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Metabolic signatures of cancer unveiled by NMR spectroscopy of human biofluids

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ABSTRACT

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Abbreviations: AL, acute leukaemia; Apo-IV and ApoA-I, apolipoproteins; AUC, area under curve; BE, Barret's esophagus; BMRB, Biological Magnetic Resonance Data Bank; BPH, benign prostatic hyperplasia; CA 19-9, CEA, CA 27.29, cancer antigens; CC, cholangiocarcinoma; CCA, canonical correlation analysis; CLL, chronic lymphocytic leukaemia; COSY, Correlation Spectroscopy; CPMG, Carr–Purcell–Meiboom–Gill; CRC, colorectal cancer; CSF, cerebrospinal fluid; CT, Computed Tomography; DA, discriminant analysis; DART-MS, direct analysis in real time Mass Spectrometry; DIGE, differential gel electrophoresis; DSS, 4,4-dimethyl-4-silapentane-1-sulfonic acid; EAC, esophageal adenocarcinoma; EOC, epithelial ovarian cancer; ER, estrogen receptor; FDA, Food and Drug Administration; GC×GC–MS, bidimensional Gas Chromatography coupled to Mass Spectrometry; HCA, Hierarchical Clustering Analysis; HCC, hepatocellular carcinoma; HDL, high density lipoproteins; HGD, high-grade dysplasia; HMBC, Heteronuclear Multiple Bond Correlation; HMDB, Human Metabolome Database; HRMAS, High Resolution Magic Angle Spinning; HSQC, Heteronuclear Single Quantum Correlation; LC, liver cirrhosis; LC–MS, Liquid Chromatography coupled to Mass Spectrometry; LDL, low density lipoproteins; LG, logistic regression; M-/U-IGHV, mutated/unmutated immunoglobulin heavy chain variable region genes; MCCV, Monte Carlo cross validation; MS, Mass Spectrometry; MVA, multivariate analysis; NED, no evidence of disease; NLD, non-liver disease; NMLD, non-malignant liver diseases; NMR, Nuclear Magnetic Resonance; OLK, oral leucoplakia; OPLS, orthogonal projections to latent structures; OPLS-DA, orthogonal projections to latent structures discriminant analysis; OSC, orthogonal signal correction; OSCC, oral squamous cell carcinoma; OSC-PLS-DA, orthogonal signal correction partial least squares discriminant analysis; PCA, Principal Component Analysis; PLS, partial least squares; PLS-DA, partial least squares discriminant analysis; PLS-DF, partial least squares discriminant function; PR, progesterone receptor; PSA, prostate specific antigen; RCC, renal cell carcinoma; ROC, receiver operating characteristic; SIMCA, Soft Independent Modeling of Class Analogy; STOCSY, Statistical Total Correlation Spectroscopy; SVM, Support Vector Machines; TCA, tricarboxylic acid; TOCSY, Total Correlation Spectroscopy; TSP, 3-(trimethylsilyl)-propionic acid; UBC, urinary bladder cancer; VIP, Variable Importance in the Projection; VLDL, very low density lipoproteins; WOS, Web of Science.

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1. Introduction

Metabonomics entails the comprehensive analysis of low molecular weight molecules (metabolites) involved in intermediary metabolism, through advanced profiling techniques, such as Nuclear Magnetic Resonance (NMR) spectroscopy and Mass Spectrometry (MS)-based methods, combined with multivariate statistical analysis [1,2]. The general aim of this approach is to determine fluctuations on the levels of endogenous metabolites, through the analysis of a given biological matrix (e.g. tissue, biofluid, cultured cells), and to mine for consistent relationships between those metabolic variations and specific pathophysiological conditions or external perturbations, such as disease, diet or therapeutic intervention. By reflecting the upstream activity of genes and proteins, as well as being modulated by factors unrelated to the genome (e.g. interaction with commensal microorganisms and environmental agents, nutritional and other lifestyle-related aspects), metabolite levels closely express cellular function, constituting a sensitive probe for homeostasis and its regulation. As pathological conditions usually disrupt normal metabolism and homeostasis, leading to altered metabolite levels and/or profiles, metabonomics holds great potential in disease diagnosis and monitoring. Indeed, metabonomics has been increasingly recognized as a valuable complementary approach to other well-established 'omic' sciences (genomics and proteomics), being part of a wide biomarkers development effort, promoted by the Food and Drug Administration (FDA), to aid in the assessment of disease and toxicity (www.fda.gov/nctr/science/centers/metabolomics).

Several pathologies have been extensively investigated through metabonomics including cancer, diabetes, cardiovascular and neurological diseases [3–6]. Cancer in particular has been the focus of an increasingly large number of studies in recent years (Fig. 1). Cancer cells show distinct metabolic behavior to face limited nutrient and oxygen availability and to meet the high energetic and biosynthetic demands for aberrant cell proliferation. Examples of known key metabolic shifts in cancer include enhanced glucose uptake and glycolytic activity (with concomitant lactate production), increased *de novo* biosynthesis of nucleotides via the pentose phosphate pathway, increased glutaminolysis and a shift in citrate metabolism from oxidation to lipogenesis [7–10]. Direct evidence of alterations in these pathways has been provided through metabolic profiling of tumor cells and tissues [11]. Moreover, as metabonomics enables the holistic non-selective assessment of metabolites, new hypothesis concerning cancer metabolic reprogramming could emerge. Tissue metabonomics, employing mainly ^{1}H High Resolution Magic Angle Spinning (HRMAS) NMR for direct sample analysis, has enabled general and specific metabolic markers of malignancy to be defined for a range of different cancer types [12–14]. This approach has also been explored for cancer characterization, for instance concerning tumor grading [15] and correlation to prognostic factors [16], both aspects being crucial in disease evaluation and treatment planning.

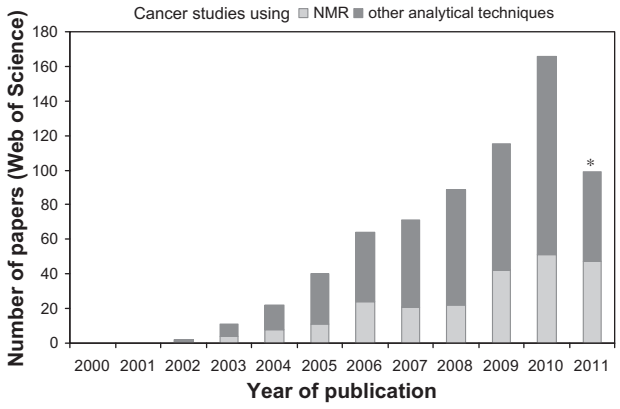

Fig. 1. Number of cancer-related metabonomics publications per year. The literature review was performed in the Web of Science (WOS) database, using the keywords cancer, metabolomic(s) or metabonomic(s), and Nuclear Magnetic Resonance or NMR. *The data was collected up to July 2011.

While analysis of tumor tissues and cells represents a direct window to cancer cellular altered metabolism, metabolic profiling of biofluids has the potential to assess the complex dynamic interaction between tumor and host, a relationship which is likely to play a critical role in defining clinical outcomes and response to therapy. Furthermore, biofluids such as blood or urine are easily obtained through minimally-invasive or non-invasive collection, thus representing increasingly attractive sources for biomarkers. Indeed, in recent years, there has been a substantial upsurge of studies focused on the metabonomic investigation of biofluids, mostly blood serum/plasma and urine, aimed at achieving various goals such as early cancer screening, improved diagnostic accuracy or prediction of response to therapy.

A significant part of these studies has made use of high resolution NMR spectroscopy for characterizing the metabolic composition of biofluids. Among all available analytical platforms in metabonomics [17,18], NMR stands out as the most robust and reliable technique, presenting unparalleled analytical reproducibility [19] and generally requiring minimal sample preparation, thus preserving the native form of biofluids and even allowing for their recovery, as measurements are non-destructive. Another distinct advantage of NMR is the ability to simultaneously provide structural and quantitative information. Direct and unequivocal identification of metabolites, including those which are unexpected or unknown, can often be attained, thus assisting the biochemical interpretation of the results. Moreover, NMR is intrinsically quantitative with peak integrals directly reflecting concentrations. The greatest limitation of NMR, however, lies in its inherent low sensitivity, which precludes the detection of metabolites present in levels below a few micromolar, at least in the 500–600 MHz spectrometers commonly used in metabonomics studies. Although not yet routinely employed, advances in NMR instrumentation, including the introduction of cryogenically cooled probes and microcoil probes, together with specific methods, such as isotope labeling and 1D selective Total Correlation Spectroscopy (TOCSY),