Human-induced pluripotent stem cells as a model for studying sporadic Alzheimer's disease

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Abstract

The discovery of induced pluripotent stem cell (iPSC) technology has the potential to accelerate scientific research for Alzheimer’s disease (AD). iPSCs are therefore increasingly considered for AD modeling and drug development. Nevertheless, most of the work conducted so far has mainly focused on iPSC models from patients with familial AD (fAD), while actually sporadic AD (sAD) is more prevalent and represents over 90% of the AD cases in the population. The development of more sAD models is therefore key for studying this multifactorial disorder. In fact, probing the unique genomes of sAD patients and their interaction with AD-associated environmental factors could contribute to a better understanding of this disease. However, initial iPSC-based models for sAD have shown a high degree of variability and inconsistencies in terms of AD hallmarks. In this review, we provide an overview of the studies that have been conducted for sAD so far. In addition, we critically assess important sources of variability related to the model in addition to those that might be explained by the heterogeneous nature of sAD. These considerations might aid in developing more consistent iPSC models of sAD, which could help in developing a better understanding of the molecular mechanisms underlying the disease.

1. Introduction

With the increasing life span of the general population, Alzheimer’s disease (AD), which is an age-related neurodegenerative disorder, is becoming a major disease burden and socio-economic challenge worldwide. It was estimated in 2015 that 46.8 million people in the world are living with AD or a related form of dementia and it is expected that this number will double by 2040 (Clay, Zhou, Yi, Zhai, & Toumi, 2019). The vast majority, i.e. more than 95%, of the AD cases are characterized by a late-onset form and sporadic development of the disease. Its origin is multi-factorial, having both genetic and environmental factors, as well as their complex interactions, contributing to the onset and course of the disease. On the other hand, fAD, which represent the early-onset form, is known to arise from mutations in genes such as amyloid precursor protein (APP), presenilin 1 (PSEN1) and presenilin 2 (PSEN2) (Chakrabarti et al., 2015). Although distinguishable based on their time of onset and mode of transmission, both types of AD appear identical in light of clinical features and neuropathological hallmarks. Lesions of accumulated amyloid beta protein (Aβ) and neurofibrillary tangles consisting of hyperphosphorylated tau protein are typically observed in AD brains, and are known to contribute to the neuronal cell death and immune activation, leading to region-specific atrophy. The loss of neural integrity in regional neurotransmitter systems is thought to underlie the clinical symptoms, such as cognitive decline and learning deficits, which are typically observed in AD patients.

In the past decades, our knowledge on the pathophysiology that underlies AD has advanced tremendously. In particular, the neurotoxicity of aberrant Aβ and neurofibrillary tangles are now much better understood. However, efforts to translate these insights into clinical success have floundered. Although researchers have conducted a large number of clinical trials for potential treatments for AD, almost none of the drugs have been brought to the market. Despite the blame being placed on a variety of factors, one of the main concerns has been the animal and cellular models that were used in the initial stages of drug development (Laurijssens, Aujard, & Rahman, 2013). Most of these have relied on transgenic models harboring genetic mutations associated with fAD. Despite the fact that these have proven to be...
instrumental for basic research, they only partially reflect AD phenotypes and have thus far have been unable to explain the complex and heterogeneous nature of sAD. It has therefore been debated that more accurate models for sAD harboring the enormous amounts of individual genetic backgrounds within the patient population are required to further improve our insights into sAD development and progression. Moreover, it has even been hypothesized that such models would potentially be able to aid in the identification of novel biomarkers for early detection of sAD, as well as in the discovery of new and much needed therapeutics.

With the foundation of human induced pluripotent stem cell (iPSCs) over a decade ago, new doors for AD-research have been opened (Takahashi & Yamanaka, 2006). Since their discovery, human iPSCs have been offering a promising avenue to fill the gap between animal and human research, providing a new platform for disease modelling and drug testing that has the potential to broaden our knowledge on the underlying pathophysiology. Pluripotent stem cell technology is currently providing an unlimited source of patient-derived cells that can be differentiated into disease-relevant cells, including neurons and glia (reviewed in Shi, Inoue, Wu, & Yamanaka, 2017; Riemens, van den Hove, Esteller, & Delgado-Morales, 2018). The majority of the conducted work so far in AD has, however, used iPSC-cells derived from patients with fAD where, similar to previous animal studies, specific genetic mutations were studied that drive the disease onset. The establishment of more iPSC-based models from sAD patients is therefore necessary and could potentially benefit research into disease etiology and development of therapeutic strategies. However, developing iPSC models of sAD remains challenging due to the multifactorial nature of the disease, the live-long disease progression and the high degree of inter-individual heterogeneity that might be reflected in cells derived from sAD patients. Furthermore, aside from the variability associated with the disease, several technological challenges currently remain that could further impact on the variability that is observed in these sAD-iPSC models. In this review, we therefore provide an overview of the studies that have been conducted thus far and we critically assess important sources of variability related to the model in addition to those that might be explained by the heterogeneous nature of the disease.

2. PSC-derived models for sAD

To date, a limited number of studies has been performed with iPSC-derived neurons from sAD patients (Table 1). Modeling AD using patient-derived iPSCs was initiated from fAD cases with known mutations in disease-causing genes, such as APP, PSEN1 and PSEN2. iPSC-derived neurons from sAD patients in these studies were often studied in parallel, with the main goal of seeking to find AD-associated cellular phenotypes for the validation of their potential for sAD modeling. For this reason, many of these reports specifically look into the presence of AD-associated hallmarks such as amyloid beta, hyperphosphorylated tau and elevated levels of GSK-3β. The very first study in 2012, which is a comparative study using iPSC-derived neurons from fAD and sAD patients (Israel et al., 2012), demonstrated increased levels of Aβ(1–40) that were found to be secreted from sAD neurons in comparison to non-AD controls. These findings in the sAD neurons were in line with the observations in neurons from fAD patients. However, this was only observed using iPSC-derived neurons from one out of two sAD patients. Secreted levels of Aβ(1–40) using iPSC-derived neurons from a second patient were similar to levels obtained using iPSC-derived neurons from two non-demented control subjects. Also other hallmarks of AD, i.e. phosphorylated tau and active GSK-3β, were elevated in the iPSC-derived neurons that exhibited elevated Aβ(1–40) secretion, while the levels of these markers for the other sAD patient-derived neurons were again close to those observed in non-demented controls. Overall, this study suggests that iPSC-derived neurons from sAD patients can display similar hallmarks to fAD lines, but that these are not evident for every sAD patient. Interestingly, this heterogeneity corresponds with the complexity of sAD and our general understanding on disease causation and progression. In a later study, Kondo and colleagues reported that iPSC-derived neurons from 2 distinct sAD patients did not show elevated secretion of Aβ(1–40) and Aβ(1–42) compared to iPSC-derived neurons obtained from 3 control subjects (Kondo et al., 2013). Instead, neurons derived from one sAD patient displayed elevated levels of intracellular Aβ fragments, while this was not observed for the second sAD patient line. Finally, Foveau et al. reported the generation of 3 iPSC-derived neural models from sAD patients and 3 control lines from non-cognitively impaired individuals (Foveau et al., 2019). Secreted levels of Aβ(1–40) and Aβ(1–42) tended to be lower in sAD derived neural models although this was not statistically significant. No changes in the ratio Aβ(1–42)/Aβ(1–40) were observed for sAD derived neural models compared to controls.

In addition, several studies have also directly focused on patient-derived iPSC lines that harbor known sAD genetic risk factors. In the study by Duan and coworkers, iPSC-derived neurons were generated from 3 sAD patients all carrying the APOE3/E4 genotype (Duan et al., 2014). In this study, increased extracellular levels of Aβ(1–42) were reported for two out of three patient-derived neural models, while Aβ(1–40) levels remained unchanged compared to a panel of iPSC-derived neurons from control subjects. Ochalek and coworkers found increased extracellular levels of both Aβ(1–40) and Aβ(1–42) in neurons derived from 4 sAD patients compared to control neurons, while the ratio Aβ(1–42)/Aβ(1–40) did not change relative to controls (Ochalek et al., 2017). In addition, hyperphosphorylation of tau, increased GSK3β activity, APP synthesis and APP C-terminal cleavage was detected in neurons from sAD compared to neurons obtained from iPSC control lines. The study performed by Young et al. focused on SORL1, which is an endocytic trafficking factor whose levels modulate the

Table 1

| Study            | Identifier | Aβ(1–40)   | Aβ(1–42)   | Aβ42/40 ratio | intracellular Aβ | p-TAU | aGSK3β   | Gender | #iPCS lines per patient |
|------------------|------------|------------|------------|---------------|------------------|-------|----------|--------|------------------------|
| Israel et al. (2012) | sAD1      | no change  | N.R.       | N.R.          | N.R.             | no change | no change | F      | 3                      |
|                  | sAD2      | increased  | N.R.       | N.R.          | N.R.             | increased | increased | M      | 5                      |
| Kondo et al. (2013) | A03E211   | no change  | no change  | no change     | no change        | no change | no change | N.R.   | M 1                    |
|                  | A08K213   | no change  | no change  | no change     | increased        | increased | no change | N.R.   | M 1                    |
| Duan et al. (2014) | AG04402   | N.R.       | Increased  | Increased     | N.R.             | N.R.     | N.R.     | N.R.   | M 1                    |
|                  | AG11141   | N.R.       | Increased  | Increased     | N.R.             | N.R.     | N.R.     | M 1    | M 1                    |
|                  | AG05810   | N.R.       | no change  | no change     | N.R.             | N.R.     | N.R.     | F 1    | F 2                    |
| Ochalek et al. (2017) | BIOT-0904-LOAD | Increased | Increased | no change     | N.R.             | Increased | Increased | F 2    | F 2                    |
|                  | BIOT-0630-LOAD | Increased | Increased | no change     | N.R.             | Increased | Increased | M 1    | M 1                    |
|                  | BIOT-4828-LOAD | Increased | Increased | no change     | N.R.             | Increased | Increased | F 1    | F 2                    |
|                  | BIOT-0726-LOAD | Increased | Increased | no change     | N.R.             | Increased | Increased | M 2    | M 2                    |
| Foveau et al. (2019) | CQ2      | no change  | no change  | no change     | N.R.             | N.R.     | N.R.     | M 1    | M 1                    |
|                  | CQ3      | no change  | no change  | no change     | N.R.             | N.R.     | N.R.     | M 2    | M 2                    |
|                  | CQ6      | no change  | no change  | no change     | N.R.             | N.R.     | N.R.     | M 2    | M 2                    |
processing of APP to Aβ and other proteolytic products implicated in sAD (Young et al., 2015). Loss of SORL1 expression has been documented in sAD cases and has been associated with sAD in both candidate gene and GWAS analyses. By studying patient iPSC-derived neurons, this latter study confirmed the importance of the SORL1/APP pathway in sAD, and their findings corroborated previous studies in cell and animal models (Young et al., 2015). In another study by Hossini et al., sAD iPSC-derived neurons were analyzed to assess their reflection of disease phenotype in gene expression patterns and the expression of typical AD proteins (Hossini et al., 2015). The differentiated neurons reflected sAD phenotypes due to the presence of phosphorylated tau proteins and the upregulation of GSK-3β. Further analysis of the neurons revealed significant changes in the expression of other genes associated with AD, including subunits of the proteasome complex. Moreover, a disease-specific protein association network that models AD pathology on the transcriptome level could be generated from the AD-iPSC lines.

Taken together, these studies have demonstrated that sAD patients’ iPSC-derived neurons are able to recapitulate neuropathological processes of the disease, which represent critical first steps in assessing the potential of using iPSCs in sAD research. Simultaneously, these studies have also proven that a high degree of variability in terms of disease hallmarks in iPSC-derived neurons from sAD patients is common. Variability between cell lines in terms of the Aβ type, i.e. Aβ(1–42) or Aβ(1–40), which is altered in sAD derived neurons, as well as variability between iPSC-derived neurons from different sAD patients within a study (Table 1). While some of this might be explained due to heterogeneous nature of sAD, it is likely that other source of variability related to the model play a role in this as well.

Until now, most of the described work on sAD models has been conducted using iPSC-derived neurons, mainly because protocols to generate human iPSC-derived microglia and astrocytes were absent or laborious. However, over recent years, several protocols have been published for differentiating iPSC into microglia (Abud et al., 2017; Haenseler et al., 2017; Muffat et al., 2016; Pandya et al., 2017). This has been a breakthrough for studying the function of microglia in human disease as human microglia appear very distinct from its murine counterpart especially in regards to immune activation (Friedman et al., 2018; Geirsdottir et al., 2019). Human microglia were found to have higher expression of genes involved in pathways related to longevity and anti-inflammatory responses compared to rodent microglia (Geirsdottir et al., 2019). This suggests that the function of human microglia may not be well recapitulated in murine AD models.

Applying iPSC-derived microglia, Lin et al investigated the role of the most significant AD risk gene, APOE4 on human microglia function and gene expression (Lin et al., 2018). For this an APOE3/3 iPSC line was edited using CRISPR/Cas9 technology to create an isogenic APOE4/4 line. This modification was found to result in reduced phagocytic activity of the microglia and altered gene expression levels of over 1,000 genes. In a reciprocal approach, gene editing of a patient-derived APOE4/4 bearing iPSC line into an APOE3/3 genotype restored the impaired phagocytic phenotype.

A series of studies illustrates the importance of studying another AD risk gene, triggering receptor expressed on myeloid cells-2 (TREM2) in a human microglia model. In humans a heterozygous TREM2 R47H mutation confers increased AD risk, almost to a similar degree as a single APOE4 e4 allele (Guerreiro et al., 2013; Jonsson et al., 2013). Mice bearing a heterozygous TREM R47H mutation exhibited aberrant TREM2 mRNA splicing and reduced expression of TREM2 mRNA and protein (Xiang et al., 2018). Strikingly, TREM2 mRNA levels and splicing were normal in human iPSC-derived microglia carrying the TREM2 R47H mutation. In line with this, Claes and colleagues found that heterozygous TREM2 R47H iPSC-derived human microglia displayed normal phagocytosis capacity in contrast to TREM2 heterozygous and homozygous knock-out microglia (Claes et al., 2019). This indicates that TREM2 R47H phenotypes found in murine models cannot be translated to humans, and highlights the need for adequate human microglia models to study sAD.

Differentiation protocols to generate human iPSC-derived astrocytes from iPSC lines have been published nearly a decade ago (Kondo et al., 2013; Krenick & Zhang, 2011). However, these protocols were laborious and yielded only a low percentage of differentiated astrocytes. Nevertheless, using this method it was found that astrocytes differentiated from sAD and fAD patient-derived iPSC lines displayed Aβ accumulation (Kondo et al., 2013). Using a slightly modified protocol, Oksanen et al. generated astrocytes using iPSC lines from three fAD patients with PSEN1 exon 9 deletion (Oksanen et al., 2017). The astrocyte cultures from fAD patients showed elevated secretion of Aβ(1–42), while Aβ(1–40) secretion was unaltered in comparison to astrocyte cultures obtained from healthy control lines or gene-corrected isogenic controls. In addition, the iPSC-derived astrocytes from fAD patients showed increased oxidative stress and altered metabolism. Finally, Jones et al. describe a protocol that can generate enriched populations of mature cortical astrocytes (> 95%), with 30 days of induction starting from NPCs (Jones, Atkinson-Dell, Verkhratsky, & Mohamet, 2017). Aβ levels were not investigated in this study, however, iPSC-derived astrocytes from both sAD and fAD patients displayed altered morphological appearance, aberrant localization of astrocyte markers and an overall atrophic profiles.

Differentiation of neurons, astrocytes and microglia from patient-derived iPSC offers a powerful toolbox for studying sAD. Nevertheless, in vitro cultures of these cell types often lack essential aspects from their natural environment including cell–cell contact between multiple cell types, which negatively impacts on the maturity and functionality of these cells. To overcome these limitations researchers have been largely using two strategies; growing iPSC-derived cells in 3 dimensional (3D) structures, including spheres and organoid models or by transplanting iPSC-derived cells into the brain of mice. For instance, astrocyte maturation can be greatly improved by cultivating iPSC-derived astrocytes in 3D human cortical spheroids (hCSs) (Pasca et al., 2015) in direct contact with neurons for prolonged periods of time (Sloan et al., 2017). Astrocytes in long-term cultures (over 20 months) display gene expression patterns and functional properties of matured astrocytes (Sloan et al., 2017). Also microglia function can be enhanced by allowing interactions from a 3D brain environment (reviewed in Claes, Van den Daele, & Verfaillie, 2018). For example, Abud and coworkers showed that culturing human iPSC-derived microglia (iMGs) on hiPSC 3D brain organoids (BORGs) results in migration and engraftment of the iMGs (Abud et al., 2017). Integrated iMGs display a more mature and ramified morphology and respond to injury similar as microglia in brain tissue. Trujillo et al. describe the generation of functional cortical organoids from NPCs (Trujillo et al., 2019). By applying single-cell transcriptional profiling and functional validation over a 10-month period of time, the authors could demonstrate that long-term development of cortical organoids is a highly dynamic process involving a range of different cell types. In addition to cellular maturation, the cortical organoids displayed increased electrophysiological activity. Eventually the organoids displayed electro-physiological network activity that was reminiscent of the spontaneous and synchronized activity in developing human neocortex (Trujillo et al., 2019).

These examples illustrate how the 3D microenvironment in organoid structures can affect maturity and functionality of cells of the CNS (for a more comprehensive review on brain organoids (Benito-Kwicinski & Lancaster, 2020). A powerful alternative way to grow iPSC-derived cells within the context of a CNS microenvironment relies on transplanting cells into the brain of mice. For example, Espuny-Camacho et al. transplanted human iPSC-derived cortical neuronal precursors into the brain of a murine AD model and could show that these precursor cells differentiated into mature human neurons that are integrated into the murine brain (Espuny-Camacho et al., 2017). These neurons express 3R/4R Tau
splice forms, display aberrant changes in phosphorylation and conformational of Tau, and undergo neurodegeneration. Hasselmann and colleagues, transplanted hiPSC-derived hematopoietic progenitor cells into the postnatal brain of humanized, immune-compromised mice (Hasselmann et al., 2019). This resulted in context-dependent differentiation of the HPCs into microglia and other CNS macrophages. Transcriptome analysis revealed a high degree of clustering between human microglia obtained through transplantation in mice and ex vivo human microglia. This suggest that the murine brain environment promotes differentiation of HPCs into human microglia. Human microglia surrounding Aβ plaques in transplanted mice display robust transcriptional response that are partly distinct from murine microglia. Linaro et al. found that cortical pyramidal neurons derived from human ESC line H9 can integrate as single cells in neonatal mouse cortex upon transplantation (Linaro et al., 2019). In particular, the transplanted neurons displayed robust maturation at the single-cell level and strong interactions with the host neurons. Moreover, sensory stimulation resulted in physiological responses with good resemblance to that of native cortical neurons.

All together, these studies show great potential for chimeric xenograft models for studying diseases like sAD. Transplantation of patient-derived and/or genetically modified cell types and assessing their in vivo function in a natural microenvironment may lead to a more complete understanding of sAD.

3. Sources of variability in iPSC-derived neural models for sAD

The ability to generate iPSC-derived cell models of sAD patients harvests great potential for studying the etiology of sAD as well as for the development of biomarkers or therapeutic targets for sAD (Fig. 1A). More specifically, this platform provides a valuable tool in exploring the complex heterogeneous nature in the etiology of sAD through the interrogation of functional effects of genetic, epigenetic and transcriptional variants linked to risk, as well as protective environmental factors. However, there are several issues associated with iPSC models that require further investigation before the actual potential of this technology in the context of sAD becomes more evident. Here we will discuss for several of these issues, and how these could impact the interpretation of the findings in relation to sAD.

3.1. iPSC generation and differentiation

Generation of iPSCs from sAD patients and differentiation into disease relevant neural lineages with high reproducibility is a key factor for developing robust sAD models. However, iPSC models and derived neurons have their limitations and using them to study sAD is particularly challenging. First of all, it is well known that reprogramming of somatic cells into iPSC can give rise to random genomic alterations including copy number variants, indels and karyotypic aberrations that were not present in the parental somatic cells (Fig. 1B) (reviewed in Assou, Bouchenheimer, & De Vos, 2018). While it is relatively easy to select iPSC lines with normal karyotypes it is less straightforward to avoid smaller rearrangements or indels. Evidently, any alteration that is not present in somatic cells is undesirable and could potentially affect study outcome.

Second, evidence supports that not all iPSC lines have equal potency to differentiate into the desired mature phenotype (Fig. 1B) (Hu et al., 2010; Kim et al., 2011). Hu et al. report reduced and more variable neural differentiation efficiencies using a panel of human iPSC lines (Hu et al., 2010). This was not related to technical or cell culture related issues since various human embryonic stem cells used in parallel revealed robust and efficient differentiation. All tested iPSC lines in this study were generated by reprogramming of fibroblasts. In addition, the tissue of origin from which iPSC lines are derived can also affect differentiation potential. Profound differences in differentiation efficiency were observed for iPSC lines that originated from umbilical cord blood cells (CB-iPSCs) and foreskin keratinocytes (K-iPSCs) (Kim et al., 2011). While CB-iPSCs were more efficient in generating hematopoietic cell types than K-iPSCs, K-iPSCs differentiated more efficiently into keratinocytes compared to CB-iPSCs. This study suggests that iPSCs can retain epigenetic marks from the original tissue the iPSCs were derived from. It will therefore be necessary to assess how variability between different neuronal lines derived from donor cells of distinct developmental lineages could affect the interpretation of disease phenotypes that might be observed in sAD lines.

Third, reprogramming somatic cells into iPSCs has been associated with loss of aging traits such as certain epigenetic chromatin marks, telomerase shortening and altered mitochondrial metabolism (Lapasset et al., 2011; Mertens et al., 2015). These aging hallmarks are likely of high relevance for the development of an age-related disease like sAD. Certain phenotypes associated with sAD might not be discovered in the absence of aging. Brain cells derived from iPSCs might therefore not be the most optimal model for studying sAD, but this requires further investigation. Efforts to create neurons that have preserved the aging signature have led to direct reprogramming of fibroblasts into neurons. However, it is unclear how other neural traits are affected by direct reprogramming or what the impact of direct reprogramming is on genomic integrity. In addition, limited neurons can be produced since there’s no cell with self-renewing capacity (Hu et al., 2015; Mertens et al., 2015). Whether neurons with a preserved aging signature presents a better in vitro cell model for studying sAD compared to iPSC-derived neurons remains to be investigated. For this reason, a wealth of research in currently focused on developing physiologically complete models of accelerating aging and defining methods for the concept of age-preservation during reprogramming. In this regard, one could also think of age accelerating compounds, prolonged cultures times or the production of cellular intermediates that allow expansion before differentiation, while maintaining their aging hallmarks (Mertens, Reid, Lau, Kim, & Gage, 2018). As such, these efforts might provide a suitable solution to this issue.

Fourth, aside from the fact that the current differentiation protocols remain limited in the generation of all neural cell types found in the brain (reviewed in (Riemens et al., 2018), great variability in the final neuronal phenotype is introduced by the extents and complexity of the differentiation protocols themselves (Fig. 1B). A variety of protocols have been developed to generate neuronal cells from iPSCs (reviewed in (McCaughy-Chapman & Connor, 2018) (Riemens et al., 2018) and can be obtained by directed differentiation using signaling molecules or by artificial overexpression of lineage-specific transcription factors. The lack of consensus in differentiation methodology complicates study comparisons. However, this does not account for all variability that is introduced by culturing practices of iPSC models. A multicenter study investigated the transcriptome and proteome of two human iPSC lines at two time points during differentiation into cortical projection neurons using the same standard operating procedure in three independent inductions (Volpato et al., 2018). The authors report poor reproducibility in differential gene expression signatures between these two lines across the five test sites. These results illustrate that even when inter-laboratory variability is acceptable, huge inter-laboratory variability can exist. Despite the use of a standard operating procedure and exactly the same iPSC lines, very subtle differences in culturing conditions between laboratories can lead to large differences in phenotypic outcome.

3.2. Control cells

A completely distinct problem that complicates interpretation of studies using iPSC sAD lines besides the issues already described above lies in the identification of adequate control cell lines. It is not trivial to define a healthy or non-diseased control. Most studies utilizing iPSC-derived disease models apply iPSC lines from unrelated healthy donors as controls, since isogenic non-diseased controls are not available or
Fig. 1. Generation of human induced pluripotent stem cell (iPSC)-derived cell models for sporadic Alzheimer’s disease (sAD). (A) Somatic cells can be reprogrammed to iPSCs. Skin fibroblasts or peripheral blood lymphocytes (PBLs) are frequently used as source of somatic cells. Final neuronal cell types of interest can be generated through directed differentiation of iPSCs using signaling molecules or by exogenous expression of lineage-specific transcription factors. (B) Potential sources of variability in iPSC-derived cell models. Reprogramming can induce genomic alterations that might stay undetected in case of small alterations (Left panel). Human somatic cells might not be as equipotent as previously assumed. Selection of superior iPSC clones can bias the ultimate disease model (middle panel). Long and complex differentiation schemes can influence robustness of the ultimate disease model. Variability can occur in terms of the maturity of the desired cellular phenotype, the percentage of cells that obtained the correct phenotype and the percentage of unwanted phenotypes created during differentiation (left panel). (C) Somatic mosaicism is frequently detected in brains of patients with neurological diseases like AD (indicated in blue). Generated iPSC models from fibroblasts or PBLs might lack these unique genotypes that could be essential for an accurate sAD iPSC-derived cell model. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
impossible to produce. A genome-wide association studies performed by the 1000 genomes project consortium revealed that a set of control cell lines obtained from multiple individuals contained 50 to 100 disease-associated genetic variants (Genomes Project, C. et al., 2010). This illustrates the problem we face in finding good reference models. Other initiatives have shown that the most prominent source of variability in a reprogrammed iPSC is related to its own genetic background (Carcamo-Orive et al., 2017; DeBoever et al., 2017; Kilpinen et al., 2017; Pashos et al., 2017; Warren et al., 2017). Studying a sufficiently large reference panel may help to reduce the noise that results from the heterogeneity in the genetic background from individuals. Ultimately this will be beneficial for the discovery of subtle, but genuine disease causing factors.

3.3. Somatic mosaicism

In the recent years, multiple studies have found proof for the presence of de novo somatic mutations in neurological disease (reviewed in D’Gama & Walsh, 2018). Somatic mutations can arise postzygotically during embryonic development or during postnatal life. The impact of this so-called somatic mosaicism is depending on the time in life and the cell type in which these somatic mutations occur. Somatic mutations that take place early in life will be present in the majority of cells in the human body. Whereas somatic mutations that occur late will only be present in a small subset of cells and may be restricted to a single tissue. It has been found that somatic mutations with alternate allele frequencies as little as 1% can cause disease (D’Gama et al., 2017). If indeed somatic mutations in the human brain play an important role in onset and progression of sAD than it is important that the iPSC also carry these somatic mutations. In a study by Bushman and coworkers it was reported that neurons isolated from sAD patients displayed increases in DNA contents and elevated APP gene copy numbers (up to 12 copies per neuron) that were not detectable in lymphocytes from the same patients (Bushman et al., 2015). Later the same group found that APP mosaicism in the brains of sAD patients was caused by integration of APP genomic complementary DNAs into the genome (Lee et al., 2018). Both wild-type sequences as well as smaller, exon lacking variants were found to be incorporated in the genome of sAD neurons. Some of these APP mRNA variants encoded proteins can cause neurotoxicity. It remains to be investigated whether APP mosaicism in the brains of sAD patients is a cause or an effect of AD.

However, in order to generate adequate disease models for sAD it is important to determine whether somatic mosaicism in the brains of sAD patients plays a causal role or whether this occurs as consequence of AD. In case that somatic mosaicism is driving sAD development then the iPSC-derived disease model should also carry this genetic variation. If somatic mosaicism occurs as a result of AD then the genetic variation is not essential in the iPSC-derived disease model (Fig. 1C). Whether it is possible to generate an iPSC line bearing such a somatic mosaicism is highly questionable for several reasons. First, the somatic mosaicism needs to be present in the tissue from which iPSC are generated. This can be problematic, as the study by Bushman and coworkers showed that somatic variants in the brain were absent in lymphocytes (Bushman et al., 2015). Second, such relatively large genetic alteration may be tolerable in post-mitotic neurons, but is likely not compatible with cell proliferation.

Generating iPSCs from patients with somatic mosaicism can in certain situation also be advantageous. In cases where the disease causing somatic mutations are known, iPSC lines can be generated for mutant and wild-type from the same patient (van der Wal et al., 2019). This offers the unique opportunity to generate an isogenic pair of iPSC clones, thereby reducing some of the noise associated with generic iPSC control lines.

3.4. Reprogramming capacity

For a long time it was thought that all individual somatic cells had the same reprogramming capacity or so called clonal equipotency (Hanna et al., 2009). However, a recent study revealed that a small subset of “elite” clones dominates reprogramming of somatic mouse cells intro pluripotent stem cells (Shakiba et al., 2019) (Fig. 1B). Expression of Wnt1 was identified as a key hallmark of elite cells. If this phenomenon also applies for humans, than this will have important consequences for the iPSC-derived cell models generated with these selected “elite” clones. This raises the question to what degree the selected “elite” clones can capture the disease-associated genotype and thus how representative these models are for the disease. Potentially, somatic mosaicism could affect reprogramming capacity, and lead to counter selection against the desired iPSC genotype. Worst-case scenario is that the disease-associated genotype cannot be captured by reprogramming somatic cells into iPSCs. This is crucially important for accurate disease modelling and drug screening studies.

4. Conclusions and future perspectives

The development of iPSC technology has great potential for studying complex multifactorial diseases like sAD. Although hallmarks of AD can be detected in iPSC-derived cell models, there is a high degree of variability in terms of their presence and severity between different sAD lines. This variability can mask important, but subtle genetic differences between individuals. As the number of studies using iPSC-derived sAD models increases, it will be important to ensure highly reproducible and informative data. The compilation of a large reference panel representing non-demented control subjects will be instrumental for adequate interpretation of genomic variants found in sAD patients. It will be important to better understand clonal selection during reprogramming, and the relevance of somatic mosaicism and aging in sAD, to ensure representative iPSCs-derived cell models of sAD.

In order to increase translatability of pre-clinical findings it will be important to continue to develop and improve sAD models that reflect the brains natural environment. Sophisticated iPSC-derived cell models should consist of multiple cell types, in 3D conformations allowing cell–cell interactions. Further development of more accurate models for studying sAD like long-term organoid cultures and chimeric xenograft models will be essential in the coming years.

While it is well established that genetic alterations contribute to the pathophysiology of sAD, detailed multi-omics characterization of iPSC-based models and the role of environmental factors associated with sAD pathophysiology remain currently underexplored. It is recognized that in the aging brain, environmental risk factors associated with complex gene-environment interactions, are playing crucial roles in reinforcing sAD pathogenesis. Recent studies have shown that exposure to these risk factors can bring about epigenomic, transcriptomic, proteomic and metabolomic changes related to AD, which can bring about sustained alterations in molecular processes leading into the manifestation of the full-blown disease (Lahiri, Maloney, & Zawia, 2009). Furthermore, many AD researchers tend to suggest that these environmental risk factors operate during the pre-clinical phase of AD, even decades before the appearance of the first clinical symptoms. In either way, there is overwhelming support for environmental and extra-genetic risk factors as inducers of sAD pathogenesis. Nevertheless, the exact molecular nature of these interactions, as well as their temporal relationship with the development of the disease, remain largely unknown. Further research from experimental and epidemiological studies that focus on the interaction of these factors with the aforementioned multi-omics modalities therefore remains necessary to develop a better understanding about their contribution to the course and development of the disease. In this regard, iPSC-derived systems might provide exciting opportunities for modelling these interactions, by allowing one to expose cells derived from healthy individuals and sAD patients with
different genetic backgrounds to environmental insults and study their cellular and molecular responses in an in vitro setting.

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