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STEM CELL RESEARCH ITALY

Rosalba Monica Ferraro
d.b.: 16/06/1989
phone: +39 333 3554628
mail: rosalba.ferraro@unibs.it

(YIA)

Induced pluripotent stem cell-derived 3D Brain organoids cultured in a dynamic bioreactor as an *in vitro* model for the study of microcephaly in Aicardi Goutières Syndrome.

Ferraro Rosalba Monica[1], Manganelli Michele[1], Mazzoldi Elena Laura[1], Parigi Marta[1], Bugatti Mattia[2], Galli Jessica[3], Cattalini Marco[4], Piovani Giovanna[5], Poliani Pietro Luigi[6], Vermi William[6], Fazzi Elisa[3], Giliani Silvia[1].

[1] "Angelo Nocivelli" Institute for Molecular Medicine. Department of Molecular and Translational Medicine, University of Brescia, Italy. ASST Spedali Civili, Brescia, Italy.
[2] ASST Spedali Civili, University of Brescia, Brescia, Italy.
[3] Child Neurology and Psychiatry Unit, ASST Spedali Civili, Brescia, Italy. Department of Clinical and Experimental Sciences, University of Brescia, Italy.
[4] Pediatric Clinic. ASST Spedali Civili, Brescia, Italy. Department of Clinical and Experimental Sciences, University of Brescia, Italy.
[5] Biology and Genetics Division. Department of Molecular and Translational Medicine, University of Brescia, Italy.
[6] Pathology Unit. ASST Spedali Civili, Brescia, Italy. Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy.

OBJECTIVE:
Neurological disorder is the kind of genetic disease that can benefit the most from 3D modelling for its capability to generate an organized neuronal and glial network, otherwise only available from post-mortem samples. We exploited this possibility to create a 3D neural *in vitro* model of disease to investigate the Aicardi Goutières Syndrome (AGS). AGS is a severe neuro-inflammatory disorder with onset in early infancy. AGS patients exhibit psychomotor retardation, and microcephaly with demyelination and calcification. To date, 9 genes have been identified responsible of the disease.

MATERIALS AND METHODS:
We have deepened the *in vitro* 2D neuronal differentiation, generating and characterizing NSCs and neurons from iPSCs of three patients, mutated in: *RNaseH2B, IFIH1, and TREX1*. We didn't observe significant differences between AGS and control-derived neurons in terms of gene and protein expression of typical markers. As one of the features of AGS is the profound microcephaly, we generated iPSC-derived cortical cerebral organoids using a bioreactor that let organoids grew in a dynamic suspension as a better *in vitro* model to explore the cytoarchitectural alteration of the disease. Mini-brains were generated from optimizing the protocol described by Lancaster. The workflow consists in iPSC-derived embryoid bodies generation, neuroectodermal induction, matrix embedding for the neuroepithelium expansion, and cerebral organoids maturation. After 6 weeks of maturation the resulting mini-brains were analyzed in terms of dimension, shape, and expression of neuro-markers by qPCR and immunohistochemical analysis.

RESULTS:
The presence of rosette-like structures, typical of cortical-like regions, containing neuroepithelial stem cells (PAX6+) organized into polarized radial structures with a lumen was evaluated. More

mature organoids also displayed the presence of cells expressing cortical and glial layer markers (DCX+/ Synaptophysin+/GFAP+). Neural rosettes were positive for the expected markers in all samples and resemble the folding structure of the cerebral ventricles. In particular, AGS organoids showed a smaller size and irregular shape in comparison to control.

CONCLUSIONS:
Since to date the description of AGS iPSCs-derived brain organoids is documented only for *TREX1* mutated-samples, we expanded the study cohort to investigate the pathogenetic contributions and interaction between neurons and glia also in other AGS-derived brain organoids characterized by specific genetic mutations.