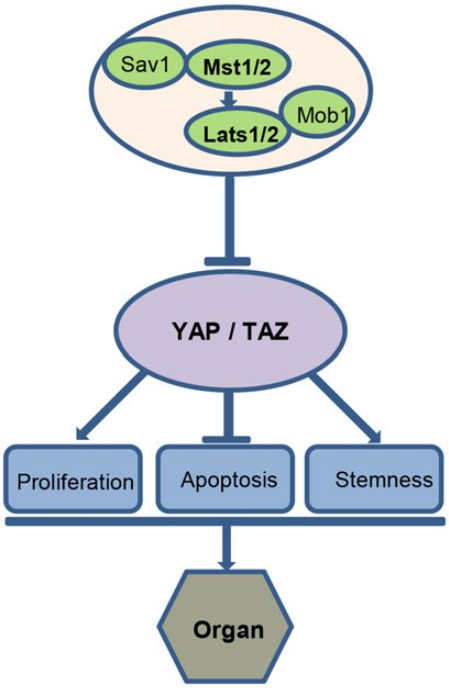

Figure 1. The Hippo pathway regulates organ size by controlling cell proliferation, apoptosis, and stemness in mammals

Table 1. The Hippo pathway regulates mammary gland development

Gene	Phenotype	Mechanism	Reference
Sav1	Epithelial cells are less differentiated in late pregnancy stage	YAP	[64]
Lats1	Epithelial tissue reduced	Prolactin and LH decreased	[65]
Yap	Alveolar structure are reduced in pregnancy stage	Unclear	[64]
Taz	Number and complexity of branches reduced in post-pubertal virgin stage	Abnormal epithelial lineage-specific gene expression	[66]

LH, luteinizing hormone.

the fat pad before birth. After birth, mammary development keeps quiescent until puberty. At puberty stage, ducts begin to elongate and undergo secondary branching. During pregnancy, mammary epithelial cells proliferate rapidly and differentiate in response to hormones, including estrogen, progesterone, and prolactin. Lipid droplets are formed at late pregnancy. Alveoli secrete milk through ducts and nipples during lactation. At involution, a large number of mammary epithelial cells undergo apoptosis. At the end of involution, mammary gland returns to a puberty-like state.

Several key components of the Hippo pathway, such as Sav1, Lats1, Yap, and Taz, have been reported to regulate mammary gland development (**Table 1**).

Sav1 is a negative regulator for YAP. Breast specific *Sav1* knockout 6- and 8-week-old virgin mice did not show defects in terminal end bud formation, ductal growth, or ductal branching [64]. In agreement with this, YAP transgenic does not affect mammary gland development in virgin stage [64]. However, there are no lipid droplets in the mammary gland alveoli of *Sav1*-deficient mice in late pregnancy stage, at P16.5 and P18.5, due to a defect in the ability of the epithelial cells to differentiate [64]. YAP over-expression showed a similar phenotype to *Sav1* knockout [64]. Thus, *Sav1* may specifically affect mammary gland terminal differentiation through YAP [64].

Lats1 is the protein kinase for YAP. *Lats1* plays a crucial role in mammary gland development. *Lats1*-deficient female mice showed reduced amount of breast epithelial tissue, and even nipple in some cases [65]. In the mammary glands of *Lats1* knockout female mice, there is frequently no epithelial component in mammary gland fat pads [65]. The defects of mammary gland development may be accounted by the reduction of the prolactin and luteinizing hormone levels [65]. Breast specific *Lats1* knockout mouse model is required to illustrate the intrinsic role of *Lats1* in mammary gland development.

Yap plays an essential role in promoting the survival of mammary epithelial cells during late pregnancy. *Yap*-deficient mouse mammary glands increase apoptosis but have no effect on cell proliferation. Furthermore, the alveolar structure is reduced at P16.5 and P18.5 [64]. This is different from *Sav1*-deficient mammary glands, which shows normal alveolar structure during pregnancy [64]. Thus, *Yap* is indispensable for mammary epithelial cell survival in the pregnancy stage [64]. Importantly, *Yap* knockout can rescue the mammary gland phenotype induced by the *Sav1* deficiency in late pregnancy stage. *Sav1/Yap* double knockout mammary glands show reduced alveolar structure but normal terminal differentiation [64]. This result further confirmed that YAP is a critical downstream target of Sav1.

Taz is a YAP-like transcription co-activator. *Taz*-deficient mouse mammary glands are normal in pubescent virgin (5–8 weeks old), like *Yap*-deficient mice. However, the number and complexity of mammary gland branches were reduced in post-pubertal virgin (16 weeks old) stage [66]. This is different from the *Yap*-deficient mouse mammary glands. The morphologic defect in *Taz*-deficient mice may be caused by the reduction of basal cells [66]. Knockdown of TAZ in basal cells does not inhibit cell proliferation; instead, it induces luminal differentiation [66]. Additionally, over-expression of TAZ can reprogram luminal cells into basal-like cells. Thus, TAZ may determine the balance and fate of basal and luminal cells in mammary glands.

The Hippo Pathway Plays Important Roles in Breast Cancer

The role of YAP in breast cancer

Accumulated evidence suggests that YAP is an oncoprotein that promotes breast cancer tumorigenesis and progression. The *Yap* gene is amplified in breast tumors of *Brca1/p53*-deficient mice [67]. YAP promotes breast cancer cell proliferation and survival [67,68]. PyMT-induced mammary tumors showed increased expression of YAP and loss of YAP suppresses PyMT-induced tumor growth [64]. High YAP expression is associated with the E-cadherin-deficient invasive lobular breast cancers [69]. Another study revealed that positive YAP expression is associated with shorter survival in HER2-positive breast cancer patients [70]. Consistently, YAP over-expression has been demonstrated to promote breast cancer cell growth *in vitro* and *in vivo* [68]. YAP has also been shown to promote breast cancer cell migration, invasion, epithelial-to-mesenchymal (EMT) transition, and metastasis. YAP was reported to promote breast cancer cell migration

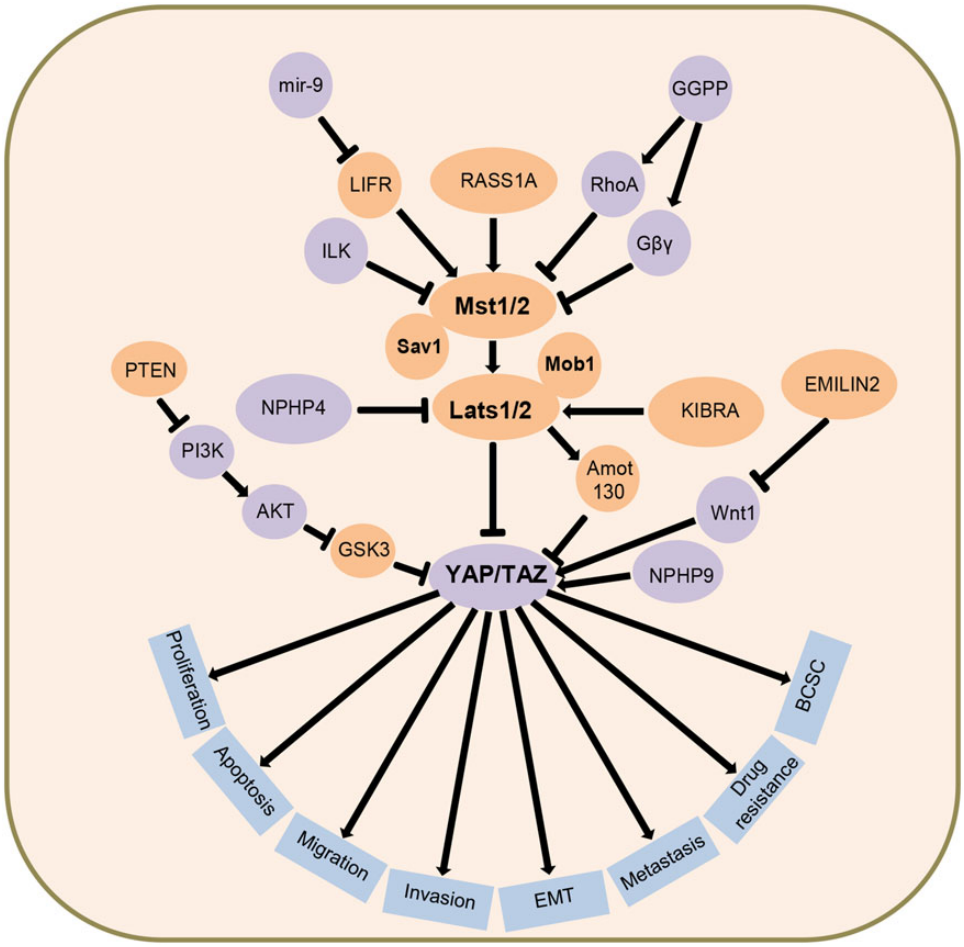

Figure 2. The regulation of YAP/TAZ has broad effects on breast cancer cell phenotypes GGPP, geranylgeranyl pyrophosphate; BCSC, breast cancer stem cell.

and invasion by promoting the gene transcription of receptor for hyaluronan-mediated motility [71].

YAP promotes breast cancer cell growth and progression predominately through interacting with TEAD transcription factors [71–73]. In addition, it has been found that YAP increases the protein stability of KLF5, an oncogenic transcription factor that promotes breast cell proliferation and survival [74–76]. YAP interacts with KLF5 and prevents its ubiquitination by E3 ubiquitin ligase WWP1 [77]. YAP overexpression increases the protein level of KLF5 and its downstream target genes, including *FGF-BP* and *ITGB2* [77]. Depletion of YAP in MCF10A and SW527 cells decreases the expression levels of KLF5, FGF-BP, and ITGB2, induces apoptosis and suppresses cell proliferation and tumor growth [77].

Several studies about YAP upstream regulators also support that YAP is an oncogene in breast cancer (**Fig. 2**). Integrin-linked kinase (ILK) has been shown to promote YAP nuclear translocation. Inhibition of ILK results in YAP phosphorylation, cytoplasm retention, and tumor growth inhibition [72]. Serum starvation induces phosphorylation of 130-kDa isoform of angiomotin (Amot130) by LATS1/2 and recruitment of an E3 ubiquitin ligase AIP4/ITCH, which promotes YAP ubiquitination and degradation [78]. In agreement with this finding, Amot130 inhibits breast cancer cell growth [78]. However, Amot80 promotes breast cell growth through activating ERK [79]. KIBRA induces LATS and YAP phosphorylation. KIBRA over-expression inhibits YAP-induced EMT in MCF10A cells [80]. Leukemia inhibitory factor receptor also inhibits YAP activation and breast cancer metastasis through activating Mst1/2–Lats1 cascade [81,82]. Recently, it has been found that TGF-β signaling regulates YAP to regulate metastasis of breast cancer cells [83]. YAP/TEAD bind with pSMAD2/3 that induces the expression of target genes, *NEGR1* and *UCA1*, to promote breast cancer cell anchorage-independent growth and migration [83].

However, a few studies suggest that YAP may function as a tumor suppressor in breast cancer. It was reported that the expression of YAP is decreased in breast tumor tissues compared with normal breast tissues [84,85]. Yuan *et al.* [86] found that YAP knockdown protected MDA-MB-231 cells from anoikis and promoted cell migration, invasion, and tumor growth. Consistent with this, nuclear YAP1 can bind with p73 tumor suppressor and induce the expression of proapoptotic gene *Puma* in breast cancer [87]. Thus, the role of YAP in breast cancer may be context dependent. Nevertheless, the evidence from *Yap* knockout mouse model suggests that YAP is more likely to play an oncogenic role in breast cancer.

TAZ promotes breast cancer

TAZ promotes breast cancer cell proliferation, migration, invasion, EMT, and metastasis. TAZ is over-expressed in breast cancer, especially in high-grade and metastatic breast cancer [52–54,88]. Several studies showed that TAZ is over-expressed in triple-negative breast cancer [66,89]. Moreover, TAZ expression negatively correlates with disease-free survival in breast cancer patients [53].

It was demonstrated that the depletion of TAZ in HCC1937 breast cancer cell line dramatically inhibited tumor growth [90]. TAZ over-expression promotes and TAZ knockdown inhibits breast cancer cell migration and invasion [91,92]. The expression level of TAZ is higher in breast CSCs than that in differentiated breast cancer cells. Knockdown of TAZ in breast CSCs inhibits migration and metastasis. Over-expression of TAZ in differentiated breast cancer cells induces migration and metastasis [53]. Consistently, TAZ over-expression not only promotes migration, but also induces EMT in MCF10A [93]. TAZ promotes breast cancer cell migration, invasion, and EMT predominately through interacting with TEADs [22,83,89,93]. The TAZ/TEAD complexes induce the transcription of AREG to promote cell migration. Knockdown of AREG partially reduces the TAZ-dependent migration. Moreover, the expression of TAZ and AREG is positively correlated in breast cancer tissues [93]. Our previous study showed that TAZ increases the protein stability of KLF5, which in turn promotes the *FGF-BP* gene transcription and tumor growth [90].

TAZ also promotes breast cancer drug resistance, a major obstacle in breast cancer chemotherapeutics [94,95]. The Hippo pathway plays an important role in drug resistance of breast cancer [25,53,94,96,97]. Taxol (paclitaxel) is a first-line chemotherapeutic drug used for breast cancer [96]. TAZ is necessary for Taxol resistance in human breast cancer cells [96]. TAZ contributes to Taxol resistance by inducing the transcription of *Cyr61* and *CTGF* [96]. In addition, TAZ also causes the doxorubicin resistance in breast cancer cells [52].

Studies about TAZ upstream regulators also support that TAZ is an oncogene in breast cancer (**Fig. 2**). NPHP4, a known cilia-associated protein, interacts with LATS1 and inhibits TAZ and YAP phosphorylation. Knockdown of NPHP4 inhibits breast cancer cell proliferation [98]. NPHP9, another nephronophthisis family member, competes with 14-3-3 to bind with TAZ and induces TAZ nuclear translocation. Knockdown of NPHP9 inhibits the TAZ-dependent breast cancer cell proliferation [99]. The PTEN tumor suppressor promotes TAZ protein degradation through the PI3K/AKT/GSK3 pathway [100]. In MCF10A, knockdown of PTEN induces EMT [100]. EMILIN2, an extracellular matrix protein, inhibits the TAZ activity and breast cancer cell motility [101]. Inhibition of geranylgeranylation of Gβγ and RhoA enhances phosphorylation of MST1/2 and LATS1, inhibits the activation of TAZ, and reduces the breast cancer cell migration [92].

The Hippo pathway regulates breast cancer stem cells

Substantial evidence supports that Hippo pathway plays a crucial role in regulation of stem cell self-renewal and differentiation. Both YAP and TAZ are required for maintaining mouse and human embryonic stem cells [23,102–104]. In breast cancer, TAZ has been shown to play an essential role in maintaining CSCs *in vitro* [55]. Over-expression of TAZ in MCF10A cells promotes mammosphere formation and knockdown of TAZ inhibits mammosphere formation [52]. Inhibition of geranylgeranylation of Gβγ and RhoA inhibits the activation of TAZ and reduces the self-renewal of breast CSCs [105]. This phenotype can be rescued by over-expressing TAZ-S89A, a constitutive active TAZ [105]. Interestingly, EMT can activate TAZ through Scribble, which is required for MST2 to interact with LATS/TAZ complex, and increase self-renewal of breast CSCs [52].

Summary and Perspective

Overwhelming evidence supports the critical role of Hippo signaling in breast cancer development. YAP and TAZ, two core components of the Hippo pathway, promote breast cancer cell proliferation, survival, migration, and invasion. The therapeutic targeting of components of the Hippo pathway is therefore highly promising for treating breast and other cancers.

Although the Hippo pathway is well established to regulate breast and breast cancer development, the function and mechanism of the Hippo pathway have not been fully addressed. For examples, the physiological and pathological roles of several key components, such as MST1/2 and Mob, in breast and breast cancer development have not been investigated in transgenic mouse models. The YAP/TAZ upstream regulatory components of the Hippo pathway and downstream target genes have not been completely identified. It would be interesting to study the roles of these new components in breast and breast cancer development. Additionally, the development of breast and breast cancer is determined by multiple factors, including genetic factors and environmental factors. The crosstalk between the Hippo pathway and other signaling pathways has not been completely understood.

Nevertheless, the Hippo pathway could provide therapeutic targets for breast cancer treatment. Recent progresses in the discovery of drugs specific for the Hippo pathway are encouraging. A number of small molecules have been identified to regulate the hippo pathway and inhibit tumor growth [55]. YAP-positive breast cancer cells are sensitive to verteporfin, a YAP inhibitor that interferes with the interaction between YAP and TEADs [64]. VGLL4 also inhibits the complex formation of YAP–TEAD. A peptide mimicking VGLL4 effectively suppresses tumor growth [106,107]. GPCRs also regulate the Hippo pathway [12]. GPCRs are great candidates for anti-cancer drug target [108].

In conclusion, the Hippo pathway plays critical roles not only in mammary gland development but also in breast cancer. It is well established that the Hippo pathway regulates mammary gland morphology and differentiation, although the mechanism is still unclear. YAP may have a context-dependent role in breast cancer. More studies are required to investigate the function and mechanism of the Hippo pathway in mammary gland development and breast cancer. It is expected that novel therapeutic approaches targeting the Hippo pathway will be developed to treat breast cancer.

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