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Research article

Generation of disease-specific autopsy-confirmed iPSCs lines from postmortem isolated Peripheral Blood Mononuclear Cells

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HIGHLIGHTS

- IPSC can be derived rapidly from patient samples retrieved after death.
- Postmortem blood drawn within 20 h after death can serve as reprogramming material.
- Autopsy confirmed patient samples are the gold standard for modeling heterogeneous and idiopathic disease.

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ABSTRACT

Understanding the molecular mechanisms that underlie neurodegenerative disorders has been hampered by a lack of readily available model systems that replicate the complexity of the human disease. Recent advances in stem cell technology have facilitated the derivation of patient-specific stem cells from a variety of differentiated cell types. These induced pluripotent stem cells (iPSCs) are attractive disease models since they can be grown and differentiated to produce large numbers of disease-relevant cell types. However, most iPSC lines are derived in advance of, and without the benefit of, neuropathological confirmation of the donor – the gold standard for many disease classifications and measurement of disease severity. While others have reported the generation of autopsy-confirmed iPSC lines from patient explants, these methods require outgrowth of cadaver tissue, which require additional time and is often only successful ~50% of the time. Here we report the rapid generation of autopsy-confirmed iPSC lines from peripheral blood mononuclear cells (PBMCs) drawn postmortem. Since this approach doesn't require the propagation of previously frozen cadaver tissue, iPSC can be rapidly and efficiently produced from patients with autopsy-confirmed pathology. These matched iPSC-derived patient-specific neurons and postmortem brain tissue will support studies of specific mechanisms that drive the pathogenesis of neurodegenerative diseases.

1. Introduction

Since the advent of induced pluripotent stem cell (iPSC) technology, multiple studies have used iPSC [1–3] and the resultant differentiated neural progenitor cells (NPCs) [4] and neurons as in vitro models of human neurodegenerative disease [16–18,20,22]. Most of these studies have focused on samples obtained from individuals with well-defined, disease-associated genetic variants, inherited in a Mendelian fashion [2,5,6]. However, these Mendelian cases represent only a minority (~5–10%) of those affected with a given neurodegenerative disease, with the majority of cases being idiopathic [7]. Understanding the molecular mechanisms that underlie the disease in idiopathic cases can be complicated. Different contributions by disease susceptibility genes, genetic and pathologic heterogeneity can confound the clinical diagnosis.

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Indeed, the classification of neurodegenerative diseases based on their clinical presentations can be problematic due to overlapping and sometimes confounding symptoms. For example, progressive supranuclear palsy, multiple system atrophy, Lewy body dementia, and even Alzheimer disease have been clinically misdiagnosed as Parkinson disease (PD) [8]. In a recent study the clinical diagnosis of PD by neurological movement specialists was only 84% accurate when compared to subsequent autopsy results [9,26]. Pathological confirmation remains the gold standard for neurodegenerative disease diagnosis [27,28]. Therefore, disease models that consider both neuropathological and genetic heterogeneity are more likely to provide results more closely resembling the disease process in humans.

Several recent studies have described the derivation of iPSC from postmortem tissue, including the derivation of iPSC from frozen brain samples [10,23,24]. Although this approach broadens the use of potential materials that can be used for the derivation of iPSC, it has been hampered by the need for explant outgrowth that was successful on only a fraction of stored samples (44–56%) [12], while PBMC from blood draws have a 100% reprogrammability in our hands [21]. In addition to the difficulties associated with cadaver tissue outgrowth, the efficiency and robustness of fibroblast reprogramming is affected by patient age [11,12]. While our lab has noticed significant decreases in the efficacy of reprogramming fibroblasts obtained from older individuals, we do not see the same variance in efficiencies related to patient age when reprogramming PBMCs. *PBMCs* isolated from whole blood obtained at the time of autopsy represent an alternative source of cells for the derivation of iPSC. These PBMCs can be readily and inexpensively isolated and cryopreserved for use following the neuropathological confirmation of disease. iPSC lines derived from these PBMCs can be differentiated into disease relevant neuronal cells for functional characterization, and compared to neuropathologically examined brain tissue samples. These matched iPSC and autopsy brain samples represent a unique resource that allows for the modeling of the neurodegenerative process in cells which can be directly compared to the genetically identical postmortem brain tissue.

2. Methods and materials

2.1. Brain tissue sections and immunohistochemistry

Participants were previously consented and enrolled in an ongoing autopsy program. At the time of death the brain was removed and preserved for subsequent neuropathological review. Brain tissue samples were stored at −80 °C degrees. Brain samples were sectioned at 60 μm using the Cryostat and mounted on microscope slides. Immunofluorescence staining utilized a standard protocol. Sections were permeated with 0.3% Triton x-100 in 1x PBS for 25 min at RT and blocked with 5% BSA/0.1% Triton x-100 in 1x PBS for 1 h at RT. Samples were stored at 4 °C in primary antibody (FOXA2/ATH) diluted in 5% BSA/1x PBS. Sections were 3x washed with 1x PBS. Secondary antibodies (anti-mouse-488 and anti-rabbit-546) were added diluted in 5% BSA/1x PBS for 1 h at RT. Sections were washed 3x with 1x PBS. DAPI diluted in 1x PBS was added for 20 min. Sections were washed 3x with 1x PBS. Mounting was done using Cytoseal XYL mounting media. The images were acquired on an LSM710 Confocal AxioObserver Inverted Automated Microscope and analyzed using FIJI software. α-synuclein immunostaining was performed on formalin fixed paraffin embedded tissues by Integrated Oncology (New York; USA). Digital images for α-synuclein were captured using a Tissue Scope™ LE digital pathology scanner (Huron Digital Pathology).

A

Patient	Tissue source	Clinical Diagnosis	post mortem interval (h)
HCT-15-HAX	post mortem PBMCs	Unaffected	19
HBHN	post mortem PBMCs	Parkinsonism/Dementia	20

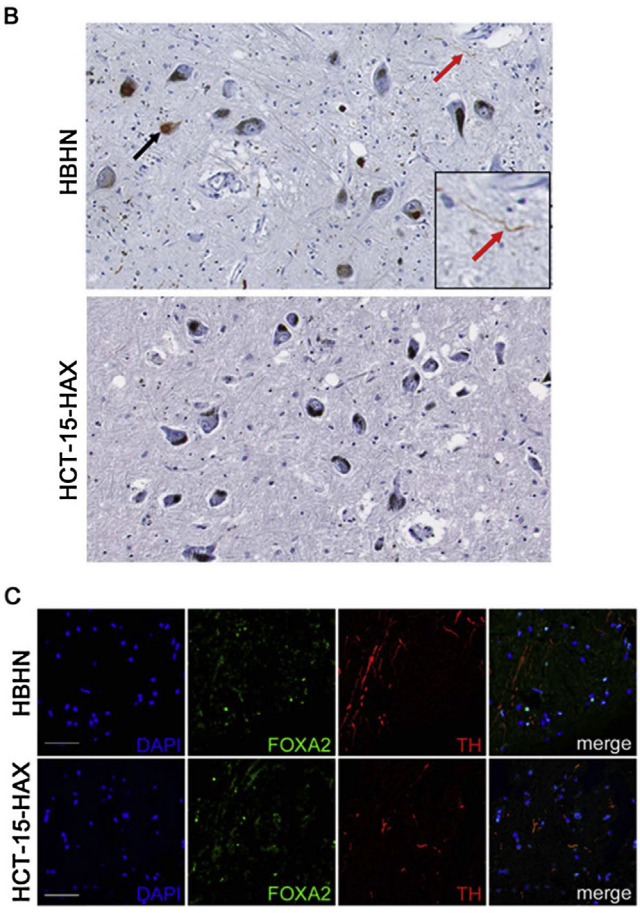

Fig. 1. Neuropathological confirmation of samples used for reprogramming from postmortem Peripheral Blood Mononuclear Cells (PBMCs). Whole blood samples obtained at the time of autopsy from two donors – one clinically diagnosed with PD with dementia and a cogitatively normal individual – were reprogrammed into iPSC lines. A) Patient characteristics including clinical diagnosis and postmortem interval. B) Digital pathscan of α-synuclein immunostaining on paraffin embedded sections of substantia nigra from patient HBHN (above) and HCT-15-HAX control (below). α-synuclein-rich Lewy bodies (black arrow) and Lewy neurites (red arrows) are observed in the presence of nigral degeneration. 400× magnification; inset is 5× original magnification. C) Immunocytochemical (ICC) analysis of patient brain punches confirm extraction of substantia nigra tissue with both punches – affected (HBHN) and unaffected HCT-15-HAX – stained positively for TH and FOXA2. (Scale bars = 50 μm).

2.2. Isolation of Peripheral Blood Mononuclear Cells (PBMCS) from a post −mortem blood sample

All blood samples in this study were drawn and isolated within 20 h of death (Fig. 1A). To isolate peripheral blood mononuclear cells from whole blood, the SEPMATE® (StemCell Technologies) protocol was performed with modifications. Postmortem whole blood starts to coagulate upon death, leaving the sample more viscous than ante-mortem blood samples. To successfully isolate large numbers of PBMCs for reprogramming, whole blood was diluted with equal volume PBS (without calcium and magnesium) containing 4 mM EDTA and 2% FBS. The sample was then added to a SEPMATE-50 tube containing density gradient (Lymphoprep) medium (StemCell Technologies) and centrifuged at 1200×g for 10 min. The top-layer or supernatant is enriched for PBMCs which