JOURNALS IN SCIENCE AND MEDICINE

Cell

Volume 110, Number 3
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Directed differentiation of embryonic stem cells into motor neurons

Hynek Wichterle et al.

Inductive signals and transcription factors involved in motor neuron generation have been identified, raising the question of whether these developmental insights can be used to direct stem cells to a motor neuron fate. We show that developmentally relevant signaling factors can induce mouse embryonic stem (ES) cells to differentiate into spinal progenitor cells, and subsequently into motor neurons, through a pathway recapitulating that used in vivo. ES-cell-derived motor neurons can populate the embryonic spinal cord, extend axons, and form synapses with target muscles. Thus, inductive signals involved in normal pathways of neurogenesis can direct ES cells to form specific classes of CNS neurons.

Journal of the American Medical Association

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Risks and Benefits of Estrogen Plus Progestin in Healthy Postmenopausal Women: Principal Results From the Women's Health Initiative Randomized Controlled Trial

Writing Group for the Women's Health Initiative Investigators

Overall health risks exceeded benefits from use of combined estrogen plus progestin for an average 5.2-year follow-up among healthy postmenopausal US women. All-cause mortality was not affected during the trial. The risk-benefit profile found in this trial is not consistent with the requirements for a viable intervention for primary prevention of chronic diseases, and the results indicate that this regimen should not be initiated or continued for primary prevention of CHD.

Menopausal Hormone Replacement Therapy and Risk of Ovarian Cancer

James V. Lacey, Jr., Ph.D. et al.

Women who used estrogen-only replacement therapy, particularly for 10 or more years, were at significantly increased risk of ovarian cancer in this study. Women who used short-term estrogen-progestin–only replacement therapy were not at increased risk, but risk associated with short-term and longer-term estrogen-progestin replacement therapy warrants further investigation.

Volume 288, Number 6
August 14, 2002

Effect of Mandatory Parental Notification on Adolescent Girls' Use of Sexual Health Care Services

Diane M. Reddy, Ph.D., Raymond Fleming, Ph.D., and Carolyne Swain

Mandatory parental notification for prescribed contraceptives would impede girls' use of sexual health care services, potentially increasing teen pregnancies and the spread of STDs.

Effect of Daily Vitamin E and Multivitamin-Mineral Supplementation on Acute Respiratory Tract Infections in Elderly Persons: A Randomized Controlled Trial

Judith M. Graat, Evert G. Schouten, M.D., Ph.D., and Frans J. Kok, Ph.D.

Neither daily multivitamin-mineral supplementation at physiological dose nor 200 mg of vitamin E showed a favorable effect on incidence and severity of acute respiratory tract infections in well-nourished noninstitutionalized elderly individuals. Instead we observed adverse effects of vitamin E on illness severity.

Journal of Clinical Investigation

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Long-term persistence of donor nuclei in a Duchenne muscular dystrophy patient receiving bone marrow transplantation

Louis M. Kunkel et al.

Duchenne muscular dystrophy (DMD) is a severe progressive muscle-wasting disorder caused by mutations in the *dystrophin* gene. Studies have shown that bone marrow cells transplanted into lethally irradiated *mdx* mice, the mouse model of DMD, can become part of skeletal muscle myofibers. Whether human marrow cells also have this ability is unknown. Here we report the analysis of muscle biopsies from a DMD patient (DMD-BMT1) who received bone marrow transplantation at age 1 year for X-linked severe combined immune deficiency and who was diagnosed with DMD at age 12 years. Analysis of muscle biopsies from DMD-BMT1 revealed the presence of donor nuclei within a small number of muscle myofibers (0.5-0.9%). The majority of the myofibers produce a truncated, in-frame isoform of dystrophin lacking exons 44 and 45 (not wild-type). The presence of bone marrow-derived donor nuclei in the muscle of this patient documents the ability of exogenous human bone marrow cells to fuse into skeletal muscle and persist up to 13 years after transplantation.

Journal of Endocrinology

Volume 174, Number 2
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Heterotopic uterine transplantation by vascular anastomosis in the mouse

R. Racho El-Akouri et al.

A method of heterotopic uterine transplantation was developed in the mouse as a model system for studies of uterine function and transplant immunology of the uterus. The model involved transplantation of the right uterine horn and the cervix by vascular anastomosis to a donor animal with the intact native uterus remaining in situ. F1-hybrids of inbred C57BL/6xCBA/ca (B6 CBAF1) mice of

six to eight weeks of age (n=42) were used. The specific pelvic vascular anatomy of these mice was first examined by intra-aortal injection of a two-component silicon-rubber curing agent. The surgery of the donor animal involved microsurgical isolation of the right uterine horn and the cervix, with preserved vascular supply from the aorta through the right uterine artery. After isolation of the uterine horn with vascular supply and venous drainage, including approximately three mm of the inferior vena cava and aorta, the organ was put on ice. The recipient animal was prepared by exposing and mobilizing the infrarenal part of the aorta and the vena cava. The grafted uterus was placed in the abdomen on the left side, and the aorta and vena cava of the graft were anastomosed end-to-side to the aorta and vena cava of the recipient animal with 11-0 sutures. The total time for these procedures declined with time and was 125+/-4 min for the last twenty-eight operations. Viability of the uterus was confirmed, several days later, by demonstrating a blood flow similar to that of the native uterus, and histology of the grafted uterus demonstrated normal morphology, including intact ultrastructure of the endothelial cells. The overall survival rate of the recipient animals increased with learning from approximately forty percent in animals 1–21 to seventy-one percent in animals 22–42. The proportion of viable grafts, as judged by normal blood flow and histology among the surviving mice was twenty-five percent in animals 1–21 and eighty-seven percent in animals 22–42. An undisturbed function of the transplanted uterus horn was finally demonstrated by its ability to implant inserted blastocysts and to carry pregnancy with fetal weight being similar to that of fetuses in the native uterus and controls. In conclusion, this is the first report of successful transplantation of the uterus with proven functionality in the mouse. The model should be useful for many aspects of research in uterine physiology and pathophysiology.

Nature

Volume 418, Number 6897
August 1, 2002

Oogenesis: Maturation of mouse fetal germ cells in vitro

Yayoi Obata, Tomohiro Kono, and Izuho Hatada

Nuclear reprogramming is essential during gametogenesis for the production of totipotent zygotes. Here we show that premeiotic female germ cells derived from mouse fetuses as early as 12.5 days post coitum are able to complete meiosis and genomic imprinting in vitro and that these matured oocytes are highly competent in supporting development to full term after nuclear transfer and in vitro fertilization. To our knowledge, this is the first time that complete oogenesis has been successfully accomplished in vitro.

Volume 418, Number 6899
August 15, 2002

Sperm from neonatal mammalian testes grafted in mice

A. Honaramooz et al.

Spermatogenesis is a productive and highly organized process that generates virtually unlimited numbers of sperm during adulthood. Continuous proliferation and differentiation of germ cells occur in a delicate balance with other testicular compartments, especially the supporting Sertoli cells. Many complex aspects of testis function in humans and large animals have remained elusive because of a lack of suitable in vitro or in vivo models. Germ cell transplantation has produced complete donor-derived spermatogenesis in rodents but not in other mammalian species. Production of sperm in grafted tissue from immature mammalian testes and across species has not yet been accom-

plished. Here we report the establishment of complete spermatogenesis by grafting testis tissue from newborn mice, pigs or goats into mouse hosts. This approach maintains structural integrity and provides the accessibility that is essential for studying and manipulating the function of testes and for preserving the male germ line. Our results indicate that this approach is applicable to diverse mammalian species.

Nature Biotechnology

Volume 20, Number 9
September 2002

Cloned transchromosomic calves producing human immunoglobulin

Yoshimi Kuroiwa et al.

Human polyclonal antibodies (hPABs) are useful therapeutics, but because they are available only from human donors, their supply and application is limited. To address this need, we prepared a human artificial chromosome (HAC) vector containing the entire unrearranged sequences of the human immunoglobulin (h*Ig*) heavy-chain (*H*) and lambda light-chain loci. The HAC vector was introduced into bovine primary fetal fibroblasts using a microcell-mediated chromosome transfer (MMCT) approach. Primary selection was carried out, and the cells were used to produce cloned bovine fetuses. Secondary selection was done on the regenerated fetal cell lines, which were then used to produce four healthy transchromosomic (Tc) calves. The HAC was retained at a high rate (78–100% of cells) in calves and the h*Ig* loci underwent rearrangement and expressed diversified transcripts. Human immunoglobulin proteins were detected in the blood of newborn calves. The production of Tc calves is an important step in the development of a system for producing therapeutic hPABs.

Human feeders support prolonged undifferentiated growth of human inner cell masses and embryonic stem cells

Mark Richards et al.

Previous reports have demonstrated the growth of undifferentiated human embryonic stem (HES) cells on mouse embryonic fibroblast (MEF) feeders and on laminin- or Matrigel-coated plastic surfaces supplemented with MEF-conditioned medium. These xenosupport systems run the risk of cross-transfer of animal pathogens from the animal feeder, matrix, or conditioned medium to the HES cells, thus compromising later clinical application. Here we show that human fetal and adult fibroblast feeders support prolonged undifferentiated HES cell growth of existing cell lines and are superior to cell-free matrices (collagen I, human extracellular matrix, Matrigel, and laminin) supplemented with human or MEF feeder-conditioned medium. Additionally, we report the derivation and establishment of a new HES cell line in completely animal-free conditions. Like HES cells cultured on MEF feeders, the HES cells grown on human feeders had normal karyotypes, tested positive for alkaline phosphatase activity, expressed Oct-4 and cell surface markers including SSEA-3, SSEA-4, Tra 1-60, and GCTM-2, formed teratomas in severely combined immunodeficient (SCID) mice, and retained all key morphological characteristics. Human feeder-supported HES cells should provide a safer alternative to existing HES cell lines in therapeutic applications.

Nature Medicine

Volume 8, Number 9
September 2002

Bone marrow-derived stem cells target retinal astrocytes and can promote or inhibit retinal angiogenesis

A. Otani et al.

Adult bone marrow (BM) contains cells capable of differentiating along hematopoietic (Lin(+)) or nonhematopoietic (Lin(-)) lineages. Lin(-) hematopoietic stem cells (HSCs) have recently been shown to contain a population of endothelial precursor cells (EPCs) capable of forming blood vessels. Here we show that intravitreally injected Lin(-) BM cells selectively target retinal astrocytes, cells that serve as a template for both developmental and injury-associated retinal angiogenesis. When Lin(-) BM cells were injected into neonatal mouse eyes, they extensively and stably incorporated into forming retinal vasculature. When EPC-enriched HSCs were injected into the eyes of neonatal rd/rd mice, whose vasculature ordinarily degenerates with age, they rescued and maintained a normal vasculature. In contrast, normal retinal angiogenesis was inhibited when EPCs expressing a potent angiostatic protein were injected. We have demonstrated that Lin(-) BM cells and astrocytes specifically interact with one another during normal angiogenesis and pathological vascular degeneration in the retina. Selective targeting with Lin(-) HSC may be a useful therapeutic approach for the treatment of many ocular diseases.

Damaged epithelia regenerated by bone marrow-derived cells in the human gastrointestinal tract

Ryuichi Okamoto et al.

Studies have shown that bone marrow cells have the potential to differentiate into a variety of cell types. Here we show that bone marrow cells can repopulate the epithelia of the human gastrointestinal tract. Epithelial cells of male donor origin were distributed in every part of the gastrointestinal tract of female bone marrow transplant recipients. Donor-derived epithelial cells substantially repopulated the gastrointestinal tract during epithelial regeneration after graft-versus-host disease or ulcer formation. Regeneration of gastrointestinal epithelia with donor-derived cells in humans shows a potential clinical application of bone-marrow-derived cells for repairing severely damaged epithelia, not only in the gastrointestinal tract but also in other tissues.

Neurology

Volume 59, Number 4
August 27, 2002

Human marrow stromal cell therapy for stroke in rat: Neurotrophins and functional recovery.

Y. Li et al.

OBJECTIVE: To test the effect of IV-injected human bone marrow stromal cells (hMSC) on neurologic functional deficits after stroke in rats.

METHODS: Rats were subjected to transient middle cerebral artery occlusion and IV injected with 3 x 10(6) hMSC one day after stroke. Functional outcome was measured before, and one, seven, and fourteen days after stroke. Mixed lymphocyte reaction and the development of cytotoxic T lymphocytes measured the immune rejection of hMSC. A monoclonal antibody specific to human cellular nuclei (mAb1281) was used to identify hMSC and to measure neural phenotype. ELISA analyzed neurotrophin levels in cere-

bral tissue from hMSC-treated or nontreated rats. Bromodeoxyuridine injections were used to identify newly formed cells.

RESULTS: Significant recovery of function was found in rats treated with hMSC at fourteen days compared with control rats with ischemia. Few (one to five percent) hMSC expressed proteins phenotypic of brain parenchymal cells. Brain-derived neurotrophic factor and nerve growth factor significantly increased, and apoptotic cells significantly decreased in the ischemic boundary zone; significantly more bromodeoxyuridine-reactive cells were detected in the subventricular zone of the ischemic hemisphere of rats treated with hMSC. hMSC induced proliferation of lymphocytes without the induction of cytotoxic T lymphocytes.

CONCLUSION: Neurologic benefit resulting from hMSC treatment of stroke in rats may derive from the increase of growth factors in the ischemic tissue, the reduction of apoptosis in the penumbral zone of the lesion, and the proliferation of endogenous cells in the subventricular zone.

The New England Journal of Medicine

Volume 346, Number 23
June 6, 2002

A Randomized Comparison of a Sirolimus-Eluting Stent with a Standard Stent for Coronary Revascularization

Marie-Claude Morice, M.D. et al.

As compared with a standard coronary stent, a sirolimus-eluting stent shows considerable promise for the prevention of neointimal proliferation, restenosis, and associated clinical events.

Results of Screening Colonoscopy among Persons 40 to 49 Years of Age

Thomas F. Imperiale, M.D. et al.

Colonoscopic detection of colorectal cancer is uncommon in asymptomatic persons 40 to 49 years of age. The noncancerous lesions are equally distributed proximally and distally. The low yield of screening colonoscopy in this age group is consistent with current recommendations about the age at which to begin screening in persons at average risk.

Cost Effectiveness of Aspirin, Clopidogrel, or Both for Secondary Prevention of Coronary Heart Disease

Jean-Michel Gaspoz, M.D. et al.

Increased prescription of aspirin for secondary prevention of coronary heart disease is attractive from a cost-effectiveness perspective. Because clopidogrel is more costly, its incremental cost effectiveness is currently unattractive, unless its use is restricted to patients who are ineligible for aspirin.

Volume 346, Number 24
June 13, 2002

Antiretroviral Therapy during Pregnancy and the Risk of an Adverse Outcome

Ruth E. Tuomala, M.D. et al.

As compared with no antiretroviral therapy or monotherapy, combination therapy for HIV-1 infection in pregnant women is not associated with increased rates of premature delivery or with low birth weight, low Apgar scores, or stillbirth in their infants. The association between combination therapy with protease inhibitors and an increased risk of very low birth weight requires confirmation.

Volume 346, Number 26
June 27, 2002

Oral Contraceptives and the Risk of Breast Cancer

Polly A. Marchbanks, Ph.D. et al.

Among women from 35 to 64 years of age, current or former oral-contraceptive use was not associated with a significantly increased risk of breast cancer.

Volume 347, Number 1
July 4, 2002

Widespread Coronary Inflammation in Unstable Angina

Antonino Buffon, M.D. et al.

The widespread activation of neutrophils across the coronary vascular bed in patients with unstable angina, regardless of the location of the culprit stenosis, challenges the concept of a single vulnerable plaque in unstable coronary syndromes.

Comparative Efficacy of Insect Repellents against Mosquito Bites

Mark S. Fradin, M.D. and John F. Day, Ph.D.

Currently available non-DEET repellents do not provide protection for durations similar to those of DEET-based repellents and cannot be relied on to provide prolonged protection in environments where mosquito-borne diseases are a substantial threat.

Volume 347, Number 2
July 11, 2002

A Controlled Trial of Arthroscopic Surgery for Osteoarthritis of the Knee

J. Bruce Moseley, M.D. et al.

In this controlled trial involving patients with osteoarthritis of the knee, the outcomes after arthroscopic lavage or arthroscopic débridement were no better than those after a placebo procedure.

Volume 347, Number 4
July 25, 2002

Kidney Transplantation from Donors without a Heartbeat

Markus Weber, M.D. et al.

Although the incidence of delayed graft function is significantly higher with kidneys from donors without a heartbeat than with kidneys from donors with a heartbeat, there is no difference in long-term outcome between the two types of graft.

Volume 347, Number 9
August 29, 2002

Insurance Coverage and Outcomes of in Vitro Fertilization

Tarun Jain, M.D. et al.

State-mandated insurance coverage for in vitro fertilization services is associated with increased utilization of these services but with decreases in the number of embryos transferred per cycle, the percentage of cycles resulting in pregnancy, and the percentage of pregnancies with three or more fetuses.

Proceedings of the National Academy of the Sciences

Volume 99, Number 16
August 6, 2002

C1qRp defines a new human stem cell population with hematopoietic and hepatic potential

Guénahel H. Danet et al.

The characterization of two distinct classes of hematopoietic stem cells based on CD34 expression and the ability of human bone marrow (BM) cells to differentiate into nonhematopoietic cells introduced new levels of complexity within the stem cell compartment. Here we report the identification and purification of a rare human stem cell population with hematopoietic and hepatic potential based on the expression of a receptor for the complement molecule C1q (C1qRp). We show that C1qRp is a positive marker of all BM-repopulating stem cells because it is expressed on both CD34 and CD34+ stem cells from umbilical cord blood and adult BM. In addition, we show that highly purified lineage-negative CD45+CD38CD34+orC1qR cells not only have BM-repopulating capacity but also can differentiate into human hepatocytes in vivo. The identification of human hepatocytes in mouse livers indicates that the NOD/SCID (nonobese diabetic/severe combined immunodeficient) mouse model can be a valuable tool to study the differentiation potential of adult human stem cells. These findings may have important scientific and clinical implications in the field of human stem cell biology and transplantation.

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Abnormal gene expression in cloned mice derived from embryonic stem cell and cumulus cell nuclei

David Humpherys et al.

To assess the extent of abnormal gene expression in clones, we assessed global gene expression by microarray analysis on RNA from the placentas and livers of neonatal cloned mice derived by nuclear transfer (NT) from both cultured embryonic stem cells and freshly isolated cumulus cells. Direct comparison of gene expression profiles of more than ten thousand genes showed that for both donor cell types four percent of the expressed genes in the NT placentas differed dramatically in expression levels from those in controls and that the majority of abnormally expressed genes were common to both types of clones. Importantly, however, the expression of a smaller set of genes differed between the embryonic-stem-cell- and cumulus-cell-derived clones. The livers of the cloned mice also showed abnormal gene expression, although to a lesser extent, and with a different set of affected genes, than seen in the placentas. Our results demonstrate frequent abnormal gene expression in clones, in which most expression abnormalities appear common to the NT procedure whereas others appear to reflect the particular donor nucleus.

Science

Volume 297, Number 5586
August 30, 2002

Identification of a Potential Ejaculation Generator in the Spinal Cord

William A. Truitt and Lique M. Coolen

We tested the significance of a population of lumbar spinothalamic cells for male sexual behavior in rats. These cells are positioned to relay ejaculation-related signals from reproductive organs to the brain, and they express neurokinin-1 receptors. Ablation of these neurons by the selective toxin SSP-

saporin resulted in a complete disruption of ejaculatory behavior. In contrast, other components of sexual behavior remained intact. These results suggest that this population of spinothalamic cells plays a pivotal role in generation of ejaculatory behavior and may be part of a spinal ejaculation generator.

Published online September 5, 2002

Little Evidence for Developmental Plasticity of Adult Hematopoietic Stem Cells

Amy J. Wagers et al.

To rigorously test the in vivo cell fate specificity of bone marrow (BM) hematopoietic stem cells (HSCs), we generated chimeric animals by transplantation of a single green fluorescent protein (GFP)-marked HSC into lethally irradiated nontransgenic recipients. Single HSCs robustly reconstituted peripheral blood leukocytes in these animals, but did not contribute appreciably to nonhematopoietic tissues, including brain, kidney, gut, liver, and muscle. Similarly, in GFP+:GFP- parabiotic mice, we found significant chimerism of hematopoietic but not nonhematopoietic cells. These data indicate that "transdifferentiation" of circulating HSCs and/or their progeny is an extremely rare event, if it occurs at all.

JOURNALS IN PHILOSOPHY AND THEOLOGY

American Journal of Bioethics

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Determining Moral Status

Ronald M. Green

In this chapter, I review some of the background thinking concerning matters of moral status that I had developed in previous years and that I would not bring to the work of the Human Embryo Research Panel. Two ideas were at the forefront of my thinking. First, that biology usually offers not decisive "events" but only continuous processes of development. Second, in making status determinations, we do not so much "identify" a point on a developmental continuum where moral respect should be accorded as "choose" that point. These choices are "balancing decisions" in which the community of moral agents weighs its interests in protecting an entity against the burdens of doing so. After illustrating these two contentions, I consider some of the reasons why thinkers on the "right" and "left" of our bioethics debates have resisted or missed this basic insight.