BMB Reports - Manuscript Submission

Manuscript Draft

Manuscript Number: BMB-16-196

Title: Interacting network of Hippo, Wnt/β-catenin and Notch signaling represses liver tumor formation

Article Type: Perspective (Invited Only)

Keywords: Wnt/β-catenin; Hippo; Notch pathways; HCC; Yap/Taz

Corresponding Author: Yingzi Yang

Authors: Wantae Kim[1], Sanjoy Kumar Khan[1], Yingzi Yang[1,*]

Institution: [1]Department of Developmental Biology, Harvard School of Dental Medicine, 188 Longwood Ave. Boston, MA 02115, USA,

Interacting network of Hippo, Wnt/β-catenin and Notch signaling represses liver tumor formation

Wantae Kim, Sanjoy Kumar Khan & Yingzi Yang*

Department of Developmental Biology, Harvard School of Dental Medicine, 188 Longwood Ave. Boston, MA 02115, USA

*Correspondence author
Yingzi Yang, Ph.D.
Department of Developmental Biology
Harvard School of Dental Medicine,
188 Longwood Ave.
Boston, MA 02115
Tel:(617) 432-8304
Email: yingzi_yang@hsdm.harvard.edu

Keywords: Wnt/β-catenin, Hippo, Notch pathways, HCC, Yap/Taz

ABSTRACT

Acquiring a selective growth advantage by breaking the proliferation barrier established by gatekeeper genes is a centrally important event in tumor formation. Removal of mammalian Hippo kinase Mst1 and Mst2 in hepatocytes leads to very rapid hepatocellular carcinoma (HCC) formation, indicating that the Hippo signaling pathway is a critical gatekeeper in hepatocytes that restrains abnormal growth. By rigorous genetic approaches, we have identified an interacting network of the Hippo, Wnt/β-catenin and Notch signaling pathways that controls organ size and HCC development. We found that in hepatocytes, loss of Mst1/2 led to activation of Notch signaling, which forms a positive feedback loop with Yap/Taz, transcription factors controlled by Mst1/2. This positive feedback loop resulted in severe liver enlargement and rapid HCC formation. Blocking the Yap/Taz-Notch positive feedback loop by Notch inhibition in vivo significantly reduced Yap/Taz activities, hepatocyte proliferation and tumor formation. Furthermore, we have uncovered a surprising inhibitory role of Wnt/β-catenin signaling to Yap/Taz activities that is important in tumor initiation. Genetic removal of β-catenin in the liver of the Mst1/2 mutants significantly accelerates tumoriogenesis. Therefore, Wnt/β-catenin signaling that itself is known for oncogenic property exerts an unexpected function in restricting Yap/Taz and Notch activities in HCC initiation. The molecular interplay between the three signaling pathways identified in our study will provide new insights in developing new therapeutic strategies to treat liver tumors.

The liver has remarkable property to regenerate after injury and to adjust to its original size (El-Serag, *N Engl J Med* 2011, 365: 1118-1127). The mechanism of how the liver can accomplish such capabilities is one of the most-wondering questions remained elusive. HCC is the fifth most frequent malignant cancer and the second leading cause of cancer-related death worldwide (Torre *et al*., *CA: a cancer journal for clinician* 2012, 65: 87-108). There are several risk factors including genetic mutations, long-term virus infection and cirrhosis that can predispose to HCC (Forner *et al*., *Lancet* 2012, 379: 1245-1255). Therefore, there is a need for uncovering proper molecular targets and developing new therapeutic strategies to improve patient survival. Understanding genetic and molecular lesions that cause liver cancer progression could provide fundamental insights for clinical applications.

A recently discovered Hippo signaling plays essential roles in organ size control and tumor suppression (Dong *et al*., *Cell* 2007, 130:1120-1133). Several lines of evidence has reported that miss-regulation of Hippo signaling or Yap/Taz, the transcription factors controlled by Hippo kinases Mst1/2, is involved in diverse human diseases, including liver cancer (Song *et al.*, *Proc Natl Acad Sci USA* 2010, 26:1431-1436). Recent reports suggested that approximately 30% of HCCs exhibit inactivation of tumor suppressor Mst1 and Mst2 and subsequent high Yap/Taz activity, indicating the contribution of Hippo in HCC (Zhou *et al*., *Cancer Cell* 2009, 16:425-438).

Wnts are secreted proteins that regulate many important developmental and physiological processes. β-catenin is a centrally important transcription co-activator that activates Wnt target gene expression in the Wnt/β-catenin pathway (Kim *et al*., *Biochem J* 2013, 15:9-21). Abnormal activation of the Wnt/β-catenin pathway causes many types of tumors in human including hepatoblastoma and HCC (de La Coste et al., *Proc Natl Acad Sci USA* 1998, 95:8847-51). The Wnt and Hippo signaling pathways interact differentially in distinct cell compartments. In the nucleus, YAP co-operates with β-catenin to regulate gene expression in heart size control or tumor transformation and maintenance (Heallen *et al*., *Science* 2011, 332:458-461). However, YAP/TAZ can also inhibit Wnt/β-catenin activities by regulating Dishevelled (Dvl) phosphorylation and nuclear localization of Dvl or β-catenin in the cytosol (Hansen *et al*., *Trends Cell Biol* 2015, 25:499-513). It is still unknown whether the mode of interaction between

YAP/TAZ and the Wnt/β-catenin signaling pathways is cell type specific. We and others have found previously that loss of Mst1/2 in hepatocytes leads to tumor formation and activation of Wnt/β-catenin signaling in the liver, but the role of activated Wnt/β-catenin signaling in liver enlargement and tumor formation caused by inactivation of the Hippo pathway remained unknown.

The Notch signaling pathway is also critically important in both liver development and tumor formation. Notch signaling promotes formation of the oval cell, the liver stem cell (Tanimizu and Mitaka, Organogenesis 2014, 10:208-2015). Notch receptor and ligand expression are highly regulated and Notch signaling is activated in HCC patients (Geisler and Strazzabosco, *Hepatology* 2015, 61:382-92). In mice, constitutively expressed NICD (Notch intracellular domain) in liver causes liver tumor formation (Zender *et al*., *Cancer Cell* 2013, 23:784-795). The Notch pathway is activated by direct cell-cell contact which allows direct binding of Notch receptors and their membrane-bound ligands (Jagged and Delta-like). Notch ligand binding induces sequential proteolytic cleavage of Notch receptors by ADAM family and γ-secretase complex. As a result, NICD is generated and enters the nucleus where it forms ternary complex with co-factor RBP-j and Mastermind to participate in the transcriptional regulation of target genes.

In the current study, we have found that in hepatocytes, loss of Mst1/2 led to activation of Notch signaling, which forms a positive feedback loop with Yap/Taz. This positive feedback loop resulted in severe liver enlargement and rapid HCC formation. Furthermore, we have uncovered a surprising inhibitory role of Wnt/β-catenin signaling to Yap/Taz activities that is important in tumor initiation. Genetic removal of β-catenin in the liver of the Mst1/2 null mutants significantly increased the number of tumor nodules that also appeared at younger ages. Mechanistically, we have identified that increased generation of the Notch intracellular domain (NICD) by Yap/Taz activation also stabilized Taz by inhibiting its binding to β-TrCP. Wnt/β-catenin signaling suppressed the positive feedback loop between Notch and Taz through promoting DP1, known as a dimerization partner of E2F transcriptional factors, nuclear localization to inhibit Notch activity. Breaking down the Yap/Taz-Notch positive feedback loop by Notch inhibition in vivo significantly reduced Yap/Taz activities, hepatocyte proliferation and tumor

formation. Therefore, Wnt/β-catenin signaling which itself is known for oncogenic property exerts an unexpected function in restricting Yap/Taz and Notch activities in HCC initiation. Overall, our results have uncovered a molecular interplay between the three signaling pathways and our studies have shed new insights on developing therapeutic strategies to treat liver tumors.

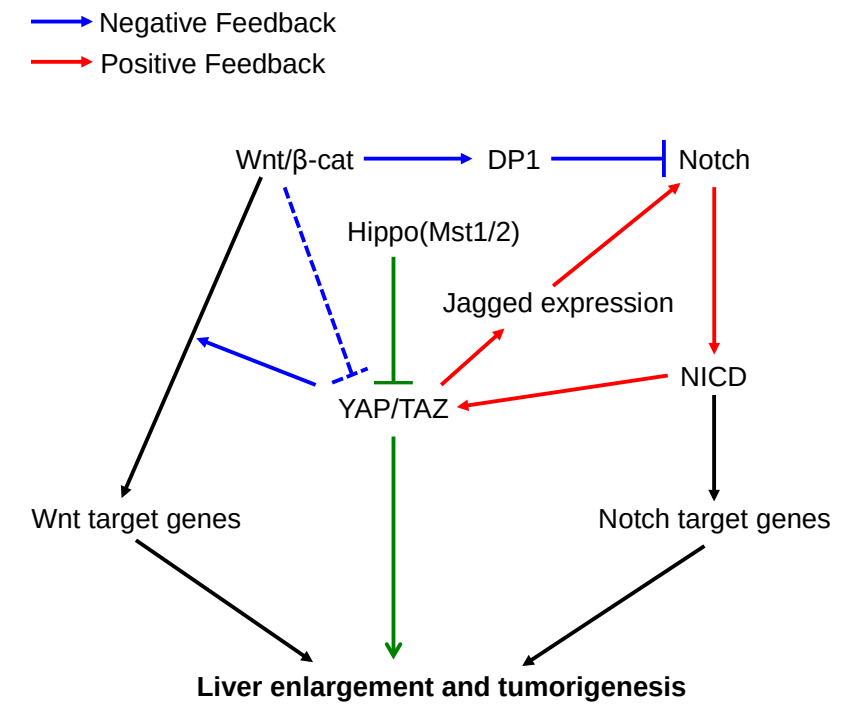

Fig 1. Yap/Taz forms a critical positive feedback loop with Notch signaling to promote liver enlargement and turmorigenesis (red). Breaking this positive feedback-loop led to reduced hepatomegaly and tumor progression. The inhibitory role of Wnt/β-catenin in the liver tumor caused by the viscous positive feedback is at least in part through the DP1-mediated inhibition of Notch signaling (blue).

ACKNOWLEDGEMENTS

This study is supported by the intramural research program of National Human Genome Research Institute (NHGRI) and Harvard School of Dental Medicine. WK (HI13C1274) is supported by the Korean Visiting Scientist Training Award (KVSTA) fellowship from Korea Health Industry Development Institute (KHIDI).