PNAS Plus Significance Statements

Contributions of microbiome and mechanical deformation to intestinal bacterial overgrowth and inflammation in a human gut-on-a-chip

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The main advance of this study is the development of a microengineered model of human intestinal inflammation and bacterial overgrowth that permits analysis of individual contributors to the pathophysiology of intestinal diseases, such as ileus and inflammatory bowel disease, over a period of weeks in vitro. By studying living human intestinal epithelium, with or without vascular and lymphatic endothelium, immune cells, and mechanical deformation, as well as living microbiome and pathogenic microbes, we identified previously unknown contributions of specific cytokines, mechanical motions, and microbiome to intestinal inflammation, bacterial overgrowth, and control of barrier function. We provide proof-of-principle to show that the microfluidic gut-on-a-chip device can be used to create human intestinal disease models and gain new insights into gut pathophysiology. (See pp. E7–E15.)

Harnessing RNAi-based nanomedicines for therapeutic gene silencing in B-cell malignancies

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RNA interference (RNAi) holds great promise as a novel therapeutic approach. Small interfering RNAs (siRNAs) that manipulate gene expression in leukocytes could be used to treat blood cancers. However, the lack of strategies for delivering siRNAs to leukocytes systemically has hampered the development of RNAi-based therapeutics. Here, we show that lipid-based nanoparticles coated with anti-CD38 monoclonal antibodies specifically target mantle cell lymphoma (MCL) cells and induce cell-specific therapeutic gene silencing in vivo. CD38-targeted nanoparticles that contain cyclin D1 siRNAs prolong survival of mice bearing MCL lymphomas in the bone marrow. This strategy opens a new avenue for treating MCL that might be applied to other hematological malignancies. (See pp. E16–E22.)

Oxidization without substrate unfolding triggers proteolysis of the peroxide-sensor, PerR

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Life in aerobic environments inevitably generates reactive oxygen species (ROS), which damage proteins and other cellular components, often irreversibly. Cells must degrade these potentially harmful, damaged macromolecules. A bacterial ROS sensor, the transcriptional repressor PerR, is itself controlled by irreversible oxidation of its metal-binding site. We found that active PerR is stable, but that this oxidation marks PerR for degradation by the protease LonA. We identified a LonA-binding site in PerR, which is exposed in structures of unliganded or oxidized PerR, but buried in the liganded active form. Therefore, we propose that an oxidation-induced and activity-coupled conformational change in PerR triggers its degradation. Similar allostery may explain how site-specific and mild oxidation can commit proteins to degradation. (See pp. E23–E31.)

Nuclear deformability and telomere dynamics are regulated by cell geometric constraints

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Physical properties of the cell nucleus are important for various cellular functions. However, the role of cell geometry and active cytoskeletal forces in regulating nuclear dynamics and chromatin dynamics is not well understood. Our results show cells with reduced matrix constraints have short actomyosin structures. These dynamic structures together with lower lamin A/C levels, resulting in softer nuclei, may provide the driving force for nuclear fluctuations. Furthermore, we observed increased dynamics of heterochromatin and telomere structures under such reduced cell–matrix interactions. We conclude that extracellular matrix signals alter cytoskeletal organization and lamin A/C expression levels, which together lead to nuclear and chromatin dynamics. These results highlight the importance of matrix constraints in regulating gene expression and maintaining genome integrity. (See pp. E32–E40.)

Endosidin2 targets conserved exocyst complex subunit EXO70 to inhibit exocytosis

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The exocyst complex is a conserved protein complex that tethers the secretory vesicles to the site of membrane fusion during exocytosis, an essential cellular process that transports molecules, such as protein, to the cell surface or extracellular space. We identified a small molecule that targets the EXO70 (exocyst component of 70 kDa) subunit of the exocyst complex to

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inhibit exocytosis. This compound made it possible to control the dynamics of the exocytosis process in a dosage-dependent manner in different organisms and overcame the mutant lethality and genetic redundancy issues in studying mechanisms of exocyst complex regulation. Further design of molecules with higher affinity and more potent activity may make it possible to use drugs to control human diseases related to exocytosis, such as cancer and diabetes. (See pp. E41–E50.)

The histone H2A deubiquitinase Usp16 regulates hematopoiesis and hematopoietic stem cell function

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Polycomb repressive complex 1 (PRC1) represents an important epigenetic regulator, which exerts its effect on gene expression via histone H2A ubiquitination (ubH2A). We developed a conditional *Usp16* knockout mouse model and demonstrated that *Usp16* is indispensable for hematopoiesis and hematopoietic stem cell (HSC) lineage commitment. We identified Usp16 to be a H2A deubiquitinase that counterbalances the PRC1 ubiquitin ligase to control ubH2A level in the hematopoietic system. Conditional *Usp16* deletion led to altered expression of many regulators of chromatin organization and hematopoiesis. In addition, Usp16 maintains normal HSC cell cycle status via repressing the expression of *Cdkn1a*, which encodes p21cip1, an inhibitor of cell cycle entry. This study provides novel insights into the epigenetic mechanism that regulates hematopoiesis and HSC function. (See pp. E51–E60.)

Limitations of GCTA as a solution to the missing heritability problem

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The genetic contribution to a phenotype is frequently measured by heritability, the fraction of trait variation explained by genetic differences. Hundreds of publications have found DNA polymorphisms that are statistically associated with diseases or quantitative traits [genome-wide association studies (GWASs)]. Genome-wide complex trait analysis (GCTA), a recent method of analyzing such data, finds high heritabilities for such phenotypes. We analyze GCTA and show that the heritability estimates it produces are highly sensitive to the structure of the genetic relatedness matrix, to the sampling of phenotypes and subjects, and to the accuracy of phenotype measurements. Plausible modifications of the method aimed at increasing stability yield much smaller heritabilities. It is essential to reevaluate the many published heritability estimates based on GCTA. (See pp. E61–E70.)

Dysregulated YAP1/TAZ and TGF-β signaling mediate hepatocarcinogenesis in *Mob1a/1b*-deficient mice

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Patients with intrahepatic cholangiocellular carcinoma (ICC) and combined hepatocellular and cholangiocarcinoma (cHC-CC) have worse prognoses than those with hepatocellular carcinoma and rarely show clinical responses to drugs. Our analyses of mice with liver-specific deletions of Mps One Binder Kinase Activator (MOB)1A/1B reveal that MOB1A/1B constitute the most important hub of Hippo signaling in mammalian liver. MOB1A/1B maintain hepatocyte stem/progenitor cell quiescence and are potent tumor suppressors, especially in cHC-CCs and ICCs. Because these functions depend on the Hippo target *Yap1/Taz* and the *Yap1/Taz* targets *Tgfb*s, our data point to a new therapeutic approach for liver cancer based on inhibition of MOB1-YAP1/TAZ and/or TGF-βs–SMADs signaling. Our demonstration that well-tolerated and already-approved antiparasitic drugs inhibit YAP1 signaling may point to a new route of treatment for these cancers that can be rapidly tested and implemented. (See pp. E71–E80.)

Transplantation of human embryonic stem cell-derived retinal tissue in two primate models of retinal degeneration

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We first confirmed the ability of human embryonic stem cell-derived retina (hESC-retina) to form structured mature photoreceptor layers after transplantation into nude rats. We then developed two monkey models of retinal degeneration and evaluated their utility as host models for transplantation studies. Finally, we performed a pilot study of hESC-retina transplantation in the developed models and conducted in vivo monitoring studies using clinical devices and subsequently confirmed structured graft maturation and the potential formation of synaptic contacts between graft and host cells. This study demonstrates the competency of hESC-retina as a graft source and the eligibility of two newly developed monkey models that may be useful in future, long-term, functional studies of retinal transplantation. (See pp. E81–E90.)

Regulation of neural gene transcription by optogenetic inhibition of the RE1-silencing transcription factor

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Repressor element 1-silencing transcription factor (REST) is a transcriptional repressor that regulates nervous system development. Normally expressed at low levels by mature neurons, REST is up-regulated in various brain pathologies. Using a light-sensitive domain from the oat plant (*Avena sativa*), we engineered novel optogenetic proteins that inhibit REST activity when illuminated by blue light, thus obtaining the spatial and temporal control of the transcription of REST target genes. This approach may have an impact in the development of new therapies for all the diseases in which REST is dysregulated, such as epilepsy, ischemia, and cancers of various origin, as such therapies may counteract the long-term changes in gene expression that take place in the context of the pathological brain. (See pp. E91–E100.)