Science Abstracts

Cell

L. M. Cox et al., **Altering the Intestinal Microbiota during a Critical Developmental Window Has Lasting Metabolic Consequences**, *Cell* 158.4 (August 14, 2014): 705–721 • Acquisition of the intestinal microbiota begins at birth, and a stable microbial community develops from a succession of key organisms. Disruption of the microbiota during maturation by low-dose antibiotic exposure can alter host metabolism and adiposity. We now show that low-dose penicillin (LDP), delivered from birth, induces metabolic alterations and affects ileal expression of genes involved in immunity. LDP that is limited to early life transiently perturbs the microbiota, which is sufficient to induce sustained effects on body composition, indicating that microbiota interactions in infancy may be critical determinants of long-term host metabolic effects. In addition, LDP enhances the effect of high-fat diet induced obesity. The growth promotion phenotype is transferrable to germ-free hosts by LDP-selected microbiota, showing that the altered microbiota, not antibiotics per se, play a causal role. These studies characterize important variables in early-life microbe-host metabolic interaction and identify several taxa consistently linked with metabolic alterations.

H. Qin et al., **Systematic Identification of Barriers to Human iPSC Generation**, *Cell* 158.2 (July 17, 2014): 449–461 • Reprogramming of somatic cells to induced pluripotent stem cells (iPSCs) holds enormous promise for regenerative medicine. To elucidate endogenous barriers limiting this process, we systematically dissected human cellular reprogramming by combining a genome-wide RNAi screen, innovative computational methods, extensive single-hit validation, and mechanistic investigation of relevant pathways and networks. We identify reprogramming barriers, including genes involved in transcription, chromatin regulation, ubiquitination, dephosphorylation, vesicular transport, and cell adhesion. Specific a disintegrin and metalloproteinase (ADAM) proteins inhibit reprogramming, and the disintegrin domain of ADAM29 is necessary and sufficient for this function. Clathrin-mediated endocytosis can be targeted with small molecules and opposes reprogramming by positively regulating TGF-β signaling. Genetic interaction studies of endocytosis or ubiquitination reveal that barrier pathways can act in linear, parallel, or feedforward loop architectures to antagonize reprogramming. These results provide a global view of barriers to human cellular reprogramming.

J. Riddell et al., **Reprogramming Committed Murine Blood Cells to Induced Hematopoietic Stem Cells with Defined Factors**, *Cell* 157.3 (April 24, 2014): 549–564 • Hematopoietic stem cells (HSCs) sustain blood formation throughout life and are the functional units of bone marrow transplantation. We show that transient expression of six transcription factors Run1t1, Hlf, Lmo2, Prdm5, Pbx1, and Zfp37 imparts multilineage transplantation potential onto otherwise committed lymphoid and myeloid progenitors and myeloid effector cells. Inclusion of Mycn and Meis1 and use of polycistronic viruses increase reprogramming efficacy. The reprogrammed cells, designated induced-HSCs (iHSCs), possess clonal multilineage differentiation potential, reconstitute stem/progenitor compartments, and are serially transplantable. Single-cell analysis revealed that iHSCs derived under optimal conditions exhibit a gene expression profile that is highly similar to endogenous HSCs. These findings demonstrate that expression of a set of defined factors is sufficient to activate the gene networks governing

HSC functional identity in committed blood cells. Our results raise the prospect that blood cell reprogramming may be a strategy for derivation of transplantable stem cells for clinical application.

Cell Metabolism

M. Petruzzelli et al., **A Switch from White to Brown Fat Increases Energy Expenditure in Cancer-Associated Cachexia**, *Cell Metab* 20.3 (September 2, 2014): 433–447 • Cancer-associated cachexia (CAC) is a wasting syndrome characterized by systemic inflammation, body weight loss, atrophy of white adipose tissue (WAT) and skeletal muscle. Limited therapeutic options are available and the underlying mechanisms are poorly defined. Here we show that a phenotypic switch from WAT to brown fat, a phenomenon termed WAT browning, takes place in the initial stages of CAC, before skeletal muscle atrophy. WAT browning is associated with increased expression of uncoupling protein 1 (UCP1), which uncouples mitochondrial respiration toward thermogenesis instead of ATP synthesis, leading to increased lipid mobilization and energy expenditure in cachectic mice. Chronic inflammation and the cytokine interleukin-6 increase UCP1 expression in WAT, and treatments that reduce inflammation or β-adrenergic blockade reduce WAT browning and ameliorate the severity of cachexia. Importantly, UCP1 staining is observed in WAT from CAC patients. Thus, inhibition of WAT browning represents a promising approach to ameliorate cachexia in cancer patients.

Human Molecular Genetics

I. Ezkurdia et al., **Multiple Evidence Strands Suggest That There May Be as Few as 19,000 Human Protein-Coding Genes**, *Hum Mol Genet*, e-pub June 16, 2014, doi: 10.1093/hmg/ddu309 • Determining the full complement of protein-coding genes is a key goal of genome annotation. The most powerful approach for confirming protein-coding potential is the detection of cellular protein expression through peptide mass spectrometry (MS) experiments. Here, we mapped peptides detected in seven large-scale proteomics studies to almost 60% of the protein-coding genes in the GENCODE annotation of the human genome. We found a strong relationship between detection in proteomics experiments and both gene family age and cross-species conservation. Most of the genes for which we detected peptides were highly conserved. We found peptides for >96% of genes that evolved before bilateria. At the opposite end of the scale, we identified almost no peptides for genes that have appeared since primates, for genes that did not have any protein-like features or for genes with poor cross-species conservation. These results motivated us to describe a set of 2001 potential non-coding genes based on features such as weak conservation, a lack of protein features, or ambiguous annotations from major databases, all of which correlated with low peptide detection across the seven experiments. We identified peptides for just 3% of these genes. We show that many of these genes behave more like non-coding genes than protein-coding genes and suggest that most are unlikely to code for proteins under normal circumstances. We believe that their inclusion in the human protein-coding gene catalogue should be revised as part of the ongoing human genome annotation effort.

Nature

N. Kaukua et al., **Glial Origin of Mesenchymal Stem Cells in a Tooth Model System**, *Nature* 513.7519 (September 25, 2014): 551–554 • Mesenchymal stem cells occupy niches in stromal tissues where they provide sources of cells for specialized mesenchymal derivatives during growth and repair. The origins of mesenchymal stem cells have been the subject of considerable discussion, and current consensus holds that perivascular cells form mesenchymal stem cells in most tissues. The continuously growing mouse incisor tooth offers an excellent model to address the origin of mesenchymal stem cells. These stem cells dwell in a niche at the tooth apex where they produce a variety of differentiated derivatives. Cells constituting the tooth are mostly derived from two embryonic sources: neural crest ectomesenchyme and

ectodermal epithelium. It has been thought for decades that the dental mesenchymal stem cells giving rise to pulp cells and odontoblasts derive from neural crest cells after their migration in the early head and formation of ectomesenchymal tissue. Here we show that a significant population of mesenchymal stem cells during development, self-renewal and repair of a tooth are derived from peripheral nerve-associated glia. Glial cells generate multipotent mesenchymal stem cells that produce pulp cells and odontoblasts. By combining a clonal colour-coding technique with tracing of peripheral glia, we provide new insights into the dynamics of tooth organogenesis and growth.

S. Kir et al., **Tumour-Derived PTH-Related Protein Triggers Adipose Tissue Browning and Cancer Cachexia**, *Nature* 513.7516 (September 4, 2014): 100–104 • Cachexia is a wasting disorder of adipose and skeletal muscle tissues that leads to profound weight loss and frailty. About half of all cancer patients suffer from cachexia, which impairs quality of life, limits cancer therapy and decreases survival. One key characteristic of cachexia is higher resting energy expenditure levels than in healthy individuals, which has been linked to greater thermogenesis by brown fat. How tumours induce brown fat activity is unknown. Here, using a Lewis lung carcinoma model of cancer cachexia, we show that tumour-derived parathyroid-hormone-related protein (PTHrP) has an important role in wasting, through driving the expression of genes involved in thermogenesis in adipose tissues. Neutralization of PTHrP in tumour-bearing mice blocked adipose tissue browning and the loss of muscle mass and strength. Our results demonstrate that PTHrP mediates energy wasting in fat tissues and contributes to the broader aspects of cancer cachexia. Thus, neutralization of PTHrP might hold promise for ameliorating cancer cachexia and improving patient survival.

B. R. Ksander et al., **ABCB5 is a Limbal Stem Cell Gene Required for Corneal Development and Repair**, *Nature* 511.7509 (July 17, 2014): 353–357 • Corneal epithelial homeostasis and regeneration are sustained by limbal stem cells (LSCs), and LSC deficiency is a major cause of blindness worldwide. Transplantation is often the only therapeutic option available to patients with LSC deficiency. However, while transplant success depends foremost on LSC frequency within grafts, a gene allowing for prospective LSC enrichment has not been identified so far. Here we show that ATP-binding cassette, sub-family B, member 5 (ABCB5) marks LSCs and is required for LSC maintenance, corneal development and repair. Furthermore, we demonstrate that prospectively isolated human or murine ABCB5-positive LSCs possess the exclusive capacity to fully restore the cornea upon grafting to LSC-deficient mice in xenogeneic or syngeneic transplantation models. ABCB5 is preferentially expressed on label-retaining LSCs in mice and p63α-positive LSCs in humans. Consistent with these findings, ABCB5-positive LSC frequency is reduced in LSC-deficient patients. Abcb5 loss of function in Abcb5 knockout mice causes depletion of quiescent LSCs due to enhanced proliferation and apoptosis, and results in defective corneal differentiation and wound healing. Our results from gene knockout studies, LSC tracing and transplantation models, as well as phenotypic and functional analyses of human biopsy specimens, provide converging lines of evidence that ABCB5 identifies mammalian LSCs. Identification and prospective isolation of molecularly defined LSCs with essential functions in corneal development and repair has important implications for the treatment of corneal disease, particularly corneal blindness due to LSC deficiency.

R. L. Redondo et al., **Bidirectional Switch of the Valence Associated with a Hippocampal Contextual Memory Engram**, *Nature* 513.7518 (September 18, 2014): 426–430 • The valence of memories is malleable because of their intrinsic reconstructive property. This property of memory has been used clinically to treat maladaptive behaviours. However, the neuronal mechanisms and brain circuits that enable the switching of the valence of memories remain largely

unknown. Here we investigated these mechanisms by applying the recently developed memory engram cell-manipulation technique. We labelled with channelrhodopsin-2 (ChR2) a population of cells in either the dorsal dentate gyrus (DG) of the hippocampus or the basolateral complex of the amygdala (BLA) that were specifically activated during contextual fear or reward conditioning. Both groups of fear-conditioned mice displayed aversive light-dependent responses in an optogenetic place avoidance test, whereas both DG- and BLA-labelled mice that underwent reward conditioning exhibited an appetitive response in an optogenetic place preference test. Next, in an attempt to reverse the valence of memory within a subject, mice whose DG or BLA engram had initially been labelled by contextual fear or reward conditioning were subjected to a second conditioning of the opposite valence while their original DG or BLA engram was reactivated by blue light. Subsequent optogenetic place avoidance and preference tests revealed that although the DG-engram group displayed a response indicating a switch of the memory valence, the BLA-engram group did not. This switch was also evident at the cellular level by a change in functional connectivity between DG engram-bearing cells and BLA engram-bearing cells. Thus, we found that in the DG, the neurons carrying the memory engram of a given neutral context have plasticity such that the valence of a conditioned response evoked by their reactivation can be reversed by re-associating this contextual memory engram with a new unconditioned stimulus of an opposite valence. Our present work provides new insight into the functional neural circuits underlying the malleability of emotional memory.

Nature Communications

R. Bouchi et al., **FOXO1 Inhibition Yields Functional Insulin-Producing Cells in Human Gut Organoid Cultures**, *Nat Comm* 5 (June 30, 2014), doi: 10.1038/ncomms5242 • Generation of surrogate sources of insulin-producing β-cells remains a goal of diabetes therapy. While most efforts have been directed at differentiating embryonic or induced pluripotent stem (iPS) cells into β-like-cells through endodermal progenitors, we have shown that gut endocrine progenitor cells of mice can be differentiated into glucose-responsive, insulin-producing cells by ablation of transcription factor Foxo1. Here we show that FOXO1 is present in human gut endocrine progenitor and serotonin-producing cells. Using gut organoids derived from human iPS cells, we show that FOXO1 inhibition using a dominant-negative mutant or lentivirus-encoded small hairpin RNA promotes generation of insulin-positive cells that express all markers of mature pancreatic β-cells, release C-peptide in response to secretagogues and survive in vivo following transplantation into mice. The findings raise the possibility of using gut-targeted FOXO1 inhibition or gut organoids as a source of insulin-producing cells to treat human diabetes.

Nature Medicine

T.A. Berendsen et al., **Supercooling Enables Long-Term Transplantation Survival Following 4 Days of Liver Preservation**, *Nat Med* 20.7 (July 2014): 790–793 • The realization of long-term human organ preservation will have groundbreaking effects on the current practice of transplantation. Herein we present a new technique based on subzero nonfreezing preservation and extracorporeal machine perfusion that allows transplantation of rat livers preserved for up to four days, thereby tripling the viable preservation duration.

J. H. Kang et al., **An Extracorporeal Blood-Cleansing Device for Sepsis Therapy**, *Nat Med,* e-pub September 14, 2014, doi:10.1038/nm.3640 • Here we describe a blood-cleansing device for sepsis therapy inspired by the spleen, which can continuously remove pathogens and toxins from blood without first identifying the infectious agent. Blood flowing from an infected individual is mixed with magnetic nanobeads coated with an engineered human opsonin—mannose-binding lectin (MBL)—that captures a broad range of pathogens and toxins without activating

complement factors or coagulation. Magnets pull the opsonin-bound pathogens and toxins from the blood; the cleansed blood is then returned back to the individual. The biospleen efficiently removes multiple Gram-negative and Gram-positive bacteria, fungi and endotoxins from whole human blood flowing through a single biospleen unit at up to 1.25 liters per h *in vitro*. In rats infected with *Staphylococcus aureus* or *Escherichia coli*, the biospleen cleared >90% of bacteria from blood, reduced pathogen and immune cell infiltration in multiple organs and decreased inflammatory cytokine levels. In a model of endotoxemic shock, the biospleen increased survival rates after a 5-h treatment.

Nature Neuroscience

H. Cai et al., **Central Amygdala PKC-δ$^+$ Neurons Mediate the Influence of Multiple Anorexigenic Signals**, *Nat Neurosci* 17.9 (September 2014): 1240–1248 • Feeding can be inhibited by multiple cues, including those associated with satiety, sickness or unpalatable food. How such anorexigenic signals inhibit feeding at the neural circuit level is not completely understood. Although some inhibitory circuits have been identified, it is not yet clear whether distinct anorexigenic influences are processed in a convergent or parallel manner. The amygdala central nucleus (CEA) has been implicated in feeding control, but its role is controversial. The lateral subdivision of CEA (CEl) contains a subpopulation of GABAergic neurons that are marked by protein kinase C-δ (PKC-δ). We found that CEl PKC-δ(+) neurons in mice were activated by diverse anorexigenic signals in vivo, were required for the inhibition of feeding by such signals and strongly suppressed food intake when activated. They received presynaptic inputs from anatomically distributed neurons activated by different anorexigenic agents. Our data suggest that CEl PKC-δ(+) neurons constitute an important node that mediates the influence of multiple anorexigenic signals.

PLOS Biology

M. Gouti et al., **In Vitro Generation of Neuromesodermal Progenitors Reveals Distinct Roles for Wnt Signalling in the Specification of Spinal Cord and Paraxial Mesoderm Identity**, *PLoS Biol* 12.8 (August 26, 2014): e1001937 • Cells of the spinal cord and somites arise from shared, dual-fated precursors, located towards the posterior of the elongating embryo. Here we show that these neuromesodermal progenitors (NMPs) can readily be generated in vitro from mouse and human pluripotent stem cells by activating Wnt and Fgf signalling, timed to emulate in vivo development. Similar to NMPs in vivo, these cells co-express the neural factor Sox2 and the mesodermal factor Brachyury and differentiate into neural and paraxial mesoderm in vitro and in vivo. The neural cells produced by NMPs have spinal cord but not anterior neural identity and can differentiate into spinal cord motor neurons. This is consistent with the shared origin of spinal cord and somites and the distinct ontogeny of the anterior and posterior nervous system. Systematic analysis of the transcriptome during differentiation identifies the molecular correlates of each of the cell identities and the routes by which they are obtained. Moreover, we take advantage of the system to provide evidence that Brachyury represses neural differentiation and that signals from mesoderm are not necessary to induce the posterior identity of spinal cord cells. This indicates that the mesoderm inducing and posteriorising functions of Wnt signalling represent two molecularly separate activities. Together the data illustrate how reverse engineering normal developmental mechanisms allows the differentiation of specific cell types in vitro and the analysis of previous difficult to access aspects of embryo development.

PLOS One

V. Voon et al., **Neural Correlates of Sexual Cue Reactivity in Individuals with and without Compulsive Sexual Behaviours**, *PLoS One* 9.7 (July 11, 2014): e102419 • Although compulsive sexual behaviour (CSB) has been conceptualized as a "behavioural" addiction and common or overlapping neural circuits may govern the processing of natural and drug rewards, little

is known regarding the responses to sexually explicit materials in individuals with and without CSB. Here, the processing of cues of varying sexual content was assessed in individuals with and without CSB, focusing on neural regions identified in prior studies of drug-cue reactivity. 19 CSB subjects and 19 healthy volunteers were assessed using functional MRI comparing sexually explicit videos with non-sexual exciting videos. Ratings of sexual desire and liking were obtained. Relative to healthy volunteers, CSB subjects had greater desire but similar liking scores in response to the sexually explicit videos. Exposure to sexually explicit cues in CSB compared to non-CSB subjects was associated with activation of the dorsal anterior cingulate, ventral striatum and amygdala. Functional connectivity of the dorsal anterior cingulate-ventral striatum-amygdala network was associated with subjective sexual desire (but not liking) to a greater degree in CSB relative to non-CSB subjects. The dissociation between desire or wanting and liking is consistent with theories of incentive motivation underlying CSB as in drug addictions. Neural differences in the processing of sexual-cue reactivity were identified in CSB subjects in regions previously implicated in drug-cue reactivity studies. The greater engagement of corticostriatal limbic circuitry in CSB following exposure to sexual cues suggests neural mechanisms underlying CSB and potential biological targets for interventions.

PNAS

H. S. Lee et al., **Astrocytes Contribute to Gamma Oscillations and Recognition Memory**, *PNAS* 111.32 (August 12, 2014): E3343–E3352 • Glial cells are an integral part of functional communication in the brain. Here we show that astrocytes contribute to the fast dynamics of neural circuits that underlie normal cognitive behaviors. In particular, we found that the selective expression of tetanus neurotoxin (TeNT) in astrocytes significantly reduced the duration of carbachol-induced gamma oscillations in hippocampal slices. These data prompted us to develop a novel transgenic mouse model, specifically with inducible tetanus toxin expression in astrocytes. In this in vivo model, we found evidence of a marked decrease in electroencephalographic (EEG) power in the gamma frequency range in awake-behaving mice, whereas neuronal synaptic activity remained intact. The reduction in cortical gamma oscillations was accompanied by impaired behavioral performance in the novel object recognition test, whereas other forms of memory, including working memory and fear conditioning, remained unchanged. These results support a key role for gamma oscillations in recognition memory. Both EEG alterations and behavioral deficits in novel object recognition were reversed by suppression of tetanus toxin expression. These data reveal an unexpected role for astrocytes as essential contributors to information processing and cognitive behavior.

Stem Cells

H. W. Choi et al., **Neural Stem Cells Differentiated From iPS Cells Spontaneously Regain Pluripotency**, *Stem Cells* 32.10 (October 2014): 2596–2604 • Differentiated somatic cells can be reprogrammed into pluripotent stem cells by transduction of exogenous reprogramming factors. After induced pluripotent stem (iPS) cells are established, exogenous genes are silenced. In the pluripotent state, retroviral genes integrated in the host genome are kept inactive through epigenetic transcriptional regulation. In this study, we tried to determine whether exogenous genes remain silenced or are reactivated upon loss of pluripotency or on differentiation using an in vitro system. We induced differentiation of iPS cells into neural stem cells (NSCs) in vitro; the NSCs appeared morphologically indistinguishable from brain-derived NSCs and stained positive for the NSC markers Nestin and Sox2. These iPS cell-derived NSCs (iPS-NSCs) were also capable of differentiating into all three neural subtypes. Interestingly, iPS-NSCs spontaneously formed aggregates on long-term culture and showed reactivation of the *Oct4*-GFP marker, which was followed by the formation of embryonic stem cell-like colonies. The spontaneously reverted green fluorescent protein (GFP)-positive (iPS-NSC-GFP$^+$)

cells expressed high levels of pluripotency markers (Oct4 and Nanog) and formed germline chimeras, indicating that iPS-NSC-GFP$^+$ cells had the same pluripotency as the original iPS cells. The reactivation of silenced exogenous genes was tightly correlated with the downregulation of DNA methyltransferases (Dnmts) during differentiation of iPS cells. This phenomenon was not observed in doxycycline-inducible iPS cells, where the reactivation of exogenous genes could be induced only by doxycycline treatment. These results indicate that pluripotency can be regained through reactivation of exogenous genes, which is associated with dynamic change of Dnmt levels during differentiation of iPS cells.

M. B. Preda et al., **Remote Transplantation of Mesenchymal Stem Cells Protects the Heart against Ischemia-Reperfusion Injury**, *Stem Cells* 32.8 (August 2014): 2123–2134 • Cardioprotection can be evoked through extracardiac approaches. This prompted us to investigate whether remote transplantation of stem cells confers protection of the heart against ischemic injury. The cardioprotective effect of subcutaneous transplantation of naïve versus heme oxygenase-1 (HMOX-1)-overexpressing mouse mesenchymal stem cells (MSC) to mice was investigated in hearts subjected to ischemia-reperfusion in a Langendorff perfusion system. Mice were transplanted into the interscapular region with naïve or HMOX-1 transfected MSC isolated from transgenic luciferase reporter mice and compared to sham-treated animals. The fate of transplanted cells was followed by in vivo bioluminescence imaging, revealing that MSC proliferated, but did not migrate detectably from the injection site. Ex vivo analysis of the hearts showed that remote transplantation of mouse adipose-derived MSC (mASC) resulted in smaller infarcts and improved cardiac function after ischemia-reperfusion compared to sham-treated mice. Although HMOX-1 overexpression conferred cytoprotective effects on mASC against oxidative stress in vitro, no additive beneficial effect of HMOX-1 transfection was noted on the ischemic heart. Subcutaneous transplantation of MSC also improved left ventricular function when transplanted in vivo after myocardial infarction. Plasma analysis and gene expression profile of naïve- and HMOX-1-mASC after transplantation pointed toward pentraxin 3 as a possible factor involved in the remote cardioprotective effect of mASC. These results have significant implications for understanding the behavior of stem cells after transplantation and development of safe and noninvasive cellular therapies with clinical applications. Remote transplantation of MSC can be considered as an alternative procedure to induce cardioprotection.

J. Pulecio et al., **Conversion of Human Fibroblasts into Monocyte-Like Progenitor Cells**, *Stem Cells* 32.11 (November 2014): 2923–2938 • Reprogramming technologies have emerged as a promising approach for future regenerative medicine. Here we report on the establishment of a novel methodology allowing for the conversion of human fibroblasts into Hematopoietic Progenitor-like Cells (HPC) with macrophage differentiation potential. *SOX2* overexpression in human fibroblasts, a gene found to be upregulated during hematopoietic reconstitution in mice, induced the rapid appearance of CD34+ cells with a concomitant upregulation of mesoderm-related markers. Profiling of Cord Blood hematopoietic progenitor cell populations identified miR-125b as a factor facilitating commitment of *SOX2*-generated CD34+ cells to immature hematopoietic-like progenitor cells with grafting potential. Further differentiation towards the monocytic lineage resulted in the appearance of CD14+ cells with functional phagocytic capacity. In vivo transplantation of SOX2/miR-125b-generated CD34+ cells facilitated the maturation of the engrafted cells towards CD45+ cells and ultimately the monocytic/macrophage lineage. Altogether, our results indicate that strategies combining lineage conversion and further lineage specification by in vivo or in vitro approaches could help to circumvent long-standing obstacles for the reprogramming of human cells into hematopoietic cells with clinical potential.