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Profiling the tumor microenvironment proteome in prostate cancer using laser capture microdissection coupled to LC–MS—A technical report

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ABSTRACT

Laser capture microdissection (LCM) allows microscopic procurement of specific cell types from tissue sections. Here, we present an optimized workflow for coupling LCM to LC–MS/MS including: sectioning of tissue, a standard LCM workflow, protein digestion and advanced LC–MS/MS. Soluble proteins extracted from benign epithelial cells, their associated stroma, tumor epithelial cells and their associated stromal cells from a single patient tissue sample were digested and profiled using advanced LC–MS/MS. The correlation between technical replicates was $R^2 = 0.99$ with a mean % CV of $9.55\% \pm 8.73$. The correlation between sample replicates was $R^2 = 0.97$ with a mean % CV of $13.83\% \pm 10.17$. This represents a robust, systematic approach for profiling of the tumor microenvironment using LCM coupled to label-free LC–MS/MS.

1. Introduction

A workflow using laser capture microdissection (LCM) that would allow for both targeted and unbiased proteomic profiling of specific target cells in tissue (that may also include, for example, immuno-MRM) could be invaluable to several experimental and clinical fields. Since its establishment, LCM has predominantly been coupled with genomic and transcriptomic analysis for large-scale studies, whereas proteomic analysis has largely lagged behind in this area due to the limited amount of sample routinely acquired using LCM. Today, while some may still argue that LCM is too challenging and labor intensive for the resulting low protein yields, the sensitivity of mass spectrometers has increased exponentially in the last number of years allowing analysis of scarce protein samples and even single cell analysis [1] as well as global proteome mapping [2]. Therefore it is now reasonable to perform large-scale LCM using limited sample amounts for global proteome analysis to complement those that are routinely performed using genomics and transcriptomic technologies. Several laboratories have studied differential protein expression in microdissected tumor tissue specimens in an effort to discover novel tumor markers [3–5]. However, the semi-quantitative approaches used in these studies may have limited the number of potential markers identified as well as the reliability of protein quantification. In order to minimize technical variations and improve reliability of protein quantification, a variety of sophisticated stable isotope labeling techniques have been developed for MS-based proteomics including chemical, metabolic, and enzymatic labeling techniques. Isotope-coded affinity tags (ICAT), isobaric tags for relative and absolute quantification (iTRAQ) and O^{18} labeling coupled with mass spectrometry provide a means of post-harvest protein labeling for protein quantification whereby relative protein expression levels are determined by the ratio of the ion intensities of the isotopically labeled peptide pairs and have successfully been applied to LCM material [6–10]. However, such labeling strategies require a relatively large amount of sample (100 μg), which requires enormous amounts of sectioned tissue for LCM not to mention the vast amount of LCM time. In addition such strategies require extensive sample handling and manipulation

Abbreviations: LCM, Laser capture microdissection; LC–MS/MS, Liquid chromatography tandem mass spectrometry.

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that can increase sample loss and contamination. Similarly, for label-free approaches in particular where peptide abundance information is critical for comparative proteome analysis, it is imperative that sample handling and manipulation be kept to a minimum. Moreover, while these efforts demonstrate significant promise, their scale is modest and undertaking larger scale analysis of individual patient tissue samples remains a formidable challenge [11].

This paper describes a robust systematic approach to coupling LCM with advanced LC–MS/MS using a telepathology approach for the proteomic profiling of the tumor microenvironment (Fig. 1). LCM requires accurate identification of the cells to be targeted and hence the pathologist has a central role in LCM-based experiments. As such, the limiting factor in LCM is generally the availability of an expert pathologist to guide the tissue micro-dissection. The telepathology approach ensures that pathological evaluation is central to the identification and annotation of the correct target cells for downstream proteomic analysis as well as recording any morphological changes as sequential sections are cut through the tissue (Fig. 1). The use of short-range separation allows for the concentration of low protein quantities into a single gel plug for digestion, helps minimize protein loss by minimal sample handling and manipulation and facilitates the removal of SDS for subsequent MS analysis.

In order to establish the effects of protein concentration for sufficient protein identifications, increasing protein yields were concentrated using short range SDS-PAGE gel electrophoresis and subjected to LC–MS/MS. Fig. 2 shows the separation of 0.5 μg-15 μg of crude prostate tissue protein lysate separated based on molecular weight using a 6% SDS-PAGE gel (Fig. 2A) followed by Coomassie Blue staining. As shown by the graph in Fig. 2B, shorter separation resulted in no significant increase in the number of proteins identified from 2 μg as to 4 μg. Furthermore, loading greater than 2 μg runs the risk of causing blockages in the column. Therefore, it is preferable, and indeed feasible, to aim to obtain good protein identifications with only 2 μg total protein. In order to demonstrate the feasibility of the approach LCM analysis of discrete regions within prostate tissue was conducted. For LCM 12 tissue sections from a single patient specimen were used in order to harvest benign epithelium, its associated stroma and tumor epithelium and its associated stroma using our systematic workflow. Each step upstream and downstream of the LCM procedure, from tissue preservation to the planning of LCM sessions, is crucial to ensure accurate cell population accrual. Using digital annotation software with a rigorous annotation system allows for pre-planned LCM sessions as well as real-time viewing of annotated images ensuring that the correct cells (and regions) are acquired for downstream analysis. For this reason the "telepathology" approach was chosen; whereby top, middle and tail sections were brought forward for pathological review as shown in Fig. 1, thus ensuring documentation of changes in tissue morphology as the tissue was sectioned through, and also allowing digital pathological annotation through Spectrum software. Online viewing of annotated slides allows planning of LCM sessions as well as real time viewing of annotated images while performing LCM.

The overall aim of this work was to assess the optimsed LCM-proteomics workflow for the proteomic profiling of laser captured microdissected material. To achieve this, three technical replicates and four sample replicates were profiled using label-free nLC–MS/

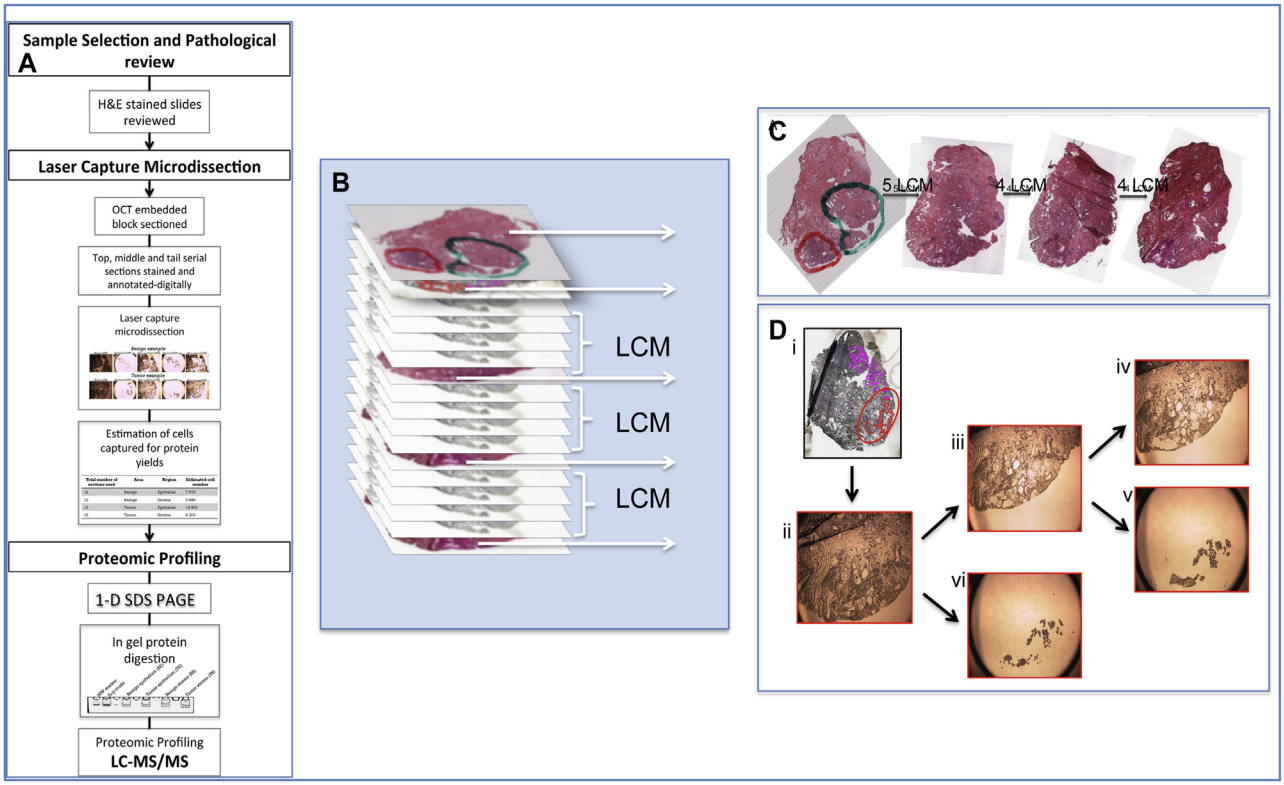

Fig. 1. Systematic workflow for the coupling of LCM to advanced LC–MS. Fig.1(A) shows a schematic illustration of the optimized workflow from sample selection and pathology review, using annotated images for correct cellular accrual to proteomic profiling using short range SDS-PAGE and LC–MS. Fig. 1B, C and D illustrate the telapathology approach implemented as part of the optimized workflow. Fig. 1(C) shows serial H&E stained sections taken from a patient sample. Panel A shows the first H&E section taken at the Dana Faber and posted to UCD. Panel B shows the sixth H&E cut section taken at St. James' Hospital. Panel C and D show the eleventh and sixteenth sections, respectively. Fig. 1(D) depicts the LCM of tumor epithelium and associated stroma from one cut section. The annotated cresyl violet-stained section is shown in D(i), before LCM is shown in D(ii), tumor epithelial cells after LCM are shown in D(iii) and associated stroma are shown in D(iv). Laser captured tumor epithelial cells are shown in D (v) and captured associated stroma are shown in D(vi).