Training the Next Generation of Physician Researchers

UT COMC
Nuts and Bolts Research Methods Symposium
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Overview

1. “Bench to Bedside” – clinical applications of bench research?
2. Finding a research project that you are passionate about
3. Proposal writing and the regulations
4. Stem cells in translational and personalized research – what’s on the medical horizon?
1) Translational Medicine - patient-centric

Basic science in lab, molecular insights, Find disease signatures

develop diagnostics and drugs

Clinical Research - Patients - biospecimens
Basic Science Research

- Why? Because we are curious about nature. How does something work? What causes disease?
- Satisfies our desire to explain nature and understand the fundamental principles and mechanisms of how stuff works!
- Results may not have direct commercial benefits
- Progress based on controlled experiments
- It is the foundation for many commercial products and applied research
What is clinical research?

NIH’s definition = Patient-oriented research

The investigator or a collaborator uses human subjects or uses materials from humans (such as tissue samples, specimens and cognitive phenomena). This research can include:

- A scientific search to discover the **origins** and **mechanisms** of human disease
- Studies of **therapies** or interventions for disease
- **Clinical trials**
- Studies to develop **new technology** related to disease
1. A scientific search to discover the **origins** and **mechanisms** of disease

2. The identification of and insight into specific biological events, biomarkers, or pathways of disease

3. The use of such insights to systematically discover and develop **new diagnostics** and **therapeutic** methods and **products**

4. The adoption of such new diagnostic and therapeutic approaches **into the routine standard of care**

Source: http://www.biobankcentral.org/translational/whatis.php
2) Finding a research project that you are passionate about
How to come up with a concept?

- Disease with personal impact.
- Where is medical knowledge falling short? Where are current clinical treatments and applications lacking?
- Could there be new and better ways to **prevent, detect, diagnose, control, and treat these diseases**?
- Researcher need to interpret the data and form an opinion about the underlying principles of disease.
Finding a research project you are passionate about

- Structure your question in a way that you can test it in the lab or clinic – your question determines nature and type of experiment.

- Scientific research - devise a methodical study to prove or disprove a hypothesis or answer a specific question.

- “The scientific definition of research generally states that a variable must be manipulated, although case studies and purely observational science do not always comply with this norm.” Source: Experiment-Resources.com

***Note this is different from literature review or a case study.
In Vitro Fertilization Research, Penn Dept of Obstetrics and Gynecology Clinical Research Program, Principal Investigator: Kurt Barnhart, MD, MSCE

Blood and follicular fluid samples from patients of Penn Fertility Care who are undergoing in vitro fertilization are being collected and analyzed to investigate ways to predict and enhance IVF success rates, and to try to find reasons for “unexplained” infertility. Factors being studied include blood-clotting factors, amino acids, and markers of egg aging.
Adair CD, Buckalew VM, Kipikasa J, Torres C, Stallings SP, Briery CM.

Department of Obstetrics & Gynecology, The Section on Maternal-Fetal Medicine, The University of Tennessee College of Medicine, Chattanooga

Repeated dosing of digoxin-fragmented antibody in preterm eclampsia.
Early onset eclampsia has significant morbidity and mortality for both the mother and fetus. No effective treatment exists at present except delivery and seizure prophylaxis with magnesium sulfate. We report the novel use of a fragmented ovine antibody against digoxin for the treatment of eclampsia. A 16-year-old primagravida at 29 weeks 5/7 days gestation presented with clinical diagnosis of eclampsia and was treated with compassionate off-label use of digoxin-fragmented ovine antibody (Digibind Glaxo Smith Kline, Research Triangle Park, NC, USA). Improvement of her underlying disorder during a 48 h treatment window was noted without adverse maternal or neonatal outcome. We suggest digoxin-fragmented ovine antibody as a possible intervention in preterm pregnancies complicated by pre-eclampsia or eclampsia.
3) Proposal Writing and the Regulations

[Southpark Studio]

Still not heading home any time soon....
Key Ingredients of a Proposal/Grant

- Sell the idea – Need
- Sell the institution
- Sell yourself
- Simple projects can evolve
The institution...

- Erlanger/UTCOMC collaboration among residents, practicing physicians, and specialists
- Initiate collaboration with basic research labs at UTC, UTK and UT Memphis
- Emergency medicine, Family medicine, Internal medicine, Obstetrics and Gynecology, Orthopedic surgery, Pediatrics, Plastic surgery, Surgery

**RESEARCH AREAS and INTERESTS:** Nanotechnology, Cultural Competence and Health Disparities, Orthopaedics, Emergency Medicine (Hyperbaric Oxygen in Brain Injuries and Hypothermia in Cardiac Arrest), Smoking Cessation, Plastic Surgery, Obstetrics/Gynecology, Oncology Surgery and Surgical Simulation in a High-Fidelity, Human Patient Simulator Lab, Pediatrics, Internal Medicine, Geriatrics and the Center for Independent Living Research Initiative (CILVRI), Family Medicine
Federal Requirements for Proposals

- UTCOMC/Erlanger has established an Institutional Review Board
  - Review projects and ensure the protection of human subjects. Obtain informed consent.
  - Investigators are required to attend meetings to present new proposals and revisions to currently approved protocols.

- B W. Ruffner, M.D. – Chairman

- Stacey Hendricks, CIM, IRB Administrator
You have help. UTCOMC has:

- **Director of Research** - Greg W. Heath, DHSc, MPH

- **Scientific Review Committee (SRC)**
  - Review the scientific validity of the hypothesis and study design
  - **Chair**: Dr. Eric Gratias. **Vice Chair**: Dr. Billy Arant
Research Compliance Officer: Sylvia Friedl, BA, CIP

- Offers consultation during protocol preparation.

- Pre-reviews protocols, consent forms, and other compliance-related documents; identifies where modifications need to be made; and assists responsible parties in making those modifications before approval is sought from appropriate compliance entities.
4) Stem cells in translational and personalized research – what’s on the medical horizon?

Stem cells are an emerging area in biomedicine.

Stem cells are a perfect example of how basic research can transition into clinical medicine.

How will stem cells be used in creating new therapies and preventative interventions?
-About 25,000 genes are needed to build a human being with about 200 histologically distinct cell types interacting through complicated organ systems
Pluripotent stem cells

- Pluripotent cells renew themselves through cell division for extensive periods and can be induced to become a cell with specialized structure and function under certain physiological or experimental conditions.

- Where do they come from? (1) ES cells from inner cell mass of blastocyst (2) teratocarcinoma (3) aborted fetus (4) adult iPS cells.
The Blastocyst
A.K.A the “Pre-implantation embryo”

4 days after fertilization

Outer layer of ciliated trophoblasts, hollow sphere of cells, becomes placenta

Inner cell mass of pluripotent stem cells in the blastocyst – form fetus (all the tissues in the body)
induced pluripotent stem cells (iPS) technology

- Adult Stem Cells
- No embryos used
- Reprogramming of terminally differentiated somatic cells into pluripotency stem cells
- Skin punch – grow cells – transform/reprogram
- Bypass ethical issues
Oct-4 (an abbreviation of Octamer-4) is a homeodomain transcription factor which functions in self-renewal of undifferentiated stem cells.

Sox2 or SRY (sex determining region Y)-box 2 is critical to maintain self-renewal of undifferentiated stem cells.

KLF4, gut-enriched Krüppel-like factor, is a transcriptional activator or repressor involved in cell proliferation, differentiation and survival.

cMYC encodes a protein that binds to the DNA of other genes and regulates expression of 15% of genes. When it is mutated, or upregulated, it can cause cancer.
(A) Create new cell culture models of human diseases for which no models or no good models currently exist. These *in vitro* models are key for investigating underlying molecular mechanisms of disease.

(B) Use these disease-specific cell culture models to serve as a platform for developing, screening and discovering drugs to treat these diseases; and,

(C) Develop replacement strategies to rebuild tissues and restore critical functions of the diseased or damaged human body **without the risk of immune rejection**. Enormous THERAPUETIC Potential. Couple documented cases...
What kinds of diseases have immediate iPS clinical applications?

- Type 1 diabetes mellitus
- Parkinson's disease
- Amyotrophic Lateral Sclerosis (ALS)
- chronic liver disease
- spinal cord injuries
- ischemic stroke
- multiple sclerosis
- blindness
- heart disease
- known inheritable diseases – genetically corrected
1. Prepare disease-specific fibroblast preparations and BANK

2. Reprogram into iPS cells using viruses containing 4 factors

3. Characterize iPS cells via ICC, RT-PCR, metaphase spread, and teratoma formation

4. If iPS cells pass QC, BANK and disseminate

CHiPS Lab
Spinal cord injury

- The world’s first clinical trial using stem cells Geron Corporation
- Made oligodendrocyte progenitor cells from hES cells
- Injected into a patient’s injured spinal cord with the goal of restoring spinal cord function.

Oligodendrocytes – insulate axons of CNS forms Myelin sheath
How to use Tf-induced reprogramming combined with gene and cell therapy for disease treatment???
Mouse Model - βS (sickle) globin genes. Develop typical disease symptoms such as severe anemia due to erythrocyte sickling, splenic infarcts, urine concentration defects, and overall poor health.
Genetic disease characterized by short stature, skeletal anomalies, increased incidence of solid tumors and leukemias, bone marrow failure (aplastic anemia), and cellular sensitivity to DNA damaging agents. Therapy is the hematopoietic stem cell transplantation if matching donor.

- July 2009: Somatic cells from Fanconi anaemia patients were reprogrammed and genetically corrected with lentiviruses.

- Corrected Fanconi-anaemia-specific iPS cells gave rise to haematopoietic progenitors of the myeloid and erythroid lineages that are disease-free. NO IMMUNE REJECTION

- These data offer proof-of-concept that iPS cell technology can be used for the generation of disease-corrected, patient-specific cells with potential value for cell therapy application.
In vivo reprogramming of adult pancreatic exocrine cells to β-cells

Qiao Zhou¹, Juliana Brown², Andrew Kanarek¹, Jayaraj Rajagopal¹ & Douglas A. Melton¹

- 2008, Short-cut between specialized states.
- Exocrine cells in the mouse pancreas → (that secrete digestive enzymes like amylase) into insulin-secreting beta cells.
- Injected the pancreas of living mice with an adenovirus vector carrying 3 genes for transcription factors.
- When the experiments were done with mice deliberately made diabetic (by destroying their pre-existing β-cells), the newly-created β-cells secreted enough insulin to bring blood sugar levels closer to normal.
A combination of three transcription factors induces insulin+ cells in adult mouse pancreas \textit{in vivo}.

\textbf{Supplementary Information}

- \textbf{Supplementary Figure S1}
  - WT versus pAd-M3
  - Bar graph showing percentage of GFP+ cells that are insulin+ for different conditions.
Neurons derived from reprogrammed fibroblasts functionally integrate into the fetal brain and improve symptoms of rats with Parkinson’s disease

April 2008. iPS → neuronal precursor cells - Upon transplantation into the fetal mouse brain, the cells migrate into various brain regions and differentiate into glia and neurons, including glutamatergic, GABAergic, and catecholaminergic subtypes. Neuronal cell replacement of dopa-neurons in rat model of PD.

March 2009: iPS cells from PD patients → differentiated into dopamine-releasing cells