The Mitotic Kinase Bubr1 is Critical for Human Brain Tumor Survival

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Glioblastoma multiforme (GBM) is a highly aggressive, treatment resistant, and almost invariably fatal brain cancer involving neuro-protective glial cells. Recently, brain tumor-initiating cells (BTIC) have been identified and are believed to be the stem cells that drive GBM. Cancer therapies that target BTIC are likely to increase patient survival to this otherwise devastating disease. However, the proteins that are involved in GBM tumor maintenance mostly remain enigmatic.

Kinases influence nearly every cellular process. Therefore, the Paddison Lab (Human Biology Division) and the Olson Lab (Clinical Research Division) recently pioneered a screen to uncover key kinases functioning in BTIC to promote GBM tumor survival. The results of this work were recently published in a paper appearing in the journal *Cancer Discovery*, the lead author of which was postdoctoral associate Dr. Yu Ding of the Paddison Lab. The authors' screen was based on the depletion of 713 human kinases (by shRNA) and the analysis of defective*in vitro* BTIC expansion. Ding *et al.* identified about 48 candidate kinases that influence BTIC proliferation. By analyzing gene expression and other parameters directly from GBM tumor samples, the researchers narrowed the list to 37 proteins in the GBM network. Intriguingly, the largest GBM subnetwork contained four mitotic kinases with BubR1 scoring as the top ranked screen hit that was required for BTIC proliferation. In order for BubR1 to be a good pharmacological candidate, it should play an essential function in BTIC but not control non-tumorigenic neural stem cells (NSC). Indeed, BubR1 inhibition significantly halted BTIC growth but did not affect the proliferation of NSC. BubR1 therefore appeared to be a prime protein for BTIC-specific survival. This result suggested that some defect in mitosis underlies GBM, and it motivated the authors to answer the specific question: What aspect of BubR1 function is required in BTIC?

Mitosis is the cell cycle stage devoted to chromosome segregation, in which BubR1 plays multiple roles. Prior to mitosis, each chromosome is duplicated and is physically paired to its "twin"; these attached chromosomes are called sister chromatids. Each "twin" builds a kinetochore, resulting in the formation of two sister kinetochores on each paired sister chromatid. The kinetochore (KT) is a megadalton-sized protein complex that binds to mitotic spindle microtubules (MT) and to chromosomes to mediate chromosome segregation. BubR1 localizes to kinetochores, leading the

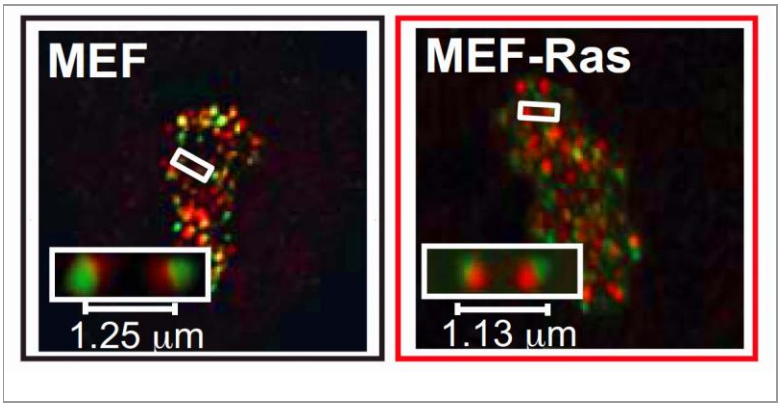
authors to posit that the necessity for BubR1 in BTIC was due to impaired KT-MT dynamics in those cells. Indeed, Ding *et al.* found that BTIC, but not NSC, depleted of BubR1 exhibited chromosome congression and segregation defects, and decreased separation of sister kinetochores (termed IKD for inter-kinetochore distance). Interestingly, the authors found that the IKD were significantly shorter in the shBubR1-sensitive BTIC compared to control cells or even to two BTIC isolates that were insensitive to shBubR1. These data indicate that BTIC have altered kinetochore activity that requires BubR1 function for cell survival.

The authors also asked whether the change in IKD was specific to BTICs or if other transformed cells exhibited a similar phenotype. They were surprised to find that IKD of Ras-transformed (oncogenic) mouse embryonic fibroblasts (MEF) were shorter than untransformed control MEF, with the transformed MEF being sensitive to BubR1 levels and exhibiting reduced proliferation upon BubR1 knockdown.

BubR1 is a multi-domain protein, and the authors wanted to determine which region of BubR1 was required for BTIC and Ras-transformed MEF viability. By assessing the ability of BubR1 deletion mutants to rescue the proliferation defects of BubR1-depleted cells, the kinetochore-targeting GLEB but not kinase domain of BubR1 was shown to be essential for BTIC and oncogenic MEF survival. This indicated a critical role for BubR1 at the kinetochore in transformed cells. Lastly, the researchers injected mice with either control or BubR1-depleted BTIC, after which they assessed the ability of the BTIC to form tumors. 90% of mice that were injected with control BTIC had succumbed to tumors by day 250 post-injection, while none of the mice injected with BubR1-depleted BTIC had died.

Altogether, these data indicate that BubR1 acts in a kinase-independent way at the kinetochore of BTIC and other transformed cells to facilitate a kinetochore function that is distinctly required upon oncogenic transformation. Future investigations will uncover the key kinetochore proteins to which BubR1 binds to promote chromosome segregation and viability in tumors. It is also exciting to speculate that BubR1 inhibition may have an anti-tumorigenic effect in human cancer, particularly those that exhibit decreased IKD.

Ding Y, Hubert CG, Herman J, Corrin P, Toledo CM, Skutt-Kakaria K, Vazquez J, Basom R, Zhang B, Risler JK, Pollard SM, Nam DH, Delrow JJ, Zhu J, Lee J, Deluca J, Olson JM, Paddison PJ. 2012. Cancer-specific requirement for BUB1B/BubR1 in human brain tumor isolates and genetically transformed cells. Cancer Discov., Epub ahead of print, doi:10.1158/2159-8290.CD-12-0353.



Modified from the manuscript

Wild-type mouse embryonic fibroblast (MEF, left) and Ras-transformed oncogenic MEF (right) stained with kinetochore antibodies (green and red). The inset shows a pair of sister kinetochores (a single kinetochore is depicted by one green and red focus). The distance between sister kinetochores is significantly shorter in Ras-transformed MEF indicating kinetochore dysfunction upon oncogenic transformation.