nature reviews cancer

https://doi.org/10.1038/s41568-024-00734-2

Perspective

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The evolutionary theory of cancer: challenges and potential solutions

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Abstract

The clonal evolution model of cancer was developed in the 1950s–1970s and became central to cancer biology in the twenty-first century, largely through studies of cancer genetics. Although it has proven its worth, its structure has been challenged by observations of phenotypic plasticity, non-genetic forms of inheritance, non-genetic determinants of clone fitness and non-tree-like transmission of genes. There is even confusion about the definition of a clone, which we aim to resolve. The performance and value of the clonal evolution model depends on the empirical extent to which evolutionary processes are involved in cancer, and on its theoretical ability to account for those evolutionary processes. Here, we identify limits in the theoretical performance of the clonal evolution model and provide solutions to overcome those limits. Although we do not claim that clonal evolution can explain everything about cancer, we show how many of the complexities that have been identified in the dynamics of cancer can be integrated into the model to improve our current understanding of cancer.

Sections

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Introduction

Cancers are populations of cells that are heterogeneous across space and through time. This diversity is currently a major clinical issue, limiting the efficiency of most cancer treatments as there is often a subset of cells that is resistant to whatever treatment is being used[1,2]. The diversity also limits the accuracy of prognosis and our ability to predict how it will respond to an intervention because a biopsy may not be representative of the entire neoplasm and there is a strong stochastic component to how it changes through time. A better understanding of the mechanisms involved in this diversification are, thus, urgently needed to better manage cancer. An explanatory mechanism of cell diversification that has gained traction in the twenty-first century is clonal evolution: cancer cells diversify through the accumulation of genetic and epigenetic alterations, which can change the relative fitness of cells and consequently lead to clonal expansion or contraction by natural selection. The principles of evolution and the tools of population genetics can be and have been successfully applied to cancer cells[3,4]. However, the evolutionary view of cancer progression has been challenged[5], and it was shown that phenotypic heterogeneity within cancers can be largely independent of the genetics of clones[6]. Given that evolution by natural selection acts on the phenotype and relies on its heritability, these data question the relevance of the clonal evolution model. It is high time that we evaluate the strengths, weaknesses and opportunities for improving the clonal evolution model.

To achieve this, we first explore what is known about the evolution of cancer cells through a historical and contemporary review of the literature. We then analyse the theoretical structure of the clonal evolution model by highlighting its underlying assumptions. By doing so, we intend to locate the theoretical and conceptual tensions and difficulties inherent to the current model. We conclude with suggestions and perspectives that can help address some of the issues and enhance the explanatory relevance of clonal evolution in cancer.

What is the clonal evolution model?

Historical perspective

The clonal evolution model is an abstract model depicting the evolution of cancer cells in a patient. It is often attributed to Peter Nowell, but Nowell himself always made it clear that he was summarizing ideas that were developed by the community[7]. The observation of intratumoural heterogeneity, and the idea that cancer cells evolve was already present before 1976, and its success occurred later.

In the 1950s, the notion of evolution was often used in oncology to describe cancer progression (see, for example, refs. 8,9), although not always suggesting a Darwinian process[10]. During this time, studies on the cancer cell karyotype have supported the hypothesis first introduced by Theodor Boveri that tumours could emerge from chromosomal defects caused by abnormal mitoses, which predicted the clonal origin of cancer[11]. Karyotyping also led to the observation of a variation in the number of chromosomes contained by cancer cells and, as a single-cell whole-genome assay, had a crucial role in revealing the evolution of cancer cells. Karyotypic observations directly raised the question of whether tumours are composed of multiple strains of cells, each having a fixed number of chromosomes, or whether the karyotype of tumour cells could change (see, for example, refs. 12,13) and if so, whether such changes were stochastic or heritable and selected (see, for example, refs. 14,15). T. S. Hauschka directly refers to "mutation-selection sequence analogous to phylogeny" and argues that "specific tumour karyotypes have competitive survival value"[15]. Many techniques were used to resolve the question of the clonal origin of cancer, including the discovery of the Philadelphia chromosome[16], random somatic inactivation of one of the two alleles of glucose-6-phosphate dehydrogenase enzyme in women[17,18], or immunoglobulin heavy chain rearrangements[19,20].

All of those experiments converged towards the hypothesis of a clonal origin of cancer, with intratumoural heterogeneity originating from subsequent variations that may undergo selection. In most of these papers, evolutionary notions are kept in the background[10], but some directly discussed evolutionary principles applied to cancer. The English cytogeneticist C. E. Ford dedicated a paper to selective pressure in healthy, irradiated, or cancerous somatic cells defending the hypothesis that "unbalanced karyotypic changes have an effect on the probability of survival and proliferation" and, thus, that "the karyotypic structure of a cell population would then be the resultant of the operation of selective forces on the variability arising within it"[21]. J. Lejeune argued that the hypothesis of selection was necessary to the coherence of the clonal evolution model and explicitly mentioned the use of karyotypes to reconstruct the "natural history of the clone", comparing such reconstruction to "the approach of paleontologists reconstructing, from form to form, the history of the phylum"[22] (p. 76, translated from French).

After Nowell's elegant summary of the evolutionary model of cancer, we got... silence. The evolutionary biology of cancer lay dormant for decades with only a few scientists[23–28] building on the framework of Nowell. It was not until the twenty-first century that the field gained momentum. It is probably not a coincidence that interest in cancer evolution accelerated when high-throughput sequencing started generating extensive amounts of genetic data from cancers. Observations of the clinical importance of intratumoural heterogeneity[29–34] and clonal expansions[35–38] soon followed, along with the confirmation that therapy often selects for pre-existing clones with mutations that render them resistant to therapy[1,39–44]. Although clinical impact remains limited, further studies have demonstrated that an evolutionary approach to cancer therapy can lead to dramatic improvements in time to progression and overall survival[45–51]. Amidst all this, reviews of how evolutionary biology and ecology could be productively applied to cancer[29,52–55] may have helped to lay a foundation for future progress.

How do cancer cells evolve?

Any population of entities with a diversity of heritable properties that can result in differential fitness between entities can evolve by natural selection[56]. Somatic cells are such entities and can be subject to evolution by natural selection because mutations cause heritable diversity among cells and (at least some) can alter cell fitness. The open question is whether and to what extent they evolve by natural selection, a question that has been debated by scientists and philosophers[57–60].

It is important to recognize that cancer cells can evolve through a plurality of mechanisms. Although they can evolve by natural selection, some tumours show little evidence of natural selection and appear to be mainly evolving by neutral evolution[61–64]. A mathematical neutral evolution model is consistent with genetic data in approximately one-third of solid tumours[4], of multiple myeloma[65] and of chronic myelomonocytic leukaemia[66]. Another pan-analysis has also highlighted that negative selection, which is predominant in the germline, is nearly absent in cancer and somatic evolution[64]. Although clonal evolution was initially considered as a continuous gradual process, it has been shown that bursts of changes can also happen[67–70]. Clonal evolution can occur through stasis, gradualism or punctuation, with different molecular clocks ticking at different speeds[71,72], and can proceed by

linear evolution or branched evolution[31,73–77]. There is a diversity of processes that underlie the clonal evolution of cancer cells.

In addition, although the clonal evolution model traditionally focuses on the evolution of cancer (stem) cells as the unit of selection[78,79], selection may occur at higher levels (Fig. 1a), on groups of cells rather than individual cells. For example, there is a debate on whether there is evolution by natural selection between metastases, each metastasis counting as a reproductive entity, generating further secondary metastases[59,60,80]. Higher-level selection could also select among colonies of cancer stem cells and their non-stem cell progeny[81] or among epithelial proliferative units such as colonic crypts, which may divide and die[52,82]. Selection in cancer may also occur at levels below the cell, through the evolution of transposable elements[83], or extrachromosomal DNA[84] and perhaps micronuclei[85], if they can replicate independently of cell replication (Fig. 1b). The fact that selection may act at additional levels, above and below the cell, does not violate the clonal evolution model. It adds complexity to it, in the same way that multilevel selection[86,87], above and below the level of the organism, does not violate the theory of evolution but rather adds complexity to it.

What is the value of the clonal evolution model?

The clonal evolution model integrates current knowledge about cancer cell evolution. It is a highly theoretical framework. It aims not just at describing but also at reconstructing past evolution, predicting potential future evolution (for example, resistance to treatment), explaining phenomena occurring in patients, and providing the foundation for novel therapeutic interventions. We refer to these properties as the theoretical value of the clonal evolution model.

A primary theoretical value of the model is to explain how and why cancers change over time and in response to therapy[53,88]. Phylogenetic reconstruction provides a description of the natural history of a cancer. For example, clonal mutations (also known as 'truncal' mutations) tend to be different from subclonal mutations (also known as mutations that only appear on a 'branch'). Phylogenetic reconstructions can highlight changes in exposures and mutational processes over time, exemplified by the loss of the aflatoxin B1-related mutational signature in African migrants with hepatocellular carcinoma in the years after they arrived in France[89]. It also showed that contrary to expectation, metastases can occur early in carcinogenesis[75,90–92]. Lessons learned from these phylogenetic reconstructions also facilitate drug targeting (for example, targeting a clonal mutation rather than a subclonal one) and help predict the risk of therapeutic resistance through selection of resistant mutations, such as the T790M mutation of epidermal growth factor receptor (EGFR) that causes resistance to first-generation EGFR inhibitors in lung cancer[93]. Many mutations have been associated with resistance to treatment in ways that now allow clinicians to better decide which treatment to apply. The clonal evolution model has also been used in risk stratification and prognosis, using measures of the clonal evolution, such as intratumoural heterogeneity, to predict which precancers tend to progress to invasive disease and which cancers tend to be lethal[1,94–96].

The clonal evolution model predicts that different environments will change the ability of mutated clones to expand. In the haematopoietic system, for example, mutated clones emerge with ageing, a process referred to as clonal haematopoiesis. Different environmental changes may lead to clonal haematopoiesis, such as ageing, chemotherapy, infections and smoking. However, each tend to select different types of mutations[97]. Clonal haematopoiesis also comes with various mutations and various dynamics through age[98,99]. This also helps to explain the late occurrence of cancer, as the decrease of healthy cell fitness with ageing provides weaker competitors for the selection of mutated clones[100–103]. Note that the promotion theory is sometime thought as in opposition to the somatic mutation theory, but these two theories are compatible in the context of clonal evolution, as fitness depends on both intrinsic cell properties and extrinsic properties[104,105].

More fundamentally, the clonal evolution model generated a profound conceptual switch that views cancer as a dynamic process. As such, it contributed to discrediting the therapeutic strategy of searching for a magic bullet and required changes in research and treatment practices. The model predicts that most advanced cancers tend to be able to escape any treatment. This has led to various propositions for how to develop therapies based on evolutionary principles, such as

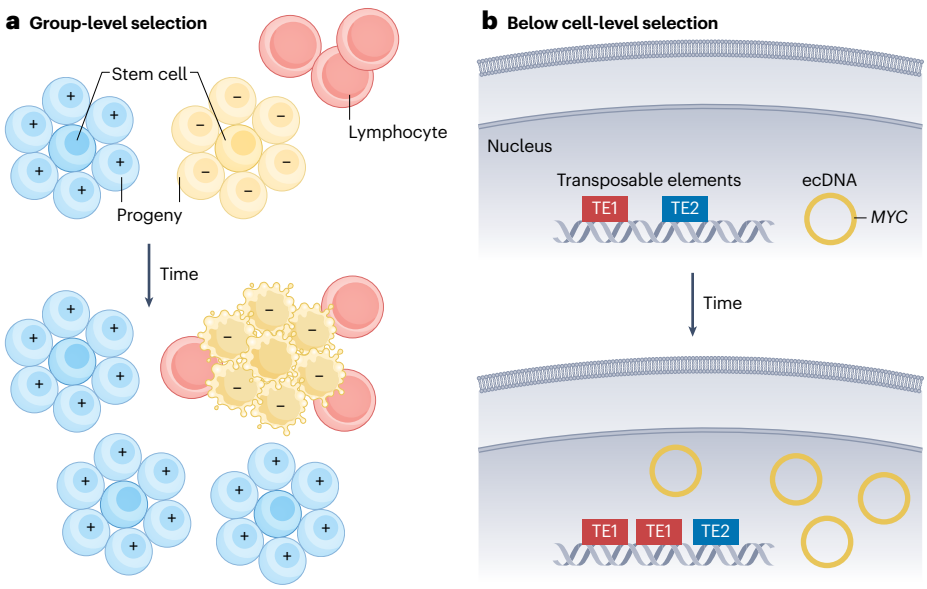

Fig. 1 | Cancer evolution by natural selection can operate at multiple levels of biological organization at once. a, There may be natural selection on groups of cells, here shown as stem cells and their non-stem cell progeny. In this example, the blue non-stem cells have a property (marked by '+') that protects their stem cells from destruction by lymphocytes and allows the colonies of blue cells to replicate. Yellow progeny lack (−) these properties, leading to the destruction of the yellow population by the lymphocytes. **b**, Below the level of the cell, extrachromosomal DNA (ecDNA) may increase the copy number of cancer-related genes (for example, *MYC*) independent of the chromosomes. Similarly, transposable elements (TE1 and TE2) in the genome may replicate within the genome.

drug holidays, changing drugs through time and adaptive therapies that manage the clonal dynamics in order to prevent therapeutically resistant clones from expanding out of control[46–49].

Years of observations and experiments testing aspects of the clonal evolution model have supported its theoretical value. However, it does not explain everything about the phenotype of cancer. Putting cancer cells in a different microenvironment gets them to behave differently. An extreme illustration of this are the examples wherein placing cancer cells in a healthy environment can 'normalize' their behaviour[106–111]. Phenotypic intratumoural heterogeneity may largely be independent of the genotype[6], and phenotypic plasticity is increasingly recognized as an important contributor to intratumoural heterogeneity and treatment escape[112–115].

Model assumptions and limitations

The performance of the clonal evolution model depends on two different aspects, one factual, the extent to which evolutionary processes contribute to cancer, and one theoretical, the extent to which the current clonal evolution model captures these processes. The model's premise is that evolutionary processes do have a role in cancer. This is supported by decades of evidence. However, the importance of the model is an open question. The more evolutionary processes influence cancer, the more relevant the clonal evolution model becomes. The theoretical performance of the clonal evolution model is a different issue. The current model may only partially capture the actual involvement of evolutionary processes in cancer. It is important to separate the challenges related to the factual involvement of evolutionary processes from those related to the model's theoretical performance. The latter can be addressed by extending the current model.

We see two important but questionable underlying assumptions in the current clonal evolution model: (1) the phenotype of cancer cells, in particular their fitness, is assumed to largely rely on their genotype (studies of clonal evolution have been historically dominated by genetic approaches); (2) the ancestral relationships between cancer cells are assumed to form a branching tree of cells, neither of which is actually necessary for a system to evolve. We will analyse both assumptions in detail, searching for limits and critiques to the model and distinguishing those that are real limitations to the extent to which evolutionary theories can explain cancer (factual extent), and those that are invitations to improve the current clonal evolution model (theoretical limitations).

Assumption 1: fitness relies largely on genotype

The clonal evolution model draws from the modern evolutionary synthesis, which is a quantitative genetic theory of Darwinian evolution defining evolution as changes in the frequencies of genetic variants in a population through space and time. As a mathematical and theoretical framework, it requires some simplifying assumptions, in particular the organism is reduced to its genetics. In oncology, specific genetic alterations have been identified and characterized for their key functional roles in the development and evolution of the disease, which led to the success of the notion of oncogenes and tumour suppressor genes in the 1980s (refs. 116,117). However, it is well known from developmental biology that the same genotype can produce widely different phenotypes in normal cells[116,117] and this also applies to cancer cells[118]. The assumption that the fitness of cancer cells largely relies on their genetics faces a number of challenges.

Identifying key clonal evolution genes is challenging. Large consortiums have invested a lot of effort in sequencing tumours to identify cancer genes and several confounding factors have been identified, such as the size of the genes and their location in fragile sites[64,119–121]. The statistical significance of the presence of some mutations needs to be evaluated according to some background factors such as the mutation rate of each nucleotide, structural variations and purity of the sample. Given that mutations in typical cancer driver genes are present in somatic healthy tissues, comparing the frequency of mutations in cancer to their frequency in non-cancerous tissues also appears crucial. For example, *NOTCH1* mutations are common in oesophageal cancer and skin cancer, leading to the assumption that it is a driver gene in those cancers. However, because *NOTCH1* mutations are more common in normal skin and oesophageal epithelium than in the cancers of those tissues, NOTCH1 mutations may actually have a protective role against both skin and oesophageal cancer[122,123]. Thus, identifying driver mutations is not trivial.

There is also a conceptual problem in identifying cancer genes[124]. The same mutation can be neutral or even deleterious (deleterious mutation) in one context and become a driver in another, and vice versa. The concept of the cell of origin illustrates this phenomenon: the same mutation may have varying impact depending on which cell it occurs in[125]. Similarly, therapies change the environment of the cells which can select for clones with specific resistant mutations that expand during treatment and regress at treatment withdrawal[126]. Treatments induce drastic and rapid selective pressures, but slower changes are also possible, with the same consequences. Ageing, for example, changes the fitness landscape of the cells so that a mutation could be neutral or even deleterious in a young person but be advantageous (advantageous mutation) in the context of ageing[101–103,127]. Fitness is by definition always relative to a particular environment. The environment of cancer cells changes, throughout the natural development of the disease but also owing to ageing, interventions and other changes in exposures. The difficulties in identifying driver mutations complicate efforts to define clones based on driver mutations (see Box 1).

Genes are not the only inherited material. It is now well appreciated that many epigenetic properties are heritable at cell division and can contribute to clonal evolution[128–137], including the evolution of metastasis[138,139] and resistance to drugs[133,140,141]. However, epigenetics encompasses a broad range of phenomena, some of which are random and heritable, whereas others are under the control of the cell and may change in response to some form of signalling, for example, during differentiation. The heritable alterations could be integrated in clonal evolution as epimutations[142]. Studies have found a concordance between genetic and epigenetic clonal evolution, which makes sense given that there is one unifying lineage of the cancer cells[143–145]. Additionally, there can be multiple epigenetic subclones within one genetic clone, or vice versa[146,147]. This again raises the question of what information to use to delineate and count clones (Fig. 2 and Box 1). Epigenetic alterations challenge the theoretical accuracy of the clonal evolution model when the model relies only on genetic mutations, but it does not challenge its factual extent.

Heritable properties may extend beyond the genetic material and its epigenetic alteration. As cell reproduction happens through cell division, daughter cells inherit a portion of the cytoplasm of the mother cell, including organelles, RNA and proteins. They also inherit the microenvironment that the mother cell may have modified[148–150]. This may generate short-term heritability of the phenotype of cells with timescales depending on the fluctuations and turnover rate of those molecules and the microenvironment. In bacteria, the use of

Box 1 | What is a clone?

The model of clonal evolution is based on the analysis, in time and space, of cells belonging to different clones. The concept of the clone is, therefore, central but is rarely explicitly defined. There is an implicit consensus to see cancer clones as populations of cells that share a common identity inherited from a common ancestor[273]. However, the alterations people use to identify a clone differs from study to study.

Traditionally, the identity of a clone is based on the genotype, or rather parts of it. A typical definition of the clone is, for example, "a set of cells that descend from a common ancestor and thus share genetic features"[274]. For practical reasons, clonal evolution studies are often based on driver mutations. Some studies use techniques allowing broader analysis of the genome such as whole-exome sequencing, or even whole-genome sequencing, which does not rely on a priori knowledge of which mutations are involved in cancer. Finally, some studies favour the use of neutral mutations as an unbiased way to track clonal evolution[275].

There is also a more fundamental ontological and epistemic challenge on the identification of a clone. The clonal evolution model relies on reconstruction of clones from incomplete information: we have access to neither all the cancer cells nor all the heritable properties of each single cell. This can lead to 'clonal illusion', when a subclonal mutation appears to be clonal because a biopsy was only taken within the subclone[276]. It also relies on choices made by investigators as to which alterations are relevant for defining a clone and which can be ignored[124]. Changing the list of mutations that are deemed relevant and, thus, used to reconstruct clonal evolution affects that reconstruction. The number of clones identified, for example, will depend on the number of genes studied and the depth of sequencing[95]. As an extreme illustration of this issue, one may define a clone as the set of cells that are genomically identical. However, whole-genome sequencing at the single-cell level would probably reveal that each individual cancer cell is unique and, therefore, count as a new clone, making the concept of a clone useless[258]. The history of the clonal evolution model shows that these choices of what alterations should be used to define a clone have changed through time. This historical contingency highlights the arbitrariness in definitions of clones. In a pluralistic approach, one could argue that which clone delineation is the right or best one will depend on the perspectives and aims one adopts. Whether and the extent to which clone delineation is an issue largely depend on what is expected from the clonal evolution model. It is, for example, an issue for any study that quantifies intratumoural heterogeneity by counting clones. It is much less of an issue for those who may want to reconstruct phylogenetic relationships between various cancer cells (for example, between metastases at different locations).

There are some alternative views of clonal identity: several studies have now been using epigenetics in their reconstruction of cancer evolution[27,37,129,143,144,147,277]. A few have supported a phenotypic or functional clonal identity. For example, a clone was defined as "a group of cells with the same phenotype, which have expressed that phenotype consistently since their most recent common ancestor"[63]. That definition might call a set of stem cells a clone if they shared the same phenotype and had a recent common ancestor, but it would exclude all the non-stem cells that were part of the same branch on the cell lineage tree. By contrast, in developmental biology, a clone has been defined as "the in vivo descendants of a single ancestral cell", equivalent to a monophyletic clade in a phylogeny[278].

Under every cancer is a cell lineage tree describing the relationships and history of the cells within the cancer[74,171]. Defining clones is a matter of dividing up that evolutionary tree (Fig. 2).

'sister machines' that separate the two daughter cells in independent growth channels, revealed that different non-genetic traits present different 'memory' patterns under different timescales, from two to ten generations[151]. In a melanoma cell line, 227 genes that show transient heritable high expression (around 40 h, sometimes reaching 5 days) were identified[152], of which 162 were previously associated with resistance to therapy[153]. Cells sorted for high expression of two of these transiently heritable genes, specifically EGFR and nerve growth factor receptor (NGFR), showed a much higher level of resistance to the MEK inhibitor trametinib than unsorted cells.

The inheritance of properties at different timescales remains largely ignored; however, given the short duration of therapeutic interventions, these alternative forms of inheritance may be of clinical relevance. As will be discussed in the 'Inheritance and timescale' section, improving our understanding of inheritance might improve the theoretical performance of clonal evolution.

One genotype can take on many phenotypes. Normal somatic cells in multicellular organisms are phenotypically plastic. Phenotypic plasticity has long been studied in the field of development and is increasingly recognized as important in cancer as well. From a single genotype, cells can take on different phenotypes as a result of at least three non-exclusive processes: differentiation, response to other extrinsic signals, and stochastic fluctuations (of gene products and epigenetic modifications). The latter two can include dedifferentiation, transdifferentiation or other changes in cell states such as metabolic changes. Cancer cells inherit and modify the phenotypic plasticity of the normal cells from which they evolve.

Currently, phenotypic variations that are independent of the genotype are disregarded by the clonal evolution model. Thus, plasticity is taken as a challenge to the factual extent of the clonal evolution model: the more non-heritable plasticity is causally involved in cancer, the less evolutionary processes are. For example, a study of dormancy and relapse in oestrogen receptor-positive breast cancer treated with adjuvant endocrine therapies has shown that the treatment stochastically induces a dormant state, which was unstable, in a fraction of cancer cells from a random diversity of clones[133]. In such cases, evolution by natural selection does not have much role in cancer cells ability to survive treatment. However, conceptual fuzziness about phenotypic plasticity obscures the debate[154]. Some cases of plasticity might be heritable and in fact only be a theoretical challenge which may be incorporated to improve the clonal evolution model.

First, the phenotype of cancer cells often relies on non-genetic, yet heritable, properties, such as epimutations. Such epigenetic-driven phenotypic diversity can easily be integrated in the clonal evolution model by incorporating epimutations. The same should apply to any other non-genetic heritable property that contributes to the cell phenotype.

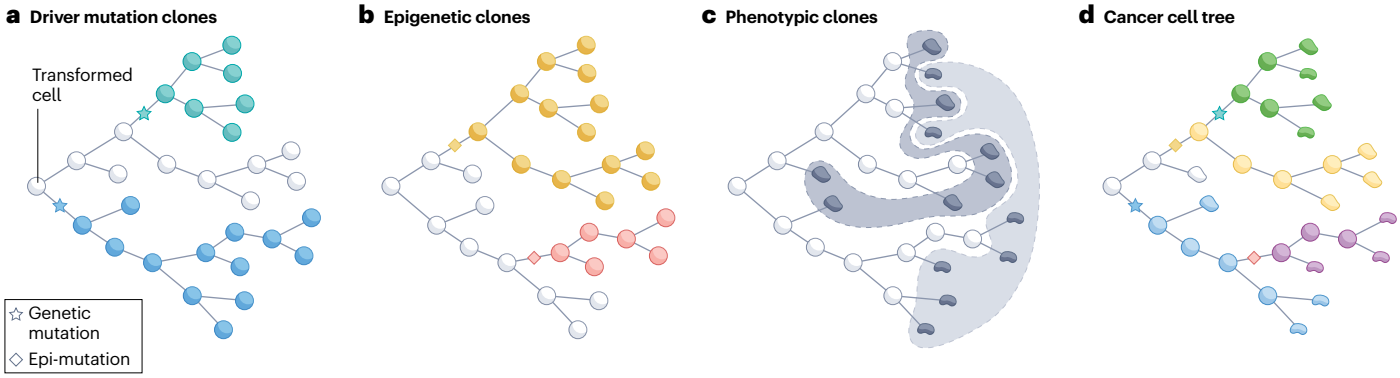

Fig. 2 | Defining clones is a matter of dividing up the cancer cell tree. a, The cancer cell tree with clones identified through driver mutations (marked by a star). Three clones are identified: the first clone (white) carries all the mutations the transformed cell had, the second clone (blue) marks the occurrence of the mutation indicated by the blue star, and the third clone (turquoise) indicates another mutation marked by the turquoise star. **b**, The cancer cell tree with clones identified through epigenetic alterations. Three clones are identified: the first clone (white) indicates the epigenetic state of the first transformed cell, the second clone (red) indicates an epigenetic state mediated by a heritable epigenetic alteration (indicated by the red rhombus), and the third clone (yellow) marks cells with a distinct epigenetic alteration (yellow rhombus). They are not the same clones as the genetic clones. **c**, Phenotypic clones. When only phenotypic characteristics are considered, there are two clones emerging marked by the grey shaded areas. **d**, The tree of cancer cells, with genetic, epigenetic and phenotypic information overlaid. Genetic, epigenetic or phenotypic evolution may operate simultaneously at different timescales. The turquoise genetic clone is mixed and contains both cell types, whereas the white and blue genetic clones contain a different cell type. The yellow epigenetic clone is mixed, whereas the red and white epigenetic clones contain the same cell type. Are there three, five or more clones? Note that these various forms of evolution can also be more or less prominent at various stages or locations.

Second, the degree of phenotypic plasticity can be heritable, encoded in the genome or epigenome (reviewed in ref. 154). Certain cell lineages may be more plastic than others, providing them an advantage in changing microenvironments. Other cells have phenotypes that are remarkably stable, such as fully differentiated cells. Some (epi)mutations may increase cell plasticity, and selection on those (epi)mutations will flow indirectly through direct selection on the phenotypes they produce. There is relevant literature on the evolution of mutation rates[155–158] and the evolution of phenotypic plasticity[159–161] that might be used to integrate phenotypic plasticity into the clonal evolution model.

Third, phenotypic plasticity includes a variety of phenomena such as differentiation (a robust, channelled, predictable process in multicellular organisms), response to extrinsic signals (also partly predictable, although conditional on microenvironmental signals), and stochastic fluctuations (unpredictable). The differences between these processes matters if one wants to address the empirical limitations of evolutionary accounts of cancers. The reason that these types of plasticity need to be conceptually distinguished can be illustrated by two hypothetical contrasting examples. First, when a treatment selects stem cells owing to their upregulation of efflux pumps, selection for efflux pumps will maintain the clonal composition. This is because all genetic clones contain cancer stem cells, although there may be changes in clone size based on the proportions of cancer stem cells in the different clones[81]. Second, when a cell cycle-specific treatment selects for slow-cycling or non-proliferating cells, and if quiescence is induced by a particular microenvironment, then clones that happen to be in that microenvironment will survive. These two cases present different challenges to the clonal evolution model. In the first case, the maintenance of all genetic clones may hide the selection of stem cells. In the second case, resistance might wrongly be attributed to the genetics of the clone when it is its location that caused the resistance. Other scenarios involving plasticity may also challenge the clonal evolution model. For example, the clonal evolution model takes the cancer stem cells to be the units of selection[79], but dedifferentiation may occur[162,163], allowing non-stem cells to contribute to the evolutionary dynamics[81,164].

Although phenotypic plasticity in response to external stimuli represents a major limitation to the factual involvement of clonal evolution, differentiation-based and stochastic-based types of plasticity represent theoretical challenges. As long as differentiation remains robust and predictable, this type of plasticity could be included in mathematical models of clonal evolution[81]. Similarly, stochastic plasticity is amenable to mathematical modelling that can be introduced in evolutionary models of cancer[165].

Many genotypes can produce the same phenotype. Such phenomena can be cases of convergent evolution, phenotypic plasticity or non-heritable factors. Selective pressures may select for the same phenotype in independent lineages, which is well accounted for by the evolutionary models of cancer. We have argued that the hallmarks of cancer are cases of convergent evolution that occur owing to natural selection acting on unrelated cells[58]. This challenge to the genetic evolutionary model of cancer led to the hypothesis that we gain more clarity and control by focusing on the evolution of phenotypes[166]. As we have argued above, differentiation processes and responses to environmental signals can result in similar phenotypes regardless of the underlying genetics. There are other non-heritable determinants of phenotype that may be independent of the genetics of clones. For example, cancer cells can transfer proteins, lipids and nucleic acids via extracellular vesicles which can contribute to the malignant phenotype[167]. In a mouse model of gliomas, it was shown that cancer cells with mutated EGFR can transfer the truncated oncogenic form EGFRvIII to cancer cells lacking the mutation, leading to the acquisition of the mutated phenotype by these cells[168]. Distinguishing between heritable and non-heritable determinants of phenotypes is key, as the second is a direct

challenge to the factual extent of the clonal evolution model, although evolutionary theory can account for non-heritable determinants of phenotype as a form of noise by including measures of the heritability or evolvability of traits in models of phenotypic evolution[169,170].

Assumption 2: a cancer cell lineage is a tree

The clonal evolution model assumes a tree shape of the cell lineages[171], wherein cell lineages divide and die but do not fuse. Clones are defined by descent from a single common ancestor cell (Box 1). Inheritance is vertical, from the parental cell. Moreover, basing the clonal evolution model on cell lineages implicitly assumes that cells are the only unit of selection. Several observations question these assumptions.

Cell fusion and cancer cell modes of reproduction. Cancer cells can fuse into viable, reproducing cells that can give rise to clonal populations of cells[172,173]. Cell fusion in cancer is considered rare and has been largely neglected. However, several studies have reported fusions between cancer cells and non-cancer cells (hybridomas being a famous case of such fusion), or between different cancer cells. In patients, fusions have been reported with haematopoietic cells in the context of bone marrow transplantations that offer markers to track cell fusions[172,174,175]. As an example, a mother who underwent bone marrow transplantation from her son later developed a renal carcinoma, with a fraction of the cancer cells containing both chromosomal alterations of the cancer cells and a Y chromosome from the cells of her son[174]. Another study has analysed tumour biopsies from seven patients with various cancers (pancreatic ductal adenocarcinoma, renal cell carcinoma, head and neck squamous cell carcinoma and lung adenocarcinoma) and found evidence of fusions between cancer cells and leukocytes in all of them[172]. Following up on this result, the researchers searched for circulating hybrid cells in patients with pancreatic cancer and found that the quantity of circulating hybrid cells correlated with advanced disease and was associated with poor prognosis, which was not the case of non-fused circulating tumour cells, raising the possibility that fusions may increase the risk of cancer progression.

The role of cell fusion in cancer cell evolution has been studied in vitro and in animal models. Hybrids of cancer cell lines and macrophages, once transplanted into mice, had a shorter doubling time than the maternal cancer cell line, suggesting that cell fusion can increase the fitness of the cells[172]. Cell fusion can also provide cancer cells with new properties. For example, fusion with haematopoietic cells such as macrophages can endow the fused cancer cells with migrating abilities[172,176–180]. Several studies discuss fusion as a possible alternative mechanism to (but not mutually exclusive with) the Darwinian clonal evolution explanation of metastasis according to which the ability to metastasize is gained through a process of evolution by natural selection[179,180]. Other studies have shown that fusion between cancer cell lines can generate new cell lines that are more malignant and therapeutically resistant than the original cell lines[172,173]. Cell fusion has also been discussed as a possible mechanism to produce cancer stem cells[181,182]. Last but not least, cell fusion is a mechanism that can generate diversity through subsequent stochastic loss of genetic material[183].

Cell fusions do not undermine the factual extent of clonal evolution but add a level of complexity regarding processes involved in their evolution that is not currently taken into account by the clonal evolution model.

Horizontal gene transfer. The clonal evolution model assumes that inheritance is vertical with cell division. However, several mechanisms of horizontal gene transfer such as exchange of mitochondria and DNA have been described. Co-culture of a lung cancer cell line depleted of mitochondrial DNA (mtDNA) with bone marrow non-haematopoietic cells or skin fibroblast[184], or transplantation of breast cancer cell lines depleted of mtDNA[185], showed rescue of mitochondrial function through mitochondrial transfer. Introduction of mitochondria from mesenchymal stromal cells (MSCs) into cancer cells led to an increase in oxidative phosphorylation, ATP production, and migration and proliferation of the cancer cells[186]. Horizontal transfer of mitochondria through tunnelling nanotubes from endothelial cells to MCF7 breast cancer cells was shown to improve chemoresistance to doxorubicin in vitro[187]. Similar results were also obtained through co-culture with bone marrow stromal cells in vitro or after engraftment of acute myeloid leukaemia blast cells from patients or cell lines in mice[188]. Co-culture of glioblastoma stem cells (GSCs) and MSCs also led to metabolic rewiring of GSCs following mitochondria transfer from MSCs to GSCs, resulting in increased proliferation and resistance to temozolomide in GSCs[189].

Horizontal gene transfer can also occur in cancer owing to the uptake of DNA from extracellular vesicles, including apoptotic bodies from dying cancer cells[190]. This has been shown in vitro[183,184,190,191] and in mouse experiments[192] and can be prevented by treatment with DNAses[192]. Microvesicles have been shown to carry oncogenes such as *MYC* or *HRAS*^{V12} (refs. 191,193,194) that can be taken up by other cells. The extent and importance of horizontal gene transfer in cancer through extracellular vesicles remain an open question.

Horizontal gene transfer does not contradict an evolutionary model of cancer. Instead, it adds complexity to the model. Adaptation through natural selection can occur through horizontal transfer in addition to vertical inheritance.

Trogocytosis and horizontal transfer. Beside gene transfer, phenotypic properties can also be horizontally transferred through trogocytosis, a mechanism of membrane fragment transfer between cells. Trogocytosis is a relevant phenomenon for cancer cell evolution as it allows cells to acquire new phenotypic properties independently of their genetics and epigenetics, leading to a mismatch between the genotype and phenotype. For example, in a colon cancer mouse model using patient-derived xenografts, cancer cells were able to acquire lymphocyte membrane proteins including lymphocyte cellular markers, as well as immune regulatory surface proteins, which suppress activation of immune cells[195]. Similar results were obtained using a mouse model of leukaemia[196], although in this study, natural killer (NK) cells and CD8$^+$ T cells acquire the checkpoint receptor programmed cell death protein 1 (PD1) from leukaemia cells, which results in the suppression of NK cell antitumour immunity. Trogocytosis has been implicated in chimeric antigen receptor-T (CAR-T) cell and CAR-NK cell escape[197,198]. Trogocytosis can only be meaningful to clonal evolution if the phenotype it induces can be transmitted through at least one cell division. Cell surface materials are partly maintained through cell division, but the symmetry of inheritance to daughter cells is unknown, as well as the number of divisions after which the phenotype is lost.

Cell cannibalism is another process that can provide properties to cells that change their fitness under particular selective pressure. For example, metastatic melanoma cells can cannibalize T cells, which allow them to survive under serum deprivation[199]. Similarly, a breast cancer cell line was shown to cannibalize mesenchymal stromal cells, which also enhanced their survival in the context of starvation, through the induction of a dormant state[200]. Again, its contribution to clonal evolution depends on the heritability of those phenotypes.

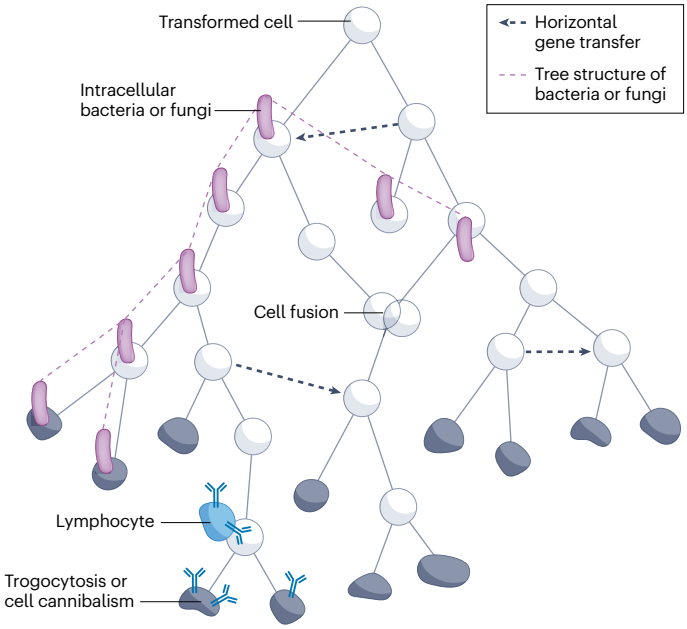

Fig. 3 | Clonal evolution is more complex than a bifurcating tree of cells. Clones can use horizontal gene transfer (dashed arrows) to transfer heritable information, thereby breaking the assumption of vertical inheritance. The purple bean-shaped cells represent intracellular microorganisms, which evolve and have their own lineages indicated by the purple dashed lines. Elements of the cells can also be transmitted horizontally from cells, including non-malignant cells, to cancer cells through absorption of extracellular vesicles, tunnelling nanotubes, trogocytosis or cell cannibalism, which is represented by the transfer of cell surface receptors from the blue cell (for example, a lymphocyte) to the grey cancer cell.

Horizontal transmission of phenotypes owing to trogocytosis or cell cannibalism reduces the heritability of those phenotypes and so reduces the factual extent of the clonal evolution model.

Intracellular microorganisms. Although clonal evolution refers to the evolution of cancer cells, presence of microorganisms (such as viruses, bacteria and fungi) inside cancer cells may call for a multispecies view of clonal evolution[201]. Viruses have been implicated in carcinogenesis for a long time, and in cases wherein they directly cause carcinogenesis and even integrate into the cancer genomes, they become part of the genetic material that is evolving in cancers[202,203]. Recent studies have observed that bacteria and fungi are not only around some cancer cells but that they can also reside inside them[204–208], and it was even suggested that most intratumoural bacteria are intracellular, residing in both cancer cells and immune cells[205].

Intracellular microorganisms can change their host cell functional properties and fitness, thereby contributing to clonal evolution. They can increase the fitness of cancer cells; for example, in colorectal cancers, *Fusobacterium nucleatum* bacteria have been associated with increased tumorigenicity[209,210]. *Fusobacterium*-positive tumours engrafted in patient-derived xenograft models whereas *Fusobacterium*-negative ones did not, a difference that was reversed by antibiotic treatment[204]. Bacteria can inhibit or elicit immune responses, for example, through peptide presentation[206,211], and can also mediate cancer cells resistance to therapies, for example, through metabolization of chemotherapies into an inactive form[212]. Human papilloma virus interferes with multiple tumour suppressor mechanisms and increases the fitness of the neoplastic cells that they infect[213].

Thus, intracellular microorganisms may contribute to clonal evolution in predictable ways that could be implemented in the clonal evolution model. The heterogeneous and focal distribution of intracellular bacteria lead to the notion of 'microbial intra-clonal diversity' whereby different fitness of cancer cells of the same genetic clone is dependent on the presence of intracellular bacteria[201]. There are, however, theoretical challenges to the integration of the role of microorganisms in clonal evolution. One of them is the mode of transmission of these microorganisms. They may be transmitted vertically and, thus, simply represent a subclone of infected cancer cells, as well as horizontally by transiting from one cell to another. In the second case, they question the tree-shape of inheritance patterns among cancer cells. Moreover, bacteria themselves may undergo their own evolutionary dynamics. Bioinformatic analysis of the metabolic activity of intratumoural bacteria has suggested that bacteria are under their own selective pressures shaped by the tumour cells and their microenvironment[205]. Thus various selective processes may act on cancer cell lineages and microbial lineages[201].

The evolution of cancer cells may, thus, not be a simple branching tree of cells but might involve fusion and transmission between branches (Fig. 3). Genetic and non-genetic inheritance may not just be vertical but may also sometimes be horizontal. Additionally, clonal evolution might concern not only cancer cells but also intracellular bacteria and fungi that may change the fitness of cancer cells. These mechanisms challenge the theoretical efficacy of the current clonal evolution model rather than the factual extent of the involvement of cancer cell evolution. They, thus, open avenues to improve the theoretical power of the clonal evolution model.

Improving the theory of cancer

The challenges and limitations of the clonal evolution model discussed in the section 'Model assumptions and limitations' suggest opportunities for improving the model. We will focus on four potential extensions to the clonal evolution model: the diversity and timescale of inheritance beyond genetics, phenotypic plasticity, reticulating modes of evolution, and the concept of clone.

Inheritance and timescale

Inheritance is a central concept for any evolutionary theory of cancer. There cannot be evolution without some sort of inheritance. However, inheritance is loosely defined, and its definition is subject to debate.

Several classifications of forms of inheritance have been proposed[214]. This includes the distinction of internal from external channels of inheritance[215]. Internal inheritance comprises factors that are transmitted through cell division (or cell fusion) such as genetic and epigenetic inheritance. External inheritance contains every transmission that is not passed through cell division. Internal inheritance can be traced through cell lineages whereas external inheritance cannot.

It is relatively easy to extend genetic models of inheritance to epigenetic models, at least for randomly mutating epigenetic states[143,216,217]. However, internal inheritance can also extend beyond genetics and epigenetics. One approach to studying non-genetic and non-epigenetic heritable phenotypes could be to quantify the heritability of those phenotypes using the breeder's equation[218]. By measuring change in

cancer cell phenotype (in vitro or in vivo) over different timescales and selective pressures, cancer biologists could quantify the degree of heritability of a phenotype of interest. This would account for both internal and external modes of inheritance, including non-(epi)genetic inheritance of the cytoplasm and niche construction. Note that these measures of heritability depend on the degree of heritable variation in the population. A phenotype could be heritable but would appear to be not if the population did not include variation in the substrates that encode that phenotype.

There is external inheritance in cancer, at least through niche construction[219]. There is a form of co-evolution between organisms and the niches that they alter. This co-evolution can be represented in formal models of evolution[220]. When neoplastic cells engage in niche construction, by activating fibroblasts[221,222], recruiting immune cells[223–225], inducing angiogenesis[226] and otherwise altering their microenvironment[148–150,227], they generate a form of external inheritance that changes the selection pressures on themselves and, thereby, changes their own evolutionary trajectories[220]. The timescale of such external inheritance can be long. For example, activated fibroblasts can maintain their pro-tumour phenotype even in the absence of cancer cells, through autocrine loops, epigenetic alterations and even genetic alterations[228]. Efforts have been made to start integrating ecology in the clonal evolution model[94], but accounting for niche construction remains challenging.

There have also been debates about which kind of inheritance matters more[229–231]. One argument is that there is a causal asymmetry between factors transmitted over many generations and those transmitted over one generation, the former having a more substantial role in evolution because they can accumulate over long periods of time by natural selection[232]. In cancer, however, selective pressures change at very different timescales. Ageing slowly reshapes the selective landscape over decades[101–103]. The hallmarks of cancer, which are necessary for transformation, must last for the time span of neoplastic progression, which can take decades[147]. Short-term forms of inheritance cannot meaningfully contribute to these long processes of evolution by natural selection[232]. However, there are other barriers to neoplastic progression, such as survival in circulation during metastasis, which probably only needs to be overcome for a matter of hours or days. In that case, inheriting some key proteins from a parental cell, or a niche, such as a cluster of neoplastic cells that may safely travel through the bloodstream, may last long enough to deliver the neoplastic cell to a new metastatic microenvironment. Similarly, treatments such as radiotherapy and chemotherapies, which impose intense selective pressures on cancer cells, are often applied for the duration of only a few cell generations, similar to the timescale of cytoplasmic inheritance.

The focus on genetic clonal evolution can only partially capture the short timescale events of evolution and several avenues could help improve the model in this regard. One approach involves reconstructing clonal evolution using a variety of molecular clocks. DNA, copy number variants (CNVs) and single nucleotide variants (SNVs) mostly change over years[233,234]. Methylation of CpG sites have faster clock rates than SNVs, although those rates may vary from site to site in the genome[165]. Other epigenetic modifications to histones[235,236] or changes to cell state through signal transduction and transitions to new attractor states in the genetic regulatory network[237,238] can occur rapidly and may require their own molecular clocks in clonal evolution models.

Future research should characterize the timescales of different mechanisms of inheritance (Fig. 4). Any phenotypic change that lasts less than the cell lifespan cannot be a meaningful heritable property for clonal evolution. Any phenotypic change that lasts less than the timescale of a selective pressure will have little effect on the evolution of a cell population in response to that pressure. By contrast, mechanisms of inheritance that produce phenotypic changes that last as long or longer than selective pressures will be important to the response of a population to these pressures, as long as there is relevant variation in the cell population. There are mathematical methods for disentangling the different forms of inheritance, including genetic, epigenetic and ecological inheritance[239].

Phenotypic plasticity

Phenotypic plasticity can weaken the relationship between genotypes and phenotypes, reducing the heritability of the phenotype and, thereby, dampening the effects of natural selection. However, phenotypic plasticity itself can be an adaptive phenotype and has evolved multiple times by natural selection[159–161]. At least one historical theoretical framework supports a contribution of phenotypic plasticity to genetic evolution: the Baldwin effect, a process by which an initial phenotypic accommodation is later reinforced by genetic adaptation.

The Baldwin effect, in its simplified form[240,241], refers to a three-step process: first, some individuals (in our case, cancer cells) accommodate environmental changes through phenotypic plasticity. This initial phase of phenotypic plasticity allows some individuals to avoid

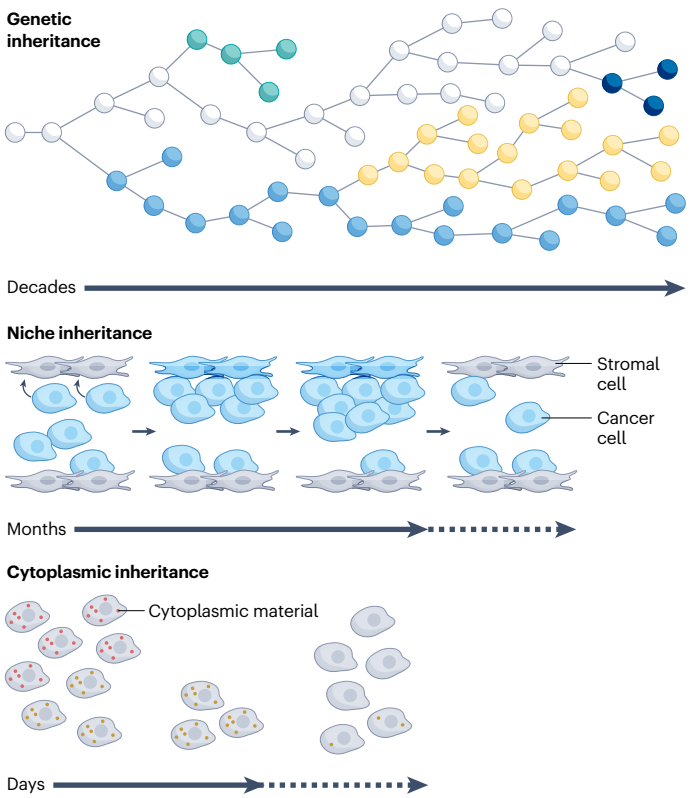

Fig. 4 | Types of inheritance over various timescales. Genetic inheritance may contribute at any timescale and persist throughout life. Niche construction by cancer cells may have selective effects lasting for days to months, though the exact timescale is unknown. Some entities such as cytoplasmic and cell surface proteins may be inherited for a very short period of time, probably just days. They may be important contributors to evolution by natural selection in case of abrupt and short changes in selective pressures such as cancer therapies.

Glossary

Advantageous mutation
A mutation that increases the fitness of an organism or cell, also known as a driver mutation.

Branched evolution
Evolution in which lineages split such that multiple lineages coexist and can be represented as a tree.

Breeder's equation
$\Delta Z = h^2 S$, describes the change in the mean phenotype (Z) in the population as a function of its heritability (h) and the selection pressure (S) placed on that phenotype.

Chronic myelomonocytic leukaemia
A type of chronic leukaemia affecting the myeloid cells that is characterized by a persisting monocytosis.

Clades
A monophyletic group, that is, a group of all the individuals who derive from a common ancestor.

Clonal expansion
An increase in the number of cells deriving from a common ancestor that defines the clone.

Clonal mutations
Mutations that derive from a single ancestral cell and is shared among all its descendants.

Convergent evolution
Independent evolution of a similar phenotype in different lineages.

Deleterious mutation
A mutation that decreases the fitness of an organism or cell.

Driver genes
Genes in which particular genetic alterations increase the fitness of a cell, causing a clonal expansion.

Epimutations
Non-genetic heritable epigenetic changes in DNA methylation or the chromatin.

Epistemic challenge
A challenge that relates to our knowledge.

Epithelial proliferative units
Organizational sub-structures of epithelial tissues consisting of one or a few stem cells along with their partially and fully differentiated progeny.

Fitness
The ability of a cell to survive and proliferate in its current microenvironment.

Gradualism
Evolution through slow continuous small changes in the phenotypes of organisms over time.

Horizontal gene transfer
The transmission of genetic material from organisms or cells that are not direct ancestors of the recipient.

Hybrid cells
Cells that directly derive from more than one ancestor.

Hybridomas
Cell lines coming from the fusion of B cells with immortal myeloma cell lines that are used to produce monoclonal antibodies.

Intratumoural heterogeneity
Variation between cells within a neoplasm.

Karyotype
The set of chromosomes of a cell, including any large-scale abnormalities visible in a mitotic spread.

Lineage tree
A type of branching graph diagramming ancestral relationships with only a single path between any two given nodes in the tree.

Linear evolution
Evolution characterized by a sequence of fixation events wherein one genotype is replaced by another in the population, such that change over time can be described by a single sequence of genotypes.

Microvesicles
Lipid bilayer-delimited particles that are released from the cell membrane.

Molecular clocks
Molecular processes that change proportional to time and can, thus, be used to infer the time elapsed since past events.

Monophyletic clade
The set of all the species that descended from a common ancestor.

Negative selection
The removal of mutations from a population owing to their negative effect on the fitness of the organism or cell.

Neoplastic progression
The process of change from normal tissue to cancer and on to metastatic disease.

Neutral evolution
Changes in allele frequency in a population owing to chance rather than fitness.

Niche construction
Modification of the environment by an organism, generally to the benefit of that organism.

Phenotypic plasticity
The ability of a cell to adopt different phenotypes without changing its genotype.

Phylogenetic reconstruction
The process of inferring the ancestral relationships between organisms or cells.

Punctuation
Evolution through sudden important changes in the phenotypes of organisms.

Reticulate evolution
Transmission of heritable properties from one lineage to another through genetic exchange mechanisms such as hybridization or horizontal gene transfer.

Stasis
Long periods during which no or few evolutionary changes occur.

Subclonal mutations
Mutations that are present in only a subset of the cells within a clone.

Transformation
The change from a normal cell state to a malignant state.

Trogocytosis
Transfer of plasma membrane fragments from one cell to another.

Vertical inheritance
The passage of properties from parents to offspring.

extinction. Second, some genetic (and, thus, heritable) changes occur in the surviving population that allow them to survive in the new environment without having to pay the cost of phenotypic plasticity. Third, these genetically determined phenotypes are favoured by natural selection and finally spread in the population because they achieve the same or more fit phenotypes as the phenotypically plastic individuals without having to pay the cost of plasticity, which may be a metabolically demanding process. Hence, the Baldwin effect is a particular case wherein an adaptation is initially individual and non-heritable and becomes hereditary and selected.

Several studies suggest mechanisms of initial phenotypic adaptation that are secondarily genetically selected[133,242,243]. For example, when

exposed to EGFR inhibitors, most *EGFR*-mutated lung cancer cells die, but a few can survive through a phenotypic accommodation called 'persister' state. Sequencing and drug screening of relapsed clones developing from persistent cells of a single initial *EGFR*-mutated clone revealed secondary selection of heterogeneous resistance mechanisms[242], suggesting a case of Baldwin effect. Similarly, it was observed that the $EGFR^{T790M}$ resistance mutation is not present at the time of therapy in some cases and can evolve from persister cells[243]. Persister cells do not need to be quiescent. Brief exposure to vincristine can activate efflux pumps, which protect cells from the drug, allowing them to persist in an actively proliferating state. That activation is stably inherited by epigenetic modifications[5] which may later be stabilized by genetic alterations.

The Baldwin effect has long been neglected in evolutionary biology as a minor phenomenon that simply 'buys time'[244] and, thus, is of interest only in cases wherein selective pressures change too fast for the traditional variation and selection process to produce adaptation[149]. Cancer is precisely one of those situations wherein selective pressure may change quickly (for example, the onset of treatment), and buying time might be a crucial evolutionary mechanism that could be incorporated in the clonal evolution model.

Reticulation, introgression and open lineages

Cell lineages are usually assumed to form a tree, but as discussed in 'Assumption 2: a cancer cell lineage is a tree' section, cancer cell fusion can occur and inheritance can also be horizontal, with possible transfer of mitochondria, extrachromosomal DNA, pieces of cells such as membranes, or intracellular bacteria and fungi. Those processes all violate the tree assumption and impact phylogenetic reconstruction, as the cell tree not necessarily matches the gene trees (Fig. 3). It also impacts clonal deconvolution of bulk assays[245,246] because horizontal gene transfer and cell fusion violate many of the assumptions those algorithms are based on. Multiple discordant gene trees may exist within a cell lineage, reflecting the diverse flows of genes through all these independent phenomena. Similar lineage violations are common in prokaryotes, giving rise to the concept of reticulate evolution. Reticulation has led many to argue that the tree of life is a misrepresentation[233,234,239]. It may be more appropriate to represent evolution as a network or web of interlaced branches[247–249]. Several measures have been developed and used such as the concordance factors[250] and diversity indices for unrooted trees[251], and new computational methods and statistics have been developed to identify lateral transfer and analyse phylogenetic networks[252,253]. In general, the revised view of evolution that developed around reticulate evolution calls for a multilevel perspective, sometimes also referred to as integrative evolution[251,254], wherein multiple genetic worlds contribute to the observed diversity[255].

These conceptual and computational tools represent opportunities to improve our understanding of clonal evolution. Given that horizontal genetic transfers can provide evolutionary short-cuts to acquire functional adaptations such as the ability to metastasize or multidrug resistance, it is important to evaluate the implications of such reticulation events in cancer progression and to adopt an integrative clonal evolution model.

Quantifying clonal diversity

Counting clones as a proxy of intratumoural heterogeneity and cancer cell evolution is based on two implicit assumptions: that the cells within a clone are homogeneous (intra-clonal homogeneity) and distinct from those of other clones (inter-clonal heterogeneity), so that their number reflects the diversity of the tumour cell population. However, as discussed in Box 1, these assumptions might be disputable (Fig. 2). If we use mutations to delineate clones (Fig. 2a), there are three clones and, thus, three relevant populations of cancer cells with distinctive characteristics. However, there is a different delineation of clones with epigenetics (Fig. 2b), and their integration (Fig. 2d) suggests that there are more than three relevant populations of cancer cells.

One can measure the degree of intra-clonal homogeneity and inter-clonal heterogeneity. This could be done by measuring the distance between any two cells inside and between clones for any phenotypic or genotypic measure. For example, a measure of transcriptomic diversity inside barcoded lineages in three mouse models of acute myeloid leukaemia[256] observed that clones are clearly not homogeneous, as single-cell RNA sequencing often reveals smears of cells across phenotype space rather than tight clusters. By contrast, another study has observed high intra-clonal homogeneity inside barcoded lineages and inter-clonal heterogeneity between barcoded lineages in a human melanoma cell line[257]. Single-cell genomics analysis can also be used to measure clonal diversity[171,258–263]. One analysis has found evidence for the existence of just a few clones, with little variation within clones and a large degree of differences between clones based on copy number profiling of two breast cancer cases[171]. By contrast, the same team found massive genomic diversity in point mutations in two other breast cancer cases, leading the authors to question the concept of a clone as no two tumour cells were found to be genetically identical[258].

It is possible to abandon the counting of clones altogether. Because they are a proxy for measuring the structure and degree of genetic diversity in a neoplasm, we may replace the counting of clones with other methods of characterizing that diversity. For example, if the evolutionary relationships between samples can be reconstructed into a tree, then there are a variety of tree statistics that may actually be more sensitive and representative of the genetic diversity than the number of clones[264]. In fact, some of these measures provide information on the population dynamics and selection within the neoplasm[264].

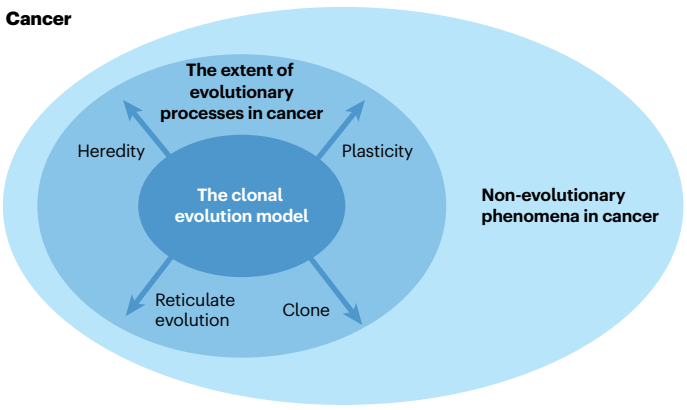

Fig. 5 | Explanatory power of clonal evolution. When aiming to explain cancer in its entirety, evolutionary processes can explain only some cancer phenomena. Other theories, such as the cancer stem cell theory or some versions of the persister cell models provide complementary explanations for various phenomena in cancer. The clonal evolution model only captures parts of the causal role of evolutionary processes in cancer. There are, thus, opportunities to increase our current ability to provide evolutionary accounts of cancer, by integrating heredity, plasticity, reticulate evolution and clone diversity into the clonal evolution model. The proportion of cancer phenomena that can be explained by evolution is unknown.

Conclusions

There is no doubt that somatic cells undergo evolutionary processes, including both genetic drift and natural selection. They acquire heritable alterations, some of which affect their reproduction and survival. The fact that cancer cells evolve was realized in the 1950s and mostly investigated since the twenty-first century. However, the extent to which evolutionary processes explain cancer remains open to debate. The success of the evolutionary theory of cancer for explaining many of the phenomena of cancer, and for developing better interventions, clearly shows the utility of the theory. However, it is also clear that non-evolutionary mechanisms contribute to cancer. The field is currently gauging the respective role of evolutionary and non-evolutionary mechanisms in cancer. However, this cannot be done if we lack clarity on the boundaries between evolutionary and non-evolutionary mechanisms. There are many theories and models of cancer today[265] whose integration is highly challenging[266]. It is unclear which ones are compatible or not with evolutionary theories. In general, other theories of cancer, such as the cancer stem cell theory[267], the atavistic theory[268,269] and the wound that will not heal[270–272], describe the biological constraints and affordances within which somatic cells evolve. To address this issue, we focused on the boundaries of evolutionary processes, distinguishing two kinds of boundaries: theoretical and factual (Fig. 5). The factual boundaries of the evolutionary dynamics of cancer extend beyond the current theory, and the evolutionary theory of cancer needs to be expanded to account for phenotypic plasticity, alternative modes of inheritance, horizontal gene transfer, cell lineage fusions and better measures of clonal diversity. Much more remains to be discovered about those phenomena and their relative importance in cancer. An improved evolutionary theory of cancer should lead to improvements in our prediction, prognosis and treatment of cancer.

Published online: 10 September 2024

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Acknowledgements

This work has been funded by CNRS MITI through the 80|Prime programme, Cancéropôle IDF (n°2021-1-EMERG-54-CNRS DR 5-1), SIRIC (INCa-DGOS-Inserm-ITMO Cancer_18002), McDonnell Foundation, Ligue Nationale Contre le Cancer EL2020 (to LL), and by National Institutes of Health grants U54 CA217376, U2C CA233254, R21 CA257980 and R01 CA140657 (to C.C.M.), as well as CDMRP Breast Cancer Research Program Award BC132057 and the Arizona Biomedical Research Commission grant ADHS18-198847 (to C.C.M.). The findings, opinions and recommendations expressed here are those of the authors and not necessarily those of the universities where the research was performed or the National Institutes of Health.

Author contributions

Both authors contributed equally to the preparation of this manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Peer review information *Nature Reviews Cancer* thanks Moritz Gerstung, Luca Magnani and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.