

## From Integrative Genomics to Therapeutic Targets

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### Abstract

Combinatorial approaches that integrate conventional pathology with genomic profiling and functional genomics have begun to enhance our understanding of the genetic basis of breast cancer. These methods have identified key genotypic–phenotypic correlations in different breast cancer subtypes that have led to the discovery of genetic dependencies that drive their behavior. Moreover, this knowledge has been applied to define novel tailored therapies for these groups of patients with cancer. With the current emphasis on characterizing the mutational repertoire of breast cancers by next-generation sequencing, the question remains as to what constitutes a driver event. By focusing efforts on homogenous subgroups of breast cancer and integrating orthogonal data-types combined with functional approaches, we can begin to unravel the heterogeneity and identify aberrations that can be therapeutically targeted. *Cancer Res*; 73(12); 3483–8. ©2013 AACR.

### Introduction

Traditionally, breast cancers have been characterized into biologically and clinically meaningful subgroups according to histologic grade and type (i.e., growth pattern; ref. 1), with the majority of breast cancers being classified by histologic exclusion, that is, invasive carcinomas of no special type (IC-NST). On the other hand, the remaining tumors can be histologically classified according to their distinctive growth patterns and are termed "special" histologic types. Over the past decade, seminal class discovery expression profiling studies have identified a number of molecular subtypes of breast cancer defined at the transcriptomic level that are characterized by distinct histologic features, clinical behaviors, and responses to therapy (2). Indeed, different breast cancer subtypes (both histologic and molecular) harbor distinct patterns of genetic aberrations and are driven by alterations in distinct molecular pathways and networks (3, 4). It is now widely accepted that breast cancer heterogeneity may be underpinned by myriad mechanisms of genetic aberration (e.g., gene amplifications, in-frame fusion genes or mutations and homozygous deletions, disrupting fusions, or deleterious mutations causing gene activation or inactivation, respectively), and that phenotypic subgroups harbor distinct patterns of genomic aberrations (3, 5). Moreover, targeting these genomic alterations has proved an effective way of developing tailored therapies for subgroups of breast cancers (5–8).

The use of high-throughput technologies has enabled the investigation of biologic phenomena and allowed its correla-

tion to specific disease behavior. Research has focused on integrative approaches combining high-throughput genomic data through the use of microarray-based comparative genomic hybridization (aCGH), gene expression profiling, and more recently the use of next-generation sequencing to define the genetic underpinning of different subtypes of cancer with the ultimate goal of identifying novel therapeutic targets. However, the main challenges for the translation of the genetic alterations identified by massively parallel sequencing into benefit for patients with cancer lie in the identification of biologically relevant aberrations among the deluge of sequencing data being produced, which can be used as therapeutic targets or predictive biomarkers.

### Exploring Genotypic–Phenotypic Correlations

There is evidence to suggest that at least some subtypes of breast cancer are underpinned by distinct arrays of genomic alterations. In fact, some special histologic types of breast cancer harbor specific pathognomonic alterations such as the *ETV6–NTRK3* oncogenic fusion gene in secretory carcinomas, the *MYB–NFIB* fusion gene in adenoid cystic carcinomas, and inactivation of E-cadherin through mutation and gene methylation in lobular carcinomas of the breast [for a review on special histologic types of breast cancer see (9)]. Perhaps the best example in breast cancer is the characterization of *ERBB2* (*HER2*) as the driver of the 17q12 amplification, which has spurred the hunt for additional amplified driver events. We and others have explored the genotypic–phenotypic correlations of different molecular subgroups of breast cancers through the use of high-throughput genomic analyses using aCGH (3, 5). Through aCGH profiling of a series of 95 high-grade breast cancers, we have shown that distinct patterns of copy number alterations are found in different molecular subtypes (5). These analyses highlight the genotypic–phenotypic association between specific amplifications and subtypes of breast cancer (3, 4).

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## Integrating Data-Types to Identify Therapeutic Targets

By using a combination of aCGH and gene expression profiling, we have shown that canonical pathways involved in estrogen receptor (ER) signaling, proliferation, and DNA repair are enriched for genes whose expression is driven by copy number in basal-like, HER2, and luminal tumors (3), suggesting that the diversity of breast cancer and the molecular subtypes may stem, to some degree, from the different patterns of genetic aberrations found in these cancers. Moreover, biologic phenomena characteristic of each subtype (e.g., proliferation, HER2, and ER signaling) may be driven by specific patterns of copy number aberrations. This approach has also led to the identification of genes that are consistently overexpressed when amplified, which are considered potential "amplicon drivers." However, not all genes within an amplicon are overexpressed, and an amplicon may harbor more than one driver (6). The expression of some driver genes is also more pervasive, in that these genes are overexpressed by other mechanisms in addition to amplification. However, such approaches have been successful in identifying novel targets for subgroups of breast cancer by exploiting the concepts of oncogene addiction. For instance, *fibroblast growth factor receptor (FGFR1)*, one of the genes mapping to the 8p11-p12 amplicon, is amplified in 10% to 15% of breast cancers and is associated with ER-positive disease and poor survival (10). FGFR1 is consistently overexpressed in tumors harboring *FGFR1* amplification both of which have been shown to constitute a mechanism of resistance to endocrine therapy (8). A phase II clinical trial is currently testing the efficacy of small-molecule FGFR inhibitors for these patients. By conducting genome-wide correlations between amplifications in different subgroups of breast cancer, we have identified a number of subgroup-specific amplifications. This approach, coupled with integrating these data with matched gene expression data, led to the identification of *PPM1D* as a putative amplicon driver (5). RNA interference (RNAi)-induced silencing and chemical inhibition of *PPM1D* in a panel of phenotypically matched *PPM1D*-amplified and -nonamplified cells showed that *PPM1D* expression and phosphatase activity is selectively required for the survival of cells harboring *PPM1D* gene amplification (5, 11). These data suggest that *PPM1D* may prove a viable therapeutic target for the subset of HER2-positive breast cancers harboring amplification at 17q23.2. Through a similar approach, we identified 38 genes that were significantly overexpressed when amplified in a series of 56 triple-negative breast cancers, including *FGFR2* amplifications in approximately 4%. Our work showed that cancer cells harboring *FGFR2* amplification are exquisitely sensitive to inhibition of FGFR2 *in vitro* and *in vivo* through the use of RNAi and treatment with FGFR small-molecule inhibitors suggesting that FGFR inhibitors may constitute a tailored therapy approach for a subgroup of triple-negative tumors (7, 8). More recently, we have shown that 5% of ER-negative high-grade breast cancers that harbor amplification of *CCNE1* within the 19q12 amplicon are dependent on *CCNE1* and CDK2 kinase activity for their survival. Cancer cells with *CCNE1* gene amplification are sensitive to CDK2 inhibitors, providing a rationale for the testing of these chemical inhibi-

tors in a subgroup of patients with ER-negative grade III breast cancers in the context of clinical trials (6).

As well as using genetic and transcriptomic data to identify potential therapeutic targets in a candidate-driven approach, integrating functional profiling data offers an unbiased way of identifying genetic dependencies. This approach has been used to identify additional amplicon drivers in *HER2*-amplified tumors by systematically assessing cell viability in a panel of *HER2*-amplified cell lines after silencing of all genes that were significantly overexpressed when identified in a cohort of primary *HER2*-amplified breast cancers. This approach identified the transcription factor *TFAP2C* as a novel genetic dependency in 5% of *HER2*-amplified breast cancer cells (12). Although such screening approaches as these can identify novel amplicon drivers, many of the targets identified (e.g., transcription factors) are not directly targetable. By exploiting the concept of synthetic lethality [(13); where loss of either gene is compatible with cell survival, however, loss or inhibition of both genes results in cell death], the alterations in the cells' physiology that arise as a consequence of aberrant activation of oncogenes or tumor-suppressor gene loss, rather than oncogene/tumor-suppressor proteins themselves, are targeted to achieve tumor selectivity. This concept has been successfully applied to identify novel therapeutic targets, including PARP inhibitors in *BRCA1/2*-mutant patients, further corroborated by the identification of a resistance mechanism to PARP inhibitors (14). High-throughput RNAi screening of the kinome (i.e., pharmacologically tractable genes) in a panel of commonly used breast cancer cell line models identified a series of novel genetic dependencies in basal, luminal, and HER2 subgroups (15). This approach also led to the identification of genetic dependencies of cells with specific mutations. For example, PTEN-null breast tumor cells were found to be dependent on signaling through mitotic checkpoint kinases. Integration of viability data with transcript and protein profiling also identified a correlation between sensitivity to ADCK2 silencing and high ADCK2 mRNA and protein levels in ER-positive cells (15). Such unbiased approaches provide a framework upon which additional dependencies and candidate therapeutic targets may be identified.

## The Next Generation

The advent of next-generation sequencing has increased our understanding of the complexity of cancer genomes tremendously and has identified a number of subtype-specific mutations associated with different cancer types. Massively parallel sequencing studies in breast cancer have identified a plethora of novel mutations, including *MAP3K1* mutations in ER-positive cancers (16) and *PARK2* mutations in triple-negative disease (17). In addition, RNA-sequencing studies have enabled the identification of novel recurrent targetable expressed fusion genes involving the *MAST* kinase and *NOTCH* gene family members (18). Large-scale sequencing efforts currently being undertaken with consortia such as The International Cancer Genome Consortium (ICGC) and The Cancer Genome Atlas (TCGA) are leading to an unprecedented amount of data. However, the main challenges that lie ahead are for the translation of the genetic alterations identified by next-

generation sequencing into benefits for patients with cancer. These mainly depend on (i) the identification of "driver" mutations, and (ii) the targeting of "driver" genetic aberrations. Although the identification of "driver" genetic aberrations so far has been largely based on statistical algorithms (19), the targeting of the "driver" aberrations has proven difficult. However, the majority of the novel mutations observed in the common types of breast cancer are at relatively low frequency, and the main challenge lies in the distinction of what constitutes a "driver" mutation event versus a "passenger" event (i.e., has no biologic significance on the cell harboring its mutation at a given point in time; ref. 13).

Traditionally, the identification of driver events stems from the fact that they are recurrent at a significant frequency above the background mutation rate within the tumor cohort studied. We can integrate different sorts of genetic alterations to aid the identification of recurrent activation or tumor-suppressive events, such as mutations and homozygous deletions, gross DNA rearrangements of a tumor-suppressor gene, or amplification and activating mutations of an oncogene (Fig. 1). We have used this approach to identify novel candidate cancer genes in *BRCAl*-mutant tumors by integrating a list of mutations identified from whole-genome sequencing with published aCGH data for the presence of homozygous deletions (20). This strategy can also be taken further to look for functional recurrences in the form of genetic alterations in members of the same gene family or members of the same signaling network or pathway. For example, mutations in chromatin remodeling genes seem to be a common alteration in many types of solid tumors (19, 21), and identifying ways of targeting these tumors with chromatin remodeling defects is a key challenge that needs to be explored in future studies. A number of computational tools are available to predict the functional effect of a mutation of interest on a protein and to identify pathways that are deregulated in cancer and therefore are likely to contain significant driver genes. Algorithms that identify key transcriptional regulators of oncogenic programs can be used to prioritize mutations for follow-up studies (for a review of these see ref. 22). Algorithms that predict the pathogenicity of somatic mutations based on the selection pressure and type of mutation have also been developed (19, 23). However, novel predicted "drivers" still need to be functionally investigated in appropriate model systems before they can be definitively defined as driver events. The recent functional validation of *HER2* mutations in breast carcinomas without *HER2* amplification has highlighted the importance of this step (24). Through the use of functional genomic screens, we can begin to identify driver events in a high-throughput manner. This can be achieved in a number of ways, from cross-species comparative approaches identifying driver genes as those that are conserved in human and mouse tumors, high-throughput insertional mutagenesis screens, or whole-genome short hairpin RNA (shRNA) screens (22). Perhaps a more intuitive approach, which aids in the identification of both tumor-suppressor genes and oncogenic drivers, lies in the generation of cancer genome-focused screens by generation of overexpressed libraries of mutant open

reading frames (ORF) and shRNA that target the same set of genes identified by sequencing primary tumor samples. These libraries can then be screened for their ability to transform premalignant cells. In addition, wild-type ORF libraries generated from primary tumors without prior knowledge of the mutations may also provide an effective approach for gain-of-function screens.

Because many mutations and fusion genes identified may not be directly targetable, synthetic lethality approaches constitute an alternative for the identification of novel targets. These approaches can be achieved through screening of isogenic cell line models with and without the genomic alteration of interest and/or a panel of heterogeneous cell lines with and without the alteration, with si/shRNA screens of druggable genes and high-throughput small-molecule drug screens. Through drug screens using small-molecule inhibitors that are already approved by the U.S. Food and Drug Administration, the time needed from target identification to phase II clinical trials is much shorter. In fact, a plethora of small-molecule inhibitors are available that have no useful predictive biomarker. Identifying these biomarkers through these integrated approaches would ultimately lead to patient benefit more quickly. Concerted efforts within the scientific community are being aimed at addressing these issues, and interrogation of systematic pharmacogenomic screening data for an aberration of interest is becoming a reality (25, 26). In parallel, the growing field of metabolomics is yielding interesting possibilities for classifying tumors based on their metabolic signatures and in identifying pathways related to drug resistance or toxicity through metabolomic profiling. Furthermore, metabolic dependencies resulting from specific genomic alterations offer novel therapeutic opportunities. Readers are directed to excellent reviews on the subject (27). In addition, other factors, such as the importance of epigenetic mechanisms (including methylation and acetylation; ref. 28), noncoding RNAs upon gene regulation (29), and the role of the tumor microenvironment, also need to be considered (30, 31).

However, not all recurrent mutations and fusion genes are represented by the available breast cancer cell line models, and pathognomonic events underlying some types of breast cancer can only be studied in the context of forced expression models, making the use of synthetic lethal approaches limited. Such models may not recapitulate the network state space of primary tumors harboring the genetic aberration of interest. These caveats must be kept in mind when interpreting pre-clinical functional validation data. However, there is an abundance of common genetic aberrations that are not directly targetable (e.g., *TP53* and *KRAS* mutations, and *PTEN* loss-of-function) and for which adequate models are available. This provides an opportunity to leverage the power of synthetic lethal screens in multiple isogenic models, thereby providing some control for the context-dependent nature of many genetic dependencies. Furthermore, by subjecting samples with and without the mutation of interest to deep sequencing one might identify a pattern of comutation (e.g., is there a set of genes that are frequently mutated in *TP53*-mutant triple-negative breast cancers but not in *TP53* wild-type cancers), which could be modeled *in vitro* through synthetic lethal

screens to interrogate potential cooperative interactions. Systems biology approaches would likely prove invaluable in these strategies.

Finally, next-generation sequencing studies have highlighted the scope (32) and important role of intratumor genetic heterogeneity in cancer evolution and emergence of drug resistance (33). High-depth multiregion sequencing and single-cell sequencing can be used to characterize the repertoire of somatic variants or patterns of copy number changes in nonmodal clones within a tumor. Of course, not all these mutations will be biologically relevant. Integration of these data with pathway analysis tools and online resources such as the Connectivity Map (34), which identifies connections between drugs, disease, and genes, aids prioritization of mutations and subsequent compound library screening, using chemical libraries of drugs currently in clinical trials. This approach would identify which mutations confer resistance to which drug; the ideal scenario would then be to analyze pre- and posttreatment samples from neoadjuvant trials to confirm the role of these nonmodal clones in the evolution of drug resistance. Focused high-depth sequencing could be effectively used as a screening strategy to exclude patients from treatment with agents they are likely to develop resistance to, or early relapse after.

## Summary

Integration of multiple data-types is becoming increasingly useful for the identification of therapeutic targets within different subtypes of breast cancer. With the advent of next-generation sequencing technologies and the vast amounts of data being generated, it is possible to identify recurrent mutational patterns within breast cancer. However, given the rel-

atively low frequency of novel mutations and fusion genes in breast cancer and to fully understand the biology and therapeutic responses of some patients, the clonal genotypes of the individual tumors will need to be determined. It is evident that these large-scale sequencing projects need to be integrated with functional screens to achieve the goal of developing novel therapeutic strategies. For functional screening to be useful in identifying key driver events, researchers need to account for the fact that many gene alterations will be context dependent, either through epistatic interactions or dependence on a particular developmental stage of the tumor. It will be necessary to develop more complex models to assess interactions in a more network-driven approach. The goal of individualized patient management will be a step closer with the inception of clinical trials designed to conduct genome-wide or targeted sequencing of cancers to identify targetable aberrations and to determine the mechanisms of resistance to specific therapeutic agents.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

## Authors' Contributions

**Conception and design:** R. Natrajan

**Writing, review, and/or revision of the manuscript:** R. Natrajan, P. Wilkerson

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