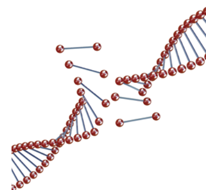
DNA Repair

Major finding: FBXW7 facilitates NHEJ-mediated repair of DNA DSBs in a DNA-PKcs– and ATM-dependent manner.

Mechanism: Phosphorylated FBXW7 binds to DSBs and induces K63 ubiquitylation of XRCC4 to promote NHEJ.

Impact: Inactivation of FBXW7 by mutations or inhibitors may radiosensitize human cancers.

FBXW7 PROMOTES NONHOMOLOGOUS END-JOINING REPAIR AND RADIORESISTANCE



DNA double-strand breaks (DSB), which can cause genomic instability and tumorigenesis if uncorrected, are primarily repaired in eukaryotic cells by one of two mechanisms, homologous recombination (HR) or nonhomologous end-joining (NHEJ). Loss-of-function mutations of the tumor suppressor F-box and WD repeat domain containing 7 (*FBXW7*), which encodes the substrate recognition component of the SKP1–Cullin1–F-box protein (SCF^{FBXW7}) E3 ubiquitin ligase, result in increased genomic instability by an unknown mechanism. To elucidate the role of FBXW7 in DSB repair, Zhang and colleagues analyzed the effects of ionizing radiation (IR), the major exogenous cause of DSBs, on FBXW7. IR resulted in the rapid recruitment of FBXW7 to DNA damage sites after phosphorylation of the serine 26 (S26) residue on FBXW7 by ATM. Depletion of FBXW7 in both cancer and normal cells had no effect on HR but significantly inhibited NHEJ and increased radiosensitization. Immunoprecipitation analyses revealed that FBXW7 bound to X-ray repair cross-complementing protein 4 (XRCC4), a component of the complex that mediates NHEJ, and that this interaction was dependent on S325 and S326 within the putative FBXW7 binding motif on XRCC4. Moreover, FBXW7 promoted lysine 63 (K63)–linked polyubiquitylation of XRCC4 after IR, leading to enhanced interaction of XRCC4 with the Ku70/80 heterodimer to facilitate NHEJ. Inactivation of DNA-dependent protein kinase catalytic subunit (DNA-PKcs), which is known to phosphorylate S325 and S326 on XRCC4, or ATM ablated FBXW7-mediated polyubiquitylation of XRCC4. K296 was found to be the ubiquitylated lysine residue on XRCC4, and ubiquitylation at K296 increased chromatin binding of XRCC4, NHEJ repair, and radioresistance. Pretreatment with MLN4924, a small-molecule inhibitor of SCF^{FBXW7} currently in clinical trials, ablated FBXW7-driven polyubiquitylation of XRCC4 and inhibited NHEJ in response to IR. Together, these results show that FBXW7 promotes NHEJ repair of DSBs and cancer cell survival following radiation, and identify FBXW7 as a potential therapeutic target to enhance tumor radiosensitivity. ■

Zhang Q, Karnak D, Tan M, Lawrence TS, Morgan MA, and Sun Y. FBXW7 facilitates nonhomologous end-joining via K63-linked polyubiquitylation of XRCC4. Mol Cell 2016;61:419–33.

Metabolism

Major finding: PI5P4Kβ is a sensor of GTP concentration that activates lipid second messenger signaling.

Mechanism: PI5P4Kβ preferentially uses GTP over ATP to phosphorylate PI(5)P to produce PI(4,5)P_2.

Impact: GTP sensing by PI5P4Kβ spurs tumorigenesis, and may be a potential therapeutic target in cancer.

PI5P4Kβ IS A LIPID KINASE GTP SENSOR THAT PROMOTES TUMORIGENESIS

ATP and GTP levels vary in different tissue types and under different conditions, and concentration changes can be detected by sensor molecules. These sensors can bind to the metabolite, have a K_M that allows its activity to be regulated by changes in metabolite concentration, and can modulate cellular functions. Although ATP sensors have been identified, GTP sensors remain unknown. To identify candidate GTP sensors, Sumita, Lo, Takeuchi, and colleagues performed a screen for GTP-binding proteins, and identified the phosphatidylinositol 5-phosphate 4-kinases (PI5P4K). PI5P4Kβ was determined to be a good candidate GTP sensor, as it bound directly to GTP more strongly than ATP. Excess ATP could compete with GTP for binding, indicating that GTP bound to the same site as ATP, but PI5P4Kβ was able to hydrolyze GTP five times faster than ATP. *In vitro* kinase assays demonstrated that, under physiologically relevant conditions, PI5P4Kβ utilized GTP to phosphorylate its substrate, the lipid second messenger PI(5)P, with approximately half of the resulting PI(4,5)P_2 produced by using GTP. X-ray crystallography structures of PI5P4Kβ in complex with non-hydrolyzable GTP and ATP analogues indicated that GTP binds to the PI5P4Kβ hydrophobic groove, and were used to engineer a PI5P4Kβ mutant ($PI5P4K\beta^{F205L}$) that disrupted GTP binding, but maintained ATP binding. The $PI5P4K\beta^{F205L}$ mutant lost its GTP-sensing ability, and cells expressing $PI5P4K\beta^{F205L}$ had higher levels of PI(5)P. $PI5P4K\beta^{F205L}$ expression resulted in formation of fewer colonies in anchorage-independent growth assays, compared with wild-type cells, and impaired tumor formation in *in vivo* allograft assays. These findings indicate that PI5P4Kβ is an intracellular GTP sensor that acts via phosphorylation of PI(5)P to regulate cell metabolism, and suggest that the GTP-sensing function of PI5P4Kβ promotes tumor growth *in vivo* and may be exploitable in the development of cancer therapeutics. ■

Sumita K, Lo Y-H, Takeuchi K, Senda M, Kofuji S, Ikeda Y, et al. The lipid kinase PI5P4Kβ is an intracellular GTP sensor for metabolism and tumorigenesis. Mol Cell 2016;61:187–98.

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