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Invited review

Targeting the Hippo pathway: Clinical implications and therapeutics

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ABSTRACT

The Hippo pathway plays a critical role in tissue and organ size regulation by restraining cell proliferation and apoptosis under homeostatic conditions. Deregulation of this pathway can promote tumorigenesis in multiple malignant human tumor types, including sarcoma, breast, lung and liver cancers. In this review, we summarize the current understanding of Hippo pathway function, it's role in human cancer, and address the potential of Hippo pathway member proteins as therapeutic targets for a variety of tumors.

Contents

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1. Introduction

The Hippo pathway was first identified in *Drosophila melanogaster* using genetic screens to characterize regulators of cell growth. It is a highly conserved signal transduction pathway in mammals. To date, studies have demonstrated the fundamental role of the Hippo signaling pathway is organ size control, regeneration, stem cell self-renewal and tumorigenesis [1–3].

The goal of this review is to highlight the molecular regulation and effectors of the Hippo pathway as potential novel targets for the treatment of cancer. We will provide an overview of the Hippo pathway, its disruption in human malignancies, and Hippo pathway targets currently being pursued for therapeutic intervention.

We will focus on the complex signaling that regulates Hippo-mediated cellular responses to external stimuli and discuss the Hippo pathway as a key mediator in normal homeostasis and pathogenic phenotypes. We examine several key areas including upstream signaling, through Fat, Kibra/Expanded/Merlin complex, the role of apical-basal proteins in cell–cell adhesion inputs into the Hippo kinase cascade, and other mechanisms that mediate proliferation and cell apoptosis in human cancers by controlling YAP translocation and crosstalk with pathways like GPCR signaling, EGFR-PI3K signaling and mevalonate pathway.

2. Overview of Hippo signaling pathway

The Hippo pathway consists of a core kinase cascade downstream of a variety of key regulators, which signal in response to cell contact inhibition, transmembrane receptors and unknown factors. The core mammalian kinases include: MST1/2, which directly phosphorylates a second set of kinases, LATS1/2 at LATS1 T1079 and LATS2 T1041 [4]. The adaptor protein SAV1 facilitates the physical proximity of MST1/2 and LATS1/2, promoting phosphorylation [5]. Additional adapter molecules MOB1A and MOB1B promote LATS1/2 kinase activity [6]. The most critical effectors of the Hippo pathway are Yes-activated protein (YAP) and transcriptional co-activator with PDZ-binding motif (TAZ). These two transcriptional activators regulate expression of many Hippo pathway targets. The active hippo kinase cascade results in LATS1/2-mediated YAP phosphorylation at S127 (S89 in TAZ) and S381 (S311 in TAZ). Phosphorylated YAP/TAZ is sequestered via 14-3-3 binding and degraded in the cytoplasm preventing nuclear translocation [7]. Therefore, to successfully localize to the nucleus, YAP/TAZ must remain unphosphorylated. Thus the Hippo pathway kinase cascade must be effectively "turned off" for YAP/TAZ to translocate to the nucleus where they activate a predominantly pro-proliferation transcriptional program [8].

3. Deregulation of the Hippo pathway in human malignancy

3.1. Upstream regulators of the Hippo pathway

Recently, our knowledge of the Hippo signaling pathway has increased substantially. However, the upstream factors regulating initiation of core kinase activity remain unclear. Here, we summarize what is currently known about the upstream modulators of Hippo signaling as shown in Table 1.

3.1.1. Fat signalling in mammals

Fat, initially identified in *Drosophila*, is a potential upstream player in the Hippo pathway [9]. In mammals, there are four fat-related atypical cadherins, Ft1-4. Defects in Ft4 expression result in the loss of oriented cell division and planar cell polarity signaling [10]. Ft1 and Ft4 are reported as tumor suppressor candidate genes in oral cancer and breast cancer, respectively [11,12].

3.1.2. The Kibra–Expanded–Merlin complex

Another key upstream regulatory module is the Kibra–Expanded–Merlin complex (KIBRA/MER/EX). Expanded and Merlin are FERM domain proteins that regulate two different upstream signaling branches in the Hippo signaling pathway. Fat may signal through Expanded [13], whereas Kibra physically interacts with Merlin and signals through a separate mechanism to the core kinase cascade [14]. KIBRA/MER/EX are three adaptor proteins which provide a direct link from the apical membrane to core Hippo proteins [13]. In mammals, the interaction between KIBRA and MER leads to enhanced phosphorylation of LATS1/2 in vitro [15]. KIBRA and LATS2 physically associate, which prevents LATS ubiquitination and subsequent proteasomal degradation, leading to decreased proliferation. KIBRA overexpression restrains proliferation by inducing phosphorylation of YAP in both murine and human cells [16]. Loss of KIBRA expression is associated with the claudin-low subtype of breast cancer, an aggressive sub-group with EMT features and a poor prognosis [17]. These data suggest that upregulation of KIBRA, through an as yet unknown mechanism may suppress proliferation in some cancers and therefore be a clinically useful paradigm.

3.1.3. Regulation of Hippo pathway by apical-basal polarity proteins

Crumbs (CRB), a transmembrane apical-basal polarity determinant, is a cell surface regulator of the Hippo pathway in *Drosophila*. CRB binds to EX through its FERM binding motif and regulates activation of the core kinase cascade. Both intracellular and extracellular domains of CRB are necessary for recruitment of EX. The interaction of CRB and EX modulates EX localization and stability [18]. Recently, Varelas et al. reported that the CRB polarity complex interacts with YAP/TAZ and stimulates their phosphorylation in response to cell density information [19]. Polarity regulator atypical protein kinase (aPKC)-PAR6-PAR3 is another apical-basolateral regulator complex that interacts with Hippo pathway components in *Drosophila* and mammals [20]. Upregulation of aPKC activity is required for the CRB overexpression phenotype [21] and is associated with non-small-cell lung cancers, Basal cell carcinoma and membrane protein Smoothened (Smo)-inhibitor resistant cancers [22,23]. Myristoylated aPKC peptide inhibitor (PSI), a pan PKC inhibitor can block proliferation of (BCC) cell lines and Smo-inhibitor resistant cancers [23].

Additionally, the apical basal polarity module Scribble (Scrib)/Disc Large (Dlg)/Lethal giant larvae (Lgl) has been shown to inhibit Hippo pathway signaling in *Drosophila*, leading to increased YAP activity [24]. Consistent with this finding, the Scrib/Dlg/Lgl polarity module has been linked to the development of mammalian tumors. However, the role of this polarity module is tissue type dependent. Whereas several groups showed that this module can be influenced by oncoviral proteins, such as E6 which mediates the onset of HPV-induced cervical cancer through increased YAP activity [25,26], other groups have shown that Scrib/Dlg are tumor suppressors in colorectal cancer. In colorectal cancer expression of Scrib and Dlg is lost during tumor progression, which is associated with loss of epithelial cell polarity and disorganized tissue architecture in colon carcinoma [27]. Collectively, these findings show that deregulation of apical-basal polarity components contributes to cancer progression through YAP. However, the role of apical-basal proteins in regulating Hippo pathway signaling is context-dependent and should be evaluated carefully.