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Theoretical Article

Alzheimer's disease mechanisms in peripheral cells: Promises and challenges

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Abstract

Introduction: Development of efficacious therapeutic interventions for Alzheimer's disease (AD) is hampered by the lack of understanding early disease mechanisms, biomarkers, and models that mimic complex pathophysiology of human disease.

Methods: This article aims to assess to what extent peripheral cells recapitulate molecular mechanisms altered in the brain and could be used as translational models for the development of individualized medicine for AD.

Results: Multiple studies suggest that AD is a systemic disorder with an active crosstalk between brain and periphery where multiple pathways altered in the brain cells are also affected in plasma, cerebrospinal fluid, and other peripheral cells of AD patients.

Discussion: Additional studies to validate molecular mechanisms in peripheral cells using advanced system biology techniques and well-characterized cohorts of AD patients together with the development of standardized protocols should be considered to support the application of peripheral cells in AD research.

Keywords: Alzheimer's disease; Brain-periphery axis; Primary skin fibroblasts; Mitochondria; Metabolism; Bioenergetics; Biomarkers; Inflammation; Calcium signaling; Neurotransmitters

1. Objective

Alzheimer's disease (AD) is the leading form of dementia where underlying molecular mechanisms are poorly understood. Therapeutic strategies designed to reduce levels of amyloid beta (Aβ) plaques or hyperphosphorylated tau (p-tau) containing tangles, two hallmarks of AD, have failed in clinical trials [1–3]. Factors contributing to this failure include limited understanding of early disease mechanisms and associated biomarkers, and poor translation of preclinical research conducted in model organisms [4,5]. Familial AD (FAD) accounts for ~5% of all cases and is linked to mutations in amyloid precursor protein (APP), presenilin 1 (PSEN1), and presenilin 2 (PSEN2) genes [6]. Most AD cases are sporadic late-onset AD (LOAD) with age being the greatest risk factor [6]. Recent clinical investigations using systems biology approaches and imaging techniques suggest that AD is a complex disorder where changes in multiple pathways occur years before the onset of clinical symptoms [7]. Moreover, the disease differentially affects males and females presenting additional challenges for biomarker and drug discovery [8]. Animal models currently used for preclinical therapeutic development do not recapitulate the complexity of sporadic AD. Thus, there is an urgent need to identify translational models that better represent AD mechanisms and could complement existing animal models to test novel therapeutic approaches and develop panels of disease stage- and sex-specific diagnostic and prognostic biomarkers.

There are no conflicts of interest.

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This article aims to test the hypothesis that AD is a systemic disorder where peripheral cells recapitulate major molecular mechanisms affected in the brain. The hypothesis predicts that alterations in pathways shown fundamentally important in the etiology of AD including inflammation, abnormal calcium signaling, amyloid precursor protein processing, Aβ and p-tau accumulation, altered oxidative metabolism, mitochondrial dysfunction, and abnormal cellular energetics will be detected in peripheral cells and biofluids of AD patients providing a unique opportunity to study/manipulate these mechanisms in a contest of the individuals' genetic, epigenetic, and metabolic background. Here, we will (1) provide the rationale for this hypothesis reviewing evidence that peripheral cells and biofluids recapitulate mechanisms affected in the brain; (2) discuss opportunities and challenges associated with the utilization of peripheral cells in AD research; and (3) review advantages and limitations of the hypothesis including the next steps required for its validation.

2. The rationale for the hypothesis and linkage to other major theories

Traditionally, AD has been viewed as a central nervous system disorder where the amyloid cascade hypothesis was broadly used to connect the accumulation of amyloid plaques and neurodegeneration [9]. In recent years, new research, clinical, and epidemiological evidence together with the consistent failure of clinical trials focused on Aβ production and clearance have prompted the reassessment of molecular mechanisms involved in AD pathogenesis [10,11]. New investigations conducted using human tissue, biofluids and advanced omics, computational, and network biology approaches to establish etiological mechanisms of AD suggest a multifactorial nature where the disease stage- and sex-specific changes in multiple interconnected pathways play a key role [12,13]. Furthermore, it is increasingly recognized that AD mechanisms extend outside of the brain where connections between cardiac, metabolic, and gut microbiota abnormalities, among others, may contribute to the development of sporadic AD [10,14–18]. Based on observations generated in red blood cells, platelets, skin fibroblasts, and lymphocytes from AD patients demonstrating defects in calcium homeostasis, membrane trafficking, and metabolic functions including glucose oxidation, ideas that AD is a systemic disorder were put forward as early as in 1980s [19,20]. More recent investigations reinforced these observations formulating "the erythrocytic hypothesis of AD" [21] where age-dependent decrease in energy production and altered ability of red blood cell to transfer oxygen to brain cells were linked to inadequate oxygenation and abnormal glucose/energy metabolism, oxidative stress, and increased neuronal damage instigating the development of AD. Further support for metabolic dysfunction as an underlying mechanism of AD came from multiple studies demonstrating that type 2 diabetes was associated with increased risk of developing AD. These studies linked peripheral changes in glucose metabolism and brain function and provided a foundation for the "metabolic hypothesis of AD" [22]. Changes in glucose availability and utilization in the brain induced by either local or systemic alterations were shown to affect levels of lipids, proteins, glycogen, and neurotransmitters including γ-aminobutyric acid (GABA) [23], glutamate [24], and acetylcholine [25]. Changes in concentrations of these important molecules directly affect neuronal homeostasis contributing to excitotoxic cell death, abnormal calcium signaling, and synaptic dysfunction. The systematic studies conducted since 1960s in the brain tissue of patients with AD clearly defined a substantial presynaptic cholinergic deficit manifested in reduced choline uptake, acetylcholine release, and loss of cholinergic perikarya from the nucleus basalis of Meynert [26]. These studies and the recognized importance of acetylcholine in learning and memory provided a foundation for the "cholinergic hypothesis of AD" [27], which led to the development of cholinesterase inhibitors that are among a few medications that the FDA approved for treatment of AD. Unfortunately, this approach is not disease-modifying emphasizing a need for further AD research [28].

Furthermore, substantial evidence supports the crosstalk between the brain and periphery to maintain proper energy homeostasis [29]. Specialized neuronal networks in the brain coordinate adaptive changes in food intake and energy expenditure in response to changes in plasma levels of a key metabolic hormones and nutrients [30]. Because the brain has exceptionally high-energy requirements, age- or disease-related alterations in energy metabolism that occur throughout the body could have a direct effect on the brain. Recent evidence suggests that early changes underlying AD pathogenesis, LOAD in particular, are associated with impaired mitochondrial function, which directly affects energy homeostasis [31–33]. The capacity of mitochondria to produce energy and sustain stress could determine the survival of brain cells [34]. Changes in mitochondrial dynamics and function affect multiple cellular processes including level of oxidative stress, energy production, generation of important signaling molecules involved in protective stress response, and epigenetic modifications, which could independently determine the course of the disease [35]. Indeed, the "mitochondrial cascade hypothesis" proposes that an individual's genetic predisposition, environmental exposure, and lifestyle could affect mitochondrial function and mediate, drive, and/or contribute to a variety of AD pathologies [31]. These mitochondrial alterations are not restricted to the brain and could be detected in the periphery including cerebrospinal fluid (CSF), plasma,

lymphocytes, and fibroblasts [32,36,37]. Importantly, altered mitochondrial behavior could affect APP processing, Aβ production, and tau phosphorylation exacerbating AD phenotype [38]. Furthermore, abnormal calcium homeostasis associated with increased levels of Aβ and mitochondrial dysfunction has been shown to affect multiple pathways in AD including neuronal development, synaptic transmission and plasticity, and the regulation of various metabolic pathways [39]. Consequentially, increased levels of oxidative stress that induces lipid peroxidation and mitochondrial and nuclear DNA damage have been considered as an important contributing factor to the development of age-related diseases including AD supporting the concept of a vicious cycle where with age, accumulating dysfunctions in multiple interconnected pathways contribute to the onset and exacerbate the disease development [38,39]. Finally, a sustained inflammatory response well documented in the brain tissue of patients with AD has been considered as an early and central feature of neurodegenerative process, the "inflammation hypothesis of AD" [40]. This devastating process is not limited to the brain. In recent study, a presence of peripheral chronic low-grade inflammation in patients carrying the *APOE* ε4 allele, a risk factor for AD development, has been shown to shorten latency for onset of AD [41]. The compromised integrity of the blood-brain barrier in AD allows the migration of peripheral immune cells to and from the brain inducing inflammatory response throughout the body [42].

Taken together, there is a strong evidence of the cross-talk between brain and periphery where multiple molecular mechanisms identified as the key contributing factors to the development of neurodegeneration in AD might be also present in periphery. If peripheral cells indeed share the complexity of mechanisms altered in the brain, they could provide valuable models for AD research.

3. Early experimental evidence to support the hypothesis

Independent studies have demonstrated that neuronal mechanisms altered in AD including signal transduction pathways, oxidative metabolism, APP processing, mitochondrial dynamics and function, calcium homeostasis, and inflammation are also affected in fibroblasts, erythrocytes, platelets, urine, plasma, and CSF [19,43–51]. For biomarker discovery, plasma represents the best source for repeated measures while the utilization of skin fibroblasts provides an outstanding opportunities for longitudinal mechanistic studies because they could be kept in culture for a long time, do not need to be transformed, and could be differentiated into disease- and patient-specific neural cell lines using inducible pluripotent stem cell (iPSC) technology [52]. Below, we will review current literature that highlights to what extent mechanisms important for the development of AD in the brain are present in primary human cells, fibroblasts in particular.

3.1. Altered energy homeostasis and mitochondrial dysfunction

Reduced glucose utilization in the brain of patients with mild cognitive impairment (MCI), a prodromal stage of AD, and AD patients detected using fluorodeoxyglucose positron emission tomography indicates a decline in neuronal cellular metabolism that is secondary to mitochondrial dysfunction. Similar changes were identified in peripheral cells. In 1990, Parker and colleagues reported decreased cytochrome oxidase (COX) activity indicative of mitochondrial dysfunction in platelets from AD subjects [53]. Around the same time, Sims and colleagues demonstrated reduced alpha-ketoglutarate dehydrogenase complex activity and altered patterns of glucose utilization in AD fibroblasts [49]. Using stable isotopes, the authors determined that the glycolytic capacity in AD fibroblasts was increased while glutamine metabolism was significantly inhibited compared to healthy controls [50]. Additional studies confirmed altered mitochondrial function in glucose and glutamine oxidation in fibroblasts from patients with sporadic AD [54]. Functional analysis conducted using an Extracellular Flux Analyzer in intact cells provided additional evidence that LOAD fibroblasts have increased glycolytic capacity, impaired mitochondrial metabolic potential associated with decreased nicotinamide adenine dinucleotide metabolites and reduced activity of the tricarboxylic acid cycle compared to control cells [33]. Lactate levels were higher in LOAD fibroblasts but not in healthy age-matched and young fibroblasts suggesting that increased glycolysis was specific to the disease and not aging [33]. The authors concluded that the increase in glycolysis and the abnormal mitochondrial metabolic potential in LOAD fibroblasts appeared to be intrinsic further supporting the hypothesis that impairment in multiple components of bioenergetics may be a key mechanism contributing to the risk and pathophysiology of LOAD. Furthermore, reduced removal of damaged mitochondria via autophagy/mitophagy pathways was demonstrated in patient-derived AD fibroblasts and neurons from iPSCs harboring the familial *PSEN1* mutation [45]. This mitophagy impairment and associated lysosomal impairment resulted in the accumulation of dysfunctional mitochondria [45]. Similar alterations in the impairment of autophagy/mitophagy pathways leading to the accumulation of dysfunctional mitochondrial were observed in fibroblasts and postmortem brain tissue from LOAD patients [55]. These findings suggest that altered mitochondrial dynamics and function represent a common nexus between familial and sporadic cases of the disease where human skin fibroblasts share mitochondrial defects observed in the AD brain [45]. Changes in mitochondrial function in AD fibroblasts also recapitulate metabolic alterations in energy pathways that we and others identified in the CSF and plasma from patients with MCI and AD [32,51,56]. These findings support the hypothesis that impairment of bioenergetics, mitochondrial dynamics

and function, and cell metabolism occur throughout the body and contribute to the pathophysiology of AD providing a rationale for the analysis of mitochondrial bioenergetics in peripheral tissues as a promising strategy to develop new diagnostic methods for AD [33].

3.2. Oxidative stress and calcium homeostasis

Similar to AD neurons that exhibit increased susceptibility to oxidative stress, excessive oxidative DNA damage and the accumulation of oxidative marker 8-oxo-guanine were found in patient-derived AD fibroblasts [57]. Application of microarray gene expression profiling in AD or control fibroblasts treated to simulate conditions of oxidative stress revealed pathways that could play a critical role in the etiology and/or pathology of AD [57]. However, in another study, the authors found a greater resistance of AD fibroblasts to the acute H_2O_2 treatment that generates reactive oxygen species, DNA damage, and apoptosis [58]. The protective mechanism was related to an impairment of H_2O_2-induced cell cycle arrest and characterized by an accelerated re-entry into the cell cycle and a diminished induction of apoptosis. Fibroblasts from AD patients also had a profound impairment in the H_2O_2-activated, p53-dependent pathway, which resulted in a lack of activation of *p53*- or *p53*-target genes, including *p21, GADD45,* and *bax*. This study demonstrates a specific alteration of an intracellular pathway involved in sensing and repairing DNA damage in peripheral cells from AD patients [58]. Calcium homeostasis was altered to a greater extent in fibroblasts from AD patients compared to aged controls [59]. Total bound calcium in fibroblasts was elevated in normal aging (+52%) but was elevated even further in AD (+197%). The authors connected this increase with other processes including reduced mitochondrial function and altered biosynthesis that depends on mitochondria, such as glucose or glutamine incorporation into proteins and lipids, which also paralleled mitochondrial dysfunction. Interestingly, cytosolic and nuclear processes such as leucine incorporation into proteins and thymidine into DNA were depressed more by aging than AD. The authors concluded that calcium homeostasis and mitochondrial functions were affected to a greater extent by AD compared to normal aging [59].

3.3. Aβ and p-tau

Increased levels of Aβ42 and p-tau were reported in multiple studies that examined fibroblasts from patients with FAD and LOAD and in iPSC-derived neuronal cells from a donor with sporadic AD [60–64]. Increase in Aβ was detected along with a reduction in ATP production associated with altered mitochondrial respiration and diminished mitochondrial content in fibroblasts from AD patients with *PSEN1* mutations [60]. Similarly, fibroblasts from patients carrying a familiar Swedish *APP670/671* mutation release significantly more Aβ compared to healthy controls [63]. In the brain, the enzyme activity of protein kinase C ε (PKCε) is associated with neuroprotective functions including reducing levels of Aβ oligomers [65]. In AD skin fibroblasts, levels of PKCε were lower compared to control subjects, which might explain increased Aβ levels [65]. Furthermore, an increase in Aβ processing and tau phosphorylation in AD fibroblasts could be attributed to the activation of the mitogen-activated protein kinases Erk1 and Erk2, which is dependent on PKC activity. Activation of Erk1/2 is well documented in susceptible neurons in mild and severe AD cases (Braak stages III-VI) [66] further supporting a cross-talk between brain and periphery. Furthermore, in neuronal cells differentiated from the iPSCs derived from fibroblasts of an 82-year-old female patient affected by sporadic AD, the expression of p-tau and GSK3β, a physiological kinase of tau, was significantly increased [64]. A similar increase in GSK3β activity has been observed in the brains of AD patients [67]. Given that GSK3β activity has been linked to most pronounced AD phenotype including cognitive impairment, inflammation, increased production of p-tau, mitochondrial dysfunction, and neuronal death, this fibroblast-derived model may provide an outstanding tool to study the underlying molecular basis of sporadic AD and a platform for drug screening and toxicology studies. Indeed, the authors were able to use this system to demonstrate that treatment with an inhibitor of gamma-secretase resulted in the downregulation of p-tau supporting a mechanistic connection between p-tau, GSK3β, and Aβ pathology [68]. In addition, transcriptome analysis conducted in these fibroblast-iPSC-derived neuronal cells revealed significant changes in the expression of genes reminiscent of changes in subregions within the AD brain [64].

3.4. Inflammation

AD now is increasingly recognized as a chronic inflammatory disease where inflammation most likely plays a causative role [41,42,69]. Bidirectional activation of immune response could be facilitated by a release of soluble inflammatory mediators (cytokines, chemokines, and reactive oxygen species) that could act on periphery. Indeed, multiple peripheral inflammatory markers including interleukin (IL)-1β, IL-2, IL-6, IL-18, interferon-γ, homocysteine, high-sensitivity C-reactive protein, C-X-C motif chemokine-10, epidermal growth factor, vascular cell adhesion molecule-1, tumor necrosis factor–α converting enzyme, soluble tumor necrosis factor receptors 1 and 2, α1-antichymotrypsin, and decreased IL-1 receptor antagonist and leptin were elevated in patients with AD compared to controls and inversely correlated with cognitive scores [70]. However, most of these markers were detected in blood, CSF, or postmortem brain tissue from AD patients [32,71,72]. Currently, it is unknown whether fibroblasts from AD patients have increased inflammation.

3.5. Peripheral cells and biofluids in biomarker and drug discovery

To date, a handful of papers describe the utilization of primary fibroblasts for drug discovery for AD. One of these reports capitalized on the previous observation that altered PKC signaling, critical for the nontoxic degradation of APP and inhibition of GSK3β in neurons, was present in fibroblasts from AD patients [73]. The authors tested whether modulation of PKC with bryostatin and a potent synthetic analog picolog could affect PKC signaling mechanism in AD primary fibroblasts. The outcomes included increased alpha-secretase activity that accounted for lowering the amount of toxic Aβ produced in AD cells. Both bryostatin and picolog increased the secretion of the alpha-secretase product (s-APP-alpha) of APP at sub-nanomolar to nanomolar concentrations. Furthermore, both of these PKC activators were shown to convert the AD Erk1/2 phenotype of fibroblasts into the phenotype of "normal" control skin fibroblasts [73]. Further experiments also demonstrated the utility of human AD fibroblasts to modulate the dysfunction associated with Erk1/2 signaling where the detection of AD-specific differences in MAP kinase in peripheral tissues provided an efficient means for early diagnosis of AD as well as helped to identify therapeutic targets for drug discovery [74]. In recent years, the use of primary human AD fibroblasts was extended via generating the iPSCs and further differentiation into neuronal cells for drug discovery [75–77]. The main advantages of these models include the ability to work with disease-relevant human cells to conduct high-throughput screening of thousands of compounds with disease-specific outcomes [78]. However, technical difficulties and high cost of these experiments represent substantial disadvantages.

While the majority of work on biomarker discovery for AD was traditionally conducted in biofluids [78,79], the utilization of fibroblasts as a source of biomarkers for AD has also been proposed [80]. One of the examples includes the identification of a proteolytic dysfunction in AD cells that produces altered isoelectrophoretic forms of the enzyme transketolase (TK-alkaline bands) that could be used for an early diagnosis [81]. The TK profile conducted in fibroblasts from clinically diagnosed probable LOAD patients, their asymptomatic relatives, neurological non-AD patients, early-onset AD patients, and control individuals demonstrated the usefulness of cultured fibroblasts as an excellent in vitro model for the study of the pathogenic processes of AD and as a low-cost laboratory tool useful for supporting AD differential diagnosis [81]. Similarly, AD fibroblasts were found to have the upregulation of the lysosomal system including increased levels of glycohydrolases (α-D-mannosidase, β-D-hexosaminidase, and β-D-galactosidase). These changes were found in AD patients affected by either sporadic or familial forms of the disease and also in presymptomatic subjects carrying the familial mutations but healthy at the time of skin biopsy [82]. Along with the development of novel biomarkers, this work also provided the foundation for the identification of early molecular mechanism of AD that could be studied in fibroblasts. Other biomarkers proposed for early diagnosis in AD fibroblasts include quantitatively measured aggregation rate that is increased in AD cases. This biomarker was successfully cross-validated with two more assays, AD-Index, based on the imbalances of ERK1/2, and Morphology, based on network dynamics, and showed 92% overlap. A significant number of cases tested with this biomarker were freshly obtained where 82% of the cases were validated with other clinical biomarkers including autopsy and/or genetic confirmation of AD [83,84]. Taken together, this evidence supports the notion that peripheral cells represent a tool to study the underlying molecular mechanisms of sporadic AD and could be used for the development of diagnostic and prognostic biomarkers and therapeutic strategies.

4. Major challenges to the hypothesis and future experiments

Although multiple lines of evidence support the hypothesis that peripheral cells could be successfully used in AD research, there are certain challenges that require further clarification. From conceptual perspective, the definitive demonstration that peripheral cells mimic complex mechanistic alterations present in brain cells of AD patients remains to be done. Brain cells, especially nondividing neurons with unique cellular architecture, and peripheral dividing cells differ in mechanisms essential for AD development including metabolic regulation, mitochondrial dynamics and function, and neurotransmitter pathways. In support of the hypothesis, it would be important to conduct experiments to demonstrate what mechanisms and biomarkers are similarly affected/expressed in particular subsets of brain cells versus peripheral cells versus biofluids. Such extensive and well-controlled experiments may not be feasible to do in living individuals but the use of animal models might be instrumental because the comparison of changes in neuronal versus peripheral cells could be done in respect to disease development and progression. Furthermore, application of systems biology approaches such as next generation sequencing, metabolomics, and epigenetics could be very useful in the identification of longitudinal changes in peripheral cells for biomarker and drug discovery. These types of studies are now actively supported by the National Institute of Health (NIH) through multiple consortia focused on the development of new animal models for AD research and application of systems biology techniques. It is feasible that only a subset of molecular mechanisms affected in AD could be recapitulated in peripheral cells,

but the ability to develop sex- and disease-stage-specific individualized treatments even for a limited subset of altered functions could be very important. To further support the hypothesis, it would be important to demonstrate whether human skin fibroblasts recapitulate genetic, epigenetic, and metabolic changes established in the brain of AD patients; how these changes are affected depending on age, sex, and disease severity; what are the cross-sectional and longitudinal changes in pathways directly linked to the development and progression of AD; to what extent sex influences pathological mechanisms that fibroblasts share with neuronal cells. For example, longitudinal studies could be designed to establish the hierarchy of the affected pathways since peripheral cells appear to mimic alterations in energy pathways detected in the brain. This is especially important because changes in fibroblasts could be correlated with changes in cognitive performance and biomarkers including fluorodeoxyglucose positron emission tomography imaging reflective of energy utilization, levels of Aβ, and p-tau to establish early mechanisms underlying the disease. Consequently, individualized approaches based on pharmacogenomics of the particular individual could be developed and tested in human cells before evaluating efficacy *in vivo*. The ability to interrogate specific mechanistic pathways as we discussed previously for oxidative DNA damage or PKCε offers unmatched opportunities to follow up with changes in mechanisms that could help to confirm or even foster new theories to advance the development of disease-modifying therapeutics.

From technical perspective, the methodology for assaying AD mechanisms in cultured primary cells has not been established, and the availability of well-characterized human cells for reproducible and rigorous research is limited. Currently, the primary source of human AD fibroblasts and other peripheral cells is the Coriell Institute Biobanks (Camden, NJ; https://www.coriell.org/). Coriell Biobanks establish, verify, maintain, and distribute cell cultures and DNA to the research community. These collections are supported by the NIH and several foundations and have cell lines from patients with various diseases including familial and sporadic AD. However, different from other biorepositories such as the Alzheimer's Disease Neuroimaging Initiative where samples collected from MCI and AD patients and unaffected individuals have detailed demographic information along with the genetic characterization, neuroimaging data, and other biomarkers (e.g., Aβ, p-tau, metabolomics data), patient information for human fibroblasts in almost all cases is limited to age, sex, and race. Important questions that are currently under active investigation in AD field including sex-, age-, disease stage–specific differences, contribution of risk factors to the progression and severity of the phenotype, pathogenic interactions, and mixed pathology could only be addressed using peripheral cells if sufficient number of cell lines with in-depth sample characterization will become available along with the establishment of standardized experimental procedures [85].

An important caveat for validation of research conducted in cultured cells involves the requirement to have standard operating procedures that allow the direct comparison of outcomes between individual laboratories. It is well known that cultured cells could change their phenotype with each passage ultimately reaching age-related senescence stage. This process is associated with fluctuations in metabolism, production of reactive oxygen species, mitochondrial function, and gene expression. Thus, some of the parameters detected in cultured cells could be acquired independently of the disease phenotype. Indeed, as we have recently shown, technical approaches implemented for cell culturing, harvesting, and storage directly affect cell metabolism resulting in variable metabolic profile [86]. It is imperative that careful consideration will be given to experimental design and quality control to avoid data misinterpretation. Experiments aimed to produce rigorous and reproducible results in fibroblasts have to include matching disease and control cells based on age, sex of the donors, and biological age in culture, that is, cumulative population doubling level and percentage of life span completed. Quality control should be in place and standard operating procedures have to include steps where cell age, number of passages, and cell divisions will be taken into account as confounding factors that may affect endophenotype. Furthermore, to date, there are no data that clearly presents the advantages of using human skin fibroblasts for translational drug discovery where outcomes are confirmed in *in vivo* models. The demonstration that fibroblasts could be directly used to design individualized treatment for the central nervous system disorders could offer relatively inexpensive tool that may significantly reduce the high cost of alternative approaches (e.g., iPSC-derived neuronal cells or multiple animal models) [87]. Nevertheless, high translational potential of outcomes generated in peripheral cells for a development of biomarkers and targeted therapeutics, the opportunity to conduct extensive evaluations using systems biology approaches to determine mechanisms and complex functional connections in the context of the individuals' genetic and epigenetic makeup, the ability to collect peripheral cells longitudinally from the same individuals, and relatively low cost associated with the utilization of human cells could provide complementary or even primary tools for AD research and clinical applications.

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RESEARCH IN CONTEXT

1. Systematic review: The author reviewed the literature using traditional sources. Several studies have investigated the extent to which peripheral cells recapitulate molecular mechanisms altered in the brain of Alzheimer's disease (AD) patients. These relevant articles are appropriately cited.
2. Interpretation: AD is a systemic disorder with an active crosstalk between the brain and periphery implying that peripheral cells could provide insight into early disease mechanisms offering translational model for the discovery of new therapeutic approaches and biomarkers for disease development, diagnosis, prognosis, and monitoring the therapeutic efficacy.
3. Future directions: Longitudinal and cross-sectional identification of molecular mechanisms in peripheral cells using advanced system biology techniques and well-characterized cohorts of AD patients together with the development of standardized protocols should be considered to support the application of peripheral cells in AD research.

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