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News & views

Tumour biology

A heritable, non-genetic road to cancer evolution

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Treatment for leukaemia can fail for reasons that are not fully clear. Tracking the progress of individual cellular lineages for this type of cancer offers a way to investigate this phenomenon.

The complexity of a tumour's evolving cellular population poses an obstacle to successful therapy. Writing in *Nature*, Fennell *et al.*[1] present data that pinpoint some of the mechanisms underlying the ability of cancers to evolve to a more aggressive and treatment-resistant form.

Cancer evolution constitutes a formidable obstacle to successful treatment. As the population of malignant cells grows, it diversifies, challenging therapeutics to face not one disease, but rather thousands of variants of the disease in each patient. This phenomenon is best exemplified by cancer cells acquiring mutations in gene sequences encoding protein regions that are binding pockets for drugs. Such alterations endow cells with the ability to resist targeted therapies. These mutations are strongly selected for under therapeutic pressure, resulting in disease relapse due to a genetic and heritable mechanism (Fig. 1a).

Nonetheless, a large proportion of relapsed cancers lack the 'smoking gun' of a clear genetic mechanism of resistance. Indeed, current data challenge the genome-centric vision of cancer evolution and treatment resistance. Studies[2–4] indicate that some tumour cells can assume a drug-tolerant state after chemotherapy that is described as a senescent-like state (such cells typically do not divide). Thus, a small percentage of tumour cells stochastically (randomly) acquire characteristics that enable them to survive treatment (Fig. 1b). This echoes the phenomenon in which a subset of cells in bacterial populations enters a 'persister' state that enables them to overcome antibiotic challenge[5]. An important distinction between tumour resistance facilitated by gene mutations and this drug-tolerant state is that the latter is thought to arise in a stochastic and non-heritable manner. Thus, such a population of cancer cells reverts to its previous state after the therapeutic attack, to repopulate the tumour.

However, intriguing data accumulated lately suggest an alternative middle path of heritable, yet non-genetic, mechanisms of cancer evolution. In acute myeloid leukaemia (AML), leukaemia stem cells (a subset of the cancer cells with stem-cell characteristics) are specifically selected for under therapeutic pressure in a heritable fashion, and yet this happens against the backdrop of distinct tumoral genetic backgrounds[6]. Non-genetic, heritable resistance to treatment has also been observed[7] in lung cancer in response to therapeutic inhibition of the protein EGFR. And melanoma cancer cells can acquire particular drug-tolerant transcriptional profiles, which exhibit some degree of heritability[8]. These *in vitro* treatment-resistant transcriptional patterns might be heritable, yet arise through non-genetic mechanisms.

Cancer treatments are often administered in cycles over many months. Heritable resistance characteristics might therefore be particularly advantageous to malignant cell populations. Thus, it is crucial to uncover such mechanisms to enable the development of future therapeutic strategies to target cancer evolution.

To definitively address the question of whether cancer evolution can be driven by heritable, yet non-genetic, mechanisms, Fennell *et al.* designed a system that they term SPLINTR. In this approach, AML cells are individually tagged using DNA 'barcodes' that can be identified through single-cell RNA sequencing. These barcodes enable the descendants of individual AML cells to be tracked both *in vitro*

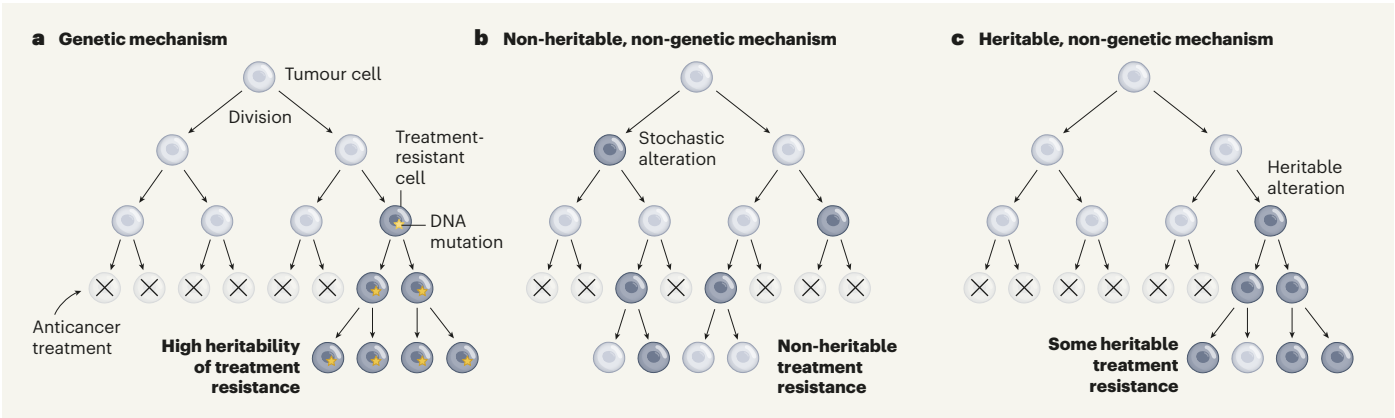

Figure 1 | Mechanisms of resistance to anticancer treatment. a, If a tumour cell acquires a mutation that enables it to evade destruction by anticancer therapy, this provides a stable and heritable way for the cancer to evolve into a treatment-resistant form (dark grey cell). **b**, Another way in which tumours can survive treatment is for some cells to randomly (stochastically) enter a treatment-resistant state. This process is not mediated by heritable, genetic mechanisms. **c**, Fennell *et al.*[1] report an analysis of leukaemia in a mouse model of cancer that points to another non-genetic mechanism of cancer evolution. These data suggest that treatment resistance might also follow a non-genetic, yet heritable, path, albeit with a degree of heritability that is not as high as that for DNA mutations.

and *in vivo* (the latter by monitoring cancer cells transplanted into mice), and the fortunes of these cell lineages (their clonal dynamics) to be determined (Fig. 1c). Crucially, the authors used mouse AML cells from genetically homogeneous and stable cell lineages, which are an optimal system in which to evaluate non-genetic mechanisms.

Fennell and colleagues found that both AML stem cells and more-mature versions of stem cells called progenitor cells caused disease with similar dynamics. That is, both of these cell types proliferated at much the same rate after transplantation into mice. Therefore, clonal differences in fitness between tumour cells after transplantation could not be explained by the nature of the tumour cell of origin (stem cells or progenitor cells). In a surprising twist, Fennell *et al.* observed that clonal descendants of the same individual barcoded cell rose to dominance across different mice, pointing to a non-genetic, yet heritable, cell property.

Some of the fittest clones had a gene-expression signature reminiscent of leukaemic stem cells, providing a clue to how the heritable identities provide an advantage. Furthermore, the authors observed that dominant clones exhibited high expression of the gene *Slpi*, which encodes secretory leukocyte peptidase inhibitor, a protein that has an anti-inflammatory function. These clones also had lower than normal expression of certain immune-system-related genes, such as those encoding components of the major histocompatibility (MHC) system. The MHC system has a function in triggering immune defence, which suggests that clonal fitness might be related to the capacity of the cells to remain hidden from the immune system.

Notably, this gene signature was present even before the cancer cells were transplanted into mice, and it remained stable after the cancer clones had grown in the animals — even in secondary transplants into other mice — reinforcing the idea of inheritance of these characteristics (phenotype). Nevertheless, the gene-expression profiles of dominant clones were not homogeneous, suggesting that, although these profiles are heritable, their stability over the course of repeated cell divisions is less than that observed for genetic factors underlying cancer evolution.

In contrast to the heritable propagation of non-genetic phenotypes, when the authors gave chemotherapy to mice with cancer, only a subset of cancer cells adopted a transcriptional program associated with the senescent-like state, consistent with previous reports[9–12]. However, the likelihood of adopting this state differed between clones, suggesting a complex interplay between heritable pre-existing characteristics and drug tolerance actively gained after exposure to treatment.

The demonstration of non-genetic yet heritable changes in cancer cells raises the question of what mechanism propagates these changes from parent to daughter cell. The immediate candidates are changes to the nuclear complex of DNA and protein called chromatin. Such epigenetic alterations include the addition of methyl groups (methylation) to DNA and modifications to the DNA-binding histone proteins. A high level of DNA methylation can inactivate genes that help to suppress tumour formation[13]. And the removal of methyl groups from histone H3 protein (at a site called H3K4) modifies chromatin that has been altered as a result of the therapeutic use of inhibitors of EGFR, and triggers the emergence of treatment-resistant persister cells[12].

In addition, key transcription factors might enable the stable propagation of rare, treatment-resistant cellular phenotypes[8]. Data implicating other post-translational protein modifications in resistance further expand the number of potential explanations to consider[10].

These intriguing findings point to the need for a more holistic view of cancer evolution. This would help to define how genetic and non-genetic mechanisms jointly contribute to cellular phenotype, which is the substrate for selection. Attempts to meet this challenge will be greatly aided by methods such as 'multi-omic' technologies that can capture multiple layers of information at single-cell resolution — the 'atomic unit' of the evolutionary process[14]. For example, capturing genetic, epigenetic and transcriptional information from the same individual cell will allow researchers to define the relative contribution of these features to evolving populations of cancer cells.

Moreover, translating animal studies into analyses of primary human samples will require innovations in clonal tracking that do not rely on artificial cell engineering, but instead use already-existing native, heritable changes to the DNA, which can be captured at the level of single cells[15,16]. These exciting new horizons might give us an integrated perspective on cancer evolution, with insights into disease progression and relapse. This could pave the way for new therapeutic approaches to directly anticipating and addressing cancer evolution, which remains a central obstacle to achieving a cure[9–12].

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