KIAA1524/CIP2A promotes cancer growth by coordinating the activities of MTORC1 and MYC

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KIAA1524/CIP2A/cancerous inhibitor of protein phosphatase 2A is a cancer-promoting protein that stabilizes the MYC proto-oncogene protein by inhibiting its dephosphorylation. Our recent report demonstrates that KIAA1524/CIP2A supports cancer cell growth also at the level of the mechanistic target of rapamycin complex 1 (MTORC1), a key signaling module that drives cell growth by stimulating protein synthesis and inhibiting autophagy. KIAA1524/CIP2A suppresses MTORC1-associated protein phosphatase 2A (PP2A) activity in an allosteric manner thereby stabilizing the phosphorylation of MTORC1 substrates and keeping the cell in an anabolic mode. In the absence of growth stimulating signals or nutrients, reduced MTORC1 activity triggers SQSTM1/p62-dependent autophagic degradation of KIAA1524/CIP2A enhancing the PP2A-mediated dephosphorylation of MTORC1 substrates and MYC. Thus, KIAA1524/CIP2A emerges as an oncprotein that can coordinate the growth-promoting activities of MTORC1 and MYC in response to environmental and intrinsic cues.

Autophagy, a process of lysosome-dependent self-eating, is a key actor in the maintenance of cellular homeostasis. MTORC1 kinase serves as a signaling nexus that integrates information regarding the availability of nutrients and growth factors to the maintenance of the appropriate balance between autophagy and protein synthesis. Contrary to our growing knowledge of autophagy-regulating kinases, the role of phosphatases in this process has remained largely unexplored. Our recently published RNAi-based phosphatome screens identified 61 negative and 17 positive regulators of autophagosome accumulation. These data will hopefully stimulate the research in phosphatases in autophagy signaling.

Prompted by 4 PP2A-related genes identified in our screens and PP2A’s prominent role as a tumor suppressor, we focused our subsequent studies on this abundant Ser/Thr protein phosphatase. PP2A is composed of a scaffolding A-subunit (PPP2R1A or PPP2R1B), a catalytic C-subunit (PPP2CA or PPP2CB), and a variable substrate-determining B-subunit that together generate numerous trimeric PP2A holoenzymes with spatially and temporally determined specific functions. In line with independent functions of distinct PP2A holoenzymes, our data demonstrated that PP2A acts both as a positive and a negative regulator of autophagy depending on the composition of the holoenzyme. Adding to the complexity of the PP2A function, PP2A holoenzymes can also associate with various inhibitory proteins. One such inhibitory protein, the KIAA1524/CIP2A oncoprotein, was identified as a potent autophagy inhibitor in our screen. Notably, KIAA1524/CIP2A is not a universal PP2A inhibitor but acts only in context with a limited number of phosphorylated PP2A substrates, such as MYC, E2F1, AKT, and DAPK1 (death-associated protein kinase 1; Fig. 1).

Our analysis identified MTORC1-associated PP2A as the autophagy-regulating target of KIAA1524/CIP2A. Immunoprecipitation studies reveal a strong PP2A-dependent association of...
KIAA1524/CIP2A and MTORC1, and immunocytochemistry shows a partial colocalization of KIAA1524/CIP2A and MTORC1 in MCF7 breast cancer cells. Supporting the activity of KIAA1524/CIP2A in the MTORC1-PP2A complex, the massive accumulation of autophagosomes and increased autophagic flux in KIAA1524/CIP2A-depleted cancer cells is associated with a significant reduction in the phosphorylation of well-established MTORC1 target sites in RPS6KB1 and EIF4EBP1 without notable changes in the phosphorylation status of AKT. KIAA1524/CIP2A protein expression shows also a highly significant positive correlation with phosphorylated RPS6KB1 in an extensive primary breast cancer tissue microarray. These data strongly support the enhancement of MTORC1 signaling as a mechanism by which KIAA1524/CIP2A promotes cancer growth and inhibits autophagy. It remains to be studied whether KIAA1524/CIP2A and PP2A regulate the activity of MTORC1 directly or merely by controlling the phosphorylation status of its substrates. To this end, it is interesting to note that 2 recent papers show that lack of amino acids triggers a rapid deactivation of MTORC1 by a mechanism involving the recruitment of its negative regulator, the tumor suppressor complex TSC1-TSC2. Thus, KIAA1524/CIP2A may be essential in defining the kinetics of the subsequent PP2A-mediated deactivation of the MTORC1 substrates.

In order to enlighten the molecular basis of the KIAA1524/CIP2A-mediated inhibition of PP2A, we analyzed the activity of MTORC1-associated PP2A toward an artificial phosphopeptide substrate following amino acid starvation or KIAA1524/CIP2A depletion. Amino acid starvation, which led to an almost complete dephosphorylation of MTORC1 substrates, failed to displace KIAA1524/CIP2A from the MTORC1-PP2A complex and increased MTORC1-bound PP2A activity only marginally. Similarly, MTORC1-associated PP2A activity was not affected by KIAA1524/CIP2A depletion in spite of a marked dephosphorylation of MTORC1 substrates in KIAA1524/CIP2A-depleted cells. These data do not support the suggested ability of KIAA1524/CIP2A to function as a direct inhibitor of PP2A. An indirect mechanism was further supported by the existence of abundant PP2A activity in KIAA1524/CIP2A immunoprecipitates and the inability of recombinant KIAA1524/CIP2A to inhibit the activity of purified PP2A holoenzyme in vitro. As an unstructured scaffold protein, KIAA1524/CIP2A is thus likely to modulate the MTORC1-associated PP2A activity in an allosteric manner rather than as a direct inhibitor.

Our studies also revealed an interesting KIAA1524/CIP2A-regulated positive feedback loop where the KIAA1524/CIP2A-mediated enhancement of MTORC1 activity stabilizes KIAA1524/CIP2A protein, thereby further enhancing MTORC1 activity (Fig. 1). The inhibition of MTORC1 by rapamycin or amino acid deprivation leads to a rapid and selective autophagic degradation of KIAA1524/CIP2A, which depends on ULK1 kinase, the SQSTM1 autophagy receptor and lysosomal function. Consistently, KIAA1524/CIP2A co-immunoprecipitates with SQSTM1 and colocalizes with LC3- and SQSTM1-positive vesicles especially when autophagic flux is impaired by lysosomal inhibitors. In line with the reported ubiquitin-dependent target recognition by SQSTM1, ubiquitinated KIAA1524/CIP2A accumulates in cells upon inhibition of autophagic flux. The enzymes involved and the functional significance of KIAA1524/CIP2A ubiquitination in autophagy-mediated degradation of KIAA1524/CIP2A remain to be confirmed. It is, however, interesting to

Figure 1. Proposed model for the regulation of tumor growth by KIAA1524/CIP2A and autophagy. KIAA1524/CIP2A inhibits PP2A-mediated dephosphorylation of MTORC1 substrates RPS6KB1 and EIF4EBP1 thereby enhancing the MTORC1 signaling pathway. Together with previously reported effects of KIAA1524/CIP2A on stabilization of MYC, activation of E2F1 and AKT as well as inhibition of DAPK1, KIAA1524/CIP2A enhances tumor cell growth by stimulating protein synthesis, cell metabolism and proliferation as well as inhibiting apoptosis. Conversely, MTORC1 inhibition leads to autophagic degradation of KIAA1524/CIP2A and reversal of its tumor-promoting activities.
note that STUB1/CHIP ubiquitin ligase was recently identified as a KIAA1524/CIP2A-associated protein making it a strong candidate for a KIAA1524/CIP2A E3-ligase. The identification of KIAA1524/CIP2A as a selective autophagy target provides a plausible explanation for previously reported KIAA1524/CIP2A downregulation in response to the rapamycin analog temsirolimus, proteasome inhibitors bortezomib and MG132, the EGFR inhibitor erlotinib, the TOP/topoisomerase inhibitor etoposide and celastrol, all of which are potent autophagy inducers.

Taken together, we uncovered enhanced MTORC1 activity as a novel mechanism by which KIAA1524/CIP2A promotes tumor growth, and the subsequent inhibition of autophagy as a positive feedback loop that stabilizes KIAA1524/CIP2A. These data should be carefully considered in the context of autophagy inhibiting cancer therapies that may result in the accumulation of KIAA1524/CIP2A and enhanced cancer growth via KIAA1524/CIP2A-mediated enhanced phosphorylation of its cancer-promoting targets (Fig. 1).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.