EDITORIAL

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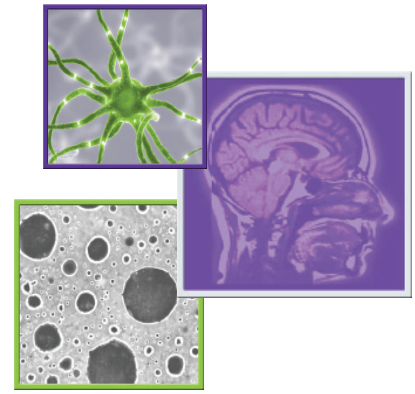
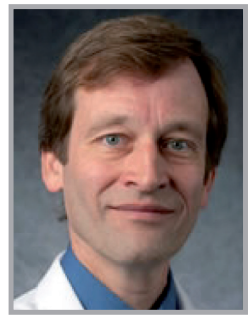


The Hippo signaling pathway and translational opportunities for brain cancers





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"...the evidence points to the Hippo pathway having an important role in driving tumor formation in multiple types of tumors."

The Hippo signaling pathway is a critical regulator of tissue homeostasis, organ size control and stem cell renewal [1]. The pathway exerts its tumor suppression activity by restraining cell proliferation and promoting apoptosis. Hippo and core pathway components were first identified in *Drosophila melanogaster* and are functionally conserved in mammals [1,2]. The mammalian Hippo pathway is comprised of a sequential phosphorylation cascade of MST1/2 and LATS1/2 that culminates with the phosphorylation and inactivation of YAP1 and TAZ. Cytoplasmic phospho-YAP1 and TAZ are then ubiquitinated and further degraded or bound to 14-3-3 leading to YAP1/TAZ cytoplasmic retention [3]. When the pathway is inactivated the transcription unphosphorylated co-activators YAP1/TAZ translocate to the nucleus, bind to TEAD1/4 transcription factors and promote the expression of key target genes that increase cell proliferation and prevent apoptosis [4].

Merlin/NF2 tumor suppressor protein is a cytoskeleton-binding protein that modulates the activity of the Hippo pathway [5]. Investigating the activity of YAP1 in the mouse liver, Zhang and colleagues defined a functional role between Merlin/NF2 tumor suppressor and the Hippo pathway. The inactivation of *Nf2* in the mouse liver led to YAP1 activation and to the formation of hepatocellular carcinoma [6]. Loss of *NF2* tumor suppressor gene is a well-characterized genetic alteration in several cancers, largely in cancers of the CNS [7], prompting the attention on the importance of the Hippo pathway in the development of these cancers. In meningiomas, loss of Merlin activity is also associated with nuclear localization and YAP1 activation, and leads to the development of cells with a characteristic transformed phenotype [8]. In addition, deregulation of the Hippo pathway seems to be associated with several human cancers not associated with *NF2* loss. Genetic and epigenetic mechanisms

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have been reported to drive the deregulation of the Hippo pathway [9,10]. Amplification and overexpression of YAP1 have been identified and are thought to be relevant oncogenic mechanisms [11,12]. Overall, the evidence points to the Hippo pathway having an important role in driving tumor formation in multiple types of tumors.

Hippo signaling pathway in brain cancers

The Hippo pathway, in particular the expression and activity of YAP1, has been investigated in adult and pediatric brain tumors. Orr *et al.* analyzed YAP1 expression and subcellular localization in 264 brain tumors [13]. Nuclear YAP1 expression was found to be heterogeneous in medulloblastomas and markedly prominent in high-grade gliomas. In addition, analysis of YAP1 expression in fetal and adult brain autopsy specimens showed high levels of YAP1 expression in the subventricular zone and in the external granular cell layer of the cerebellum. These findings suggest that YAP1 expression and activity might be essential for maintenance and expansion of neural stem cells. This also supports the hypothesis that the Hippo pathway plays an important role in controlling cell fate and survival in certain tumors of neural crest origin [13]. Furthermore, recent studies in medulloblastomas revealed high YAP1 expression in these tumors, in particular in sonic hedgehog-associated subtypes [14]. Importantly, Fernandez and colleagues demonstrated that YAP1 expression is upregulated by the sonic hedgehog signaling in proliferating cerebellar granule neural precursors, the proposed cells of origin for some medulloblastoma subtypes [14]. In meningiomas, YAP1 was found highly expressed and localized to the nucleus [15]. Moreover, it has been demonstrated that *NF2* loss confers proliferative advantage to meningioma cells, and that *YAP1* knockdown in NF2-mutant cells rescues, in part, the effect of Merlin loss on cell proliferation [8]. Data generated in our laboratory demonstrated that YAP1 expression induces a transformed phenotype in meningioma cells by modulating cell proliferation and motility, and by restraining cisplatin-induced apoptosis. Moreover, overexpression of YAP1 in non-neoplastic arachnoidal cells led to the development of tumor-like growth in athymic nude mice [15]. In summary, these studies demonstrate an oncogenic role for YAP1 in brain tumors.

> "...better understanding of the complex oncogenic functions of the pathway, cell-type specificities and tissue-specific transcriptional programs might give greater insight into the biological functions and novel cancer therapeutic targets within the Hippo pathway."

Hippo signaling pathway & cancer stem cells

The stem cell concept has been developed over recent years and it is defined by the existence of a small subpopulation of undifferentiated progenitor cells, confined to organ-specific compartments and accountable for cell fate decisions and renewal, as well as organ size control [16,17]. Evidence supports the hypothesis that the Hippo cascade is one of the pathways responsible for modulating tissue homeostasis by controlling stem cell proliferation and expansion [2]. For instance, it has been observed that YAP1 expression is markedly intense in precursor cells from small intestine crypts, and that YAP1 expression induces a loss of differentiation and expansion of these progenitor cells [2]. Similarly, YAP1 expression was observed in mouse single-layered basal epidermal progenitors and seemed to be responsible for the undifferentiated state of these epithelial progenitors [18]. Importantly, YAP1 expression and activity has also been observed in cancer stem cell precursors, including brain cancers [14]. Considering the fact that cancer stem cells are thought to be the cells with greater capacity for tumor repopulation and expansion and are involved in drug resistance [16], it is reasonable to speculate that targeting the Hippo pathway will be relevant for treating certain cancers.

Targeting the Hippo signaling pathway for cancer treatment

Recent studies have demonstrated that strategies targeting YAP1 activity might indeed prove to be useful for therapeutic purposes. Using cell-based assays, Bao *et al.* found that dobutamine, a β-adrenergic receptor agonist, induces cytoplasmic retention of YAP1 by a mechanism that is yet to be determined [19]. Additionally, *in vitro* treatment with dobutamine was shown to increase phospho-S127 of YAP1 and to inhibit YAP1-dependent TEAD reporter activity [19]. This might suggest the use of dobutamine to inactivate YAP1, although further characterization and *in vivo* testing will be necessary to clarify the efficacy of dobutamine in targeting YAP1. More recently, Liu-Chittenden *et al.* identified compounds of the porphyrin family that potently inhibited YAP1–TEAD interaction, therefore inhibiting the transcriptional activity of this complex [20]. One of the porphyrin compounds, verteporfin, inhibited YAP1 oncoprotein activity in mouse models, generated by either inducing

YAP1 overexpression or by deletion of the *Nf2* gene in the liver. These studies showed that verteporfin was efficacious in inhibiting YAP1 transcriptional activity, therefore preventing tissue overgrowth in both models, but with no evident effects on liver homeostasis [20]. If the efficacy of verteporfin in other tumor models is confirmed, especially in shrinking pre-established tumors, therapeutic strategies using porphyrin compounds might be very attractive for certain tumors driven by YAP1 oncogenic activity.

In conclusion, the Hippo tumor suppressor signaling pathway is deregulated in a variety of human cancers. Progress has been made in identifying the major players in the pathway and its functionality, although key points are still to be elucidated. For instance, better understanding of the complex oncogenic functions of the pathway, cell-type specificities and tissue-specific transcriptional programs might give greater insight into the biological functions and novel cancer therapeutic targets within the Hippo pathway.

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