Abstract # P6-07-16

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

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Background

- 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 gene amplification, as determined by fluorescence in situ hybridization (FISH) [4,5].
- 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).

Methods

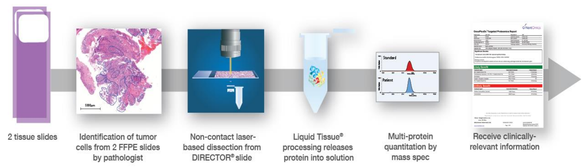


Figure 1: FFPE tissue sections from breast (n=20), esophageal (n=1), gastric (n=1), lung (n=3), and endometrial (n=1) tumors were marked by a board-certified pathologist, which were microdissected and solubilized. A mass spectrometry-based proteomic assay was used to quantitate protein expression levels 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).

Results

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

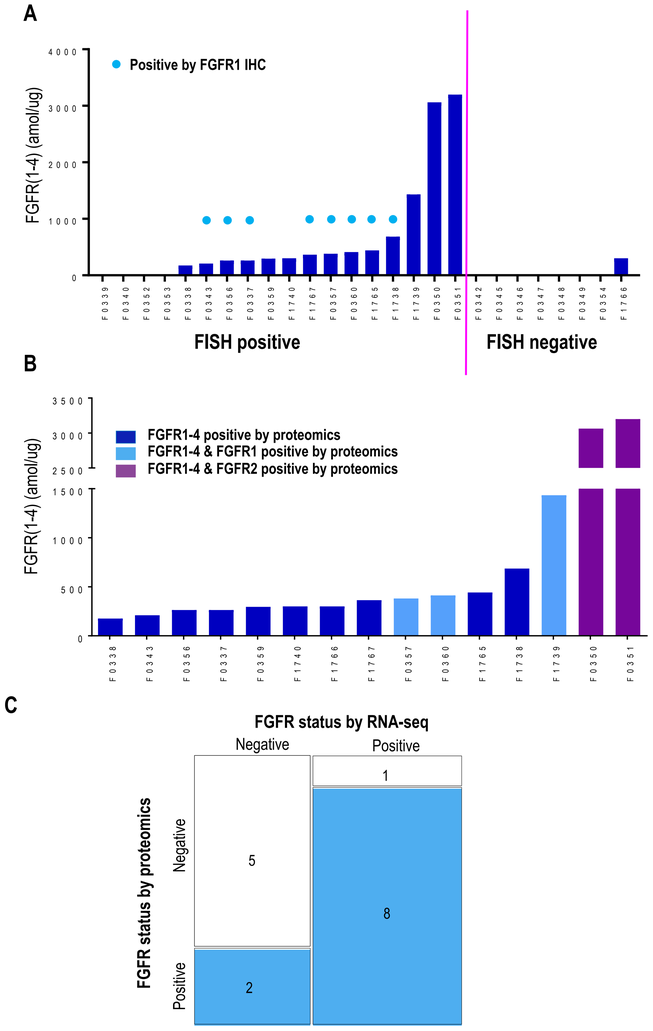
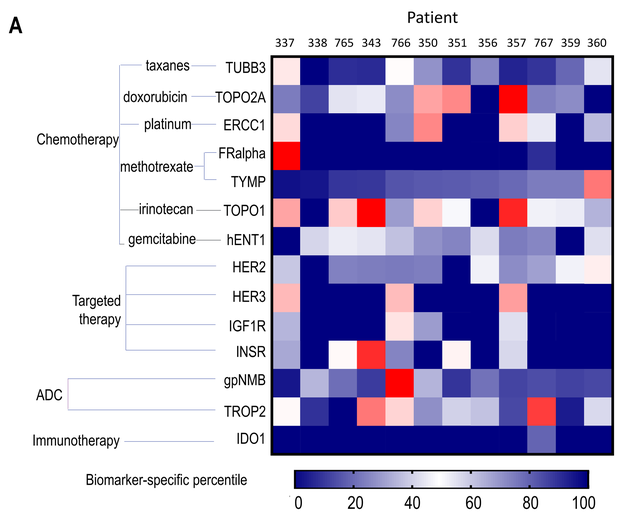


Figure 2. A. The FGFR1-4 proteomic assay detected FGFR proteins in 15 of 26 tumors analyzed; 14 samples (93%) were FGFR amplified by FISH and 8 (61%) showed FGFR overexpression by IHC. FGFR protein was undetectable in 11 samples, of which 4 (36%) were FGFR1 amplified by FISH. A single non-amplified case overexpressed FGFR by proteomics and by RNA-seq. All samples were tested for FGFR1 by FISH, except F0350 and F0351 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%.

Targeted proteomics identifies potential combinational therapies for FGFR-positive patients



Targeted proteomics identifies potential therapy combinations for FGFR-negative patients

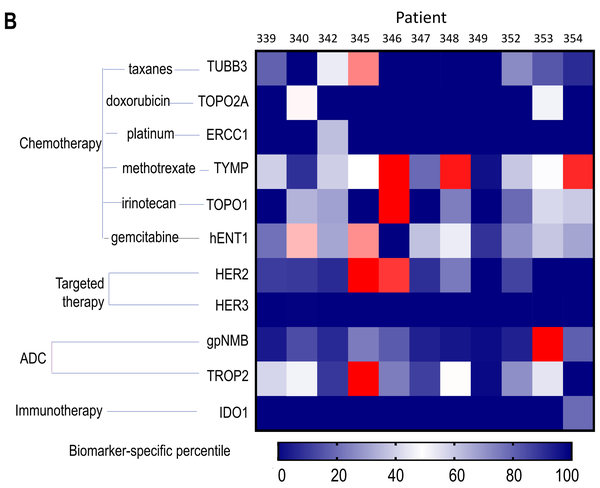


Figure 3. A, B. Multiplexed expression analysis of therapy-associated protein biomarkers clustered by therapy type. Percentile scale is specific to each biomarker and based on hundreds of samples tested. ADC= antibody-drug conjugates (IMMU-132: anti-TROP-2 antibody conjugated with irinotecan; glembatumumab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

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 like to benefit from FGFR inhibitors, other targeted therapies, immunotherapies and chemotherapies.

References

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