Exploration of Stage and Grade-Related Biomarkers of Prostate Cancer by Novel Proteomic Approach Using Laser-Microdissected Formalin-Fixed and Paraffin-Embedded Tissues

Introduction and Objective: New biomarkers of prostate cancer (PCa) which distinguish between aggressive cancers with metastatic potential and cancers with low risk for progression are required. Although proteomic analysis is useful for discovery of cancer biomarkers, PCa has some difficulties. Collecting fresh and homogeneous cancer tissue from prostatectomy (RP) specimens is difficult because PCa is multifocal and heterogeneous and accuracy of pathological diagnosis of frozen section is limited. In the present study, we performed shotgun LC/MS-based global proteomics using recently developed technique, laser-microdissection (LMD) of formalin-fixed and paraffin-embedded (FFPE) tissues to identify grade and stage related biomarkers from low and high Gleason score (GS) and metastatic PCa.
Materials and Methods: The nanoLC-MS/MS proteomics was performed using paraffin blocks of FFPE tissues obtained from RP specimens with low GS, high GS and biopsy specimens with metastatic cancer (n=5). Benign normal epithelium from RP specimens were used as control. Targeted cells were microdissected and proteins were extracted, digested and subjected to LC/MS. Identified proteins were semi-quantified by spectral counting and subjected further to statistical evaluation. Candidate proteins were extracted from 3D-scatter plot analysis based on *G*-test. Extracted candidates were validated using immunohistochemistry (IHC) and SRM MS assay.
Results: A total of 371 proteins were identified. According to 3D-scatter plot analysis, we have extracted 11 proteins as low GS specific marker candidates and 23 proteins as high GS specific marker candidates. Validation analysis using IHC and SRM MS assay revealed that methylcrotonoyl-CoA carboxylase b (MCCC2) and fatty acid synthase (FASN) were significantly higher expressed in cancer cells comparing to normal cells.
Conclusions: We established the global clinical shotgun proteomics that could work excellently within the small patients number feasibility study in PCa. To achieve such performance, combination of LMD of FFPE specimens, high-resolution mass spectrometry, spectral counting method and 3D-scatter plot analysis are indispensable.