Mass spectrometry-based proteomic analysis may improve identification of patients sensitive to FGFR inhibitor therapy

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Abstract

Fibroblast growth factor receptors 1 and 2 (FGFR1 and FGFR2) are amplified in multiple tumor types including breast, lung and gastric [1-3]. FGFR inhibitor therapies have shown only modest efficacy in patients with FGFR protein amplification, as determined by fluorescence in situ hybridization (FISH) [4,8]. Gene copy number is not an optimal therapeutic biomarker because the targets of FGFR inhibitors are FGFR proteins; recent findings suggest that direct measurement of FGFR proteins may be necessary to identify patients likely to respond to FGFR inhibitor therapies [5,6].

Multiplexed mass spectrometry-based proteomic analysis objectively quantifies FGFR proteins and other actionable protein biomarkers from two formalin-fixed, paraffin-embedded (FFPE) tissue sections.

In archived patient tumor samples, we sought to correlate FGFR1, FGFR2 and FGFR1-4 proteins measured by mass spectrometry-based proteomics with FGFR gene amplification determined by FISH and RNA-seq, and with FGFR protein overexpression determined by immunohistochemistry (IHC).

Results

FGFR protein quantity, by status of FGFR FISH and IHC (N=26)

Targeted proteomics identifies potential combinational therapies for FGFR-positive patients

FGFR status by IHC

FGFR status by RNA-seq

Targeted proteomics identifies potential therapy combinations for FGFR-negative patients

Conclusions

Quantitative proteomics objectively measures FGFR proteins in FFPE tumor samples. A subset of FGFR-amplified tumors do not express FGFR protein when assessed by highly-sensitive mass spectrometry. A previous study in lung cancer tumors found that elevated FGFR1 mRNA and/or protein expression occurred independently of FGFR1 gene amplification [6]. Our findings are important because patients whose tumors do not express FGFR protein are not likely to respond to FGFR inhibitor therapy. In a study of lung cancer cell lines, ponatinib sensitivity correlated with FGFR1 protein expression, but not with FGFR gene copy number [7]. RNA-seq identified isoforms specific to FGFR inhibiting agents. An approach combining quantitative proteomic and genomics analysis may accurately identify patients most likely to respond to specific FGFR inhibitors.

Multiplexed proteomics simultaneously quantitates up to 60 different target proteins to identify cancer patients mostly likely to benefit from FGFR inhibitors, other targeted therapies, immunotherapies and chemotherapies.

References


Methods

Figure 1: FFPE tissue sections from breast (n=20), esophageal (n=1), gastric (n=1), lung (n=3), and endometrial (n=1) tumors were marked by a board-certified pathologist, who were microdissected and solubilized. A mass spectrometry-based proteomic assay was used to quantify protein expression levels of FGFR1, FGFR2, FGFR1-4, and other targetable proteins, including HER2, IDO1 and gpNMB. We compared FGFR protein levels with IHC and with FGFR amplification by FISH (FGFR to CEP ratio >2.2) and by RNA-seq (>147 transcripts per million TPM).

Figure 2. A. The FGFR1-4 proteomic assay detected FGFR proteins in 15 of 26 tumors analyzed; 14 samples (93%) were FGFR amplified by FISH and 8 (61%) showed FGFR overexpression by IHC. FGFR protein was undetectable in 11 samples, of which 4 (36%) were FGFR1 amplified by FISH. A single non-amplified case overexpressed FGFR by proteins and by RNA-seq. All samples were tested for FGFR1 by FISH, except F050 and F031 that were tested for FGFR2 FISH. B. Sensitivity of the FGFR1-4 assay was superior to the single FGFR1 assay, but 2 of 2 FGFR2-amplified cases showed high FGFR2 protein expression C. In 16 tumors analyzed by RNA-seq, the agreement rate between the FGFR proteomic assay and RNA-seq was 81%.

Figure 3. A, B. Multiplexed expression analysis of therapy-associated protein biomarkers clustered by therapy type. Percentile scale is specific to each biomarker and based on hundreds of samples tested. ADC= antibody-drug conjugates (IMMU-132: anti-TROP-2 antibody conjugated with irinotecan; glembratumumab vedotin: anti-gpNMB linked to monomethyl auristatin E).

Figure 4. A. Targeted proteomics identified potential combinational therapies for FGFR-positive patients. B. Targeted proteomics identified potential therapy combinations for FGFR-negative patients.