From salamanders to stem cells: key role for veterinary and medical doctors in advancing regenerative medicine

Andy Hoffman, D.V.M., D.V.Sc., Diplomate A.C.V.I.M
Professor, Large Animal Internal Medicine
Director, Regenerative Medicine Laboratory
Cummings School of Veterinary Medicine,
Tufts University, North Grafton MA
andrew.hoffman@tufts.edu

no conflicts of interest to declare
Challenges for public health

Many incurable conditions that are managed but not cured

• Inherited diseases
• Physical ailments (infectious, non-infectious, acute, chronic)
• Mental illness
• Obesity-related illness
• Addiction and related illnesses
What is ‘Regenerative Medicine’?

“process of replacing, engineering or regenerating cells, tissues or organs to restore or establish normal function”

What is ‘Regenerative Medicine’?

e.g.
- Bone marrow transplants
- Pure stem cell transplants
- Tissue grafts (skin, cornea)
- Bioengineered organs (ear, bladder)
- Gene therapy (DNA, RNA)

Skin Graft
Regenerative Medicine

The guiding motto in the life of every natural philosopher should be, 'Seek simplicity and distrust it.” -- Alfred North Whitehead
Regenerative Medicine

Potential solutions

One drug, one target
Regenerative Medicine

Potential solutions

One drug, one target vs Regen Med approach, multiple targets
Regenerative Medicine

Potential solutions: One drug, one target vs Regen Med approach, multiple targets

Complex disease problems: Autoimmune diseases, heart failure, neurodegenerative diseases (e.g., Alzheimer’s)
Goals of this new discipline

- Address natural complexity
- Leverage natural healing ability
- Employ interdisciplinary approaches
- Improve efficacy, benefit : cost, and safety
Goals of this new discipline

- Address natural complexity
- Leverage natural healing ability
- Employ interdisciplinary approaches
- Improve efficacy, benefit : cost, and safety

Veterinary medicine
Regenerative Medicine

‘...potentially disruptive technology...’ replacing major pharmaceuticals, devices, prostheses.

Guiding principles of regenerative medicine

“Study nature, love nature, stay close to nature. It will never fail you.”

Frank Lloyd Wright
Guiding principles of regenerative medicine

• Evolution

• Developmental biology

• Regeneration biology in animals

http://elitetrack.com/evolution-strength-training-personal-perspective-51-years-experiences-part-three
Guiding principles of regenerative medicine

Regeneration in the animal kingdom

Urodeles

Lizards

Arthropods

Coelenterates

Echinoderms
Guiding principles of regenerative medicine

- Fish
  Tail, fins, organs

- Mammals
  Cervid antlers

Yin, Current Opin Genetics and Develop 2008
Gyurjan, Mol Genet Genomics 2007
Humans and veterinary species: have evolved with limited regenerative capacity

**Regeneration**
- Tips of digits
- Blood system
- +/- Ear holes

**Quasi-regeneration (compensatory growth)**
- Liver, kidney, and lung in children only
Salamanders – ‘experts’ at regeneration

Mexican salamander
Order: Caudata (urodeles):

• Shed tail or leg to get away from predator (autotomy)
• Regenerate limbs, eye, spinal cord, tail, skin, etc.
• Retain many juvenile traits (neoteny)

http://www.arkive.org/axolotl/ambystoma-mexicanum/image-G19629.html
Key events in re-growing a limb (in salamanders)

1. Rapidly close skin defect (<1 d)
2. Quell inflammation, remodel
3. Stem cells arise, divide
4. Specialized growth factors pattern growth
‘Next generation regeneration’ – which approach? how will these be tested?

- Stem cells
- Biological chemicals (DNA, RNA, proteins)
- Bio-mimetic scaffolds
- Nanoparticles
Initial testing in the dish

- Cells (including stem cells)
- ‘Organs on a chip’
- Organoids


http://www.ted.com/talks/geraldine_hamilton_body_parts_on_a_chip.html
Animal models

- Zebra fish
- Rodents
- Non-human primates
- Large animals
Naturally occurring disease, i.e. veterinary patients, are underutilized in biomedical research

- Dogs (78M), Cats (80M), Horses (6M), Birds (8M)
- Veterinarians: 67,000 (AVMA 2008)
- University Teaching Hospitals: >27
  
  *e.g. Caseload @ Tufts University: >40,000/yr*

- Specialists, referral centers, clinical trial consortium
## Mouse vs. Dog vs. Human

<table>
<thead>
<tr>
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<th>Mouse</th>
<th>Dog</th>
<th>Humans</th>
</tr>
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<tbody>
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<td>environment</td>
<td>-</td>
<td>+++</td>
<td>+++</td>
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<tr>
<td>life expectancy</td>
<td>2-3 yr</td>
<td>~15 yr</td>
<td>76-81</td>
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<tr>
<td>chromosomes</td>
<td>40</td>
<td>78</td>
<td>46</td>
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<tr>
<td>genes</td>
<td>23,000</td>
<td>20,000</td>
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<td>#base pairs</td>
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<td>~80-90% (100%)</td>
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<tr>
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<td>0.02 kg</td>
<td>5-50 kg</td>
<td>70 kg</td>
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<tr>
<td>anatomy</td>
<td>+</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>physiology</td>
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<tr>
<td>genetic diversity</td>
<td>+</td>
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<td>natural diseases</td>
<td>+/-</td>
<td>+++</td>
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<td>+</td>
<td>+++</td>
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</tr>
<tr>
<td>patient management</td>
<td>-</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>therapeutic responses</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
</tr>
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</table>

### 'Sister species'

- Mouse vs. Dog: ~65-75% DNA sequence similarity
- Dog vs. Humans: 100% DNA sequence similarity
## Mouse vs. Dog vs. Human

<table>
<thead>
<tr>
<th>Trait</th>
<th>Mouse</th>
<th>Dog</th>
<th>Humans</th>
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<tbody>
<tr>
<td>Environment</td>
<td>-</td>
<td>+++</td>
<td>+++</td>
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<tr>
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<td>+</td>
<td>+++</td>
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10,000-12,000 years ago

'Sister species'

10% - 12,000 years ago
## OMIA - Online Mendelian Inheritance in Animals

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<thead>
<tr>
<th>Category</th>
<th>dog</th>
<th>cattle</th>
<th>cat</th>
<th>sheep</th>
<th>pig</th>
<th>horse</th>
<th>chicken</th>
<th>goat</th>
<th>rabbit</th>
<th>Japanese quail</th>
<th>golden hamster</th>
<th>Other</th>
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<tr>
<td>Total traits/disorders</td>
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<td>444</td>
<td>318</td>
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<td>221</td>
<td>208</td>
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<td>180</td>
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<td>96</td>
<td>50</td>
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<td>1087</td>
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<td>Mendelian trait/disorder, key mutation known</td>
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<td>87</td>
<td>46</td>
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<td>7</td>
<td>9</td>
<td>3</td>
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<td>37</td>
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<td>15</td>
<td>269</td>
<td>1386</td>
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</table>

http://omia.angis.org.au/home/
Are we different or just the same?
Genetic basis for disease

DNA $\rightarrow$ messenger RNA $\rightarrow$ protein...cells, tissues

coding

https://commons.wikimedia.org/wiki/File:DNA_simple2.svg
Genetic basis for disease

DNA $\rightarrow$ messenger RNA $\rightarrow$ protein...cells, tissues

- Abnormal proteins
- Diseased organs

sequence variations or mutations

https://commons.wikimedia.org/wiki/File:DNA_simple2.svg
Genetic basis for disease

- Human, canine genome projects
- Genome wide association study (GWAS)

Genetic basis for disease → Risk, Cures
Genetic basis for disease

- Human, canine genome projects
- Genome wide association study (GWAS)

Genetic basis for disease → Risk, Cures

- Type II diabetes
- Parkinson’s
- Heart disorders
- Obesity
- Crohn’s disease
- Prostate cancer
- Response to anti-depressant medication

Natural model of human ALS form, 2% (SOD1 mutation)

Spinal cord of Corgi with DM
Normal dog

Awano PNAS 2009
Genetic basis for disease

- Human, canine genome projects
- Genome wide association study (GWAS)

However, majority of GWAS studies yield links between disease and non-coding DNA (previously referred to as ‘junk DNA’).
Genetic

DNA $\rightarrow$ messenger RNA $\rightarrow$ protein...cells, tissues

coding

https://commons.wikimedia.org/wiki/File:DNA_simple2.svg
Genetic vs. Epigenetic

DNA → messenger RNA → protein...cells, tissues

coding

DNA Methylation
Histone modification

DNA → microRNA
long non-coding RNA
piwi RNA

non-coding
Our DNA is 98% non-coding, but not junk

- Human, canine genome projects
- Genome wide association study (GWAS)

DNA coding for proteins

98% genome (DNA) non-coding
Epigenetics: nature’s feedback system

- DNA → messenger RNA → protein...cells, tissues
- DNA Methylation
- Histone modification

Silence coding genes

- non-coding
- microRNA
Epigenetics: 
nature’s feedback system

DNA → messenger RNA → protein...cells, tissues

Silence coding genes

DNA Methylation
Histone modification

non-coding

DNA

microRNA

• Evolution
• Anatomy, physiology
• Expression of traits (coat color)
• Natural Diseases
Epigenetics

Gene ↔ Environment

- Phenotype (size, shape, coat, color, etc)
- Many diseases of public health importance
Exploiting epigenetic tools to treat heart disease

Zebrafish can regenerate 20% heart muscle, a mouse or human cannot...

http://www.devbio.biology.gatech.edu/?page_id=398

...however, the mouse heart can be epigenetically reprogrammed to turn on heart regeneration.

http://www.devbio.biology.gatech.edu/?page_id=398

marked reduction in scar!!

Aguirre et al., 2014, Cell Stem Cell 15, 589–604
Which strategy is most effective? cost effective?

- Stem cells
- Biological chemicals (DNA, RNA, proteins)
- Bio-mimetic scaffolds
- Nanoparticles
Stem cells

• Fertilized embryo – **totipotent** – *whole body*

• Embryonic stem cells (ESC) – **pluripotent** – *all cell types*

• Induced pluripotent stem cells (iPSC) – **pluripotent** – *all cell types*

• Adult stem cells – **multipotent** – *limited range of cell types*
Stem cells

• Fertilized embryo – totipotent – whole body

• Embryonic stem cells (ESC) – pluripotent – all cell types

• Induced pluripotent stem cells (iPSC) – pluripotent – all cell types

• Adult stem cells – multipotent – limited range of cell types
Unlocking pluripotency to make cell replacements

Ma et al.  Circ Res. 2013;112:562-574
Advantages of reprogramming to pluripotency

- Requires only a blood or skin sample
- No immune response to your self (?)
- Basic studies of development, toxicology, etc
Induced pluripotent stem cells and reproduction… e.g., saving endangered species

Inducing pluripotency in somatic cells from the snow leopard (Panthera uncia), an endangered felid
Multipotent mesenchymal stem cells (MSC) are currently the most widely used stem cells for therapies

- Derived from **uncontroversial** sources
- **No epigenetic reprogramming**
- Naturally **pro-regenerative**
- Evidence they **extend lifespan** Kim et al STEM CELLS TRANSLATIONAL MEDICINE 2015;4:1–11
- >289 clinical trials in humans vs. 1 for iPSC (Japan) and 2 for ESC (clinicaltrials.gov)
Mesenchymal stem cells (MSC) make many chemicals and nanoparticles with potent epigenetic signals.

STEM CELLS

Nanoparticle from canine MSC

Canine chorionic MSCs EVs (TEM by Vicky Yang)

Umbilical cord and placental tissue as source of stem cells and exosomes (nanoparticles)

• Underutilized, discarded tissue source

• Pre-natal (‘young’) stem cell features (multipotency, pluripotency in many reports)

• Potent ‘pro-regenerative signals’

• Animals: Identical siblings (2-10...)
General approach

Disease of:
heart
brain
gut
kidney
lung, etc

Epigenetic corrections
(umbilical cord tissue MSC, nanoparticles, genetics)

Epigenetic abnormalities

Biomarkers
General approach

Mitral valve disease

Epigenetic abnormalities:
↓ miR-29b, -30a in valve cells

↑ miRNA 29b, 30a derived from umbilical MSC, nanoparticles, genetic sources

Biomarkers
↓ blood levels of miR-29b, -30a

Human and rodent model data (Beth Israel, Dr. Saumya Das)
General approach – getting more personal…

- **Mitral valve disease**
  - Epigenetic abnormalities: ↓ miR-29b, -30a in valve cells
  - ↑ miRNA 29b, 30a derived from umbilical MSC, nanoparticles, genetic sources
  - Biomarkers: ↓ blood levels of miR-29b, -30a

THERANOSTICS

*Human and rodent model data (Beth Israel, Dr. Saumya Das)*
Process of developing clinical trial in companion animals

- Identify problem
- Articulate vision
- Scientific rationale
- Design study
- Study review
- Funding
- Recruit patients
- Screen patients
- Test / Treat patients
- Monitor patients
- Review results
Process of developing clinical trial in companion animals

- Identify problem
- Articulate vision
- Scientific rationale
- Design study
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- Recruit patients
- Screen patients
- Test / Treat patients
- Monitor patients
- Review results
Novel approach to treatment of patients after CPR
(Drs. Elizabeth Rozanski and Vicky Yang – Cummings School)

• Identify problem → CPR* success rate is very low in humans/animals
• Vision → CPR outcome can improve by protecting brain, heart
• Rationale → Stem cells shown to improve CPR outcome in rats
• Design study → Identify molecular disturbances thus potential targets in blood after CPR in dogs

*Cardiopulmonary Resuscitation
Biomarkers that appear in blood after CPR (in dogs)

<table>
<thead>
<tr>
<th>Groups:</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>CPR dogs:</td>
<td>6 dogs that survive CPR for at least 1 hr</td>
</tr>
<tr>
<td>ICU Controls:</td>
<td>6 dogs in ICU</td>
</tr>
<tr>
<td>Healthy controls:</td>
<td>6 healthy young Beagles</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Intervention:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Sampling:</td>
<td>1 and 6 hrs after CPR (return to spontaneous circulation)</td>
</tr>
</tbody>
</table>

| Owner consent: | prior to 1 hr blood sample (above) |
| Owner incentive: | Reimbursement for blood tests that would normally be taken during this period. |

| Endpoints: | Survival, neurologic outcome, and biomarkers (microRNA) |
Naturally occurring diseases shared with humans (dogs, cats) - candidates for new approaches

- Heart
- Neurodegenerative
- Stroke
- Epilepsy
- Alzheimer’s / dementia
- Auto-immune (skin, brain, etc)
- Diabetes
- Asthma
- Arthritis
- Cancer
- Disc degeneration
- Obsessive-compulsive disorder
- Age-related syndromes
Regenerative medicine / stem cell trials at Cummings

http://sites.tufts.edu/vetclinicaltrials/regenerative-medicine-stem-cell-trials/

**Stem cell trials in canine patients for:**
- Mitral valve disease and associated heart failure – **open**
- ARVC – heart disease that causes sudden death - **open**
- Allergic (atopic) dermatitis - **open**
- Inflammatory bowel disease – **open**
- Perianal fistulas / fistulizing Crohn’s Disease- **open**
- Auto-immune kidney disease - **open**
- Spinal cord compression due to disc degeneration – **pending**

**Biomarker studies in canine patients:**
- Biomarkers relating to CPR – **open**
- Biomarkers for mitral valve disease and ARVC - **open**
Evolving paradigm in regenerative medicine at Cummings

Referring Veterinarians

Clinical trial center

Clients-animals

Institutions of medicine (AVMA, AAVMC)

Philanthropic Foundations (e.g. Shipley Foundation)

NIH

Industry (Biotech, Pharma)

Education DVM, MS, PhD

Administration

databases
‘One Health’: intersection: humans, animals, & environment
Veterinary medicine – proof of principle, safety, reproducibility, translation to humans

- Public health problems
- Basic Science
- Economic drivers
- Reproducibility
- Scientific integrity, ethics and public policy
- New Therapies, Tests
- Regulation
- Safety, reliability
“What could be more gratifying than to discover, describe, and explain some basic principle that no human being has ever understood before? This is the stuff of true science. Those societies that foster and harness that passion will be the prosperous, knowledge-based economies of the future.”

*The Beginners Guide to Winning the Nobel Prize,*
*by Peter Doherty*
Discussion

- Veterinary and human doctors working together
- Better understanding of natural disease models
- Employing natural disease models in biomedical research
- Better outcomes for human and animal patients
http://vetsites.tufts.edu/rml/

**Regenerative Medicine Laboratory**
Vicky Yang, DVM, PhD: Research Assistant Professor, Assistant Director
Alisha Gruntman, DVM, PhD (candidate) – Assistant Professor
Kristen Thane, DVM: Post-Doctoral Scholar
Sarah Crain, DVM, MS: PhD Candidate
Airiel Davis, BS: Research Assistant
Dawn Meola, BS: Research Assistant (Clinical Trial Supervisor)
Christine Juhr, BS: Large Animal Technician
Diane Welsh, BS: Clinical Trial Technician

**Cummings School Faculty:** Drs. Elizabeth Rozanski (ECC), Lluis Ferrer (Derm), Andrea Lam (Derm), Cyndie Webster (GI), Mary Labato (Renal), Dominik Faissler (Neurology), Jennifer Graham (Exotics), Suzanne Cunningham and John Rush (Cardio), Nick Robinson and Arlin Rogers (Pathology)

**Collaborators outside Cummings School:**
Edward Ingenito (Brigham and Womens)  Carla Kim (Childrens, Harvard Stem Cell Inst.)
Dan Weiss (Univ VT, Pulmonary Center)  Robert Lanza (Advanced Cell Technology)
Alan Fine (BU Medical Center)  Diana Bianchi (Tufts Medical Center)
Julia Paxson (College of Holy Cross)  Saumya Das, MD, PhD (Beth Israel)
Alex Mitsialis (Children’s Hospital)  Jason Aliotta (Brown Univ, Rhode Island Hospital)
Questions?

I’ll have a double-shot!

$STEMBUCKS$
Over 1 billion cells served per customer

“Bring me a stem cell.”