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Quantification of FGFR protein expression in solid tumors by targeted mass spectrometry and correlation with FGFR gene amplification status

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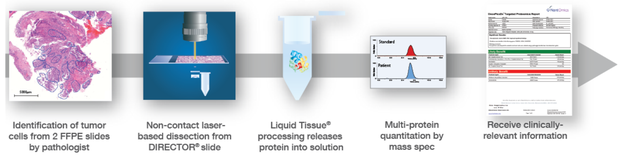
Background

- Fibroblast growth factor receptors (FGFR) have been reported as gene amplified or protein overexpressed in multiple cancers, including lung, breast and gastric.[1-3]
- However, FGFR protein inhibitor therapies have shown only modest efficacy in patients with FGFR gene amplification as determined by fluorescence in situ hybridization (FISH).[4-5]
- Recent reports suggest direct measurement of FGFR proteins may be necessary to help predict treatment efficacy of FGFR inhibitor therapies.[5-6]
- We therefore developed a quantitative LC-MS assay for FGFR proteins using SRM and stable isotope-labeled standards.
- Expression of FGFR proteins by LC-MS was compared with FGFR gene amplification status by FISH (n=15) and by RNA-seq (n=7).

Methods

- Malignant cells were identified in formalin-fixed, paraffin-embedded (FFPE) clinical tumor tissues by a board-certified pathologist and isolated by laser microdissection.
- Proteins were solubilized and digested with trypsin for subsequent LC-MS analysis using SRM (Waters nanoAcquity UPLC – Thermo Quantiva QQQ).
- Stable isotope-labeled standards and response curves enabled analytical characterization in a surrogate matrix and the assay was qualified in FFPE tissues.
- FGFR gene amplification status by FISH (FGFR/CEP ratio >2.2) and by RNA-seq was determined in a subset of matching tumor tissues.



Results

Table 1. Overview of the LC-MS assay for FGFR proteins

LOD	LLOQ	ULOQ	Linearity (R^2)	Intra-Assay Precision	Inter-Machine Precision	Maximum Carry-Over
75 amol	100 amol	25,000 amol	0.9999	< 15%	< 15%	0.21%

Figure 1. LC-MS response curve for FGFR proteins
Varying amounts of light peptide were spiked in a complex background with a constant amount of heavy peptide (5 fmol/µg) to build the standard curve (n=5).

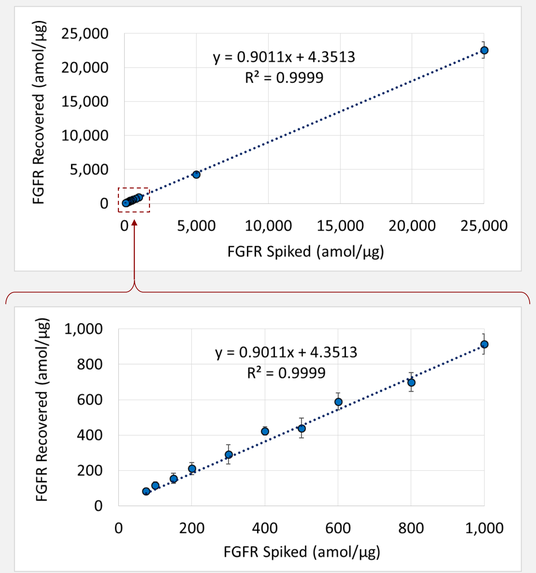


Table 2. LC-MS intra-assay precision in FFPE tissue

FGFR Spike (amol/µg)	LC-MS System 1 CV (%)	LC-MS System 2 CV (%)
200	13.0	6.9
300	10.4	8.0
400	1.6	4.8
500	4.0	5.7
600	4.6	2.4
800	14.5	5.9
5,000	6.4	3.5
10,000	3.3	0.8

Figure 2. LC-MS inter-machine precision in FFPE tissue
Ten FFPE tissue samples were spiked with different concentrations of the light peptide. Three injections of each sample were analyzed on 2 different LC-MS systems.

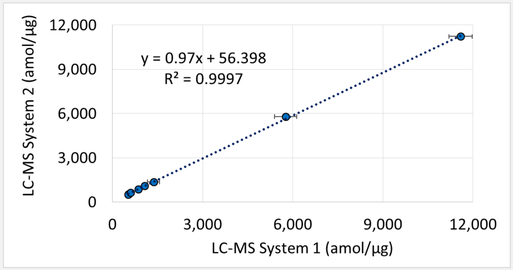


Table 3. Matching samples for LC-MS, FISH and RNA-seq

Sample ID	Tissue Type
RTZ-50	Breast
RTZ-51	Breast
RTZ-52	Breast
RTZ-53	Breast
RTZ-54	Breast
RTZ-55	Gastric
RTZ-56	Breast
RTZ-57	Lung

Sample ID	Tissue Type
RTZ-58	Breast
RTZ-59	Breast
RTZ-60	Endometrium
RTZ-61	Lung
RTZ-62	Breast
RTZ-63	Breast
RTZ-64	Breast

Figure 3. Comparison of FGFR by LC-MS and FISH
FGFR status was assessed by LC-MS and FISH in matching FFPE tumor samples (n=15). Of 12 gene-amplified samples, 3 had no detectable FGFR proteins. ND = Not Detected.

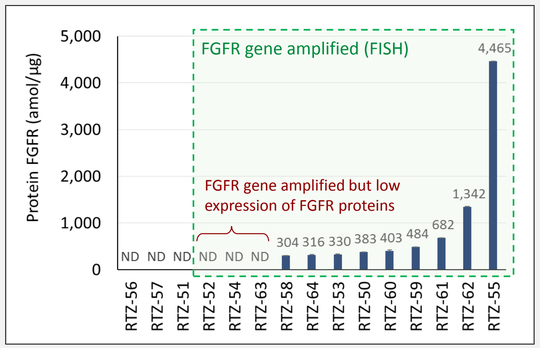


Figure 4. Comparison of FGFR by LC-MS and RNA-seq
FGFR status by LC-MS and by RNA-seq was in agreement for 5 of the 7 clinical samples available for comparison.

FGFR Status by RNA-seq \ FGFR Status by LC-MS	Positive	Negative
Positive	RTZ-50, RTZ-61, RTZ-62, RTZ-64	RTZ-63
Negative	RTZ-53	RTZ-52

Conclusions

- We developed a quantitative LC-MS assay for FGFR proteins in FFPE tissue samples with high linearity and precision across the entire dynamic range.
- Three of 12 clinical samples with FGFR gene amplification by FISH had no detectable FGFR proteins as measured by LC-MS. FGFR status by LC-MS and RNA-seq was in agreement in 5 of 7 samples.
- These findings are important because patients whose tumors express low or undetectable FGFR protein are not likely to respond to therapy with FGFR protein inhibitors.

References

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