Science

Young Brains Continue to Grow

Our brains continue to grow and develop even after birth.[1] Contrary to the popular myth that we are born with a limited number of brain neurons that dwindle throughout our lives, ample evidence shows that small numbers of new neurons continue to arise throughout our lifetimes.[2] It was also previously believed that all neuronal precursor migration and interconnection took place prior to birth. New evidence shows that a great deal of neuronal precursor migration continues up to at least five months after birth. Using both postmortem labeling of brain cells and MRI imaging of living brain tissue before and after birth, the researchers found that there was a migratory stream of young neurons from a deeper part of the brain, known as the subventricular zone, or SVZ, to the outer cortex of the developing infant brain. Once the cells reached the cortex of the brain, they displayed plasticity, that is, they could change into a number of different neuronal types and connectivities, including inhibitory neurons that balance the stimulatory and inhibitory pathways within the brain. The large migration and formation of connections even after birth indicate that this is still a crucial part of human brain development.

Able to Stomach Conversion to New Stem Cells

Chinese researchers have developed a direct, safe way to convert stomach cells to multipotent stem cells that can be used to create various types of tissues.[3] At this

1. Mercedes F. Paredes et al., "Extensive Migration of Young Neurons into the Infant Human Frontal Lobe," *Science* 354.6308 (October 7, 2016): 81, doi: 10.1126/science.aaf7073.

2. Guo-li Ming and Hongjun Song, "Adult Neurogenesis in the Mammalian Brain: Significant Answers and Significant Questions," *Neuron* 70.4 (May 26, 2011): 687–702, doi: 10.1016/j.neuron.2011.05.001.

3. Yunfang Wang et al., "Conversion of Human Gastric Epithelial Cells to Multipotent Endodermal Progenitors Using Defined Small Molecules," *Cell Stem Cell* 19.4 (October 6, 2016): 449–461, doi: 10.1016/j.stem.2016.06.006.

point, some of the most difficult tissues to form for regenerative medicine studies are those derived from the endoderm, the innermost embryonic layer that goes on to form parts of the digestive system and supporting organs. Starting with stomach epithelial cells, themselves derivatives of the endoderm, the scientists were able to transform the stomach cells into multipotent stem cells, which they labeled human-induced endodermal progenitor cells (hiEndoPCs). The process does not use genetic vectors, as is usually the case both with induced pluripotent stem cells and in many directed differentiation methods that change one differentiated cell type into another. Instead, the hiEndoPCs were formed by exposing the stomach cells to a cocktail of metabolic stimulatory small molecules in the presence of specific mesenchymal (connective tissue) cells obtained from areas of endodermal organs. The stomach epithelial cells were originally isolated from the gastric tissue of patients aged thirty-five to seventy-eight years who had undergone surgery. Conversion in the presence of the small molecules and mesenchymal tissue took approximately two weeks, after which the newly formed stem cells were grown in culture. When these stem/progenitor cells were stimulated with specific differentiation signals, they could be turned into functional hepatocytes, pancreatic endocrine cells, or intestinal epithelial cells. The hepatocytes derived from hiEndoPCs repaired the damaged liver in a mouse model. The authors also note that their hiEndoPCs do not cause teratomas in mice, unlike human embryonic stem cells, which did lead to tumor formation. They also point out that human gastric cells can be obtained with relative ease from almost any patient, so that individualized stem cells can be prepared for drug testing or potentially even for conversion and transplantation to treat various conditions.

Schwann Cells Tip the Scale for Digit Regeneration

Most mammalian tissues cannot regenerate. However, the tips of digits retain the ability to regrow. Canadian researchers have identified the mechanism responsible for mammalian digit tip regeneration.[4] The key ingredient in the mouse model was Schwann cell precursors, that is, the progenitors that turn into Schwann cells, glial cells in the peripheral nervous system that wrap themselves around large nerves somewhat like insulation. In the mouse models, the nerve axons themselves were not present during the first two weeks after digit tip amputation, while the Schwann cell precursors were present throughout. Researchers identified the precursors as the key regenerative element when their removal impaired digit tip regeneration in the mouse models. Schwann cell precursor's regenerative properties come from a secretion of growth factors that stimulates surrounding cells, causing them to proliferate and form new tissues. Two protein factors in particular, oncostatin M and platelet-derived growth factor AA, could be injected and stimulate substantial regeneration even when Schwann cell precursors were knocked out. This information provides intriguing evidence for mechanisms of regeneration that potentially could be useful for regenerating complete tissues and organs.

4. Adam P. W. Johnston et al., "Dedifferentiated Schwann Cell Precursors Secreting Paracrine Factors Are Required for Regeneration of the Mammalian Digit Tip," *Cell Stem Cell* 19.4 (October 6, 2016): 433–448, doi: 10.1016/j.stem.2016.06.002.

Helpful Mutation for Treating ß-Hemoglobin Problems, but Direct Gene Editing May Be Some Way Off

Hereditary persistence of fetal hemoglobin is a benign genetic condition, caused by mutations that prevent the cell switching from expression of genes for the fetal form of hemoglobin (containing gamma globin) to the adult form (containing beta globin); the switch in gene expression normally takes place around the time of birth. Despite the failure to express the adult form of globin genes, individuals with HPFH show no symptoms of anemia and lead normal lives. This observation has led to a hypothesis that in conditions where there is a faulty beta globin, such as sickle cell anemia and ß-thalassemia, reexpression of fetal hemoglobin gene might replace the defective beta globin and restore normal oxygen-carrying capacity to the red blood cells of affected individuals. The idea itself is not a new one. Several decades ago, researchers found that the chemotherapeutic drug hydroxyurea caused reexpression of fetal hemoglobin, helping patients who suffer from sickle cell anemia.[5] However, hydroxyurea treatment can cause toxicities and is a non-specific initiator of gene expression for many genes, not just the fetal gamma globin gene.[6] Now a multinational team led by scientists at St. Jude Children's Research Hospital has shown that gene editing tools can be used to precisely and successfully reexpress gamma globin in mutated cells in the laboratory, suggesting a potential treatment for various blood disorders.[7] The genetic engineering was relatively straightforward: CRISPR-Cas9 was used to induce a mutation in the normal beta globin genes, such that their expression was decreased or eliminated. When tested in a human laboratory cell line (HUDEP-2) that is used to model red blood cell production, the number of cells expressing fetal hemoglobin increased from 2 to 46 percent in response to the crippling of the beta globin genes. Next the technique was tested on hematopoietic, or blood-forming, bone marrow stem cells taken from healthy adults. After gene editing, the cells increased their production of fetal hemoglobin protein levels, up from 5 to 20 percent. The scientists also tested this technique on hematopoietic stem cells from patients with sickle cell disease. After using CRISPR to decrease expression of beta globin, the proportion of gamma globin–expressing cells increased to 90 percent, and the treatment reduced sickle-like morphology to 4 percent of the cells. The technique needs significant refining and testing to eliminate any off-target cutting of DNA and to ensure selection of the properly engineered cells

5. Orah S. Platt et al., "Hydroxyurea Enhances Fetal Hemoglobin Production in Sickle Cell Anemia," *Journal of Clinical Investigation* 74.2 (Aug 1984): 652–656, doi: 10.1172/JCI111464.

6. G. J. Dover et al., "Hydroxyurea Induction of Hemoglobin F Production in Sickle Cell Disease: Relationship between Cytotoxicity and F Cell Production," *Blood* 67.3 (March 1986): 735–738; and Q. Ma et al., "Fetal Hemoglobin in Sickle Cell Anemia: Genetic Determinants of Response to Hydroxyurea," *Pharmacogenomics Journal* 7.6 (December 2007): 386–394, doi:10.1038/sj.tpj.6500433.

7. Elizabeth A. Traxler et al., "A Genome-Editing Strategy to Treat β-Hemoglobinopathies that Recapitulates a Mutation Associated with a Benign Genetic Condition," *Nature Medicine* 22.9 (September 2016): 987–990, doi:10.1038/nm.4170.

in the laboratory for transplantation back into the patient, but these results provide hopeful evidence that gene editing to reexpress gamma globin genes could be used effectively to treat sickle cell anemia and similar conditions caused by faulty beta globin mutations.

While using genetic engineering to reactivate gamma globin looks like a promising treatment for sickle cell anemia and other hemoglobinopathies, directly editing the beta globin gene to correct pathological mutations is not feasible at present. A new report shows that the method has some potential but is neither efficient enough nor error-free.[8] A collaborative group led by scientists at the University of California, Berkeley, attempted to correct the single-nucleotide mutation that causes sickle cell anemia by using CRISPR to cleave the targeted DNA and replace the faulty nucleotide with the normal nucleotide. When the hematopoietic (blood-forming) stem cells from sickle cell patients were genetically manipulated with this system in the laboratory, the modification decreased the production of faulty sickling beta globin and also resulted in some small production of the corrected beta globin. After being transplanted into mice, only about 5 percent of the corrected cells produced normal hemoglobin. This level is too low for clinical utility. There is also some concern that the gene editing can result in additional DNA deletions within the engineered cells. This would only swap one problem for another, producing a different faulty beta globin. However, the new technique shows promise for future use, once it is properly controlled and refined.

Cord Blood Stem Cells a Better Transplant Alternative

For patients who must rely on a donor for an adult stem cell transplant, the first choice is usually a matched sibling. Unfortunately, around 70 percent of patients do not have a matched sibling donor. New evidence suggests that such patients fare better if they receive a transplant of stem cells from umbilical cord blood than from matched but unrelated bone marrow donors, especially if the patient has minimal residual disease left to treat at the time of transplant.[9] The investigators looked at results from 582 patients who received adult stem cell transplants for acute leukemia or a similar disease called myelodysplastic syndrome. The results for patients who received donor cord blood stem cells were as good as or better than those experienced by patients who received transplants from a matched but unrelated donor, including a much lower chance of relapse. The results are encouraging for those who do not have a matched sibling donor and indicate that umbilical cord blood stem cells are favorably tolerated in leukemic patients and are a superior alternative to bone marrow in some cases.

8. Mark A. DeWitt et al., "Selection-Free Genome Editing of the Sickle Mutation in Human Adult Hematopoietic Stem/Progenitor Cells," *Science Translational Medicine* 8.360 (October 12, 2016): 360ra134, doi: 10.1126/scitranslmed.aaf9336.

9. Filippo Milano et al., "Cord-Blood Transplantation in Patients with Minimal Residual Disease," *New England Journal of Medicine* 375.10 (September 8, 2016): 944–953, doi: 10.1056/NEJMoa1602074.

New Esophagus with Adult Stem Cells

A US medical team has used commercially available, FDA approved stents and autologous cells to regenerate a patient's esophagus and restore normal swallowing.[10] Stents coated with an extracellular matrix were sprayed with autologous platelet-rich plasma, which contained a large number of growth factors and attracted adult stem cells to the site. The report notes that seven years after the esophagus was reconstructed, and four years after the stents were removed, a functional, multi-layer, full-thickness esophagus was regenerated. The doctors report that the patient is eating a normal diet, maintaining weight, and has no swallowing problems.

Adult Stem Cells Relieve Arthritic Keen Pain

An international collaboration has found that autologous stem cells show potential to decrease pain and swelling in osteoarthritic knees.[11] In the clinical trial, which was conducted in France and Germany, eighteen patients with severe osteoarthritis of the knee received one injection of autologous adipose-derived (from fat) adult stem cells. The cell treatment, injected into the patients' knees, showed no adverse effects. Moreover, patients showed significant improvements in their pain levels and an improved range of function in the treated knees.

A similar success has been achieved for canine patients in the United States. A team from Kansas State University used autologous stromal vascular fraction (a mesenchymal stem cell–enriched mixture from fat tissue) and platelet-rich plasma in a placebo-controlled study of dogs with osteoarthritis of the hip joints. Treated dogs showed less pain and lameness than the control group.[12] These two studies strongly suggest that more human trials should be approved to address these conditions, especially in the aging population.

Tumor-Free Transplantation of Human iPS cells to Treat Diabetes

One significant practical problem with pluripotent stem cells, whether embryonic stem cells or induced pluripotent stem (iPS) cells, is their potential to form tumors. Now a group has shown that a new method produces human iPS cells that do not cause tumor formation when transplanted into mice.[13] The original protocol for

10. Kulwinder S. Dua et al., "In-Vivo Oesophageal Regeneration in a Human Being by Use of a Non-Biological Scaffold and Extracellular Matrix," *Lancet* 388.10039 (July 2, 2016): 55–61, doi: 10.1016/S0140-6736(15)01036-3.

11. Yves-Marie Pers et al., "Adipose Mesenchymal Stromal Cell-Based Therapy for Severe Osteoarthritis of the Knee: A Phase I Dose-Escalation Trial," *Stem Cells Translational Medicine* 5.7 (July 2016): 847–856, doi: 10.5966/sctm.2015-0245.

12. David A. Upchurch et al., "Effects of Administration of Adipose-Derived Stromal Vascular Fraction and Platelet-Rich Plasma to Dogs with Osteoarthritis of the Hip Joints," *American Journal of Veterinary Research* 77.9 (September 2016): 940–951, doi: 10.2460/ajvr.77.9.940.

13. Moustafa M. El Khatib et al., "Tumor-Free Transplantation of Patient-Derived Induced Pluripotent Stem Cell Progeny for Customized Islet Regeneration," *Stem Cells Translational Medicine* 5.5 (May 2016): 694–702, doi: 10.5966/sctm.2015-0017.

producing iPS cells involved adding certain protein-coding genes to normal cells. The added DNA, or transgenes, integrated into the host cell's genome and subsequently expressed the transforming factors that induce pluripotency in the cell.[14] One of the added genes was *c-Myc*, which is an oncogene (it can cause cancerous cell growth). The authors came up with a different protocol for producing iPS cells from human tissue, which uses the Sendai virus, a non-integrating viral vector, to reprogram the normal cells into iPS cells. Then they differentiated the human iPS cells into insulin-secreting pancreatic beta cells. To test the new technique, they injected derivatives from iPS cells made with the older, integrating viruses into mice. These cells developed invasive tumors, including metastatic ones, in 90 percent of the subjects. Mice that were injected with iPS cells made with the new technique were tumor-free. Pre-treating the iPS cells so that they were injected as individual cells rather than aggregates assured that the mice remained tumor-free; tumor-forming pluripotent stem cells, which can be contaminants in these injections, do not survive well as single cells. The tumor-free injections formed human pancreatic islets in the mice, although better methods to produce mature, insulin-secreting cells will be needed before this can be used in human patients with diabetes.

First Three-Parent Child Born

The debate over three-parent embryos has taken a turn that increases the concern about the ethics involved, especially regarding the scientists who are pushing this technology. We discussed some of these ethical challenges in the Autumn 2016 issue of this journal.[15] A new report has been published on a 2003 attempt to create and gestate human three-parent embryos who would not inherit their mother's mutated mitochondrial DNA.[16] An accompanying editorial points out that the report contains deficiencies and unknowns, including significant questions about the ethical review.[17] None of the embryos survived to term, though at least seven genetically manufactured embryos were created using a process called pro-nuclear transfer.[18] Now John Zhang, the same doctor who conducted the experiments on human embryos in 2003, is reporting the successful live birth of a boy created using one

14. Kazutoshi Takahashi et al., "Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors," *Cell* 131.5 (November 2007): 861–872, doi: 10.1016/j.cell.2007.11.019.

15. David A. Prentice, "Science," *National Catholic Bioethics Quarterly* 16.3 (Autumn 2016): 492–493.

16. John Zhang et al., "Pregnancy Derived from Human Zygote Pronuclear Transfer in a Patient Who Had Arrested Embryos after IVF," *Reproductive BioMedicine Online* 33.4 (October 2016): 529–533, doi: 10.1016/j.rbmo.2016.07.008.

17. Jacques Cohen and Henry Malter, "The First Clinical Nuclear Transplantation in China: New Information about a Case Reported to ASRM in 2003," *Reproductive BioMedicine Online* 33.4 (October 2016): 433–435, doi: 10.1016/j.rbmo.2016.08.002.

18. For a primer describing various methods of genetic engineering, see "'3-Parent Embryos' and 'Gene-Edited Babies': A Visual Aid," Charlotte Lozier Institute, June 26, 2015, https://www.lozierinstitute.org/.

of the three-parent nuclear transfer techniques.[19] The human genetic experimentation and subsequent birth were reported in a meeting abstract, so few details are available as of this writing. According to the report, five genetically manipulated embryos were created, though only one embryo had normal chromosomes. That single embryo was transferred to the womb and survived to term birth. At least part of the experiment was carried out in Mexico because, unlike the United States, that country does not place legal restrictions on this science. Since the child is still only a few months old, it is far too early to tell if the procedure was a success or if there will be any adverse outcomes, especially long-term, for this child. A second abstract from the same meeting provides more information on the methodology and number of embryos created and destroyed in different experiments.[20] At least thirty-four human eggs were collected from women solicited for donation, and thirty-two genetically manipulated embryos were created. Of these, only about half had normal chromosomes. This report also notes that for the one normal embryo transferred to the womb and gestated to birth, initial tests showed there was still an average level of residual mitochondrial DNA mutation of 5 percent in the sampled tissues. Few physiological problems are noticed at this level of mutated DNA within cells. However, only a few of the infant's tissues were sampled, and other laboratories have reported that the carryover of mutated DNA increases over time. This child will thus continue to be an experiment in progress, needing constant monitoring for his health because of possible increases in mutation level. Unfortunately, news stories indicate that other genetically manipulated three-parent children have been created by rogue doctors, with one more born in the Ukraine and others now gestating.[21] The Brave New World of manufactured children may be upon us.

David A. Prentice

19. John Zhang et al., "First Live Birth Using Human Oocytes Reconstituted by Spindle Nuclear Transfer for Mitochondrial DNA Mutation Causing Leigh Syndrome," *Fertility and Sterility* 106.3 suppl (September 2016): e375–e376, doi: 10.1016/j.fertnstert.2016.08.004.

20. H. Liu et al., "In Vitro Fertilization and Development of Human Oocytes Reconstituted by Spindle Nuclear Transfer to Replace Mutated Mitochondrial DNA," *Fertility and Sterility* 106.3 suppl (September 2016): e21, doi: 10.1016/j.fertnstert.2016.07.071.

21. Peter Dockrill, "World-First in Ukraine as 'Three-Parent' Baby Born to an Infertile Couple," *Sciencealert*, January 19, 2017, http://www.sciencealter.com/.

Science Abstracts

American Journal of Veterinary Research

D.A. Upchurch et al., **Effects of administration of adipose-derived stromal vascular fraction and platelet-rich plasma to dogs with osteoarthritis of the hip joints**, *Am J Vet Res* 77.9 (September 2016): 940–951, doi: 10.2460/ajvr.77.9.940 • *Objective:* To evaluate effects of simultaneous intra-articular and IV injection of autologous adipose-derived stromal vascular fraction (SVF) and platelet-rich plasma (PRP) to dogs with osteoarthritis of the hip joints. *Animals:* 22 client-owned dogs (12 placebo-treated [control] dogs and 10 treated dogs). *Procedures:* Dogs with osteoarthritis of the hip joints that caused signs of lameness or discomfort were characterized on the basis of results of orthopedic examination, goniometry, lameness score, the Canine Brief Pain Inventory (CBPI), a visual analogue scale, and results obtained by use of a pressure-sensing walkway at week 0 (baseline). Dogs received a simultaneous intra-articular and IV injection of SVF and PRP or a placebo. Dogs were examined again 4, 8, 12, and 24 weeks after injection. *Results:* CBPI scores were significantly lower for the treatment group at week 24, compared with scores for the control group. Mean visual analogue scale score for the treatment group was significantly higher at week 0 than at weeks 4, 8, or 24. Dogs with baseline peak vertical force (PVF) in the lowest 25th percentile were compared, and the treatment group had a significantly higher PVF than did the control group. After the SVF-PRP injection, fewer dogs in the treated group than in the control group had lameness confirmed during examination. *Conclusions and clinical relevance:* For dogs with osteoarthritis of the hip joints treated with SVF and PRP, improvements in CBPI and PVF were evident at some time points, compared with results for the control group.

Blood

G.J. Dover et al., **Hydroxyurea induction of hemoglobin F production in sickle cell disease: relationship between cytotoxicity and F cell production,** *Blood* 67.3 (March 1986): 735–738 • Initial alterations in fetal hemoglobin (HbF) production among eight sickle cell anemia subjects treated with hydroxyurea (Hu) are summarized. Four of these subjects had been previously treated with 5-azacytidine (5-aza). All subjects treated with Hu (50 mg/kg/d for three to five days) had suppression of their total reticulocyte counts by seven days, whereas the four subjects previously treated with 5-aza (2 mg/kg/d for three to five days) had increased reticulocyte counts at day 7. The effect of Hu on increasing the number of HbF-containing reticulocytes (F reticulocytes) is extremely variable, ranging from ten- to less than one-fold differences in maximal posttherapy v pretherapy levels. Recovery from marrow suppression did not result in greater than twofold increases in F reticulocyte counts. Mean day 7 F reticulocyte levels in the four subjects treated with both Hu and 5-aza were 4.1 X 10/microL and 15.4 X 10(4)/microL, respectively. Among Hu-treated subjects, increased F reticulocyte production was correlated with low serum creatinine levels and rapid removal of Hu from the plasma. Furthermore, suppression of CFU-E colony formation on day 2 of therapy with Hu was inversely correlated with maximal F reticulocyte response. We conclude that where Hu treatment results in marrow toxicity (decreased reticulocyte counts, decreased CFU-E colony formation) HbF production is less likely to increase. Those sickle cell anemia subjects with minimal renal dysfunction (serum creatinine level, greater than 1.0 mg/dL) exhibit the most cytotoxicity and least F reticulocyte response to Hu.

Cell

K. Takahashi et al., **Induction of pluripotent stem cells from adult human fibroblasts by defined factors**, *Cell* 131.5 (November 30, 2007): 861–872, doi: 10.1016/j.cell.2007.11.019 • Successful reprogramming of differentiated human somatic cells into a pluripotent state would allow creation of patient- and disease-specific stem cells. We previously reported generation of induced pluripotent stem (iPS) cells, capable of germline transmission, from mouse somatic cells by transduction of four defined transcription factors. Here, we demonstrate the generation of iPS cells from adult human dermal fibroblasts with the same four factors: Oct3/4, Sox2, Klf4, and c-Myc. Human iPS cells were similar to human embryonic stem (ES) cells in morphology, proliferation, surface antigens, gene expression, epigenetic status of pluripotent cell-specific genes, and telomerase activity. Furthermore, these cells could differentiate into cell types of the three germ layers in vitro and in teratomas. These findings demonstrate that iPS cells can be generated from adult human fibroblasts.

Cell Stem Cell

A. P. Johnston et al., **Dedifferentiated Schwann cell precursors secreting paracrine factors are required for regeneration of the mammalian digit tip,** *Cell Stem Cell* 19.4 (October 6, 2016): 433–448, doi: 10.1016/j.stem.2016.06.002 • Adult mammals have lost multi-tissue regenerative capacity, except for the distal digit, which is able to regenerate via mechanisms that remain largely unknown. Here, we show that, after adult mouse distal digit removal, nerve-associated Schwann cell precursors (SCPs) dedifferentiate and secrete growth factors that promote expansion of the blastema and digit regeneration. When SCPs were dysregulated or ablated, mesenchymal precursor proliferation in the blastema was decreased and nail and bone regeneration were impaired. Transplantation of exogenous SCPs rescued these regeneration defects. We found that SCPs secrete factors that promote self-renewal of mesenchymal precursors, and we used transcriptomic and proteomic analysis to define candidate factors. Two of these, oncostatin M (OSM) and platelet-derived growth factor AA (PDGF-AA), are made by SCPs in the regenerating digit and rescued the deficits in regeneration caused by loss of SCPs. As all peripheral tissues contain nerves, these results could have broad implications for mammalian tissue repair and regeneration.

Y. Wang et al., **Conversion of human gastric epithelial cells to multipotent endodermal progenitors using defined small molecules,** *Cell Stem Cell* 19.4 (October 6, 2016): 449–461, doi: 10.1016/j.stem.2016.06.006 • Endodermal stem/progenitor cells have diverse potential applications in research and regenerative medicine, so a readily available source could have widespread uses. Here we describe derivation of human induced endodermal progenitor cells (hiEndoPCs) from gastrointestinal epithelial cells using a cocktail of defined small molecules along with support from tissue-specific mesenchymal feeders. The hiEndoPCs show clonal expansion in culture and give rise to hepatocytes, pancreatic endocrine cells, and intestinal epithelial cells when treated with defined soluble molecules directing differentiation. The hiEndoPC-derived hepatocytes are able to rescue liver failure in $Fah^{-/-}Rag2^{-/-}$ mice after transplantation, and, unlike hESCs, transplanted hiEndoPCs do not give rise to teratomas. Since human gastric epithelial cells are readily available from donors of many ages, this conversion strategy can generate clonally expandable cell populations with a variety of potential applications, including personalized drug screening and therapeutic strategies for liver failure and diabetes.

Fertility and Sterility

H. Liu et al., **In vitro fertilization and development of human oocytes reconstituted by spindle nuclear transfer to replace mutated mitochondrial DNA,** *Fertil Steril* 106.3 suppl (September 2016): e21, doi: 10.1016/j.fertnstert.2016.07.071 • Mutations in mitochondrial DNA (mtDNA) are maternally inherited and can cause fatal or severely debilitating disorders with limited treatment options. Metaphase II (MII) spindle nuclear

transfer (SNT) using Sendai virus has been shown to be effective in preventing transmission of heteroplasmy mtDNA from oocytes to pre-implantation embryos. This study aimed to examine the safety and efficacy of SNT using electric pulse to initiate membrane fusion on fertilization of human oocytes and embryo development, and examine transmission of mutated mtDNA from oocytes to SNT pre-implantation embryos.

J. Zhang et al., **First live birth using human oocytes reconstituted by spindle nuclear transfer for mitochondrial DNA mutation causing Leigh syndrome,** *Fertil Steril* 106.3 suppl (September 2016): e375–e376, doi: 10.1016/j.fertnstert.2016.08.004 • Mutations in mitochondrial (mt) DNA are maternally inherited and can cause fatal or debilitating disorders without effective treatments. The severity of clinical symptoms is often associated with the mtDNA mutation load in heteroplasmy. Experimental nuclear transfer in metaphase II (MII) spindle oocytes or in pronuclear (PN) zygotes, also called mitochondrial replacement therapy, has been shown to be a novel technology in minimizing mutated mtDNA transmission from oocytes to pre-implantation embryos. Here we report the first live birth of a boy following spindle nuclear transfer (SNT).

Journal of Clinical Investigation

O. S. Platt et al., **Hydroxyurea enhances fetal hemoglobin production in sickle cell anemia**, *J Clin Invest* 74.2 (August 1984): 652–656, doi: 10.1172/JCI111464 • Hydroxyurea, a widely used cytotoxic/cytostatic agent that does not influence methylation of DNA bases, increases fetal hemoglobin production in anemic monkeys. To determine its effect in sickle cell anemia, we treated two patients with a total of four, 5-d courses (50 mg/kg per d, divided into three oral doses). With each course, fetal reticulocytes increased within 48-72 h, peaked in 7-11 d, and fell by 18-21 d. In patient I, fetal reticulocytes increased from 16.0 +/- 2.0% to peaks of 37.7 +/- 1.2, 40.0 +/- 2.0, and 32.0 +/- 1.4% in three successive courses. In patient II the increase was from 8.7 +/- 1.2 to 50.0 +/- 2.0%. Fetal hemoglobin increased from 7.9 to 12.3% in patient I and from 5.3 to 7.4% in patient II. Hemoglobin of patient I increased from 9.0 to 10.5 g/dl and in patient II from 6.7 to 9.9 g/dl. Additional single-day courses of hydroxyurea every 7-20 d maintained the fetal hemoglobin of patient I t 10.8-14.4%, and the total hemoglobin at 8.7-10.8 g/dl for an additional 60 d. The lowest absolute granulocyte count was 1,600/mm3; the lowest platelet count was 390,000/mm3. The amount of fetal hemoglobin per erythroid burst colony-forming unit (BFU-E)-derived colony cell was unchanged, but the number of cells per BFU-E-derived colony increased. Although examination of DNA synthesis in erythroid marrow cells in vitro revealed no decreased methylcytidine incorporation, Eco RI + Hpa II digestion of DNA revealed that hypomethylation of gamma-genes had taken place in vivo after treatment. This observation suggests that hydroxyurea is a potentially useful agent for the treatment of sickle cell anemia and that demethylation of the gamma-globin genes accompanies increased gamma-globin gene activity.

Lancet

K. S. Dua et al., **In-vivo oesophageal regeneration in a human being by use of a non-biological scaffold and extracellular matrix,** *Lancet* 388.10039 (July 2, 2016): 55–61, 10.1016/S0140-6736(15)01036-3 • *Background:* Tissue-engineered extracellular matrix populated with autologous pluripotent cells can result in de-novo organogenesis, but the technique is complex, not widely available, and has not yet been used to repair large oesophageal defects in human beings. We aimed to use readily available stents and extracellular matrix to regenerate the oesophagus in vivo in a human being to re-establish swallowing function. *Methods:* In a patient aged 24 years, we endoscopically placed a readily available, fully covered, self-expanding, metal stent (diameter 18 mm, length 120 mm) to bridge a 5 cm full-thickness oesophageal segment destroyed by a mediastinal abscess and leading to direct communication between the hypopharynx and the mediastinum. A commercially available

extracellular matrix was used to cover the stent and was sprayed with autologous platelet rich plasma adhesive gel. The sternocleidomastoid muscle was placed over the matrix. After 4 weeks, stent removal was needed due to stent migration, and was replaced with three stents telescopically aligned to improve anchoring. The stents were removed after 3.5 years and the oesophagus was assessed by endoscopy, biopsy, endoscopic ultrasonography, and high-resolution impedance manometry. *Findings:* After stent removal we saw full-thickness regeneration of the oesophagus with stratified squamous epithelium, a normal five-layer wall, and peristaltic motility with bolus transit. 4 years after stent removal, the patient was eating a normal diet and maintaining a steady weight. *Interpretation:* Maintenance of the structural morphology of the oesophagus with off-the-shelf non-biological scaffold and stimulation of regeneration with commercially available extracellular matrix led to de-novo structural and functional regeneration of the oesophagus.

Nature Medicine

E. A. Traxler et al., **A genome-editing strategy to treat β-hemoglobinopathies that recapitulates a mutation associated with a benign genetic condition,** *Nat Med* 22.9 (September 2016): 987–990, doi: 10.1038/nm.4170 • Disorders resulting from mutations in the hemoglobin subunit beta gene (*HBB*; which encodes β-globin), mainly sickle cell disease (SCD) and β-thalassemia, become symptomatic postnatally as fetal λ-globin expression from two paralogous genes, hemoglobin subunit gamma 1 (*HBG1*) and *HBG2*, decreases and adult β-globin expression increases, thereby shifting red blood cell (RBC) hemoglobin from the fetal (referred to as HbF or α2λ2) to adult (referred to as HbA or α2β2) form. These disorders are alleviated when postnatal expression of fetal λ-globin is maintained. For example, in hereditary persistence of fetal hemoglobin (HPFH), a benign genetic condition, mutations attenuate λ-globin-to-β-globin switching, causing high-level HbF expression throughout life. Co-inheritance of HPFH with β-thalassemia- or SCD-associated gene mutations alleviates their clinical manifestations. Here we performed CRISPR–Cas9-mediated genome editing of human blood progenitors to mutate a 13-nt sequence that is present in the promoters of the *HBG1* and *HBG2* genes, thereby recapitulating a naturally occurring HPFH-associated mutation. Edited progenitors produced RBCs with increased HbF levels that were sufficient to inhibit the pathological hypoxia-induced RBC morphology found in SCD. Our findings identify a potential DNA target for genome-editing-mediated therapy of β-hemoglobinopathies.

Neuron

G. L. Ming and H. Song, **Adult neurogenesis in the mammalian brain: significant answers and significant questions**, *Neuron* 70.4 (May 26, 2011): 687–702, doi: 10.1016/j.neuron.2011.05.001 • Adult neurogenesis, a process of generating functional neurons from adult neural precursors, occurs throughout life in restricted brain regions in mammals. The past decade has witnessed tremendous progress in addressing questions related to almost every aspect of adult neurogenesis in the mammalian brain. Here we review major advances in our understanding of adult mammalian neurogenesis in the dentate gyrus of the hippocampus and from the subventricular zone of the lateral ventricle, the rostral migratory stream to the olfactory bulb. We highlight emerging principles that have significant implications for stem cell biology, developmental neurobiology, neural plasticity, and disease mechanisms. We also discuss remaining questions related to adult neural stem cells and their niches, underlying regulatory mechanisms, and potential functions of newborn neurons in the adult brain. Building upon the recent progress and aided by new technologies, the adult neurogenesis field is poised to leap forward in the next decade.

New England Journal of Medicine

F. Milano et al., **Cord-blood transplantation in patients with minimal residual disease**, *N Engl J Med* 375.10 (September 8, 2016): 944–953, doi: 10.1056/NEJMoa1602074 • *Background:* The majority of patients in

need of a hematopoietic-cell transplant do not have a matched related donor. Data are needed to inform the choice among various alternative donor-cell sources. *Methods:* In this retrospective analysis, we compared outcomes in 582 consecutive patients with acute leukemia or the myelodysplastic syndrome who received a first myeloablative hematopoietic-cell transplant from an unrelated cord-blood donor (140 patients), an HLA-matched unrelated donor (344), or an HLA-mismatched unrelated donor (98). *Results:* The relative risks of death and relapse between the cord-blood group and the two other unrelated-donor groups appeared to vary according to the presence of minimal residual disease status before transplantation. Among patients with minimal residual disease, the risk of death was higher in the HLA-mismatched group than in the cord-blood group (hazard ratio, 2.92; 95% confidence interval [CI], 1.52 to 5.63; P = 0.001); the risk was also higher in the HLA-matched group than in the cord-blood group but not significantly so (hazard ratio, 1.69; 95% CI, 0.94 to 3.02; P = 0.08). Among patients without minimal residual disease, the hazard ratios were lower (hazard ratio in the HLA-mismatched group, 1.36; 95% CI, 0.76 to 2.46; P = 0.30; hazard ratio in the HLA-matched group, 0.78; 95% CI, 0.48 to 1.28; P = 0.33). The risk of relapse among patients with minimal residual disease was significantly higher in the two unrelated-donor groups than in the cord blood group (hazard ratio in the HLA-mismatched group, 3.01; 95% CI, 1.22 to 7.38; P = 0.02; hazard ratio in the HLA-matched group, 2.92; 95% CI, 1.34 to 6.35; P = 0.007). Among patients without minimal residual disease, the magnitude of these associations was lower (hazard ratio in the HLA-mismatched group, 1.28; 95% CI, 0.51 to 3.25; P = 0.60; hazard ratio in the HLA-matched group, 1.30; 95% CI, 0.65 to 2.58; P = 0.46). *Conclusions:* Our data suggest that among patients with pre-transplantation minimal residual disease, the probability of overall survival after receipt of a transplant from a cord-blood donor was at least as favorable as that after receipt of a transplant from an HLA matched unrelated donor and was significantly higher than the probability after receipt of a transplant from an HLA-mismatched unrelated donor. Furthermore, the probability of relapse was lower in the cord-blood group than in either of the other groups.

Pharmacogenomics Journal

Q. Ma et al., **Fetal hemoglobin in sickle cell anemia: genetic determinants of response to hydroxyurea,** *Pharmacogenomics J* 7.6 (December 2007): 386–394, doi: 10.1038 /sj.tpj.6500433 • The increase in fetal hemoglobin (HbF) in response to hydroxyurea (HU) varies among patients with sickle cell anemia. Twenty-nine candidate genes within loci previously reported to be linked to HbF level (6q22.3–q23.2, 8q11–q12 and Xp22.2–p22.3), involved in metabolism of HU and related to erythroid progenitor proliferation were studied in 137 sickle cell anemia patients treated with HU. Three-hundred and twenty tagging single nucleotide polymorphisms (SNPs) for genotyping were selected based on HapMap data. Multiple linear regression and the nonlinear regression Random Forest method were used to investigate the association between SNPs and the change in HbF level after 2 years of treatment with HU. Both methods revealed that SNPs in genes within the 6q22.3–23.2 and 8q11–q12 linkage peaks, and also the *ARG2*, *FLT1*, *HAO2* and *NOS1* genes were associated with the HbF response to HU. Polymorphisms in genes regulating HbF expression, HU metabolism and erythroid progenitor proliferation might modulate the patient response to HU.

Reproductive BioMedicine Online

J. Cohen and H. Malter, **The first clinical nuclear transplantation in China: new information about a case reported to ASRM in 2003,** *Reprod BioMed Online* 33.4 (October 2016): 433–435, doi: 10.1016/j .rbmo.2016.08.002 • Before there was public debate on mitochondrial replacement therapy (MRT) to treat mothers at risk of transmitting mitochondrial disease (Hyslop et al., 2016), and before there was a publicly

traded company called OvaScience aiming to use nuclear transplantation and stem-cell technologies to treat infertility (Woods and Tilly, 2015), there was a solitary case report from China about the use of nuclear transplantation with donor oocyte-derived cytoplasm at the zygote stage to overcome cleavage arrest in a patient's embryos (Zhang et al., 2003). The landmark abstract, presented to the 2003 annual meeting of the American Society for Reproductive Medicine (ASRM), was received with excitement and concern, sentiments that promptly seeped into the lay press. This was not surprising considering the topic and the risk-sensitive audience of reproductive scientists listening to the presentation given by the lead author, Dr John Zhang.

J. Zhang et al., **Pregnancy derived from human zygote pronuclear transfer in a patient who had arrested embryos after IVF**, *Reprod BioMed Online* 33.4 (October 2016): 529–533, doi: 10.1016/j .rbmo.2016.07.008 • Nuclear transfer of an oocyte into the cytoplasm of another enucleated oocyte has shown that embryogenesis and implantation are influenced by cytoplasmic factors. We report a case of a 30-year-old nulligravida woman who had two failed IVF cycles characterized by all her embryos arresting at the two-cell stage and ultimately had pronuclear transfer using donor oocytes. After her third IVF cycle, eight out of 12 patient oocytes and 12 out of 15 donor oocytes were fertilized. The patient's pronuclei were transferred subzonally into an enucleated donor cytoplasm resulting in seven reconstructed zygotes. Five viable reconstructed embryos were transferred into the patient's uterus resulting in a triplet pregnancy with fetal heartbeats, normal karyotypes and nuclear genetic fingerprinting matching the mother's genetic fingerprinting. Fetal mitochondrial DNA profiles were identical to those from donor cytoplasm with no detection of patient's mitochondrial DNA. This report suggests that a potentially viable pregnancy with normal karyotype can be achieved through pronuclear transfer. Ongoing work to establish the efficacy and safety of pronuclear transfer will result in its use as an aid for human reproduction.

Science

M. F. Paredes et al., **Extensive migration of young neurons into the infant human frontal lobe**, *Science* 354.6308 (October 7, 2016): 81, doi: 10.1126/science.aaf7073 • The first few months after birth, when a child begins to interact with the environment, are critical to human brain development. The human frontal lobe is important for social behavior and executive function; it has increased in size and complexity relative to other species, but the processes that have contributed to this expansion are unknown. Our studies of postmortem infant human brains revealed a collection of neurons that migrate and integrate widely into the frontal lobe during infancy. Chains of young neurons move tangentially close to the walls of the lateral ventricles and along blood vessels. These cells then individually disperse long distances to reach cortical tissue, where they differentiate and contribute to inhibitory circuits. Late-arriving interneurons could contribute to developmental plasticity, and the disruption of their postnatal migration or differentiation may underlie neurodevelopmental disorders.

Science Translational Medicine

M. A. DeWitt et al., **Selection-free genome editing of the sickle mutation in human adult hematopoietic stem/progenitor cells,** *Sci Transl Med* 8.360 (October 12, 2016): 360ra134, doi: 10.1126/scitranslmed .aaf9336 • Genetic diseases of blood cells are prime candidates for treatment through ex vivo gene editing of CD34+ hematopoietic stem/progenitor cells (HSPCs), and a variety of technologies have been proposed to treat these disorders. Sickle cell disease (SCD) is a recessive genetic disorder caused by a single-nucleotide polymorphism in the β-globin gene (HBB). Sickle hemoglobin damages erythrocytes, causing vasoocclusion, severe pain, progressive organ damage, and premature death. We optimize design and delivery parameters of a ribonucleoprotein (RNP) complex comprising Cas9 protein and unmodified single guide RNA, togetherwith a single-stranded DNA oligonucleotide donor

(ssODN), to enable efficient replacement of the SCD mutation in human HSPCs. Corrected HSPCs from SCD patients produced less sickle hemoglobin RNA and protein and correspondingly increased wild-type hemoglobin when differentiated into erythroblasts. When engrafted into immunocompromised mice, ex vivo treated human HSPCs maintain SCD gene edits throughout 16 weeks at a level likely to have clinical benefit. These results demonstrate that an accessible approach combining Cas9 RNP with an ssODN can mediate efficient HSPC genome editing, enables investigator-led exploration of gene editing reagents in primary hematopoietic stem cells, and suggests a path toward the development of new gene editing treatments for SCD and other hematopoietic diseases.

Stem Cells Translational Medicine

M.M. El Khatib et al., **Tumor-Free Transplantation of Patient-Derived Induced Pluripotent Stem Cell Progeny for Customized Islet Regeneration**, *Stem Cells Transl Med* 5.5 (May 2016): 694–702, doi: 10.5966/sctm.2015-0017 • Human induced pluripotent stem cells (iPSCs) and derived progeny provide invaluable regenerative platforms, yet their clinical translation has been compromised by their biosafety concern. Here, we assessed the safety of transplanting patient-derived iPSC-generated pancreatic endoderm/ progenitor cells. Transplantation of progenitors from iPSCs reprogrammed by lentiviral vectors (LV-iPSCs) led to the formation of invasive teratocarcinoma-like tumors in more than 90% of immunodeficient mice. Moreover, removal of primary tumors from LV-iPSC progeny-transplanted hosts generated secondary and metastatic tumors. Combined transgene-free (TGF) reprogramming and elimination of residual pluripotent cells by enzymatic dissociation ensured tumor-free transplantation, ultimately enabling regeneration of type 1 diabetes-specific human islet structures in vivo. The incidence of tumor formation in TGF-iPSCs was titratable, depending on the oncogenic load, with reintegration of the cMYC expressing vector abolishing tumor-free transplantation. Thus, transgene free cMYC-independent reprogramming and elimination of residual pluripotent cells are mandatory steps in achieving transplantation of iPSC progeny for customized and safe islet regeneration in vivo.

Y.M. Pers et al., **Adipose mesenchymal stromal cell-based therapy for severe osteoarthritis of the knee: a phase I dose-escalation trial**, *Stem Cells Transl Med* 5.7 (July 2016): 847–856, doi: 10.5966 /sctm.2015-0245 • Osteoarthritis (OA) is the most widespread musculoskeletal disorder in adults. It leads to cartilage damage associated with subchondral bone changes and synovial inflammation, causing pain and disability. The present study aimed at evaluating the safety of a dose-escalation protocol of intra-articular injected adipose-derived stromal cells (ASCs) in patients with knee OA, as well as clinical efficacy as secondary endpoint. A bicentric, uncontrolled, open phase I clinical trial was conducted in France and Germany with regulatory agency approval for ASC expansion procedure in both countries. From April 2012 to December 2013, 18 consecutive patients with symptomatic and severe knee OA were treated with a single intra-articular injection of autologous ASCs. The study design consisted of three consecutive cohorts (six patients each) with dose escalation: low dose (2×10^6 cells), medium dose (10×10^6), and high dose (50×10^6). The primary outcome parameter was safety evaluated by recording adverse events throughout the trial, and secondary parameters were pain and function subscales of the Western Ontario and McMaster Universities Arthritis Index. After 6 months of follow-up, the procedure was found to be safe, and no serious adverse events were reported. Four patients experienced transient knee joint pain and swelling after local injection. Interestingly, patients treated with low-dose ASCs experienced significant improvements in pain levels and function compared with baseline. Our data suggest that the intraarticular injection of ASCs is a safe therapeutic alternative to treat severe knee OA patients. A placebo-controlled double-blind phase IIb study is being initiated to assess clinical and structural efficacy.