Distribution of Receptor Tyrosine Kinase (RTK) nsSNPs in Breast Cancer (BC) Patients using Next-Generation Sequencing (NGS)

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BACKGROUND

Receptor tyrosine kinase (RTK) pathways are aberrantly activated in cancer, and gain-of-function mutations and alterations in RTKs serve as attractive drug targets.

Comprising 20 families, the 58 known human RTKs each contain an extracellular domain (ECD), single transmembrane helix (TM), and cytoplasmic domain with juxtamembrane (JM) and C-terminal (CT) regulatory regions flanking a tyrosine kinase domain (TKD).[1]

Tumor profiling with next-generation sequencing (NGS) can reveal novel non-synonymous single nucleotide polymorphisms (nsSNPs) along RTKs' entire amino acid sequence that need further classification.

We sought to classify nsSNPs within RTKs among breast cancers identified by NGS.

METHODS

Institutional review board approval was obtained.
A database of breast cancer patients treated at West Cancer Center (Memphis, TN) from 2013-2015 was reviewed.

Inclusion Criteria:

- Known ER, PR, HER2 status
- Received tumor profiling including NGS with a 592 cancer-related gene panel from Caris Life Sciences[2]

Caris NGS interrogated 29 RTKs. All mutations test-defined as either pathogenic (PATH) or nsSNPs deemed variants of undetermined significance (VUS) were included. All variants had >99% detection confidence based upon allele frequency and amplicon coverage.[2]

nsSNPs were arranged by amino acid location including the ECD, TM, JM, TKD, or CT.

In order to classify VUS, nsSNPs underwent *in silico* analysis using PolyPhen 2 (Harvard)[3,4] to predict pathogenicity, denoted pnsSNP.

RESULTS

VARIABLE	NUMBER PATIENTS (%)
Total number of patients	77
Median age	58 yo (range 32-83)
Mean age	57 yo
Gender	
Female	76 (99%)
Male	1 (1%)
Race	
Caucasian/White	46 (60%)
African American/Black	29 (38%)
Hispanic	1 (1%)
Asian	1 (1%)
Hormone Receptor Expression	
ER+/HER2-	35 (46%)
ER-/HER2+	10 (13%)
Triple-negative (ER-/PR-/HER2-)	24 (31%)
Triple-positive (ER+/PR+/HER2-)	8 (10%)
Number of nSNPs per Patient	
0	25 (32%)
1	33 (43%)
2	13 (17%)
3	4 (5%)
4	2 (3%)

Table 1. Summary of patient characteristics, demographics, and number of nSNPs per patient

- 78 nsSNPs and 1 PATH were found.
- 52/79 (66%) of patients had ≥ 1 RTK nsSNP (range 0-4).
- 28/79 (35%) of patients had an nsSNP predicted to be damaging (pnsSNP) via i*n silico* analysis, with 3 patients having 2 pnsSNPs.
- 40% of all nsSNPs were predicted damaging (pnsSNPs)
- 28/29 RTKs had ≥ 1 nsSNP with median of 2 (range 0-15).
- 17/29 RTKs had pnsSNPs, median 1 (range 0-9).
- The most commonly mutated RTKs were ROS1 (9/15 variants were pnsSNPs), ALK (3/4), EPHA5 (3/3), FLT4 (2/5), c-KIT (2/4), and ERBB4 (2/3).
- nsSNPs were spread in all 5 RTK regions: 58% localized to the ECD (20/45 pnsSNPs), 17% TKD (8/13), 9% CT (2/7), 9% TM (1/7), and 8% JM (1/6) lesions were found. Of 9 ROS1 pnsSNPs, 7 were ECD, 1 CT, and 1 TKD

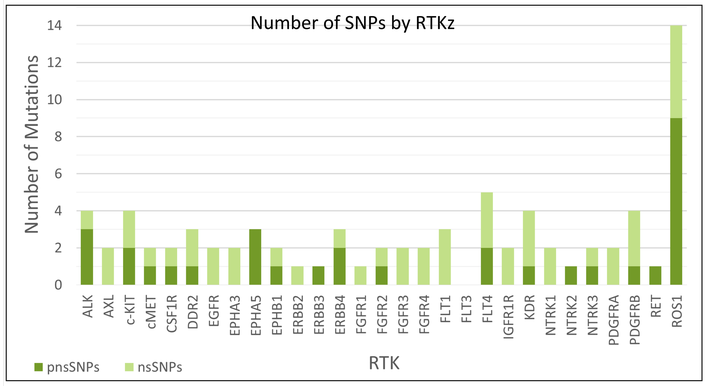

Figure 1. Distribution of pnsSNP and nsSNPs test-defined as VUS, by RTK type

- 100% triple-positive patients (6/8 were pnsSNPs), 69% ER+/HER2- (18/35), 60% ER-/HER2+ (2/10) and 58% triple-negative (3/24) had RTK nsSNPs.
- ROS1 and ALK nsSNPs were most frequently seen in ER+/HER2- patients (9/10 were pnsSNPs) and triple-positive patients (3/4). ER-/HER2+ patients (0/2) and triple-negative patients (0/3) were less frequently seen.

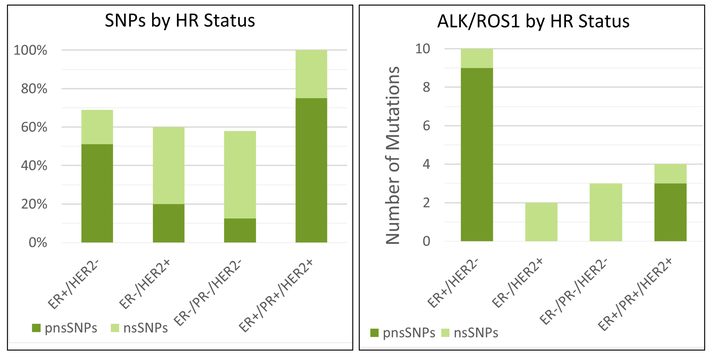

Figure 2-3: Left, percentage of patients with nsSNPs and pnsSNPs by hormone receptor expression. Right, distribution of ALK + ROS1 nsSNPs and pnsSNPs by hormone receptor expression

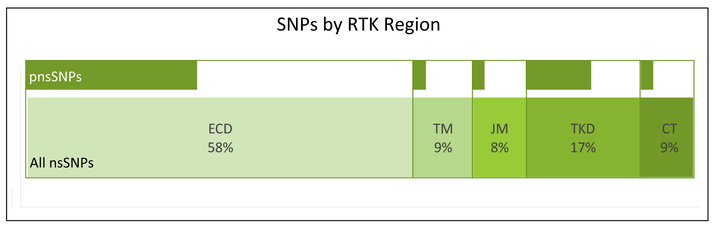

Figure 4: Percentage of total nsSNPs occurring in each region of the RTK (large, lower bar), with proportion occurring as pnsSNPs by in silico *analysis (narrow, upper bar)*

CONCLUSIONS

- 66% of breast cancer patients carried ≥1 RTK nsSNP, with 35% patients harboring a predicted-damaging lesion (pnsSNP) following *in silico* analysis with PolyPhen 2.
- RTK nsSNPs were distributed in various breast cancer phenotypes and included frequent mutations in potentially actionable genes such as ROS1 and ALK.
- 26% (9/35) of ER+/HER2- patients had pnsSNPs in ROS1 or ALK.
- nsSNPs in the ECD or TKD were most frequent and also most likely to be damaging.

Novel RTK pnsSNPs identified in breast cancer patients warrant further classification and collaboration.

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