Neurotrophic receptor tyrosine kinase family members in secretory and non-secretory breast carcinomas

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Abstract

BACKGROUND
Breast cancer is the most common female cancer and a major cause of morbidity and mortality. Progress in breast cancer therapeutics has been attained with the introduction of targeted therapies for specific sub-sets. However, other subsets lack targeted interventions and thus there is persisting need for identification and characterization of molecular targets in order to advance breast cancer therapeutics.

AIM
To analyze the role of lesions in neurotrophic receptor tyrosine kinase (NTRK) genes in breast cancers.

METHODS
Analysis of publicly available genomic breast cancer datasets was performed for identification and characterization of cases with fusions and other molecular abnormalities involving NTRK1, NTRK2 and NTRK3 genes.

RESULTS
NTRK fusions are present in a small number of breast cancers at the extensive GENIE project data set which contains more than 10000 breast cancers. These cases are not identified as secretory in the database, suggesting that the histologic characterization is not always evident. In the breast cancer The Cancer Genome Atlas (TCGA) cohort the more common molecular lesion in NTRK genes is amplification of NTRK1 observed in 7.9% of breast cancers.

CONCLUSION
Neurotrophin receptors molecular lesions other than fusions are observed more often than fusions. However, currently available NTRK inhibitors are effective...
mainly for fusion lesions. Amplifications of \( \text{NTRK1} \), being more frequent in breast cancers, could be a viable therapeutic target if inhibitors efficacious for them become available.

**Key Words**: Neurotrophic receptor tyrosine kinases; Breast cancer; Amplifications; Fusions; Tropomyosin related kinases

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**Core Tip**: Molecular lesions in neurotrophic receptor tyrosine kinase (NTRK) receptors have been brought to the forefront of cancer therapy with the introduction of specific inhibitors which are effective in cancers with fusions involving the family of receptors. In breast cancer fusions involving the NTRK receptors are rarely seen and concern exclusively the secretory sub-type. In non-secretory breast carcinomas amplifications are observed in a minority of cases.

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**INTRODUCTION**

Breast cancer is the most common neoplasm in women and a significant cause of morbidity and mortality. In United States alone, an estimated 284000 cases of breast cancer will occur in 2021, with more than 44000 resulting deaths [1]. Breast cancers represent about 30% of all cancers diagnosed in women. Despite recent advances in breast cancer therapies produced by an improved understanding of molecular pathogenesis, the disease remains difficult to treat when metastatic. It is currently well established that breast cancer does not represent a single entity, but several sub-types exist with implications for prognosis and treatment [2]. The most frequent sub-type is estrogen receptor (ER) positive and negative for the epidermal growth factor receptor (EGFR) family receptor 2, known as human EGFR receptor 2 (HER2) and is further divided in a more indolent disease corresponding with the genomic luminal A sub-type and a more aggressive, less estrogen-dependent disease aligning with the genomic luminal B classification [3]. Another breast cancer sub-type is positive for the HER2 receptor, while the triple negative sub-type is negative for both ER and HER2, as well as the receptor for Progesterone, PR, which is positive in most ER positive cases. Treatments for each sub-type has evolved to differ significantly and therapeutic decisions in breast cancer depend on the sub-type. HER2 positive cancers, for example, are treated with monoclonal antibodies, small molecule kinase inhibitors and antibody drug conjugates targeting HER2 [4].

Several other receptor tyrosine kinases have been implicated in cancer pathogenesis and progression and treatments targeting some of them exist. These include the angiogenesis kinase receptor vascular endothelial growth factor receptor (VEGFR) and its ligand VEGF, EGFR, fibroblast growth factor receptor (FGFR) and c-Met, the receptor for hepatocyte growth factor (HGF). In breast cancer, besides HER2, no receptor tyrosine kinase inhibitors have been approved. Recently, inhibitors of the neurotrophic kinase family of tyrosine kinase receptors have become available and confirmed to be effective in cancers with fusions involving these receptors [5]. These fusions are observed in the majority of rare histologic types of cancers such as mammary analogue secretory carcinomas of the salivary glands and secretory breast cancers but are exceedingly rare in more common histologic subtypes of cancers [6]. The current analysis examines fusions and other molecular lesions of neurotrophin receptors in secretory and non-secretory carcinomas of the breast using publicly available genomic data.

**MATERIALS AND METHODS**

**Study design and data collection**

Published genomic studies of molecular lesions in neurotrophic receptor tyrosine kinases (NTRKs) in breast cancer were interrogated in the cBioportal platform ([http://www.cbioportal.org](http://www.cbioportal.org)). cBioportal is a platform freely available to investigators containing molecular studies and corresponding clinical data [7,8]. The platform allows for a multi-dimensional interrogation of genomic data from publicly available studies. cBioportal also provides the opportunity to associate data of molecular lesions (mutations, fusions, copy number alterations and mRNA hyperexpression or hypo-expression) of any gene of
interest in studies from the Cancer Genome Atlas (TCGA) and other studies with patient clinical characteristics and survival outcomes [7]. The current analysis is based on the TCGA breast cancer study and on the project GENIE study, both included in the cBioportal and providing open access to their data [9, 10]. The analysis of copy number alterations (CNAs) in TCGA is performed with the algorithm Genomic Identification of Significant Targets in Cancer (GISTIC) [11]. In GISTIC, putative amplification of a given gene is defined as a score of 2 or above. TCGA provides an aneuploidy score (AS) as a measure of chromosomal instability of each sample. AS is calculated as the sum of the number of chromosome arms in each sample that have copy number alterations (gains or losses). A chromosome arm is considered copy number altered, either gained or lost, if there is a somatic copy number alteration in more than 80% of the length of the arm as calculated by an algorithm called ABSOLUTE based on Affymetrix 6.0 SNP arrays [12]. Chromosomal arms with somatic copy number alterations in 20% to 80% of the arm length are considered not evaluable and chromosomal arms with somatic copy number alterations in less than 20% of the arm length are considered not altered. mRNA expression grids in cBioportal are constructed and normalized using the RSEM (RNA-Seq by Expectation Maximization) algorithm [13].

Expression of NTRK proteins in breast cancer were evaluated using publicly available data from the Human Protein Atlas (www.proteinatlas.org), a database of protein expressions in human normal and neoplastic tissues [14]. The Human Protein Atlas contains a semi-quantitative immunohistochemistry-based evaluation of the expression of proteins of interest.

The effect of mRNA expression level of NTRK1 gene on survival of breast cancer patients was examined with data derived from the online publicly available platform Kaplan Meier Plotter (www.kmplot.com) [15]. The cut-off of amplified and non-amplified samples for each gene was set at the higher quartile of amplification, which is the closer cut-off provided by the platform to the percentage of breast cancer cases with NTRK1 amplifications.

Statistical analysis
The Fisher’s exact test or the $\chi^2$ test and the $t$ test, respectively, are used to compare categorical and continuous data. Kaplan-Meier survival curves were compared using the Log Rank test. All statistical comparisons were considered significant if $P < 0.05$.

RESULTS
Among 11,886 patients with breast cancer in the project GENIE cohort, 27 patients (0.22%) had fusions in one of the three NTRK genes. Two male patients were included among the 27 NTRK fusion positive breast cancer patients. Most patients with information for race were White, while 2 patients were Black and one was Asian. Histologically, 25 patients were diagnosed with ductal carcinomas or breast carcinomas not otherwise specified and one patient had an invasive lobular carcinoma. Interestingly, only one case was diagnosed as juvenile secretory carcinoma, in a male patient. A total of 32 fusions involving the 3 NTRK genes were present in the 27 patients (5 patients had more than one different fusions). The most frequent fusions involved NTRK3, in 16 samples, followed by NTRK1 in 13 samples and NTRK2 in 3 samples. Fusions in seven cases were intragenic and a variety of partners were involved in other fusions. Recurring partner genes include ETV6 with NTRK3 and LMNA with NTRK1. Frequent mutations observed in breast cancers such as in TP53, PIK3CA and GATA3 genes are also observed in cases with NTRK gene fusions. with a frequency not statistically significant different compared with cases without NTRK fusions. Similarly, common amplifications in breast cancers of CCND1 at 11q13.3, ERBB2 at 17q12, NSD3 at 8p11.23 and c-MYC at 8q24.21 are encountered in cases with NTRK fusions in frequencies comparable to cancers without NTRK fusions.

In TCGA breast cancer cohort, no fusions involving the 3 NTRK genes were observed. The most common molecular lesions were amplifications that were observed in 9.8% of patients, most commonly in NTRK1 (7.9%) and more rarely in NTRK3 (1.9%) and in NTRK2 (0.2%). NTRK amplified cancers have a distribution of histologic types (ductal, lobular, mixed, other) that is similar to non-amplified cases. NTRK amplified cancers are basally more common than NTRK non-amplified cancers (31.1% vs 14.1%, $\chi^2 P < 0.001$) but less commonly of the luminal B phenotype (11.3% vs 18.9% in non-amplified cancers, $\chi^2 P = 0.05$) (Figure 1). Total tumor mutation burden (TMB) was not different between NTRK amplified and non-amplified breast cancers with about 10% of cases in each group displaying a TMB above 120. In contrast, NTRK amplified cancers had more commonly chromosomal instability as measured by an AS of 4 or greater ($\chi^2 P < 0.001$) (Figure 2). Among cancer-associated genes frequently mutated in breast cancer, tumor suppressor TP53 was mutated in 41.5% of NTRK amplified cancers and in 31.6% of non-amplified cancers ($\chi^2 P = 0.03$) (Figure 3). In contrast, oncogene PIK3CA was more often mutated in NTRK non-amplified cases (33.5% vs 23.6% in NTRK amplified cases), although the difference did not reach statistical significance ($\chi^2 P = 0.08$). Amplifications of oncogene c-MYC were more common in NTRK amplified cases (24.5% vs 14% in NTRK non-amplified cases, $\chi^2 P = 0.005$).

In breast cancer cases with amplifications of NTRK genes, expression of the respective mRNAs is not increased, except in rare cases. However, breast cancers with increased NTRK1 mRNA expression at the upper quartile tend to have a better overall survival (OS) than cancers with low NTRK1 mRNA
expression, falling in the three lower quartiles (Figure 4A). This holds true also for an analysis restricted to basal breast cancers (Figure 4B).

Protein expression of TrkA is present in most breast cancers as evaluated by immunohistochemistry. The Human Protein Atlas has found moderate to high expression of the protein in 72.7% (8 of 11 samples) and 63.6% (7 of 11 samples) using two different antibodies (HPA035799 rabbit polyclonal antibody from Sigma-Aldrich and CAB004606 mouse monoclonal antibody from Santa Cruz Biotechnology). One and three additional cases have shown a low intensity of staining. In contrast, almost all cases of breast cancer examined showed low or absent staining for TrkB and TrkC in the Human Protein Atlas.

DISCUSSION

The human neurotrophin system consists of four ligands, nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), neurotrophin 3 (NT-3) and neurotrophin 4/5 (NT-4/5), and three Trk receptors, TrkA, TrkB, TrkC, as well as two additional non-Trk receptors, p75NTR and sortilin[16]. NGF is the ligand for receptor TrkA, encoded by gene NTRK1, BDNF and NT-4/5 are the ligands for TrkB encoded by gene NTRK2 and NT-5 is the ligand for TrkC, encoded by gene NTRK3. NT-5 can also ligate
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Figure 3 Percentage of mutations in the most common breast cancer associated genes in breast cancers with and without neurotrophic receptor tyrosine kinase amplifications. Data are from TCGA breast cancer cohort. Black columns: Neurotrophic receptor tyrosine kinase amplified; Grey columns: Neurotrophic receptor tyrosine kinase non-amplified.

Figure 4 Overall survival of breast cancers according to NTRK1 mRNA expression. A: Overall survival curves of all breast cancer patients with high NTRK1 mRNA expression vs low NTRK1 mRNA expression, across sub-types. Log-rank \( P = 0.023 \). B: Overall survival curves of basal sub-type patients with high NTRK1 mRNA expression vs low NTRK1 mRNA expression. Log-rank \( P = 0.047 \).

All four ligands ligate the common neurotrophin receptor p75NTR, which also serves as a receptor for the precursor forms of the four ligands pro-NGF, pro-BDNF, pro-NT-3 and pro-NT-4/5 and possesses an intracellular death domain[17]. All precursor forms ligate, additionally, sortilin, also known as neurotensin receptor 3 (NTSR3). The three Trk kinase receptors have a common structure with an extracellular part consisting of two cysteine-rich domains separated by three leucine-rich repeats. Closer to the cell membrane, the three Trk receptors possess two immunoglobulin-like domains. The intracellular part of the receptors holds the tyrosine kinase domain with five conserved tyrosines, three of which are phosphorylated when the receptors are activated by ligand binding. Downstream of receptors activation, the MAPK and PI3K/Akt kinase cascades are activated transmitting signals of proliferation and apoptosis inhibition. The physiologic role of neurotrophins signaling is best described in the nervous system, both in peripheral nervous system and the brain[18].

In peripheral nervous system, NGF signaling stimulates neurite growth and prevents apoptosis. NGF is also functional in brain where BDNF is also present and involved in signal transduction through binding and induction of phosphorylation of TrkB resulting in neuron survival and differentiation[18, 19].

Similarities of downstream signaling between the neurotrophin receptors and the EGFR family of tyrosine kinase receptors, which are well-known oncogenes, have led to the exploration of the role of Trk receptors in cancer[18, 20]. Expression of receptor TrkA is observed in breast cancer cell lines and in breast cancer tissues, where it is more prominent than surrounding normal breast epithelium[21]. TrkA signaling enhances breast cancer cell proliferation and anchorage independent growth. Moreover, TrkA
inhibits apoptosis and anoikis in metastatic sites. In breast cancer cells, activation of TrkA transmits signals through the MAPK pathway consistent with the physiologic circuit of the receptor[18]. Pro-NGF derived from cancer cells also binds TrkA and signals in an autocrine manner[22]. TrkA ligation promotes interaction with another receptor kinase EphA2 and downstream activation of Src and Akt kinases, resulting in phosphorylation and activation of these kinases and stimulation of cell growth[22]. In contrast, pharmacologic or small interfering RNA inhibition of TrkA or EphA2 decreases tumor growth and metastases of breast cancer in an in vivo mouse model. Immunohistochemical studies showed that Pro-NGF is expressed at higher levels in breast cancer tissues compared with normal breast tissues and benign lesions[23]. Receptor p75NTR is also expressed in breast cancer cells and leads to increased proliferation and inhibition of apoptosis through activation of transcription factor NF-κB[24]. p75NTR interacts with NF-κB through mediator TRADD (TNFR Associated Death Domain) when MCF-7 breast cancer cells are exposed to NGF and absence of TRADD promotes apoptosis in the same conditions[24].

BDNF and its receptor TrkB are expressed in breast cancer cells and promote cell survival and proliferation[25,26]. Knock down of BDNF or pharmacologic inhibition of TrkB reduced the viability of breast cancer cells in vitro. TrkB is expressed in cancer stem cells with the CD44 positive phenotype derived from triple negative breast cancers and participates in a paracrine circuit ligated by BDNF derived from differentiated breast cancer cells[27]. BDNF stimulation induces stemness core factor KL4 and promotes stemness phenotype and stem cell renewal. In addition, TrkB expression predicts relapse of localized triple negative breast cancers[27]. Related to stemness, epithelial to mesenchymal transition (EMT) is a process that promotes cancer cell metastasis[28]. TrkB is a target of micro-RNA miR-200c, which protects against EMT and preserves breast cancer cell epithelial phenotype, further supporting the role of TrkB in breast cancer promotion[29].

Further interest in neurotrophin family receptors in cancer was stimulated by the discovery of rare fusions involving the receptor genes NTRK1, NTRK2 and NTRK3 that act as oncogenic drivers and can be neutralized by NTRK inhibitors[30]. Fusions involving most frequently NTRK3 were confirmed to be the underlying molecular mechanism of secretory mammary carcinomas but were absent in other more common breast cancer histologies[30,31]. The carcinogenic mechanism of the fusion involves deregulated activation of the RAS-MAPK and PI3K-Akt pathways that are the physiologic targets of neurotrophic kinases signaling[32]. In contrast, these fusions do not affect the differentiation programs of cells, as suggested by their presence in diverse cancers, in addition to secretory breast carcinomas, including nephromas, infant fibrosarcomas, mammary analogue secretory carcinomas of the salivary glands and acute myeloblastic leukemias[33,34].

Breast secretory carcinoma is a rare type of breast cancer malignancy, accounting for 0.15% to 0.2% of all breast cancers[35]. It has generally an indolent clinical behavior, with late local recurrence and prolonged survival, even with lymph node involvement, that occurs at nearly 30% of cases[36]. It was initially described by Mc Divitt and Stewart as a variation of mammary carcinoma in the pediatric population, also called “juvenile carcinoma”[37]. In 1980, Tavassoli and Norris[38] reported several cases presenting at variable ages and recommended to replace the previous definition with the term “secretory carcinoma”. Secretory breast cancer displays a particular phenotype related to the presence of large amounts of intracellular and extracellular secretory material. Although it has been described as a sub-family of basal-like triple negative breast cancers with cytokeratin 5/6 expression and no expression of either hormone receptors or HER2, this tumor has a unique immunophenotypic profile among triple negative breast carcinomas. It stains diffusely for mammaglobin and gross cystic disease fluid protein (GCDFP) and displays strong and diffuse SOX10 and S-100 Labeling[39,40]. The hallmark genetic alteration of this cancer is a t(12;15) translocation, resulting in the ETV6-NTRK3 gene fusion which is pathognomonic, detected in more than 90% of secretory breast carcinomas. The same translocation is also identified in the mammary analog secretory carcinoma, its salivary gland counterpart[30,39,41]. Given that NTRK3 protein expression is not detected in breast epithelial cells physiologically, immunohistochemical analysis using pan-Trk antibodies has been shown to constitute a sensitive and specific approach for the detection of NTRK3 rearrangements in breast secretory carcinomas[42]. Absence of Trk receptor expression in non-secretory breast cancer was also observed in a micro-array study of 339 breast cancer patients, using a pan-Trk rabbit monoclonal antibody[43]. However, since TrkA is expressed in a sub-set of non-secretory breast cancers, as shown in the human Protein Atlas, the intensity of staining should be taken into consideration and confirmatory molecular detection of fusions should be standard practice.

Patients with tumors that harbor NTRK fusions as a specific molecular alteration may benefit from treatment with selective tyrosine kinase inhibitors or antagonistic monoclonal antibodies. There is accumulating evidence for the efficacy of Trk inhibitors in the control of the disease in these patients. Two Trk inhibitors, larotrectinib and entrectinib, are both approved by the American Food and Drug Administration (FDA) and the European Medicines Agency (EMA). Larotrectinib is a selective inhibitor of the Trk proteins (including TrkA, TrkB, and TrkC) approved for the treatment of locally advanced and metastatic solid tumors with NTRK fusions, in both adult and pediatric cancer patients. Its efficacy and safety have been studied in three multicenter, phase 1/2, open-label, single-arm clinical trials[44-46]. An objective response rate (ORR) of 78% [95% confidence interval (CI): 71%-84%] regardless of histology, age, and type of NTRK fusion was shown in the joint analysis of these studies. The median
The current study confirms the rarity of NTRK fusions in breast cancer with a prevalence rate of less than 1%. Amplifications of NTRK1 are more common and are associated with basal cancers and TP53...
mutations which are most common in this breast cancer type. Although amplification of the NTRK1 gene does not always lead to mRNA over-expression, TrkA protein is expressed in many breast cancers and thus can be a therapeutic target. In contrast to our results, another study that examined expression of TrkA at the protein level suggested that the receptor is more often expressed in HER2 positive cancers compared to luminal and basal carcinomas[64]. This study also confirmed discrepancies between gene dosage and protein expression. Clearly, transcription and post-transcription regulations are critical for TrkA expression in breast cancer.

ARTICLE HIGHLIGHTS

Research background
Breast cancer is a common female cancer. It constitutes a major cause of morbidity and mortality. Progress in breast cancer therapeutics has been attained with the introduction of targeted therapies for specific sub-sets. However, other breast cancer subsets lack targeted therapeutics.

Research motivation
Personalized therapies for breast cancer have the potential to improve outcomes. These therapies take consideration of specific molecular abnormalities that could be targeted for optimal results.

Research objectives
This article aims to analyze the landscape of neurotrophic receptor tyrosine kinase (NTRK) abnormalities in breast cancer. These are molecular lesions in a family of receptor tyrosine kinases. Specific drug inhibitors have been developed and have obtained regulatory approval, paving the way for effective patient treatment.

Research methods
An analysis of publicly available genomic breast cancer datasets was performed for identification and characterization of cases with fusions and other molecular abnormalities involving NTRK1, NTRK2 and NTRK3 genes.

Research results
NTRK fusions are rare in breast cancers as a whole. They are present in a small number of breast cancers at the extensive GENIE project data set which contains more than 10000 breast cancers. These cases are not identified as secretory in the database.

Research conclusions
NTRK lesions other than fusions are more common than fusions in breast cancers. However, confirmation of efficacy for the currently available inhibitors exist only for NTRK fusions.

Research perspectives
Amplifications of NTRK receptor genes are more common than fusions in breast cancers. Inhibitors effective for interfering with the activity of amplified NTRK receptors could advance therapeutics in this subset of breast cancers.

FOOTNOTES

Author contributions: Both authors contributed to the conception of the study, literature review, data analysis, writing of the article and revising the article.

Institutional review board statement: No institutional review board approval was required or obtained as the study was not a clinical trial.

Clinical trial registration statement: This study was not registered as a clinical trial as it includes only data from publicly available previously published studies and no new patient information.

Informed consent statement: No informed consents have been obtained for this study as no new patients were included. The study analyzed publicly available data from previously published studies.

Conflict-of-interest statement: The authors declare no conflicts of interest.

Data sharing statement: No additional data are available.
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