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**Translating the Genomic
Architecture of Breast Cancer into
Clinical Applications**

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PersPective

BREAST CANCER

Translating the Genomic Architecture of Breast Cancer into Clinical Applications

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ABSTRACT

The genetic alterations in breast cancer have in recent years been studied through a variety of techniques: analysis of alterations in individual oncogenes and tumor suppressor genes; gene expression profiling of both messenger RNA and microRNA; global analysis of DNA copy number changes; and most recently, whole-genome sequence analysis. Analysis of the association between genetic alterations and gene expression profiles with prognosis and response to specific treatments will lead to improved possibilities for patient-tailored treatment. Russnes et al. now add an additional view on the complex genetic makeup of breast carcinomas by developing algorithms that can be used to subclassify tumors based on their patterns of genome-wide DNA copy number gains and losses, which vary from very simple (only a few gains and losses) to complex. The algorithms provide indices that can be used in conjunction with results from other genetic analyses to subclassify breast cancer, with the aim of defining subgroups of patients that differ with respect to prognosis and response to therapy.

INTRODUCTION

Breast cancer is markedly heterogeneous with respect to distinctive biological characteristics and clinical behavior; this attribute is also reflected by the heterogeneity in genetic alterations that have been identified by analyzing large series of tumors. Breast cancer, like all malignancies, arises from a multistep process of genetic alterations in oncogenes and tumor suppressor genes that affect the function of individual genes and cellular processes (1).

DNA copy number alterations are a reflection of the genetic aberrations of tumors: An increase from the two copies of a gene present in a normal diploid genome to several copies (usually 10 or more) represents gene amplification, producing a “gain of function” in the affected tumor cell, and is one way to activate a “normal” proto-oncogene to become an oncogene. Inactivation of a tumor suppressor gene by the mutation of one tumor suppressor gene allele and complete loss of the second allele, producing a “loss of function” in the tumor cell, is another step contributing to a malignant phenotype (2).

Because genetic alterations are the cause of cancer development, it is expected that the combination of specific genetic alterations in tumors will be predictive of clinical behavior (3–5). The need to recognize genetically defined subtypes of breast cancer is enhanced by the increasing availability of specific and more effective therapy regimens. One important example of a genetically defined tumor type that guides treatment is human epidermal growth factor receptor 2 (HER2)–positive breast cancer; a positive HER2 status predicts the response to HER2 targeted therapy such as trastuzumab (Herceptin) (6). Meticulous selection of patients for specific therapies could lead to improved treatment outcomes. Determining the molecular mechanism of primary or acquired drug resistance can be critical for identifying patients that will fail to respond to therapy and might help to design more efficient treatment protocols (7). Genome-wide approaches for the identification of molecular genetic changes therefore provide powerful instruments to study cancer. Relevant molecular techniques include cytogenetic banding, spectral karyotyping, analysis of loss of heterozygosity, fluorescent and chromogenic *in situ* hybridization, and comparative genomic hybridization (CGH). In this issue of *Science Translational Medicine*, Russnes et al. (8) describe two algorithms to measure changes in genomic architecture using data from CGH experiments; one measurement independently predicts breast cancer outcome.

CGH

Array-based CGH (aCGH) offers a good approach to screen whole genomes for a detailed analysis of DNA copy alterations (9). In a CGH experiment, total genomic DNA is isolated from test cell populations (tumor tissue) and reference cell populations (normal tissue), differentially labeled with green and red fluorescent dyes, mixed in a 1:1 ratio, and hybridized to a microarray containing DNA fragments representing the whole genome, which allows the binding of sequences at different genomic locations to be distinguished (9). Unlabeled human Cot1 DNA (placental DNA that is enriched in repetitive DNA sequences) is contained in the mix to block nonspecific hybridization. Data processing of the scanned microarray slide includes signal intensity measurements with specialized image software and a fluorescent microscope. Deviation from 1:1 log-scaled intensity ratios (green/red) is counted as a change in DNA copy number. Normally, a threshold is set (gain, ratio 1.2; loss, 0.75), and statistical

verification is applied (95 to 99% confidence intervals) (10).

The sensitivity of aCGH methods depends on the proportion of tumor cells in the tissue (a desirable proportion is > 70%) and the extent of the aberration. Smaller alterations in size [for example, a few hundred base pairs (bp)] and copy number are more difficult to detect than larger changes. The sensitivity of the technique has improved with the advent of high-resolution aCGH platforms. The resolution for the identification of genomic gains and losses is determined by the distance between two contiguous probes and varies depending on the type of probe: for example, bacterial artificial chromosome (BAC) clones (with a length of 100 to 200 kb), cDNA clones (~100 to 1000 bp), or oligonucleotides (30 to 100 bp). A drawback of aCGH is that it only recognizes physical changes in DNA copy number and is unable to identify smaller variations in DNA sequence or balanced chromosomal translocations (rearrangements that do not involve the loss or gain of any genetic material). aCGH has multiple applications; it can be used (i) to identify genomic regions that harbor oncogenes and tumor suppressor genes that have been amplified or deleted, and (ii) to build class discovery tools for categorizing independent breast cancers (11).

IDENTIFYING ABERRANT CHROMOSOMAL REGIONS

An important challenge during the analysis of aCGH data is the detection of regions of concentrated high or low fluorescence ratios—that is, aberrant chromosomal regions specific to the problem under study.

Broadly, there are two obstacles: (i) determining the statistical significance of the alteration and (ii) defining the boundaries of the alteration. To reach these goals, different approaches and algorithms have been used (12, 13).

The first approach uses only aCGH data. Amplifications and deletions in each sample are individually identified, and common aberrations between the samples are sought. The identification of amplifications and deletions can be simply done by setting a threshold and determining which DNA probes (which can be BAC clones, cDNA clones, or oligonucleotides) result in hybridization signals that exceed the threshold. These regions are considered to be amplified or deleted (14). More complex algorithms employ the fact that copy number changes involve chromosome segments; ratios at contiguous sets of probes should be identical, except for occasional abrupt steps to another level (indicating a chromosomal breakpoint) (13). Identification of these breakpoints is referred to as “segmentation” and produces “segmented data.”

A second approach to detect aberrations across samples is to use gene expression data together with the chromosomal location of the genes (15). This approach assumes that amplification directly affects gene expression. Therefore, the genes in an amplified region should have a detectable common overexpression. Similarly, the genes located in a deleted region would have a detectable underexpression. Because the alteration in expression may be caused by mechanisms other than a change in copy number, the potentially underlying chromosomal aberrations would need to be verified either by polymerase chain reaction or fluorescent in situ hybridization, if the number of loci to be tested is tractable; otherwise, the alterations would need to be confirmed by aCGH data.

A third approach combines aCGH and expression data to detect regions of chromosomal aberrations. The stepwise linkage analysis of microarray signatures (or SLAM) algorithm (16)

is an excellent example of this approach. First, significance analysis of microarrays (or SAM) analysis (17) is applied to the aCGH data to identify the DNA probes that distinguish tumor versus normal DNA. Then, the focus is on the DNA probes that display hybridization patterns that are correlated with the gene expression pattern. An algorithm to study this correlation is supervised identification of regions of aberration (or SIRAC) of aCGH data sets (12), which has been used to identify chromosomal regions associated with the classes of breast tumors defined by prognostic gene expression signatures or clinical and pathological characteristics.

GENOMIC ALTERATIONS IN BREAST CANCER

Using these different approaches and algorithms, frequently observed genomic alterations in breast carcinomas include the gain of chromosomal regions 1q, 8q, 16p, 17q, and 20q; the loss of 16q and 17p; and DNA amplification in 8q12-24, 11q11-13, 17q1221, 17q22-24, and 20q13-ter [reviewed by Reis-Filho et al. (5)]. The histological grade of the tumor is strongly associated with the amount and complexity of the genomic aberrations; tumors with higher histological grades harbor more chromosomal alterations. Well-differentiated tumors (grade 1) often show only gain at 1q and loss of 16q, whereas poorly differentiated tumors (grade 3) exhibit more amplifications and less frequent loss of 16q (5).

aCGH data have also been correlated with the prognosis of patients with breast cancer. Tumors from patients with a poor prognosis exhibited significantly more changes than those from patients with a good prognosis. Additionally, DNA gains and losses have been shown to vary between tumors with different prognostic gene expression signatures and clinical and pathological features. For example, the previously identified 70-gene signature indicating poor prognosis is associated with the gain of 3q26-27, 8q22-24, and 17q24-25; the 70-gene good prognosis profile is associated with the loss of 16q11-24 (18).

The wealth of data derived from aCGH experiments has the potential to improve biological understanding of breast cancer development and progression, and in combination with well-known clinical and pathological prognostic markers may also result in improved prediction of prognosis and response to breast cancer therapy (19).

Despite these ample data, translation into clinical practice remains a challenge. Until a resultant model can be of practical use, some limiting factors may hamper progress: (i) The size of sample sets available for microarray-based studies has so far been limited, (ii) studies often include a heterogeneous mix of patients with respect to clinical stage and treatment received, (iii) combining data sets to increase their size has been challenging because various types of array platforms have been used, (iv) validation of molecular classification in independent data sets has so far been limited, (v) analyses of high dimensional data are complex, and (vi) new genetic factors should demonstrate improved prediction accuracy over the combination of standard prognostic factors (20, 21).

PATTERNS OF GENOMIC REARRANGEMENTS

In the article published in this issue of *Science Translational Medicine*, a team of Norwegian, U.S., Swedish, and British scientists collaborated to overcome some of these obstacles. Russnes et al. (8) developed an objective estimate of genome-wide architectural distortion and investigated the ability of this marker of genomic complexity to provide prognostic information. They used aCGH data from 4 clinical cohorts, including 595 breast carcinomas. Their aim to develop such an estimate was inspired by three different patterns of segmented genome profiles—"Simplex," "Firestorm," and "Sawtooth" (Fig. 1)—that were previously visually recognized by Hicks et al. (22). These patterns were presented as tools for distinguishing distinct processes of genomic rearrangement. Simplex has broad segments of duplications and deletions, usually comprising entire chromosomes or chromosome arms, with occasional isolated narrow peaks of amplification (Fig. 1A). Sawtooth is characterized by many narrow segments of duplications and deletions, more or less affecting all the chromosomes. Typically, the events in these tumors do not involve high copy number amplification, although little of the genome remains at the normal copy number (Fig. 1B). Firestorm resembles the Simplex type, except that the genomes contain at least one localized region of clustered, relatively narrow peaks of amplification, with each cluster confined to a single chromosome arm. In these tumors, the amplifications often occur at high copy number (Fig. 1C) (22).

Russnes et al. (8) developed two new algorithms to estimate genomic complexity objectively using segmented aCGH data: (i) whole-arm aberration index (WAAI) and (ii) complex armwise aberration index (CAAI). This approach to classifying tumors is new, because the criteria include not only specific genomic regions but also the architectural type of rearrangement, such as the gain or loss of whole chromosome arms. Segmentation was performed on data from three different studies that each used slightly different methods to obtain DNA copy number data. Because the authors aimed to pool all the segmented aCGH profiles, they scaled different parameters to obtain roughly equal segmentation resolutions for the three studies.

The WAAI score was designed to capture events that involve whole chromosome arms rather than more localized gains and losses of DNA; this was done to reflect underlying defects in DNA maintenance, such as processes that lead to the formation of isochromosomes (which lack one arm and contain a duplication of the remaining arm) and translocations with a breakpoint close to the centromere. Russnes et al. (8) defined chromosomal arms with a WAAI score ≥ 0.8 as whole arms, and arms with a score of ≤ -0.8 as whole-arm losses. In contrast, CAAI measures local distortion to recognize regions with structural complexity. For each breakpoint found by the segmentation algorithm, three scores were calculated: (i) the proximity to neighboring breakpoints, (ii) the magnitude of change, and (iii) a weight of importance. Areas of complex rearrangements were found by selecting chromosome arms with a CAAI score ≥ 0.5 .

To define subgroups based on genomic architecture, the authors first distinguished four groups of tumors, based on previously identified genomic alterations that tend to occur in different breast cancer subtypes: (i) those with whole-arm gain of 1q and/or loss of 16q (group A), (ii) those with regional loss on 5q and/or gain on 10p (group B), (iii) those with both group A and group B alterations (group AB), and (iv) those with neither (group C). The subgroups displayed pronounced differences with respect to the number of whole-

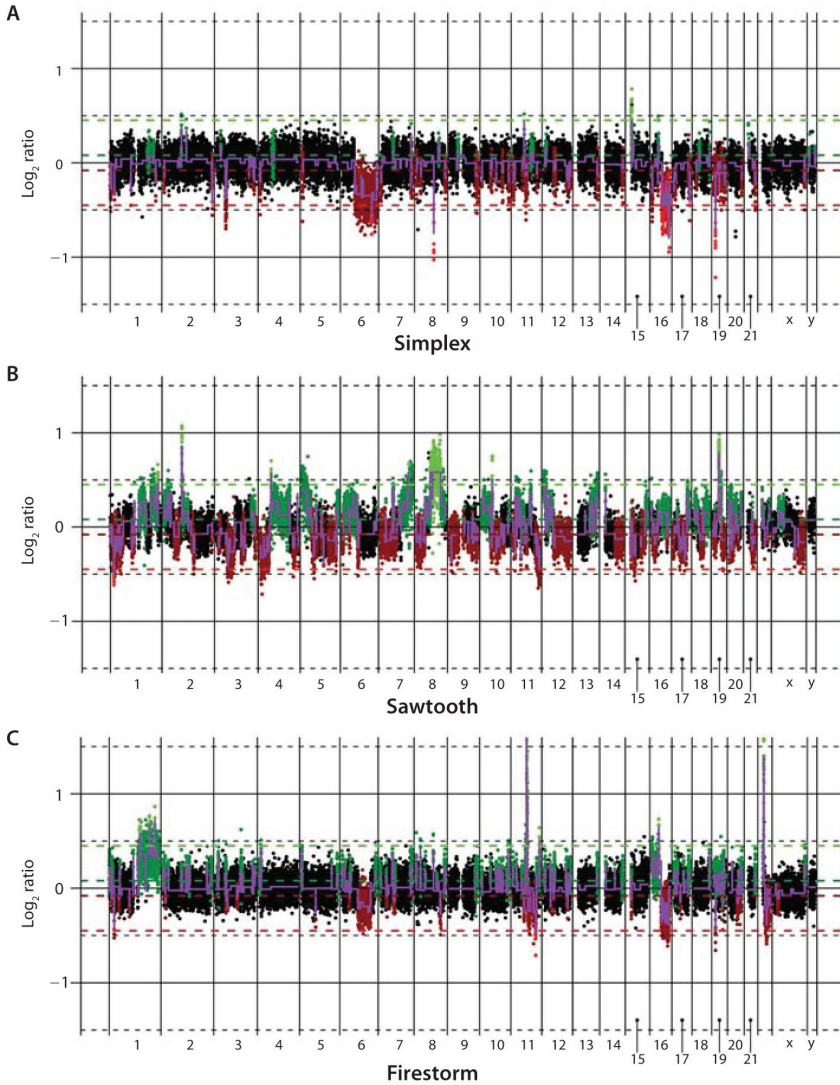


Fig. 1. Segmented genome profiles. Examples of patterns representing simplex (A), sawtooth (B), and Firestorm (C). the y axis displays the geometric mean value of two experiments on a log scale. chromosomes 1 to 22, plus X and Y, are displayed in order from left to right, according to the probe position.

chromosome arm loss or gain events. To characterize these groups further, each was split into two CAAI subgroups, depending on the level of complex rearrangement: those with CAAI < 0.5 for all arms (low-level CAAI; A1, B1, AB1, and C1) and those with CAAI ≥ 0.5 for at least one arm (high-level CAAI; A2, B2, AB2, and C2). The results show that all tumors with complex rearrangements had more whole arms affected than those without complex rearrangements.

These subgroups tend to have other characteristics in common. Breast tumors can be classified into several subtypes based on their hormone receptor [estrogen receptor (ER) and progesterone receptor (PR)] and HER2 status; based on gene expression profiling, luminal A, luminal B, HER2-enriched, basal-like, and normal-like tumors can be distinguished. The type A tumor class was dominated by ER-positive, luminal A tumors. These tumor genomes had high-magnitude WAAI scores, as well as 1q gain and 16q loss. As compared to A1 tumors, genomes from A2 tumors had chromosomes with more arms with high-magnitude WAAI scores and were more frequently aneuploid. A2 tumors tended to be of high grade and were associated with worse outcomes than A1 tumors. The B1 class was dominated by the basal-like subtype. Type B tumors had different and more heterogeneous genomic patterns; B1 tumor genomes were dominated by losses. A majority of HER2-enriched and normal-like tumors were classified as C tumors, and almost 30% of all basal-like tumors were classified as C tumors.

Next, the CAAI score was shown to have independent prognostic power in 451 breast carcinomas with clinical follow-up data. Patients with a B type tumor had a twofold increased risk of dying of breast cancer as compared with those with the A type, independent of lymph node status, tumor size, histological grade, and treatment. Individuals with tumors with a high-magnitude CAAI score had a twofold increased risk of dying of breast cancer as compared to those with a low-magnitude CAAI score, independent of lymph node status, tumor size, histological grade, and WAAI class.

CONCLUSION

The analyses presented by Russnes et al. (8) illustrate the complexity of the types of analysis that are needed to integrate large-scale genomic analysis with clinical and pathological parameters. In recent years, it has become possible to give tailored therapy to many breast cancer patients as a result of the multitude of choices for surgical, radiotherapy, and adjuvant or neoadjuvant systemic treatment. (Adjuvant therapy is given after all detectable disease has been removed to reduce the risk of relapse; neoadjuvant therapy is given before the primary treatment.) The prognostic and predictive factors on which these choices are presently based include clinical and pathological factors (Fig. 2). Prognostic factors (defined as factors that predict the course of the disease) and predictive factors (defined as factors that predict the response to specific therapies) are used to guide treatment in individual patients (23). With increasing knowledge of specific genetic alterations and gene expression profiles of tumors, and the prognostic and predictive value of these genetic tumor characteristics, more refined patient therapy is starting to be possible. Axillary lymph node status, tumor size, histological grade, histological subtype, HER2 status, and hormone receptor status are still the most important factors for determining treatment (23). The St. Gallen (24), National Institutes of Health (25), and Nottingham Prognostic Index guidelines (26), as well as the Adjuvant Online! decision-making tool (27), use a combination of these prognostic factors to guide decision-making about adjuvant systemic treatment of patients with early breast cancer. However, using these guidelines, a substantial proportion of breast cancer patients who would also survive without adjuvant systemic therapy undergo systemic therapy and suffer from its side effects without gaining any benefit (28, 29). In addition, more (genetic) tests are urgently needed to predict the responsiveness of tumors to chemotherapy and targeted therapies.

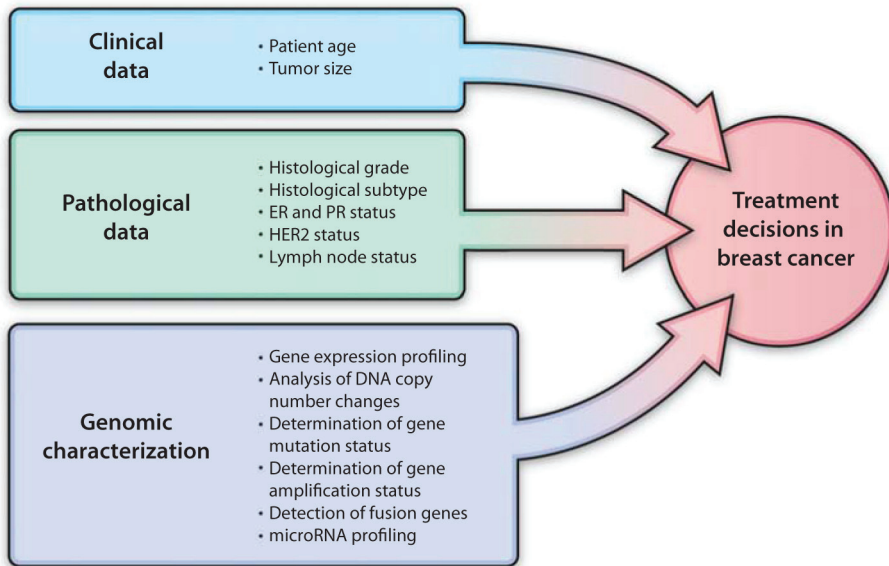


Fig. 2. Better decisions. Integration of clinical, pathological, and possible genetic factors to improve treatment decisions in breast cancer.

To a progressively increasing extent, genetic factors are being added to clinical and pathological characteristics to derive individualized predictions of disease outcome and response to therapy. The information derived from gene expression profiling, aCGH, and more recently, massive parallel sequencing has been used for these reasons. Translation into the clinical area has so far been limited by (i) the heterogeneity of the studies, (ii) the complexity of breast cancer biology, (iii) the complex analyses, (iv) small sample sizes and lack of independent validation, and (v) the relatively rare occurrence of most genetic alterations in breast cancer (most alterations occur in only 10 to 20% of breast carcinomas); if subgroups of tumors are defined on the basis of combinations of genetic alterations, these subgroups become very small.

The study by Russnes et al. (8) demonstrates a correlation between structural genomic alterations, molecular subtypes, and clinical outcomes and reveals that an objective score of genomic complexity can give independent prognostic information in breast cancer. In this way, an additional tool has been added to the arsenal of analysis algorithms for genetic data that is currently being used to decipher the association between the genetic makeup of breast carcinomas and their clinical behavior.

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