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Cell

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Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors

Kazutoshi Takahashi et al.

Successful reprogramming of differentiated human somatic cells into a pluripotent state would allow creation of patient- and disease-specific stem cells. The authors previously reported generation of induced pluripotent stem (iPS) cells, capable of germline transmission, from mouse somaic cells by transduction of four defined transcription factors. Here, they 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 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.

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Semen-Derived Amyloid Fibrils Drastically Enhance HIV Infection

Jan Münch et al.

Sexual intercourse is the major route of HIV transmission. To identify endogenous factors that affect the efficiency of sexual viral transmission, the authors screened a complex peptide/protein library derived from human semen. They show that naturally occurring fragments of the abundant semen marker prostatic acidic phosphatase form amyloid fibrils. These fibrils, termed semen-derived enhancer of virus infection (SEVI), capture HIV virions and promote their attachment to target cells, thereby enhancing the infectious virus titer by several orders of magnitude. Physiological concentrations of SEVI amplified HIV infection of T cells, macrophages, ex vivo human tonsillar tissues, and transgenic rats in vivo, as well as trans-HIV infection of T cells by dendritic or epithelial cells. Amyloidogenic prostatic acidic phosphatase fragments are abundant in seminal fluid and boost semen-mediated enhancement of HIV infection. Thus, they may play an important role in sexual transmission of HIV and could represent new targets for its prevention.

Cell Stem Cell

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Developmental Study of Fragile X Syndrome Using Human Embryonic Stem Cells Derived from Preimplantation Genetically Diagnosed Embryos

Rachel Eiges et al.

The authors report on the establishment of a human embryonic stem cell line from a preimplantation fragile X–affected embryo and demonstrate its value as an appropriate model to study developmentally regulated events that are involved in the pathogenesis of this disorder. Fragile X syndrome results from FMR1 gene inactivation due to a CGG expansion at the 50UTR region of the gene. Early events in FMR1 silencing have not been fully characterized because of the lack of appropriate animal or cellular models. Here the authors show that, despite the presence of a full mutation, affected undifferentiated HESCs express FMR1 and are DNA unmethylated. However, epigenetic

silencing by DNA methylation and histone modification occurs upon differentiation. Their unique cell system allows the dissection of the sequence by which these epigenetic changes are acquired and illustrates the importance of human embryonic stem cells in unraveling developmentally regulated mechanisms associated with human genetic disorders.

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Restoration of Human Dystrophin Following Transplantation of Exon-Skipping-Engineered DMD Patient Stem Cells into Dystrophic Mice

Rachid Benchaouir et al.

Duchenne muscular dystrophy (DMD) is a hereditary disease caused by mutations that disrupt the dystrophin mRNA reading frame. In some cases, forced exclusion (skipping) of a single exon can restore the reading frame, giving rise to a shorter, but still functional, protein. In this study, the authors constructed lentiviral vectors expressing antisense oligonucleotides in order to induce an efficient exon skipping and to correct the initial frameshift caused by the DMD deletion of CD133+ stem cells. The intramuscular and intra-arterial delivery of genetically corrected CD133 expressing myogenic progenitors isolated from the blood and muscle of DMD patients results in a significant recovery of muscle morphology, function, and dystrophin expression in scid/mdx mice. These data demonstrate that autologous engrafting of blood or muscle-derived CD133+ cells, previously genetically modified to re-express a functional dystrophin, represents a promising approach for DMD.

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C-Myc Is Dispensable for Direct Reprogramming of Mouse Fibroblasts

Marius Wernig et al.

Retroviral transduction of the four transcription factors—Oct4, Sox2, Klf4, and c-Myc—has been shown to initiate a reprogramming process that results in the transformation of mouse fibroblasts into embryonic stem–like cells designated as induced pluripotent stem (iPS) cells (Maherali et al., 2007, Meissner et al., 2007, Okita et al., 2007, Takahashi et al., 2006, Wernig et al., 2007). The promise of somatic reprogramming is the possibility to generate pluripotent stem cells that are patient specific and can be used as a unique source for autologous cell types for transplantation therapy (Jaenisch, 2004, Yamanaka, 2007). Many iPS cell-derived animals develop tumors due to the reactivation of the c-Myc virus (Okita et al., 2007), and this represents a major safety concern if we want to translate this approach to humans. It is thus of great importance to achieve reprogramming without this particular oncogene in the future. Here the authors show that fibroblasts can be reprogrammed to a pluripotent state by Oct4, Sox2, and Klf4 in the absence of c-Myc.

Journal of Neuroscience

Volume 27, Number 44
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Neural Stem Cells Improve Memory in an Inducible Mouse Model of Neuronal Loss

Tritia R. Yamasaki et al.

Neuronal loss is a major pathological outcome of many common neurological disorders, including ischemia, traumatic brain injury, and Alzheimer's disease. Stem cell–based approaches have received considerable attention as a potential means of treatment, although it remains to be determined whether stem cells can ameliorate memory dysfunction, a devastating component of these disorders. The authors generated a transgenic mouse model in which the tetracycline-off system is used to regulate expression of diphtheria toxin A chain. After induction, they found progressive neuronal loss primarily within the hippocampus, leading to specific

impairments in memory. They found that neural stem cells transplanted into the brain after neuronal ablation survive, migrate, differentiate and, most significantly, improve memory. These results show that stem cells may have therapeutic value in diseases and conditions that result in memory loss.

Nature

Volume 450, Number 7169
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Producing Primate Embryonic Stem Cells by Somatic Cell Nuclear Transfer

J. A. Byrne et al.

Derivation of embryonic stem cells genetically identical to a patient by somatic cell nuclear transfer (SCNT) holds the potential to cure or alleviate the symptoms of many degenerative diseases while circumventing concerns regarding rejection by the host immune system. However, the concept has only been achieved in the mouse, whereas inefficient reprogramming and poor embryonic development characterizes the results obtained in primates. Here, the authors used a modified SCNT approach to produce rhesus macaque blastocysts from adult skin fibroblasts, and successfully isolated two embryonic stem cell lines from these embryos. DNA analysis confirmed that nuclear DNA was identical to donor somatic cells and that mitochondrial DNA originated from oocytes. Both cell lines exhibited normal embryonic stem cell morphology, expressed key stem cell markers, were transcriptionally similar to control embryonic stem cells, and differentiated into multiple cell types in vitro and in vivo. Their results represent successful nuclear reprogramming of adult somatic cells into pluripotent embryonic stem cells and demonstrate proof of concept for therapeutic cloning in primates.

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Engraftment of Connexin 43–Expressing Cells Prevents Post-Infarct Arrhythmia

Willhelm Roell et al.

Ventricular tachyarrhythmias are the main cause of sudden death in patients after myocardial infarction. The authors show that transplantation of embryonic cardiomyocytes (eCMs) in myocardial infarcts protects against the induction of ventricular tachycardia (VT) in mice. Engraftment of eCMs, but not skeletal myoblasts (SMs), bone marrow cells, or cardiac myofibroblasts markedly decreased the incidence of VT induced by in vivo pacing. eCM engraftment results in improved electrical coupling between the surrounding myocardium and the infarct region, and Ca2+ signals from engrafted eCMs expressing a genetically encoded Ca2+ indicator could be entrained during sinoatrial cardiac activation in vivo. eCM grafts also increased conduction velocity and decreased the incidence of conduction block within the infarct. VT protection is critically dependent on expression of the gap-junction protein connexin 43 (Cx43, also known as Gja1): SMs genetically engineered to express Cx43 conferred a similar protection to that of eCMs against induced VT. Thus, engraftment of Cx43-expressing myocytes has the potential to reduce life-threatening post-infarct arrhythmias through the augmentation of intercellular coupling, suggesting autologous strategies for cardiac cell-based therapy.

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Reprogramming of Human Somatic Cells to Pluripotency with Defined Factors

In-Hyun Park et al.

Pluripotency pertains to the cells of early embryos that can generate all of the tissues in the organism. Embryonic stem cells are embryo-derived cell lines that retain pluripotency and represent invaluable tools for research into the mechanisms of tissue

formation. Recently, murine fibroblasts have been reprogrammed directly to pluripotency by ectopic expression of four transcription factors (Oct4, Sox2, Klf4 and Myc) to yield induced pluripotent stem (iPS) cells. Using these same factors, we have derived iPS cells from fetal, neonatal and adult human primary cells, including dermal fibroblasts isolated from a skin biopsy of a healthy research subject. Human iPS cells resemble embryonic stem cells in morphology and gene expression and in the capacity to form teratomas in immune-deficient mice. These data demonstrate that defined factors can reprogramme human cells to pluripotency, and establish a method whereby patient-specific cells might be established in culture.

Nature Biotechnology

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Cardiomyocytes Derived from Human Embryonic Stem Cells in Pro-Survival Factors Enhance Function of Infarcted Rat Hearts

Michael A. Laflamme et al.

Cardiomyocytes derived from human embryonic stem cells potentially offer large numbers of cells to facilitate repair of the infarcted heart. However, this approach has been limited by inefficient differentiation of human embryonic stem cells into cardiomyocytes, insufficient purity of cardiomyocyte preparations and poor survival of human embryonic stem cell-derived myocytes after transplantation. Seeking to overcome these challenges, the authors generated highly purified human cardiomyocytes using a readily scalable system for directed differentiation that relies on activin A and BMP4. They then identified a cocktail of pro-survival factors that limits cardiomyocyte death after transplantation. These techniques enabled consistent formation of myocardial grafts in the infarcted rat heart. The engrafted human myocardium attenuated ventricular dilation and preserved regional and global contractile function after myocardial infarction compared with controls receiving noncardiac human embryonic stem cell derivatives or vehicle. The ability of human embryonic stem cell-derived cardiomyocytes to partially remuscularize myocardial infarcts and attenuate heart failure encourages their study under conditions that closely match human disease.

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Generation of Induced Pluripotent Stem Cells without Myc from Mouse and Human Fibroblasts

Masato Nakagawa et al.

Direct reprogramming of somatic cells provides an opportunity to generate patient- or disease-specific pluripotent stem cells. Such induced pluripotent stem (iPS) cells were generated from mouse fibroblasts by retroviral transduction of four transcription factors: Oct3/4, Sox2, Klf4 and c-Myc. Mouse iPS cells are indistinguishable from embryonic stem cells in many respects and produce germline-competent chimeras. Reactivation of the c-Myc retrovirus, however, increases tumorigenicity in the chimeras and progeny mice, hindering clinical applications. The authors describe a modified protocol for the generation of iPS cells that does not require the Myc retrovirus. With this protocol, they obtained significantly fewer non-iPS background cells, and the iPS cells generated were consistently of high quality. Mice derived from Myc(-) iPS cells did not develop tumors during the study period. The protocol also enabled efficient isolation of iPS cells without drug selection. Furthermore, the author generated human iPS cells from adult dermal fibroblasts without MYC.

Nature Neuroscience

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A Glial Amino-Acid Transporter Controls Synapse Strength and Courtship in *Drosophila*

Yael Grosjean et al.

Mate choice is an evolutionarily critical decision that requires the detection of multiple sex-specific signals followed by central integration of these signals to direct appropriate behavior. The mechanisms controlling mate choice remain poorly understood. The authors show that the glial amino-acid transporter genderblind controls whether *Drosophila melanogaster* males will attempt to mate with other males. Genderblind (gb) mutant males showed no alteration in heterosexual courtship or copulation, but were attracted to normally unappealing male species-specific chemosensory cues. As a result, genderblind mutant males courted and attempted to copulate with other *Drosophila* males. This homosexual behavior could be induced within hours using inducible RNAi, suggesting that genderblind controls nervous system function rather than its development. Consistent with this, and indicating that glial genderblind regulates ambient extracellular glutamate to suppress glutamatergic synapse strength in vivo, homosexual behavior could be turned on and off by altering glutamatergic transmission pharmacologically or genetically.

PLoS Biology

Volume 5, Number 10
September 4, 2007

The Diploid Genome Sequence of an Individual Human

Samuel Levy et al.

Presented here is a genome sequence of an individual human. It was produced from less than thirty-two million random DNA fragments, sequenced by Sanger dideoxy technology and assembled into 4,528 scaffolds, comprising 2,810 million bases (Mb) of contiguous sequence with approximately 7.5-fold coverage for any given region. The authors developed a modified version of the Celera assembler to facilitate the identification and comparison of alternate alleles within this individual diploid genome. Comparison of this genome and the National Center for Biotechnology Information human reference assembly revealed more than 4.1 million DNA variants, encompassing 12.3 Mb. These variants (of which 1,288,319 were novel) included 3,213,401 single nucleotide polymorphisms (SNPs), 53,823 block substitutions (2–206 bp [base pairs]), 292,102 heterozygous insertion/deletion events (indels)(1–571 bp), 559,473 homozygous indels (1–82,711 bp), and 90 inversions, as well as numerous segmental duplications and copy number variation regions. Non-SNP DNA variation accounts for 22 percent of all events identified in the donor; however, they involve 74 percent of all variant bases. This suggests an important role for non-SNP genetic alterations in defining the diploid genome structure. Moreover, 44 percent of genes were heterozygous for one or more variants. Using a novel haplotype assembly strategy, the authors were able to span 1.5 Gb [giga base pairs] of genome sequence in segments greater than 200 kb, providing further precision to the diploid nature of the genome. These data depict a definitive molecular portrait of a diploid human genome that provides a starting point for future genome comparisons and enables an era of individualized genomic information.

PNAS: Proceedings of the National Academy of Sciences USA

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Bone Marrow Cells Adopt the Cardiomyogenic Fate In Vivo

Marcello Rota et al.

The possibility that adult bone marrow cells (BMCs) retain a remarkable degree of developmental plasticity and acquire the cardiomyocyte lineage after infarction has been challenged, and the notion of BMC transdifferentiation has been questioned. The center of the controversy is the lack of unequivocal evidence in favor of myocardial regeneration by the injection of BMCs in the infarcted heart. Because of the interest in cell-based therapy for heart failure, several approaches including gene reporter assay, genetic tagging, cell genotyping, PCR-based detection of donor genes, and direct immunofluorescence with quantum dots were used to prove or disprove BMC transdifferentiation. The results indicate that BMCs engraft, survive, and grow within the spared myocardium after infarction by forming junctional complexes with resident myocytes. BMCs and myocytes express at their interface connexin 43 and N-cadherin, and this interaction may be critical for BMCs to adopt the cardiomyogenic fate. With time, a large number of myocytes and coronary vessels are generated. Myocytes show a diploid DNA content and carry, at most, two sex chromosomes. Old and new myocytes show synchronicity in calcium transients, providing strong evidence in favor of the functional coupling of these two cell populations. Thus, BMCs transdifferentiate and acquire the cardiomyogenic and vascular phenotypes restoring the infarcted heart. Together, our studies reveal that locally delivered BMCs generate de novo myocardium composed of integrated cardiomyocytes and coronary vessels. This process occurs independently of cell fusion and ameliorates structurally and functionally the outcome of the heart after infarction.

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Modulation of Metabolic Brain Networks after Subthalamic Gene Therapy for Parkinson's Disease

Andrew Feigin et al.

Parkinson's disease (PD) is characterized by elevated expression of an abnormal metabolic brain network that is reduced by clinically effective treatment. The authors used fluorodeoxyglucose (FDG) positron emission tomography (PET) to determine the basis for motor improvement in twelve PD patients receiving unilateral subthalamic nucleus (STN) infusion of an adenoassociated virus vector expressing glutamic acid decarboxylase (AAV-GAD). After gene therapy, they observed significant reductions in thalamic metabolism on the operated side as well as concurrent metabolic increases in ipsilateral motor and premotor cortical regions. Abnormal elevations in the activity of metabolic networks associated with motor and cognitive functioning in PD patients were evident at baseline. The activity of the motor-related network declined after surgery and persisted at one year. These network changes correlated with improved clinical disability ratings. By contrast, the activity of the cognition-related network did not change after gene transfer. This suggests that modulation of abnormal network activity underlies the clinical outcome observed after unilateral STN AAV-GAD gene therapy. Network biomarkers may be used as physiological assays in early-phase trials of experimental therapies for PD and other neurodegenerative disease.

Science

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Induced Pluripotent Stem Cell Lines Derived from Human Somatic Cells

Junying Yu et al.

Somatic cell nuclear transfer allows trans-acting factors present in the mammalian oocyte to reprogram somatic cell nuclei to an undif-

ferentiated state. The authors show that four factors (OCT4, SOX2, NANOG, and LIN28) are sufficient to reprogram human somatic cells to pluripotent stem cells that exhibit the essential characteristics of embryonic stem cells. These human induced pluripotent stem cells have normal karyotypes, express telomerase activity, express cell surface markers and genes that characterize human ES cells, and maintain the developmental potential to differentiate into advanced derivatives of all three primary germ layers. Such human induced pluripotent cell lines should be useful in the production of new disease models and in drug development as well as application in transplantation medicine once technical limitations (for example, mutation through viral integration) are eliminated.

Volume 318, Number 5854
November 23, 2007

Efficient Transplantation via Antibody-Based Clearance of Hematopoietic Stem Cell Niches

Agnieszka Czechowicz et al.

Upon intravenous transplantation, hematopoietic stem cells (HSCs) can home to specialized niches, yet most HSCs fail to engraft unless recipients are subjected to toxic preconditioning. The authors provide evidence that, aside from immune barriers, donor HSC engraftment is restricted by occupancy of appropriate niches by host HSCs. Administration of ACK2, an antibody that blocks c-kit function, led to the transient removal of more than 98 percent of endogenous HSCs in immunodeficient mice. Subsequent transplantation of these mice with donor HSCs led to chimerism levels of up to 90 percent. Extrapolation of these methods to humans may enable mild but effective conditioning regimens for transplantation.

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Treatment of Sickle Cell Anemia Mouse Model with iPS Cells Generated from Autologous Skin

Jacob Hanna et al.

It has recently been demonstrated that mouse and human fibroblasts can be reprogrammed into an embryonic stem cell–like state by introducing combinations of four transcription factors. However, the therapeutic potential of such induced pluripotent stem (iPS) cells remained undefined. By using a humanized sickle cell anemia mouse model, the authors show that mice can be rescued after transplantation with hematopoietic progenitors obtained in vitro from autologous iPS cells. This was achieved after correction of the human sickle hemoglobin allele by gene-specific targeting. Their results provide proof of principle for using transcription factor–induced reprogramming combined with gene and cell therapy for disease treatment in mice. The problems associated with using retroviruses and oncogenes for reprogramming need to be resolved before iPS cells can be considered for human therapy.