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Cell

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Interaction between *Oct3/4* and *Cdx2* Determines Trophectoderm Differentiation

Hitoshi Niwa et al.

Trophectoderm, the first differentiated cell lineage of mammalian embryogenesis, forms the placenta, a structure unique to mammalian development. The differentiation of trophectoderm is a hallmark event in early mammalian development, but molecular mechanisms underlying this first differentiation event remain obscure. Embryonic stem (ES) cells can be induced to differentiate into the trophectoderm lineage by forced repression of the POU-family transcription factor, *Oct3/4*. The authors show that this event can be mimicked by overexpression of *Caudal-related homeobox 2* (*Cdx2*), which is sufficient to generate proper trophoblast stem (TS) cells. *Cdx2* is dispensable for trophectoderm differentiation induced by *Oct3/4* repression but essential for TS cell self-renewal. In preimplantation embryos, *Cdx2* is initially coexpressed with *Oct3/4* and they form a complex for the reciprocal repression of their target genes in ES cells. This suggests that reciprocal inhibition between lineage-specific transcription factors might be involved in the first differentiation event of mammalian development.

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Regulation of ADMP and BMP2/4/7 at Opposite Embryonic Poles Generates a Self-Regulating Morphogenetic Field

Bruno Reversade and E. M. De Robertis

Embryos have the ability to self-regulate and regenerate normal structures after being sectioned in half. How is such a morphogenetic field established? The authors discovered that quadruple knockdown of ADMP [anti-dorsalizing morphogenetic protein] and BMP [bone morphogenetic protein] 2/4/7 in *Xenopus* embryos eliminates self-regulation, causing ubiquitous neural induction throughout the ectoderm. ADMP transcription in the Spemann organizer is activated at low BMP levels. When ventral BMP2/4/7 signals are depleted, *Admp* expression increases, allowing for self-regulation. ADMP has BMP-like activity and signals via the ALK-2 receptor. It is unable to signal dorsally because of inhibition by Chordin. The ventral BMP antagonists Sizzled and Bambi further refine the pattern. By transplanting dorsal or ventral wild-type grafts into ADMP/BMP2/4/7-depleted hosts, the authors demonstrate that both poles serve as signaling centers that can induce histotypic differentiation over considerable distances. They conclude that dorsal and ventral BMP signals and their extracellular antagonists expressed under opposing transcriptional regulation provide a molecular mechanism for embryonic self-regulation.

Journal of Agricultural and Food Chemistry

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Transgenic Expression of Bean alpha-Amylase Inhibitor in Peas Results in Altered Structure and Immunogenicity

Vanessa E. Prescott et al.

The development of modern gene technologies allows for the expression of recombinant proteins in non-native hosts. Diversity in translational and post-translational modification pathways between species could potentially lead to discrete changes in the molecular architecture of the expressed protein and subsequent cellular function and antigenicity. The authors show that transgenic expression of a plant protein (alpha-amylase inhibitor-1 from the common bean, *Phaseolus vulgaris* L. cv. Tendergreen) in a non-native host (transgenic pea, *Pisum sativum* L.) led to the synthesis of a structurally modified form of this inhibitor. Employing models of inflammation, they demonstrated in mice that consumption of the modified alpha-AI and not the native form predisposed to antigen-specific $CD4^+$ Th_2-type inflammation. Furthermore, consumption of the modified alpha-AI concurrently with other heterogeneous proteins promoted immunological cross priming, which then elicited specific immunoreactivity of these proteins. Thus, transgenic expression of non-native proteins in plants may lead to the synthesis of structural variants possessing altered immunogenicity.

Molecular Biology of the Cell

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Induction of Dedifferentiation, Genomewide Transcriptional Programming, and Epigenetic Reprogramming by Extracts of Carcinoma and Embryonic Stem Cells

Christel K. Taranger et al.

Functional reprogramming of a differentiated cell toward pluripotency may have long-term applications in regenerative medicine. The authors report the induction of dedifferentiation, associated with genome-wide programming of gene expression and epigenetic reprogramming of an embryonic gene, in epithelial 293T cells treated with an extract of undifferentiated human NCCIT carcinoma cells. 293T cells exposed for one hour to extract of NCCIT cells, but not of 293T or Jurkat T-cells, form defined colonies that are maintained for at least twenty-three passages in culture. Microarray and quantitative analyses of gene expression reveal that the transition from a 293T to a pluripotent cell phenotype involves a dynamic up-regulation of hundreds of NCCIT genes, concomitant with down-regulation of 293T genes and of indicators of differentiation such as A-type lamins. Up-regulated genes encompass embryonic and stem cell markers, including *OCT4*, *SOX2*, *NANOG*, and Oct4-responsive genes. *OCT4* activation is associated with DNA demethylation in the OCT4 promoter and nuclear targeting of Oct4 protein. In fibroblasts exposed to extract of mouse embryonic stem cells, Oct4 activation is biphasic and RNA-PolII dependent, with the first transient rise of Oct4 up-regulation being necessary for the second, long-term activation of Oct4. Genes characteristic of multi-lineage differentiation potential are also up-regulated in NCCIT extract-treated cells, suggesting the establishment of "multi-lineage priming." Retinoic acid triggers Oct4 down-regulation, de novo activa-

tion of A-type lamins, and nestin. Furthermore, the cells can be induced to differentiate toward neurogenic, adipogenic, osteogenic, and endothelial lineages. The data provide a proof-of-concept that an extract of undifferentiated carcinoma cells can elicit differentiation plasticity in an otherwise more developmentally restricted cell type.

Nature

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Embryonic and Extraembryonic Stem Cell Lines Derived from Single Mouse Blastomeres

Young Chung et al.

The most basic objection to human embryonic stem (ES) cell research is rooted in the fact that ES cell derivation deprives embryos of any further potential to develop into a complete human being. ES cell lines are conventionally isolated from the inner cell mass of blastocysts and, in a few instances, from cleavage stage embryos. So far, there have been no reports in the literature of stem cell lines derived using an approach that does not require embryo destruction. Here the authors report an alternative method of establishing ES cell lines—using a technique of single-cell embryo biopsy similar to that used in pre-implantation genetic diagnosis of genetic defects—that does not interfere with the developmental potential of embryos. Five putative ES and seven trophoblast stem (TS) cell lines were produced from single blastomeres, which maintained normal karyotype and markers of pluripotency or TS cells for up to more than fifty passages. The ES cells differentiated into derivatives of all three germ layers in vitro and in teratomas, and showed germ-line transmission. Single-blastomere-biopsied embryos developed to term without a reduction in their developmental capacity. The ability to generate human ES cells without the destruction of *ex utero* embryos would reduce or eliminate the ethical concerns of many.

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Generation of Nuclear Transfer-Derived Pluripotent ES Cells from Cloned *Cdx2*-Deficient Blastocysts

Alexander Meissner and Rudolf Jaenisch

The derivation of embryonic stem (ES) cells by nuclear transfer holds great promise for research and therapy but involves the destruction of cloned human blastocysts. Proof of principle experiments have shown that "customized" ES cells derived by nuclear transfer (NT-ESCs) can be used to correct immunodeficiency in mice. Importantly, the feasibility of the approach has been demonstrated recently in humans, bringing the clinical application of NT-ESCs within reach. Altered nuclear transfer has been proposed as a variation of nuclear transfer because it would create abnormal nuclear transfer blastocysts that are inherently unable to implant into the uterus but would be capable of generating customized ES cells. To assess the experimental validity of this concept the authors used nuclear transfer to derive mouse blastocysts from donor fibroblasts that carried a short hairpin RNA construct targeting *Cdx2*. Cloned blastocysts were morphologically abnormal, lacked functional trophoblast, and failed to implant into the uterus. However, they efficiently generated pluripotent embryonic stem cells when explanted into culture.

Nature Materials

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Light-Induced Gene Transfer from Packaged DNA Enveloped in a Dendrimeric Photosensitizer

Nobuhiro Nishiyama et al.

The control of gene transfection in the body is a core issue in gene therapy. Photochemical internalization is a technology that allows light-induced delivery of DNA, drugs, or other biological factors directly inside cells.

Usually it requires that a photosensitizer be added to the drug-delivery system to photochemically destabilize the endosomal membrane. The authors present a system for in vivo DNA delivery in which these two components are assembled into one structure. This is a ternary complex composed of a core containing DNA packaged with cationic peptides and enveloped in the anionic dendrimer phthalocyanine, which provides the photosensitizing action. The ternary complex showed more than one-hundred-fold photochemical enhancement of transgene expression in vitro with reduced photocytotoxicity. In an animal experiment, subconjuctival injection of the ternary complex followed by laser irradiation resulted in transgene expression only in the laser-irradiated site. This work demonstrates a new biomedical application for dendrimers, and the first success in the photochemical-internalization-mediated gene delivery in vivo.

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Gene Therapy for Progeny of Mito-Mice Carrying Pathogenic mtDNA by Nuclear Transplantation

Akitsugu Sato et al.

Pathogenic mutations in mtDNAs have been shown to be responsible for expression of respiration defects and resultant expression of mitochondrial diseases. This study directly addressed the issue of gene therapy of mitochondrial diseases by using nuclear transplantation of zygotes of transmitochondria mice (mito-mice). Mito-mice expressed respiration defects and mitochondrial diseases due to accumulation of mtDNA carrying a large-scale deletion (delta-mtDNA). Second polar bodies were used as biopsy samples for diagnosis of mtDNA genotypes of mito-mouse zygotes. Nuclear transplantation was carried out from mito-mouse zygotes to enucleated normal zygotes and was shown to rescue all of the F_0 progeny from expression of respiration defects throughout their lives. This procedure should be applicable to patients with mitochondrial diseases for preventing their children from developing the diseases.

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Implanted Hair Follicle Stem Cells Form Schwann Cells That Support Repair of Severed Peripheral Nerves

Yasuyuki Amoh et al.

The hair follicle bulge area is an abundant, easily accessible source of actively growing, pluripotent adult stem cells. Nestin, a protein marker for neural stem cells, also is expressed in follicle stem cells and their immediate, differentiated progeny. The fluorescent protein GFP, whose expression is driven by the nestin regulatory element in transgenic mice, served to mark the follicle cell fate. The pluripotent nestin-driven GFP stem cells are positive for the stem cell marker CD34 but negative for keratinocyte marker keratin 15, suggesting their relatively undifferentiated state. These cells can differentiate into neurons, glia, keratinocytes, smooth muscle cells, and melanocytes in vitro. In vivo studies show the nestin-driven GFP hair follicle stem cells can differentiate into blood vessels and neural tissue after transplantation to the subcutis of nude mice. Equivalent hair follicle stem cells derived from transgenic mice with beta-actin-driven GFP implanted into the gap region of a severed sciatic nerve greatly enhance the rate of nerve regeneration and the restoration of nerve function. The follicle cells transdifferentiate largely into Schwann cells, which are known to support neuron regrowth. Function of the rejoined sciatic nerve was measured by contraction of the gastrocnemius muscle upon electrical stimulation. After severing the tibial nerve and subsequent transplantation of hair follicle stem cells, walking print length and intermediate toe spread significantly recovered, indicat-

ing that the transplanted mice recovered the ability to walk normally. These results suggest that hair follicle stem cells provide an important, accessible, autologous source of adult stem cells for regenerative medicine.

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Global Gene Expression Profiles Reveal Significant Nuclear Reprogramming by the Blastocyst Stage after Cloning

Sadie L. Smith et al.

Nuclear transfer (NT) has potential applications in agriculture and biomedicine, but the technology is hindered by low efficiency. Global gene expression analysis of clones is important for the comprehensive study of nuclear reprogramming. Here, the authors compared global gene expression profiles of individual bovine NT blastocysts with their somatic donor cells and fertilized control embryos using cDNA microarray technology. The NT embryos' gene expression profiles were drastically different from those of their donor cells and closely resembled those of the naturally fertilized embryos. The findings demonstrate that the NT embryos have undergone significant nuclear reprogramming by the blastocyst stage; however, problems may occur during redifferentiation for tissue genesis and organogenesis, and small reprogramming errors may be magnified downstream in development.

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Development of Functional Human Embryonic Stem Cell-Derived Neurons in Mouse Brain

Alysson R. Muotri et al.

Human embryonic stem cells are pluripotent entities, theoretically capable of generating a whole-body spectrum of distinct cell types. However, differentiation of these cells has been observed only in culture or during teratoma formation. The authors' results show that human embryonic stem cells implanted in the brain ventricles of embryonic mice can differentiate into functional neural lineages and generate mature, active human neurons that successfully integrate into the adult mouse forebrain. Moreover, this study reveals the conservation and recognition of common signals for neural differentiation throughout mammalian evolution. The chimeric model will permit the study of human neural development in a live environment, paving the way for the generation of new models of human neurodegenerative and psychiatric diseases. The model also has the potential to speed up the screening process for therapeutic drugs.

Reproductive BioMedicine Online

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Reprogramming of Human Somatic Cells by Embryonic Stem Cell Cytoplast

N. Strelchenko et al.

Somatic cell nuclear transfer (SCNT) provides the basis for the development of patient-specific stem cell lines. Recent progress in SCNT suggested the presence of reprogramming factors in human embryonic stem (hES) cells, although no method is currently available for replacement of nuclei of hES cells by somatic cell nuclei. An original technique has been developed, involving the fusion of different types of somatic cells with hES cells, which allowed a complete replacement of the nuclei of hES cells by nuclei of somatic cells. The resulting "cybrids" were shown to have the genotype of the donor somatic cells and "stemness" of the recipient hES cells. However, the colonies isolated from the resulting fusion contained a mixture of these cybrid cells with the cells with the recipient nuclei, as well as hybrid cells containing both donor and recipient nuclei, so future purification will be necessary before the technique can be considered for future practical application.