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Current Drug Targets, **2016**, *17*, 000-000

The Hippo Pathway As Drug Targets in Cancer Therapy and Regenerative Medicine

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Abstract: Yes-associated protein 1 (YAP1) and transcriptional co-activator with PDZ-binding motif (TAZ) co-operate with numerous transcription factors to regulate gene transcriptions. YAP1 and TAZ are negatively regulated by the tumor suppressive Hippo pathway. In human cancers, the Hippo pathway is frequently deregulated and YAP1 and TAZ escape the inhibition by the Hippo pathway. The up-regulation of YAP1 and TAZ induces epithelial-mesenchymal transition and increases drug resistance in cancer cells. TAZ is implicated in cancer stemness. In consequence cancers with hyperactive YAP1 and TAZ are associated with poor clinical prognosis. Inhibitors of YAP1 and TAZ are reasoned to be beneficial in cancer therapy. On the other hand, since YAP1 and TAZ play important roles in the regulation of various tissue stem cells and in tissue repair, activators of YAP1 and TAZ are useful in the regenerative medicine. We discuss the potential application of inhibitors and activators of YAP1 and TAZ in human diseases and review the progress of drug screenings to search for them.

Keywords: Cancer, drug screening, regeneration, sarcopenia, tumor suppressor.

INTRODUCTION

Drosophila Hippo pathway started with four founding members (Hippo, Salvador, Mats, and Warts) [1, 2]. Hippo and Warts are protein kinases. Hippo phosphorylates and activates Warts. Salvador and Mats interact with Hippo and Warts to promote the Hippo-mediated activation of Warts. Flies with the mutations of these genes show tumorous phenotypes, indicating that these gene products form the tumor suppressive kinase cassette. Accordingly the researchers formulated "the Hippo pathway" as the name of the new pathway. Yorkie was thereafter identified as a Warts-interacting protein, and turned out to be the substrate of Warts [3]. Yorkie co-operates with Scalloped, a transcription activator, and regulates transcription of cell cycle-promoting and anti-apoptotic genes. The phosphorylation by Warts induces the translocation of Yorkie from the nucleus to the cytoplasm and triggers its degradation. That is, the Hippo pathway negatively regulates Yorkie and the deregulation of the Hippo pathway causes hyperactivity of Yorkie, resulting in cell over-proliferation. Human genome harbors homologues of all the components that were identified in *Drosophila* [4-6] (Fig. **1**). Mammalian Ste20-like kinase (MST) 1 and -2 and large tumor-suppressive kinase (LATS) 1 and -2 are the homologues of Hippo and Warts, respectively. Yes-associated protein 1 (YAP1) and transcriptional co-activator with PDZ-binding motif (TAZ) are Yorkie homologues. LATS1/2 phosphorylate and inhibit YAP1 and TAZ, which interact with TEA domain family member (TEAD)1 to -4, homologues of Scalloped. The deregulation of the Hippo pathway is frequent in human cancers, supporting that the Hippo pathway plays a tumor suppressive role in human as well as in *Drosophila* [7, 8]. At the initial stage the MST1/2-LATS1/2-YAP1/TAZ-TEAD axis was the focus of the study on the Hippo pathway. The late coming studies revealed the regulation of YAP1 and TAZ independent of this axis. They are regulated by the interaction with cell junction proteins and the mechanic stimuli and in response to metabolic state [5, 9-13]. YAP1 and TAZ cross-talk with other signaling such as the Wnt pathway and regulate microRNA biogenesis [10, 14-16]. Proteomics approaches for the Hippo pathway have revealed the enormous protein network, in which YAP1 and TAZ are embedded [17-21]. We do not expound all interacting proteins (readers are requested to refer to the recent reviews) [5, 6], but it is clear that YAP1 and TAZ play many roles independently of MST1/2, LATS1/2, and TEAD. Thus, YAP1 and TAZ have outshined MST1/2 and play the first fiddle in the Hippo pathway. We discuss under the title "the Hippo pathway as drug targets" YAP1/TAZ inhibitors and activators in this review.

2. YAP1/TAZ INHIBITORS IN CANCER THERAPY

Clinical data underscore the importance of YAP1 and TAZ in human cancer. Many studies demonstrate the association of the high nuclear expression of YAP1 and TAZ with the shorter disease-free survival in cancer patients [7, 22]. The up-regulation of YAP1 and TAZ induces epithelial-mesenchymal transition and enhances drug resistance [23-25]. The inhibition of YAP1 and TAZ by the Hippo pathway

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1389-4501/16 $58.00+.00

suppresses growth in tetraploid cells and counteracts its oncogenic effect, while the deregulation of the Hippo pathway causes the bypass of the checkpoint and enhances the genomic instability [26]. TAZ confers cancer stemness to breast and oral cancers [27]. The suppression of YAP1 and TAZ blocks *in vitro* tumor sphere formation, 3D-matrigel growth, and migration of certain cancer cells. It can be deduced that YAP1/TAZ inhibitors prevent metastasis and recurrence and improve the clinical prognosis.

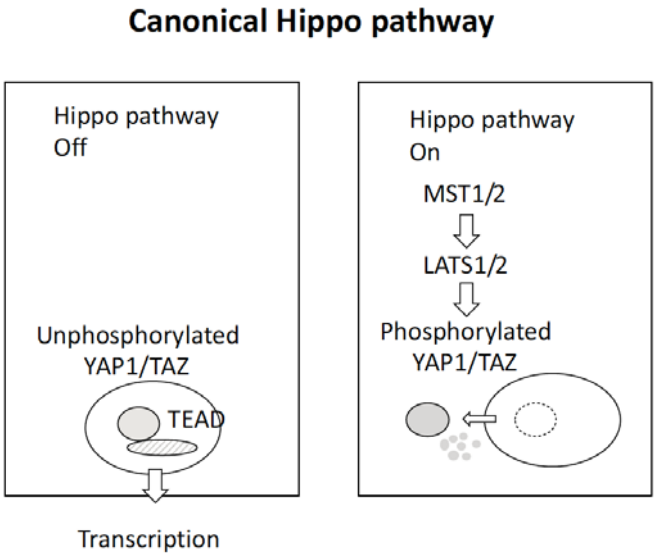

Fig. (1). Canonical Hippo pathway in mammals. The core kinase cassette of mammalian Hippo pathway is composed of mammalian Ste20-like kinases (MST1/2) and large tumor suppressor kinases (LATS1/2). When the Hippo pathway is off, YAP1 and TAZ remain in the nucleus and co-operate with TEAD to up-regulate gene transcription. When cells become confluent or cells are exposed to DNA damage, the Hippo pathway is switched on. Then MST1/2 phosphorylate and activate LATS1/2, which subsequently phosphorylate YAP1 and TAZ. Phosphorylated YAP1 and TAZ are recruited from the nucleus to the cytoplasm and undergo protein degradation. This scheme represents the canonical Hippo pathway.

More recently YAP1 has gathered attention as a therapeutic target in cancers with Ras mutants [28-30]. In the Ki-Ras mutant-driven mouse model of pancreatic cancer, YAP1 is activated down-stream of Ki-Ras, and eventually even after withdrawal of Ki-Ras mutant, cancer escapes oncogenic Ki-Ras addiction and survives. YAP1 promotes resistance against RAF- and MEK-inhibitors in cancer cells with BRAF and Ki-Ras mutations [31]. YAP1 knockdown recovers the sensitivity. These findings open the possibility that YAP1 inhibitors are effective against cancers with Ras mutants.

The deregulation of the Hippo pathway and hyperactive YAP1/TAZ are frequently observed in patients with various cancers, and not limited to the small number of patients. It raises hopes for the big impact of YAP1/TAZ inhibitors in cancer therapy. Experimentally researchers express LATS1/2-target site-deficient YAP1 and TAZ mutants in animals to develop cancers. These mutants, above all the mutants with alanine replacement at all sites, are highly oncogenic. The findings obtained from the experiments using YAP1 and TAZ mutants prompt us to regard YAP1 and TAZ activation as the initial event in oncogenesis. However, it should be noted that these mutants are totally artificial. Although there exist the reports of mutations in YAP1 and TAZ, they are extremely rare and not necessarily related to cancer [5]. In human cancers, YAP1 and TAZ without any mutation are activated by the loss of the Hippo pathway or gene amplification. The above mentioned experiments using Ki-Ras-driven mouse models imply that YAP1 is secondarily activated down-stream of Ki-Ras. It is reported that YAP1 activation is detected in human liver dysplastic nodules and hepatocellular adenomas, but the clinical data supporting that YAP1/TAZ are activated prior to cancers are still limited [32]. It is possible that cancers gain hyperactive YAP1 and TAZ during evolution and become more malignant at the later stage. We need to clarify how activated YAP1 and TAZ without mutations contribute to oncogenesis in human cancers. This knowledge is crucial to determine how to use YAP1/TAZ inhibitors and to evaluate their therapeutic effects. The attempt to develop a magic bullet that obliterates cancer has succeeded in certain cases but has limitations. We need to develop anti-cancer drugs that can be widely used for a majority of patients to prolong survival and improve quality of life. If we expect YAP1/TAZ inhibitors to undertake such a role, it may be necessary to use them in combination with other drugs and to evaluate the effect not only on the reduction of the primary tumor size but also on the frequency of metastasis and recurrence.

3. YAP1 ACTIVATORS IN CANCER THERAPY

The early studies before the emergence of the Hippo pathway revealed that YAP1 interacts with p73 and plays a tumor suppressive role [33]. A recent paper dealing with myeloma has revived this view [34]. Myeloma cells exhibit DNA damage, which should normally induces ABL1-YAP1-p73-dependent cell death. DNA damage triggers the nuclear translocation ABL1. ABL1 phosphorylates YAP1 at a tyrosine residue and promotes the p73-depedent transcription. However, as YAP1 is suppressed, myeloma cells escape apoptosis. The inactivation of MST1 or the re-expression of YAP1 recovers apoptosis and inhibits cell proliferation. These findings imply that YAP1 activators can be one of, choices in the therapy of myeloma.

4. YAP1/TAZ ACTIVATORS IN REGENERATIVE MEDICINE

Unleashed YAP1 and TAZ incite unlimited cell growth. We hope the inhibitors of YAP1 and TAZ to control cancers. On the other hand, cell division is essential for animals to grow and for tissues to be maintained and repaired. Not surprisingly YAP1/TAZ activators are expected to be useful in regenerative medicine [35]. *Yap1* deletion compromises heart regeneration after ischemia, whereas the inhibition of the Hippo pathway improves cardiac function after infarction in adult mice [36, 37]. YAP1 is activated after bile duct ligation and hepatectomy [38, 39]. *Yap1* deletion blocks bile duct cell proliferation and enhances hepatocyte necrosis. *Yap1* deletion also impairs intestinal regeneration and skin wound healing [40, 41]. These findings suggest that YAP1 activators can promote tissue repair after acute injuries. TAZ enhances osteogenesis and inhibits adipogenesis in mesenchymal stem cells [42]. TAZ is activated after muscle inju-

ries [43]. Enforced expression of TAZ promotes myogenesis in mouse myoblast C2C12 cells and enhances MyoD-mediated myogenesis in mouse mesenchymal C3H10T1/2 cells. Accordingly kaempferol, (-)-epicatechin gallate, and phorbaketal A, which are natural products, stimulate osteogenesis and inhibits adipogenesis *via* TAZ [44-47]. KM-62980, a peroxisome proliferator activated receptors γ (PPARγ) agonist, augments the interaction between TAZ and PPARγ to exhibit the anti-adipogenic activity [48]. TM-25659 that was identified thorough the high-throughput screening for TAZ modulators, enhances osteogenesis in C3H10T1/2 cells and inhibits adipogenesis in 3T3-L1 cells [49]. TM-53 and TM-54 accelerate myogenesis in C2C12 cells in non-muscle cells, and enhance muscle regeneration in the sciatic nerve injury model [50]. We identified IBS008738 as a compound that activates TAZ and promotes myogenesis in C2C12 cells [51]. IBS008738 increases Pax7-positive satellite cells and facilitates muscle repair after cardiotoxin-induced muscle injury, and prevents steroid-induced muscle atrophy in mice. We also found that ethacridine, a widely used antiseptic and abortifacient, increases the unphosphorylated nuclear TAZ and inhibits adipogenesis in C3H10T1/2 cells [52].

These findings give life to the assumption that TAZ activators prevent osteoporosis, obesity, and muscle atrophy, but we want to argue that the application for muscle atrophy in old people is the most realistic. TAZ is an oncogene. To prevent osteoporosis and obesity, we need to use TAZ activators systemically and for a long term. It might lead to oncogenesis and exacerbate indolent cancers. When we use TAZ activators against sarcopenia, we can avoid a systemic and long-term application. People lose skeletal muscle volume and power with ageing. In Japan the frequency of sarcopenia among community-dwelling adults older than 65 is almost 20%, and 4% people show low physical performance [53]. Hospitalization is associated with a more advanced sarcopenia. In Germany 18.7% of hospitalized people older than 65 are severely sarcopenic [54]. Nutritional support and exercise are key to prevent and treat sarcopenia. However, when old people stay in bed, they further lose skeletal muscles in lower limb and become disabled. The muscle loss hampers rehabilitation. If we can maintain muscle volume by use of TAZ activators during bed-confinement, old patients can start physical training smoothly and once old patients start exercises, TAZ activators can be discontinued. Moreover the local application of TAZ activators to lower limb muscles is sufficient to improve the quality of life of old people. Ethacridine inhibits adipogenesis at 0.5 μM *via* TAZ, while it is used at 3 μM as an antiseptic [52, 55]. It follows that TAZ is activated, even though it may be a collateral effect, when ethacridine is used as an antiseptic. Ethacridine has a long history, and there is no report that ethacridine causes cancer. We expect that TAZ activators can be safely used if we stipulate to a short-term local application.

The Hippo pathway is a barrier for reprogramming to pluripotency [56]. LATS2 knockdown in human fibroblasts enhances the induction of iPS cells, while the additional knockdown of TAZ abolishes the enhancement, implying that TAZ activation promotes reprogramming. YAP1 protein expression is up-regulated in human iPS cells, and conversely the overexpression of YAP1 increases the reprogramming efficiency [57]. YAP1/TAZ activators can be the reagents that improve reprogramming efficiency.

5. CURRENT STATE OF YAP1/TAZ INHIBITORS AND ACTIVATORS

Several patent applications related to YAP1 and TAZ are published. For example, in WO 2009045179A1, TAZ is claimed as a therapeutic and diagnostic target in cancers by Hong W & Chan S.W. Guan K-L, Yu F, and Ding S. provide novel YAP1/TAZ inhibitors as the method of preventing and inhibiting the cancer growth (WO 2013188138A1). They also propose the activators and inhibitors of G protein-coupled receptors and the reagents that modulate the intracellular cyclic AMP concentration as the method that inhibits YAP1/TAZ. Binding agents like antibodies and soluble receptors that modulate the Hippo pathway are claimed in US 2014005680 (Gurney A.L. & Chartier-Courtaud C). Phenyltetrazole derivatives are claimed as TAZ activators that prevent and treat osteoporosis and obesity in US 20130123297 (Kim NJ *et al.*). In this section, we focus on the journal articles that are viewable on PubMed and summarize how YAP1/TAZ inhibitors and activators are screened.

5.1. Reagents That Inhibit the Interaction Between YAP1 and TEAD (Fig. 2)

Sudol *et al.* discuss YAP1 as a promising target for new anti-cancer drugs and predict potential drugs based on the structure of YAP1 [58]. Although YAP1 interacts with various molecules, the YAP1-TEAD interaction is the most intensely studied [59-62]. TEAD proteins bind to the N-terminal region of YAP1 [23]. They play an important role in epithelial-mesenchymal transition. In one of the aforementioned papers that studied Ki-Ras-driven pancreatic cancer model, the co-operation of YAP1 and TEAD2 was highlighted in Ki-Ras-independent tumor survival [28]. Thus reagents that inhibit the interaction between YAP1 and TEAD are the reasonable choice as anti-cancer drugs. Vestigial-like (Vgll) proteins interact with TEAD and compete with YAP1 for TEAD-binding [63]. Based on the sequences of the interacting domains of TEAD, YAP1, and Vgll, two types of synthetic peptides are designed [64-66]. One is the chimera peptides composed of the interacting domains of Vgll and YAP1 and the other is the cyclic peptides derived from YAP1. The Vgll-YAP1 peptide suppresses gastric tumor growth in mouse. Verteporfin was developed as a photosensitizer for photodynamic therapy [67]. Liu-Chittenden *et al.* expressed YAP1, TEAD4 fused to GAD4, and upstream activation sequence (UAS)-driven luciferase in HEK293 cells and measured the luciferase activity to evaluate the interaction between YAP1 and TEAD4. Thus they identified verteporfin as an inhibitor of the interaction between YAP1 and TEAD. Verteporfin is now frequently used in the study of the Hippo pathway.

5.2. Reagents That Modulate YAP1/TAZ-mediated Gene Transcriptions (Fig. 3)

Basu *et al.* used TEAD-responsive luciferase reporter and searched for the compounds that suppress the reporter activity [68]. They obtained the compound C19 that inhibits not only the Hippo signaling but also the Wnt and TGF-β signal-

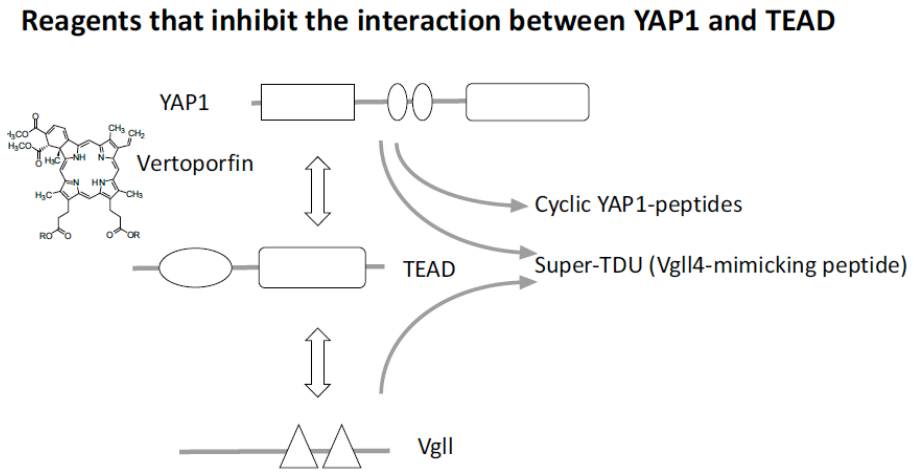

Fig. (2). Reagents that inhibit the interaction between YAP1 and TEAD. YAP1 and Vestigial-like (Vgll) proteins compete with each other for the binding to TEAD proteins. Based on the sequences of YAP1 and Vgll4 that are involved in the interaction with TEAD, cyclic YAP1 peptides and the synthetic peptide named Super-TDU, which is composed of the chimera of Vgll4 and YAP1, are designed. Verteporfin was identified by use of the GAL4-UAS system (Fig. **3**) and was found to inhibit the interaction between YAP1 and TEAD.

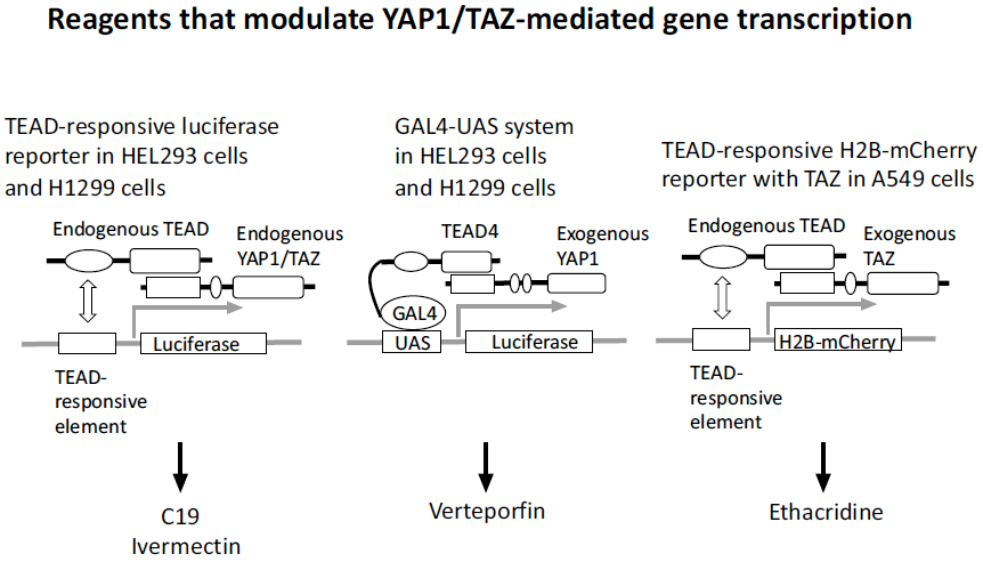

Fig. (3). Reagents that modulate YAP1/TAZ-mediated gene transcription. Basu *et al.* and Nishio *et al.* used the TEAD-responsive luciferase reporter in HEK293 cells and in H1299 cells. They reported C19 and ivermetctin as TAZ inhibitor and YAP1/TAZ inhibitor, respectively. Liu-Chittenden *et al.* expressed GAL4-fused TEAD4, YAP1, and UAS-luciferase in HEK293 cells and identified Verteporfin as YAP1-TEAD inhibitor. Kawano *et al.* expressed TEAD-responsive H2B-mCherry and TAZ in A549 cells and revealed that ethacridine is a TAZ activator.

ing. The direct target of C19 is not yet clear, but its effect partly depends on MST1, LATS1, and AMPK. We expressed TAZ and a reporter that expresses mCherry fused to histone2b (H2B-mCherry) under the promoter harboring eight copies of TEAD-binding motif in A549 cells [52]. We searched for small molecule compounds that enhance the signals and revealed that ethacridine activates TAZ. Nishio *et al.* expressed luciferase reporter under ten copies of the TEAD-binding sequence derived from *CTGF* gene in human lung cancer H1299 cells to search for the compounds that suppress the reporter activity and found that ivermectin, an antiparasitic drug, and its derivative, milbemycin D, inhibit YAP1/TAZ activity and suppress tumor growth in MOB1-deficient mice [69].

5.3. Reagents That Modulate the Subcellular Localization of YAP1/TAZ (Fig. 4)

In the canonical Hippo pathway, unphosphorylated YAP1 and TAZ reside in the nucleus and regulate transcriptions, while phosphorylated YAP1 and TAZ degrade in the cytoplasm. In fact the subcellular localization of YAP1/TAZ correlates with the activity. We applied small chemical compounds to U2OS cells expressing green fluorescent protein-fused YAP1 (GFP-YAP1) and found that dobutamine, an agonist of β-adrenergic receptor, recruits GFP-YAP1 from the nucleus to the cytoplasm [70]. Jang *et al.* used COS7 cells expressing GFP-TAZ and identified novel TAZ modulators [49]. Sorrentino *et al.* immunostained endogenous

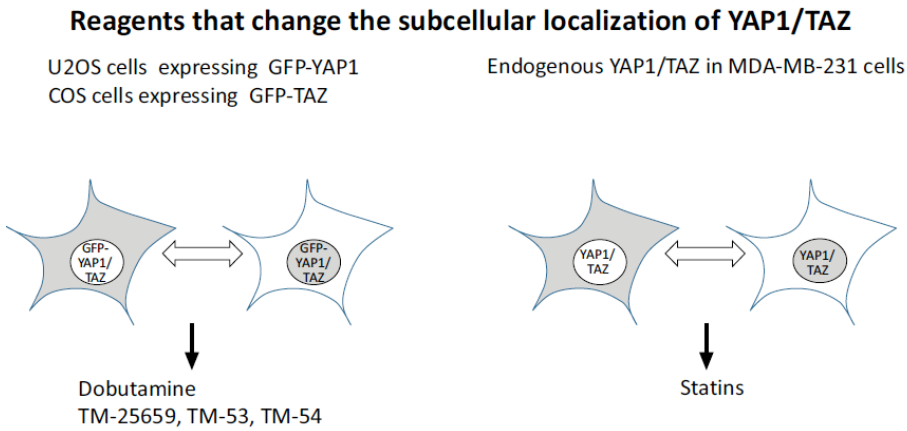

Fig. (4). Reagents that change the subcellular localization of YAP1/TAZ. Bao *et al.* used U2OS cells expressing GFP-YAP1 and found that dobutamine recruits GFP-YAP1 from the nucleus to the cytoplasm. Jang *et al.* and Park *et al.* identified TAZ modulators by use of COS cells expressing GFP-TAZ. Sorrentino *et al.* immunostained endogenous YAP1 and TAZ in MDA-MB-231 cells and revealed the inhibitory effect of statins on YAP1 and TAZ.

YAP1 and TAZ in MDA-MB-231 cells [71]. They discovered that statins inhibit the synthesis of geranylgeranyl pyrophosphate, which is necessary for Rho activation, and eventually opposes nuclear localization of YAP1 and TAZ.

5.4. Reagents That Induce TAZ-dependent Sphere Formation in MCF10A Cells (Fig. 5)

We subjected MCF10A cells expressing the wild type of TAZ (MCF10A-TAZ) to the mammosphere-forming condition [51]. MCF10A-TAZ cells form spheres only when TAZ is activated. We could obtain TAZ activators by using the sphere formation as the read-out of TAZ activity. Among them we further selected the compounds that promote myogenesis in C2C12 cells and finally reported IBS008738.

5.5. Other Approaches

Tacolli *et al.* created a database named Mutations and Drugs Portal, which links the cell-based screenings of chemical compounds to the mutations in cancer cells [72]. By use of this database, they identified that statins and dasatinib, a tyrosine kinase inhibitor, are effective against cancer cell lines harboring *NF2* mutations. *NF2* encodes Merlin, which is an essential component of the Hippo pathway, and the mutations cause hyperactivation of YAP1/TAZ. Therefore, the compounds that suppress cancers with *NF2* mutations are expected to inhibit YAP1/TAZ. Accordingly they experimentally confirmed that the combination of statins and dasatinib inhibits YAP1/TAZ activity.

6. PERSPECTIVES

Mounting evidence underscores the important roles of YAP1 and TAZ in the pathology of cancers and in tissue regeneration. Even so whether YAP1/TAZ modulators can be used as drugs in patients is still elusive. As YAP1 and TAZ play versatile roles, YAP1/TAZ modulators may bring unexpected side effects. This concern daunts researchers. The Hippo pathway has multiple layers and is composed of quite a few components. The screenings by use of the subcellular localization of YAP1/TAZ and the YAP1/TAZ-dependent reporter activity as read-outs provide compounds with diverse target molecules. The specificity of those candidate compounds is not guaranteed and the risk of off-target effects cannot be excluded unless the direct target of each compound is identified. For instance, ivermectin exhibits strong effects on the Hippo pathway-defective tumors [69]. But currently the direct target is unknown and it is difficult to predict the side effect. On the other hands, even if the targets are identified, there exist problems to be considered. In many cancers, the Hippo pathway is deregulated. Dobutamine activates LATS kinases *via* β-adrenergic receptor and suppresses YAP1 activity [70]. Integrin-linked kinase inhibitor blocks the inactivation of myosin-phosphatase MYPT1-PP1 to promote dephosphorylation of Merlin and activates MST1 and LATS1 [73]. PDK1 inhibitors block the dissociation of PDK1 from the core complex of the Hippo pathway including MST kinases, LATS1, and Sav1 to maintain the phosphorylation of YAP1even under the growth factor treatment [74]. Potentially all these compounds could suppress YAP1/TAZ in cancer cells. However, they will fail to inhibit YAP1/TAZ in cancers lacking their targets. Integrin kinase inhibitors will not work in cancers with *NF2* mutations. Dobutamine and PDK1 inhibitors cannot inhibit YAP1 in cancers with down-regulated LATS kinases. It is essential to select appropriate cancers for each compound. The mechanism how statins suppress YAP1 is relatively clear [71]. They interfere with the lipid modification of Rho proteins and subsequently suppress Rho signallings, which play a variety of roles. As statins are widely used, their application may be safe, but unexpected effects are possible, when used as anti-cancer drugs. To minimize side effects, the disruption of the interaction between YAP1/TAZ and TEAD is a reasonable strategy to develop anti-cancer drugs. Pobbati *et al.* have taken one step further. They focused on the structure of TEAD and revealed that flufenamates, non-steroidal anti-inflammatory drugs, bind to the central pocket of TEAD [75]. Flufenamates inhibit YAP1-TEAD-mediated transcription without the disruption of the interaction between TEAD and YAP1. The molecular mechanism how flufenamates compromise TEAD function is not yet clear, but the direct inhibitors of TEAD may be the better choice as anti-cancer

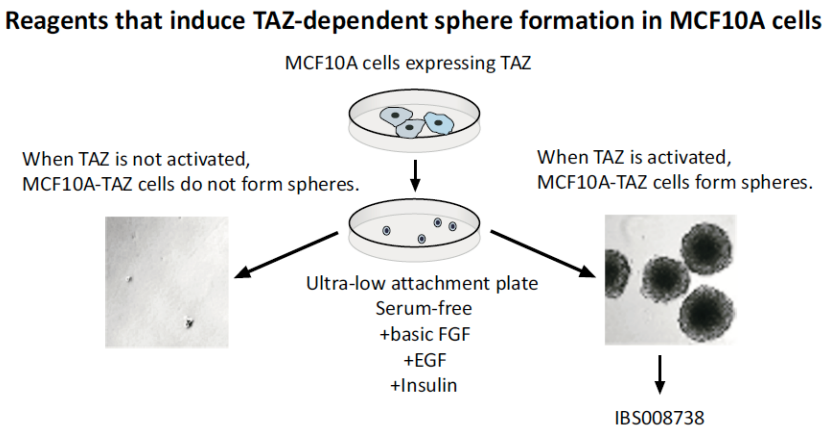

Fig. (5). Reagents that induce TAZ-dependent sphere formation in MCF10A cells. MCF10A cells overexpressing TAZ form spheres under the sphere-forming condition when TAZ is activated. Yang *et al*. searched for the compounds that induce the sphere formation in MCF10A-TAZ cells and identified IBS008738 as a TAZ activator that promotes myogenesis in C2C12 cells.

drugs. TEAD is palmitoylated in the central pocket and the palmitoylation is necessary for the stabilization [76]. The palmitoylation of TEAD, although not dynamic, may be targetable.

In the application of YAP1/TAZ activators in regenerative medicine, we need to consider how they are used. As described above, we expect that the application in sarcopenia is realistic if we use them locally and for the short term. YAP1 activators may be used in the similar manner in patients with cardiac infarction. However, we also need to further study how YAP1/TAZ regulate tissue stem cells. YAP1/TAZ are reported to interact with the Nucleosome Remodeling Deacetylase (NuRD) complex and to regulate the balance between self-renewal and differentiation in stem cells [77, 78]. To get the foremost achievement in tissue regeneration, it is essential to promote cell differentiation without exhausting stem cells. To this end, we need to understand more precisely how YAP1/TAZ interact with and play a role in the NuRD complex. It is not yet clear at which stage of differentiation YAP1/TAZ interact with which component of the NuRD complex. We may need to use YAP1/TAZ activators in the appropriate stage of tissue regeneration.

In conclusion, to evaluate the usefulness and the risks of YAP1/TAZ modulators, we have to test them in animal models. Unexpected side effects remain to be unexpected until tested. To fulfil the test, we have to get YAP1/TAZ modulators that are worthwhile to test. Fortunately reports of YAP1/TAZ modulators are increasing steadily. We hope that the studies using these candidate compounds settle the discussion in the near future and pave the way to the development of new drugs.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

This work was supported by research grants from Japan Society for the Promotion of Science (JSPS) (26293061), and the Mitsubishi Foundation.

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Received: July 13, 2015 **Revised: January 06, 2016** **Accepted: January 11, 2016**