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Archives of Sexual Behavior

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Genetic and Environmental Effects on Same-Sex Sexual Behavior: A Population Study of Twins in Sweden

Niklas Långström et al.

There is still uncertainty about the relative importance of genes and environments on human sexual orientation. One reason is that previous studies employed self-selected, opportunistic, or small population-based samples. The authors used data from a truly population-based 2005–2006 survey of all adult twins (twenty to forty seven years) in Sweden to conduct the largest twin study of same-sex sexual behavior attempted so far. The authors performed biometric modeling with data on any and total number of lifetime same-sex sexual partners, respectively. The analyses were conducted separately by sex. Twin resemblance was moderate for the 3,826 studied monozygotic and dizygotic same-sex twin pairs. Biometric modeling revealed that, in men, genetic effects explained .34–.39 of the variance, the shared environment .00, and the individual-specific environment .61–.66 of the variance. Corresponding estimates among women were .18–.19 for genetic factors, .16–.17 for shared environmental, and .64–.66 for unique environmental factors. Although wide confidence intervals suggest cautious interpretation, the results are consistent with moderate, primarily genetic, familial effects, and moderate to large effects of the nonshared environment (social and biological) on same-sex sexual behavior.

Cell

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Ronin Is Essential for Embryogenesis and the Pluripotency of Mouse Embryonic Stem Cells

Marion Dejosez et al.

Pluripotency is a unique biological state that allows cells to differentiate into any tissue type. Here the authors describe a candidate pluripotency factor, Ronin, that possesses a THAP domain, which is associated with sequence-specific DNA binding and epigenetic silencing of gene expression. Ronin is expressed primarily during the earliest stages of murine embryonic development, and its deficiency in mice produces periimplantational lethality and defects in the inner cell mass. Conditional knockout of Ronin prevents the growth of ES cells while forced expression of Ronin allows ES cells to proliferate without differentiation under conditions that normally do not promote self-renewal. Ectopic expression also partly compensates for the effects of Oct4 knockdown. The authors demonstrate that Ronin binds directly to HCF-1, a key transcriptional regulator. Our findings identify Ronin as an essential factor underlying embryogenesis and ES cell pluripotency. Its association with HCF-1 suggests an epigenetic mechanism of gene repression in pluripotent cells.

Volume 134, Number 1
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Highly Efficient, Functional Engraftment of Skeletal Muscle Stem Cells in Dystrophic Muscles

Massimiliano Cerletti et al.

Satellite cells reside beneath the basal lamina of skeletal muscle fibers and include cells that act as precursors for muscle growth and repair. Although they share a common anatomical localization and typically are considered a homogeneous population, satellite cells actually exhibit substantial heteroge-

neity. The authors used cell-surface marker expression to purify from the satellite cell pool a distinct population of skeletal muscle precursors (SMPs) that function as muscle stem cells. When engrafted into muscle of dystrophin-deficient mdx mice, purified SMPs contributed to up to 94 percent of myofibers, restoring dystrophin expression and significantly improving muscle histology and contractile function. Transplanted SMPs also entered the satellite cell compartment, renewing the endogenous stem cell pool and participating in subsequent rounds of injury repair. Together, these studies indicate the presence in adult skeletal muscle of prospectively isolatable muscle-forming stem cells and directly demonstrate the efficacy of myogenic stem cell transplant for treating muscle degenerative disease.

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An RNAi Screen of Chromatin Proteins Identifies Tip60-p400 as a Regulator of Embryonic Stem Cell Identity

Thomas G. Fazzio et al.

Proper regulation of chromatin structure is necessary for the maintenance of cell type-specific gene expression patterns. The embryonic stem cell (ESC) expression pattern governs self-renewal and pluripotency. Here, the authors present an RNAi screen in mouse ESCs of 1008 loci encoding chromatin proteins. They identified sixty eight proteins that exhibit diverse phenotypes upon knockdown (KD), including seven subunits of the Tip60-p400 complex. Phenotypic analyses revealed that Tip60-p400 is necessary to maintain characteristic features of ESCs. The authors show that p400 localization to the promoters of both silent and active genes is dependent upon histone H3 lysine 4 trimethylation (H3K4me3). Furthermore, the Tip60-p400 KD gene expression profile is enriched for developmental regulators and significantly overlaps with that of the transcription factor Nanog. Depletion of Nanog reduces p400 binding to target promoters without affecting H3K4me3 levels. Together, these data indicate that Tip60-p400 integrates signals from Nanog and H3K4me3 to regulate gene expression in ESCs.

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Disease-Specific Induced Pluripotent Stem Cells

I. H. Park et al.

Tissue culture of immortal cell strains from diseased patients is an invaluable resource for medical research but is largely limited to tumor cell lines or transformed derivatives of native tissues. Here we describe the generation of induced pluripotent stem (iPS) cells from patients with a variety of genetic diseases with either Mendelian or complex inheritance; these diseases include adenosine deaminase deficiency-related severe combined immunodeficiency (ADA-SCID), Shwachman-Bodian-Diamond syndrome (SBDS), Gaucher disease (GD) type III, Duchenne (DMD) and Becker muscular dystrophy (BMD), Parkinson disease (PD), Huntington disease (HD), juvenile-onset, type 1 diabetes mellitus (JDM), Down syndrome (DS)/trisomy 21, and the carrier state of Lesch-Nyhan syndrome. Such disease-specific stem cells offer an unprecedented opportunity to recapitulate both normal and pathologic human tissue formation in vitro, thereby enabling disease investigation and drug development.

Cell Stem Cell

Volume 3, Number 1
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Efficient Hematopoietic Differentiation of Human Embryonic Stem Cells on Stromal Cells Derived from Hematopoietic Niches

Maria Ledran et al.

Hematopoietic stem cells derived from human embryonic stem cells (hESCs) could

provide a therapeutic alternative to bone marrow transplants, but the efficiency of currently available derivation protocols is low. In this study, the authors investigated whether coculture with monolayers of cells derived from mouse AGM and fetal liver, or with stromal cell lines derived from these tissues, can enhance hESC hematopoietic differentiation. We found that under such conditions hESC-derived differentiating cells formed early hematopoietic progenitors, with a peak at day eighteen to twenty one of differentiation that corresponded to the highest CD34 expression. These hESC-derived hematopoietic cells were capable of primary and secondary hematopoietic engraftment into immunocompromised mice at substantially higher levels than described previously. Transcriptional and functional analysis identified TGF-β1 and TGF-β3 as positive enhancers of hESC hematopoietic differentiation that can further stimulate this process when added to the culture. Overall, the authors' findings represent significant progress toward the goal of deriving functional hematopoietic stem cells from hESCs.

Volume 3, Number 1
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Extensive Hematopoietic Stem Cell Generation in the AGM Region via Maturation of VE-Cadherin+CD45+ Pre-Definitive HSCs

Samir Taoudi et al.

Elucidating the mechanisms underlying hematopoietic stem cell (HSC) specification and expansion in the embryo has been hampered by the lack of analytical cell culture systems that recapitulate in vivo development. Here, the authors describe an ex vivo model that facilitates a rapid and robust emergence of multipotent long-term repopulating HSCs in the embryonic AGM region. Because this method includes a cell dissociation step prior to reconstruction of a three-dimensional functional tissue and preserves both stromal and hematopoietic elements, it allowed the authors to identify the direct ancestry of the rapidly expanding HSC pool. They demonstrate that extensive generation of definitive HSCs in the AGM occurs predominantly through the acquisition of stem characteristics by the VE-cadherin+CD45+ population.

Volume 3, Number 3
September 11, 2008

A Drug-Inducible System for Direct Reprogramming of Human Somatic Cells to Pluripotency

Dirk Hockemeyer et al.

Current approaches to reprogram human somatic cells to pluripotent iPSCs utilize viral transduction of different combinations of transcription factors. These protocols are highly inefficient because only a small fraction of cells carry the appropriate number and stoichiometry of proviral insertions to initiate the reprogramming process. Here the authors have generated genetically homogeneous "secondary" somatic cells, which carry the reprogramming factors as defined doxycycline (DOX)-inducible transgenes. These cells were obtained by infecting fibroblasts with DOX-inducible lentiviruses, isolating "primary" iPSCs in the presence of the drug, and finally differentiating to "secondary" fibroblasts. When "secondary" fibroblast lines were cultured in the presence of DOX without further viral infection, up to 2 percent of the cells were reprogrammed to pluripotent "secondary" human iPSCs. This system will facilitate the characterization of the reprogramming process and provides a unique platform for genetic or chemical screens to enhance reprogramming or replace individual factors.

Volume 3, Number 3
September 11, 2008

A High-Efficiency System for the Generation and Study of Human Induced Pluripotent Stem Cells

Nimet Maherali et al.

Direct reprogramming of human fibroblasts to a pluripotent state has been achieved through ectopic expression of the transcrip-

tion factors OCT4, SOX2, and either cMYC and KLF4 or NANOG and LIN28. Little is known, however, about the mechanisms by which reprogramming occurs, which is in part limited by the low efficiency of conversion. To this end, the authors sought to create a doxycycline-inducible lentiviral system to convert primary human fibroblasts and keratinocytes into human induced pluripotent stem cells (hiPSCs). hiPSCs generated with this system were molecularly and functionally similar to human embryonic stem cells (hESCs), demonstrated by gene expression profiles, DNA methylation status, and differentiation potential. While expression of the viral transgenes was required for several weeks in fibroblasts, The authors found that ten days was sufficient for the reprogramming of keratinocytes. Using our inducible system, they developed a strategy to induce hiPSC formation at high frequency. Upon addition of doxycycline to hiPSC-derived differentiated cells, the authors obtained "secondary" hiPSCs at a frequency at least one hundred-fold greater than the initial conversion. The ability to reprogram cells at high efficiency provides a unique platform to dissect the underlying molecular and biochemical processes that accompany nuclear reprogramming

Journal of Biological Chemistry

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Generation of Insulin-Secreting Islet-Like Clusters from Human Skin Fibroblasts

Keisuke Tateishi et al.

Increasing evidence suggests that islet cell transplantation for patients with type I diabetes holds great promise for achieving insulin independence. However, the extreme shortage of matched organ donors and the necessity for chronic immunosuppression has made it impossible for this treatment to be used for the general diabetic population. Recent success in generating insulin-secreting islet-like cells from human ES cells, in combination with the success in deriving human ES cell-like induced pluripotent stem (iPS) cells from human fibroblasts by defined factors have raised the possibility that patient-specific insulin-secreting islet-like cells might be derived from somatic cells through cell fate reprogramming using defined factors. Here the authors confirm that human ES-like iPS cells can be derived from human skin cells by retroviral expression of OCT4, SOX2, c-MYC, and KLF4. Importantly, using a serum-free protocol, they successfully generated insulin-producing islet-like clusters (ILCs) from the iPS cells under feeder-free conditions. The authors demonstrate that, like human ES cells, skin fibroblasts-derived iPS cells have the potential to be differentiated into islet-like clusters through definitive and pancreatic endoderm. The iPS-derived ILCs not only contain C-peptide positive and glucagon positive cells, but also release C-peptide upon glucose stimulation. Thus, their study provides evidence that insulin-secreting ILCs can be generated from skin fibroblasts, raising the possibility that patient-specific iPS cells could potentially provide a treatment for diabetes in the future.

Nature

Volume 454, Number 7200
July 3, 2008

Dissecting Direct Reprogramming through Integrative Genomic Analysis

T.S. Mikkelsen et al.

Somatic cells can be reprogrammed to a pluripotent state through the ectopic expression of defined transcription factors. Understanding the mechanism and kinetics of this transformation may shed light on the nature of developmental potency and suggest strategies with improved efficiency or safety. Here the authors report an integrative genomic analysis of reprogramming of mouse fibroblasts and B lymphocytes.

Lineage-committed cells show a complex response to the ectopic expression involving induction of genes downstream of individual reprogramming factors. Fully reprogrammed cells show gene expression and epigenetic states that are highly similar to embryonic stem cells. In contrast, stable partially reprogrammed cell lines show reactivation of a distinctive subset of stem-cell-related genes, incomplete repression of lineage-specifying transcription factors, and DNA hypermethylation at pluripotency-related loci. These observations suggest that some cells may become trapped in partially reprogrammed states owing to incomplete repression of transcription factors, and that DNA de-methylation is an inefficient step in the transition to pluripotency. The authors demonstrate that RNA inhibition of transcription factors can facilitate reprogramming, and that treatment with DNA methyltransferase inhibitors can improve the overall efficiency of the reprogramming process.

Volume 454, Number 7205
August 7, 2008

Genome-Scale DNA Methylation Maps of Pluripotent and Differentiated Cells

Alexander Meissner et al.

DNA methylation is essential for normal development and has been implicated in many pathologies including cancer. The authors' knowledge about the genome-wide distribution of DNA methylation, how it changes during cellular differentiation and how it relates to histone methylation and other chromatin modifications in mammals remains limited. Here we report the generation and analysis of genome-scale DNA methylation profiles at nucleotide resolution in mammalian cells. Using high-throughput reduced representation bisulphite sequencing and single-molecule-based sequencing, the authors generated DNA methylation maps covering most CpG islands, and a representative sampling of conserved non-coding elements, transposons and other genomic features, for mouse embryonic stem cells, embryonic-stem-cell-derived and primary neural cells, and eight other primary tissues. Several key findings emerge from the data. First, DNA methylation patterns are better correlated with histone methylation patterns than with the underlying genome sequence context. Second, methylation of CpGs are dynamic epigenetic marks that undergo extensive changes during cellular differentiation, particularly in regulatory regions outside of core promoters. Third, analysis of embryonic-stem-cell-derived and primary cells reveals that "weak" CpG islands associated with a specific set of developmentally regulated genes undergo aberrant hypermethylation during extended proliferation in vitro, in a pattern reminiscent of that reported in some primary tumours. More generally, the results establish reduced representation bisulphite sequencing as a powerful technology for epigenetic profiling of cell populations relevant to developmental biology, cancer and regenerative medicine.

Volume 455, Number 7211
September 18, 2008

Regulatory Networks Define Phenotypic Classes of Human Stem Cell Lines

Franz-Josef Müller et al.

Stem cells are defined as self-renewing cell populations that can differentiate into multiple distinct cell types. However, hundreds of different human cell lines from embryonic, fetal and adult sources have been called stem cells, even though they range from pluripotent cells—typified by embryonic stem cells, which are capable of virtually unlimited proliferation and differentiation—to adult stem cell lines, which can generate a far more limited repertoire of differentiated cell types. The rapid increase in reports of new sources of stem cells and their anticipated value to regenerative medicine has highlighted the need for a general, reproducible method for classification of these cells. The authors report here the creation and analysis of a database of global gene expression profiles, which they call the 'stem cell matrix', that enables the classification of cultured human stem cells in the context of a wide variety of pluripotent, multipotent and differentiated cell types. Using an unsupervised clustering method to

categorize a collection of approximately one hundred and fifty cell samples, the authors discovered that pluripotent stem cell lines group together, whereas other cell types, including brain-derived neural stem cell lines, are very diverse. Using further bioinformatic analysis the authors uncovered a protein-protein network (PluriNet) that is shared by the pluripotent cells (embryonic stem cells, embryonal carcinomas and induced pluripotent cells). Analysis of published data showed that the PluriNet seems to be a common characteristic of pluripotent cells, including mouse embryonic stem and induced pluripotent cells and human oocytes. The authors' results offer a new strategy for classifying stem cells and support the idea that pluripotency and self-renewal are under tight control by specific molecular networks.

Volume 455, Number 7213
October 2, 2008

In Vivo Reprogramming of Adult Pancreatic Exocrine Cells to β-Cells

Quiao Zhou et al.

One goal of regenerative medicine is to instructively convert adult cells into other cell types for tissue repair and regeneration. Although isolated examples of adult cell reprogramming are known, there is no general understanding of how to turn one cell type into another in a controlled manner. Here, using a strategy of re-expressing key developmental regulators in vivo, the authors identify a specific combination of three transcription factors (Ngn3, also known as Neurog3, Pdx1 and Mafa) that reprograms differentiated pancreatic exocrine cells in adult mice into cells that closely resemble beta-cells. The induced beta-cells are indistinguishable from endogenous islet beta-cells in size, shape and ultrastructure. They express genes essential for beta-cell function and can ameliorate hyperglycaemia by remodelling local vasculature and secreting insulin. This study provides an example of cellular reprogramming using defined factors in an adult organ and suggests a general paradigm for directing cell reprogramming without reversion to a pluripotent stem cell state.

Nature Biotechnology

Volume 26, Number 8
August 2008

A Drug-Inducible Transgenic System for Direct Reprogramming of Multiple Somatic Cell Types

Marius Wernig et al.

The study of induced pluripotency is complicated by the need for infection with high-titer retroviral vectors, which results in genetically heterogeneous cell populations. The authors generated genetically homogeneous "secondary" somatic cells that carry the reprogramming factors as defined doxycycline (dox)-inducible transgenes. These cells were produced by infecting fibroblasts with dox-inducible lentiviruses, reprogramming by dox addition, selecting induced pluripotent stem cells and producing chimeric mice. Cells derived from these chimeras reprogram upon dox exposure without the need for viral infection with efficiencies 25- to 50-fold greater than those observed using direct infection and drug selection for pluripotency marker reactivation. The authors demonstrate that (1) various induction levels of the reprogramming factors can induce pluripotency, (2) the duration of transgene activity directly correlates with reprogramming efficiency, (3) cells from many somatic tissues can be reprogrammed and (4) different cell types require different induction levels. This system facilitates the characterization of reprogramming and provides a tool for genetic or chemical screens to enhance reprogramming.

Nature Medicine

Volume 14, Number 8
August 1, 2008

Mouse Embryonic Stem Cell–Based Functional Assay to Evaluate Mutations in BRCA2

Sergey G. Kuznetsov et al.

Individuals with mutations in breast cancer susceptibility genes BRCA1 and BRCA2 have up to an 80 percent risk of developing breast cancer by the age of seventy. Sequencing-based genetic tests are now available to identify mutation carriers in an effort to reduce mortality through prevention and early diagnosis. However, lack of a suitable functional assay hinders the risk assessment of more than nineteen hundred BRCA1 and BRCA2 variants in the Breast Cancer Information Core database that do not clearly disrupt the gene product. The authors have established a simple, versatile and reliable assay to test for the functional significance of mutations in BRCA2 using mouse embryonic stem cells (ES cells) and bacterial artificial chromosomes and have used it to classify seventeen sequence variants. The assay is based on the ability of human BRCA2 to complement the loss of endogenous Brca2 in mouse ES cells. This technique may also serve as a paradigm for functional analysis of mutations found in other genes linked to human diseases.

PLoS One

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June 18, 2008

Sexually Antagonistic Selection in Human Male Homosexuality

Andrea Camperio Ciani, Paolo Cermielli, and Giovanni Zanzotto

Several lines of evidence indicate the existence of genetic factors influencing male homosexuality and bisexuality. In spite of its relatively low frequency, the stable permanence in all human populations of this apparently detrimental trait constitutes a puzzling "Darwinian paradox." Furthermore, several studies have pointed out relevant asymmetries in the distribution of both male homosexuality and of female fecundity in the parental lines of homosexual verses heterosexual males. A number of hypotheses have attempted to give an evolutionary explanation for the long-standing persistence of this trait, and for its asymmetric distribution in family lines; however a satisfactory understanding of the population genetics of male homosexuality is lacking at present. The authors perform a systematic mathematical analysis of the propagation and equilibrium of the putative genetic factors for male homosexuality in the population, based on the selection equation for one or two diallelic loci and Bayesian statistics for pedigree investigation. They show that only the two-locus genetic model with at least one locus on the X chromosome, and in which gene expression is sexually antagonistic (increasing female fitness but decreasing male fitness), accounts for all known empirical data. The authors' results help clarify the basic evolutionary dynamics of male homosexuality, establishing this as a clearly ascertained sexually antagonistic human trait.

PNAS: Proceedings of the National Academy of Sciences USA

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PET and MRI Show Sifferences in Cerebral Asymmetry and Functional Connectivity between Homo- and Heterosexual Subjects

Ivanka Savic and Per Lindström

Cerebral responses to putative pheromones and objects of sexual attraction were recently found to differ between homo- and heterosexual subjects. Although this observation may merely mirror perceptional differences, it raises the intriguing question as to whether

certain sexually dimorphic features in the brain may differ between individuals of the same sex but different sexual orientation. The authors addressed this issue by studying hemispheric asymmetry and functional connectivity, two parameters that in previous publications have shown specific sex differences. Ninety subjects [twenty five heterosexual men (HeM) and women (HeW), and twenty homosexual men (HoM) and women (HoW)] were investigated with magnetic resonance volumetry of cerebral and cerebellar hemispheres. Fifty of them also participated in PET measurements of cerebral blood flow, used for analyses of functional connections from the right and left amygdalae. HeM and HoW showed a rightward cerebral asymmetry, whereas volumes of the cerebral hemispheres were symmetrical in HoM and HeW. No cerebellar asymmetries were found. Homosexual subjects also showed sex-atypical amygdala connections. In HoM, as in HeW, the connections were more widespread from the left amygdala; in HoW and HeM, on the other hand, from the right amygdala. Furthermore, in HoM and HeW the connections were primarily displayed with the contralateral amygdala and the anterior cingulate, in HeM and HoW with the caudate, putamen, and the prefrontal cortex. The present study shows sex-atypical cerebral asymmetry and functional connections in homosexual subjects. The results cannot be primarily ascribed to learned effects, and they suggest a linkage to neurobiological entities.