Notable advances 2015

This year saw a whirlwind of insights gleaned into topics ranging from heart cell proliferation to organoid modeling. Here are a few of the research papers detailing some of these intriguing discoveries.

■ Obesity

Expression of risk

Genome-wide association studies for human obesity have consistently identified single-nucleotide polymorphisms (SNPs) in the locus of the fat mass and obesity–associated (*FTO*) gene, which encodes an mRNA methylase. However, it is not clear how the SNPs increase the risk of obesity. Results from a study by an international group of researchers that included teams at Harvard Medical School and at the Massachusetts Institute of Technology help to resolve this uncertainty (*N. Engl. J. Med.* **373**, 895–907, 2015).

Data, including those of genetic co-expression, from human preadipocytes led the researchers to propose a mechanism by which some noncoding variants in *FTO* contribute to obesity by preventing the binding of the transcriptional repressor ARID5B to a site on the *FTO* locus. This site would normally repress the expression of two nearby genes: iroquois homeobox 3 (*IRX3*) and 5 (*IRX5*). *IRX3* and *IRX5* encode transcription factors that, in the early phases of fat cell differentiation, act to suppress key genes involved in thermogenesis while also upregulating those that are involved in lipid storage. The team validated the causality of the risk variants by using CRISPR (clustered regularly interspaced short palindromic repeats)-Cas9 technology to correct the SNP, which reverts the cells' gene expression to that of cells with the non-risk allele and vice versa. These results suggest that obesity-associated variants in the *FTO* locus may result in a skewing of fat progenitor cells during their maturation into lipid-storing white adipocytes rather than into fat-burning beige adipocytes. The results also support the importance of beige fat in protecting humans from obesity.

■ Hepatology

New liver cells

Given the worldwide shortage in organs available for transplantation, cell-based therapy for advanced liver disease is an attractive potential alternative. To this end, three separate studies this year reported newly identified populations of liver cells that arise either under healthy conditions or when the organ is injured. Scientists at the Stanford University School of Medicine in California performed lineage-tracing studies showing that a population of cells that respond to the Wnt protein exists around the central vein of the liver; these cells give birth to new hepatocytes during homeostasis. They also showed that endothelial cells along the central vein are the source of the Wnt proteins that maintain these cells, which thus constitute the progenitor cell niche (*Nature* **524,** 180–185, 2015).

A team of British and Japanese researchers, meanwhile, developed a model of severe liver injury by deleting the E3 ubiquitin ligase MDM2 in the hepatocytes of adult mice, which resulted in the loss or senescence of these cells. This manipulation led to a rapid and extensive activation of hepatic progenitor cells, which repopulated the entire liver and restored normal architecture and function. By using various surface markers and cell sorting, the team then isolated these progenitor cells and expanded them in culture. Their findings showed that injection of the cells into mice with liver injury resulted in the repopulation of the organ and improvements in its function (*Nat. Cell Biol.* **17,** 971–983, 2015).

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An experiment by a team that included researchers at the University of California, San Diego, revealed the existence of hybrid hepatocytes in the periportal region. These cells are dubbed hybrids because they express genes enriched in bile duct cells but give rise to new hepatocytes upon liver injury. The scientists isolated, expanded and injected these hybrid hepatocytes into mice with liver injuries, and they showed that these cells could repopulate the liver. In spite of their high proliferative capacity, these hybrid hepatocytes did not give rise to cancerous cells in the three mouse models of liver cancer that the researchers tested (*Cell* **162,** 766–779, 2015).

■ Infectious Disease

Ebola vaccine hope

The Ebola virus outbreak in West Africa infected more than 28,000 individuals and caused more than 11,000 deaths. But a vaccine study conducted this year provides real hope that the spread of the virus can be halted. In August, *The Lancet* published the interim results of the first randomized phase 3 clinical trial of an Ebola virus vaccine called rVSV-ZEBOV. The trial was conducted in Guinea by a group of international organizations that included the World Health Organization and Doctors Without Borders (*Lancet* **386**, 857–866, 2015).

The trial is the first for Ebola to use a ring vaccination approach. This method involved identifying clusters of people at high risk of infection because of close contact with an individual who had a confirmed case of the disease. They were then randomized to receive vaccination either immediately or after a 21-day delay.

The results show that no one in either arm of the trial developed Ebola more than six days after vaccination. The results were so encouraging that the data and safety board monitoring the trial recommended that the delayed arm be dropped and that immediate vaccination be given to any new clusters identified in the trial.

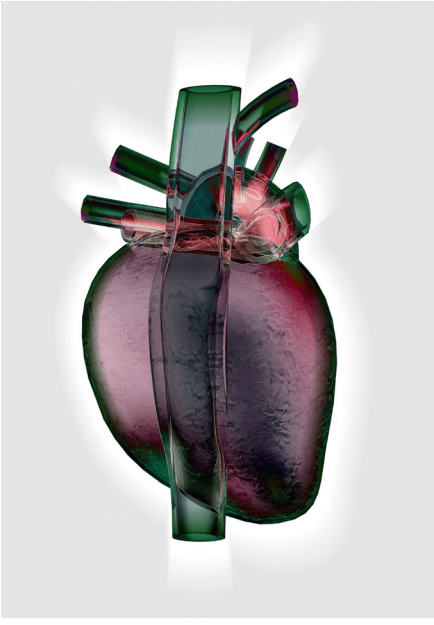
More studies are needed to determine whether the vaccine delivers long-lived protection, but its effectiveness in this trial suggests that it could prevent the spread of Ebola virus in a disease outbreak setting. In a study published shortly afterward, a team that included scientists from the US National Institute of Allergy and Infectious Diseases treated a small group of cynomolgus macaques with the same vaccine. The researchers showed that the immunization may have helped to protect the animals by stimulating the innate immune system to keep viral replication in check during the first days after infection (*Science* **349**, 739–742, 2015).

■ Cardiology

Awakening the heart

Regenerative therapy for the heart will require a solid grasp of both the endogenous potential of cardiomyocytes to proliferate and of mechanisms that could spur this inherent capacity. This year, researchers at the Karolinska Institute conducted a comprehensive analysis of cell generation in the human heart. The team found that although cardiomyocyte numbers do not change over the course of a lifetime, there is a low rate of cardiomyocyte renewal in adults (*Cell* **161**, 1566–1575, 2015). By studying adult mouse hearts, scientists at the University of Texas Southwestern Medical Center identified a rare subpopulation of proliferative cardiomyocytes that reside in a hypoxic microenvironment (*Nature* **523**, 226–230, 2015).

Two other studies zeroed in on the role of the epicardium—a layer of epithelial cells that surrounds the myocardium—in heart regeneration. In a zebrafish study, a group at Duke University Medical Center found that the epicardium is needed for cardiomyocyte regeneration and that the epicardium itself is capable of regeneration via the action of the Hedgehog signaling pathway (*Nature* **522**, 226–230, 2015). In the second study, researchers at the Stanford University School of Medicine and at the University of California, San Diego, identified the protein follistatin-like 1 (FSTL1) as an epicardially derived protein that, when applied via an epicardial patch, stimulates cardiomyocyte proliferation in both mouse and pig models of myocardial infarction (*Nature* **525**, 479–485, 2015).



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■ Neuroscience

Brain drain

The central nervous system was thought to be devoid of a classic lymphatic system, a network of vessels that moves immune cells throughout the body and aids in the clearance of excess fluid, macromolecules and waste. Two studies have overturned this assumption by identifying lymphatic vessels underneath the skulls of mice.

One group of researchers from the University of Virginia (*Nature* **523**, 337–341, 2015) found vessels in the meninges—a layer of tissue between the skull and the brain—that express markers that are specifically associated with lymphatic vessels. These vessels can transport a fluorescent dye injected into the ventricles of the brain to the deep cervical lymph nodes (dCLNs). Removal of a dCLN resulted in the accumulation of T cells in these vessels, whereas ligation of the vessel adjacent to a dCLN increased the diameter of these vessels, suggesting that these lymphatic vessels are involved in the drainage of cerebrospinal fluid and the exit of immune cells from the brain.

An independent group from the University of Helsinki (*J. Exp. Med.* **212**, 991–999, 2015) revealed these lymphatic vessels using reporter mice that expressed markers of lymphatic vessels. The team found that clearance of fluorescently labeled macromolecules is impaired in transgenic mice lacking these lymphatic vessels. Whether these vessels contribute to immune cell surveillance and disorders of the central nervous system awaits further investigation.

■ Cancer

Metabolic tug-of-war

Tumor cells are known to rewire their metabolic pathways in order to sustain fast-paced growth and to adapt to different nutrient environments. This year, two papers expanded on the role of metabolic reprogramming in cancer by showing how it contributes to the dampening of anti-tumor immune responses.

A group from the Yale University School of Medicine illustrated how tumors indirectly suppress the function of T cells in their milieu by depriving them of glucose, an essential nutrient. By using a mouse model of melanoma, the authors demonstrated that the high metabolic rate of tumors restricts the availability of glucose for these T cells nearby (*Cell* **162**, 1217–1228, 2015). They further characterized the effects of glucose deprivation on T cells, deciphering how the glycolytic metabolite phosphoenolpyruvate (PEP) regulates the intracellular calcium flux needed to trigger the activation of T effector cells. By enhancing the production of PEP, the authors demonstrated that exogenous metabolic reprogramming can boost the anti-tumor function of T cells, and they postulated that such a strategy could be harnessed to increase the efficacy of adoptive T cell therapy.

In a paper published simultaneously, a group from the Washington University School of Medicine in St. Louis approached the idea of targeting metabolic reprogramming to increase anti-tumor immunity and bolster immunotherapy from the opposite perspective—the tumor cell side (*Cell* **162**, 1229–1241, 2015). The authors show in mouse sarcoma models that enhanced glycolytic activity by tumor cells decreases T cell function independently of the antigenic stimulation of the T cells. Furthermore, they show that the nutrient competition between tumor cells and immune cells is regulated via immune checkpoint signaling.

The authors discovered that the upregulation by tumor cells of the immune checkpoint protein programmed death ligand 1 (PD-L1), an action that is known to protect them from death via immune-mediated killing, can also foster their glycolytic metabolism. The new findings suggest that checkpoint blockade, a promising form of immunotherapy, can tilt the nutrient competition in favor of T cell activation against tumors.

■ Immuno-oncology

Going viral

DNA methyltransferase inhibitors such as 5-azacitidine have shown efficacy in some hematological cancers, but it is still unclear how they slow tumor progression. New data from two groups point to the immune response as a key mediator of the beneficial effects of 5-azacitidine in a variety of tumor types (*Cell* **162**, 961–973, 2015; *Cell* **162**, 974–986, 2015). Scientists exposed human colorectal and ovarian cancer cell lines to 5-azacitidine, and they found that the drug activated an inflammatory response that is dependent on MDA5, a host defense protein responsible for detecting viral double-stranded RNA (dsRNA). The drug also boosted the expression of endogenous retroviruses.

Knockdown of MDA5 or of its downstream signaling components within either tumor cell lines or primary tumor samples abolished the ability of 5-azacitidine to suppress tumor cell growth and self-renewal *in vitro*. RNA-seq data from tumor samples from people with melanoma, who had been treated with a therapy known as anti-CTLA4 immune checkpoint blockade, revealed a strong correlation between the abundance of viral defense transcripts and the duration of clinical response, suggesting the relevance of patient response to immunotherapy.

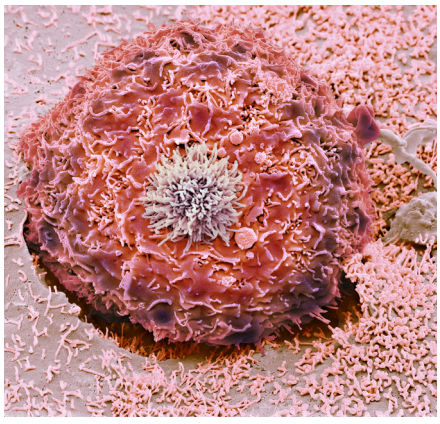
■ Cancer

Improved modeling

This year, scientists exploited new methods of genetic engineering and new organoids to better assess how mutations influence the development of cancer. Parsing out the cancer-associated mutations that actually drive cancer from those that arise inconsequentially is becoming a more and more challenging task because of the sheer amount of mutations that have been identified.

Organoids can recapitulate many features of human organ architecture that are not possible to replicate in conventional cell culture, in addition to mimicking relevant genetic diversity, and they also provide human context not available from animal models. Researchers from the Hubrecht Institute in the Netherlands successfully modeled ductal pancreatic cancer by deriving pancreatic organoids from both neoplastic and normal pancreatic tissue (*Cell* **160**, 324–338, 2015). Scientists from the University of Toronto (*Nature Med.*, doi:10.1038/nm.3973, 2015) also tracked how gene mutations influenced the progression of pancreatic cancer organoid growth.

Organoids have also been used in conjunction with the CRISPR-Cas9 gene-editing system to tease out the roles of individual mutations in the development and progression of cancer. By using this system, scientists from Keio University in Japan were able to probe mutations that drive colorectal cancer in human intestinal organoids,. They found that these mutations, in addition to canonical mutations, were necessary for the progression to invasive cancer (*Nature Med.* **21**, 256–262, 2015).



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■ Neuroscience

Rescued from repeats

One of the most common causes of familial amyotrophic lateral sclerosis (ALS) and of frontotemporal dementia (FTD) are tandem-repeat mutations that occur in a six-letter stretch of nucleotides, known as hexanucleotide repeat expansions (HREs), in the gene *C9ORF72*. However, the mechanisms underlying *C9ORF72*-associated ALS/FTD have remained elusive. This autumn, three independent studies provided converging evidence in support of the hypothesis that HREs in *C9ORF72* cause nuclear transport deficits that may contribute to cellular dysfunction and, ultimately, lead to neurodegeneration.

In a study involving genetic manipulations in fruit flies, research teams at Johns Hopkins University in Baltimore discovered that activating mutations in the nuclear transport factor RanGAP could rescue eye degeneration induced by the transgenic expression of a 30-repeat HRE (*Nature* **525**, 56–61, 2015). Similarly, a collaboration between research groups at the St. Jude Children's Research Hospital in Memphis and at the University of Massachusetts used fly genetics to screen for modifiers of an HRE-induced eye-degeneration phenotype; they also identified nuclear import, nuclear export and nuclear pore complex genes (*Nature* **525**, 129–133, 2015). During an unconventional form of protein translation, mRNAs transcribed from the mutant HRE-*C9ORF72* gene can produce aggregate-prone dipeptide repeat proteins (DPRs) that might contribute to cellular toxicity. In a third report, a team that included scientists from the Stanford University School of Medicine identified particular members of the karyopherin family of nuclear import factors that were capable of suppressing toxicity induced by the expression of an arginine-rich DPR in yeast (*Nature Neurosci.* **18**, 1226–1229, 2015).

Each of these studies also provided evidence of dysfunctional nuclear transport in induced pluripotent stem cell–derived neurons from humans with ALS. In addition to identifying a cellular mechanism that may contribute to neurodegeneration in *C9ORF72*-associated ALS or FTD, these findings suggest that the nuclear transport machinery may be an attractive candidate for future therapeutic development.

■ Cancer

Microbes and immunotherapy

The role of the microbiome in modulating disease is under intense investigation, and recently, commensal gut organisms were shown to affect the efficacy of cancer chemotherapy in mice. In November, two groups reported that distinct species of gut bacteria can improve the effects of two cancer immunotherapies in these rodents (*Science* doi:10.1126/science.aac4255, 2015; *Science*, doi:10.1126/science.aad1329, 2015).

In a study of mice, researchers from the University of Chicago found an association between different gut flora and patterns of tumor growth. In one experiment, they transferred fecal material from mice raised at the Jackson Laboratories into mice from the Taconic Farms facility; these two mouse groups are known to differ in their gut microbe populations. Fecal transfer dramatically improved the antitumor efficacy of an immunotherapy called anti-programmed death ligand 1 (anti–PD-L1). The improvements in tumor control were associated with an increased recruitment of T cells to the tumors, which in turn was linked to *Bifidobacterium* species in the fecal flora. Oral treatment with a cocktail of *Bifidobacterium* species also improved the efficacy of the therapy and this effect required CD8 T cells in the mice. The research suggests that *Bifidobacterium* improved dendritic cell function, resulting in the increased expansion and activity of tumor-specific CD8 T cells.

In a similar study, scientists at the Gustave Roussy Cancer Campus found that anti–cytotoxic T lymphocyte antigen 4 (anti–CTLA-4) therapy failed to control tumor growth in both germ-free and broad-spectrum antibiotic–treated mice. Oral treatment with *Bacteroides fragilis*, however, could rescue the effects of the immunotherapy. The mechanisms by which discrete gut bacteria exert a systemic effect on the immune system—and whether these bacteria confer clinical benefit to people treated with immunotherapies—must now be determined.

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