Efficient Generation of Induced Pluripotent Stem and Neural Progenitor Cells From Acutely Harvested Dura Mater Obtained During Ventriculoperitoneal Shunt Surgery

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■ BACKGROUND: **The dura mater can be easily biopsied during most cranial neurosurgical operations. We describe a protocol that allows for robust generation of induced pluripotent stem cells (iPSCs) and neural progenitors from acutely harvested dura mater.**

■ OBJECTIVE: **To generate iPSCs and neural progenitor cells from dura mater obtained during ventriculoperitoneal shunt surgery.**

■ METHODS: **Dura was obtained during ventriculoperitoneal shunt surgery for normal pressure hydrocephalus from a 60-year-old patient with severe cognitive impairment. Fibroblasts were isolated from the dural matrix and transduced with nonintegrating Sendai virus for iPSC induction. A subset of successfully generated iPSC clones underwent immunocytochemical analysis, teratoma assay, karyotyping, and targeted neural differentiation.**

■ RESULTS: **Eleven iPSC clones were obtained from the transduction of an estimated 600,000 dural fibroblasts after 3 passages. Three clones underwent immunocytochemical analysis and were shown to express the transcription factors OCT-4, SOX2, and the embryonic cell markers SSEA-4, TRA-1-60, and Nanog. Two clones were tested for pluripotency and formed teratomas at the injection site in immunodeficient mice. Three clones underwent chromosomal analysis and were found to have a normal metaphase spread and karyotype. One clone underwent targeted neural differentiation and formed neural rosettes as well as TuJ1/SOX1-positive neural progenitor cells.**

■ CONCLUSIONS: **IPSCs and neural progenitor cells can be efficiently derived from the dura of patients who need to undergo cranial neurosurgical operations. IPSCs were obtained with a nonintegrating virus and exhibited a normal karyotype, making them candidates for future autotransplantation after targeted differentiation to treat functional deficits.**

INTRODUCTION

Cell restoration therapy with neural stem and progenitor cells holds great promise for the treatment of various neurological and neurosurgical diseases such as stroke, traumatic brain injury, Alzheimer disease or Parkinson disease (2, 3, 11, 12). Transplantation of human neural stem cells from adult donors has proven inefficient in replacing lost neurons after stroke. In contrast to fetal-derived progenitor cells, adult neural progenitor cells predominantly differentiate down a glial lineage pathway and have poor capacity to generate neurons in vivo (14). Even neural progenitor cells derived from the developing cortex lose their ability to form neurons over time and eventually undergo senescence after expansion in vitro (24).

Induced pluripotent stem cells (iPSCs) provide a better means of generating neurons, as these cells are capable of replicating the

Key words
- Dura
- Fibroblast
- Induced pluripotent stem cell
- Neural progenitor cell
- Regenerative medicine
- Shunt surgery

Abbreviations and Acronyms
bFGF: Basic fibroblast growth factor
cDNA: Complementary deoxyribonucleic acid
DMEM: Dulbecco's modified eagle medium
DPBS: Dulbecco's PBS
FBS: Fetal bovine serum
GMP: Good manufacturing practices
Ig: Immunoglobulin
iPSC: Induced pluripotent stem cell
MEF: Mouse embryonic fibroblasts
PBS: Phosphate-buffered saline
RNA: Ribonucleic acid

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period of fetal neuronal production, which is largely complete by midgestation (19). Often, iPSCs are derived from skin fibroblasts (7) or from hematopoietic progenitor cells (26). Neurosurgeons can easily obtain a dural sample during shunt surgery (e.g., hydrocephalus after aneurysmal subarachnoid hemorrhage, hydrocephalus after germinal matrix hemorrhage) or during craniotomies (e.g., hemicraniectomy for traumatic brain injury or stroke). This raises the question whether iPSCs and neural progenitor cells can reliably be generated from dura mater tissue obtained during surgery.

Although the generation of iPSCs from postmortem dura mater has been described before (18), the derivation of iPSCs from a small piece of acutely harvested dura mater for therapeutic purposes has not yet been published. We present the efficient production of iPSCs and neural progenitor cells with normal karyotype from dura mater fibroblasts of a 60-year-old man who underwent ventriculoperitoneal shunt surgery for normal pressure hydrocephalus. Because the patient also suffered from failure to thrive resulting in modified Rankin scale 3 disability, he may be considered as an example of a patient who could benefit in the future from transplantation of his own iPSCs-derived neural progenitor cells using this technique.

METHODS

Dural Harvest

The local institutional review board (#364524-7) and the stem cell research oversight (#1106) committees approved the dural harvest to generate iPSCs. The patient was a 60-year-old man with impaired cognition and a modified Rankin scale of 3 due to failure to thrive in the setting of traumatic brain injury and chronic alcoholism. He cannot read or write and never held a job. He underwent a craniotomy for right frontal hematoma evacuation in 2012 at an outside hospital. He was subsequently diagnosed with normal pressure hydrocephalus because he had ventriculomegaly on magnetic resonance imaging, and his gait improved after a large-volume lumbar puncture. He was scheduled for placement of a left frontal ventriculoperitoneal shunt. The family consented to the dural harvest. During surgery, a left frontal burr hole was placed with a perforator. The edges of the burr hole were undercut with a No. 4 Kerrison punch (Aesculap, Center Valley, Pennsylvania, USA). The dura was not coagulated before the harvest. The dura was harvested as a 1-cm piece with a No. 11 scalpel by cutting the dura in a circular fashion along the edges of the burr hole. The dural specimen was placed in Hank's buffered solution (GIBCO, Grand Island, New York, USA) and stored overnight at 4°C. The sample was frozen to −90°C with a Gordinier Electronics controlled rate freezer (Roseville, Michigan, USA) in 10% dimethyl sulfoxide (Sigma, St. Louis, Missouri, USA), 45% fetal bovine serum (FBS; JR Scientific, Woodland, California, USA), and 45% dura fibroblast medium consisting of 10% FBS, 2 mM glutamax (GIBCO), 0.1 mM 2-mercaptoethanol (GIBCO), and 100 U/mL—0.1 mg/mL penicillin-streptomycin (HyClone, Logan, Utah, USA) in Dulbecco's modified eagle medium: nutrient mixture F12 (DMEM/F12; HyClone).

Fibroblast Isolation and Passaging

The sample was thawed for 1 minute at 37°C and washed twice in phosphate-buffered saline (PBS; HyClone), followed by 2 washes in DMEM (HyClone). The sample was cut into pieces measuring 100 μm to 3 mm using a sterile scalpel. One drop of sterile silicon grease (Corning, Tewksbury, Massachusetts, USA) was added to the center of the wells of a 6-well plate (Corning), and 4—5 pieces of the dura were placed around each drop. A coverslip (Fisher Scientific, Waltham, Massachusetts, USA) was placed on top of the silicon and samples and 2 mL of biopsy culture media consisting of 10% FBS, 2 mM glutamax, 0.1 mM 2-mercaptoethanol, 0.1 mM Minimal Essential Medium Non-Essential Amino Acids Solution (GIBCO), 1× antibiotic-antimycotic solution (GIBCO), and 1× nucleosides (Millipore, Temecula, California, USA) in DMEM was added to each well. The sample was incubated for 6 days at 37°C in 5% CO_2. After 6 days, the medium was switched to 2 mL/well of fibroblast medium, and medium was changed every other day. After 16 days, fibroblasts were confluent and all coverslips were lifted. Fibroblast medium was removed, and cells were rinsed with PBS. Fibroblasts were incubated for 5 minutes with 500 μL TrypLE (1×; Life Technologies, Carlsbad, California, USA), then diluted with 500 μL fibroblast medium and replated in T25 flasks (Corning). Fibroblasts were passaged 3 times before transduction. Fibroblasts were cryopreserved after centrifugation at 1000 rpm for 5 minutes at room temperature and resuspension in 10% dimethyl sulfoxide/45% FBS/45% dura fibroblast medium.

Transduction of Fibroblasts into iPSCs

Fibroblasts were plated into 2 wells of a 6-well plate at a concentration of 300,000 cells per well with fibroblast medium. Cells were transduced 1 day later with the CytoTune 2.0 Sendai virus (Life Technologies) at a multiple of infection of 5:5:3 (KOS:c-Myc:Klf4) and incubated at 37°C overnight. Day 1 after transduction, the medium was replaced with fresh fibroblast medium to remove the CytoTune 2.0 Sendai reprogramming virus (Life Technologies). On days 3 and 5 after transduction, the medium was again replaced with fresh fibroblast medium. On day 6, mouse embryonic fibroblast (MEF) dishes were prepared. Twelve 100-mm dishes were coated for 1 hour at room temperature with 0.1% gelatin (Life Technologies). The gelatin was then aspirated and 10 mL of MEF medium containing 10% FBS (JR Scientific), 2 mM glutamax in DMEM High Glucose (HyClone) with 1.6×10^6 mouse embryonic fibroblasts (GlobalStem, Gaithersburg, Maryland, USA) was added to each dish. On day 7 after transduction, the human fibroblast cells were washed once with Dulbecco's PBS (DPBS) and subsequently lifted from the 6-well plates with 0.5 mL TrypLE Select reagent (Life Technologies) at 37°C. After 2 minutes, 2 mL of fibroblast medium was added, and cells were centrifuged for 4 minutes at 1000 rpm in 15 mL conical tubes (Corning). Cells were resuspended in fibroblast medium and plated at a concentration of 217,500 fibroblasts per 100-mm MEF dish in a total of 12 dishes. One day later, the medium was changed to iPSC medium (20% KnockOut Serum Replacement, 100 nM Minimal Essential Medium Non-Essential Amino Acids Solution, 1% glutamax, 55 nM β-mercaptoethanol, and 30 ng/mL basic fibroblast growth factor (bFGF) in Knockout DMEM/F12; Life Technologies). The iPSC medium was changed every other day for 10 days. Twelve days after plating the human fibroblasts on MEF dishes, 32 iPSC-like colonies were collected manually with a 10-μL pipette tip. They were plated as individual clones in their own wells in 12-well plates. Eleven of the original 32 iPSC-like clones were passaged 6

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