GENOMICS

One cell at a time

> these two breast cancers evolved through "punctuated clonal evolution"

Genomic analyses of tumour samples can provide insight into tumour progression but they are complicated to interpret as such samples are genetically heterogeneous and can be comprised of various cell types. So, Michael Wigler and colleagues used single nucleus sequencing (SNS) of cells from two human breast cancers to investigate tumour progression.

SNS involves the isolation of nuclei by flow cytometry and then amplification of the DNA for massively parallel sequencing: these data are then used to ascertain copy number alterations (CNAs). Having validated the sensitivity and reproducibility of SNS using various control cells, Navin and colleagues analysed a genetically heterogeneous, high-grade, triple-negative ductal carcinoma. The tumour was split into 12 sectors to preserve its anatomy and then cells were sorted from six sectors. They found four main distributions of ploidy (hypodiploid, diploid and two subtetraploid fractions) and they selected 100 nuclei for SNS. The diploid fraction showed few genetic alterations although ~65% had small deletions in T cell receptor loci or immunoglobulin variable regions, indicating that these cells were infiltrating immune cells within the tumour, which was consistent with histological analysis. Next, they used integer copy number profiles to produce a neighbour-joining tree and found three clonal tumour subpopulations from the hypodiploid and the two subtetraploid fractions. Cells within each tumour subpopulation shared CNAs and so probably represent a clonal expansion as the tumour developed. Furthermore, there were similarities in CNAs between each subpopulation but each had developed unique attributes (for example, one subpopulation had *KRAS* amplification). This indicates that these subpopulations are related to each other and therefore probably represent divergent populations as the tumour has developed.

The authors next investigated a genetically homogeneous high-grade, triple-negative ductal carcinoma and its liver metastasis. The tumours were split into six sectors and flow cytometry analysis showed that there was a diploid and an aneuploid fraction at about equal proportions, which was consistent with histological analysis. Analyses of the SNS data revealed limited divergence within the aneuploid subpopulation, indicating that in this case the primary tumour formed from a single clonal expansion and that one of these cells seeded the metastasis, which did not subsequently diverge much more.

Interestingly, the diploid fraction from each of the primary tumours contained numerous pseudodiploid cells that were identified by SNS. These had diverse chromosome gains and losses that were unique to each cell and that were not found in cells of the other subpopulations. These cells were not found in the metastasis, and the authors speculate that the pseudodiploid cells arise from a genomically unstable diploid subpopulation of tumour cells.

The authors propose that these two breast cancers evolved through "punctuated clonal evolution", whereby a clonal population with a substantial growth advantage suddenly emerges from a genomically unstable precursor. Whether this is generally applicable to other cancers requires further investigation.

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ORIGINAL RESEARCH PAPER Navin, N. *et al.* Tumour evolution inferred by single-cell sequencing. *Nature* 13 Mar 2011 (doi:10.1038/nature09807)