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Abstract

Multilayer Immunohistochemical Analysis of Brain Tissue in Severe Traumatic Brain Injury †

Chloe L. T. Kam [1], Cengiz Guner [1], Shumaila Hasan [2], Christopher E. G. Uff [1,2] and Ping K. Yip [1,*]

[1] Centre for Neuroscience, Surgery and Trauma, Blizard Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London E1 2AT, UK; chloekam2004@gmail.com (C.L.T.K.); cngzgner@hotmail.com (C.G.); c.uff@qmul.ac.uk (C.E.G.U.)
[2] Department of Neurosurgery, Royal London Hospital, Whitechapel, London E1 1FR, UK
* Correspondence: p.yip@qmul.ac.uk
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1. Background and Aims

Severe traumatic brain injury (TBI) is a complex disease, and understanding its injury-induced cellular pathobiology is vital to predicting outcomes and providing effective treatment and precision healthcare. This can be achieved through studying brain tissue obtained from severe TBI patients at biopsy soon after injury, which has been proven to be a safe procedure. Distinguishing the various cellular markers within the biopsy tissue can provide us with a better understanding of the complex cellular interactions involved in TBI.

2. Methods

Using multilayer immunohistochemistry, 12 immunostaining markers were applied on brain tissue obtained at biopsy (n = 2). One was from a patient who recovered, and one from a patient who died. The cryostat-sectioned tissue was stained with two immunostaining markers per staining procedure and imaged using a fluorescence microscope. Thereafter, the immunostaining was removed using a stripping buffer, and the successful removal of immunostaining was confirmed by the absence of the previous immunostains. The staining and stripping process was then repeated to achieve an additional 5 sets of double immunostaining.

3. Results

The multilayer immunohistochemistry using markers for neurones (NeuN, N52, SMI31, SMI32, MAP2, somatostatin), oligodendrocytes (CNPase), astrocytes (GFAP), microglia (Iba1, P2Y12), and vasculature (claudin5, vWF) successfully stained the same brain tissue section. Merging the immunostaining images together allowed for the visualisation of the complex cellular interactions, and the increased or decreased expression levels of immunostaining markers between a patient with a good functional outcome and another patient with a poor functional outcome.

4. Conclusions

Multilayer immunohistochemistry enabled the identification and simultaneous visualisation of 12 immunostaining markers on a single brain biopsy. This is the first study to conduct a cheap multilayer immunohistochemistry technique on brain biopsies from patients with severe TBI.

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Informed Consent Statement: All patients were unconscious at the time of enrolment and in line with the declaration of Helsinki, the 2005 UK Mental Capacity Act and the REC approved protocol, the next of kin were asked to act as a personal consultee, or if none were available, a senior doctor was asked to act as an Independent Healthcare Professional (IHP) consultee.

Data Availability Statement: Data available on request.

Conflicts of Interest: The authors declare no conflict of interest.