Stem cell-derived three-dimensional (organoid) models of Alzheimer's disease: a precision medicine approach

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The major challenges of the "brain disorders" field – dementia, schizophrenia, other neuropsychiatric disorders – are that these are defined by clinical phenotypes whose underlying biology is poorly understood. There is great variability in definition, prognosis, trajectory, and treatment response indicating that the next step is defining subgroups by combining clinical and biologic information at the level of the individual. These challenges are especially relevant and urgent in the case of dementia and related disorders.

Prevalence of dementia due to Alzheimer's disease (AD) is growing globally and the disease is associated with severe disability, mortality, adverse effects on families/caregivers, and huge societal costs. While the pathogenesis of AD is incompletely understood, two general forms are recognized: familial Alzheimer's disease (FAD) and sporadic Alzheimer's disease (SAD). Familial AD patients, only 2–5% of cases, develop symptoms before age 65. FAD follows autosomal dominant "deterministic" inheritance linked to mutations in three key genes: amyloid precursor protein (*APP*), presenilin 1 (*PSEN1*), and presenilin 2 (*PSEN2*). SAD is the most common form of AD with typical onset later in life (median ~78) often without a family history. The most common gene associated with SAD risk is the allele 4 of apolipoprotein E (Saunders et al., 1993). The leading hypothesis regarding the etiology of AD is that accumulation of pathogenetic amyloid β (Aβ) protein, derived from APP, triggers a cascade of downstream events, involving formation of intracellular "tau" protein neurofibrillary tangles, followed by neuronal cell death, and ultimately dementia.

Multiple efforts have been underway for decades to prevent or cure AD based on the amyloid hypothesis. These have failed to produce a disease-modifying therapy which has called this hypothesis into serious question. More recent thinking attributes this failure to approaching AD as a unitary biologic entity for which a single type of treatment – "anti-amyloid" – will be effective for all. Instead, novel approaches to subtyping AD are needed based on evidence that while amyloid beta and tau are central disease components, other genes, stress, inflammation, insulin resistance, vascular disease, and co-morbidities strongly influence the onset, expression, and progression of clinical disease (Khoury and Grossberg, 2020). Better understanding of variables that affect AD progression before and after onset of clinical manifestations will improve our ability to define individual pathways to dementia and develop clues for interventions tailored to individual pathways.

With the above in mind, Precision Medicine naturally emerges as the best direction for the field to take with the goal of producing effective treatments tailored to individuals. Also known as Personalized Medicine, this approach aims to deliver the right treatment to the right patient at the right time in the most efficient fashion. It focuses on linking the best available treatments at any given point in time with the right patient subgroup that will most benefit from that treatment. In order to succeed in the AD field, Precision Medicine will rely on biologic tools that will define and characterize disease subtypes at the individual level (Hampel et al., 2016). Ideally, these tools will be based on human as opposed to animal models, which have not been proven to be successful models for treatment developments in the field.

The advent of human induced pluripotent stem cell (hiPSC) lines derived from persons with unique genomes has revolutionized our approach to human disease modeling and drug screening. Human iPSC technologies afford the opportunity to generate any type of cell from individuals so as to study disease mechanisms, develop novel diagnostic tools, or test therapies at the individual level. *In vitro* models like these combine the advantage of human biology (as in traditional neuropathology) with the potential for mechanistic studies (as in transgenic animal models). In addition, such models allow an unprecedented level of cellular resolution and great experimental versatility.

Despite earlier predictions holding that these breakthroughs would be primarily relevant to developmental disorders, there have been a number of reported applications of hiPSCs as models of neurodegenerative diseases, such as AD. Reprogramming of fibroblasts or blood cells from patients with AD resulted in the generation of multiple cell culture models of FAD and SAD. These include neurons differentiated from iPSCs derived from patients with known AD mutations (FAD patients) (Mahairaki et al., 2014; Muratore et al., 2014) and overexpression of mutant AD-related genes (such as APP, PS1, and hyperphosphorylated tau) in cells from healthy donor by viral transduction (Yagi et al., 2011; Koch et al., 2012) or CRISPR/Cas9 mediated gene editing (Huang et al., 2017; Kwart et al., 2019). Many of these models in two-dimensional cell culture system have recapitulated some features of AD pathology, such as elevated levels of toxic amyloid-β species and phosphorylated tau, but did not demonstrate amyloid-β plaques or neurofibrillary tangles. They also have limitations presenting various regulatory functions by glial cells as in the human brain. Unfortunately, these models have not been geared towards understanding individual variability in the activity of neurons and other relevant brain cells derived from iPSCs. The promise of this methodology in the future is based on developing iPSC-derived cell models of individuals in large numbers to study disease mechanisms, test therapies, and ultimately deliver these therapies tailored to individuals using Precision Medicine.

Organ-like three-dimensional (3D) tissue cultures, such as brain organoids or cerebral organoids, are proving a powerful complementary approach, allowing the effects of new therapies to be predicted more accurately by closely replicating aspects of the brain environment. Lancaster et al. first described the formation of self-organizing cerebral organoids with organized structures composed of progenitor and neuronal cells, resembling distinct regions of the brain (Lancaster et al., 2013). In these culture systems, patterns of gene expression as well as cell morphologies and functions are similar to ones encountered *in vivo*. Nerve cells cultured in 3D configurations better reflect the complexity of nervous tissue, where 3D neurites form functional synapses, have network properties and perform essential cellular physiological processes such as DNA damage repair, as our group have recently shown (Das et al., 2020).

Choi et al. (2014) first generated 3D-differentiated neuronal cells to model AD by genetically modulating the expression of FAD mutations (APP and PS1) that recapitulate extracellular amyloid aggregation and tau pathology as well as higher neuronal apoptosis rates in AD cerebral organoids. They used the 3D culture model to investigate the impact of an experimental drug on regulation of β-amyloid-mediated tau phosphorylation showing the potential to serve as drug screening platform. More recently, our group (**Figure 1**) – as well as others – have developed iPSC-derived organoids from FAD patients and found AD-like accumulation of Aβ and tau aggregates as well as markers of apoptosis (Raja et al., 2016; Gonzalez et al., 2018). Raja et al. (2016) reported that treatment of FAD-derived organoids with β- or γ-secretase inhibitors partially reversed both amyloid and tau pathologies. Therefore, brain organoids are of immense interest as more precise human neural cell model for AD because of their ability to recapitulate fundamental pathogenic features and mechanisms. Moreover, the neural tissue-like cell-cell interactions in these systems promote the survival of nerve cells over long periods of time that are essential for the formation of Aβ deposits. Once again, the real power of this approach will be to understand disease subtypes at the individual level and develop individual level diagnostic and drug testing platforms.

Despite the great promise of hiPSC-derived brain organoids, we cannot overlook technical limitations of these methods. Making these models effective for clinical applications in a personalized level will require reductions in cost and time frames. A major limitation of the current 3D cultures is the insufficient maturation and aging of neural cells, which are typically at a fetal developmental stage. This is particularly challenging when neurodegenerative diseases are being investigated in which age is a major risk factor for disease development. While several studies have demonstrated the use of iPSCs to model age-related disorders, such as AD, the fact that

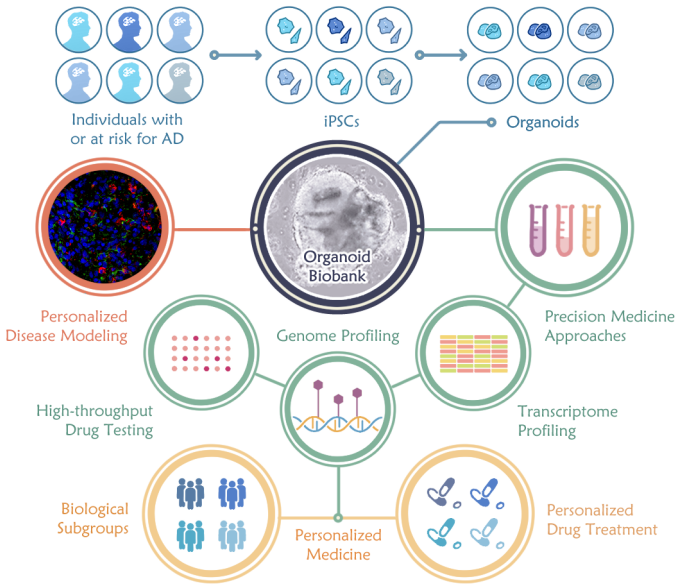

Figure 1 | **Applications of cerebral organoids in Precision Medicine for AD.**
Schematic of cerebral organoids generation from patients at risk or with AD (upper) and their potential application for the development of personalized drug treatment (lower).Representative images of AD patient-derived cerebral organoid generated in the authors' lab (bright-field; central) and amyloid-β deposits in 120-day organoid (Fluorescence confocal image; left); (green, Tuj1; blue, nuclei; red, 4G8 antibody, extracellular amyloid-β deposits). AD: Alzheimer's disease; iPSCs: induced pluripotent stem cells.

reprogramming to iPSC reverts cells to a fetal state could limit the potential of this model to recapitulate fully the pathophysiology of AD. However, several "induced aging" strategies, such as expression of progerin to model late-onset diseases have been reported. One more constraint that the 3D culture models have faced is the low optical transparency during high-resolution imaging due to the thickness of the tissue (Hernandez-Sapiens et al., 2020). However, novel approaches like tissue clearing techniques and advanced microscopic methods such as confocal and multiphoton microscopy and light-sheet fluorescence microscopy ensure the detailed high-resolution imaging of the organoids (Dekkers at al., 2019). Another challenge is the lack of vascularization that is critical for maturation of cells and non-neuronal components. In this regard, microglia, and immune system cells all could contribute to the generation of brain organoids with amyloidogenic properties. Recent work though has demonstrated the feasibility of combining brain organoids with microglia-like cells (Song et al., 2019), as well with blood vessels (Mansour et al., 2018).

In view of several recent negative trials for AD, the field is in dire need of novel, relatively simple, accessible screening platforms in which large numbers of anti-amyloid and neuroprotective compounds can be rapidly tested. The availability of stem cell-derived 3D models of AD, offer novel tools for diagnostics and therapeutics, including drug screening, chemical toxicity assessment and personalized therapies. A key advantage in drug development using these *in vitro* models is the potential of determining time windows of drug efficacy. Another advantage is the opportunity to demonstrate the efficacy of new drugs on Aβ-mediated neurotoxicity. Both types of capabilities address key needs in the field of AD therapeutics.

Given the substantial heterogeneity of AD, Precision Medicine is the best direction for the field to take with the goal of producing individually tailored treatments for patients with subtypes of AD. While in the distant future better understanding of brain biology will bring about more impactful, perhaps curative, medications for AD, until that time it is essential to use the best currently available experimental models that closely mimic brain architecture and AD pathology. The potential generation of a biobank of brain organoids from individual AD patients will serve as accessible screening platforms in which large numbers of promising therapies could be rapidly tested at the individual level. The availability of such AD models offer novel tools for diagnostics and therapeutics, including drug screening, chemical toxicity assessment and personalized therapies.

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