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Mammalian Hippo signalling: a kinase network regulated by protein-protein interactions

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Abstract

The Hippo signal transduction cascade controls cell growth, proliferation, and death, all of which are frequently deregulated in tumour cells. Since initial studies in *Drosophila melanogaster* were instrumental in defining Hippo signalling, the machinery was named after the central Ste20-like kinase Hippo. Moreover, given that loss of Hippo signalling components Hippo, Warts, and Mats resulted in uncontrolled tissue overgrowth, Hippo signalling was defined as a tumour suppressor cascade. Significantly, all core factors of Hippo signalling have mammalian orthologues that functionally compensate for loss of their counterparts in flies. Furthermore, studies in flies and mammalian cell systems showed that Hippo signalling represents a kinase cascade that is tightly regulated by protein-protein interactions (PPIs). Several Hippo signalling molecules contain SARAH domains that mediate specific PPIs, thereby influencing the activities of MST1/2 kinases, the human Hippo orthologues. Moreover, WW domains are present in several Hippo factors, and these domains also serve as interaction surfaces for regulatory PPIs in Hippo signalling. Finally, the kinase activities of LATS1/2, the human counterparts of Warts, are controlled by binding to hMOB1, the human Mats. Therefore, Hippo signalling is regulated by PPIs on several levels. Here we review our current understanding of how these regulatory PPIs are regulated and contribute to the functionality of Hippo signalling.

Keywords

Hippo signalling pathways; Protein protein interactions; Phosphorylation; Protein kinase signal transduction; Mps one binder

Introduction

Various molecular processes need to be deregulated in cancer cells in order to ensure the long-term survival of cancerous material. Hanahan and Weinberg recently updated their Hallmarks of Cancer review [1] pointing out that in addition to hallmarks such as resistance to cell death, sustained proliferative potential, and evasion of growth suppression, researchers should also consider genomic instability as an enabling characteristic, and deregulation of cellular energetics as a novel emerging hallmark of cancer. Given the ongoing fight against human cancer it is not surprising that intensive research efforts are ongoing to understand signal transduction machineries that play key roles in the control of all these cancer-driving and/or -enabling cellular processes. Significantly, numerous signal transduction cascades transmit extra- and intracellular inputs through protein kinases, which represent one of the largest superfamilies found in the human genome [2]. In particular members of the AGC kinase subfamily of protein kinases have diverse and crucial cellular

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functions that are deregulated in cancer cells [3]. Specifically, members of a subgroup of the AGC group of protein kinases termed the LATS (large tumour suppressor) / NDR (nuclear Dbf2-related) family of kinases have recently attracted more attention due to their involvement in the regulation of cell division, genomic stability, cell growth, cell proliferation and controlled cell death/apoptosis [4, 5]. Research over the past decade has revealed that LATS/NDR kinases are central parts of complex signal transduction cascades in multicellular eukaryotes [6-11].

Our current understanding of the regulation of these core components can be briefly summarised as follows: members of the MST (mammalian Ste20-like serine/threonine) kinase family activate LATS/NDR kinases by phosphorylation [5], a process which seems to require the protein-protein interaction (PPI) of LATS/NDR with members of the Mps one binder (MOB) protein family [12]. Activated LATS/NDR kinases then target downstream factors such as the proto-oncogene YAP [7, 11] or the CDK inhibitor p21 protein [13], respectively. In particular MST/LATS/YAP signalling has attracted much attention, since deregulated expression of these components can result in cellular transformation [3, 7, 8, 10, 11].

Noteworthy studies in *Drosophila* were instrumental in defining Hippo/Warts/Yorkie signalling (the counterpart of human MST/LATS/YAP signalling), hence MST/LATS/YAP signalling was named after the central Ste20-like kinase Hippo. Therefore, we will refer to MST/LATS/YAP signalling as mammalian Hippo signalling from hereafter. Significantly, fly geneticists not only provided an excellent name for this novel signal transduction cascade, but they were also the first to demonstrate that several components of Hippo signalling are actually tumour suppressor proteins. For example, loss of Hippo, Warts, or Mats (the counterparts of human MST1/2, LATS1/2, and MOB1, respectively) resulted in uncontrolled tissue overgrowth in flies [14-16]. Even more importantly, loss of Hippo, Warts or Mats could be functionally compensated by mammalian MST2, LATS1 and MOB1A, respectively [17-19]. Studies of transgenic mice also showed that loss of MST1/2 or LATS1 results in the development of cancers [7, 20-22]. Together these studies strongly suggest that the core components of mammalian Hippo signalling represent tumour suppressor proteins. Last, but not least, one must further mention that fly genetics also provided leads in how Hippo signalling can be regulated by protein-protein interactions (PPIs) [9]. Here we will give a brief overview of our current understanding of the importance and complexity of these PPIs in mammalian Hippo signalling.

Protein-Protein interactions in mammalian Hippo signalling – an overview

Over the past years research in flies and mammalian cell systems demonstrated that Hippo kinase signalling can be regulated on different levels by PPIs. Intriguingly, the vast majority of these PPIs are mediated by three types of modular protein domains: (1) SARAH domains that are important for Hippo/MST activation, (2) WW domains that can interact with PPXY motifs, and (3) conserved stretches of hydrophobic and basic residues in LATS/NDR kinases (also termed N-terminal regulatory domains; NTRs) that mediate the binding of LATS/NDR to MOB proteins. Hippo signalling can also be controlled by other types of PPIs [6, 9-11]. However, given that these three subgroups of PPIs clearly represent the best studied PPIs in mammalian Hippo signalling, we will focus our discussion on the functionality of SARAH, WW and NTR domains in mammalian Hippo signalling.

Hippo signalling and SARAH domain containing proteins

As already mentioned, members of the MST kinase family phosphorylate and thereby activate LATS/NDR kinases [5]. In particular the MST1 and MST2 kinases play an important role in the activation of LATS/NDR kinases [22-25]. Intriguingly, MST1/2

contain a C-terminal domain that is not present in the other three MST kinase family members [5, 26]. Since this C-terminal domain was also found in Salvador/WW45 and RASSF1A, two regulators of MST1/2 activity [26], this domain was termed SARAH domain for Salvador-RASSF1A-Hippo/MST [27]. Current evidence suggests that the SARAH domain of MST1/2 is required for their auto-phosphorylation activity, plays a key role in the PPI of MST1/2 with the tumour suppressor protein RASSF1A, and is required for the interaction of MST1/2 with the scaffolding protein Salvador/WW45 [26]. The interaction of MST1/2 with RASSF1A through their SARAH domains appears to regulate the activation of LATS/NDR kinases by MST1/2 [25, 28]. The association of Salvador/WW45 with MST1/2 through their SARAH domains has been shown to be essential for the terminal differentiation of epithelial cells [29]. Of note, RASSF1A and Salvador can also form a complex which seems to function independent of their interaction with MST1/2 [30]. Significantly, MST1/2 kinases can also function without their SARAH domains. On one hand, caspase-cleaved MST1/2, that is hyperactive in spite of the deletion of the SARAH domains, is the predominantly expressed form in hepatocytes [22]. On the other hand, the phosphorylation and activation of NDR kinase by MST1 in S-phase does not require the presence of a SARAH domain in MST1 [24]. Furthermore, MST3 kinase, which does not contain a SARAH domain, plays a major role in the phosphorylation and activation of NDR during G1/S cell cycle progression [31].

In conclusion, MST1/2 kinase activities are influenced by signalling components containing SARAH domains. RASSF1A and Salvador can activate MST1/2 by forming SARAH domain dependent complexes, suggesting that the SARAH domains of MST1/2 are required for MST1/2 function in certain biological settings. Nevertheless, RASSF1A and Salvador also seem to have MST1/2 independent roles that potentially do not involve SARAH-dependent signalling. In this context, it is also important to mention that MST1/2 kinases can function completely independent of their SARAH domains in some biological settings. These observations illustrate that we have not yet fully understood how MST1/2 kinases can be regulated independent of and dependent on PPIs. The identification of conserved SARAH domains has been very helpful in defining how RASSF1A and Salvador can activate the core cassette of mammalian Hippo signalling, but recent evidence suggests that various MST1/2 signalling events should be re-addressed with regard to their dependency on SARAH domains, and possibly also other motifs such as the PPXF motifs in MST1/2. In our opinion SARAH-independent protein domains of MST1/2 need to be studied in more detail, since we can currently not exclude the possibility that MST1/2 are regulated by PPIs that are completely independent of these SARAH domains.

Hippo signalling and WW domain containing factors

Since Sudol and Harvey recently summarised the importance of the WW module in Hippo signalling [9], we will give here only a brief overview on WW domains. Nevertheless, we will take the time to highlight very recent progress in our understanding of WW domain dependent Hippo signalling.

The WW domain is a small modular protein domain of about 40 amino acids in length, which contains two highly conserved tryptophan (W) residues [32]. The majority of WW domains recognise short PPXY motifs, and the phosphorylation of tyrosine (Y) in these PPXY motifs has been shown to block WW/PPXY interactions. Considering that several Hippo signalling components contain either WW domains or PPXY motifs [9], WW/PPXY mediated PPIs have been studied the most extensively among all known PPIs currently known to play a role in Hippo signalling. LATS1/2 kinases regulate the proto-oncogene YAP through WW/PPXY mediated interactions [33, 34], where the LATS/YAP interaction seems to serve two purposes: (1) phosphorylation of YAP by LATS1/2 results in the

inactivation of YAP by creating a 14-3-3 anchor site and changing the subcellular localisation of YAP [33, 35-37], and (2) the direct protein-protein complex formation between LATS1/2 and YAP can further negatively affect YAP activity [33, 34]. Significantly, Angiomotin-like protein 1 (AMOTL1) and 2 (AMOTL2) can also bind to the YAP WW domain with their PPXY motifs [38-40]. Similarly to LATS1/2, AMOTL1/2 negatively regulate the nuclear activity of YAP by altering the subcellular localisation YAP. Furthermore, Camargo and colleagues recently showed that the activity of YAP is negatively controlled by α -catenin binding in keratinocytes [41]. More specifically, they provided evidence suggesting that the binding of α -catenin to the YAP WW domain can maintain YAP in its phosphorylated and hence inactive state, while MST/LATS signalling was dispensable for efficient YAP regulation in this setting [41]. Last, but not least, one should also note that the WW domain containing protein Kibra interacts with LATS1/2 [42-44]. The association of the PPXY motif of LATS1/2 with the WW domains of Kibra appears to play a role in the activation of LATS1/2 kinases [45], but the precise mechanism is not well understood yet, since the activation of LATS1/2 by Kibra seems to be MST1/2 independent [45].

Taken together, PPIs of WW domains with PPXY motifs are a common feature of mammalian Hippo signalling. Using WW/PPXY interactions the nuclear activity of the proto-oncogene YAP can be influenced by direct binding of YAP to LATS1/2, AMOTL1/2, and α -catenin. Given the wide occurrence of WW domains and PPXY motifs in all these Hippo signalling components, we fully agree with Sudol and Harvey [9] that it is very likely that more factors playing a role in mammalian Hippo signalling will be identified in the near future based on sequencing comparison approaches.

Regulation of Hippo signalling by MOB proteins

As mentioned earlier, PPIs also play a role in the regulation of LATS/NDR kinase activities. In addition to the binding of LATS1/2 to Kibra [45], members of the highly conserved family of Mps One binder (MOB) co-activator proteins can associate with LATS/NDR kinases. More specifically, human MOB1 (hMOB1) can bind to all four human LATS/NDR kinases [12]. Combined structural and biochemical studies have shown that a stretch of conserved hydrophobic and positively charged residues N-terminal of the catalytic domains of LATS/NDR kinases is required for the association of hMOB1 with LATS/NDR kinases, while a conserved cluster of negatively charged residues on hMOB1 is needed for the interaction of hMOB1 with LATS/NDR kinases [12]. The binding of hMOB1 to NDR1/2 kinases through these conserved domains triggers auto-phosphorylation of NDR1/2 on the activation segment (also known as T-loop phosphorylation), and also facilitates the phosphorylation of NDR1/2 on the hydrophobic motif (HM phosphorylation) by MST kinases [12]. In contrast, binding of hMOB1 to LATS1/2 kinases seems to be only required for T-loop phosphorylation, while HM phosphorylation is independent of hMOB1/LATS complex formation [46]. This illustrates that hMOB1/NDR and hMOB1/LATS complexes are not equal, thereby allowing cells to trigger different biological outputs through hMOB1 signalling [12]. Of importance, the regulation of LATS/NDR kinases by hMOB1 is even more complicated. hMOB1 is phosphorylated on Thr12 and Thr35 by MST1 and MST2, which results in increased affinity of phospho-hMOB1 for NDR/LATS kinases [46]. This increased formation of hMOB1/NDR and hMOB1/LATS complexes results in increased phosphorylation of LATS/NDR kinases. Therefore, hMOB1 regulated signalling through binding to LATS/NDR can be regulated by MST kinase activity.

Another level of complexity between hMOB1/NDR and hMOB1/LATS complexes is how they can be influenced by hMOB2. In spite of the highly conserved hMOB1 binding domain shared between NDR and LATS kinases [47, 48], hMOB2 interacts with NDR1/2 kinases

through the hMOB1 binding domain, but does not associate with LATS1/2 kinases [49]. Significantly, binding of hMOB2 to NDR1/2 kinases inhibits the phosphorylation of NDR and thereby blocks kinase activation [49]. This inhibition is most likely caused by competition between hMOB1 and hMOB2 for direct binding to the same domain within NDR1/2 kinases, since a hMOB2 mutant deficient in NDR1/2 binding could not interfere with the activation of NDR by hMOB1 [49].

Taken together, the PPI of hMOB1 with LATS/NDR kinases is crucial for the phosphorylation and activation of LATS/NDR kinases. The efficiency of hMOB1 binding to LATS/NDR kinases can be influenced by MST1/2-mediated phosphorylation of hMOB1. A second human MOB protein, hMOB2, also plays a role in the regulation of NDR/LATS kinase signalling, more specifically in the inhibition of NDR signalling. Therefore, hMOB2 has been classified as an inhibitor of NDR signalling, while hMOB1 has been classified as a co-activator of NDR/LATS signalling cascades.

Conclusions

In summary, mammalian Hippo signalling is controlled by various PPIs that can influence the activities of MST and LATS/NDR kinases, as well as alter the subcellular localisations of the proto-oncogenes YAP and TAZ (Figure 1). Currently we are aware of three main types of modular protein domains that are repeatedly utilised for PPIs regulating Hippo signalling. Firstly, SARAH domains are important for the activation of MST1/2 kinases by the tumour suppressor proteins RASSF1A and Salvador/WW45. Secondly, the WW domain-containing proteins Kibra, YAP and TAZ can bind to PPXY motifs in LATS1/2 kinases and AMOTL1/2, respectively. Thirdly, the association of LATS/NDR kinases with MOB1/2 proteins stimulates or inhibits kinase activation. The latter PPI is influenced by MST1/2 kinase activities. hMOB1 phosphorylated by MST1/2 binds more readily to LATS/NDR kinases, thereby leading to increased LATS/NDR phosphorylation and activation. The tyrosine in the PPXY motif might also be a target of tyrosine protein kinases, which could significantly affect formation of Kibra/LATS, YAP/LATS, TAZ/LATS, AMOTL/YAP, and AMOTL/TAZ complexes. Last, but not least, it is also possible that the phosphorylation of the SARAH domain could influence the formation of RASSF1A/MST and Salvador/MST complexes. However, according to our knowledge the regulation of the PPXY motif or the SARAH domain by phosphorylation has not yet been reported in mammalian Hippo signalling.

Mammalian Hippo signalling plays crucial roles in various cellular processes. To a certain degree the importance of selected PPIs has already been addressed. For example, the modification of hMOB1 by MST1/2, which influences the formation of hMOB1/LATS and hMOB1/NDR complexes, has been studied in cell proliferation [46], apoptosis signalling [22], and the control of the proto-oncogene YAP [22]. Nevertheless, in spite of such progress, we feel that the scientific community has only just started to study the role of PPIs in Hippo signalling in more detail. In particular, the binding patterns of Hippo signalling components containing more than one modular protein domain are not completely understood. For example, Salvador/WW45 contains two WW domains and one C-terminal SARAH domain, while LATS kinases contain one NTR domain and at least one PPXY motif. Definitely different binding partners should be addressed in parallel, in order to allow comprehensive snap shots of a range of PPIs. Taken together, this would suggest the need to study as many PPIs as possible when analysing mammalian Hippo signalling. In particular, novel functions of YAP signalling (such as the role in stem cell renewal [50]) and its regulation by PPIs will be of outmost importance.

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Abbreviations

LATS	<u>l</u> arge <u>t</u> umour <u>s</u> uppressor kinase
NDR	<u>n</u> uclear <u>D</u> bf2-related kinase
MST	<u>m</u> ammalian <u>S</u> te20-like serine/threonine kinase
MOB	<u>M</u> ps <u>o</u> ne <u>b</u> inder protein
PPI	<u>p</u> rotein- <u>p</u> rotein interaction
SARAH	<u>S</u> alvador- <u>R</u> ASSF1A- <u>H</u> ippo domain
HM	<u>h</u> ydrophobic <u>m</u> otif

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Figure 1.

The complexity of selected PPIs in mammalian Hippo signalling. MST1/2 kinases are activated by binding to RASSF1A and Salvador through conserved SARAH domains. MST1/2 associate with and activate LATS/NDR kinases by phosphorylation. LATS/NDR activities are controlled by binding to hMOB1/2 proteins using the NTR domains of LATS/NDR. Kibra also influences LATS activity through a WW/PPXY interaction. LATS associates with YAP and TAZ using WW/PPXY domains, which plays a role in the inhibition of YAP and TAZ. AMOTL1/2 proteins further negatively regulate YAP and TAZ activities through WW/PPXY mediated PPIs.