A SRM/MMR based targeted proteomics strategy for quantification of potential biomarkers of TKI sensitivity in EGFR mutated lung adenocarcinoma

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Introduction
Lung cancer remains the leading cause of cancer-related deaths worldwide. Epidemiological growth factor receptor (EGFR) is the primary driver oncogene in 10-30% lung adenocarcinoma. Kinase domain mutations in EGFR (L858R and/or T790M deletion) provide sensitivity to EGFR-specific tyrosine kinase inhibitors (TKIs). Despite good tumor inhibitory effects, after approximately a year, patients acquire resistance to EGFR TKIs. About 60% of the acquired resistance can be attributed to a mutation in the gatekeeper residue of the kinase domain of the receptor (Tyr992). There are limited options for circumventing acquired resistance to the 1st generation EGFR-TKIs, gefitinib or erlotinib. Recently, the FDA approved the 3rd generation EGFR TKI, osimertinib, for treatment of patients harboring EGFR T790M mutation. However, acquired resistance to osimertinib is inevitable, underscoring the need of identifying potential markers of EGFR TKI response and resistance. We have previously identified several kinases and adaptor proteins from SILAC-based quantitative MS methods that were significantly differentially expressed, suggesting that these are a subset of potential biomarkers of TKI response. We are currently carrying out verification studies utilizing the MS method most often used to complement shotgun approach known as selected/multiple reaction monitoring (SRM/MMR) because of its high sensitivity, speed and dynamic range.

Although, the MS-based shotgun proteomics approaches have revolutionized the pace of protein identification and relative quantification in complex samples, several technical limitations exist that make it unsuitable for absolute quantification, i.e. only the high abundant part of the proteome is reproducibly identified, protein targets present in lower stoichiometric amounts are stochastically identified leading to missing data and unreliable quantification. In order to overcome some of the limitations of these data dependent acquisition methods, we have utilized a more robust method of targeted proteomics via multiple reaction monitoring to carry out quantification of these potential biomarkers in the lung adenocarcinoma cell lines.

Methods
We performed SRM/MMR MS using a nano-flow liquid chromatography instrument (Agilent Infinity 1500 series) coupled to a nano-electrospray ionization mass spectrometer source attached to a Triple Quadrupole MS (Agilent 6495). In this method, digested proteins are chromatographically separated and ionized in an Agilent ChipLC, followed by mass analysis in a triple quadrupole MS.

Results and discussion
Round 1 nanoLC MS/MS: SRM Signal Intensity Rank and Fragmentation Fingerprint using Skyline 3.5

- Spectral library from individual peptide injections on the triple Q, digested peptides with transition list parameters (pro Precursor, m/z Product, Protein, iRT)
- Export methods with transition list parameters (pro Precursor, m/z Product, Protein, iRT)

Round 2 nanoLC MS/MS: RT Calibration and retention time normalization

- Export methods for 3rd generation TKI with or without treatment with 1st and 3rd generation TKI

Round 3 nanoLC MS/MS: Scheduling MRMA assays for optimum duty cycle and sensitivity

- Scheduling MRMA assays for optimum duty cycle and sensitivity

Conclusions
- We have shown the development of quantification assays using MS-based targeted proteomics methods for testing potential biomarkers of drug response.
- An external multi-point calibration curve will be used in conjunction with these optimized assays for accurate and precise quantification of these potential biomarkers.
- We are currently validating these assays in human lung adenocarcinoma cell lines with known EGFR mutations with or without treatment with 1st and 3rd generation TKI.

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