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stem cell research&therapy

Do hypoxia/normoxia culturing conditions change the neuroregulatory profile of Wharton Jelly mesenchymal stem cell secretome?

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Presented by:

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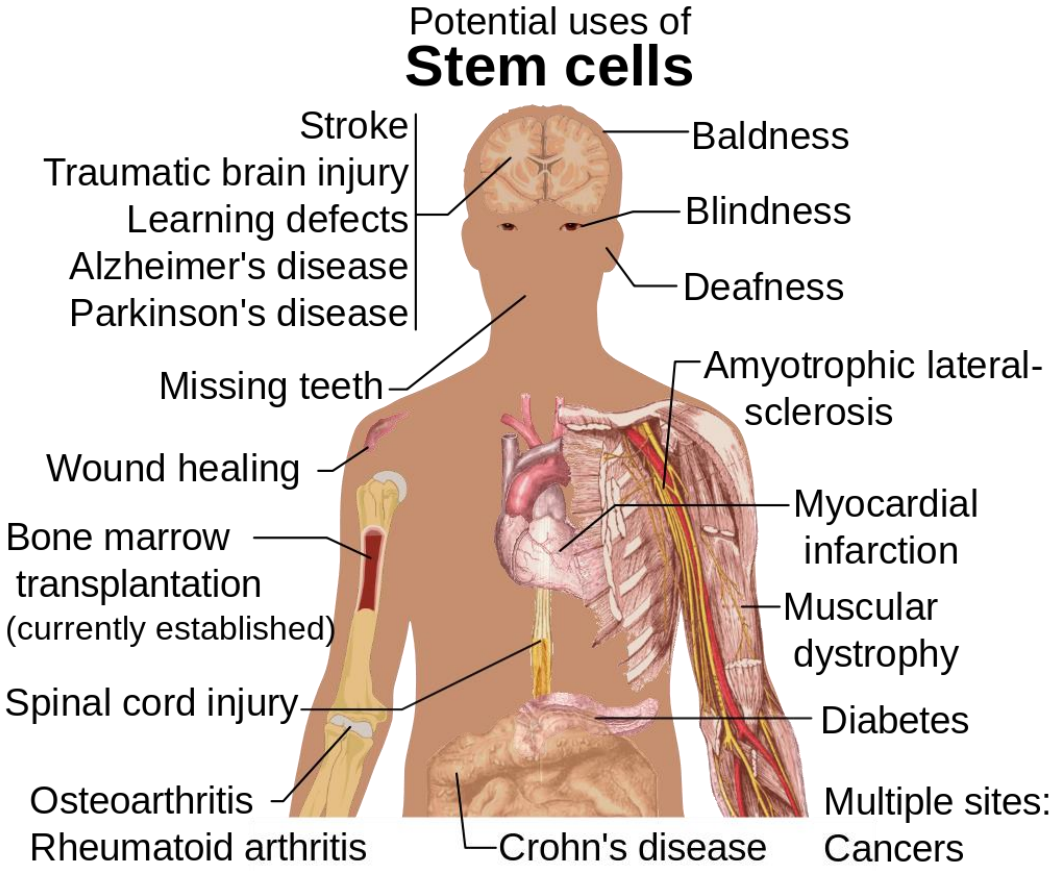
Stem Cells

- undifferentiated cells that are able to differentiate into specialized cells
- Classical abilities: self-renewel and potency
- Two types of stem cells:
  - embryonic stem cells →isolated from blastocysts (4-5 days post fertilization
  - adult stem cells → in various tissues →"repair system of the body"
    - Sources of autologous adult stem cells: bone marrow, adipose tissue, blood, umbilical cord
- Treatment:
  - Bone marrow transplant → for patients with cancers of blood or bone marrow (multiple myeloma or leukemia)
    - Problems:Immunosuppression, no specific cell type →pluripotency, some stem cells form tumors

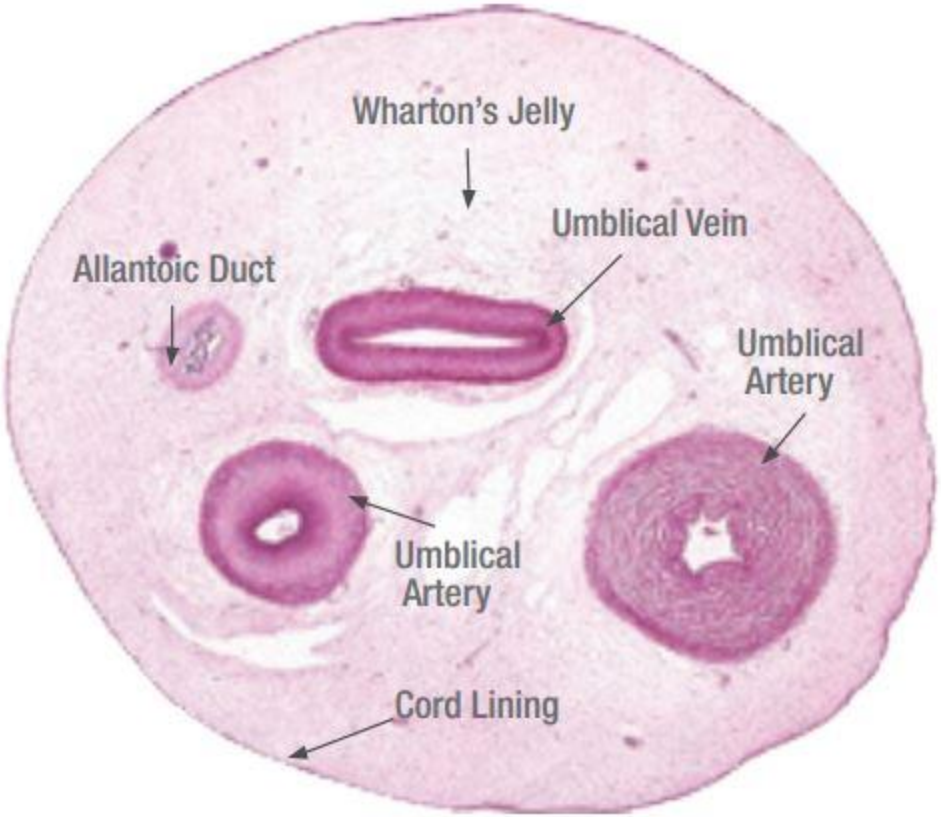
Stem cells

Research



Wikipedia

Wharton Jelly Mesenchymal Stem Cells



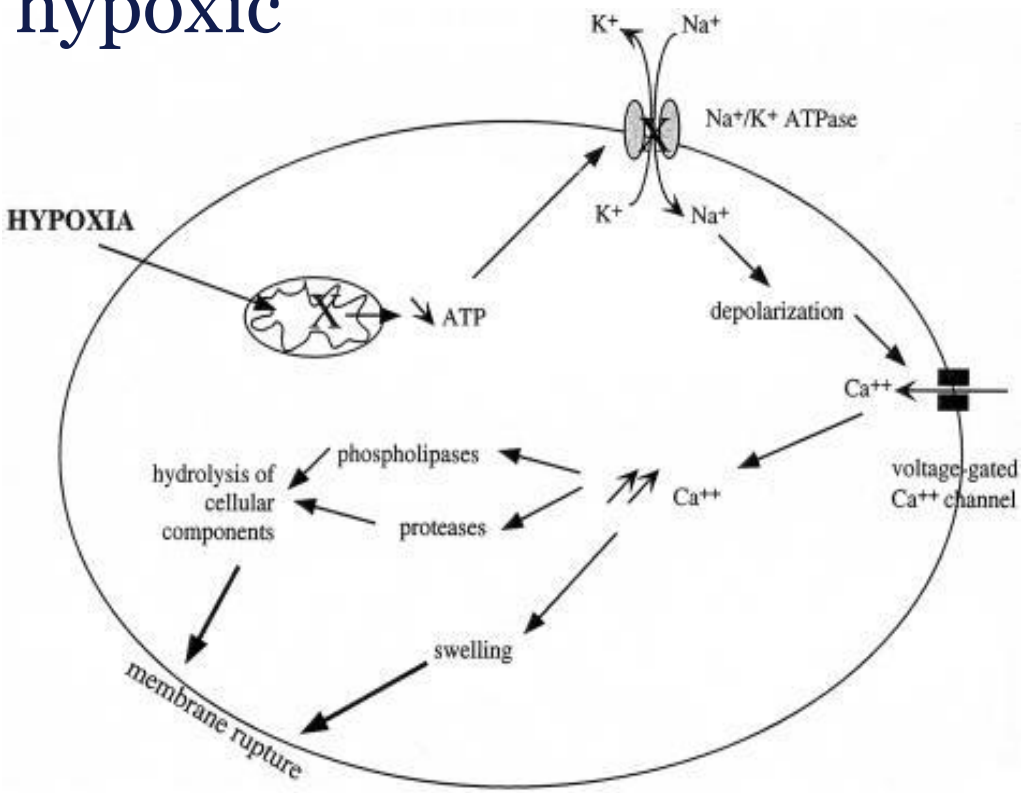
Cross-section of an umbilical cord

Wharton Jelly → gelatinous substance within the umbilical cord

Mesenchymal Stem Cells (MSC) → able to differentiate into bone, nerve, cornea, heart, fat and cartilage cells

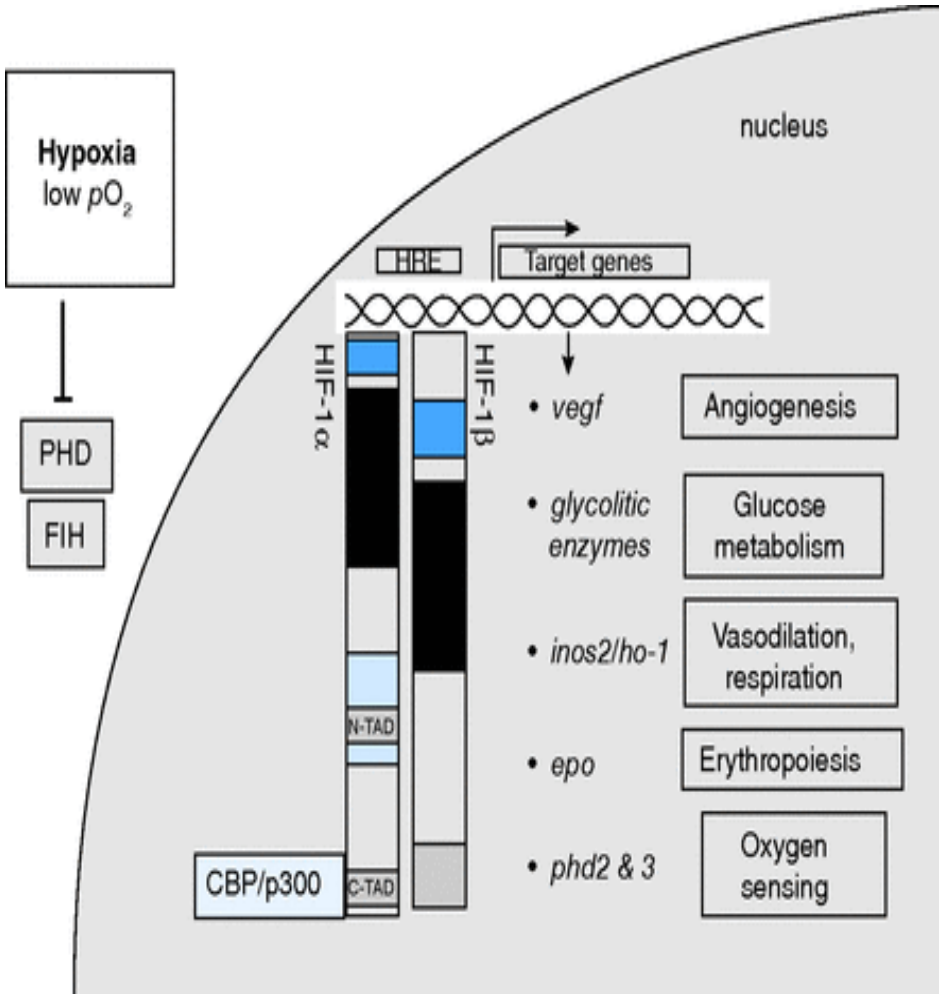
→sources of MSC: umbilical cord,
bone marrow
adipose tissue
tooth pulp

Reaction of cells in hypoxic conditions:



- most important parameter in healthy cells are a high amount of ATP
- Hypoxia → Mitochondria produces less and less ATP → failure of Na^+/K^+ ATPase → membrane depolarization → Ca^{2+} influx → activation of calcium dependent-phospholipases and proteases → cell swelling, hydrolysis of cellular components or cell necrosis

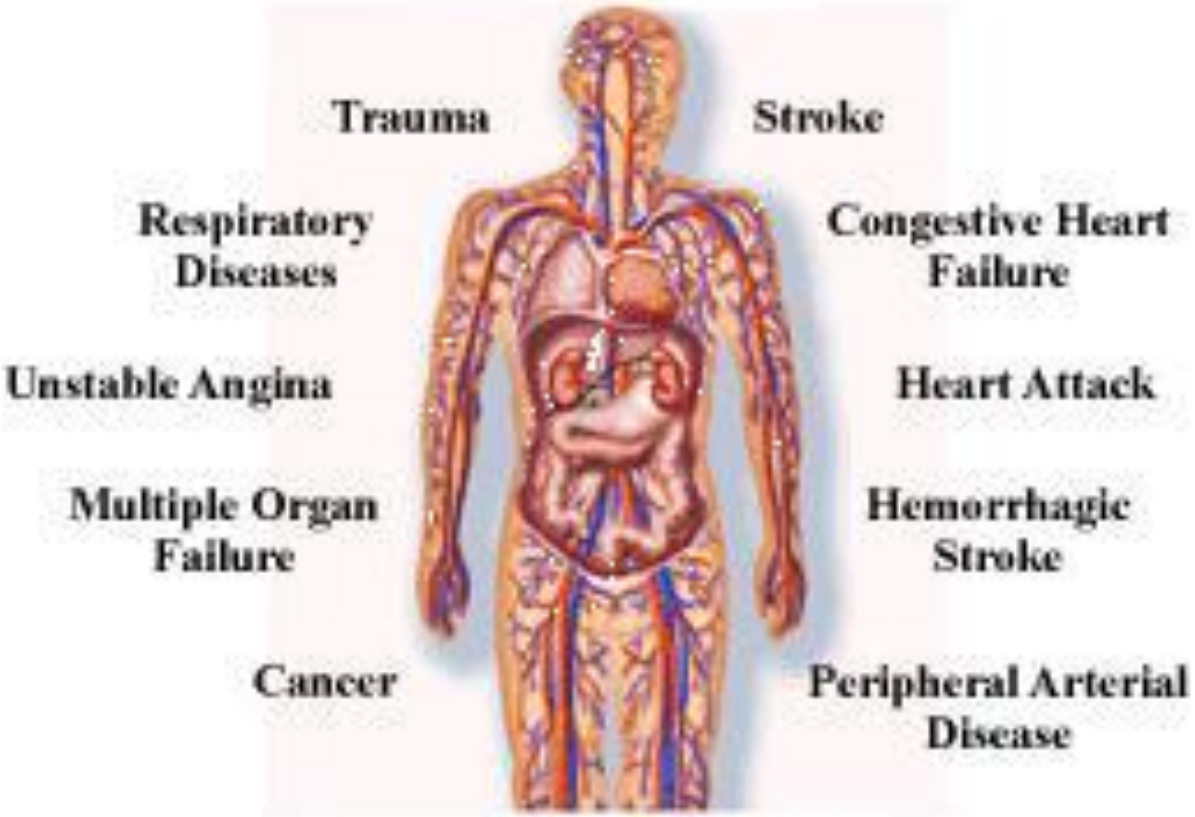
Effect of hypoxia on gene expression:



- Hypoxia inhibits the oxygen sensor PHD and FIH →HIF-alpha is stable →translocates to the nucleus →dimerizes with HIF-1ß →binds to DNA at a HRE (hormone response element) → activates the transcription of genes

PHD = Prolyl-4-hydroxylase
FIH = Factor inhibiting HIF
HIF-a = Hypoxia-inducible factor 1-alpha
VEGF = vascular endothelial growth factor
Inos2= inducible nitric oxide synthase
ho1 = haemoxgenase-1
epo =erythropoetin

Hypoxic Conditions



Introduction

- human Mesenchymal Stem Cells (hMSC) → important role in regenerative medicine
- The problem → low amount of cells after isolation → difficult to apply in clinical conditions
- hMSC → normal in static culture and in medium with 21% oxygen tension
- BUT hMSC are in the body at much lower oxygen tension (bone marrow 1-7%, adipose tissue 10-15%, umbilical cord 1,5-8%)
- The study from Rhijn et al. (2013) showed that hypoxic conditioning of hMSC improve the regenerative potential and so preserve their immunosuppressive ability.
- The study from Tsai et al. (2011) used 1 % oxygen that showed the reduction of their senescence and augmentation of their proliferation

Introduction

- angiogenic potencial of MSCs is affected by modulating the oxygen concentration → a higher level of VEGF, bFGF and HGF

(**VEGF**=vascular endothelial growth factor, **bFGF** = beta-fibroblast growth factor, **HGF** =hepatocyte growth factor)

- cell death at long exposure to hypoxic conditions (Volkmer et al., 2008)
- Higher oxygen tension might be toxic → oxidative stress caused by production of ROS (=reactive oxygen species)

Methods

- DASGIP Parallel Bioreactor System:
  - growth of human Wharton Jelly Mesenchymal Stem Cells
  - contains Teflon 4-paddle impellers
  - siliconized with Sigmacote and autoclaved of vessels
  - Hold at 37 ˚C with a heating jacket
  - 100% dissolved oxygen for normoxic conditions
  - 21% dissolved oxygen for the hypoxic conditions
  - pH =7,4
  - Agitation of 52 rpm using a magnetic stir plate under the bioreactor

https://www.genengnews.com/gen-articles/in-search-of-a-reliable-scale-down-model/3337

Methods

microcarriers

- utilizing Cytodex 3 microcarriers
- 1,0 g (per condition) of microcarriers hydrated in 50 ml 1x phosphate-buffered saline (PBS) in 2 x 125ml Erlenmeyer flasks at room temperature for one night
- 2-3 drops of Tween 80 → for breaking surface tension and guarantee proper wetting and sedimentation
- 3 x times washing of microcarriers with 1xPBS and autoclaved
- incubated with fetal bovine serum (FBS) for 6 h at 37 ˚C for coating the microcarriers with serum-attachment factors

Methods

Preparation of microcarriers:

- after 6h, microcarriers washed with serum-free medium (PPRF-msc6)
- occulated in 275 ml of serum-free medium for 4 h at controlled culture conditions

Methods

- hWJ-MSCs:
  - expanded in Nunc T-flask with the gelatine solution (including „ Typ B bovine gelatine“)
  - In static culture for two passages
  - harvested with trypsin-EDTA and inoculate in bioreactors
  - Volume of the bioreactors →325ml for the first 24 h and then to 500ml increased
  - Bioreactors removed from DASGIP system and placed for 10 min a biosafety cabinet
  - Cells were cultured on the microcarriers for 72 h!

Methods

- the supernatant was removed from the bioreactors
- microcarriers washed with Neurobasal®-A medium
- 500ml of Neurobasal®-A medium with 1 % kanamycine were added in the bioreactors
- again for 24 h in the DASGIP control system
- then removed from the system and for 10 min in the biosafety cabinet
- supernatant was harvested →centrifuged at 300g for 10 min to take off the cell debris

Methods

Human telencephalon neural precursor cell (hNPCs)

- hNPCs
  - isolated from telecephalon area of a 10-week fetus and cryopreserved
  - melted at 37˚C and placed into a Nunc T-25 flask including 5 ml of serum-free medium (^PPRF-h2)
  - after 2 day → cell collected and mechanically dissociated into single cell suspension
  - every 4 days 40 % of the medium was replaced by fresh growth medium
  - after 14-20 days hNPCs were passaged and plated into precoated laminin 24-well plates including the hWJ-MSC condition medium from normoxic and hypoxic condition
  - Neurobasal®-A was the control group
  - the plates → placed in a incubator with 37˚C, 5% CO_2, 95% air and 90% relative humidity

Methods

Immunostaining

- hNPCs:
  - fixed in 4% paraformaldehyde for 15 min
  - permeabilized by incubation with 0,1% Triton X-100 in 1xPhosphate buffered saline (PBS)
  - washed 3 times in 1xPBS
  - blocked with 10 % fetal calf serum in 1xPBS
  - 1 h incubation with primary antibodies
    - rabbit anti-doublecortin (DCX) → detect immature neurons
    - mouse antiratmicrotubule associated protein-2 (MAP-2) → detect mature neurons

Methods

Immunostaining

- hNPCs:
  - 3 times washed in 1xPBS
  - incubated with the secondary antibodies: Alexa Fluor 488 goat anti-rabbit and Alexa Fluor 594 goat anti-mouse immunoglobulin G for 1 h at 37˚C
  - 10 min with 4-6-diamidino-2-phenylindole-dihydrrochloride
  - stained and then observed under an Olympus BX-61 Fluorescence Microscope
  - normalizing the data with presenting the results in percentage of cells → calculated by counting the cells positive for MAP-2/DCX markers and dividing it by total number of cells/field

Methods

Sample preparation and quantification

- Vivaspin 20 sample concentrator → ultracentrifugation at 3000g for 45 min
- Condition medium → precipitated with Trichochloroacetic – acetone
- Samples were evaluated with 2D-Quant Kit
- Samples were digested with trypsin at 37˚C
- peptides were dried by rotary evaporation
- Triple TOF 5600System →analyzed the samples (mass spectrometry)

Results / Discussion

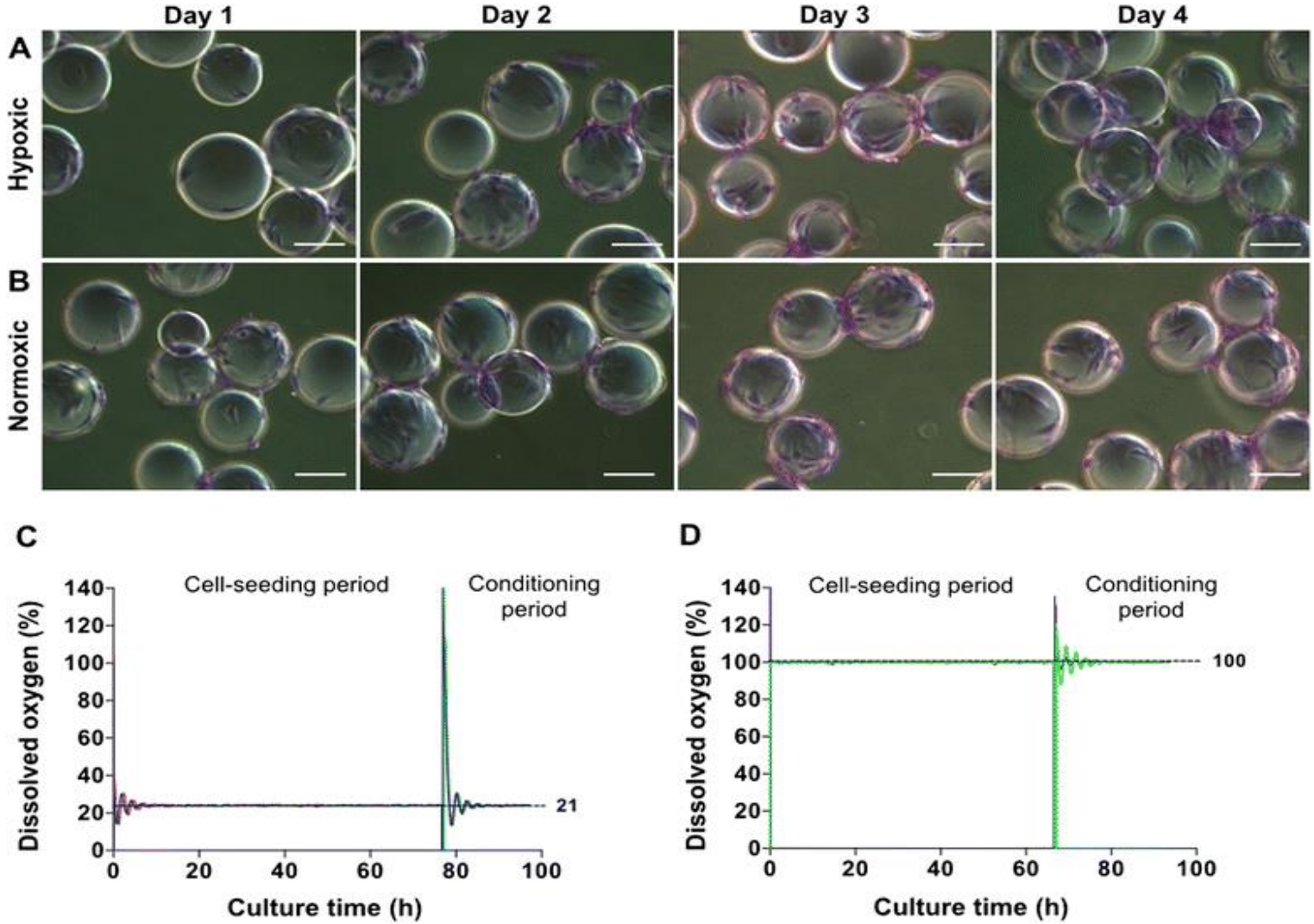


Fig.1 (A,B) → hWJ-MSCs could attach to and grow on Cytodex 3 microcarriers

Results / Discussion

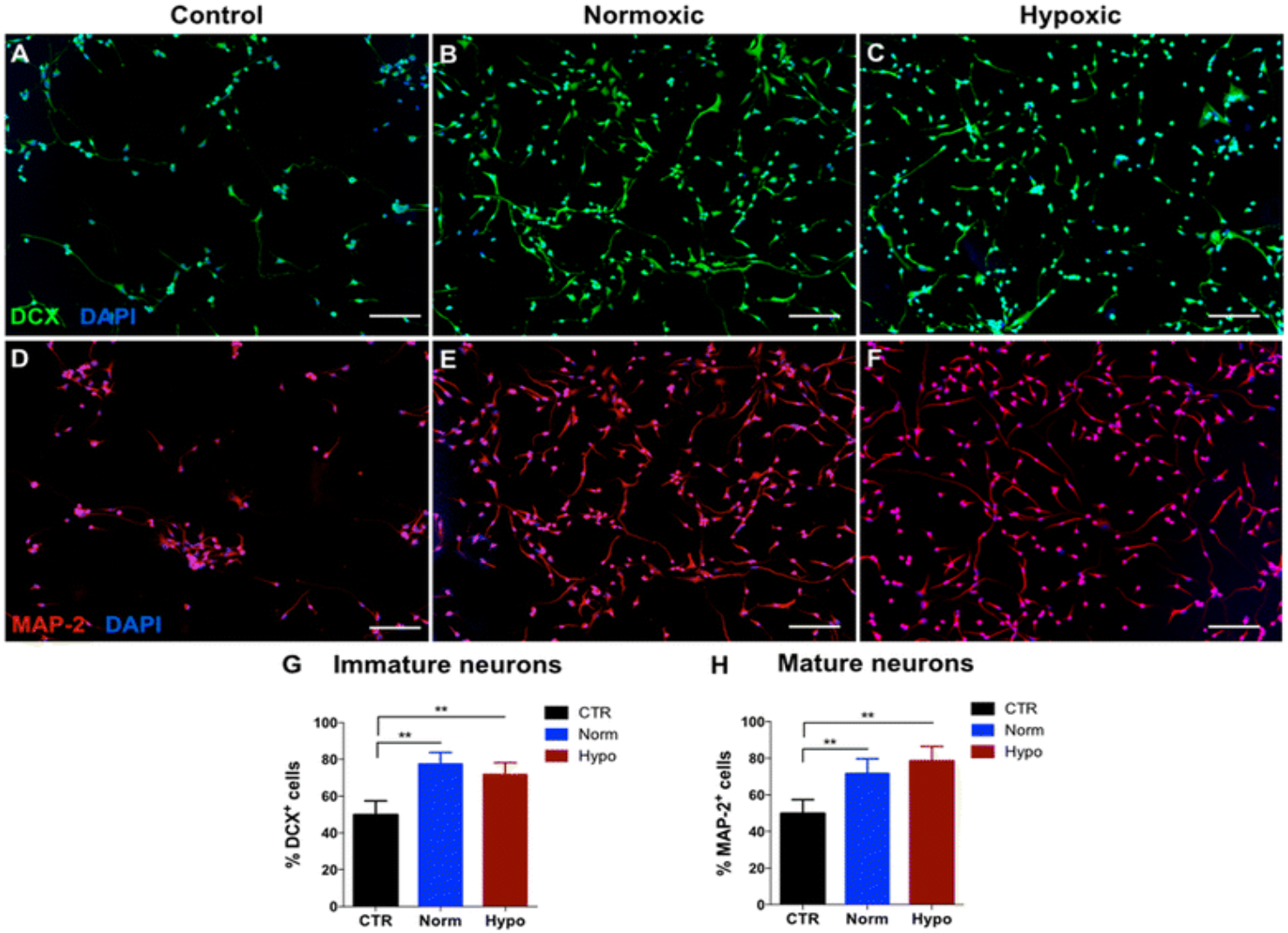


Fig.2 →expansion of immature and mature neurons in hypoxic and normoxic conditions

Results / Discussion

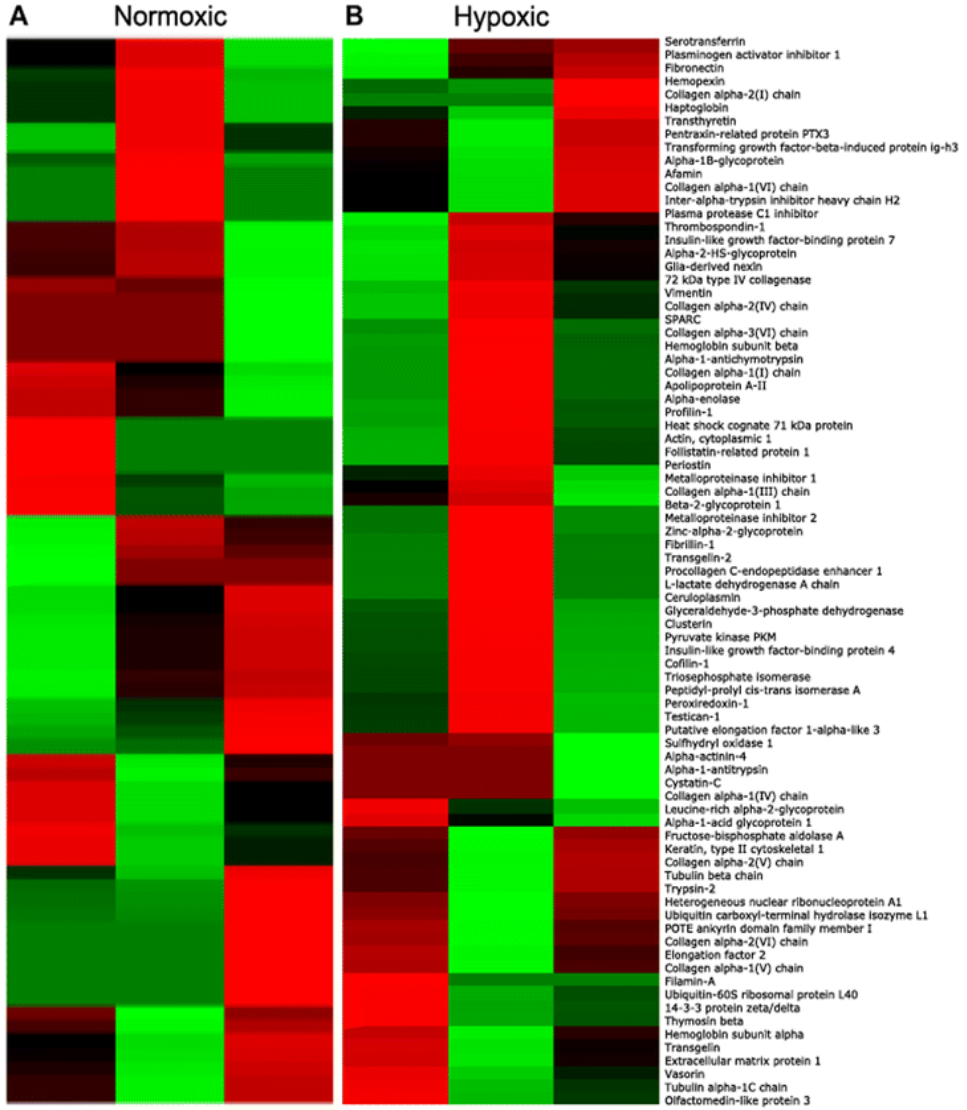
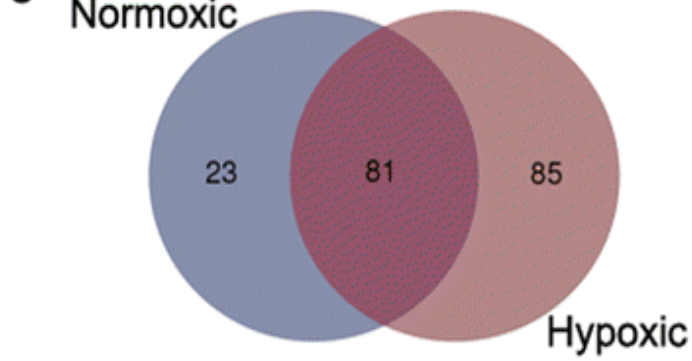


Fig.3 → WJ-MSC CM proteomic analysis by mass spectrometry



Venn diagram:
→ more proteins in in hWJ-MSC CM from hypoxic conditions

Red → low expression
Green →high expression

Results / Discussion

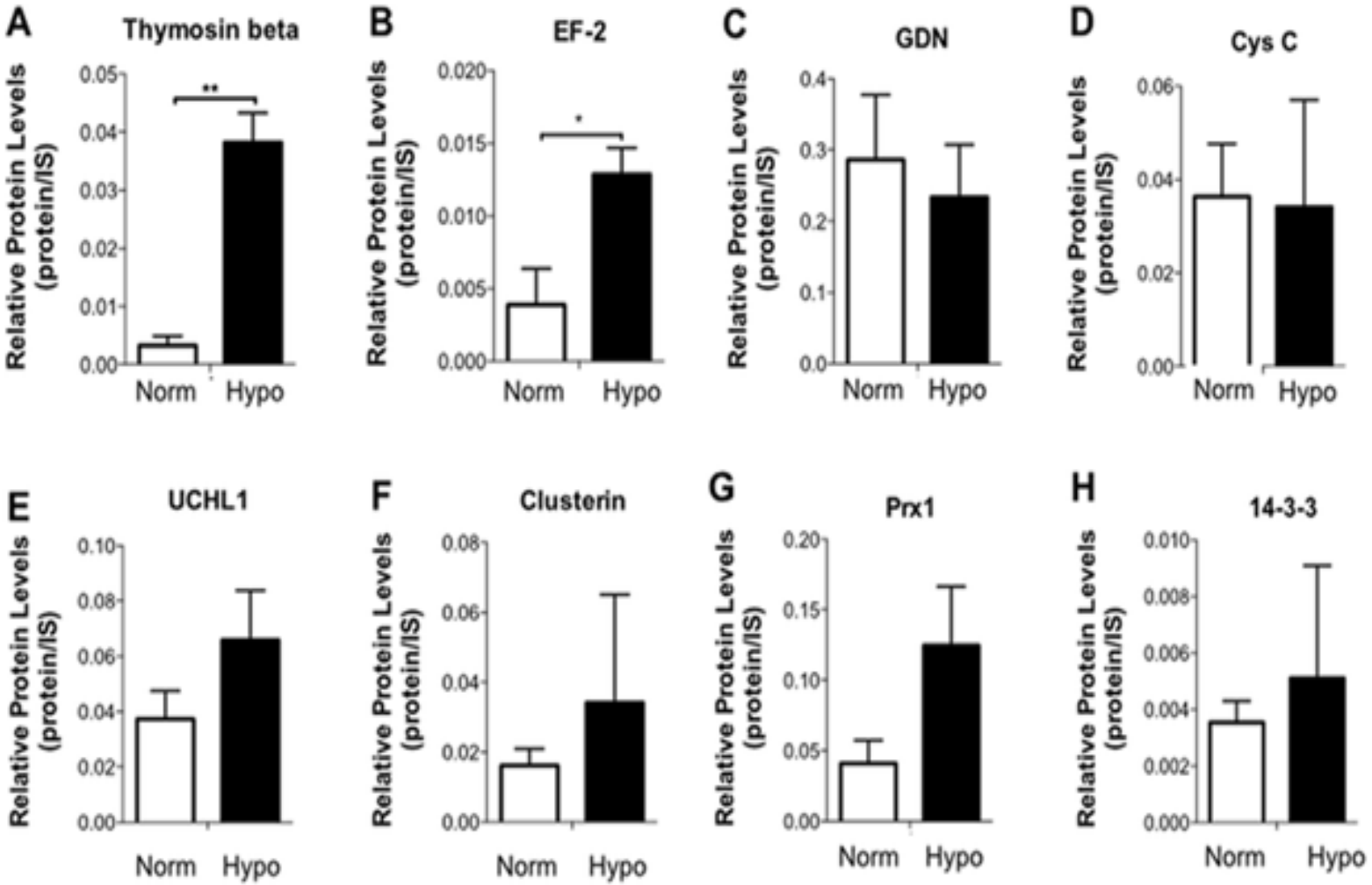


Fig. 4→both hWJ-MSC secretomes were analyzed for specific proteins with neuroregulatory potential

Results / Discussion

Normoxic conditions

- Vironectin
- Cadherin-2
- MRP-1

Hypoxic conditions

- PEDF
- IGF-2
- MIF
- Hsp 70
- semaphorin-7A
- moesin

Results/ Discussion

- hypothesized normoxic conditions could cause a reduction in therapeutic potential of human Mesenchymal Stem Cells

  →in vivo physiologic oxygen concentration is lower than 21 % oxygen tension

- this study showed in both conditions that hWJ-MSCs can be cultivated and induce differention of hNPCs

Discussion

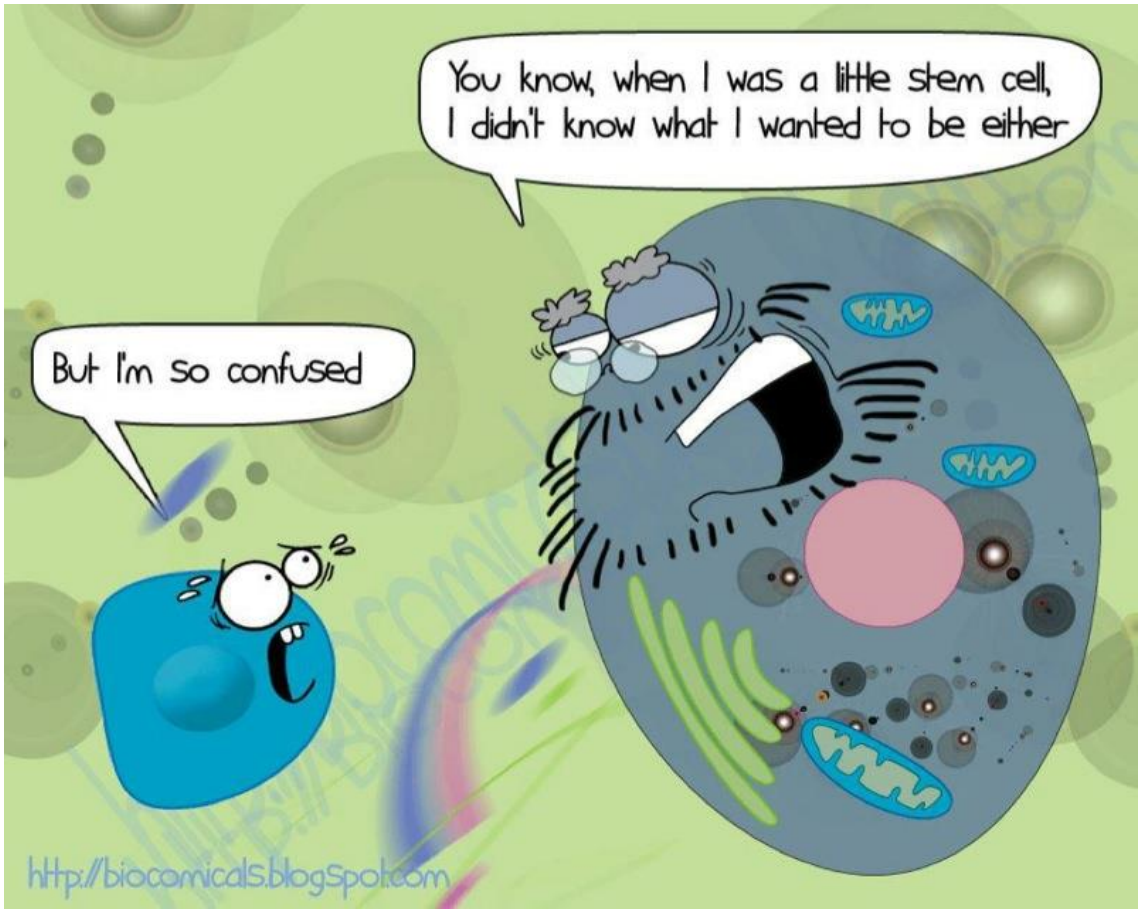
- Pro:
  - reproducibility → very detailed methods
  - illustrations understandable, except of Fig. 1 → C, D

- Cons:
  - lack of definition of mature and immature neurons
  - Glia cells ?

Discussion:

- Other studies:
  - from Rhijn et al. (2013)→"Effects of hypoxia on immunomodulatory properties of adipose tissue-derived mesenchymal stem cells":
    - Effect of adipose tissue-derived mesenchymal stem cells (ASC) in hypoxic (1% oxygen) culture conditions → no immunophenotypical changes →no effect of proliferation of ASC in 1% and 20% oxygen!
  - from Cicione et al. (2013) →"Effects of Severe Hypoxia on Bone Marrow Mesenchymal Stem Cells Differentiation Potential":
    - absence of differentiation in severe hypoxic conditions compared to normoxic condition→ severe culture condition with 1% oxygen

Any questions?



Thank you for your attention!

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