

Mutations in the gene *CIB2* contribute to Usher syndrome and non-syndromic deafness

Individuals with the hereditary disorder Usher syndrome suffer from hearing loss. Associated genetic mutations impair function of the inner ear, where sensory cells fail to convert sound waves into electrical signals. Riazuddin and co-authors have determined that mutations in the gene *CIB2* contribute to Usher syndrome and non-syndromic deafness. *CIB2* encodes calcium and integrin binding protein 2, which is widely expressed in human and mouse tissue. In the mouse inner ear, the protein localizes to the tips of stereocilia of inner ear cells. When deflected by sound waves, ion channels in these hair-like projections open, triggering a mechano-electrical signaling cascade. *CIB2*

interacts with whirlin, a protein that organizes molecular complexes which maintain stereocilia structure and growth. Suppression of *CIB2* expression in zebrafish disrupted responses to acoustic stimuli and caused abnormal balance during movement. Overexpression of *CIB2* in cultured cells decreased the release of calcium from intracellular stores. *CIB2* may help to maintain intracellular calcium homeostasis in inner ear cells by sequestering calcium and influencing the release of stored calcium during mechano-electrical signal transduction.

Nat Genet 2012; 44: 1465

Eitan Israeli

Phosphorylation of NLRC4 is critical for inflammasome activation

NLRC4 is a cytosolic member of the NOD-like receptor family that is expressed in innate immune cells. It senses indirectly bacterial flagellin and type III secretion systems, and responds by assembling an inflammasome complex that promotes caspase-1 activation and pyroptosis. Qu et al. used knock-in mice expressing NLRC4 with a carboxy-terminal 3×Flag tag to identify phosphorylation of NLRC4 on a single, evolutionarily conserved residue, Ser 533, following infection of macrophages with *Salmonella enterica* serovar Typhimurium (also known as *Salmonella typhimurium*). Western blotting with a NLRC4 phospho-Ser533 antibody confirmed that this post-translational modification occurs only in the presence of stimuli known to engage NLRC4 and not the related protein NLRP3 or AIM2. *Nlrc4*^{-/-} macrophages reconstituted with NLRC4 mutant S533A, unlike those reconstituted with wild-type NLRC4, did not activate caspase-1 and pyroptosis in response to *S. typhimurium*, indicating that S533 phosphorylation

is critical for NLRC4 inflammasome function. Conversely, phosphomimetic NLRC4 S533D caused rapid macrophage pyroptosis without infection. Biochemical purification of the NLRC4-phosphorylating activity and a screen of kinase inhibitors identified PRKCD (PKC δ) as a candidate NLRC4 kinase. Recombinant PKC δ phosphorylated NLRC4 S533 in vitro, immunodepletion of PKC δ from macrophage lysates blocked NLRC4 S533 phosphorylation in vitro, and *Prkcd*^{-/-} macrophages exhibited greatly attenuated caspase-1 activation and IL-1 β secretion specifically in response to *S. typhimurium*. Phosphorylation-defective NLRC4 S533A failed to recruit procaspase-1 and did not assemble inflammasome specks during *S. typhimurium* infection, so phosphorylation of NLRC4 S533 probably drives conformational changes necessary for NLRC4 inflammasome activity and host innate immunity.

Nature 2012; 490: 539

Eitan Israeli

Structure of the agonist-bound neurotensin receptor

Neurotensin (NTS) is a 13-amino acid peptide that functions as both a neurotransmitter and a hormone through the activation of the neurotensin receptor NTSR1, a G protein-coupled receptor (GPCR). In the brain, NTS modulates the activity of dopaminergic systems, opioid-independent analgesia, and the inhibition of food intake; in the gut, NTS regulates a range of digestive processes. White et al. present the structure at 2.8Å resolution of *Rattus norvegicus* NTSR1 in an active-like state, bound to NTS₈₋₁₃, the carboxy terminal portion of NTS responsible for agonist-induced

activation of the receptor. The peptide agonist binds to NTSR1 in an extended conformation nearly perpendicular to the membrane plane, with the C terminus oriented towards the receptor core. These findings provide, to our knowledge, the first insight into the binding mode of a peptide agonist to a GPCR and may support the development of non-peptide ligands that could be useful in the treatment of neurological disorders, cancer and obesity.

Nature 2012; doi:10.1038/nature11558

Eitan Israeli

Melanomas resist T cell therapy through inflammation-induced reversible dedifferentiation

Adoptive cell transfer therapies (ACTs) with cytotoxic T cells that target melanocytic antigens can achieve remissions in patients with metastatic melanomas, but tumors frequently relapse. Hypotheses explaining the acquired resistance to ACTs include the selection of antigen-deficient tumor cell variants and the induction of T cell tolerance. However, the lack of appropriate experimental melanoma models has so far impeded clear insights into the underlying mechanisms. Landsberg and co-workers established an effective ACT protocol in a genetically engineered mouse melanoma model that recapitulates tumor regression, remission and relapse as seen in patients. They report the unexpected observation that melanomas acquire ACT resistance through an inflammation-induced reversible loss of melanocytic antigens. In serial transplantation experiments, melanoma cells switch between a differentiated and a dedifferentiated phenotype in response to T cell-driven inflammatory stimuli. The authors identified the pro-inflammatory cytokine

tumor necrosis factor-alpha (TNF α) as a crucial factor that directly caused reversible dedifferentiation of mouse and human melanoma cells. Tumor cells exposed to TNF α were poorly recognized by T cells specific for melanocytic antigens, whereas recognition by T cells specific for non-melanocytic antigens was unaffected or even increased. Our results demonstrate that the phenotypic plasticity of melanoma cells in an inflammatory microenvironment contributes to tumor relapse after initially successful T cell immunotherapy. On the basis of our work, they propose that future ACT protocols simultaneously target melanocytic and non-melanocytic antigens to ensure broad recognition of both differentiated and dedifferentiated melanoma cells, and include strategies to sustain T cell effector functions by blocking immune-inhibitory mechanisms in the tumor microenvironment.

Nature 2012; doi:10.1038/nature11538

Eitan Israeli

“Often the search proves more profitable than the goal”

E. L. Konigsburg (born 1930), American author and illustrator of children’s books and young adult fiction

A FOXO3-IRF7 gene regulatory circuit limits inflammatory sequelae of antiviral responses

Antiviral responses must be tightly regulated to defend rapidly against infection while minimizing inflammatory damage. Type 1 interferons (IFN-I) are crucial mediators of antiviral responses and their transcription is regulated by a variety of transcription factors; principal among these is the family of interferon regulatory factors (IRFs). The IRF gene regulatory networks are complex and contain multiple feedback loops. The tools of systems biology are well suited to elucidate the complex interactions that give rise to precise coordination of the interferon response. Litval et al. have used an unbiased systems approach to predict that a member of the forkhead family of transcription factors, FOXO3, is a negative regulator of a subset of antiviral genes.

This prediction was validated using macrophages isolated from *Foxo3*-null mice. Genome-wide location analysis combined with gene deletion studies identified the *Irf7* gene as a critical target of FOXO3. FOXO3 was identified as a negative regulator of *Irf7* transcription and the researchers have further demonstrated that FOXO3, IRF7 and IFN-I form a coherent feed-forward regulatory circuit. These data suggest that the FOXO3-IRF7 regulatory circuit represents a novel mechanism for establishing the requisite set points in the interferon pathway that balances the beneficial effects and deleterious sequelae of the antiviral response.

Nature 2012; 490: 421

Eitan Israeli

“A decent provision for the poor is the true test of civilization”

Samuel Johnson (1709-1784), English lexicographer

Black mamba venom peptides target acid-sensing ion channels to abolish pain

Polypeptide toxins have played a central part in the understanding of physiological and physiopathological functions of ion channels. In the field of pain, they led to important advances in basic research and even to clinical applications. Acid-sensing ion channels (ASICs) are generally considered principal players in the pain pathway, including in humans. A snake toxin activating peripheral ASICs in nociceptive neurons was recently shown to evoke pain. Diochot et al. show that a new class of three-finger peptides from another snake, the black mamba, is able to abolish pain through inhibition of ASICs expressed either in central or peripheral neurons. These peptides, called mambalgins, are not toxic in mice but show a potent analgesic effect upon

central and peripheral injection that can be as strong as morphine. This effect is, however, resistant to naloxone, and mambalgins cause much less tolerance than morphine and no respiratory distress. Pharmacological inhibition by mambalgins combined with the use of knockdown and knockout animals indicates that blockade of heteromeric channels made of ASIC1a and ASIC2a subunits in central neurons and of ASIC1b-containing channels in nociceptors is involved in the analgesic effect of mambalgins. These findings identify new potential therapeutic targets for pain and introduce natural peptides that block them to produce a potent analgesia.

Nature 2012; 490: 552

Eitan Israeli

Generation of functional thyroid from embryonic stem cells

The primary function of the thyroid gland is to metabolize iodide by synthesizing thyroid hormones, which are critical regulators of growth, development and metabolism in almost all tissues. So far, research on thyroid morphogenesis has been missing an efficient stem cell model system that allows for the *in vitro* recapitulation of the molecular and morphogenic events regulating thyroid follicular cell differentiation and subsequent assembly into functional thyroid follicles. Antonica et al. report that a transient overexpression of the transcription factors NKX2-1 and PAX8 is sufficient to direct mouse embryonic stem

cell differentiation into thyroid follicular cells that organize into three-dimensional follicular structures when treated with thyrotropin. These *in vitro*-derived follicles showed appreciable iodide organification activity. Importantly, when grafted *in vivo* into athyroid mice, these follicles rescued thyroid hormone plasma levels and promoted subsequent symptomatic recovery. Thus, mouse embryonic stem cells can be induced to differentiate into thyroid follicular cells *in vitro* and generate functional thyroid tissue.

Nature 2012; doi:10.1038/nature11525

Eitan Israeli

“The trees that are slow to grow bear the best fruit”

Moliere (1622-1673), French actor and playwright considered one of the greatest masters of comedy in Western literature

Capsule

An ultraviolet radiation-independent pathway to melanoma carcinogenesis in the red hair/fair skin background

People with pale skin, red hair, freckles and an inability to tan – the ‘red hair/fair skin’ phenotype – are at highest risk of developing melanoma, compared to all other pigmentation types. Genetically, this phenotype is frequently the product of inactivating polymorphisms in the melanocortin 1 receptor (*MC1R*) gene. *MC1R* encodes a cyclic AMP-stimulating G protein-coupled receptor that controls pigment production. Minimal receptor activity, as in red hair/fair skin polymorphisms, produces the red/yellow pheomelanin pigment, whereas increasing *MC1R* activity stimulates the production of black/brown eumelanin. Pheomelanin has weak shielding capacity against ultraviolet radiation relative to eumelanin, and has been shown to amplify ultraviolet A-induced reactive oxygen species. Several observations, however, complicate the assumption that melanoma risk is completely ultraviolet radiation dependent. For example, unlike non-melanoma skin cancers, melanoma is not restricted to sun-exposed skin and ultraviolet radiation signature mutations are infrequently oncogenic drivers. Although linkage of melanoma risk to ultraviolet radiation exposure is beyond doubt, ultraviolet radiation-independent events are likely to play a significant role. Mitra and co-

workers introduced a conditional, melanocyte-targeted allele of the most common melanoma oncoprotein, BRAF^{V600E}, into mice carrying an inactivating mutation in the *MC1R* gene (these mice have a phenotype analogous to red hair/fair skin humans). The authors observed a high incidence of invasive melanomas without providing additional gene aberrations or ultraviolet radiation exposure. To investigate the mechanism of ultraviolet radiation-independent carcinogenesis, they introduced an albino allele, which ablates all pigment production on the *Mc1re/e* background. Selective absence of pheomelanin synthesis was protective against melanoma development. In addition, normal *Mc1re/e* mouse skin was found to have significantly greater oxidative DNA and lipid damage than albino-*Mc1re/e* mouse skin. These data suggest that the pheomelanin pigment pathway produces ultraviolet radiation-independent carcinogenic contributions to melanomagenesis by a mechanism of oxidative damage. Although protection from ultraviolet radiation remains important, additional strategies may be required for optimal melanoma prevention.

Nature 2012; 491: 449

Eitan Israeli

Capsule

Clonal allelic predetermination of immunoglobulin-κ rearrangement

Although most genes are expressed biallelically, a number of key genomic sites – including immune and olfactory receptor regions – are controlled monoallelically in a stochastic manner, with some cells expressing the maternal allele and others the paternal allele in the target tissue. Very little is known about how this phenomenon is regulated and programmed during development. Using mouse immunoglobulin-κ (*Igκ*) as a model system, Farago and team demonstrate that although individual hematopoietic stem cells are characterized by allelic

plasticity, early lymphoid lineage cells become committed to the choice of a single allele, and this decision is then stably maintained in a clonal manner that predetermines monoallelic rearrangement in B cells. This is accompanied at the molecular level by underlying allelic changes in asynchronous replication timing patterns at the κ locus. These experiments may serve to define a new concept of stem cell plasticity.

Nature 2012; 490: 561

Eitan Israeli

Androgenetic haploid embryonic stem cells produce live transgenic mice

Haploids and double haploids are important resources for studying recessive traits and have large impacts on crop breeding, but natural haploids are rare in animals. Mammalian haploids are restricted to germline cells and are occasionally found in tumors with massive chromosome loss. Recent success in establishing haploid embryonic stem (ES) cells in medaka fish and mice raised the possibility of using engineered mammalian haploid cells in genetic studies. However, the availability and functional characterization of mammalian haploid ES cells are still limited. Li et al. show that mouse androgenetic haploid ES (ahES) cell lines can be established by transferring sperm into an enucleated oocyte. The ahES cells maintain haploidy and stable growth over 30 passages,

express pluripotent markers, possess the ability to differentiate into all three germ layers in vitro and in vivo, and contribute to germlines of chimeras when injected into blastocysts. Although epigenetically distinct from sperm cells, the ahES cells can produce viable and fertile progenies after intracytoplasmic injection into mature oocytes. The oocyte injection procedure can also produce viable transgenic mice from genetically engineered ahES cells. These findings show the developmental pluripotency of androgenetic haploids and provide a new tool to quickly produce genetic models for recessive traits. They may also shed new light on assisted reproduction.

Nature 2012; 490: 407

Eitan Israeli

MR1 presents microbial vitamin B metabolites to MAIT cells

Antigen-presenting molecules, encoded by the major histocompatibility complex (MHC) and CD1 family, bind peptide- and lipid-based antigens, respectively, for recognition by T cells. Mucosal-associated invariant T (MAIT) cells are an abundant population of innate-like T cells in humans that are activated by an antigen(s) bound to the MHC class I-like molecule MR1. Although the identity of MR1-restricted antigen(s) is unknown, it is present in numerous bacteria and yeast. Kjer-Nielsen et al. show that the structure and chemistry within the antigen-binding cleft of MR1 is distinct from the MHC and CD1 families. MR1 is ideally suited to bind ligands originating from vitamin metabolites. The structure of MR1 in complex with

6-formyl pterin, a folic acid (vitamin B9) metabolite, shows the pterin ring sequestered within MR1. Furthermore, the authors characterize related MR1-restricted vitamin derivatives, originating from the bacterial riboflavin (vitamin B2) biosynthetic pathway, which specifically and potently activate MAIT cells. Accordingly, they show that metabolites of vitamin B represent a class of antigen that are presented by MR1 for MAIT-cell immunosurveillance. As many vitamin biosynthetic pathways are unique to bacteria and yeast, these data suggest that MAIT cells use these metabolites to detect microbial infection.

Nature 2012; doi:10.1038/nature11605

Eitan Israeli

A live impaired-fidelity coronavirus vaccine protects in an aged, immunocompromised mouse model of lethal disease

Live, attenuated RNA virus vaccines are efficacious but subject to reversion to virulence. Among RNA viruses, replication fidelity is recognized as a key determinant of virulence and escape from antiviral therapy; increased fidelity is attenuating for some viruses. Coronavirus (CoV) replication fidelity is approximately 20-fold greater than that of other RNA viruses and is mediated by a 3'5' exonuclease (ExoN) activity that probably functions in RNA proofreading. In this study Graham and colleagues demonstrate that engineered inactivation of severe acute respiratory syndrome (SARS)-

CoV ExoN activity results in a stable mutator phenotype with profoundly decreased fidelity in vivo and attenuation of pathogenesis in young, aged and immunocompromised mice. The ExoN inactivation genotype and mutator phenotype are stable and do not revert to virulence, even after serial passage or long-term persistent infection in vivo. ExoN inactivation has potential for broad applications in the stable attenuation of CoVs and, perhaps, other RNA viruses.

Nature Med 2012; 18: 1820

Eitan Israeli

Targeting VEGF-B as a novel treatment for insulin resistance and type 2 diabetes

The prevalence of type 2 diabetes is rapidly increasing, with severe socioeconomic impacts. Excess lipid deposition in peripheral tissues impairs insulin sensitivity and glucose uptake, and has been proposed to contribute to the pathology of type 2 diabetes. However, few treatment options exist that directly target ectopic lipid accumulation. Recently it was found that vascular endothelial growth factor B (VEGF-B) controls endothelial uptake and transport of fatty acids in heart and skeletal muscle. Hagberg and co-scientists show that decreased VEGF-B signaling in rodent models of type 2 diabetes restores insulin sensitivity and improves glucose tolerance. Genetic deletion of *Vegfb* in diabetic *db/db* mice prevented ectopic lipid deposition, increased muscle glucose uptake and maintained normoglycemia. Pharmacological inhibition of VEGF-B signaling by antibody administration to *db/db* mice enhanced glucose tolerance, preserved

pancreatic islet architecture, improved B cell function and ameliorated dyslipidemia – key elements of type 2 diabetes and the metabolic syndrome. The potential use of VEGF-B neutralization in type 2 diabetes was further elucidated in rats fed a high fat diet, in which it normalized insulin sensitivity and increased glucose uptake in skeletal muscle and heart. Their results demonstrate that the vascular endothelium can function as an efficient barrier to excess muscle lipid uptake even under conditions of severe obesity and type 2 diabetes, and that this barrier can be maintained by inhibition of VEGF-B signaling. The authors propose VEGF-B antagonism as a novel pharmacological approach for type 2 diabetes, targeting the lipid-transport properties of the endothelium to improve muscle insulin sensitivity and glucose disposal.

Nature 2012; 490: 426

Eitan Israeli