Development of a Quantitative Gastroesophageal Cancer Selected Reaction Monitoring Mass Spectrometric Multi-plex Assay for Use in FFPE Tissues

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Abstract

Introduction: Aberrant over-expression of receptor tyrosine kinases, including the MET, HER, FGFR, and IGFR families along with other critical oncogenic mediators including KRAS, PI3 Kinase and SRC are known drivers of gastroesophageal adenocarcinoma (GEC), subdividing the disease into distinct molecular subsets. Inter/intrapatient tumor heterogeneity suggests that an expedient, reliable, medium throughput oncogene protein expression profiling will provide vital information to better personalize cancer care. To date, clinical quantification of protein in formalin fixed paraffin embedded (FFPE) tissues is limited to immunohistochemistry (IHC), which is semi-quantitative at best. Moreover, IHC of multiple proteins of interest is laborious, time consuming, wasteful of scarce tissue, and costly. Other protein quantification methods (ELISA, ECL) would require non-standard tissue processing for analysis. We present a quantitative mass spectrometric (MS) assay for GEC utilizing Liquid Tissue – Selected Reaction Monitoring (SRM), with subsequent multiplex quantification of relevant oncoproteins in a panel of gastroesophageal cancer (GEC) cell lines and tissues.**Methods:** Using trypsin digestion mapping of recombinant oncoproteins, we identified unique peptide sequences, and built quantitative MS assays which could be multiplexed into a single SRM analysis of 1µg of tumor protein. Assays were preclinically validated on 10 different formalin fixed (FF) cell lines. We then tested the 'GEC-plex' MS assay using a panel of FF GEC cell lines previously characterized by immunoblot (IB), IHC FFPE pellet, and gene copy number by fluorescence in situ hybridization (FISH). In addition to RON, we multiplexed SRM quantification of Met, EGFR, HER2, HER3, IGF1R, FGFR2, KRAS and cSRC. We evaluated 17 GEC lines including three AGS lines: wild type (AGS-WT), scrambled shRNA (AGS-SC) and RON shRNA knockdown (AGS-KD) to assess 'post-treatment' changes in oncogene expression profiles. We then evaluated 100 GEC human cancer tissues with paired peritoneal metastases when available, and select paraneoplastic normal tissues using laser capture microdissection of the target material from a single unstained 10µm thick section per sample. **Results:** Validation of the GEC-plex SRM assay on GEC cell lines revealed very high concordance when compared to IB and IHC measurement. The AGS-WT/SC cells showed comparable levels of RON (284/323 amol/µg cell protein), while RON was not detected in AGS-KD cells, as expected. Measurement of each oncoprotein in the GEC cell lines and tissues correlated well with IHC and FISH data. Multiplex oncogene quantification of all cell lines and tissues, along with expression profile changes in the AGS RON KD line compared to AGS-WT/SC will be presented.**Conclusions:**Taken together, these data demonstrate a sensitive, accurate, and quantitative assay to measure relevant actionable oncoproteins in FF cells. The GEC-plex multiplexed oncogene expression of these tumors was feasible and expedient using limited tissue from clinical samples, and is a novel clinically applicable approach for tumor characterization for baseline and post-treatment assessment.

Methods

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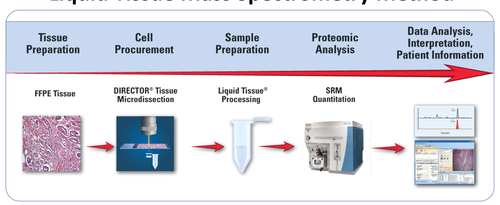
GEC cultured cells were washed, fixed with formalin, and subjected to Liquid Tissue® processing. FFPE tumor tissue blocks were cut on DIRECTOR® slides and processed using standard histological procedures. Tissues were laser microdissected on a Leica LMD-6000, collected in tubes and solubilized to tryptic peptides using Liquid Tissue® technology. FISH and IHC assays were performed as previously described.

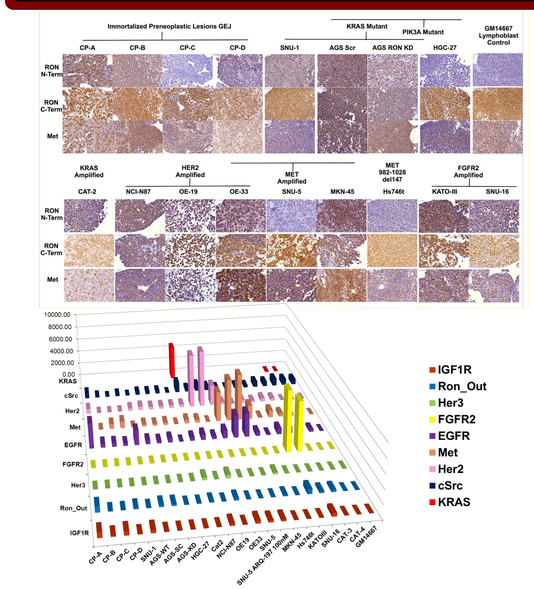
Liquid Tissue Mass Spectrometry Method



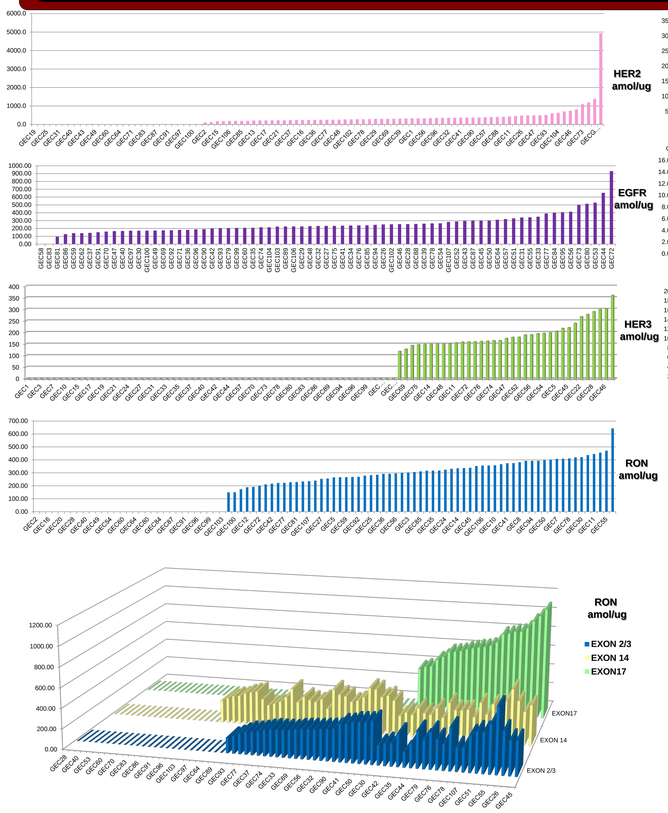
References:

1. Hembrough et al. *J Clin Proteomics* 2012.[1] 2. Catenacci et al. *Cancer Discovery* 2011.[2]

Validation in GEC Cell Lines



FFPE Tissue Results - Univariate



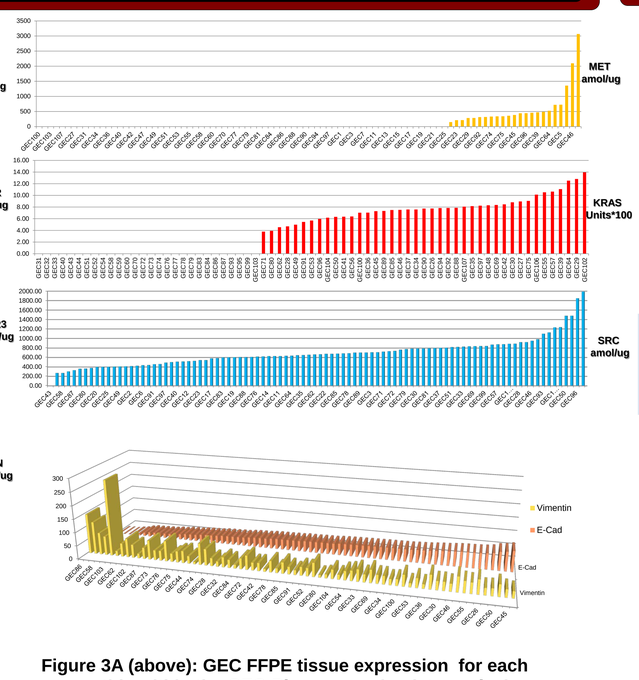
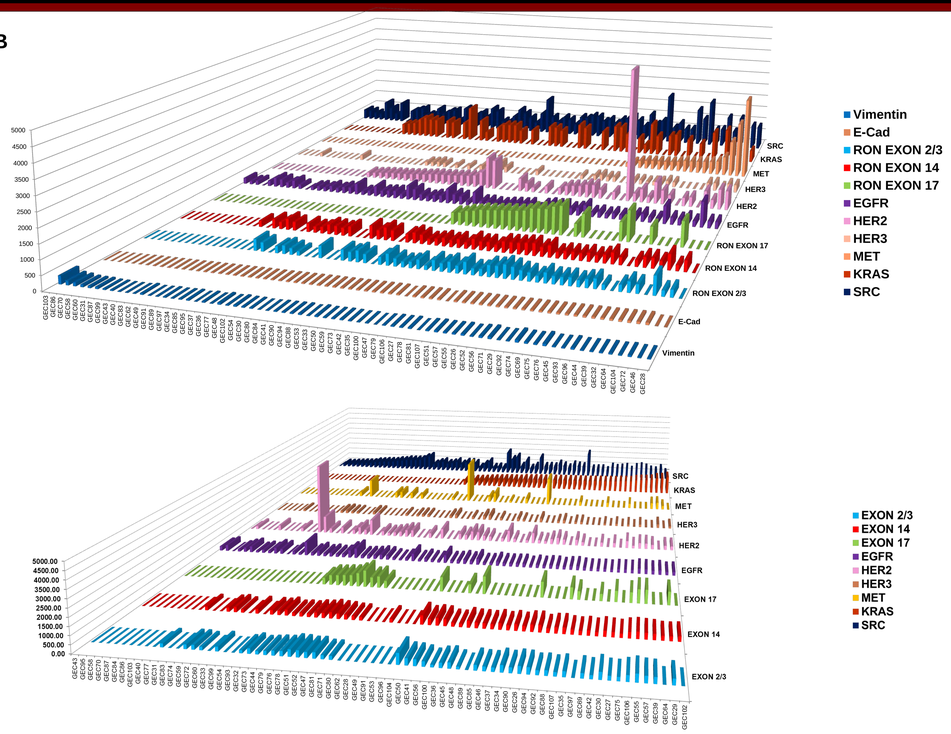


Figure 3A (above): GEC FFPE tissue expression for each peptide within the GEC-Plex as a univariate analysis from lowest to highest expression, left to right.

Figure 3B (below): GEC FFPE tissue expression for each peptide within the GEC-Plex as a multivariate analysis, sorted by MET expression from low to high, left to right (above) and KRAS (below).

FFPE Tissue Results - Multivariate

Figure 3B



SRM GEC-Plex Correlation with IHC and FISH

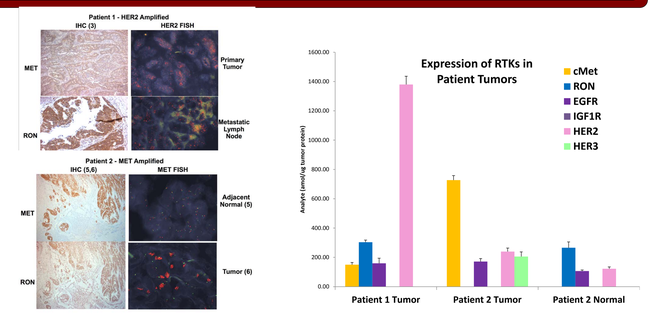
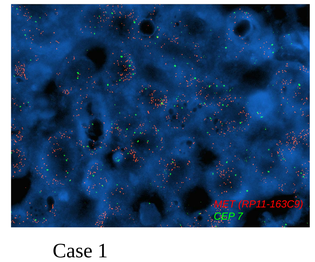
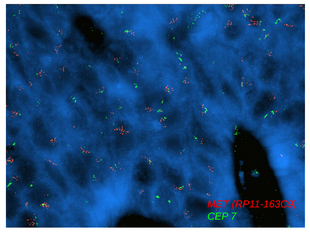


Figure 4. Comparison of IHC and FISH tissue characterization with SRM results in select patient samples. The left column of the left figure represents MET and RON IHC in two patient tumor samples. The right column demonstrates FISH for HER2 (patient 1: amplified in tumor and lymph node) and MET (paient 2: amplified in tumor not adjacent normal. The right graph shows multiplex SRM results of these samples, and concordance of HER2, MET, and RON expression.



Case 1



- Case 2

Correlation of protein expression

Pairwise Correlation of Protein Expression by SRM

	Egfr	Her2	Her3	Met	RON:ex2	ex14	ex17	KRAS	Src
Egfr	1.0000								
Her2	0.0740	1.0000							
Her3	**0.1546**	**0.2823**	1.0000						
Met	**0.2045**	**0.1622**	**0.4605**	1.0000					
RONexon2	-0.0173	**0.2217**	**0.2050**	0.0355	1.0000				
RONexon14	0.0231	**0.1940**	**0.3058**	0.0566	**0.8398**	1.0000			
RONexon17	0.0501	0.0042	0.0521	**-0.0584**	**0.5500**	**0.4264**	1.0000		
KRAS2A	**-0.1261**	**-0.0989**	**0.1600**	0.1311	**0.3311**	**0.5089**	**0.1267**	1.0000	
Src	-0.0102	0.1162	**0.3755**	**0.2582**	**0.3700**	**0.4199**	**0.1892**	**0.2642**	1.000

Conclusions

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- We have developed multiple specific and quantitative SRM assays for oncoproteins important in GEC. This was first shown in GEC cell lines demonstrating concordance with IB and IHC, before proceding with FFPE tumor tissue using our Liquid Tissue-SRM platform.
- Multiplexed oncogene expression of these FFPE tumors was feasible and expedient using limited tissue, and is a novel clinically applicable approach for tumor characterization for baseline and post-treatment assessment. Correlation of protein expression within the GEC-plex with each other was informative.
- Outcome based analysis of larger cohorts has been initiated, in an attempt to assess the role of multiple receptor tyrosine kinases in prognosis, as well as drug sensitivity and resistance.