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Sequencing efforts help to refine the molecular classification of breast cancer

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Gene-expression studies of patients with breast cancer demonstrated that tumors can be organized into subtypes on the basis of patterns of gene expression that differ in clinical outcome. Importantly, successes that have been achieved in the treatment of breast cancer are in part because of this fundamental appreciation that breast cancer does not represent a single pathologic entity. Depending on the genes and pathways responsible for driving a specific subtype, the therapeutic approaches should be and are significantly different. These transcriptional profiling studies have led to the identification of four clinically useful major molecular subtypes: luminal A, luminal B, triple-negative/basal-like and human epidermal growth factor receptor 2 (HER2) type. However, recent studies using high-throughput sequencing techniques to profile DNA and RNA in breast cancer samples suggest that the heterogeneity of breast cancer is actually much larger than initially anticipated [1–3]. Massively parallel sequencing has brought a new level of genomic resolution and now not only provides us with a more comprehensive picture of breast cancer's genetic diversity, but also suggests the need for a refined tumor classification.

Over the last year, multiple articles have been published which describe the application of massively parallel sequencing techniques to hundreds of breast cancer samples [1–3]. These studies provide us with an unprecedented comprehensive catalog of a large number of somatic gene mutations and copy-number variations found in breast cancer. For example, Stephens *et al.* [1] recently examined the genomes of 100 breast cancers for mutations and somatic copy-number changes in the coding exons of protein-coding genes and identified over 7000 point mutations, among which 4737 were predicted to generate missense mutations (which code for a different amino acid), 422 nonsense mutations (which code for a stop and can truncate a protein) and 1637 silent mutations (no change in protein sequence). Moreover, analyses of gene copy-number changes yielded 1712 homozygous deletions and 1751 regions of increased copy number. The genomic complexity of breast cancer appears to be daunting and points to a higher degree of

intertumor heterogeneity between individual breast cancers than initially anticipated.

Numerous statistical methods to identify driver gene mutations have been described. Some are based on the frequency of mutations in an individual gene compared with the mutation frequency of other genes in the same or related tumors after correction for sequence context and gene size. Significantly mutated genes can be identified based on the fact that they occur at a rate that is above what would be expected by chance, and therefore are likely to be mutational events that drive the disease process. Other methods are based on the predicted effect of a mutation on the encoded protein, as inferred from preclinical mechanistic studies. All of these methods are useful for prioritizing genes that are most likely to promote a selective growth advantage when mutated. In contrast, passenger gene mutations have not been subject to selection as they are biologically neutral and do not confer growth advantage. Importantly, the majority of the recently discovered mutations in breast cancer are thought to be passenger gene mutations rather than potentially targetable driver gene mutations. Driver gene mutations were found in approximately 40 genes in the genomes of these 100 breast cancers examined by Stephens *et al.* [1]. However, only seven of the 40 cancer genes (*TP53*, *PIK3CA*, *ERBB2*, *MYC*, *FGFR1*, *ZNF703*, *GATA3* and *CCND1*) were altered in more than 10% of cases. Other driver gene mutations were relatively infrequent and each only found in 1–2% of the samples. The Cancer Genome Atlas (TCGA) Network identified 35 significantly mutated genes among 510 tumors that were subjected to whole-exome sequencing. In addition to identifying nearly all, more common driver gene mutations previously

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implicated in breast cancer (PIK3CA, PTEN, AKT1, TP53, GATA3, CDH1, RB1, MLL1, MAP3K1 and CDKN1B), a number of novel significantly mutated genes were identified albeit each at very low frequency between 1 and 2% [2]. Another recent study analyzed the copy-number aberrations that have coincident abnormal gene expression in 2000 breast cancer tumors and revealed 45 regions as putative driver genes [3]. More commonly amplified driver genes included MYC, HER2, CCND1 and ZNF703, and more commonly deleted driver genes included PPP2R2A, MTAP, PTEN and MAP2K4. This large sequencing study also illuminated rare but potentially significant events including IGF1R, KRAS and EGFR amplifications and CDKN2B, BRCA2, RB1 and ATM homozygous deletions; however, some of these events had very low overall frequencies (<1% of patients) [3].

These new insights into the true molecular heterogeneity of breast cancer may make it necessary to further divide the existing breast cancer subtypes into more fragmented subgroups, as each of these evolving new genomic features may require distinct treatment approaches to further improve the treatment outcomes. For example, TCGA data suggest that clinical HER2-positive disease may be divided into two groups. One HER2-positive subgroup was associated with high levels of EGFR and HER2 protein phosphorylation, and a tendency to be estrogen receptor negative, whereas the second group had lower level DNA amplification and lower protein-based signaling, and tended to be estrogen receptor positive/luminal. Similar subdivisions have recently also been made for triple-negative/basal-like breast cancer. Lehmann *et al.* [4] analyzed the gene-expression profiles from 21 breast cancer datasets and identified 587 triple-negative breast cancer cases. Cluster analysis identified six triple-negative breast cancer subtypes displaying unique gene-expression profiles and ontologies, including two basal-like (BL1 and BL2), an immunomodulatory, a mesenchymal, a mesenchymal stem-like and a luminal androgen receptor subtype. It is very likely that future therapeutic approaches will need to take these differences into account to improve the outcomes for triple-negative/basal-like breast cancer.

This issue of *Current Opinion in Obstetrics and Gynecology* contains four comprehensive reviews that will discuss the implications of these and other recent developments for the treatment of patients diagnosed with breast cancer. Peintinger [5] updates us on the molecular profiling assays such the 70-gene assay (MammaPrint, Agendia, Amsterdam, The Netherlands), the 21-gene assay (Oncotype DX, Genomic Health, Redwood City, California, USA)

and the 50-gene assay (PAM50, NanoString, Seattle, Washington, USA), and how they have been used to tailor treatments for patients diagnosed with breast cancer. Williams and Harris [6] describe how the aforementioned discoveries of new genomic targets have led to new treatment strategies for hormone-receptor-positive breast cancer. Hirshfield and Ganesan [7] depict the new molecular subtypes of triple-negative/basal-like breast cancer and how these findings may build novel targeted treatment strategies. Lastly, Kim *et al.* [8] illustrate how the management of HER2-positive breast cancer has recently evolved and how the treatment paradigm for HER2-positive breast cancer is shifting toward a dual anti-HER2 therapeutic approach.

Our new understanding of the genomic landscape of breast cancer suggests that individual breast cancers typically carry a few consistent molecular abnormalities, along with thousands of other changes that are rare or unique to the individual tumor. As such, the genomic landscape of breast cancer consists of a small number of 'mountains' (genes altered in a high percentage of tumors) and a much larger number of 'hills' (genes altered infrequently) [9]. The low frequency of some of these driver gene mutations poses a new challenge as their low frequency may make it difficult to study their clinical relevance within the realm of a clinical trial. However, this obstacle may be less relevant for breast cancer when compared with other cancers. For example, HER2 is mutated in only about 1.6% of HER2 nonamplified breast cancers [3,10]. Although this is considered rare, breast cancer is so common that a 2% incidence still produces an estimated 4000 cases a year, comparable to other diseases routinely treated with tyrosine kinase inhibitors such as chronic myeloid leukemia [10,11]. As such, further analyses and mining of available genomic data will help us improve our understanding of new driver gene mutations and how they affect the signaling pathways in breast cancer and will ultimately allow us to develop a refined and more accurate molecular classification of the disease.

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Conflicts of interest

The author has no conflicts of interest to declare.

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