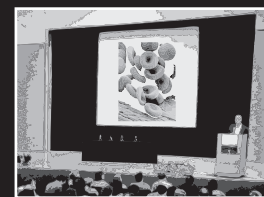


Conference Scene

Laser microdissection: a day for answers



Laser microdissection: a day for answers

BioPark, Hertfordshire, Welwyn Garden City, UK, 17 June 2010

'Laser microdissection: a day for answers conference,' organized by EuroSciCon (Hertfordshire, UK), hosted a large group of scientists from across Europe. Laser-capture microdissection (LCM) is a state-of-the-art technique that has made a large impact amongst the scientific community in recent times. Despite the usefulness of this technique, LCM is still relatively underused and, thus, the main objective of this conference was to increase awareness, to provide a timely overview of the diverse applications of LCM in biological research and to discuss the potential future directions of this technique. Topics covered during the day included the application of LCM in parasitology, forensic science and oncological research. In this report, we present the key summary of the day's events and the ensuing question and answer session.

The meeting was opened by the conference chair, David Whitehouse (scientific consultant, D-Gen Ltd, London, UK) (FIGURE 1), who emphasized the large impact that laser-capture microdissection (LCM) has had in recent times. There are currently 200–300 publications on LCM published every year, whilst just over a decade ago this number averaged at 20–30.

Laser microdissection in parasitology

The conference commenced with a fascinating talk given by Rory Post, a medical entomologist and parasitologist based at the Natural History Museum, London, UK. He discussed his work on parasitic worms that cause river blindness in tropical Africa and the blood sucking blackflies (*Simulium*) that transmit them.

Professor Post gave a brief introduction to river blindness, also known as onchocerciasis, which is estimated to claim 951,000 healthy life years in the world each year; it is a tropical epidemic commonly found in African countries. In his words "it's not the adult worm but the larval microfilaria moving through the skin that causes either skin disease or blindness." The only drug available for treatment and control of this disease is ivermectin, which is distributed to the entire population of Africa with the sponsorship of the WHO/African Programme for Onchocerciasis Control.

He discussed his exciting work on the population biology of parasites – a challenging task because adult parasites are found deep inside the body and are very inaccessible. An important parameter to be

considered is the number of adult parasites infecting the host, as well as their reproductive rate. One way of doing this is to obtain the larval offspring from a tiny skin biopsy and DNA fingerprinting the biopsy to work out how many families of parasites and, hence, how many adults there are. However, the larval parasites are difficult to handle and have a tough cuticle. LCM enables reliable isolation of parasites and improved isolation of DNA by disrupting the cuticle to release the DNA.

Professor Post also conducted studies on the genomics of the vector *Simulium*. During these studies, he found genes from an endosymbiotic bacterium *Wolbachia* inside the vector. He characterized around 20 genes of *Wolbachia*, which is known to exhibit various peculiar effects, such as host speciation, parasite immunity and population suppression. *Wolbachia*, however, can be integrated into the host genome from prior infections. To investigate whether they were current or previous infections, he used LCM to localize the genes by cutting out the nucleus, and identified that the genes were not present in the blackfly nucleus.

He also reported his work on ribosomal DNA in blackflies. It was an exciting insight that the blackfly, alone, consists of 55 species, solely as a result of various chromosome variations and inversions, which could be cut out and DNA fingerprinted. He raised the various technical issues of using LCM for his studies: small quantities of DNA, electrostatic problems, preservatives and staining. He also illustrated the various solutions to these technical difficulties including, whole-genome amplification, cutting

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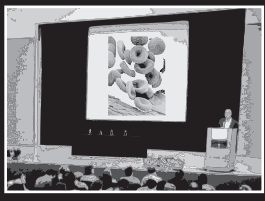


Figure 1. Speakers at Laser microdissection meeting. From left to right: Mr Ajay Joseph, Professor Rory Post, Dr David Whitehouse, Dr Tatjana Crnogorac-Jurcevic and Ms Trees Lepez.

Taken with permission from the Euroscicon News website (www.eurosciconnews.com/blog/_archives/2010/6/21/4556375.html)

out multiple objects, investigating multi-copy DNA, use of frozen and unstained material, and many other solutions.

Transcriptional profiling of formalin-fixed paraffin-embedded tissue in prostate cancer biopsies using LCM

Ajay Joseph (University of Cambridge, Cambridge, UK) discussed how the coupling of LCM with transcript profiling of formalin-fixed paraffin-embedded (FFPE) prostate cancer (PCa) specimens can improve our understanding of PCa biology. He emphasized the fact that PCa is the second-leading cause of cancer-associated death, with over 36,000 men diagnosed each year. The number of patients diagnosed with PCa has been on the increase since the advent of prostate-specific antigen blood testing.

Men with suspected PCa generally undergo a needle biopsy to obtain a sample for diagnosis. Histologically, PCa is characterized by the progressive loss of the normal prostatic glandular architecture. “Despite the fact that we know cancer is a group of cells displaying uncontrolled growth or division beyond normal limits,

simply put, prostate malignant glands show a loss of the normal architectural growth pattern. The architecture becomes progressively more disorganized and correlates with the aggressiveness of the cancer,” explained Joseph.

He also pointed out that Gleason grading was a method of scoring this architecture and that it aids clinicians in evaluating the prognosis of men diagnosed with PCa. This also allows an informed decision to be made alongside other clinical tests regarding the choice of different treatment modalities, such as active surveillance, surgery, radiotherapy and androgen deprivation. It is apparent that some men naturally have a more aggressive disease course than others and the key challenge is to correctly identify these high-risk men at the point of diagnosis.

Transrectal ultrasound-guided needle biopsies, obtained from diagnosis in PCa, are often the only tissue available before a treatment decision. These tissues are routinely fixed in FFPE; however, fixation results in a high risk of RNA degradation. FFPE tissues are a rich source of molecular information that can potentially be useful in predicting outcome.



Recently, most studies have compared expression levels in tumor and non-tumor tissues using whole prostate tissue samples, which contain mixed cell populations, such as prostate epithelia and stroma, which have distinctly different physiological roles.

Laser-capture microdissection allows enriched tumor cell populations to be accurately isolated from heterogeneous complex tissue, hence, facilitating multi-component analysis from a single tissue sample. Studying gene expression patterns between these different histopathological cell types can elucidate their underlying crosstalk. By briefly illustrating the methodological flowchart of LCM technique, he summarized by stating that the combination of LCM and transcript profiling in FFPE PCa specimens can improve the understanding of molecular-level characteristics of PCa, which may subsequently help to develop an accurate diagnostic test that is technically simple and applicable for routine clinical use for patient-specific treatment in PCa.

Laser microdissection in forensic sciences

Trees Lepez (Ghent University, Ghent, Belgium) began by explaining the importance of isolating male cells from a mixture of female cells. Single tandem-repeat profiling in cases of sexual assault, where male cells are found in trace amounts, need to be isolated to produce a full DNA profile.

Lepez demonstrated that traditional methods of differential extraction of spermatozoa from vaginal cells had proved to be ineffective owing to the challenge of contamination of the perpetrator's DNA with victim's DNA. Y-chromosome short tandem-repeat analysis, which was used to detect the male component, also failed to provide encouraging results, owing to its lower discriminatory power. Lepez and her research group had developed an automated screening method to detect male cells after FISH in suspension using a Y-chromosome-specific probe.

She reported striking experimental results by employing LCM in the precise separation of male cells from a mixture with female cells. Using this technique, she reported that a full DNA profile could be obtained by fluorescent detection of

spermatozoa using a monoclonal antibody against proteins in the human sperm heads. The automated detection of spermatozoa can then be collected by a defined laser pulse using the Palm laser-pressure catapulting function and subjected to DNA analysis.

The detected male cells were collected by laser-pressure catapulting, using a pulsed nitrogen UV-A laser. The high energy generated by the laser was used to catapult the male cells into the cap of a standard microfuge tube, containing DNA extraction buffer. This contact-free method helps to overcome the risk of contamination of the sample.

Lepez demonstrated that the FISH-in-suspension protocol has no major adverse effects on the recovery of DNA from male cells. She concluded that the LCM method, used in conjunction with automatic detection by the FISH-in-suspension signal, enables a fast and sensitive procedure for DNA profiling of male contributor in forensic samples containing an unfavorable mixture of male and female cells.

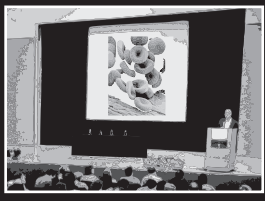
Analysis of metastatic disease in PCa: use of laser microdissection to procure

■ Lymph node metastases

Tatjana Crnogorac-Jurcevic (Bart's Institute of Cancer, Queen Mary University of London, UK) reviewed her work on pancreatic ductal adenocarcinoma, estimated to be the fifth-leading cause of cancer-related deaths in the industrialized world. She emphasized the devastating nature of the disease with a dismal prognosis and less than 5% survival rate.

In the UK, more than 7000 people are diagnosed with the disease each year and an almost equal number of patients succumb to it. She stated that this is largely because the disease remains undiagnosed until a late stage, when the cancer has already spread locally or to the distant organs, and curative surgery is no longer possible.

In addition, there are currently no effective therapies for pancreatic ductal adenocarcinoma metastatic disease, and understanding the underlying molecular changes is, therefore, of paramount importance. Her work is mainly focused on finding novel therapeutic targets that can elucidate



the molecular mechanisms of metastatic behavior. She presented basic elegant research on the proteomic analysis of primary lesions and lymphatic metastases. Frozen specimens are unavailable in these cases, yet FFPE tissues have been demonstrated to be promising specimens for the study. She stressed the critical importance of using LCM in pancreatic ductal adenocarcinoma owing to the fact that it is a highly desmoplastic tumor. LCM was performed using the Palm instrument. Dissected material was processed using proprietary liquid tissue (expression pathology); proteins were digested by trypsin and analyzed by multidimensional protein identification technology.

She was able to successfully identify a number of proteins belonging to various signaling pathways that could contribute to the metastatic spread of pancreatic cancer and may explain the poor prognosis of this disease. Several selected candidate proteins are currently being verified by immunohistochemistry.

She summarized the talk by demonstrating that laser microdissection coupled to downstream proteomics analysis is a valid approach for molecular analysis of pancreatic adenocarcinoma and its associated lymph node metastases.

Conclusion

The conference was extremely well layered, ranging from the chromosomal, transcriptional, proteomic and cellular applications of LCM. The presentations demonstrated that LCM is a powerful tool for isolating a highly pure cell population from a complex heterogeneous tissue via direct visualization. The meeting fulfilled the chair's aim by expanding the knowledge and application of LCM to a broader area of science. Many technically challenging questions from the LCM users were addressed by the expert's panel at the end of the meeting.

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