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The Role of Protein Kinase CK2 in Glioblastoma Development

Haitao Ji¹ and Zhimin Lu^{1,2,3,*}

¹Brain Tumor Center and Department of Neuro-Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA

²Department of Molecular and Cellular Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA

³The University of Texas Graduate School of Biomedical Sciences at Houston, Houston, TX 77030, USA

SUMMARY

Glioblastoma (GBM) is the most prevalent and malignant primary brain tumor in adults, and its response to current therapies is limited. Protein kinase CK2 is overexpressed in GBM and regulates GBM cell survival, proliferation, and migration and brain tumorigenesis. Targeting CK2 for GBM treatment may benefit GBM patients.

In this issue of *Clinical Cancer Research*, Zheng and colleagues found that *CSNK2A1*, the gene encoding for CK2 α , was amplified in 33.7% of 537 glioblastoma (GBM) samples via gene copy number variation analyses of The Cancer Genome Atlas (TCGA) database (1). GBM is a highly malignant and aggressive disease and is the most common primary intrinsic brain tumor in adults. Patients diagnosed with GBM have a mean survival time of only 15 months (2). GBM can arise either *de novo* or from lower-grade astrocytomas. According to differing patterns of patients' gene expression profiles, GBM is classified into four subgroups: proneural, neural, classical, and mesenchymal (2). The report by Zheng et al. posits that *CSNK2A1* amplification is more common in classical GBM (50.7%) than in non-classical GBM (21.3%).

Protein kinase CK2, which has more than 300 substrates, is an important and highly pleiotropic protein kinase, and elevated CK2 kinase activity is frequently observed in many types of human tumors. CK2 is a dual-specificity protein kinase and phosphorylates substrates at serine, threonine, or tyrosine residues with a S/T-X-X-D/E phosphorylation motif (where X is any residue except basic residues). CK2 can function as a tetramer composed of two catalytic subunits—CK2 α and/or CK2 α' (i.e., $\alpha\alpha$, $\alpha\alpha'$, $\alpha'\alpha'$)—and two regulatory subunits—CK2 β . The two catalytic subunits in the holoenzyme make no contacts with each other (3). Although CK2 is traditionally regarded as a constitutively active kinase, studies have shown that ERK phosphorylates CK2 α at Thr360/Ser362 and activates CK2 α in response to growth factor stimuli, including epidermal growth factor (EGF) (4).

CK2 regulates multiple signaling pathways that play instrumental roles in many important cellular activities (Figure 1). CK2 regulates Wnt/ β -catenin signaling cascades via several mechanisms: CK2 phosphorylates α -catenin and disrupts the complex of β -catenin and α -catenin, thereby abrogating the inhibitory effect of α -catenin on β -catenin transactivation induced by EGF (4); CK2 phosphorylates LEF-1 and enhances its binding to nuclear β -

*Correspondence: zhiminlu@mdanderson.org.

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catenin in Wnt-signaling cells (5); and CK2 can either increase or decrease cytoplasmic β -catenin stability by phosphorylating β -catenin at different residues or the E2 ubiquitin-conjugating enzyme UBC3B (6, 7). In addition, CK2 can positively regulate PI3K/AKT activity by directly phosphorylating AKT at Ser129 (8). Moreover, CK2 activates the NF- κ B pathway by promoting I κ B degradation and phosphorylating p65/RelA at Ser529 to enhance the DNA binding ability of p65/RelA (9). CK2 also phosphorylates Janus kinase (JAK) 1 and 2 and promotes JAK-STAT pathway activation (10). Furthermore, CK2 inhibition decreases hypoxia-inducible factor-1 (HIF-1) transcription activity without changes in HIF-1 α protein level (11). Given that CK2 regulates multiple signaling pathways involved in tumor cell survival, proliferation, migration, and invasion, downregulation of CK2 by chemical inhibitors or genetic approaches promotes cell apoptosis and inhibits tumor cell migration and tumor growth (12).

Notably, activation of the Wnt/ β -catenin, PI3K/AKT, JAK/STAT, and NF- κ B pathways has been strongly implicated in GBM development (2). For instance, EGF receptor, the activation of which upregulates CK2 activity by ERK-dependent phosphorylations is overexpressed or mutated in about 50% of GBMs, which makes it the most frequent genetic alteration associated with GBM (2, 4). In addition, 36% of GBM samples have mutations or homozygous deletions of PTEN and alterations in genes encoding subunits of PI3K (PIK3R1 and PIK3CA) (2). STAT3, which promotes tumor cell survival, proliferation, and angiogenesis, is overexpressed and constitutively activated in GBM. Furthermore, the NF- κ B signaling pathway is also highly activated in GBM (2). Zheng et al. revealed that the CK2 α gene is amplified in a large percentage of GBMs, and inhibition of CK2 with CX-4945, which is a selective, orally bioavailable CK2 inhibitor and currently in a clinical trial for treating multiple myeloma, inhibits GBM growth in mice, indicating that CK2 is an attractive therapeutic target for GBM.

Zheng et al. demonstrated that treatment of human GBM cells with CX-4945, reduced JAK2 expression level, inhibited EGF-, interleukin (IL)-6-, or IL-6 family member oncostatin M-induced STAT3 activation and EGF-induced expression of c-Myc, which is downstream from STAT3 activation. Depletion of CK2 α , CK2 α' , or CK2 β expression by siRNA resulted in reduced IL-6-induced STAT-3 activation, with depletion of CK2 α or CK2 α' having the most pronounced effect. In addition, CX-4945 largely blocked EGF-induced STAT5 activation, indicating that CK2 regulates both STAT3 and STAT5 activation in response to EGF stimulation. Besides the effect of the inhibition of CK2 on STAT activation, the inhibition of CK2 by CX-4945 or knockdown of CK2 α , CK2 α' , or CK2 β expression reduced TNF α - or IL-1 β -induced phosphorylation of p65 at Ser529 and the expression of downstream targets, I κ B α and IL-8. Furthermore, constitutive phosphorylation of AKT at Ser129 and Ser473 in GBM cells was inhibited by CX-4945 in a dose-dependent manner. These findings indicate that CK2 inhibition reduces the activities of STAT3, NF- κ B, and AKT in human GBM cells.

The functional studies revealed that GBM cells became retracted and rounded and had disorganized actin stress fibers after CX-4945 treatment or expression of the siRNA for CK2 α and CK2 α' downregulation. In addition, CX-4945 inhibited GBM cell migration. These results indicate that adhesion and migration of GBM cells require CK2 activation. In agreement with these findings, PCR array assay and immunoblotting analyses revealed that integrin α 4 (encoded by *ITGA4*) and integrin β 1 (a fibronectin receptor encoded by *ITGB1*) were downregulated by CX-4945 treatment. Consistent with the role of CK2 in cell survival and proliferation, CK2 inhibition led to cell cycle arrest, cell senescence, and apoptosis. Using both subcutaneously and intracranially implanted xenograft models, the authors showed that administration of CX-4945, which reduced STAT-3, NF- κ B, and AKT activities in xenograft tumor cells, reduced tumor growth and prolonged the survival of

tumor-bearing mice. Importantly, CX-4945 treatment did not affect the mice's body weights, the number of white or red blood cells, or the levels of hemoglobin, indicating minor side effects of CX-4945 on mice.

In summary, CK2, the expression of which is amplified in GBM, regulates several important signaling pathways involved in GBM cell survival, proliferation, and migration and brain tumorigenesis. Treating GBM with CX-4945 or other specific CK2 inhibitors could be an attractive targeted approach and may benefit GBM patients.

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Figure 1. Scheme for CK2 activation and CK2-regulated signaling pathways

CK2 activity can be upregulated by gene amplification-induced protein expression and stimulation of growth factors and cytokines. ERK downstream from growth factor signaling phosphorylates and activates CK2. Activated CK2 regulates β -catenin, AKT, STAT3, and NF- κ B pathways to promote brain tumor cell proliferation, survival, angiogenesis, and migration.