A NOVEL ORGANOID MODEL FOR SENOLYTIC DRUG DISCOVERY

Stefanie Siebert-Krassnig, Tina Loeffler, Izaskun Itxaso, Irene Schilcher, Irantzu Perez Ruiz, Magdalena Daurer, Manuela Prokesch

scantox

For further information and inquiries contact office-austria@scantox.com

Background

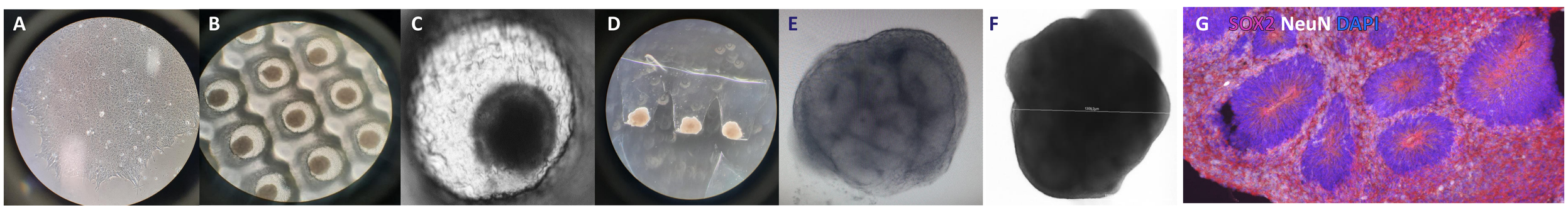
Age-related accumulation of senescent cells in the nervous system is relevant for the onset and progression of neurodegenerative diseases and has recently even been addressed in clinical Alzheimer's disease trials. Since most neurodegenerative diseases are age-associated, the use of translatable models that reflect aspects of senescence and aging are of high relevance in the field. Brain organoids, three-dimensional structures derived from human iPSCs, offer a novel approach to study brain development and disease. However, organoid research faces challenges as cells are juvenile and may not be fully mature for a certain time span. Thus, the aim of this study was to accelerate aging in organoids to create a model system more accurately reflecting key aspects of neurodegenerative diseases.

Materials and Methods

To address this, we induced accelerated aging and senescence in brain organoids using D-galactose (D-Gal) treatment. Cerebral organoids, derived from a healthy control human iPSC line, were treated with D-Gal for 1 to 3 weeks in presence or absence of senolytic drugs. The size of organoids as well as secretion of neurofilament light chain (NFL), analyzed with Uman Diagnostics ELISA, was monitored over time. Additionally, levels of senescence markers p53 and p21 as well as markers for different cellular subpopulations, including PAX6 (neuronal progenitor), NeuN (mature neurons), GFAP (astrocytes) and SNAP25 (synapse marker) were analyzed in RIPA lysates that were harvested 1 week after application of D-Gal and evaluated by WES.

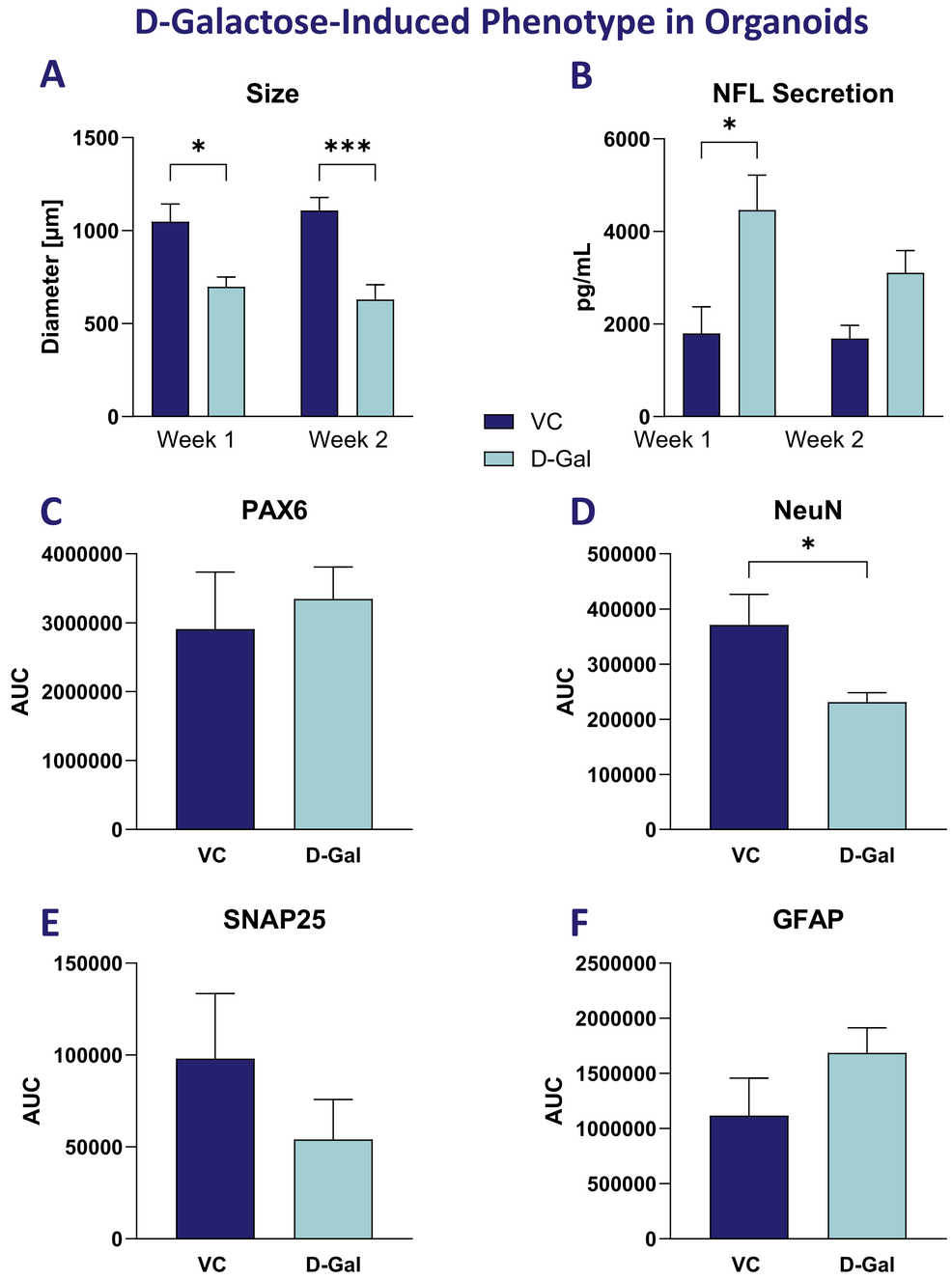
Results

Development of Cerebral Organoids



▲ **Figure 1. Representative images of the development of cerebral organoids from human-derived iPSCs. A**: Colony of human iPSCs from a healthy individual. **B**: DIV2 of embryoid bodies seeded in round bottom plates. **C**: Embryoid body at DIV7. Formation of neuroectoderm is represented as a halo around the embryoid body. **D**: Embryoid bodies at DIV10 embedded in Matrigel that supports the formation of the neural epithelium. **E**: Cerebral organoid at DIV13. Neural rosettes are present as the representation of differentiation of stem cells into neural progenitor cells. **F**: Cerebral organoids at DIV34. Diameter of the organoids is >1,000 µm. **G**: Immunohistochemical staining for neuronal precursor SOX2 (red) and mature neurons NeuN (white) in DIV35 organoid.

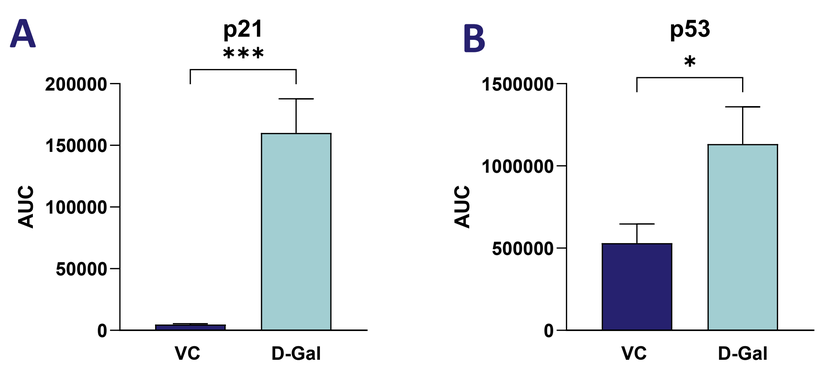
D-Galactose-Induced Phenotype in Organoids



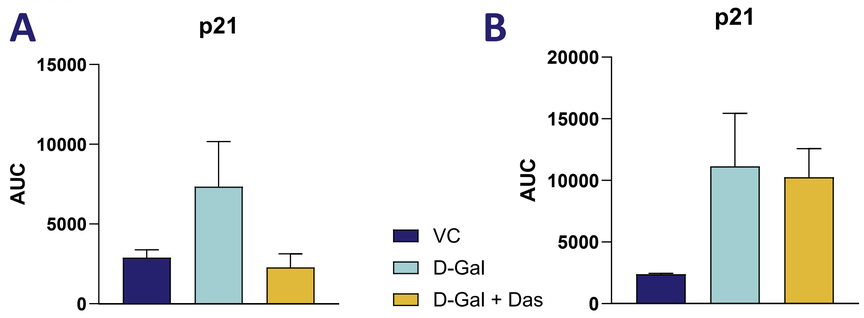
▲ **Figure 2. Analysis of D-Gal-induced changes in growth, neuronal maturity and astrocytic activation in cerebral organoids.** Analysis of changes in size (**A**) given as the diameter of the organoids in µm and (**B**) NFL secretion at two time points after start of D-Gal treatment. Protein level of neural progenitor marker PAX6 (**C**), NeuN as marker for mature neurons (**D**), SNAP25 involved in synaptic vesicle release (E) and GFAP as marker for astrocytes and astrocyte activation (**F**) in RIPA lysates of organoids lesioned with D-Gal for 1 week. Analyses were performed via automated western blot WES (Bio-Techne). Mean + SEM; n=6 per group. **A-B**: Two-way ANOVA followed by Šídák's multiple comparisons test comparing vehicle control (VC) versus D-Gal-treated condition at different time points. **C-F**: Unpaired t-test. *p<0.05, ***p<0.001.

The significant reduction in size across the organoids treated with D-Gal suggests a general trend of cellular atrophy or diminished proliferation. This atrophy correlates with an observed reduction in NeuN levels, indicating a decrease in mature neuronal density or health. The concomitant increase in NFL secretion at the 1-week time point aligns with this finding, as elevated NFL is indicative of neuronal stress or degeneration. No significant changes were observed for PAX6, SNAP25, or GFAP levels.

D-Gal-Induced Senescence



▲ **Figure 3. Analysis of senescence and autophagy markers in D-Gal-treated cerebral organoids.** Organoids were treated with 60 mg/mL D-Gal on DIV35 in medium for 1 week and harvested thereafter in RIPA buffer for analysis of senescence marker p21 (**A**), a cyclin-dependent kinase inhibitor that is tightly regulated by the tumor suppressor protein p53. It plays a key role in cell cycle arrest, particularly in response to DNA damage or other stress signals, and tumor suppressor protein p53 (**B**) and autophagy marker via automated western blot WES (Bio-Techne). Both markers were significantly upregulated in the D-Gal-treated cerebral organoids as shown in the automated western blot analysis, indicating that D-Gal induced senescence via upregulation of p21 and p53. Mean + SEM (n=4-6 per group). Unpaired t-test. *p<0.05, ***p<0.001.



▲ **Figure 4. Analysis of senescence in D-Gal-treated cerebral organoids in presence or absence of senolytic drug.** Organoids were treated with 60 mg/mL D-Gal and D-Gal +25 nM dasatinib (Das) on DIV38 in medium for 2 weeks (A) and 3 weeks (B) and harvested thereafter in RIPA buffer for analysis of senescence marker p21 via automated western blot WES (Bio-Techne). The p21 marker was upregulated in the D-Gal-treated cerebral organoids and a reverse effect of dasatinib was observed as being more obvious after 2 weeks of treatment. Mean + SEM; n=4 per group. One way ANOVA followed by Dunnet´s multiple comparison test.

Conclusion

Overall d-galactose treatment of cerebral organoids leads to increased senescence and changes in age-associated biomarkers as analyzed by different methods. Accelerated aging of cerebral organoids can serve as a valuable tool in drug development by providing a faster and more efficient means of testing potential therapeutics for age-related neurological conditions such as Alzheimer's disease.

For more information about the models please visit:
www.scantox.com
Scantox Neuro GmbH, Parkring 12, 8074 Grambach, Austria