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Review article

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HIPPO PATHWAY - BRIEF OVERVIEW OF ITS RELEVANCE IN CANCER

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The Hippo pathway is the major regulator of organ growth and proliferation. Described initially in *Drosophila*, it is now recognized as one of the most conserved molecular pathways in all metazoan. Recent studies have revealed the Hippo signalling pathway might contribute to tumorigenesis and cancer development. The core components of the Hippo pathway include the mammalian sterile 20-like kinases (MSTs), large tumour suppressor kinases (LATSs), the adaptor proteins Salvador homologue 1 (SAV1, also called WW45) and Mps One Binder kinase activator proteins. The major target of the Hippo core kinases is the mammalian transcriptional activator Yes-associated protein (YAP) and transcriptional co-activator with PDZ-binding motif (TAZ). In cancer, the Hippo signalling is inactivated and YAP and TAZ are activated and free to translocate into the nucleus to promote cell proliferation. Nuclear YAP/TAZ activate or suppress transcription factors that regulate target genes involved in cell proliferation, tissue growth, control of organ size and shape or metastasis. The Hippo signalling pathway that controls the most important cellular processes like growth and division appears to be a very promising research subject in the field of cell biology and tissue engineering. It consists of elements that in the cell play the roles of tumour suppressors as well as oncogenes. This 'Janus like' - an opposite activity hidden within one and the same signalling pathway represents a significant obstacle for studying it. This property of the Hippo pathway is worth remembering, as it will appear several times during the discussion of its properties. Here, we will review certain data regarding biology of the Hippo signalling and its interplay with other prominent signalling pathways in the cell, its relevance in cancer development and therapies that might target elements of the Hippo pathway in most human cancers.

Key words: *Hippo signalling pathway, apoptosis, proliferation, cancer, cell polarity, cell signalling*

WHAT HAVE WE LEARNED FROM FLIES?

Contemporary biological sciences, in spite of the increasing specialization in the field, are trying to answer the most essential and basic questions about the processes that control the cell life, growth and differentiation. One of the pivotal issues dealt with recently is the answer to the question how the tissue 'knows' when to stop growing and how it is 'able to' implement this knowledge to control cell divisions. Moreover, it still remains a mystery how the process of inhibition of the cell division can be temporarily abolished and then restarted during controlled tissue growth in case of its damage or organ injury. For example, if more than a half of mouse liver is surgically removed, the remaining part almost immediately starts to grow in the process of regeneration to reach the original size within a few days and then the growth stops (1). Over the years, a surplus of 'revered' signalling pathways was considered to be a perpetrator of this process but the information has not been confirmed.

Everything changed in 1995 when the discovery of growth-suppressing genes became evident (2, 3) during a study of genetic mosaics in *Drosophila melanogaster*. This finding triggered a real 'avalanche' of discoveries, revealing the up-stream elements of the Hippo signalling pathway. The 'core', consisting of four paramount members of this pathway: Warts (Wts) protein kinase, a member of NDR family, the Salvador (Sav) WW domain-containing protein, the Ste20-like protein kinase Hippo (Hpo) and the Mob-as tumour-suppressor (Mats) an adaptor protein, was discovered when studying *Drosophila melanogaster* loss-of-function mutants of these genes (4-7). The results were very surprising showing that decay of the activity of any of these four genes due to a mutation or loss of function led to a massive tissue overgrowth with declined apoptosis rate. Additionally, Tapon *et al.* (8) have discovered that salvador (*Sav*) gene product and its human ortholog WW45 possessed the ability of regulating both cell cycle exit and apoptosis. Year later, Pantalacci *et al*. (9) identified Hippo (Hpo) as a partner of Sav, stabilizing its function and regulating the process of apoptosis control during the *Drosophila* development. The restriction of tissue growth has been achieved through quantity control of *Drosophila* inhibitor of apoptosis protein (DIAP-1), which is Hpo dependent. The elements of the pathway, mentioned above, orchestrated as a network of dependent kinases, where Hpo-Sav as a complex is able to phosphorylate and, in consequence, activate the Wts-Mats kinases, as well as constitute a major portion of the scaffold of the Hippo signalling pathway (10, 11). The activated Wts-Mats kinases or more precisely Wts, phosphorylate the 'prey' - a

transcriptional co-activator and oncogene - Yorkie (Yki), inactivating it in Hpo dependent manner and promoting its cytoplasmic retention and degradation (12). This abolishes the growth promoting function of the transcription co-activator. And that is how in general outline the Hippo pathway emerged from the studies carried out in *Drosophila melanogaster*. The elaboration of this scheme has become a strong motivation factor to investigate its functioning in mammals. Its general organization has been presented in the *Fig. 1*.

The Hippo pathway has become extensively studied in mammalian tissues and results of these studies have corroborated the conservative features of its composition, for example the 'core kinases grid' known from *Drosophila*, but, at the same time, many differences and astonishing complexity of its mammalian components have been revealed.

MAMMALS AND INSECTS - ANY SIMILARITIES?

An analysis of the Hippo 'core' kinase system components in the mammalian genome revealed the presence of a pair of *Drosophila melanogaster* Hpo homologs - MST1/STK4 and MST2/STK3 (mammalian Ste20-like serine/threonine kinases 1/2), the activity of which is enhanced through interaction with WW45/Sav1, a homolog of *Drosophila* Sav. WW45/Sav1, acting like a coupler, is able to hold MST1/2 and LATS1/2 (Large tumour suppressor 1/2 serine/threonine protein kinases) together in complex. Both MST1/2 and Sav1 utilize similar SARAH (Salvador/Sav1-WW45, Rassf/ Hippo/MST1/MST2) domains (*Fig. 2*) (13). Additionally, MST1/2 might also be activated through direct phosphorylation by TAOK1, thousand-and-one amino acids kinase 1, which can function as a MAP3K (MAP kinase kinase kinase), capable of direct phosphorylation of MAP2K3 (mitogen-activated protein kinase kinase kinase 2) and MAP2K6, what, as a consequence, results in activating direct phosphorylation of p38 MAPKs (*Fig*. 2). Moreover, TAOK3, also known as JIK, has the capability to inhibit JNK activation (14, 15). This 'regulatory connection' of MST1/2 with the TAO kinases family significantly expands the fields of control and modulation of the Hippo pathway elements (16-18). Next, two *Drosophila* Wts homologs (large tumour suppressor 1 and 2, LATS1 and LATS2), and two Mats homologs, MOB (Mps-one binder) kinase activator 1A and 1B (MOBKL1A and MOBKL1B - also called Mob1) have been identified in the mammalian cells (19-21). MOBKL1A, a natural substrate of kinases MST1/2, is able to, when phosphorylated, activate a phosphorylation loop of LATS1/2 by binding and covering their auto-inhibitory motif. This action leads to amplification of LATS activation and enhancement of their kinase activity (*Fig. 1*) (22).

Yki homologs - TAZ (transcriptional co-activator with PDZ-binding motif) and YAP (Yes-associated protein encoded by YAP1) cloned and characterized by Sudol (23) as a proline-rich phosphoprotein that binds to the SH3 domain of the Yes proto-oncogene product as well as other signalling molecules containing the same domain like Nck, Crk and Src, the downstream components of the Hippo pathway, are being phosphorylated on multiple HxRxxS motifs and, due to this action, inactivated as a response to the cell density signals, leading to their cytoplasmic sequestration and following proteasomal degradation. The extensive studies of mammalian YAP gene have revealed its conserved nature among the higher eukaryotes and almost ubiquitous expression on the RNA level in the adult human tissues. YAP protein, depending on alternative splicing, contains one or two WW domains, the prominent feature of which is the presence of two tryptophans and also a PDZ interaction motif, an SH3 binding motif, and a coiled-coil domain (24). In the mechanism of the cytoplasmic - nuclear YAP/TAZ trafficking, phosphorylation of YAP at S127 and TAZ at S89 plays a pivotal role, turning HxRxxS motifs, associated with them, to 14-3-3 protein binding sites, which function as an off-switch in cell density-dependent manner and are absolutely indispensable for the contact inhibition phenomenon in mammalian cells (*Fig. 2*) (25, 26). Recent studies have established an additional way of YAP/TAZ cytoplasmic - nuclear translocation control. Zhao *et al*. (26) have confirmed that LATS 1/2 dependent phosphorylation of HxRxxS motif at S381 in YAP leads to its consecutive phosphorylation by casein kinase 1 (CK1 delta/epsilon) and, consequently, to the ubiquitin-mediated degradation of YAP through the recruitment of the β-transducin repeat-containing proteins, a subunit of the SCF ubiquitin E3 ligase (27). But still, the most efficient regulatory mechanism for YAP/TAZ is the phosphorylation by upstream kinases, which affects YAP/TAZ protein nuclear translocation and jeopardizes its stability (*Fig. 2*). Additionally, as shown by Oka *et al*. (28), at least one of its three major isoforms, YAP-2, might be translocated to the nucleus as a complex with ZO-2 protein (zonula occludens 2). Complex formation is possible due to the interaction of a C-terminally located PDZ domain-binding motifs of both protein (29). Moreover, Oka and Sudol (30) have documented that the PDZ-binding motif is absolutely indispensable for the stabilization of p73 and for promoting apoptosis. Dominguez-Calderon *et al*. (31) presented evidence that the absence of ZO-2 protein in the MDCK renal epithelial cells triggers YAP nuclear accumulation and its elevated transcriptional activity. This resulted in decreased expression of phosphatase and tension homologue (PTEN), leading to an increased concentration of phosphatidylinositol (3,4,5)-triphosphate (PIP3) and in consequence Akt/mTORC1 transactivation. The presence, in a N-terminally located PDZ domain, of one SH3 (Src homology 3) domain, a GUK (guanylate kinase) homology domain and a proline-rich C-terminal region are the general characteristics of junction-associated ZO proteins belonging to the MAGUK (membrane-associated guanylate kinase) protein family (31). In the absence of the nuclear co-activators YAP/TAZ, TEAD transcription factor associates with VGLL4 transcription co-activator and that results in the down-regulation of the pro-proliferative, pro-EMT (epithelial to mesenchymal transition) and anti-apoptotic target genes under the control of this nuclear factor (*Fig. 1*).

A lack of intrinsic DNA-binding domains in YAP/TAZ proteins has resulted in the different indirect manner of target genes regulation. YAP/TAZ proteins bind to the promoters of the target genes in a roundabout way by interacting with transcription factors that directly bind to DNA. In this way, YAP and TAZ proteins are targeting a diversity of transcription factors that control tissue growth and cell viability. The preeminent mediators of the growth and the carcinogenesis, which belong to YAP/TAZ distinct targets, are for example TEADs (TEA domain family members also known as TEFs) and SMAD2/3/4, member of p53 family - p73, PPARγ and thyroid transcription factor-1 or ErbB4 (*Fig. 2*) (32-37).

Considering the wide range of YAP partners, the TEAD/TEF family of transcription factors seemed to be a prime destination in downstream signalling of the Hippo pathway. TEAD transcription factors are being expressed in every kind of tissue. Moreover, almost in all differentiated tissues at least one member of TEAD protein was found to be expressed (38). Additionally, TEAD1/TEAD2 together with YAP were shown to be responsible for the regulation of overlapping target genes sets and any disruption of this interplay resulted in a decreased ability of YAP/TAZ to promote epithelial-mesenchymal transition or anchorage-independent growth (39). Results of recent works of Sidor *et al*. (40) complemented our knowledge

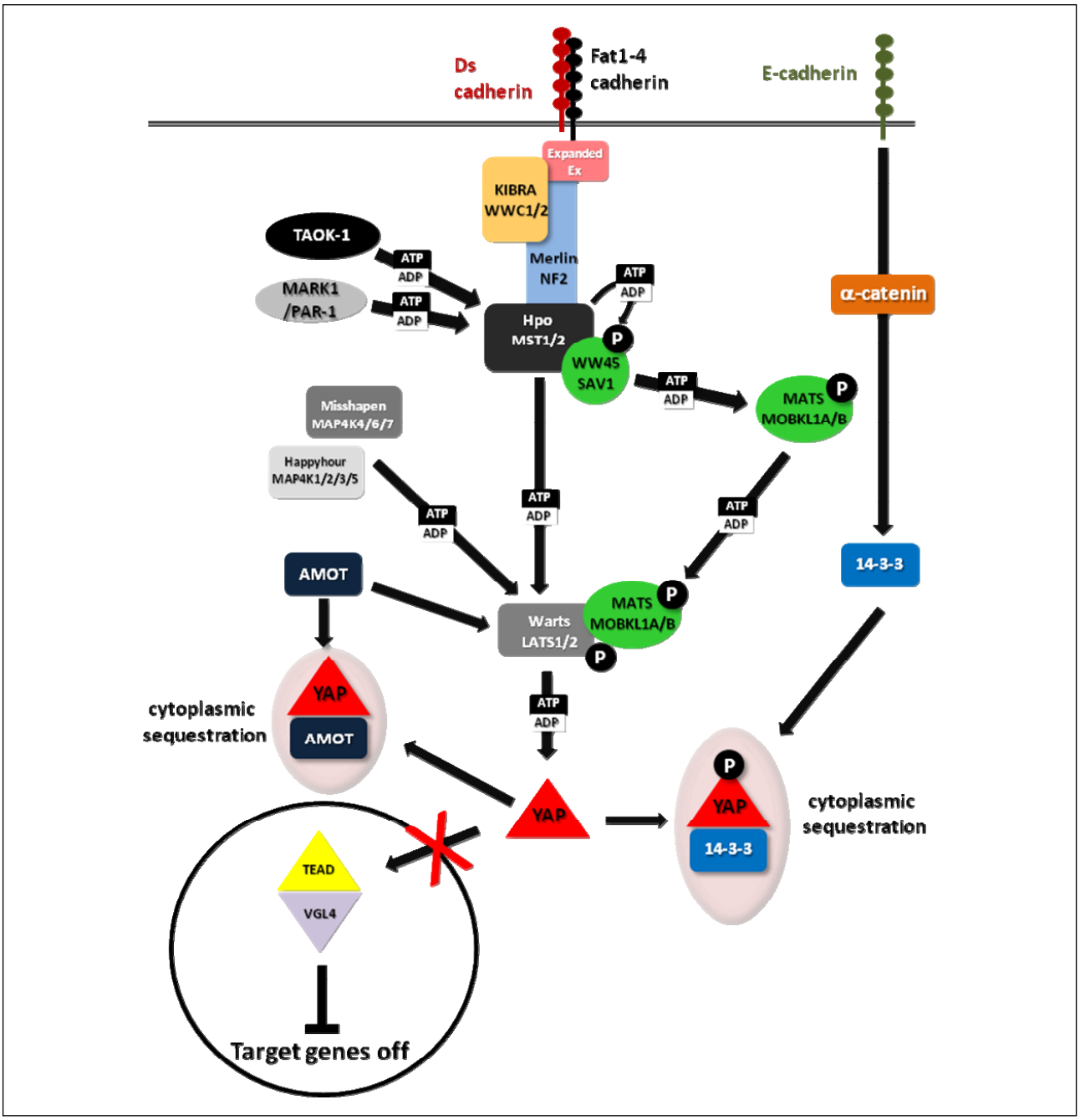

Fig. 1. Diagram presenting the general features of the *Drosophila's* and mammalian Hippo signalling pathway. Central axis of the entire pathway contains the 'core kinases' interplaying with the up-stream modulators and specific adhesion molecules. Merlin/NF2 employing Kibra/WWC1/2 proteins interacts with a variety of cellular signalling pathways like Ras, STATs, or PI3K as well as cellular receptors, like cadherins, CD44 - hyaluronic acid receptor or adherens and tight junctions. This leads to the apical co-localization of Kibra-FRMD6/Ex1-Merlin/NF2 and recruitment of the Hippo pathway kinases MST1/2, LATS to the plasma membrane. Active 'core kinases' phosphorylate YAP (Yes-associated protein encoded by YAP1) resulting in sequestration YAP-1in the cytoplasm of the cell and ultimately down-regulation activity of the pathway. MST1 and MST2 activity, is enhanced through interaction with WW45/Sav1. WW45/Sav1, acting like a coupler, of MST1/2 and LATS1/2 complexes. Additionally, MST1/2 might also be activated through direct phosphorylation by TAOK1, thousand-and-one amino acids kinase 1, which can function as a MAP3K. MOBKL1A, a natural substrate of kinases MST1/2, when phosphorylated, activate a phosphorylation loop of LATS1/2 by binding and covering their auto-inhibitory motif resulting in enhancement of their kinase activity. Active LATS1/2 phosphorylate YAP. Phosphorylated YAP is bound and inactivated in the complex with 14-3-3 protein binding. Another mechanism of YAP cytoplasmic sequestration involves angiomotin (Amot) and angiomotin-related AmotL1 and AmotL2 which were identified as negative regulators of YAP and TAZ by preventing their nuclear translocation. In the absence of the nuclear co-activators YAP/TAZ, TEAD transcription factor associates with VGLL4 transcription co-activator and that results in the down-regulation of the pro-proliferative, pro-EMT (epithelial to mesenchymal transition) and anti-apoptotic target genes under the control of this nuclear factor. Processes of activation of different components of the pathway have been marked using arrow headlines (→). Involvement of specific phosphorylation has been emphasized with ATP/ADP sign on the line. Processes of inhibition or deactivation have been marked using blunt lines (—|). The precise explanation can be found in the text of the article.

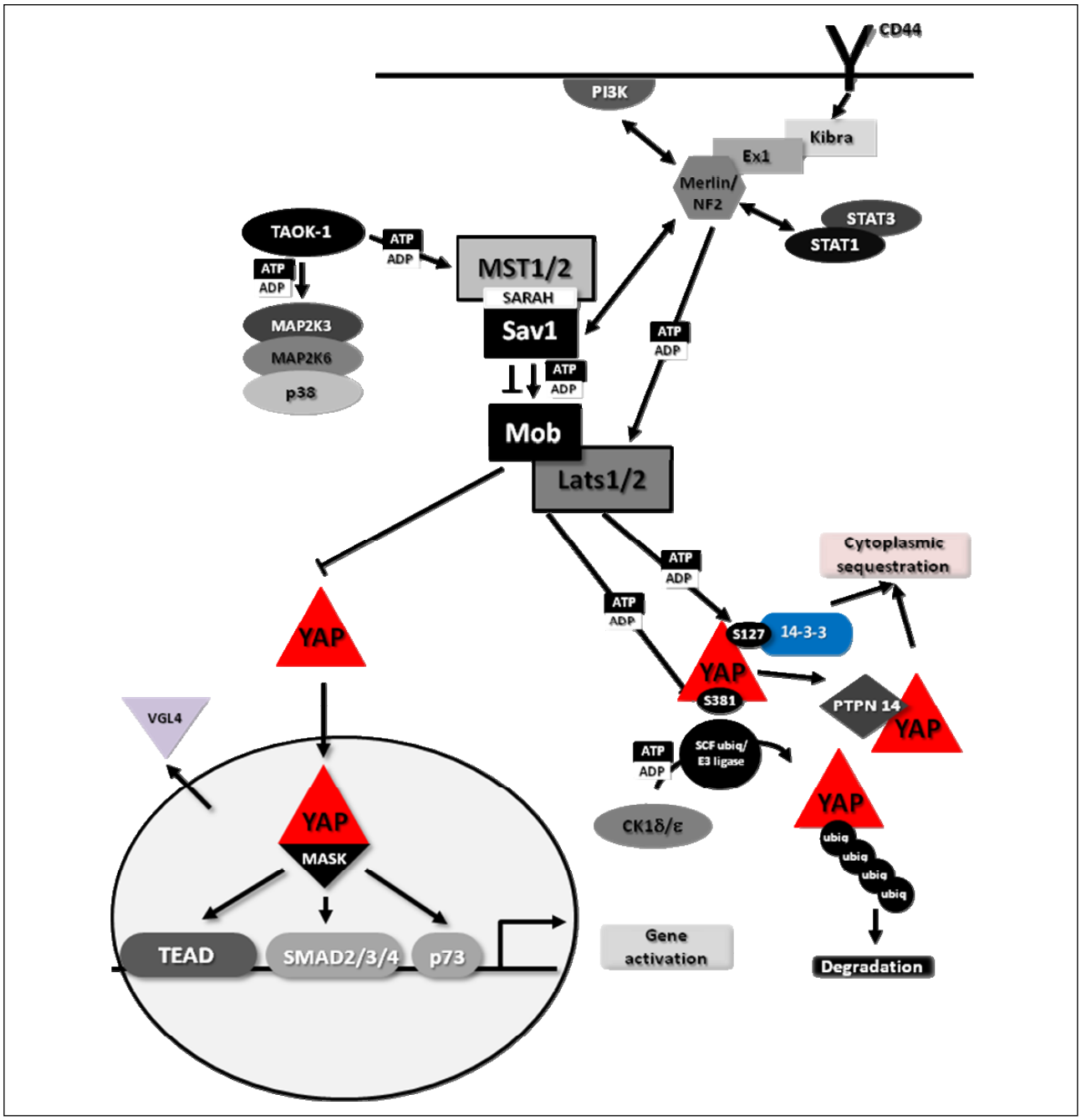

Fig. 2. Diagram presenting the detailed interaction of the 'core' elements of the Hippo signalling pathway during the activation or inhibition of its down-stream, effector elements and Hippo dependent genes. The most efficient regulatory mechanism for YAP/TAZ is the phosphorylation by upstream kinases, which affects YAP/TAZ protein nuclear translocation and jeopardizes its stability. However, LATS 1/2 dependent phosphorylation of HxRxxS motif at S381 in YAP leads to its consecutive phosphorylation by casein kinase 1 (CK1 delta/epsilon) and to the its ubiquitin-mediated degradation through the recruitment of the β-transducin repeat-containing proteins, a subunit of the SCF ubiquitin E3 ligase. A lack of intrinsic DNA-binding domains in YAP/TAZ proteins has resulted in the different indirect manner of target genes regulation. YAP/TAZ proteins bind to the promoters of the target genes in a roundabout way by interacting with transcription factors that directly bind to DNA. In this way, YAP and TAZ proteins are targeting a diversity of transcription factors that control tissue growth and cell viability. The preeminent mediators of the growth and the carcinogenesis, which belong to YAP/TAZ distinct targets, are for example TEADs and SMAD2/3/4, member of p53 family - p73, PPAR γ and thyroid transcription factor-1 or ErbB4. Processes of activation of different components of the pathway have been marked using arrow headlines (➡). Involvement of specific phosphorylation has been emphasized with ATP/ADP sign on the line. Processes of inhibition or deactivation have been marked using blunt lines (⊣). The precise explanation can be found in the text of the article.

of YAP-TAED interaction. They have documented a completely new member of the Hippo pathway component involved in the positive modulation of YAP or Yorki activity. Multiple ankyrin repeats single KH domain (MASK) and MASK human homologues were identified as ANKHD1 and ANKRD17, which have been purified from YAP-TAED complexes, were found to

be mandatory co-activators of YAP in the interaction with TEAD. Both YAP and MASK exhibit the coinciding pattern of cellular distribution in response to several stimuli (41, 42).

All this data point out that an 'ungoverned' YAP/TAZ activity may hamper basic tumour suppressor checkpoints in the cell cycle (43).

YAP and TAZ activity might be controlled not only through its phosphorylation and repression in the so-called 'canonical manner'. Many of upstream elements of the Hippo pathway are able to bypass the 'core' kinase network and create complexes, in which YAP/TAZ is trapped and simply swept out from cytoplasm preventing its nuclear translocation (35). This kind of YAP/TAZ control has been associated with the upstream to the 'core' kinase cassette members of the Hippo pathway, connected with the intracellular parts of membrane receptors or cytoskeleton related proteins (*Fig. 1*).

An example of such action might be angiomotin (Amot) and angiomotin-related AmotL1 and AmotL2 which were identified as negative regulators of YAP and TAZ by preventing their nuclear translocation. This might be achieved through direct interaction using Amot PPXY motifs to complex with WW domains of YAP/TAZ to either sequester YAP/TAZ in the cytoplasm or pin it to the tight junction. Amot proteins were described as scaffold proteins capable of coordinating the functional integration of several pathways. Amot proteins are composed of different domains like N-terminal region containing two PPXY motifs and one PPXY-like LPTY (T, Thr) motif that interact with WW domains in YAP; the central coiled-coil regions that interact with Merlin and indirectly with Kibra and the C-terminal part enriched with type II PDZ-binding motif interacting with the PDZ domains (44, 45).

Kim *et al.* (46) were able to demonstrate that in hepatocytes Mst1/2 knockout Notch pathway signalling components form a positive feedback loop with YAP/TAZ accelerating rapid liver enlargement and hepatocellular carcinoma formation. Inhibition of Notch substantially reduced Yap/Taz activities followed by reduced hepatocyte proliferation rate. Moreover, silencing of β-catenin in Mst1/2 knockouts additionally accelerated liver carcinogenesis showing unexpected connection of Wnt/β-catenin and the Hippo signalling pathway, in the process of liver carcinogenesis initiation. Heallen *et al.* (47) have shown that YAP interacts with β-catenin, resulting in an inhibition of β-catenin translocation to the nucleus and regulation of Sox2 and Snai2 target genes. This observation reveals interaction at the nuclear level between Hippo and Wnt pathways in the process of restriction of cardiomyocyte proliferation and heart size control. The interaction between upstream elements of the Hippo and Wnt/β-catenin pathways was confirmed with the observation that Merlin can inhibit Wnt/β-catenin signalling *via* interfering with LRP6. Kim *et al.* (48) demonstrated that Merlin interacted with LRP6 blocking its phosphorylation and suppressing Wnt/β-catenin signalling. Cells isolated from NF2 patients with schwannomas possessing Merlin mutations showed significantly increased level of β-catenin, as compared to that in normal adjacent tissues. PAK1 (p21 activated kinase) dependent phosphorylation of Merlin on Ser 518 initiate its detachment from LRP6 and in consequence phosphorylation of LRP6, thereby restoring Wnt/β-catenin signalling (49) (*Table 1*).

HIPPO DEPENDENT CONTROL OF CELL POLARITY

Almost all of *Drosophila's* Hippo pathway homologs were identified in the mammalian cells with only one exception of *Drosophila's* Dachs. An analysis of mammalian genomes revealed even more genes than those discovered in case of *Drosophila*, the products of which are involved in functioning of the upstream to the Hippo 'core' kinase cassette network. Most of these proteins were found to be playing a crucial role in maintaining the Hippo signalling, thus no loss-of-function mutations were found that could be used for the analysis. This group of genes includes two known homologs of Kibra (WWC1,WWC2), two homologs of Ex (Ex1 and Ex2), one homolog of Merlin (Merlin/NF2) and one of Ft (Fat4/Fat-j). Moreover, two homologs of Ds (Dchs1 and Dchs2), one Fj (Fjx1), two Lft (Lix1 and Lix1-like), and at least three Crb (Crb1, Crb2 and Crb3) homologs were described (*Fig. 1* and *Fig. 2*) (50-52). One product of these genes, Merlin/NF2, has been known as tumour suppressor for almost 20 years. Its mutation was observed in patients suffering from benign schwannomas, but it is also the main manifestation of an inherited disease called neurofibromatosis 2 (53, 54). Consistent and reliable results of long-period studies gave scientific credence to the hypothesis that Merlin/NF2 interacts with a variety of cellular signalling pathways like Ras, STATs, or PI3K (55, 56). But probably the most important function of Merlin/NF2, regarding the Hippo role with reference to controlling the phenomenon of cell-cell contact inhibition, is its essential role in governing the cellular receptors, like CD44 - hyaluronic acid receptor or adherens and tight junctions (*Fig. 2*) (57, 58). Recent works of Genevet *et al.* (58) and Yu *et al.* (59) shed some light on the proposed networking of the upstream Hippo components like Kibra-FRMD6/Ex1-Merlin/NF2, with regards to its physical interaction and complex formation with canonical pathway members, like LATS1/2, stimulating its phosphorylation leading to activation of Wts and inhibition of YAP activity. It has been shown that the upstream components of the Hippo pathway react in a different manner depending on the target membrane protein, which helps the cell stratify its cytoplasm into apical and basolateral domains. Distinguishing the cell polarity is one of the most fundamental processes during tissue growth and differentiation. Apical co-localization of Kibra-FRMD6/Ex1-Merlin/NF2, observed in epithelial cells, might suggest participation of at least some members of this complex in the control of actin cytoskeleton reorganization or dynamics of its attachments to the apical plasma membrane of the cell (60, 61). This hypothesis is authenticated by the fact that at least one of the complex constituents - Kibra - possesses a C2 domain and there is physical evidence for its interaction with

Table 1. Hippo pathway components in *Drosophila* and its mammalian orthologs.

Drosophila	Hippo Hpo	Salvador Sav	Warts	Mats	Yorkie Yki
Mammalian	Ste20-like kinase Mst1 and Mst2	WW45	Large tumor suppressor Lats1/2	Mps one binder kinase activator-like 1 Mob1A/B	Yes-associated protein YAP

plasma membrane phospholipids (62). It is worth noting that Sav and Hpo have been found to physically associate with Merlin/NF2 and Ex, and, again, as was mentioned above, Kibra participates in Wts complexing (*Fig. 1* and *Fig. 2*). Moreover, a direct interaction of adherens junction related enzyme, non-receptor PTPN14 - protein tyrosine phosphatase 14, with YAP has been confirmed by several laboratories. To form a stabile complex PTPN14 and YAP utilizes PPxY motifs and WW domains respectively. YAP 'imprisoned' in this complex is incapable of nuclear translocation, and that decreases its regulatory activity (63-65).

Taken together, it might suggest that apical co-localization of Kibra-FRMD6/Ex1-Merlin/NF2 or adherens junction complex with PTPN14 recruit the Hippo pathway kinases MST1/2, LATS or effector protein YAP to the plasma membrane or sequester them in the cytoplasm of the cell and might be ultimately responsible for the entire down-regulation activity of the pathway.

Some recent observations of Zhao *et al.* (65) and Dupont *et al*. (66) indicate that Hippo effectors YAP/TAZ proteins might be involved in controlling the process of cell attachment-detachment to the extracellular matrix component. YAP activity was shown to be controlled dependently on the rigidity of extracellular matrix. In cells seeded on rigid matrix substratum, YAP/TAZ remained active, while in the cells transferred to the soft matrix the culture became inactivated. Moreover, the cell attachment-detachment process was demonstrated to be linked with YAP/TAZ induction or repression respectively. The detachment dependent repression of YAP/TAZ was followed by cell anoikis. In view of the fact that Rho GTPases are strongly involved in controlling the actin cytoskeleton reorganization during cell proliferation, its association with dynamics of Hippo effectors YAP/TAZ should not be surprising. Zhao *et al*. (65) have demonstrated that RhoA plays a role of a strong enhancer of YAP/TAZ in proliferating cells. The YAP/TAZ activity was regulated through its phosphorylation dependent on actin cytoskeleton rearrangement or microtubules dynamic status change. Recent studies give support to the thesis that dynamics of cytoskeleton might be a prominent regulatory signal for activation or deactivation of the Hippo pathway during the process of tissue growth and differentiation. A new line of evidence indicates that not only actin but also spectrin cytoskeleton seems to be involved in the regulation of YAP/TAZ cellular activity, playing an important role as a mechanical signal dependent up-stream regulator of the Hippo signalling. The membrane bound spectrin cytoskeleton is known to be very conservative in all eukaryotes. This heterotetrameric protein, composed of α and β subunits, can crosslink with F-actin filaments (67). In a recent paper Fletcher *et al*. (68) have proven that in dense confluent epithelial cell monolayers it is enough to knock-down the SPTAN1 or SPTBN1 gene, encoding α-spectrin and β-spectrin, to abolish the phosphorylation of LATS1 and, consequently, YAP, which results in YAP nuclear translocation and uncontrollable cell divisions. The same authors, having analysed *Drosophila melanogaster* mutants of α-spectrin and β-spectrin genes, demonstrated early over-proliferation and multilayering in the follicular epithelium of mutants. This may be a strong piece of evidence for a highly conservative control mechanism of the Hippo pathway by elements of the cell cytoskeleton and mechanical stimuli (68). Deng *et al.* (69) go even further, suggesting that, regardless of its location, whether apical or basolateral, spectrin cytoskeleton participates in the regulation of the Hippo pathway activity involving actin-myosin filaments, which greatly expands the number of regulatory elements associated with the cytoskeleton and involved in the control of the Hippo signalling (69).

HIPPO RELATED NETWORK

There are many examples supporting the connection of the Hippo signalling with the control of the cell division and the cell cycle rather than the induction of apoptosis. The results of Ganem *et al.* (74) have extended the scope of the Hippo pathway regulatory features, showing that the LATS2 gene is necessary for the cell cycle arrest in tetraploid cells, which is not connected with the DNA damage. A small interfering RNA knock-down of LATS2 in tetraploid cells resulted in the cell cycle progression while a re-expression of wild-type LATS2 restored the cell cycle arrest. Moreover, in tetraploid arrested cells, YAP has been found to be phosphorylated and sequestered in the cytoplasm and, at the same time, LATS2 has been shown to co-localize with the negative regulator of p53, MDM2. This action led to the p53 stabilization and, together with the massive DNA damage signal, it forces the cell to undergo apoptosis under the supervision of a well-established p53 dependent mechanism (74-76).

On the other hand, we have to disclose that the 'dark side' of the Hippo pathway regarding apoptosis also exists. A growing body of evidence supports a contradictory action of Hippo components to the one described above. And we are not talking of its physiological activity, when, under the condition of low-density of cells, it suppresses pro-apoptotic target genes (39, 77). As revealed by Reuven *et al*. (78), apoptosis induced by DNA-damage might be abolished by the Hippo components even at high cell density conditions and due to inhibition of tyrosine kinase c-Abl, which is known as a prominent inducer of cell death under DNA damage. This is caused by a direct interaction of kinase LATS2 with c-Abl, which results in a diminished activity of c-Abl as well as down-regulation of c-Abl down-stream substrates phosphorylation. These results shed a light on the observation, which has been a mystery for decades - the radio-resistance of dense cellular cultures (78). But, at the same time, Basu *et al*. (79) have demonstrated that YAP functions as a coactivator of transcription factor p73 and might play an important role in triggering apoptosis. DNA damage dependent induction of Bax expression is mediated by p73. But p73 requires YAP as a coactivator. Any attenuation of YAP function due to the phosphorylation may consequently suppress the induction of pro-apoptotic genes leading to inhibition of cell-damage dependent apoptosis. One possible kinase, not belonging to Hippo pathway, responsible for YAP phosphorylation was shown to be protein kinase B/Akt (79) (*Fig. 1*). The mechanism standing behind the phenomenon described is the substrate sequence recognized by Akt, which has been identified as RxRxxS/T (80). This motif, when phosphorylated, partially overlap with RssS/T(phos)xP binding motifs of 14-3-3 proteins (81) causing cytoplasmic YAP sequestration in complexes with 14-3-3.

A complex formation is a well-known and efficient molecular mechanism of modulation in case of many regulatory signalling pathway proteins. MST1/2 do not differ from any other in this respect. Possessing specific SARAH (Salvador/Sav1-WW45, Rassf/ Hippo/MST1/MST2) domains opens up the possibility of heterodimers or even heterotrimers formation between MST1/2 and extensive array of other regulatory proteins involved in cellular mechanisms of signal transduction.

The family of RASSF (Ras association (RA) domain family)/Nore1 consists of proteins encoded by six mammalian genes and has been characterized as tumour suppressors. This activity in the most of epithelial malignant neoplasms happened to be abolished due to gene loss and/or epigenetic mechanisms. Their function under physiological conditions focus on the cell cycle delay in G1 or M phase and the promotion of caspase-dependent or -independent apoptosis. Moreover, some members

of the RASSF family - RASSF6, are able to bind MDM2 and, in this way, regulate the expression of p53. A direct protein-protein interaction of RASSF6 with Ras and Modulator of apoptosis 1 (MOAP1), as well as with NFκB and JNK signalling pathway, places it among the most essential regulators of the apoptosis in the cell (82). The presence of two specific fragments - the Ras association (RA) domain and the SARAH domain, located at the C-terminal part of protein is a characteristic feature of all members of the RASSF/Nore1 family. Utilizing its RA domain, Nore1A exhibits a high affinity binding ability to the active (GTP 'loaded') forms of Ras, Rap-1, and other Ras GTPases. Although Nore1A and RASSF1 share more than 50% of their RA domain homology, RASSF1 demonstrates a significantly lower binding affinity for Ras-like GTPases, but, in contrast to Nore1, it interacts with Ras GTPases indirectly. Additionally, Nore1 is capable of blocking the auto-activation of MST2, but once MST2 becomes active, Nore1 cannot block it anymore. This entails disruption of the interaction with RASSF1A and releases it from the deactivating 'embrace' of Raf-1. This, in turn, enables a normal interaction between MSTs and LATS and consequently induces apoptosis in the cell. MST1/2 using SARAH domain are capable of heterodimerization with both Nore1A and RASSF1A through their RA domains and the entire complex was shown to associate with endogenous Ras in response to the addition of serum (*Fig. 3*) (83-85). In this assembly MST1/2 remain active. Moreover, this kind of interaction may lead to the deliverance of MST2 from its physiological inhibitor such as the kinase Raf-1, possessing the ability of binding to MST2, what precludes both its activation and, in consequence, the pro-apoptotic signalling. Releasing of MST2 from its inhibitor Raf1 by RASSF1A might be a critical step in RASSF1A-induced apoptosis, leading to the augmentation of MST2 interplay with its natural substrate, LATS1, a downstream Hippo kinases member. The resultant nuclear translocation of YAP caused by LATS1 dependent phosphorylation and association with p73 impels the transcription of a pro-apoptotic gene - *puma* (85). Mob1 and LATS1/2, other components of the Hippo pathway, have been found to be responsible for the phosphorylation of a divergent scope of cellular targets-substrates, such as histone H2B or transcription factors FOXO1 and FOXO3 as well as the LATS1/2-related kinases Ndr1/Ndr2. It occurs mostly as a response to apoptosis inducing stimuli (*Fig. 3* for details) (86-90).

MST1/2, Hippo pathway downstream kinases activators, exhibit apoptosis inducing features. Unlike the *Drosophila* Hpo, MST1/2 possess cleavage sites, specific for caspase 3, located between catalytic domain and SARAH. A caspase-dependent cleavage leads to the liberation of 35KDa N-terminal fragment of MST1/2. An additional auto-phosphorylation of this MST1/2 fragment on Ser183 is required for its full catalytic activity and nuclear translocation, where its natural substrate, histone H2B, becomes the target of phosphorylation as well as histone H2AX, which become phosphorylated at Ser-139. This kind of action promotes DNA degradation in the stress-induced apoptosis, controlled by the Hippo pathway components and is followed by inactivation of YAP through its phosphorylation in LATS1/2 - independent manner (*Fig. 3*) (70-72, 91).

The involvement of Hippo in conveying pro-apoptotic signals was supported by earlier findings demonstrating that death receptors and DNA damaging agents might activate core kinases of the pathway (71). Some members of Mst kinases family have been shown to be activated by non-physiological stress such as high temperature heat shock and high concentrations of staurosporine or sodium arsenite (92), as well as by cellular stressors like inflammatory cytokines such as TNF-α. As demonstrated by Lee *et al*. (93), MST1 was strongly activated during Fas-mediated apoptosis (93). Similarly to MST, in case of p21-activated kinase (PAK2) and STE20-like kinase (SLK), caspase-dependent cleavage and influencing the apoptotic morphology suggest that SPS1/STE20 family kinases are involved in the induction of apoptosis, due to targeting the same or similar molecules targets. Involvement of MST1/2 in the suppression of apoptosis was revealed by O'Neill *et al*. (94). They have presented evidence that proto-oncogene RAF1, through the direct interaction, inhibits MST2 dimerization and autophosphorylation. Moreover, it recruits a phosphatase inactivating MST in a kinase-independent fashion, leading to a protective effect against apoptosis (94). Later studies of Matallanas *et al*. (85) have confirmed that LATS1 and RASSF1A play a role of MST2 interactors in propagation of the pro-apoptotic downstream signal of RAF1 upon stimulation. Moreover, the authors presented evidence that RASSF1A is capable of dissociating the RAF1-MST2 complex and subsequent activation of MST2 kinase activity (85). Based on conflicting results the nature of interactions between RASSF1A and MST is still debatable. Avruch's group and others have indicated that RASSF1A prevented MST2 activation (84, 90, 94) while Wen *et al*. confirmed the role of RASSF1A as an activator of MST2 kinase activity (91, 96, 97). Moreover, RASSF1A-MST2 interaction might influence LATS1 activity resulting in YAP1 phosphorylation on different residues than Ser127 (85). Additionally, as shown *in vivo* by Su *et al*. (98) in mice models also YAP was able to mediate a pro-apoptotic signal in hepatocytes (98).

On the other hand, an opposite effect on MSTs kinases activity has been shown by Romano *et al.* (99). They have presented evidence that phosphorylation of MST2 by Akt may confine its activity by blocking its binding ability to RASSF1A and, at the same time, preserve its association with Raf-1 in the stabile inhibitory complex. Moreover, phosphorylation of MST2 by Akt impedes homodimerization and auto-phosphorylation of MST2, which seems to be obligatory for its activation. Under these circumstances detachment of the MST2 from the complex with its inhibitor Raf-1 results in mitogenic signalling and limitation of plausible apoptotic reaction (95, 99, 100). These results show the complexity of interactions between the Hippo pathway components and the elements of different regulators of the cellular key process - apoptosis.

SIZE MATTERS! HIPPO DEPENDENT CONTROL OF ORGAN SIZE

Controlling the size of internal organs is a mysterious process. We have known, for quite a long time, about some observations suggesting that the control of individual organs is autonomous as it is in the case of thymus transplantation into the mouse fetus - all implanted organs during development of the adult mouse reach the size of a typical mature thymus (101). However, in the same mouse developmental model, implantation of the multiple spleens to one individual results in the limitation of their growth. As a consequence, the total mass of all transplanted spleens equals to one mature organ (102). This observation, in turn, implies a lack of autonomy in the regulation of growth of individual organs. So, what is the truth about this process and what is responsible for its control? The first signals came from the observations made in *Drosophila melanogaster*. The loss of function mutations induced in almost any gene of the set constituting 'the core' of the Hippo signalling pathway like *Warts*, *Hippo*, *Salvador*, *Mats* or dysregulation of the up-stream components namely *Merlin* or *Expanded* has led to a dramatic and uncontrolled tissue growth and the wings, the legs, or thorax hypertrophy. This phenomenon, as it turned out, was 'executed' due to an elevated expression of cyclin E and the cell death inhibitor DIAP1, resulting in the loss of cell cycle control and

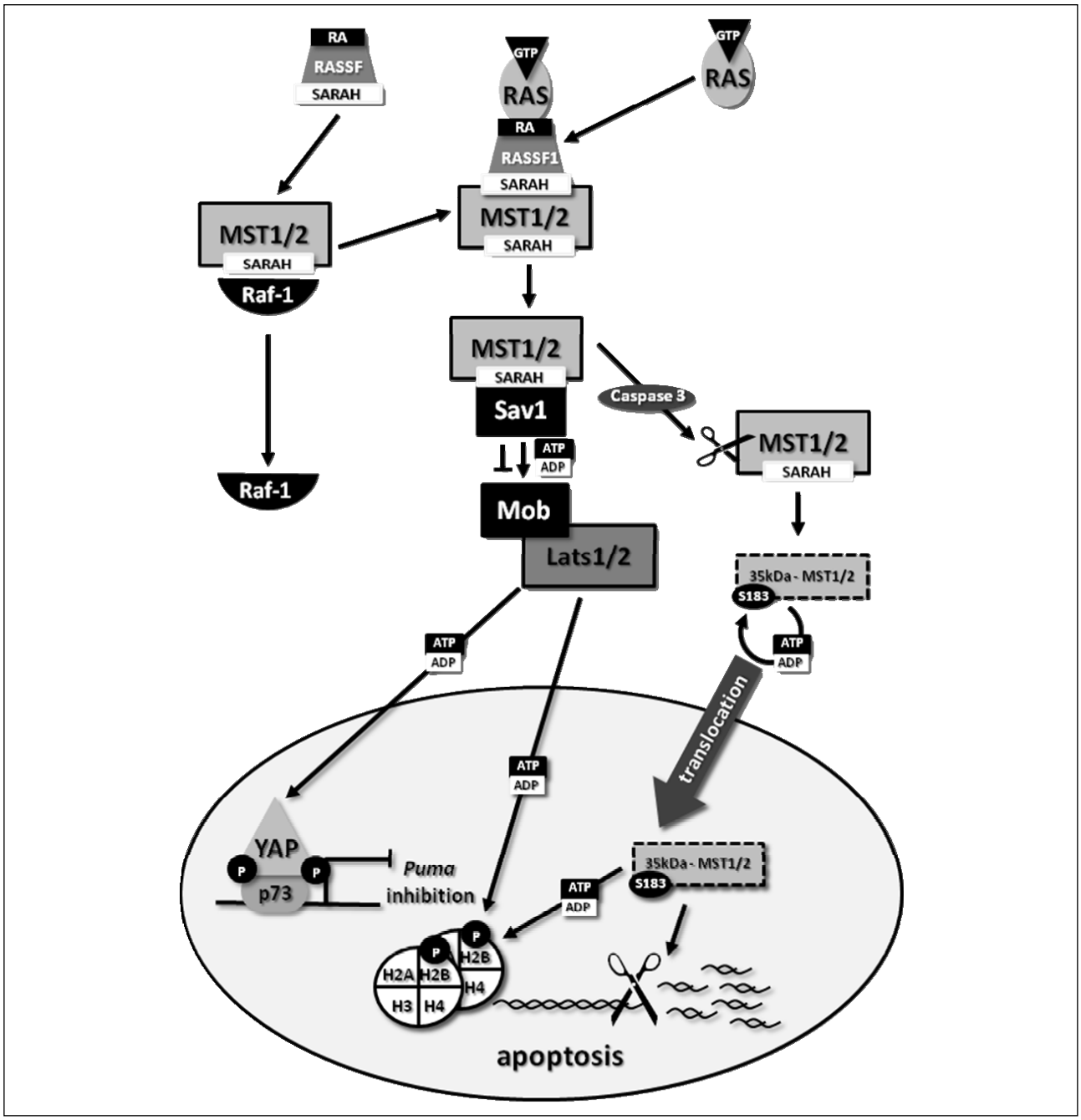

Fig. 3. Diagram showing the involvement of the Hippo elements in the regulation of apoptosis. MST1/2 use SARAH domains for heterodimers or even heterotrimers formation between and extensive array of other regulatory proteins involved in cellular mechanisms of signal transduction. MST1/2 using SARAH domain are capable of heterodimerization with both Nore1A and RASSF1A through their RA domains and the entire complex was shown to associate with endogenous Ras. The presence of two specific fragments - the Ras association (RA) domain and the SARAH domain, located at the C-terminal part of protein is a characteristic feature of all members of the RASSF/Nore1 family. In this assembly MST1/2 remain active. Releasing of MST2 from its inhibitor Raf1 by RASSF1A might be a critical step in RASSF1A-induced apoptosis, leading to the augmentation of MST2 interplay with its natural substrate, LATS1, a downstream Hippo kinases member. RASSF1A-MST2 interaction might influence LATS1 activity resulting in YAP1 phosphorylation on different residues than Ser127. The resultant nuclear translocation of YAP caused by LATS1 dependent phosphorylation and association with p73 impels the transcription of a pro-apoptotic gene - *puma*. Mob1 and LATS1/2 have been found to be responsible for the phosphorylation of a divergent scope of cellular targets-substrates, such as histone H2B or transcription factors FOXO1 and FOXO3 as well as the LATS1/2-related kinases Ndr1/Ndr2. It occurs mostly as a response to apoptosis inducing stimuli. Auto-phosphorylation of this MST1/2 fragment on Ser183 is required for its full catalytic activity and nuclear translocation, where its natural substrate, histone H2B, becomes the target of phosphorylation as well as histone H2AX, which become phosphorylated at Ser-139. This kind of action promotes DNA degradation in the stress-induced apoptosis, controlled by the Hippo pathway components and is followed by inactivation of YAP through its phosphorylation in LATS1/2 - independent manner. Processes of activation of different components of the pathway have been marked using arrow headlines (➡). Involvement of specific phosphorylation has been emphasized with ATP/ADP sign on the line. Processes of inhibition or deactivation have been marked using blunt lines (▬). The precise explanation can be found in the text of the article.

inhibition of apoptosis respectively, and, in consequence, the overall amplified cell proliferation (4, 6, 103). In all metazoans the Hippo pathway displays strongly conservative features, so it was not a surprise that transgenic mice experiments and directed mutagenesis of the components of the mammalian Hippo pathway revealed a lot of similarities with *Drosophila's*, manifested with tissue overgrowth phenotypes (104). The mammalian liver growth ratio was shown to be dependent on YAP activity and loss of function mutation of one or both of YAP alleles abolished liver enlargement. The effect of organ enlargement was achieved due to the increased cell number, not the cell size. This results was confirmed with the observation of elevated Ki-67 index and increased PCNA level in the liver tissue. Moreover, hepatocytes with overexpressed YAP-1 were unresponsive to Fas-mediated apoptosis. The same was true for *Mst1* and *Mst2* genes, mammalian Hippo orthologs. Their activity loss was sufficient to initiate hepatocyte proliferation and, in consequence, liver overgrowth as well as hepatocytes resistance to pro-apoptotic stimuli, leading to the development of hepatocellular carcinoma. The mechanism standing behind this phenomenon was the lack of inhibitory Ser127 phosphorylation of the Yorkie ortholog, Yap-1 due to inactivate M1/2, resulting in the absence of MATS (Mob1 as tumour Suppressor) phosphorylation, which, in turn, prevented autophosphorylation of LATS1/2 (105, 106).

The mammalian target of rapamycin serine/threonine protein kinase (mTOR), belonging to the superfamily of phosphatidylinositol 3-kinase (PI3K)-kinase-related kinase (PIKK), with its two distinct complexes TORC1 and TORC2, has been shown to act, through the integration of many intracellular signalling pathway, as a sensor of vital cellular signals. Apart from the energy related stimuli like level of amino acids and glucose, it is also capable of sensing growth-regulating signals like hormones, growth factors and responding with activation to different cellular stress signals, especially to heat shock, hypoxia, DNA damage, as well as osmotic and oxidative stress (107). Each of the multi-protein mTOR complexes mTORC1 and mTORC2, possesses a diverse structure and is responsible for the regulation of distinct cellular processes.

mTORC1 is more sensitive to inhibitory action of rapamycin, which, through engagement of FK506 binding protein 12kDa (FKBP12), is capable of potent mTOR deactivation. mTORC1, which might be described as a principal regulator of cell growth and metabolism, is composed of five elements (proline-rich AKT substrate 40 kDa (PRAS40); mammalian lethal with Sec13 protein 8 (mLST8); regulatory-associated protein of mTOR (Raptor) and DEP-domain-containing mTOR-interacting protein (Deptor)) and oversees processes like: 1) protein synthesis by phosphorylation of eIF4E-binding protein 1 and the p70 ribosomal S6 kinase 1; 2) lipid synthesis through the up-regulation of sterol regulatory element binding protein 1 (SREBP1) and peroxisome proliferator-activated receptor-γ (PPARγ); 3) response to pro-inflammatory as well as WNT-originated stimuli *via* inactivation of TSC1 or TSC2, leading as a consequence to mTORC1 activation (108-112). The control over such a number of significant cellular processes makes mTORC1 one of the most important factors in managing cell cycle, cell growth and cell division.

As presented by Sarbassov *et al*. (113), mTORC2 is absolutely indispensable for the full activation of AKT, which requires phosphorylation at Ser308 by phosphoinositide-dependent kinase 1 (PDK1) and simultaneous phosphorylation of Ser473 by mTORC2. This allows us to place mTORC2 among the supervisors of cell survival and proliferation (113).

Interaction of Hippo and mTOR has been confirmed and is associated with the development of meningioma and schwannoma, where deletion or loss of function mutation in *NF2* gene resulted in the activation of mTORC1, followed by an unrestrained cell growth and divisions. The same happened to be true in case of malignant mesothelioma cell lines (114, 115). But maybe the most prominent connection between these two regulatory pathways is disclosed through the interaction between MST1/2 and mTORC1 and mTORC2. This connection has been revealed by Zhou *et al.* (106) showing that any loss of *MST1* or *MST2* gene results in an immediate increase of mTORC1 activity, but does not alter mTORC2 or the Ras-MAPK pathway. Another member of the 'core kinases' of the Hippo pathway - LATS2, in the YAP independent manner, was found to conjoin with pRB in silencing the E2F controlled genes, which was followed by the cell cycle arrest (116). These facts indicate the 'entanglement' of both pathways, Hippo and mTOR, in the monitoring of cell growth and organ size.

HIPPO AND CELL SIGNALLING

All information presented above clearly indicates the involvement of the Hippo pathway in the regulation of cellular processes in response to more or less intra cellular signals. But can external stimuli reaching through cell surface receptors directly activate Hippo signalling? Surprisingly, it has been found that neither insulin nor EGF, well-known potent stimulators of cell division, had a significant influence on the activity of Hippo manifested by the YAP/TAZ phosphorylation status (25). However, Chen *et al*. (117) have demonstrated experimental data indicating that in diabetic mouse renal proximal tubule epithelial cells EGF receptor knock-down or application of erlotinib, a specific inhibitor of EGFR tyrosine kinase, were sufficient to increase *YAP* gene expression and phosphorylation of its product simultaneously. But, on the other hand, the authors reported that activation of an EGFR-PI3K-Akt-CREB signalling pathway has resulted in the elevated ration of YAP nuclear translocation and activation of TEAD-dependent genes expression and tissue overgrowth. siRNA silencing of the *YAP* gene restored tissue equilibrium, even in response to high glucose conditions indicating functional association between Hippo and EGFR-PI3K-Akt-CREB axis (117). Yu *et al.* (118) have presented the results pointing out that a large extent of surface G-protein-coupled receptors are capable of influencing the activity of the Hippo pathway components. They have employed transient transfection technology and demonstrated that purinergic receptor 1 (P2YR1) and the platelet-activating factor receptor (PTAFR) promoted YAP/TAZ de-phosphorylation, and, at the same time, endothelin receptor type A, dopamine receptor D1 and glucagon receptor induced YAP phosphorylation (118). Additionally, the data presented by Yu *et al*. (118) and the Miller's group confirmed that lysophosphatidic acid (LPA) and sphingosine-1-phosphate (S1P), being the most plentiful phospholipids represented in the serum, binding to their membrane receptors belonging to the surface G-protein-coupled receptor family, may activate YAP/TAZ (119). Mo *et al*. (120) demonstrated diminished phosphorylation and enhanced nuclear translocation of YAP/TAZ after the stimulation of a protease-activated receptor 1 (PAR1) due to the inhibition of LAT1/2. Protease-activated receptors become irreversibly active under proteolytic cleavage of its N-terminus by thrombin - serine protease (120). Expression of thrombin as well as that of PAR1 have been found to be substantially up-regulated in invasive cancer cells. But, at the same time, thrombin as a physiological stimulus for the wound healing process is released in extensive amount at the sites of tissue injury (121, 122).

This data strongly supports a possible mechanism, in which agonists of G-protein-coupled receptors linked with Gα12/13,

Gαq/11 and Gαi/o proteins act like LATS1/2 repressors, activating Rho GTPases, which results in the inhibition of LATS1/2. On the other hand, in response to their ligands, Gαs-coupled receptors, engaging cAMP-PKA pathway, indirectly induce LATS1/2 leading to YAP/TAZ inactivation. This hypothesis finds the support in the data showing that LATS might be directly phosphorylated by cAMP dependent PKA at (R/K)(R/K)xS/T motifs in response to stimuli like high cell density or disruption of cytoskeleton structures. This kind of LATS activation is followed by YAP phosphorylation at Ser381 and its subsequent degradation at proteasome (123, 124). As it was shown above, RASSF1A is a direct activator of the MST/Hippo pathway. Donninger *et al*. (125) and others confirmed that KRAS proteins bind to RASSF1A. What was interesting only KRAS isoform, not HRAS or NRAS, was able to form the complex (125-127). Findings of Romano *et al*. (127) demonstrated selective KRAS/RASSF1A/MST2 interaction and complex formation dependent on the tyrosine kinases, connected to the receptors. It has been showed that EGF dependent activation of wild type KRAS promotes the interaction of RASSF1A and MST2 without activation of LATS1 (127). Property of RAF1 as a direct regulator of MST/Hippo proteins has pointed out to its possible crosstalk with the MAPK pathway. Rauch *et al*. (128) revealed that only ARAF binds to MST2 preventing its activation. This result is not surprising since both RAF family members use the same region between amino acids 151 and 303 of RAF1 to form the complex with MST2. And this region happens to be the most divergent among the RAF isoforms, what might be the reason for the lack of interaction between MST2 and BRAF (129). Although the wild form of the BRAF does not exhibit the ability to form the complex with MST, the most common oncogenic mutant of BRAF - BRAFV600E successfully forms the complex with C-terminal of MST1 inhibiting its kinase function in papillary thyroid carcinoma (130). This result confirming that BRAFV600E is necessary for inhibition of the pro-apoptotic MST/Hippo pathway lend credence to the idea that both pathways are part of the same signalling network. Another proof for the MST/Hippo and MAPK pathways interactions mediated at different levels is the fact that MST2-RAF1 complexes effectively prevent RAF-1 from the binding to MEK. Moreover, RAF-1 being complexed with MST2 was shown to be phosphorylated on Ser259. But for successful activation of ERK pathway RAF1 cannot remain phosphorylated on Ser259. Dephosphorylation of this residue is a prerequisite for physiological RAF1 phosphorylation on Ser338. Being a Ser259 kinase LATS1 is playing a pivotal role in regulation of both the MST2 and ERK pathways (131).

Phosphorylation of MST1/2 by AKT, the main downstream effector of phosphoinositide 3-kinase (PI3K), reveals connections between these two pathways. It has been shown that phosphorylation of MST2 on at least two residues, Thr117 and Thr384 dispersed between its C- and N-terminus, by AKT led to downregulation of its pro-apoptotic activity and this downregulation is not due to the formation of complex with RAF1 (99, 132). Additionally, AKT dependent phosphorylation of MST2 was shown to be promoted by KRAS , leading in consequence to the inhibition of the pro-apoptotic signal mediated by the MST/Hippo pathway (133). On the other hand, Cinar *et al*. (134) demonstrated that AKT can be directly phosphorylated by MST, which results in its inhibition and, in consequence, prevention of the pro-survival signal mediated by this protein (134). Another level of PI3K/MST/Hippo pathways entanglement seems to involve YAP. Basu *et al*. (79) showed that AKT can phosphorylate YAP at Ser127 promoting its association with 14-3-3 and its consequent cytoplasmic sequestration. YAP in YAP/14-3-3 complex is unable to act as a coactivator of p73, attenuating p73 induced cell death (79).

Recently, Iorns *et al*. (135) have postulated that LIFR may act as a tumour suppressor based on the information that its overexpression in breast cancer cells abolished tumour growth while silencing the LIFR gene resulted in a following transformation of normal mammary cells. This thesis has found the support in the paper of Chen *et al*. (136), who proposed a plausible mechanism of involving increased phosphorylation of YAP upon LIF administration. Taken together with the fact that an elevated phosphorylation ratio of both Hippo core kinases - MST/LATS was described in the cells overexpressing LIFR, this might suggest a real functional connexion of LIFR signalling with the canonical Hippo pathway (136).

TO WHAT EXTENT THE HIPPO PATHWAY MIGHT BE INVOLVED IN CARCINOGENESIS?

Based on the analysis of the results of many clinical trials, the last few years have proven that the employment of a targeted therapy for cancer treatment results in patients' better response and changing of profile of the adverse side effects of treatment due to an expected higher molecular specificity of its action. Targeted therapies focus on the specific molecular targets known to be associated with the cancer growth or spread, in contrast to standard anticancer drugs that act on all rapidly dividing cells, without differentiating them as normal or cancerous. Several different strategies of targeted therapies have been developed and the therapeutic approach stands out among them. We can divide them, depending on the molecular targets, into groups of: inhibitors of signal transduction pathway elements, modulators of the gene expression, inducers of apoptosis, inhibitors of angiogenesis, immunotherapy modulators, and monoclonal antibodies delivering toxic molecules. Year after year, the variety of substances used has been expanded, covering, at this point, a wide range of specific antibodies as well as small molecule inhibitors of enzymes involved in the post-receptor signal transduction in the selected signalling cascades, known to be altered in tumour cells. These alterations might be due to genetic or somatic mutations converting products of the defective genes into inactive as well as hyperactive state. This might manifest as a total or partial loss of function of the protein or its uncontrolled activity independent on the presence or absence of its inducer. It has been shown that deregulation of certain elements of the Hippo signalling pathway may be demonstrated in several types of cancer.

For several years some abnormality found in the Hippo pathway components has been reported in most human cancers. The most frequent observation, based generally on an immunohistochemical analysis of cancer tissue, was nuclear translocation of the YAP protein. Its increased nuclear localization, comparing to normal tissue, has been reported in ovarian and colorectal cancers as well as non-small cell lung cancer s and hepatocellular carcinomas (137-141). Since Hippo core kinases down-regulate YAP/TAZ nuclear translocation by its phosphorylation, the phenomenon of exclusive YAP localization within cancer cell nuclei might point to some disorder upstream from YAP in the Hippo pathway, resulting in the lack of inhibitory phosphorylation of YAP. But we are still missing data to draw a brighter image of the Hippo signalling disturbances in cancer. A direct connection between the Hippo pathway components and the development of human cancers remain unclear. But at least one human cancer has been undoubtedly correlated with the defective, belonging to the Hippo pathway, gene encoding Merlin/NF2. Type 2 neurofibromatosis is an autosomal dominant syndrome with inherited mutation in *NF2* gene, which manifests as benign schwannomas, meningiomas or neuromas (142, 143). Although

acquired somatic mutations or amplifications have been reported in case of *NF2*, *YAP1*, as well as in a transmembrane cadherin-like receptor, product of *DCHS* gene has been reported in meningiomas, mesotheliomas or colorectal cancer. Moreover, recent data suggests that in some human cancers *MST1/2* or *LATS1/2* genes are more likely inactivated by epigenetic silencing rather than by mutational mechanism (144-148). At the same time, there is a growing number of evidence of the abnormalities in the control of genes encoding the Hippo pathway components, affecting, in many ways, the functioning of the entire pathway in different human cancers. For example, *MST1/2* as well as *LATS 1* expression levels have been found significantly reduced in tumours with lymph node metastasis, especially in gastric cancer and gene amplification followed with over-expression of YAP was often observed in hepatocellular carcinoma (8, 100). Moreover, mutation in *AV1* gene was identified in colon and renal cancer cell lines and mutations in *FAT4* gene were described in more than 5% of patients suffering from gastric cancer (149, 150).

The Hippo pathway, acting as a signal integrator of many signalling pathways in the cell, might also be involved in the development of human cancers in the indirect but influential manner due to the known multiple cross-talk points with other important cellular signalling pathways like WNT, Hedgehog, Notch or mTOR.

Based on the description provided above, taking into account the nature of enzymatic activity of its components, the Hippo signalling pathway might be divided into two general sections: 1) with tumour suppressor activity composed of a highly conserved group of serine/threonine kinases and their regulators; 2) negatively regulated by the upstream components oncogenes, being transcription co-activators with not-known catalytic activity what so ever. Since the downstream elements of the pathway might be recognized as potentially ‘dangerous’ and involved in tumourigenesis, its inhibition seems to be the major task of anticancer action. But the lack of catalytic activity of YAP/TAZ might be a serious impediment in case of designing the targeted drug. Considering this fact, repression of protein-protein interactions seems to be the most promising direction with regards to the attempts to develop effective inhibition methods of the Hippo pathway down-stream elements in cancer cells.

The strategy to interfere with the YAP/TAZ during formation transcriptional complexes with TEAD or p73 in case of tumour formation, development or metastasis has a strong confirmation in the clinical observations. Frequent for the malignant transformation deregulation of YAP/TAZ, followed by the deposition of these proteins in nucleus, results in epithelial to mesenchymal transition - which is enhanced with cells proliferation, resistance to pro-apoptotic stimuli, one of prerequisites for tumourigenesis, leading in consequence to abolishment of senescence.

Recent publications suggest that this approach to destabilizing YAP/TAZ-TEAD complexes resulted in tangible effects. The first observation reporting possible clinical benefits of YAP/TAZ complexes destabilization was made by Liu-Chittenden *et al*. (151) in 2012. The authors have confirmed that administration of verteporin, benzoporphyrin derivative, diminished liver overgrowth caused by YAP over-expression or by NF2 knockout, without obvious adverse effects in other organs (152). An unique new feature of verteporfin, a photosensitizer commonly use in photodynamic therapy of neovascular macular degeneration was revealed. The authors have noticed that verteporfin in a dose dependent manner reduced proliferation and viability of human retinoblastoma cells, even without activation by light. They established that this phenomenon was due to the disruption of YAP-TEAD complex and subsequent substantial down-regulation of the YAP-TEAD transcriptionally regulated proto-oncogenes c-myc, Axl, known to be controlled by the Hippo pathway (152).

Lately, Sorrentino *et al*. (153) have published results which seem to authenticate the earlier concept that dysregulation of the mevalonate metabolic pathway might be involved in the anchorage-independent growth and tumourigenesis progression. In the series of experiments exploiting several specific inhibitors of the elements of the mevalonate metabolic pathway, (for example statins), like 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA reductase) as well as geranylgeranyl transferase, the authors have proven that the increased levels of mevalonic acid in the tumour cells promoted YAP/TAZ activation and this process was independent of LATS1/2 kinases (153). This, in part, corroborated the supposition made by Oku *et al*. that statins combined with dasatinib (Bcr-Abl tyrosine kinase inhibitor and Src family tyrosine kinase inhibitor) or pazopanib (receptor tyrosine kinase inhibitor of vascular endothelial growth factor (VEGFR), platelet-derived growth factor (PDGFR) and stem cell factor receptor (c-KIT) decreased nuclear localization of YAP/TAZ and promoted the proteasomal degradation of the entire complex in breast cancer cells, efficiently reducing their viability. The mechanism proposed by the authors combined inhibitory action of statins on the HMG-CoA reductase, which, in consequence, led to the impairment of geranylgeranylation of Rho-A protein with the known influence of dasatinib on the dynamics of actin cytoskeleton and observed restoration of YAP phosphorylation as a result of direct inhibition of protein geranylgeranylation (154, 155). The facts presented above militate in favour of the attempts made to pharmacologically destabilize the complexes of YAP/TAZ as a potential new approach in cancer treatment.

HIPPO IN CASE OF HUMAN CANCERS

A dysfunction of the Hippo pathway occurs very often in human malignant neoplasms and is associated with a poor prognosis. The neurofibromatosis tumour suppressor *NF2* (*Mer*) is the best known human tumour suppressor gene in the Hippo signalling pathway. Mutations found in *NF2* are characteristic in such tumours like schwannomas, meningiomas, ependymomas, astrocytomas, neurofibromas. *NF2* mutations are detected as frequently as in 1 for 25 000 people. Half of these mutations were described as spontaneous somatic, but the rest of them might be classified as genetic, inherited in the germline (150). *NF2* gene mutation is only one of many others observed in different cancer disturbances of the Hippo pathway components. Here, we would like to make a brief overview of the changes observed in the Hippo pathway components in case of most common human cancers.

HEPATOCELLULAR CARCINOMA (HCC)

Hepatocellular carcinoma is the fifth most common malignancy worldwide with a fatal prognosis and chemo resistance. One of the reasons of its resistance to anticancer drugs might be related to the Hippo signalling pathway abnormality. According to Huo *et al*. (156) over-expression of YAP resulted in resistance against doxorubicin-induced apoptosis in hepatocellular carcinoma cell lines. But suppression of the endogenous YAP expression employing RNA interference techniques leads to overcoming this resistance.

Markers of Hippo pathway activity such as MST1/2 and nuclear YAP concurrently constitute prognostic markers related to more aggressive tumour-phenotype in HCC. Immunoreactivity

of MST1/2 and YAP is associated with higher tumour grading, positive lymph node status, older age at diagnosis in HCC. Moreover, MST1/2 is detected in HCC and not in the other liver lesions. Immunoreactivity of MST1/2 is strongly associated with shorter survival. In turn, immunopositivity of nuclear YAP correlates with higher Ki67 indices and a trend to lower rates of apoptosis. Positive immunoreactivity for nuclear YAP and negative for phospho TAZ prove HCC diagnosis and - in second order - hepatocellular adenoma (157).

Both YAP and mitogen-activated protein kinase kinase 1 (MAP2K1/MEK 1) act as downstream elements of the Hippo signalling pathway. The interaction between YAP and MEK1 is indispensable for hepatocarcinogenesis *in vitro* and *in vivo* (158). The over-expression of YAP is an early event in liver tumourigenesis and is pivotal for the invasion of carcinogen-initiated hepatocytes and oval cells (159). The expression of YAP is significantly positively correlated with Edmondson symptom assessment system, serum AFP level, and HCC prognosis (160). Amphiregulin (AREG) is a member of the epidermal growth factor family, and a direct target gene of YAP/TAZ. The abundance of serum AREG is higher than serum AFP level in patients with HCC and is related to Edmondson symptom assessment system (160).

The over-expression of TAZ was also found in HCC (161, 162). Up-regulation of TAZ correlates with a lower overall survival rate of HCC patients after hepatic resection (162). On the other hand, TAZ knockdown decreases growth of hepatocarcinoma cells, but unfortunately, it induces the compensatory expression of YAP. TAZ knockdown with 5-fluorouracil treatment significantly increases chemoresistance of HCC (161).

YAP-TEAD complex is a key factor for the YAP-driven oncogenic activity. Due to this reason disruption of the YAP-TEAD interaction (for example using a small molecule verteporfin) might be very important and promising target against tumourigenesis (159, 163).

YAP/TAZ regulates amino acid metabolism by upregulating expression of the amino acid transporters SLC38A1 and SLC7A5. Furthermore, high expression of SLC38A1 and SLC7A5 is significantly related to a worse prognosis in HCC. Inhibition of the transporters and mTORC1, cell growth and cell proliferation regulators leads to diminishing of YAP1/TAZ - mediated tumourigenesis in the liver. It makes SLC38A1 and SLC7A5 transporters as well as mTORC1 also the potential therapeutic targets (164).

β1-integrin family consists of four members (α1 β1, α2 β1, α10 β1, α11 β1) and play a role of collagen receptors. The integrin α2 β1 modulates the Hippo core kinases and activates YAP. Extracellular matrix regulates cellular processes such as division and migration. Binding of α2 β1 integrin to extracellular matrix collagen IV inhibits the function of MST1 and LATS1 and activates the transcriptional activity of YAP. MST1 binding to the cytoplasmic tail of integrin α2 β1 is a downstream target of this integrin. Focal adhesion kinase (FAK) participates in the integrin-extracellular matrix mediated signalling pathway. FAK activation promotes integrin α2 - mediated activation of the Hippo pathway in HHC cells. ITGA2 (integrin α2 gene) expression correlates with HCC progression and co-expression with YAP targeted genes (e.g. AXL receptor tyrosine kinase) and is associated with shorter overall survival of patients with HCC (165).

LATS2 rs 7317471 C>T polymorphism correlates with a lower risk of death in HCC patients, especially in those who are younger (below 53 years), females, smokers, drinkers. The association is also strongly showed in untreated patients (no prior chemotherapy or transcatheter hepatic arterial chemoembolization) and with tumour stage B according to Barcelona Clinic Liver Cancer. Thus, LATS2 rs 7317471 could be a potential predictor of HCC prognosis (166).

Proteins belonging to the angiomotin family (Amot) might cooperate with YAP to either stimulate or inhibit its activity. Experiments employing biochemical analysis of the interaction nature of Amot-YAP complexes have demonstrated that one of splicing isoforms of Amot (Amot-p130)-p130 and YAP are able to interact in both the nuclear or cytoplasmic pool of hepatic epithelial cell. Amot-p130 is associated with the transcriptionally competent YAP-TEADs complex in the nucleus. Amot-p 130 is proposed to be related to tumourigenesis through its proven participation in the regulation of a wide spectrum of YAP target genes. In the cytoplasm, Amot-p130 blocks access to the WW domains in such a way that kinase LATS1 has no contact with dependent prevention of YAP phosphorylation, resulting in Amot-p130 (167).

Malat1 (metastasis-associated lung adenocarcinoma trascript1) has been shown to be also over-expressed in liver cancer. Malat1 increases cell migration or proliferation. It has been shown that YAP up-regulates Malat1 expression at both transcriptional and post-transcriptional levels. Serine/ arginine-rich splicing factor 1 (SRSF1) down-regulates Malat1 expression. SRSF1 inhibits YAP activity by preventing its co-occupation with TCF/β-catenin on the Malat1 promoter. YAP over-expression impairs the nuclear retention of SRSF1 and its interaction with angiomotin. A higher level of YAP expression is consistent with a lower SRSF1 nuclear accumulation in human liver cancer tissues. As shown, YAP over-expression with a knockdown of SRSF1 results in enhanced tumour growth and loss of body weight in tail vein-injected mouse models (168).

Concluding, the inactivation of the Hippo pathway leads to TAZ/YAP over-expression, and this might be correlated with a shorter survival of patients with HCC (169).

OVARIAN CANCER

Ovarian cancer is a major cause of death in case of gynaecological cancer patients. Surgery and platinum-based chemotherapy is the standard treatment but its efficiency is still insufficient and unsatisfactory.

A high level of YAP expression strongly correlated with TEAD4 gene expression has been found to be associated with a poor overall survival in ovarian cancer patients. Mutations of all five inhibitory phosphorylation sites of YAP promotes its hyper-activation, which results in increased ovarian cancer cell proliferation, migration and resistance to chemotherapeutic drugs. According to Xia *et al*. (170, 171) YAP initiates ovarian cancer cell growth and tumourigenesis. On the other hand, activation of YAP has been associated with a longer overall survival in ovarian cancer. The YAP expression signature has positive correlation with benefit from taxane-based adjuvant chemotherapy (172).

Lysophosphatidic acid (LPA) is one of the most abundant serum phospholipid which was found to influence tumourigenesis and metastasis of ovarian cancer through activation of G protein-coupled receptors. MEK-ERK pathway in R182 human epithelial ovarian cancer cells has an important role in LPA-induced cell migration and mRNA expression of TAZ protein. Interfering RNA-mediated inhibition of TAZ expression and attenuated LPA-stimulated migration of R182 human epithelial ovarian cancer cells have been reported and linked together. Briefly, TAZ influences the ovarian cancer development through LPA-stimulated migration of ovarian cancer cells (173).

MiR-129-5p directly inhibits YAP and TAZ expression and, consequently, leads to the inactivation of the TEA domain (TEAD) transcription factor, leading to the downregulation of

the Hippo dependent downstream genes. The down-regulation of miR-129-5p in ovarian cancer is associated with a shorter survival because miR-129-5p represses ovarian cancer cell proliferation and survival (174).

Ann over-expression of YAP leads to carcinogenesis in ovarian epithelial cells and induces growth of cancer cells *in vivo* and *in vitro*. As shown, YAP stimulates the expression of epidermal growth factor (EGF) receptors (EGFR, ERBB3) and production of EGF-like ligands (HBEGF, NRG1 and NRG2). HBEGF or NRG1 subsequently induces YAP and stimulates cancer cell growth. Taken all together, YAP interacts with the ERBB signalling pathways to regulate the initiation and progression of ovarian cancer (175).

Hypoxia is a vital micro-environmental factor that induces carcinogenesis. Hypoxic conditions (1% O2 or hypoxia mimics) decrease the phosphorylation ratio of YAP, but increase the total YAP expression in epithelial ovarian cancer cell lines and upregulate phosphorylated TAZ. LATS1 and Akt - TAZ kinases do not interact with TAZ during hypoxic conditions, which suggests that there is, still obscured, a regulatory mechanism of TAZ and YAP in cancer cells standing behind the hypoxic conditions (176).

The Hippo signalling pathway has a critical role in the initiation and progression of fallopian tube and ovarian high-grade serous carcinoma. An over-expression of YAP occurs in inflammatory and cancerous fallopian tube tissues and promotes cell proliferation, migration, colony formation and tumourigenesis. The Hippo and the fibroblast growth factor (FGF) signalling pathways induce an autocrine/paracrine-positive feedback loop to drive the progression of the Fallopian tube secretory epithelial cells-derived ovarian high-grade serous carcinoma (177).

GRANULOSA CELL TUMOUR

According to the medical statistics 5 – 8% of all diagnosed ovarian cancers are associated with ovarian granulosa cell tumours. This kind of tumours is characterized by an enlargement of the ovary and a tendency for late recurrence even many decades after the radical treatment. They have a potential for malignancy and, in consequence, metastases.

Granulosa cell tumours show higher levels of YAP expression in comparison with age-matched normal ovaries. YAP regulates cell proliferation, migration and steroidogenesis in this kind of ovary tumours (178).

GASTRIC CANCER

The Hippo signalling pathway is known to have been associated with the development, progression and metastasis of human gastric cancer.

Zhou *et al*. (179) have demonstrated that the expression of the genes encoding Hippo members on the mRNA level, - such as: *MST1, LATS1, Oct4, YAP1, TAZ, TEAD1* and *CDX2* has shown a significant correlation with tumour staging and metastases to the lymph nodes in gastric cancer. Moreover, protein expression of MST1, LATS1, YAP1, TAZ, TEAD1 and CDX2 has a close correlation between each of them.

An elevated nuclear accumulation of the YAP protein was detected in one quarter of gastric cancer cases. Patients with gastric cancer and with YAP positive nuclear accumulation tumours have significantly shorter overall survival time than those observed in the accumulation-negative group. The nuclear accumulation of YAP is an independent negative prognostic factor, especially for patients with intestinal-type gastric cancer (180).

3,3-diindolylmethane (DIM) has antineoplasm effects in both *in vivo* and *in vitro* tumour models. The analysis of the cytotoxicity of DIM in human gastric cancer cells lines: SNU-1 and SNU-484 shows that DIM inhibited the proliferation in the cultures of both cell lines and the effect was dose-dependent. Moreover, DIM reduces expression of CDK2, CDK4, CDK6 and cyclin D1 on the protein level. At the same time, DIM increases phosphoLATS1, Mob1, phosphoMob1, phosphoYAP and Ras association domain family 1 (RASSF1) protein levels and reduces YAP protein synthesis levels. DIM stimulates the binding and stabilizes the complex of RASSF1 with the MST1/2-LATS1-Mob1 complex. It leads to the activation of the Hippo signalling resulting in YAP phosphorylation and consecutive degradation which is followed by the inhibition of gastric cancer cell proliferation (181).

According to Jiao *et al*. (182), VGLL4 (vestigial-like family member 4), transcriptional regulator inhibits activity of YAP through a direct binding with TEAD in gastric cancer cells. Peptides possessing function of VGLL4 could suppress malignant cell growth *in vitro* and *in vivo*.

β-catenin is a component of cell adhesion molecules complex which plays an important role in tumour development and progression. An over-expression of TAZ is directly correlated with an abnormal nuclear β-catenin localization in adenocarcinoma of the esophagogastric junction (AEG). The nuclear and membrane-bound β-catenin localization as well as TAZ nuclear translocation are significantly higher in adenocarcinoma esophagogastric junction and dysplasia compared to the normal mucosa. The over-expression of TAZ and abnormal β-catenin (nuclear and membranous) are related to shorter overall survival in patients with the esophagogastric junction. Targeting TAZ and β-catenin is a new therapeutic strategy for the treatment of AEG because abnormal expression of TAZ and TAZ as well as β-catenin (nuclear and membranous) are independent prognostic factors (183).

The other molecule involved in the Hippo signalling pathway is FAT atypical cadherin 4 (FAT4) (*Fig. 1*). Loss of FAT4 expression is significantly related to the invasiveness in gastric cancer. A correlation has been found between the loss of FAT4 expression and perineural invasion, high pathologic T stage, high tumour-node-metastasis stage, and shortened disease-free survival time (184).

Fujimoto *et al*. (185) have shown a connection between the protease-activated receptor 1 (PAR1) signalling pathway and the Hippo-YAP pathway in gastric cancer stem-like cells. PAR1 activation inhibits the Hippo-YAP pathway kinase LATS *via* Rho GTPase, which leads to enhanced nuclear localization of dephosphorylated YAP. The Hippo-YAP pathway correlates with epithelial mesenchymal transition, which is induced by PAR1 activation. PAR1 signalling contributes to the ability of multidrug resistance and carcinogenesis through interactions with the Hippo-YAP pathway signalling in gastric cancer stem-like cells.

The Zheng *et al*. Study (186) has presented the inhibitory effects of dobutamine on cell growth, migration - cell invasion and reduced abundance of phosphorylated YAP in the cytosol. Moreover, dobutamine enhances apoptosis of gastric adenocarcinoma cells, which suggests that dobutamine may be used in neoadjuvant therapy in gastric cancer.

COLORECTAL CANCER

Colon cancer is one of the most common cancers worldwide. Admittedly, nowadays overall survival in this disease has been prolonged, but it is still one of the leading causes of cancer-related deaths in the world. It is a well-known fact that the

expression levels of the Hippo pathway components are associated with colorectal cancer differentiation, and TNM stage, and that they could be employed as prognostic indicators (138). It has been demonstrated that YAP activation is a characteristic feature of tubular adenomas in familial adenomatous polyposis (inherited syndrome with multiple colorectal polyps) and necessary for the development of adenomatous polyposis coli-deficient adenomas (187).

A strong and diffuse YAP expression has been confirmed in the colon carcinoma cells, especially in the nuclei, in over half of the cases. There is a high correlation between YAP expression and pathological staging of colorectal cancer. It mean that YAP expression is stronger within a bigger tumour, involving regional lymph nodes metastases. In addition, YAP expression is significantly associated with the cyclin D1 activity. Colorectal cancer patients with presenting YAP-positive nuclear translocation and β-catenin-positive expression have shorter overall survival and progression-free survival. However, cytoplasmic manifestation of phosphorylated YAP (pYAP) is correlated with the inhibition of the cell proliferation and negatively correlates with nuclear phosphorylated extracellular signal-regulated kinase (pERK) localization in colorectal cancer (188). The expression of YAP and phosphorylated YAP might be an independent prognostic indicator of colorectal cancer (187-189).

Inactivated YAP in patients with stage IV colorectal cancer and wild-type KRAS correlates with longer progression-free survival after cetuximab monotherapy, comparing to colorectal cancer patients with activated YAP. In case of KRAS mutations colorectal patients treated with cetuximab monotherapy, progression-free survival is similar in activated and inactivated YAP patient groups (190). Furthermore, a positive feedback loop between activated YAP and increased EGFR/KRAS is associated with the colorectal cancer progression and resistance to epidermal growth factor receptor (EGFR) inhibitors. The expression level of YAP in wild-type KRAS colorectal cancer might be then used as a marker of cetuximab therapy effectiveness (191).

TEAD4, as a nuclear target of YAP, controls genes responsible for induction of cell adhesion and up-regulation of the epithelial-mesenchymal transition-related changes in colorectal cancer cells. Increased expression and nuclear localization of TEAD4 is related to metastasis and a poor prognosis in colorectal cancer. Thereby, elevated nuclear manifestation of TEAD4 might be used as a progression biomarker for colorectal cancer (192).

Both YAP/TAZ and Ki-67 are coactivators of liver metastases in colon cancer and a high level of YAP/TAZ correlates with the proliferation marker Ki-67. YAP/TAZ is the independent factor of shorter disease free survival and overall survival after 5Fu-based chemotherapy given due to residual liver metastases in colon cancer. Additionally, YAP/TAZ is linked with Cyclin E1/c- Myc and CREB signalling cascades in the regulation of 5Fu chemoresistance. YAP/TAZ plays an important role in the control of the proliferation/quiescence switch in liver metastases (193).

Single nucleotide polymorphisms in genes involved in the Hippo pathway influence the recurrence rate in stage II and III colon cancer treated with 5Fu-based adjuvant chemotherapy. In left-sided cancers a polymorphism located in MST1 rs 17420378 is related to the recurrence probability. Female patients with left-sided tumours with TAZ rs 3811715 CT or TT genotypes have lower 3-year recurrence rate than patients with a CC genotype. RASSF1A rs2236947 AA genotype has higher 3-year recurrence rate than patients with CA/CC genotypes. In turn, Rassf1a rs 2236947 is the key polymorphism associated with recurrence probability in right-sided colon cancer patients (194).

NON-SMALL CELL LUNG CANCER

High mortality is still, unfortunately, a characteristic of a lung cancer, especially in its advanced stages, with the 5-year survival lower than 15%. Alterations of the Hippo/YAP intracellular signalling pathway are responsible for tumourigenesis, metastasis and drug resistance in non-small cell lung cancer (195-199). Knockdown of TAZ or YAP decreases lung cancer cell migration and metastasis (197). Thyroid transcription factor 1 (TTF1), interacting with TAZ/YAP and TAZ/YAP transcriptional targets such as AREG, ERP (epiregulin), Cyr61, AXL, is involved in lung tumorigenesis. In turn, LKB1 and RASSF1A have been identified as the upstream regulators of the core Hippo pathway (197). Moreover, transforming growth factor-β (TGF-β) signalling pathway, which also influences development and the progression of lung cancer, interplays with the Hippo pathway (198). On the other hand, extracellular signal-regulated protein kinase ERK1/2 inhibitors decrease the YAP protein level and, consequently, inhibit metastases of non-small cell lung cancer (200).

Similar as in case of gastric cancer cells, an elevated level of VGLL4 expression correlates with significant inhibition of the growth of lung cancer cells *in vitro*. The mechanism of direct competition between VGLL4 and YAP for binding site in TEAD stand behind this observation. VGLL4, as an irreversible competitor abolish activity of YAP completely and that might be the explanation for this phenomenon (201).

High cytoplasmic YAP localization is correlated with the low pathological staging and histological grading for squamous cell lung cancer. In case of adenocarcinomas, a high level of nuclear YAP accumulation is related to an increased cyclin A expression and activity. Additionally, a high YAP expression might serve as a predictor of epidermal growth factor receptor gene amplification. The diverse effects of YAP expression in squamous cell carcinomas and adenocarcinomas suggest that YAP may play a crucial role of a molecular switch - in various pathways and in distinct tumour subtypes (202).

LATS1 expression is significantly lower in non-small cell lung cancer tissues than in normal lung tissues. The expression of LATS1 is strongly associated with pathological stage and lymph node metastasis. The loss of LATS1 expression correlates with a short overall survival in lung cancer. On the other hand, an over-expression of LATS1 inhibits cell proliferation (203). LATS1/2 are down-regulated in most cases of the non-small cell lung cancer (197).

The mechanism of primary resistance towards tyrosine kinase inhibitors of the receptor (EGFR-TKIs) involves various driver mutations present e.g. in the KRAS oncogene. Changes in signal transduction in EGFR mutant lung cancers make acquired resistance. YAP/ERK signalling contributes to more resistance to EGFR-TKI of lung cancer. One of the strategies to overcome resistance to EGFR-TKI is to modulate the alternative pathways. Increased nuclear YAP expression is observed in lung cancer after acquiring EGFR-TKI resistance (gefitinib resistance). YAP influences EGFR-TKI resistance through different mechanisms (e.g. by activating the receptor kinase tyrosine AXL). YAP inhibitor and EGFR-TKI could overcome the EGRF-TKI resistance in lung adenocarcinoma (204).

The T790M mutation of epithelial growth factor receptor (EGFR) is the main cause of the acquired resistance to EGFR tyrosine kinase inhibitors therapy for lung cancer. A high TAZ expression in lung cancer cells is an intrinsic mechanism of the T790M - it induced resistance in response to EGFR - tyrosine kinase inhibitors. Doubled EGFR and TAZ target could increase the efficacy of EGFR tyrosine kinase inhibitors in case of acquired resistance of lung cancer (205).

According to Dhanasekaran *et al*. (206), the Hippo pathway fusions, NRG1 (neuregulin1) fusion/outliner expression, NF1

(neuofibromatosis 1) mutations and c-MET exon skipping are responsible for approximately 16% for driver-unknown non-small cell lung cancers. High number of fusions (based on distribution percentiles over 18) correlated significantly with shorter median overall survival after adjusting for histological subtype, stage, age, gender, TP53, KRAS, EGFR mutation status and smoking status (206).

CLEAR CELL RENAL CANCER

Despite of the new targeted therapies, the results of metastatic clear cell renal cancer treatment have still remained unsatisfactory. The neurofibromin2 (NF2)-Hippo-Yap pathway is used as a therapeutic target for renal cell carcinoma patients who progressed under the treatment with vascular endothelial growth factor receptor (VEGFR) tyrosine kinase inhibitors (207).

One of the members of the Hippo pathway, SAV1, participates in the pathogenesis of high grade clear cell renal cancer. SAV1 has been shown to be down-regulated and related to the presence of YAP in the nucleus of clear cell renal cancer (208, 209). Nuclear extensive localization of YAP is one of molecular hallmarks of clear cell renal carcinoma cells. The expression of YAP protein in clear cell renal carcinoma tissues is strongly correlated with clinical stage and differentiation. In clear cell renal carcinoma, in which YAP is decreased, inhibition of cell proliferation was observed, cell cycle arrest at the G1 and increased apoptosis (210).

SAV1 downregulation promotes the proliferation of renal tubular epithelial cells through transcriptional activation of YAP1. Loss of Sav1 in renal tubules enhances the proliferation of renal tubular cells in mice and, at the same time, it does not alter the proliferation in the tubes (209). Sav1 depletion correlates with nuclear localization of YAP (209, 210). Sav1 deficiency in renal tubules cells leads to structural and cellular abnormalities such as large, irregular nuclei and the formation of renal cysts. In turn, nuclear atypia and high cellularity are observed in the tubular cysts. Therefore, Sav1 is absolutely necessary for the growth, nuclear size and normal architecture of the renal tubules *via* activation of the Hippo pathway (209).

Abrogated YAP activity by shRNA-mediated knockdown leads to inhibition of the proliferation and migration of clear cell renal cancer cells *in vitro*. Knockdown of YAP decreases tumour growth both *in vitro* and *in vivo*. YAP knockdown shows downregulation of CYR61, c-Myc, endothelin 1, endothelin 2 in clear cell renal cancer cells. Therefore, CYR61, c-Myc and the endothelin axis signalling are actual downstream effectors of YAP (210).

The expression of LATS1 is markedly reduced in renal cell carcinoma cells in comparison to the normal kidney tissues. Moreover, the expression of LATS1 is related to pathological grade and clinical stage of renal cell carcinoma. LATS1 demethylation, as well as over-expression of LATS1 down-regulates the expression of YAP, inhibits cell proliferation, induces cell apoptosis and cell cycle G1 arrest. LATS1 can be used as early diagnosis biomarker and target for therapy of renal cell carcinoma (212).

MESOTHELIOMA

Malignant mesothelioma is a very aggressive malignant tumour which arises from serosal surfaces of the pleural, peritoneal and pericardial cavities. It is often diagnosed at advanced stage. Asbestos exposure plays the most important role in the development of this cancer. Furthermore, erionite, the simian virus 40 (SV40) and some genes such as LATS2, SAV and the Hippo signalling pathway are implicated in the tumourigenesis of malignant mesothelioma (213, 214). A lack of cytostatic anti-cancer drugs effectiveness in mesothelioma explains the turn to targeted therapies.

The tumour suppressor protein Merlin is encoded in the neurofibromatosis type 2 (*NF2*) gene. Under-phosphorylated Merlin regulates the Hippo pathway in mesothelioma cells. The Merlin-Hippo signalling pathway is frequently inactivated in mesotheliomas, which leads to YAP activation and, in consequence, elevated transcription rate of multiple genes promoting cancer (215). The purpose of new therapeutic strategies is the YAP activity abolishment *via* upstream signalling: activating stem signalling, thrombin/PAR 1 and lysophosphatidic acid/ lysophosphatidic acid receptor (216).

According to the Takana *et al*. findings (214), AJUBA, a binding partner of LATS2 negatively regulates YAP activity through the LATS family in mesothelioma cells. Inactivation of AJUBA is an important mechanism in mesothelioma cell proliferation. Additionally, chromosomal translocation between the LATS1 and presenilin-1 (PSEN1) genes is observed in malignant mesothelioma cell line. The LATS1-PSEN1 fusion gene causes a lack of ability to phosphorylate YAP and, in consequence, to inhibit the growth of a malignant mesothelioma cell line (217).

MELANOMA

Melanoma is a very aggressive tumour associated with a high susceptibility to metastasis. This tumour is characterized by high chemo- and radio-resistance. Melanocytes, melanoma cell lines and cells of melanocytic lesions express YAP and TAZ, which are suspected to be responsible for invasiveness and metastatic capacity of melanoma (218-220). An analysis of signal pathways in melanoma cell lines resistant to BRAF inhibitors shows that treatment with the histone deacetylases inhibitor of bromodomain and extra-terminal proteins inhibitor strongly downregulated the expression of anti-apoptotic proteins as well as the protein components in the AKT and Hippo pathways (220). Especially some polymorphic variants of the Hippo pathway genes, such as *YAP1* rs11225163 CC, *TEAD1* rs7944031 AG+GG and *TEAD4* rs1990330 CA + AA, have a potentially predictive role in the prognosis of cutaneous melanoma (221).

UVEAL MELANOMA

Uveal melanoma is the most common ocular malignant tumour in case of adults. Approximately 80% of all uveal melanomas have somatic activating mutations in GNAQ or GNA11 (encoding Gq or G11, respectively). Gq/11 mutants induce YAP, and the connection to the G protein-coupled receptor signalling pathway has been presented and therefore it might influence melanoma development (223, 224). Moreover, Gαq activates the YAP-dependent growth of uveal melanoma cells. And this is why YAP might be treated as a potential new therapeutic target in uveal melanoma treatment (225).

BRAIN TUMORS

Overexpression of TAZ is observed in high grades (III/IV) more than in low grade (I/II) of gliomas. It suggests that TAZ participates in tumourigenesis and progression of brain tumours. TAZ expression is restricted to cytoplasm in low grade brain tumours and it occurs in cytoplasm and nucleus in high grade gliomas. Moreover, TAZ expression in nucleus positively

correlates with tumour grade. Glioma cell growth is enhanced in TAZ overexpressed cells (226).

Frizzled-7 (FZD7) is a seven-pass transmembrane Wnt receptor and the most known among ten human Frizzled receptors. High level FZD7 expression in glioma positively correlates with advanced tumour stage and shorter overall survival. Additionally, the higher Ki-67proliferation index correlates with higher FZD7 expression. In turn, no significant association between FZD7 and age, gender, performance status, treatment and tumour differentiation was observed. High level of FZD7 induces glioma cell proliferation *via* upregulation of TAZ (227).

GLIOBLASTOMA MULTIFORME

Glioblastoma multiforme is recognised as one of the most aggressive and currently incurable brain tumours. Similar to melanoma, glioblastoma multiforme is a chemo- and radio-resistant malignant neoplasm. A cellular receptor CD44 - hyaluronian receptor is a cancer stem cell marker. It implicates diverse cellular functions. Depletion of CD44 blocks glioblastoma multiforme proliferation and leads to sensitization of tumour cells to cytotoxic chemotherapeutics. Moreover, CD44 functions upstream of the Hippo pathway and protects glioblastoma multiforme cells from reactive oxygen species and a cytotoxic agent-induced stress and apoptosis by decreasing the activity of the Hippo signalling pathway (228). CD44 antagonists (e.g. sh RNAs against CD44, hs CD44-Fc fusion proteins) seem to have a potential capacity as an anti-glioma agents with high efficacy of action, but it still requires further research.

Knocking down the expression of 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMGCR) - enzyme of the mevalonate pathway, inhibits the growth, migration and metastasis of glioblastoma cells. Moreover, HMGCR positively regulated the expression of TAZ protein, suggesting that HMGCR might activate the Hippo pathway in glioblastoma cells (229). Histone deacetylase 9 (HDAC9) is also over-expressed in glioblastomas and is related to a worse prognosis for the patient. Acceleration of the cell cycle in part by potentiating the EGFR signalling pathway is one of the HDAC9 functions. Furthermore, knockdown of HDAC9 decreases the expression of TAZ. HDAC9 promotes glioblastoma formation *via* TAZ-mediated EGFR pathway activation, which makes it a new potential target for the therapy of this kind of brain tumour (230).

Inhibition of miR-130b induces down-regulation of the YAP/TAZ and decreases expression of the Hippo signalling downstream genes like CTGF and the pluripotency markers such as CD133, SOX2, Nanog, MYC and BMI1. This cascade attenuates the promotion of glioblastoma stem cell phenotype (231). According to the Yuan *et al.* study, miR-125a-5p is an important regulator of TAZ in glioma cells by targeting the TAZ 3' UTR and, in this way, MiR-125a-5p inhibits the expression of TAZ target genes, cell proliferation and prompts the differentiation of glioblastoma cells (232).

Dysregulation of the Hippo and mTOR signalling pathways is vital in gliomagenesis, but relations between these pathways still remain unclear. AMOTL2 is phosphorylated at serine 760 by mTORC2. A mutation of AMOTL2 mimicking constitutive Ser 760 phosphorylation blocks its ability to bind and inhibit YAP, leading to enhanced expression of YAP gene targets. An over-expression of AMOTL2 or a non-phosphorylable AMOTL2-S760A mutant inhibits YAP-induced transcription, foci formation, growth, and metastatic properties, whereas an over-expression of a phosphomimetic AMOTL2-S760E mutant does not block the effects of AMOTL2 in glioblastoma cells *in vitro*. The drugs co-targeting mTORC2/AMOTL2/YAP may be useful as a therapeutic tool in treatment of gliomas (233).

BREAST CANCER

Breast cancer is the most frequent malignancy diagnosed in women all around the world. The Hippo signalling pathway is a key factor in mammary gland and breast cancer development (234). Hyper-activation of YAP in mammary epithelia leads to a defect in its terminal differentiation. Loss of YAP potently suppresses oncogene-induced mammary tumours. Thereby, YAP antagonists could be considered for the targeted therapies dedicated to breast cancer (235). Moreover, TAZ/YAP complex contributes to breast cancer tumourigenesis, which makes it a feasible therapeutic target (236, 237). Loss of TAZ in breast cancer stem cells severely impaired metastatic process and acquired chemoresistance. A high expression level of TAZ is associated with transformation and induces cell migration of breast cancer cells and it increases chemoresistance. Its over-expression contributes to a shorter disease-free survival, overall survival and higher probability of recurrence in breast cancer patients (237, 238). Moreover, TAZ is highly over-expressed in the cytoplasmic and nuclear compartments in triple negative breast cancer (239, 240). According to Diaz-Martin *et al.* (237), nuclear expression of TAZ correlates with the triple negative phenotype of breast cancer (60.5% TAZ-positive), the basal-like subtype (70.8% TAZ-positive) and metaplastic breast carcinomas (90% TAZ-positive). TEAD4 is expressed in breast cancer cell lines, especially in triple negative breast cancers cell lines. TEAD4 binds to *KLF5*, which is an oncogenic transcription factor in breast cancer. Over-expression of TEAD4 leads to proliferation *in vitro* and breast cancer growth in mice. Due to this reason, TEAD4 with KLF5 contribute to triple negative breast cancer cell proliferation and tumour growth (241).

Vestigial-like 1 (VGLL1) is a gene encoding a transcriptional co-activator structurally homologous to TAZ and YAP. The nuclear expression of VGLL1 is revealed in 13% of sporadic breast cancers, 17% of HER2-positive, over 40% of triple negative and over 50% of BRCA1-associated triple negative breast cancers. VGLL1 is observed in 0.7% of luminal A and 5.6% of luminal B breast cancers. VGLL1 expression correlates with a shorter overall survival (242).

TAZ and YAP expression have both predictive and prognostic significance in triple negative breast cancer patients treated with neoadjuvant chemotherapy. The expression of TAZ and YAP impact shorter disease-free survival in these group of patients. Expression of YAP in triple negative breast cancer cells and non-lymphatic stromal cells is predictive factor of decreased pathological complete response (pCR) on chemotherapy (243).

Paclitaxel is an anticancer drug widely used in advanced and metastatic breast cancer. TAZ, Cyr61 and CTGF - the Hippo pathway downstream transcriptional targets are responsible for paclitaxel resistance in breast cancer cells with mechanism still not elucidated (244). The TAZ-TAED-Cyr61/CTGF signalling pathway is a novel target for treatment development in paclitaxel-resistant breast cancer.

Breast cancer progression promotes the changes in the biophysical properties of the extracellular matrix, which contributes to the HER2-targeted kinase inhibitor lapatinib resistance. A downregulation of YAP and TAZ expression, by siRNA or activity with the small molecule YAP/TEAD inhibitor verteporfin, eliminates lapatinib resistance. Decreasing of YAP delays the growth of HER2-positive breast cancers and enhances sensitivity to lapatinib in mice (245).

The presence of oestrogen receptor (ER+) and progesterone receptor (PR+) in breast cancer correlates with better prognosis (longer disease free survival and overall survival). A positive hormonal status is a predictive factor for response to anti-hormonal therapies (e.g. tamoxifen, fulvestrant). In their study Tufail *et al.* (246) revealed that YAP expression was

significantly reduced in invasive breast cancer cells and high level of YAP expression was showed in normal breast tissue. A decreased expression of YAP in invasive breast cancer cells was significantly related to absence of oestrogen and progesterone receptors (ER–, PR–). These results suggest that YAP is a tumour suppressor in invasive breast cancer and it could be a molecular marker for negative hormonal breast cancers (246).

The Hippo signalling pathway influences bone metastases growth in breast cancer. TAZ and WW domain-containing oxido-redutase (Wwox) activate binding of hypoxia inducible factor-1 (HIF-1) to the promoter of E-cadherin (247). E-cadherin and Wwox are expressed in bone metastasis, but not in breast cancer. HIF-1α and TAZ are found predominantly in nuclei of metastasis and in all cell compartments of breast cancer. The peroxisome proliferator-activated receptor γ (PPARγ) through interruption of E-cadherin transactivation leads to the prevention of Wwox and TAZ functions. Methylation of Wwox and TAZ affects the Hippo pathway and, in consequence, it modifies metastatic phenotype (248). Nuclear translocation of HIF-1α regulated by Wwox under hypoxic conditions, through E-cadherin target gene, might play an important role in bone metastasis colonization (248). ABL kinases regulate tumour-bone interaction by regulating the crosstalk between tumour cells and the bone microenvironment and by increasing the Hippo pathway effector TAZ and the expression of TAZ-dependent target genes, which initiates bone metastasis. ABL kinases knockdown or treatment with ABL - a specific inhibitor impairs osteolytic metastasis of breast cancer cells in mice. These findings contribute to using ABL - specific inhibitors to limit breast cancer metastasis to bone (249).

Protein geranylgeranylation (GGylation) is an important biochemical process for cancer cell survival. The Hippo signalling pathway induces GGylation-dependent cell proliferation and migration in breast cancer cells. GGylation inhibition leads to phosphorylation of MST1/2 and LATS1, and respective inhibition YAP and TAZ activity as well as YAP/TAZ transcription. The YAP/TAZ complex is pivotal for GGylation-dependent cancer cell proliferation and migration (250). An increased transcriptional activity of YAP/TAZ is associated with glycolysis. In case of blocking glucose metabolism, protumourigenic function of YAP/TAZ is attenuated. The gene expression regulated by glucose metabolism in breast cancer cells is strongly associated with YAP/TAZ activation and with the cancer progression (251).

Angiomotin (Amot) is a protein that among other physiological processes also influences angiogenesis. This protein is presented in breast cancer cells, but not in the normal breast tissues. The expression level of Amot is associated with level of Ki-67. Amot knockdown in MCF-7 cells contributes to a significant decrease of the YAP, YAP/TAZ and LATS1 expression, leading in consequence to the diminution of cell proliferation and cancer invasiveness (252).

High Mobility Group A1 (HMGA1) is an architectural chromatin factor that induces neoplastic transformation and progression. Cyclin E2 (CCNE2) interplays with HMGA1 to regulate invasiveness of basal-like breast cancer cells by promoting the nuclear localization and activity of YAP. High levels of HMGA1 and CCNE2 expression correlate with the YAP/TAZ activity in breast cancer patients. Cyclin-dependent kinases inhibitors induce the translocation of YAP from the nucleus to the cytoplasm, what leads to a decrease in the activity of YAP dependent genes (253).

The epithelial-mesenchymal transition-inducing transcriptional repressor ZEB1 is a pivotal factor of metastasis and therapy resistance. It is also related to the aggressive types of many malignant tumours (e.g. breast cancer). As a result of a direct interaction between ZEB1 and YAP, ZEB1 changes its function that of a transcriptional co-activator of a ‘common ZEB1/YAP target gene set’, which is a predictor of poor survival, chemoresistance and increased metastatic risk in breast cancer (254).

In Yuan *et al.*’s study (255) over a half (63.4%) of infiltrating ductal breast cancers had loss of YAP expression. A correlation between YAP expression and prognostic factors such as tumor grade, ER , HER2 and lymph node status were not observed. Breast cancer cells with YAP suppression migrated further and in a wider range than control cells. YAP silencing protects cells death from anoikis and increases colony formation. Neither promoter methylation nor mutation was confirmed as possible mechanism for loss of YAP in breast cancer cells. Probably YAP requires loss of heterozygosity (LOH) to cause protein loss in breast cancer (255).

Increased nuclear TAZ/YAP overcomes TGF-β mediated tumour suppressive functions in early stage of cancer (e.g. inducing cytostatic signals). Concomitantly, TAZ/YAP coordinate tumorigenic transcriptional events in the nucleus by promoting the activity of TEAD-SMAD complex. TAZ/YAP and TGF-β induce the expression of NEGR1 (neuronal growth factor 1) and UCA1 (urothelial cancer associated 1). CTGF expression is associated with the presence TAZ/YAP, TEADs, TGF-β signalling (256).

PANCREATIC CANCER

Pancreatic cancer is one of the most lethal and one of the most chemo-resistant malignancies. In adult mouse pancreatic acinar cells are transformed in pancreatitis-induced acinar-to-metaplasia by oncogenic activation of KRAS. What leads to activation of the transcriptional regulators YAP1 and TAZ. A downstream effector of KRAS signalling *via* YAP1 and TAZ is the (Janus Kinase/ Signal Transducers and Activators of Transcription) JAK-STAT3 pathway. Both YAP1 and TAZ influence transcriptional activation of several in the JAK-STAT3 signalling pathway what induces development of pancreatic ductal adenocarcinoma (257).

Inhibition of miR-181c induces down-regulation of YAP/TAZ and decreased expression of the Hippo signalling genes CTGF, BIRC5 and BLC2L1. The consequence of this is survival shortening of pancreatic cancer cells, chemosensitivity to gemcitabine treatment *in vitro* and *in vivo* and remission. In turn, upregulation of miR-181c significantly correlates with more advanced primary tumours and poorer overall survival in pancreatic cancer patients (258).

The majority of pancreatic cancers and early pancreatic intraepithelial neoplasia have KRAS mutations. YAP is necessary for pancreatic intraepithelial neoplasia progression into pancreatic cancer. YAP induces the proliferation of KRAS mutant pancreatic ductal cells. Yap inactivation in Kras or Kras: Trp53 resulted in inhibition of pancreatic intraepithelial neoplasia progression *in vivo*. Oncogenic KRAS modulates the transcriptional activity of YAP through the MAPK pathway that is mediated by ERK and its downstream targets (259).

SARCOMAS

Sarcomas account about 1% of all malignancies in adults and 15% of all childhood neoplastic diseases (260). They are of mesenchymal origin.

Osteosarcoma is the most common primary bone sarcoma. High YAP1 protein expression is revealed in osteosarcoma in comparison with surrounding non-osteosarcoma tissues and it correlates with more advanced staging of tumour (261). Suppression of YAP decreased osteosarcoma growth. YAP

upregulation is modulated by SOX2 (the stem cell transcription factor). In murine osteosarcoma cell line, Sox2 inhibits the Hippo activators Kibra and Nf2 what leads to increased YAP (260). In turn, RASSF5 is downregulated in osteosarcoma and low expression positively correlates with metastasis (262).

Embryonal and alveolar rhabdomyosarcomas are paediatric soft-tissue sarcomas. High YAP1 expression occurs in embryonal than alveolar type. Moreover, high YAP1 activity is associated with higher stage and poorer prognosis in embryonal rhabdomyosarcoma but not in alveolar type. In turn, YAP suppression inhibits proliferation and promotes differentiation (263).

Expression of the paired box 3-forkhead box protein O1 (PAX3-FOXO1) fusion oncogene is feature of alveolar rhabdomyosarcoma. RASSF4 is a PAX3-FOXO1 transcriptional target gene. PAX3-FOXO1-positive alveolar rhabdomyosarcomas have significantly higher RASSF4 levels, what correlates with shorter overall survival. RASSF4 blocks phosphorylation of MST1 in alveolar rhabdomyosarcomas and it leads to inhibition the Hippo pathway. PAX3-FOXO1-RASSF4-MST1 pathway has pivotal role in alveolar rhabdomyosarcomas (264).

FURTHER PERSPECTIVES AND FINAL CONCLUSIONS

The involvement of more than five hundred interactions within over three hundred distinctive proteins, which create the core of protein-protein interaction ‘apparatus’ of the Hippo pathway, have been described so far. But the global amalgamation of the mammalian Hippo signalling pathway still remains unclear (265). A complete understanding of the mechanisms controlling the Hippo pathway might contribute to the development of effective anti-cancer drugs. It seems that pharmacological inhibitors of YAP and TAZ could be these medicaments in the nearest future. It might be especially the case as sometimes a scientific approach reveals some unexpected aspects of known drugs, commonly used for years, like proton pump inhibitors (PPI’s) in preventing hypergastrinemia-associated carcinogenesis of gastrointestinal tract or verteporin as YAP inhibitor (266). We should not disregard novel and sometimes unorthodox ideas. This kind of approach has resulted in the development of such methods like successful employment of fecal microbiota in the treatment of gastrointestinal and extra-gastrointestinal diseases (267) or usage of tissue plasminogen activator as a prognostic and differentiation factor in patients with pancreatic cancer and chronic pancreatitis (268). However, the specificity of the Hippo pathway will rather impose the sophisticated activities in the field of pharmacology, considering the fact that every link of this pathway may be the potential target of the therapy. It is very important to select the proper group of oncological patients who are most likely to obtain clinical benefit from blocking the Hippo activity. Toxicity of this ‘ideal’ drug should be acceptable and easily manageable especially in context of cancer patients comfort during the treatment (269). Moreover, the involvement of other cells types, like lymphocytes or macrophages, in the process of cancer development and spreading should not be neglected (270). Besides the obvious influence on the tumourigenesis, the Hippo pathway acts as the essential constituent in the response of the neoplastic cell to anti-cancer drugs and, in that manner, it contributes to the chemo-resistance which has not been broken so far.

Abbreviations: Akt1, RAC-alpha serine/threonine-protein kinase1 or protein kinase B; Amot, angiomotin family; ANKHD, ankyrin repeat and KH domain containing; AREG, amphiregulin; c-Abl, Abelson murine leukemia viral oncogene homolog 1; CCNE2, cyclin E2; CK1, casein kinase 1; CREB, cAMP response element-binding protein; CTGF, connective tissue growth factor; DCHS, Dachsous homolog; Deptor, DEP-domain-containing mTOR-interacting protein; DIM, 3,3-diindolylmethane; EGFR, epidermal growth factor receptor; EMT, epithelial to mesenchymal transition; ErbB4, erythroblastic leukemia viral oncogene homolog 4; ERK1/2, extracellular signal-regulated protein kinase; FGF, fibroblast growth factor; FOXO, Forkhead box protein O; FRMD6, FERM (F for 4.1 protein, E for ezrin, R for radixin and M for moesin) domain-containing protein 6; Ggylation, geranylgeranylation; HDAC9, histone deacetylase 9; HIF-1, hypoxia inducible factor-1; HMGCR, 3-hydroxy-3-methyl-glutaryl-CoA reductase; JAK-STAT3, Janus kinase/signal transducers and activators of transcription; JNKs, c-Jun N-terminal kinases; LATSs, large tumour suppressor kinases; LIF, leukemia inhibitory factor; LIFR, LIF receptor; LPA, lysophosphatidic acid; LST8, mammalian lethal with Sec13 protein 8; Malat1, metastasis-associated lung adenocarcinoma transcript 1; MAP2K3, mitogen-activated protein kinase kinase kinase 2; MAP3K, mitogen-activated protein kinase kinase kinase; MASK, multiple ankyrin repeats single KH domain; Mats, adaptor protein Mob-as tumor-suppressor; Merlin protein, moesin-ezrin-radixin-like protein; MDM2, mouse double minute 2 homolog; MOAP1, modulator of apoptosis 1; MOB, Mps-one binder; MOBKL1, MOB kinase activator 1; MSTs, mammalian sterile 20-like kinases; mTOR, mammalian target of rapamycin serine/threonine protein kinase; mTORC, mTOR complexes; NF2, neurofibromatosis type 2; P2YR1, purinergic receptor 1; PAR1, protease-activated receptor 1; PDGFR, platelet-derived growth factor receptor; PDK1, phosphoinositide-dependent kinase 1; pERK, phosphorylated extracellular signal-regulated kinase; PI3K, phosphatidylinositol 3-kinase; PPARγ, peroxisome proliferator-activated receptor γ; PSEN1, presenilin-1; PTAFR, platelet-activating factor receptor; pYAP, phosphorylated Yes-associated protein; RA, Ras association; RAF, rapidly accelerated fibrosarcoma; Raptor, regulatory-associated protein of mTOR; RASSF1, Ras association domain family 1; RB, retinoblastoma; RhoA, Ras homolog gene family member A; SARAH, Salvador/Sav1-WW45, Rassf/ Hippo/MST1/MST2; SAV1 also called WW45, adaptor proteins Salvador homologue 1; SPTAN1, α-spectrin; SPTBN1, β-spectrin; SREBP1, sterol regulatory element binding protein 1; SRSF1, serine/arginine-rich splicing factor 1; STAT, signal transducer and activator of transcription; TAOK1, thousand-and-one amino acids kinase 1; TAZ, transcriptional co-activator with PDZ-binding motif; TEADs, TEA domain family members; UTR, untranslated region; VEGFR, vascular endothelial growth factor receptor; VGLL4, vestigial-like family member 4; Wts, Warts; WNT, wingless-related integration site; Wwox, WW domain-containing oxidoredutase; YAP, Yes-associated protein; Yki, Yorkie; ZEB1, Zinc finger E-box-binding homeobox 1.

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