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Survey section contribution—Basic/translational research

Commentary on "Programmable base editing of A-T to G-C in genomic DNA without DNA cleavage." Gaudelli NM, Komor AC, Rees HA, Packer MS, Badran AH, Bryson DI, et al. *Department of Chemistry and Chemical Biology, Harvard University, Cambridge, MA*.

Nature 2017;551:464-71.

Abstract

The spontaneous deamination of cytosine is a major source of transitions from C•G to T•A base pairs, which account for half of known pathogenic point mutations in humans. The ability to efficiently convert targeted A•T base pairs to G•C could therefore advance the study and treatment of genetic diseases. The deamination of adenine yields inosine, which is treated as guanine by polymerases, but no enzymes are known to deaminate adenine in DNA. Here we describe adenine base editors (ABEs) that mediate the conversion of A•T to G•C in genomic DNA. We evolved a transfer RNA adenosine deaminase to operate on DNA when fused to a catalytically impaired CRISPR-Cas9 mutant. Extensive directed evolution and protein engineering resulted in seventh-generation ABEs that convert targeted A•T base pairs efficiently to G•C (approximately 50% efficiency in human cells) with high product purity (typically at least 99.9%) and low rates of indels (typically no more than 0.1%). ABEs introduce point mutations more efficiently and cleanly, and with less off-target genome modification, than a current Cas9 nuclease-based method, and can install disease-correcting or disease-suppressing mutations in human cells. Together with previous base editors, ABEs enable the direct, programmable introduction of all four transition mutations without double-stranded DNA cleavage.

Commentary

The imagination is the limit when it comes to possible applications of gene editing. The CRISPR/Cas9 system is the most commonly used laboratory technique for gene editing and relies on sequence-directed double strand breaks to disrupt genes or add genetic sequences. This paper describes a gene editing technique that does not require double-stranded DNA breaks but chemically converts 1 base pair to another at a target genomic locus. The authors engineered an adenine base editor that can convert an A-T base pair to a G-C base pair. This enzyme is directed to a specific genomic locus using a CRISPR/Cas9 nuclease-based method that is catalytically impaired and unable to produce strand breaks. This technology can cleanly introduce point mutations with approximately 50% efficiency in human cells. Other previously described editors can convert C-G to T-A. Therefore, it is now possible to make all possible nucleotide transitions in the genome. A potential application of this technology is to permanently correct diseases caused by more than 32,000 pathogenic single nucleotide polymorphisms. As next generation sequencing becomes more common place, we will not only be able to better understand the biologic implications of our genetic sequence, but we will have an increasing array of tools to manipulate these sequences.

Commentary on "Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors." Routy B, Le Chatelier E, Derosa L, Duong CPM, Alou MT, Daillere R, et al., *Gustave Roussy Cancer Campus, Villejuif, France*.

Science 2018 Jan 5;359(6371):91-97.

Abstract

Immune checkpoint inhibitors (ICIs) targeting the PD-1/PD-L1 axis induce sustained clinical responses in a sizable minority of cancer patients. We found that primary resistance to ICIs can be attributed to abnormal gut microbiome composition. Antibiotics inhibited the clinical benefit of ICIs in patients with advanced cancer. Fecal microbiota transplantation (FMT) from cancer patients who responded to ICIs into germ-free or antibiotic-treated mice ameliorated the antitumor effects of PD-1 blockade, whereas FMT from nonresponding patients failed to do so. Metagenomics of patient stool samples at diagnosis revealed correlations between clinical responses to ICIs and the relative abundance of *Akkermansia muciniphila* Oral supplementation with *A. muciniphila* after FMT with nonresponder feces restored the efficacy of PD-1 blockade in an interleukin-12-dependent manner by increasing the recruitment of CCR9+CXCR3+CD4+ T lymphocytes into mouse tumor beds.

Commentary

Checkpoint inhibitors are effective against solid tumors including renal cell carcinoma and urothelial carcinoma. However, the majority of patients treated with checkpoint inhibitors do not have a clinical response. This study identifies the gut microbiome as a potential mediator of response to immunotherapy targeting programmed cell death protein 1 (PD-1) or its ligand PD-L1. In tumor models, mice that received broad spectrum antibiotics had a decreased response to anti-PD-1-based therapy. In a retrospective review of patient outcomes, history of antibiotic use was associated with shorter survival for non−small-cell lung cancer and renal cell carcinoma, independent of classic clinical predictors of survival. The gut microbiota from patients before and after starting anti-PD-1 therapy was examined using a shotgun sequencing approach to quantify DNA sequences from bacteria. The best clinical response to anti-PD-1 therapy was associated with relative abundance of *Akkermansia muciniphila* and with presence of T cells directed again it. In mouse models, the response to anti-PD-1 therapy could be improved with fecal microbiota transplantation from patients who responded to anti-PD-1 therapy or from oral supplementation with *A. muciniphila*. In their mouse models, *A. muciniphila* promoted IL12 production by dendritic cells, which in turn enhanced the recruitment of effector lymphocytes to the tumor bed. These exciting results suggest not only a potential predictive marker for response to anti-PD1 therapy but also a strategy to improve treatment response. It also suggests caution against indiscriminate use of antibiotics in patients receiving check point inhibitors.

Commentary on "Targeting latency-associated peptide promotes antitumor immunity." Gabriely G, da Cunha AP, Rezende RM, Kenyon B, Madi A, Vandeventer T, et al., *Ann Romney Center for Neurologic Disease, Evergrande Center for Immunologic Diseases, Brigham and Women's Hospital, Harvard Medical School, Boston, MA*.

Sci Immunol 2017 19;2(11).

Abstract

Regulatory T cells (Tregs) promote cancer by suppressing antitumor immune responses. We found that anti-LAP antibody, which targets the latency-associated peptide (LAP)/transforming growth factor-β (TGF-β) complex on Tregs and other cells, enhances antitumor immune responses and reduces tumor growth in models of melanoma, colorectal carcinoma, and glioblastoma. Anti-LAP decreases LAP+ Tregs, tolerogenic dendritic cells, and TGF-β secretion and is associated with CD8+ T cell activation. Anti-LAP increases infiltration of tumors by cytotoxic CD8+ T cells and reduces CD103+ CD8 T cells in draining lymph nodes and the spleen. We identified a role for CD103+ CD8 T cells in cancer. Tumor-associated CD103+ CD8 T cells have a tolerogenic phenotype with increased expression of CTLA-4 and interleukin-10 and decreased expression of interferon-γ, tumor necrosis factor-α, and granzymes. Adoptive transfer of CD103+ CD8 T cells promotes tumor growth, whereas CD103 blockade limits tumorigenesis. Thus, anti-LAP targets multiple immunoregulatory pathways and represents a potential approach for cancer immunotherapy.

Commentary

Immunotherapies that target checkpoint inhibitors are effective against an ever-growing list of solid tumors. The search is on for novel and perhaps even more effective checkpoint inhibitors. The authors present preclinical data for a novel cancer immunotherapy, which is an antibody against latency-associated peptide (LAP). Regulatory T cells (Tregs) are master regulators of the immune system and a potential target for immunotherapy. However, the classic marker for Tregs, FoxP3, is intracellular and not amenable to antibody therapy. LAP is a lesser known marker of Tregs, which has some overlap with FoxP3 expression in T cells. LAP is a cell surface protein that complexes with transforming growth factor-β. The authors show that

in multiple mouse tumor models, treatment with anti-LAP antibody decreased tumor growth and improved survival. The treatment effect on the immune system included decrease in tolerogenic dendritic cells, decrease in TGF-β secretion, and increase in CD8 T cell activation. Its effects were mediated in part by decreasing tolerogenic CD8 cells, which were marked by CD103 expression. Tolerogenic cells promote immune tolerance to tumor rather than cytotoxicity. The authors also present a preclinical model where anti-LAP therapy is used in combination with a dendritic cell vaccine to produce a highly effective antitumor response. These promising results pave the way for future clinical trials to establish the safety and antitumor efficacy of anti-LAP-based therapy in patients.

Commentary on "NF-κB c-Rel is crucial for the regulatory T cell immune checkpoint in cancer." Grinberg-Bleyer Y, Oh H, Desrichard A, Bhatt DM, Caron R, Chan TA, et al., *Department of Microbiology & Immunology, College of Physicians & Surgeons, Columbia University, New York, NY.*

Cell 2017;170:1096-108 e13.

Abstract

Regulatory T cells (Tregs) play a pivotal role in the inhibition of anti-tumor immune responses. Understanding the mechanisms governing Treg homeostasis may therefore be important for development of effective tumor immunotherapy. We have recently demonstrated a key role for the canonical nuclear factor κB (NF-κB) subunits, p65 and c-Rel, in Treg identity and function. In this report, we show that NF-κB c-Rel ablation specifically impairs the generation and maintenance of the activated Treg (aTreg) subset, which is known to be enriched at sites of tumors. Using mouse models, we demonstrate that melanoma growth is drastically reduced in mice lacking c-Rel, but not p65, in Tregs. Moreover, chemical inhibition of c-Rel function delayed melanoma growth by impairing aTreg-mediated immunosuppression and potentiated the effects of anti-PD-1 immunotherapy. Our studies therefore establish inhibition of NF-κB c-Rel as a viable therapeutic approach for enhancing checkpoint-targeting immunotherapy protocols.

Commentary

NF-κB (nuclear factor kappa-light-chain enhancer of activated B cells) is a major transcription factor in B and T-cells. It is expressed in nearly all cells, and attempts to target NF-κB for cancer treatment have been limited by toxicity. However, the NF-κB pathway can activate through multiple pathways. Therefore, it is possible that targeting one of the pathways more specifically may achieve the desired effects while limiting toxicity. In this study, the authors explore one of the NF-κB pathways. They show that by targeting the c-Rel subunit of NF-κB they are able to specifically target the generation and maintenance of activated regulatory T cells (Tregs). In mouse models, knock out of c-Rel in Tregs decreases growth of melanoma. Pharmacologic inhibition of c-Rel also had an antitumor effect that was mediated by CD8 expressing lymphocytes and was additive to the effects of anti-PD-1 therapy. This is a wonderful example of basic science studies that will illustrate immune mechanisms that can be targeted for immunotherapy. A more detailed understanding of immune activation and regulation will allow us to move beyond what is currently achievable with PD-1/PD-L1 targeting agents and anti-CTLA4.

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