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Authors

Yuan, Hai-Xin

Guan, Kun-Liang

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The Sin1 PH domain connects mTORC2 to PI3K

Hai-Xin Yuan¹ and Kun-Liang Guan²

¹Institutes of Biomedical Sciences, Shanghai Medical College, Fudan University, Shanghai 200032, China

²Department of Pharmacology and Moores Cancer Center, University of California San Diego, La Jolla, CA 92093

Summary

The mechanistic target of rapamycin (mTOR) complex 2, mTORC2, is a critical downstream effector of PI3K to stimulate AGC kinase members, including Akt, PKC, and SGK. Liu et al reported that the PH domain of Sin1, an essential component of mTORC2, directly binds the PI3K product PtdIns(3,4,5)P₃ to promote mTORC2 kinase activation and membrane localization, thereby revealing a mechanistic link between PI3K and mTORC2.

The mechanistic target of rapamycin (mTOR) is a central cell growth modulating kinase in all eukaryotes. mTOR exists in two different complexes, mTORC1 and mTORC2, which are distinguished by unique accessory protein Raptor and Rictor, respectively. In addition, Sin1 is also a component uniquely present in the mTORC2 and is required for mTORC2 complex formation and function (1–3). Compared to the well-defined regulation and function of mTORC1, mTORC2 is less understood. Akt/PKB, a member of the AGC family kinases, is a key signaling hub in the PI3K pathway and regulates multiple cellular processes such as growth, proliferation, metabolism, and survival. Akt is one of the most important substrates of mTORC2, which promotes Akt activation by directly phosphorylating its hydrophobic motif (Ser473), a site required for AKT maximum activation (4). Phosphorylation at Ser473 primes Akt for further phosphorylation at Thr308 in the catalytic domain by phosphoinositide-dependent protein kinase 1 (PDK1), and thus leading to full activation of Akt. mTORC2 also phosphorylates other AGC kinases, including serum- and glucocorticoid-regulated kinase (SGK) (5) and protein kinase C (PKC) (6). It is generally accepted that mTORC2 is activated by growth factors via PI3K signaling, however, the precise molecular mechanism of mTORC2 activation remains elusive thus far.

Accumulating evidence suggests that Sin1 may play a key role in the regulation of mTORC2. It was found that alternatively spliced Sin1 isoforms in the cell defines distinct mTORC2 pools; of these, only two are regulated by insulin (1). This suggests that Sin1 may function as a mediator between growth factor signaling and mTORC2. Sin1 contains a

Corresponding Author: Kun-Liang Guan, Department of Pharmacology and Moores Cancer Center, University of California San Diego, La Jolla, CA 92093. Phone 858-822-7945; kuguan@ucsd.edu.

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phospholipid-binding pleckstrin homology (PH) domain that may facilitate the association of mTORC2 with membranes (7).

Recently, Wei and colleagues reported that mTORC2 complex is directly regulated by mTORC1-S6K axis through Sin1 phosphorylation (Fig.1) (8). Phosphorylation of Sin1 at Thr86 and Thr398 by either S6K or Akt dissociates Sin1 from mTORC2, thus resulting mTORC2 inhibition. Importantly, the authors also demonstrated a link between Sin1 phosphorylation and a cancer-derived mutation R81T in Sin1, providing clinical significance of Sin1 phosphorylation in cell growth control. R81T mutation attenuates Sin1 Thr86 phosphorylation and sustains mTORC2 activity and Akt Ser473 phosphorylation upon physiological stimulation, thus bypassing Sin1 phosphorylation-mediated negative regulation of mTORC2 activity in response to upstream PI3K activation (Fig.1) (8).

In the current issue of Cancer Discovery, Liu et al from the same group reported a direct molecular mechanism through which Sin1 regulates mTORC2 kinase activity by PI3K signaling (Fig.1) (9). Although Sin1 is required for mTORC2 integrity and kinase activity, surprisingly the authors found that the PH domain of Sin1 actually binds to mTOR kinase domain and inhibits mTOR activity towards Akt Ser473 phosphorylation. Overexpression of Sin1-PH domain is sufficient to suppress the phosphorylation of Akt Ser473. Interestingly, substitution of Akt-PH with Sin1-PH was able to functionally reconstitute the phosphorylation of Akt Ser473, indicating that the Sin1-PH domain shares biochemical property similar to the Akt PH domain capable of binding PtdIns(3,4,5) P_3 .

Inspired by the mechanism of PtdIns(3,4,5) P_3 -mediated Akt activation, the authors indeed demonstrated that PtdIns(3,4,5) P_3 , a product of PI3K at plasma membrane, is able to interact with Sin1-PH domain (Fig.1). By comparing the Akt1-PH/Ins(1,3,4,5) P_4 crystal structure with a computer modeling of Sin1-PH/Ins(1,3,4,5) P_4 structure, the authors identified three critical residues including R395, K428 and K464 that mediate PtdIns(3,4,5) P_3 binding to Sin1-PH. Mechanistically, increased PtdIns(3,4,5) P_3 levels upon insulin or growth stimulation compete with mTOR kinase domain in binding to Sin1-PH, and thus release the Sin-PH inhibition on the mTORC2 activity. Moreover, PtdIns(3,4,5) P_3 association with Sin1-PH is also responsible for the recruitment of mTORC2 complex to plasma membrane where mTORC2 can phosphorylate the membrane associated Akt or other physiological substrates.

The significance of this study is further expanded by genetic analysis of cancer-associated mutations in the Sin-PH domain. Analysis of TCGA database revealed several somatic mutations in the Sin1-PH domain. Biochemical characterizations showed that these cancer-associated mutations compromise Sin1-PH binding to mTOR kinase domain, thereby leading to increased mTORC2 activity towards Akt-Ser473 phosphorylation (Fig.1). Among these mutations, D412G shows the most robust enhancement on mTORC2 activity even under non-stimulation conditions, thus identifying a key residue important for interaction with mTOR kinase domain. Interestingly, the cancer mutation analyses show that D412G mutation and R81T mutation, which compromises Sin1 Thr86 phosphorylation (8), are mutually exclusive with either *Akt1* gene amplification or *PIK3CA* oncogenic mutations (9).

Collectively, these observations support a notion that mutations in PI3K, Akt, and Sin1 affect a common pathway important for cancer development.

Finally, compared to Sin1-WT, introduction of Sin1-D412G mutant into ovarian cancer cells that is depleted of endogenous Sin1 significantly increased Akt phosphorylation. These cells also show stronger oncogenic features, such as resistance to apoptosis-inducing and chemotherapeutic drugs, enhanced colony formation ability, and increased tumor growth in xenograft. Given that Sin1 phosphorylation is also compromised by a cancer-patient-derived mutation R81T, these findings provide important clues for Sin1 dysregulation in tumorigenesis. Further genetic and biochemical studies are needed to elucidate the mechanisms and relevant contribution of Sin1 mutations in different cancer types.

In summary, Sin1 can regulate mTORC2 in multiple ways: (i) it helps mTORC2 complex formation (1–3); (ii) it is responsible for recruiting certain mTORC2 substrates, including Akt and SGK (10); (iii) its PH domain directly receives the PI3K signal to activate mTORC2 (9); (iv) its PH domain recruits mTORC2 to plasma membrane (9). Notably, Sin1 regulates mTORC2 activity in both positive and negative manners. The switch between negative and positive is dictated by PtdIns(3,4,5) P_3 . Upon activation of PI3K, binding of the Sin1-PH domain by PtdIns(3,4,5) P_3 not only relieves its inhibition on the mTOR kinase but also promotes mTORC2 translocation to plasma membrane for phosphorylation of its physiological substrates. The current study together with previous results establishes Sin1 as a critical signaling integrator for mTORC2 activity.

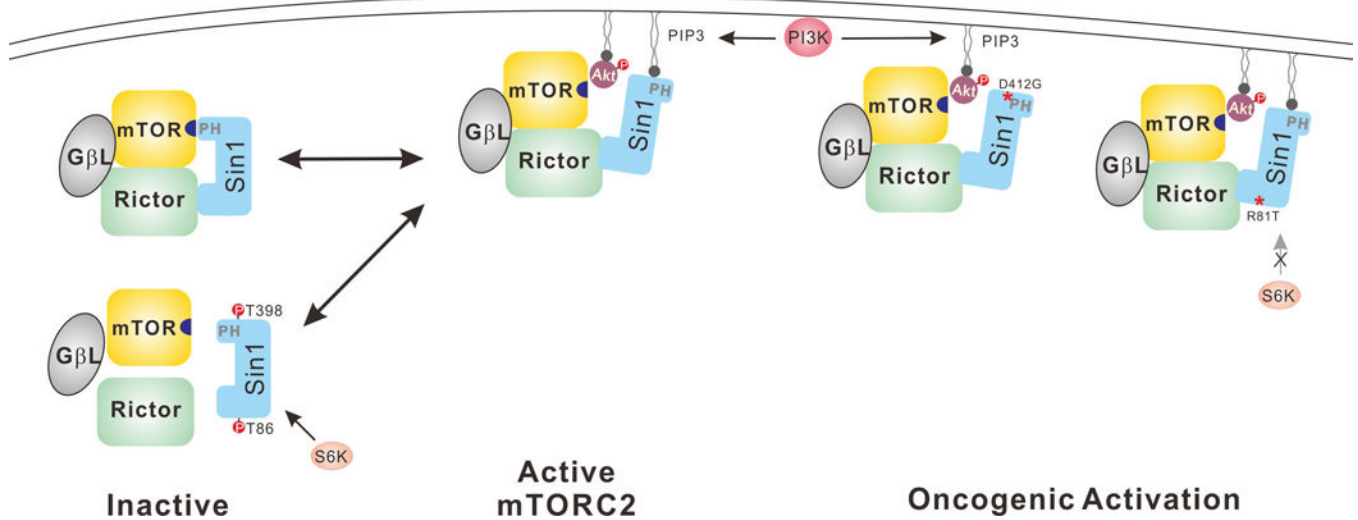
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**Figure 1.**

Dual role of Sin1 in mTORC2 regulation. In the absence of stimulation, the PH domain of Sin1 binds to and suppresses mTORC2 activity. Upon PI3K activation, the increased PtdIns(3,4,5) P_3 binds to Sin1 PH domain, leading to membrane recruitment as well as relieve of inhibition of mTORC2 by Sin1. The active mTORC2 phosphorylates and activates the membrane associated Akt. Sin1 can be phosphorylated by S6K and Akt to constitute a negative regulation. Cancer associated mutations in Sin1 can potentiate mTORC1 activity by disrupting the inhibitory phosphorylation in Sin1 or interaction with mTOR.