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Nontargeted Metabolite Profiling of Induced Pluripotent Stem Cells (iPSCs) Derived Neural Cells: Insights Into Mechanisms of Brain Diseases

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Additional information is available at the end of the chapter

Abstract

Since the discovery that introduction of four specific genes encoding transcription factors convert mature human somatic cells into induced pluripotent stem cells (iPSCs), there has been an enormous increase in the applications of iPSC technologies to medical sciences, especially in modeling human diseases. In this review, we summarize recent advances in applying human iPSC reprogramming to generate patient-specific neural subtypes in order to reveal molecular pathways affected in various neurodegenerative diseases. Metabolites provide a functional readout of various cellular states enabling identification of biomarker candidates for early diagnosis, segregation of patient cohorts, and to follow-up disease progression or disease responses to novel therapies. With emerging technologies, that is, mass spectrometry and nuclear magnetic resonance applications to metabolomics and various software solutions of bioinformatics, it has become possible to measure thousands of metabolites simultaneously. These fascinating new techniques provide a powerful tool for setting scientific hypotheses and linking cellular pathways to biological mechanism. This review focuses on mass spectrometry-based metabolomics as a tool for iPSC research.

Keywords: iPS cell-derived neural cells, metabolomics, mass spectrometry, Alzheimer's disease, Parkinson's disease

1. Introduction

Stem cell reprogramming has provided a completely new way to explore human development and diseases. This novel technology is especially useful when investigating diseases in which affected tissue are not easily accessible prior to autopsy. The central nervous system is a good example of a tissue that falls into this category. Using induced pluripotent stem cells (iPSCs) to model human brain diseases has remarkable potential to generate insights into understanding disease mechanisms and opening new avenues for the development of effective clinical intervention. Importantly, iPSCs may allow for searching potential biomarkers of brain diseases, especially neurodegenerative diseases which are well known to be diagnosed far too late to be efficiently treated. Disease-specific iPSCs are now available from patients of several major neurodegenerative diseases.

Metabolomics combines strategies to identify and quantify endogenous small molecules that are products of biochemical reactions, and thereby to reveal connections between different pathways that operate within a living cell. Nontargeted metabolite profiling has become a powerful analytical tool to reveal molecular mechanism in various physiological and pathophysiological stages. Even though there are a lot of publications related to these techniques, there are only a few reports combining iPSCs technologies and nontargeted metabolite profiling.

In this chapter, we first describe the use of nervous system disease-specific iPSCs to model human neurodegenerative diseases. Next, we summarize key metabolites from human cohort studies of Alzheimer's disease (AD) and Parkinson's disease (PD). Finally, the recent findings in mass spectrometry-based nontargeted metabolite profiling are discussed to reveal the details of cellular mechanism in iPS cell-derived neurons and astrocytes.

2. Human-induced pluripotent stem cells (iPSCs) in studies of nervous system diseases

2.1. Application of iPSCs in neurodegenerative diseases

Chronic neurodegenerative diseases are characterized by the slow and progressive loss of neuronal functions, which in turn results in memory loss, cognitive deficits, and/or motor coordination impairment or even loss of motor functions. Neurodegenerative diseases, such as AD, amyotrophic lateral sclerosis (ALS), Huntington's disease (HD), PD, and spinal muscular atrophy (SMA), are typically of sporadic origin or are caused by very rare gene mutations (**Table 1**). Although intensive efforts have been made, effective treatments for neurodegenerative diseases have not been yet discovered.

Disease	Gene	Cell type	Phenotype	References
Alzheimer's disease	APP, PS1, PS2, sporadic	Cortical neurons	Increased deposition of amyloid β; activated GSK-3β; endosomal abnormalities; APP mutations increased total and pTau protein, whereas PS1 mutations did not	[1–6]
Amyotropic lateral sclerosis	SOD1, VAPB, TDP43, FUS, C9ORF72, sporadic	Motor neurons, astroglia	Reduced survival, cytoplasmic and nuclear protein aggregation, altered expression in genes encoding cytoskeletal proteins, neurofilament aggregation, mitochondrial defects, increased oxidative stress, activation of ER and UPR	[7–16]
Huntington's disease	HTT	Glutamatergic neurons, GABAergic neurons, astrocytes	Reduced survival, neurite outgrowth and firing capacity, enhanced lysosomal activity, changes in actin cytoskeleton, decreased cell-cell adhesion properties, and decreased intracellular ATP, decreased ATP/ADP ratios; increased susceptibility to stressors, vacuolation phenotype of astrocytes	[17–23]
Parkinson's disease	LRRK2, GBA, PINK1, SNCA, PARKIN, sporadic	Dopaminergic neurons	Reduced number of neurons and their branches, mitochondrial dysfunction, elevated α-synuclein, reduced synthesis and release of dopamine, increased MAOB expression, impaired autophagy, increased susceptibility to stressors, activated ERK, impaired intrinsic network activity, reduced GBA activity	[24–32]
Spinal muscular atrophy	SMN1, CAG repeats	Motor neurons	Reduced number of neurons, abnormality in neurite outgrowth, impaired clustering of AchR	[33–35]

AchR, acetylcholine receptor; APP, amyloid precursor protein; C9ORF72, chromosome 9 open reading frame 72; ER, endoplasmic reticulum; ERK, extracellular signal-regulated kinases; FUS, fused in sarcoma; GBA, glucosidase beta acid; GSK-3β, glycogen synthase kinase-3 beta; HTT, hungtintin; LRRK2, leucine-rich repeat kinase 2; MAOB, monoamine oxidase B; PINK1, PTEN-induced putative kinase 1; PS1, presenilin 1; PS2, presenilin 2; SMN1, survival of motor neuron 1; SNCA, alpha-synuclein; SOD1, superoxide dismutase; TDP43, transactive response DNA-binding protein 43; UPR, unfolded protein response; VAPB, vesicle-associated protein B.

Table 1. Neurodegenerative diseases modeled with iPSCs.

Possible reasons for failing to develop efficient therapies include the lack of appropriate disease models of human neurons and a limited understanding of the etiological and neurobiological mechanisms of neurodegenerative diseases. Recent advances in PSC research have now opened the path to the generation of iPSCs starting from somatic cells, thus offering an unlimited source of patient-specific disease-relevant neuronal cells. By applying sophisticated differentiation protocols, iPSCs can be directed to differentiate to functional neuronal sub-types, such as glutamatergic cortical neurons, striatal GABAergic neurons, midbrain dopaminergic (DA) neurons, or motor neurons of the spinal ventral horn. Since patient-specific cells are used to generate neuronal population, the resulting phenotype of these cells can be used to model the pathology of neurons or other neural cells within the same individual.

2.1.1. Modeling AD with disease-specific iPSCs

The central nervous system has a limited capacity to regenerate after acute injuries or during chronic degenerative diseases. Neurodegeneration is probably most studied and characterized in AD and PD.

AD is the most prevalent age-related disorder characterized by dysfunction and deterioration of neurons within the neocortex and limbic system, resulting in gradual progressive memory loss and cognitive decline. More than 48 million patients are afflicted with AD worldwide. AD is the most common cause of dementia and may contribute to 60–70% of the cases. There are currently no medications for preventing the disease progression. Even though the pathogen-

esis of AD has been extensively studied during the last decades, the mechanisms underlying the neuronal defects and synaptic damage in this disease are still unclear. Mutations in the presenilin 1 (PS1), presenilin 2 (PS2), and the amyloid precursor protein (APP) genes account for most of the familial early onset cases of AD by enhancing the production of pathological amyloid beta (Aβ), especially Aβ42, which has a greater tendency to form fibrillary amyloid deposits. The large amounts of Aβ accumulate and form senile plaques in the brains of AD patients.

The "amyloid hypothesis" states that extracellular Aβ deposits are the cause of the disease [36]. This theory has been difficult to verify in living neurons of patients, using ordinary models and technologies. Fortunately, iPSCs technology and the recent method's development toward AD iPS cell-derived cortical neurons provides access to cell types that were previously unobtainable [37]. Presenilin-1 (PS1) A246E and presenilin-2 (PS2) N141I iPSC-derived neurons in conventional 2D cultures produced twice the normal level of highly toxic Aβ42 [1]. In one report, the Aβ accumulation was also seen in neuronal cells derived from sporadic AD patient-derived iPSCs [2]. In addition to Aβ, the accumulation of phosphorylated Tau was observed in neurons of sporadic AD patients, but not in iPS cell-derived neurons that had a PS1 or PS2 gene mutation [2]. Moreover, cerebral neurons derived from iPSCs of APP-mutant patients (E693D, V717L) and sporadic AD patients showed an accumulation of Aβ oligomers associated with endoplasmic reticulum and increased oxidative stress [3]. Intracellular stress and neuronal death were inhibited with the unsaturated fatty acid docosahexaenoic acid (C22:6n3) in neurons derived from one patient carrying mutant APP and one patient with sporadic AD. These findings point out a diversity of pathologies in AD and indicate the need for individual treatment strategies. In line with this conclusion go also the results from a very recent study where neurons derived from patients with various forms of AD differed in APP processing [6]. While in iPSC models, APP mutations appeared to increase the levels of total and phosphorylated Tau, PS1 mutations failed to do so. Finally, AD pathology was recapitulated in a single 3D human neural cell culture system where human neural progenitor cells overexpressed mutant APP or both mutant APP and mutant PS1. These mutations induced robust deposition of Aβ and exhibited high levels of pTau [4]. ApoE4 is the major known genetic risk factor for sporadic AD. Defining the precise functions of ApoE that lead to AD pathology is likely to allow for more specific therapeutic treatment. Combination of iPSC technology and genome editing may shed light on the role of ApoE in AD.

In summary, disease-specific iPSCs have provided the field of neurodegeneration with an exciting tool for research. Revealing the mechanisms underlying AD and other brain diseases is essential for advancing our understanding of those disorders with the goal of finding effective therapies that would restrain or stop disease progression.

2.1.2. Modeling PD with disease-specific iPS cells

PD is the third most common neurodegenerative disorder after AD and dementia with Lewy bodies and affects approximately 1–2% of the population over 60. The pathology is characterized by the progressive degeneration of dopaminergic (DA) neurons in the *substantia nigra pars compacta* and by intracellular inclusions known as Lewy bodies [38]. The loss of DA neurons

results in motor symptoms, including resting tremor, cogwheel rigidity, bradykinesia, and later in the course of the disease, loss of postural reflexes. Despite extensive research, there is no cure for this devastating disorder. The majority of PD cases are sporadic and of unknown origin, with up to 10% of patients presenting with familial monogenetic forms of the disease. Combination of genetic and environmental factors is likely to play an important role in the pathogenesis of PD. Although monogenic forms of PD account for a small percentage of PD cases, the most successful reports involving PD modeling to date utilize DA neurons from patients with monogenetic mutations in which the gene mutation has been characterized (**Table 1**). Understanding how mutations of these genes cause the degeneration of DA neurons is critically important for the mechanistic studies as well as for the identification of disease-modifying drugs. In human molecular genetic studies, at least 18 loci and 11 genes leading to the development of PD have been identified. Mutations in SNCA, UCHL1, LRRK2, PINK1, DJ-1, and ATP13A2 can lead to monogenic forms of PD [39]. Furthermore, mutations in genes including those that code for SNCA, LRRK2, and GBA have been found to be risk factors for sporadic PD [40], and in many cases people carrying these mutations will develop PD. Overall, the most extensively studied PD-related genes are SNCA and LRRK2. DA neurons derived from iPSCs of PD patients with triplication of the SNCA gene produced double the amount of α-synuclein protein when compared with the normal controls [24]. Also, DA neurons bearing the LRRK2 G2019S mutation showed an increased level of α-synuclein and greater oxidative stress [25]. These diseased DA neurons also exhibit an increased number of autophagy vacuoles and impaired synaptic and neuritic morphology [35]. In the latest study, gene correction of LRRK2 G2019S mutation in iPSCs resulted in phenotypic rescue of differentiated DA neurons [30].

In summary, studies of PD using iPSCs technology have shown the presence of PD-associated abnormalities in mitochondrial function, autophagy/lysosomal pathway, axonal transport, and neurite extension. These findings open up an opportunity to use iPSC-derived human midbrain DA neurons in studies of disease mechanisms, biomarker search, and drug development, even in much larger scale.

3. Metabolomics

The metabolome of an organism refers to the complete set of endogenous and exogenous molecules present in biological sample. Metabolite concentrations reflect the phenotype of tissue or cells and provide insights into the biochemistries of the disease [41, 42]. There are two general approaches used in metabolomics study; targeted and nontargeted metabolomics. The targeted approach measures only a defined set of metabolites, whereas the nontargeted profiling, or global approach, measures as many metabolites as possible [41, 43]. The nontargeted metabolomics cover very wide concentration range of the metabolites present in the cells (i.e., from millimolar (mM, 10^{-3} mol/L) to attomolar (aM, 10^{-18} mol/L)) with the different chemical and structural diversities of compounds. Recent developments in mass spectrometry (MS) and bioinformatics-related software solutions have enabled to measure thousands of metabolites simultaneously from biological sample.

MS is an analytical technique that sorts molecules as ions, based on their mass-to-charge (m/z) ratio. As a detection method, MS provides the quantitation with extremely high selectivity and sensitivity, reproducibility, and a wide dynamic range from highly complex biological samples such as iPSC-derived neurons and astrocytes. In addition, MS has the ability to give a specific information about molecular structure, which together with the above-mentioned qualities makes MS the technique of choice for metabolomics [43, 44]. Biological samples with extreme molecular complexity can be detected by MS combined with gas chromatography (GC) or liquid chromatography (LC) [45–49]. In addition to chromatographic techniques, MS has been combined to capillary electrophoresis (CE) and flow injection analysis (FIA) used in shotgun lipidomics [45, 46].

Targeted approach measurements are hypothesis-driven and focus on one or more metabolic pathways of interest [41]. A specified list of metabolites is measured by highly optimized protocols in regard to sampling, sample preparation, and instrumental analysis of these metabolic classes [52–54]. The most widely used MS technique for these types of analyses is the triple quadrupole mass spectrometer in combination with atmospheric pressure ionization techniques (i.e., electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) [43–55].

Nontargeted metabolite profiling aims to simultaneously measure as many metabolites as possible from a biological specimen, in this case from iPSC-derived neurons or glia. These studies are often hypothesis-generating, and therefore it is important to carefully construct an experimental design that maximizes the number of metabolites detected and their quantitative reproducibility [41]. The workflow of nontargeted metabolite profiling used in our laboratory to study iPSC-derived neurons and astrocytes is presented in **Figure 1**. We adapted previously published protocols to generate iPSC-derived mesencephalic dopaminergic neurons [56, 57] and astrocytes [58]. To maximize the number of molecular features obtained from cell culture samples, it is possible to optimize several steps in the nontargeted metabolite profiling workflow (i.e., sample extraction, chromatographic separation, ionization, mass filtering, and detection of metabolites) [42, 43, 55, 59, 60]. Samples are measured with ultra-high performance liquid chromatography (UHPLC), combined with high accuracy and resolution mass spectrometers with fast scanning capabilities, like the quadrupole time-of-flight (QTOF) mass spectrometer [59, 61]. In our laboratory, two chromatographic separation techniques (i.e., reversed phase (RP) and hydrophilic interaction chromatography (HILIC)) are used to cover both hydrophilic and hydrophobic metabolites. In addition, both ionization polarities (i.e., positive and negative) in ESI are employed to widen the coverage of metabolites present in the sample. After data acquisition, the molecular features are extracted and the results delivered for downstream univariate and multivariate statistical analyses. The identification of metabolites is based on the comparison of the accurate mass and isotope information (i.e., ratios, abundances, and spacing), as well as product ion spectra (MS/MS) to metabolite databases (e.g., the Human Metabolome Database (HMDB) and METLIN). This tentative identification is subsequently confirmed by comparing tandem mass spectrometry (MS/MS) data, together with chromatographic retention time with reference standards.
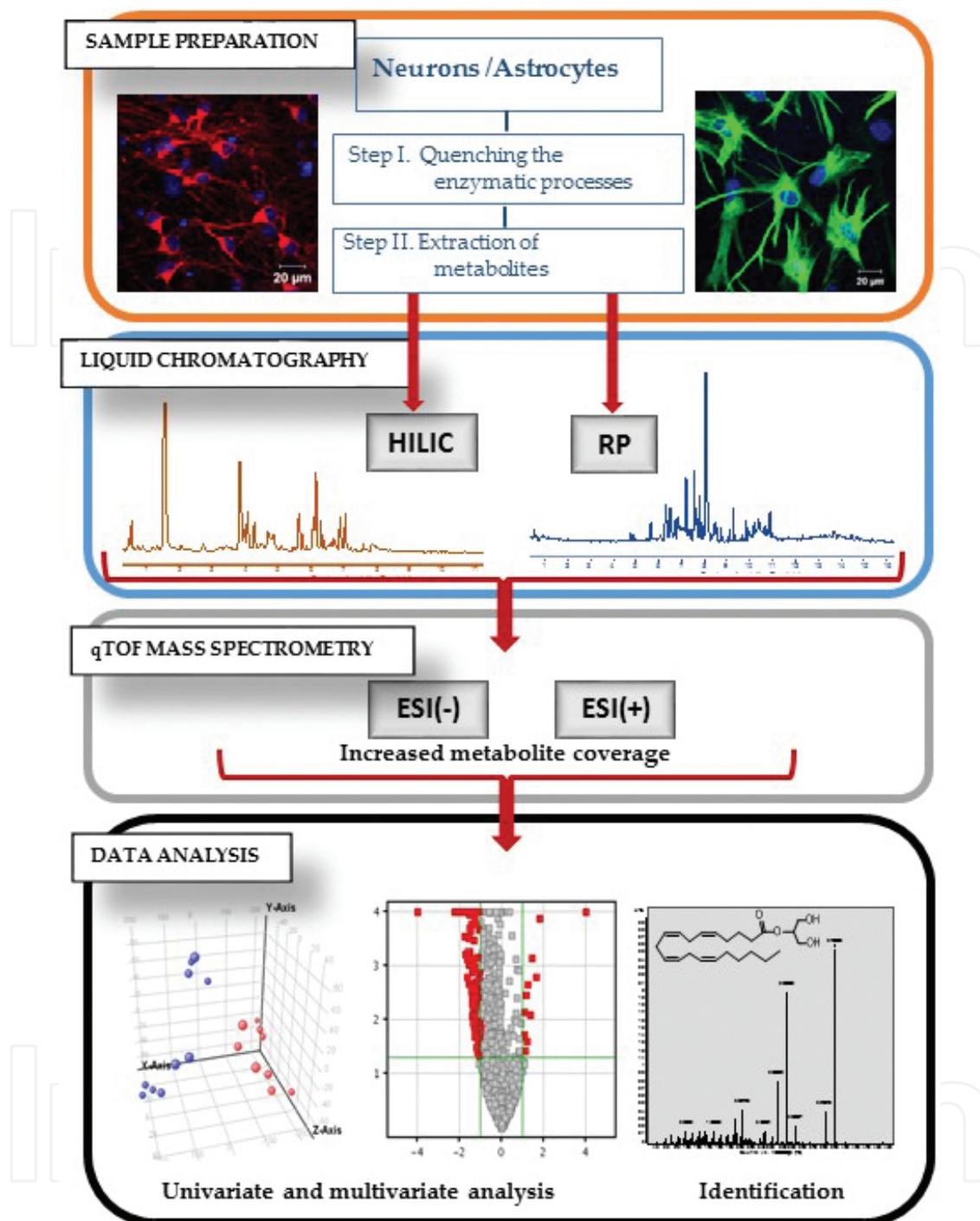
Figure 1. The proposed workflow. The proposed workflow of the nontargeted metabolite profiling for iPS cell-derived neurons and astrocytes. After data acquisition, the results are processed by bioinformatics software for peak-picking, nonlinear retention time alignment, visualization, relative quantification, and statistical analysis. ESI(−), negative mode electrospray ionization; ESI(+), positive mode electrospray ionization; HILIC, hydrophilic interaction chromatography; RP, reversed phase.

Using nontargeted metabolite profiling, in concert with the iPSC technology, presents the new opportunity to better understand the biological processes inside of a cell and extend our view of AD and PD pathophysiology. In our hands, this technique provides thousands of molecular

features from the neurons and astrocytes (unpublished data). In conclusion, MS-based metabolite profiling has a central role in revealing the metabolic pathways in healthy and disease-specific iPSCs.

3.1. Metabolomic profiling of AD and PD

Metabolomics is a powerful tool measuring the downstream products of the –omics cascade that reflects genomic, transcriptomic, and proteomic changes in a given biological sample. Metabolomics characterizes the endogenous small molecules and reveals connection between different pathways within a living cell in health and disease [41]. More than 3000 metabolites have been reported, but this number is not ultimate, as many metabolites need to be still identified and characterized. Out of 3000 metabolites, approximately 550 metabolites are of particular interest in studies of brain diseases [62]. Among the metabolites of interest are obviously neurotransmitters, but it is especially phosphates and lipids that are likely to reveal novel features of neurodegenerative diseases with the potential to help drug discovery or identification of novel biomarkers for brain diseases.

Disease	Sample	Instrumentation	Key metabolites	Ref.
Alzheimer's disease	Brain (post mortem)	UPLC-QTOF-MS	Unidentified metabolites	[49]
	CSF (post mortem)	HPLC-EC	Up: methoxy-hydroxymandelate, 5-hydroxytryptophan, methoxy-hydroxyphenylglycol, Down: alpha tocopherol, norepinephrine, ascorbate, 3-methoxytyramine	[64]
	CSF	GC–MS LC–MS/MS	Up: pyruvate, creatinine, cysteine, tyrosine, serine, phenylalanine, methionine, cortisol, dopamine Down: uridine	[45]
	CSF	CE–MS	Up: choline, valine, serine Down: carnitine	[51]
	CSF	HPLC-EC	Up: 5-hydroxyindoleacetic acid, methionine, xanthosine, vanillylmandelic acid, gluthatione Down: gluthatione/ methionine, 5-hydroxyindoleacetic acid/5-hydroxytryptophan	[65]
	Plasma	MDMS-SL	Up: ceramide down: sphingomyelin	[50]
	Plasma	UPLC-MS/ GCxGC-MS	Up: 2,4-dihydroxybutanoic acidDown: ether phospholipids, phosphatidylcholines, sphingomyelins, sterols	[46]
	Plasma	LC–MS/MS UPLC-QTOF-MS	Down: serotonin, phenylalanine, proline, lysine, taurine, phosphatidylcholine, acylcarnitine	[47]
Parkinson's disease	CSF (post mortem)	UHPLC-MS/MS GS–MS	Up: 3-hydroxykynurenine down: oxidized glutathione, N-acetylated amino acids, trimethylglycine, corticosteron	[48]
	Plasma	HPLC-EC	Up: glutathione, 2-hydroxy-2-deoxyguanosine Down: uric acid	[67]
	Plasma	HPLC-EC	Down: hypoxanthine, uric acid, hypoxanthine/xanthosine, xanthosine/xanthine, hypoxanthine/uric acid	[68]

CE–MS, capillary electrophoresis–mass spectrometry; CSF, cerebrospinal fluid; GC–MS, gas chromatography–mass spectrometry; HPLC-EC, high-performance liquid chromatography electrochemical detector; LC–MS/MS, liquid chromatography-tandem mass spectrometry; MDMS-SL, multidimensional mass spectrometry-based shotgun lipidomics; UPLC-QTOF-MS, ultra-performance liquid chromatography quadrupole time-of-flight mass spectrometry.

Table 2. Summary of key metabolites from human cohort studies of AD and PD patients.

Studies utilizing different analytical platforms have already identified several AD pathology-related metabolites in cerebrospinal fluid (CSF) and plasma from human cohorts (**Table 2**). Importantly, by using metabolomics set enrichment analyses, it is possible to identify and quantify metabolite changes [63]. The changes of neurotransmitters (e.g., dopamine, adrenaline, and noradrenaline) are tightly linked to phenylalanine and tyrosine metabolism pathways and have been found to be altered in CSF of AD patients [45]. While the decrease of noradrenaline may correlate with the severity of the disease [64], the increased levels of vanillylmandelic acid seem to correspond to a degradation of xanthine and dopamine [65]. The studies using serum of AD patients have revealed the decrease in several lipid classes, especially sphingomyelins [46, 50]. The most recent study followed elderly adults for 4 years, at a time by which some from them had developed AD [47]. The authors identified 10 metabolite profile features, phospholipids that play a role in integrity and functionality of cell membranes and were predictive of the occurrence of mild cognitive impairment or AD within 2–3 year time frame. The finding supported the link between dyslipidemia in AD and APOE4 variant as a major risk allele for AD that leads to disruption in sphingolipid metabolism [66]. It is reasonable to assume that global metabolite profiling directly from the brain cells (i.e., iPSC-derived material) of corresponding patients with AD or carrying APOE4 gene variant would tremendously increase the sensitivity of the method.

It is known that different areas of brain are affected during progression of neurodegenerative diseases. The composition of metabolites in CSF reflects the neuronal integrity as well as the underlying pathology of brain that leads to changes in brain metabolism. Studies from CSF of postmortem PD patients have shown the activation of kynurenine pathway by increase of 3-hydroxykynurenine concentration [48].

While 3-hydroxykynurenine contributes to oxidative stress, glutathione is known for its antioxidant proprieties. The levels of glutathione were found to be increased in PD plasma samples reinforcing the contribution of oxidative stress [67]. In the same study, the levels of uric acid were negatively associated with PD progression. The aberrations in the purine pathway that might appear upstream from uric acid have been studied in LRRK2 PD and sporadic PD [68]. Based on the plasmatic changes of nine metabolites involved in purine metabolism, the authors were able to predict whether the PD was secondary to LRRK2 mutation or of sporadic origin. A key perspective in metabolic phenotyping of brain diseases like AD and PD depends on the metabolic features interpretation: a single metabolite is unlikely to be a biomarker of a disease, while combinations of altered metabolites could form a diagnostic metabolic signature of the disease.

3.2. Cell-type specific metabolomics

Nontargeted metabolite profiling so far has focused to predict diseases by measuring the changes in specific metabolites in brain and CSF or systemically from plasma or urine. Essentially, the next step would be to move to the study of cell-type specific metabolic variations. iPS cell-derived neurons and astrocytes of AD and PD patients can be used to study cell-type specific metabolites which could determine whether the brain pathology changes

originate from one cell type or another. This approach will also provide insights into neuron/glia cell co-metabolome and will help to refine more targeted treatments for those diseases.

The causes of AD and PD diseases suggest a combination of genetic and environmental factors, with possible mechanisms ranging from mitochondrial dysfunction, oxidative stress, protein aggregation, impaired protein degradation, dysregulated autophagy, and inflammation. So far, there are only unpublished data available from one metabolome analysis of PARK2 iPS cell-derived neurons briefly discussed in a review by Okano and Yamanaka [69]. The changes seen in glycolytic pathways, the tricarboxylic acid cycle (TCA), and pentose phosphate pathways suggest that mitochondria are the sites of dysfunctions in neurons harboring PARK2 mutation (H.O. unpublished data). Abnormalities in mitochondria were further confirmed by morphology and impaired mitochondrial turnover (unpublished data). As mitochondria are required for the energy demands of the brain cells including neurons, mitochondrial alterations can promote neuron degeneration. Mitochondrial complex I activity and its regulation by transcriptional factors are both seen to be altered also in PD patients [70]. Reduced level of mitochondrial α-ketoglutarate dehydrogenase, a rate-limiting enzyme in TCA cycle, was reported in the brain of PD patients [71–73], suggesting the involvement of bioenergetics defects as well reduced mitochondrial complex activity in PD pathology. The results from postmitotic human dopaminergic neurons (DA) exposed to mitochondrial respiratory chain inhibitor MPP+ (1-methyl-4-phenylpyridinium) are in line with those findings. Altogether, 190 metabolites have been found altered in MPP+-treated DA neurons [74]. In energy metabolism, decreased levels of intracellular glucose leads to increase of pyruvate and lactate, while consumption of phosphorylated creatine and accumulation of creatine suggest depletion of the cellular energy accompanied by gradual increase of ADP, AMP, and adenine [74]. Decreased total intracellular ATP levels have also been found from fibroblast cells of PD patients with mutations in LRRK2 accompanied with decreased mitochondrial membrane potential [75]. Moreover, increase in methionine sulfoxide formation in MPP+-treated DA neurons suggested an increase in oxidative stress and ROS production [74]. To better understand the effects of oxidative stress pathways on cellular metabolism, we propose to apply a combined strategy, consisting of nontargeted metabolite profiling together with gene expression analyses to characterize DA neurons and astrocytes derived from iPSCs of PD patients, both sporadic and familiar form. This will allow us to explore how the cells of interest generate reducing potential to compensate oxidative stress and how this could be impaired in genetically perturbed cells.

Several studies have suggested an important role of mitochondria also in the onset and progression of AD [76]. The fact that mitochondria are prime targets for APP as well for Aβ [77] has been underlined in studies of neuronal cells overexpressing APP and Aβ that leads to abnormal mitochondrial dynamics via modulation of mitochondrial fission/fusion proteins [78]. PET imaging has shown decreased levels of brain glucose metabolism in AD brain [79], while another study has demonstrated decreased expression of genes involved in glucose delivery, oxidative phosphorylation, and energy consumption in the brains of AD patients [80]. Postmortem studies on AD brain and fibroblasts from AD patients have revealed changes in TCA, pyruvate dehydrogenase, isocitrate dehydrogenase, and α-ketoglutarate dehydro-

genase [81–83]. In addition, the levels of ATP and activities of cytochrome oxidase were reported to be decreased. Apart from direct mitochondrial respiratory chain defects, increased autophagic degradation of mitochondria has also been reported [84].

While AD and PD pathology from diseased patients show late stages of these neurodegenerative diseases, patient-specific derived iPSCs have a potential to model early presymptomatic stage of AD and PD. In 2D cultures where the cells are grown in a monolayer, cell phenotype might be amplified and seen much more earlier compared to the moment when patients are diagnosed. On the other hand, 2D system lacks interaction between the other cells present normally in human brain. In 3D culture models, cells interact with each other, but culture is rather heterogeneous and compensation capability is higher than that in 2D culture. Therefore, it would be necessary to study the metabolic changes in neurons and astrocytes first separately as well as in co-culture, in order to understand the mechanisms behind the pathology. Genetic background needs to be taken into account as well. Genetic correction of patient mutations or introduction of mutation to healthy line allows validation of the role of mutations in the observed phenotype.

4. Conclusions

In this review, we have described the current status of using disease-specific iPSCs to model the onset and initial stages of neurodegenerative diseases such as AD and PD. Human iPSCs improve disease modeling in previously inaccessible cells, such as neurons and astrocytes that are the key cell types of the brain. Emerging evidence suggests that brain energy metabolism goes beyond synthesis of neurotransmitters and that different pathways within neuronal cells contribute to brain disorders. Combining the technologies of iPCS and untargeted metabolomics may provide a powerful tool to better understand the biological processes inside of cell. In the future, brain metabolite profiles could be correlated to DNA polymorphisms, and together with transcriptomics/proteomics data could provide a platform for new neuroprotective strategies.

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