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Extended Abstract

Differential proteomics by label-free quantification for early diagnosis and prognosis of cancers

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Abstract

In the last few years, differential proteomics has gained popularity due to its ability to distinguish proteome of different states by comparative analysis. This has a greater significance in identifying disease vs. healthy condition, and thereby advanced further to the application of early detection, diagnosis and prognosis of diseases using mass spectrometry (MS)-based protein quantification.Several strategies using labeling and label-free approaches have been established for both relative and absolute quantification of proteins. Recent developments in the MS instrumentation, extensive advances in bioinformatics and computing power facilitated protein quantification by label-free methods. Label-free quantification overcomes the expensive and extensive workflows required in the labeling techniques.In our laboratory, we are using a label-free quantification approach called spectral counting for the identification of disease-specific biomarkers for early diagnosis and prognosis of cancers, specifically glioblastomamultiforme (GBM) and endometrial cancer (EC). In this study, tumor biopsies and plasma/serum samples were analyzed by SDS-PAGE for minimizing the complexity of the proteome before analyzing by MS using nanoAquity UPLC-LTQ OrbitrapVelos MS. Data analysis was carried out using SEQUEST algorithm for protein identification and Visualize software for quantification of identified proteins using spectral counting method. In the GBM study, we identified 2214 � 121 proteins in tissue biopsies and 853 � 52 proteins in plasma samples, and found 883 � 71 in tumor and 363 � 56 proteins in plasma to be differentially modified ($p \leq 0.05$). In GBM patients, 46 and 21 proteins were identified exclusively in tumors and plasma, respectively compared to controls. We further characterized two of the potential biomarkers pigment epithelium derived factor (PEDF) and brevican core protein (BCAN) using Western blotting and MS. We observed that the protein expression and possible posttranslational modifications (PTM) of these candidate biomarkers to be altered among GBM patients. Similarly, in the EC study, we identified an average of 1048 � 209 proteins in serum samples, and an average of 389 � 39 proteins with significant differential expression between pre- and post- surgery samples ($p<0.05$). Of these, nine proteins were absent in pre- surgery control samples but present in pre- surgery patient samples, which are enlisted to be the potential biomarkers for the early diagnosis of endometrial cancer. Using label-free quantification MS method, we identified tumor-specific proteins in patient samples, which could be the potential biomarkers for early diagnosis in EC and prognostic markers in GBM. The emerging new knowledge of protein markers and their PTMs identified from this study is expected to represent a major step forward for better understanding of the fundamental biology of cancers and for giving deeper insight into the pathogenesis of disease, thereby identifying novel therapeutic targets for optimal therapy.

Breast cancer (BC) is a major public health problem worldwide. Despite the widespread use of mammographic screening, which has contributed to reduced mortality, BC is still the most common form of cancer among women. It can only be detected using mammography if there is a visible, detectible abnormality with architectural distortion or calcification, which correlates with the presence of several hundred thousand tumor cells. Once BC has been biopsied and the diagnosis has been confirmed pathologically, the tumor is surgically excised. The complexity and heterogeneity of individual tumors play an important role in therapeutic decision making. Pathological examination is still the gold standard for diagnosis and assessment of prognostic indicators in BC which include tumor size, grade (degree of tumor cell differentiation), presence or absence of positive lymph nodes (metastases), immunohistochemical expression of key proteins such as estrogen receptor (ER), progesterone receptor (PR) and HER2.

Although advances in BC diagnosis have been made in the last decade, there are still many BC patients who cannot be diagnosed in the early stages of disease or monitored adequately for tumor recurrence using current techniques. To reduce morbidity and mortality from BC, novel approaches must be considered for screening, early detection and prevention, as well as for monitoring cancer progression or recurrence. The early detection of ductal carcinoma in-situ (DCIS) or invasive breast cancer (IBC) may prevent the development of life threatening metastatic disease. Additionally, monitoring metastatic progression could identify early BC recurrence and help guide therapeutic decision making.

Human urine is one of the most interesting and useful bio-fluids for clinical proteomics studies. Advances in proteomics, especially in mass spectrometry (MS)] have rapidly changed our knowledge of urine proteins which have simultaneously led to the identification and quantification of thousands of unique proteins and peptides in a complex biological fluid. Proteomic

studies of urine are highly informative, and have been successfully used to discover novel markers for cancer diagnosis and surveillance [6–9] as well as for monitoring cancer progression [10,11]. Technological development combined with the addition of urine screening would increase the knowledge about patient status and further assist assessment and treatment in clinical practice. Proteomic analysis of urine holds the potential to apply a non-invasive method to identify novel biomarkers of BC. However, investigation of urinary proteins from different stages of BC patients using a liquid chromatography tandem mass spectrometry (LC-MS/MS) proteomic approach has not been reported to date. In this study, we used a label free LC-MS/MS technique to test the feasibility of urine as a source for BC biomarkers and identify the urinary proteins for BC diagnosis and monitoring progression. One potential marker (extracellular matrix protein 1 (ECM1) previously identified and associated with BC), and two novel potential protein markers (MAST4-microtubule associated serine/threonine kinase family member 4 and filaggrin) identified from BC urine were validated in BC cell lines and MAST4 was validated in a small number of primary BC tissues and in the individual human BC urine samples, demonstrating the link of these proteins with BC. However, a larger cohort of BC patients' samples is needed for the validation of the identified potential markers in the following studies. The proteins identified showed significant differences in abundance between the different BC disease stages which provide a useful reservoir of biomarkers for the detection of early and advanced BC.

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