Synthetic biology

Retrofitted *E. coli* genome confers phage resistance

Recoded genomes could mitigate bacteriophage infections in cell cultures that produce biologic agents and reduce the likelihood of undesired horizontal gene transfer events. A study in *Nature* by Nyerges et al. generated new phage-resistant *Escherichia coli* strains that are engineered for dependence on a synthetic amino acid, thus nullifying their selective advantage in the absence of specific growth conditions.

Nyerges et al. interrogate sequenced viral genomes for identification of mobile tRNA genes that could generate functional serine tRNAs when heterologously expressed in an *E. coli* strain in which endogenous serine codons and the TAG stop codon are replaced with synonymous codons. The authors screened diverse environmental samples for new phages that could lyse the *E. coli* tRNA screening strain, indicating the presence of functional tRNA operons containing genes that could rescue the serine-less strain.

They exploited this natural phage resistance to successfully promote insertion of leucine instead of serine in viral proteins by using viral tRNAs that recognize the modified serine codons but actually carry leucine. The authors used a similar approach to ensure the phage-resistant strain is fully dependent on the synthetic amino acid BipA for growth. Codons were engineered to force incorporation of BipA into the polypeptide chain. Because BipA does not naturally occur in the environment, the engineered strains are auxotrophic — thus serving as a biosecurity feature.

Breaking down the genetic code to retrofit the genome of an organism has applications in biotechnology and in preventing transgene escape in genetically modified organisms.

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COVID-19

Long COVID immune phenotypes identified for blockade

Symptoms of severe long COVID may include lung fibrosis, and a precise, detailed profile of associated immunopathology has been missing until now. Writing in *Proceedings of the National Academy of Sciences USA*, Cui et al. use published single-cell transcriptomics data from human lung-tissue samples to explore the onset of fibrosis, alongside a new humanized mouse model for long COVID. A series of unique immune hallmarks serve as a basis to investigate potential therapeutic strategies for the ever-growing cohort of patients with long COVID.

Leukocytes and pulmonary fibroblasts from patients with long COVID had unique gene-expression profiles compared to those from healthy, matched control individuals. Pathways related to the pro-inflammatory cytokine and anti-inflammatory myokine interleukin-6 (IL-6) were upregulated in alveolar macrophages from these patients, consistent with previous reports of cytokine storms. Neutrophils and lung fibroblasts were enriched for profibrotic pathways and associated with increased deposition of extracellular matrix proteins. Immunofluorescence assays validated the enrichment of proteins involved in innate immune activation and profibrosis pathways in lung tissue from patients with long COVID, probably mediated by the transcription factor JUN.

Next, the authors developed a humanized mouse model of SARS-CoV-2 infection. Fibrosis symptoms appeared in 75% of treated mice four weeks after exposure. Neutrophil and macrophage recruitment further recapitulated patient pathology in the mouse model. Treatments using anti-IL-6 and anti-CD47 antibodies were administered for two weeks, and this was sufficient to restore lung morphology, reduce fibrosis and ameliorate recruitment of neutrophils and macrophages to the affected tissue.

This study pinpoints the role of fibrosis and inflammation pathways in initial long-COVID pathology and offers clinical targets related to the innate immune response.

Jared B. Fudge
Nature Biotechnology

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Applied immunology

Base editor restores CD3δ-dependent SCID mutation

Approvals for gene therapies are gaining traction for monogenic diseases and show promise for treating some immune disorders. A paper in *Cell* by McAuley et al. reports a base-editing strategy to correct a pathogenic mutation responsible for CD3δ-dependent severe combined immunodeficiency (SCID). Delivery of an adenine base editor (ABE) and guide RNA to hematopoietic and stem and progenitor cells derived from a single patient with SCID corrected pathogenic CD3δ mutations in 71% of cells, restored T cell maturation in 88% of cells collected from bone marrow 16 weeks after xenografting into mice, and displayed normal T cell receptor repertoires in an artificial thymic organoid model.

Safe and effective potential gene therapies rely on the editing strategy having no bystander edits or off-target mutations, and a minimally immunogenic vector. To test these, McAuley et al. first developed transgenic human and mouse T cell lines modeling CD3δ-dependent SCID mutations. Transfection with ABEs showed markedly greater editing efficiencies of the pathogenic mutations over Cas9 strategies that were sufficient to restore normal CD3 surface expression in 29–85% of cells. Bystander edits varied according to editing machinery and nucleotide position: ABEmax-NRTH emerged as ideal for safe and efficient correction. Off-target mutations were detected at 5 of 200 predicted candidate sites in cells in which pathogenic CD3δ was successfully edited.

Following experiments in artificial thymus organoids to successfully recapitulate T cell maturation from stem cells in vitro, $CD34^+$ bone-marrow-derived stem cells were obtained from an infant with CD3δ-dependent SCID for editing. Of the edited cells from this patient, 71.2% (±7.85%) reverted to non-pathogenic CD3δ sequences and the corresponding differentiated T cells exhibited normal maturation and T cell receptor repertoires in the organoid system.

Pending successful clinical trial outcomes, the new strategy is a potential 'one-and-done' gene therapy for patients with CD3δ-dependent SCID.

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