Capsule

A Cas9-guide RNA complex preorganized for target DNA recognition

Bacterial adaptive immunity uses CRISPR (clustered regularly interspaced short palindromic repeats)-associated (Cas) proteins together with CRISPR transcripts for foreign DNA degradation. In type II CRISPR-Cas systems, activation of Cas9 endonuclease for DNA recognition upon guide RNA binding occurs by an unknown mechanism. Crystal structures of Cas9 bound to single-guide RNA reveal a conformation distinct from both the apo and DNA-bound states, in which the 10-nucleotide RNA "seed" sequence required for initial DNA interrogation is preordered in an A-form conformation. This segment of the guide RNA is essential for Cas9 to form a DNA recognition-competent structure that is poised to engage double-stranded DNA target sequences. This convergent evolution of a "seed" mechanism is reminiscent of that used by Argonaute proteins during RNA interference in eukaryotes.

Science 2015; 348: 1477

Eitan Israeli

Capsule

Mechanical induction of the tumorigenic β-catenin pathway by tumor growth pressure

The tumor microenvironment may contribute to tumorigenesis owing to mechanical forces such as fibrotic stiffness or mechanical pressure caused by the expansion of hyper-proliferative cells. Fernández-Sánchez et al. explore the contribution of the mechanical pressure exerted by tumor growth onto non-tumorous adjacent epithelium. In the early stage of mouse colon tumor development in the Notch+Apc+/^{1638N} mouse model, we observed mechanistic pressure stress in the non-tumorous epithelial cells caused by hyperproliferative adjacent crypts overexpressing active Notch, which is associated with increased Ret and β-catenin signaling. The authors thus developed a method that allows the delivery of a defined mechanical pressure in vivo, by subcutaneously inserting a magnet close to the mouse colon. The implanted magnet generated a magnetic force on ultramagnetic liposomes, stabilized in the mesenchymal cells of the connective tissue surrounding colonic crypts after intravenous injection. The magnetically induced pressure quantitatively mimicked the endogenous early tumor growth stress in the order of 1200 Pa, without affecting tissue stiffness, as monitored by ultrasound strain imaging and shear wave elastography. The exertion of pressure mimicking that of tumor growth led to rapid Ret activation and downstream phosphorylation of β-catenin on Tyr654, impairing its interaction with the E-cadherin in adherens junctions, followed by β-catenin nuclear translocation after 15 days. As a consequence, increased expression of β-catenin-target genes was observed at 1 month, together with crypt enlargement accompanying the formation of early tumorous aberrant crypt foci. Mechanical activation of the tumorigenic β-catenin pathway suggests unexplored modes of tumor propagation based on mechanical signaling pathways in healthy epithelial cells surrounding the tumor, which may contribute to tumor heterogeneity.

Nature 2015; 523: 92

Eitan Israeli

Capsule

Viremia suppressed in HIV-1-infected humans by broadly neutralizing antibody 3BNC117

HIV-1 immunotherapy with a combination of first-generation monoclonal antibodies was largely ineffective in pre-clinical and clinical settings and was therefore abandoned. However, recently developed single cell-based antibody cloning methods have uncovered a new generation of far more potent broadly neutralizing antibodies to HIV-1. These antibodies can prevent infection and suppress viremia in humanized mice and non-human primates, but their potential for human HIV-1 immunotherapy has not been evaluated. Caskey et al. report the results of a first-in-man dose escalation phase 1 clinical trial of 3BNC117, a potent human CD4 binding site antibody, in uninfected and HIV-1-infected individuals. 3BNC117 infusion was well tolerated and demonstrated favoable pharmacokinetics. A single 30 mg kg-1 infusion of 3BNC117 reduced the viral load in HIV-1-infected individuals by 0.8–2.5 log10 and viramia remained significantly reduced for 28 days. Emergence of resistant viral strains was variable, with some individuals remaining sensitive to 3BNC117 for a period of 28 days. The authors conclude that, as a single agent, 3BNC117 is safe and effective in reducing HIV-1 viremia, and that immunotherapy should be explored as a new modality for HIV-1 prevention, therapy and cure.

Nature 2015; 522: 487

Eitan Israeli

"A designer knows he has achieved perfection not when there is nothing left to add, but when there is nothing left to take away"

Antoine de Saint-Exupery (1900-1944), French aristocrat, writer, poet, and pioneering aviator. He became a laureate of several of France's highest literary awards and also won the U.S. National Book Award. He is best remembered for his novella *The Little Prince* (*Le Petit Prince*)

Capsule

Selective enhancement of endothelial BMPR-II with BMP9 reverses pulmonary arterial hypertension

Genetic evidence implicates the loss of bone morphogenetic protein type II receptor (BMPR-II) signaling in the endothelium as an initiating factor in pulmonary arterial hypertension (PAH). However, selective targeting of this signaling pathway using BMP ligands has not yet been explored as a therapeutic strategy. Long and fellow-researchers identified BMP9 as the preferred ligand for preventing apoptosis and enhancing monolayer integrity in both pulmonary arterial endothelial cells and blood outgrowth endothelial cells from subjects with PAH who bear mutations in the gene encoding BMPR-II, *BMPR2*. Mice bearing a heterozygous knock-in allele of a human *BMPR2* mutation, R899X, which the authors generated as an animal model of PAH caused by BMPR-II deficiency, spontaneously developed PAH. Administration of BMP9 reversed established PAH in these mice, as well as in two other experimental PAH models, in which PAH develops in response to either monocrotaline or VEGF receptor inhibition combined with chronic hypoxia. These results demonstrate the promise of direct enhancement of endothelial BMP signaling as a new therapeutic strategy for PAH.

Nature Med 2015; 21: 777

Eitan Israeli

Capsule

HIV vaccination steps in the right direction

HIV-1 mutates rapidly, making it difficult to design a vaccine that will protect people against all of the virus' iterations. A potential successful vaccine design might protect by eliciting broadly neutralizing antibodies (bNAbs), which target specific regions on HIV-1's trimeric envelope glycoprotein (Env). Jardine et al. (*Science* 2015; 349: 139) used mice engineered to express germline-reverted heavy chains of a particular bNAb and immunized them with an Env-based immunogen designed to bind to precursors of that bNAb. Sanders et al. (10.1126/*science*.aac4223) compared rabbits and monkeys immunized with Env trimers that adopt a native-like conformation. In both cases, immunized animals produced antibodies that shared similarities with bNAbs. Boosting these animals with other immunogens may drive these antibodies to further mutate into the longsought bNAbs. Chen et al. (p.156) report that retaining the cytoplasmic domain of Env proteins may be important to attract bNAbs. Removing the cytoplasmic domain may distract the immune response and instead generate antibodies that target epitopes on Env that would not lead to protection.

Eitan Israeli

Capsule

Diabetes primes neutrophils to undergo NETosis, which impairs wound healing

Wound healing is impaired in diabetes, resulting in significant morbidity and mortality. Neutrophils are the main leukocytes involved in the early phase of healing. As part of their antimicrobial defense, neutrophils form extracellular traps (NETs) by releasing decondensed chromatin lined with cytotoxic proteins. NETs, however, can also induce tissue damage. Wong et al. show that neutrophils isolated from type 1 and type 2 diabetic humans and mice were primed to produce NETs (a process termed NETosis). Expression of peptidylarginine deiminase 4 (*Padi4*, encoded by *Padi4* in mice), an enzyme important in chromatin decondensation, was elevated in neutrophils from individuals with diabetes. When subjected to excisional skin wounds, wild-type (WT) mice produced large quantities of NETs in wounds, but this was not observed in *Padi4*$^{-/-}$ mice. In diabetic mice, higher levels of citrullinated histone H3 (H3Cit, a NET marker) were found in their wounds than in normoglycemic mice and healing was delayed. Wound healing was accelerated in *Padi4*$^{-/-}$ mice as compared to WT mice, and it was not compromised by diabetes. DNase 1, which disrupts NETs, accelerated wound healing in diabetic and normoglycemic WT mice. Thus, NETs impair wound healing, particularly in diabetes, in which neutrophils are more susceptible to NETosis. Inhibiting NETosis or cleaving NETs may improve wound healing and reduce NET-driven chronic inflammation in diabetes.

Nature Med 2015; 21: 815

Eitan Israeli

Capsule

Structure of a sterol sensor

The aberrant accumulation of sterols contributes to heart attack and stroke. Two proteins embedded in the membrane of the endoplasmic reticulum, Insig-1 and Insig-2, are key players in the cellular pathway that regulates cellular sterol levels. Ren et al. report the structure of a mycobacterial homolog of Insig. The structure, together with biochemical experiments, suggests how Insig interacts with other components of the sterol regulatory pathway.

Science 2015; 349: 187

Eitan Israeli

"There are two kinds of truth: the truth that lights the way and the truth that warms the heart. The first of these is science, and the second is art. Neither is independent of the other or more important than the other. Without art science would be as useless as a pair of high forceps in the hands of a plumber. Without science art would become a crude mess of folklore and emotional quackery. The truth of art keeps science from becoming inhuman, and the truth of science keeps art from becoming ridiculous"

Raymond Thornton Chandler (1888-1959), American novelist and screenwriter, considered one of the finest writers of detective fiction

Capsule

Inflammasome-independent role of AIM2 in suppressing colon tumorigenesis via DNA-PK and Akt

The inflammasome activates caspase-1 and the release of interleukin-1β (IL-1β) and IL-18, and several inflammasomes protect against intestinal inflammation and colitis-associated colon cancer (CAC) in animal models. The absent in melanoma 2 (AIM2) inflammasome is activated by double-stranded DNA, and AIM2 expression is reduced in several types of cancer, but the mechanism by which *AIM2* restricts tumor growth remains unclear. Wilson et al. found that Aim2-deficient mice had greater tumor load than Asc-deficient mice in the azoxymethane/dextran sodium sulfate (AOM/DSS) model of colorectal cancer. Tumor burden was also higher in $Aim2^{-/-}$/$Apc^{Min/+}$ than in $APC^{Min/+}$ mice. The effects of AIM2 on CAC were independent of inflammasome activation and IL-1β and were primarily mediated by a non-bone marrow source of AIM2. In resting cells, AIM2 physically interacted with and limited activation of DNA-dependent protein kinase (DNA-PK), a PI3K-related family member that promotes Akt phosphorylation, whereas loss of AIM2 promoted DNA-PK-mediated Akt activation. AIM2 reduced Akt activation and tumor burden in colorectal cancer models, while an Akt inhibitor reduced tumor load in $Aim2^{-/-}$ mice. These findings suggest that Akt inhibitors could be used to treat AIM2-deficient human cancers.

Nature Med 2015; 21: 906

Eitan Israeli

"I want to put a ding in the universe"

Steve Jobs (1955-2011), American pioneer of the personal computer revolution of the 1970s (along with engineer, inventor and Apple Computer co-founder, Steve Wozniak). He was described as the "creative entrepreneur whose passion for perfection and ferocious drive revolutionized six industries: personal computers, animated movies, music, phones, tablet computing, and digital publishing"

Capsule

Nicotinamide *N*-methyltransferase regulates hepatic nutrient metabolism through Sirt1 protein stabilization

Nicotinamide *N*-methyltransferase (Nnmt) methylates nicotinamide, a form of vitamin B3, to produce N^1-methylnicotinamide (MNAM). Nnmt has emerged as a metabolic regulator in adipocytes, but its role in the liver, the tissue with the strongest *Nnmt* expression, is not known. In spite of its overall high expression, Hong et al. find that hepatic expression of Nnmt is highly variable and correlates with multiple metabolic parameters in mice and humans. Further, the authors find that suppression of hepatic Nnmt expression in vivo alters glucose and cholesterol metabolism and that the metabolic effects of *Nnmt* in the liver are mediated by its product MNAM. Supplementation of high fat diet with MNAM decreases serum and liver cholesterol and liver triglycerides levels in mice. Mechanistically, increasing Nnmt expression or *MNAM* levels stabilizes sirtuin 1 protein, an effect that is required for their metabolic benefits. In summary, they describe here a novel regulatory pathway for vitamin B3 that could provide a new opportunity for metabolic disease therapy.

Nature Med 2015; 21: 887

Eitan Israeli

Capsule

Preconditioning allows engraftment of mouse and human embryonic lung cells, enabling lung repair in mice

Repair of injured lungs represents a longstanding therapeutic challenge. Rosen and co-workers show that human and mouse embryonic lung tissue from the canalicular stage of development [20–22 weeks of gestation for humans, and embryonic day 15–16 (E15–E16) for mouse] are enriched with progenitors residing in distinct niches. On the basis of the marked analogy to progenitor niches in bone marrow (BM), the authors attempted strategies similar to BM transplantation, employing sublethal radiation to vacate lung progenitor niches and to reduce stem cell competition. Intravenous infusion of a single cell suspension of canalicular lung tissue from GFP-marked mice or human fetal donors into naphthalene-injured and irradiated syngeneic or SCID mice, respectively, induced marked long-term lung chimerism. Donor-type structures or 'patches' contained epithelial, mesenchymal and endothelial cells. Transplantation of differentially labeled E16 mouse lung cells indicated that these patches were probably of clonal origin from the donor. Recipients of the single cell suspension transplant exhibited marked improvement in lung compliance and tissue damping, reflecting the energy dissipation in the lung tissues. This study provides proof of concept for lung reconstitution by canalicular-stage human lung cells after preconditioning of the pulmonary niche.

Nature Med 2015; 21: 869

Eitan Israeli

Capsule

Protecting neurons from amyloid β

In the developing nervous system, the secreted protein Reelin helps to guide migrating neurons to their correct destination. In the adult nervous system, Reelin enhances synaptic plasticity and protects isolated neurons from amyloid β toxicity. Accumulation of amyloid β causes the neurodegeneration characteristic of Alzheimer's disease. To avoid the developmental defects associated with Reelin deficiency, Lane-Donovan et al. generated mice with an inducible knockout of Reelin. Mice that lacked Reelin as adults showed defects in synaptic plasticity, learning, and memory in response to amyloid β accumulation. Thus, Reelin can protect against amyloid β neurotoxicity in vivo.

Sci Signal 2015; 8: ra67

Eitan Israeli

Capsule

Detecting gram-negative bacteria

Invariant molecules specific to different classes of microbes, but not expressed by eukaryotic cells, alert the immune system to a potential invader. Gaudet et al. identified one such molecule expressed by a variety of gram-negative bacteria: the monosaccharide heptose-1,7-bisphosphate (HBP). HBP is an intermediate in the synthesis of lipopolysaccharide, a major component of bacterial cell walls. Rather than alerting the immune system through traditional pathogen detection pathways, such as Toll-like receptors, HBP signals through the host protein TIFA (TRAF-interacting protein with forkhead-associated domain), which activates both innate and adaptive immune responses to controlthe infection.

Science 2015; 348: 1251

Eitan Israeli

"It's fine to celebrate success but it is more important to heed the lessons of failure"

Bill Gates (born 1955), American business magnate, philanthropist, investor, computer programmer, inventor, and co-founder of Microsoft, the world's largest PC software company. He has pursued a number of philanthropic endeavors, donating large amounts of money to various charitable organizations and scientific research programs through the Bill & Melinda Gates Foundation

Capsule

Geometry and FLU virus evolution

Meyer and Wilke analyzed how the hemagglutinin protein of the H3 subtype of influenza has evolved, to learn how mutation allows this virus to escape host immune surveillance. This method combines sequencing data with data on protein structure and present and past antigenic sites (i.e., sites recognized by antibodies) on hemagglutinin. Surprisingly, antigenic information revealed little, but the geometrical changes wrought by mutations in the host cell receptor-binding site did. This analysis indicates that mutation in sites that we understand to be antigenic may not influence how influenza evolves as much as previously assumed.

PLOS Pathol 2015; 11: e1004940

Eitan Israeli

"It is not the mountain we conquer but ourselves"

Edmund Hillary (1919-2008), New Zealand mountaineer, explorer and philanthropist. On 29 May 1953, Hillary and Nepalese Sherpa mountaineer Tenzing Norgay became the first climbers to reach the summit of Mount Everest