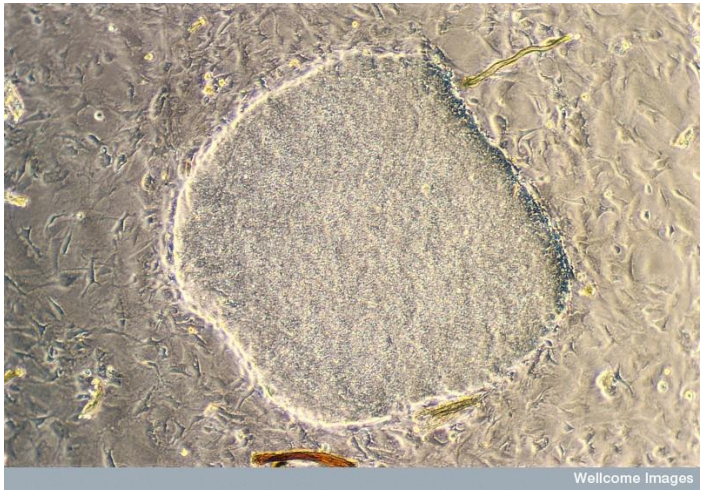
Neural stem cells: therapeutic potential



(Jenny Nichols, Wellcome Images)

What is a stem cell?

- A stem cell can self-renew

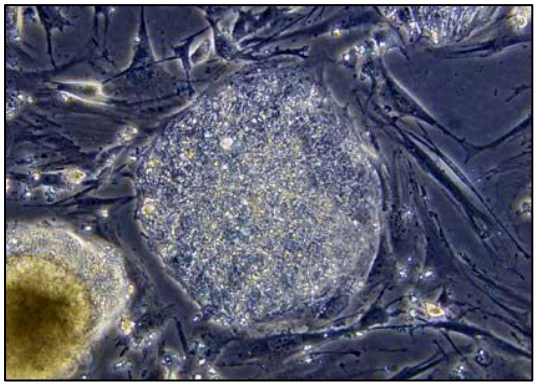
(divide via mitosis producing at least 1 daughter cell that is the same as the mother stem cell)

- And is multipotent

i.e. Generate >1 cell type

all the cells of an embryo or a tissue

(depending on the type of stem cell more later)



Stem cells: defining behaviour

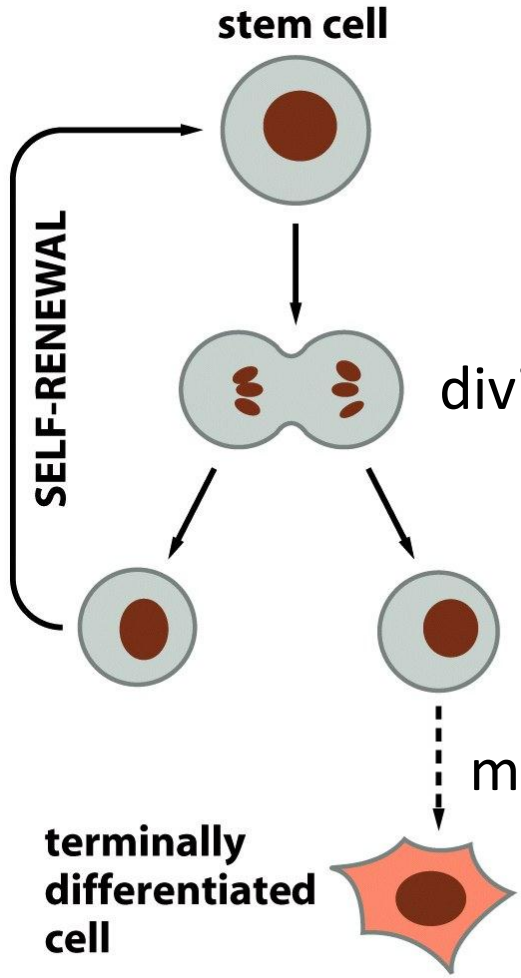


Figure 23-5 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Some ways we can use stem cells

As tools:

to understand MCB and developmental biology

to investigate the cause/s of disease

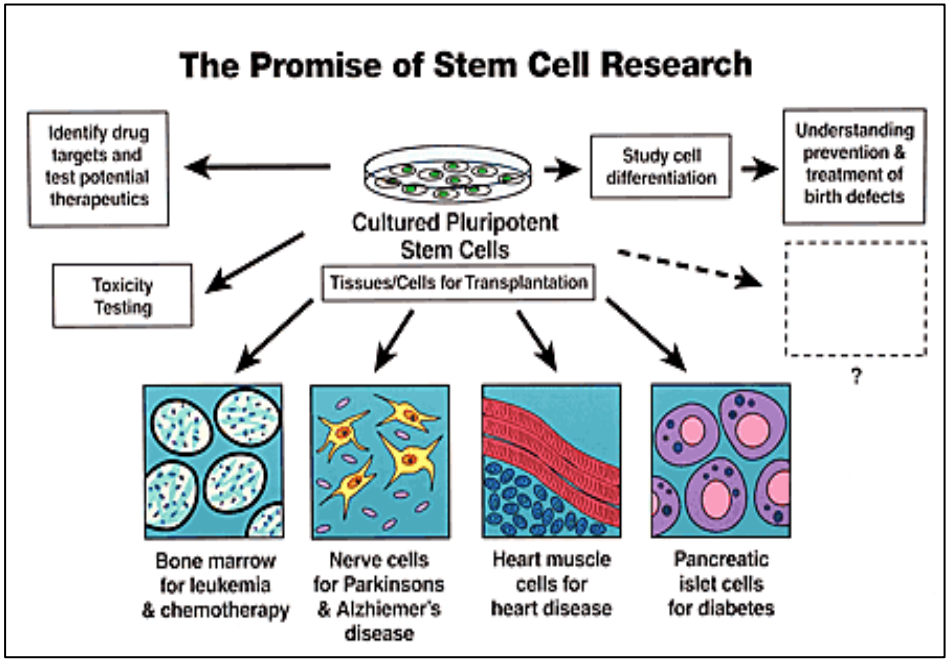
to test drugs and other therapeutics on

As therapies:

to repair damaged/diseased tissues

to replace diseased/dying cells

(ideally with no immune response)



(returned to later)

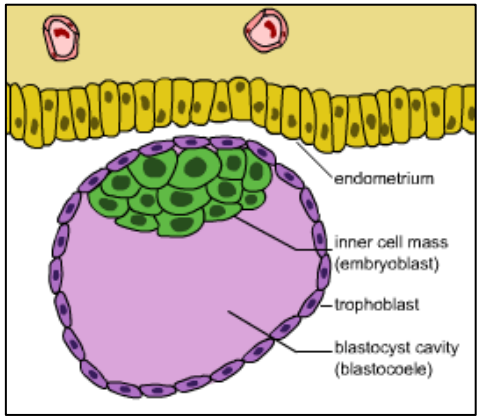
Types of stem cells

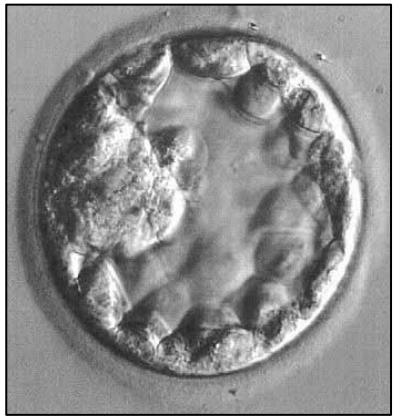
1. Embryonic stem cells (ES)
2. Induced pluripotent stem cells (iPS/iPSCs)
3. Embryonic or adult tissue specific stem cells

Differ in their 'potency' = the number of cell types that they can produce

Types of stem cells: 1. Embryonic stem cells (ES)

A preimplantation embryo 4-5 days after fertilisation ('blastocyst') = cells that allow implantation into the womb + inner cell mass ('ICM')



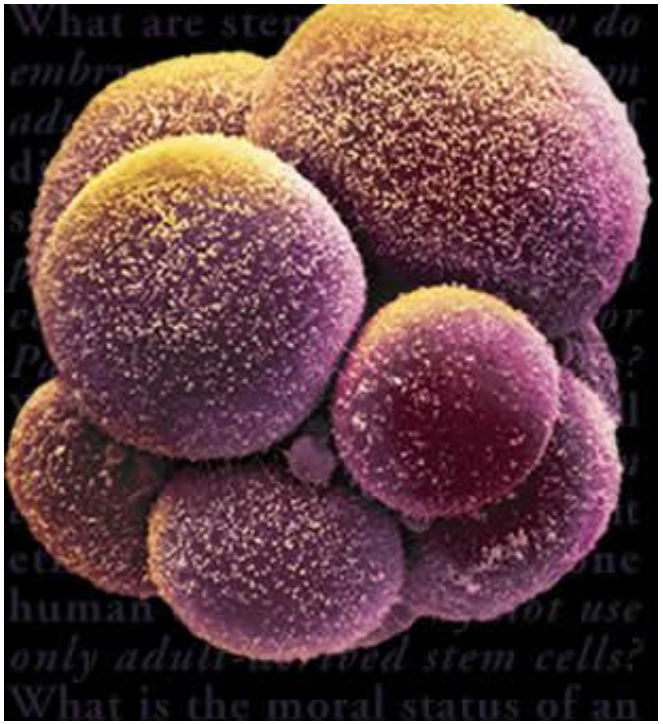


- ES cells come from the ICM
- They are pluripotent and can differentiate into all the cells of the embryo (all 3 germ layers)

NB: cells before 4-5 days post-fertilisation are 'totipotent' as can also develop into extra embryonic cells (part of placenta)

Ethical issues behind using ES cells

- Almost always involves destroying a preimplantation embryo
- USA ban on ES cell research



Drive to find other stem cells as useful as ES cells

- What is the basis of ES pluripotency?
- Need to stop them differentiating

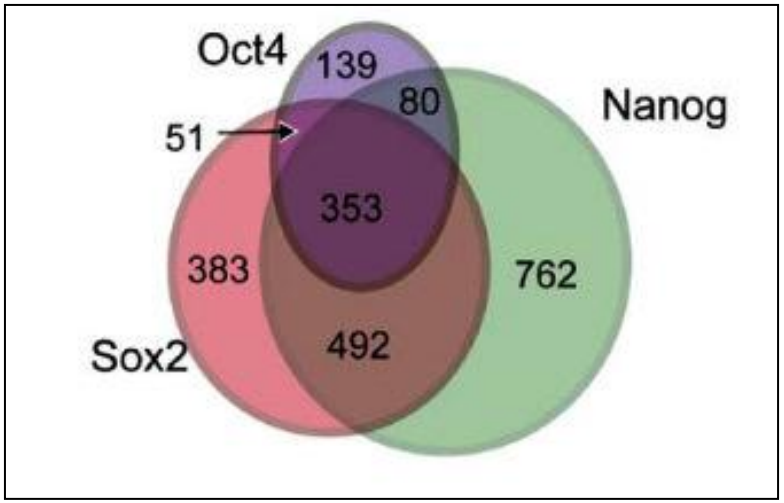
Boyer et al., Cell, 2005

Just a few transcription factors can control pluripotency in human ES cells:

Oct4

Nanog

Sox2



Boyer et al., continued

- Oct4, Nanog and Sox2 activate genes involved in pluripotency (e.g. chromatin remodellers or Stat3 involved in self-renewal)
- and down regulate genes involved in differentiation (e.g. those involved in neuronal differentiation)

- Feedback loops among these 3 and their targets can allow sensitive/quick responses to outside signals

Huge breakthrough! Takahashi & Yamanaka, Cell 2006

- Introduced Oct3/4, Sox2, c-Myc, and Klf4 into mouse embryonic or adult fibroblasts (i.e. differentiated somatic cells)

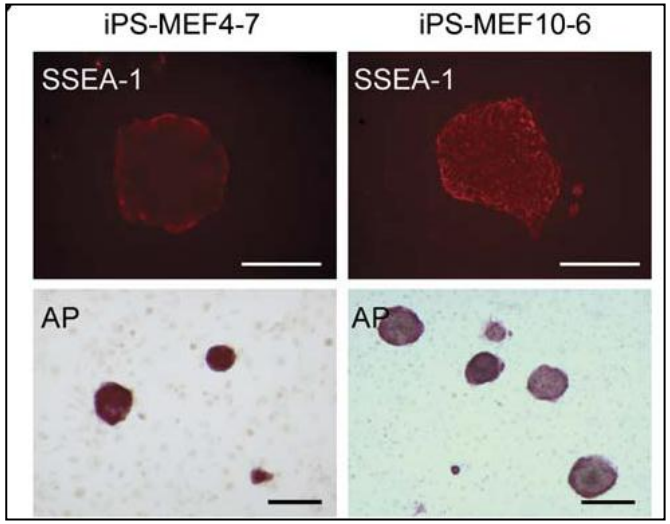
Resulting cells behaved like ES cells (in many NOT ALL ways)

- Called Induced pluripotent stem cells (iPS/iPSCs)
- No ethical barriers or immune reactions

Are they really like ES cells?

Need to pass a set of tests

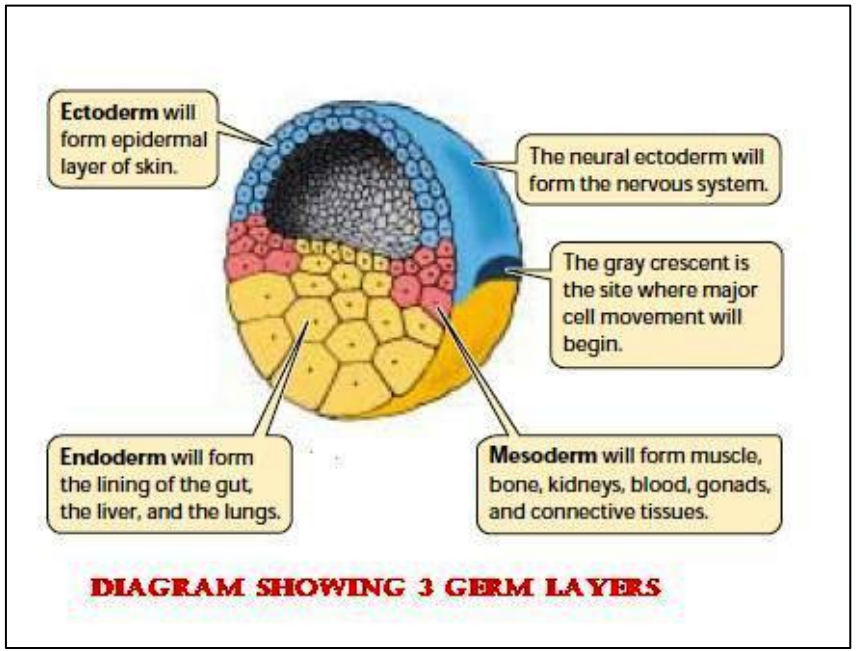
- Looked like ES cells, divided and self-renewed
- Expressed many ES cell genes (DNA microarrays) NOT all ES genes
- Expressed ES marker proteins:

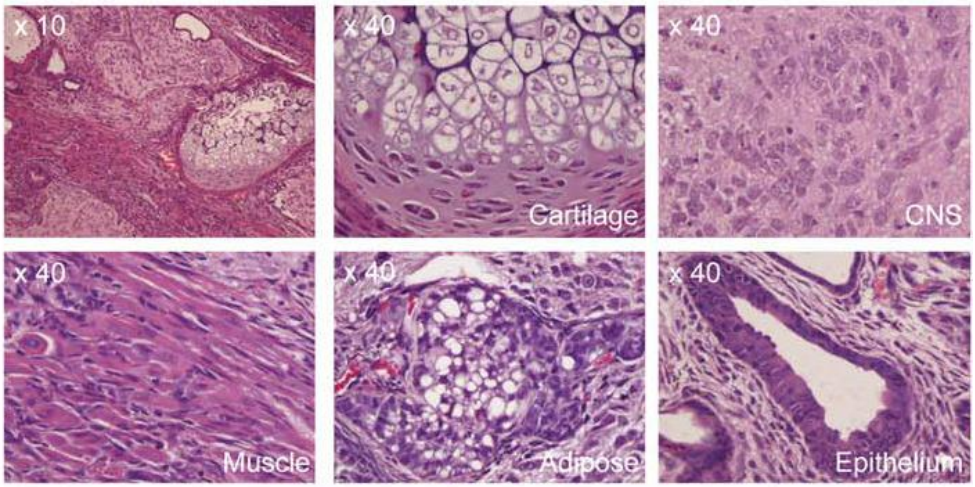


The teratoma test

- Injected the iPS cells into nude mice – they could form teratomas with a mixture of cell types from each of 3 germ layers

(Reminder from prev. Lecture: Germ layers = cell/tissue layers with different fates)

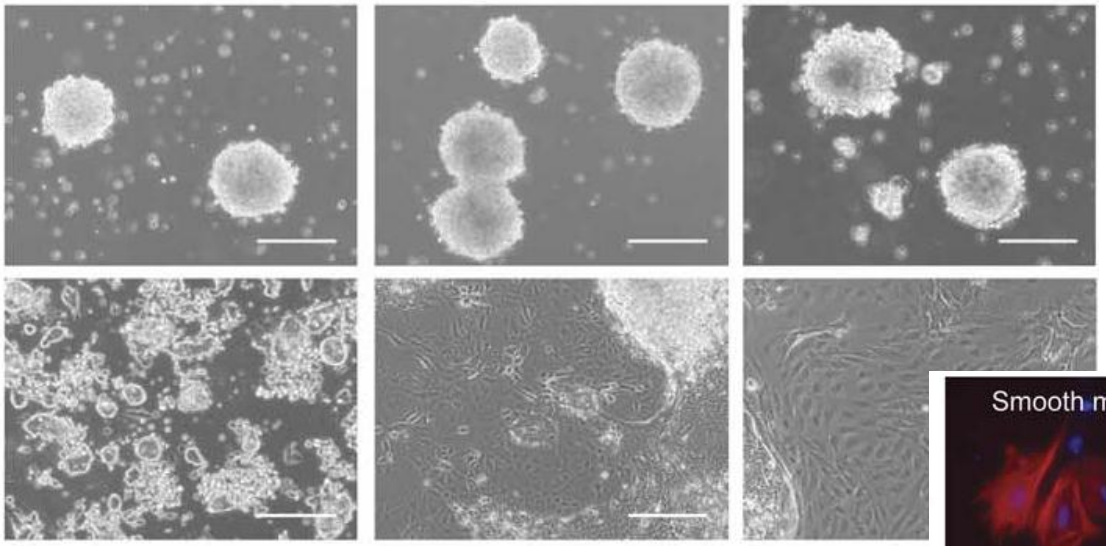


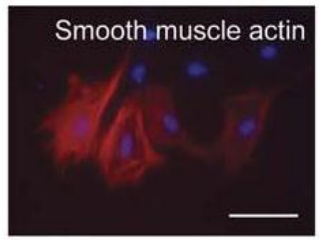


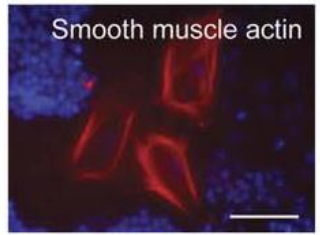
Further tests

- Behaved like ES cells in vitro e.g. formed embryoid bodies

Embryoid bodies
don't let the cells stick down
they aggregate
(dividing centre,
differentiated outer layer)
mimic some embryonic MCB
but disordered



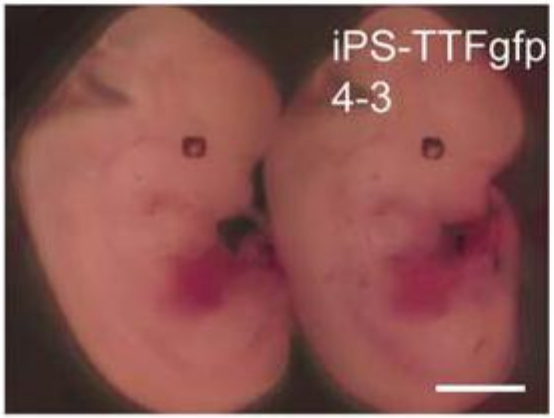


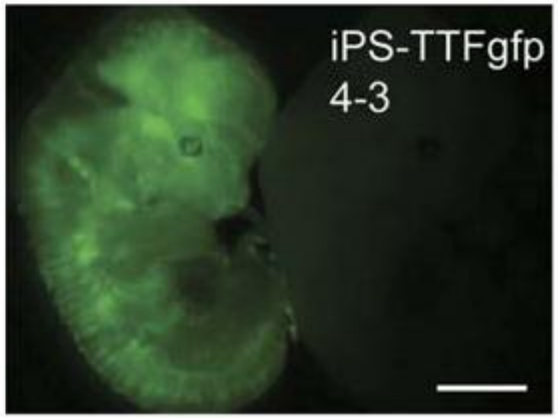


More tests

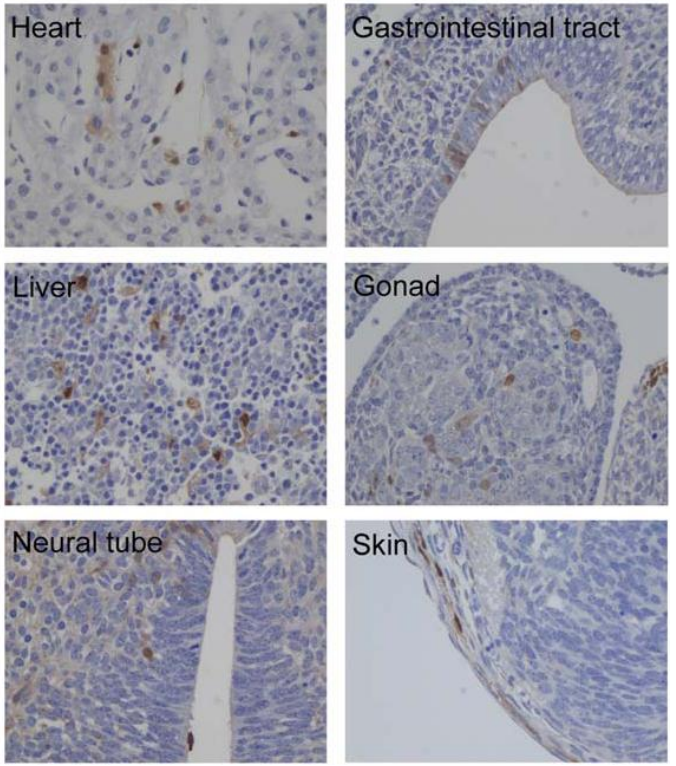
- Promoters and histones of pluripotency genes demethylated (i.e. active)
- The iPS (labelled with GFP) could contribute to an embryo (microinject the cells into

blastocyst and trace later):



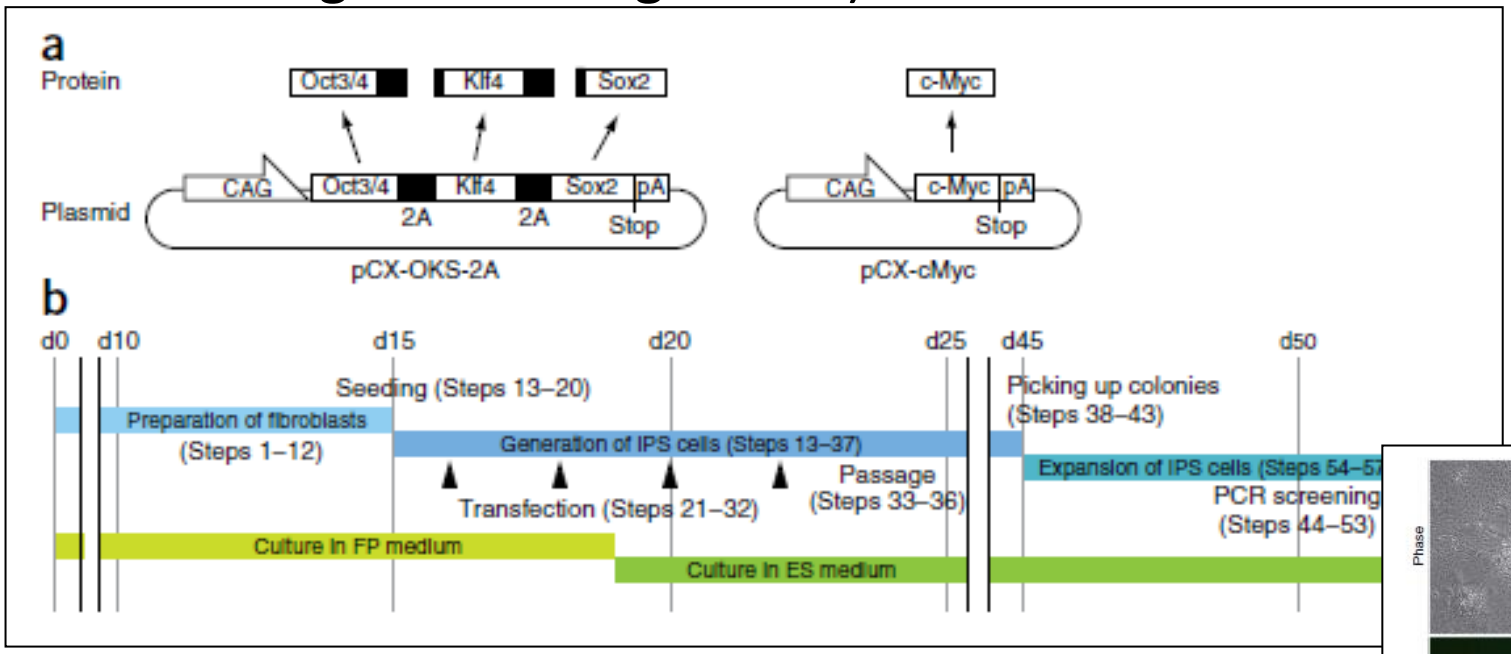


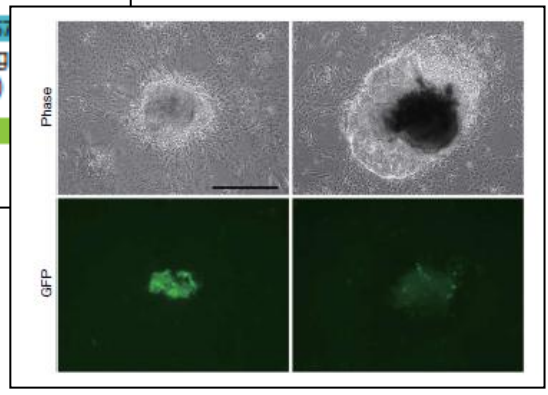
brown cells are the GFP+ iPS derivatives:



Safety concerns: recent iPSC developments

- (iPS induce teratomas)
- Did use viruses: Need to avoid cancer development (esp. c-Myc driven)
- Now non-viral introduction of genes (less efficient but can check for non-integration into genome)





Resultant ES-like colonies (Nanog-GFP+):
Okita et al., Nature protocols 2010

Still to discover

- How the cell of origin can influence iPS behaviour
- iPS derived mice are more likely to die of cancer than those made from ES cells. Why?
- Why DNA modifications may vary

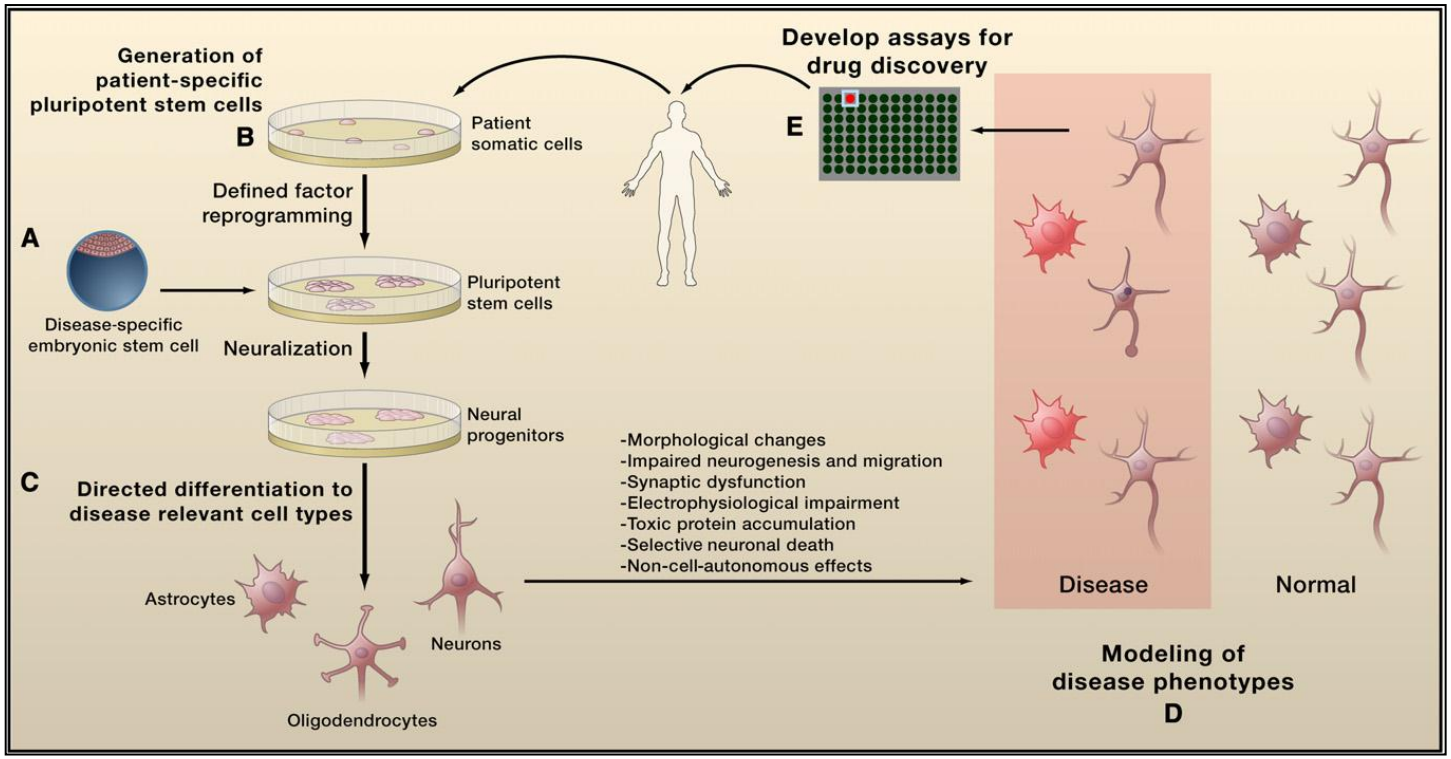
Chimeric mouse with iPS-derived grey hair Okita et al., Nature protocols 2010

Type of pluripotent Cell Line	Inoculation Site	Teratoma Efficiency	Teratoma Latency (days)	Histological Analysis (3 germ layers staining)
Human ESCs	subcutaneous	13/16 (81%)	59±36	YES (meso, ecto & endoderm)
	intratesticular	13/14 (94%)	66±30	YES (meso, ecto & endoderm)
Human iPSCs	subcutaneous	8/8 (100%)	31±10	YES (meso, ecto & endoderm)
	intratesticular	8/8 (100%)	49±19	YES (meso, ecto & endoderm)

Gutierrez-Aranda et al., 2010

Uses of iPS cells in Neuroscience

1. iPS cells from a patient - could use to model the disease, test treatments, grow and engineer replacement cells

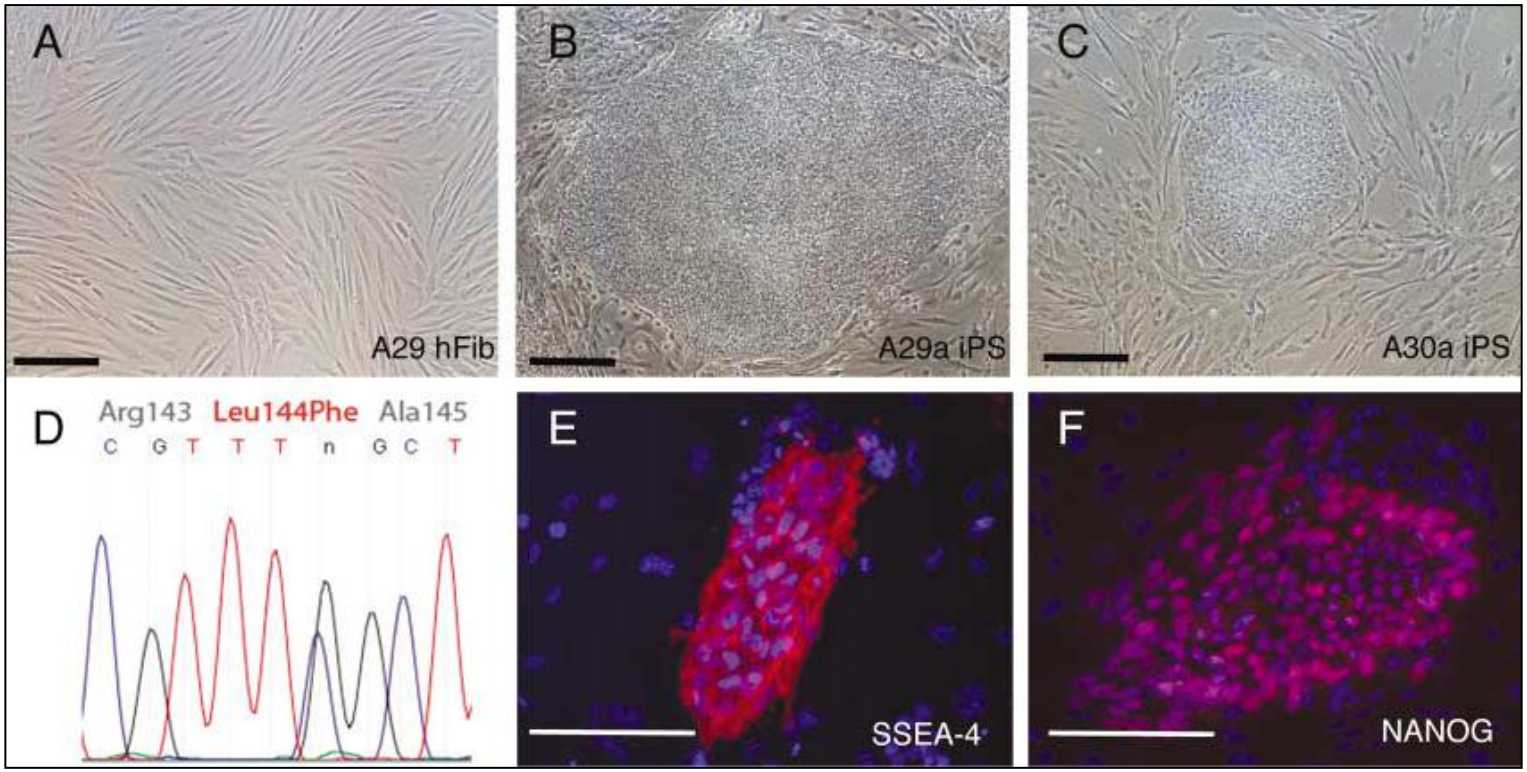


(Han et al., Neuron 2011)

E.g. Demos et al., Science, 2008

- iPS from an Amyotrophic lateral sclerosis patient (ALS, type of motor neuron disease)
- motor neurons in spinal cord and motor cortex die
- ? Why yet

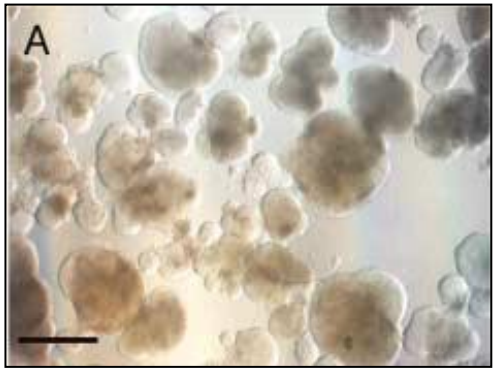
82 yr. old ALS patient: from simple skin biopsy to iPS



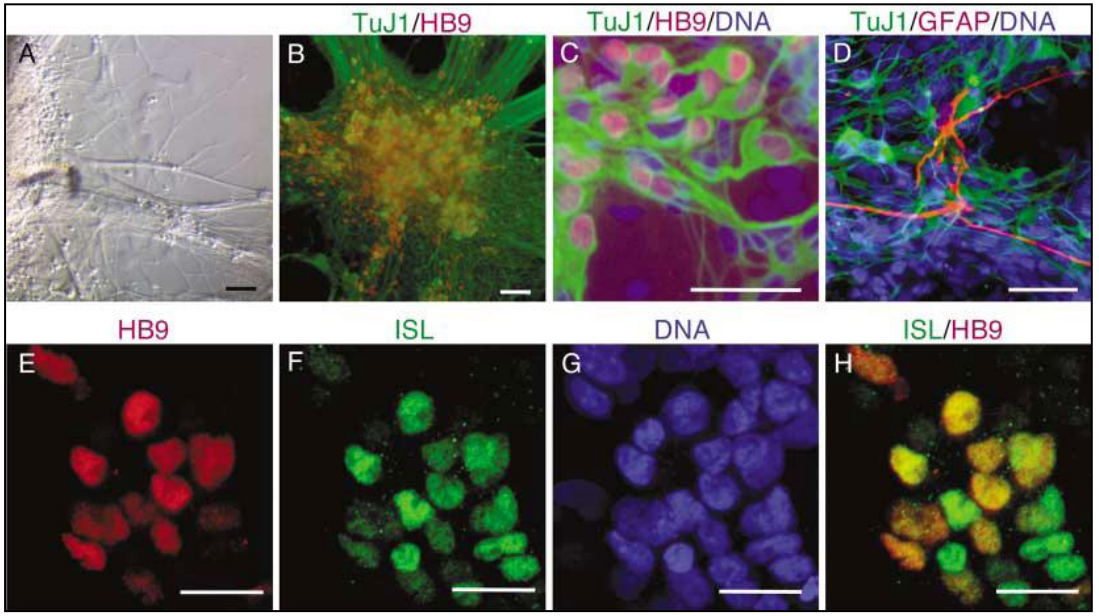
D = Sequencing of the iPS cells showing rare L144F (Leu144 → Phe) dominant allele of the superoxide dismutase (SOD1): associated with her slowly progressing form of ALS

Can differentiate these iPS into neurons

1. Take embryoid bodies formed from the iPS



2. Treat with a Shh and RA agonist, grow on laminin
3. The cells differentiated into motor neurons (expressed MN-specific transcription factors:



Another example of patient-specific iPS in neuroscience

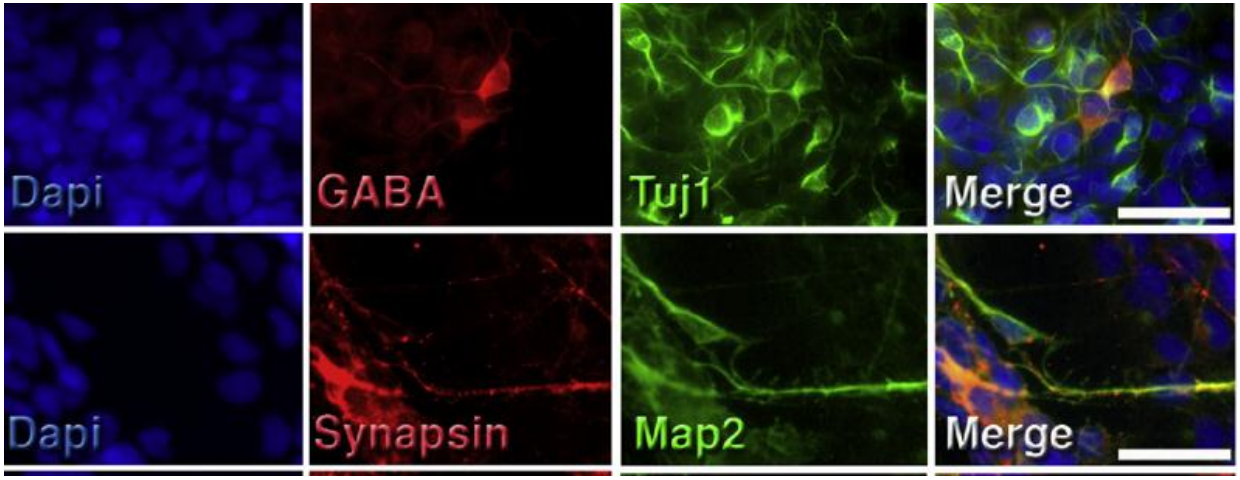
Rett syndrome

- Progressive neurological disorder (X-linked gene encoding MeCP2)
- Normal development until 18m old, then motor decline, regression, weak muscles, seizures, autistic behaviour

Marchetto et al., Cell, 2010

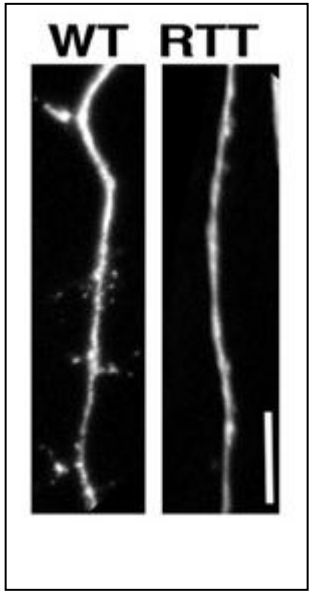
Developed iPS from Rett patient skin biopsies

Can differentiate into neurons



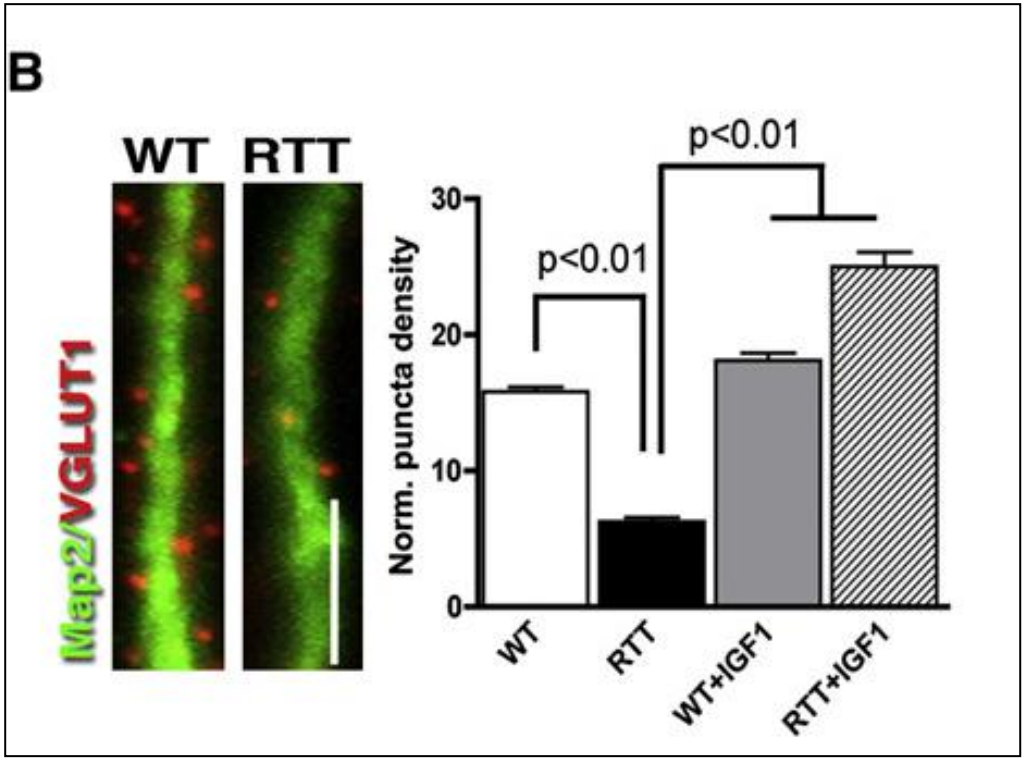
Found that Rett iPS derived neurons have:

- Fewer spines and synapses than ‘WT’
- Altered calcium signals
- Electrophysiology changes



Testing therapies on these iPS

Could ‘repair’ the gene defect (e.g. with IGF-1 treatment) and see some rescue of normal characteristics:



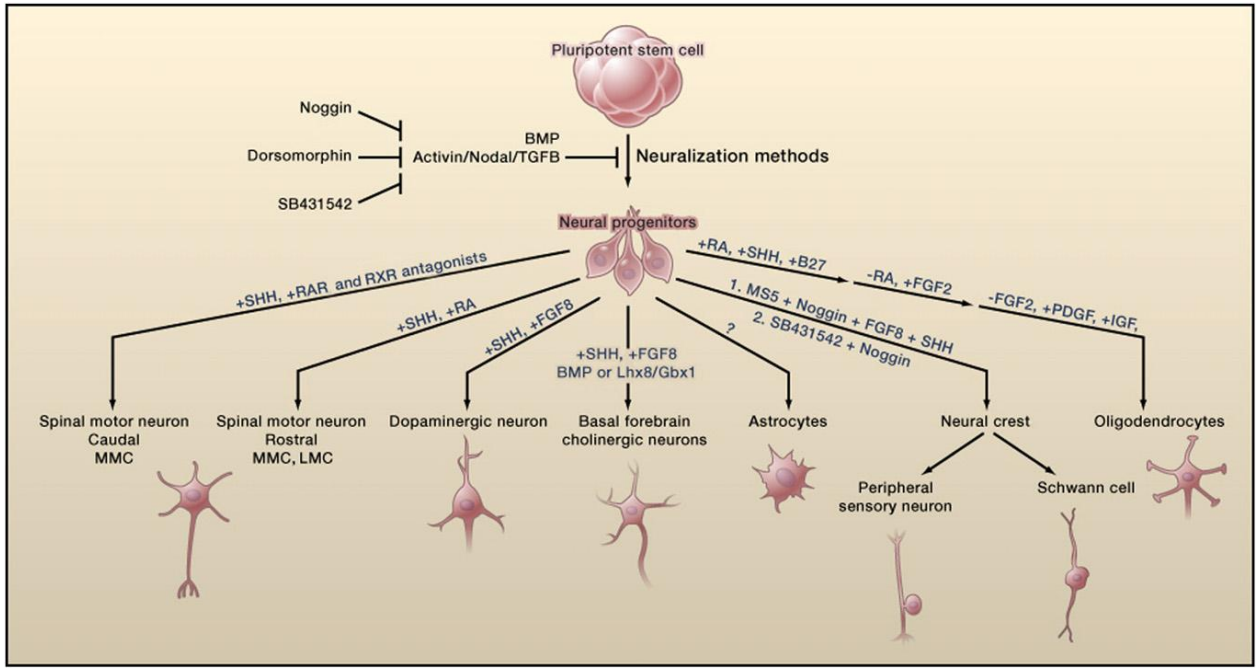
Future uses of these neurons

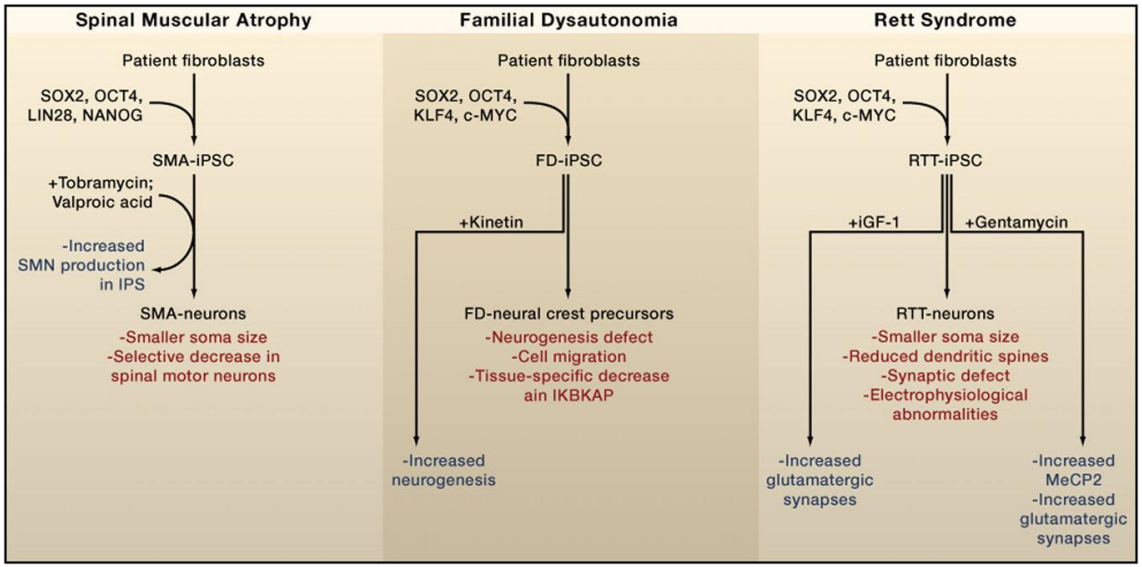
How does the disease causing mutation affect:

- Their survival?
- Interaction with other cells?
- Response to stresses?

- Response to therapies in development?

Summaries of disease modelling





(Review by Han et al., Neuron 2011)

15 minute break

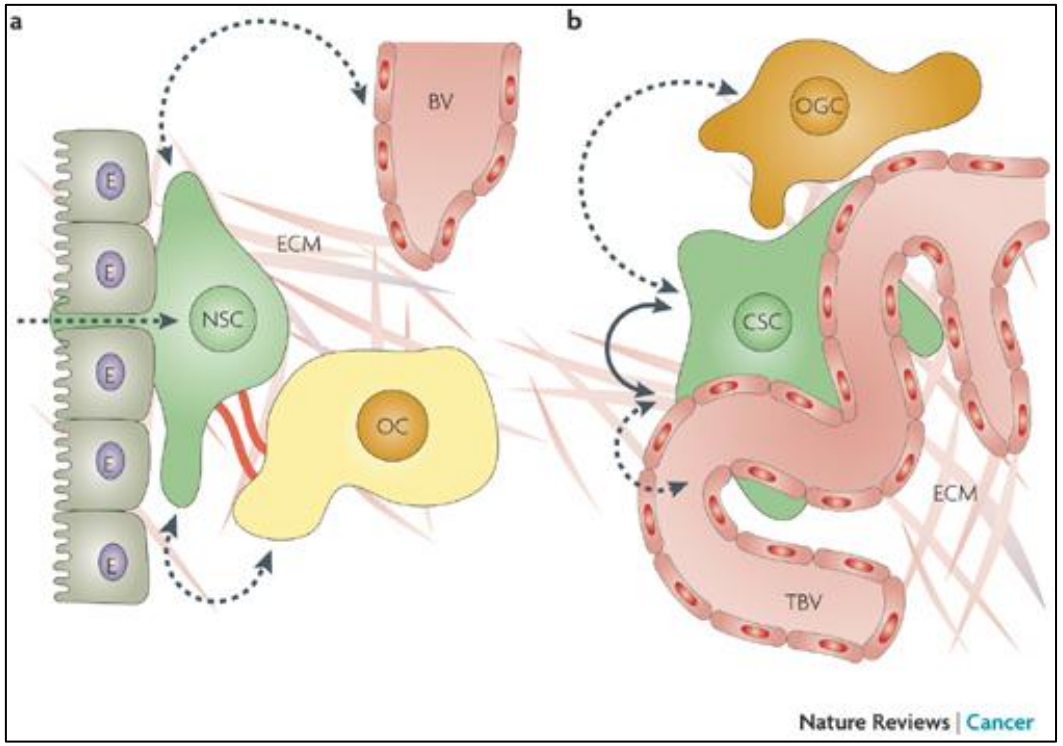
Relax
Meet your neighbour
Review your notes
Ask questions

Another useful concept: The stem cell niche

= the environment surrounding the stem cell

- Contains the signals that keep it as a stem cell and stop it differentiating (i.e. stops depletion)
- Includes signals from support cells, extracellu7lar matrix, other stem cells etc.

- Differentiation can be triggered by leaving the niche



Tissue-specific stem cells inc. in the nervous system

= undifferentiated cells in a differentiated tissue or organ

- Can self-renew and/or produce offspring that can differentiate into the cell types of that tissue

Role:

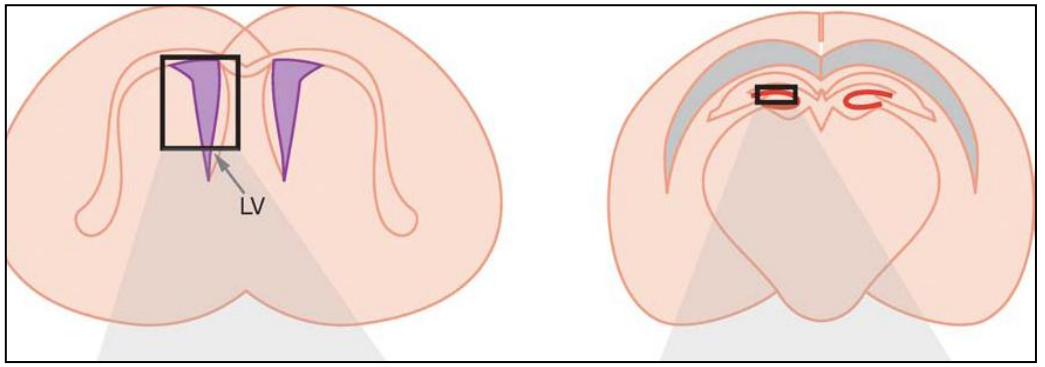
- Embryo: generate enough cells for each tissue
- Adult: repair and replace

Examples: in your brain, blood, gut, muscle

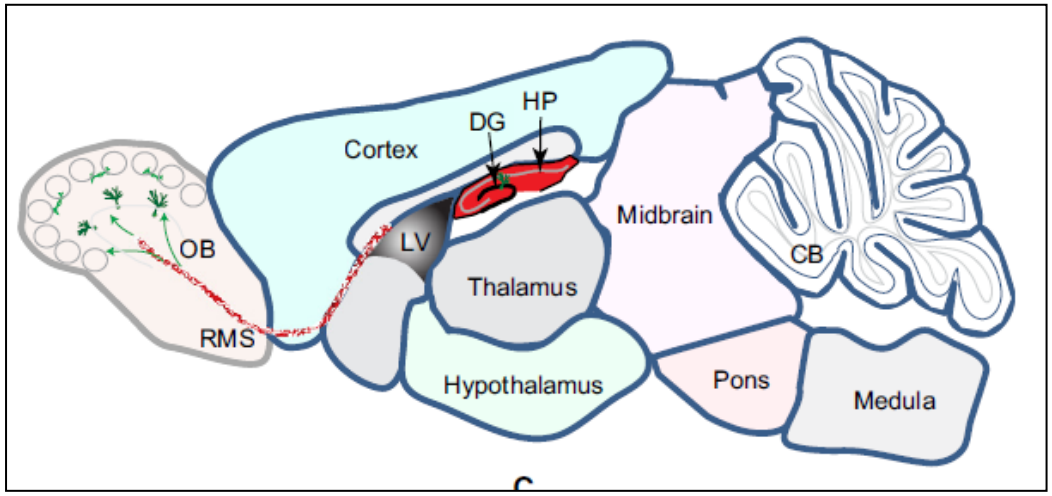
Hugely important target of research for regenerative medicine

(Endogenous) Neural stem cells in the adult brain

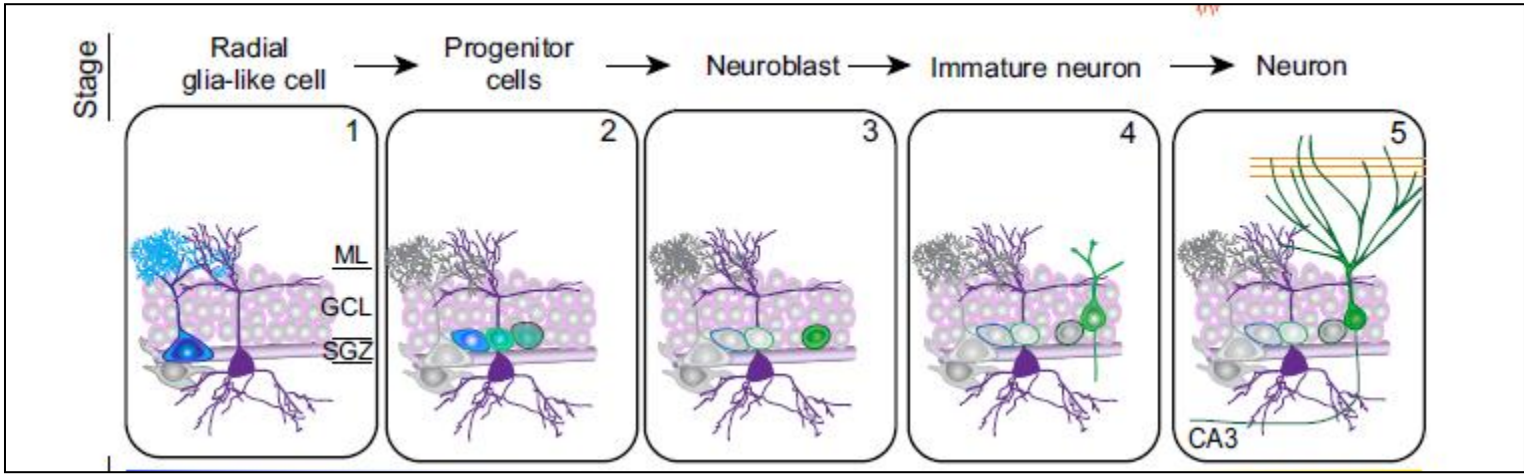
- Previous 2 Lectures looked at NSCs in embryo
- But they also exist in the adult
- limited expts in human e.g. see Knoth et al., PLoS One, 2010
  much more known about non-human primates and rodents
- 2 regions: striatal subventricular zone and dentate gyrus of the hippocampus:



More on adult neurogenic regions



Mapped
e.g. Adult
dentate gyrus:



(Ming and Song Review, Neuron, 2011)

Therapeutic potential of stem cells in the CNS

- MS, stroke, tumours, spinal cord damage etc
- Stem cells can migrate to sites of injury
- Are multipotent

- Need to reach correct site, integrate, survive, function and not cause tumours and ideally not trigger an immune reaction
- Also don’t want abnormal connections

Brain tumours

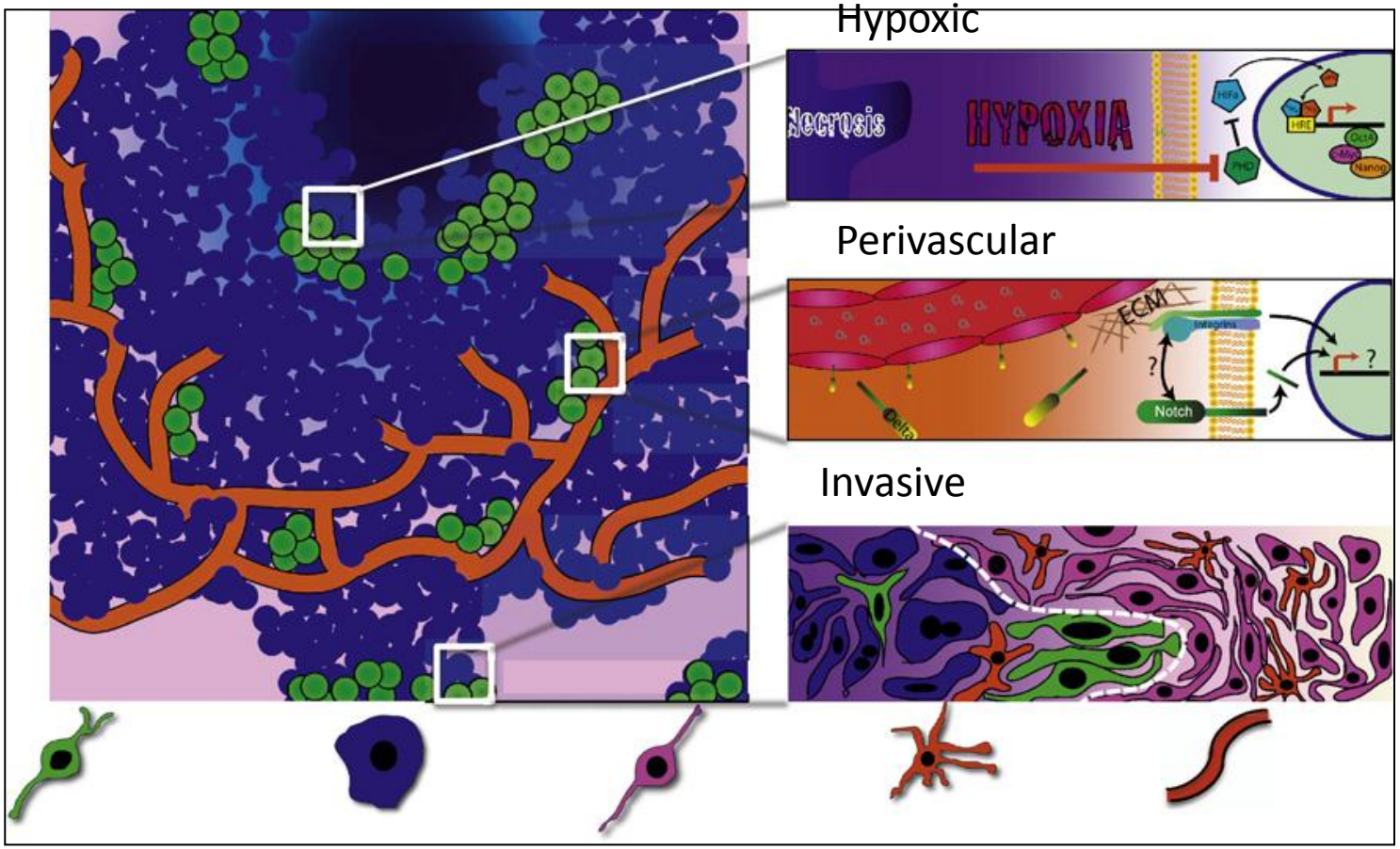
- subventricular zone and the dentate gyrus reportedly more prone to viral and chemical oncogenesis
- Do brain tumours contain/produce cancer stem cells? i.e. cells that can self-renew and produce offspring that don't rarely differentiate

E.g. Glioblastoma Multiforme (GBM)

- most common and lethal of human primary CNS tumours, median survival of 14-16m despite optimal surgery, radiation and chemotherapy
- Have profiled whole tumours – seen changes in e.g. PDGFRec and EGFRec

- Current mouse models target many cells in GBM need to search for and study any stem cells here to identify origin of these tumours

Possible glioma stem cell niches

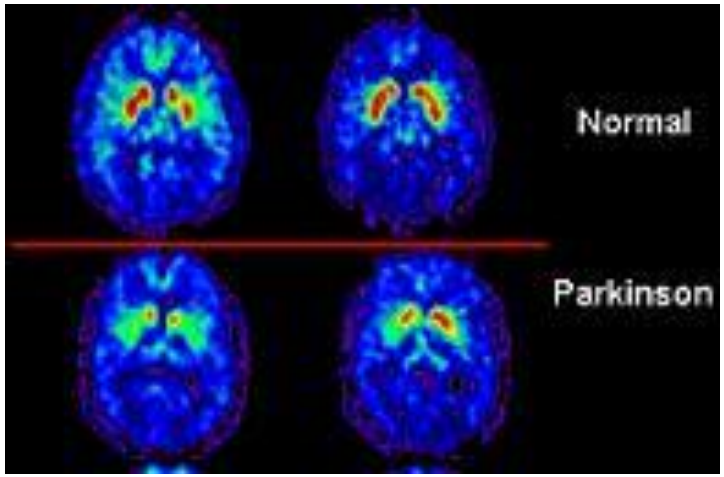


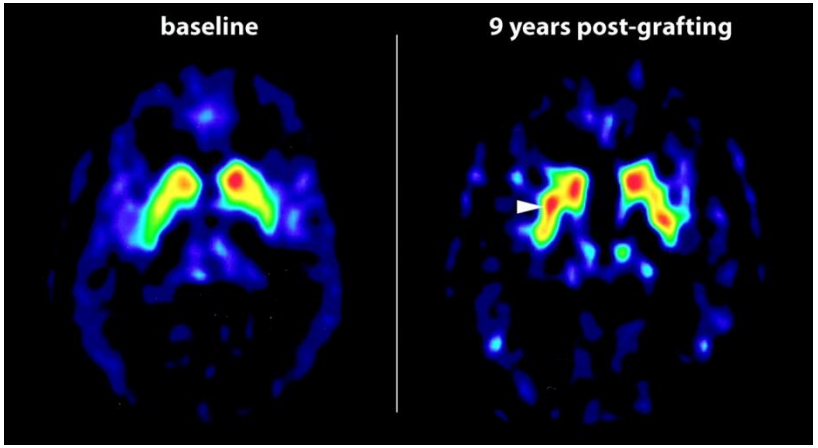
Glioma SC Glioma Brain stromal Immune cell Blood vessel

(Lathia et al., Review in Cell stem Cell, 2011)

Stem cell derived neural transplants e.g. Parkinson's disease

- PD is… (see later Lecture too)
- Current treatments
- Inc. foetal cell transplants





Strategy: Replace diseased dopaminergic neurons

Use clues from Developmental Biology (previous Lectures)

Nat Biotechnol. 2009 Mar;27(3):275-80. **Highly efficient neural conversion of human ES and iPS cells by dual inhibition of SMAD signaling.** Chambers SM, Fasano CA, Papapetrou EP, Tomishima M, Sadelain M, Studer L. (e.g. mimic neural plate inhibition BMP signalling)

Mol Cell Neurosci. 2010 Nov;45(3):258-66. Epub 2010 Jul 24. **Differentiation of human ES and Parkinson's disease iPS cells into ventral midbrain dopaminergic neurons requires a high activity form of SHH, FGF8a and specific regionalization by retinoic acid.** Cooper O, Hargus G, Deleidi M, Blak A, Osborn T, Marlow E, Lee K, Levy A, Perez-Torres E, Yow A, Isacson O.

Need to push SCs along the lineage you want and then test their characteristics
Currently testing which stage of cells to use

Another SC based strategy: neuroprotection (transplant supporting cells)

- Again, use knowledge of neurobiology

J Neurosci. 2000 May 1;20(9):3182-90.
Glial cell line-derived neurotrophic factor is essential for postnatal survival of midbrain dopamine neurons.
Granholm AC, Reyland M, Albeck D, Sanders L, Gerhardt G, Hoernig G, Shen L, Westphal H, Hoffer B.

J Neurosci. 2001 Oct 15;21(20):8108-18.
Neuroprotection through delivery of glial cell line-derived neurotrophic factor by neural stem cells in a mouse model of Parkinson's disease.
Akerud P, Canals JM, Snyder EY, Arenas E.

Eur J Neurosci. 2005 Dec;22(11):2755-64.
Ex vivo gene delivery of GDNF using primary astrocytes transduced with a lentiviral vector provides neuroprotection in a rat model of Parkinson's disease.
Ericson C, Georgievska B, Lundberg C.

Stem cell based Anti CNS ageing therapy?

- Local and/or systemic factors?

Brack et al., Science, 2007

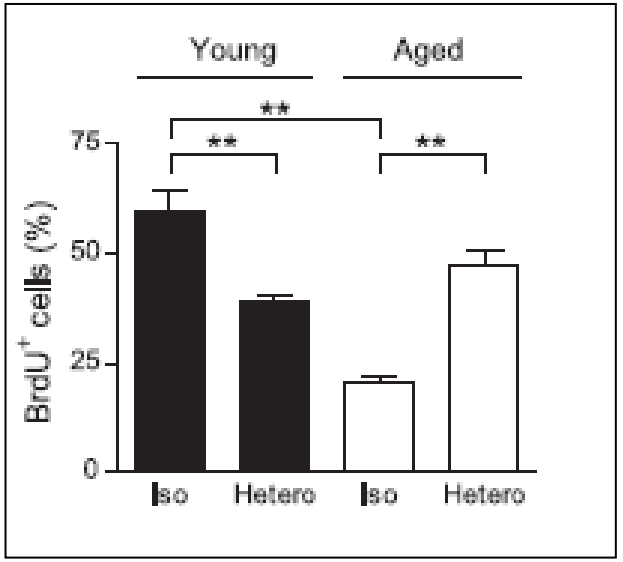
Notes: 'Parabiosis' = sharing a circulation

old muscles: stem cells less proliferative and often generate fibrosis generating cells rather than muscle

Parabiosis expt: could 'rejuvenate' muscle stem cells

It was due to Wnt inhibition

by the younger serum



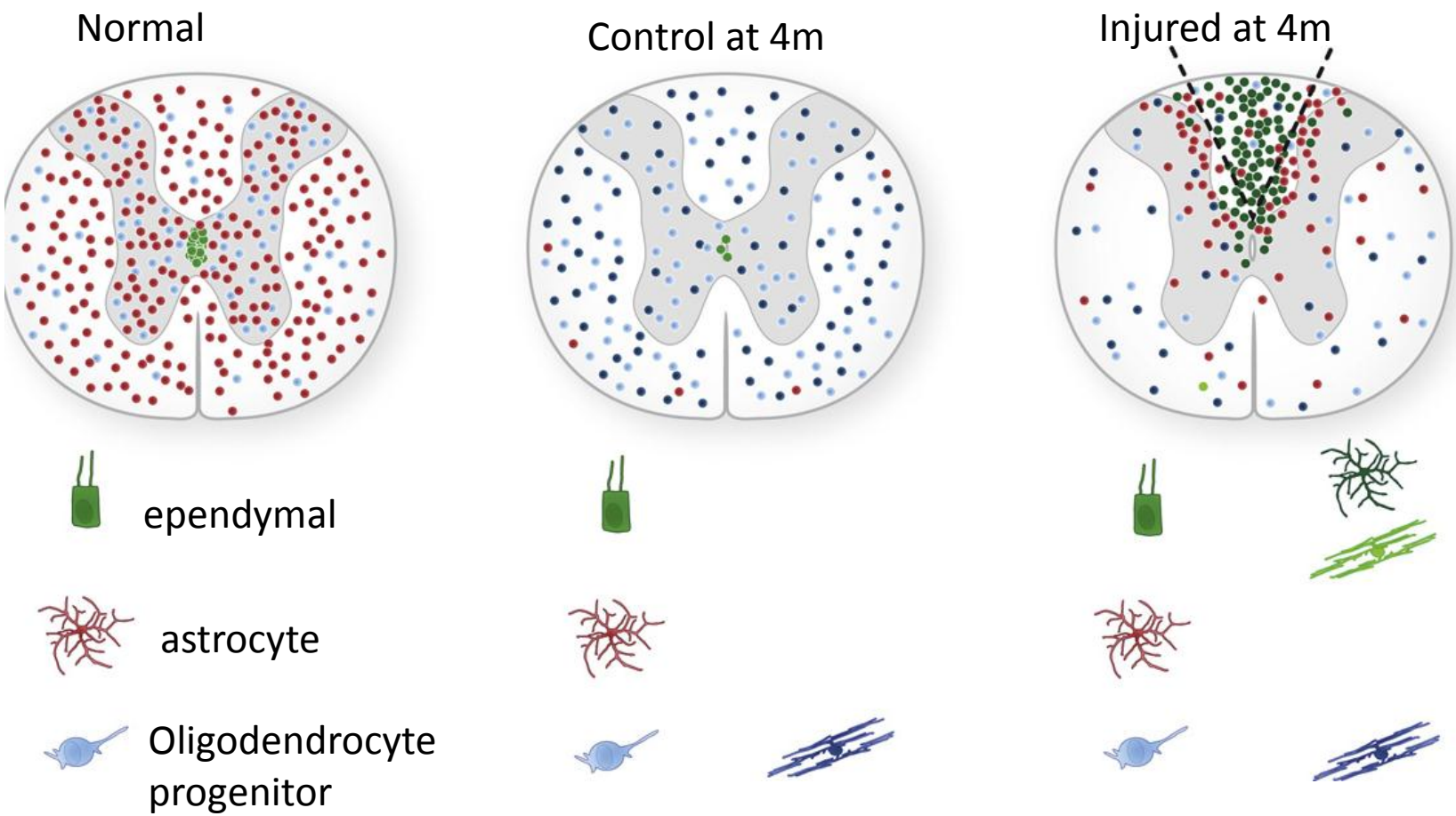
Glia stem cells

- Recent finding: Oligodendrocytes are still produced in adulthood
- From NG2+ cells
- After gliotoxin injury these can also generate Schwann cells and possibly astrocytes (not neurons)
- Remyelination possible using these?

(Reviewed in Richardson WD et al., Neuron 2011)

Translating SC studies into medicine

New basic biology of spinal cord injury e.g. Barnabe –Heider et al., Cell Stem cell 2010 :



Ependymal cells can generate astrocyte scar cells (bad) and oligodendrocytes (good)

Some are at clinical trial stage already

(See also Review Aboody et al., Neuron, 2011)

Table 1 Selected stem cell therapies entering the clinic

Company	Cell type	Development progress	Indication
ReNeuron	ReN001 (adult neural stem cells derived from 12-week-old fetus tissue). Committed, not pluripotent. Genetically engineered to be conditionally immortal. Some technology licensed from StemCells	Phase 1. First patient has been treated	Ischemic stroke. Six months to two years after injury
StemCells	HuCNS-SC (adult stem cells derived from fetal tissue). Cultured but not altered. Same cells used for both Batten's and cord injury	Phase 1. First patient to be treated in early 2011 in Phase 1b	Chronic spinal cord injury
			Batten's disease
Neuralstem	NSI-566RSC, human spinal cord–derived neural stem cell lines	Phase 1	Amyotrophic lateral sclerosis
		IND filed	Chronic spinal cord injury
Geron	GRNOPC1 (oligodendrocyte progenitor cells, derived and differentiated from hESCs)	Phase 1	Spinal cord injury (acute—7 to 14 days after injury)

IND, investigational new drug; source: company websites

(Nat Biotechnol. 2011 Feb;29(2):95-7)

Learning Outcomes of this Lecture

What defines a stem cell?

Different types of stem cells: ES, iPS, Tissue-specific

Potential uses of stem cells

The concept of a stem cell 'niche'

Neural stem cells in the adult

Neural stem cell-based therapy development inc. cancer, neurodegeneration, genetic disease, ageing (and your own suggestions) and current clinical trials

NB: there are many links between this Lecture and the other teaching in the course