**IN THE SPOTLIGHT**

**Driver Oncogenes but Not as We Know Them: Targetable Fusion Genes in Breast Cancer**

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**Summary:** Two reports in this issue of Cancer Discovery outline how the genomic composition of tumors, including the presence of intragenic gene fusions, could inform the selection of treatment approaches in aggressive forms of the disease. Cancer Discov; 8(3); 272–5. © 2018 AACR.

See related article by Matissek et al., p. 336 (7).
See related article by Liu et al., p. 354 (8).

Despite the implementation of targeted therapy approaches in breast cancer, managing many of the aggressive forms of the disease still remains a challenge. For example, despite the improvements in survival that estrogen deprivation therapy has delivered for those with estrogen receptor–positive (ER-positive) breast cancers, managing endocrine therapy-resistant disease is complex. Likewise, identifying targeted approaches that are effective in those breast cancers that lack estrogen receptor, progesterone receptor, and expression of the ERBB2 receptor, the so-called “triple-negative breast cancers” (TNBC), also remains challenging. One approach to these issues has been to take a precision medicine approach by customizing the treatment strategy based upon the precise molecular makeup of each individual’s disease.

Although the delineation of breast cancer genomes and transcriptomes has heralded an era where this precision medicine approach can be much better implemented, one particular mutation type, intergenic fusions, that is, chromosomal rearrangements that lead to fusions between two distinct genes, has received somewhat less attention than might be expected. This is largely because fusions were historically considered to be a feature of leukemias and sarcomas, and to have less influence upon carcinomas. Furthermore, the technical challenges associated with discriminating real fusion events from false positives are considerable and have limited large-scale discovery of intergenic fusions. This scenario has changed somewhat, with advances in techniques including anchored multiplex PCR (AMP), next-generation sequencing, and sequencing analytic pipelines now making fusion identification in breast cancers a more profitable exercise (1). For example, secretory carcinomas of the breast, a rare yet distinct form of TNBC, are driven by a t(12;15)(p13;q25) chromosomal translocation, which encodes a transforming ETV6–NTRK3 gene fusion (Fig. 1; ref. 2). Likewise, adenoid cystic carcinomas of the breast are driven by an MYB–NFIB fusion caused by a t(6;9)(q22–23:p23–24) translocation (3). Other rare, yet recurrent, rearrangements in invasive ductal carcinomas of the breast have also been identified, including those associated with the MAST kinase and NOTCH gene family members (1). More recently, recurrent gain-of-function fusions involving the gene that encodes the alpha isoform of the estrogen receptor, ESR1 (e.g., ESR1–CCDC170), have been identified in aggressive ER-positive, luminal B breast cancers; these fusions likely cause constitutive activity of ERα (4). Next-generation sequencing studies have also identified a number of breast cancer gene fusions associated with other well-established cancer driver genes, including BRAF and MET (Fig. 1; refs. 5, 6).

In this issue of Cancer Discovery, the laboratories of Ellisen (7) and Candeiy (8) describe the identification of novel, therapeutically tractable oncogenic fusion genes in ER-positive and triple-negative breast cancers, respectively. Importantly, both groups make the critical leap from identifying fusion events to functionally assessing how these mutations alter therapeutic responses.

In the first report, Matissek and colleagues used AMP to isolate fusion genes associated with 54 candidate genes in 110 patients with ER-positive breast cancer. This identified intragenic fusions associated with the kinase-coding genes PIK3CA, AKT3, RAF1, and also ESR1, consistent with prior reports (1). After confirming some of the fusion events in both primary and metastatic lesions by an orthogonal technology (FISH), Matissek and colleagues used a panel of functional assays to establish that some of the fusions involving the RAF1, PIK3CA, and AKT3 genes modulated phosphoprotein signaling via elevated phosphorylation of the downstream substrate RPS6 as well as estrogen-dependent growth in three-dimensional in vitro cultures of breast cancer. Importantly, the RPS6K1–AKT3 fusion also conferred resistance to estrogen withdrawal in mice bearing ER-positive tumor cell line xenografts. An analysis of clinical data suggests that fusions associated with PIK3CA, ESR1, RAF1, or AKT3 are relatively uncommon in primary disease but more frequent in metastatic ER-positive cancers. Furthermore, those with fusion-positive tumors had a shorter overall survival when compared with those without fusions. In totality, Matissek...
Figure 1. Gene fusions in breast cancer. Schematic diagrams of oncogenic gene fusions identified in breast cancers. The breast cancer subtype and functional domains preserved in the gene fusion are noted. 5′ and 3′ untranslated regions (UTR) are depicted by lighter colors. Vertical line, chromosomal breakpoint.
and colleagues’ work suggests that oncogenic fusions in ER-positive breast cancer could function as predictive biomarkers of clinical resistance to endocrine therapy. One interesting aspect of the work of Matissek and colleagues is the enhanced frequency of fusion events in metastatic disease when compared with primary ER-positive cancer; this suggests that fusion genes might, in themselves, be a biomarker of advanced and aggressive disease. This association with metastatic disease might be expected, given the enhanced level of genomic alterations in this setting when compared with primary disease. Furthermore, a number of the fusion events that were private to metastatic lesions, and not found in patient-matched primary lesions, were present prior to any treatment in the metastatic setting. Whether these are selected for by treatment of primary disease or exist in clones that have other fitness advantages remains to be seen. For many, the therapeutic aspects of this work will be of most interest; although the RPS6K1–AKT3 fusion was associated with endocrine therapy resistance, the combination of estrogen deprivation with the CDK4/6 inhibitor palbociclib caused significant tumor growth suppression in vivo (7), suggesting that looking for this and similar fusions in biopsies from clinical trials assessing CDK4/6 inhibitors with endocrine therapies might be informative.

Matissek and colleagues’ work is also notable in that, although most of the literature in the area of fusion genes in breast cancer has focused upon identifying fusions via DNA/RNA sequencing, there is much less emphasis given to demonstrating that the fusions identified are oncogenic or modulate therapy response. This is perhaps expected, given the technical challenges faced in experimentally recapitulating chromosomal translocations and the gene fusions they cause. The expression of cDNAs from expression constructs is normally the approach used to interrogate fusion gene function, and this can often be very informative. However, the ectopic pattern of gene expression generated from a cDNA expression construct often does not replicate that seen in tumor cells when the gene is transcribed in its chromosomal context. Endogenous gene promoters are rarely used with cDNAs, and therefore cDNAs are not transcribed from within the genomic context of the fusion gene. In models of acute myeloid leukemia and Ewing sarcoma, chromosomal translocations and the fusion genes they cause have been engineered into cells by CRISPR/Cas9-mediated gene editing (9), suggesting that this might also be an approach that could be applied to the study of breast cancer gene fusions.

Compared with ER-positive cancers, where targeted endocrine therapy is widespread but drug resistance is a major challenge, in TNBC identifying targeted therapies that work in significant numbers of patients is the significant issue. There are a number of reasons for this; perhaps chief among these is the relative absence of unifying and targetable molecular features, such as ERα or ERBB2 amplification, in the wide variety of patients with TNBC. Although TNBCs do display some relatively recurrent molecular alterations (e.g., TP53, RB, NF1, BRCA1, PTEN, or PIK3CA mutations; a suite of copy-number alterations; a basal-like transcriptomic signature, etc.), strategies to target most of these do not exist. Instead, intertumoral molecular heterogeneity is the prevailing characteristic of TNBC; this has led to propositions that the most effective approach to treating this disease is to take a fully personalized approach and target the almost-unique set of molecular aberrations in each patient, rather than seeking therapeutic approaches that might work in many.

Liu and colleagues test the hypothesis that some of the relatively private gene mutations that occur in individual TNBCs provide therapeutic vulnerabilities (8). To do this, Liu and colleagues carried out whole-exome DNA sequencing and RNA sequencing from more than 80 TNBCs that developed in genetically engineered mice with either a breast lineage-specific Trp53 mutation or a combination of Trp53 and Brca1 mutations (8). Similar to previous studies using the same mouse models (10), the DNA/RNA profiling of TNBCs by Liu and colleagues identified a recurrent basal-like transcriptomic signature and Met oncogene amplification, and in Trp53/Brca1–mutant mouse tumors an elevated level of genomic rearrangements. Reassuringly, the mutational spectrum seen in Trp53/Brca1–mutant tumors was most similar to the signature 3 profile associated with BRCA1 or BRCA2 mutations in human tumors. The two most notable discoveries, however, focused upon (i) fusion genes associated with a series of potentially oncogenic and targetable protein kinases, such as Fgfr2 or Braf; and (ii) different tumors exhibiting distinct routes to the same dysregulated pathways, namely MAPK or PI3K signaling, a potential form of parallel evolution. In some cases, these fusions were shown to be essential for tumor-cell fitness and also therapeutically exploitable; for example, in mice with Fgfr2 fusions, clinical FGFR inhibitors impeded tumor growth (8).

One interesting aspect of the work of Liu and colleagues is that tumors with multiple distinct alterations, such as a tumor with both an Fgfr2 fusion and a Brca1 mutation, showed a better response to combination therapies that targeted both oncogenic drivers (e.g., a FGFR inhibitor plus a PARP inhibitor) than single-agent approaches that targeted only one driver gene. The rationale for such an approach might appear at first sight to be self-evident, in that two drugs with distinct mechanisms of action, targeting different driver effects, are less unlikely to have overlapping mechanisms of resistance. However, although much of the field focuses upon identifying synergistic drug combinations that could be effective as cancer treatments, the approach of simultaneously targeting multiple driver lesions in the same tumor with additive combinations might be as, or even more, effective.

Both of these reports highlight the potential of fusion genes as bona fide driver events and determinants of targeted therapy responses in breast cancer. The increasing technical advances in sequencing methodologies, especially those that aim to increase DNA read lengths, will undoubtedly enhance the ability to detect these events in the future. Moreover, incorporating fusion gene detection into the analysis of cell-free DNA/circulating tumor cell DNA sequencing will provide the opportunity to monitor these events through the course of an individual patient’s clinical journey, information that could prove critical in the optimal selection and adjustment of treatment.

Disclosure of Potential Conflicts of Interest

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REFERENCES


