Stem Cells

UNSW AUSTRALIA

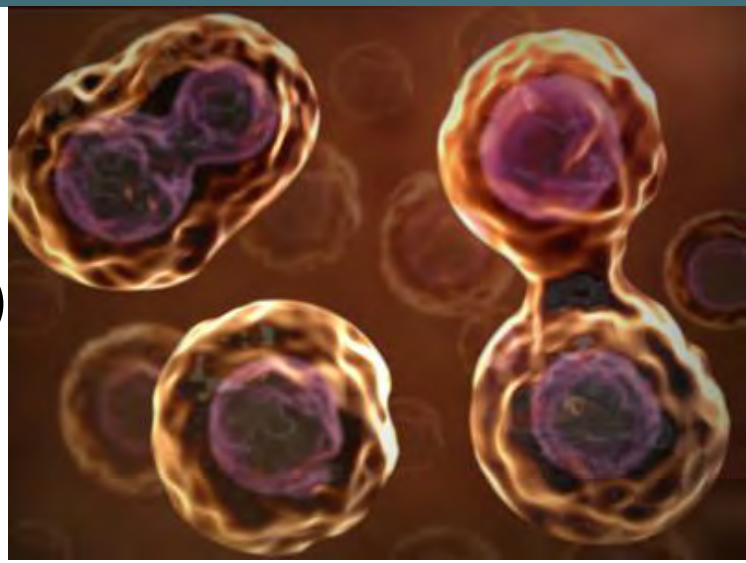
Never Stand Still | Medicine | School of Medical Sciences

Prof. Edna Hardeman (e.hardeman@unsw.edu.au)

Neuromuscular and Regenerative Medicine Unit

School of Medical Sciences

UNSW



Mesenchymal stem cells
Photo courtesy of Mesoblast

Stem Cells

At the end of this lecture you should be able to:

- Define what is meant by the term ‘stem cell’
- Understand the difference between embryonic and adult stem cells
- Know the landmark discoveries in stem cell research
- Understand the ‘decision-making steps’ of a stem cell
- Understand where stem cells reside – the stem cell niche
- Understand the advances in biomedical research due to stem cells
- Understand the therapeutic promise and limitations of stem cells

Stem Cells – The Definition

Stem cell = An unspecialised cell characterised by the ability to self-renew by mitosis and the capacity to give rise to various specialised/differentiated cell types.

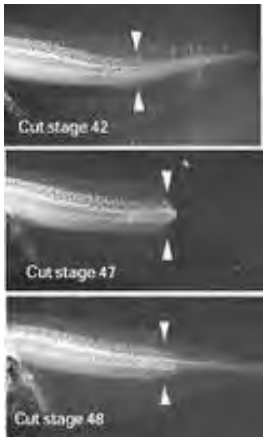
Evidence for the Existence of Stem Cells

Regeneration in lower organisms

Crayfish
claw, leg

Earthworms
body

Tadpoles
tail



Newts
leg
www.youtube.com/watch?v=4exOh6swPp8

Planaria (flatworm)
whole organism
http://www.youtube.com/watch?v=vXN_5SPBPtM

Indirect Evidence of the Existence of Stem Cells

Regeneration of adult tissues in humans

LIVER – regrows 75%

BLOOD – always renewing

BONE – repairs breaks

SKIN – always renewing

MUSCLE – repairs damage [crush
tears, cuts, genetic diseases (dystrophies)]

Direct Evidence of the Existence of Stem Cells

Regeneration of blood cells in humans

1960s - Till & McCulloch (Canada)

- 'Fathers of stem cell research'
- Gave lethal doses of radiation to mice that killed bone marrow
- Rescued with bone marrow transplantation
- Discovered - single cell from bone marrow → copy itself (self-renewal) → amplify numbers (transit-amplifying) → make all blood cell types
- Discovered adult blood stem cells

Stem Cells – The Definition

Stem cell = An unspecialised cell characterised by the ability to self-renew by mitosis and the capacity to give rise to various specialised/differentiated cell types.

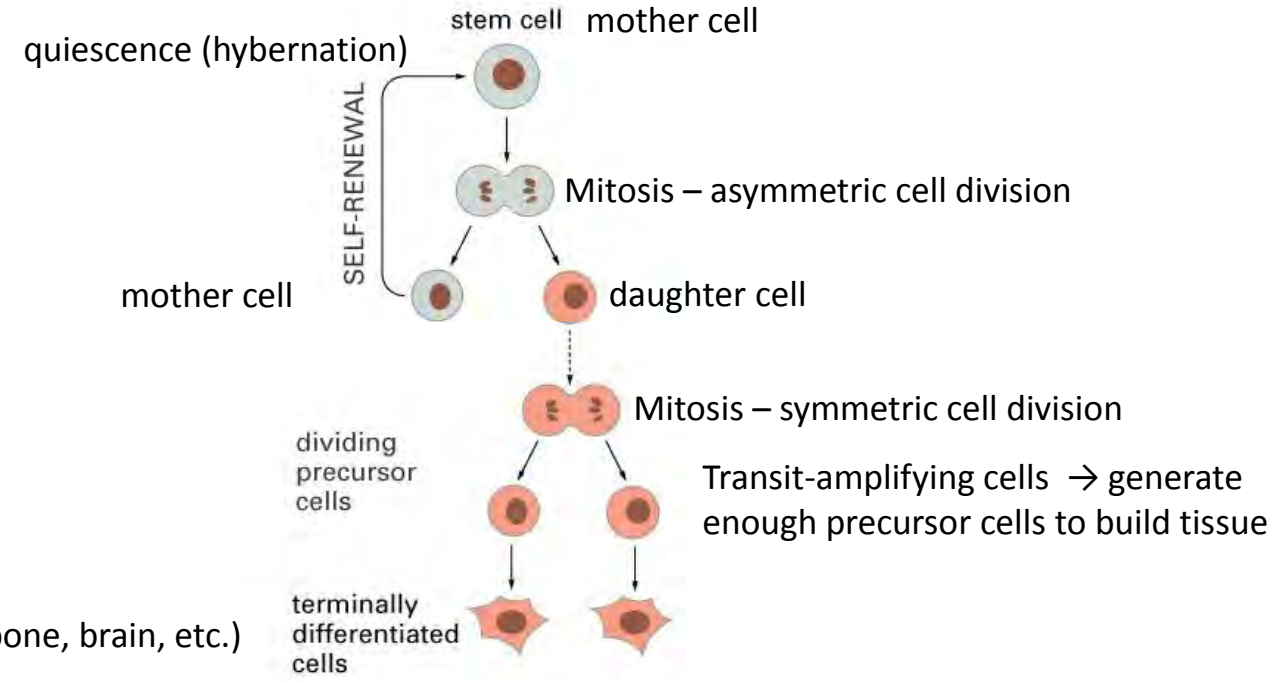
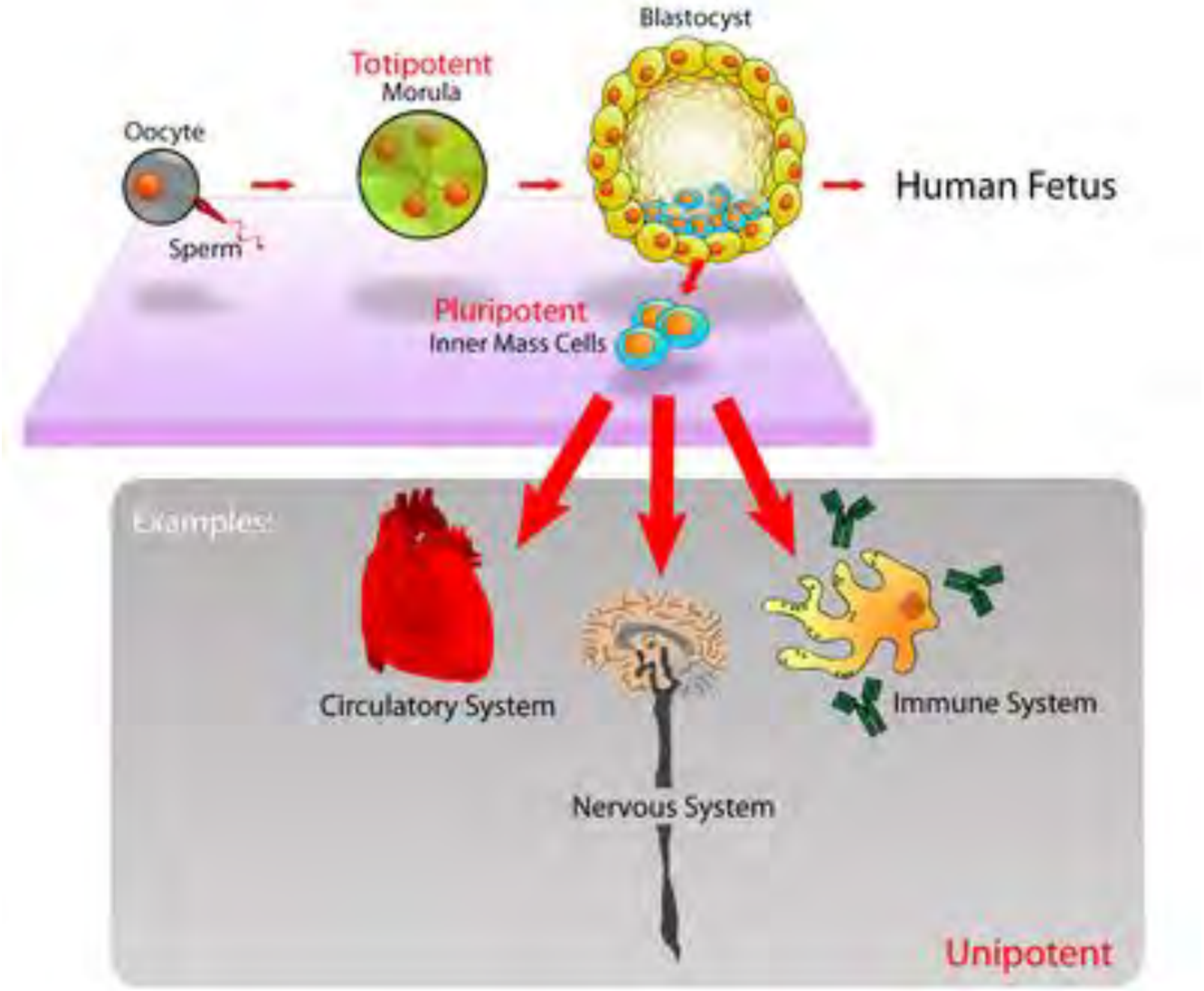


Figure 21-35 Essential Cell Biology, 2/e. (© 2004 Garland Science)

Stem Cells – Different types based on hierarchy of 'potential'



Stem Cells – Definitions of different types based on hierarchy of 'potential'

Stem cell = An unspecialised cell characterised by the ability to self-renew by mitosis and the capacity to give rise to various specialised/differentiated cell types.

- **Totipotent stem cell** = can give rise to all of the >200 cell types within the body + extraembryonic tissue (e.g. fertilised egg, embryo within the first couple of cell divisions)
- **Pluripotent stem cell** = can give rise to all of the >200 cell types within the body (e.g. inner mass cells = embryonic stem cells; induced pluripotent stem cells = iPS cells)
- **Multipotent stem cell** = can give rise to more than one cell type (e.g. haematopoietic stem cells → all adult blood cell types)
- **Uni-potent stem cell (tissue precursor)** = can only give rise to one cell type (e.g. muscle stem cell or 'satellite' cell)

Stem Cells – The Decisions

Should I remain in hibernation? = Quiescence

Should I die? = Apoptosis

Should I activate? = Proliferation

Should I self-renew? = Re-enter Quiescence

Should I divide enough times to generate a tissue? = Transit-amplification

Should I become a tissue/organ? = Differentiation

Stem Cells – The Decisions

Should I remain in hibernation? = Quiescence

Should I die? = Apoptosis

Should I activate? = Proliferation

Should I self-renew? = Re-enter Quiescence

Should I divide enough times to generate a tissue? = Transit-amplification

Should I become a tissue/organ? = Differentiation

These decisions are made in the stem cell niche.

Signalling Pathways within Cells

Important Concepts

- Signalling pathways exist within cells to coordinate specific cellular activities, e.g. cell division.
- Signalling between cells typically involves one cell providing a ‘ligand’ that interacts with a ‘receptor’ on the surface of the receiving cell.
- ‘Ligand-receptor’ interaction leads to a cascade of events in the receiving cell with a specific outcome, e.g. cell division.

Where are stem cells found in the body?

The Stem Cell Niche

Important Concepts

- Stem cells do not live in isolation; they live within a community of cells.
- There are a variety of cells in the local environment of a stem cell that comprise the ‘stem cell niche’.
- These cells ‘communicate’ with (signal to) the stem to make the right decision.

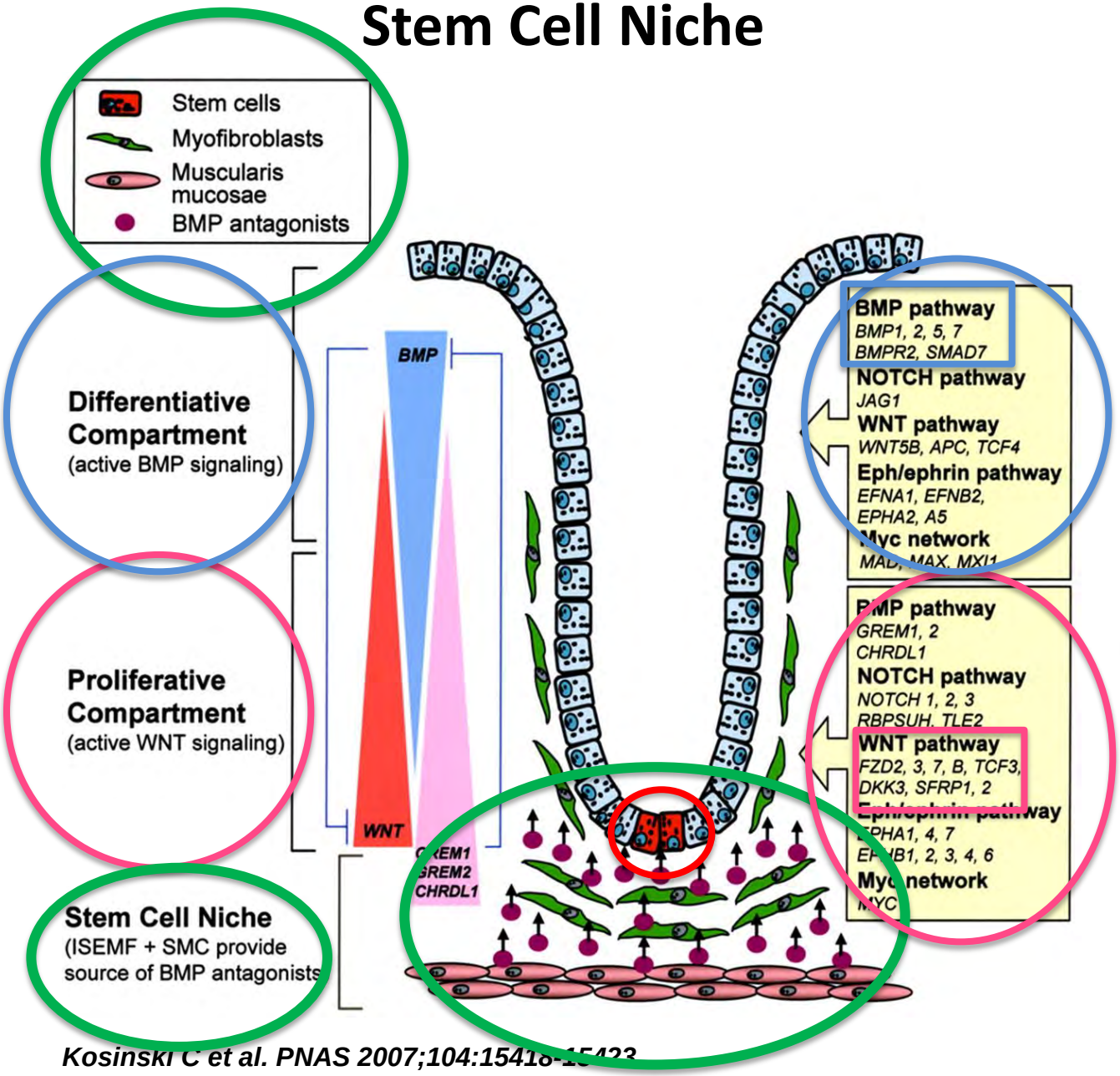
The Stem Cell Niche

Prominent Signalling Pathways

Important Concepts

- Key signalling pathways are involved in the decision-making steps in the stem cell niche.
- The same pathway can have different effects on stem cells in different tissues/organs.
- These signalling pathways are characterised by short distance communication, i.e. support cell that provides ligand is relatively close to the stem cell with its receptors.

Cell Fate Decision-Making in the Intestinal Epithelial Stem Cell Niche



Kosinski C et al. PNAS 2007;104:15418-15423

The Stem Cell Niche

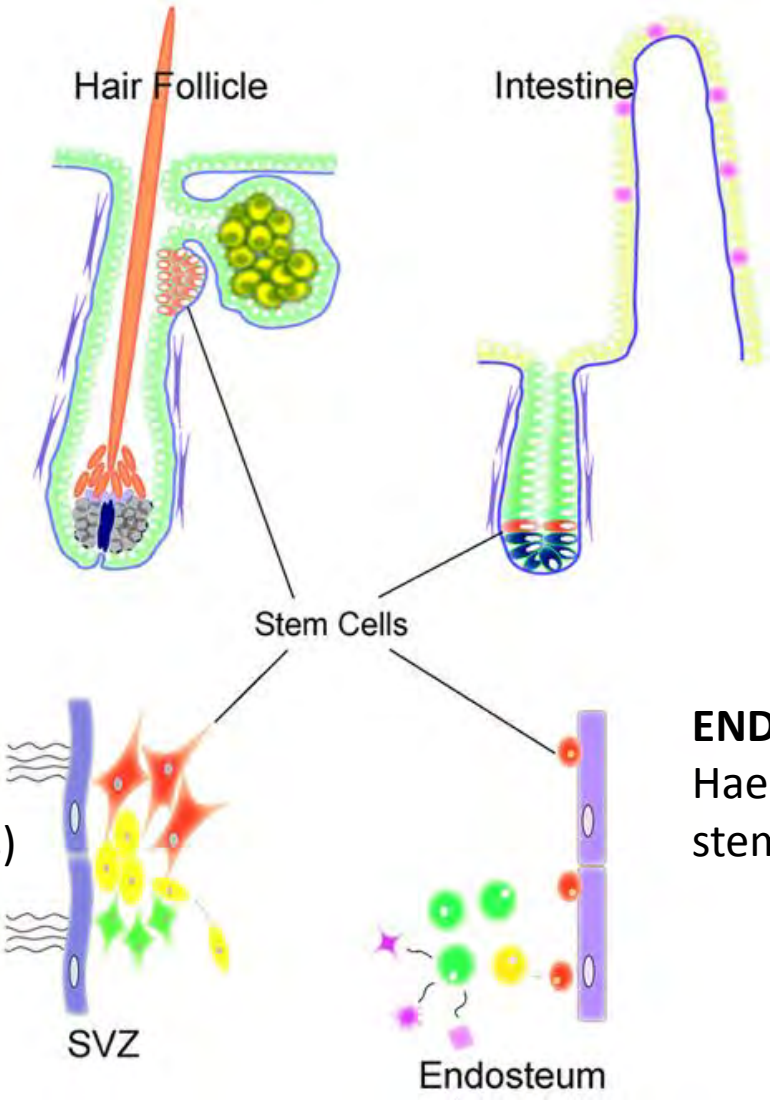
Prominent Signalling Pathways

Important Concepts

- Key signalling pathways are involved in the decision making steps in the stem cell niche.
- The same pathway can have different effects on stem cells in different tissues/organs.
- These signalling pathways are characterised by short distance communication, i.e. support cell that provides ligand is relatively close to the stem cell with its receptors.

Four stem cell niches where the same signalling pathways have different effects

SKIN
Hair follicle stem cell (HFSCs)

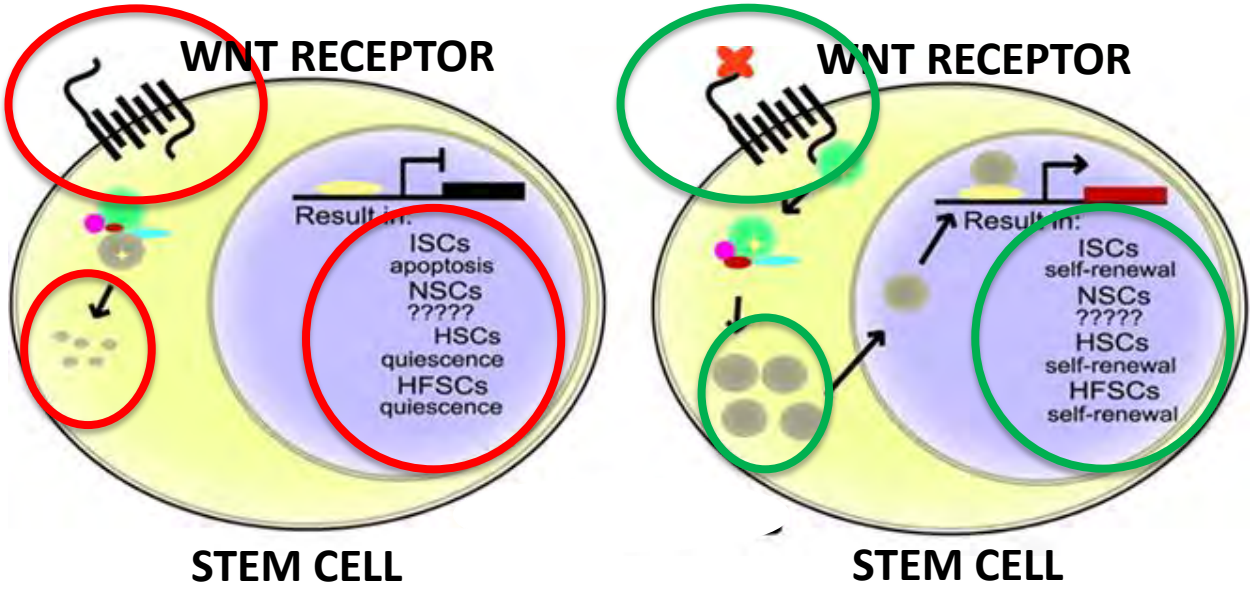


INTESTINE
Intestinal stem cell s(ISCs)

BRAIN
(sub-ventricular zone)
Neural stem cells (NSCs)

ENDOSTEAL BONE
Haematopoietic stem cells (HSCs)

Wnt Signalling



- WNT LIGAND

WNT messenger is degraded

Can't travel to nucleus

Differential stem cell fate

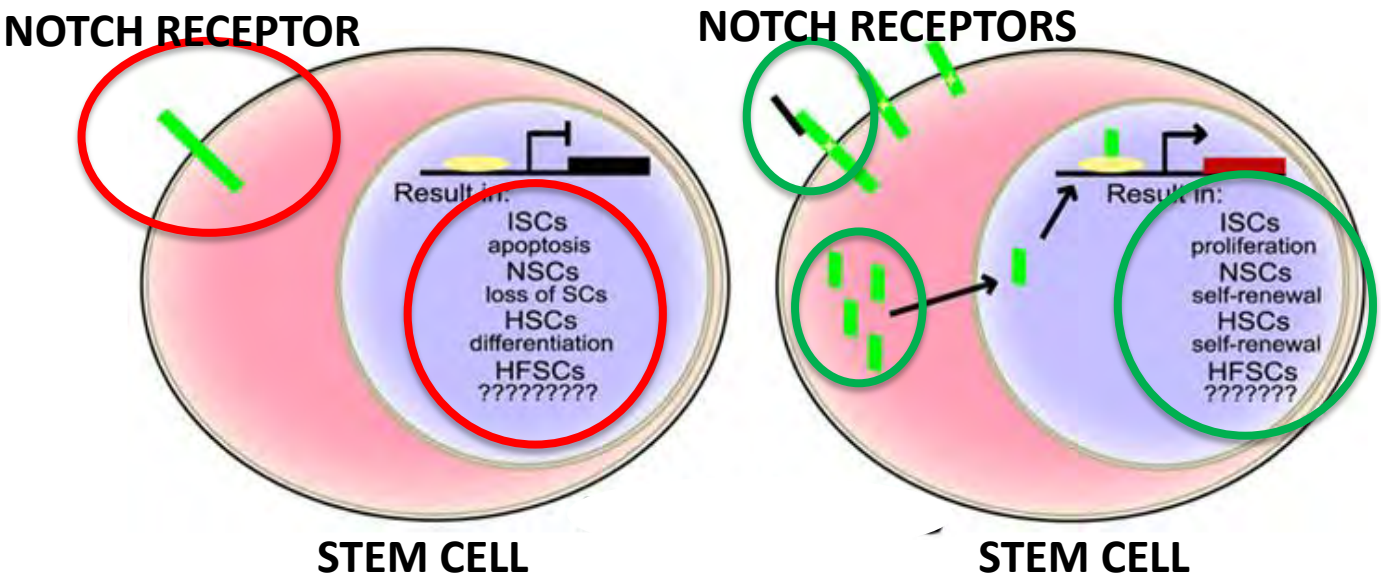
+ WNT LIGAND

WNT messenger is stabilised

Travels to the nucleus

Similar stem cell fate = self-renewal

Notch Signalling



- NOTCH LIGAND

NOTCH remains in the membrane

Can't travel to nucleus

Differential stem cell fate

+ NOTCH LIGAND

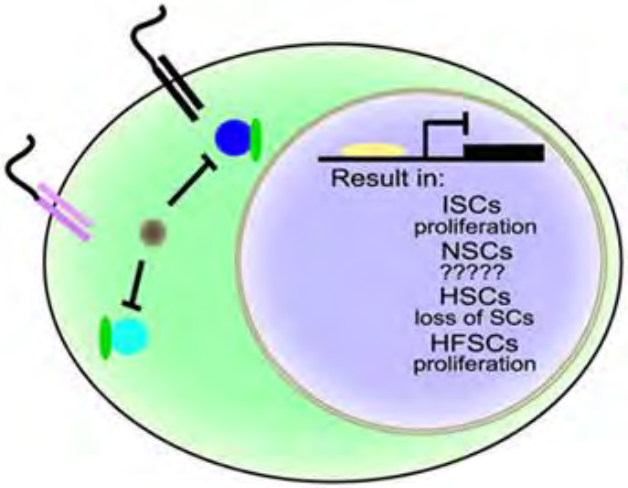
NOTCH is internalised

Travels to nucleus

Differential stem cell fate

Tgf-beta Signalling

TGF-BETA RECEPTORS



STEM CELL

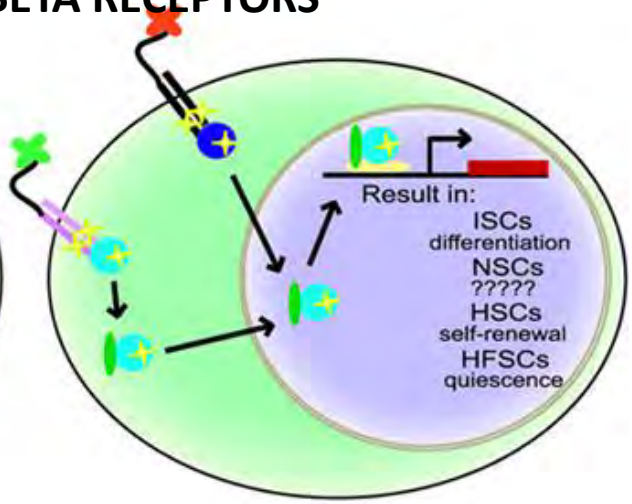
- TGF-BETA LIGAND

TGF-BETA messengers inhibited

Can't travel to nucleus

Differential stem cell fate

TGF-BETA RECEPTORS



STEM CELL

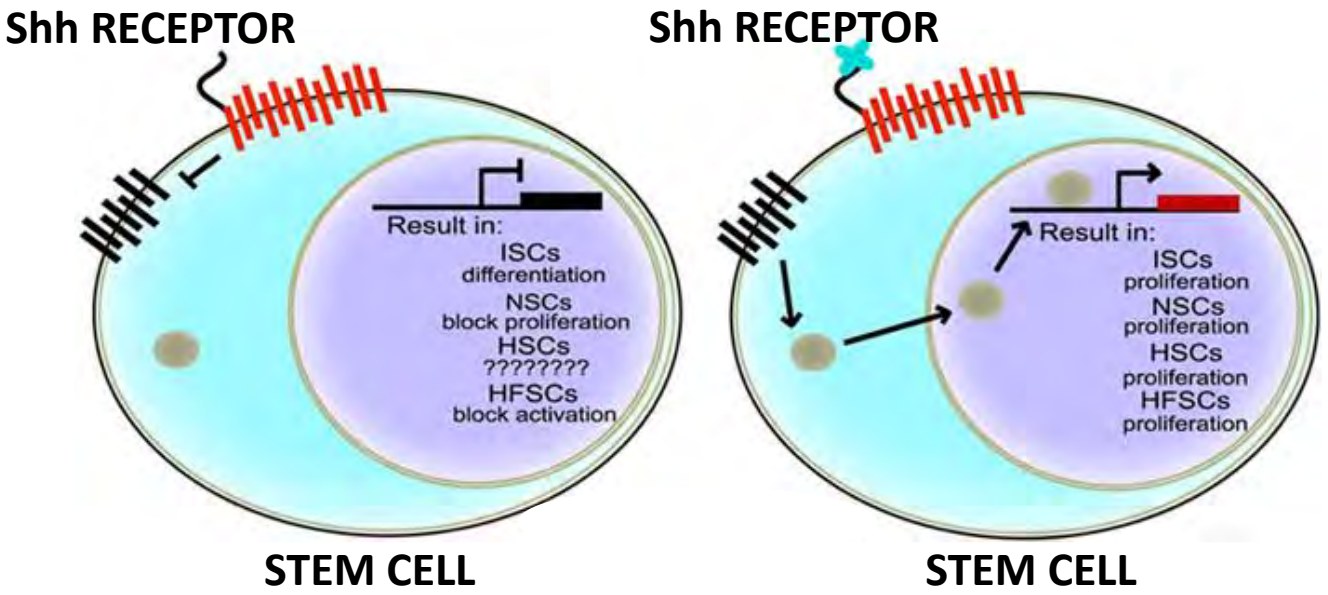
+ TGF-BETA LIGAND

TGF-BETA messengers are activated

Travel to nucleus

Differential stem cell fate

Shh (sonic hedgehog) Signalling



- Shh LIGAND

Shh messengers inhibited

Can't travel to nucleus

Differential stem cell fate

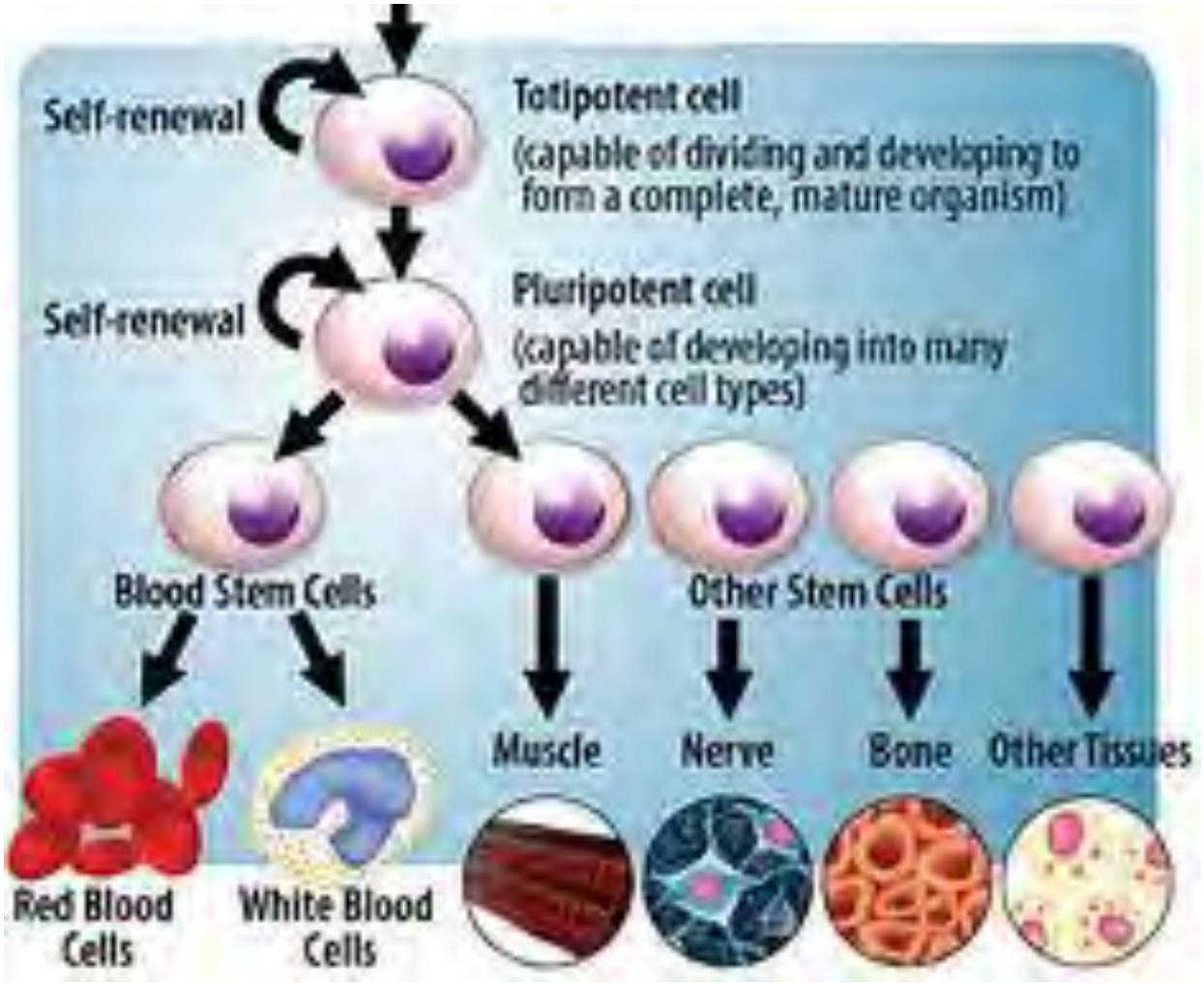
+ Shh LIGAND

Shh messengers are activated

Travel to nucleus

Similar stem cell fate

Hierarchy of Stem Cells – Progression of step-wise decisions that restrict genetic potential



How do Cells Lose Their Genetic Potential?

Important Concepts

1. Altered gene expression (most common)
   - only those genes specific for a tissue/organ are expressed
   - specific transcription factors are expressed
2. Terminal differentiation
   - loss of cell division capacity (e.g. muscle, neurons)
3. Gross DNA rearrangement or loss (rare)
   - immunoglobulin genes arise out of DNA splicing
   - mammalian red blood cells lose their nucleus

Stem Cell Decision-Making Reversal
Reprogramming

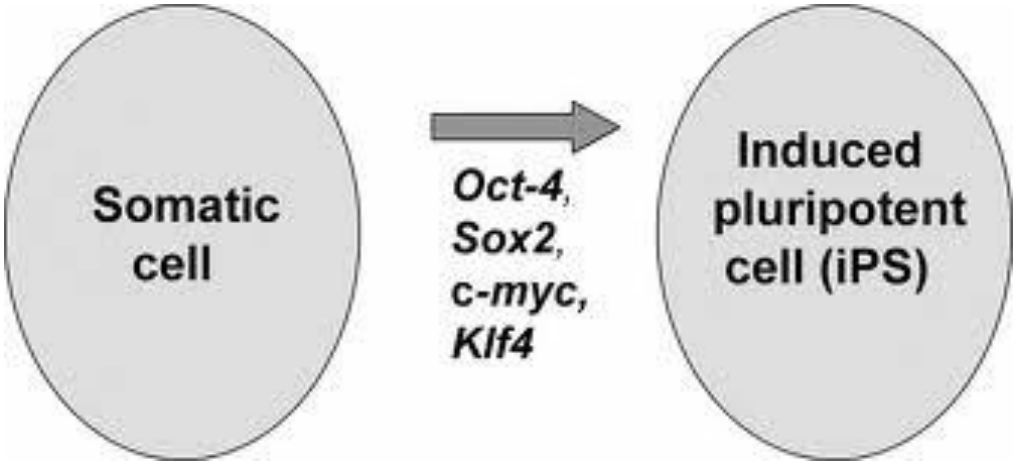
1958 – John Gurdon (UK)

- Cloned a frog – enucleated frog egg + nucleus from tadpole intestine → frog

Stem Cell Decision-Making Reversal
Reprogramming

2006 – Shinya Yamanaka (Japan)

- Turned an adult cell into an embryonic stem cell – adult cell + Yamanaka factors (Oct3/4, Sox2, c-Myc, Klf4) → embryonic stem cell



2012 Nobel Prize in Medicine

"for the discovery that mature cells can be reprogrammed to become pluripotent"

The Problems with Reprogramming

- Clones are relatively difficult to generate.
- Clones have shorter lives.
- Clones may have compromised ‘fitness’.
- DNA of iPS cells may retain modifications obtained during development.

DNA in adult cells may retain a ‘memory’ of developmental history = may retain a ‘memory’ of age. May accumulate mutations that are difficult to erase.

Stem Cells
&
Biomedical Research
&
Regenerative Medicine

Embryonic Stem Cells & Biomedical Research

Key Discoveries

1981 – Martin Evans & Matthew Kaufman (UK)
Gail Martin (USA)

- First isolated mouse embryonic stem (ES) cells from the inner cell mass of cultured blastocysts.

1989 – Mario Capecchi (USA), Oliver Smithies (USA),
Martin Evans(UK)

- Developed the technology to genetically manipulate mouse ES cells – remove genes (**KNOCKOUT MICE**), add mutated genes (**KNOCK-IN MICE**) → make mouse models of human genetic diseases

2007 Nobel Prize in Medicine

"for their discoveries of principles for introducing specific gene modifications in mice by the use of embryonic stem cells"

How to Make a KO/K-In Mouse

Manipulate **DNA** to contain specific disease-causing mutation

Harvest & culture ES cells from inner cell mass of mouse blastocyst

Transfer **DNA** w/ mutation into ES cells

DNA replaces corresponding mouse DNA (homologous recombination)

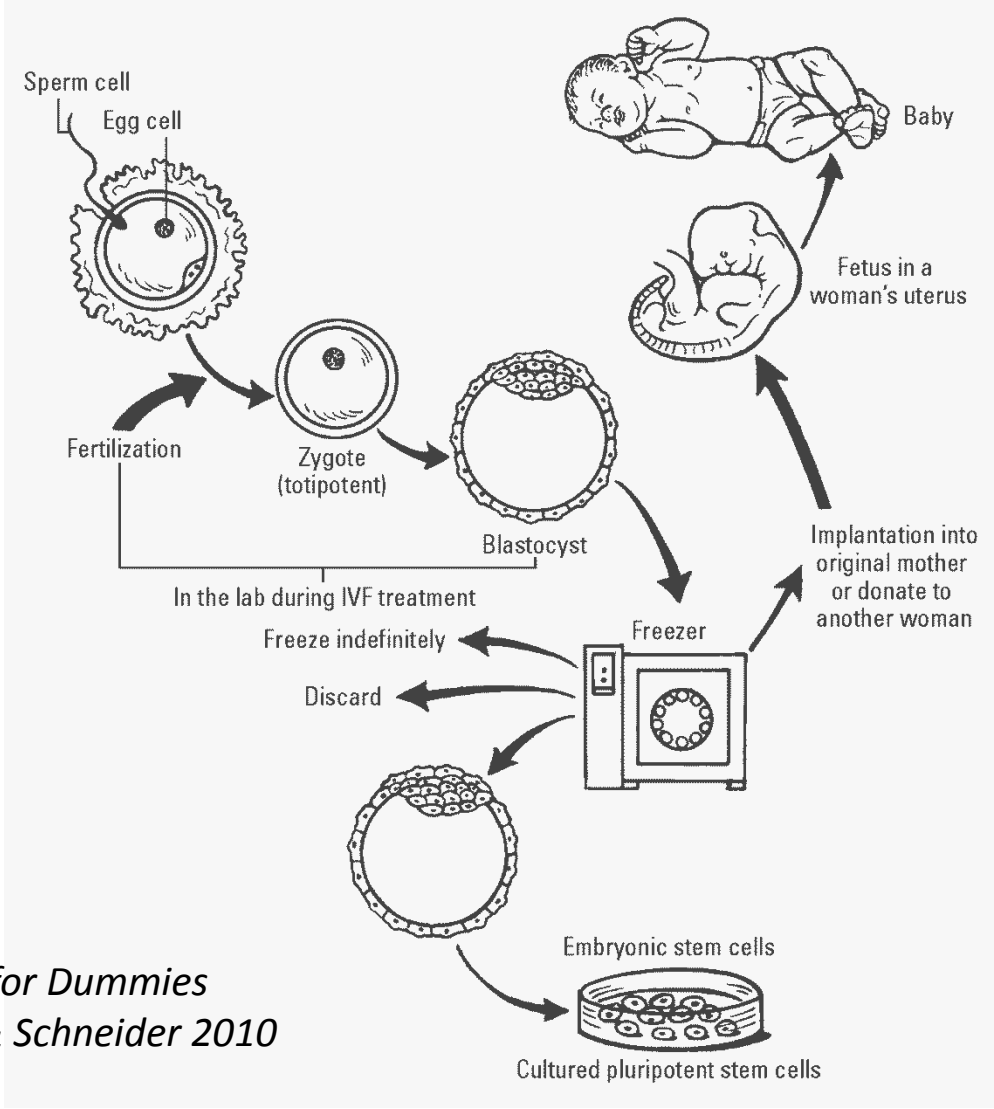
Grow only those **ES cells** with the **DNA** (selective growth media)

Transplant the **ES cells** into a blastocyst

Mouse w/ human muscle disease

Sources of Stem Cells for Research & Therapy

Pluripotent – IVF-derived Embryonic Stem Cells



Stem Cells for Dummies
Goldstein & Schneider 2010

Sources of Stem Cells for Research & Therapy

Pluripotent – Cloning-derived Embryonic Stem Cells

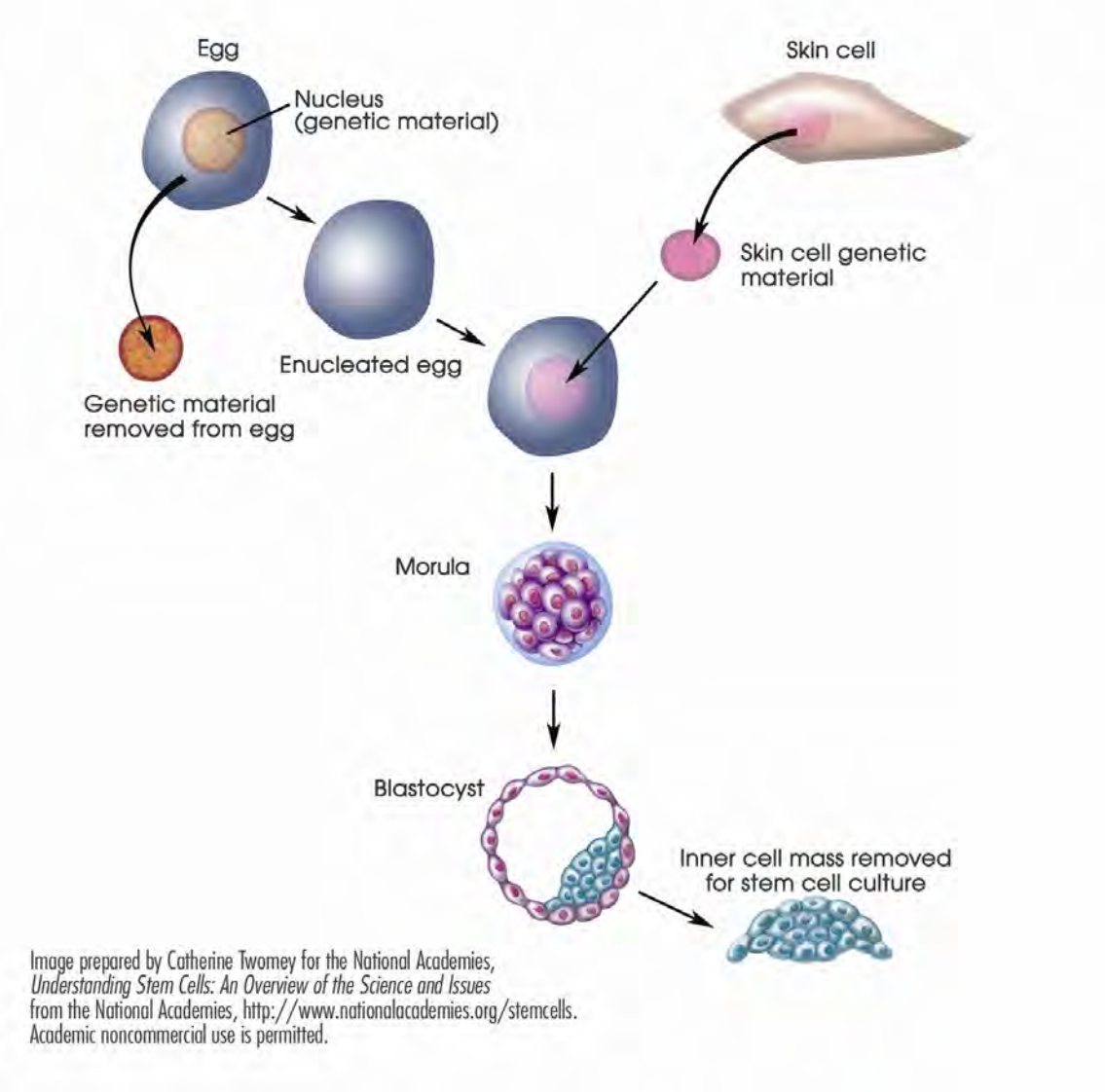
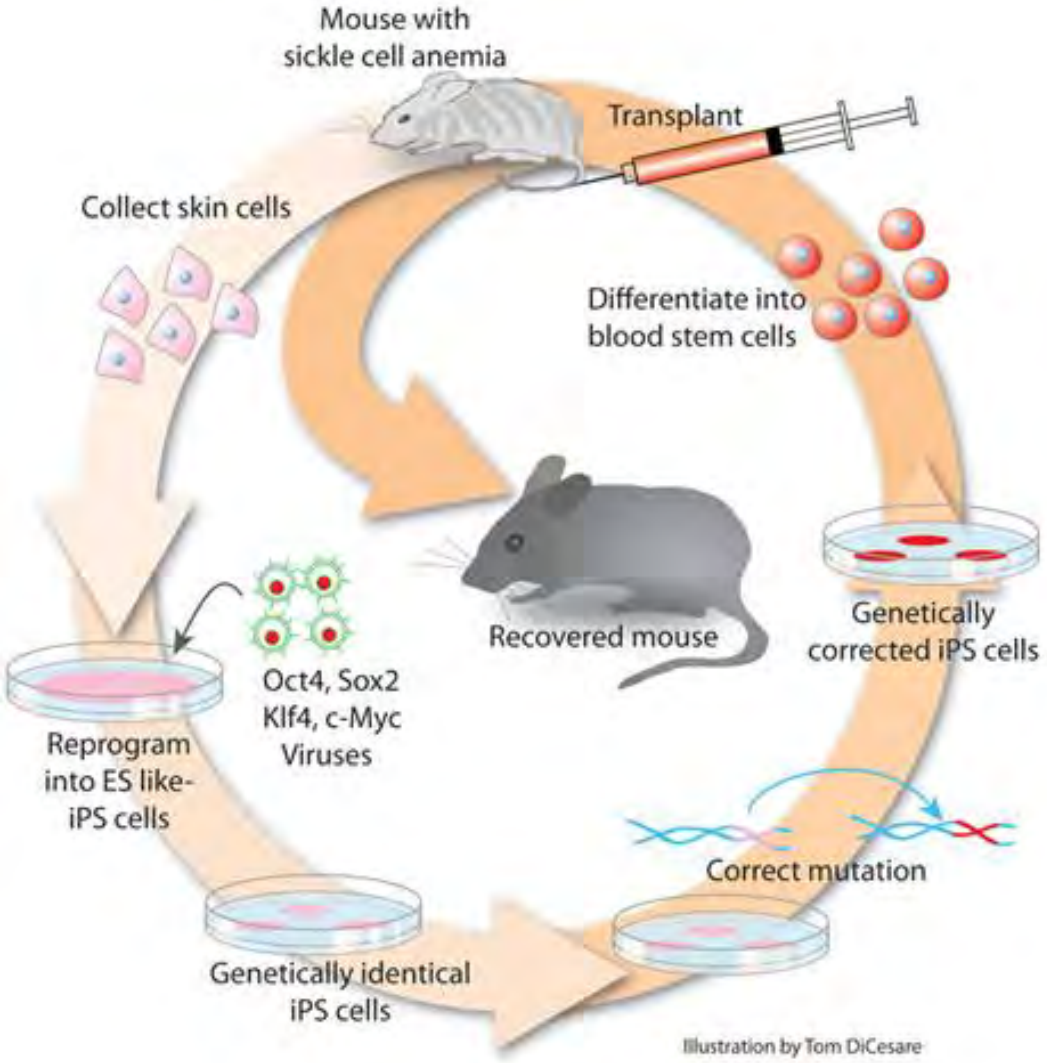


Image prepared by Catherine Twomey for the National Academies,
Understanding Stem Cells: An Overview of the Science and Issues
from the National Academies, http://www.nationalacademies.org/stemcells.
Academic noncommercial use is permitted.

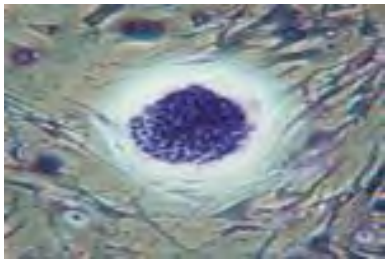
Sources of Stem Cells for Research & Therapy

Pluripotent – Induced Pluripotent Stem Cells

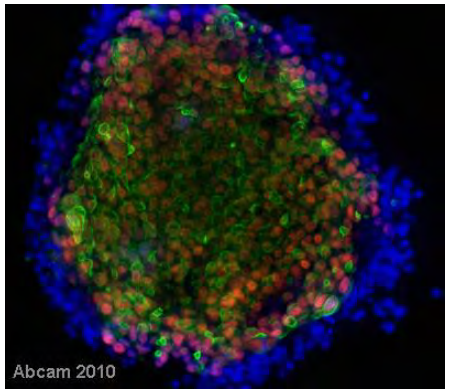


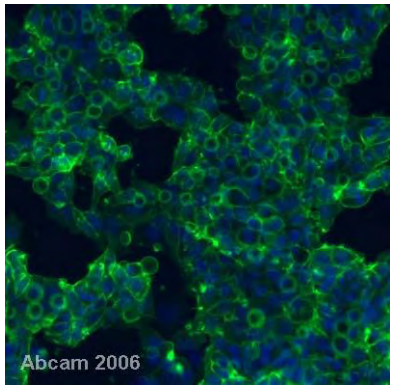
Sources of Stem Cells

Verification Markers of Pluripotent Stem Cells



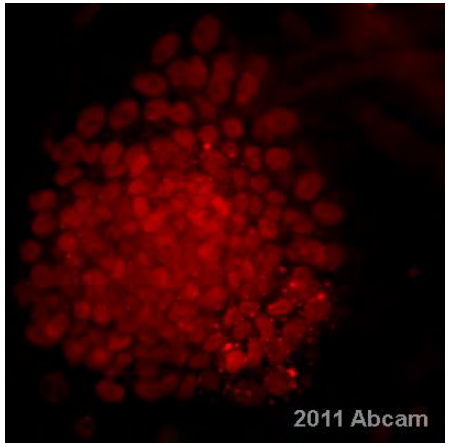
Oct4 = homeobox transcription factor; involved in embryonic patterning; critical for self-renewal

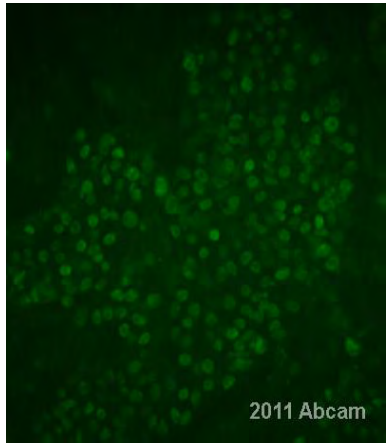




SSEA4 = carbohydrate attached to a lipid (glycolipid) found on early cleavage stage embryos

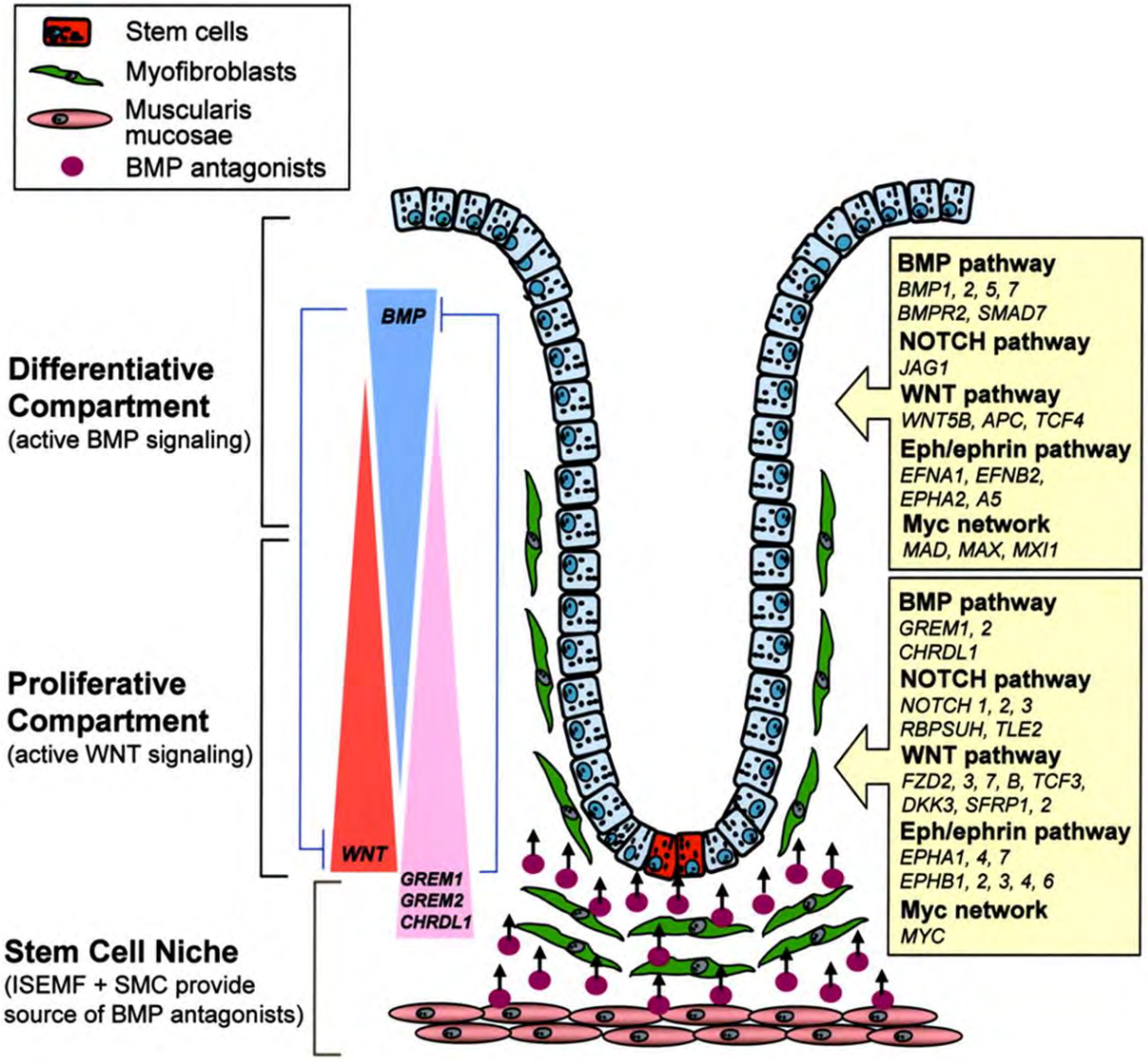
SOX2 = transcription factor; interacts with Oct4 to regulate cell cycle genes





Tra-1-60 = keratin sulfate; sulfated structural carbohydrate

Adult Stem Cells – Isolate from tissue source



Kosinski C et al. PNAS 2007;104:15418-15423

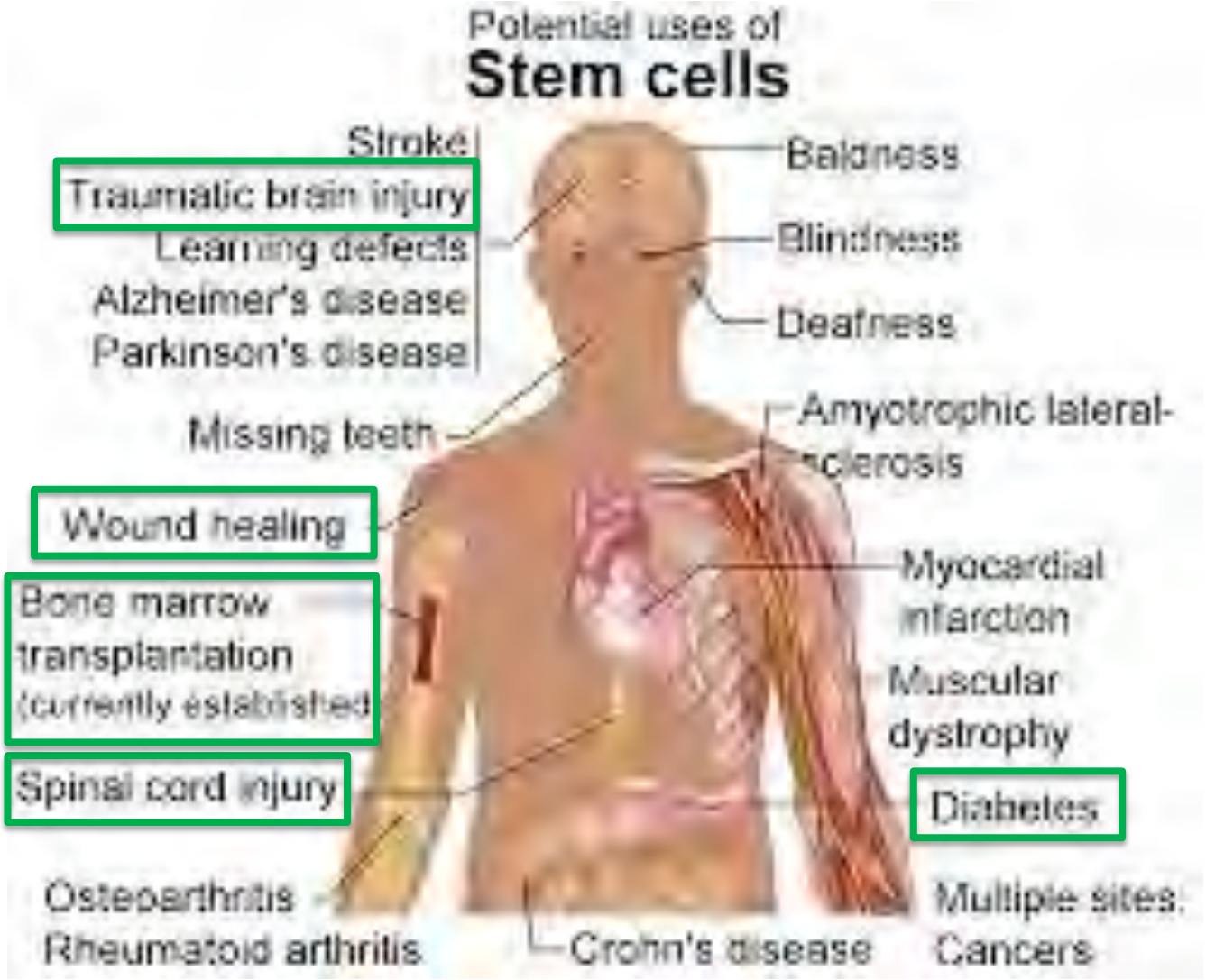
Stem Cells – The Sources

COMPARISON OF THE DIFFERENT SOURCES OF STEM CELLS

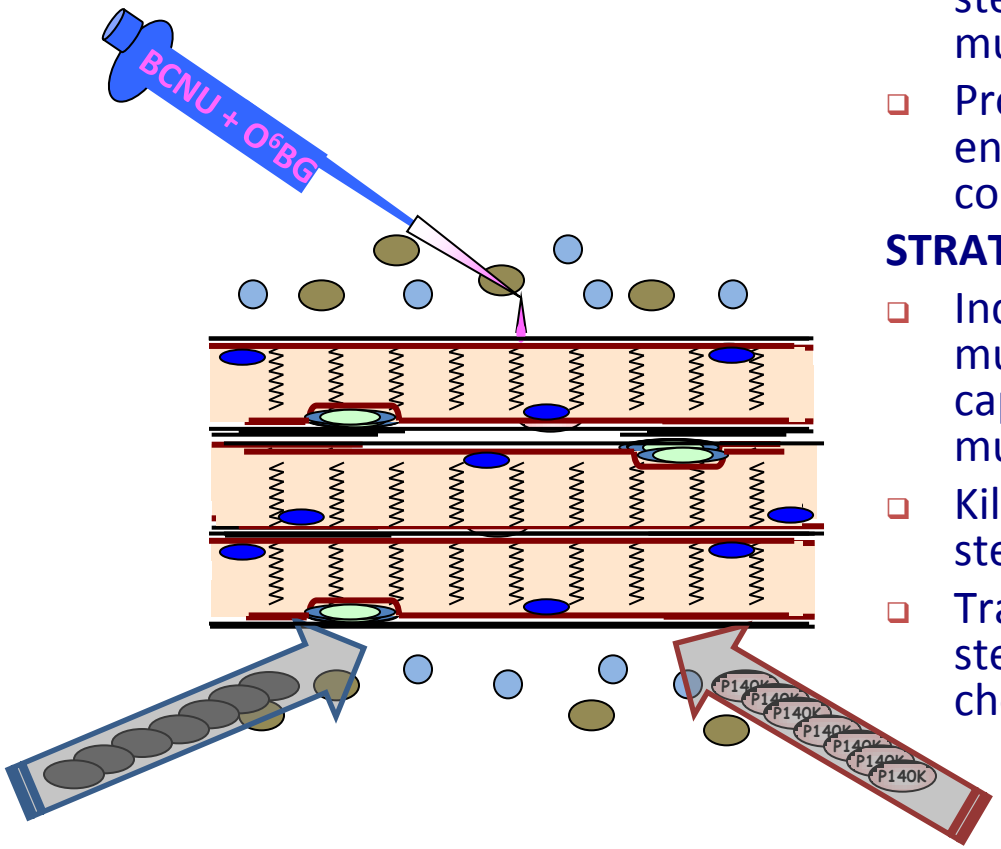
	Embryonic Stem Cells		Adult Stem Cells	iPS Cells
	 **In Vitro Fertilization**	 **Nuclear Transfer**	 **Adult Tissues**	**Reprogramming of Somatic Cell**
Attributes	• can produce all cell types • relatively easy to identify, isolate, maintain, and grow in the laboratory • large source of "excess" blastocysts from IVF clinics	• can produce all cell types • relatively easy to identify, isolate, maintain, and grow in the laboratory • stem cells may be genetically matched to patient	• demonstrated success in some treatments • stem cells may be genetically matched to patient	• **can produce all cell types** • **relatively easy to generate, maintain and grow in the laboratory** • **stem cells may be genetically matched to patient**
Limitations	• limited number of cell lines available for federally funded research • risk of creating teratomas (tumors) from implanting undifferentiated stem cells	• not yet achieved with human cells • risk of creating teratomas (tumors) from implanting undifferentiated stem cells	• produce limited number of cell types • not found in all tissues • difficult to identify, isolate, maintain, and grow in the laboratory	• **risk of creating teratomas if these are indeed true ES cells** • **may retain the age of the parent cell**
Ethical Concerns	• destruction of human blastocysts • donation of blastocysts requires informed consent	• destruction of human blastocysts • donation of eggs requires informed consent • concern about misapplication for reproductive cloning	• no major ethical concerns have been raised	• **risk of creating teratomas if these are indeed true ES cells** • **may retain the age of the parent cell**

http://thescienceofstemcells.com/home.html

Stem Cells – The Applications



Just for Fun - Enhanced Muscle Stem Cell Engraftment Strategy



- Goal = replace defective muscle stem cells that carry a disease mutation with healthy stem cells
- Problem = need to get rid of endogenous stem cells → competition for the niche

STRATEGY

- Induce regeneration in skeletal muscle through injury – it has the capacity to regenerate via its adult muscle cells
- Kill off proliferating endogenous stem cells with chemotherapy drugs
- Transplant genetically engineered stem cells that are resistant to chemotherapy

Rebuilding a muscle with chemotherapy drug-resistant adult muscle stem cells