Science

Gene Editing of Cells for Correction of Muscular Dystrophy

Gene editing of a patient's own cells, to correct the mutated dystrophin gene seen in muscular dystrophies, would be a potential path to treatment of many diseases, including muscular dystrophy. One potential route for such a genetically engineered treatment would be first to create iPS cells (induced pluripotent stem cells) from the patient. Starting with the patient's cells could potentially avoid any problems with transplant matching and rejection. The correction of the mutated gene could also take place in the iPS cells under controlled conditions in the laboratory dish, before the cells with the corrected genes are re-inserted into the patient. A Japanese group earlier showed proof-of-principle results that such a technique is feasible.[1] They started by creating iPS cells from a patient with muscular dystrophy, and verified that the newly created stem cells retained the genetic problem in the dystrophin gene. The group then investigated use of two different gene-editing enzyme systems—TALENs and CRISPR-Cas9—and three different genetic correction techniques to develop genetically corrected iPS cells. In laboratory tests, the corrected cells were able to produce normal dystrophin protein successfully, paving the way to preclinical tests for efficacy.

Yongping Yue et al. recently showed in a preclinical test that genetic therapy might indeed work for muscular dystrophy treatment.[2] However, their approach differed in that they did not use iPS cells as the vehicle of correction, but instead used

1. Hongmei Lisa Li et al., "Precise Correction of the Dystrophin Gene in Duchenne Muscular Dystrophy Patient Induced Pluripotent Stem Cells by TALEN and CRISPR-Cas9," *Stem Cell Reports* 4.1 (January 13, 2015): 143–154, doi: 10.1016/j.stemcr.2014.10.013.

2. Yongping Yue et al., "Safe and Bodywide Muscle Transduction in Young Adult Duchenne Muscular Dystrophy Dogs with Adeno-Associated Virus," *Human Molecular Genetics* 24.20 (October 15, 2015): 5880–5890, doi: 10.1093/hmg/ddv310.

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whole-body infection with a virus containing a shortened but functional version of the dystrophin gene. A shortened version of the gene was used because the full-size gene is extremely large and difficult to package in a virus for delivery to a cell. The hope was that the addition of this functional gene would overcome the dystrophin protein deficits in genetically altered cells and tissues and produce a therapeutic benefit. Three young dogs with muscular dystrophy were tested, receiving sufficient injections of virus containing the engineered gene to give systemic (body-wide) exposure. Expression of the functional gene was observed in skeletal muscle and in cardiac muscle, and overall improvement in muscle histology was seen. The study indicates that a body-wide approach to delivery of genetic therapy could be useful.

Three other groups now report success in genetic correction of cells with muscular dystrophy mutations. In these studies, all three groups used slight variations of a more direct and targeted approach to genetic therapy than discussed above, with the gene-editing correction of mutations taking place directly within the affected cells of the living body. Chengzu Long et al. used an adenovirus vector that preferentially infects cardiac and skeletal muscle to deliver the components of CRISPR-Cas9, a gene targeting-and-cutting enzyme complex, into muscle cells of postnatal *mdx* mice.[3] The *mdx* mouse is a good genetic model for human Duchenne muscular dystrophy, exhibiting similar mutations as well as disease symptoms. This group found that the virus targeted muscle tissue, delivering the gene-editing enzyme which then carried out the snipping and replacement of a specific portion (exon-23) of the mutated dystrophin gene within the targeted cells. While the levels of successful genetic correction were low (a few percent of the muscle cells), even these low levels of functional dystrophin gene expression produced enhanced muscle function for the treated mice.

Christopher Nelson et al. used a very similar adenovirus delivery system for gene editing within adult and newborn *mdx* mice, also targeting removal of mutated exon 23 within the large dystrophin gene.[4] The delivery system in their experiment not only targeted skeletal and cardiac muscle but also delivered the gene-editing machinery throughout the body. The group found the gene-editing treatment produced recovery of dystrophin protein up to 8 percent of the level found in normal muscle. Not only was functional dystrophin expressed in both skeletal and cardiac muscle, but they observed a significant improvement in muscle force in the treated mice.

Mohammadsharif Tabebordbar et al. used a one-time treatment with adenovirus to deliver CRISPR-Cas9 components to cells of living *mdx* mice, again testing the gene-editing therapy in both newborn and adult mice.[5] Also targeting the genetic

3. Chengzu Long et al., "Postnatal Genome Editing Partially Restores Dystrophin Expression in a Mouse Model of Muscular Dystrophy," *Science* 351.6271 (January 22, 2016): 400–403, doi: 10.1126/science.aad5725.

4. Christopher E. Nelson et al., "In Vivo Genome Editing Improves Muscle Function in a Mouse Model of Duchenne Muscular Dystrophy," *Science* 351.6271 (January 22, 2016): 403–407, doi: 10.1126/science.aad5143.

5. Mohammadsharif Tabebordbar et al., "In Vivo Gene Editing in Dystrophic Mouse Muscle and Muscle Stem Cells," *Science* 351.6271 (January 22, 2016): 407–411, doi: 10.1126/science.aad5177.

correction to remove the mutated exon-23 stretch of DNA within the dystrophin gene of these dystrophic mice, they found that removal of the mutated portion of DNA was successful in 3 to 18 percent of various muscles tested in the treated mice, and these mice showed significant improvement in muscle force after treatment. This group also examined the effects of treatment on satellite cells, the stem cells associated with the creation and repair of muscle, and found that these adult stem cells could be similarly repaired through the gene-editing method. This is noteworthy, as long-term correction of the gene mutation would likely rely on repair of the stem cells that supply skeletal muscle.

Induced Pluripotent Stem Cells as Good as Embryonic Stem Cells

From the moment of their first production in 2007 from human cells,[6] iPS cells have been compared to embryonic stem cells. While seemingly identical in their characteristics, the derivation of iPS cells (by reprogramming normal cells with a few genes or proteins) gave these cells a distinct advantage, not only in the policy debates but also in the potential to produce—ethically, easily, and inexpensively—patient-specific stem cells for laboratory study or possibly even therapeutic use.

Despite evidence over the years, there has been persistent skepticism that iPS cells were "close enough" to embryonic stem cells to be truly equivalent. Now a new study will hopefully put this debate to rest.[7] A US-Italian research group started with two well-characterized human embryonic stem cell lines (which had been derived by the destruction of young human embryos). These cell lines were differentiated in the laboratory into fibroblast-like cells, resembling normal human connective tissue cells. Their specialization was confirmed by checking the gene expression of the cells, showing that they did indeed express fibroblast genes and no longer expressed pluripotent stem cell genes. The fibroblast cells were then reprogrammed into iPS cells using a standard protocol that expresses the reprogramming factors from a Sendai virus vector. Sendai virus gene vectors have the advantage over earlier methods in that they do not integrate into the cell's DNA and so can be completely removed from the system after reprogramming, leaving no genetic traces or mutations.

The result was human iPS cells that were genetically matched to the human embryonic stem cells. After extensive tests for gene expression, growth, and differentiation ability, the group found that the iPS cells and embryonic stem cells were molecularly and functionally equivalent, and were indistinguishable based on characteristics including gene expression. They also concluded that previous differences detected between iPS cells and embryonic stem cells in other studies could

6. Kazutoshi 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; and Junying Yu et al., "Induced Pluripotent Stem Cell Lines Derived from Human Somatic Cells," *Science* 318.5858 (December 21, 2007): 1917–1920, doi: 10.1126/science.1151526.

7. Jiho Choi et al., "A Comparison of Genetically Matched Cell Lines Reveals the Equivalence of Human iPSCs and ESCs," *Nature Biotechnology* 33.11 (November 2015): 1173–1181, doi:10.1038/nbt.3388.

be explained by genetic variability and lack of genetic matching in the cells tested. These results should open the door to full-scale utilization of iPS cells, with no further need for embryonic stem cells even in laboratory tests.

Using iPS Cells to Make a Muscle

One practical test of the utility of iPS cells is whether functional tissues, and more than one type of functional tissue, can be derived from the pluripotent stem cells. An international team has now shown that not only can iPS cells flex their potential and form functional muscle cells, but they also show flexibility and can form both functional skeletal muscle as well as functional cardiac muscle.[8] This flexibility could potentially hold great promise for some forms of muscular dystrophy.

While this disease targets and degenerates primarily skeletal muscle, some forms also affect cardiac muscle. The research team made use of mesoangioblasts, cells associated with the walls of large blood vessels that have a normal propensity both to form endothelial cells to line blood vessels and to form mesodermal tissue, which can be a precursor to both skeletal and cardiac muscle. Starting with mouse cells, the team found that iPS cells produced from normal mesangioblasts retained a greater ability to turn from stem cells into mesodermal tissue, including muscle. They were also able to isolate a specific subset of the mouse iPS cells, which they termed "mesodermal iPSC-derived progenitors" (MiPs) that had significant ability to form both skeletal muscle and cardiac muscle. But despite their flexibility in the culture dish, could these cells actually form functional tissue and regenerate damaged muscle? When the MiPs from normal mice were injected as a donor transplant into a mouse with muscular dystrophy, the MiPs did indeed repair both damaged skeletal muscle and damaged cardiac muscle. After eight weeks, the treated mice showed substantial improvement in cardiac output as well as treadmill time and muscle force measurement. Thus, this iPS cell sub-population shows promise for eventual dual use to regenerate damaged muscles in muscular dystrophy. Moreover, the team was able to repeat the derivation and isolation of MiPs from dog cells and from human cells, and in both cases the cells were able to produce both skeletal and cardiac muscle.

Direct Conversion of Cell Types Expands and Improves

Regenerating muscle tissue is not only the province of iPS cells or gene-editing. The relatively new area of direct cell conversion has seen evidence of potential application of this technique for production and repair of muscles. In the direct conversion technique, specialized cells are not de-differentiated into pluripotent stem cells like iPS cells before re-differentiation into new cell types, but rather are directly changed into the new cell type without an intermediate stem cell stage. Examples of direct conversion of cells from one type into another continue to multiply in the literature, with ever-increasing numbers of examples for different cellular end products (some of which were mentioned in recent editions of these Science Notes). Like a modern

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8. Mattia Quattrocelli et al., "Mesodermal iPSC–Derived Progenitor Cells Functionally Regenerate Cardiac and Skeletal Muscle," *Journal of Clinical Investigation* 125.12 (December 2015): 4463–4482, doi: 10.1172/JCI82735.

biological version of transmuting lead into gold, direct conversion transforms normal cells of one tissue into valuable, functional cells for a different tissue or organ. This is much more than a laboratory oddity, however, because this method has tremendous potential to provide large numbers of cells for regenerative medicine in a reproducible manner, and without the concern for potential tumor formation that is seen with pluripotent cell types such as embryonic stem cells or even iPS cells.

A Texas-based group has been working on methods to convert fibroblasts (generic connective tissue cells) directly into cardiac cells and cardiomyocytes (cardiac precursors). Conversion had been achieved previously by expressing specific cardiac genes within fibroblast cells, but the conversion was slow and inefficient. Now the researchers report that addition of a protein kinase (which phosphorylates other proteins to activate metabolic pathways) called Akt1/protein kinase B to the expression mix significantly improved the efficiency of conversion into cardiac precursor cells.[9] In the laboratory, approximately 50 percent of reprogrammed mouse fibroblasts treated with the kinase-containing mixture were spontaneously beating after three weeks. This raises the possibility of using this method to regenerate functional cardiac cells after heart damage and to provide information about the metabolic pathways important for creation and maintenance of cardiac cells.

A Chinese group took a different route to direct conversion of cells into cardiac precursors, treating cells directly with specific proteins rather than adding genes.[10] Protein induction could be advantageous because it would leave no vestiges of virus or DNA behind in the converted cells. The group started with normal human skin cells (fibroblasts) and exposed the cells to a cocktail of cardiac-specific proteins. The technique reprogrammed the cells into cardiac precursors that expressed cardiac genes. When treated with additional factors to stimulate differentiation, beating cardiac cells were produced within twenty days, showing that the precursor cells were functionally competent. In addition, when injected into damaged hearts of rats, the protein-induced cardiac precursors improved heart function.

Several groups have reported recent success using direct conversion to turn various cell types directly into functioning neurons. These previous studies used addition of specific genes and their subsequent expression to induce the direct cell conversion. Lei Zhang et al. have now shown that they can reprogram human astroglia, a type of support cell in the brain, directly into functioning neurons.[11] This group's previous work used the addition of a single gene to change the one brain cell type into another. This newest study showed that use of a few small molecules (e.g., retinoic

9. Huanyu Zhou et al., "Akt1/Protein Kinase B Enhances Transcriptional Reprogramming of Fibroblasts to Functional Cardiomyocytes," *Proceedings of the Natonal Academy of Sciences USA* 112.38 (September 22, 2015): 11864–11869, doi: 10.1073/pnas.1516237112.

10. Xiao-Hong Li et al., "Generation of Functional Human Cardiac Progenitor Cells by High-Efficiency Protein Transduction," *Stem Cells Translational Medicine* 4.12 (December 2015): 1415–1424, doi: 10.5966/sctm.2015-0136.

11. Lei Zhang et al., "Small Molecules Efficiently Reprogram Human Astroglial Cells into Functional Neurons," *Cell Stem Cell* 17.6 (December 3, 2015): 735–747, doi: 10.1016/j.stem.2015.09.012.

acid, valproic acid) could accomplish the same task, directly converting astroglia into neurons. As discussed before for the cardiac precursors, the use of nongenetic methods could provide an advantage in production of functional cell types that do not retain any mutations caused by genetic vectors for conversion.

Serotonergic neurons have numerous functions in the brain, and dysfunction of this subset of neurons has been associated with depression, anxiety, and compulsive disorders. A collaboration among Chinese and American researchers has demonstrated that they can convert human fibroblast cells in culture into serotonergic neurons by induction with four specific genes.[12] The genes were chosen because they are expressed normally during the development and maintenance of serotonergic neurons in the body. As this technique currently stands, the cells potentially could be used as laboratory models for drug testing.

Separately, another international collaboration tackled the use of direct conversion methodology to form a different neuronal subset within the brain, creating functional GABAergic interneurons.[13] The GABAergic interneurons have roles in the brain to balance excitatory stimuli, playing inhibitory roles including in the cerebral cortex and hippocampus. The imbalance between excitatory and inhibitory signaling can lead to disorders such as epilepsy. Using the genes for a set of five factors that they had identified as particular signals for development of these cells, this research group was able to successfully form functional GABAergic interneurons in the laboratory. The method succeeded starting both with mouse fibroblast cells and with human iPS cells. These lab-generated interneurons could be very valuable for drug development.

Appropriate laboratory models for the study of human aging are difficult to obtain, especially in the study of aging in the human brain. Live-tissue samples of human neuronal tissue at different ages are unavailable. Jerome Mertens et al. have published a study that finds the new functional cell types created by direct conversion technique still retain aging-related molecular characteristics, making them excellent model systems for aging studies.[14] The group created neurons from skin fibroblasts obtained from donors aged newborn to eighty-nine years old, via two different processes. One set was created by producing iPS cells from the skin cells, then differentiating these stem cells into neurons in the laboratory. Another set was produced by direct conversion of fibroblasts into neurons, using a procedure which this group had already shown to be successful, in which two gene factors and some small molecules supplement the conversion into functional neurons. The conversions showed no age-related differences in efficiency of neuron formation. After verifying

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12. Z. Xu et al., "Direct Conversion of Human Fibroblasts to Induced Serotonergic Neurons," *Molecular Psychiatry* 21.1 (January 2016): 62–70, doi: 10.1038/mp.2015.101.

13. Gaia Colasante et al., "Rapid Conversion of Fibroblasts into Functional Forebrain GABAergic Interneurons by Direct Genetic Reprogramming," *Cell Stem Cell* 17.6 (December 3, 2015): 719–734, doi: 10.1016/j.stem.2015.09.002.

14. Jerome Mertens et al., "Directly Reprogrammed Human Neurons Retain Aging-Associated Transcriptomic Signatures and Reveal Age-Related Nucleocytoplasmic Defects," *Cell Stem Cell* 17.6 (December 3, 2015): 705–718, doi: 10.1016/j.stem.2015.09.001.

the functional neuronal state of each sample, they then assayed each set of neurons for gene expression and age-related changes. They found that the neurons made by the iPS cell route had "reset" or rejuvenated their gene expression, and there were no differences related to the age of the original cells. However, the neurons created by direct conversion did show age-related differences in the expression of some genes, as well as age-associated decline in a protein that is used for transport from the nucleus to the cytoplasm. The cells showing age-related changes in gene expression were compared with brain autopsy samples from subjects aged newborn to eighty-nine years, and there was a high degree of correlation in similarly aged samples. This elegant study shows that direct conversion can provide the most appropriate models for studies of aging.

Giving Voice Back with Bioengineered Vocal Folds

Vocal impairment, known as dysphonia, affects approximately twenty million people in the United States. A majority of these have some damage to their vocal folds, which is very difficult to repair. Changying Ling et al. report that they have constructed a bioengineered vocal fold using adult human tissue that shows normal morphology and function.[15] Adult human tissue was obtained from cadaver or patients undergoing laryngectomy for other conditions. Both epithelial cells (the specialized tissue lining) and fibroblast cells (the connective tissue) were isolated, and the cells were cultured separately. Bioengineered vocal folds were constructed by co-culture of the epithelial and fibroblast cells on a collagen matrix. Within ten to fourteen days, the cells had organized and grown into structures resembling native vocal folds. Vocal folds grafted into canine hosts showed normal morphology and generated vibrations and acoustic output. When grafted into humanized mice (mice with a human immune system, in this case constructed using human adult stem cells), the vocal folds were tolerated and not rejected. These bioengineered vocal folds show promise for the treatment of human patients in the near future.

Engineering Human Lungs for the Body, and Airways on a Chip

A breath of fresh air is not always easy to obtain, especially if your lungs have been damaged by a chronic pulmonary disease. While lung transplants can address this problem, long-term complications can ensue because of immunosuppression, and the availability of transplantable lungs is insufficient to meet current needs. Harvard researchers have shown the potential of bioengineered lungs to meet these needs.[16] Their method for construction of fresh lung tissue is actually simple and straightforward, and relies on two main components: de-cellularized lungs and autologous cells. By starting with de-cellularized lung tissue, they were able to provide the proper basic framework structurally but also retain tissue-specific proteins that direct the binding and differentiation of cells into lung-specific tissue. Cadaveric rat or human lungs

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15. Changying Ling et al., "Bioengineered Vocal Fold Mucosa for Voice Restoration," *Science Translational Medicine* 7.314 (November 18, 2015): 314ra187, doi: 10.1126/scitranslmed.aab4014.

16. Xi Ren et al., "Engineering Pulmonary Vasculature in Decellularized Rat and Human Lungs," *Nature Biotechnology* 33.10 (October 2015): 1097–1102, doi: 10.1038/nbt.3354.

were perfused with a mild detergent solution to remove all cells but leave the protein scaffold intact. Mixtures of cells were then perfused into the de-cellularized lungs, and the cells were allowed time for binding. The newly cellularized lungs were then perfused to maintain nutrient and oxygenation levels, and allowed to mature. Initial cell mixtures contained human adult bone marrow mesenchymal stem cells and human umbilical vein endothelial cells (to reconstruct blood vessels in the re-cellularized lungs). A second test system used endothelial and perivascular cells derived from human iPS cells. These initial tests using the de-cellularized rat lung showed successful binding of the human cells to the lung matrix, and eventually approximately 75 percent coverage of tissue as compared to native rat lung. Transplantation of the bioengineered lungs into rats showed success at maintaining pressure within the lung tissue for up to three days. De-cellularized human lung was also tested, and the team showed that they could produce viable lung tissue and structures in this system as well. These results are very promising for the potential to create replacement lungs using a patient's own matching cells for transplant.

Kambez Benam et al. took a different route for constructing bioengineered lung tissue. In an effort to study the biochemistry of human lung airway as well as potential drug development to treat chronic lung diseases, they developed a human "small airway-on-a-chip."[17] They started with lithography of a silicon wafer, similar to that used for computer chips, and created a channel with a size similar to the radius of a human bronchiole. Primary human airway epithelial cells were then cultured on one surface of the chip in liquid medium, then one side was changed to an air interface along with addition of specific chemicals to trigger differentiation of the airway epithelial cells. Human endothelial cells were cultured on the opposite side of the chip membrane to create blood vessel tissues and a tissue–tissue interface similar to that seen in native lung. As a test of functionality, exposure of the lung epithelium on the chip to interleukin-13 stimulated cellular responses similar to those seen in patients with asthma. When small airway chips were constructed using epithelial cells from patients with chronic obstructive pulmonary disease, the cellular and biochemical responses seen with this condition could be replicated on the chip. This organ-on-a-chip will be a valuable model for investigating human lung inflammatory diseases.

Rested Adult Stem Cells Engraft Better

The secrets to a successful adult stem cell transplant are still largely unknown, but Asya Rolls et al. have now shown that a sleep-deprived donor's stem cells are much less effective.[18] Testing their hypothesis in mice, they found that four hours of sleep deprivation before donation of hematopoietic adult stem cells reduced the ability of the stem cells to engraft and reconstitute the bone marrow of an irradiated stem cell

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17. Kambez H. Benam et al., "Small Airway-on-a-Chip Enables Analysis of Human Lung Inflammation and Drug Responses In Vitro," *Nature Methods* 13.2 (February 2016): 151–157, doi: 10.1038/nmeth.3697.

18. Asya Rolls et al., "Sleep Disruption Impairs Haematopoietic Stem Cell Transplantation in Mice," *Nature Communications* 6.8516 (October 14, 2015): 1–9, doi: 10.1038/ncomms9516.

recipient by more than 50 percent. They were able to trace the decreased effectiveness of the stem cells to a decreased ability of the cells to migrate and home to the bone marrow, where they would normally engraft and begin blood cell production. At a molecular level, they showed that the affected stem cells had increased levels of suppressor of cytokine signaling (SOCS) genes, which inhibit bone marrow stem cell migration. The effect was temporary, as transplanted mice were subsequently able to donate effective stem cells. The results are important given our current busy society, demonstrating that for an adult stem cell donor, even short-term sleep deprivation can compromise the effectiveness of an adult stem cell transplant.

David A. Prentice

SCIENCE ABSTRACTS

Cell Stem Cell

G. Colasante et al., **Rapid Conversion of Fibroblasts into Functional Forebrain GABAergic Interneurons by Direct Genetic Reprogramming**, *Cell Stem Cell* 17.6 (December 3, 2015): 719–734, doi: 10.1016/j.stem.2015.09.002 • Transplantation of GABAergic interneurons (INs) can provide long-term functional benefits in animal models of epilepsy and other neurological disorders. Whereas GABAergic INs can be differentiated from embryonic stem cells, alternative sources of GABAergic INs may be more tractable for disease modeling and transplantation. We identified five factors (Foxg1, Sox2, Ascl1, Dlx5, and Lhx6) that convert mouse fibroblasts into induced GABAergic INs (iGABA-INs) possessing molecular signatures of telencephalic INs. Factor overexpression activates transcriptional networks required for GABAergic fate specification. iGABA-INs display progressively maturing firing patterns comparable to cortical INs, form functional synapses, and release GABA. Importantly, iGABA-INs survive and mature upon being grafted into mouse hippocampus. Optogenetic stimulation demonstrated functional integration of grafted iGABAINs into host circuitry, triggering inhibition of host granule neuron activity. These five factors also converted human cells into functional GABAergic INs. These properties suggest that iGABAINs have potential for disease modeling and cellbased therapeutic approaches to neurological disorders.

J. Mertens et al., **Directly Reprogrammed Human Neurons Retain Aging-Associated Transcriptomic Signatures and Reveal Age-Related Nucleocytoplasmic Defects**, *Cell Stem Cell* 17.6 (December 3, 2015): 705–718, doi: 10.1016/j.stem.2015.09.001 • Aging is a major risk factor for many human diseases, and in vitro generation of human neurons is an attractive approach for modeling aging-related brain disorders. However, modeling aging in differentiated human neurons has proved challenging. We generated neurons from human donors across a broad range of ages, either by iPSC-based reprogramming and differentiation or by direct conversion into induced neurons (iNs). While iPSCs and derived neurons did not retain aging-associated gene signatures, iNs displayed age-specific transcriptional profiles and revealed age-associated decreases in the nuclear transport receptor RanBP17. We detected an age-dependent loss of nucleocytoplasmic compartmentalization (NCC) in donor fibroblasts and corresponding iNs and found that reduced RanBP17 impaired NCC in young cells, while iPSC rejuvenation restored NCC in aged cells. These results show that iNs retain important aging-related signatures, thus allowing modeling of the aging process in vitro, and they identify impaired NCC as an important factor in human aging.

L. Zhang et al., **Small Molecules Efficiently Reprogram Human Astroglial Cells into Functional Neurons**, *Cell Stem Cell* 17.6 (December 3, 2015): 735–747, doi: 10.1016/j.stem.2015.09.012 • We have recently demonstrated that reactive glial cells can be directly reprogrammed into functional neurons by a single neural transcription factor, NeuroD1. Here we report that a combination of small molecules can also reprogram human astrocytes in culture into fully functional neurons. We demonstrate that sequential exposure of human astrocytes to a cocktail of nine small molecules that inhibit glial but activate neuronal signaling pathways can successfully reprogram astrocytes into neurons in 8–10 days. This chemical reprogramming is mediated through epigenetic regulation and involves transcriptional activation of NEUROD1 and

NEUROGENIN2. The human astrocyte-converted neurons can survive for >5 months in culture and form functional synaptic networks with synchronous burst activities. The chemically reprogrammed human neurons can also survive for >1 month in the mouse brain in vivo and integrate into local circuits. Our study opens a new avenue using chemical compounds to reprogram reactive glial cells into functional neurons.

Human Molecular Genetics

Y. Yue et al., **Safe and Bodywide Muscle Transduction in Young Adult Duchenne Muscular Dystrophy Dogs with Adeno-Associated Virus**, *Hum Mol Genet* 24.20 (October 15, 2015): 5880–5890, doi: 10.1093/hmg/ddv310 • The ultimate goal of muscular dystrophy gene therapy is to treat all muscles in the body. Global gene delivery was demonstrated in dystrophic mice more than a decade ago using adeno-associated virus (AAV). However, translation to affected large mammals has been challenging. The only reported attempt was performed in newborn Duchenne muscular dystrophy (DMD) dogs. Unfortunately, AAV injection resulted in growth delay, muscle atrophy and contracture. Here we report safe and bodywide AAV delivery in juvenile DMD dogs. Three ~2-m-old affected dogs received intravenous injection of a tyrosine-engineered AAV-9 reporter or micro-dystrophin (μDys) vector at the doses of 1.92–6.2 × 1014 viral genome particles/kg under transient or sustained immune suppression. DMD dogs tolerated injection well and their growth was not altered. Hematology and blood biochemistry were unremarkable. No adverse reactions were observed. Widespread muscle transduction was seen in skeletal muscle, the diaphragm and heart for at least 4 months (the end of the study). Nominal expression was detected in internal organs. Improvement in muscle histology was observed in μDys-treated dogs. In summary, systemic AAV gene transfer is safe and efficient in young adult dystrophic large mammals. This may translate to bodywide gene therapy in pediatric patients in the future.

Journal of Clinical Investigation

M. Quattrocelli et al., **Mesodermal iPSC–Derived Progenitor Cells Functionally Regenerate Cardiac and Skeletal Muscle**, *J Clin Invest* 125.12 (December 2015): 4463–4482, doi: 10.1172/JCI82735 • Conditions such as muscular dystrophies (MDs) that affect both cardiac and skeletal muscles would benefit from therapeutic strategies that enable regeneration of both of these striated muscle types. Protocols have been developed to promote induced pluripotent stem cells (iPSCs) to differentiate toward cardiac or skeletal muscle; however, there are currently no strategies to simultaneously target both muscle types. Tissues exhibit specific epigenetic alterations; therefore, source-related lineage biases have the potential to improve iPSC-driven multilineage differentiation. Here, we determined that differential myogenic propensity influences the commitment of isogenic iPSCs and a specifically isolated pool of mesodermal iPSC-derived progenitors (MiPs) toward the striated muscle lineages. Differential myogenic propensity did not influence pluripotency, but did selectively enhance chimerism of MiP-derived tissue in both fetal and adult skeletal muscle. When injected into dystrophic mice, MiPs engrafted and repaired both skeletal and cardiac muscle, reducing functional defects. Similarly, engraftment into dystrophic mice of canine MiPs from dystrophic dogs that had undergone TALEN-mediated correction of the MD-associated mutation also resulted in functional striatal muscle regeneration. Moreover, human MiPs exhibited the same capacity for the dual differentiation observed in murine and canine MiPs. The findings of this study suggest that MiPs should be further explored for combined therapy of cardiac and skeletal muscles.

Molecular Psychiatry

Z. Xu et al., **Direct Conversion of Human Fibroblasts to Induced Serotonergic Neurons**, *Mol Psychiatry* 21.1 (January 2016): 62–70, doi: 10.1038/mp.2015.101 • Serotonergic (5HT) neurons exert diverse and

widespread functions in the brain. Dysfunction of the serotonergic system gives rise to a variety of mental illnesses including depression, anxiety, obsessive compulsive disorder, autism and eating disorders. Here we show that human primary fibroblasts were directly converted to induced serotonergic (i5HT) neurons by the expression of Ascl1, Foxa2, Lmx1b and FEV. The transdifferentiation was enhanced by p53 knockdown and appropriate culture conditions including hypoxia. The i5HT neurons expressed markers for mature serotonergic neurons, had Ca2+-dependent 5HT release and selective 5HT uptake, exhibited spontaneous action potentials and spontaneous excitatory postsynaptic currents. Application of serotonin significantly increased the firing rate of spontaneous action potentials, demonstrating the functional utility of i5HT neurons for studying serotonergic neurotransmission. The availability of human i5HT neurons will be very useful for research and drug discovery on many serotonin-related mental disorders.

Nature Biotechnology

J. Choi et al., **A Comparison of Genetically Matched Cell Lines Reveals the Equivalence of Human iPSCs and ESCs**, *Nat Biotechnol* 33.11 (November 2015): 1173–1181, doi: 10.1038/nbt.3388 • The equivalence of human induced pluripotent stem cells (hiPSCs) and human embryonic stem cells (hESCs) remains controversial. Here we use genetically matched hESC and hiPSC lines to assess the contribution of cellular origin (hESC vs. hiPSC), the Sendai virus (SeV) reprogramming method and genetic background to transcriptional and DNA methylation patterns while controlling for cell line clonality and sex. We find that transcriptional and epigenetic variation originating from genetic background dominates over variation due to cellular origin or SeV infection. Moreover, the 49 differentially expressed genes we detect between genetically matched hESCs and hiPSCs neither predict functional outcome nor distinguish an independently derived, larger set of unmatched hESC and hiPSC lines. We conclude that hESCs and hiPSCs are molecularly and functionally equivalent and cannot be distinguished by a consistent gene expression signature. Our data further imply that genetic background variation is a major confounding factor for transcriptional and epigenetic comparisons of pluripotent cell lines, explaining some of the previously observed differences between genetically unmatched hESCs and hiPSCs.

X. Ren et al., **Engineering Pulmonary Vasculature in Decellularized Rat and Human Lungs**, *Nat Biotechnol* 33.10 (October 2015): 1097–1102, doi: 10.1038/nbt.3354 • Bioengineered lungs produced from patient-derived cells may one day provide an alternative to donor lungs for transplantation therapy. Here we report the regeneration of functional pulmonary vasculature by repopulating the vascular compartment of decellularized rat and human lung scaffolds with human cells, including endothelial and perivascular cells derived from induced pluripotent stem cells. We describe improved methods for delivering cells into the lung scaffold and for maturing newly formed endothelium through co-seeding of endothelial and perivascular cells and a two-phase culture protocol. Using these methods we achieved ~75% endothelial coverage in the rat lung scaffold relative to that of native lung. The regenerated endothelium showed reduced vascular resistance and improved barrier function over the course of in vitro culture and remained patent for 3 days after orthotopic transplantation in rats. Finally, we scaled our approach to the human lung lobe and achieved efficient cell delivery, maintenance of cell viability and establishment of perfusable vascular lumens.

Nature Communications

A. Rolls et al., **Sleep Disruption Impairs Haematopoietic Stem Cell Transplantation in Mice**, *Nat Commun* 6.8516 (October 14, 2015): 1–9, doi: 10.1038/ncomms 9516 • Many of the factors affecting the success of haematopoietic cell transplantation are still unknown. Here we show in mice that donor sleep deprivation reduces

the ability of its haematopoietic stem cells (HSCs) to engraft and reconstitute the blood and bone marrow of an irradiated recipient by more than 50%. We demonstrate that sleep deprivation downregulates the expression of microRNA (miR)-19b, a negative regulator of the suppressor of cytokine signalling (SOCS) genes, which inhibit HSC migration and homing. Accordingly, HSCs from sleep-deprived mice have higher levels of SOCS genes expression, lower migration capacity in vitro and reduced homing to the bone marrow in vivo. Recovery of sleep after sleep deprivation restored the reconstitution potential of the HSCs. Taken together, this study provides insights into cellular and molecular mechanisms underlying the effects of sleep deprivation on HSCs, emphasizing the potentially critical role of donor sleep in the success of bone marrow transplantation.

Nature Methods

K. H. Benam et al., **Small Airway-on-a-Chip Enables Analysis of Human Lung Inflammation and Drug Responses In Vitro**, *Nat Methods* 13.2 (February 2016): 151–157, doi: 10.1038/nmeth.3697 • Here we describe the development of a human lung 'small airway-on-a-chip' containing a differentiated, mucociliary bronchiolar epithelium and an underlying microvascular endothelium that experiences fluid flow, which allows for analysis of organ-level lung pathophysiology in vitro. Exposure of the epithelium to interleukin-13 (ILIL-13) reconstituted the goblet cell hyperplasia, cytokine hypersecretion and decreased ciliary function of asthmatics. Small airway chips lined with epithelial cells from individuals with chronic obstructive pulmonary disease recapitulated features of the disease such as selective cytokine hypersecretion, increased neutrophil recruitment and clinical exacerbation by exposure to viral and bacterial infections. With this robust in vitro method for modeling human lung inflammatory disorders, it is possible to detect synergistic effects of lung endothelium and epithelium on cytokine secretion, identify new biomarkers of disease exacerbation and measure responses to anti-inflammatory compounds that inhibit cytokine-induced recruitment of circulating neutrophils under flow.

Proceedings of the National Academy of Sciences USA

H. Zhou et al., **Akt1/Protein Kinase B Enhances Transcriptional Reprogramming of Fibroblasts to Functional Cardiomyocytes**, *Proc Natl Acad Sci USA* 112.38 (September 22, 2015): 11864–11869, doi: 10.1073/pnas.1516237112 • Conversion of fibroblasts to functional cardiomyocytes represents a potential approach for restoring cardiac function after myocardial injury, but the technique thus far has been slow and inefficient. To improve the efficiency of reprogramming fibroblasts to cardiac-like myocytes (iCMs) by cardiac transcription factors [Gata4, Hand2, Mef2c, and Tbx5 (GHMT)], we screened 192 protein kinases and discovered that Akt/protein kinase B dramatically accelerates and amplifies this process in three different types of fibroblasts (mouse embryo, adult cardiac, and tail tip). Approximately 50% of reprogrammed mouse embryo fibroblasts displayed spontaneous beating after 3 wk of induction by Akt plus GHMT. Furthermore, addition of Akt1 to GHMT evoked a more mature cardiac phenotype for iCMs, as seen by enhanced polynucleation, cellular hypertrophy, gene expression, and metabolic reprogramming. Insulin-like growth factor 1 (IGF1) and phosphoinositol 3-kinase (PI3K) acted upstream of Akt whereas the mitochondrial target of rapamycin complex 1 (mTORC1) and forkhead box o3 (Foxo3a) acted downstream of Akt to influence fibroblast-to-cardiomyocyte reprogramming. These findings provide insights into the molecular basis of cardiac reprogramming and represent an important step toward further application of this technique.

Science

C. Long et al., **Postnatal Genome Editing Partially Restores Dystrophin Expression in a Mouse Model of Muscular Dystrophy**, *Science* 351.6271 (January 22, 2016): 400–403, doi: 10.1126/science.aad5725 • CRISPR/Cas9-mediated genome

editing holds clinical potential for treating genetic diseases, such as Duchenne muscular dystrophy (DMD), which is caused by mutations in the dystrophin gene. To correct DMD by skipping mutant dystrophin exons in postnatal muscle tissue in vivo, we used adeno-associated virus-9 (AAV9) to deliver gene editing components to postnatal mdx mice, a model of DMD. Different modes of AAV9 delivery were systematically tested, including intra-peritoneal at postnatal day (P) 1, intra-muscular at P12, and retro-orbital at P18. Each of these methods restored dystrophin protein expression in cardiac and skeletal muscle to varying degrees and expression increased from 3 to 12 weeks post-injection. Postnatal gene editing also enhanced skeletal muscle function, measured by grip strength tests 4 weeks post-injection. This method provides a potential means of correcting mutations responsible for DMD and other monogenic disorders after birth.

C. E. Nelson et al., **In Vivo Genome Editing Improves Muscle Function in a Mouse Model of Duchenne Muscular Dystrophy**, *Science* 351.6271 (January 22, 2016): 403–407, doi: 10.1126/science.aad5143 • Duchenne muscular dystrophy (DMD) is a devastating disease affecting about 1 out of 5000 male births and caused by mutations in the dystrophin gene. Genome editing has the potential to restore expression of a modified dystrophin gene from the native locus to modulate disease progression. In this study, adeno-associated virus was used to deliver the CRISPR/Cas9 system to the mdx mouse model of DMD to remove the mutated exon 23 from the dystrophin gene. This includes local and systemic delivery to adult mice and systemic delivery to neonatal mice. Exon 23 deletion by CRISPR/Cas9 resulted in expression of the modified dystrophin gene, partial recovery of functional dystrophin protein in skeletal myofibers and cardiac muscle, improvement of muscle biochemistry, and significant enhancement of muscle force. This work establishes CRISPR/Cas9-based genome editing as a potential therapy to treat DMD.

M. Tabebordbar et al., **In Vivo Gene Editing in Dystrophic Mouse Muscle and Muscle Stem Cells**, *Science* 351.6271 (January 22, 2016): 407–411, doi: 10.1126/science.aad5177 • Frame-disrupting mutations in the DMD gene, encoding dystrophin, compromise myofiber integrity and drive muscle deterioration in Duchenne muscular dystrophy (DMD). Removing one or more exons from the mutated transcript can produce an in-frame mRNA and a truncated, but still functional, protein. In this study, we develop and test a direct gene-editing approach to induce exon deletion and recover dystrophin expression in the mdx mouse model of DMD. Delivery by adeno-associated virus (AAV) of clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 endonucleases coupled with paired guide RNAs flanking the mutated Dmd exon23 resulted in excision of intervening DNA and restored Dystrophin reading frame in myofibers, cardiomyocytes, and muscle stem cells following local or systemic delivery. AAV-Dmd CRISPR-treatment partially recovered muscle functional deficiencies and generated a pool of endogenously corrected myogenic precursors in mdx mouse muscle.

Science Translational Medicine

C. Ling et al., **Bioengineered Vocal Fold Mucosa for Voice Restoration**, *Sci Transl Med* 7.314 (November 18, 2015): 314ra187, doi: 10.1126/scitranslmed.aab4014 • Patients with voice impairment caused by advanced vocal fold (VF) fibrosis or tissue loss have few treatment options. A transplantable, bioengineered VF mucosa would address the individual and societal costs of voice-related communication loss. Such a tissue must be biomechanically capable of aerodynamic-to-acoustic energy transfer and high frequency vibration and physiologically capable of maintaining a barrier against the airway lumen. We isolated primary human VF fibroblasts and epithelial cells and cocultured them under organotypic conditions. The resulting engineered mucosae

showed morphologic features of native tissue, proteome-level evidence of mucosal morphogenesis and emerging extracellular matrix complexity, and rudimentary barrier function in vitro. When grafted into canine larynges ex vivo, the mucosae generated vibratory behavior and acoustic output that were indistinguishable from those of native VF tissue. When grafted into humanized mice in vivo, the mucosae survived and were well tolerated by the human adaptive immune system. This tissue engineering approach has the potential to restore voice function in patients with otherwise untreatable VF mucosal disease.

Stem Cell Reports

H.L. Li et al., **Precise Correction of the Dystrophin Gene in Duchenne Muscular Dystrophy Patient Induced Pluripotent Stem Cells by TALEN and CRISPR-Cas9**, *Stem Cell Reports* 4.1 (January 13, 2015): 143–154, doi: 10.1016/j.stemcr.2014.10.013 • Duchenne muscular dystrophy (DMD) is a severe muscle-degenerative disease caused by a mutation in the dystrophin gene. Genetic correction of patient-derived induced pluripotent stem cells (iPSCs) by TALENs or CRISPR-Cas9 holds promise for DMD gene therapy; however, the safety of such nuclease treatment must be determined. Using a unique k-mer database, we systematically identified a unique target region that reduces off-target sites. To restore the dystrophin protein, we performed three correction methods (exon skipping, frameshifting, and exon knockin) in DMD-patient-derived iPSCs, and found that exon knockin was the most effective approach. We further investigated the genomic integrity by karyotyping, copy number variation array, and exome sequencing to identify clones with a minimal mutation load. Finally, we differentiated the corrected iPSCs toward skeletal muscle cells and successfully detected the expression of full-length dystrophin protein. These results provide an important framework for developing iPSC-based gene therapy for genetic disorders using programmable nucleases.

Stem Cells Translational Medicine

X.-H. Li et al., **Generation of Functional Human Cardiac Progenitor Cells by High-Efficiency Protein Transduction**, *Stem Cells Transl Med* 4.12 (December 2015): 1415–1424, doi: 10.5966/sctm.2015-0136 • The reprogramming of fibroblasts to induced pluripotent stem cells raises the possibility that somatic cells could be directly reprogrammed to cardiac progenitor cells (CPCs). The present study aimed to assess highly efficient protein-based approaches to reduce or eliminate the genetic manipulations to generate CPCs for cardiac regeneration therapy. A combination of QQ-reagent-modified Gata4, Hand2, Mef2c, and Tbx5 and three cytokines rapidly and efficiently reprogrammed human dermal fibroblasts (HDFs) into CPCs. This reprogramming process enriched trimethylated histone H3 lysine 4, monoacetylated histone H3 lysine 9, and Baf60c at the Nkx2.5 cardiac enhancer region by the chromatin immunoprecipitation quantitative polymerase chain reaction assay. Protein-induced CPCs transplanted into rat hearts after myocardial infarction improved cardiac function, and this was related to differentiation into cardiomyocyte-like cells. These findings demonstrate that the highly efficient protein-transduction method can directly reprogram HDFs into CPCs. This protein reprogramming strategy lays the foundation for future refinements both in vitro and in vivo and might provide a source of CPCs for regenerative approaches.