STEM CELLS IN NEUROSURGEY

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The Fundamental axiom of neuroscience

"the adult human brain, in contrast to other organs such as skin and liver, lacked the capacity for self repair and regeneration."

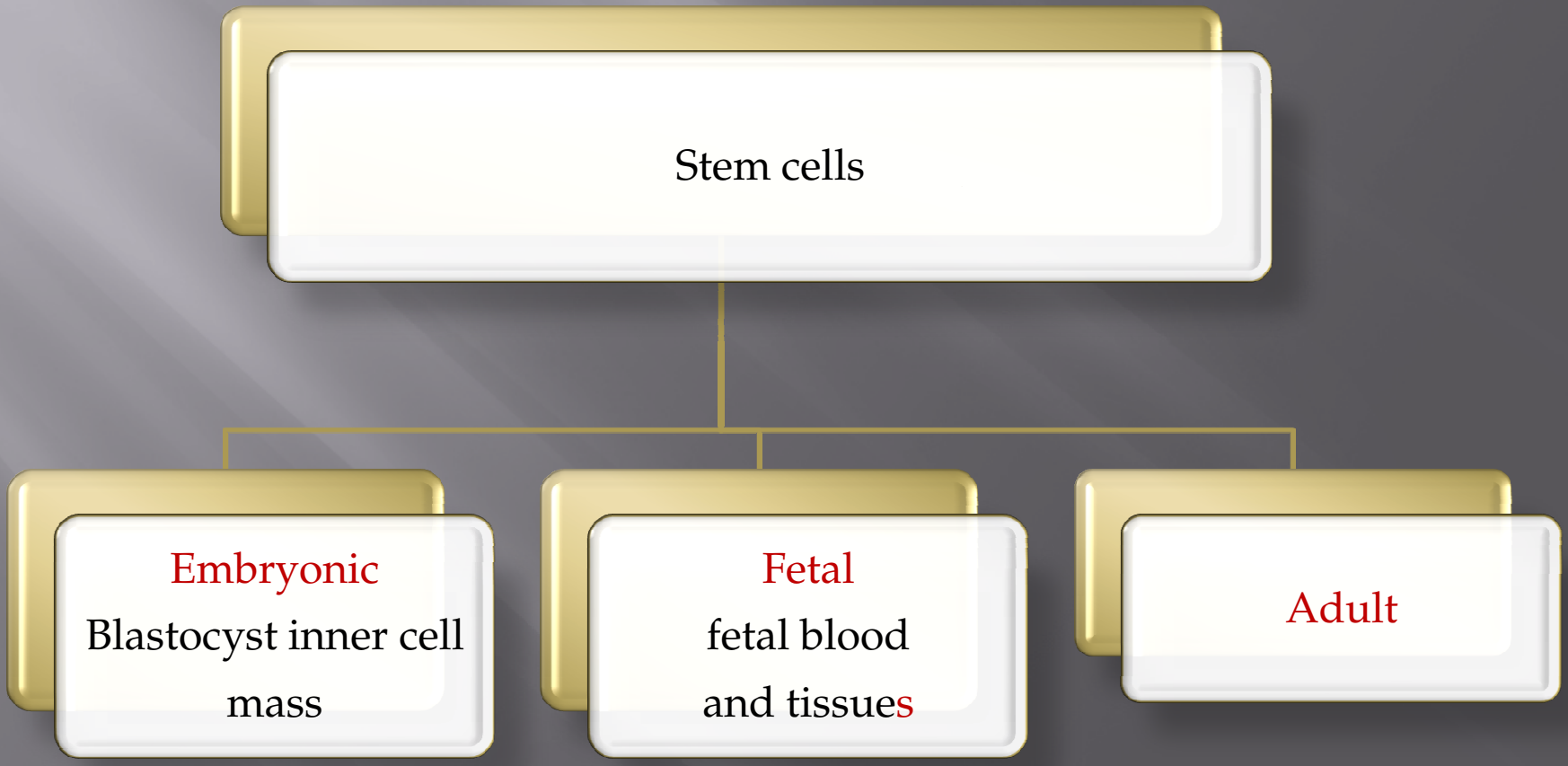
no longer considered true.

- Cell replacement therapy is an exciting research area

- Offers potential treatment for
  developmental
  traumatic &
  degenerative neurological
  diseases for which there is currently no cure.

Stem cells

- Defined by two main properties: self-renewal and multipotency



Embryonic Stem cells

- Cells derived from the inner cell mass
- Can be propagated indefinitely in culture
- Characteristic set of markers
- Lack of contact inhibition
- Atypical cell cycle regulation
- Form teratocarcinomas in nude mice
- Ability to differentiate

Fetal Stem Cells

- The "building block" cells of blood, tissue and organs
- Earliest cells found in the fetus, which then replicate efficiently and rapidly to create more blood, liver tissue, heart tissue
- Umbilical cord blood is a rich source

Adult stem cells

- Potential source of autologous cells for transplantation therapies that eliminates immunological complications.
- First recognized in the hematopoietic system with the development of bone marrow transplantations.
- Discovery of unexpected plasticity and regenerative capabilities in the adult CNS made it the first solid organ system shown to possess somatic stem cells.

Neural Stem Cells

- First inferred from evidence of neuronal turnover in the olfactory bulb and hippocampus
- Cells with more restricted neural differentiation capabilities committed to specific subpopulation lineages
- Currently, there is still no set of markers or protein expression profiles that precisely define and fully characterize undifferentiated NSCs

Induced pluripotent stem cells

- Type of pluripotent stem cell artificially derived from a non-pluripotent cell, typically an adult somatic cell, by inducing a "forced" expression of certain genes.
- Are believed to be identical to natural pluripotent stem cells
- first produced in 2006 from mouse cells and in 2007 from human cells.

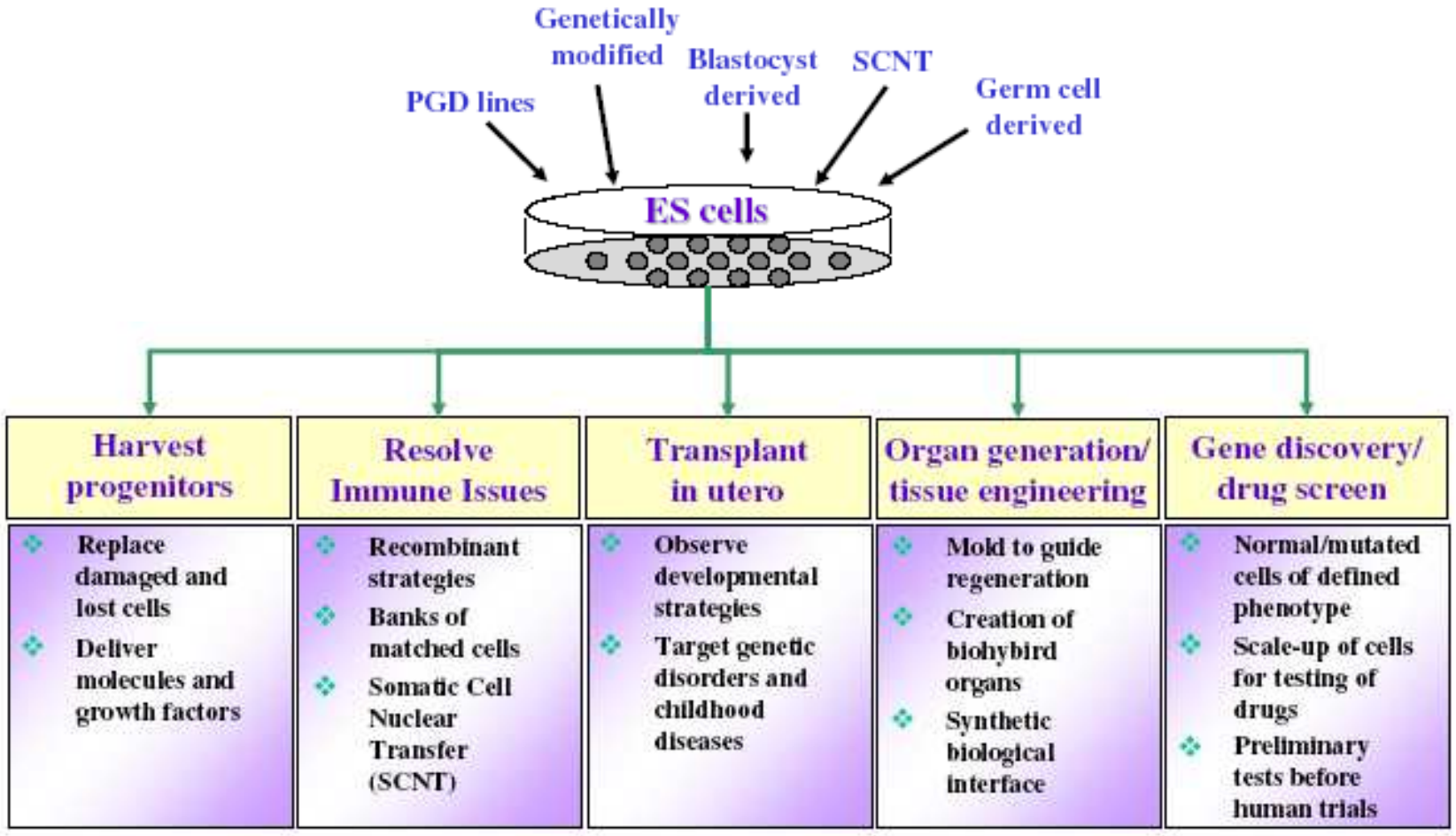
Yu J, Vodyanik MA, et al. | Induced Pluripotent Stem Cell Lines Derived from Human Somatic Cells | Science DOI: 10.1126/science.1151526

- Oncogenecity is a concern

Takahashi, K. & Yamanaka, S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 2006;126:663–676

Cell Type	Donor Source	Advantages	Disadvantages
ESC	embryo	unlimited expansion in undifferentiated state, enormous differentiation potential	difficulty in directing in vitro differentiation, potential of teratoma formation
neural stem/ progenitor cells	fetal brain	long-term in vitro expansion, large differentiation potential, safe	lack of ability to differentiate into projection neurons
neuronal-restricted progenitor cells	embryo or fetal brain	predictable differentiation to neuronal phenotype, safe	restricted neuronal differentiation potential, very limited expandability
primary neural cells	fetal brain	predictable phenotypic differentiation, safe	lack of expandability, restricted differentiation potential, require stage-specific embryonic source, mixed/inconsistent cell types
adult stem cells	adult "self" neural or nonneural	avoids ethical & immunological constraints, expandable in culture, possible autologous transplant, safe	greatly restricted potential in neural differentiation, questionable functional differentiation & integration
xenotransplant cells	nonhuman	readily available, possible genetic modification	tendency of immune rejection, possible zoonoses
iPSCs	adult somatic cells with nuclear transfer	immunosuppression avoided, large differentiation potential, expandable	difficulty in directing in vitro differentiation, potential of teratoma formation

Uses


Genetically modified
PGD lines
Blastocyst derived
SCNT
Germ cell derived
ES cells
Harvest progenitors
Replace damaged and lost cells
Deliver molecules and growth factors
Resolve Immune Issues
Recombinant strategies
Banks of matched cells
Somatic Cell Nuclear Transfer (SCNT)
Transplant in utero
Observe developmental strategies
Target genetic disorders and childhood diseases
Organ generation/ tissue engineering
Mold to guide regeneration
Creation of biohybird organs
Synthetic biological interface
Gene discovery/ drug screen
Normal/mutated cells of defined phenotype
Scale-up of cells for testing of drugs
Preliminary tests before human trials

Treatment of Brain Cancer

- Malignant gliomas represent a significant challenge
- Urgent need for the development of more effective therapies
- A therapeutic delivery system that could ‘‘seek out’’ dispersed tumor cells
- One promising method of meeting this challenge is the use of neural stem cells (NSCs) and mesenchymal stem cells (MSCs).

Stem cells as delivery vehicles

- Ability of stem cells to ‘‘home in’’ to areas of intracranial pathologic change

  Aboody KS, Brown A, Rainov NG, et al. Neural stem cells display extensive tropism for pathology in adult brain: evidence from intracranial gliomas. Proc Natl Acad Sci USA 2000;97:12846–51

- Drug delivery to disseminated tumor cells

Immunostimulatory cytokines

- Retroviral mediated gene transfer to induce expression of mouse interleukin (IL)-4 (C57.npr.IL-4) or the lysosomal enzyme, galactocerebrosidase (C57.npr.GALC)

  Benedetti S, Pirola B, Pollo B, et al. Gene therapy of experimental brain tumors using neural progenitor cells. Nat Med 2000;6:447–50.

- NSC tropism for glioma could be exploited for therapeutic benefit by engineering NSCs to express and deliver IL-12

Prodrug activation enzymes

- Prodrugs are compounds on chemical modification by specific enzymes, converted to physiologically active molecules

- NSCs could be engineered to express the bioactive transgene for CD, an enzyme that converts the nontoxic prodrug 5-FC to 5-FU

Aboody KS, Brown A, Rainov NG, et al. Neural stem cells display extensive tropism for pathology in adult brain: evidence from intracranial gliomas. Proc Natl Acad Sci USA 2000;97:12846–51

- NSCs permitted the delivery of a prodrug-activating enzyme directly to tumor cell , enhancing the tumor-toxic effect & minimizing local toxicity

Viral vectors

- Cellular vehicles for the delivery of replication-conditional oncolytic herpes simplex virus

  Herrlinger U, Woiciechowski C, Sena-Esteves M,et al. Neural precursor cells for delivery of replication-conditional HSV-1 vectors to intracerebral gliomas.Mol Ther 2000;1:347–57.

- An important tool for a targeted therapeutic attack against disseminated glioma

Proapoptotic proteins

- Members of the tumor necrosis factor (TNF)family
- TNF-related apoptosis-inducing ligand (TRAIL), has been shown to induce apoptosis selectively in malignant glial cells while sparing normal tissue
- Inoculation of TRAIL secreting NSCs into gliomatous brain

Ehtesham M, Kabos P, Gutierrez MA, et al. Induction of glioblastoma apoptosis using neural stem cell-mediated delivery of tumor necrosis factor-related apoptosis-inducing ligand. Cancer Res 2002;62:7170–4.

Inherent antitumor activity of neural stem cells

- Benedetti and colleagues first presented evidence suggesting that exogenously administered unmodified NSCs may be independently capable of inhibiting glioma proliferation in vivo

  Benedetti S, Pirola B, Pollo B, et al. Gene therapy of experimental brain tumors using neural progenitor cells. Nat Med 2000;6:447–50

- The exact mechanisms remain unclear.

- NSCs may elaborate certain factors, such as transforming growth factor (TGF)-b

  Staflin K, Honeth G, Kalliomaki S, et al. Neural progenitor cell lines inhibit rat tumor growth invivo. Cancer Res 2004;64:5347–54

Future perspectives and challenges

- Preliminary evidence has established the promise of stem cell therapy for malignant glioma.
- Significant concerns must be addressed :
  ethical, immunological etc.
- An optimal stem cell candidate must not only be readily accessible but should possess significant migratory capabilities and display robust tumor tropism.
- Additional sources must be explored

Traumatic Brain Injury

- Many head-injured patients incure permanent neurologic impairment.
- Neurogenesis increases in response to mechanical brain injury in multiple areas of the adult mammalian brain.
- Studies have shown posttraumatic neurogenesis occur in hippocampus, subventricular zone and some extent in cortex

Kernie SG, Erwin TM, Parada LF. Brain remodeling due to neuronal and astrocytic proliferation after controlled cortical injury in mice. J Neurosci Res 2001;66(3):317–26.

- The autologous transplantation of putative NPCs harvested from and reintroduced into patients with open brain trauma was recently reported
- The harvest of NPCs from neocortical regions and subsequent improvement in neurologic function after NPC reintroduction, as measured by imaging and clinical outcome scores
- There is clearly much work to be done in verifying these results.

Zhu J, Wu X, Zhang HL. Adult neural stem cell therapy: expansion in vitro, tracking in vivo and clinical transplantation. Curr Drug Targets 2005;6(1):97–110

Spinal Cord Injury

- Once thought impossible, nervous system repair is now entering the realm of feasibility.

(1) It is not necessary to cure a nervous system injury.

(2) A disproportionate return of function can result from a small degree of regeneration.

(3) Substantial loss of spinal cord tissue, particularly gray matter, does not preclude near-normal long-tract function.

- Growing evidence indicates substantial but limited cellular and molecular mechanisms for self-repair do exist.

- In animal models of SCI proliferation in the ependymal and periependymal canal gives rise to precursor cells that differentiate toward glial lineages

Yang H, Lu P, McKay HM, et al. Endogenous neurogenesis replace oligodendrocytes and astrocytes after primate spinal cord injury. J Neurosci 2006; 26(8):2157–66.

- Neurons unregulated their expression of

1) Regeneration-associated proteins like growth associated protein-43 (GAP-43), b1-tubulin and bII-tubulin

2) Cell adhesion molecules, including L1

3) Growth-promoting molecules, such as fibroblast growth factor-2 , ciliary neurotrophic factor, glial growth factor-2 , glial-derived neurotrophic factor & vascular endothelium growth factor (VEGF),

Barriers to regeneration and common strategies of spinal cord repair

(A) Prevention of progression of secondary injury: necrotic and apoptotic cell death can be prevented by antiexcitotoxic drugs and antiapoptotic treatments.

(B) Compensation for demyelination: chemicals that prevent conduction block in demyelinated areas and agents that encourage surviving oligodendrocytes to remyelinate axons can be provided. Lost oligodendrocytes can be replenished

(C) Removal of inhibition: agents that block the actions of natural inhibitors of regeneration or drugs that down regulate expression of inhibitory proteins can be provided.

(D) Promotion of axonal regeneration: growth factors that promote regeneration (sprouting) of new axons can be provided

(E) Direction of axons to proper targets: guidance molecules can be provided, or their expression can be increased in host cells

(F) Creation of bridges: bridges would be implanted into the cyst, which would provide directional scaffolding that encourages axon growth.

(G) Replacement of lost cells: cells capable of generation of all cell types (progenitor cells or ES cells) would be implanted. Substances that induce undifferentiated cells to replace dead cells would be provided. Transplanted cells to deliver regenerative molecules would also be used.

Barriers	Mechanisms	Strategies
Tissue lost (injury epicenter, typically cystic)	Cell replacement	Transplantation
	Bridging the gap	Peripheral nerve transposition
	Endogenous cell replacement	ABRT to stimulate endogenous cell birth and survival
	Appropriate cell differentiation	Deliver growth factors or ABRT to induce neuronal differentiation
Demyelination	Cell replacement	Transplantation
	Stimulation of endogenous oligodendrocyte precursors to myelinate	ABRT or growth factor delivery
	Overcome conduction block	Delivery drugs that enhance conduction (ie, 4-AP, HP-184)
Scar and inhibitory molecules	Antibody therapy	IN-1 antibody
	Infusion of enzymes	Chondroitinase ABC, sialidase, hyaluronidase
		ABRT to inhibit expression of inhibitory effects
Lack of growth molecules	Restoration of key molecules	Overexpression of growth factors by genetically altered transplanted cells
		ABRT to stimulate cellular release
Guidance molecules	Cell transplantation	Overexpression of growth factors by transplanted cells
	Induction of endogenous expression	Deliver of agents to induce signaling
		ABRT to induce molecular expression

Transplanting embryonic stem cells as therapy for spinal cord injury

- Unique features make ES cells primary candidates

- Can replicate indefinitely while maintaining genetic stability
- Being pluripotent, can differentiate into every cell type
- Cells can be genetically manipulated in unprecedented ways
- Low immunogenicity

Previous experiments involving murine neuronal progenitors have shown that ES cells

(1) Survive for weeks after transplantation

(2) Do not form teratomas in the spinal cord

(3) Differentiate into the three cell types of the neuronal lineage (neurons, oligodendrocytes, and astrocytes)

(4) Induce functional improvements

McDonald JW, Liu XZ, Qu Y, et al. Transplantedembryonic stem cells survive, differentiate and promoter ecovery in injured rat spinal cord. Nat Med1999;5(12):1410–2

Cummings BJ, Uchida N, Tamaki SJ, et al. Human neural stem cells differentiate and promote locomotor recovery in spinal cord-injured mice. Proc NatlAcad Sci USA 2005;102(39):14069–74

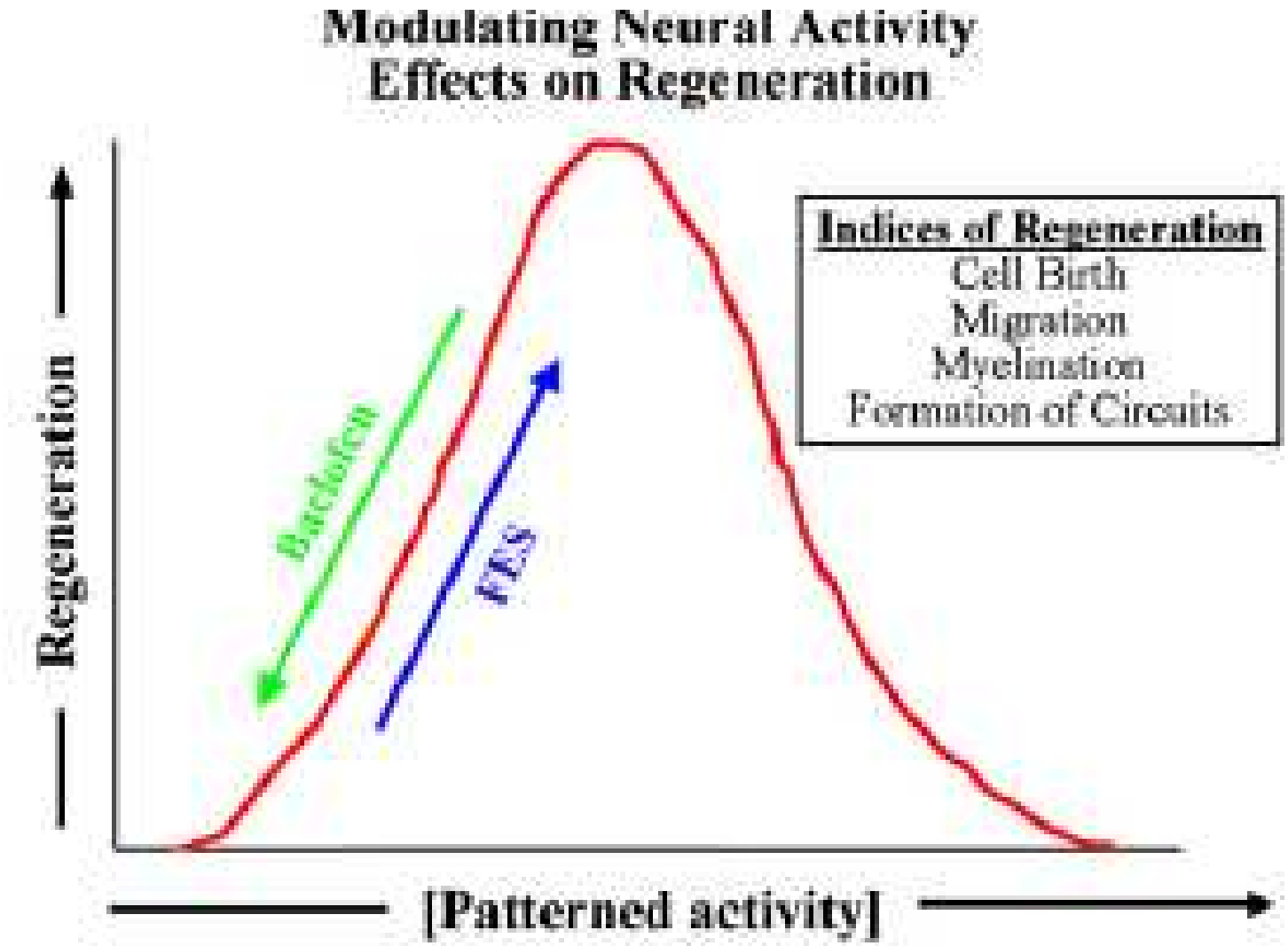
Possible mechanisms for improvement

- ES cells can secrete neurotrophic factors at the injury site
- Differentiated derivatives of ES cells can remyelinate surviving axons as well as regenerating axons
- Improve function by integrating themselves into a host's motor and sensory circuits.

Activity-based restoration therapy

- The spinal cord below the level of injury experiences the consequences of reduced neural activity after SCI
- Optimal neural activity is required to maximize spontaneous recovery of function after nervous system injury.
- Rehabilitative therapy needs to precede any molecular or cellular therapeutic intervention to recover function after SCI to optimize curative efforts.

Cummings BJ, Uchida N, Tamaki SJ, et al. Human neural stem cells differentiate and promote locomotorrecovery in spinal cord-injured mice. Proc NatlAcad Sci USA 2005;102(39):14069–74.


Modulating Neural Activity
Effects on Regeneration
Regeneration
[Patterned activity]
Baclofen
FES
Indices of Regeneration
Cell Birth
Migration
Myelination
Formation of Circuits

Peripheral nerve injury

- Capacity for regeneration in peripheral nerve is higher than that of the central nervous system
- Even then, complete recovery is fairly infrequent, misdirected, or associated with debilitating neuropathic pain.
- Combined approaches with cells or trophic factors within synthetic tubes may extend their functionality

Possible sources for peripheral nerve repair

- Embryonic neural stem cells
- Bone marrow cells
- Adipose tissue
- The skin and its associated structures

Considerations for Optimizing Stem Cell Therapy for Peripheral Nerve Repair

- Optimization strategies should take the number of delivered cells into account

  (Mosahebi A, Woodward B, Wiberg M, et al: Retroviral labeling of Schwann cells: in vitro characterization and in vivo transplantation to improve peripheral nerve regeneration. **Glia34:8–17, 2001)**

- Direct microinjection , suspension within artificial tubes and seeding within devitalized muscle or nerve grafts

- It is expected that cells will be prompted by the microenvironment to differentiate into the required cell type as shown by invitro studies

  (Brannvall K, Corell M, Forsberg-Nilsson K, et al: Environmental cues from CNS, PNS, and ENS cells regulate CNS progenitor differentiation. **Neuroreport 19:1283–1289, 2008)**

- Choosing to predifferentiate stem cells toward a desired phenotype prior to delivery into the repair site

- Survival and effectiveness of transplanted cells can be improved by ex vivo genetic manipulation or concomitant delivery of protective agents or trophic factors

  Pan HC, Chen CJ, Cheng FC, et al: Combination of G-CSF administration and human amniotic fluid mesenchymal stem cell transplantation promotes peripheral nerve regeneration.**Neurochem Res , 2008**

- Differences in the material in which stem cells are delivered have demonstrated varying capacities to support long-term cell survival

  Cao F, Sadrzadeh Rafie AH, Abilez OJ, et al: In vivo imaging and evaluation of different biomatrices for improvement of stem cell survival. **J Tissue Eng Regen Med 1:465–468,**2007

- Immunosuppressive regimens

  Parr AM, Kulbatski I, Wang XH, et al: Fate of transplanted adult neural stem/progenitor cells and bone marrow-derived mesenchymal stromal cells in the injured adult rat spinal cord and impact on functional recovery. **Surg Neurol 70:600–607,** 2008

Cell Source/ Type	Authors & Year	Donor/ Host Animal	No. of Cells Injected	Delivery Method	Cell Survival Time	% Survival	Phenotype	Regenerative Advantage Conferred Over Vehicle
bone marrow aspirate/ mesenchymal stem cell	Hu et al., 2007	Rhesus monkey	2×10^7	proximal/distal side of acellular allograft	ND	ND	ND	↑ no. of NF+ axons, improved CMAP amp/latency
	Keilhoff et al., 2006[25]	Wistar rat	2×10^6/ ml	devitalized muscle conduits	≥6 wks	ND	MBP+, bipolar morphology in predifferentiated cells only	↑ no. of myelinated fibers; faster return of thermosensitivity
	Chen et al., 2007	Sprague-Dawley rat	10^6 cells	in gelatin w/in lumen of silicone tube; 15-mm gap	unable to detect due to label loss	ND	express neurotrophins; not P0, PMP22	improved SFI, improved CMAP amp/ latency
C17.2 neonatal cerebellar granule cells ± overexpression of GDNF	Heine et al., 2004	mouse cell line/ Sprague-Dawley rat	5×10^5 cells	subepineural injection into chronically denervated nerve	up to 4 mos	0.5–1%	most remained distal to repair site, very few GFAP or NF+; mesenchymal tumor	↑ no. of axons, improved CMAP amp/ latency
hippocampal E17 neuronal progentitor cells	Murakami et al., 2003	Fischer rat	10^5 cells	in collagen gel w/ in silicone tube; 15-mm gap	up to 10 wks	ND	some cells positive for S100/p75	superior electrophysiological recovery
E11 DRG/ boundary cap neural crest stem cells	Aquino et al., 2006	Rosa 26 mouse (lac-Z+)/ Sprague-Dawley rat	4×10^3 cells	intact nerve; cultured in 12-mm silicone tube & implanted in nerve gap	up to 90 days; only predifferentiated cells survived	ND	69.7–94.6% GFAP+ after 13 & 60 days, respectively; MBP+ transplanted cells ensheathed axons in tube	ND
neonatal skin/ neural crestlike precursors	Marchesi et al., 2007	Wistar rat	10^6 cells	in PBS in lumen of collagen guide; 16-mm gap	up to 2 mos	25–38%	4.5% S100+, 6.1% GFAP+	improved CMAP, SFI, no. of myelinated fibers
vibrissal follicles	Amoh et al., 2005	C57/B6-GFP/C57/ B6 mouse	ND	transplanted btwn severed sciatic/ tibial nerve stumps	detected after 2 mos	ND	GFAP+; envelop βIII tubulin+ axons	improved SFI, contraction of gastrocnemius
amniotic fluid/ mesenchymal stem cells	Murakami et al., 2003	Sprague-Dawley rat	1.5×10^4 cells	in fibrin glue around crushed sciatic nerve	up to 10 days, none at 4 wks	ND	NT-3 and CNTF+; no expression of GFAP/ S100β	motor function recovery, improved CMAP

Effect of bone marrow-derived mononuclear cells on nerve regeneration in the transection model of the rat sciatic nerve

Journal of Clinical Neuroscience, Volume 16, Issue 9, September 2009, Pages 1211-1217
Rohit Kumar Goel, Vaishali **Suri**, Ashish **Suri**, Chitra Sarkar, Sujata Mohanty, Meher Chand Sharma, Pradeep Kumar Yadav, Arti Srivastava

- Study demonstrated that local delivery of BM-MNCs (which can be isolated easily from bone marrow aspirates) into injured peripheral nerve increases the rate and degree of nerve regeneration

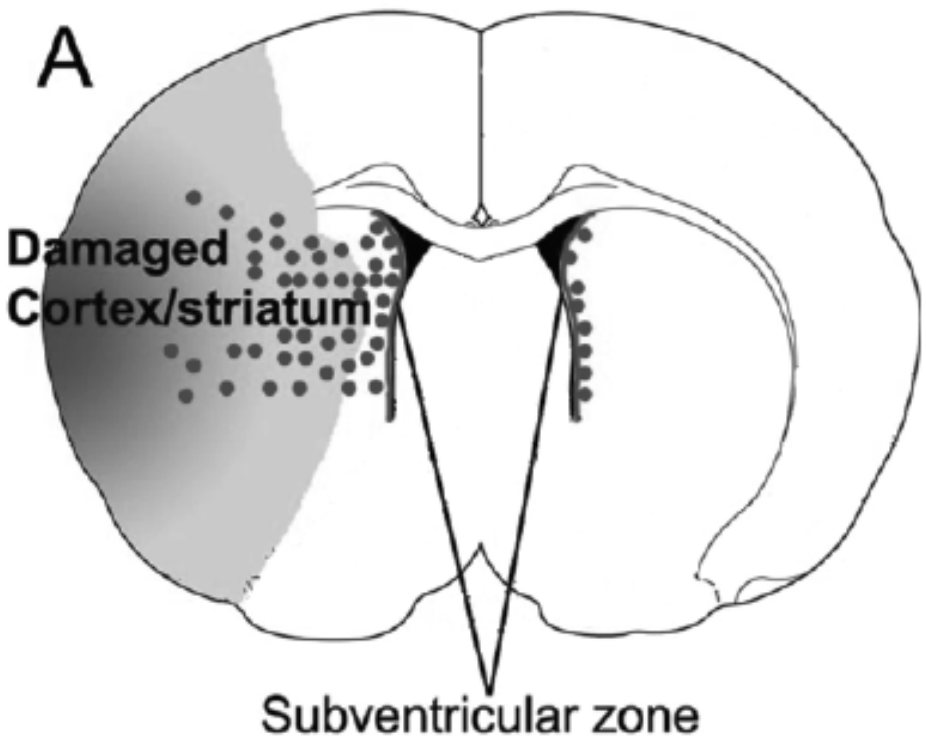
- Nilesh K ,Suri A etal.

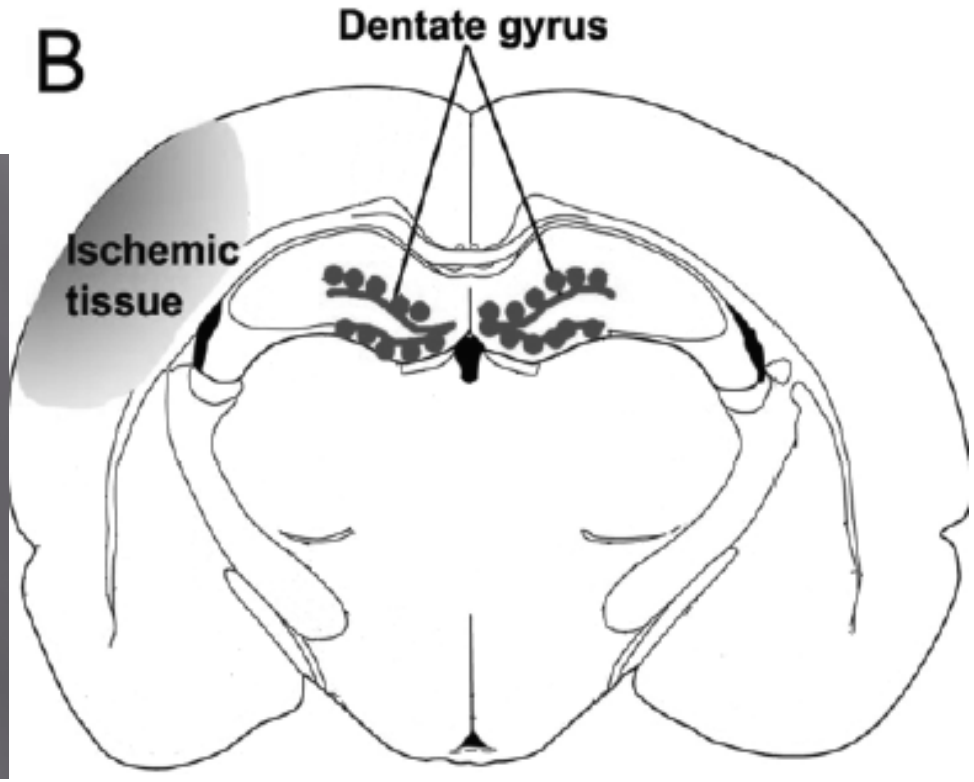
  Semi quantitative and quantitative assessment of the effect of bone marrow derived mononuclear cells (BM-MNC) on early and late phase of nerve regeneration in rat sciatic nerve model.

- Observed statistically significant difference in the number of fibers, percentage of axons, percentage of myelinated fibers and axon diameter, fiber diameter myelin thickness in the two groups. These differences were evident at the repair site and at intermediate distal site at 15 days and at the distal most site at the end of 60 days

Stroke

- Stroke is the leading cause of death and disability in the United States
- Treatment of stroke has emphasized prevention and protection, but little has been done in the brain to repair stroke.
- An approach to functional repair would be replacing the damaged tissue with new cells


A
Damaged
Cortex/striatum
Subventricular zone


B
Dentate gyrus
Ischemic
tissue

- The neuronal cells could improve neurological function through a number of different mechanisms.
- Provision of neurotrophic support (acting as local pumps to support cell function)
- Provision of neurotransmitters
- Reestablishment of local interneuronal connections cell differentiation and integration
- Improvement of regional oxygen tension

- Ongoing study by Dr. K prasad etal. Dept of neurology ,AIIMS
- Injected stem cells taken from the patient's bone marrow back into his antecubital vein
- 50% in the stem cell group became free of deficits like weakness of one limb and inability to walk as against 30% in the control arm
- Large-scale DBT-funded multi-centric study is on.

Parkinson's Disease

- Medical treatment and even surgical approaches are not permanent solutions, given the increasing side effects that arise over time.

- Stem cell therapy holds tremendous promise for being one of the most exciting therapeutic avenues of the future

- Cell culture systems in which stem cells are differentiated into dopaminergic cells that are then transplanted into the SN or its target areas.
- This may offer a stable long term solution in terms of motor symptom suppression
- Initially realized by proof-of-principle studies with fetal brain transplants.

Fetal VM tissue transplantation

- Freed et al.

Forty patients

Robust graft survival was demonstrated by PET and confirmed on postmortem.

Study failed to meet its primary endpoint of clinical improvement

Troubling off-period dyskinesias were observed in 15% of the patients receiving neural transplantation.

Freed CR, Greene PE, Breeze RE, et al. Transplantation of embryonic dopamine neurons for severe Parkinson's disease. N Engl J Med 2001;344:710–9.

Olanow et al.

- 34 patients
- Striatal fluorodopa uptake was significantly increased
- There was no significant improvement in the motor component
- Off-medication dyskinesias were observed in as many as 56% of the patients who received transplants.

Olanow CW, Goetz CG, Kordower JH, et al. A double- blind controlled trial of bilateral fetal nigral transplantation in Parkinson's disease. Ann Neurol 2003;54:403–14.

Generating dopaminergic neurons from stem cells

Requirements

1. Reliably survive in a high percentage

2. Secrete a standardized amount of dopamine for consistent efficacy

3. Avoid rejection or induction of an inflammatory response

4. Appropriately connect with the target cells in the striatum

- Most experience so far has supported the notion that only ES cells and fetal brain neural stem cells (NSCs) are reliable sources for dopaminergic neurons

- Embryonic stem cells showing the more promising results than NSC

The choice of location for stem cell transplantation?

- In every animal experiment, it has become the standard to transplant cells into the striatum
- It is important in the long run to transplant dopaminergic cells into the SN.

Stem cells as delivery tools

- May serve as vehicles to deliver a wide variety of proteins to specific areas in the brain in an attempt to repair defective neurons rather than replacing them
- These include trophic factors, enzymes of the dopamine synthesis pathway, or proteins that are defective in the genetic causes of PD

Future Directions, Toward Clinical Applications

- Clinical trials have provided proof of Principle that transplantation of fetal DA neurons can Improve patients' neurological symptoms

- 2 "double-blind" clinical trials have shown benefits in subgroups of patients.

- **Long-term Evaluation of Bilateral Fetal Nigral Transplantation in Parkinson Disease** Robert A. Hauser, MD etal. *Arch Neurol.* 1999;56:179-187.

Six patients with advanced PD underwent bilateral fetal nigral transplantation

Conclusions: Consistent long-term clinical benefit and increased fluorodopa uptake on positron emission tomography. Clinical improvement appears to be related to the survival and function of transplanted fetal tissue

Concerns

- Cell survival
- Loss of dopaminergic phenotype
- Long term recovery prospects
- Side effects like dyskinesias
- Immunogenicity
- Quality of the stem cells cross-species contamination,
- Ethical issues

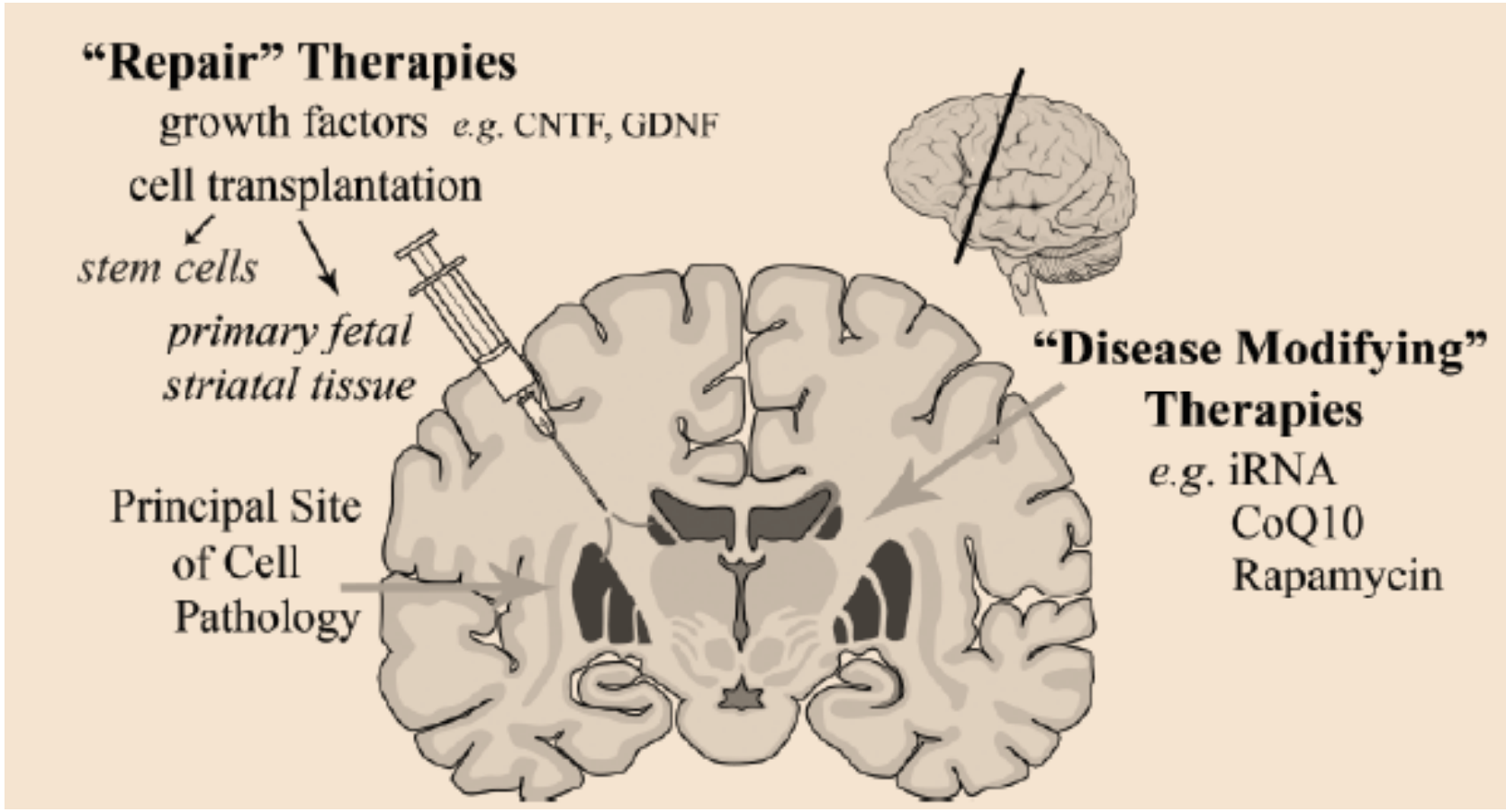
Cell therapy in Huntington disease

- 3 approaches to HD cell therapy includes

1) The potential for self-repair through the manipulation of endogenous stem cells and/or neurogenesis.

2) The use of fetal or stem cell transplantation as a cell replacement strategy.

3) The administration of neurotrophic factors to protect susceptible neuronal populations.


"Repair" Therapies
growth factors e.g. CNTF, GDNF
cell transplantation
stem cells
primary fetal striatal tissue
Principal Site of Cell Pathology
"Disease Modifying" Therapies
e.g. iRNA
CoQ10
Rapamycin

- Transplantation of fetal-derived cells into degenerative striatal regions has proven safe and at least partially effective in patients with HD.

- The use of both stem cell– and growth factor–based therapies, although in its early stages, appears likely to contribute to future clinical strategies

Other uses

- Amyotrophic lateral sclerosis
- Lysosomal storage disorders
- *Retinal Degenerative Diseases*

  vitreoretinal degeneration

  age-related macular degeneration,

  retinitis pigmentosa,

  numerous forms of retinopathy
- Regeneration of the intervertebral disc

Methods of stem cell implantation

- Stem Cell Implantation using Brain Stereotactic Surgery
- Direct injection to the injury site
- Subarachnoid Stem Cell Implantation via Lumbar Puncture
- Intravenous transplantation

  Hiroki Takeuch etal. Neuroscience Letters ,Volume 426, Issue 2, 16 October 2007, Pages 69-74

- Intra-nasal cell delivery

  Danielyan,L.,etal.,Intranasaldeliveryofcellstothebrain.Eur.J.CellBiol.(2009),

Problems with use of neural stem cells

- Logistic and Ethical problems
- The use of allogeneic cells would necessitate immunosuppressive therapy
- The potential tumorigenicity of transplanted NSCs
- Ideal cellular therapy should comprise autologous cells that can be harvested without difficulty, processed efficiently in vitro, and reinoculated into the same patient.

Alternate sources of stem cells

- BM an alternative and much more clinically accessible pool of stem cells
- Eliminate the ethical concerns and logistic issues related to the use of allogeneic fetal-derived NSCs
- Relative ease of accessibility
- BM-derived stem cells represent a potentially important step

- Adipose tissue

  Comparable phenotypic profile to the bone marrow stromal cells

  Kingham PJ, Kalbermatten DF, Mahay D, et al: Adiposederived stem cells differentiate into a Schwann cell phenotype and promote neurite outgrowth in vitro. **Exp Neurol 207:267–274, 2007**

- Skin

  neural crest stem cells has been found in the bulge area of hair and whisker follicles

  Sieber-Blum M, Grim M, Hu YF, et al: Pluripotent neural crest stem cells in the adult hair follicle. **Dev Dyn 231:258–269,**2004

Stem cells: Ethics, law, and policy

- Ethics and policy debates centered on the moral status of the embryo—whether the 2- to 4-day-old blastocyst is a person
- Each country has different moral and regulatory frameworks—one permissive, one restrictive.
- Stem cell research involves balancing the interests of 2 groups

1) the cell, gamete, and embryo donors and

2) The volunteers participating in clinical trials

- First-in-human trials are high-reward, high-risk endeavors and are full of uncertainty.
- Investigators and sponsors should present review committees with a clear and thoughtful research plan that will satisfy requirements to “do no harm.”
- Ethicists and nonspecialists should take time to familiarize themselves with the basics of stem cell biology and the predictive power of animal models
- The world is watching these experiments to see if the field can live up to its bold promise.

Indian Scenario

- ICMR and department of biotechnology has come up with "Guidelines for stem cell research and therapy "in 2007.
- These guidelines address both ethical and scientific concerns to encourage responsible practices in the area of stem cell research and therapy
- National Apex Committee for Stem Cell Research and Therapy (NAC-SCRT) and Institutional Committee for Stem Cell Research and Therapy (IC-SCRT) formed to review and monitor stem cell research .

Use of stem cells for therapeutic purposes

- As of date, there is no approved indication for stem cell therapy as a part of routine medical practice, other than Bone Marrow Transplantation (BMT)

- All other usages are experimental and should be done with prior permission from the regulatory body.

Safeguards for Use of Human Embryonic Stem (HES) Cells

Safeguards	Tests/Methods
Screening Donors	HIV, HBV, HCV, CMV, HTLV, VDRL
Derivation and culture	Use control and standardized practices and procedures for establishment of human embryonic stem cell lines. GMP clean room is must if ES cells are to be used for clinical application
Animal source	Develop alternatives to culturing ES cells on animal derived feeder cells and serum.
Characterization	Perform detail characterization of stem cell lines
	Cellular markers: SSEA-1, SSEA-3, SSEA-1, OCT-4, TRA-1-60, TRA-1-81, Alaline phosphatase, ABCG2,
	Molecular markers: OCT-4, SOX2, NANOG, REX1, TERT, UTF-1, DPPA5, FGF4, FOXD3, TDGF1, BCRP1, ABCG2, GCTM2, Genesis, GDF3, GCNF
Karyotyping	Traditional karyotyping of FISH including sex chromosome
HLA Typing	A, B, DR
Microarray	Growth factors, cytokines and ECM molecules
Immunophenotyping	**CD markers:** DC4, CD8, CD14, CD24, CD31, CD34, CD45, CD90, CD73, DC105, CD133
Analysis of differentitation properties	Minimum 3 markers for Ectoderm, Endoderm and Mesoderm should be checked. Each cell line should be checked for differentiation potential.
Teratoma Formation	Required SCID mouse
Comprehensive toxicity	Endotoxin, mycoplasma, aerobic, anaerobic cultures for sterility; and acute, sub-acute and chronic toxicity testing


Thank You!