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# Anchoring proteins encounter mitotic kinases

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**Abbreviations:** AKAP, A-kinase anchoring protein; SSeCKS, src-suppressed C-kinase substrate; Plk1, Polo-like kinase 1; CDK1, cyclin-dependent kinase 1; PKC, protein kinase C; FISH, fluorescence in situ hybridization

Defective progression of cells through mitosis permits an accumulation of genetic defects that lead to aneuploidy. Cells in a state of aneuploidy acquire mutations that eventually cause their transformation from normal cells into cancer.[1] Recent evidence suggests that perturbing the spatial and temporal pattern of cell division can enhance such oncogenic states. This can occur at the level of anchoring and scaffolding proteins that target signaling enzymes, such as protein kinases and phosphatases to specific sites within cells.[2] One example is the A-kinase anchoring protein Gravin (also known as AKAP12, AKAP250 or SSeCKS), which is believed to participate in the modulation of signaling events directing cell division and cellular transformation.[3] Recently, we discovered a complex of Gravin and the mitotic kinase Plk1 that controls aspects of mitotic progression and genomic stability[4] (**Fig. 1**).

We first asked what effect depletion of Gravin had on tumor formation in vivo. Immunodeficient mice were injected subcutaneously with human glioma cells stably expressing control shRNA or a pool of shRNAs targeting the anchoring protein, and tumor volume was measured over time. Although the results were subtle, we found that tumors depleted of Gravin grew to a larger volume than their control counterparts. Importantly, we also noted a disproportionate increase in mitotic cells within the Gravin shRNA tumors. This led to the notion that depletion of the anchoring protein was causing cells to become stalled in mitosis. Consequently, we explored the possibility that Gravin played direct roles in mitotic progression. In keeping with this postulate, a phosphoproteomic screen revealed that Gravin is phosphorylated by the central mitotic kinase, CDK1/Cyclin B1, on numerous residues both in vitro and inside cells. One highly conserved residue, Thr766, conforms to a canonical phosphorylation site for this kinase. We generated a phospho-specific antibody against Thr766 and validated that the antibody only detected the anchoring protein when phosphorylated at this residue.

Using this new tool, we examined the phosphorylation of the anchoring protein as cells synchronously progressed from $G_1$/S into mitosis. Gravin was phosphorylated concurrently with the mitotic marker phospho-Ser10-histone H3, and phospho-Thr766 Gravin localized to the centrosomes and spindle in dividing cells. This result was confirmed biochemically by showing that phospho-Gravin was enriched in isolated mitotic spindles and co-purified with the mitotic kinase, Polo-like kinase 1 (Plk1). Since Plk1 plays key roles in mitotic entry and progression via phosphorylation-dependent interaction through its Polo-box domain[5] (PBD), we were intrigued by the possibility that Gravin could scaffold the kinase. We established that Gravin and Plk1 form a complex in mitotic cells that requires priming phosphorylation by CDK1/Cyclin B1 on Gravin Thr766. Furthermore, we showed that the Gravin and Plk1 co-localize in the nucleus during prophase and at the centrosomes/mitotic spindle during metaphase. This interaction appears to be transient in nature, since Plk1 redistributes to the midzone during anaphase, while phospho-Thr766 Gravin remains localized at the centrosome.

We next wanted to examine the effect of Gravin depletion or disruption of the Gravin-Plk1 complex on mitotic progression and genomic integrity. Using live-cell imaging, spindle profiles and FISH analysis, we found that cells depleted of Gravin exhibit mitotic defects, including misalignment of chromosomes, prometaphase stall and aneuploidy. We believe that this prometaphase stall may explain the significant increase in mitotic cells observed in Gravin knockdown tumors during our xenograft assay. Interestingly, we also found that both knockdown of the anchoring protein and disruption of Gravin-Plk1 binding using a Gravin-Thr766Ala mutant resulted in decreased cell proliferation using growth curves and colony-formation assays. Taken together, these results suggest that disruption of the Gravin-Plk1 complex impacts mitotic progression.

Since knockdown of Gravin leads to aneuploidy, we anticipated that the anchoring protein would be downregulated in primary tumors. Consistent with this postulate, we showed that Gravin mRNA and protein levels were decreased within highly aneuploid clinical sections of human glioblastoma multiforme. This data suggest that the levels of the anchoring protein may vary with the degree of tumor aneuploidy. Despite reduced levels of the total protein, the remaining Gravin was robustly phosphorylated at Thr766 and co-localized with both phospho-histone H3 and Plk1. Previous reports have documented that increased levels of Plk1 are a poor prognostic indicator in numerous cancers.[6] Our results raise the possibility that levels of the Gravin/Plk1 complex may be linked to tumor aggressiveness in glioblastoma multiforme and could therefore provide additional prognostic value.

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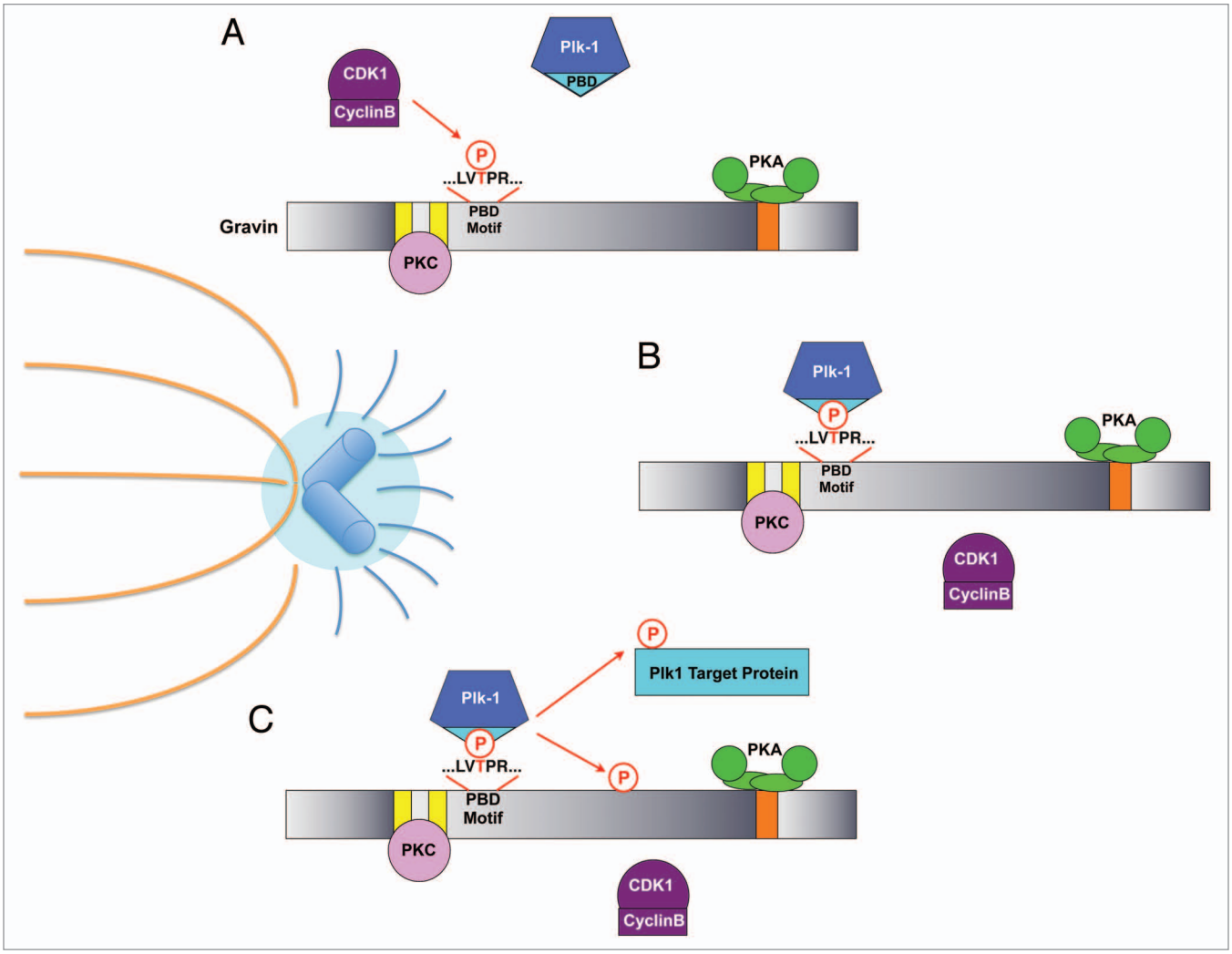


**Figure 1.** Schematic model illustrating the Gravin-Plk1 complex assembly during mitosis. Gravin (gray) is a 1781 amino acid protein capable of anchoring PKA (green), PKC (pink) and Plk1 (dark blue). (**A**) During mitosis, CDK1/Cyclin B1 phosphorylates Gravin at numerous residues, including Thr766. (**B**) Phosphorylation of ThrT766 leads to the recruitment of Plk1, which engages Gravin via its polo-box domain (PBD; light blue). (**C**) Active Plk1 is now able to phosphorylate appropriate target substrates, possibly including Gravin itself. The centrosome (blue) and mitotic spindle (orange) are also depicted.

An emerging notion is that anchoring and scaffolding proteins are valid therapeutic targets. For example, we recently demonstrated that AKAP79/150 modifies the pharmacological profile of anchored PKC.[7] This study demonstrates that AKAPs play previously unrecognized roles in scaffolding kinases that control mitotic entry, and that these signaling complexes may be altered in certain cancers. Moreover, there is considerable interest in Plk1 as a therapeutic target, as its inhibition can block the uncontrolled cell division that is prevalent in tumors. One potential target for the next generation of Plk1 inhibitors could be the enzyme when associated with Gravin, as a means to selectively manage facets of uncontrolled mitotic progression and genomic integrity. Thus, targeting the Gravin-Plk1 complex could provide a new means to regulate aberrant mitotic kinase activity.

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