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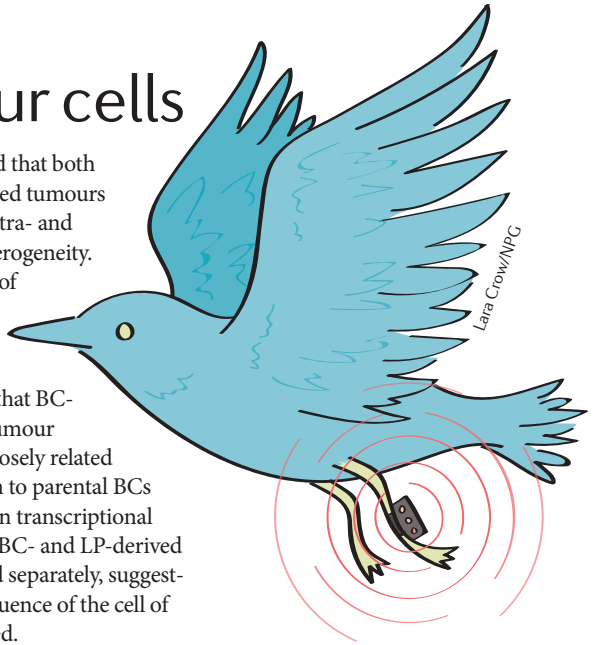
TUMORIGENESIS

Tracking early tumour cells



Most cancers are genomically and phenotypically diverse at diagnosis, making the study of early tumorigenesis and tumour evolution challenging. Although our understanding of these processes has improved through the use of technologies such as lineage tracing in mice and deep sequencing of cancer genomes, there is still much that is not known.

Nguyen, Pellacani *et al.* devised a mouse modelling system in which the early stages of breast tumour development could be monitored. They separated reduction mammoplasty-derived human mammary cells into populations of basal cells (BCs), luminal progenitors (LPs), non-clonogenic luminal cells (LCs) and stromal cells (SCs), and induced expression of different combinations of oncogenic KRAS, PIK3CA (the PI3K catalytic subunit-α) and p53 mutants in each population. Cells also expressed DNA barcodes to enable clonal tracking. Cells were implanted subcutaneously or subrenally into immunocompromised mice to study tumour development.

Expression of all three oncogenes in BCs or LPs (triply transduced cells) produced tumours that resembled invasive ductal carcinomas within 8 weeks, whereas no tumours developed from LCs or SCs. Analysis of different oncogene combinations revealed that expression of KRAS-G12D only was crucial for efficient tumour formation. Immunohistochemical analyses of various markers in tumour sections and gene expression analyses indicated that both BC- and LP-derived tumours had substantial intra- and inter-tumour heterogeneity. RNA sequencing of tumours derived from triply transduced BCs or LPs suggested that BC- and LP-derived tumour cells were more closely related to each other than to parental BCs or LPs, although in transcriptional profile clustering BC- and LP-derived tumours clustered separately, suggesting that some influence of the cell of origin was retained.

Heterogeneity was also observed in the clonal content of different tumours, with the total number of clones per tumour ranging from ~30 to ~1,700 (using a threshold of 70 cells per clone). Furthermore, relative clone sizes within a tumour were broadly distributed and did not seem to be dependent on the original cells transduced, the oncogene(s) introduced or the transplantation site. Similar results were observed when cells from the primary tumours were transplanted into secondary recipient mice. Interestingly, however, 75% of the clones detected in secondary tumours were not present in the initial primary tumours, and there was also large variation in the clones in different secondary tumours derived from the same primary tumour. When the authors looked at an earlier time point (2 weeks), the numbers of clones and relative sizes were surprisingly similar to those detected at 8 weeks.

> “a slow multi-step evolutionary process might not be required”

Overall, these data suggest that a slow multi-step evolutionary process might not be required to generate tumours following KRAS-G12D expression, and they provide an experimental example of rapid accumulation of transformants that display biologically heterogeneous properties regardless of the cell type initially transduced. The data also raise many interesting questions. For example, would breast tumour cells grown orthotopically and/or in immunocompetent mice have similar properties, and do the features revealed in this experimental KRAS-G12D model extend to more common modes of breast cancer development or other KRAS-mutant human tumours?

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ORIGINAL ARTICLE Nguyen, L. V., Pellacani, D. *et al.* Barcoding reveals complex clonal dynamics of *de novo* transformed human mammary cells. *Nature* **528**, 267–271 (2015)