Drug Development

Major finding: Cysteine-reactive allosteric inhibitors irreversibly bind a pocket on the KRAS^{G12C}-mutant protein.

Mechanism: Inhibitor binding increases the preference of KRAS^{G12C} for GDP and impairs binding to RAS effectors.

Impact: The identification of an allosteric regulatory site on KRAS may guide design of allele-specific inhibitors.

ALLOSTERIC INHIBITORS TARGET KRAS IN A MUTANT-SPECIFIC MANNER

Activating mutations in *KRAS* are among the most frequent genetic events in human cancer and are predictive of poor response to many cytotoxic and targeted therapies. *KRAS*^{G12C} is one of the most prevalent oncogenic *KRAS* alleles and is the most common *KRAS* mutation in non–small cell lung cancer. Ostrem and colleagues devised a small molecule screening strategy to identify selective inhibitors of the G12C mutant protein that exploited the unique ability of cysteine to react with disulphide-containing molecules. Binding of the hit compounds, which did not interact with wild-type KRAS, was not affected by GDP but was impaired by GTP, suggesting that the compounds preferentially bind an allosteric site in the inactive form of KRAS. Indeed, a crystal structure of the G12C mutant protein in complex with a derivative of one of the identified compounds showed that the compound did not bind in the nucleotide pocket but bound a previously uncharacterized adjacent pocket. The authors then synthesized carbon-based electrophile, acrylamide, and vinyl sulphonamide analogues that potently and irreversibly bound the G12C mutant protein. These compounds, which disrupted the conformation of the switch-I and switch-II regions that mediate interactions with nucleotides and effector proteins, shifted the native nucleotide preference of the G12C mutant protein from GTP to GDP and decreased binding to the RAS effector proteins BRAF and CRAF in *KRAS*^{G12C}-mutant lung cancer cell lines. Moreover, treatment with one such compound specifically decreased the viability of *KRAS*^{G12C}-mutant lung cancer cell lines but had little effect on *KRAS*-mutant cell lines with non-G12C mutations. These findings suggest that compounds that exploit specific features of mutant KRAS proteins may selectively block oncogenic KRAS signaling and provide a structural and biochemical framework for further development of allele-specific KRAS inhibitors. ■

Ostrem JM, Peters U, Sos ML, Wells JA, Shokat KM. K-Ras(G12C) inhibitors allosterically control GTP affinity and effector interactions. Nature 2013;503:548–51.

Animal Models

Major finding: Tumor growth is slowed in mice housed at thermoneutral temperatures (30 to 31°C).

Concept: Cold stress induced at standard housing temperatures (20 to 26°C) may suppress antitumor immunity.

Impact: Ambient temperatures should be considered when modeling cancer and responses to therapy in mice.

HOUSING TEMPERATURE AFFECTS TUMOR GROWTH IN MICE

To ensure the comfort of animal care technicians and reduce the need for frequent cage cleaning, the National Research Council mandates that mice be housed at a constant temperature between 20 and 26°C. In this range, mice can maintain a normal body temperature, but thermogenesis is required because basal metabolism is only sufficient to maintain normal body temperature at 30 to 31°C (a state known as thermoneutrality). Concerned that chronic cold stress caused by standard, subthermoneutral housing temperatures might affect outcomes of *in vivo* experiments, Kokolus and colleagues compared tumor formation, growth, and metastasis in several commonly used mouse models housed at either standard temperature (approximately 22 to 23°C) or thermoneutral temperature (approximately 30 to 31°C). Remarkably, the tumor growth rate and metastatic burden of mice housed at thermoneutral temperature were significantly reduced compared with those housed at standard temperature. In contrast, differences in tumor growth were not observed in immunodeficient mice, suggesting that subthermoneutral temperatures may affect antitumor immune responses. Indeed, fewer active cytotoxic CD8$^+$ T cells were found in the tumor microenvironment and more immunosuppressive myeloid-derived suppressor cells were observed in the spleen at standard temperature than at thermoneutral temperature. At standard temperature, but not thermoneutral temperature, the body temperature of mice bearing large tumors also fell by 1 to 2 degrees, indicating that tumor growth impairs thermoregulation under subthermoneutral conditions. Moreover, unlike tumor-free mice, which preferred thermoneutral temperature over standard temperature in a temperature preference assay, tumor-bearing mice preferred the warmest temperature available (38°C), an example of heat-seeking behavior consistent with elevated cold stress and an increased requirement for thermogenesis. Collectively, these findings raise the possibility that at standard animal housing temperatures, murine tumor model experiments are performed in the context of cold stress-induced metabolic changes and immunosuppression and may not accurately model antitumor immune responses. ■

Kokolus KM, Capitano ML, Lee CT, Eng JW, Waight JD, Hylander BL, et al. Baseline tumor growth and immune control in laboratory mice are significantly influenced by subthermoneutral temperature. Proc Natl Acad Sci U S A 2013;110:20176–81.