ITSI-Life Science NEWSFLASH

Highlighting Recent Technologies and Approaches used in Biomedical Research.

iTRAQ detects eEF1A1 as a candidate biomarker for Metastatic Progression of Prostate Cancer

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Prostate cancer ranks as the second most diagnosed cancer and the third major cause of cancer related death in men[1]. PSA screening is one of the currently available tests for prostate cancer[2]. However, PSA is plagued with controversies and is unreliable because of its potential to provide false positive and false negative results[3]. Hence additional biomarkers are needed to replace or compliment PSA to improve detection, diagnosis and prognostication of prostate cancer.

Investigators from the University of Sheffield, Sheffield, UK (Rehman *et al*[4]) report for the first time the use of Isobaric Tags for Relative and Absolute Quantitation (iTRAQ) technology to identify Eukaryotic translation elongation factor 1 alpha 1 (eEF1A1) as a candidate biomarker for metastatic stage of prostate cancer. Metastatic prostate cancer affects mainly the bones and results in bone pain, spinal cord compression and marrow failure. To identify the biomarkers, the authors classified patients into four groups (n = 5 / groups) —benign prostatic hyperplasia (BPH), non-progressing prostate cancer, progressive prostate cancer and bone metastatic patients. Patient selection was also narrowed down based on the PSA levels. Serum samples were collected from the groups and were immunodepleted using the IgY-14 column. The depleted samples were labeled with 117 (BPH); 116 (nonprogressing cancer); 115 (progressing cancer); 114 (metastatic disease). Labeled sample was SCX fractionated and analyzed by LC-MS/MS. 75 unique proteins with ≥ 2 peptides were quantified. Among them 25 proteins were either up regulated or down regulated in progressive vs. non progressive. Eukaryotic translation elongation factor 1 alpha 1 (eEF1A1) was significantly increased during different stages of the diseases and also during metastatic condition. Rehman *et al*[4] confirmed the up regulation of eEF1A1 with immunohistochemistry. Intense immunoexpression of eEF1A1 was seen in osteoblasts and in particular those osteoblasts in the vicinity of metastatic prostate tumor cells compared to osteoblasts in control bone samples. 11 human prostate cancer cell lines and an osteosarcoma cell line offered similar observations.

The paper by Rehman *et al*[4] is possibly the first article to describe the use of the iTRAQ technique to identify biomarkers of prostate cancer progression and metastasis, underscoring the potential of this powerful quantitative proteomics technology in cancer research. iTRAQ is a non-gel based method for simultaneous detection and quantitation of proteins. iTRAQ is a chemical labeling method that can be used to label proteins from biological samples. iTRAQ label has three parts - N-methyl piperazine reporter group (variable masses from 113 to 121), a balance group which breaks during peptide fragmentation, a -hydroxy succinimide ester group that reacts with the primary amines of the proteins. The protein samples are reduced and alkylated to break the disulfide groups and trypsin digested into peptides. The primary amino groups of the proteins are labeled with iTRAQ tags that vary in molecular weight. Equal micrograms of the labeled peptides are mixed. The mixed peptides are fractionated by Strong Cation eXchange (SCX) to reduce the sample complexity and are analyzed by mass spectrometry. During MS/MS fragmentation the labeled peptide fragments into reporter ions and peptides undergo traditional backbone fragmentation. The identity of the peptides comes from the backbone fragmentation and concentration is deduced from the intensity of the individual reporter ions. Up to eight samples can be run in a multiplexed assay and the relative changes in protein levels can be quantified simultaneously (Fig. 1). iTRAQ is becoming the method of choice for non-gel based biomarker identification from serum or plasma, and more than 145 papers have been published so far in 2012 alone.

ITSI - Biosciences (www.itsibio.com) offers iTRAQ as a service. There has been a 100% increase in the demand for this service in the first quarter of 2012. All biological and clinical samples can be analyzed and 2-plex to 8-plex assays can be performed.

For more information: contact ITSI - Biosciences, Johnstown, PA 15901 via Email: itsi@itsibio.com, Phone: 814-262-7331 or Fax 814-262-7334.

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ITSI-Biosciences is utilizing iTRAQ (5-plex assay) to simultaneously detect and quantify selenium (Se) responsive proteins in human plasma samples collected at five different time points (0 months, 3 months, 6 months, 9 months and 12 months) by investigators from Pennsylvania State University Cancer Center. (Fig 2) The typical turnaround time is 2 weeks for most experiments. As little as 5 µg to 100 µg of protein can be analyzed. The deliverable includes raw and analyzed data, fold-difference of proteins that show difference in expression between the test and reference samples and gene ontology classification of the identified proteins.

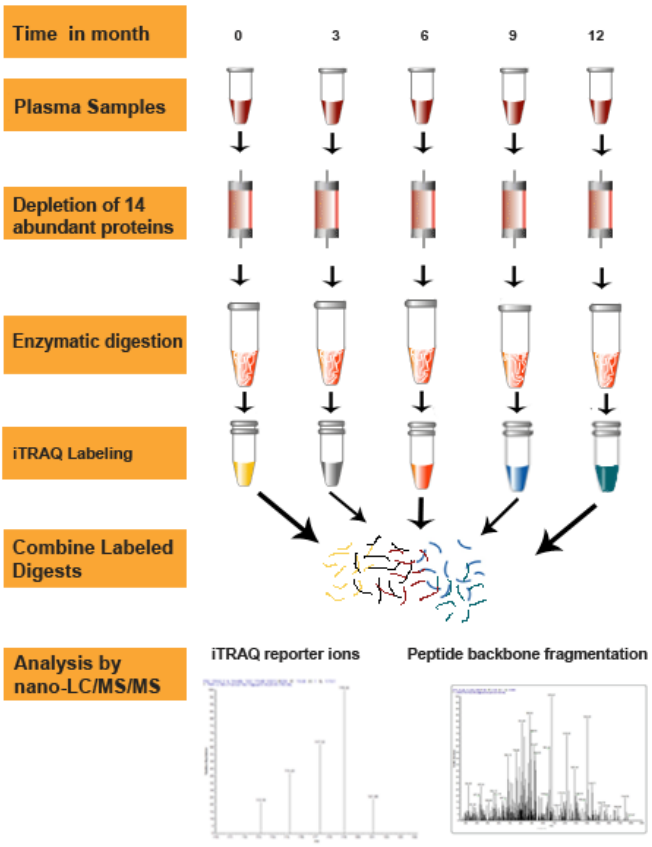

Fig1: Workflow process for 5-plex iTRAQ based quantitation of plasma proteins that show differential expression over time.

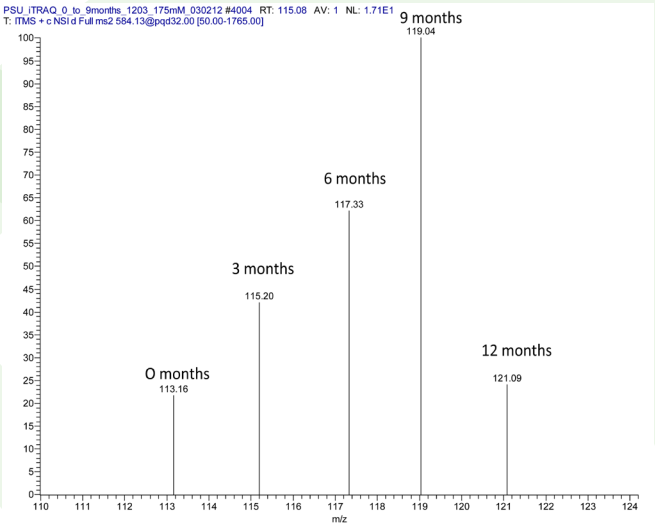

Fig 2: 5-Plex iTRAQ data showing the relative amounts of protein x in plasma of human donor receiving Se-supplementation between 0 months and 9 months. Note that the values decrease to baseline levels in 12 months

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