Multiplexed Selected-Reaction-Monitoring (SRM) of Formalin-Fixed Paraffin-Embedded (FFPE) Samples From Clinical Trial Patient Biopsies

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Overview

Formalin fixation is the widely practiced method for long-term preservation of biopsies. Using EPI's patented Liquid Tissue process we have shown that it is possible to analyze the proteins in formalin-fixed paraffin-embedded (FFPE) tissue. Comparison of proteomic data from FFPE and frozen tissue has shown comparable results in quantity and quality of the data. Additionally, it was shown that the fidelity of phosphorylation and glycosylation is not lost following formalin fixation. In this study we conducted selected reaction monitoring (SRM) analysis of multiple analytes in blinded FFPE tissue obtained from patients with colon cancer enrolled in the COIN trial. Overall in the COIN trial, Cetuximab (anti-EGFR) did not show any survival benefits over standard treatment. One of the striking observations was that in some samples where EGFR levels were low, IGF-1R levels were high indicating a resistance/compensatory mechanism. This information can be used for patient stratification.

Introduction

Mass spectrometry based proteomic approaches can be successfully employed on FFPE (Hood et al. 2005, Mann Lab, 2010).

SRM was recently used to quantitate proteins from FFPE clinical tissue (Kawamura et al. 2010, Guzel et al. 2011).

We applied the Liquid Tissue protocol on clinical biopsies obtained from the COIN trial to concurrently quantitate multiple analytes in a single run using SRM methodology.

The COIN trial was designed to determine the effectiveness of Cetuximab (anti-EGFR monoclonal antibody, Imclone and Bristol-Meyers Squibb) in combination with standard chemotherapy drugs for the treatment of advanced colorectal cancer.

Overall, there was no improvement in survival following the addition of Cetuximab to standard chemotherapy therapy. However, some patients showed a trend of benefit.

It has been reported that IGF-1R and cMet are possible candidates that confer resistance to anti-EGFR treatment.

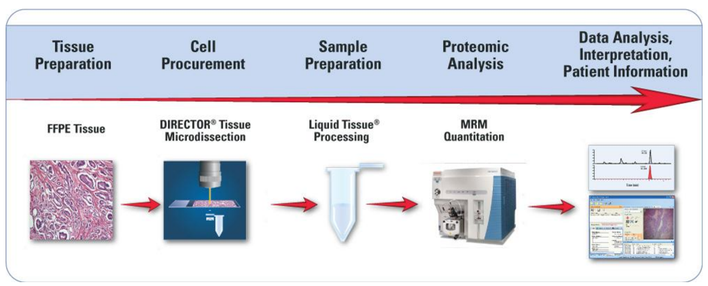

Figure 1. Liquid Tissue-SRM workflow for analysis of proteins from FFPE tissue

Hood BL et al. Molecular and Cellular Proteomics, Nov; 4(11): 1741-53
Ostasiewicz P, Mann M et al. 2010. J. Proteome Research Jul 2; 9(7): 3688-700
Kawamura T et al. J. Proteomics , Apr 18; 73(6): 1089-99
Guzel C et al. 2010, J. Proteome Research Mar 24

Methods

Forty two colorectal cancer tissue blocks from the COIN trial were cut onto DIRECTOR slides and processed using standard histological procedures.

Tumor was microdissected using laser micro-dissection and then solubulized to tryptic peptides using Liquid Tissue technology (Fig. 1)
Absolute quantitation was accomplished through the use of heavy labeled peptides identical to endogenous analytical targets (EGFR, IGF1R, cMet, etc.)

Triplicate injections of 0.5 ug of sample and 1 fmol of heavy labeled peptides were injected using a splitless nanoHPLC (NanoAcquity, Waters) into a triple quadrupole (TSQ Vantage, Thermo)

The triple quad was operated at 2.2kV, FWHM was set at 0.2 for Q1, 0.7 for Q3, with a scan width of 0.002 and scan time of 10 ms.

Data analysis was done using Pinpoint (Thermo)

Clinical Validation of Multiplex SRM Assay

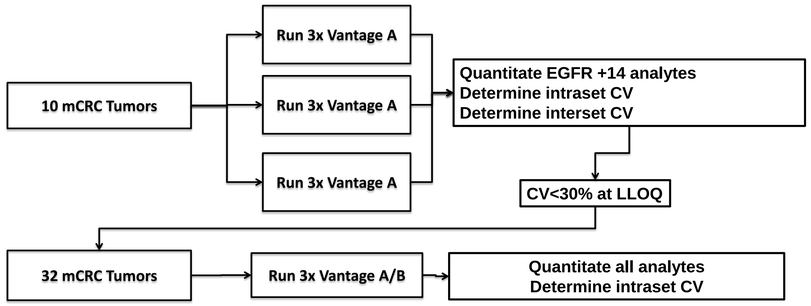

Figure 2. Assay Validation Strategy. 10 FFPE colorectal tumor samples were selected for initial assay validation. Intraset and interset CV's on the 10 validation samples, run in triplicates, were <15% except when analyte concentration approached the lower limit of quantitation (*LLOQ) where the CV approached 30%. Any samples with a CV in excess of 30% were rerun.

Results

Quality Control Analysis of CRC clinical biopsies

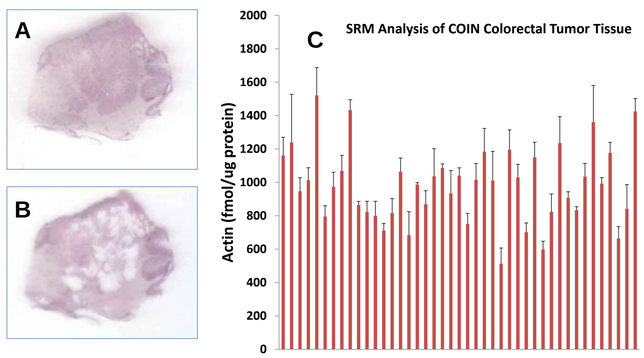

Figure 3. Colorectal carcinoma tissue before (A) and after (B) laser microdissection. C. Quantitation of Actin in mCRC clinical samples. Actin, tubulin and GAPDH are used to assess the cellularity of the tissue. In this particular subset, there were no gross deviations among samples.

Quantitative Analysis of Cancer Drug Targets

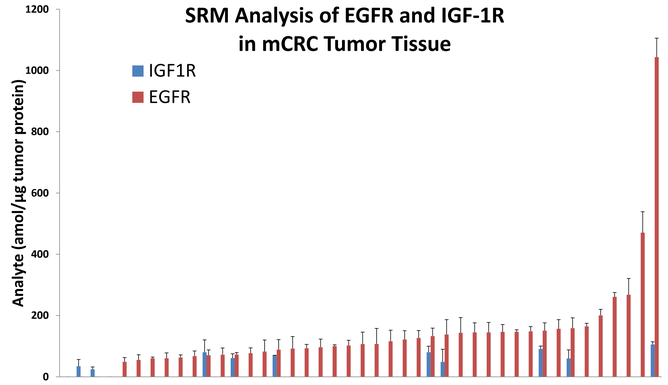

Fig. 4. Multiplexed quantitation of EGFR and IGF-1R in mCRC tumor tissue. Quantitation of IGF-1R in mCRC tumor samples. Expression levels of IGF-1R are co-plotted with EGFR expression data. IGF-1R is hypothesized to be a resistance marker in patients receiving EGFR targeted therapies (e.g. cetuximab in mCRC). Identifying the expression of alternate drug targets could help define which targeted therapy has the best chance of clinical success..

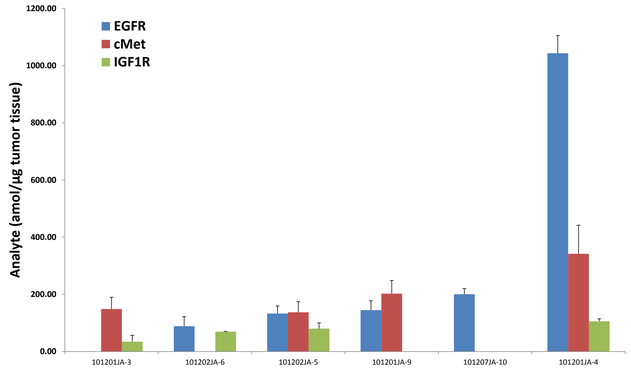

Figure 5. Comparison of EGFR, cMet and IGF-1R Expression in mCRC Clinical Trial Tissues. Quantifying EGFR, IGF-1R and cMet in single tumors may help define the role of these candidate resistance markers in colorectal cancer.

Conclusions

- A multiplexed Liquid Tissue-SRM assay was used to quantify 12-15 different proteins in FFPE Colorectal Cancer tissue from the COIN trial.
- Quantifying EGFR, IGF-1R and cMet in single tumors will allow clinicians to assess the likely sensitivity of a tumor to multiple targeted oncology therapies, and make the best decisions about which drugs will have the greatest chance of showing efficacy.

Expression Pathology

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