ISRE:tdTomato reporter system to map type I IFN responses in human brain organoids

Isabella Zafferri[1,2], Elisabetta De Gasperi[1], Agustina Ranieri[1], Chiara Riccardi[1] and Veronica Krenn[2]
E-mail: Isabella.zafferri@unimib.it
[1] University of Milano Bicocca, Italy
[2] Experimental medicine PhD, University of Milano, Italy

Keywords: Neurodevelopmental disorders, Brain organoids, Maternal infection, Type I IFNs, ISGs

Abstract:
Neurodevelopmental disorders (NDDs) are prevalent conditions with lifelong consequences and limited therapeutic options. Maternal infection and inflammation during pregnancy have emerged as important risk factors, but how they may disrupt early brain development is largely unknown. In this project, we focused on how elevated cytokines shape human neurodevelopment, using type I interferons (IFN-I) signalling, cytokines potently induced by infections, as a test case. Towards this aim, we built an IFN-responsive genetic reporter system in human brain organoids, in vitro human models of early brain architecture.

The reporter consists of tdTomato under the control of the ISRE element from the ISG15 promoter (ISRE:tdTomato), integrated at the AAVS1 safe harbour locus. Validation of this reporter in A549 lung epithelial cells, a standard model for IFN studies, showed that acute stimulation with IFNα2 or IFNβ induced a clear, dose-dependent increase in reporter activity, quantified by flow cytometry and confocal microscopy. Furthermore, we showed that reporter activation closely matches endogenous ISG15 expression kinetics measured by RT–qPCR and western blotting, confirming ISRE:tdTomato as a reliable readout of IFN-I pathway activation.

Next, we generated an H9 human embryonic stem cell line carrying the same ISRE:tdTomato cassette and used it to derive neural progenitor cells and cortical brain organoids. Using stimulation conditions calibrated in A549 cells, we confirmed a potent detection of ISRE:tdTomato induction in response to both IFNα2 and IFNβ. Fluorescence imaging and flow cytometry of ISRE:tdTomato organoids revealed a strong dose-dependent effect on heterogeneity in type I IFN responsiveness in neural progenitor cells as well as in the magnitude of the response. These observations indicate that cytokine dose is a major determinant of this variability and point to a substantial heterogeneity in cytokine responsiveness of neural progenitor cells.

Overall, this work establishes ISRE:tdTomato as a robust reporter to study IFN-I signalling in human cells and brain organoids and provides a methodological basis to investigate how distinct IFN-I exposure patterns may contribute to neurodevelopmental vulnerability and NDD risk.