Causal Mutation Homework Assignment

Most of the SNP variations associated with diseases in genome-wide association studies do not cause the disease, but instead, these SNPs serve as genetic markers that are linked to genes which are involved in the disease. Ongoing research is attempting to sequence these genes in patients and in controls to find the actual variations in these genes that do in fact, cause the disease.

For this assignment I would like you to choose a simple Mendelian inherited disease other than those mentioned in class (Huntingsons, diabetes, Parkinsons, cystic fibrosis, sickle cell, etc.) and describe what is known about the genetic variations that cause that disease.

You may search OMIM, dbSNP, dbVAR, HGMD, HGVS, ClinVar, SwissVar and other database of genome variations that are associated with specific diseases to find an example of the kinds of mutations associated with the disease. Please describe how each of these variations cause the disease.

Is it by:

1) mutating the coding region of the protein
2) altering the gene expression by affecting the promoter
3) altering gene expression by affecting a transcription factor binding site
4) altering gene expression indirectly by mutating a transcription factor itself
5) altering copy number, hence changing gene expression levels
6) altering other regulatory sites (miRNA targets)
7) altering splice signals

etc.

Often there will be several types of mutations that can cause the disease. Please comment on all types that are known for your chosen disease.
Welcome to Henry Stewart Talks Online Collections

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Series: Stem Cells
Recent advances in understanding and utilizing

TOPICS COVERED

- Embryonic stem cells in perspective
- The advent of direct reprogramming
- Embryonic stem cells: derivation and properties
- Pluripotency and disease modeling: insights into reprogramming mechanisms
- Stem cells and regeneration: The physiological function of mesenchymal stem cells
- Niche regulation of stem cell function: stem cells and tissue homeostasis
- RNA regulation and stem cells: microRNA regulation of pluripotency
- The aging of mitotic cells: regeneration and aging
- Stem cells and cancer: lineage tracing in normal stem cells and cancer
- Stem cells derived from amniotic fluid and placenta
- Cord blood stem cells
- Mesenchymal stem cells derived from bone marrow
- Hematopoietic stem cells
- Stem cells derived from peripheral blood
- Stem cells derived from fat
- Skeletal muscle stem cells
- Epithelial skin stem cells
- Stem cells and heart disease
- Islet cell therapy and pancreatic stem cells
- Cell therapy of liver disease: from hepatocytes to stem cells
HUMBIO 157: The Biology of Stem Cells (DBIO 257)

The role of stem cells in human development and potential for treating disease. Guest lectures by biologists, ethicists, and legal scholars. Prerequisites: HumBio 2A and 3A, or the equivalent in the BioCore in Biological Sciences.

Terms: Spr | Units: 3 | UG Reqs: WAY-SMA | Grading: Letter or Credit/No Credit
Instructors: Fuller, M. (PI) ; Nusse, R. (PI)

Schedule for HUMBIO 157

2014-2015 Spring

HUMBIO 157 | 3 units | UG Reqs: WAY-SMA | Class # 20511 | Section 01 | Grading: Letter or Credit/No Credit | LEC
03/30/2015 - 06/03/2015 Tue, Thu 2:15 PM - 3:45 PM with Fuller, M. (PI); Nusse, R. (PI)
Instructors: Fuller, M. (PI); Nusse, R. (PI)
Early Embryo Development

Wong et al. 2010 Nat. Biotech. 28; 115-1121
Differentiation of Human Tissues

- **Ectoderm (external layer)**
  - Skin cells of epidermis
  - Neuron of brain
  - Pigment cell
  - Cardiac muscle
  - Skeletal muscle cells
  - Tubule cell of the kidney
  - Red blood cells
  - Smooth muscle (in gut)
  - Pancreatic cell
  - Thyroid cell
  - Lung cell (alveolar Cell)
- **Mesoderm (middle layer)**
- **Endoderm (internal layer)**
- **Germ cells**

Courtesy Paul Berg
Embryonic Stem Cell Cultures

Inner Cell Mass Cells Continue to Proliferate Indefinitely in Culture

Dissociate

Subculture

Freeze

Pluripotent Embryonic Stem Cells

Thaw
Pluripotent Stem Cells Differentiate into many Cell Types

Add different growth factors

Muscle

Blood

Nerve

Courtesy Paul Berg
Basic Problems of Stem Cell Therapy

• HOW TO DIRECT DIFFERENTIATION OF CELLS DOWN SPECIFIC PATHWAYS?
  e.g. all into muscle or all into nerve; different “cocktails” of growth factors

• HOW TO OVERCOME IMMUNE REJECTION?
  e.g. alter histocompatibility genes; therapeutic cloning for “customized” lines

• HOW TO MAKE AN ORGAN?
  e.g. combine different cell types in three dimensional arrangements.
Methods to Generate Pluripotent Stem Cells

Defined Transcription Factors

Nanog-Mediated Enhancement of Reprogramming by Fusion

## Five Transcription Factors Needed to Maintain Pluripotency


<table>
<thead>
<tr>
<th>Factor</th>
<th>Knockout ES Cells</th>
<th>Knockout Embryos</th>
<th>Overexpression in ES Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oct-3/4</td>
<td>Cannot be established</td>
<td>No epiblast</td>
<td>Induces differentiation</td>
</tr>
<tr>
<td>Niwa et al., 2000</td>
<td>Nichols et al., 1998</td>
<td>Niwa et al., 2000</td>
<td></td>
</tr>
<tr>
<td>Sox2</td>
<td>Cannot be established</td>
<td>No epiblast</td>
<td>Does not induce differentiation</td>
</tr>
<tr>
<td>Masui et al., 2007</td>
<td>Avilion et al., 2003</td>
<td>Does not induce LIF independency</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>M. Nakagawa and S.Y., unpublished data</td>
<td></td>
</tr>
<tr>
<td>c-Myc</td>
<td>Can be established</td>
<td>Normal epiblast</td>
<td>Does not induce differentiation</td>
</tr>
<tr>
<td></td>
<td>Normal self-renewal</td>
<td>Induces LIF independency</td>
<td></td>
</tr>
<tr>
<td>Davis et al., 1993</td>
<td>Davis et al., 1993</td>
<td>Cartwright et al., 2005</td>
<td></td>
</tr>
<tr>
<td>KLF4</td>
<td>Not reported</td>
<td>Normal epiblast</td>
<td>Does not induce differentiation</td>
</tr>
<tr>
<td></td>
<td>Katz et al., 2002</td>
<td>Induces LIF independency</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Y. Tokuzawa, M. Nakagawa, and S.Y., unpublished data</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nanog</td>
<td>Can be established</td>
<td>No epiblast</td>
<td>Does not induce differentiation</td>
</tr>
<tr>
<td></td>
<td>Spontaneous differentiation</td>
<td>Induces LIF independency</td>
<td></td>
</tr>
<tr>
<td>Mitsui et al., 2003</td>
<td>Mitsui et al., 2003</td>
<td>Chambers et al., 2003; Mitsui et al., 2003</td>
<td></td>
</tr>
</tbody>
</table>
Induction of Pluripotent Stem Cells (iPS) from Somatic Stem Cells

Adipose Tissue Provides iPSC Efficiently

'Liposuction leftovers' easily converted to iPSC cells, study shows

BY KRISTA CONGER

Globs of human fat removed during liposuction conceal versatile cells that are more quickly and easily coaxed to become induced pluripotent stem cells, or iPSC cells, than are the skin cells most often used by researchers, according to a new study from Stanford's School of Medicine.

“We’ve identified a great natural resource,” said Stanford surgery professor and co-author of the research, Michael Longaker, MD, who has called the readily available liposuction leftovers “liquid gold.” Reprogramming adult cells to function like embryonic stem cells is one way researchers hope to create patient-specific cell lines to regenerate tissue or to study specific diseases in the laboratory.

Using CRE – Recombinase to Remove Viral Transforming DNA from iPSCs

Parkinson’s Disease Patient-Derived Induced Pluripotent Stem Cells Free of Viral Reprogramming Factors

Frank Soldner,1,4 Dirk Hockemeyer,1,4 Caroline Beard,1 Qing Gao,1 George W. Bell,1 Elizabeth G. Cook,1 Gunnar Hargus,3 Alexandra Blak,3 Oliver Cooper,3 Maisam Mitalipova,1 Ole Isacson,3 and Rudolf Jaenisch1,2,*  
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4These authors contributed equally to this work  
*Correspondence: jaenisch@wi.mit.edu  
DOI 10.1016/j.cell.2009.02.013
Cre-Lox Recombination to Remove Viral DNA

Figure 1. A pair of \textit{lox} P sites (yellow ovals) flanking the target DNA (purple) to be deleted.

Figure 2. After the cre enzyme has excised the target DNA, one \textit{lox} P site is left behind and the two flanking fragments of DNA are spliced together. The target DNA is excised and degraded.
Inducing iPSCs using Transcription Factor Proteins

Generation of Human Induced Pluripotent Stem Cells by Direct Delivery of Reprogramming Proteins

Dohoon Kim,¹,⁵ Chun-Hyung Kim,¹,⁵ Jung-II Moon,¹ Young-Gie Chung,³ Mi-Yoon Chang,¹ Baek-Soo Han,¹ Sanghyeok Ko,¹ Eungi Yang,¹ Kwang Yul Cha,⁴ Robert Lanza,³,* and Kwang-Soo Kim¹,²,⁴,*
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DOI 10.1016/j.stem.2009.05.005
We recently showed that defined sets of transcription factors are sufficient to convert mouse and human fibroblasts directly into cells resembling functional neurons, referred to as “induced neuronal” (iN) cells. For some applications however, it would be desirable to convert fibroblasts into proliferative neural precursor cells (NPCs) instead of neurons. We hypothesized that NPC-like cells may be induced using the same principal approach used for generating iN cells. Toward this goal, we infected mouse embryonic fibroblasts derived from Sox2-EGFP mice with a set of 11 transcription factors highly expressed in NPCs. Twenty-four days after transgene induction, Sox2-EGFP+ colonies emerged that expressed NPC-specific genes and differentiated into neuronal and astrocytic cells. Using stepwise elimination, we found that Sox2 and FoxG1 are capable of generating clonal self-renewing, bipotent induced NPCs that gave rise to astrocytes and functional neurons. When we added the Pou and Homeobox domain-containing transcription factor Brn2 to Sox2 and FoxG1, we were able to induce tripotent NPCs that could be differentiated not only into neurons and astrocytes but also into oligodendrocytes. The transcription factors FoxG1 and Brn2 alone also were capable of inducing NPC-like cells; however, these cells generated less mature neurons, although they did produce astrocytes and even oligodendrocytes capable of integration into dysmyelinated Shiverer brain. Our data demonstrate that direct lineage reprogramming using target cell-type-specific transcription factors can be used to induce NPC-like cells that potentially could be used for autologous cell transplantation-based therapies in the brain or spinal cord.
Direct Cell Reprogramming *in vivo* & *in vitro*

http://www.the-scientist.com/?articles.view/articleNo/39241/title/A-Twist-of-Fate/
Alternate Stem Cell Fates

Embryonic Stem Cells

Adult Stem Cells

Adult Stem Cells

stem cell proliferation

asymmetric division

stem cell loss

Courtesy of Minx Fuller
signals from niches maintain adult stem cells and tissues

Courtesy of Roel Nusse
In the absence of niche signals, adult stem cells will differentiate, by default.

1. Self-renewal is proliferation coupled to blocking differentiation, controlled by signals.
2. Signals are local; niches have a limited capacity and cells compete for the signals.
3. The signals control tissue homeostasis, also after damage.

Courtesy of Roel Nusse
Cell-Cell Interactions at Oocyte Niche

Li and Xie, Ann. Rev. Dev. Biol. 2005, 605-663
Drosophila Spermatogonial Niche

Li and Xie, Ann. Rev. Dev. Biol. 2005, 605-663
Cell-Cell Interactions at the Spermatogonial Niche
Summary of Stem Cell Niche Signals

Physical Contact
- Tight Junction: N, I
- Adherens Junction: D, N
- Notch Signaling: C, N, H, I
- Gap Junction: D
- Basement Membrane: N, E, I
- Extracellular Matrix: D, N

Diffusible Factors
- Pathway:
  - Wnt: C, E, H, I
  - BMP: D, N, E, I
  - JAK/STAT: D
- Growth Factors: N
- Hedgehog: I
- PGE2: I
- O2: H

Transcription Factor
Activation
Signal Transduction
Hair Follicle Niche

Li and Xie, Ann. Rev. Dev. Biol. 2005, 605-663
Intestinal Stem Cells in Crypts
Rainbow Villi
Asymmetric stem cell divisions

**Extrinsic factor(s)**

**Niche**

Nguyen (2007) Genomics & Medicine
review article

Mutation selection and the natural history of cancer

John Cairns*

Survival of the rapidly renewing tissues of long-lived animals like man requires that they be protected against the natural selection of fitter variant cells (that is, the spontaneous appearance of cancer). This article discusses three possible protective mechanisms and shows how they could explain various features of the natural history of certain common cancers of man.
Motivation for Asymmetric Strand Segregation

- Adult rat contains $6 \times 10^{10}$ cells
- In its small intestine, a rat sheds over $10^{13}$ epithelial cells during its lifetime.
- Requires $10^3$ symmetric cell doublings from embryo to adult followed by $10^{13}$ asymmetric cell doublings during its lifetime
- How do epithelial cells minimize mutations that lead to cancer?

Cairns (1975) Nature 255, 197
Asymmetric Segregation of Parental DNA Strands

Rando (2007) Cell 129 1239
Asymmetric Stem Cell Growth with Asymmetric Parental Strand Segregation

Rando (2007) Cell 129 1239
Asymmetric DNA Labeling Patterns

First round of DNA replication, cell division

- DNA Replication
- CldU

Second round of DNA replication, cell division

- DNA Replication
- IdU

Random Segregation
- Daughters indistinguishable

Non-Random Segregation
- Daughters distinguishable

Rando (2007) Cell 129 1239
Duplicating Muscle Cell Pairs Display Asymmetric DNA Labeling Patterns

**Figure 2.** Evidence of Co-Segregation of DNA Template Strands during Muscle Progenitor Cell Division
(B) Cell pairs were immunostained for CldU and IdU. Shown is a representative photograph of an immunostained pair of cells, in which both daughter cells were labeled with the second label, IdU (green), but only one daughter inherited the first label, CldU (red).
Asymmetric Stem Cell Growth with Asymmetric Parental Strand Segregation

Rando (2007) Cell 129 1239
Wnt signaling

Courtesy of Roel Nusse
Wnt Signaling Pathway
Jak Stat Pathway

http://www.biocarta.com/pathfiles/h_stat3Pathway.asp
The Hedgehog pathway

Ligand

Receptor

7-pass membrane transducer

Cytoplasmic Negative Regulator

Transcription Factor

Target Genes: Gli1 and Ptc1
The primary cilium: A specialized compartment for signal transduction

Cilia as sensors for Shh: Shh binds to its receptor Patched1 at primary cilia in live cells.
Smo moves to cilia and when the Hedgehog pathway is activated.
Smo activates downstream signaling components in cilia
Smo as a model for signal-regulated protein transport at primary cilia
Models for ciliary protein transport

1: Direct Trafficking

2: Trafficking to plasma membrane

2a: Lateral Transport

2b: Recycling Mediated

Post Golgi Vesicle

Plasma Membrane

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Induction of Apoptosis via DR3 and DR4/5 Death Receptors

http://www.biocarta.com/pathfiles/h_deathPathway.asp
Cloning Procedures

Blastocyst

Early Embryo

Fetus

Recipient Egg

Remove Nucleus

Injection

Fusion

Implantation into Surrogate Uterus

Progeny

Culture Cells

Adult POSSIBLE DONORS

How Cloning might be used Therapeutically

Anucleate Unfertilized Egg from Donor

Adult Cell from Patient

Nucleus Transfer

Courtesy Paul Berg
Direct versus indirect Cell Reprogramming

Cellular Reprogramming

For the better part of the past decade, researchers have been reprogramming adult cell types, either into induced pluripotent stem cells (iPSCs), which themselves can give rise to diverse cell types, or directly into other differentiated cell types through a process called direct reprogramming. Such approaches support the switching of diverse cell types once believed to be permanently locked in their differentiated form.

Traditionally, relevant transcription factors encoded by genetic material were carried by retro- or lentivirus vectors and integrated into the host cell genome. More recently, the use of nonintegrating vectors, RNA, or small molecules have been developed to minimize the chance of harmful mutations.

Fibroblasts were the first and remain the most common type of cell to be reprogrammed, but other cells, such as lymphocytes, which can be isolated from blood, are also proving to be successful starting points for stem-cell generation.

Open Chromatin

Transfected transcription factors, such as Oct4, induce the expression of pluripotency-related genes, such as Nanog, or cell-type-specific genes in the case of direct reprogramming.

Closed Chromatin

Sequences from pioneer factors, such as the myogenic factor MyoD, are also employed to increase reprogramming efficiency in the face of closed chromatin, which can inhibit access of the transfected transcription factors to their target genes.