Welcome to
STEM CELL DIALOGUES

Beyond Transplants:
Stem Cells and Organ Regeneration

Tonight’s session is being videotaped and will soon be available at www.ucdmc.ucdavis.edu

April 30, 2010
Solid Organ Transplantation: Success and Limitations

Stem Cell Dialogues
April 30, 2010

Brian Gallay, M.D., Ph.D.
Associate Professor, Internal Medicine
Transplant Nephrology Section
Why Transplant?

- End stage organ disease
- Organ failure is fatal or life-limiting
- Function restored by transplant
US Patients Awaiting Transplant

- Total: 106816
- Kidney: 84355
- Liver: 15970
- Heart: 3172
- Kidney-pancreas: 2198
- Lung: 1838
- Pancreas: 1469
- Intestine: 245
- Heart-lung: 72
Transplantation is Successful.....

- Life-saving
- Improved quality of life
- Cheaper
- Development of novel immunologic therapies
But It’s Not Perfect

- Many more candidates than available organs
- Not all patients are candidates
- Not a cure
The Donor-Recipient Gap: Kidney
The Donor-Recipient Gap: Liver
Not all Patients are Candidates

84,000 candidates

580,000 ESRD patients
Donor and Recipient Factors

- Increasing donor age
- More obese patients
- Donor organ must be matched to recipient by size for heart, lung, and liver
Transplant is Not a Cure

- Kidney transplants do not last indefinitely
- Chronic immunosuppressive medications
- Chronic monitoring for rejection, recurrent disease
Kidneys Don’t Last Forever

Optn Registry data

- Cadaveric ($t_1/2=9.7$)
- Living related ($t_1/2=15.2$)
- Living distant/unrelated ($t_1/2=15.5$)
Chronic Kidney Disease in the US

Number of Patients

<table>
<thead>
<tr>
<th>CKD Stage</th>
<th>Number of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>5900000</td>
</tr>
<tr>
<td>II</td>
<td>5300000</td>
</tr>
<tr>
<td>III</td>
<td>7600000</td>
</tr>
<tr>
<td>IV</td>
<td>400000</td>
</tr>
<tr>
<td>V</td>
<td>300000</td>
</tr>
</tbody>
</table>

Death from Cardiovascular Disease
Cost of CKD: Medicare Expenditure

<table>
<thead>
<tr>
<th>Year</th>
<th>CKD</th>
<th>Total</th>
<th>CKD</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1993</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2007</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Acute Kidney Failure

- Occurs in 5-20% of hospitalized patients
- 40-60% mortality
- Longer hospital stay
- Higher hospital cost
- Increased risk of CKD
Promise of Regenerative Therapy

- Avoid complications of transplantation
- Increase access to therapy
- Improve patient survival
- Decrease impact of acute kidney failure
- Long term health care cost savings
The Use of Stem Cells in Organ Bioengineering at UC Davis

Stem Cell Dialogues
April 30, 2010

Jan A. Nolta, Ph.D.
Professor, Internal Medicine
Director, UC Davis Institute for Regenerative Cures
UC Davis is leading the way in regenerative medicine

- ‘Bench to bedside,’ patient-centered research
- Stem cell ‘disease teams’
- Unique, new facilities for research and clinical collaboration
- Focused on turning stem cells into cures
Stem cell types

- **Adult stem cells:**
  - Hematopoietic – blood-forming
  - Mesenchymal – supporting cells

- **Pluripotent cells:**
  - Embryonic stem cell lines
  - Induced pluripotent stem cells
Growth and Differentiation of Stem Cells

Stem Cell
1) Self-renewal
2) Multi-potential
3) Highly proliferative

Differentiation and Commitment
Differentiation of Human Embryonic Stem Cells - Kidney Cells

Directed differentiation

Tarantal Laboratory, UC Davis 2010
Kidney progenitor cells are damaged in many diseases

- Best methods to grow them from stem cells?
- Best Method to implant new cells into the damaged organ?

Pediatric Research 66:448-454, 2009
Tissue Engineering – Kidney Regeneration

Different cell populations under study to regenerate kidneys using special scaffolds and matrices on which to grow the cells

Tissue Engineering, in press, 2010
Cells growing on a synthetic scaffold of proteins -
More “normal” for them than a flat surface
Scaffolds for implanting cells

**Synthetic matrix scaffold**
- Several types available
- Does not have the innate structure of the organ

**Decellularized matrix scaffold**
- All cells have been stripped away
- No immune system attack if done properly
- Intricate structure is maintained by the supporting proteins
- “Nature’s platform” to rebuild the organ
Decellularized scaffolds

Heart

Heart during the “washing” process-
Cells are removed

Decellularized Heart

Image description:
- Decellularized Heart
- Heart
- Heart during the “washing” process—Cells are removed

[Image of heart specimen at different stages of decellularization process]
Decellularized liver

Zhou and Wu, Nolta and Zern labs 2010
Human Liver Cells Growing in Decellularized Liver Matrix Prior to Transplantation
**Human hepatocytes viewed inside living mice:**
Cells implanted on decellularized liver scaffold retain good survival and function

A) Cells implanted on decellularized liver scaffold:
Excellent retention – 3 months

B) Cells transplanted by injection-no scaffold:
Scattering/poor retention

Zhou, Nolta, Zern, Wu – Manuscript submitted 2010
Neo-organs from human tissue grown in mice
Human cells in matrix after removal from mouse model
Induced Pluripotent Stem Cells (iPSC)

- Generated from a patient’s own tissues: skin or the root of a plucked hair
- Resemble embryonic stem cells
- Have the potential to directly generate all tissues, including kidney and liver cells
Developing Cell and Organ Replacement for Individual Patients

- Skin fibroblasts - from the patient
- Induced pluripotent stem cells
- Patient-specific human Hepatocytes in culture
- Transplant Into patient
- Place on scaffold
Pediatric Urology Laboratory

Stem Cells & Bioengineering Bladder

Eric A. Kurzrock, M.D., F.A.A.P.
Associate Professor of Urology and Pediatrics
Dept. of Urology, UC Davis School of Medicine
Bladder & Stem Cells

- Bladder disorders
  - Spinal cord problems
  - Cancer
- Bioengineering bladder
- Stem cell research
  - Better bioengineering
  - Cancer treatments
People who need bladder replacement or enlargement
Spinal Cord Anomalies

- Spina Bifida
- Spinal cord injury
  - Nerves going to
    - Legs
    - Bladder
Neurogenic Bladder

- Bladder dysfunction
  - Kidney damage
  - Urine incontinence

Kurzrock et al: Journal of Spinal Cord Medicine, 30: 30-34, 2007
Bladder Cancer

- 4th most common cancer
  - 15% become invasive
  - 15,000 deaths last year
  - Most expensive cancer to treat
    - Highly recurrent

- Causes
  - Genetic predisposition
  - Environmental toxins
Bladder Cancer Treatments

- **Treatments**
  - Endoscopic resection – >50% recurrence
  - Bladder removal +/- chemotherapy
    - Construction of urinary conduit
    - Construction of new bladder

**Urostomy**
Bladder Exstrophy

- Birth defect with bladder outside the body
Bladder Reconstruction

- Bladder augmentation and replacement
  - Ileum, colon, stomach and ureter
Complications

- Combining urine with bowel
  - Chemical imbalance
  - Urine infection
  - Urine stones
  - Cancer

Kurzrock *et al.*: World J Urology, 27: 2009
Bladder Wall Substitutes

- **Synthetic materials**
  - Teflon, vicryl, collagen

- **Autologous (patient’s) tissue**
  - Peritoneum, omentum

- **Complications**
  - Contraction
  - Leakage
  - Stone formation
Doctors grow organs (bladders) from patients' own cells
Bioengineered Bladder

- Bladder grafts made from synthetic matrix seeded with patient’s own cells
  - 2 out of 9 patients had benefit (larger bladder)

- Advantages
  - ? Less graft contraction
Why Study Bladder Stem Cells?

- Bioengineering bladder
- Determining the causes of bladder cancer
- Improving bladder cancer treatments
- Gene Therapy
  - Bladder factories
Cells for Tissue Engineering

- **Normal adult cells**
  - Short lived
  - Persistence of acquired malfunction
  - Carry genetic defects (oncogenes) or actual cancer

- **Adult stem cells**
  - Survive and proliferate longer
  - Possibly free of oncogenes

- **Embryonic stem cells**
  - Can become any tissue type – urothelium or muscle
  - Free of oncogenes
Bioengineering Questions

- Do transplanted cells survive?
  - Is the graft replaced with patients’ cells?
  - Does it matter?

- Does the tissue construct have stem cells?
  - Does it matter?

- What are the limits of using patients’ own cells?
  - Cancer
  - Congenital disorders
What happens to transplanted bladder cells?

- Transgender bladder wall transplant
  - Best model for bioengineered graft
  - Syngeneic (shared genes) rat siblings
  - Male to Female bladder dome transplant

Male donor

Female host

Kurzrock et al: Tissue Engineering 2010
Transgender Transplant

Kurzrock et al: Tissue Engineering 2010
Transgender Transplant

Male bladder urothelium with Y-chromosome marker (red dot)

Male bladder muscle cells with Y-chromosome marker

Female bladder urothelium with X-chromosome marker

Kurzrock et al: Tissue Engineering 2010
Transgender Transplant

Results
- Urothelium slowly replaced
- Muscle cells survive long term
- Nerves and Blood Vessels?

Is this a model for bioengineered grafts?
- Would stem cells perform better?

Kurzrock et al: Tissue Engineering 2010
Normal Urothelial Development

- Embryonic stem cell
- Adult stem cell
- Mature cell

Genes → Differentiation → Genes

Cancer stem cell (CSC)
Objectives of Urology
Bioengineering & Stem Cell Laboratory

- Adult Urothelial Stem Cells
  - Identify
  - Characterize
  - Isolate and enrich
- Human Embryonic Stem Cells
  - Induction into urothelial cells
  - Determine mechanisms and markers
Identifying Adult Urothelial Stem Cells

- Label Retaining Cells in the Bladder
  - Labeled nucleotide incorporated into the DNA

Identifying Urothelial Stem Cells in Culture

Kurzrock et al: Cell Proliferation, 42: 770, 2009

Long-term Culture of Porcine Urothelial Cells

Total Urothelial Cell Output at Day 70

Kurzrock et al: Cell Proliferation, 42: 770, 2009
Isolating Adult Urothelial Stem Cells

- Isolation of bladder stem cells
  - Tagged-antibodies attached to stem cell markers allow separation of cells
  - Unique excretion of toxic dyes by stem cells also allows their separation by flow

Characterizing Adult Urothelial Stem Cells

CK14
CK18
Beta1
Beta4
p63
Bcl-2

Human Embryonic Stem Cells

- Induction of hESCs into Urothelium
Present and Future

- Characterization and enrichment of adult urothelial stem cells
  - Evaluate their pluripotentiality or plasticity
- Induction of hESCs into urothelium
  - Determine critical genes
  - Test new strategies for transplanting these cells
- Evaluate new models for bladder bioengineering and transplantation
Bladder Factories of the Future

- Bioengineered bladder stem cells
- Production of proteins
- The perfect organ for protein synthesis and excretion and collection
Bladder & Stem Cells

- Bladder disorders
  - Spinal cord problems
  - Cancer
- Bioengineering bladder
- Stem cell research
  - Better bioengineering
  - Cancer treatments
A Virtual Tour –
UC Davis Institute for Regenerative Cures

Stem Cell Dialogues
April 30, 2010

Jan A. Nolta, Ph.D.
Professor, Internal Medicine
Director, UC Davis Institute for Regenerative Cures
UC Davis Institute for Regenerative Cures
Opened March 2010

- More than 145 basic, translational and clinical faculty working together in teams
- Developing stem cell therapies to treat tissue damage and a variety of diseases
- Comparing adult, postnatal and induced pluripotent stem cells
Good Manufacturing Practice (“clean room”) Facility

Growing stem cells for clinical trials at Stanford, industry and other institutions

- Gerhard Bauer, director
  - Two decades of experience with cell therapy trials
UC Davis GMP facility- 6 suites
Cell harvest from patient

GMP Facility

Clinical trial with UC Davis GMP Facility

Transplantation

Quality Control and Quality Assurance

Stem Cell expansion And place on scaffold
Stem Cell Clinical Trials Ongoing/Pending at UC Davis

- Non-union bone fractures *Started in 2008*
- Cardiac infarction *Started Sept. 2009*
- Peripheral vascular disease *2010*
- Retinal occlusion, causing blindness *pending*
- Gene-modified MSC for Huntington’s disease and ALS *pending*
- Liver disease *pending*
Nolta lab tour guides at Institute for Regenerative Cures grand opening event
Thank you for attending

STEM CELL DIALOGUES

Tonight’s session is being videotaped and will soon will be available at www.ucdmc.ucdavis.edu

April 30, 2010