ImmunoTools *special* Award 2018

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Modeling Fragile X syndrome with iPSC-derived neurons as a purpose of deciphering the molecular mechanisms and the neurobiological phenotypes associated with the pathology

The aim of our study is to create an *in vitro* model based on human induced pluripotent stem cells (hiPSCs) and test new potential drugs for Fragile X syndrome (FXS). FXS is the most common inherited form of human mental retardation and patients suffer different symptoms, including moderate to severe intellectual disability, obsessive-compulsive and autistic behaviors. FXS is caused by deficiency in fragile X mental retardation protein (FMRP) expression due to an unstable expansion of a CGG trinucleotide repeat inside the fragile X mental retardation-1 gene (FMR1). The resulting epigenetic silencing causes the loss of FMRP with defects in the regulation of dendritic spine morphology and synaptogenesis.

Progresses in understanding the pathophysiological mechanisms contributing to FXS have increased optimism that drug interventions can provide significant therapeutic benefits and there are evidences that inhibitors of glycogen synthase kinase-3 (GSK3) may contribute to the therapeutic treatment of FXS; indeed significant improvements in several FX-related phenotypes have been obtained in FX mice following the administration of GSK3 inhibitors *(Mines and Jope, 2011).*

Growing evidence demonstrates how aspects of human development and disease can be accurately modelled *in vitro* using hiPSCs *(Passier et al., 2016).* With the purpose of deciphering the molecular mechanisms and the neurobiological phenotypes associated with FXS, the creation of a robust *in vitro* model based on hiPSCs can be used to study FXS in a time frame that is relevant to the disease, understand its mechanisms and allow for therapeutic testing, all in cells carrying the genetic background of individual patients.

Two types of neurons could be used as *in vitro* models of FXS: cortical neurons and hippocampal neurons and we are currently setting up conditions for neuron differentiation from human iPSCs lines with the Fragile X syndrome mutation and one line with the FMR1 premutation (range from 55 to 200 repeats). Previous work by the Bhattacharyya lab demonstrated that FXS-iPSCs can be differentiated into $FOXG1^{+}$ forebrain neurons *(Doers et al., 2014)* and the mutation doesn't impair the differentiation capacity of these cells.

It has been demonstrated that FXS-iPSC-derived forebrain neurons exhibited neurite outgrowth defects, in particular FXS forebrain neurons extended significantly fewer processes that were significantly shorter relative to controls *(Doers et al., 2014)*.

Our goal is first of all set up of a cortical neuron differentiation protocol and therefore, for the optimization of the differentiation, we will test some molecules that can improve the efficiency of the differentiation; so we will treat neural precursor cells with survival (SCF, FGF-b, SHH, FGF-1, FGF-2, FGF-7, FGF-19, FGF-22, FGF-23, FGF-8) and neurotrophic factors (BDNF, GDNF, NGF, CNTF, Neuregulin-1a, Neuregulin-1b) to achieve a functionally mature culture.

Once obtained a robust method of differentiation, we want to study the electrophysiological properties and axon growth dynamics of FXS-iPSCs derived neurons and the possible rescue function obtained by GSK3b inhibitors. In particular, we want to analyze voltage gated channels and firing properties of FXS-iPSCs derived neurons and synaptic activity on neuronal networks *in vitro*, using patchclamp and ion imaging recordings.

Preliminary calcium imaging and patch clamp recordings suggested a good degree of maturation of iPSC-derived neurons, which form a connected and partially synchronized network. The use of neurotrophic factors and ImmunoTools human cytokines will contribute to improve the efficiency of our differentiation protocol and to characterize the related phenotypic profile respect to WT cells and cross-validate GSK3-b hypothesis by challenging the phenotypic profile of FXS neurons with selected and validated inhibitors.

Doers ME, Musser MT, Nichol R, Berndt ER, Baker M, Gomez TM, Zhang SC, Abbeduto L, Bhattacharyya A. iPSC-derived forebrain neurons from FXS individuals show defects in initial neurite outgrowth. Stem Cells Dev. 2014 Aug 1;23(15):1777-87.

Mines MA, Jope RS. Glycogen synthase kinase-3: a promising therapeutic target for fragile x syndrome. Front Mol Neurosci. 2011 Nov 1;4:35.

Passier R, Orlova V, Mummery C. Complex Tissue and Disease Modeling using hiPSCs. Cell Stem Cell. 2016 Mar 3;18(3):309-21.

ImmunoTools *special* AWARD for **Carlo Brighi** includes 24 reagents

recombinant human cytokines**:**
rh BDNF, rh CNTF, rh EGF, rh FGF-a / FGF-1, rh FGF-b / FGF-2, rh FGF-7, rh FGF-8, rh FGF-19, rh FGF-23, rh G-CSF, rh GDNF, rh GH, rh IGF-I, Neuregulin-1a, Neuregulin-1b, rh beta NGF, rh Noggin, rh PDGF-BB, rh RANTES/CCL5, rh SCF, rh SHH, rh SDF-1 a, rh TNFa, rh VEGF-A/VEGF-165

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