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Protein chip array profiling analysis of sera from neuroblastoma patients

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Abstract

Neuroblastoma, the most common extracranial solid tumour in children, is characterised by highly heterogeneous clinical behaviour; patients are stratified into risk categories according to a combination of clinical and biological markers. However, identifying non-invasive prognostic markers predicting outcome independently from current risk-stratification features remains critical for better disease monitoring. Using the SELDI-TOF-MS technology (surface-enhanced laser desorption/ionization time-of-flight mass spectrometry), we found a serum biomarker that strongly correlates with prognosis in neuroblastoma patients. Subsequent peptide mapping identified this biomarker as SAA protein. In support of this observation, high SAA levels were detected by ELISA in the sera of patients with poor prognosis neuroblastoma. Based on this finding, promises and limitations of the approach are discussed.

Keywords: Neuroblastoma; SELDI-TOF-MS; SAA; Serum; ProteinChip arrays

1. Introduction

Over the past few years, advances in proteomic profiling technologies, such as SELDI-TOF-MS, have allowed preliminary profiling and identification of tumour markers in the biological fluids of cancer patients. Such developments are of crucial interest to enhance early detection, diagnosis, prognosis and monitoring of neoplasms. Serum proteomic profiling by SELDI-TOF is one of the most promising novel approaches for cancer diagnostics, and impressive sensitivities and specificities have been reported for some frequent tumour types such as ovarian, prostate, and breast cancers [1–4]. As an example, with the objective to aid early ovarian cancer diagnosis, the SELDI profiling of sera from 50 ovarian cancer patients and 50 unaffected women (healthy controls and patients with benign ovarian diseases) allowed to identify a discriminatory proteomic pattern exhibiting a sensitivity of 100% and a specificity of 95%. Data were validated with a subsequent blind set consisting of a novel series of 50 ovarian cancer patients,

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including 18 patients with stage I disease, and 66 unaffected or benign cases [1]. So far, the majority of SELDI-based studies have examined the diagnostic utility of the approach, but, equally, other potential uses such as predicting or monitoring response to therapy are likely to be fruitful.

Neuroblastoma (NB) stands out among paediatric solid tumours because of its relative frequency, intriguing natural history, prognostic biologic features, and therapeutic challenges. It is the most common extracranial paediatric solid tumour, accounting for 7–10% of all childhood cancers, and the most common neoplasm in infancy. It is characterised by highly heterogeneous clinical behaviour, ranging from spontaneous regression without treatment to aggressive outcome in spite of high-dose chemotherapy. At present, patients are stratified into risk categories on the basis of a combination of clinical and biological markers. These include age at diagnosis, stage of disease, histopathology findings, and amplification of the proto-oncogene N-myc (*MYCN*). *MYCN* amplification, observed in about 25% of primary tumours, is strongly associated with rapid tumour progression and poor outcome, independently from the stage of the tumour or the age of the patient, and has thus become an important factor in clinical decision-making and therapy. Nevertheless, a subset of patients with *MYCN* amplification will be cured by aggressive therapy, whereas others without amplification will ultimately prove to have aggressive, incurable disease. Furthermore, patients who have the most advanced disease and are at the highest risk for tumour-related death are often those from whom little or no tumour is submitted for biologic studies. Indeed, up-front surgical resection is usually not indicated in advanced localised neuroblastomas or metastatic neuroblastomas, because of the associated risk of clinical complications; besides, surgical or percutaneous core biopsies do not always provide enough material for the analysis of biological markers [5]. Therefore, the identification of non-invasive prognostic markers capable to predict outcome independently from current risk-stratification features is a priority. A variety of serum markers have been associated with adverse prognosis, including high serum levels of lactate dehydrogenase (a marker for *MYCN*-amplified and rapidly proliferating disease), neuron-specific enolase, and ferritin. However, all suffer from a lack of sensitivity and specificity [6–8]. In the search for novel prognostic factors, we thus carried out a SELDI TOF analysis of serum samples collected from neuroblastoma patients at diagnosis with the aim to identify a proteomic pattern capable to discriminate between good and poor prognosis neuroblastomas.

2. Principle of the SELDI TOF-MS procedure

The significant technological advances in protein chemistry of the last two decades have established mass spectrometry as an indispensable tool for protein study, including discovery, identification and structural characterisation. Ciphergen Biosystems Inc. (Fremont, CA) has developed the SELDI-TOF technology to facilitate the high-throughput analysis of proteins in complex biological specimens such as serum. The SELDI-TOF technology uses chip-based protein sample arrays with different chromatographic surfaces to selectively bind proteins with specific chemical properties, before generating mass/charge profiles of the applied sample. The initial separation is performed on patented ProteinChip Arrays that provide a variety of surface chemistries with different chromatographic properties, including hydrophobic, hydrophilic, anion exchange, cation exchange, and immobilised-metal affinity surfaces. An energy-absorbing matrix is added to the ProteinChip so that, when laser energy is applied to the sample, the proteins present become ionised, and their mass can be measured from the time they need to travel through a vacuum to the mass detector. Usually, studies designed to identify specific protein profiles with diagnostic or prognostic value are performed in three successive steps: (i) at the so-called ‘discovery phase’, a limited number of serum samples are analysed on different ProteinChip® Arrays in order to identify the most effective conditions of retention. The number of significant peaks and the patterns of these peaks determine the choice of the conditions that will be used in the subsequent phase; (ii) during the ‘validation phase’, a large number of samples are analysed in the condition retained in the previous stage; (iii) the last step is the identification of the protein, usually by in-gel trypsin digestion and peptide fingerprinting.