(31) Enhancing Sensitivity of Top-Down LC/MS-based Cardiac Troponin Assay

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Cardiac troponin (cTn) is a complex of three regulatory proteins, cTnI, cTnT and cTnC, which plays critical roles in cardiac muscle contraction and relaxation. cTnI and cTnT are widely recognized as gold-standard biomarkers in patients with cardiac disease. High-sensitivity ELISA-based assays are available for detecting these biomarkers at low concentration but unable to identify their proteoforms arising from post-translational modifications (PTMs) and sequence variations. PTMs play critical regulatory roles in cell signaling and disease pathophysiology. Our previous study has identified phosphorylation of cTnI as a candidate biomarker for chronic heart failure using immunoaffinity purification coupled with top-down mass spectrometry (MS). Herein, we aim to develop a high sensitivity top-down LC/MS-based cTn assay for comprehensive analysis of their proteoforms. In a general top-down LC/MS-based proteomics assay, proteins are extracted from cell or tissue lysates, separated by LC, and directly analyzed by MS for a complete view of all proteoforms including those with PTMs and sequence variations. In order to further improve the sensitivity of cTn assay, we focus on the optimization of (1) direct infusion using four ionization sources, and (2) online LC/ MS parameters to achieve low limit of detection (LOD) and limit of quantification (LOQ) of cTn. On the other hand, the sensitivity of top-down LC/MS-based cTn assay would significant benefit from the optimal parameters on a nano-scale LC coupled with nano-ESI ion source. Affinity purified cardiac troponin complex was analyzed under the optimized condition to evaluate its LOD/LOQ with different ionization sources on both FTICR and Q-TOF mass spectrometers. Furthermore, the possibility of capillary electrophoresis-mass spectrometry (CE-MS) assay analysis is also being investigated to achieve efficient separation with extremely low cTn protein loading amount. Our goal is to develop an ultra-sensitivity top-down LC/MS-based car assay to analyze the cTn level in the limited amount of human clinical sample.