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News and Commentary

Elementary: breast cancer culprits leave their signatures on the double helix

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Sherlock Holmes was renowned for his ability to discern subtle clues imprinted on people's history to solve a case. A study recently published in *Nature* identified breast cancer culprits, scrutinising the genome of a large number of patients.[1] The authors found specific genomic 'prints' or 'signatures' left by mutational processes in the DNA, providing valuable insights into the enigmatic genesis of breast cancer. The mutational landscape of 560 breast cancer patients was determined using whole-genome sequencing, RNAseq, miRNAseq and DNA methylation arrays.[1] Malignant tissues, mostly obtained from drug-naïve patients, were compared with non-malignant tissues (lymphocytes, adjacent normal tissue or skin). The power of this study is that the resulting catalogue of somatic mutations was used not only to reveal driver mutations that confer clonal advantage, but also to empirically identify the mutational processes that generate these somatic mutations.

In their analysis, Nik-Zainal *et al.* identified five new breast cancer-driver genes: *MED23, FOXP1, MLLT4, XBP1* and *ZFP36L1* (Figure 1). Most of these genes have been previously associated with tumorigenesis in various cancer types, including breast cancer.[2–6] It would be interesting to know whether their expression is correlated with patient outcome or associated with specific molecular subtypes. In non-coding DNA, the authors reported that only three promoters showed more mutations than expected by chance (*PLEKHS1, WDR74* and *TBC1D12*), consistent with the recent observation that the number of somatic mutations in promoter regions is lower in breast cancer compared with other cancer types.[7] Mutations in the promoters of *PLEKHS1* and *WDR74* have previously been described using a large-scale sequencing analysis of non-coding regulatory mutations using multiple datasets, including The Cancer Genome Atlas.[8] A growing number of recurrent promoter mutations have been identified in cancer, but with the exception of *TERT*,[9,10] whether they have a role as tumorigenic drivers has not been established. Further validation of these promoter mutations-for instance using targeted mutation analysis-will be required to determine the relevance of these findings.

It has previously been established that different mutational processes, such as DNA damage by carcinogens or mutations in DNA repair pathways, leave mutational patterns in the genome in the form of biases in the distribution of mutated tri-nucleotides, referred as 'mutational signatures'. In a smaller study from the same team, five mutational signatures were identified in breast cancer.[11] Subsequently, 20 distinct mutational signatures were found using data from various cancers.[12] In the cohort of 560 breast cancer patients, 12 mutational signatures involving single nucleotides were described. Of these, five had been observed previously in breast cancer, five in other cancers, and two new signatures were identified (Figure 1). Other types of mutations have now also been included, revealing two indels and six rearrangement signatures. The latter are characterised by enrichment for particular types, sizes and clustering of genomic rearrangements. This extension to other mutation types is interesting since it has revealed new association of signatures with BRCA-, p53- and oestrogen receptor-status. Certainly, expanding this analysis to other types of cancer could reveal more mutational signatures, and correlate their presence with specific oncogenic factors.

Mismatch repair deficiency, homologous recombinational repair deficiency, kataegis (or localised hypermutations) and APOBEC-related mutagenesis were associated with some of these specific mutational signatures,[1] suggesting that the origin of these signatures could be predicted. For instance, rearrangement signature 3, substitution signatures 3 and 8 were associated with BRCA1 breast cancer; rearrangement signature 5 was associated with *BRCA1* and *BRCA2* mutations; rearrangement signature 1 and substitution signature 3 were associated with p53 mutations, and rearrangement signature 2 with ER-positive cancers with a quiet copy number profile (Figure 1). Interestingly, a subset of breast cancer showing rearrangement signatures 5, with substitution signatures 3 and 8 did not show any abnormality in BRCA1 and BRCA2, thus suggesting that other genes involved in the DNA repair pathway may carry mutations. However, none of the potential gene candidates *ATM, ATR, PALB2, RAD51C, RAD50, TP53, CHEK2* and *BRIP1* showed any relationship with the identified mutational patterns. This important information may imply that patients with tumours harbouring rearrangement signatures 5 and substitution signatures 3 and 8 may

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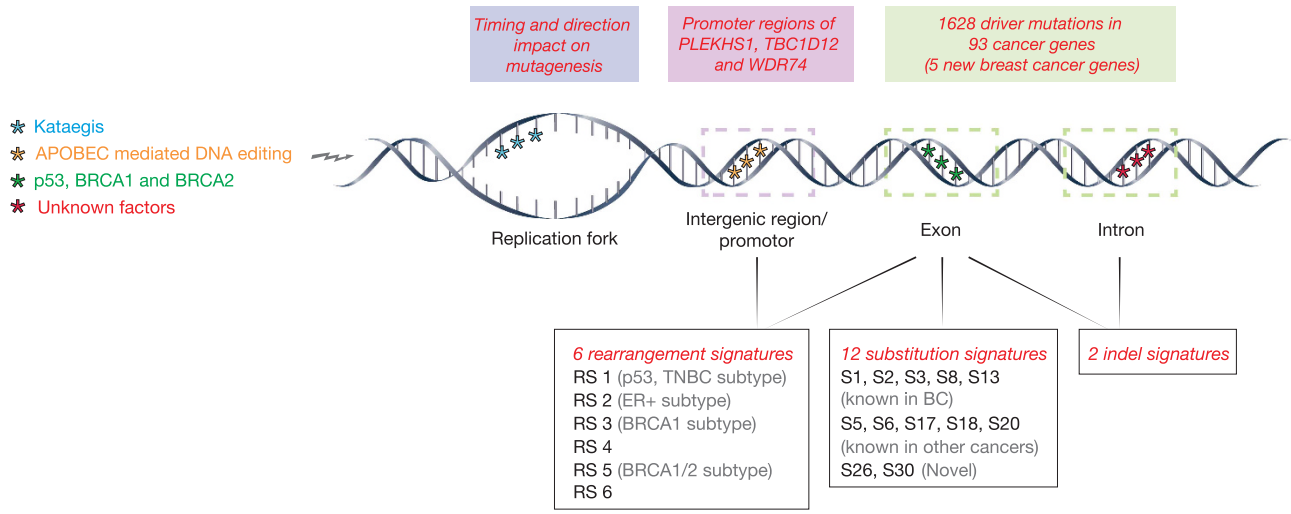

Figure 1 Identification of new somatic mutations and related mutational signatures in 560 breast cancer patients. The beauty of the analysis of Nik-Zainal *et al.* is that it goes beyond looking for recurrent mutations in protein coding regions of genes, and examines the patterns of mutations in intronic and intergenic regions. They found that various processes such as APOBEC-mediated DNA editing, p53, BRCA1/2 mutations or Kataegis mediated hypermutation can leave footprints in the genome. BC, breast cancer; RS, rearrangement signatures; S, substitution; TNBC, triple negative breast cancer

benefit from treatments given to patients with *BRCA1* or *BRCA2* mutations, such as Cisplatin and PARP inhibitors. Furthermore, it would be interesting to know whether patients with a strong family history of breast cancer who don't carry *BRCA1* or *BRCA2* germline mutations (~50% of cases) present these signatures. Although the authors described rearrangement signatures according to the molecular subtypes (Figure 1), little is known about the correlation of the base substitution and indel signatures with respect to breast cancer subtypes.

This study not only provided a new analytical framework, but also fascinating insights into mutagenesis, as explored in a second publication, relating mutational signatures with replication timing, transcription strand and nucleosome position (Figure 1).[13] Base substitution and rearrangement signatures increase in mutation density during time of replication, but at their own speed, whereas somatic deletions seem to be enriched later in replication. The level of asymmetry between strands varies between mutational signatures, with some signatures associated with the transcriptional strand, and some with the 'non-transcribed' strand. Remarkably, this replication time and strand specificity may vary according to the mutational mechanisms, but are similar regardless of the molecular subtype of breast cancer. These findings rely on using the ER-positive cell line MCF-7, and it will be interesting to determine whether the same conclusions apply in patient samples, particularly considering different molecular subtypes. In addition, the way each mutation was labelled by a specific mutation signature that most likely produced it might be refined by future studies. Nevertheless, this study provides valuable clues about the 'modus operandi' of mutational processes in breast cancer.

Conclusion

Nik-Zainal *et al.* have unlocked mysteries on the genomic landscape of breast cancer patients and opened a Pandora's box: can mutational signatures be used to predict patient outcome and response to treatments? Are these signatures present in neo-plastic tissues? How would chemotherapy such as DNA damaging agents impact the shaping of the mutational landscape? Finally, how are these patterns evolving during tumour progression? This study provides important insights into breast cancer evolution, holding promise for patient stratification and precision medicine. Analysis of larger breast cancer cohorts from the Molecular Taxonomy of Breast Cancer International Consortium (METABRIC) has identified 10 molecular subgroups with distinct clinical outcome.[14,15] Future studies integrating these new mutational signatures with long-term clinical follow-up are needed to further resolve the heterogeneity of existing molecular classifications.

Conflict of Interest

The authors declare no conflict of interest.

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