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JB Commentary

# Exploration of a new drug that targets YAP

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**Yes-associated protein (YAP) has been shown to play a critical role in the growth of various tumours. Phosphorylation of Ser127 of YAP leads to the inhibition of YAP translocation into nucleus and subsequent failure to regulate the expression of target genes that induce cell proliferation. Chemical manipulation of YAP localization or expression may provide an efficient method for cancer treatment. In a recent work published by Bao *et al.* (*J. Biochem.* 2011;150:199–208), various compounds were screened in human osteosarcoma cells that stably express Green Fluorescent Protein-labeled YAP by monitoring subcellular localization of GFP-YAP. Using this cell-based assay, they found that dobutamine, a β-adrenergic receptor agonist, attenuated YAP-dependent transcription by inhibiting its nuclear translocation. The authors suggest dobutamine as a possible drug for cancer treatment.**

*Keywords*: β-Adrenergic receptor/dobutamine/Hippo signaling/osteosarcoma cells/Yes-associated protein (YAP).

*Abbreviation*: AREG, amphiregulin; CTGF, connective tissue growth factor; GFP, Green Fluorescent Protein; TEAD, TEA-domain family member; YAP, Yes-associated protein.

Recent intensive studies of Hippo signaling and its downstream target, Yes-associated protein (YAP), revealed that this pathway regulates cell growth and organ size and plays a critical role in human disease. Hippo signaling components, including Hippo, Salvador and Warts, were originally identified in *Drosophila* and were the subject of previous reviews (*1*–*3*). Homologues of these components are highly conserved in mammals and include MST1/2 (mammalian Ste20-like serine/threonine kinase 1/2; homologous to *Drosophila* Hippo) and LATS1/2 (large tumor suppressor 1/2; homologous to *Drosophila* Warts). Proteins such as Merlin (encoded by the neurofibromatosis Type 2 gene; *NF2*) were found to activate Hippo signaling in mammals. This pathway responds to cell density and regulates YAP phosphorylation and localization. At low cell density, YAP is predominantly found in the nucleus, whereas at high density it is translocated to the cytoplasm (*4*) following phosphorylation at Ser 127 by LATS, leading to attenuated transcription of YAP target genes. Conversely, dephosphorylation of YAP leads to its nuclear accumulation. Mutation of Ser 127 (S127A) prevents YAP phosphorylation and allows its continued nuclear localization (*4*).

Although YAP does not directly bind to DNA, it works as a transcriptional co-activator in the nucleus by associating with the TEA-domain family member (TEAD) (*5*), which has a DNA-binding domain and regulates the expression of target genes, such as connective tissue growth factor (CTGF) (*6*, *7*), amphiregulin (AREG) (*8*) and cyclin D1 (*9*), thereby inducing cell proliferation. Thus, nuclear localization of YAP can determine cell fate in development and disease.

Owing to its significant role as a tumor suppressor, disturbance in NF2 or Hippo pathway components has often been linked to cancer. Mutation or deletion of NF2 was found in various sporadic tumors of the nervous system, including almost all schwannomas, 50–60% of meningiomas and 30% of ependymomas (10). Genetic inactivation of *NF2*, *SAV1* (*Salvador 1*) and/or *LATS2* was observed in 75% of malignant mesothelioma cells (*11*), and YAP nuclear translocation was observed in malignant mesothelioma cells both *in vitro* and *in vivo* (*7*, *11*).

Amplification of the *YAP* gene locus 11q22 has been reported in several human cancers including ependymoma (*12*), hepatocellular carcinomas (*13*), malignant mesothelioma (*14*) and esophageal squamous cell carcinomas (*15*). Moreover, YAP overexpression has been reported in tumors of the colon, lung, ovary (*16*), squamous cells (*17*), liver and prostate (*4*, *18*). Indeed, mouse models demonstrate that MST1/2 deficiency in the liver results in loss of YAP Ser127 phosphorylation, uncontrolled liver growth and development of hepatocellular carcinoma (*19*). Observations of activated YAP in the skin of transgenic mice carrying a YAP (S127A) mutation, which induces nuclear translocalization, revealed that YAP activation can lead to abnormally thick epidermis, hyper-keratinization and tumor formation (*20*). These data suggest that YAP expression and nuclear localization strongly influence cell proliferation and tumor promotion. Hence, inhibition of YAP nuclear translocation and prevention of target gene transcription may prevent tumor progression.

In a recent report, Bao *et al.* (*21*) established a cell-based fluorescence assay using stable transfection of GFP-labeled YAP to monitor its subcellular localization in human osteosarcoma U2OS cells following treatment with various chemical agents. In U2OS cells, GFP-YAP was evenly distributed in the cytosol and in the nucleus at low cell density, but accumulated in the cytosol at high density, as observed in other

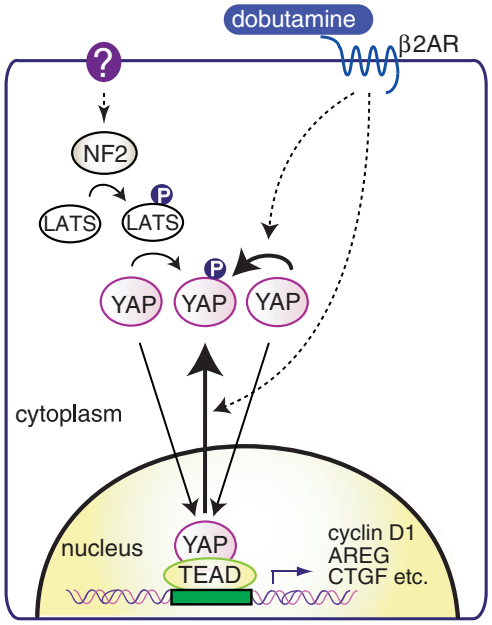


**Fig. 1** Schematic model of the regulation of YAP subcellular localization in U2OS cells. Dobutamine binds to β-adrenergic receptors (β2AR) and promotes phosphorylation of YAP at Ser127. Subsequently, YAP is translocated into the cytoplasm through mechanisms that are independent of Hippo signaling or the Akt pathway. β2AR, β-adrenergic receptors.

types of cells (*4*). This study confirmed that Hippo signaling could regulate the GFP-YAP localization in U2OS cells. This was indicted by accumulation of GFP-YAP in the cytosol following $H_2O_2$-dependent activation of MST kinase and nuclear accumulation of YAP due to knockdown of LATS1/2.

Forty-eight chemical compounds were screened by monitoring cytoplasmic translocation of GFP-YAP. Among these, dobutamine was the most effective at inducing phosphorylation of YAP at Ser127 and subsequent cytoplasmic translocalization. Dobutamine is an agonist of the G-protein-coupled β-adrenergic receptor and is mostly used in the treatment of congestive heart failure and low cardiac output. A recent report shows that stimulation of acute β-adrenergic receptors significantly induced the PI3K/Akt pathway in mouse heart, but not in lungs or livers (*22*). Bao *et al*. have shown that the β-adrenergic receptor is responsible for dobutamine-induced translocation of GFP-YAP to the cytoplasm through phosphorylation of YAP at Ser127 (Fig. 1) and inhibition of YAP-dependent gene transcription in both U2OS cells and HEK293FT cells (*21*). However, dobutamine did not cause phosphorylation of LATS1/2 or Akt in U2OS cells, suggesting that it acts independently of the Hippo signaling pathway. Hence, this work indicates a novel β-adrenergic receptor-mediated pathway for YAP inactivation.

Recently, a growing number of proteins have been related to the Hippo signaling cascade. Since YAP is a practical effector that is translocated into the nucleus and *trans*-activates target genes, removing YAP from the nucleus is theoretically the most efficient way to suppress its action regardless of its dependency on the Hippo signaling cascade. This is the first work to identify a single drug treatment that induces YAP translocation to the cytoplasm and that offers clinical potential to treat specific cancer types that use disturbance of Hippo signaling and/or overexpression of the YAP as the main route of cell growth. Further studies are urgently required to determine whether the dopamine-β-adrenergic receptor pathway suppresses development of tumors in cell types that regulate cell growth through the Hippo signaling pathway and/or YAP. Since intracellular signaling activated by the dobtamine-β-adrenergic receptor pathway has not been clarified, exploring the mechanism of YAP phosphorylation through this pathway may help in elucidating the function of this drug.

This work established a cell-based assay system for screening a large number of drugs and compounds efficiently. Since this screening assay is based on simple monitoring of the subcellular localization of YAP, this method may also be effective in exploring the presence of other mechanisms that regulate YAP localization even without phosphorylation at Ser127. The authors are continuing to screen compounds that have potential to inhibit tumor progression using their U2OS-GFP-YAP assay system. The identification of new compounds that inhibit YAP activation or stimulate the Hippo pathway may provide as new drug inventions for cancer therapy and further comprehension of the related signaling pathways.

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**Conflict of interest**
None declared.

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