Cell Death

Major finding: The mechanisms of action of diverse ferroptosis-inducing compounds converge on GPX4 inhibition.

Mechanism: Ferroptosis-inducing compounds inactivate GPX4 by direct binding or by depleting glutathione.

Impact: Small molecules that induce ferroptosis may have therapeutic applications as antitumor agents.

GPX4 IS A KEY REGULATOR OF FERROPTOSIS

Ferroptosis is a form of nonapoptotic cell death involving the iron-dependent production of reactive oxygen species (ROS). Several small molecules that selectively induce ferroptosis in oncogenic RAS (RAS^{V12})-expressing cells have been described, but whether these compounds target a common regulatory pathway is unclear. Yang and colleagues found that erastin, a ferroptosis-inducing compound, caused glutathione depletion, which was necessary for selective lethality of erastin and erastin-induced generation of cytosolic and lipid ROS in RAS^{V12}-expressing cells. The depletion of glutathione in erastin-treated cells inactivated glutathione-dependent peroxidases (GPX), which catalyze the reduction of hydrogen peroxide and organic hydroperoxides and require glutathione as a cofactor. Because RSL3, another ferroptosis-inducing compound, increased lipid ROS production in the absence of glutathione depletion, a chemoproteomic approach was used to identify RSL3 targets in RAS^{V12}-expressing cells. RSL3 was found to bind GPX4 and inactivate its peroxidase activity, and knockdown of GPX4 induced selective ferroptotic cell death of RAS^{V12}-expressing cells, indicating that GPX4 is a central regulator of both erastin- and RSL3-induced ferroptosis. Consistent with these findings, 10 additional structurally diverse compounds exhibiting RAS^{V12}-selective ferroptosis were identified and found to similarly inhibit GPX4 and induce lipid ROS accumulation, further indicating that modulation of GPX4 is a common mechanism shared by ferroptosis-inducing small molecules. Moreover, both RSL3 and erastin induced ferroptosis *in vivo* in xenograft mouse tumor models driven by oncogenic RAS. Of note, sensitivity profiling across a panel of cancer cell lines identified diffuse large B-cell lymphomas (DLBCL) and renal cell carcinomas (RCC) as being particularly ferroptosis sensitive. Erastin induced lipid ROS generation in both DLBCL and RCC cells, and knockdown of GPX4 alone was sufficient to induce ferroptosis of RCC cells. Together, these findings identify GPX4 as an essential regulator of ferroptosis and suggest that ferroptosis-inducing compounds may have therapeutic applications in diverse cancer types. ■

Yang WS, SriRamaratnam R, Welsch ME, Shimada K, Skouta R, Viswanathan VS, et al. Regulation of ferroptotic cell death by GPX4. Cell 2014;156:317–31.

Immunotherapy

Major finding: Tumor-specific antibodies fused with IFN-β effectively treat antibody-resistant tumors.

Mechanism: The anti-EGFR–IFN-β fusion antibody leads to dendritic cell–mediated $CD8^+$ T-cell restimulation.

Impact: Tumor-targeted delivery of IFN-β may increase the efficacy of antibody-based cancer therapy.

TUMOR-TARGETED IFN-β TREATMENT OVERCOMES ANTIBODY RESISTANCE

Resistance often develops after extended tumor-specific antibody treatment. Yang and colleagues explored a strategy to treat antibody-resistant tumors based on reactivation of antitumor immune responses instead of tumor-intrinsic mechanisms. The authors established that type I IFNs mediate the antitumor responses of antibody treatment by showing that the efficacy of antibody treatment on antibody-sensitive tumors could be disrupted by blocking IFN receptor signaling. Additionally, local IFN-β expression sufficiently controlled tumor growth in antibody-resistant tumors. Based on these findings, a fusion protein of IFN-β and an EGF receptor (EGFR)–specific antibody was created to deliver IFN-β directly to tumors, as systemic administration of IFN-β has problematic side effects. Not only was the fusion antibody more effective than the first-generation anti-EGFR antibody cetuximab in several models of antibody-resistant tumors, including a *KRAS*-mutant model, it was also effective in breaking tolerance in EGFR-tolerized hosts. However, the fusion antibody was unable to control tumor growth in tumor-bearing mice lacking $CD8^+$ T cells, suggesting that the effectiveness of anti-EGFR–IFN-β treatment depends on adaptive immunity and not induction of tumor cell apoptosis. Anti-EGFR–IFN-β–treated antigen presenting cells induced significantly more IFN-γ production by $CD8^+$ T cells than anti-EGFR treated cells, indicating that IFN receptor–expressing dendritic cells are the primary target cells for anti-EGFR–IFN-β treatment, which stimulates them to reactivate antitumor responses by $CD8^+$ T cells. Anti-EGFR–IFN-β treatment significantly increased expression of the T-cell inhibitory molecule programmed death-ligand 1 (PD-L1) in tumors, providing a potential explanation for why anti-EGFR–IFN-β–treated tumors eventually relapsed and raising the possibility that PD-L1 blockade could enhance the long-term efficacy of anti-EGFR–IFN-β. Indeed, the combination of anti-EGFR–IFN-β and anti-PD-L1 completely blocked tumor growth and enhanced the antitumor T-cell response. These findings provide a framework to increase tumor-specific antibody efficacy and circumvent antibody resistance by reactivating adaptive antitumor responses. ■

Yang X, Zhang X, Fu ML, Weichselbaum RR, Gajewski TF, Guo Y, et al. Targeting the tumor microenvironment with interferon-β bridges innate and adaptive immune responses. Cancer Cell 2014;25:37–48.