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Clinical Cancer Research

Molecular Pathways

Molecular Pathways: YAP and TAZ Take Center Stage in Organ Growth and Tumorigenesis

Stefano Piccolo, Michelangelo Cordenonsi, and Sirio Dupont

Abstract

The evolution of a solid tumor is fueled by genetic aberrations. Yet, the tumor environment often dominates over the effects of genetics: normal tissues have powerful tumor-suppressive properties that constantly tame or eliminate cells carrying transforming mutations. Critical elements of such a suppressive microenvironment are structural characteristics of normal cells and tissues, such as cell polarity, attachment to the extracellular matrix (ECM), and epithelial organization. Once these tissue-level checkpoints have been overcome, tumor growth is enhanced by recruitment of stromal cells and remodeling of the ECM. Genetic inactivation in mouse models indicates the Hippo pathway as a fundamental inhibitor of organ growth during development and as a critical tumor suppressor in epithelial tissues, such as the liver, skin, and ovaries, and soft tissues. At the centerpiece of this pathway lie two related transcriptional coactivators, YAP and TAZ, that promote tissue proliferation and the self-renewal of normal and cancer stem cells, and incite metastasis. Strikingly, YAP and TAZ are controlled by the same architectural features that first inhibit and then foster cancer growth, such as ECM elasticity, cell shape, and epithelial-to-mesenchymal transition. These findings open unexpected opportunities for the development of new cancer therapeutics targeting key YAP/TAZ regulatory inputs such as Wnt signaling, cytoskeletal contractility, G-protein–coupled receptors, or YAP/TAZ-regulated transcription. *Clin Cancer Res; 19(18); 4925–30. ©2013 AACR.*

Background

Tumors as organs

In the past 40 years, the study of the genetic lesions that fuel neoplastic transformation has been at the centerpiece of cancer research (1). However, it also clear that tumor cells do entertain, and actually need, their own social life (2, 3), and tumor growth is driven by its microenvironment as much as it is by genetic mutations (1, 3). In addition to nutrients, oxygen levels, and growth factors, key ingredients of the microenvironment are structural and architectural elements such as the tridimensional arrangement of the extracellular matrix (ECM) and of the surrounding cells, the organization and polarity of junctions by which individual cells connect to their surroundings, and the corresponding tensional forces of the actin cytoskeleton that keep cells, tissues, and organs in a certain shape (4–7). In normal tissues, this "physical" microenvironment is a potent tumor suppressor; indeed, the progressive loss of spatial control over proliferation is also often associated with deranged tissue architecture, cell polarity, and cell shape (8, 9).

The growth of a solid tumor hijacks aspects of normal organ development and tissue regeneration processes. Indeed, tumors are often hierarchically organized in stem and non–stem cell populations, the first endowed with the potential of tumor initiation and self-renewal (10). Tumor progression parallels with the capacity of tumors to build their own extracellular niches (11), to establish a number of physical and paracrine interactions with the surrounding stroma (2), and with an increased cancer stem cell content (12, 13). Although our understanding of these processes remains scant, it is increasingly clear that nurturing inputs represented by contacts with other cells and by a permissive ECM are key factors to promote and maintain different aspects of tumor biology, acting in concert with, and perhaps sometimes dominating over, the genetic lesions that fuel tumorigenesis (8).

The mysteries of the biology of the tumor organs are intimately connected to an even more fundamental black box, that is, how a tissue grows and takes shape during development. Although growth of body parts can occur by cell enlargement and accrual of ECM, the size of an organ largely reflects its cell number (14). Organs seem "to know" what is their final size, inducing cells to stop dividing after the organ has reached it, in some cases even independently of the size of the host organism (15, 16) or of the total number of cells that form the organ (17). This also applies in the case of regeneration of mammalian

Authors' Affiliation: Department of Molecular Medicine, University of Padua School of Medicine, Padua, Italy

Corresponding Author: Sirio Dupont, Department of Molecular Medicine, viale Colombo 3, 35100 Padua, Italy. Phone: 00390-49827-6095; Fax: 00390-4982-76079; E-mail: dupont@bio.unipd.it

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livers, or *Drosophila* imaginal discs, after surgical cell ablation (18, 19). With this background in mind, it is clear why much excitement was generated by the discovery of the Hippo pathway, as this provided a key entry point into the molecular mechanisms that underlie organ growth control.

The Hippo pathway and the control of organ size. The mammalian Hippo cascade is composed of a highly conserved kinase cassette regulating YAP and TAZ, two closely related transcriptional regulators. In its most basic formulation, the pathway is activated when the kinases MST1 and MST2, related to the *Drosophila* Hippo kinase, phosphorylate and activate the LATS1/2 kinases, which in turn phosphorylate and inhibit YAP and TAZ (refs. 20, 21; Fig. 1). Two cofactors, Salvador (SAV or WW45) and MOB1, are also involved as MST and/or LATS partners. NF2, encoded by the type II neurofibromatosis gene, has been implied as the first upstream regulator of hippo kinases (20, 22). The mechanism of YAP/TAZ inhibition by phosphorylation is dual: degradation by the proteasome and/or sequestration in the cytoplasm through anchoring proteins. In the nucleus, YAP and TAZ do not bind DNA directly but are coactivators that regulate gene expression by associating with specific transcription factors and in particular with TEAD factors (ref. 20; Fig. 1).

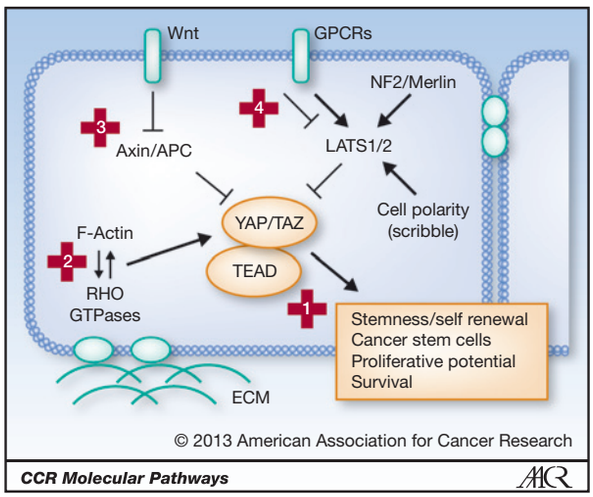

Figure 1. YAP and TAZ transcriptional coactivators lie at the core of a complex regulatory network that offers multiple potential therapeutic targets (exemplified by red crosses). YAP/TAZ cooperate in the nucleus with TEAD transcription factors to regulate gene transcription and promote malignant cell behaviors. Small molecules precluding TEAD interaction dampen YAP/TAZ activities (1). F-actin and small RHO GTPases, in response to cell–substrate adhesion (green dots) and to mechanical cues imposed by the ECM, are key inputs to sustain YAP/TAZ activity. Inhibitors of RHO and of the machinery that maintains tension of the F-actin cytoskeleton (such as ROCK or MLCK, not shown here) may serve to inactivate YAP/TAZ (2). WNT signaling inactivates the destruction complex containing the APC (adenomatous polyposis coli) and Axin proteins, thereby rescuing TAZ from degradation. Tankyrase inhibitors may reactivate Axin function and inhibit YAP/TAZ activity in cancers with Wnt activation or APC mutation (3). Apico-basal cell polarity (through Scribble) and NF2/Merlin regulate the activity of the LATS1/2 kinases, which phosphorylate and inhibit YAP/TAZ. GPCRs, by regulating LATS1/2 kinases and/or RHO GTPases, can either activate or repress YAP/TAZ activity. GPCR regulators might be used to dampen YAP/TAZ function (4).

The Hippo cascade constitutes an intrinsic regulator of organ size essential to stop cell growth when the organ reaches its final size; inactivation of this cascade causes excess of YAP/TAZ nuclear accumulation leading to organ overgrowth. The first indications in this direction involved conditional overexpression of YAP in the mouse liver, which caused an astonishing 4-fold increase in liver size (23). Notably, this increase was reversible, as if the organ "perceived" its size as abnormal and returned to a normal size after turning off YAP expression. After this landmark study, *MST1/2*- and *SAV*-knockout mice provided crucial genetic evidence that the Hippo cascade is required to limit tissue overgrowth. Liver-specific ablation of *MST1/2* or *SAV* phenocopied the giant liver phenotype observed in the YAP transgenics (24, 25), and this phenotype was rescued by concomitant removal of one YAP allele. Also in the small intestine and to some extent in the skin, *MST1/2* and *SAV* knockout, or YAP activation, all expand the progenitor cell population (26–28). The role of Hippo signaling extends to nonepithelial tissues; for example, depletion of *MST1/2* or *SAV* in the heart causes massive cardiomegaly, due to increase in the number of cardiomyocyte (29), whereas *MST1/2* inactivation in the central nervous system (CNS) promotes expansion of neural progenitors (30).

In recent years, several variations on the basic Hippo cascade have been reported, including LATS-independent phosphorylation of YAP/TAZ, MST-independent activation of LATS, and phosphorylation-independent modalities of YAP/TAZ control (25, 31–33). Thus, the reader should be aware of a potential semantic problem, as the definition of what "is" the Hippo pathway has progressively blurred to include clear non-Hippo regulations feeding on YAP/TAZ activity. For example, skin-specific depletion of *MST1/2* is surprisingly inconsequential, yet YAP overexpression does cause hyperplasia and skin thickening due to amplification of undifferentiated progenitors (33). Regardless of the exact mechanism by which YAP is regulated in each tissue, results from genetic studies on YAP strongly support the remarkable conclusion that active YAP induces proliferation and self-renewal of progenitor cells (33, 34), whereas YAP inactivation leads to cell death and differentiation in multiple tissues.

How nuclear YAP/TAZ activity controls cell and organ growth remains one of the key unanswered questions. YAP and TAZ may induce, but only in specific cell types, the transcription of genes involved in cell proliferation and survival, such as *Birc5*, *c-Myc*, or *cyclin D1*. However, none of these growth regulators have been shown to serve as downstream mediator of YAP/TAZ effects, and, despite few exceptions (35), neither overexpression nor genetic ablation of a number of cell-cycle regulators or apoptosis mediators cause overt changes in organ size. The jury is thus still out on whether a universally valid YAP/TAZ-dependent gene expression program directing organ growth does truly exist. In another scenario, YAP/TAZ may not give precise instructions: YAP/TAZ could indeed reprogram the

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cellular genome to escape terminal differentiation, and in favor of an increased, or prolonged, proliferative potential. These traits could be exploited only in presence of other intrinsic (i.e., activation of proto-oncogenes) or extrinsic growth regulators, such as nutrient availability, metabolites, insulin-like growth hormones (36), or other tissue-specific growth factors (37). This remains a tempting, untested hypothesis, but perhaps consistent with the observation that, *in vivo*, nuclear YAP/TAZ are frequently localized in stem and immature progenitor cells (ref. 38; and M. Cordenonsi, unpublished results).

YAP/TAZ regulation by cell shape and mechanical cues. Organ size control must involve control layers operating on a whole tissue and organ scale (39). Tissue regeneration occurring after experimental ablations or during normal homeostasis implies that cells are constantly informed on the size of the whole organ and that they are able to perceive what happens hundreds of cell diameters away. At the same time, organs are built and constantly regenerated with exquisite spatial accuracy, for example, by establishing sharp growth differentials over few cell diameters (i.e., activating one cell, but not its equally potent neighbors). It is hard to conceive these multiscale regulations as being mediated only by gradients of soluble factors or morphogens (40). An intriguing hypothesis involves regulation by mechanical signals, such as stretching, compression, and compliance of the ECM that are known to profoundly influence cell behavior (4–7). Indeed, mechanical cues can easily reverberate to distant cells, through a "wave-like" propagation mediated by the semiflexible and pretensed organization of the cell cytoskeleton and ECM network (41). Stem cells, in particular, are lodged in specific physical niches within tissues, making them primary targets of the mechanical inputs intrinsic to a given tissue architecture.

In light of this background, the discovery that YAP and TAZ are activated by mechanical cues and cytoskeletal tension added an entirely new dimension to our understanding of tissue biology (6, 31). A rigid ECM, a spread cell shape, shear stress, and a tense actin cytoskeleton are all potent inducers of YAP/TAZ nuclear localization and activity (31, 42–44). Crucially, YAP and TAZ are emerging not just as mechanotransducers but also as key functional mediators of the biologic effects of ECM stiffness and cell shape (31, 45). For example, endothelial cells die when forced to remain small, whereas they proliferate when allowed to spread (46). The levels of YAP and TAZ dictate these opposite behaviors: when YAP or TAZ are overexpressed in small cells, these start to proliferate, whereas attenuation of YAP and TAZ in spread cells causes them to die (31). Unveiling how cell mechanics and tension controls YAP/TAZ activity is a key challenge for the field, as the regulation of YAP and TAZ by mechanical cues appears independent from, and dominant over, the Hippo cascade (31). This also raises the exciting possibility, yet to be tested, that information arriving to YAP/TAZ from tissue structure and architecture through the cytoskeleton may be permissive for regulations from other cascades.

Clinical–Translational Advances

Several lines of evidence indicate that human cancers hijack the potent biologic properties of YAP and TAZ to foster their own growth. For example, a *YAP1*-containing amplicon has been reported in hepatocellular carcinomas and, at low frequency, in a wide spectrum of tumors (22, 47); and YAP can be found in the nucleus (active) at high frequency in hepatocellular, ovarian, colon, and non–small cell lung cancers by immunohistochemistry (20, 22).

Beside this correlative evidence, recent studies have highlighted a critical role for TAZ as a primary molecular determinant of several biologic traits typically associated to cancer stem cells, at least in breast cancer (48). Indeed, TAZ is required for self-renewal and tumor initiation capacities of breast cancer cells, as measured by the capacity of cells to grow as self-regenerating mammospheres and to form tumors once cancer cells are injected at limiting dilutions in immunocompromised mice. Once TAZ levels are experimentally induced in more differentiated tumor cells, their tumor-initiating potential is awakened to generate high-grade, undifferentiated, and chemoresistant tumors. In primary human breast cancers, TAZ activation, as defined by anti-TAZ immunohistochemistry or by bioinformatic analyses of TAZ target genes, is associated with poor survival and metastasis and identifies tumors that are resistant to chemotherapy and with a high stem cell content (48, 49). It will now be important to extend these provocative findings to other tumor types and animal models. For example, TAZ is a master gene for mesenchymal glioblastomas, impacting on invasion, differentiation, and self-renewal (50). Another very exciting area of investigation is metastasis, as TAZ is elevated in high-grade breast cancer specimens (48) and YAP has been recently shown to be instrumental for the growth of overt metastatic lesions in animal models (51, 52).

Facing this compelling set of results, sporadic mutations in Hippo pathway components are surprisingly rare in human cancers (20, 22). This suggests that either the Hippo pathway is targeted by mechanisms other than direct mutation or that YAP/TAZ nuclear localization and activity are turned on by non-Hippo mechanisms in tumors. There is evidence suggesting that both scenarios may be at work. First, TAZ protein stabilization is an essential downstream effector of epithelial-to-mesenchymal transition (EMT) in breast cancer: TAZ is dispensable for expression of mesenchymal phenotypes but required for EMT-induced self-renewal, reduced differentiation, and tumorigenesis (48). Mechanistically, EMT is intrinsically associated to mislocalization of the Scribble apicobasal cell polarity determinant; indeed, EMT inhibits the normal function of Scribble as adaptor for TAZ and the Hippo kinases on the cell membranes, leading to TAZ phosphorylation and subsequent degradation (48, 53).

Second, YAP and TAZ are activated by signaling cascades other than Hippo. Recent data indicate that TAZ stability is under control of the same destruction complex, made of APC, Axin, and GSK3 that controls β-catenin stability (54). Inhibition of this complex by Wnt growth factors or direct

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inactivation of APC triggers TAZ stabilization. In turn, TAZ is essential to mediate Wnt biologic effects, including the proliferation of colorectal cancer cells. Moreover, TAZ mediates the activation of a substantial portion of Wnt targets (54). Reinforcing this Wnt connection in colon cancer, phosphorylation of YAP by the tyrosine kinase YES1 leads to localization of a YAP/β-catenin/TBX5 complex at the promoters of antiapoptotic genes (55).

Finally, aberrant geometry, composition, and stiffness of the ECM are important regulators of tumor growth and metastasis (4, 8). The normal mammary gland is soft, whereas tumors are stiff. For tumor cells, in particular those facing the reactive tumor stroma, mechanical stresses force a reshaping of the adhesion sites and cytoskeletal organization, and, as such, a change in cell shape. As a consequence, blunting the stiffening of the ECM can oppose cancer progression in experimental models (56, 57); moreover, tumor cells seem to "mechanically select" the sites of future metastatic growths through a "preconditioning" step, namely, through secretion of lysyl oxidases that increase the rigidity of the distant metastatic niches (58). As we mentioned, ECM rigidity and cell shape exerts an overarching control on YAP and TAZ (31, 42, 43); this raises the possibility, yet to be tested, that YAP and TAZ may serve as mediators of the effect of ECM rheology in tumors. Very recently, such a link has been proposed in cancer-associated fibroblasts (CAF), where YAP/TAZ are embedded in a feed-forward loop between ECM remodeling and the contractile phenotype of CAFs (59), likely reverberating on the activation of YAP/TAZ in the tumor itself.

YAP and TAZ as therapeutic targets in cancer

Much research is still required to validate YAP and TAZ as "princes of darkness" of tumor growth and cancer stemness. Yet, the available data do suggest the possibility that YAP/TAZ activation may be a common endpoint of pathways leading to malignant progression, a notion supported by functional validation in human breast cancer and glioblastoma (48, 50), as well as by the tumorigenic phenotypes of mouse *NF2/MST/SAV/MOB* knockouts and of humans carrying *NF2* germline mutations (22, 24, 25). Time is thus ripe to take advantage of Hippo/YAP/TAZ biology to envision potentially new therapeutic avenues in exploratory clinical trials.

Direct targeting of YAP and TAZ. The tumorigenic potential of YAP, and likely that of TAZ, requires association to TEAD (60). Liu-Chittenden and colleagues recently provided the proof-of-principle that disruption of the YAP/TEAD complex is indeed a valid therapeutic option. By screening a small-molecule library, they identified members of the porphyrin family as inhibitors of YAP/TEAD-dependent transcription. Intriguingly, one of these molecules, verteporfin (VP), is already in clinical use for the photodynamic therapy of macular degeneration. In animal models, administration of VP blunted liver overgrowth caused by YAP overexpression or by *NF2* knockout, without overt adverse effects in other organs (60).

Rho and Rock inhibitors. Rho and the F-actin cytoskeleton are fundamental to sustain YAP/TAZ function. For example, inhibition of Rho, or even a mild disruption of the actin cytoskeleton, leads to dramatic downregulation of YAP/TAZ transcriptional effects and inhibition of nuclear localization (31, 42, 43). Considering the fact that this occurs in a largely Hippo-independent manner (6), these discoveries opened new and exciting therapeutic scenarios, because they at least suggest that already existing Rho-inhibiting drugs may be exploited as anti-YAP/TAZ therapeutics. For example, bisphosphonates inhibit prenylation, a lipid modification required for membrane attachment of Rho and other small G-proteins. Bisphosphonates are already in use for palliative care of bone metastases. However, when used in the adjuvant setting, some compounds have provided provocative results preventing metastasis, with improved survival (61). Are these beneficial effects associated with attenuation of YAP and TAZ? While this question remains pending, another possibility to blunt YAP/TAZ function is represented by another set of enzymes regulating actin contractility, such as ROCK, MLCK, and non-muscle myosin itself. In particular, the ROCK inhibitor Y27632 and the myosin inhibitor blebbistatin have been shown to inhibit YAP/TAZ activity (31, 42). Although applications of Y27632 have been discontinued, other ROCK inhibitors are in clinical use for cardiovascular diseases (fasudil), with more potent inhibitors (AR-12286) being tested in phase III trials for glaucoma (62). Moreover, the possible use of fasudil to attenuate tissue fibrosis might be useful to interrupt the feed-forward effects between the tumor and the progressive stiffening of the stroma.

GPCR agonist and antagonists. In keeping with the pro-YAP/TAZ role of Rho (31), recent results indicate that agonists of some G-protein–coupled receptors (GPCR), such as the bioactive lipids lysophosphatidic acid (LPA) and sphingosine-1-phosphate (S1P), promote YAP/TAZ nuclear localization by sustaining Rho signaling (63). This suggests that antibodies or small-molecule agonists mimicking LPA or S1P stimulation, or activators of the enzymes that build them, may represent a therapeutic option for subsets of tumors addicted to GPCR activation.

WNT inhibitors. In tumors characterized by mutations that inactivate *APC*, such as colorectal tumors, TAZ levels and activity are elevated and contribute to the malignant phenotype. It follows that, as recently shown, another route for attenuating TAZ, and possibly YAP, is the use of tankyrase inhibitors (54). These drugs restore the activity of Axin, the limiting factor of the β-catenin–and TAZ–degradation complex (64).

Finally, we speculate that the YAP/TAZ and the Hippo pathway may indeed represent a valuable target for cancer therapy and that more opportunities will emerge as we learn more on the role of YAP/TAZ in organ growth and tumorigenesis. That said, cancer selectivity and toxicity might turn into a phenomenal challenge for potent inhibitors. It is thus possible that milder yet better-tolerated inhibitors may hit

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an important therapeutic window: cutting "the edge" of YAP/TAZ activity required for tumor and metastasis growth while sparing normal tissues. And some of these factors may already be on our shelves.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: S. Dupont
Writing, review, and/or revision of the manuscript: S. Piccolo, M. Cordenonsi, S. Dupont

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Stefano Piccolo, Michelangelo Cordenonsi and Sirio Dupont

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