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BREAST CANCER

SUBCLONES—GO FORTH AND MUTATE

A novel DNA-sequencing method, Nuc-Seq, has been used for single-cell analysis of cells isolated from one oestrogen-receptor positive (ER^+) breast tumour and one triple-negative breast cancer (TNBC), with coverage of >90% of the whole genome. "Our data clearly show that no two single tumour cells are genetically identical, challenging the strict definition of 'clones' as 'genetically identical cells'," claims Nicholas Navin, who led the study.

Initial copy-number profiling revealed that cells within each tumour shared chromosomal abnormalities, suggesting that such aberrations evolve in punctuated bursts followed by stable clonal expansions to form a monoclonal tumour mass. "By contrast, we found that the evolution of point mutations occurs gradually over long periods of time, generating extensive clonal diversity," explains Navin. The Nuc-Seq data, validated by deep sequencing, identified many subclonal and *de novo* point mutations at low frequencies in the tumour mass, often present in <1% of cells, and in only 0.01% of cells for some. "These rare subclonal mutations may play an important part in diversifying the phenotypes of cancer cells when they are challenged by selective pressures in the tumour microenvironment, including perhaps the greatest pressure of all: chemotherapy."

Interestingly, a greater number of mutations were identified in the TNBC compared with the ER^+ tumour, and the mutation rate in the TNBC was calculated to be 13.3-fold higher than in both normal cells and the ER^+ tumour. These results have important implications for the development of chemoresistance, as the data suggest that many mutations involved in resistance probably exist at low frequencies in the tumour mass prior to therapy. Navin adds, "these data may explain why TNBCs often develop resistance to chemotherapy."

"We will need to conduct similar studies, in a larger set of patients, to determine how generalizable our findings are in breast cancer, and other human cancer types," states Yong Wang, first author of the study. The team hopes to use their tool to study many complex biological processes that are difficult to study in bulk tissue samples. For example, "we will apply Nuc-Seq to study the evolution of chemoresistance, by sequencing single cancer cells before and after chemotherapy," Wang concludes.

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Original article Wang, Y. *et al*. Clonal evolution in breast cancer revealed by single nucleus genome sequencing. *Nature* doi:10.1038/nature13600