ELECTROPHILES AND KEAP1-NRF2 PROTEIN-PROTEIN INTERACTION INHIBITORS FOR PREVENTION AND TREATMENT OF CHRONIC DISEASE

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The Kelch-like ECH-associated protein 1 - Nuclear factor erythroid 2-related factor 2 (Keap1-Nrf2) partnership is the principal regulator of the cellular redox homeostasis, allowing adaptation and survival during oxidative, metabolic, and inflammatory stress. Under homeostatic conditions, Keap1 targets Nrf2 for ubiquitination and proteasomal degradation. When Keap1 is inactivated by small molecules (termed inducers), Nrf2 accumulates and, in association with a small musculoaponeurotic fibrosarcoma (sMaf) protein, induces the expression of genes encoding cytoprotective proteins. Activation of Nrf2 is protective in many animal models of chronic disease and has shown benefits in clinical trials. Two Nrf2 activators, the electrophiles dimethyl fumarate and omaveloxolone, are now in clinical practice for the treatment of relapsing multiple sclerosis and Friedreich's ataxia, respectively. Non-electrophilic compounds that inhibit the Keap1-Nrf2 protein-protein interactions (PPI), are also in development. Compared to electrophiles, the early PPI inhibitors were generally less potent Nrf2 activators in cell-based assays, but PPI inhibitors with similar potencies to electrophiles are now emerging. Using quantitative Forster resonance energy transfer (FRET)-based multiphoton fluorescence lifetime imaging microscopy (FLIM), we have shown that PPI inhibitors, but not electrophiles, cause dissociation of Nrf2 from Keap1. Using the cellular thermal shift assay (CETSA), we found that PPI inhibitors designed to bind to the Nrf2-interacting Kelch domain of Keap1, increase the thermostability of Keap1 in cells and in vivo, demonstrating target engagement. This was further confirmed using cells stably expressing fluorescent Keap1-mCherry fusion protein, at comparable levels to the levels of endogenous Keap1. By contrast, electrophiles that bind to Keap1 cysteines covalently and reversibly, do not affect the thermostability of Keap1. Overall, FRET-FLIM and CETSA can be used to distinguish the mechanism of action of electrophiles and PPI inhibitors. The Keap1-mCherry fluorescence-based CETSA has the advantage of being significantly quicker, cheaper, and has the potential for high-throughput applications.