Neural Stem Cells

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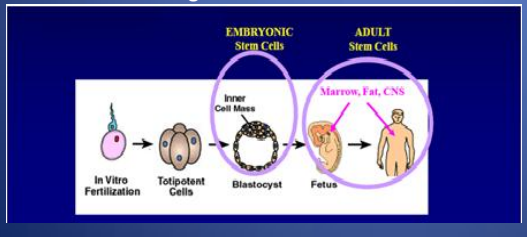
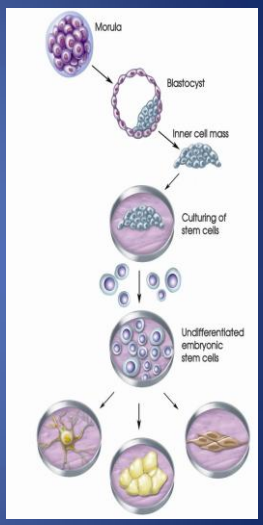
INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Support Of Establishment, Development And Mobility Of Quality Research Teams At The Charles University
CZ.1.07/2.3.00/30.0061

Stem Cells

- Stem cells are undifferentiated cells that become the kinds of cells that make up your body and replace old cells when they wear out and die.
  - An undifferentiated cell is a cell that doesn't have a job…yet.
  - To differentiate means to acquire a specific job and characteristics.
- Scientists believe that stem cells could help change how patients are treated by modern medicine.
  - Stem cells have the potential to create more individualized treatments that use the body's own abilities to repair itself in order to create new tissue and maybe even new organs.
  - Additionally, stem cells may help scientists better understand why some problems occur, increasing the likelihood of finding effective treatments.





Germ Layers

- All tissue in the body comes from the inner cell mass of a 5-7 day old blastocyst. The inner cell mass develops into three germ layers, the endoderm, the mesoderm and the ectoderm.
  - The endoderm forms soft tissues like the pancreas and liver.
  - The mesoderm becomes muscle (including the heart), blood, and bone.
  - The ectoderm forms the skin and nerve cells.
- To be a pluripotent stem cell, a stem cell must be able to become all three of these germ layers.

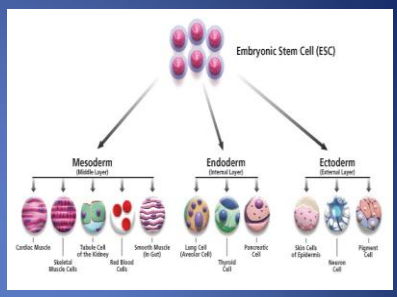
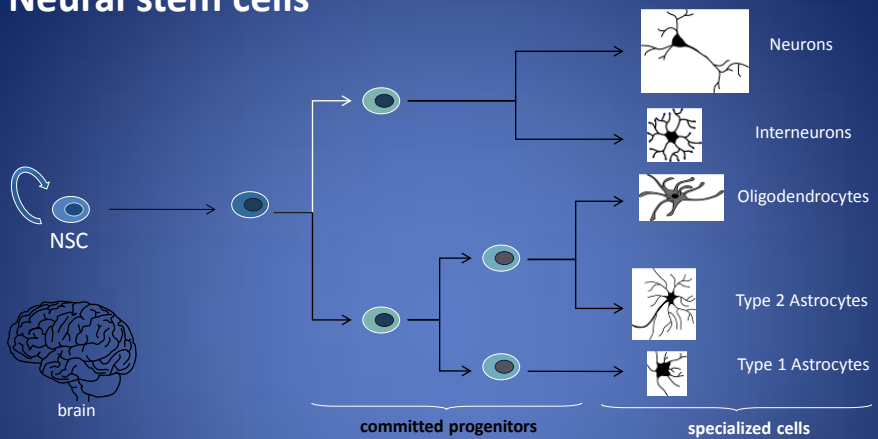
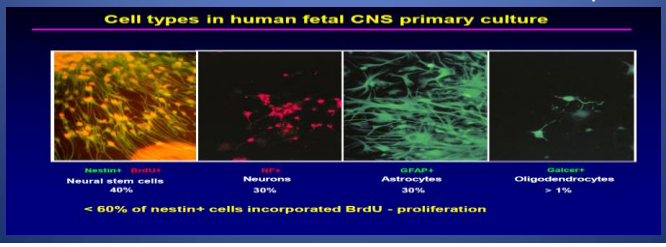


Table 1.1. Embryonic Germ Layers From Which Differentiated Tissues Develop

Embryonic Germ Layer	Differentiated Tissue
Endoderm	Thymus Thyroid, parathyroid glands Larynx, trachea, lung Urinary bladder, vagina, urethra Gastrointestinal (GI) organs (liver, pancreas) Lining of the GI tract Lining of the respiratory tract
Mesoderm	Bone marrow (blood) Adrenal cortex Lymphatic tissue Skeletal, smooth, and cardiac muscle Connective tissues (including bone, cartilage) Urogenital system Heart and blood vessels (vascular system)
Ectoderm	Skin Neural tissue (neuroectoderm) Adrenal medulla Pituitary gland Connective tissue of the head and face Eyes, ears

Neural stem cells



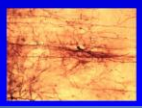


History

Stem cells in the adult nervous system … ?

"Once development was ended, the fonts of growth and regeneration of the axons and dendrites dried up irrevocably. In adult centres the nerve paths are something fixed, ended, immutable. Everything may die, nothing may be regenerated. It is for the science of the future to change, if possible, this harsh decree."

S. Ramon y Cajal, *Degeneration and regeneration of the nervous system*, 1928



Joseph Altaman and Gopal Das

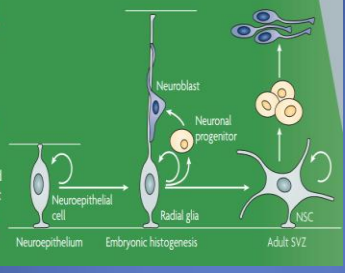
The earlier evidence of adult neurogenesis was presented by Joseph Altaman and Gopal Das in the 1960's (Altaman, 1962; Altaman et al., 1965), which totally contradicts the long held dogma of no new neurons after birth proposed originally by Cajal (Ming et al., 2005).

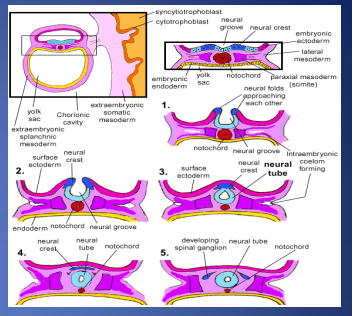
In 1989, Sally Temple described multipotent, self-renewing progenitor and stem cells in the subventricular zone (SVZ) of the mouse brain. In 1992, Brent A. Reynolds and Samuel Weiss were the first to isolate neural progenitor and stem cells from the adult striatal tissue, including the SVZ — one of the neurogenic areas — of adult mice brain tissue. In the same year the team of Constance Cepko and Evan Y. Snyder were the first to isolate multipotent cells from the mouse cerebellum and stably transfected them with the oncogene v-myc. Interestingly, this molecule is one of the genes widely used now to reprogram adult non-stem cells into pluripotent stem cells. Since then, neural progenitor and stem cells have been isolated from various areas of the adult brain, including non-neurogenic areas, such as the spinal cord, and from various species including humans.

Neurogensis

Fetal neural stem cells

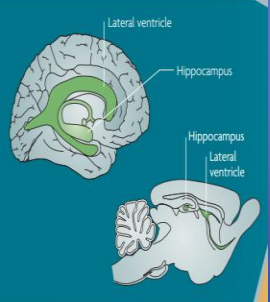
The CNS begins as a tube of neuroepithelial cells, the most primitive form of neural stem cells. In the cortex, neuroepithelial cells transition into radial glial cells, which then give rise to neural progenitors, neurons, astrocytes and oligodendrocytes. In other regions of the developing CNS[1] such as spinal cord and striatum, radial glial cells are not as prominent, and progenitors emerge from nonradial multipotent NSC populations. True NSCs are difficult to expand from fetal brain tissue. They may be better thought of as regionally pre-specified progenitor cells with characteristics of the region from which they were initially isolated.

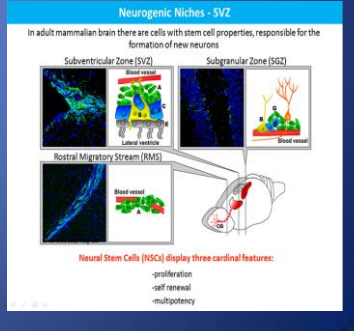




Adult neural stem cells

NSCs are located within two regions of the adult human and rodent brain (green): the subgranular zone of the hippocampus and the subventricular zone of the striatum[2]. Adult NSCs generate new neurons throughout life that integrate into hippocampal and olfactory circuits and are thought to be important for memory and olfaction. These NSCs can be isolated and expanded from rodent brains; however, they are more difficult to isolate from human brain biopsies or autopsy samples. Another type of NSC outside these two regions expresses the marker NG2 and can also proliferate *in vitro* and *in vivo*. However, this cell type does not normally give rise to new neurons *in vivo*. NG2 cells can be activated after injury and can generate new oligodendrocytes.

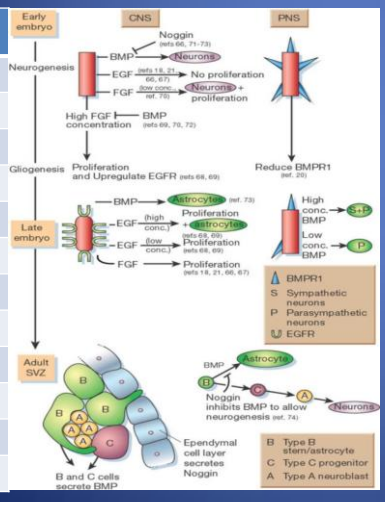


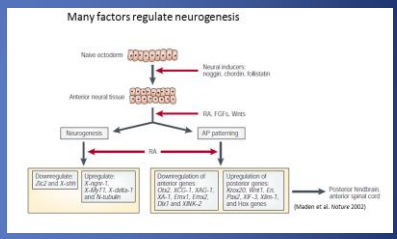


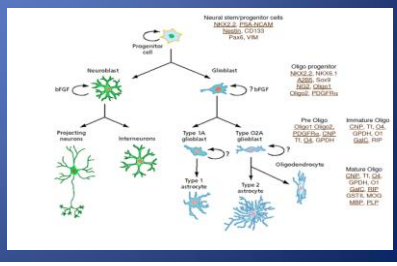
Neural Stem Cell Growth Factors

Elucidating the signaling pathways that govern differentiation of neural stem cells is central to understanding the development of the central nervous system. Research suggests the involvement of several different molecular pathways which interact to form highly complex signaling cascades. Familes of growth factors known to be important for the development of neural stem cells toward different lineages include bone morphogenetic proteins (BMPs), fibroblast growth factor (FGF), Wnt proteins, and Hedgehog proteins.
Many studies suggest a central role for BMPs in promoting neural stem cell proliferation and differentiation. These findings are often supported by the use of Noggin to antagonize BMP-4 signaling. Although the molecule cascades remain incompletely defined, FGF is commonly used to expand neural stem and progenitor cells *in vitro*. Equally, Wnt signaling is known to be important for neural stem cell function and development during embryogenesis and in adult brain. In culture, Wnt molecules and Sonic Hedgehog are thought to be essential for promoting the expansion of single cell populations into neurons, astrocytes, and oligodendrocytes.

Growth Factor Family
Activin
BMP
EGF
FGF
GDF
Hedgehog
IGF
PDGF
TGF-B
VEGF
Wnt
Others





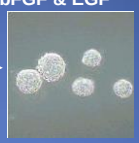


Neural Progenitor Isolation

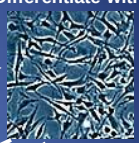
Derivation and expansion of NSCs

To convert different tissue sources into NSCs, cells derived from fetal (green box), adult (teal box) or ES/iPS (gold box) sources are cultured in media containing the mitogens EGF and FGF-2. NSCs derived from these sources can be expanded either as spherical aggregates termed 'neurospheres' or as monolayer 2D cultures. They can turn into neurons, astrocytes and oligodendrocytes, depending on the growth and differentiation factors they are exposed to during subsequent *in vitro* differentiation steps.

Expand using bFGF & EGF



Differentiate with RA



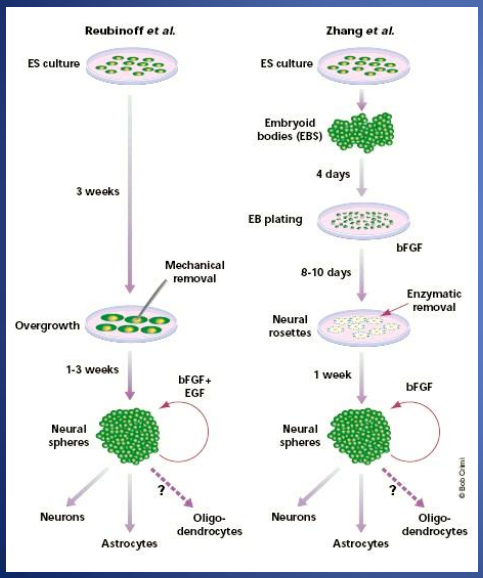
Multipotent Neural Progenitors/Nestin

Post-mitotic Neurons/ MAP2A/B

Astrocytes/GFAP

Oligodendrocytes/ bGalactocerebroside

Early Derivation of Human Neural Progenitor Cells from 3D Neurospheres

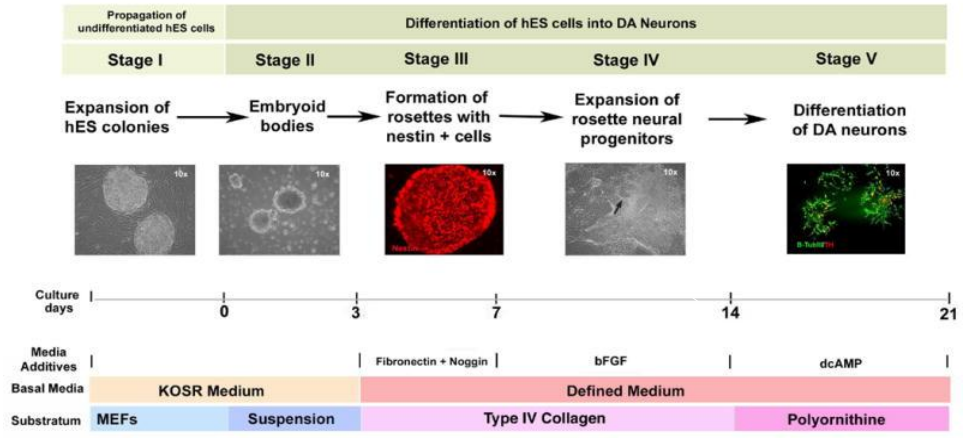


- Early derivation protocols require:
  - Progression through EB step in serum-containing medium
  - End result is free-floating cell aggregates or neurospheres

"In vitro differentiation of transplantable neural precursors from human embryonic stem cells" Zhang et al., 2001 *Nat. Biotechnology*

"Neural progenitors from human embryonic stem cells" Reubinoff et al., 2001 *Nat. Biotechnology*

A General Multi-Stage Process for the ES → NSC

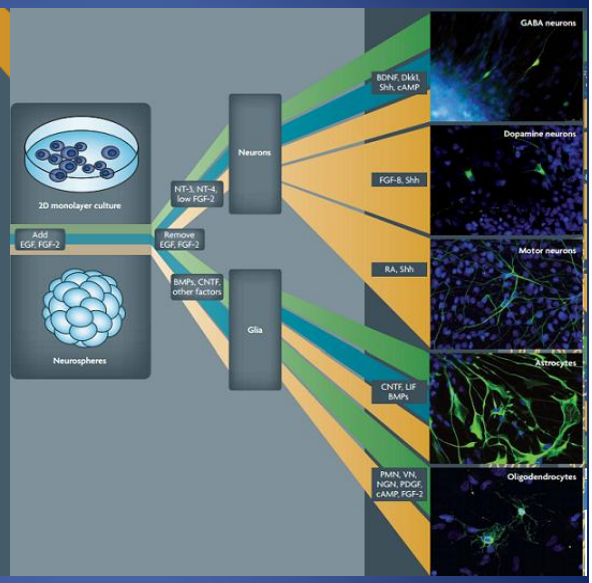


•NEURONS:
•Program EBs in 20ng/ml EGF + 20ng/ml FGF2 and switch to 1-5ng/ml FGF2 upon plating
•Switch to 10ng/ml NT-3 + 10-20ng/ml BDNF + 0.5uM Retinoic Acid upon plating

•ASTROCYTES:
•Program EBs in 20ng/ml EGF and switch to 10ng/ml CNTF + 10ng/ml BMP-4 upon plating

Differentiation

To promote NSC differentiation, EGF and FGF-2 are usually replaced with specific morphogens or growth factors that promote initial maturation into either neurons or glia. Final differentiation into specific neuron and glial types requires other morphogens and growth factors, and in some cases transcription factors. Current protocols for differentiation of specific neuron and glial types are indicated along the shaded paths. Yield varies substantially for the different cell types. In some cases only NSCs from certain sources can generate specific types of neural tissue. For example, only ES and iPS derived NSCs (gold shaded lines) can generate all types of neurons, whereas fetal and adult-derived NSCs do not easily make dopaminergic and motor neurons after expansion in culture.



Thanks