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Serum metabolomic profiles evaluated after surgery may identify patients with oestrogen receptor negative early breast cancer at increased risk of disease recurrence. Results from a retrospective study

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ABSTRACT

Purpose: Metabolomics is a global study of metabolites in biological samples. In this study we explored whether serum metabolomic spectra could distinguish between early and metastatic breast cancer patients and predict disease relapse.
Methods: Serum samples were analysed from women with metastatic ($n = 95$) and predominantly oestrogen receptor (ER) negative early stage ($n = 80$) breast cancer using high resolution nuclear magnetic resonance spectroscopy. Multivariate statistics and a Random Forest classifier were used to create a prognostic model for disease relapse in early patients.
Results: In the early breast cancer training set ($n = 40$), metabolomics correctly distinguished between early and metastatic disease in 83.7% of cases. A prognostic risk model predicted relapse with 90% sensitivity (95% CI 74.9–94.8%), 67% specificity (95% CI 63.0–73.4%) and 73% predictive accuracy (95% CI 70.6–74.8%). These results were reproduced in an independent early breast cancer set ($n = 40$), with 82% sensitivity, 72% specificity and 75% predictive accuracy. Disease relapse was associated with significantly

Abbreviations: AUC, area under the curve; CERM, Center of Magnetic Resonance; CPMG, Carr–Purcell–Meiboom–Gill; ER, oestrogen receptor; GC, gas chromatography; ^{1}H NMR, proton nuclear magnetic resonance; HER2, human epidermal growth factor receptor 2; MS, mass spectroscopy; MSKCC, Memorial Sloan-Kettering Cancer Center; NOESY1D, nuclear overhauser effect spectroscopy pulse sequence; RF, random forest; ROC, receiver operating curves.

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lower levels of histidine ($p = 0.0003$) and higher levels of glucose ($p = 0.01$), and lipids ($p = 0.0003$), compared with patients with no relapse.

Conclusions: The performance of a serum metabolomic prognostic model for disease relapse in individuals with ER-negative early stage breast cancer is promising. A confirmation study is ongoing to better define the potential of metabolomics as a host and tumour-derived prognostic tool.

1. Introduction

Breast cancer is the most common malignancy and most common cause of cancer death in women (Ferlay et al., 2010). There is marked heterogeneity in breast cancer biology and disease behaviour. Among individuals with seemingly similar disease as assessed by clinico-pathological features, immunohistochemistry and molecular platforms, outcomes can be substantially different.

The window of opportunity for curative intervention in breast cancer is in early stage disease. Following surgical excision of the breast lesion and surgical sampling and/or dissection of axillary nodes, patients might be offered loco-regional radiotherapy and/or post-operative (adjuvant) systemic therapy. The rationale behind this approach is that residual micrometastatic disease might be eradicated by chemotherapy, targeted anti-human epidermal growth factor receptor 2 (HER2) therapy and/or targeted endocrine therapy. If not eradicated, micrometastases might progress to incurable disseminated breast cancer.

In current clinical practice adjuvant therapy is indicated on the assumption of residual micrometastases. The primary disease is assessed using traditional clinico-pathologic features with or without gene profiling, and an estimation of the risk of recurrence is thus made (Paik et al., 2004; Ravdin et al., 2001). Micrometastatic disease is detectable, as circulating tumour cells in the peripheral blood and disseminated tumour cells in the bone marrow, although not all patients with micrometastases will develop clinically evident macrometastatic disease (Braun et al., 2005; Stathopoulou et al., 2002; Xenidis et al., 2003). Factors beyond the presence of micrometastases, such as tumour cell dormancy, host immunity, and the microenvironment, influence the clinical outcome.

Novel prognostic and predictive biomarkers may refine risk assessment and guide use of systemic therapy in individuals with early breast cancer. In this setting there are promising tools such as the various –omics, including metabolomics, a science dedicated to the global study of small molecules and metabolites (Nicholson, 2006). Metabolomics combines high resolution data-rich analytical methodology with advanced chemometric data interpretation. The 'metabolome', the extensive analysis of hundreds of metabolites in a biological specimen, can be considered the downstream end product of the complex interaction of genome, transcriptome and proteome. By its very nature of being downstream it may be a very sensitive tool for phenotype assessment.

The metabolome is affected by physiological, pathological and iatrogenic factors (Griffin, 2003; Urbanczyk-Wochniak et al., 2007). Breast cancer has been associated with marked metabolic shifts, which have been demonstrated in many preclinical and clinical metabolomic studies of breast cells, breast tissue, serum and urine (Aboagye and Bhujwalla, 1999; Budczies et al., 2012; Katz-Brull et al., 2002; Li et al., 2011; Mackinnon et al., 1997; Mountford et al., 2001; Singer et al., 1995). Metabolomics has been explored as a tool for diagnosis of breast cancer, refined sub-classification of breast cancer, and prediction of treatment sensitivity (Li et al., 2011; Mountford et al., 2001; Asiago et al., 2010; Borgan et al., 2010; Giskeodegard et al., 2012; Oakman et al., 2011; Slupsky et al., 2010).

A potential strength of serum and urine metabolomic analyses is that this approach provides a composite metabolomic snapshot of both the tumour and the host. By comparing samples from patients with early versus metastatic disease, host features conducive to tumour progression might be identified, with incorporation of both tumour and host factors from the outset.

In view of the need for more refined prognostic estimation in early breast cancer, we undertook this study to explore whether metabolomics can add prognostic information in individuals with early breast cancer. We assessed serum metabolomic profiles in breast cancer patients using proton nuclear magnetic resonance (^{1}H NMR) spectroscopy, with two hypotheses: (1) serum metabolomic profiles would be different between women following surgery for early breast cancer and women with metastatic disease, due to tumour-specific changes in the ^{1}H NMR detectable metabolomic profile; and (2) some patients with early breast cancer would be recognized by metabolomic analysis as having metastatic disease due to the presence of residual micrometastases.

We report serum metabolomic distinction between women following surgery for early breast cancer and women with metastatic disease, and further, we report metabolomic classification of a minority of early patients as metastatic, in whom future disease relapse was more likely.

2. Patients and methods

This was a collaborative project between the Breast Cancer Medicine Service, Memorial Sloan-Kettering Cancer Center (MSKCC), New York, United States; the Center of Magnetic Resonance (CERM), University of Florence, Sesto Fiorentino, Italy; and the 'Sandro Pitigliani' Medical Oncology Department, Hospital of Prato, Prato, Italy.