Reprogramming Neurodegeneration in the Big Data Era

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Abstract

Recent genome-wide association studies (GWAS) have identified numerous genetic risk variants for late-onset Alzheimer's disease (AD) and Parkinson's disease (PD). However, deciphering the functional consequences of GWAS data is challenging due to a lack of reliable model systems to study the genetic variants that are often of low penetrance and non-coding identities. Pluripotent stem cell (PSC) technologies offer unprecedented opportunities for molecular phenotyping of GWAS variants in human neurons and microglia. Moreover, rapid technological advances in whole-genome RNA-sequencing and epigenome mapping fuel comprehensive and unbiased investigations of molecular alterations in PSC-derived disease models. Here, we review and discuss how integrated studies that utilize PSC technologies and genome-wide approaches may bring new mechanistic insight into the pathogenesis of AD and PD.

Introduction

Alzheimer's disease (AD) and Parkinson's disease (PD) are the most common forms of neurodegenerative disease, currently without effective treatments. AD is pathologically defined by accumulation of A β plaques, neurofibrillary tangles, and severe neuronal loss [1]. Whereas PD is often characterized by deposition of α -synuclein and loss of dopaminergic (DA) neurons in the midbrain substantia nigra as well as other neurons in the brain [2]. A subgroup of AD and PD cases are early-onset familial forms caused by rare gene mutations, but the vast majority (>90-95%) of clinical cases are late-onset sporadic forms. These may originate from genetic susceptibility in combination with enigmatic environmental factors. Recent large scale GWAS and meta-analyses have identified a large number of single nucleotide polymorphisms (SNPs) located in 22 loci associated with sporadic AD (sAD), and 26 loci associated with sporadic PD (sPD) [3,4]. The current challenge confronting biomedical researchers is to decipher the mounting genetic information as well as to pinpoint the molecular mechanisms by which the genetic variants contribute to disease pathogenesis. Reprogramming of patient-derived somatic cells into induced pluripotent stem cells (iPSCs), as well as gene-editing of human embryonic stem cells (hESCs) provides unique platforms for interrogating GWAS risk variants in human neurons and other cell types that reside in the brain. Furthermore, PSC-derived disease models facilitate genome-wide transcriptome and epigenome analyses

to identify common molecular alterations and causal regulators in the development of complex neurodegenerative diseases including AD and PD (Figure 1).

Interrogating Risk Variants from GWAS

Non-coding variants in human neurons

The vast majority (98.5%) of the human genome are non-coding regions [5], which contain functional sequences including *cis*-elements that regulate gene expression. The genetic variants identified in GWAS are enriched in *cis*-regulatory elements [6], implicating *cis*-acting effects as a common factor in human diseases. In the case of sAD and sPD, the most strongly associated SNPs identified from GWAS are all located in non-coding regions. These SNPs are also often present in both patients and unaffected individuals, albeit with different frequencies. The low penetrance of GWAS risk variants also predicts their weak phenotypes. Approaches of generating transgenic animals that model coding mutations known to deterministically cause familial forms of disease, are difficult to deploy when studying the effect of GWAS-identified SNPs associated with sporadic cases. Recently, human PSC-derived neuronal models have emerged as an attractive strategy for deciphering functional effects of GWAS-identified genetic variants.

Excellent examples of such strategies are seeing the light: Young et al. applied patient iPSC to dissect the molecular phenotypes of common non-coding risk variants in the *SORL1* locus associated with sAD [7]. *SORL1* is an endocytic receptor involved in APP trafficking and subsequently affecting A β generation [8,9]. Reduced *SORL1* expression was observed in the brains of sAD patients [10], but whether and how common risk variants affect *SORL1* expression is unknown. To address these questions, Young et al. generated a cohort of 13 iPSC lines from 6 control individuals and 7 sAD patients harboring common variants in the 5'-introns of *SORL1*. In iPSC-derived neurons, all protective alleles (from 4 controls and 5 sADs) significantly increased BDNF-induced *SORL1* gene expression, whereas all homozygous risk variants (from 2 controls and 2 sADs) had no effect on BDNF-induced *SORL1* gene expression. Therefore, the detected gene-specific effects were consistent with the protective or risk haplotype of the SNPs, but were largely dissociated from the actual disease status, which are often an outcome of complex genetic and environmental factors.

The study could be strengthened by applying statistical criteria to determine sample sizes taking into account the number of protective and risk alleles included in both AD and control cases. As increased *SORL1* expression correlates to lower A β levels [7-9], the current study provides a possible functional link between *SORL1* risk haplotypes to AD pathogenesis.

Another study by Soldner et al. used efficient gene-editing based on CRISPR/Cas9 in hESCs to assess the effects of PD-associated non-coding risk variants in the α -synuclein (*SNCA*) locus [11]. They first prioritized risk variants that are located in distal enhancers based on epigenetic annotation. They subsequently introduced selected risk alleles into the genome of hESCs. In ESCs-derived human neurons, one high-risk allele increases *SNCA* expression by 10% [11]. Consistently, this risk allele impairs binding of sequence-specific transcription factors to its nearby DNA [11]. Given that 50% increase of *SNCA* causes a familial form of PD, a modest upregulation of *SNCA* over lifetime may represent a possible molecular basis of increased risk of PD.

There are two major challenges for future attempts to use non-coding GWAS variants for investigating disease mechanisms. The first challenge is prioritizing SNPs for functional studies, since typically a linkage disequilibrium (LD) block identified by GWAS contains multiple SNPs, with some or many among them being neutral mutations [12]. Therefore, prioritizing non-coding variants based on existing biological databases is probably crucial for successful identification of true disease-associated variants in the following functional studies. As shown by the work of Soldner et al. [11], current available epigenetic data (<http://www.roadmapepigenomics.org>) [13] was successfully used to filter PD associated SNPs in the

SNCA locus based on cumulative overlap with enhancer-associated markers. Overall, advances in systematic annotations of non-coding genomes are expected to help predicting hotspots where functional SNPs are more likely located[12].

The second challenge is the small effect sizes for most GWAS alleles, which implicate relatively low penetrance and weak disease phenotypes. Instead of directly probing disease-phenotypes in patient iPSC-derived cultures, one could consider that specific assays based on rational designs targeting at candidate genes are more likely to yield reliable information on the functional effects of the GWAS alleles. Take the work of Young et al, [10] for example, the same haplotypes of SNPs in the *SORL1* locus are highly consistent in their effects on induced *SORL1* gene expression, despite the fact that these SNPs have very low penetrance in association with disease phenotypes. Therefore, human iPSC-derived cultures allow the determination of gene-specific effects of non-coding variants as the first step, which should be followed up by studies exploring the involvement of candidate genes (which the SNPs are located within or flanking) in disease pathogenesis. Although finding the functional consequences of SNPs in cell-based assays does not prove causality of the SNPs to disease, the more relevant approach is to apply information generated from GWAS variants to guide the identification of specific genes and pathways, which are likely involved in disease pathology and therefore could potentially be targeted for therapeutic discovery.

Risk genes and human microglia

Microglia are the resident immune cells in the brain, which are activated in response to neuropathological factors and regulate neuroinflammation in neurodegenerative diseases [14]. Although microglia-mediated neuroinflammation has long been recognized as a hallmark feature of both AD and PD, evidence suggesting a crucial role of microglia in disease pathogenesis has only emerged recently. Network-based analysis of whole-genome transcriptome profiling data from thousands of autopsied brain tissues from sAD patients has identified microglial pathways as the most prominent causal regulator in sAD [15]. In concordance with this finding, GWAS has uncovered mutations of several immune-related genes, including *TREM2*, *CD33*, *CR1*, *EPHA1*, *ABCA7*, *SHIP1*, *APOE*, *HLA-DRB5-HLA-DRB1*, *MS4A6A*, increasing the risk to develop sAD [3,16]. Further dissecting these GWAS variants will bring essential mechanistic insights to the role of microglia in AD pathogenesis. However, studies of microglia in human disease has been hampered by lack of relevant model systems. Primary human microglia differ substantially from rodent microglia, and protocols for reliably generating such cells *in vitro* are not mainstream.

Recent studies from three independent groups are starting to open this door and show that it is possible to differentiate human iPSCs and ESCs into microglia [17-19]. The reported protocols are largely similar in that they all differentiate PSCs through a myeloid intermediate, and use colony-stimulating factor (CSF1) and interleukins (IL-34) to induce microglial cell fates. These protocols produce similarly high yields of microglia, which faithfully resemble their *in vivo* counterparts isolated from human brain in terms of phenotypic and gene expression profiles [17-19]. Notably, these work also show that it is possible to generate 3D brain organoids by integrating human microglia into a cortical-like matrix of PSC-derived neurons, astrocytes and oligodendrocytes. In the context of 3D organoids, microglia show rapid responses to neuronal injury similar to brain microglia [17,19]. The current availability of systems to generate human microglia from PSCs, along with rapid technical maturation of 3D organotypic cultures [20] holds great promise and will likely revolutionize studies that investigate microglia and their interactions with neurons and astrocytes in human disease pathogenesis.

Unveiling common pathways via transcriptome profiling

Genetic studies have identified a wide range of mutations that can either cause or increase risk for AD and PD. Despite their divergent genetic origins, AD or PD patients share a similar spectrum of clinical features and pathological manifestations, implicating that some common downstream pathways may contribute to the characteristic phenotypes of these diseases. Several studies of familial and sporadic patient iPSC-derived neurons have captured common disease-related phenotypes in genetically diverse AD or PD cases [21-25], providing a basis for using iPSC-derived neuronal models to dissect molecular pathways involved in disease pathogenesis. Recent work has resorted to whole-genome RNA-sequencing (RNA-seq) to uncover gene networks that are disturbed in patient iPSC-derived neurons.

In an effort to explore common molecular pathologies of different genetic backgrounds in PD, Lin et al. used a collection of iPSC lines from monogenic PD patients carrying distinct mutations, including LRRK2-G2019S, SNCA triplication or PARKIN-255delA, together with a sporadic PD case and healthy control individuals [21]. A comprehensive transcriptome analysis of iPSC-derived DA neurons identified hundreds of genes that are consistently deregulated in iPSC-derived DA neurons across these different PD mutations. These deregulated genes fall into major pathways including oxidative stress and synaptic transmission, which may explain related phenotypes, including increased oxidative stress susceptibility and impaired neuronal activity observed in models of PD and iPSC-derived neurons from PD patients [21]. Among the most consistently upregulated genes in iPSC-derived DA neurons from PD patients is *RBFOX1*, a splicing factor which regulates neuronal splicing networks and has been linked to various neurological diseases [21]. However, this work did not assess the functional impact of *RBFOX1* knockdown in PD neurons and the relevance of *RBFOX1* to PD-related phenotypes remains unclear. Future work should also analyze perturbed pathways and try to identify upstream genes that occupy higher hierarchical positions in the gene networks and regulate expression of downstream genes [26,27]. Subsequent work can then assess their contribution to disease-related phenotypes.

Other studies have also assessed the expression profile of iPSC-derived DA neurons from PD patients carrying LRRK2-G2019S mutation by RNA-seq [28,29]. The earlier study by Reinhardt et al. employed isogenic controls where the pathogenic mutation was corrected and identified a group of genes that are deregulated in LRRK2-G2019S neurons. Knockdown of some of the upregulated genes, including *CPNE8*, *CADPS2*, and *MAP7* ameliorated neurodegeneration of LRRK2-G2019S DA neurons *in vitro* [28]. Another study by Sandor et al. has resorted to the purification of iPSC-derived DA neurons, as to enrich for this one cell type for RNA-seq analysis [29]. Comparing gene expression profiles of LRRK2-G2019S mutant DA neurons with those of DA neurons from healthy control individuals uncovered 168 differentially expressed genes, which functionally converge onto four major pathways including oxidative stress, glycosaminoglycans, immunity NF- κ B signaling and lipopolysaccharides. Notably, the gene expression profiles of purified iPSC-derived DA neurons highly resembles that those of their counterparts from postmortem brains, validating the use of the *in vitro* differentiated and purified DA neurons for studying transcriptomic changes in PD. Curiously, the transcriptomic effects of LRRK2-G2019S backgrounds are similar to those of rotenone, a neurotoxin that blocks mitochondrial function [30,31], suggesting LRRK2 and rotenone may share pathogenic mechanisms. Moreover, the transcriptomic effects detected in LRRK2-G2019S DA neurons anti-correlate with transcriptomic responses induced by clioquinol, a compound shown to prevent DA neuronal loss as well as revert PD symptoms in certain rodent models [32,33]. These data suggest that there may be some common molecular alterations in various genetic PD

backgrounds, and they highlight the potential of using iPSC-derived neurons for identifying and characterizing therapeutics for PD.

Mapping epigenetic alterations

Epigenetics refers to changes in gene expression caused by modifications of DNA molecules or their packaging proteins without changing the DNA sequence [34]. For complex diseases, including AD and PD, which often lack causative genetic determinants, exploration of epigenetic alterations is thought to be particularly important and may explain at least in part the environmental impact on neurodegenerative disease [35]. Recent genome-wide DNA methylation analyses using postmortem tissues have revealed epigenetic alterations in AD and PD patient brains, suggesting that epigenetic changes occur in the context of these neurodegenerative disorders [36-38]. However, postmortem epigenetic analysis using brain tissues composed of mixed brain cells may underestimate or confound disease-associated epigenetic changes, as different cell populations are known to have distinct methylation landscapes [39]. In particular, the loss of neurons in AD and PD may confound postmortem epigenetic analysis by changes in the proportion of cell types. Alternatively, patient iPSC-derived neuronal cultures, along with techniques developed for purification of disease-specific subtype of neurons [29,40], could represent a new avenue for interrogating epigenetic changes associated with early disease status.

Recent work shows a genome-wide DNA methylation profiling of iPSC-derived DA neurons from monogenic PD patients carrying LRRK2-G2019S mutation, sPD patients and healthy control individuals [41]. Interestingly, LRRK2-G2019S and sPD DA neurons show similar changes in DNA methylation patterns, with abnormal methylation prominently hitting enhancer elements and transcription factor (TF) binding sites, consequently up- or down- regulating gene expression. A significant correlation between changes in DNA methylation and gene expression was confirmed by microarray-based transcriptome analysis. In total, 23 TF-binding sites were disrupted by differential DNA methylation in PD neurons. Among them, several TFs including *FOXA1*, *NR3C1*, *HNF4A* and *FOSL2* have been previously associated with the maintenance of midbrain DA neurons and with PD symptoms [42-46]. Notably, DNA methylation changes occur early in cultures of iPSC-derived DA neurons from PD patients, preceding the appearance of PD-related morphological or functional phenotypes. Such methylation changes are not detected in undifferentiated iPSCs, nor in iPSC-derived neuronal cultures that are not enriched in DA neurons [41]. These results suggest that abnormalities in DNA methylation may underlie early disease manifestations in cell types vulnerable in PD. Future work will establish if targeting DNA methylation and its related pathways is a strategy that can be explored in combating neurodegeneration. A potential caveat of using iPSC-derived cultures to study disease-related epigenetic alterations is that the reprogramming processes may involve epigenomic reconfiguration of somatic cells [47,48], which might confound the study of non-coding variants located at the hotspots for epigenomic reprogramming [47]. This should be taken into account for experimental design.

Conclusion

GWAS-identified risk variants hold important information for decoding molecular mechanisms of complex neurodegenerative diseases including AD and PD. Recently, human PSC-derived neuronal models have emerged as valuable tools for functional studies of non-coding GWAS variants. Breakthroughs in human microglia differentiation protocols have endowed us with the capacity of generating PSC-derived microglia and even combining them in 3D organoids to study immune-related AD risk genes *in vitro*. Furthermore, genome-scale RNA-seq and epigenomic profiling of PSC-derived disease models represent new avenues

for identifying gene or network perturbations associated with early disease status, which may hold promise for the development of new diagnostics and therapeutic strategies.

Conflict of interest statement

The authors declare no conflict of interest.

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