JOURNALS IN SCIENCE

Cell Stem Cell

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Generation of Induced Pluripotent Stem Cells in the Absence of Drug Selection

R. Blelloch et al.

Reprogramming of somatic cells to the pluripotent stem cell state may allow the development of in vitro models of disease and could provide a mechanism for the generation of patient-specific cells of therapeutic interest. Reprogramming of mouse fibroblasts into induced pluripotent stem cells, or iPS cells, has been achieved with overexpression of *oct4*, *sox2*, *klf4*, and c-*myc* and drug selection for the reactivation of a marker of pluripotency. Here the authors show that n-*myc* can substitute for c-*myc* and that drug selection is dispensable for reprogramming of fibroblasts to pluripotent stem cells. They show that serum-free conditions facilitate reprogramming and that the resulting induced pluripotent stem cells contribute extensively to teratomas and chimeras. Their findings greatly simplify the method for induction of pluripotency and bring it one step closer to clinical applications.

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Recombination Signatures Distinguish Embryonic Stem Cells Derived by Parthenogenesis and Somatic Cell Nuclear Transfer

K. Kim et al.

Parthenogenesis and somatic cell nuclear transfer (SCNT) are two methods for deriving embryonic stem (ES) cells that are genetically matched to the oocyte donor or somatic cell donor, respectively. Using genome-wide single nucleotide polymorphism (SNP) analysis, the authors demonstrate distinct signatures of genetic recombination that distinguish parthenogenetic ES cells from those generated by SCNT. They applied SNP analysis to the human ES cell line SCNT-hES-1, previously claimed to have been derived by SCNT, and present evidence that it represents a human parthenogenetic ES cell line. Genome-wide SNP analysis represents a means to validate the genetic provenance of an ES cell line.

Cloning and Stem Cells

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September 2007

Patient-Specific Stem Cell Lines Derived from Human Parthenogenetic Blastocysts

E. S. Revazova et al.

Parthenogenetic activation of human oocytes may be one way to produce histocompatiblecells for cell-based therapy. The authors report the successful derivation of six pluripotent human embryonic stem cell (hESC) lines from blastocysts of parthenogenetic origin. The parthenogenetic human embryonic stem cells (phESC) demonstrate typical hESC morphology, express appropriate markers, and possess high levels of alkaline phosphatase and telomerase activity. The phESC lines have a normal 46,XX karyotype, except one cell line, and have been cultured from between twenty-one to thirty-five passages. The phESC lines form embryoid bodies in suspension culture and teratomas after injection into immunodeficient animals and give differentiated derivatives of all three embryonic germ layers. DNA profiling of all six phESC lines demonstrates that they are MHC (major histocompatibility complex) matched with the oocyte donors. The study of imprinted genes demonstrated further evidence of the parthenogenetic origin of the phESC

lines. The authors' research has resulted in a protocol for the production of human parthenogenetic embryos and the derivation of stem cell lines from them, which minimizes the presence of animal-derived components, making the derived phESC lines more suitable for potential clinical use.

Human Reproduction

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July 2007

Reprogramming following Somatic Cell Nuclear Transfer in Primates Is Dependent upon Nuclear Remodeling

S.M. Mitalipov et al.

Background: Somatic cell nuclear transfer (SCNT) requires cytoplast-mediated reprogramming of the donor nucleus. Cytoplast factors such as maturation-promoting factor are implicated based on their involvement in nuclear envelope breakdown (NEBD) and premature chromosome condensation (PCC). Given prior difficulties in SCNT in primates using conventional protocols, the authors hypothesized that the ability of cytoplasts to induce nuclear remodeling was instrumental in efficient reprogramming. *Methods*: NEBD and PCC in monkey (*Macaca mulatta*) SCNT embryos were monitored by lamin A/C immunolabeling. *Results*: Initially, a persistent lamin A/C signal from donor cell nuclei after fusion with cytoplasts was observed indicative of incomplete NEBD following SCNT and predictive of developmental arrest. The authors then identified fluorochrome-assisted enucleation and donor cell electrofusion as likely candidates for inducing premature cytoplast activation and a consequent lack of nuclear remodeling. Modified protocols designed to prevent premature cytoplast activation during SCNT showed robust NEBD and PCC. Coincidently, over 20 percent of SCNT embryos reconstructed with fetal fibroblasts progressed to blastocysts. Similar results were obtained with other somatic cells. Reconstructed blastocysts displayed patterns of Oct-4 expression similar to fertilized embryos reflecting successful reprogramming. *Conclusions*: The authors' results represent a significant breakthrough in elucidating the role of nuclear remodeling events in reprogramming following SCNT.

Volume 22, Number 10
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Allograft of Ovarian Cortex between Two Genetically Non-Identical Sisters: Case Report

J. Donnez et al.

Aggressive chemotherapy and radiotherapy generally result in the loss of both endocrine and reproductive functions. In 1990, a woman aged twenty years, presenting with beta-thalassemia major, underwent chemotherapy (busulfan and cyclophosphamide) and total body irradiation (TBI) before bone marrow transplantation (BMT), the donor being her seventeen-year-old HLA-compatible sister. The treatment resulted in premature ovarian failure. In 2006, after excision of ovarian cortical fragments from the HLA-compatible sister, these fragments were immediately sutured to the ovarian medulla of the patient. Both procedures were performed by laparoscopy. Six months after reimplantation, vaginal ultrasonography and hormone concentrations indicated recovery of ovarian secretion and function. From six to eleven months, the patient experienced menstrual bleeding and the development of a follicle concomitant with high estradiol levels. Eleven months after reimplantation, two follicles were detected and punctured under vaginal ultrasonographic control. Two mature oocytes were retrieved and inseminated by intracytoplasmic sperm injection. Two embryos (two- and three-cell) were obtained. Allotransplantation of fresh ovarian tissue was laparoscopically performed between two genetically nonidentical sisters. Restoration of ovarian function was achieved after six months. Oocyte retrieval and embryo development were demonstrated.

Nature

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Histone Arginine Methylation Regulates Pluripotency in the Early Mouse Embryo

M. E. Torres-Padilla et al.

It has been generally accepted that the mammalian embryo starts its development with all cells identical, and only when inside and outside cells form do differences between cells first emerge. However, recent findings show that cells in the mouse embryo can differ in their developmental fate and potency as early as the four-cell stage. These differences depend on the orientation and order of the cleavage divisions that generated them. Because epigenetic marks are suggested to be involved in sustaining pluripotency, the authors considered that such developmental properties might be achieved through epigenetic mechanisms. Here they show that modification of histone H3, through the methylation of specific arginine residues, is correlated with cell fate and potency. Levels of H3 methylation at specific arginine residues are maximal in four-cell blastomeres that will contribute to the inner cell mass (ICM) and polar trophectoderm and undertake full development when combined together in chimeras. Arginine methylation of H3 is minimal in cells whose progeny contributes more to the mural trophectoderm and that show compromised development when combined in chimeras. This suggests that higher levels of H3 arginine methylation predispose blastomeres to contribute to the pluripotent cells of the ICM. The authors confirm this prediction by overexpressing the H3-specific arginine methyltransferase CARM1 in individual blastomeres and show that this directs their progeny to the ICM and results in a dramatic upregulation of Nanog and Sox2. Thus, their results identify specific histone modifications as the earliest known epigenetic marker contributing to development of ICM and show that manipulation of epigenetic information influences cell fate determination.

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July 12, 2007

Derivation of Pluripotent Epiblast Stem Cells from Mammalian Embryos

I. G. M. Brons et al.

Although the first mouse embryonic stem (ES) cell lines were derived twenty-five years ago using feeder-layer-based blastocyst cultures, subsequent efforts to extend the approach to other mammals, including both laboratory and domestic species, have been relatively unsuccessful. The most notable exceptions were the derivation of nonhuman primate ES cell lines followed shortly thereafter by the derivation of human ES cells. Despite the apparent common origin and the similar pluripotency of mouse and human embryonic stem cells, recent studies have revealed that they use different signaling pathways to maintain their pluripotent status. Mouse ES cells depend on leukemia inhibitory factor and bone morphogenetic protein, whereas their human counterparts rely on activin (INHBA)/nodal (NODAL) and fibroblast growth factor (FGF). Here the authors show that pluripotent stem cells can be derived from the late epiblast layer of postimplantation mouse and rat embryos using chemically defined, activin-containing culture medium that is sufficient for long-term maintenance of human embryonic stem cells. Their results demonstrate that activin/nodal signaling has an evolutionarily conserved role in the derivation and the maintenance of pluripotency in these novel stem cells. Epiblast stem cells provide a valuable experimental system for determining whether distinctions between mouse and human embryonic stem cells reflect species differences or diverse temporal origins.

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New Cell Lines from Mouse Epiblast Share Defining Features with Human Embryonic Stem Cells

P. J. Tesar et al.

The application of human embryonic stem (ES) cells in medicine and biology has an inherent reliance on understanding the starting

cell population. Human ES cells differ from mouse ES cells, and the specific embryonic origin of both cell types is unclear. Previous work suggested that mouse ES cells could only be obtained from the embryo before implantation in the uterus. Here the authors show that cell lines can be derived from the epiblast, a tissue of the postimplantation embryo that generates the embryo proper. These cells, which we refer to as EpiSCs (postimplantation epiblast-derived stem cells), express transcription factors known to regulate pluripotency, maintain their genomic integrity, and robustly differentiate into the major somatic cell types as well as primordial germ cells. The EpiSC lines are distinct from mouse ES cells in their epigenetic state and the signals controlling their differentiation. Furthermore, EpiSC and human ES cells share patterns of gene expression and signalling responses that normally function in the epiblast. These results show that epiblast cells can be maintained as stable cell lines and interrogated to understand how pluripotent cells generate distinct fates during early development.

Nature Biotechnology

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Characterization of Human Embryonic Stem Cell Lines by the International Stem Cell Initiative

The International Stem Cell Initiative

The International Stem Cell Initiative characterized fifty-nine human embryonic stem cell lines from seventeen laboratories worldwide. Despite diverse genotypes and different techniques used for derivation and maintenance, all lines exhibited similar expression patterns for several markers of human embryonic stem cells. They expressed the glycolipid antigens SSEA3 and SSEA4, the keratin sulfate antigens TRA-1-60, TRA-1-81, GCTM2 and GCT343, and the protein antigens CD9, Thy1 (also known as CD90), tissue-nonspecific alkaline phosphatase and class 1 HLA, as well as the strongly developmentally regulated genes *NANOG*, *POU5F1* (formerly known as *OCT4*), *TDGF1*, *DNMT3B*, *GABRB3*, and *GDF3*. Nevertheless, the lines were not identical: differences in expression of several lineage markers were evident, and several imprinted genes showed generally similar allele-specific expression patterns, but some gene-dependent variation was observed. Also, some female lines expressed readily detectable levels of *XIST* whereas others did not. No significant contamination of the lines with mycoplasma, bacteria, or cytopathic viruses was detected.

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

M.A. Laflamme et al.

Cardiomyocytes derived from human embryonic stem (hES) cells potentially offer large numbers of cells to facilitate repair of the infarcted heart. However, this approach has been limited by inefficient differentiation of hES cells into cardiomyocytes, insufficient purity of cardiomyocyte preparations, and poor survival of hES-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 non-cardiac hES cell derivatives or vehicle. The ability of hES-cell-derived cardiomyocytes to partially remuscularize myocardial infarcts and attenuate heart failure encourages their study under conditions that closely match human disease.

Published online
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Direct Reprogramming of Genetically Unmodified Fibroblasts into Pluripotent Stem Cells

A. Meissner, M. Wernig, and R. Jaenisch

In vitro reprogramming of somatic cells into a pluripotent embryonic-stem-cell-like state has been achieved through retroviral transduction of murine fibroblasts with Oct4, Sox2, c-myc and Klf4. In these experiments, the rare "induced pluripotent stem" (iPS) cells were isolated by stringent selection for activation of a neomycin-resistance gene inserted into the endogenous *Oct4* (also known as *Pou5f1*) or *Nanog* loci. Direct isolation of pluripotent cells from cultured somatic cells is of potential therapeutic interest, but translation to human systems would be hindered by the requirement for transgenic donors in the present iPS isolation protocol. Here the authors demonstrate that reprogrammed pluripotent cells can be isolated from genetically unmodified somatic donor cells solely based on morphological criteria.

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High-Frequency Generation of Viable Mice from Engineered Bi-Maternal Embryos

M. Kawahara et al.

Mammalian development to adulthood typically requires both maternal and paternal development, strictly precluding parthenogenesis. Here the authors report the generation of bi-maternal embryos that develop at a high success rate equivalent to the rate obtained with in vitro fertilization of normal embryos. These bi-maternal mice developed into viable and fertile female adults. The bi-maternal embryos, distinct from parthenogenetic or gynogenetic conceptuses, were produced by the construction of oocytes from fully grown oocytes and nongrowing oocytes that contain double deletions in the H19 differentially methylated region (DMR) and the Dlk1-Dio3 intergenic-germline-derived DMR. The results provide conclusive evidence that imprinted genes regulated by these two paternally methylated imprinting-control regions are the only paternal barrier that prevents the normal development of bi-maternal mouse fetuses to term.

Nature Medicine

Volume 13, Number 8
September 2007

Evidence from a Genetic Fate-Mapping Study That Stem Cells Refresh Adult Mammalian Cardiomyocytes after Injury

P. C. Hsieh et al.

An emerging concept is that the mammalian myocardium has the potential to regenerate, but that regeneration might be too inefficient to repair the extensive myocardial injury that is typical of human disease. However, the degree to which stem cells or precursor cells contribute to the renewal of adult mammalian cardiomyocytes remains controversial. Here the authors report evidence that stem cells or precursor cells contribute to the replacement of adult mammalian cardiomyocytes after injury but do not contribute significantly to cardiomyocyte renewal during normal aging. The authors generated double-transgenic mice to track the fate of adult cardiomyocytes in a "pulse-chase" fashion: after a 4-OH-tamoxifen pulse, green fluorescent protein (GFP) expression was induced only in cardiomyocytes, with 82.7 percent of cardiomyocytes expressing GFP. During normal aging up to one year, the percentage of GFP+ cardiomyocytes remained unchanged, indicating that stem or precursor cells did not refresh uninjured cardiomyocytes at a significant rate during this period of time. By contrast, after myocardial infarction or pressure overload, the percentage of GFP+ cardiomyocytes decreased from 82.8 percent in heart tissue from sham-treated mice to 67.5 percent in areas bordering a myocardial infarction, 76.6 percent in areas away from a myocardial infarction, and 75.7 percent in hearts subjected to pressure over-

load, indicating that stem cells or precursor cells had refreshed the cardiomyocytes.

PloS Biology

Volume 5, Number 7
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Generalized Reciprocity in Rats

C. Rutte and M. Taborsky

The evolution of cooperation among nonrelatives has been explained by direct, indirect, and strong reciprocity. Animals should base the decision to help others on expected future help, which they may judge from past behavior of their partner. Although many examples of cooperative behavior exist in nature where reciprocity may be involved, experimental evidence for strategies predicted by direct reciprocity models remains controversial; and indirect and strong reciprocity have been found only in humans so far. Here the authors show experimentally that cooperative behavior of female rats is influenced by prior receipt of help, irrespective of the identity of the partner. Rats that were trained in an instrumental cooperative task (pulling a stick in order to produce food for a partner) pulled more often for an unknown partner after they were helped than if they had not received help before. This alternative mechanism, called generalized reciprocity, requires no specific knowledge about the partner and may promote the evolution of cooperation among unfamiliar nonrelatives.

Volume 5, Number 7
July 3, 2007

Spontaneous Altruism by Chimpanzees and Young Children

F. Warneken et al.

People often act on behalf of others. They do so without immediate personal gain, at cost to themselves, and even toward unfamiliar individuals. Many researchers have claimed that such altruism emanates from a species-unique psychology not found in humans' closest living evolutionary relatives, such as the chimpanzee. In favor of this view, the few experimental studies on altruism in chimpanzees have produced mostly negative results. In contrast, the authors report experimental evidence that chimpanzees perform basic forms of helping in the absence of rewards spontaneously and repeatedly toward humans and conspecifics [members of the same species]. In two comparative studies, semi-free-ranging chimpanzees helped an unfamiliar human to the same degree as did human infants, irrespective of being rewarded (experiment 1) or whether the helping was costly (experiment 2). In a third study, chimpanzees helped an unrelated conspecific gain access to food in a novel situation that required subjects to use a newly acquired skill on behalf of another individual. These results indicate that chimpanzees share crucial aspects of altruism with humans, suggesting that the roots of human altruism may go deeper than previous experimental evidence suggested.

Science

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The New Synthesis in Moral Psychology

J. Haidt

People are selfish, yet morally motivated. Morality is universal, yet culturally variable. Such apparent contradictions are dissolving as research from many disciplines converges on a few shared principles, including the importance of moral intuitions, the socially functional (rather than truth-seeking) nature of moral thinking, and the coevolution of moral minds with cultural practices and institutions that create diverse moral communities. The author proposes a fourth principle to guide future research: morality is about more than harm and fairness. More research is needed on the collective and religious parts of the moral domain, such as loyalty, authority, and spiritual purity.

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Retraction of Deb et al., Science 311 (5763) 992–996

R. M. Roberts, M. Sivaguru, and H. Y. Yong

Note: *The retracted paper, by Kaushik Deb, Mayandi Sivaguru, Hwan Yul Yong, and R. Michael Roberts, appeared in the February 17, 2006, issue of* Science. *The article was discussed and its abstract included in the* Quarterly's *Notes & Abstracts for Summer 2006 (6.2: 354-355, 357).*

The authors wish to retract their report "CDX2 Gene Expression and Trophectoderm Lineage Specification in Mouse Embryos." Allegations of research misconduct were received by the University of Missouri–Columbia (MU) provost, and an investigation found that the first author (K.D.) engaged in research misconduct by intentionally falsifying and fabricating digital images in the preparation of Figs. 4I; 4N; 4S; 2G; 3, J to L; S2, V to X; and S6, I to K, accompanying the *Science* article. In addition, the original raw image files for the majority of the figures in the paper have not been located (the exceptions being the confocal scanning images in Figs. S1, S3, S4, S5, and S6), raising the possibility that the data they represent may also be suspect. The authors have decided to withdraw the article in its entirety in view of the fact that the paper was founded at least in part on falsified or fabricated images. The corresponding author (R.M.R.) takes responsibility for placing excessive trust in his coworker and for not assuring that a complete set of raw data existed at the time the questions first arose about the paper. The authors deeply regret any scientific misconceptions that have resulted from the publication of this article. The first author resigned from MU shortly after the allegations of research misconduct were received, and could not be found to sign.

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Willingness to Donate Frozen Embryos for Stem Cell Research

A. D. Lyerly and R. R. Faden

A national survey indicates that, for many infertility patients facing decisions about embryo disposition, research is the morally preferred option.

Volume 317, Number 5838
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Genome Transplantation in Bacteria: Changing One Species to Another

C. Lartigue et al.

As a step toward propagation of synthetic genomes, the authors completely replaced the genome of a bacterial cell with one from another species by transplanting a whole genome as naked DNA. Intact genomic DNA from *Mycoplasma mycoides* large colony (LC), virtually free of protein, was transplanted into *Mycoplasma capricolum* cells by polyethylene-glycol-mediated transformation. Cells selected for tetracycline resistance, carried by the *M. mycoides* LC chromosome, contain the complete donor genome and are free of detectable recipient genomic sequences. These cells that result from genome transplantation are phenotypically identical to the *M. mycoides* LC donor strain as judged by several criteria.