IN BRIEF

GALLBLADDER DISORDERS

Targeting NETs to treat gallstones

The mechanisms that lead to the generation of gallstones remain unclear. By analysing human biliary sludge (small stones in biliary fluid) and larger gallstones, Munoz et al. identify key signs of the presence of neutrophil extracellular traps (NETs), including deposits of extracellular DNA and robust neutrophil elastase activity (involved in NET formation). A series of studies using human neutrophils and gallstones confirmed a role of NETs in gallstone formation and growth. Genetic and pharmaceutical inhibition of NET formation or neutrophil activity effectively prevented and treated gallstones in mice.

ORIGINAL ARTICLE Munoz, L. E. et al. Neutrophil extracellular traps initiate gallstone formation. *Immunity* **51**, 1–8 (2019)

PAIN

Orphan GPCR exhibits anti-opioid activity

Improved understanding of the mammalian μ-opioid receptor (MOR) system is vital for the development of safer opioid analgesics. Here, Wang et al. generate transgenic *Caenorhabditis elegans* expressing the mammalian MOR (tgMOR) for unbiased genetic discovery of opioid modulators. By engineering mutations in ~2,500 tgMOR animals, they ultimately identified the orphan receptor, GPR139, as a negative regulator of MOR signalling. GPR139 is coexpressed with MOR in opioid-sensitive brain regions and influences MOR trafficking and signalling properties. GPR139 deletion in mice enhanced opioid-induced inhibition of neuronal firing, increased the analgesic and rewarding effects of morphine and reduced withdrawal.

ORIGINAL ARTICLE Wang, D. et al. Genetic behavioral screen identifies an orphan anti-opioid system. *Science* https://doi.org/10.1126/science.aau2078 (2019)

IMMUNOTHERAPY

DuoCAR-T cells eliminate HIV

Chimeric antigen receptor (CAR)-T cells, which have shown success in the treatment of blood cancers, could potentially eradicate HIV infection. Anthony-Gonda et al. have designed HIV-1-specific CAR-T cells based on a two-molecule CAR architecture, termed duoCAR, which targets multiple sites on the HIV envelope glycoprotein. In vitro, the duoCAR-T cells exerted broad and potent killing of HIV-infected blood cells. Notably, the duoCAR-T cells themselves were resistant to HIV infection. In a humanized mouse model of intrasplenic HIV infection, the duoCAR-T cells exerted long-term control of HIV infection and prevented loss of $CD4^+$ T cells.

ORIGINAL ARTICLE Anthony-Gonda, K. et al. Multispecific anti-HIV duoCAR-T cells display broad in vitro antiviral activity and potent in vivo elimination of HIV-infected cells in a humanized mouse model. *Sci. Transl Med.* **11**, eaav5685 (2019)

INFECTIOUS DISEASE

Profiling the malaria transcriptome

Malaria-causing parasites display remarkable cellular plasticity during their complex life cycle, but remain relatively uncharacterized at the molecular level. Here, Howick et al. use a modified Smart-seq2 approach, to profile the single-cell transcriptomes of thousands of individual malaria parasites, spanning the entire life-cycle of *Plasmodium berghei*. Droplet sequencing was applied to further characterize cells from red blood cell stages of *P. berghei* and three additional species, *P. falciparum*, *P. malariae* and *P. knowlesi*, including parasites taken from patients with malaria. The resulting freely accessible data set, the Malaria Cell Atlas, provides insight into gene usage and function, and may inform future drug and vaccine development.

ORIGINAL ARTICLE Howick, V. M. et al. The Malaria Cell Atlas: single parasite transcriptomes across the complete *Plasmodium* life cycle. *Science* **365**, eaaw2619 (2019)

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NEUROLOGICAL DISORDERS

Reversing Rett syndrome

There are currently no treatments for the neurodevelopmental disorder Rett syndrome (RTT), which is caused by mutations in the X-linked gene *MECP2*, a transcription factor that binds to methylated DNA and regulates gene expression. Now, writing in *Science Translational Medicine*, Jaenisch, Sur and colleagues have identified small-molecule compounds that ameliorate RTT symptoms in mice by enhancing the expression of the K^+–Cl^- cotransporter 2 (KCC2).

KCC2 plays a pivotal role in the balance between excitatory and inhibitory neural activities (E/I balance). In previous work, the authors reported that KCC2 expression is reduced both in neurons derived from patients with RTT and in brains of the *Mecp2*-mutant mouse model of RTT. The authors found that such a decrease in KCC2 expression resulted in a depolarizing shift in the GABA reversal potential (E_{GABA}), which reflects impaired GABAergic inhibition. Increasing KCC2 expression to restore brain E/I balance therefore represents an attractive therapeutic approach to treat RTT.

With this in mind, Jaenisch, Sur, and colleagues first developed a platform to screen for KCC2 expression-enhancing compounds (KEECs) in human neurons. To do this, they applied CRISPR–Cas9 genome-editing technology to insert a 2A-luciferase reporter gene directly before the stop codon of the endogenous *KCC2* locus in human embryonic stem cells, which were then differentiated into KCC2 expression reporter human neurons.

Next, using the reporter neurons, the authors screened a collection of 929 small-molecule compounds compiled from several drug libraries and identified 30 compounds as hit KEECs, which included FDA-approved inhibitors of the FMS-like tyrosine kinase 3 (FLT3) or glycogen synthase kinase 3β (GSK3β) pathways and activators of the sirtuin 1 (SIRT1) and transient receptor potential cation channel subfamily V member 1 (TRPV1) pathways.

The KEECs enhanced KCC2 mRNA and protein expression in organotypic mouse brain slices and in human wild-type and isogenic *MECP2*-mutant RTT neurons. Further studies, using siRNA and pharmacology, confirmed that inhibition of the FLT3 or GSK3β pathways or activation of the SIRT1 pathway or the TRPV1 channel enhances KCC2 expression in human RTT neurons.

The KEECs also rescued electrophysiological and morphological abnormalities of RTT. Treatment of cultured human RTT neurons with KEECs induced a hyperpolarizing shift in E_{GABA} and increased the frequency of miniature excitatory postsynaptic currents. Furthermore, the compounds reversed deficits in nuclei size and neurite complexity and branching — hallmarks of RTT neurons.

Finally, the authors assessed the in vivo efficacy of identified KEECs. In a *Mecp2*-mutant mouse model of RTT, daily injection with the FLT3 inhibitor KW-2449 or the TRPV1 agonist piperine (the molecule responsible for the pungency of black pepper) ameliorated severe RTT symptoms — apnoea and locomotor activity reduction.

These findings support further investigation and optimization of KEECs, many of which have already been proved safe for human consumption, for clinical trials to treat Rett syndrome. Given that KCC2 expression is reduced in other brain disorders — including epilepsy, schizophrenia, spinal cord injury and stroke — the small-molecule compounds identified in this study may have diverse applications.

Sarah Crunkhorn

ORIGINAL ARTICLE Tang, X. et al. Pharmacological enhancement of *KCC2* gene expression exerts therapeutic effects on human Rett syndrome neurons and *Mecp2* mutant mice. *Sci. Transl Med.* **11**, eaau0164 (2019)