Brain cells Transformed from blood: a cell model for investigating autism

BrainsTorm Study FINAL REPORT

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Brain cells Transformed from blood: a cell model for investigating autism

EXECUTIVE SUMMARY

This study used cutting edge technology to transform white blood cells into induced pluripotent stem (iPS) cells, to provide a neurological cell model that will help us identify cellular differences in an individual with autism. The reasoning behind analyses of neurological cells is to improve the chances of detecting cell differences related to autism. In this study, the following primary outcomes were achieved:

- Staff were upskilled and educated in iPS cell technology and the subsequent generation of neurological cells.
- Our team has established the capability for investigating cellular models of autism
- The study identified and recruited a family that has 1 member on the autism spectrum, and 1 unaffected sibling.
- Blood from the affected and unaffected siblings was used to generate and validate iPS cells, which provide a valuable resource for ongoing studies into the cellular and molecular differences associated with autism.

The outcomes demonstrate the feasibility of our approach, as well as the capability of our team for successfully generating iPS cells from an individual with autism and an unaffected sibling. In addition, the results of this study provide the potential for leveraging future external grant funding of subsequent cohort studies in which varying clinical phenotypes can be identified and analysed. This approach has the potential to increase our knowledge of the molecular defects that underlie the aetiology of autism, allowing the opportunity to sub-classify the disorder and to provide better informed advice about prognosis, as well as seek therapeutic approaches to treatment. In addition, our iPS cell studies, also have the potential to complement the DNA sequence analyses and the RNA profiling from blood, being undertaken by the Autism CRC, as well as provide valuable cellular models of the disorder which can be used to seek pre-clinical testing of small molecule drugs. This project has successfully served to develop the described protocols and analysis procedures.

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The Cooperative Research Centre for Living with Autism (Autism CRC)

The Cooperative Research Centre for Living with Autism (Autism CRC) is the world's first national, cooperative research effort focused on autism. Taking a whole of life approach to autism focusing on diagnosis, education and adult life, Autism CRC researchers are working with end-users to provide evidence-based outcomes which can be translated into practical solutions for governments, service providers, education and health professionals, families and people with autism.

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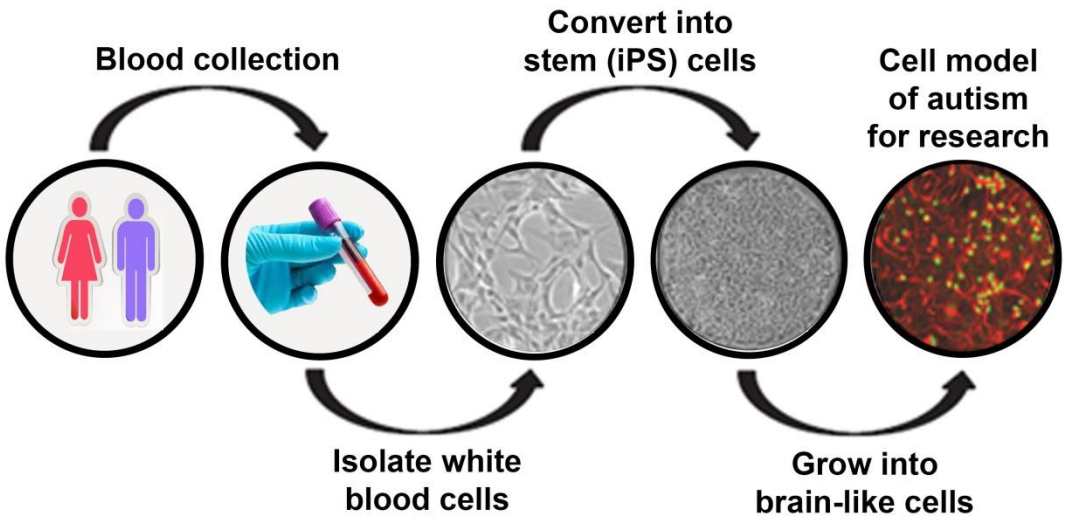
BACKGROUND

The social and financial impact of autism on families and society cannot be underestimated. Accordingly, research is important for working towards understanding the biological basis of autism, which may lead to future therapeutic approaches for reducing the impact of this condition on individuals, their families and carers.

Regardless of cause, the characteristics of autism are manifest in altered function of brain cells, so these are the logical cells for research. Clearly, it is not feasible to obtain brain tissue for research purposes, so we need to consider other strategies for studying brain cells. One approach is to use cutting edge technology to transform blood cells into brain-like cells. This technology involves multiple steps, including the isolation of white blood cells, which are then converted into stem cells. In the body, stem cells are a basic type of cell that can be converted into a range of different cells during development, including brain cells. So the initial steps in our procedure are the reverse of what occurs in the body, with white bloods converted back to stem cells, which can then be converted into almost any cell type, including brain cells. The stem cells generated with this research approach are term “induced pluripotent stem cells” or “iPS cells”.



Human induced pluripotent stem (iPS) cells are emerging as potential cellular models to recapitulate disturbed steps in neurodevelopment that lead to neuropathy. To date, neurological diseases modelled with iPS cells include schizophrenia, Rett’s syndrome, Huntington’s disease, spinal muscular atrophy and Parkinson’s disease (1). Using established protocols that have been previously used for investigating other neurological conditions, this study aimed to generate iPS cells from white blood cells for use as a neurological cell model of autism. The reasoning behind analyses of neurological cells is to improve the chances of detecting underlying differences at a cellular level related to autism.

Whilst there are limitations of interpreting the outcomes of iPS cell research to the *in vivo* situation (within the living organism), it is important to note that similar iPS cell studies have provided suitable models of certain neurological diseases, as well as

providing cellular models for therapeutic drug testing. For example, spinal muscular atrophy (3), Rett's syndrome (4), familial dysautomia (5) and schizophrenia (6). These studies highlight the value of iPS cell research for investigating certain neurological disorders, and warrant investigations of iPS cells to study autism. In this report, we describe the key outcomes of the study, including: i) upskilling and educating staff in iPS cell technology; and ii) generating iPC cells for autism research.

METHODOLOGY

An outline of the methodology is described below:

1. **Staff education and training in iPS cell technology.**
   - Staff undertake approved iPS training courses, and day to day hands-on laboratory training and education.

2. **Ethics, Governance and Legal approvals.**
   - Obtain approvals from the Mater Medical Research Institute, Mater Health Services, Children's Health Queensland Hospital and Health Service, and The University of Queensland.

3. **Patient Recruitment and Blood Sample Collection**
   - Identify and recruit a family that has 1 child with autism and 1 unaffected sibling. Draw 20ml blood from both children.

4. **Cell Reprogramming**
   - Day 1: Transduce cells using Cytotune iPS 2.0 sendai reprogramming kit (Invitrogen A16517) with cell densities of 10k, 50K and 100k (2 wells each).
   - Day 3: Medium change with ReproTeSR.
   - Day 4: Seed Cell on mouse embryonic fibroblast (MEF) feeder layer.
   - Day 5: Media change
   - Day 10: Confirm small clusters of cells via light microscopy.

5. **Selection and Cryopreservation of Clones**
   - Over 2 weeks: Isolate cell clones from each child. Cryopreserve cells using vitrification straw in liquid nitrogen as a back-up stock.
   - Ongoing: Weekly passaging for maintenance of clones.

6. **Pluripotency marker testing**
   - TRA-1-60 (extracellular pluripotency marker) staining was performed using anti-tra160 (Millipore #MAB4360) and alexa fluor 488 goat anti—mouse IgM (Invitrogen #A-21042). Each week, cells were manually passaged.
   - Isolate genomic DNA from clones, as well as white blood cells from each child using a BIOLINE gDNA kit. (ISOLATE II Genomic DNA Kit 50 prep BIOLINE Cat no: BIO-52066). DNA was analysed by Fingerprint testing, to determine identity between the starting material (PBMCs) and the generated clones. Karyotyping was used to ensure normal karyotypes of each clone.
   - Pluripotency was determined by PCR analysis of pluripotency genes, FACS and Immunostaining of cell surface markers and trilineage differentiation capacity in embryoid bodies and teratomas.

OUTCOMES

This study upskilled staff in iPS cell technology, which was applied to transform white blood cells into iPS cells. Outcomes are listed below:

1. **Education and training of a staff member in iPS cell technology.**
   - Completion of an intensive 3 day training program at The University of Queensland.
   - Day to day hands-on training and education (from the published literature) over 1 year

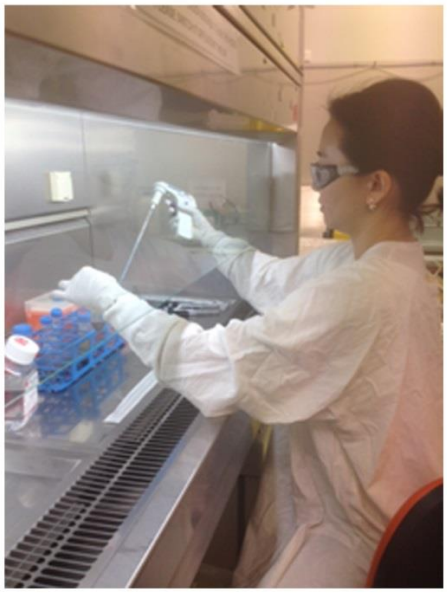
2. **Ethics, Governance and Legal approvals obtained.**
   - Mater Human Research Ethics approval: HREC/14/MHS/211
   - Mater Health Services and Mater Medical Research Governance – Site specific authorization: RG-15-036
   - Children's Health Queensland Hospital and Health Service Governance – Site specific authorization: SSA/15/QRCH/66
   - The University of Queensland Institutional Human Research Ethics Approval: 2015000130.

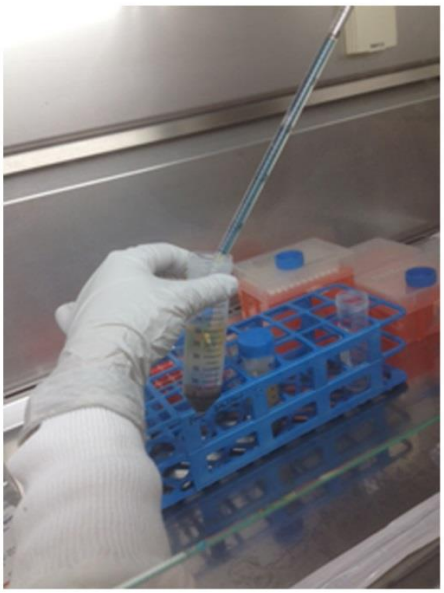
3. **Patient Recruitment and Blood Sample Collection**
   - Parents provided informed consent for their children to donate a blood sample for testing and storage of cells.
   - 20ml blood was collected from each of two children, 1 child with autism and 1 unaffected sibling. White blood cells (PBMCs) were isolated and erythroid progenitor cells (EPCs) were further purified. A portion of the PBMCs was stored in liquid nitrogen for future testing.

4. **Cell Reprogramming**
   - Following cell transduction, and seeding on MEF feeder layers, small clusters of cells were visible by Day 10.

5. **Selection and Cryopreservation of Clones**
   - A total of 40 clones were generated (20 from each child). Portions of these clones were cryopreserved in liquid nitrogen.
   - Based on cell morphology (shape and structure of cells), 12/40 clones were further characterized.

6. **Pluripotency marker testing**
   - Of 40 clones, 30 (15 from each child) were selected (based on morphology) to carry forward.
   - From a total of 30 clones, genomic DNA was isolated from 12 clones (6 from each child), as well as stored frozen PBMCs from each child. DNA Fingerprint testing showed conserved identity between the starting material (PBMCs) and the generated clones. Numbering and classification of the chromosomes (karyotyping) was performed on 8 clones (4 from each child), of which all were confirmed to be normal karyotype.
   - Pluripotency testing confirmed the clones were iPS cells.





Blood samples are processed in the laboratory, with the initial step being isolation of the white blood cells.

SUMMARY

This study upskilled laboratory staff and researchers in iPS cell technology, which was applied to transform white blood cells into iPS cells, an undertaking that has not previously been explored for autism. These iPS cells provide a valuable resource for further studies, whereby selected iPS cell clones from each individual, will be directed to differentiate into brain-like cells (neurons and glial cells) using established techniques. The outcomes confirm the feasibility of our approach for generating iPS cells from blood, and thereby the potential for undertaking subsequent cohort studies in which varying clinical phenotypes of autistic individuals can be identified and analysed between families. This approach has the potential to link specific cellular abnormalities to certain neurological features, thereby helping us to further understand the clinical spectrum of autism which is still poorly understood.

In addition, this approach has the potential to increase our knowledge of the molecular differences that underlie the aetiology of autism, allowing the opportunity to sub-classify the disorder and to provide better informed advice about prognosis, as well as seek therapeutic approaches to treatment. In addition, our iPS cell studies, also have the potential to complement the DNA sequence analyses and the RNA profiling from blood, being undertaken by the Autism CRC, as well as provide valuable cellular models of the disorder which can be used to seek pre-clinical testing of small molecule drugs. This project has successfully served to develop the described protocols and analysis procedures.

References

(1) Robinton, D.A. and G.Q. Daley, The promise of induced pluripotent stem cells in research and therapy. Nature, 2012. 481(7381): p. 295-305.
(2) Volterra, A. and J. Meldolesi, Astrocytes, from brain glue to communication elements: the revolution continues. Nat. Rev. Neurosci., 2005. 6(8): p. 626-640.
(3) Chang, T., et al., Brief report: phenotypic rescue of induced pluripotent stem cell-derived motoneurons of a spinal muscular atrophy patient. Stem Cells, 2011. 29(12): p. 2090-2093.
(4) Marchetto, M.C., et al., A model for neural development and treatment of Rett syndrome using human induced pluripotent stem cells. Cell, 2010. 143(4): p. 527-539.
(5) Lee, G. and L. Studer, Modelling familial dysautonomia in human induced pluripotent stem cells. Philos. Trans. R. Soc. Lond. B. Biol. Sci., 2011. 366(1575): p. 2286-2296.
(6) Brennand, K.J., et al., Modelling schizophrenia using human induced pluripotent stem cells. Nature, 2011. 473(7346): p. 221-225.