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UMF UNIVERSITATEA DE MEDICINĂ ȘI FARMACIE IULIU HAȚIEGANU CLUJ-NAPOCA

The Education, Scholarships, Apprenticeships and Youth Entrepreneurship

F-SEE-117/12.2018
Project No: SEE 21-COP-0049
Project title: Strategic interuniversity cooperation to improve research abilities for ph.d. Students for higher educational quality
Strategic Interuniversity Cooperation To Improve Research Abilities For Ph.D Students For Higher Educational Quality - SEE 21-COP-0049

Activity report

Period: 08.09.2023 - 29.09.2023

Georgiana Cabau

Activity 1: **Cell culture from neural stem cells**

Period: 08. 09. 2023 - 17. 09.2023

We performed cell culture of neural stem cells from human embryos:

1. Coating - using Poly-D-lysine we coated the culture plates to ensure cell adhesion.
2. Coating - using laminin we coated the culture plates, which promoted the growth and proliferation of neural stem cells in the monolayer.
3. Defrosting a neural stem cell cluster
4. Addition of culture medium containing neuronal growth factors EGF and FGF.
5. Cell passage - due to increased proliferation of stem cells with a doubling time of 16 hours, they must be maintained in culture by subculturing and re-adapting a new culture medium with the necessary growth factors.
6. Repeat steps every 2 days.

Activity 2: **Scientific Communications Session**

Period: 18.09.2023- 19.09.2023

I attended the following scientific sessions:

Session-1 Cancer Epitranscriptomics

Session-2: Transcriptional dysregulation in cancer

Session-3 Epigenetic dysregulation in Cancer

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Session-4 Epigenetic dysregulation in Cancer

Activity 3: **Transfection of neural stem cells using the CRISPR/Cas9 system**

Period: 20.09.2023- 29.09.2023

The CRISPR/Cas9 system can be used to transfect neural stem cells and study the mechanisms involved in glioblastoma formation by precisely editing the genes involved in the formation or progression of this type of brain tumour.

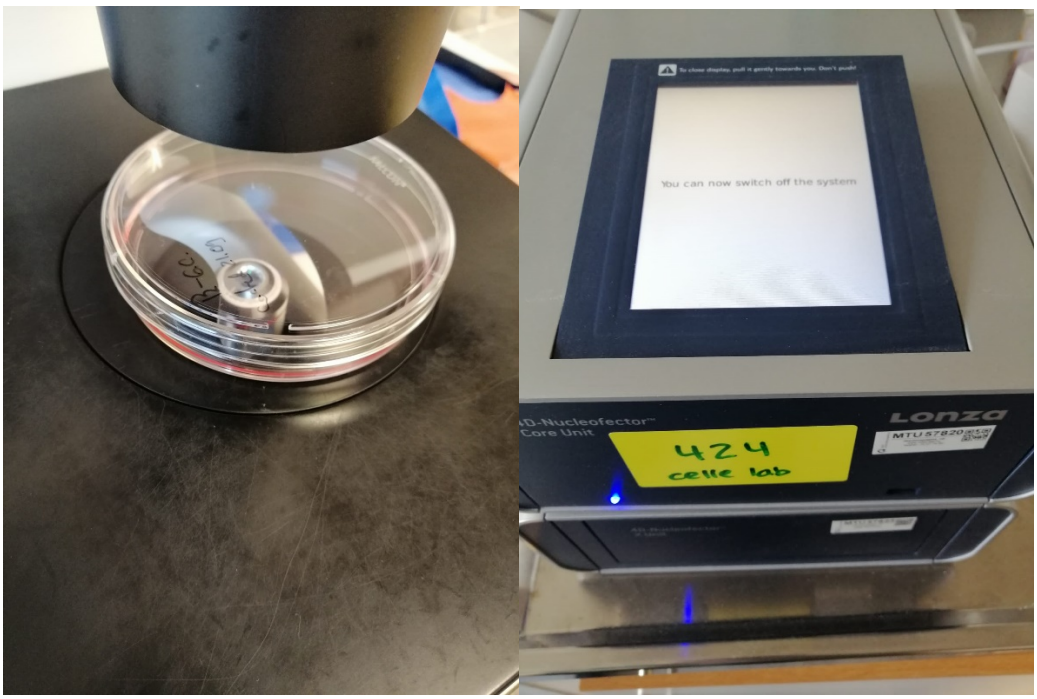
Using this system, we marked the OLIG2 transcription factor of interest by inserting a V5 peptide sequence in its vicinity. Subsequently, using antibodies against the V5 peptide sequence, we were able to determine by immunofluorescence the rate of stem cell transfection.

Cells that were efficiently transfected were isolated and cultured for proliferation.

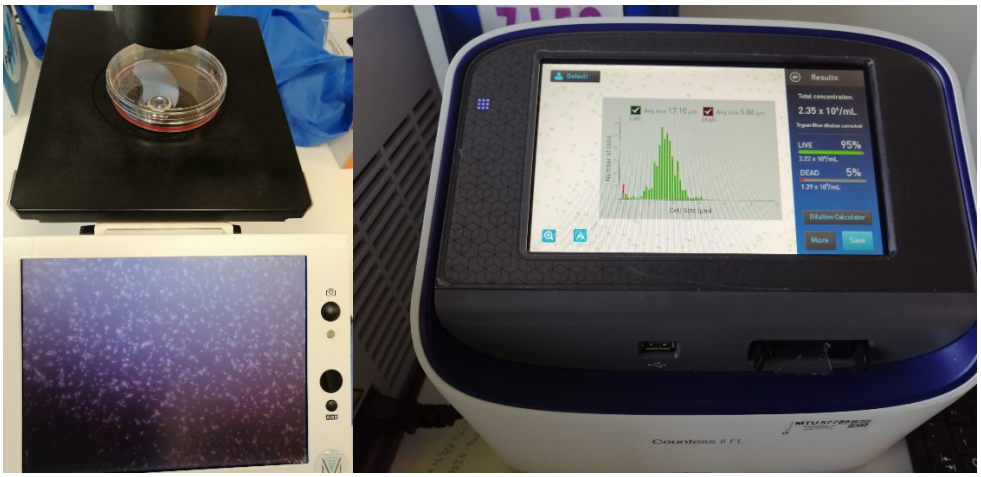
Subsequently they could be used in *in vitro* experiments, and by modulating OLIG2 activity it was possible to determine the dependence of tumour growth on OLIG2.

The main steps consisted of:

1. **Formation of the ribonucleic complex composed of Cas9 and trRNA**
2. **Cell transfection step using the Amaxa 4D System**



3. Cell culture of transfected cells



4. Immunofluorescence to determine efficacy.