Molecular Pathways: Regulation and Targeting of Kinetochores-Microtubule Attachment in Cancer

Jacob A. Herman\textsuperscript{1}, Chad M. Toledo\textsuperscript{2,3}, James M. Olson\textsuperscript{4}, Jennifer G. Deluca\textsuperscript{1}, and Patrick J. Paddison\textsuperscript{2,3}

Abstract

Kinetochores are large protein structures assembled on centromeric DNA during mitosis that bind to microtubules of the mitotic spindle to orchestrate and power chromosome movements. Deregulation of kinetochores-microtubule (KT-MT) attachments has been implicated in driving chromosome instability and cancer evolution; however, the nature and source of KT-MT attachment defects in cancer cells remain largely unknown. Here, we highlight recent findings suggesting that oncogene-driven changes in kinetochore regulation occur in glioblastoma multiforme (GBM) and possibly other cancers exhibiting chromosome instability, giving rise to novel therapeutic opportunities. In particular, we consider the GLE2p-binding sequence domains of BubR1 and the newly discovered BuGZ, two kinetochore-associated proteins, as candidate therapeutic targets for GBM. Clin Cancer Res; 21(2); 233–9.

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Background

Regulating kinetochore-microtubule attachment during mitosis

Kinetochores are large protein structures assembled on centromeric DNA during mitosis that bind to microtubules of the mitotic spindle to orchestrate and power chromosome movements. To properly segregate chromosomes during mitosis, kinetochores must attach to the dynamic plus-ends of mitotic spindle microtubules (1). Early in mitosis, attachments are unstable and labile, allowing improperly connected microtubules to be released. This prevents premature stabilization of commonly generated erroneous attachments, which can lead to chromosome mis-segregation (1, 2). Conversely, in late mitosis, KT-MT attachments are stabilized to generate forces required for chromosome movements and to silence the spindle assembly checkpoint (SAC), which prevents mitotic exit until all chromosomes are properly bi-oriented (1, 2).

Although many of the >100 proteins that comprise the vertebrate kinetochore contribute to the generation of KT-MT attachments, the core attachment factor is the "KMN network," comprised of KNL1, the MIS12 complex, and the NDC80 complex (Fig. 1; refs. 1, 3). Regulation of KT-MT attachments relies on the essential mitotic kinase Aurora B (ABK). Upon nuclear envelope breakdown, kinetochores lack spatial organization and bind microtubules indiscriminately. Thus, early in mitosis, it is common for sister kinetochore pairs to attach to microtubules emanating from the same pole (syntelic attachment) or for a single kinetochore to attach to microtubules from both poles (merotelic attachment; ref. 1). To prevent the accumulation of such attachment errors, ABK phosphorylates multiple kinetochore proteins early in mitosis, including members of the KMN network, to increase KT-MT turnover (Fig. 1A; refs. 2–5). As mitosis progresses, kinase activity decreases and phosphatase activity dominates, resulting in low levels of ABK-dependent kinetochore phosphorylation. Decreased NDC80 complex phosphorylation increases its microtubule-binding activity, resulting in stabilized KT-MT attachments (Fig. 1B; ref. 6). Defects in the ABK regulatory system can result in erroneous KT-MT attachments, which often lead to chromosome segregation errors and chromosome instability, which are observed in many cancers (7).

To prevent mitotic exit until proper KT-MT attachments have formed, the cell uses a surveillance mechanism known as the SAC. The core SAC proteins, MAD1, MAD2, BUBR1, BUB1, BUB3, and MPS1, accumulate at unattached kinetochores and generate a "wait anaphase" signal, which inhibits activation of the anaphase promoting complex/cyclosome (APC/C) and prevents mitotic exit (8, 9). The mechanism by which cells integrate both phosphorylation and SAC signals is still being characterized, yet interestingly, some SAC proteins have direct roles in KT-MT attachment, independent of their well-defined checkpoint functions. For instance, both BUBR1 and BUB1 function at this interface between KT-MT attachments and the SAC. BUBR1 recruits the phosphatase PP2A to kinetochores to dephosphorylate ABK substrates and promote KT-MT attachment stability (Fig. 1; refs. 10–12). In addition, BUB1 has been implicated in regulating KT-MT attachments both through the recruitment of ABK to centromeres via phosphorylation of histone H2A and through promotion of ABK activity at kinetochores independent of its
Figure 1.
A, early in mitosis, three GLEBS-containing proteins, BUBR1, BUGZ, and BUB1, accumulate at kinetochores through BUB3 binding to regulate KT-MT attachments. The recruitment of these proteins is dependent on MPS1 phosphorylation of MELT motifs in KNL1. BUB1 and Aurora B kinases increase kinetochore phosphorylation, particularly of the Ndc80 complex, to inhibit stable microtubule attachment. Conversely, BUBR1/PLK1 recruits PP2A to counteract kinetochore phosphorylation to facilitate KT-MT attachment stabilization. B, late in mitosis, phosphatase activity dominates and reduces kinetochore phosphorylation to stabilize microtubule attachments. Clinically relevant chemical inhibitors of kinetochore phosphoregulation are shown in blue.
centromere accumulation (Fig. 1; refs. 13–15). Although SAC activity and SAC protein levels have been commonly characterized in cancers, their secondary role in regulating KT–MT attachments has only recently been evaluated.

Rethinking SAC function in cancer

Cytologic analysis of most late-stage solid tumors such as glioblastoma multiforme (GBM) reveals dramatic numerical and structural chromosome alterations (16) and intratumoral genomic heterogeneity (16–19). All of these features can promote tumor cell evolution, invasiveness, therapy resistance, and recurrence (7, 20–22). Such chromosomal alterations often arise from aberrant mitoses (e.g., lagging chromosomes, anaphase bridges), consistent with increases in chromosome instability during tumor progression (7, 23, 24). Loss of SAC function is a common explanation proffered for increased chromosome instability and aneuploidy in cancers (25–27). This notion that loss of SAC activity promotes tumorgenesis has found support in studies of certain cancers (25, 26) and model systems (e.g., mouse knockout of certain SAC genes; refs. 23, 28). However, loss-of-function mutations in SAC genes are rare in cancers (27), and many late-stage cancers exhibit high SAC gene expression (29, 30), suggesting hyperactivity (30, 31). Furthermore, as discussed above, there is now evidence that SAC proteins play additional roles in KT–MT regulation. Thus, paradoxically, instead of loss of SAC activity causing chromosome instability and complex karyotypes observed in high-grade glioma and ducal carcinomas, it is likely that SAC proteins become increasingly required to support mitotic defects in KT–MT attachments as low-grade tumors transition to aggressive malignancies such as GBM. One hypothesis is that oncogenic signaling fundamentally alters regulation of KT–MT attachments in cancers with increased SAC protein expression, resulting in chromosome alignment defects. These defects are suppressed by the contribution of SAC proteins in regulating KT–MT attachments, an otherwise nonessential function. Data supporting this hypothesis are summarized below. If true, the allowance of otherwise lethal KT–MT attachments by SAC proteins leads to the genomic instability observed for such cancers, albeit other factors likely contribute (e.g., tetraploidy, chromothrypsis, telomere fusions, sister chromatid cohesion defects).

The KT–MT attachment activities of SAC proteins may represent much sought-after cancer-specific therapeutic targets for GBM and other refractory late-stage cancers. This particular mechanism may transcend the heterogeneity of molecular subclasses and combinations of oncogenic drivers that has thwarted most pharmacologic interventions for aggressive malignancies in the past.

KT–MT attachments are defective in GBM patient isolates

GBM, or grade IV astrocytoma, is the most aggressive and common form of brain cancer in adults (32, 33). Even with standard-of-care treatments, including surgery, radiation, and the alkylating agent temozolomide, GBM remains among the deadliest cancers, with a median survival period of 12 to 14 months (32, 33). Standard-of-care therapies fail in part due to the fact that GBM tumors are heterogeneous both in cellular composition (e.g., cell morphology and gene expression; ref. 21) and in karyotype (17).

To identify new therapeutic targets for GBM, others and we have performed functional genetic screens in patient-derived GBM stem-like cells (GSC; refs. 30, 34–39). GSCs retain tumor-initiating potential and tumor-specific genetic and epigenetic signatures over extended outgrowth periods (36, 38), under culture conditions that mimic the neural progenitor perivascular niche (40, 41). By performing parallel RNAi screens in GSCs and fetal neural stem cells (a nontransformed candidate cell of origin control), we were able to find genes that when knocked down specifically blocked GSC expansion (i.e., candidate cancer lethal genes). Among these genes were BUB1B/ BUBR1 and BUBGZ, two kinetochore-associated proteins with roles in regulating KT–MT attachment (30, 39). These studies revealed that GBM cells have two separable defects in kinetochore regulation triggered by oncogenic signaling, which BUB1R and BUBGZ suppress.

BUB1B/BUBR1 function in GBM

BUB1B encodes the highly conserved BUB1-like protein kinase. BUB1R. BUB1R has multiple functional domains that have been implicated in mitotic checkpoint control, mitotic timing, and regulating KT–MT attachment (8–10, 27, 42). These include N- and C-terminal KEN box domains required for CDC20 binding and APC/C inhibition (43), a C-terminal kinase domain required for protein stability (44), and a GLE2p-binding sequence (GLEBS) domain necessary for kinetochore localization during mitosis (45, 46). Although BUB1B is essential for mammalian development (31), its essential function is contained solely within the N-terminal KEN box (30, 47), which enables BUB1R to act as a pseudo-substrate inhibitor of APC/C (Cdc20) during G2 and preanaphase mitosis, preventing premature anaphase onset (43, 47).

By contrast, recent studies suggest that BUB1R’s GLEBS domain and kinetochore localization are not required for KT–MT attachment in normally dividing somatic cells (e.g., mouse embryonic fibroblasts, neuronal stem cells, astrocytes, retinal pigment epithelial cells). However, in approximately 60% of GBM isolates assayed, RAS-transformed cells, and HaLa cells, the GLEBS domain becomes essential to suppress lethal KT–MT attachment defects (30). BUB1R’s GLEBS domain facilitates its interaction with BUB3 and its localization to prometaphase kinetochores, where BUB1R stabilizes KT–MT attachment by recruiting PP2A to kinetochores to counteract ABK phosphorylation of outer kinetochore substrates (Fig. 1; refs. 11, 12).

Intriguingly, defects in kinetochore regulation are observed in cells that require the BUB1R GLEBS domain. BUB1R-sensitive cells invariably have shorter distances between sister kinetochores when stable end-on microtubule attachments have formed at metaphase, termed interkinetochore distances (IKD; ref. 30). This distance serves as an indirect measure of the pulling forces generated by dynamic microtubules bound to kinetochores, such that stronger attachments lead to longer IKDs and weaker attachments produce shorter IKDs. A survey of different cell types revealed that, in general, nontransformed cells, including astrocytes, fibroblasts, hematopoietic progenitors, neural stem cells, and retinal pigment epithelial cells, all have “long” IKDs (~1.24 μm), whereas other transformed cell types, including HeLa cells and RASV12-expressing MEFs, exhibit “short” IKDs (~1.12 μm). In cells harboring short IKDs, knockout of BUB1R or GLEBS domain inhibition results in profound loss of KT–MT attachment and cell death. This suggests that BUB1R-dependent kinetochore recruitment of PP2A is not essential for normal KT–MT dynamics, perhaps due to functional redundancy with other kinetochore phosphatases, or alternative PP2A recruitment mechanisms. However, in a subset of cancer cells, oncogenic signaling may lead to ABK misregulation,
which prevents stable end-on attachment of KT–MTs in the absence of BUBR1 activity.

**BUGZ function in GBM**

BUGZ was isolated from an RNAi screen targeting putative human transcription factors to identify key regulators of GSC expansion. This previously uncharacterized C2-H2 zinc-finger domain gene and putative transcription factor was originally named ZNF207 (39, 48). We renamed the gene BUGZ (Bub3 interacting GLEBS and Zinc finger domain containing protein) and demonstrated that it is a novel kinetochore component that binds to and stabilizes BUB3 during interphase and mitosis (Fig. 1A). Just like BUBR1, BUGZ binds to BUB3 through a highly conserved GLEBS domain. Inhibition of BUGZ results in loss of both BUB3 and another BUB3 binding partner, BUB1, from kinetochores, but does not result in loss of BUBR1 from kinetochores (39). Localized BUB1 kinase activity helps mediate proper KT–MT attachments through recruitment and activation of ABK to centromeres and kinetochores (13–15).

Consistent with BUGZ affecting BUB1 kinetochore localization in GBM isolates, we observe chromosome alignment defects in transformed cells with BUGZ knockdown, but not in untransformed cells. As expected, BUGZ inhibition reduced BUB1 kinase activity at kinetochores as measured by immunostaining the phosphorylated form of its substrate, histone H2AT120, which is key for ABK recruitment. We also observed decreased phosphorylation of HEC1544, a critical downstream kinetochore substrate of ABK required for regulation of KT–MT attachments. As with BUB1, we found that the cancer-specific requirement for BUGZ was limited to its GLEBS domain, which mediates BUGZ kinetochore localization.

Intriguingly, the cancer-specific requirement for BUGZ-GLEBS does not correlate to the requirement for BUBR1-GLEBS. Particularly, GBM isolates that do not require the BUBR1-GLEBS for viability do require the BUGZ-GLEBS (39). These independent requirements likely arise from their opposing mechanistic roles: BUBR1 antagonizes ABK activity and stabilizes KT–MT attachments by recruiting the counteracting phosphatase PP2A (Fig. 1B; refs. 10–12, 30). BUGZ instead activates ABK through BUB1 activity, and presumably destabilizes KT–MT attachments (Fig. 1A ref. 39). As opposing regulators, there are likely unique oncogenic pressures that drive the requirement for BUGZ and/or BUBR1. Determining how different oncogenic stresses induce these unique defects, and which, if either, is dominant in GBM will be important for translating these findings into successful therapies.

**Clinical–Translational Advances**

**GLEBS domains as therapeutic targets**

A common theme arising from analysis of mitotic defects in GBM patient isolates is the cancer-specific requirement for GLEBS domain activity of BUBR1, BUGZ, and likely BUB1 (30, 39, 49). GLEBS domains are short disordered regions of about 40 amino acids that form a series of salt bridges between the WD40 domains of BUB3 and two glutamate residues in the GLEBS domain (50). As a result of BUB3 binding, the GLEBS domain undergoes a conformational shift from a disordered to a well-ordered structure with fixed interaction points on the top face of BUB3’s WD40 propeller (50). This interaction is critical for BUB3-dependent recruitment of BUB1 and BUBR1 to kinetochores (45, 47, 51).

For example, a single amino acid change in BUBR1’s GLEBS domain (E406K in mouse; corresponds to 409K in human) is sufficient to prevent BUB3 interaction and BUBR1’s kinetochore localization (47). In addition to BUGZ, BUB1, and BUBR1, the only other known GLEBS domain–containing protein is NUP98, which binds to the RAE1 WD40 repeat domain protein but not BUB3 (52).

One possible route to new therapeutic strategies for GBM and other cancers with KT–MT attachment defects is targeting the BUB3–GLEBS domain interactions. This would require finding either a GLEBS domain small-molecule mimetic capable of binding BUB3 at its WD40–GLEBS interface and blocking one or all of BUB1/BUBR1/BUGZ GLEBS domain interactions, or alternatively, an allosteric interaction that distorts the GLEBS domain binding interface. Intriguingly, the Structural Genomics Consortium successfully campaigned to find lead compounds that antagonize the WD40 propeller binding pocket of WDR5, which disrupts WDR5 interactions with the MLL1/KMT2 SET domain methyltransferase in vitro (53). Moreover, the Tyers lab has recently found allosteric inhibitors (SCF-I2) of the WD40 domain of CDC4, which distort the substrate binding pocket (54). The ideal GLEBS domain drug may be one that would inhibit BUGZ–BUB3 interaction during interphase, which would reduce BUB3 levels by approximately 50% during interphase and prime nonmitotic tumor cells for mitotic catastrophe.

**Repurposing current therapies to target KT–MT attachments**

More conventional therapies and targets may also take advantage of oncogenically induced kinetochore defects. Both BUB1 and BUGZ function within complex regulatory pathways to affect kinetochore phosphorylation, and targeting other mitotic proteins in these pathways may yield GBM-specific cell death. These include kinase activities of MPS1 (55–57), BUB1 (13–15), PKM2 (58), and PLK1 (11). As discussed previously, BUB1 activation of ABK activity requires BUGZ through an unknown mechanism. In addition, BUB1 cannot bind kinetochores without MPS1-dependent phosphorylation of MELT motifs within the kinetochore factor KNL1 (Fig. 1A; refs. 55–57). Thus, kinase inhibitors specific for either MPS1 or BUB1 may exacerbate the same KT–MT attachment defects in GBM that induce the requirement for the BUGZ–GLEBS domain. However, because these kinases are essential for SAC signaling, it remains unclear whether dose-limiting toxicities in noncancer cells will limit the effectiveness of MPS1 or BUB1 inhibitors, albeit there have been promising preclinical trials of MPS1 inhibitors, some of which have initiated phase I trials (59–61). Although cycloalkenepyrazole inhibitors of BUB1 kinase activity have been patented (Patent WO2013167698), no cell-based or in vivo studies have been published to date.

Interestingly, PKM2, which has important roles in glycolysis and gene transcription, binds BUB3 during mitosis and phosphorylates residue Y207, a regulatory event required for BUB3–BUB1 complex recruitment to kinetochores in GBM cells (58). PKM2 inhibitors have been previously developed to metabolically target cancers (62). An interesting possibility is that these drugs may have the added effect of destabilizing compromised KT–MT attachments observed in GBM cells which require BUGZ for chromosome alignment; however, this activity has not been assayed.

PLK1 inhibitors have shown efficacy in preclinical work using GBM models (63) and have had clinical success in acute myeloid leukemia. Currently, at least six unique PLK1 inhibitors have...
reached phase I or II clinical trials for various cancers, and BI-6727 (volasertib) was recently designated a “breakthrough therapy” by the FDA in the treatment of acute myeloid leukemia after raising complete remission rates 3-fold for patients enrolled in a phase II trial. (64–67)

However, without detailed mechanistic studies, it is difficult to know if the clinical success of these targets is attributable, even in part, to mitotic disruption. As these therapies demonstrate clinical success, it will be important to expand them into cancers with documented KT–MT attachment defects. However, these targets, particularly MPS1 and PLK1, have many roles within the cell cycle, including centrosome duplication and mitotic entry, and thus may exhibit broad antimitotic effects.

Conclusions

Aneuploidy was among the first cytologic features associated with cancer cells (23), and thus chromosome segregation has long been a logical target for cancer therapies. However, our understanding of molecular drivers of chromosome instability in cancer and the interdependency of chromosome instability, tumor initiation, and evolution is only just emerging.

Antimitotic drugs, including microtubule poisons such as taxanes and vinca alkaloids, have long been instrumental in cancer therapy, but unfortunately due to their nonspecific nature can be quite toxic. Even recently developed drugs, such as Aurora kinase or KIF11/EG5 inhibitors, target all dividing cells and thus have performed poorly in clinical trials (68, 69). These failings likely result from targeting mitotic master regulators that are required in healthy cells; inhibiting a ubiquitously essential target dramatically reduces the therapeutic window and efficacy of a treatment. It is clear that the next generation of antimitotic biologic chemotherapies must capitalize on defects already present in cancer cells. Proteins and processes that have become destabilized by oncogenic signaling are ideal targets for a therapy that inhibits accessory or redundant regulators. As observed with BUBR1, healthy cells with robust kinetochore signaling survive BUBR1–GLEBS inhibition, whereas GBM cells, compromised by oncogenic signaling, cannot tolerate this loss. By targeting accessory regulators in defective pathways, healthy cells with redundant or robust regulatory mechanisms are largely unaffected, and the inhibition is amplified or exacerbated in compromised cells. Kinetochores and their dynamic attachments to microtubules are an exciting area from which to identify targets for precision cancer therapies. Mitotic factors are commonly altered in cancers through mutation, transcriptional changes, or epigenetic and posttranslational modifications. Moreover, a large body of work characterizing the complex pathways, which regulate KT–MT attachments, informs the many targets for chemical intervention. Even more exciting is the possibility of applying previously FDA-approved antimitotic therapies to specific cancers with compromised kinetochore signaling.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Authors’ Contributions

Conception and design: J.A. Herman, C.M. Toledo, J.M. Olson, P.J. Paddison
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): C.M. Toledo, J.M. Olson
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): C.M. Toledo, J.M. Olson
Writing, review, and/or revision of the manuscript: J.A. Herman, J.M. Olson, J.G. DeLuca, P.J. Paddison
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): J.G. DeLuca
Study supervision: J.M. Olson, J.G. DeLuca

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