Proteomic analysis of primary and metastatic breast cancers reveals a wide range of expression of the folate receptor, a potential drug target

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Overview

- The folate receptor alpha (FOLR1) is reported to be highly expressed in triple negative breast cancer (TNBC).
- Targeting this pathway with pemetrexed in unselected breast cancer (BC) has been a modest success.
- We used multiplexed mass spectrometry (MS) to assess the expression of the FOLR1 and other biomarkers in TNBC to identify patients who may be responsive to antifolate therapy.
- Targeted proteomics data confirms that FOLR1 expression is more common in TNBC than other BC subtypes, and showed a 10-fold range of expression in both sample sets; a range that is most likely indiscernible by IHC.

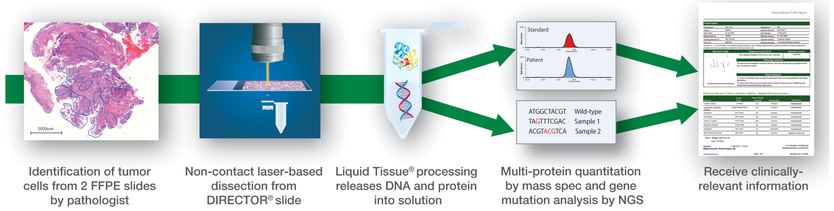


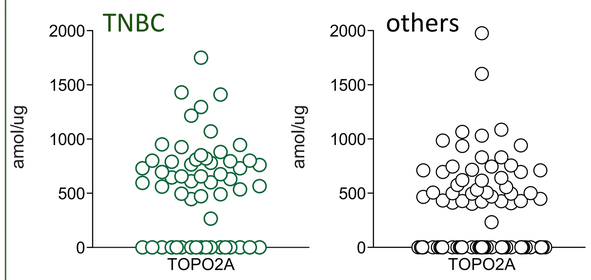
Figure 1: Liquid Tissue®-SRM workflow for analysis of proteins from FFPE tissue

- In our CAP/CLIA lab, primary and metastatic BC tissues were microdissected, solubilized and enzymatically digested following standard protocols.
- Archival BC tissues (n=173) were analyzed following GLP protocols.
- Peptides unique to proteins of interest were identified and labeled peptides were synthesized
- Calibration curves were constructed to establish linearity of the assay and lower limits of detection and quantitation for each protein
- Formalin-fixed, paraffin-embedded (FFPE) human tumor tissue was used for assay validation and testing
- Absolute quantitation of protein targets was performed using selected reaction monitoring (SRM) mass spectrometry.

OncoPlex Diagnostics Clinical Proteomic Menu

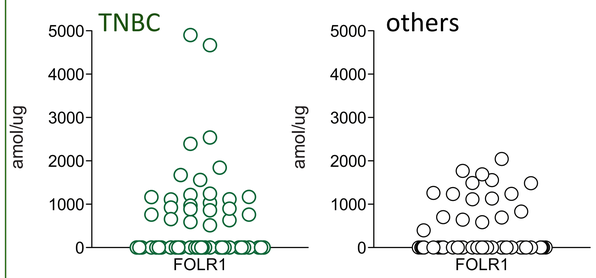
AR, ALK, AXL, CK-5, CK-7, EGFR, ERCC1, FGFR2, FOLR1, hENT1, HER2, HER3, KRAS, MET, MGMT, MSLN, PD-L1, p16, RON, ROS1, RRM1, SPARC, TOPO1, TOPO2A, TP63, TUBB3, TTF1

Multiplex Proteomic Analysis of TNBC Tissues Identifies Potential Chemotherapy Targets



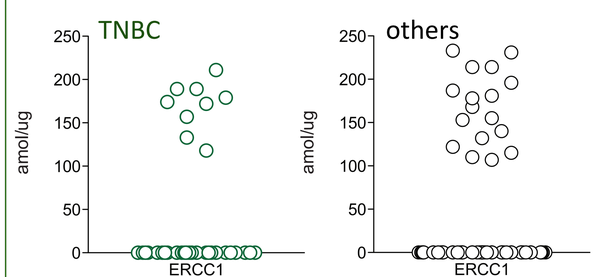
	TNBC	other
Minimum	266	233
10.00%	490	416
25.00%	600	461
50.00%	792	680
75.00%	880	774
90.00%	1295	1055
Maximum	1295	1975
Total tested	56	103
Prevalence	70%	48%

Figure 2. Distribution of TOPO2A in BC and TNBC. 30% of TNBC patients do not express measurable TOPO2A and may be less likely to demonstrate a pathological complete response to neoadjuvant AC-T treatment. More than 50% of BC patients express TOPO2A



	TNBC	other
Minimum	515	397
10.00%	614	547
25.00%	810	697
50.00%	1443	1169
75.00%	1618	1523
90.00%	3392	1825
Maximum	4900	2045
Total tested	56	117
Prevalence	45%	14%

Figure 3. Distribution of FOLR1 in BC and TNBC
FOLR1 is a biomarker for antifolate drugs. While only ~17% of all BC expresses FOLR1, 45% of TNBC do, suggesting that these patients may respond to folate targeted therapy.



	TNBC	other
Minimum	118	107
10.00%	118	109
25.00%	145	127
50.00%	170	167
75.00%	190	205
90.00%	211	231
Maximum	211	233
Total tested	35	77
Prevalence	26%	22%

Figure 4. Distribution of ERCC1 in BC and TNBC.
ERCC1 is a biomarker of resistance for Platinum therapies. A high percentage of BC and TNBC express ERCC1, suggesting that these patients may not respond to platinum therapy.

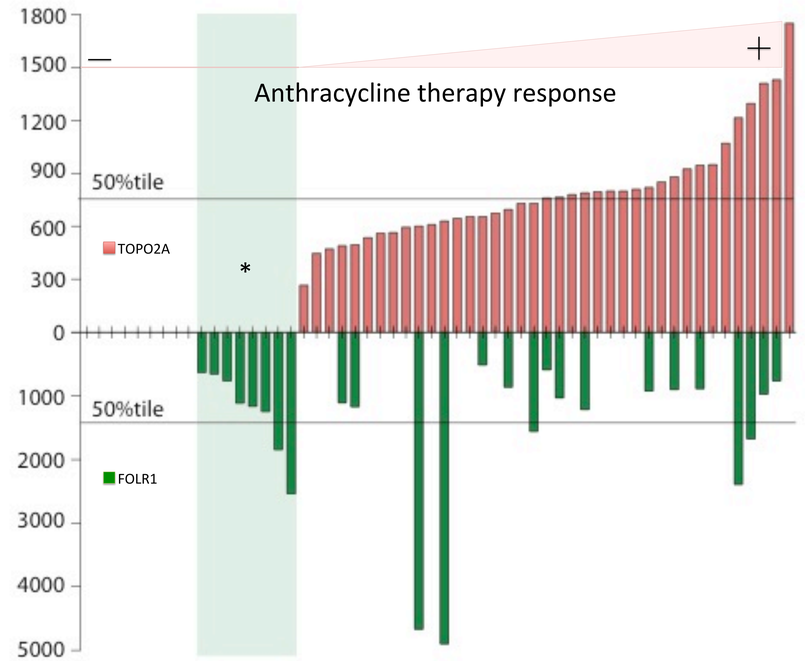


Figure 5: Lack of TOPO2A may cause poor response to anthracycline-based chemotherapies
~14% of all TNBC are both positive for FOLR1 and negative for TOPO2A (*), suggesting that these patients may respond better to folate targeted therapy than anthracycline treatment.
Multiplex targeted proteomics shows that ~30% of all TNBC are both positive for FOLR1 and TOPO2A. Simultaneous assessment of biomarkers give option for combination therapies and second line therapies.

Conclusions

- Quantitative proteomics using multiplexed mass spectrometry is extremely sensitive, uniquely precise and highly reproducible unlike existing protein analysis methods.
- A survey of TNBC patient tumors has identified protein biomarkers with highly variable expression patterns that offers insight into multiple, novel opportunities to optimizing treatment.
- Multiplex MS data confirm that FOLR1 expression is more common in TNBC and can help to identify patients who may be responsive to antifolate therapy.
- Multiplexing allows for the simultaneous assessment of biomarkers and give option for second line therapies.