Differential proteomic analysis of PLC/PRF/5 cell lines treated with various anti-cancer drugs by iTRAQ labeling and mass spectrometry

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Sorafenib (Bayer/Onyx), sunitinib (Pfizer) and tivozanib (Aveo) are oral small molecular VEGF receptor tyrosine kinase (RTK)-targeted drugs, and two of them are approved to treat anti-angiogenesis. In this study, we used isobaric tags for relative and absolute quantitation (iTRAQ) technology to investigate the protein profiles in PLC/PRF/5 cell lines treated with these three anti-cancer drugs (sorafenib, sunitinib, tivozanib). In order to reduce sample complexity, iTRAQ labeled tryptic peptides were fractionated by solution isoelectric focusing (sIEF), strong cation exchange chromatography (SCX) or basic reverse phase chromatography (bRP), followed by nano-LC tandem mass spectrometric analysis. A total of 11233 unique peptides were identified which were associated with 2010 proteins in two biological replicate experiments. The solution-IEF, SCX and basic RP methods permitted a total of 80%, 30% and 81% of proteins to be identified respectively. The results for these approaches were complementary, and allowed more than 23% more of proteins to be identified, when compared with a single fractionation strategy (bRP). Two-dimensional fractionation technique provided excellent complementary that improves both the quantification (peptide-based quantification approaches using stable isotope labeling), and confidence of protein identification. Among them, 187, 112 and 128 differential expressed proteins were selected with the treatment sorafenib, sunitinib and tivozanib for Metacore analysis. These proteins are found to be associated with oxidative phosphorylation,

cytoskeleton remodeling and transcription role of AP-1 in regulation of cellular metabolism. Some of these correlated proteins will be further validated for their biological significance in the future.