Stem Cells: The New Therapeutics Era

Dr. Diala Abu-Hassan, DDS, PhD
School of Medicine
dr.abuhassand@gmail.com

Central Nervous System
What are stem cells?

- Are primal cells common to all multicellular organisms that retain the ability to renew themselves through cell division and can be differentiated into a wide range of specialized cell types.

- All stem cells are unspecialized (undifferentiated) cells that are of the same family type (lineage).
Differentiation vs self renewal

Asymmetric division due to differential segregation of cell membrane proteins between the daughter cells

Self-renewal: The ability to go through numerous cycles of cell division while maintaining the undifferentiated state.

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HOW DOES ASYMMETRIC DIVISION OCCUR?

• DIFFERENTIAL SEGREGATION OF CELL MEMBRANE PROTEINS (SUCH AS RECEPTORS) BETWEEN THE TWO DAUGHTER CELLS.
WHAT DOES STEM CELL DIVISION PRODUCE?

• **PROGENITOR CELL**: STEM CELLS GENERATE AN INTERMEDIATE CELL TYPE OR TYPES BEFORE THEY ACHIEVE THEIR FULLY DIFFERENTIATED STATE.

Venere et al 2012
Stem cell niche

A specialized cellular environment that provides stem cells with the support needed for self-renewal (keeping stemness).
Stem cell niche-composition

**Cells only**
A single cell type, or a whole host of interacting cells. Cells outside the stem cell’s lineage, or they may derive primarily from the stem cell’s own descendants.

**Cells & ECM (extracellular matrix)**

**Secreted or cell surface factors**

Notch, Wnt, FGF, EGF, TGF-β, SCF, and chemokine families
Why stem cells need a special environment?

- Demands on stem cells necessitate special support for viability.
- Nutritive function
- Niches might be agents of feedback control (control of stem cell pool size).
- Niches are instruments of coordination among tissue compartments.
- Niches are hubs of inter-lineage coordination.
POTENCY OF STEM CELLS

• THE DIFFERENTIATION POTENTIAL OF THE STEM CELLS

TYPE OF POTENCY:

1- TOTIPOTENT (GIVES ALL CELLS OF THE BODY IN THE 3 GERM LAYERS AND EXTRAEMBRYONIC TISSUES)

2- PLEURIPOTENT (GIVES ALL CELLS OF THE BODY)

3- MULTIPOTENT (GIVES A FEW NUMBER OF CELL TYPES)

4- UNIPOTENT (GIVES ONE CELL TYPE)
Types of stem cells

Embryonic stem cells

- Are able to differentiate into all the specialized embryonic tissue

Adult stem cells

- Act as a repair system for the body replacing specialized damaged cells
Embryonic Stem Cells (ESCs)

- ES cells are derived from inner cell mass of mammalian blastocysts
- Develop before implantation in the uterus
Pluripotency of ESCs

What makes embryonic stem cells pluripotent?

Pluripotency transcription factors (TFs):

1. Oct 4
2. Nanog
3. Wnt-β-catenin signaling
4. Other TFs
The Ethical Dilemma of ESCs

To use ESCs, you have to kill embryos and take their cells, is this ethical?

Prevention or alleviation of suffering

Respect the value of human life

Morals and religion

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Induced Pluripotent Stem Cells (iPSCs)

Cells that resemble ESCs but they were made by converting differentiated cells (as fibroblasts) back into stem cells.

- **Ethical**
- **Safer**
- **Autologous**
- **Patient-specific**
iPS cells were obtained by transducing embryonic and adult fibroblasts with defined transcription factors.

- OCT3/4, SOX2, c-Myc, KLF4

Different sets of TFs were used to generate iPSCs


Yamanaka’s comparison of iPS and ES cells

iPSCs were compared to embryonic stem cells in many aspects like:

- Surface antigens
- Morphology
- Gene expression
- Telomerase activities
- In vitro differentiation
- Proliferation
- Teratoma formation
- Promoter activities
- Epigenetic status of pluripotent cell-specific genes

iPS cells are indistinguishable from ES cells in:
Undifferentiated cells found throughout the body.

Function: they divide to replenish dying cells and regenerate damaged tissue.
Types of adult stem cells

1. Bone marrow stem cells
   A. Hematopoietic stem cells that give different cells of the blood
Types of adult stem cells

1. Bone marrow stem cells
B. Somatic stem cells such as mammary stem cells and mesenchymal stem cells that give osteoblasts, chondrocytes, myocytes, adipocytes, neuronal cells.
2. Neural stem cells: **neurospheres** – floating heterogenous aggregates of cells, containing a large proportion of stem cells responsible for adult neurogenesis in **subventricular zone**, which lines the **lateral ventricles of the brain**, and the dentate gyrus of the hippocampal formations. They are important for neural tissue regeneration.
Types of adult stem cells

3. Adipose tissue-derived stem cells (ASCs).
   Present in adipose tissue.
   Can be obtained by liposuction.
   It can differentiate to several cell types.
Types of adult stem cells

4. Umbilical cord stem cells.
   Are mesenchymal stem cells and can differentiate to several cell types
Types of adult stem cells

5. Olfactory adult stem cells: found in olfactory mucosal cells
Types of adult stem cells

6. Tissue stem cells in cornea, trabecular meshwork, etc.
USES OF STEM CELLS

• TO STUDY THE SPECIFIC SIGNALS AND DIFFERENTIATION

• GENETIC THERAPY

• DRUG TESTING

• CELL BASED THERAPIES

• STEM CELLS FOR CANCER TREATMENT BY ACTIVATION OF CHEMOTHERAPEUTIC AGENTS
STEM CELL THERAPY LIMITATIONS

- STEM CELL THERAPY HAS DISADVANTAGES SUCH AS
  - CARCINOGENICITY
  - IMMUNE REJECTION
  - INFECTION

- THESE FACTORS MAKE THE USAGE OF STEM CELL LIMITED.
LIMITATIONS OF USING ADULT STEM CELLS

1- Lack of stem cell markers resulting in difficulties to separate and identify cells.

2- In vitro systems for manipulating adult stem cell populations are often not well defined.

3- In vivo: Our understanding of how adult stem cells are regulated within their niche is in its infancy.

4- Multipotency of ASCs
Why stem cell research is important?

• Functional genomic studies to understand human embryonic gene expression, genomic data mining, and bioinformatics.

• To study biological processes to understand human developmental disorders like birth defects, cancers, etc.

• Creating human disease models for drug discovery and development.

• Cell-based therapy and regenerative medicine.
Stem Cells & neurodegenerative diseases
Neurodegenerative Diseases

A wide range of acute and chronic conditions in which neurons and glial cells in the brain and spinal cord are lost.

Acute: ischemic stroke or spinal cord injury

Chronic: Parkinson disease (PD), amyotrophic lateral sclerosis (ALS), or Alzheimer disease (AD).
Neurodegenerative Diseases & Stem Cell Therapy

- Clinical trials using stem cells have already been performed or initiated (e.g., for the rare, fatal, autosomal recessive neurodegenerative disorder Batten disease)

- No stem cell–based therapy has yet been proven beneficial for any neurodegenerative condition.

- Despite this fact, unproven treatments for several neurodegenerative diseases are offered at “clinics” around the world without rationale and with poor scientific and clinical basis.

- Ethical, regulatory, societal, and economical issues need to be addressed.
Main considerations when we use stem cells to treat neurodegenerative diseases

- What is required for the stem cell-based approach to be clinically competitive
- Risks to the patient that are acceptable, depending on disease severity. Animal models may not fully predict their toxicity, occurrence of immune and other biologic responses, and risk for tumor formation after implantation in patients.
- The variability between neurodegenerative diseases in the degree of disability that they cause and in the therapeutic options that are available.

PD- symptomatic treatment

ALS- No efficient treatment
Main considerations when we use stem cells to treat neurodegenerative diseases

- The cell type to be regenerated and transplanted.

PD - dopamine neurons

ALS - motor neurons

Stroke and Alzheimer's disease - several cell types

- The stem cell-based approach should show substantial improvement of functional deficits in animal models before their use in clinical application.

- To determine the biological mechanism underlying the observed effects of a stem cell-based treatment in an animal model. E.g. reconstruction of neuronal circuitry
Stem cell technology and cellular therapy classifications

- **Somatic cell**: Cells like fibroblasts may be used as shuttles for growth factors.
- **Stem cell**: Fetal tissue grafts provide a source of progenitor cells.
- **Tissue graft**: Fetal tissue grafts provide a source of progenitor cells.

**Induced pluripotent stem (iPSC) cell**
- Reprogrammed adult somatic tissue
- Characteristics of ES cells
- Differentiated into NPCs

**Embryonic stem cell (ES cell)**
- Derived from blastocyst inner cell mass
- True "stem cell"
- Pluripotent and self-renewing
- Differentiated into NPCs

**Mesenchymal stem cell (MSC)**
- Derived from bone marrow
- Potential to transdifferentiate into neural lineages

**Neural progenitor cell (NPC)**
- Restricted to neural lineages
- Self-renewing
- Common source for neurodegenerative disease cellular therapy

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Translating a stem cell-based treatment from the bench to bed

- Research and medical ethics must be considered.
- Trials begin with cells in the dish, then small animal models, followed by large animal models and ending with clinical trials.
- No treatment with stem cells should be applied until it is approved by the Food and Drug Administration (FDA)

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### Common considerations when translating stem cell therapies to neurodegenerative disease patients

<table>
<thead>
<tr>
<th>Inclusion/exclusion criteria</th>
<th>Enrolling late-stage patients may prevent loss of quality of life. Late-stage patients may mask any positive effects due to the intervention occurring too late in the disease course.</th>
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<tbody>
<tr>
<td>Realistic expectation</td>
<td>Informed consent forms must clearly illuminate the goals of the study. Safety trials vs. efficacy trials. Expectations of therapeutic effects based on disease state at intervention.</td>
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<tr>
<td>Controlled study</td>
<td>Ideal study is a double-blind placebo study. Late-stage patients may mask any positive effects not observed due to the intervention occurring too late in disease. Original PD studies offered control arms treatment after a 1-year follow-up which confuses interpretation of efficacy.</td>
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<td>Immunosuppression</td>
<td>While the brain remains an immunologically privileged site due to the blood-brain-barrier, there is evidence that this barrier can be compromised in disease. Studies into cell graft survival demonstrate that immunosuppression increases that survival of graft tissue.</td>
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<tr>
<td>Potential side effects</td>
<td>Prevent/minimize potential side effects (i.e. meningitis, fever). Avoid exacerbation of disease and tumor formation. Risk vs. quality of life.</td>
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<tr>
<td>Safety of cellular therapy administration</td>
<td>Consider CNS accessibility and safety of delivery methods. Pros/cons of systemic delivery, lumbar puncture or stereotactic injection are important.</td>
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Abbreviations: PD, Parkinson’s disease; CNS, central nervous system.
Parkinson’s disease (PD)

Degeneration of nigrostriatal DA neurons is the main pathology

Available treatment Tx: l-DOPA, DA agonists, enzyme inhibitors, and deep brain stimulation

No Tx for dementia

iPSCs for modelling the genetically complex PD

Characteristic symptoms are rigidity, hypokinesia, tremor, and postural instability
Stem cell–based therapies for PD

Proof of principle: clinical trials with intrastriatal transplantation of human embryonic mesencephalic tissue (rich in postmitotic DA neuroblasts).

- Other types of cells were tried for PD treatment but none gave highly improving results.
**Stem cell–based therapies for PD**

<table>
<thead>
<tr>
<th>Pros</th>
<th>Cons</th>
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<tr>
<td>-The DA neurons that form from the transplanted tissue reinnervate the denervated striatum and become functionally integrated, restoring striatal DA release and giving rise to clear symptomatic relief in some patients.</td>
<td>-A small fraction of graft-derived DA neurons contain Lewy bodies (the hallmark of PD).</td>
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<td></td>
<td>- Availability of human embryonic mesencephalic tissue is limited.</td>
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<tr>
<td>11–16 years after transplantation, cell replacement remains a viable therapy.</td>
<td>Variability of functional outcome after transplantation is high.</td>
</tr>
<tr>
<td>The progression of pathology in graft-derived neurons is slow, and they are still functional after a decade.</td>
<td>Poor standardization of the transplanted cell material contributes to the high variability.</td>
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</table>
Stem cell–based therapies for PD

Hurdles that prevent stem cell therapy for PD from bench to clinic:

✓ PD is a **multisystem disorder**, if **nondopaminergic** systems are affected, they will not improve by intrastriatal DA grafts.
✓ **Substantial re-innervation** of striatum has not been demonstrated.
✓ **Restoration of DA release** in *vivo* has not been demonstrated.
✓ **Marked improvement** (50-70%) in the deficits and symptoms experienced by PD patients has not been demonstrated.
✓ **Risk of tumor formation**—even if minor, it is not acceptable.
✓ The need to inject cells at **all sites of injury**.
Alzheimer's disease (AD)

Memory impairment, cognitive decline, and dementia due to widespread and progressive pathological changes.

Loss of several cell types (tissue) adds to the complexity of the disease.

Treatments were tried using cells as well as other approaches such as acetylcholinesterase inhibitors, which enhance cholinergic function, induce some temporary improvement in AD patients. Other approaches include nerve growth factor (NGF) releasing stem cells and anti-β-amyloid antibodies or β-amyloid-degrading protease neprilysin.

Neuronal and synaptic loss, neurofibrillary tangles, and deposits of β-amyloid protein involve the basal forebrain cholinergic system, amygdala, hippocampus, and cortical areas.

www.positivehealth.com
Stem cell–based therapies for AD

Hurdles that prevent stem cell therapy for AD from bench to clinic:

✓ Stem cells have to be pre-differentiated in vitro to many different types of neuroblasts for subsequent implantation in many brain areas.

✓ For a long-lasting symptomatic benefit, cholinergic cell replacement requires intact target cells (host neurons that the new cholinergic neurons can act on) that are damaged in AD.

✓ Stem cell–based cell replacement strategies are very far from clinical application in AD.
Stroke

Ischemic stroke, caused by occlusion of a cerebral artery, leads to focal death of multiple neuron types, as well as oligodendrocytes, astrocytes, and endothelial cells.

Neuronal plasticity and reorganization of neural circuitries contribute to spontaneous recovery to varying degrees, but most patients exhibit persistent motor, sensory, or cognitive impairments.
Stem cell–based therapies for stroke

Human ES cell–derived NSCs and MSCs, grafted into rat stroke site, migrated toward the lesion and improve forelimb performance.

IV injection of human NSCs induced improvements after hemorrhagic stroke in rats, probably through antiinflammatory actions.
Stem cell–based therapies for stroke

✓ No substantial clinical improvements were detected after IV injection of autologous MSCs in patients with an ischemic lesion in the regions supplied by the middle cerebral artery (MCA).

✓ Several clinical studies using intravenous or intraarterial (into damaged territory) infusion of autologous bone marrow–derived stem cells in stroke patients are ongoing.

✓ A clinical trial in stroke patients involving transplantation of clonal, conditionally immortalized NSCs isolated from human fetal cortex.
Stem cell–based therapies for stroke

80% of neuroblasts and neurons die during the first two weeks after formation at stroke site in rats.

How can we fix this problem?
A. Improvement in cell survival by:
1. Inflammation-modulating agents
2. Caspase inhibitors
3. Neurotrophic factors

B. Promoting the migration of the new neurons to the damaged area by stromal cell–derived factor 1α [SDF-1α], monocyte chemoattractant protein–1, and matrix metalloproteinase–9.

C. Stimulation of the differentiation of cortical neurons that form in limited numbers after stroke by growth factor delivery.
Spinal cord injuries

Pathological changes after spinal cord injury are complex and include:
1. Interruption of ascending and descending pathways
2. Loss of neurons and glial cells
3. Inflammation
4. Scar formation
5. Demyelination

- Patients experience loss of movement, sensation, and autonomic control below the level of the injured spinal segment.
- Available treatments are ineffective.
- Different types of stem cells were tested and improved functional outcome in animal models through secretion of neurotrophic factors, remyelination of spared axons, or modulation of inflammation.
Several cell types and synapses were lost due to injury. Treatment with stem cells should replace:
Formation of neurons, oligodendrocytes, & astrocytes.
Formation of synapses and axons
**Remylenation**: high-purity oligodendrocyte progenitor cells (OPCs) generated from human ES cells in vitro can differentiate into oligodendrocytes (clinical trial)

Several stem cell types were tried
Stem cell–based therapies for spinal cord injuries

Before moving to clinic:

Determine how to control the proliferation of transplanted stem cells and their progeny

Determine how to enhance the differentiation of these cells to the specific types of neurons that have been lost

Determine how the resulting neurons can be directed to format appropriate synaptic contacts
Stem cell–based therapies for spinal cord injuries

Several stem cell types were tried

Problems in these trials:
1. The implanted cells were often poorly characterized.
2. The preclinical evidence of efficacy for several of these approaches was insufficient.
3. The therapeutic benefit was reported from open-label trials where patients had been subjected to physiotherapy.
4. The mechanisms underlying observed improvements were unclear.
None of the neurodegenerative disease can be treated safely and effectively by stem cells so far.

Further research has to be done to fine tune the use of stem cells as a therapy.

Ethics MUST be considered when treating patients with stem cells. DO NOT CREATE ANOTHER PROBLEM TO THE PATIENT.

For the disease we discussed, most of them has reached the stage of clinical trials but none succeeded.

Do not be a victim of the propagandas that announce successful treatments everyday. Be aware and check any treatment before you apply it on patients. BE A SAFE DOCTOR.