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Opinion

Phenotypic noise and plasticity in cancer evolution

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Non-genetic alterations can produce changes in a cell's phenotype. In cancer, these phenomena can influence a cell's fitness by conferring access to heritable, beneficial phenotypes. Herein, we argue that current discussions of 'phenotypic plasticity' in cancer evolution ignore a salient feature of the original definition: namely, that it occurs in response to an environmental change. We suggest 'phenotypic noise' be used to distinguish non-genetic changes in phenotype that occur independently from the environment. We discuss the conceptual and methodological techniques used to identify these phenomena during cancer evolution. We propose that the distinction will guide efforts to define mechanisms of phenotype change, accelerate translational work to manipulate phenotypes through treatment, and, ultimately, improve patient outcomes.

Highlights

Cancer cells change phenotype through cancer evolution; some phenotype changes are caused by underlying genetic mutations, but many are due to non-genetic mechanisms.

We suggest separating non-genetic phenotype changes into two distinct mechanisms: changes induced by microenvironmental stimuli (that we term 'phenotypic plasticity') and changes induced by stochastic cell-intrinsic effects (that we term 'phenotypic noise').

We discuss how lineage tracing methods – either experimental or those that make use of sporadic mutations to mark lineages – can be used to distinguish between phenotypic plasticity and noise.

Distinguishing between phenotypic plasticity and noise guides mechanistic work to determine the molecular causes of phenotype change, and will ultimately accelerate efforts to control or prevent phenotype changes that enable cancer cells to resist therapies.

Defining phenotypic plasticity in cancer evolution

Tumour-cell **phenotypes** (see Glossary) are the features that clinical interventions aim to repress; these include unregulated growth or immune escape. A major challenge in cancer research has been to understand how these phenotypes arise and behave.

Advancing genomic technologies have enabled us to study the genetic alterations associated with malignant phenotypes. Increased frequency of genetic variants within tumours [1] or their enrichment across tumours [2] identifies cancer 'driver mutations' that are under positive selection.

However, a cell's phenotype need not be derived exclusively from genetic changes. Indeed, specialised cell types within the human body all have the same genome because phenotype specification during development is a plastic process, not an evolutionary one. Burgeoning omics technologies now enable us to probe the full spectrum of phenotypic control, including differences in nucleotide sequences, **epigenetic** modifications of DNA and DNA 3D structure, and transcription regulatory machinery, alongside more proximal readouts of cell phenotypes in the form of RNA transcripts or high-throughput imaging. Post-translational regulation can also be studied at scale via techniques such as (phospho)proteomics.

In cancer, it is now clear that some phenotypes of clinical consequence can arise without underlying causative change in DNA sequences [3]. Molecular mechanisms include: stochastic, transient changes in transcript levels, buffering and feedback of pathways via gene regulatory networks, and epigenetic modifications of DNA and histones. The resulting phenotypes may differ in their **heritability**: the non-genetic mechanism responsible for a phenotypic difference can influence the probability of inheritance by offspring cells.

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In the field of cancer evolution, **phenotypic plasticity** is used as a catch-all term to encompass non-genetic changes that lead to a detectable phenotypic difference between tumour cells. Here, we argue that adopting the term 'plasticity' in cancer evolution to refer to any non-genetic change masks important nuances in how phenotypes are produced and maintained.

Phenotypic plasticity is a well-established concept in the field of evolutionary biology, originally defined as a non-genetic change in an organism's phenotype in response to an environmental change [4]. A textbook example is the inducible defences in the small planktonic crustaceans of the genus *Daphnia*; the environmental change involves the arrival of predators which promote the growth of morphological traits to defend against predation [5–7]. The evidence supporting plasticity is clear: the development of these defensive morphological modifications is experimentally inducible via the addition of the predators' chemical cues, they develop within a single generation, and they are reversible upon cue removal. It is easy to draw conceptual parallels between this illustrative example and how plasticity might operate in an evolving tumour; one could imagine cancer cells in place of individual *Daphnia*, cytotoxic therapy instead of a predator's chemical cues, and replacement of the rapid development of predator defences with a cancer cell's rapid phenotypic transition to a resistant state. However, important differences between the two systems deserve attention. In *Daphnia*: (i) the phenotype that exhibits plasticity is clearly defined and easy to measure, (ii) there is a clear distinction between the individual and the environment, with limited scope for feedback mechanisms, (iii) inheritance occurs via the germline, whilst the contents of somatic cells are not inherited, and (iv) the rate of environmental change is fast relative to the organism's generation time.

By contrast, in an evolving tumour, it is difficult to define and measure a cell's phenotype, the separation between an individual and the environment is often hard to delineate, cells inherit their molecular contents directly during mitosis, and environmental change and cancer-cell generation times occur on similar timescales. In cancer evolution these differences produce a unique set of challenges that likely led to the adoption of the term 'plasticity' to refer to all non-genetic changes in phenotype. Despite this, we contend that clarity around the mechanism – particularly whether it is cell-intrinsic or environmentally caused – is essential to enable development of therapies that prevent cancer adaption to treatment or tumour progression. Open questions include: when can we define observed molecular differences as discrete phenotypes? In what settings are non-genetic phenomena feasibly measurable? How heritable are phenotypes under non-genetic control, and how should we subsequently define them?

In this opinion article we discuss conceptual and methodological techniques used to identify non-genetic evolution in cancer in light of these questions.

Phenotypic noise and plasticity in normal tissue and relevance to cancer

Prior to the emergence of any aberrant, neoplastic behaviour, normal cells are capable of phenotypic plasticity. Despite sharing the same genome, any given cell must respond to the chemical and positional cues that allow a zygote to develop into a multicellular organism; hierarchies of phenotypically distinct cells give rise to organs composed of specialised tissues. Even post-development, cells must be able to respond to environmental insults (wounding) to ensure that tissue integrity and homeostasis are maintained. The cell differentiation that drives these processes is regulated by epigenetic factors and the **Waddington landscape** [8] is a conceptual tool often invoked to explain the process whereby cells are committed to these specialised lineages.

Glossary

Cell state: a group of repeatedly observed cell behaviours that are often discussed with reference to epigenetically mediated states that emerge during healthy development.
Epigenetic: molecular differences in a cell that can influence processes such as gene expression that are not DNA sequence changes, but are nonetheless heritable. Examples include DNA methylation and chromatin structure.
Heritability: the propensity of offspring (cells) to inherit traits from their parent (cell). Of note, this relaxed definition deviates from that adopted in quantitative genetics but makes room for epigenetic inheritence.
Phenotype: a collective term to refer to a single or multiple features of a cell (or related cells – a clone), including the transcriptome, proteome, morphological size, shape, and organisation.
Phenotypic noise: a difference in phenotype (either within a cell over time, or between contemporaneous cells) that occurs despite a shared genotype and independently of any environmental change.
Phenotypic plasticity: a difference in phenotype (either within a cell over time, or between contemporaneous cells) that occurs despite a shared genotype but as the direct result of an/some environmental change(s).
Phylogenetic signal: the tendency for related species to resemble each other, more than they resemble species drawn at random from a phylogenetic tree. Can be used as a tool to assess the correlation between genotype and phenotype data.
Waddington landscape: a conceptual framework describing how the epigenome state constrains possible phenotype changes.

The phenotypic plasticity of normal cells is vital; they must respond to environmental cues from the microenvironment or neighbouring cells during development and tissue maintenance. Simple feedback networks can ensure intercellular signals are converted into stable cell-type differentiation patterns, as has been shown for the lateral inhibition mechanism of intercellular delta-notch signalling that specifies cell type in the gut epithelium and other organs [9]. By contrast, there is a strong selection pressure (at the organism level) against healthy somatic cells exhibiting **phenotypic noise**, where stochastic changes in phenotype that are not environmentally controlled could lead to a breakdown in homeostasis (Figure 1).

In cancer, the fitness of a tumour cell becomes its ability to survive and proliferate in the face of changing environments. Molecular machinery that controls the phenotypic plasticity necessary for somatic cell specialisation can be co-opted during malignant evolution; the 'stemness' that previously enabled development and homeostasis provides a reservoir of rapid phenotypic innovation. Additionally, it is possible that the inhibitory mechanisms which previously prevented cells from accessing new phenotypes via phenotypic noise are lost. Further, these two phenomena are not mutually exclusive; one could imagine a phenotype which exhibits non-genetic variation within a clone of cells (noise) that subsequently responds to an external stimulus through heritable epigenetic rewiring (plasticity). Importantly, the propensity to exhibit plasticity – or switch the range of accessible phenotypes – could also itself result from underlying genetic evolution. A pressing question in cancer evolution is how frequently and to what degree these non-genetic phenomena are the driving forces of tumour evolution.

Environmental dependence

Determining the environmental dependence of cancer-cell phenotypes is important for understanding cancer biology, and has ramifications for treatment. For example, treatment effectiveness should hinge on whether the therapy induces a resistant phenotype. Approaches such as 'evolutionary steering' [10] – which aims to use treatment to drive cancer cell populations to more sensitive phenotypic states – rely on treatment-induced environmental changes generating desired cancer phenotypes.

Relevant environmental changes encountered by cancer cells include resource variability (e.g., density of the vasculature or stromal composition), pressures exerted by immune predation, and the clinical administration of cytotoxic therapies. Distinguishing plasticity from noise depends on being able to measure these environmental differences. This is particularly challenging in patient samples, where many environmental forces can be shifting simultaneously [11] and longitudinal measurement is impractical [12,13].

Timescale and heritable nature of phenotypic changes

Non-genetic phenomena can produce phenotypic changes that differ in their degree of permanence. For example, stochastic differences that arise during transcription can lead to variation in mRNA abundance that persists for only a few hours. During cell division, daughter cells inherit the 'molecular contents' of the parent cell directly; this leads to stochastic variations in the quantity of mRNA each daughter cell receives. Theoretical and experimental work has shown that these features of expression and division can drive differences in transcript counts and, ultimately, phenotypes [14–19]. If we instead consider less transient phenomena, heritable chromatin modifications can propagate through a subclone/lineage causing long-term phenotypic differences within an otherwise genetically homogeneous population [20–22] (Figure 2).

Phenotype heritability can be a function of the timescale in which the underlying molecular changes persist within a lineage. This in turn can dictate the potential for the given phenotype

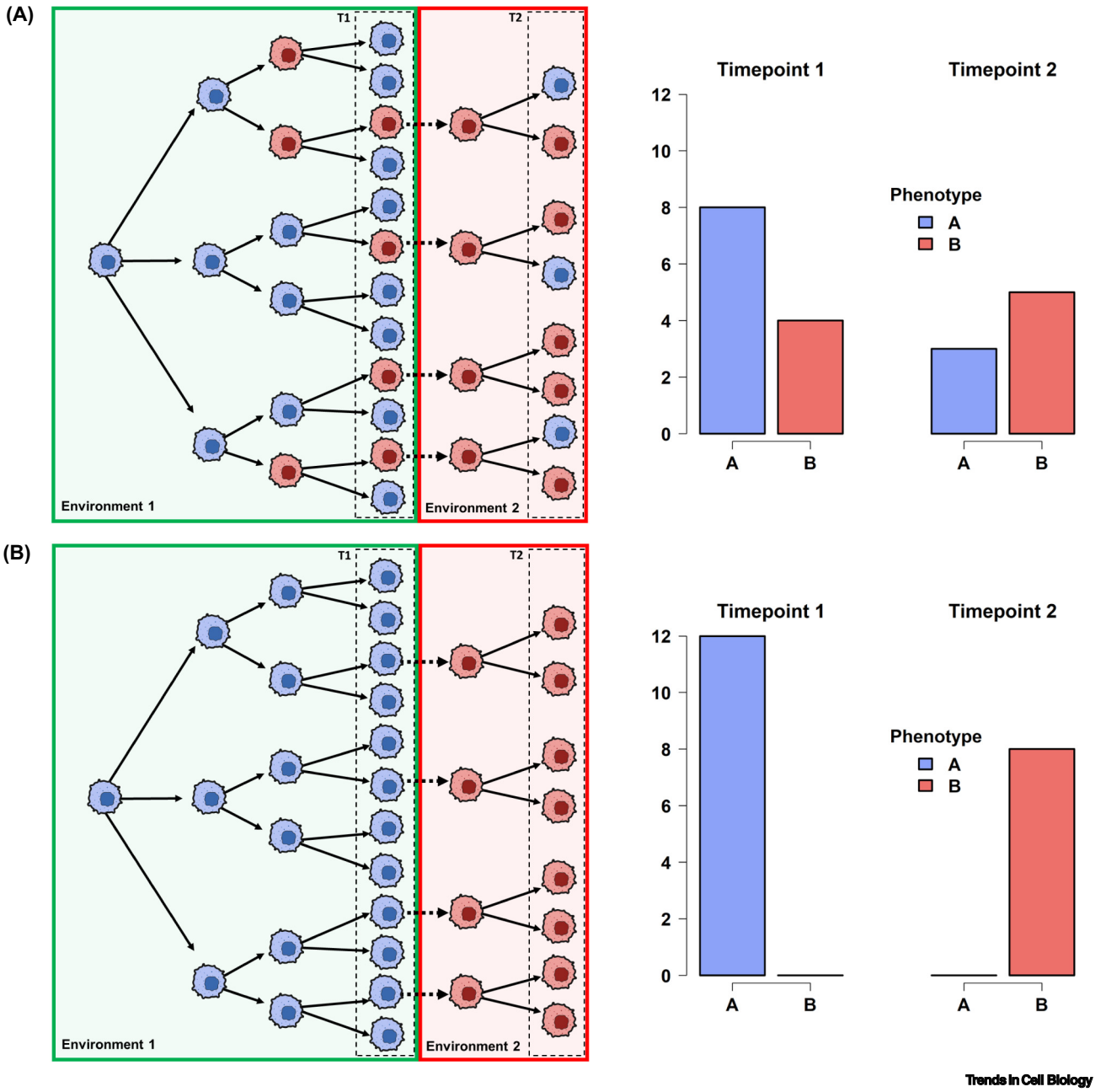

Figure 1. Primary differences between our definitions of phenotypic noise and phenotypic plasticity. (A) 'Phenotypic noise' – phenotypic diversity generated independently of genetic changes, illustrated by the diverging lineages (left panels), and despite a constant environment. As such, phenotypic diversity that confers a selective benefit in a new environment is present and selected for when the environment changes (dotted arrows). (B) 'Phenotypic plasticity' – phenotypic diversity is still generated independently of genetic changes; however, unlike phenotypic noise, it is induced by a changing environment. This influences the theoretical expectations of each phenotype prior to and following a change in selection pressure (right panels). Importantly, although illustrated as such here, these two modes of non-genetic change in phenotype are not mutually exclusive.

to benefit future generations: stable, non-genetic differences can be subject to selection. In species evolution, discussions traditionally tended to ignore adaptive changes that are not genetic. These changes might persist for a few generations but lack the permanence of DNA alterations. In cancer, however, rapid evolution and exponential growth mean that phenotypes persisting

even for a few generations still have the potential to alter disease dynamics and determine patient outcomes. When tumour cells need to respond to sudden external selection pressures (such as a pulse of chemotherapy), the ability to rapidly access a beneficial phenotype has clear fitness advantages over 'waiting' for novel genetic variants.

Further, the propensity for cells to exhibit these non-genetic phenomena – a trait that could be thought of as a 'meta phenotype' [23] – are themselves subject to selection. In cases where beneficial phenotypes have emerged via non-genetic means, the 'meta-phenotype' that controls the propensity for this shift may be caused by a genetic change (Figure 3). For example, chromatin modifier genes are mutated in numerous cancers and enable epigenetically mediated phenotypic changes [24,25] whilst mutations in gene promoters can impact levels of transcriptional noise [26], a source of phenotypic diversity.

In summary, the different timescales over which non-genetic mechanisms operate influence the extent to which phenotypes are heritable and, by extension, their ability to confer sustained adaptive changes following environmental change. Genetic alterations can influence the propensity for cells to exhibit these non-genetic behaviours and can also be subject to selection.

Identifying, measuring and understanding phenotypic plasticity and noise

Cancer is studied using a wide range of methodologies, including *in vitro* methods (cell lines, organoids), animal models, direct analysis of primary human tissue or mathematical modelling. Here we will discuss these approaches including their respective challenges, examples and opportunities for studying non-genetic evolution in cancer.

Experimental

Experimental characterisation of the dynamics of non-genetic phenotypic evolution offers many advantages: high resolution lineage tracing, tight control of selective environmental pressures, and the direct manipulation of molecular pathways.

An observation frequently taken as evidence of non-genetic phenotypic control is the rate of phenotypic change; if phenotypic transitions occur at a rate unexplainable via the generation of genetic differences alone, some alternative mechanism(s) must be responsible. A 'population-down' approach ignores the molecular machinery responsible and instead asks 'can the change in a cell's phenotype through multiple generations be explained by non-genetic phenomena?'.

Tracking the relatedness of cells over time distinguishes stable phenotypic heterogeneity in the population from ongoing phenotypic transitions within cell lineages [27]. Newer lineage tracing 'barcodes' are both stably inherited in the genome and expressed, providing a simultaneous readout of genetic relatedness and phenotype (in the form of transcript counts). As non-genetic mechanisms will generate phenotypic changes that don't correlate with genetic relatedness, the difference between relatedness as measured by barcodes and gene expression has been used to assign cells 'plasticity scores' [28] (though this score does not explicitly include microenvironmental stimulation).

A challenge of using barcode-derived lineage distributions is confidently rejecting genetic modes of evolution. Indeed, stringent lineage bottlenecks can accompany a lack of any clear genetic drivers, as was the case in a barcoded mouse model of acute myeloid leukaemia [20]. Furthermore, the statistical signatures left in lineage distributions are produced by transitions rapidly breaking the correlation between relatedness and phenotype. As such, lineage tracing data alone are biased towards finding rapid transitions: fast switching between states causes the

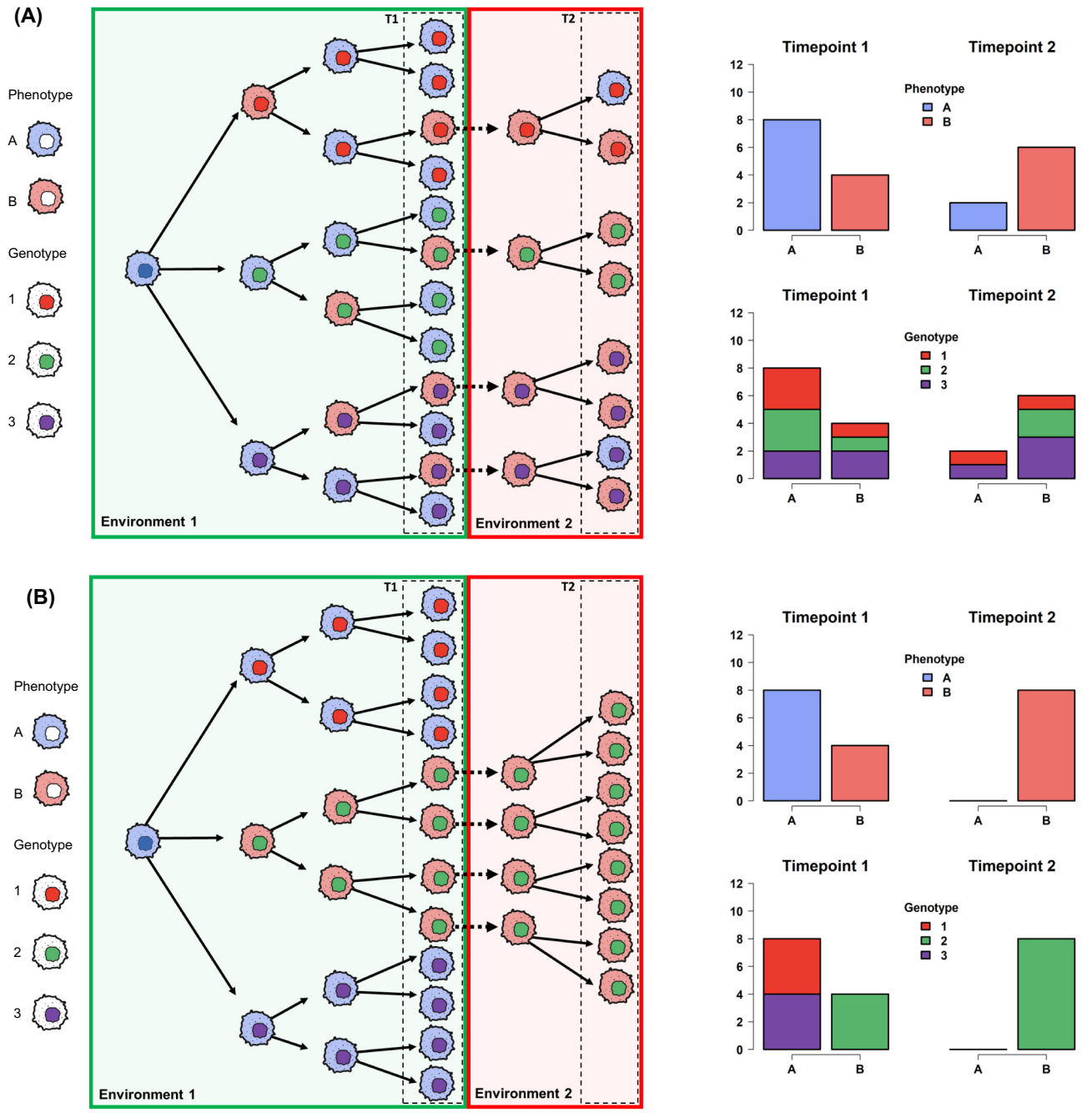

Figure 2. Differences in the timescale of non-genetic changes in phenotype. (A) Short-term changes in phenotype can result from transient non-genetic phenomena such as stochastic changes in gene expression. These changes persist for few cell divisions and occur independently of genetic lineage identity. (B) Long-term changes in phenotype, such as those controlled by stable epigenetic modifications, can persist for many cell divisions. As such, they can reside within a single lineage and can resemble positive selection for a genetic change following positive selection in a new environment. Because the differences illustrated here arise before a population experiences selection in a new environment, they are restricted to our definition of phenotypic noise.

relationship between phenotype and lineage identity to break down through cell divisions. By contrast, the patterns left by more stable, heritable epigenetic traits could easily resemble those

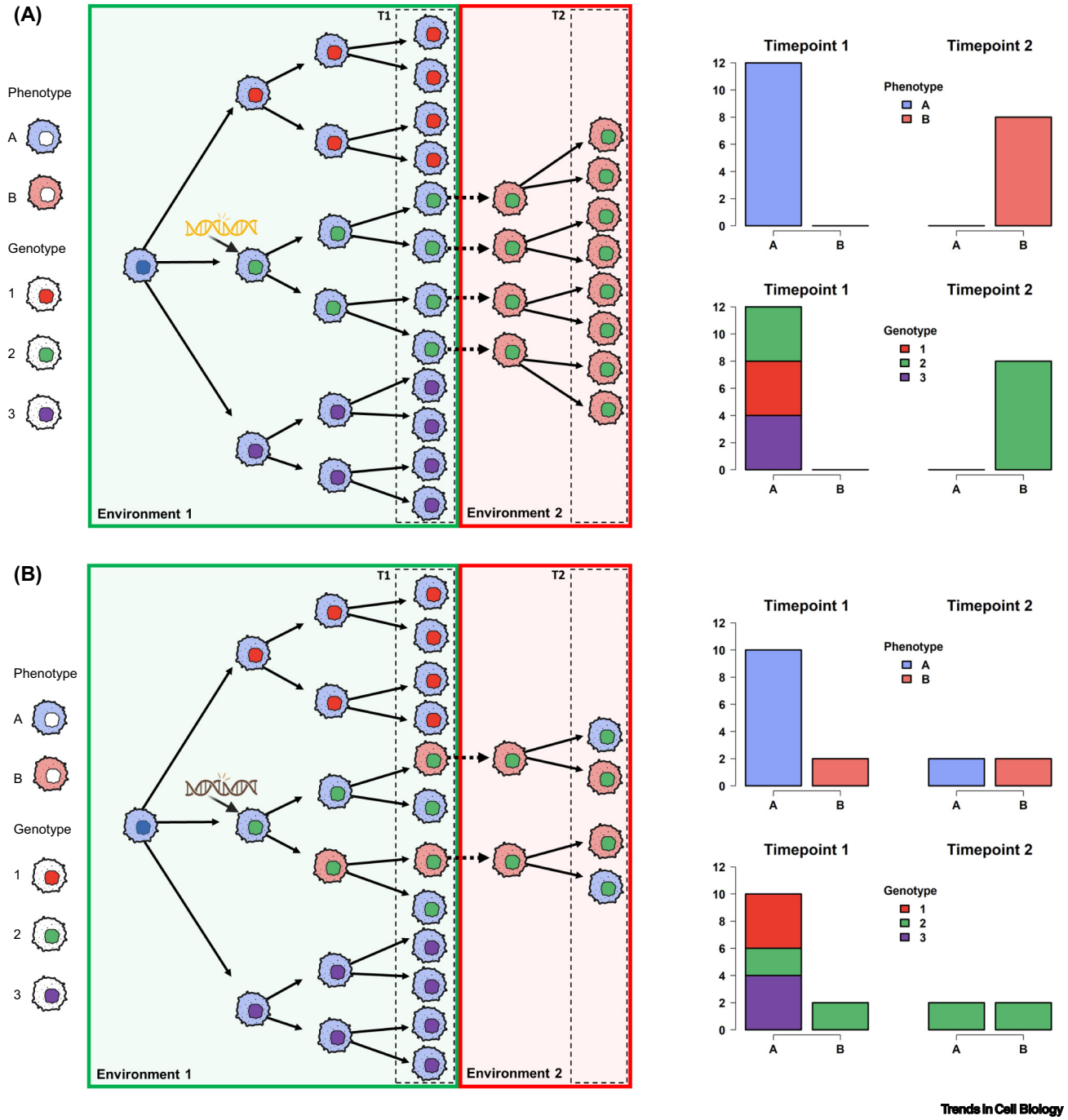

Figure 3. Genetic mutations increasing the propensity for phenotypic plasticity and phenotypic noise: (A) Whilst the change in phenotype following a shift in environmental pressures (our definition of plasticity) is not directly controlled by a genetic change, a mutation can arise (yellow mutation) that increases a lineage's propensity for phenotypic plasticity. (B) Similarly, whilst ongoing phenotypic diversity is generated despite no new genetic differences and independently from any extrinsic selection pressures, an ancestral mutation (brown mutation) can increase the propensity for cells to exhibit phenotypic noise.

of genetic mutations, and further genetic mutations can 'hitchhike' on adaptive epigenetic changes. If a phenotype under epigenetic control experiences positive selection, this lineage would increase in frequency, carrying the passenger mutations to higher frequency too (Figure 2). By developing

statistical models that generate expected lineage distributions under various evolutionary scenarios, we expect future work will be able to further leverage the quantitative information provided by lineage tracing experiments.

An alternative experimental approach is to consider the distribution of phenotypes within and between single-cell-derived clonal populations. The famous Luria–Delbruck fluctuation experiments used this approach to demonstrate that phage-resistance mutations are pre-existing [29]. Importantly, the change in environment (e.g., application of drug treatments) is precisely controlled, meaning phenotypic noise can be distinguished from plasticity. In cancer, specifically melanoma, such an approach identified a subset of resistance-associated genes transiently expressed prior to treatment [30], consistent with phenotypic noise. Additional work has shown that these transient changes can be coordinated across multiple genes, lead to phenotypic changes with consequences for survival, and are more likely to occur in a rare population of cells [31]. Conversely, changes observed in a constant environment can exclude the presence of plasticity: expansion of single-cell-derived colorectal clones showed that slow, heritable shifts in gene expression values were a function of stochastic changes in DNA methylation, providing a mechanistic explanation for phenotypic noise [21].

Experimental settings also provide the opportunity to perturb specific molecular pathways in isogenic cells, either therapeutically or via genetic modification, and to test 'bottom-up' how phenotypes are controlled. For example, deletion of DNA methylation enzymes demonstrated epigenetic regulation of hematopoietic phenotypic compartments [32], and therapeutic inhibition of chromatin modifiers (histone methyltransferases) sensitises treatment-refractory cells, supporting an epigenetic role in resistance [24,25]. That is not to say that epigenetic dysregulation in cancer cells necessarily leads to the generation of adaptive phenotypic variation. Surprisingly, a recent study showed that perturbation of epigenetic landscapes can elevate cancer cell fitness by preventing a response to environmental stimuli [33]: a phenomenon termed 'phenotypic inertia' that resembles the inverse of phenotypic plasticity.

Patient data

Limited sampling of tumours hinders the identification of phenotypic plasticity and noise in patient data; most projects analyse a single biopsy from a single time point [12,13]. Multi-region sequencing has become more commonplace due to increased interest in intratumour heterogeneity. Genetic data allow for the inference of tumour evolution via phylogenetics [34–36], and the addition of other omic approaches gives insight into non-genetic evolution.

In primary colorectal tumours, our own multiregion multi-omic sequencing revealed that most expressed genes did not show evidence of intratumour genetic control [37]. Here the presence of widespread phenotypic plasticity was implied, although the lack of direct microenvironmental measurements means that phenotypic noise could have also contributed. Further analysis revealed major changes in chromatin accessibility through progression, often independent of any genetic changes, with evidence of intratumour epigenome heterogeneity [38]. Recent studies have used single cell multi-omic sequencing to study epigenetic heterogeneity in gliomas, finding positive associations between genetic and epigenetic heterogeneity which increased through disease progression [39], and determining epigenetic **cell states** to be heritable with differences in these dynamics between glioblastoma genetic subtypes [40]. However, the inability to accurately measure microenvironmental changes in these studies makes it difficult to distinguish between phenotypic plasticity and noise.

A promising new technology to explore plasticity *in vivo* involves mapping genetic clones onto phenotypically characterised spatial data [41]. Whole-genome sequencing is first performed to determine the mutational and subclonal structure of the sample, followed by base-specific *in situ* sequencing and single-cell transcriptomics. This combines spatial context with genetic ancestry whilst integrating histological, transcriptomic, and microenvironmental features. A limitation lies in the assignment of cells/pixels to a few distinct subclones based on a limited number of mutated loci, which surely underestimates genetic heterogeneity and could lead to an overestimation of phenotypic plasticity/noise. More broadly, multiple emerging spatial transcriptomic technologies – including 10X Genomics Visium [42] and Nanostring GeoMx [43] – promise to provide unprecedented characterisation of spatially-resolved transcriptomic tumour heterogeneity (recently reviewed in [44]). Notably, the ability to infer copy-number alterations from gene expression [45,46] allows the definition of genetic subclones that can then be compared with spatially-resolved expression phenotypes. A potential confounder for distinguishing phenotypic plasticity and noise is that the expression from each 'spot' will be a combination of tumour-cell-intrinsic expression and microenvironmental infiltration, an issue that will remain until spatial transcriptomics can achieve single-cell resolution.

Profiling non-genetic heterogeneity is also possible without spatially resolved or highly sampled patient data. One such study derived single-cell transcriptomes through disease progression in a mouse model of lung cancer, and elucidated a gene expression signature of high tumour-cell plasticity (here defined by rapid switching of cell states) which was then applied to single-cell and bulk-tumour patient data [47]. In bulk transcriptome data from patient samples, this score was associated with drug resistance and patient prognosis. More recently, this cell plasticity score contributed to a model for predicting metastatic seeding in lung cancer [48].

The history of genotype–phenotype interactions reveals phenotypic plasticity, noise, and genetic control. **Phylogenetic signal** is often used in evolutionary biology to quantify phenotype heritability by computing the correlation between genetic distance and phenotype (dis)similarity [49]. Recently, a framework was implemented for measuring phylogenetic autocorrelation in single-cell RNA sequencing (scRNA-seq) data alongside a barcode-enabled phylogeny [50]. A Markov model of cell state transitions then inferred transition rates, and was applied to a murine model of pancreatic cancer to demonstrate differential transition rates between epithelial and mesenchymal cell states. Phylogenetic signal is therefore a useful tool for investigating plasticity which could be applied to patient or experimental data, but the onus is on users to appropriately define the quantitative phenotype assessed and evaluate whether any change not related to genetic ancestry is oncogenically relevant and/or is in response to external stimuli.

Mathematical modelling

Mathematical models provide a quantitative framework with which we can test mechanisms and processes that are important contributors to malignant survival and growth [51]. In a genetic setting, the cancer evolution field has borrowed heavily from traditional population genetic models to describe the behaviour of mutations in tumour populations [1,52]. These models infer evolutionary dynamics by leveraging the footprints left in the genome by clonal selection, foremost the over-representation of positively selected alterations. Features of genetic evolution – such as the stable inheritance of DNA through generations, low mutation rates, and the discrete nature of nucleotide sequences – made these models tractable. However, many of these assumptions lose relevance for non-genetic evolution. A new class of models addressing this roughly fall into two categories: population models (those modelling the behaviour of cells given non-genetic changes in phenotypes) and mechanistic models (those that model the non-genetic phenomena that cause changes in phenotypes).

Population models

Mathematical models have been used extensively to describe the behaviour of phenotypes in cancer-cell populations. Markov chains are a technique well suited to capturing the stochastic, reversible transitions between discrete phenotypic states [53] and model steady states by predicting the (average) phenotypic composition of a population over time [54]. However, without information concerning the lineage relationships of the population, care should be taken when using time-series phenotypic compositions alone to infer phenotype transition rates; other evolutionary phenomena such as growth rates that are either phenotype- and/or frequency-dependent can lead to quasi-steady state distributions without having to invoke any movement between phenotypic compartments [55].

If the ancestral relationships of cells are known, Markov chains can be imposed onto phylogenies to generate statistical predictions of lineage–phenotype relationships given different phenotypic transition rates (we note the underlying mathematics is similar to that used in phylogenetic signal methods). Studies have leveraged live-cell imaging and RNA fluorescence in situ hybridisation (RNA-FISH) [56] or expressed barcode systems that offer a joint readout of single-cell relatedness and scRNA-seq [50] to map phenotypes onto cell phylogenies and infer phenotypic switching rates. Whilst these modelling techniques offer a sophisticated approach for transition rate inference, they still assume that the discrete phenotypes can be described using end-point readouts such as scRNA-seq (Box 1).

In treatment settings, the strong selective pressure exerted on cells by treatment obviates many of the difficulties around defining phenotypes based on subtle differences, since the key phenotype is whether a cell is 'sensitive' or 'resistant' to treatment. Furthermore, treatment is one of the few environmental changes under experimental or clinical control, offering additional information

Box 1. Defining phenotypes

To identify when non-genetic mechanisms of phenotypic control are driving tumour evolution, we need to consider when to define differences between cells as distinct phenotypes. Historically, phenotypes were features that could be either observed directly or inferred from the behaviour of cancer cells, such as the rate of repopulation in a scratch assay, or the tumour growth rate in a mouse model. As such, phenotypes tended to be broad in nature. Today, single-cell sequencing technologies such as scRNA-seq and scATAC-seq have enabled high-resolution characterisation of the molecular differences between cells. However, the granularity offered by single-cell techniques means we must determine when molecular features separating cells become functionally important and warrant distinction as unique phenotypes. Additionally, the subtle changes revealed by these technologies can provide a quantitative readout of previously qualitative phenotypes. Although sometimes statistically convenient, assigning continuous features to discrete phenotypes can impact estimates of the transition rates between phenotypes, with ramifications for understanding phenotypic plasticity and noise.

Whilst static snapshots of single-cell data can identify groups of genes co-expressed in cells – commonly referred to as 'modules' or 'programs' [22,24] – defining phenotypes of interest using highly dimensional molecular data can prove challenging: the capacity of single timepoint measurements to identify meaningful biological relationships between expressed genes is limited. Newer approaches include using time-series data and functional perturbations to develop gene–gene relationships into gene regulatory networks (GRNs) that enable predictions of future behaviours given changes to external selection pressures [66,69–71]. Properties of these networks can give rise to stable cell features despite the noisy processes underpinning gene expression [63,64,72,73].

Cancer cell phenotypes are often defined with reference to normal cells, where those with recognisable features that are observed repeatedly are described as 'cell states'. These states are usually discussed within the context of the epigenetically mediated differentiation of cells during normal tissue development [21,40]. Open questions include: to what extent are new states made possible during malignant transformation, and how should this impact the way we define cancer cell phenotypes?

We propose that tumour phenotypes should be assigned with consideration of behaviours deemed important to tumour evolution: for example, with reference to the hallmarks of cancer [11]. A robust definition is paramount for describing non-genetic evolution, where accurate identification of phenomena such as phenotypic plasticity and noise hinges on the sound description of unique phenotypes.

for distinguishing phenotypic changes that occur either independently of (phenotypic noise) or in response to (phenotypic plasticity) some external selection pressure.

In bacteria, quantitative models have shown that non-genetic sources of phenotypic differences can provide rapid sources of adaptive diversity when suddenly faced with strong selection pressures such as drug treatment [57]. Similar models in cancer have tackled when the rapid phenotypic transitions consistent with non-genetic mechanisms might promote resistance. A stochastic branching process assessed how the fraction of cells that were either transiently or permanently resistant differed during treatment given transition rates that could vary in the presence of therapy [58].

As non-genetic transitions between phenotypes are relatively high, the switching dynamics modelled stochastically in Markov chains can be replaced by instead employing differential equations. Deterministic ordinary differential equations (ODEs) have shown that phenotypic switching enhances an adaptive therapy regime by re-sensitizing the population between treatment windows [59], and stochastic differential equations (SDEs) have been used to assess the efficiency of growth-rate-dependent treatments in a population where discrete phenotypes were stratified by growth rates and cells could transition between adjacent compartments [60]. Alternatively, phenotypes can be modelled as continuous traits: SDEs have been used to model the non-genetic evolution of cells through a two-dimensional phenotypic space representing survival and proliferative axes [61], where stress-induced transitions to a tolerant state in the presence of treatment were also permitted. In another study, the expression of a hypothetical gene was modelled as a continuous variable, where gene expression noise increased the variance of a cell's gene product and expression above a threshold conferred survival during treatment [62].

Mechanistic models

An alternative domain of modelling work has asked: how do molecular, non-genetic features give rise to the generation of phenotypically distinct populations?

Non-genetic mechanisms must constantly mediate the protein levels within a cell via a complex web of feedback interactions. One promising approach to investigate the rules governing these complicated gene regulatory networks is to frame them as dynamical systems. Describing the underlying structure of these gene–gene relationships can provide a window into how stochastic molecular processes such as transcription can produce recognisable cell behaviours, and how these phenomena are perturbed during cancer evolution. The topology of these gene networks – that is, the presence and magnitude of feedback relationships between genes – has been shown to engender stable phenotypic states [63], whilst models of gene expression noise show it can lead to network modifications that generate multi-modality in protein concentrations [64]. These models can use existing knowledge of gene–gene relationships to test the propensity for cells to transition between cell states, states which can influence a cell's ability to metastasise or resist treatment [65]. As these models can test how perturbations to the networks via intrinsic and extrinsic forces can shift the phenotypic composition of a cancer-cell population, they are well placed to directly probe how likely cells are to exhibit phenotypic noise and plasticity given certain evolutionary scenarios. Recently, researchers leveraged high-dimensional -omics data to simultaneously define phenotypic states, their key gene components and the structure of the underlying regulatory network [66]. The growing sophistication of these quantitative models will enable a greater understanding of how non-genetic phenomena constrain cells to stable phenotypic states in healthy somatic cells, and how the breakdown of these systems promotes growth and survival in cancer cells.

Finally, whilst not discussed in detail here, mathematical models also provide an opportunity to distinguish plasticity from noise by enabling descriptions of the interplay between cell-intrinsic and -extrinsic forces: for example, models that permit phenotypic shifts have investigated how environmental factors such as acidity and oxygen can drive metabolic evolution [67,68].

In conclusion, mathematical models allow us to formalise the behaviour of non-genetic phenomena such as phenotypic plasticity and noise. The relative paucity of existing models that tackle the evolutionary dynamics specific to these non-genetic mechanisms offer an exciting opportunity for further model development and new biological insight into the control of phenotypic plasticity and noise. We believe that there is a growing demand for models that explicitly combine population-level evolutionary dynamics with those that capture the molecular mechanisms and shifting environmental forces driving non-genetic changes in phenotype.

Concluding remarks

Enabled by a rapidly developing single-cell omics field, there is growing appreciation that non-genetic phenomena are important drivers of cancer evolution. In discussions concerning these phenomena, the term 'phenotypic plasticity' is now commonly used to mean any phenotypic change which cannot be ascribed to a difference in genotype. Here, we argue that this sweeping usage masks important biology: specifically, whether or not the change in phenotype was caused by extrinsic selection pressures. As such, we suggest the term 'phenotypic noise' to denote phenotype differences that arise independently of any environmental changes, and 'phenotypic plasticity' for situations where the environment induces phenotype change. Both processes make critical, yet mechanistically distinct, contributions to cancer evolution. The distinction will aid future research that tackles the many unanswered questions surrounding non-genetic evolution in cancer (see Outstanding questions). The high-dimensional nature of modern omics data introduces new challenges to the question of defining phenotypes, as identifying salient non-genetic phenomena requires first identifying the discrete phenotypes they control. Understanding the causes of phenotype transitions in cancer will identify new opportunities to block adaption to treatment and improve the efficacy of anticancer therapies.

Outstanding questions

How frequently and to what degree do plasticity and noise act as sources of adaptive phenotypic diversity during cancer evolution?

To what extent is non-genetic evolution in cancer characterised by the noisy dysregulation of healthy cell states versus the directed acquisition of new phenotypic states?

How confidently can we define phenotypes from single time-point molecular data? And, by extension, how well can we identify non-genetic drivers of evolution from these data?

How can we better adopt molecular and statistical tools to distinguish cases where non-genetic changes in cancer-cell phenotypes occur in response to (plasticity) or independently from (noise) the environment?

How should we learn the mechanisms responsible for non-genetic differences in phenotype? For example, can we leverage cases of phenotypic plasticity and noise identified with experimental and theoretical models to uncover the molecular changes responsible?

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Author contributions

All authors jointly wrote the article.

Declaration of interests

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