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Review

The YAP and TAZ transcription co-activators: Key downstream effectors of the mammalian Hippo pathway

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ABSTRACT

The Hippo signaling pathway was initially defined by genetic studies in *Drosophila* to regulate tissue growth and organ size [1,2]. This pathway is highly conserved in mammals and dysregulation of the Hippo pathway has been implicated in human cancer. Although the exact extracellular signal that controls the Hippo pathway is currently unknown, compelling evidence supports a critical role of the Hippo pathway in cell contact inhibition, which is a property commonly lost in cancer cells. Many molecules, such as the merlin tumor suppressor protein, have been identified as regulating the activity of the core Hippo pathway components [1,2]. Acting downstream are two key transcription co-activators, YAP and TAZ, which mediate the major gene regulation and biological functions of the Hippo pathway. This article will focus on the physiological function and molecular regulation of YAP/TAZ and its *Drosophila* homolog Yki.

Contents

1. The Hippo pathway acts to restrict YAP and TAZ in mammals

The Hippo pathway is a newly discovered and evolutionally conserved signaling cascade. It regulates organ size control and stem cell property by governing cell proliferation and apoptosis. *In vitro*, it is a major regulatory mechanism in cell contact inhibition. Alterations of this pathway are increasingly recognized to be associated with cancer development. Components of the Hippo pathway were firstly discovered by functional genetic screens in *Drosophila* and shown to be evolutionally and functionally conserved in mammals. Basically, the Hippo pathway can be divided into three interlinked parts: the upstream regulatory components, the Hippo core kinase components, and the downstream transcriptional machinery. For the upstream regulatory components, much in depth discussion can been found in recent reviews [1,2]. The Hippo core kinase cassette contains four proteins, two of which are kinases: Hpo and Wts in the fly and Mst1/2 and Lats1/2 in mammals. The other two proteins, Sav and Mats in the fly, and WW45 and Mob in mammals, act as adaptors/activators. In mammals, Mst1/2 in association with WW45 is activated by phosphorylation in response to upstream regulators. The activated Mst1/2-WW45 can phosphorylate and activate LATS1/2–Mob complex. The major target of the Hippo core kinase cascade is Yki transcription co-activator in the fly and YAP

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and TAZ which are the mammalian homologues. Phosphorylation of YAP and TAZ by the Hippo pathway leads to their sequestration in the cytoplasm by interaction with 14-3-3 proteins and ubiquitination-dependent proteosomal degradation. Therefore, the Hippo pathway acts to restrict the availability/functionality of YAP and TAZ in the nucleus by governing its distribution and protein levels. In the fly, Yki binds scalloped (Sd) and activates transcription of downstream target genes like *Diap*, *bantam* and *cycE*. In mammals, YAP and TAZ interact primarily with transcriptional factors TEAD1–4 (TEADs) and activate expression of target genes such as *CTGF*, *IGFBP3*, *ITGB2*, *Birc5*/*Survivin*, *Gli2*, and *Axl*. In this review, we will focus on the discovery of YAP and TAZ, the demonstration of their function as transcriptional co-activators, their identification as targets of Hippo pathway, their involvement in cancer, and the transcriptional outcome of their complexes with TEAD transcriptional factors.

2. Identification of YAP and TAZ

YAP was originally identified in chicken as an interacting protein of Yes protein tyrosine kinase. However, the functional significance of this interaction is still not clear. Unlike the use of routine protein-protein interacting approaches, YAP was identified by generating anti-idiotypic antibodies against the N-terminal domain of the Yes protein. The interaction was defined to be mediated by the SH3 domain of Yes and Pro-rich region (PVKQPPPLAP) of YAP. Due to its size of 65 kDa, the chicken protein was referred to as YAP65 (Yes-associated protein of 65 kDa) [3]. The human and mouse homologues were identified by using the YAP65 cDNA to probe human and mouse cDNA libraries and the study was reported one year later [4]. During the course of sequence analysis by comparison of YAP with other proteins, a conserved module was noticed to be present in several proteins of various species such as human dystrophin, yeast Rsp5p, and mammalian Nedd-4. This was named the WW domain to reflect the sequence motif containing two conserved and consistently-positioned tryptophan (W) residues [5]. The WW domain was shown to bind PPXY motif around the same time [6]. Using a functional screen of a cDNA expression library, two proteins binding to the WW domain of YAP were identified as interacting partners and named as WBP-1 and WBP-2 (for WW domain binding protein). Sequence comparison between WBP1 and WBP2 followed by interaction assays demonstrated that the PPXY motif binds WW domain with relatively high affinity and specificity. The solution structure of YAP WW domain with PPXY motif was resolved in 1996 [7]. The human YAP gene, located at 11q13, can be transcribed into at least 4 isoforms based on annotation by NCBI. Isoform1, 2, 3 and 4 have 504, 450, 488, and 326 residues in length, respectively. Two consecutive WW domains were present in all isoforms except for isoform 2 which has only one WW domain. Isoform 3 containing 488 residues and two WW domains is most thoroughly studied and the cDNA clone encoding this isoform is most widely used for analysis in the scientific community. The schematic depiction of isoform 3 of YAP is shown in Fig. 1A. The C-terminus contains a PZD-binding motif (TWL-COOH) for interaction with PDZ domain of other proteins such as ZO2 and NHERF2 [8,9].

TAZ (transcriptional co-activator with PDZ-binding motif) is also referred to as WWTR1 (WW domain containing transcription regulator 1). It was originally identified in 2000 as a 14-3-3 binding protein using immobilized 14-3-3 proteins to pull down interacting proteins derived from *in vitro* translation reactions [8]. 14-3-3 proteins are a family of seven homologous proteins having the ability to bind phosphorylated serine with certain sequence motifs and are thus involved in diverse cellular functions including differentiation, cell cycle progression and apoptosis through their ability to interact with diverse intracellular phosphoproteins involved in signal transduction network. Although NCBI annotation has indicated that the TAZ gene may be transcribed into three variants, they all have the same coding region for a protein having 400 amino acids in length. Phosphorylation of S89 was shown to be important for interaction with 14-3-3 proteins. TAZ is homologous to YAP with 46% amino acid sequence identify (with YAP isoform 3) and displaying similar domain organization but having only one WW domain (Fig. 1A). Biochemically, TAZ was shown to display transcriptional co-activator function *via* interaction with PPXY-containing transcriptional factors through its WW domain. The C-terminal region (amino acids 165–395) was shown to be responsible for the transcriptional co-activation property [8]. Several transcriptional factors such as Runx/PEBP2, AP2, C/EBP, c-Jun, Krox-20, Krox-24, MEF2B, NF-E2, Oct-4 and p73 contain proline-rich PPXY motif, are speculated to be interacting transcriptional factors for TAZ (and possibly for YAP) (reviewed in [1]). Similar to YAP, the TAZ C-terminus has a PDZ-binding motif (TWL-COOH), which has been shown to mediate interaction with the first PDZ domain of NHERF-2 [8].

Both YAP and TAZ are homologous to fly Yki, which was identified as a downstream target of the Hippo pathway in 2005 [9]. The identification of Yki and the demonstration of its sequence homology with YAP and TAZ have two important implications. Firstly, like Yki, YAP and TAZ may serve as downstream targets of potential mammalian Hippo pathway. Secondly, like YAP and TAZ, Yki may function as a transcriptional co-activator to regulate the transcriptional outcome of the Hippo pathway. Importantly, YAP can functionally substitute Yki in *Drosophila*, indicating it is a true Yki ortholog. Like YAP, Yki contains two WW domains (depicted in Fig. 1A). The N-terminal region of Yki is most homologous to YAP and TAZ. The sequence alignment of the N-terminal regions of YAP, TAZ and Yki is shown in Fig. 1B, in which the residues responsible for interaction with TEAD transcriptional factors in mammals and scalloped in the fly, respectively, are indicated (see below).

Both YAP and TAZ genes have been studied using knockout mice and revealed to have different physiological functions. Knockout of the YAP gene in mice leads to early embryonic lethality, suggesting an essential role in development [10]. Three independent knockout studies of the TAZ gene suggest an important role of TAZ in the kidney and lung as TAZ−/− mice developed renal cysts characteristic of polycystic kidney disease (PKD) [11–13]. The cellular role of TAZ may be to maintain the integrity of renal cilia to ensure the structural integrity of the kidney [11]. Furthermore, levels of calcium-permeable cation channel protein polycystin 2 (PC2) were increased in TAZ−/− kidney and TAZ was shown to link PC2 to the beta-Trcp of SCF (beta-Trcp) E3 ubiquitin ligase pathway [12]. Glis3 is recently shown to interact with TAZ to maintain the normal development and architecture of the kidney [14]. The P/LPXY motif in the C terminus of Glis3 may mediate the interaction with TAZ so that TAZ is able to function as a co-activator of Glis3-mediated gene transcription. Localization to the primary cilium and interaction with TAZ may be involved in the Glis3 signaling pathway. In addition to a role in the kidney, TAZ knockouts also exhibited defects in the lung characteristic of pulmonary emphysema [13].

3. YAP and TAZ as transcriptional co-activators

Although YAP was identified in 1995, its biochemical function remained elusive until a study 4 years later showed that YAP possesses transcriptional co-activator activity [15]. Several transcription factors such as c-Jun, AP-2, NF-E2, C/EBPalpha and PEBP2/CBF, Runx/PEBP2, Krox-20, Krox-24, MEF2B, Oct-4 and p73 contain the PPXY motif and could be potential target for YAP (reviewed in [1]). The PY motif in the transcription factor PEBP2