Biochem_4 – stem cell research

ꕥ In this lecture, we will focus on the use of stem cell therapy for Parkinson's Disease (PD), Alzheimer's, Ischemic strokes, and spinal cord injuries. All of these conditions share a common feature that is "Damage of Neurons".

> ✓ ***No stem cell–based therapy has yet been proven beneficial for any neurodegenerative condition.***
>
> ✓ ***Treatment by stem cells must be able to improve the life of the patient by a large degree to be considered an encouraging option for treatment***.

Four important questions should never leave your mind when we're talking about the use of stem cells in this field:

- Is it -in reality- **approved** to be used in the clinic?
- Is it **ethical**?
- Is it **safe**?
- Is it **efficient**?

ꕥ The most important "take-home message" from this lecture is: Don't believe the propagandas (فيها مُبالغ أخبار أو دعايات) you hear every now and then from the media. For example: when you read titles like "A research group discovered a treatment for disease X using stem cells", keep in your mind that you have to be very careful before applying this treatment in your clinic.

ꕥ The approval of any treatment -including stem cell-based therapy- has to pass through many stages that include:-Important-

- Cell cultures or organ cultures (**in vitro** experiments).
- Followed by in vivo experiments using **small animals**.
- Then moving to **higher (large) animals**; like monkeys, pigs…etc.
- After passing the previous stages, we can move on to **clinical trials**.

- Clinical trials include several stages (phases). It's important to mention that using the method under investigation on your patient (as a treatment) is not allowed in the first phases of the clinical trials.
- The first phase is usually concerned with checking the safety of the treatment (safe/toxic). Other phases include checking the required dose and frequency of usage, in addition to many other important steps.

Note: **If a treatment succeeds in animal models -even if in higher animals- it does not have to be successful in human beings**

ꕥ The cell type to be regenerated and transplanted in each disease:

- PD: dopamine neurons
- ALS: motor neurons
- Stroke and Alzheimer's disease: several cell types

- We should **assess the treated subjects on the long run** (we should check for possible long-term side effects/ complications that might develop in the patients as a result of the treatment).
- Common considerations when translating stem cell therapies to neurodegenerative disease patients include:
  - Are there going to be any complications? **(you have to tell your patient about the possible complications and risks, both; in written and oral forms)**
  - Do I need to suppress the immunity of the patient or not? (this depends on the source of the transplanted cells. Are they autologous –from the patient himself- or from nother person?)
  - Other points are illustrated in the table: (memorize the main points on the left, and just read the rest).

Inclusion/exclusion criteria	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
Realistic expectation	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
Controlled study	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
Immunosuppression	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
Potential side effects	Prevent/minimize potential side effects (i.e. meningitis, fever) Avoid exacerbation of disease and tumor formation Risk vs. quality of life
Safety of cellular therapy administration	Consider CNS accessibility and safety of delivery methods Pros/cons of systemic delivery, lumbar puncture or stereotactic injection are important

Abbreviations: PD, Parkinson's disease; CNS, central nervous system.

❖ Parkinson's Disease (PD)

- The traditional treatment:
  - L-Dopa (to compensate for the decreased dopamine), dopamine (DA) agonists, some enzyme inhibitors, *or deep brain stimulation.*
  - *There is no treatment for dementia*

- In the trial of stem cell therapy, they transplanted a special type of stem cells derived from human <u>embryonic mesencephalic tissue</u> at certain sites. The cells were monitored over time, things were okay, and <u>newly derived DA neurons</u> were formed in different regions of the brain.
- Advantages (pros):
  - The used stem cells were able to settle and attach to the tissue and produce dopamine.
  - They also stayed viable for a long period of time (up to years).

- disadvantages (cons):
  - The availability of these cells (human embryonic mesencephalic tissue) is limited.
  - The variability of functional outcome after transplantation is high among different patients.
  - <u>Poor standardization</u> of the transplanted cell material contributes to the high variability.

ꕥ Other types of stem cells that were also used in trials:

- Embryonic stem (ES) cells
- Cloned Embryonic stem cells
- Induced pluripotent stem cells (Fibroblast-derived iPSCs)
- Bone marrow stem cells
- Neuronal stem cells (NSCs) and progenitors of embryonic ventral mesencephalon
- *Adult NSCs from subventricular zone (SVZ)*.

ꕥ Hurdles (العوائق) that prevent stem cell therapy for PD from bench to clinic:

(1)PD is <u>a multisystem disorder</u>
- Different regions in the brain might be affected so we might need to inject <u>different sites</u> and that's not practical. Moreover, we might need to repeat the procedure (<u>several injections</u>). This is an example where the dose and frequency are key points in the treatment.
- *Since it's a multisystem disease, if nondopaminergic systems are affected, they will not improve by intrastriatal DA grafts.*

(2) *Substantial re-innervation of the striatum has not been demonstrated.*

(3) Restoration of <u>DA release in vivo</u> (in animal models) <u>did not show promising results</u>. (in vitro, the cells were able to secrete dopamine, but in vivo, the restoration of DA release has not been demonstrated).
- In vivo results are more important than in vitro results.

(4) *<u>Marked improvement</u> (50-70%) in the deficits and symptoms experienced by PD patients has <u>not been demonstrated.</u>*

(5) There's a <u>risk of tumor</u> formation, and even if it's minor, it is not accepted.

ꕥ Notes:
- The risk of tumor formation depends on two main factors:

1. The type of stem cells used → if you use pluripotent stem cells like ESCs and iPSCs, the risk of tumor formation will be much higher compared to the risk that accompanies the use of mesenchymal stem cells that usually do not result in cancer formation.
2. The state in which the stem cells are used → for example, if you use pluripotent stem cells in a differentiated stage (differentiate them, then inject them), the risk of tumor formation will be low, because the differentiated cells don't have an unlimited ability to proliferate like pluripotent stem cells.

- Stem cell-based bone marrow transplantation is considered safe because the adult stem cells used (mesenchymal or hematopoietic) do not have the ability to form cancer as embryonic or pluripotent stem cells.

❖ Alzheimer's Disease (AD)

☙ Possible therapy methods for AD

(1) Trials started targeting cholinergic neurons by using Acetylcholine-esterase inhibitors.
→ If we inhibit acetylcholine-esterase, acetylcholine degradation will decrease and thus, transmission and synapses will be more efficient. *(in other words, the cholinergic function will be enhanced and some temporary improvement in AD patients will be induced)*

(2) Using certain types of **stem cells** that stimulate neurogenesis or maturation of hippocampal neurons was reported in AD.

(3) It is also possible to transplant **stem cells** that release Nerve Growth Factor (NGF) to stimulate the tissue in which they were transplanted to regenerate and form more neurons and more synapses.

(4) The use of β-amyloid antibodies or certain proteases like β-amyloid degrading protease neprilysin is also possible. (to degrade the accumulated β- amyloid).

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

• The situation in Alzheimer's is more complex than Parkinson's disease because in Alzheimer's there's loss of different types of cells, and thus, if we are going to treat using stem cells, we'll have to replace all the lost types of neurons (the situation is closer to replacing a "tissue" rather than replacing a specific type of neurons).

☐*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)*

☐The location of injection must be a healthy region and that's hard to determine using the available examination techniques.

• Stem cell-based cell replacement strategies are very far from clinical application in AD. However, some clinical trials are being carried out nowadays.

❖ Strokes

☙ Stem cell-Based therapy for strokes

• When scientists injected neural stem cells, they noticed that these cells start to attach to the tissue and start forming some synapses.

• Other types used in trials were ES cells and mesenchymal stem cells.

- *Notes from the slides:*
  • Human ES cell–derived NSCs and MSCs, grafted into rat stroke site, migrated towards the lesion and improved forelimb performance.
  • IV injection of human NSCs induced improvements after hemorrhagic stroke in rats, probably through anti-inflammatory actions.

- 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).*

- Stem cell therapy is NOT considered an effective treatment when it comes to strokes- at least, for now.

❖ Spinal cord injuries

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

- Many factors contribute to the complexity of using stem cell-based therapy in spinal cord injuries. Of these:
  - We have to replace the lost neurons, re-establish synapses, re-establish (or activate) myelination, and stop or inhibit scar formation.
  - There's a high degree of variability between patients with spinal cord injuries in terms of clinical picture, age, life style, response to treatment and many other factors.

- Because we need to restore many things (neurons, synapses, myelination,...), we have to use a combination of different types of cells and molecules.
- Other stem cell types that were tried in spinal cord injuries(other than neural stem cells):
  (1) Umbilical cord blood
  (2) Bone marrow–derived HSCs
  (3) MSCs
  → *These three types have already been applied in patients with spinal cord injury, with claims of partial recovery.*

- **Problems in spinal cord injury 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**

ꕥ **Before moving the trial to the clinic, we need to**

(1) Determine how to control the proliferation of transplanted stem cells and their progeny, especially if we want to use embryonic stem cells or iPSCs.

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

(3) Determine how the resulting neurons can be directed to format appropriate synaptic contacts, induce remyelination, and inhibit/stop scar formation or degrade it by certain mechanisms.

❖ Important Take-home Messages

I. Don't believe everything you hear :)

II. Be safe doctors.

- Whatever your specialty in the future, you have to be very careful before transmitting any new technique to your clinic.

- Always remember the following four words: Safe, Ethical, Efficient, Approved.

- To regret NOT doing something is better than regretting doing something that might hurt your patients.

III. Before preceding to the clinic, any new treatment (whether stem cell-based or drug-based) must pass many steps {cell/organ culture, small animal models, large animal models, clinical trials…. (refer to page 1)}

For example, if a pharmaceutical company one day asks you to try a new drug that passed the first stages (in vitro), but did not undergo the next required steps, you should NOT accept trying this drug on your patients even if in vitro experiments showed promising results.

IV. You should be part of the awareness programs related to stem cell-based therapy.

For example, it is your responsibility to make it clear for patients that not everything they hear in the media about stem cell-based therapy is considered to be an "approved" treatment.

V. Finally, always remember that no one knows everything :)

THE END