PERSPECTIVE

Laser capture microdissection: from its principle to applications in research on neurodegeneration

History and the principle of laser capture microdissection (LCM): LCM, also known as laser microdissection and pressure catapulting, is one of the most powerful and useful techniques in various research areas where isolation of heterogeneous cell population is required. The first use of laser as a cell operation method, was originated in early 1920s (Gilbrich-Wille, 2013), and it became widely used as a microsurgical tool in early 1960s. In the need of extremely small tissue isolation, laser beam of LCM has been modified and developed over several decades from Ultraviolet (UV) laser beam to high-energy nitrogen, infrared and carbon dioxide lasers (Gilbrich-Wille, 2013). Due to the development of its hardware, which combines a laser unit and a microscope, tissue preparation technique also has improved from manual preparation to histological sections in 1980s and this has brought numerous advantages in biochemistry and molecular biology research (Gilbrich-Wille, 2013).

There are two different laser types in LCM: Infrared/(IF) and UV. Although tissue preparation and capturing methods of LCM can be different between laser types, they share common fundamental principles: visualisation and selection of tissue of interest with a microscope connected to LCM, laser transfer for excising the cells, and capturing into a collection tube. Here, we focus on the principle of LCM with UV laser system (PALM microbeam, Carl Zeiss).

Tissue preparation is important in LCM and it should be free of contaminants (*i.e.*, nucleases). Tissues can be formalin fixed and paraffin embedded, or prepared as a frozen block in optimum cutting temperature medium. Tissue blocks can be sectioned onto polyethylene naphthalate membrane coated slides and various staining methods such as hematoxyln and eosin stating, fluorescence *in situ* hybridisation, or immunohistochemistry can be applied for histological visualisation. The slides are then moved to a microdissection chamber, which is equipped with an energy adjustable UV laser, an automated slide-moving plane and a robotic arm to hold a collection tube. Followed by selection of cells of interest, the cells are excised with UV beam and a light catapult transports the cells into a collection tube. The catapult is designed to minimise potential modifications of cellular components by short catapulting (1 nanosecond), no contact with other cells, and more importantly, no extreme temperature changes. Unlike infrared laser system, which can increase temperature up to 90°C and potentially cause damages on cellular materials, keeping a constant ambient temperature is one of the greatest advantages of using UV laser. Then the cells are transferred to lysis buffer for downstream analysis.

Advantages and disadvantages of LCM: LCM has numerous advantages. It enables accurate separation of extremely small amount of cells (*i.e.*, homogeneous cell population from heterogeneous population), or a single cell isolation. Due to the recent improvement of optic resolution in LCM, even the cell organelles can be isolated these days (Chen et al., 2009; Satori et al., 2012). LCM also enables separation of live cells or a single cell in a culture dish and re-culturing them. It also can preserve the tissue morphology while dissecting. LCM is also a quicker cell separation method than other microdissection methods, which is crucial for preserving genomic molecules.

Although LCM brought a numerous advantages in biomedical research, its disadvantages still exist. Firstly, it is one of the most expensive tools. LCM with a microscope can cost more than a million dollars, and the rest of the consumables, such as nuclease free membrane slides and collecting tubes, are more than 5 times more expensive than normal consumables. Also, there is a considerable risk that the quality of microdissected tissues may not meet the standard quality for further analysis due to its exposure to fixatives and staining reagents. Hence, dehydration of the sections caused by absence of coverslips and mounting medium onto sections, also may influence the quality of cellular materials as well as the morphology of the section. Thus, researchers should consider spending substantial amount of time in training and troubleshooting in order to ensure the quality results.

Applications of LCM in neuroscience research: LCM is widely used in various medical research areas from neuroscience, cancer, forensic science research to biomarker discovery and clinical diagnostics. Its applications in these areas are mainly based on molecular biology such as genetics and proteomics. In genetics, various genetic materials such as mRNA, DNA, and microRNA, can be extracted from captured cells/tissues, and used for various applications (*i.e.*) quantitative real time polymerase chain reaction, microarray gene chip analysis, representation difference analysis, expression sequence tags, and serial analysis of gene expression (also known as SAGE) (Domazet et al., 2008). In proteomics, as an apparelled approach of genomics, proteins extracted from LCM tissues can be used for 2 dimensional polyacrylamide gel electrophoresis, mass spectrometry, western blotting and peptide sequencing (Domazet et al., 2008).

Selective vulnerability is one of the main features in many neurodegenerative diseases (Standaert, 2005). The pathogenesis of neurodegenerative diseases is mainly characterised by degeneration/disorder of a particular neuron type whereas the rest of the cells are relatively not affected. Although comprehensive understanding in degeneration mechanisms of affected neurons is required in order to study the precise pathogenesis of diseases and the development of new treatments, selective vulnerability created technical difficulties. Despite the challenges, research with LCM resolves the problem with an accessible method by

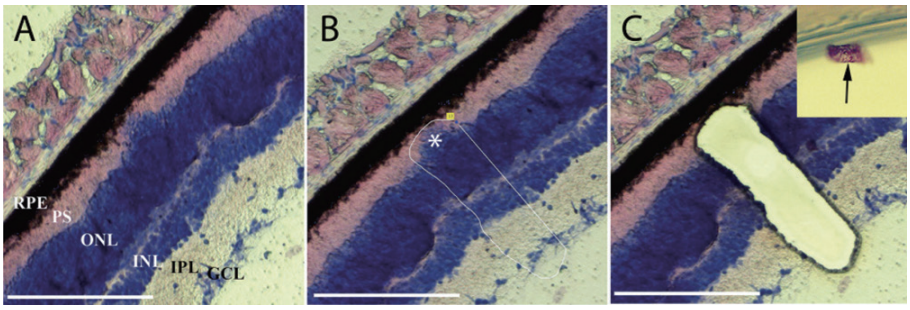

Figure 1 The figure shows the use of laser capture microdissection in the retina where selective Müller cell ablation leads to photoreceptor degeneration (Chung et al., 2013).
(A) A section was fixed with alcohol and the morphology was exposed with histogene staining solution. (B) Identification of an area where photoreceptor cell bodies protruded into the subretinal space (asterisk) before microdissection. (C) A small tissue was cut with an ultraviolet microbeam and a laser catapult was used to capture the dissected tissue into a collecting tube (inset, arrow). RPE: Retinal pigment epithelium; PS: photoreceptor segments; ONL: outer nuclear layer; INL: inner nuclear payer; IPL: inner plexiform layer; GCL: ganglion cell layer. Scale bars: 150 μm.

separating the affected cells from the tissue. Here, we present some interesting examples of applications of LCM in neurodegenerative diseases.

LCM in proteomic analysis: Alzheimer's disease is a neurological disorder, which impairs cognitive function and disturbs emotion and personality (Liao et al., 2004). Histopathological analysis revealed that accumulation of amyloid plaques in neurons in the brain plays a critical role in the pathogenesis of the disease (Liao et al., 2004). There have been, however, few limitations to perform more precise investigations on the pathogenesis of the diseases due to the previous plaque purification methods to remove the essential protein, which identifies amyloid plaque itself, from the tissue (Liao et al., 2004). Liao et al. (2004) applied LCM to isolate amyloid plaques labelled with thioflavin-S from post-mortem samples and performed liquid chromatography in combination with tandem mass spectrometry to identify protein compositions of the plaque compared with the surrounding tissues. The proteomics analysis with protein extracts from LCM samples revealed noble 26 proteins enriched in the plaques compared with the surrounding tissues, and Liao et al. (2014) also compared the proteins from the human post-mortem samples and transgenic murine model of Alzheimer's disease, which revealed possible roles of neuronal transport in neuritic degeneration in the pathogenesis of the disease. Combination of LCM with proteomic analysis in this study led to a comprehensive analysis of protein composition of the plaques and permitted the direct identification of several hundreds of proteins from less than 5 μg of total plaque proteins. By applying other types of staining methods, Liao et al. (2014) suggested possible applications of the technique to investigate other types of plaques such as diffused versus primitive forms, to determine proteins involved in each stage of plaques aggregation in addition to illustrate the molecular event that initiates the plaque formation.

LCM in diagnostics: Another interesting example of LCM application is to use it as a diagnostic tool in neural stem cell therapy. Amariglio et al. (2009) reported a patient case with ataxia telangiectasia in which the patient developed a multifocal brain tumor 4 years after receiving intracerebellar and intrathecal injection of human fetal neural stem cells. As the main concern of stem cell therapy is the development of teratocarcinoma, a type of tumors developed from transplanted stem cells, the authors used biopsy samples from the patient and performed a genomic analysis to prove that the tumor was not the host origin but from the stem cell donors. With LCM, as one of the diagnostic tools, Amariglio et al. (2009) performed a MALDI-TOF mass spectrometry to genotype a known gene mutation for ataxia telangiectasia (ATM C103T) and single nucleotide polymorphism (SNP) in order to compare genomic DNA from a number of sample groups, including peripheral blood from the patient's parents and the patient, tumor tissues and LCM-isolated tumor cell nuclei. Their results showed that DNA isolated from LCM-isolated tumor cell nuclei carried absence of ATM C103T mutant allele, and SNP revealed the patients and the parents are homozygous for A allele whereas the LCM isolated tumor cells carried G allele only, thus proving that the tumor was not host origin. Amariglio et al. (2009) appreciated the advanced molecular techniques used in the study to prove their hypothesis and raised an alarm of adverse effects of stem cell therapy. LCM, in particular, significantly contributed to the successful comparison of the genomic difference between the tumor and the patient tissues.

LCM-directed genomic analysis of retinal degeneration caused by selective Müller cell ablation in transgenic mice: In our laboratory, we used LCM to perform genomic analysis in a disease model of macular telangiectasis type 2 (MacTel Type 2). MacTel Type 2 is a bilateral disease, which includes neuronal degeneration and alterations of the macular capillary network (Charbel Issa et al., 2013). Recent histopathological analysis of post-mortem donor eyes suggests that loss of Müller cells, the principal glial cells in the retina, may contribute to MacTel type 2. We have created transgenic mice in which selective ablation of Müller cells leads to photoreceptor degeneration, vascular leak and the development of deep retinal neovascularization (Shen et al., 2012). These changes are major features observed in MacTel type 2. As the pathological changes in Müller cell knockout mice occurs mainly in areas of selective Muller cell ablation, which can be easily identified by photoreceptor protrusion into the outer retina due to the loss of support from outer limiting membrane (**Figure 1**) (Shen et al., 2012). We have applied LCM to isolate the areas of Müller cell ablation and performed genomic analysis of genes involved in the glycolytic and mammalian target of rapamycin (mTOR) signaling pathways by quantitative polymerase chain reaction (**Figure 1**) (Chung et al., 2013). Our results revealed decreased expression of a number of key genes critical for the glycolytic and mTOR signaling pathways in the area of Müller cell loss. Our data indicate that selective Müller cell ablation may cause alterations in glucose metabolism and energy production. The successful isolation of the area of Müller cell ablation using LCM in our study allowed us to conduct comprehensive genomic comparisons, thus providing a deep insight into the molecular mechanisms of photoreceptor degeneration caused by primary Müller glial dysfunction.

Conclusion: LCM is a powerful and useful tool in many areas of medical research. The technique has been tremendously developed in the past decade to a level which can produce meaningful, accurate and reproducible results. In neuroscience research, LCM could be the most beneficial and advantageous tool in the context that the area of 'selective vulnerability' can be isolated.

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***Accepted:** 2015-05-17*
***doi:**10.4103/1673-5374.158346* ***http**://www.nrronline.org/*
Chung SH, Shen W (2015) Laser capture microdissection: from its principle to applications in research on neurodegeneration. Neural Regen Res 10(6):897-898.

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