Asparaginase, the enzyme responsible for deamination of the nonessential amino acid asparagine, is a mainstay of treatment regimens for aggressive hematopoietic malignancies. Acute lymphoblastic leukemia (ALL) cells are particularly sensitive to exogenous asparaginase treatment, as they are largely dependent on exogenous asparagine for their survival. However, resistance to asparaginase can develop and is associated with a poor prognosis. Hinze and colleagues sought to identify molecular pathways necessary for ALL cell fitness upon asparaginase treatment. Two of the top hits from a genome-wide CRISPR/Cas9 loss-of-function screen in an asparaginase-resistant T-ALL cell line were Nkd2 and Lgr6, genes encoding regulators of WNT signaling, suggesting that asparaginase resistance could be reversed by WNT pathway activation. Individual knockdown experiments validated that depletion of either Nkd2 or Lgr6 increases levels of active β-catenin and sensitizes ALL cells to asparaginase treatment. Small-molecule inhibition of Gsk3, a key step in the activation of WNT/β-catenin signaling, also sensitized cells to asparaginase, but this effect was not phenocopied by activation of β-catenin, the canonical effector of WNT signaling. Instead, WNT pathway activation promoted asparaginase toxicity by inhibiting protein degradation. Overexpression of FBXW7, an E3 ubiquitin ligase that stimulates degradation of GSK3-phosphorylated proteins, rescued the asparaginase-sensitizing effect of Nkd2 or Lgr6 knockdown, further indicating that WNT activation cooperates with asparaginase treatment by affecting WNT-dependent protein stabilization. Combining a Gsk3β-specific inhibitor with asparaginase in asparaginase-resistant ALL patient-derived xenograft models was highly efficacious, well tolerated, and significantly extended survival. Collectively, these observations support the development of Gsk3 inhibitors as a combination therapy to be used with asparaginase for ALL and provide an example of using synthetic lethality to improve the therapeutic window of broad-acting cancer drugs.


Chimeric antigen receptor (CAR) T-cell therapy targeting B-cell maturation antigen (BCMA) has shown some efficacy in patients with multiple myeloma. However, BCMA expression is heterogeneous and relapse associated with BCMA downregulation remains a significant challenge, underscoring the importance of identifying additional therapeutic targets. Smith and colleagues evaluated the orphan G protein-coupled receptor, class C group 5 member D (GPRC5D) as a potential target for CAR T-cell therapy in multiple myeloma. Normally restricted to the hair follicle, GPRC5D protein was found to be expressed on the surface of greater than 50% of CD138+ multiple myeloma cells from primary bone marrow samples, independent of BCMA expression. A single-chain variable fragments phage display library screen identified 32 distinct clones reactive against GPRC5D, seven of which were engineered into CARs of various structural formats, each containing a CD28 transmembrane domain and 4-1BB and CD3ζ signaling domains. CARs containing a long spacer exhibited high GPRC5D-specific signaling, and a CAR that induced minimal antigen-independent signaling was identified. Human T cells expressing GPRC5D-specific CARs induced cytokine secretion and cytotoxicity against human multiple myeloma cell lines and eliminated primary multiple myeloma cells from bone marrow aspirates. GPRC5D CAR T cells interacted with and were specifically activated by GPRC5D on the surface of target cells. In vivo, GPRC5D-specific CAR T cells injected into a human multiple myeloma xenograft model characterized by bone marrow–predominant disease rapidly localized to the tumor, eradicated multiple myeloma cells, and increased survival, with comparable efficacy to BCMA-targeted CAR T cells. Moreover, GPRC5D-specific CAR T-cell therapy successfully rescued mice from antigen escape–mediated tumor progression following BCMA-targeted immunotherapy. In addition, GPRC5D-specific CAR T cells did not exhibit any overt on-target/off-tumor toxicity in murine and cynomolgus models. These findings identify GPRC5D as an attractive target for immunotherapy in patients with multiple myeloma, regardless of prior BCMA-targeted therapy.

GPRC5D CAR T-cell Therapy Has Antitumor Activity in Multiple Myeloma