Cerebral Organoids as a New Way to Model ADHD Pathophysiology: A Look at Molecular, Cellular and Connectivity Deficits

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ABSTRACT

The lack of a clear etiology of ADHD coupled with difficulties in recapitulating human brain development is limiting our understanding of its pathophysiology. While much remains to be clarified, it is now accepted that ADHD patients have altered brains. The Prefrontal Cortex (PFC) has risen to be of central relevance to the neuronal pathways of ADHD.

Particularly, we propose that the root cause of the PFC’s smaller structure involves a limited progenitor pool and impaired radial migration. To achieve these long-term goals, we used a novel episomal reprogramming method to generate high quality control ADHD-iPSC’s and optimize in vitro organogenesis. Our approach will facilitate examination of how disease risk is translated at the cellular and tissue levels through comparative studies of processes such as progenitor cell proliferation, migration and connectivity during development.

INTRODUCTION

-Attention deficit hyperactivity disorder (ADHD) is a common neurodevelopmental disorder, affecting between 5-10% of school-aged children.
-ADHD is highly heritable and multifac torial, and its core symptomology includes inattention, hyperactivity/impulsivity, and motivational/emotional deficits.
-While the molecular mechanisms underlying ADHD pathogenesis are unknown, recent studies place the prefrontal cortex (PFC) at the center stage of the neuronal pathway of ADHD, making it critical to understand the molecular influences that modulate PFC function in order to develop novel ADHD therapeutics.
-ADHD's pathophysiology appears to be rooted in embryonic development, establishing the need for a better model to study ADHD. Thus, prompting us to hypothesize that cerebral organoids derived from ADHD patients' induced pluripotent stem cells (iPSCs) can be developed as a platform to study ADHD's pathophysiology and to investigate the molecular and cellular phenotypes as an early ADHD brain surrogate system.

METHODS

Generation of IPS-like clones from ADHD patients' fibroblasts:
-ADHD subjects recruited for biopsy fulfilled DSM-IV criteria, had no comorbid disorders and manifested objective symptoms of hyperactivity and inattention.
-We first established fibroblast cultures from four ADHD (EB, A09, I03, I12) and four healthy (RB, A10, I01, A05) subjects following clinical evaluation and informed consent. Approximately 1 x 10^6 fibroblasts from each subject were electroporated with episomal plasmids. For three days after electroporation, cells were maintained in fibroblast media and supplemented with human PSC media for 4 days. From day 7 to day 15, cells were cultured with human iPSC media. 10 ng/ml basic fibroblast growth factor (bFGF) was added as supplementation. We picked up granulated colonies with HES-like morphologies following approximately three weeks of culture. These IPS lines were established based on morphological criteria, stable expansion (at least 28 passages) or multiple ES marker expression (e.g., AP, SSEA, Oct4, and Nanog), and no chromosomal integration was confirmed by comparative studies of processes such as progenitor cell proliferation, migration and connectivity during development.

ADHD and Control Subjects Recruitment

Four Caucasian ADHD subjects with no comorbid disorders were selected for the generation of multiple IPS-like clone candidates as well as four psychiatrically healthy gender matched siblings to be used as healthy controls. The lack of a clear etiology of ADHD coupled with difficulties in recapitulating human brain development is limiting our understanding of its pathophysiology. While much remains to be clarified, it is now accepted that ADHD patients have altered brains. The Prefrontal Cortex (PFC) has risen to be of central relevance to the neuronal pathways of ADHD.

Particularly, we propose that the root cause of the PFC’s smaller structure involves a limited progenitor pool and impaired radial migration. To achieve these long-term goals, we used a novel episomal reprogramming method to generate high quality control ADHD-iPSC’s and optimize in vitro organogenesis. Our approach will facilitate examination of how disease risk is translated at the cellular and tissue levels through comparative studies of processes such as progenitor cell proliferation, migration and connectivity during development.

REFERENCES


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Cell diversity in cortical organoids

Immunohistochemistry shows protein expression of pluripotency markers from all eight generated IPS lines (A). mRNA expression of pluripotency markers shows several fold increase of pluripotency markers in generated IPS's compared to fibroblasts (B). PCR shows no chromosomal integration of reprogramming episomal plasmids in all IPS lines generated for this study (C).

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