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GUEST EDITORIAL

Brain organoids: a next step for humanized Alzheimer's disease models?

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A major and actual issue in the field of neurodegenerative diseases is the poor translational potential of most preclinical models [1]. In the context of Alzheimer's disease (AD), a number of promising pharmaceutical approaches yielded impressive results in various in vitro systems and animal models where they almost cured the disease, but ultimately failed in providing effective protection in clinical trials [2]. Protein misfolding is a hallmark of AD, involving the accumulation of amyloid-β peptide (Aβ) and hyperphosphorylated Tau protein in discrete areas of the brain [3]. These lesions are proposed as a triggering step of a cascade of molecular events resulting in synaptic dysfunction and neuronal degeneration, leading to cognitive and memory impairment. While the vast majority of AD cases are sporadic, familial cases (FAD) are extremely aggressive with an earlier onset and are usually associated to exacerbated amyloidopathy [4]. Mutations on the genes encoding the amyloid precursor protein (APP) gene or presenilin 1 (PS1), the catalytic component of the γ-secretase complex that produce the Aβ peptide, are responsible for most of FAD cases. For this reason, widely used mouse models of AD are genetically engineered to overexpress high levels of the human APP in combination or not with human PS1, where both genes carry one or multiple FAD mutations [5]. These models produce large amounts of Aβ peptide, generally resulting in memory impairment at a young age. Transgenic mice have been invaluable in elucidating some of the molecular mechanisms underlying AD pathogenesis but they suffer from several limitations [5]. Among them, a central critic is the difficulty to reproduce Tau pathology [5, 6]. The expression of APP or PS1 human mutations in AD transgenic mice is unable to fully recapitulate this central lesion, unless a human Tau transgene is expressed concomitantly [7]. In addition, most trangenic mice develop histopathological AD features early in life, which is a major limitation to study the molecular events priming the development of the pathology during aging [5, 7]. Furthermore, despite that the murine genome contains most AD-related genes, mice do not spontaneously develop an AD phenotype during aging, suggesting that the molecular networks driving disease initiation may not be present in this specie. Thus, alternative systems are needed to study these pathological mechanisms and test the efficacy of new therapeutic strategies. The development of humanized models is emerging as a powerful complementary approach to overcome the limitations of current AD transgenic mice (see Fig. 1). In a recent issue of *Molecular Psychiatry*, Claudio Soto's group developed a consistent characterization of brains organoids from cell derived of a human FAD patient, recapitulating the major hallmarks of the disease, including the interplay between Aβ peptide accumulation and Tau phosphorylation [8].

The technical advances in human somatic cells reprogramming into induced pluripotent stem cells (iPSCs) enabled new research angles to study human disease and organ development [9]. The improvement of in vitro protocols for the differentiation of iPSCs led to the generation of "organoids", which are defined as self-organized and self patterning three-dimensional (3D) structures that share some similarities with complex organs. Brain organoids, also known as mini-brains, recapitulate the developmental process of the brain in the test tube, generating organized structures broadly resembling different regions of the brain

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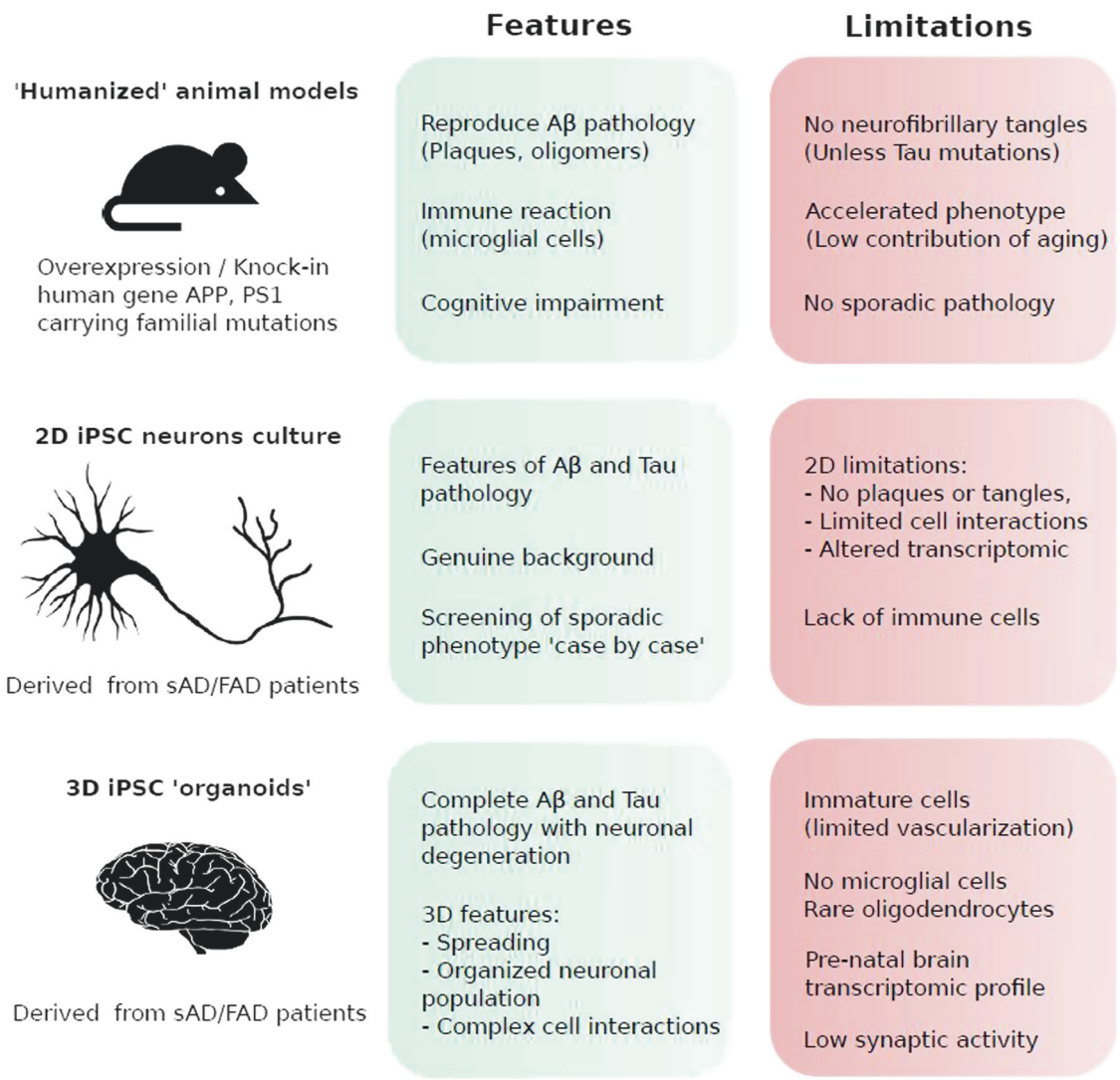

Fig. 1 Features and limitations of humanized Alzheimer's disease models. Humanized animal models have the advantage of covering multiple aspects of the pathology (including brain inflammation, metabolic alterations, or cognitive impairment) but are built with an artificial etiology and lack the specificity of the human genetic and molecular background. 2D iPSC neurons culture have a more relevant background and allow the study of unique features from sporadic cases, but the 2D system fail to reproduce complex brain cell to cell interactions which affect their transcriptomic profile and maturation. 3D brain organoids keep the same background advantages of the 2D systems while featuring complex cell interactions and cell populations. They reproduce the AD hallmarks admirably: Aβ deposits, Tau tangles, and neuronal degeneration. The most critical limitations to overcome are the lack of complete vascularization and incomplete immune system (presence of astrocytes but no microglial cells)

[10]. In addition, various methodological approaches have been established to reproduce specific and unique substructures of the brain (forebrain, midbrain, hippocampus, or retinal organoids) [11]. Mini-brain generated from patient iPSCs have been used in the last years as a model to study neurodevelopmental disorders such as microcephaly [9], autism spectrum disorder [12, 13], or Zika virus infections [14]. However, the relevance of brain organoids for late-onset pathology such as AD was unclear until very recently. Interestingly, neuronal cultures of iPSC-derived neurons from FAD and some SAD cases resulted in elevated Aβ peptide accumulation and Tau phosphorylation, combined with endosome alterations which are a key early event of the disease [15–17]. A first attempt in developing a 3D cell culture model of AD was accomplished using human neuronal progenitor cells genetically engineered to overexpress mutant APP and PS1 [18]. This approach resulted in increased extra-cellular desposits of the Aβ peptide toxic specie 1–42 and Tau phosphorylation into detergent-insoluble fibrils. Here, Gonzalez and coworkers, moved forward to develop a more physiological model of AD brain organoids generated from iPSC of FAD and Down syndrome patients [8]. Through an in vitro developmental process, AD brain organoids showed a progressive

accumulation of Aβ peptide with amyloidogenic properties, in addition to accumulate plaque-like structures, preceding the appearance of phosphorylated Tau and neurofibrillary tangles. Measurements of caspase-3 activation also indicated neuronal death in the AD brain organoids. The experimental model was highly reproducible and characterized on a quantitative manner.

Overall, the results obtained with AD brain organoids further support the close relationship between Aβ deposition and Tau phosphorylation. The use of AD mini-brains will open the possibility of validating disease mechanisms and hopefully identify new pathways contributing to AD progression. For example, the possible role of the glycogen synthase kinase 3β (GSK3β) in Tau phosphorylation could be further explored, since 2D and 3D human iPSC AD cultures resulted in Aβ, Tau, and GSK-3β activity [15, 18]. Among other molecular features linked to AD, endosomal and lysosomal alterations with altered expression of Rab5 were reported in brain organoids derived from FAD stem cells [19]. Some SAD and FAD iPSC 2D systems also reported endosomal and lysosomal alterations, along with increased expression of endoplasmic reticulum (ER) stress markers [16, 17, 20]. ER stress is proposed as a key feature of AD and others neurodegenerative disease [21, 22]. However, there is inconsistency in the identification of ER stress markers using AD transgenic mice [23, 24]. Thus, human brain organoids could open new possibilities to identify the regulatory network affected in AD brains and define strategies for their intervention. The spreading of protein misfolding by a Prion-like mechanism is also emerging as a relevant factor mediating disease progression in AD, a phenomenon that could be also studied and intervened using the human mini-brain technology [25].

Considering the demonstrated low translational potential of rodent models of AD, humanized models should be considered as a complementary platform for drug screening to support preclinical studies. Results in iPSC-derived neuronal cultures from FAD patients showed important variations compared to non-human models in the dose required to obtain significant activity of γ-secretase modulators or inhibitors [26]. In addition, undirect effects of β-secretases inhibitors on Tau protein phosphorylation were also observed [15, 27]. Studies using pharmacological modulators of the β-secretase or γ-secretase yielded convincing results in AD brain organoids, with reductions of Aβ peptide levels and also Tau pathology, in agreement with previous results in iPSC cultures [18, 19]. In addition, the interest for gene-therapy approaches or stem-cell-based regenerative strategies for AD is increasing [28–30], which could be improved and complemented using AD mini-brains. Clinical trials with gene therapy to deliver the nerve growth factor (NGF) [31] or the injection of genetically modified stem cells [32], showed overall good safety profiles. However, the therapeutic benefits obtained were limited despite the encouraging results observed in rodents and non-human primates [33, 34], highlighting again the need of new models that closely resemble the human condition to study the efficacy of such interventions.

While brain organoids have great potential for future discovery initiatives, some technical limitations still need to be overcome. Aging is the main non-genetic risk factor to develop AD, and the aging process is associated with numerous alteration including changes in gene expression [35]. However, neural cells derived from iPSC show a transcriptomic profile similar to prenatal brain [36]. In addition, the lack of vascularization in the brain organoid prevents further maturation of the neuronal cells, having adverse effects to the formation of active synapses while impairing the organoid survivability after prolonged periods of culture [11, 37]. A possible solution to this issue may lie in the use of heterotypic cultures, with endothelial and mesenchymal stem cells [38]. Alternatively, chimeric models using the mouse brain vascularization system are under development [39]. Another factor limiting the use of brain organoid systems in the study of neurodegenerative disease is the lack of microglial cells, because they are not derived from the neural progenitor cell line [40]. However, microglial cells derived from human iPSC have been successfully integrated into 3D brain organoids structures, and appeared to be active and able to respond to localized injury [41]. In addition, a 3D culture model including neurons, astrocytes and microglia have been recently developped to modelize AD [42]. Thus, all these technological advances could be applied to the model developed by Soto's group to further resemble and mimic the pathological events observed in the AD brain. In addition, because hundreds of mini-brains can be generated at the same time, the possibility of developing drug screenings, or secondary validation of larger screenings using iPSC-derived AD neurons will accelerate the identification of compounds with therapeutic efficacy. Overall, 3D culture methods still needed to be refined, but certainly promise the implementation of clinically relevant models to accelerate the discovery of a cure or a treatment not only for AD, but also for other major neurodegenerative diseases with high incidence such as Parkinson's disease. In this line of interest, a recent study used human mid-brain organoids to generate spatially organized clusters of dopaminergic neurons [43]. Finally, the possibility of studying the complex contributions of the genetic landscape of the human population in sporadic AD may be feasible in the future using brain organoids. The current study represents an inflection point for the discovery of hopefully effective approaches to treat AD.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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