CANCER GENOMICS

ECdetect hunts extrachromosomal DNA

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Oncogenes are frequently activated through gene amplification (that is, an increase in copy number), which can lead to their over-expression and drive neoplastic progression. In tumour cells, amplified genomic regions (so-called 'amplicons') can occur within chromosomes, or amplification gives rise to circular extrachromosomal DNA (ecDNA). Now, Turner *et al.* have developed a new integrative analysis pipeline that provides insights into ecDNA biology in human cancers.

DNA sequencing can identify amplicons in tumour genomes, but it cannot spatially resolve their location in the chromosomal landscape. To ensure the unbiased detection of ecDNA, the authors developed ECdetect, a semi-automated image analysis software package for cytogenetic analyses, and integrated it with whole-genome sequencing.

ECdetect quantifies ecDNA from cells in metaphase that are stained with the fluorescent DNA-binding dye DAPI (4',6-diamidino-2-phenylindole). The

software was used to quantify ecDNA in 2,572 cells from a variety of cancer types, including 72 primary cancer cell samples, 10 immortalized tumour cell lines and 8 non-cancerous control cell lines.

Notably, ecDNA was rarely seen in normal cells but was detectable in nearly half of human cancers, both in immortalized cells and primary samples, at varying frequencies depending on tumour type. Additionally, ecDNA copy number varied from cell to cell in most tumour cultures that were analysed, demonstrating the heterogeneity of cells within a tumour.

Next, the authors carried out whole-genome sequencing of 117 tumour samples and compared the resulting reads with sequences in The Cancer Genome Atlas to validate focal amplifications. Selected amplified oncogenes in tumour cells were visualized by fluorescence *in situ* hybridization (FISH) and analysed using ECdetect. Interestingly, all genes tested were located solely in ecDNA,

or in both ecDNA and in chromosomal homogeneously staining regions (which are indicative of gene amplification). Furthermore, quantitative PCR analysis of the oncogenes *MYC* and *EGFRvIII* (EGFR variant III) showed that they were highly expressed from ecDNA in tumour cells.

Together, these data suggest that common oncogenic driver amplifications in cancer are located on, and are expressed from, ecDNA. Importantly, mathematical modelling and the experimental validation of its predictions revealed that oncogenes on ecDNA increase copy number and tumour heterogeneity more than do those resulting from chromosomal amplification. These findings strongly suggest that ecDNA plays a crucial part in driving accelerated cancer evolution.

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