Breast Cancer IHC Subtypes Display Heterogeneous And Independent Targetable Signaling Pathway Activity Profiles

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INTRODUCTION

Primary breast cancer is routinely sub-typed using immunohistochemistry (IHC) staining and treatment choice is guided by the subtype.

Targeted treatment requires the targeted signaling pathway to be active and tumor driving. IHC staining does not provide reliable information on active signaling pathways, and we reported before that clinical response of ER positive breast cancer patients to neoadjuvant aromatase inhibitor therapy appears to be more closely related to ER pathway activity rather than ER/PR IHC staining.
To better understand the role of tumor driving signaling pathways within breast cancer subtypes and potentially improve precision medicine we evaluated signaling pathway activity profiles per IHC subtype, using novel assays to quantitatively measure activity of multiple main tumor driving signal transduction pathways, within tissue samples.

METHODS

mRNA-based estrogen (ER) and androgen (AR) receptor, MAPK, PI3K, Hedgehog (HH), TGFβ, Notch, Wnt signal transduction pathway assays have been previously described.

Pathway activity scores (PAS) were measured on primary untreated breast cancer samples, and compared to normal breast epithelium, using Affymetrix expression dataset GSE65194 (Institut Marie Curie), excluding the duplicate measures, containing 29 Luminal A, 30 Luminal B, 30 HER2, 41 triple negative (TN) breast cancer and 6 normal (epithelial) breast tissue (obtained from plastic surgery) sample data. Pathway activity scores exceeding the 95th percentile of normal were considered abnormally active.

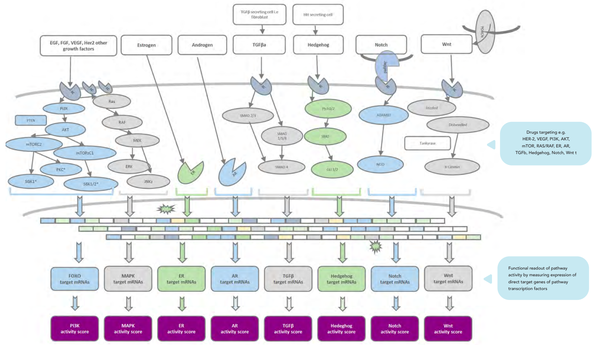

Figure 1: Colon major signaling pathways in breast cancer, measured by OncoSignal method

RESULTS

- Pathway activity scores: PAS thresholds for defining abnormally active pathway activity in breast cancer were established based on the PAS of each pathway measured in benign breast tissues, these thresholds were: MAPK 23, AR 29, ER 34, PI3K 42, HH 34, Notch 65, TGF 48, Wnt 29. Each canonical subtype of breast cancer had distinct signal pathway activation patterns.
- Luminal A breast cancer subtype was characterized by high ER PAS (n=25, 86%, mean PAS 45, SD 10).
- Luminal B cancers displayed high ER PAS (n=10, 33%; mean PAS 32, SD 11) increased PI3K PAS (n=27, 90%; mean PAS 53, SD 10) and increased HH PAS (n=23, 77% mean PAS 29, SD 7).
- HER2 enriched tumors had by high AR PAS (n=18, 60%; mean PAS 30, SD 6) increased PI3K PAS (n=19, 63%; mean PAS 48, SD8) and increased HH PAS (n=27, 90% mean PAS 30 SD 7).
- TNBC tumors displayed high PI3K PAS (n=37, 90%; mean PAS 53, SD 7) plus high developmental pathway PAS (Notch, n=6, 15%; mean PAS 53, SD 12; Wnt, n=28, 68%; mean PAS 31, SD 6).
- All breast cancer subtypes had elevated MAPK PAS.

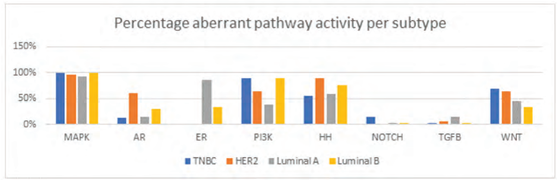

Figure 2: the % of pathways that are aberrant per BrCa subtype if all samples are counted that show pathway activity above set threshold

OBSERVATIONS

- The OncoSignal assay can accurately measure the activity of key targetable molecular driver signal pathways in breast cancer.
- OncoSignal ER PAS scores are strongly associated with luminal A, B, HER2 and TNBC breast cancer subtypes as defined by routine IHC.
- OncoSignal can measures PAS in key targetable pathways including MAPK, AR, and PI3K and may enable the identification of individual tumors where these pathways are the dominant oncogenic drivers and attractive therapeutic targets.

TARGETABLE PATHWAYS

- ER – Estrogen receptor signaling PAS was highest in the luminal A and B cancers. Luminal B showed reduced ER PAS compared to luminal A cancers.
- AR- Androgen receptor PAS was elevated across multiple subsets of tumors including HER2 enriched, TNBC and Luminal B cancers.
- TGF B- Transforming growth factor beta PAS was elevated in a sub group of luminal A as well as a group of benign breast tissues.
- MAPK PAS was elevated across all tumor types compared to benign tissue.
- Notch PAS was reduced in most tumor types compared to benign tissues.
- PI3K showed wide PAS variation with more than half of cases in all tumor types displaying elevated PAS of this signaling pathway.

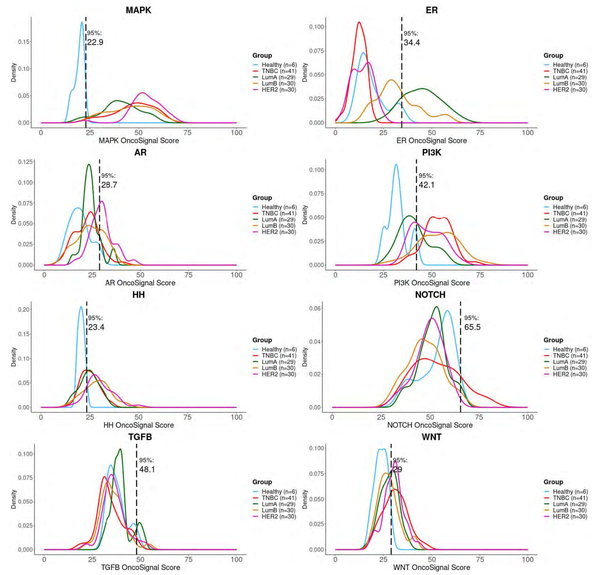

Figure 3: The distribution of pathway activity per pathway per subtype. The dotted line represents the threshold that was set to determine aberrant pathway activity.

Six benine samples were obtained from mammary plastic surgery. the tumors contained between 50–90% cancerous cells 10% was used as the cut-off for ER and PR positive cells. 30% staining of membrane for HER2-positivity, 35 LA (ER+, PR+, HER2–); 40 LB (ER+, PR+, HER2+/–); 33 HER2 (ER–, PR–, HER2+) and 46 TNBC (ER–, PR-, HER2–).

CONCLUSION

Each breast cancer subtype based on IHC, had in the overall population a typical pathway activity profile. However within each IHC subtype, a substantial range of pathway activities between patients is observed, suggesting that within same IHC subtypes, clear differences in signal pathway activation can be measured that may provide a novel approach to more effectively select patients for specific precision oncology targeted therapy treatments. The activity of Key targetable pathways including ER, AR, MAPK and PI3K can be rapidly and precisely measured using this approach. This method may identify additional therapeutic options for patients with breast cancer targeting PI3K, MAPK, HH, ER and AR in tumors where these are dominant molecular drivers.

AACR 2021 Permanent Abstract Number: 533

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