Immunotherapy

Major finding: Expression of synthetic RNA-based gene circuits in tumors induces an antitumor T-cell response.

Approach: Coactivation of tumor-specific promoters drives the coexpression of combinatorial immunomodulators.

Impact: Modular tumor-targeting RNA-based gene circuits hold promise as an immunotherapeutic approach.

RNA-BASED GENETIC CIRCUITS ARE A POTENTIAL ANTITUMOR IMMUNOTHERAPY

Recent studies in the field of synthetic biology have described the design and construction of genetic circuits as logic-driven cancer gene therapies in which the genetic circuits integrate the activities of tumor-specific synthetic promoters and generate the output protein only when both input promoters are concurrently activated. To ascertain the immunotherapeutic potential of synthetic gene circuits, Nissim, Wu, and colleagues developed a modular synthetic RNA-based gene circuit platform that integrates the activity of two ovarian cancer–specific synthetic promoters, P1 and P2, which regulate genetic modules 1 and 2, respectively. Initially, activation of the synthetic MYC promoter S(cMYC)p in module 1 drove a miRNA-driven autoinhibitory loop and prevented expression of the reporter output gene mKate2; activation of the synthetic E2F1 promoter S(E2F1)p in module 2 resulted in the production of a sponge for the miRNA in module 1 to relieve the autoinhibition of module 1 and drive the expression of mKate2 and the module 2 reporter gene EGFP. To further enhance the tumor specificity of the genetic circuit, the modules were modified so that coactivation of S(cMYC)p and S(E2F1)p resulted in the expression of GAD, a synthetic transcription factor that drives the expression of target genes under the control of the synthetic promoter GALp, in ovarian cancer cells but not in other cells. Replacement of the target genes expressed by GAD with a gene encoding an surface T-cell engager (STE) resulted in T cell–specific secretion of IFNγ *in vitro*, and replacement with genes encoding the STE and the immunomodulatory genes CCL21, IL12, and anti–PD-1 antibody resulted in T cell–mediated inhibition of tumor growth and increased survival *in vivo*. Together, these findings describe a modular synthetic biology platform approach that is versatile and exhibits potential as an immunotherapeutic strategy. ■

Nissim L, Wu M-R, Pery E, Binder-Nissim A, Suzuki HI, Stupp D, et al. Synthetic RNA-based immunomodulatory gene circuits for cancer immunotherapy. Cell 2017 Oct 19 [Epub ahead of print].

Ubiquitination

Major finding: Specific USP7 inhibitors stabilized p53 and exhibited toxicity in tumor cells *in vitro* and *in vivo*.

Approach: Co-crystal structures reveal the mechanism of inhibition of USP7 inhibitors in complex with USP7.

Impact: Specific pharmacologic inhibition of USP7 may be feasible and promote stabilization of p53 in cancer.

IDENTIFIED SELECTIVE USP7 INHIBITORS COMPETE WITH UBIQUITIN BINDING

Stabilization of reactivation of the tumor suppressor p53 is a potential strategy for cancer treatment. One potential approach to stabilize p53 is to target the ubiquitin E3 ligase MDM2, which ubiquitinates and destabilizes p53. The deubiquitinase USP7 protects MDM2 from degradation, suggesting the potential for targeting USP7 in cancer. However, specific USP7 inhibitors have been challenging to develop. Kategaya, Di Lello, Rougé, and colleagues used nuclear magnetic resonance–based screening and structure-based design to develop two selective USP7 inhibitors, GNE-6640 and GNE-6776, which attenuated ubiquitin binding to USP7, thereby suppressing its deubiquitinase activity. Both compounds increased MDM2 ubiquitination and degradation, and resulted in cytotoxicity in a number of cancer cell lines, including acute myeloid leukemia cells. Further, GNE-6640 and GNE-6776 enhanced sensitivity to chemotherapeutic agents and targeted inhibitors such as PIM kinase inhibitors. Structural analyses showed that the USP7 inhibitors bind the USP7 ubiquitin-binding site, and found that USP7 preferentially interacts with free Lys48 side chains, resulting in sequential depolymerization of Lys48-linked ubiquitin chains. In a related study, Turnbull, Ioannidis, Krajewski, and colleagues used a ubiquitin-rhodamine assay to screen 500,000 compounds to identify potential USP7 inhibitors, and further compound optimization yielded the selective inhibitors FT671 and FT827. Co-crystal structures of FT671 and FT827 with USP7 revealed plasticity in the USP domains, and showed that both compounds bound competitively at the ubiquitin binding site. In cancer cell lines FT671 destabilized MDM2, increasing levels of p53 and enhancing transcription of p53 target genes. Further, in a multiple myeloma xenograft model, FT671 was well tolerated and suppressed tumor growth. Taken together, these studies identified specific USP7 inhibitors that exhibited cytotoxicity in cancer cells, suggesting the feasibility of targeting USP7 as a strategy to stabilize p53 in cancer. ■

Kategaya L, Di Lello P, Rougé L, Pastor R, Clark KR, Drummond J, et al. USP7 small-molecule inhibitors interfere with ubiquitin binding. Nature 2017;550:534–8.

Turnbull AP, Ioannidis S, Krajewski WW, Pinto-Fernandez A, Heride C, Martin AC, et al. Molecular basis of USP7 inhibition by selective small-molecule inhibitors. Nature 2017;550:481–6.