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Chemical mapping of the colorectal cancer microenvironment via MALDI imaging mass spectrometry (MALDI-MSI) reveals novel cancer-associated field effects

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ABSTRACT

Matrix-assisted laser desorption ionisation imaging mass spectrometry (MALDI-MSI) is a rapidly advancing technique for intact tissue analysis that allows simultaneous localisation and quantification of biomolecules in different histological regions of interest. This approach can potentially offer novel insights into tumour microenvironmental (TME) biochemistry. In this study we employed MALDI-MSI to evaluate fresh frozen sections of colorectal cancer (CRC) tissue and adjacent healthy mucosa obtained from 12 consenting patients undergoing surgery for confirmed CRC. Specifically, we sought to address three objectives: (Hogan et al., 2012 Jul 1) To identify biochemical differences between different morphological regions within the CRC TME; (Straussman et al., 2012 Jul 26) To characterise the biochemical differences between cancerous and healthy colorectal tissue using MALDI-MSI; (Engelhardt et al., 2012 Mar 20) To determine whether MALDI-MSI profiling of tumour-adjacent tissue can identify novel metabolic 'field effects' associated with cancer. Our results demonstrate that CRC tissue harbours characteristic phospholipid signatures compared with healthy tissue and additionally, different tissue regions within the CRC TME reveal distinct biochemical profiles. Furthermore we observed biochemical differences between tumour-adjacent and tumour-remote healthy mucosa. We have referred to this 'field effect', exhibited by the tumour locale, as cancer-adjacent metaboplasia (CAM) and this finding builds on the established concept of field cancerisation.

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1. Introduction

1.1. Imaging mass spectrometry

The tumour microenvironment (TME) is thought to play a critical role in solid organ cancer development and progression (Hogan et al., 2012 Jul 1) and TME profiling approaches are gaining increasing attention in cancer research (Straussman et al., 2012 Jul 26; Engelhardt et al., 2012 Mar 20; Tarin and 2012 Feb 3). Imaging mass spectrometry (MSI) utilising techniques such as matrix-assisted laser-desorption ionisation (MALDI), (Caprioli et al., 1997 Dec 1; Schwamborn et al., 2010 Sep; Gruner et al., 2012) desorption electrospray ionization (DESI) (Gerbig et al., 2012 Jun; Eberlin et al., 2013 Jan 29; Takats et al., 2004 Oct 15) and secondary ion mass spectrometry (SIMS) (Passarelli et al., 2011 Nov; Cillero-Pastor et al., 2012 Nov 6) have demonstrated early promise in this area, offering a means of chemically mapping tissue sections, without the requirement for specific staining/labelling agents. The major advantage of MSI as an analytical tool lies in the ability to measure biochemical activity with direct correlation to morphological features of interest, bypassing the need for time-consuming steps such as laser-capture micro-dissection. The latter by its very nature results in significant disruption to the tumour niche, preventing visualisation of biomolecules within their native backdrop.

To date a number of studies have successfully used MSI approaches to characterize region-specific protein/peptide distribution within the cancer TME (Gruner et al., 2012; Aichler et al., 2013 Apr 16; Morgan et al., 2013 Mar; Meding et al., 2012 Mar 2). More recently, the ability of MSI to assess the localisation and concentration distribution of chemotherapeutic compounds in cancer tissue has also been demonstrated, offering the opportunity to investigate anti-cancer drug mechanisms and efficacy from an entirely novel perspective (Bouslimani et al., 2010 Feb; Rompp et al., 2011 Jul).

Of the available MSI techniques MALDI has been the most commonly employed in the recent literature (Caprioli et al., 1997 Dec 1; Schwamborn et al., 2010 Sep; Gruner et al., 2012). This technique is a 'matrix-assisted' approach that involves the application of a chemical solution (referred to as a 'matrix solution' in this context) to the tissue surface to crystallise biomolecules prior to ionisation (Caprioli et al., 1997 Dec 1). Commonly employed solutions for this purpose include α-cyano-4-hydroxycinnamic acid (CHCA) and 2,5-dihydroxybenzoic acid (DHB). (Cohen et al., 1996 Jan 1) Where employed in this study the term 'matrix', refers to this experimental solution and should not be confused with the conventional biological definition of 'matrix' that refers to the inter-cellular material found within connective tissue.

The MALDI technique is very well suited to the localisation of proteins, which occupy the upper end of the mass-to-charge (m/z) spectrum. The successful application of MALDI-based methods to profiling biomolecules at the lower end of the m/z range (<1000 m/z = metabolites/lipids) is more challenging as matrix and/or matrix-analyte cluster peaks (generated as a consequence of matrix deposition) can interfere with the detection of target low molecular weight compounds (Goto-Inoue et al., 2011 Nov). A novel method for matrix-associated peak removal has recently been proposed by our group to address this issue and facilitate effective MALDI-based profiling of lower m/z value molecular species (Fonville et al., 2012 Feb 7).

1.2. Exploring the cancer lipidome via imaging mass spectrometry

Lipid profiling in particular represents an attractive avenue for novel cancer biomarker discovery as there is growing evidence to suggest that membrane lipids play a vital role in carcinogenesis (Sparvero et al., 2012 Jul; Meriaux et al., 2010 Apr 18). Lipids are key cell membrane constituents (Quehenberger et al., 2011 Nov 10) and serve several critical physiological roles including regulation of energy metabolism, (Quehenberger et al., 2011 Nov 10) cellular signalling (Fernandis et al., 2007 Apr) and trafficking of immune cells. (Dykstra et al., 2003) Disordered lipid metabolism at the cellular level is now recognised as a hallmark feature across a variety of cancer subtypes. (Smith et al., 2008 Jan) Imaging mass spectrometry-based techniques offer a means of mapping lipid biochemistry in unparalleled detail within different tissue subtypes comprising the TME. Current methods for lipid detection and localisation such as staining with Nile Red, Oil Red O or osmium tetroxide can be applied to frozen sections (van Goor et al., 1986), and while these can reveal the distribution of a handful of specific lipid fractions, they lack the ability to provide a more holistic lipid profile, as can be achieved with MSI.

To date a limited number of studies have applied MSI-based approaches to identify specific lipid signatures with respect to CRC; Shimma et al. identified region-specific lipid profiles in CRC liver metastasis samples obtained from a single patient (Shimma et al., 2007 Aug), and more recently Thomas et al. found a panel of lipid-based biomarkers to be up- and down-regulated in CRC liver metastases (Thomas et al., 2013 Feb 8). These preliminary studies have highlighted the potential of lipid profiling in identification of disease-relevant lipid signatures for next-generation biomarker discovery.

1.3. Study objectives

In the current study we have applied MALDI-MSI analysis to determine region-specific lipid signatures in colorectal tissue samples (CRC, $n = 12$; healthy 'tumour-remote' colorectal mucosa, $n = 12$; healthy 'tumour-adjacent' colorectal mucosa, $n = 12$) obtained from patients with confirmed CRC. This study methodology is novel as until recently most studies that have employed MALDI-MSI to evaluate primary CRC tissue have focused principally on protein expression, (Djidja et al., 2010 May; Casadonte et al., 2011 Nov) with little or no consideration to downstream lipid biochemistry.

Specifically we have sought to address three primary objectives in this study:

(1) To determine whether MALDI-MSI can reveal regional differences in lipid biochemistry in different tissue types within the CRC microenvironment.
(2) To determine basic lipidomic differences between cancerous and healthy colorectal tissue via MALDI-MSI analysis.

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