Microscopy & Microtechniques Focus

INVESTIGATING THE MOLECULAR NEUROPATHOLOGY OF BATTEN DISEASE

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Around the world, the search is on for the molecular mechanisms underlying diseases. Despite some notable successes, there are very few therapeutic options for a group of more than 40 neurodegenerative illnesses.

Each is characterised by lysosomal dysfunction and the build up of undigested storage material, typically affecting children or young adults and proving fatal after a long period of disability.

> ***"WE USE THE MICRO DISSECTION TO RECOVER BOTH SINGLE CELLS AND ENTIRE POPULATIONS OF CELLS WITHIN TISSUE TO LOOK AT DIFFERENTIAL EXPRESSION PATTERNS."***

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Batten disease is an important member of this group of diseases known as lysosomal storage disorders and is the most common inherited disorder of childhood, first identified in 1826. Rather than a single disease, Batten disease is a group of at least nine closely related storage disorders, also known as the Neuronal Ceroid Lipofuscinoses (NCLs). These different forms of NCL first impact affected individuals as infants and children, but rarer forms that occur in adulthood also exist. Although starting at radically different ages, the childhood forms of NCL share many common features including blindness, increasingly more severe seizures and an inexorable decline in mental and physical function before an inevitable early death.

Each of the nine identified forms of NCL is due to a single gene defect in the genes that code either for lysosomal enzymes (congenital, infantile and late infantile forms) or novel proteins of unknown function (juvenile and variant forms) that sit within the endosomal-lysosomal system or other intracellular compartments. The end result of these gene defects is the build up of autofluorescent storage material within the lysosome, but very little is known about how these events lead to the devastating effects of the disease upon the brain and affected individual.

PSDL

The Pediatric Storage Disorders Laboratory (PSDL) is the leading laboratory internationally for the morphological analysis of human, large and small animal models of NCL and works closely together with colleagues in the US, Europe and New Zealand to understand the neurobiology of these devastating disorders. To investigate the molecular mechanisms that operate in each form of NCL we are using a variety of genetically engineered or naturally occurring mutant mice and sheep, comparing our results to the human disease. Using morphological and molecular methods we are building a detailed picture of events within the NCL brain, together with biochemical and behavioural studies of these disease models. These studies provide landmarks of disease progression, telling us where and when the brain is impacted, which can also be used to judge whether therapeutic strategies have been successful.

To obtain morphological data, our Nissl stained or immunohistochemically stained brain sections are viewed on either a Zeiss Axioskop 2MOT or Zeiss AxioPlan, equipped with AxioCam HRc digital cameras and AxioVision software. We also routinely perform Quantitative Thresholding Image Analysis on captured images using Image Pro Plus software from Media Cybernetics. Unbiased stereological estimates of a variety of parameters in the brain (e.g. Cavalieri estimates of regional volume, optical fractionator estimates of neuronal number) and the assessment of neuronal morphology in 3D dimensions are carried out on a Zeiss Axioskop 2MOT microscope equipped with high precision motorised stages, z-focus drive and StereoInvestigator and Neurolucida software from Microbrightfield.

Although the effects upon the brain in Batten disease are devastating, using these unbiased stereological approaches, we are learning that neurons are remarkably selectively affected, especially early in the disease. We have seen that subpopulations of inhibitory neurons are particularly sensitive in the cortex and hippocampus, but have more recently revealed that the thalamus is a particular focus for the effects of Batten disease displaying a variety of pathological changes before its target cortical regions. It is also clear that there is a subtle interplay between localised neuron and glial cell populations; with an early activation of astrocytes before neuron loss occurs, followed by a profound microglial response.

It appears that it is not only the type of cell, but where these cells are located that influences how any individual cell will response to the disease. Nevertheless, it is not yet clear why different neuron subpopulations of the NCL brain should display such selective effects.

DISSECTING OUT THE MOLECULAR BASIS OF VULNERABILITY

To begin understanding the molecular basis of this selective neuronal vulnerability in the NCLs, our current work involves looking at the molecular make up of susceptible cells via their altered gene expression profiles. This involves the collection of individual cells from vulnerable or resistant neuron populations and using single PCR analysis to investigate the expression of a variety of candidate genes identified from previous microarray studies.

In this work we are using the PALM® MicroBeam™ from Carl Zeiss to dissect either single cells for real time PCR analysis of gene expression or for the extensive enrichment of particular neuronal cell types for comparative micro-genomics and proteomics. This microdissection and pressure catapulting system, also known as LMPC, is ideal for this purpose allowing non-contact, and thus contamination-free, isolation of individual cells from slide mounted brain tissue sections.

The LMPC process utilises a single defocused laser pulse to optically eject laser microdissected specimens from the slide surface and into the lid of a specially prepared 500Ìl microfuge tube. Microdissected cells arrive in the microfuge tube lid with their morphology preserved and ready for the extraction of biomolecules.

An example of this process is shown in *Figure 1*, where an immunohistochemically identified microglial cell is shown *(Figure 1A)*, marked for microdissection *(Figure 1B)* and subsequently cut from the tissue section *(Figure 1C)*, arriving intact in the microfuge tube lid *(Figure 1D)* for subsequent analysis. In addition to studying neuronal vulnerability we shall be increasingly using this system to investigate the post-transplantation fate of neural stem cells and how this process may be influenced by the environment of a diseased brain.

For some approaches we want to undertake, for example microarray or proteomics (GE/MS/LCMS), we require a relatively large number of cells for adequate signal detection.

To achieve this we aim to harvest and pool together sufficiently large numbers of discretely distributed neurons. The high-throughput (HT) technology available on the PALM MicroBeam, enables us to employ advanced image recognition software with pre-programmed detection algorithms in order to scan tissue sections quickly, to identify and locate target cells across the whole tissue section area.

Large numbers of prescribed cells, such as that shown in Figure 1B, can then be automatically laser microdissected and optically ejected into a multiplex recovery device – the

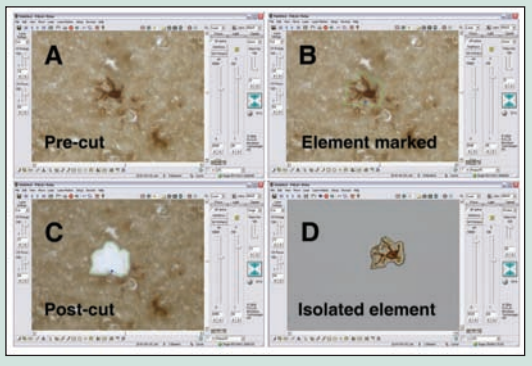

Figure 1. Laser microdissection of an immunohistochemically identified microglial cell from human NCL post mortem material.

(A) An interleukin 1-β, immunoreactive microglial cell in the cortex of a human juvenile NCL case viewed under a 40x cutting objective and the PALM® Robo software interface.

(B) As in this instance the object chosen for cutting can be selected manually (green line drawn freehand, with laser pressure catapulting point shown in blue), or alternatively recognised automatically via algorithms set with PALM MicroBeam HT™ software (with the user retaining the option to accept or reject selected objects).

(C) Following laser cutting and catapulting (RoboLPC feature) the selected element has been excised from the tissue and optically ejected.

(D) Microdissected and catapulted microglial cell visualised in the microfuge collection cap held in the RoboMover™. Isolated cells may then be subject to a variety of downstream applications.

RoboMover™ , which is capable of collecting cells into a range of capture formats. Precise positioning of the RoboMover and the RoboStage™ is synchronised via the PALM RoboSoftware, allowing complete control of recovery targets, volume, and sequence. We use the micro dissection to recover both single cells and entire populations of cells within tissue to look at differential expression patterns.

However, the ability to isolate live, single cells and cellular entities in a sterile environment is prompting other researchers to revisit laser microdissection, for example to dissect adult and embryonic stem cells that can subsequently be expanded into cell lines for implantation in animal models.

For this work, the importance of maintaining sterile conditions and eliminating cross-infection is paramount. Laser microdissection can also be used to penetrate the cell and obtain subcellular moieties. Using a high-magnification lens along with a laser provides sufficient resolution to cut and retrieve a region less than a micrometer across, which is smaller than the diameter of the nucleus inside a cell. This set-up can be used to retrieve part of metaphase chromosomes or to monitor nuclear trafficking of drugs.

CONCLUSION

Using laser microdissection it will be possible for us to combine both morphological and molecular methodologies to shed more light upon events within the Batten disease brain.

Combining these two powerful approaches should enable us to make significant steps towards understanding these profoundly disabling disorders, a process that we hope will be accelerated by recent advances in the hardware and software for laser microdissection.

Figure 2. PALM® microdissection equipment from Carl Zeiss
**Patented by PALM*

ACKNOWLEDGEMENTS

In addition to grants from the US National Institutes of Health, The Wellcome Trust and European Union, the work of the Pediatric Storage Disorder Laboratory is also supported by the UK Batten Disease Family Association (BDFA) and the Batten Disease Support and Research Association (BDSRA), its equivalent organization in the US. These non-profit organisations support and help families, carers and professionals who have contact with children and young adults with Batten disease. The BDFA and BDSRA are run primarily by volunteers who are parents of children with Batten disease. Further information about Batten disease may be found at the following websites www.bdfa-uk.org.uk and www.bdsra.org . More information about work in the Pediatric Storage Disorders Laboratory can be found at http://neuroscience.iop.kcl.ac.uk/psdl

Quick and Easy Identification of Crop Pests

Syncroscopy is pleased to announce that Auto-Montage Pro, its premier 3D image reconstruction software, is being used at a major US plant quarantine enforcement centre, Los Angeles County Department of Agricultural Commissioner/Weights and Measures, to produce perfectly in-focus images, ensuring rapid and accurate identification of crop pests.

Researchers in the Entomology Laboratory at the Los Angeles County Department of Agricultural Commissioner/Weights and Measures are using Auto-Montage Pro to generate focused images of important agricultural pests including snails, slugs, beetles, moths, weevils and ants entering California's ports and airports.

Many of these images are being used by quarantine inspectors to make crucial decisions on whether or not to quarantine agricultural crop and commodity shipments containing these pests.

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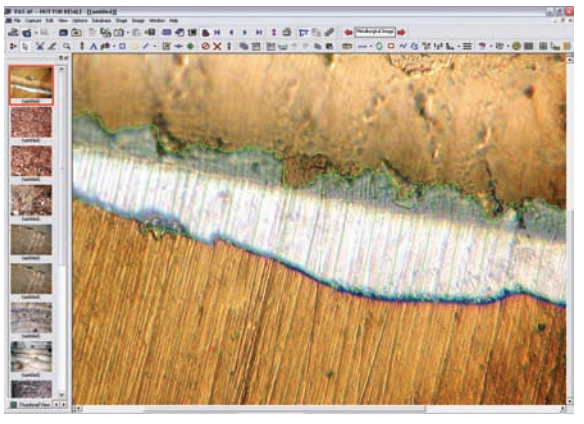

The acquisition, management and analysis of images taken with a microscope has never been easier. The introduction of Pax-it software from **Prior Scientific** offers unsurpassed flexibility in image management and storage thanks to a totally user definable database system. If you have ever asked yourself "Where's that image?", or wondered how best to keep track of an ever growing collection of images, then Pax-it is for you !

With PAX-it software you can capture and archive image files from microscopes using your existing camera or the impressive PAX-cam range of USB 2.0 Digital cameras. PAX-cam cameras are available with a range of resolutions up to 20 Mega Pixels, suitable for a vast range of applications and budgets. Being USB 2.0, power is supplied directly from the PC ensuring easy installation and less clutter on the bench.

PAX-it also permits importing of images from other sources such as digital cameras for macroscopic work or flatbed scanners for example. All files from a particular patient or project, for example, can then be held in one location. The powerful database functions then enable fast and easy location of all relevant files. To make your life even easier, images or data from PAX-it can then be incorporated into Word documents, Powerpoint presentations and Excel spreadsheets quickly and easily from within PAX-it.

PAX-it is a highly flexible system offering expanded capabilities through a series of bolt-on modules. These include measurement, image analysis and extended security which ensures compliance with stringent CFR-21 regulations. If you are considering a new microscope imaging system or simply want to upgrade an existing microscope, contact Prior to see how PAX-it can solve your imaging requirements.

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