Journal of Neuroscience Methods 268 (2016) 125–130

Contents lists available at ScienceDirect

Journal of Neuroscience Methods

journal homepage: www.elsevier.com/locate/jneumeth

Expression microdissection isolation of enriched cell populations from archival brain tissue

C. Appleby-Mallinder, M.D. Wyles, J.E. Simpson, S.B. Wharton, P.G. Ince, P.R. Heath*

Sheffield Institute for Translational Neuroscience, University of Sheffield, UK

HIGHLIGHTS

- Expression microdissection (xMD) reduces issues of laser capture microdissection.
- xMD reduces procedure length compared to LCM.
- xMD can isolate immunopositive central nervous system cells (CNS) from FFPE tissue.
- RNA integrity number suggests RNA is of sufficient quality for further analysis.
- CNS cells isolated by xMD show enriched populations, confirmed by RT-PCR analysis

ARTICLE INFO

Article history:
Received 5 January 2016
Received in revised form 26 April 2016
Accepted 4 May 2016
Available online 10 May 2016

Keywords:
Expression microdissection
Laser capture microdissection
Neurones
Astrocytes
RNA
RNA integrity number

ABSTRACT

Background: Laser capture microdissection (LCM) is an established technique for the procurement of enriched cell populations that can undergo further downstream analysis, although it does have limitations. Expression microdissection (xMD) is a new technique that begins to address these pitfalls, such as operator dependence and contamination.
New method: xMD utilises immunohistochemistry in conjunction with a chromogen to isolate specific cell types by extending the fundamental principles of LCM to create an operator-independent method for the procurement of specific CNS cell types.
Results: We report how xMD enables the isolation of specific cell populations, namely neurones and astrocytes, from rat formalin fixed-paraffin embedded (FFPE) tissue. Subsequent reverse transcriptase-polymerase chain reaction (RT-PCR) analysis confirms the enrichment of these specific populations. RIN values after xMD indicate samples are sufficient to carry out further analysis.
Comparison with existing method: xMD offers a rapid method of isolating specific CNS cell types without the need for identification by an operator, reducing the amount of unintentional contamination caused by operator error, whilst also significantly reducing the time required by the current basic LCM technique.
Conclusions: xMD is a superior method for the procurement of enriched cell populations from post-mortem tissue, which can be utilised to create transcriptome profiles, aiding our understanding of the contribution of these cells to a range of neurological diseases. xMD also addresses the issues associated with LCM, such as reliance on an operator to identify target cells, which can cause contamination, as well as addressing the time consuming nature of LCM.

1. Introduction

It has long been recognised that in order to unravel the underlying pathologies of central nervous system (CNS) disorders, it is necessary to identify the contributions of individual cell types to the overall disease process. We previously reported an immuno-LCM technique that combines rapid immunostaining with standard laser capture microdissection (LCM) to enable the isolation of an enriched population of cells from frozen post-mortem tissue (Waller et al., 2012). Using this approach, we have carried out a

Abbreviations: CNS, central nervous system; LCM, laser capture microdissection; EVA, ethylene vinyl acetate; DAB, 3′3-diaminobenzidine; xMD, expression microdissection; FFPE, formalin fixed paraffin embedded; RT-PCR, reverse transcriptase polymerase chain reaction; ABC, avidin/biotinylated complex; DEPC, diethylpyrocarbonate; TBS, Tris-buffered saline; UV, ultra violet; RIN, RNA integrity number.

* Corresponding author at: Sheffield Institute for Translational Neuroscience, 385A Glossop Road, Sheffield S10 2HQ, UK. Fax: +44 114 222 2290.
E-mail address: p.heath@sheffield.ac.uk (P.R. Heath).

http://dx.doi.org/10.1016/j.jneumeth.2016.05.007
0165-0270/

number of studies to enrich for populations of specific CNS individual cell types to compare the gene expression profile of disease and non-disease cells, including oligodendrocytes (Asi et al., 2014), astrocytes (Garwood et al., 2015) and neurones (Highley et al., 2014; Simpson et al., 2015). This approach has been useful in identifying disease-relevant gene expression related changes which can then be investigated further, but the protocols are somewhat lengthy and it can, on occasion, be difficult to confidently identify certain cells.

The development of the expression microdissection (xMD) technique (Tangrea et al., 2004), later refined (Hanson et al., 2011), offers a new approach to streamline and simplify the isolation of specific cell populations, removing the need for a microscope or cell identification by the user, making it an operator independent process. Similar to the LCM preparation process, for xMD, tissue sections undergo Immunohistochemistry for a cell-type-specific antigen. Tissue sections are then covered with an ethylene vinyl acetate (EVA) polymer film and the whole tissue is irradiated with a low energy infra-red laser. When the laser is fired over an immunopositive cell, the EVA film melts, causing a thermoplastic bond to occur between the EVA film and the immunopositive cell, adhering the cell to the EVA film. The film can then be removed from the tissue, taking with it only the immunopositive cells that have formed a thermoplastic bond with the EVA polymer, after which they can then be placed in extraction buffer for further downstream analysis (Fig. 1).

In the current study, formalin-fixed paraffin embedded (FFPE) post-mortem tissue was used to highlight how this resource can be used in conjunction with the expression microdissection technique to acquire enriched central nervous system (CNS) cell populations, namely neurones and astrocytes. For the purpose of this proof of principle study the neurone marker NeuN and a widely used astrocyte marker GFAP were the antibodies of choice. Histopathologically well-characterised archival FFPE tissue is currently an underutilised resource that has the potential to unravel underlying mechanisms contributing to disease pathogenesis. To date, two studies have been conducted utilising this method as a means to isolate specific cell types (Grover et al., 2006; Hanson et al., 2006). However, neither of these studies isolated CNS cells, making the method described here novel for these specific cell types.

This paper describes the detailed methodology of xMD which can be used to isolate enriched populations of specific cell types from FFPE tissue, and confirms this enrichment by RT-PCR. Future studies can potentially use this technique in well characterised archived FFPE cohorts to investigate the contribution of specific cell types to the pathogenesis of a range of neurological diseases.

2. Materials and methods

2.1. Rat brain tissue

Formalin fixed paraffin embedded (FFPE) post mortem rat brain tissue blocks were obtained from 6 Sprague Dawley adult rats. Cell isolation was carried out from the hippocampal region.

2.2. Immunohistochemistry

5 μm sections were collected onto sterile charged slides. Sections were dewaxed in xylene and rehydrated to DEPC-water in a graded series of alcohols (100%, 100%, 95%, 70% EtOH for 5 min each). Immunohistochemistry was used following the standard avidin/biotinylated enzyme complex (ABC) staining method (Vector Laboratories, UK). All work was carried out at room temperature (RT) under RNase-free conditions. Sections were blocked in 1.5% relevant normal serum for 30 min, then incubated in the appropriate specific antibody (glial fibrillary acidic protein [Dako catalogue number Z0334] 1:500; NeuN [Chemicon catalogue number MAB377] 1:100) diluted in blocking serum for 1 h before washing with Tris-buffered saline (TBS) for 5 min. Sections were then incubated with 0.5% biotinylated secondary antibody for 30 min, washed with TBS for 5 min, and then incubated with 2% horse-radish peroxidise conjugated ABC for 30 min and washed with TBS for 5 min. Antibody staining was visualised with 3,3′-diaminobenzidine (DAB) as chromogen (Vector Laboratories, UK) for 3 min. Sections were rinsed in DEPC-water and dehydrated in graded alcohols (70%, 95%, 100%, 100% for 15 s each) then cleared in xylene for 5 min. Sections were left to air dry in an air flow hood for approximately one hour prior to baking. Each antibody was optimised in order to ensure a specific pattern of immunoreactivity was achieved with minimal background staining. The optimisation process included the appropriate negative controls with primary antibody exclusion and an isotype control to ensure there was no non-specific reactivity.

2.3. Film and slide preparation for microdissection

CoTran 19% ethylene-vinyl acetate (EVA) film (3M, cat. No. 3M CoTran 9715) was sterilised in an ultraviolet (UV) air flow hood for 20 min. Using a sterile scalpel and forceps, the film was positioned over the slide so that all the tissue section was covered, and the film was cut to fit the slide, leaving a 2–3 mm border to prevent the film catching on the LCM system. After placing the slides in a pre-warmed oven for 10 min at 60 °C, they were taken out and pressure was applied using a roller to increase the film-section contact area. Slides were then placed back in the oven for a further 10 min.

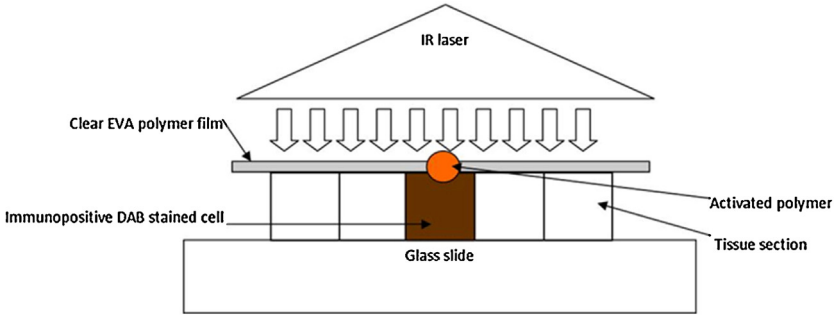

Fig. 1. Schematic representation of xMD. Immunopositive cells with the bound DAB chromogen are covered with a clear EVA polymer. The entire tissue is then irradiated with the IR laser of a LCM system. The EVA polymer melts at the site of the immunopositive cell due to the increased temperature caused by the chromagen DAB. This then bonds the EVA polymer film to the target cells.