Driver oncogenes but not as we know them - targetable fusion genes in breast cancer

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Running title: Fusion genes in breast cancer

One sentence summary:

Two reports in this edition of *Cancer Discovery* (1,2) 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.

Main text

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" (TNBCs), 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 make-up of each individual's disease.

Whilst 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, i.e. chromosomal rearrangements that lead to fusions between two distinct genes, have 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 somewhat changed, with advances in techniques including Anchored Multiplex PCR (AMP), next generation sequencing and sequencing analytical pipelines now making fusion identification in breast cancers a more profitable exercise (3). 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 (4) (**Figure 1**). Likewise, adenoid cystic carcinomas of the breast are driven by a *MYB-NFIB* fusion caused by a t(6;9)(q22–23;p23–24) translocation (5). Other rare, yet recurrent, rearrangements in invasive ductal carcinomas of the breast have also been identified, including those associated with the *MAST* (microtubule-associated serine threonine) kinase and *NOTCH* gene family members (3). 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 (6). 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 (7,8)* (**Figure 1**).

In this edition of *Cancer Discovery*, the labs of Ellisen (1) and Cantley (2) describe the identification of novel, therapeutically tractable oncogenic fusion genes in estrogen receptor 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, Ellisen 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 (3). After confirming some of the fusion events in both primary and metastatic lesions by an orthogonal technology (fluorescent in situ hybridisation (FiSH)), Ellisen and colleagues used a panel of functional assays to establish that some of the fusions involving the *RAF1, PIK3CA* and *AKT3* genes modulated phosphoprotein signalling 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 associate 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 to those without fusions. In totality, Ellison's 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 Ellisen's work is the enhanced frequency of fusion events in metastatic disease, when compared to 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 to 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* (1), suggesting that looking for this and similar fusions in biopsies from clinical trials assessing CDK4/6 inhibitors with endocrine therapies might be informative.

Ellison's work is also notable in that, whilst 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 often generated from a cDNA expression construct often does not replicate that seen in tumor cells with chromosomal translation. This is likely because endogenous gene promoters are rarely used with cDNAs, and because cDNAs are not transcribed from within the genomic context of the fusion gene. In models of acute myeloid leukaemia and Ewing's 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 fusions.

Compared to estrogen receptor-positive cancers, where targeted endocrine therapy is widespread but where drug resistance is a major challenge, in TNBC, identifying targeted therapies that work in significant numbers of patients is a significant issue. There might be a number of reasons for this; perhaps chief amongst these is the relative absence of unifying and targetable molecular features, such as ERα or *ERBB2* amplification, in the wide variety of TNBC patients. Although TNBC do

display some relatively recurrent molecular alterations (e.g. *p53*, *Rb, NF1, BRCA1, PTEN* or *PIK3CA* mutations, a series of copy number alterations, a basal-like transcriptomic signature etc.) strategies to target most of these do not exist. Instead inter-tumoral molecular heterogeneity is the pervading characteristic of TNBC; this has led many to think that the most effective approach to treating this disease is to take a fully personalised approach, and to target the almost unique set of molecular aberrations in each patient, rather than seeking therapeutic approaches that might work in many.

Cantley and colleagues test the hypothesis that some of the relatively private gene mutations that occur in individual TNBCs provide therapeutic vulnerabilities (2). To do this, Cantley and colleagues carried out whole exome DNA sequencing and RNA sequencing from over 80 TNBCs that developed in genetically engineered mice with either a breast-lineage specific *Tp53* mutation or a combination of *Tp53* and *Brca1* mutations (2). Similar to previous studies using the same mouse models (10), the DNA/RNA profiling of TNBCs by Cantley and colleagues identified a recurrent basal-like transcriptomic signature and *Met* oncogene amplification and in *Tp53/Brca1* mutant mouse tumors, an elevated level of genomic rearrangements. Reassuringly, the mutational spectrum seen in *Tp53/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, focussed 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 map-kinase (MAPK) or phopho-inositol-3-kinase (PI3K) signalling, 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 (2).

One interesting aspect of Cantley's work is that tumors with multiple distinct alterations, such as a tumor with both a *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 only targeted 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, whilst much of the field focusses 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 Ellison and Cantley reports both 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.

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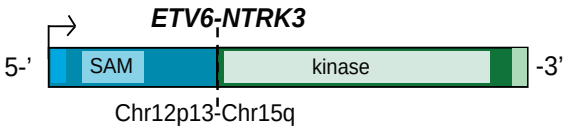
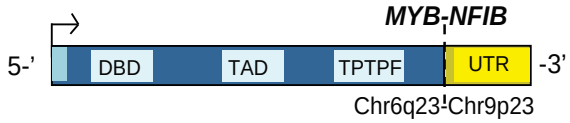
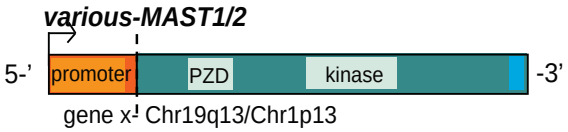
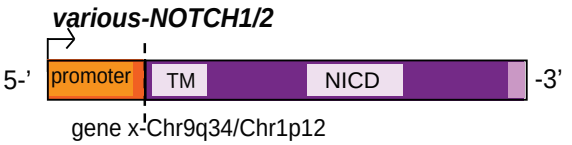
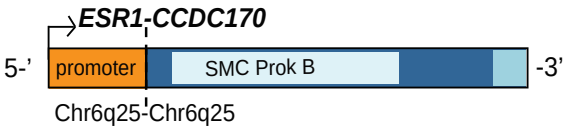
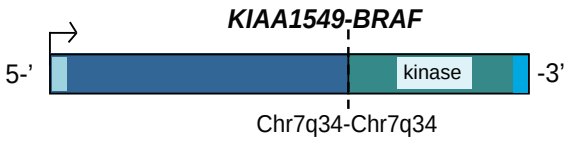
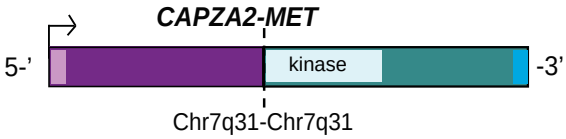
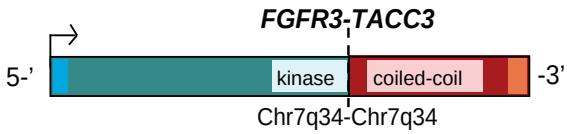
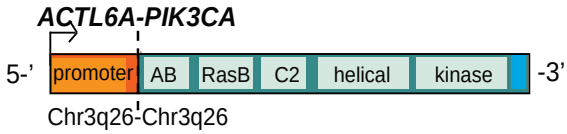
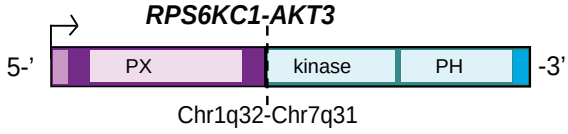
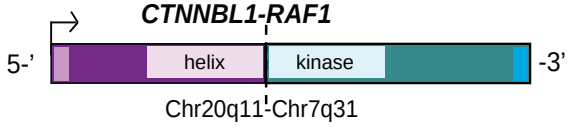
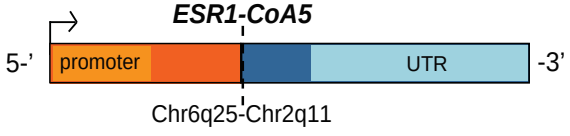
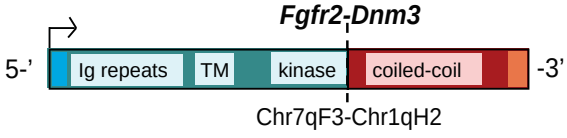
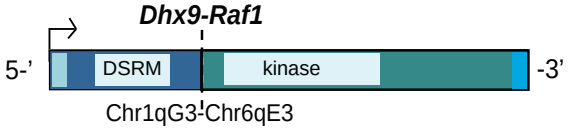
Acknowledgements

We thank Breast Cancer Now and Cancer Research UK for funding work in our laboratories.

Figure legend

Figure 1. Gene fusions in breast cancer. Schematic diagrams of oncogenic gene fusions identified in breast cancers. The breast cancer subtype and functional domains of the gene fusion are noted. 5’ and 3’ untranslated regions (UTR) are depicted by lighter colors. Vertical line indicates the chromosomal breakpoint.

Fusion gene structure	Breast Cancer subtype	Reference
	Secretory carcinomas (ER-)	Tognon et al (5)
	Adenoid-cystic carcinomas (ER-)	Persson et al (5)
	ER+/ER-	Robinson et al (3)
	ER-	Robinson et al (3)
	ER+/Luminal B	Veeraraghavan et al (6)
	Metastatic	Ross et al (7)
	ER+	Yoshihara et al (8)
	Triple negative breast cancer	Shaver et al
	ER+ advanced disease	Matiseek et al (1)
	ER+ advanced disease	Matiseek et al (1)
	ER+ advanced disease	Matiseek et al (1)
	ER+ advanced disease	Matiseek et al (1)
	*Tp53/Brca1* GEMM	Liu et al (2)
	*Tp53/Brca1* GEMM	Liu et al (2)