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Trends in Cancer

Opinion

The origin of novel traits in cancer

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The traditional view of cancer emphasizes a genes-first process. Novel cancer traits arise by genetic mutations that spread to drive phenotypic change. However, recent data support a phenotypes-first process in which nonheritable cellular variability creates novel traits that later become heritably stabilized by genetic and epigenetic changes. Single-cell measurements reinforce the idea that phenotypes lead genotypes, showing how cancer evolution follows normal developmental plasticity and creates novel traits by recombining parts of different cellular developmental programs. In parallel, studies in evolutionary biology also support a phenotypes-first process driven by developmental plasticity and developmental recombination. These advances in cancer research and evolutionary biology mutually reinforce a revolution in our understanding of how cells and organisms evolve novel traits in response to environmental challenges.

Highlights

The novel traits that drive cancer often arise by nonheritable cellular variability that is subsequently stabilized by heritable genetic and epigenetic changes – phenotypes come first and genetic changes come second.

Classic cancer genes such as *KRAS* and *TP53* increase cellular plasticity, providing the nonheritable phenotypic variability that drives cancer.

Evolutionary theory predicts that recombination of different developmental plasticity programs often creates the novelty that generates new traits.

Lung cancer studies show exactly the type of developmental plasticity and recombination predicted by evolutionary theory, creating the new cellular phenotypes that drive tumor progression.

Pancreatic cancer studies show how KRAS-driven cellular plasticity triggers the novel cellular interactions that create the tumor microenvironment.

When thinking about the origin of new traits in evolution and in cancer, traditional explanations favor a genes-first model. A mutation arises and, if beneficial, spreads, driving a change in phenotype. In cancer, a genes-first view has long been the basis for understanding carcinogenesis, metastasis, and resistance to chemotherapy. Alternatively, recent evolutionary theory argues that a new trait may first arise as a nonheritable phenotypic variant [1–4]. In this phenotypes-first model, subsequent genetic change then stabilizes and makes heritable the new trait.

Ideas about phenotypic variety are not new to cancer research [5–7]. For example, the epithelial-to-mesenchymal transition (EMT) has become part of the foundation by which we understand typical changes in cancer evolution and metastatic spread [8–15]. However, two things are new. First, our recent ability to measure cell-state changes at the single-cell level provides novel observations of the paths of evolutionary change at the level that drives cancer. These observations reveal that cellular plasticity and phenotypes-first processes are not just one special part of cancer evolution. Instead, those processes may be the fundamental drivers of the novel traits that generate tumors, spread metastases, and resist drugs [6,7,16–21].

For example, in lung and pancreatic cancer, *KRAS* (and perhaps *TP53*) mutations come first [20–22]. However, these mutations primarily drive cancer by triggering greater nonheritable cellular diversity arising from more frequent stochastic cell-state transitions that release phenotypic variety. This variety follows pathways of developmental plasticity in cells and kickstarts a phenotypes-first process for the primary changes that drive tumorigenesis. Only after the release of the initial phenotypic variety do subsequent (epi)genetic mutations heritably stabilize the novel traits that drive cancer evolution. In this scenario, the initial *KRAS* mutation enhances the overall cellular rate of the phenotypes-first process but may not by itself directly encode a novel and essential trait that drives cancer.

Similarly, ideas about the origin of resistance to chemotherapy typically emphasize pre-existing genetic variation among cancer cells. The treatment favors the spread of those pre-existing resistance variants. However, recent studies show that resistance may often arise by nonheritable cellular

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variability. In some cases the novel resistance traits may be triggered by cellular plasticity in response to the stress caused by the therapy, further supporting a phenotypes-first perspective [6].

A second novel aspect of our perspective comes from parallel advances in evolutionary biology. Recent work suggests that we are starting to understand the origin of novelty in biology in a new way. As that broader understanding of evolutionary novelty feeds back to studies of cancer, we will gain further insights into tumor progression and drug resistance. Indeed, evolutionary biology suggests that the greatest leaps in phenotypic novelty arise by recombining parts of different developmental programs [1,2]. This process of developmental recombination has recently been observed in lung cancer, in which a primary cellular driver of progression expresses parts of the developmental programs of trophoblasts, chondroblasts, and kidney tubular epithelium [22].

Here we summarize the close match between the emerging conceptual framework in evolutionary biology and emerging empirical observations in cancer research. We may be in the early stages of a revolution in how we understand the origin of novelty in biology. That new understanding may change how we study cancer and how we design treatments.

We begin with a brief overview of the evolutionary concepts. That evolutionary framework structures our subsequent review of drug resistance and cancer progression. Throughout, we emphasize how novel traits arise in response to new environmental challenges.

Origin of novel traits

A recent evolutionary synthesis suggests that most novel traits first appear by an organism's intrinsic plasticity in response to a new environmental challenge [1,2]. Development is particularly labile, with each component of the developing organism adjustings its phenotype to the environment and to other developing parts of the individual. When new environmental challenges arise, components of development and other aspects of organismal plasticity may reorganize and recombine to produce novel traits.

Novel traits that arise by phenotypic plasticity are not random variants, as is often supposed for genetic mutations. Instead, the novelty comes from the organism's intrinsic developmental processes and phenotypic plasticity which reflect the adaptive responses that have evolved through a history of natural selection. Because novel traits that arise by phenotypic responsiveness to new environments tend to be the result of prior adaptations, such environmentally initiated traits often have greater evolutionary potential than mutationally initiated traits.

When thinking about the origin of novel traits, we must begin with the source of new phenotypes. We consider the three processes that create phenotypic variety in the absence of genetic change: stochasticity, plasticity, and developmental recombination. Each plays an important role in the origin of the traits that drive cancer progression and therapy resistance. In some cases, initial genetic mutations enhance subsequent phenotypic variety. These nongenetic variants then lead the phenotypes-first evolutionary process, which takes place at a faster rate because the initial mutations release greater phenotypic diversity.

In cancer, stochasticity arises when particular proteins occur in small numbers per cell. With few copies, protein numbers fluctuate randomly [23–25]. That stochasticity may cause cellular phenotypes to vary. Some of these phenotypes may resist drugs or provide the next step in cancer progression. Plasticity arises when a novel environment induces cells to express a different phenotype. The alternative phenotype is often a programmed cellular response. Developmental recombination occurs when parts of different phenotypically plastic programs of

expression come together to create a new trait [1]. Such recombination may be particularly important in creating truly novel phenotypes, including the types of change that drive the origins of cancer.

As an example of developmental recombination, consider how primitive humans came to be fully upright, walking on two legs. In the classic view, a new trait of this kind would arise by a sequence of mutations, each moving the phenotype in the direction of the final form. For upright bipedalism, such a sequence must be complex. The novel trait requires reorganizing muscles, nerves, skeleton, and behavior. Each mutational step must provide a fitness advantage.

Alternatively, bipedalism might have arisen initially by environmental induction and recombination of pre-existing developmental programs. West-Eberhard [2] illustrates this process by describing a goat that was born with a congenital defect of its front legs and subsequently learned to walk on its two hind legs. As the goat grew in early life, the developmental processes that shaped its leg bones, muscles, and tendons adjusted to the altered forces of bipedalism, causing broad morphological changes compared with a normal goat. The novel forces during development also caused a modified thoracic skeleton and extensive changes to the pelvis. The successful two-legged phenotype arose by recombining and mutually adjusting many distinct development processes of the muscles, bones, and behavior.

Stochasticity, plasticity, and developmental recombination arise from the same underlying genome. To transform that initial phenotypic variability into a permanent evolutionary change requires that the new phenotypes become transmitted heritably (Figure 1). Some kind of transmissible genetic or epigenetic change is necessary to stabilize the favored phenotype–genetic accommodation.

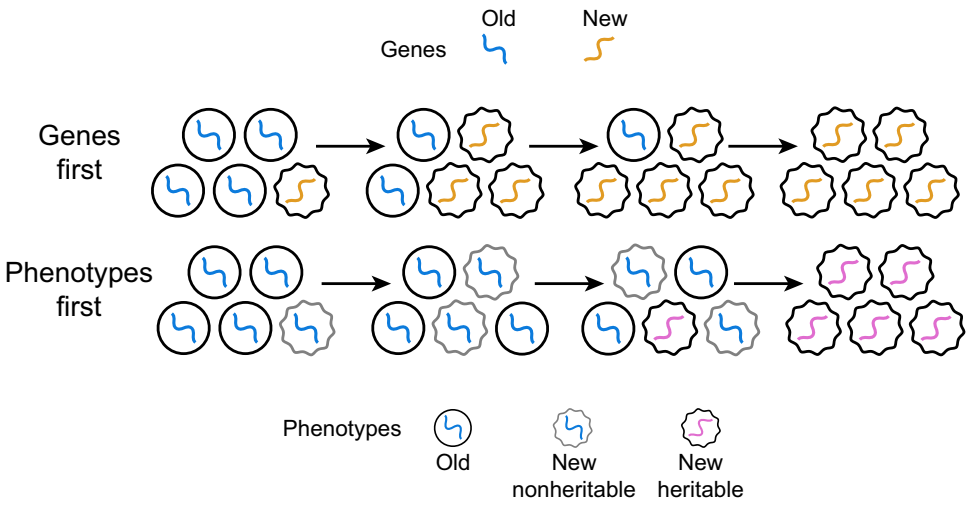

Figure 1. Genes-first versus phenotypes-first processes for the origin and spread of a new trait. In a genes-first process, the first step (upper left) is the arrival of a new mutation. If that mutation provides a reproductive advantage, the new mutation spreads until it takes over the population. In a phenotypes-first process, the first step (lower left) is the appearance of a new nonheritable trait. In this case, the novel trait arises by a stochastic and reversible fluctuation of trait expression. Moving to the right as time passes, the initial variant cell may revert back to its prior state while other cells change to the novel phenotype. In this case the novel phenotype is advantageous and therefore increases in frequency even though some cells revert back to the earlier form. Eventually, the fluctuating trait may be stabilized by a subsequent (epi)genetic change. Because the stabilized heritable trait provides a reproductive advantage, it spreads to take over the population.

Box 1 describes how phenotypes-first processes accelerate evolutionary change. Figure 2 places these phenotypes-first processes in the historical context of cancer research. In that historical context, Figure 3 suggests a new way to understand the most important genetic drivers of cancer, such as *KRAS* and *TP53*. The following sections link current research studies to that historical context, the new interpretation of key genetic drivers, and the ongoing transitions in our understanding of treatment resistance and carcinogenesis.

Phenotypes-first origin of drug resistance

Does resistance originate from mutations or nonheritable phenotypic variety [5,6,26]? In some cases, mutations start the process. For example, pre-existing mutations initiate resistance to chemotherapy in triple-negative breast cancer [27]. However, recent studies increasingly emphasize phenotypic variation or cellular plasticity as the primary initiator of resistance evolution [7] (Figure 4).

Nonheritable cell-state fluctuations initiate resistance

The initial phenotypic diversity often begins with cell-state transitions in which cancer cells can change between multiple phenotypic states without genetic alterations, enabling dynamic, reversible resistance [16]. New environments favor subsequent stabilization of beneficial states.

Box 1. A phenotypes-first process accelerates evolution

A phenotypes-first process, arising from nonheritable phenotypic variety, increases the chance of matching a novel trait to a new environmental challenge [3,4,44]. Evolutionary adaptation can take place much faster because nonheritable phenotypic variety smooths the fitness landscape. Consider two alternatives [3,45].

In the simplest genes-first scenario, each genotype always generates the same phenotype. Assume that only a very particular phenotype can survive. None of the current genotypes generates that phenotype. All die unless a new mutation happens to match the required phenotype exactly. This resembles searching for a needle in a haystack. Finding the needle requires fantastic luck, a very rare event.

In the simplest phenotypes-first scenario, different individuals with the same genotype generate a distribution of phenotypes. Phenotypes more distant from the average occur less often but they do occur. A genotype may occasionally generate the particular phenotype required for survival. The individuals that match the environment survive to the next generation.

In this phenotypes-first case, a genetic mutation does not need to land exactly on the required form to be favored. Instead, a mutation that moves the average phenotype closer to the required spot will increase the fraction of individuals that produce the required phenotype and survive. Thus, step-by-step, traditional genetic evolution can slowly improve fitness by smoothly climbing the fitness landscape. Instead of blindly searching for a needle in a haystack, this second case is like searching for a needle in a haystack when someone tells you when you are getting closer [46].

Things are even better when the phenotypic variety comes from adaptive plasticity [1,3,6,47]. Then, as with the two-legged goat, the phenotypes will tend to be good responses to the environmental challenge. All that is left is to refine and stabilize the initial plastic responses.

A study of drug resistance demonstrated that an accelerated evolutionary rate was caused by increased nonheritable phenotypic variety [48]. The experiment inserted an inducible synthetic gene circuit into the yeast *Saccharomyces cerevisiae*. The circuit could be switched on to produce an increased level of phenotypic stochasticity in the expression level of an antifungal resistance gene. Greater stochastic variability in gene expression increased the rate of drug-resistance evolution. Ultimately, resistance became heritable by subsequent genetic mutations that stabilized transmission of the resistant trait.

When an environment demands rapid evolutionary change, a genotype that generates more phenotypic variation evolves more quickly to meet the new challenge [3,6]. Thus, extreme challenge may favor more variable types with weaker homeostasis. As evolution proceeds and the phenotypic match to the environment improves, large phenotypic variety becomes a burden rather than a benefit. Wide exploration early and more narrowly focused exploitation later does best.

In the yeast drug-resistance study, cellular variants that initially had low stochasticity in gene expression evolved higher stochasticity in expression in response to the drug treatment [48]. In other words, environmental challenge favored greater phenotypic stochasticity. In the absence of the drug, low expression of the drug-resistance gene and low phenotypic heterogeneity had the highest fitness. Thus, an absence of environmental challenge favored reduced phenotypic heterogeneity.

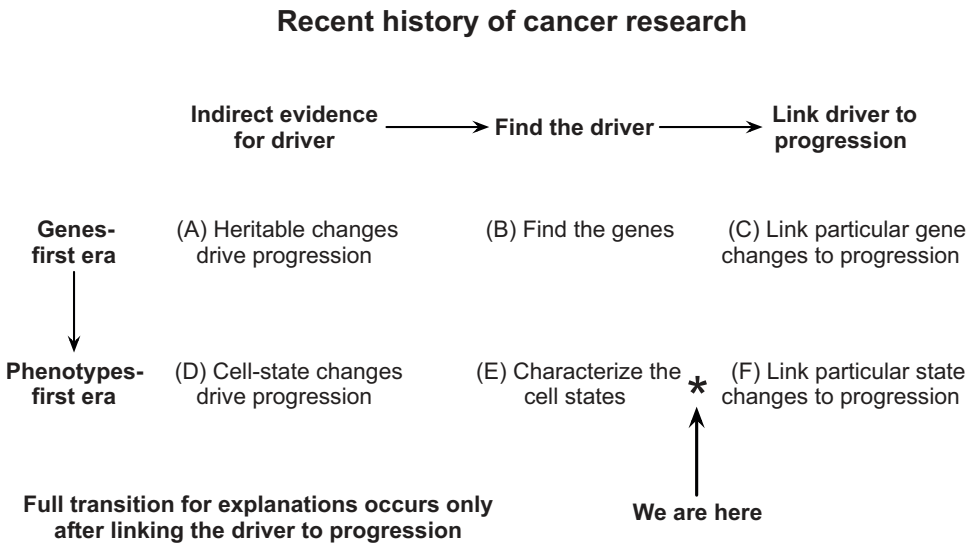

Figure 2. The rise of the phenotypes-first era in cancer research. The recently dominant genes-first era began with (A) indirect evidence for genes as drivers, suggested by heritable changes that influence progression. For example, a study in 1969 showed that a tendency to develop colorectal tumors could be transmitted within families [49]. Then (B) the genes associated with the driving process were found. In the case of colorectal cancer, inherited mutations in the *APC* gene drove progression [50,51]. Finally, (C) the particular cellular and tissue changes caused by the mutation were linked to specific steps in progression. For *APC*, the mutations associated with reduced cell death and the buildup of excess tissue were an early step in tumor formation [52]. We may currently be transitioning to a phenotypes-first era. Several studies reviewed in this article describe how (D) initially nonheritable cell-state changes associate with treatment resistance or tumor progression. Some of the studies (E) have characterized particular cell states and their association with stages in resistance or progression. Finally, (F) a few recent studies described in this review causally link particular cell states to specific changes in resistance or progression. Going forward, some cases will continue to fit within the genes-first perspective whereas others will fit within the phenotypes-first perspective. What will be the relative dominance of these alternatives? We believe that the new single-cell measurement technologies will reveal a significant and perhaps primary role for phenotypes-first processes.

For example, when ovarian cells were treated *in vitro* with a sequentially increasing dose of a poly-(ADP-ribose) polymerase (PARP) inhibitor, among the fluctuating cellular states the dominant state changed with each increase in dose [16]. Specifically, the dominant cell state of the ovarian cancer cells underwent five sequential changes as the cell lineage evolved increasing resistance to chemotherapy. Broad cellular reprogramming of phenotypically plastic stress-response mechanisms drove the increasing resistance. Changes in transcription factors (TFs) stabilized the fluctuating phenotypic variation of cellular state. For example, open chromatin was increasingly enriched for TF binding sites of the global stress regulators AP1, NRF2, and ATF4.

This study of ovarian cancer cell resistance to treatment also observed EMT. In normal development, specialized epithelial cells arise through a sequence of cell-state transitions. A mesenchymal cell type typically occurs as an earlier step in the pathway of cell transitions. In cancer evolution, EMT frequently reverses the common developmental pathway – in essence leading to dedifferentiation of specialized terminal epithelia to an earlier and less specialized mesenchyme [8–13]. Simplifying a little, mesenchyme is a more flexible and general type of cell that can take on a wider variety of phenotypes, transit to other cell states, and promote changes that allow tumors to progress.

This study monitored and controlled EMT with SOX17, a TF that represses EMT, providing direct evidence for the effect of EMT plasticity on drug resistance [10]. In the short term, reduced SOX17 enhanced EMT and slightly increased drug resistance. In the long term, EMT accelerated drug-

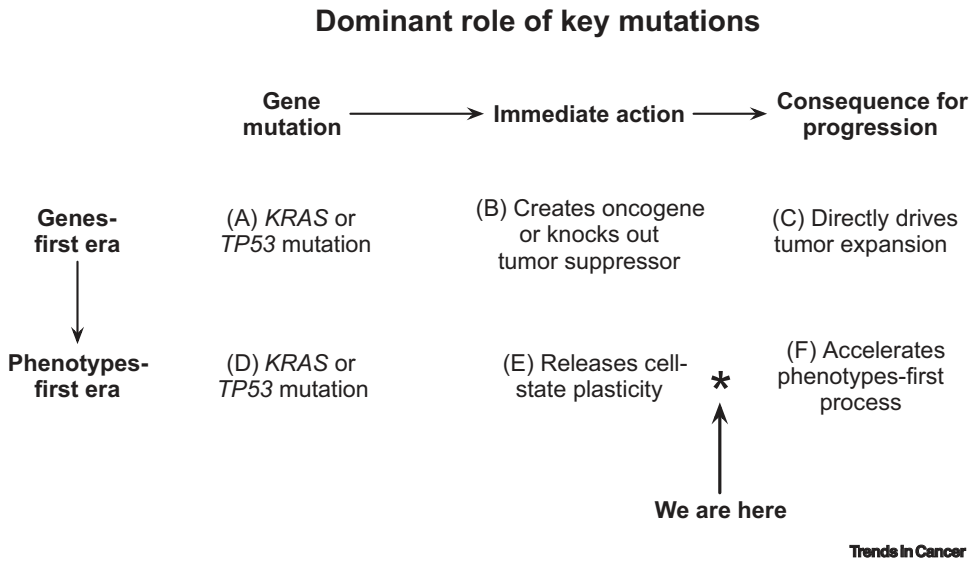

Figure 3. Key mutations release cell-state variability, accelerating phenotypes-first processes. In the genes-first interpretation, (A) key mutations often take place in *KRAS*, *TP53*, or other genes that are widely associated with cancer. (B) *KRAS* mutation creates an oncogene that speeds up cell division, thus promoting tumor formation. *TP53* mutation knocks out normal tumor-suppressor activity, such as apoptosis. Other key mutations often fall into the oncogene or tumor-suppressor classes. (C) Faster cell division or slower cell death directly drive tumor expansion. In the phenotypes-first interpretation, the roles of (D) key mutations in genes such as *KRAS* and *TP53* differ from their roles under the genes-first interpretation. Most importantly, (E) such mutations release cell-state plasticity, creating a broad pool of fluctuating and initially nonheritable phenotypic variety. That phenotypic variety (F) accelerates phenotypes-first processes, the primary force that drives cancer progression. Several studies summarized in this review show how *KRAS* and sometimes *TP53* mutations release cell-state plasticity, as in (E,F). These studies also provide hints about the final link to (F), the consequences for progression.

resistance evolution. The enhanced cellular variability and plasticity of the mesenchymal cell state may have increased the opportunity for surviving in the short term and for broadly reprogramming cellular state in the long term.

Stochastic protein fluctuations accelerate resistance

Melanoma provides a good example of how stochastic generation of phenotypic variety drives the evolution of drug resistance in a phenotypes-first manner. In this case, stochasticity arises by random fluctuations of cellular TF protein levels, creating nonheritable fluctuations in cellular state [17,18].

In normal development, neural crest cells form a migratory pool of precursors that differentiate into a variety of specialized cell types. Increased expression of microphthalmia-associated transcription factor (MITF) transforms neural crest cells into melanocytes. As melanocytes evolve into melanoma, MITF is necessary to maintain or accelerate cellular proliferation. Low expression of MITF dedifferentiates the cellular state back toward the more stem-like neural crest phenotype. In melanoma, the dedifferentiated cells divide rarely, are more invasive, and resist stress and harsh environments. These characteristics also increase resistance to chemotherapy [17,18].

A population of melanoma cells typically contains some cells with low MITF. Low-expressor cells may arise by chance fluctuations in MITF levels, a state that can revert to normal by a subsequent stochastic rise in expression. Low expressors may also occur in response to local environments or signals that cause cells to reduce MITF expression, although it is not clear exactly what these

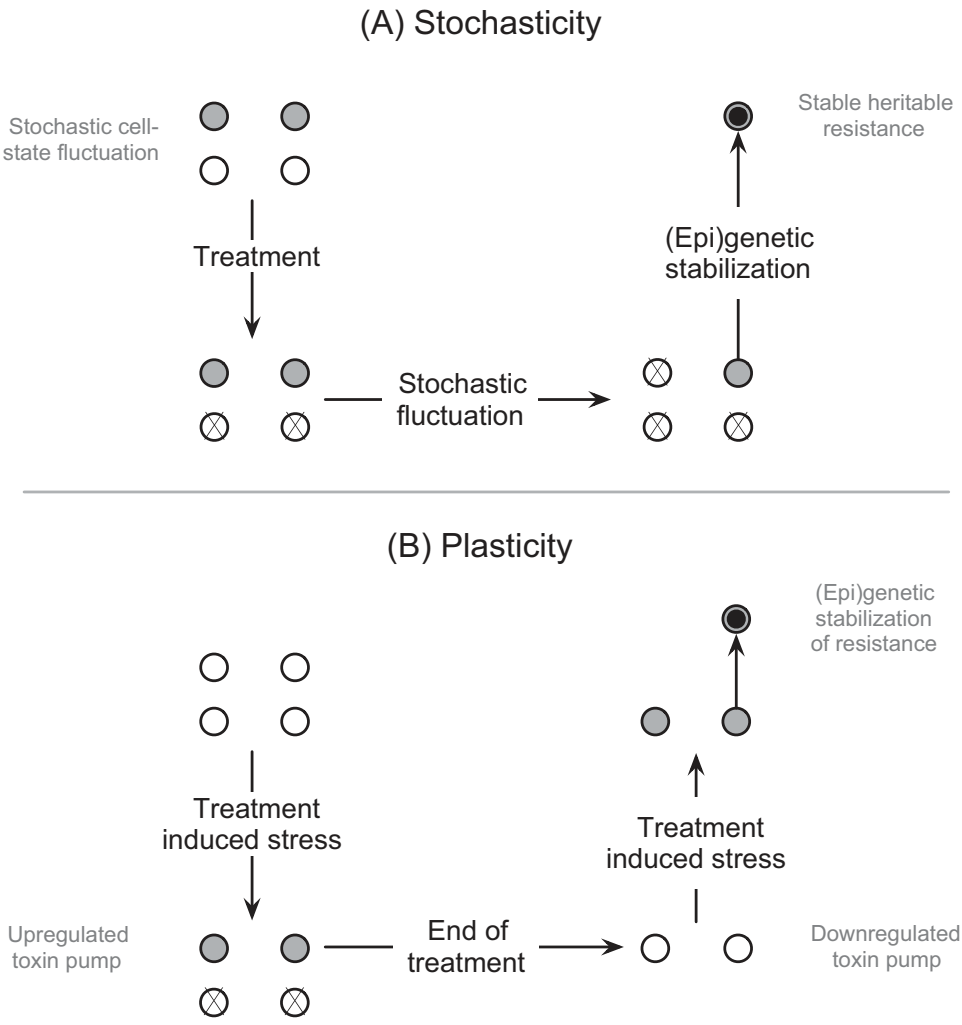

Figure 4. Phenotypes-first processes in treatment resistance. (A) Before treatment, cellular phenotype fluctuates stochastically. For example, the white cells may have relatively low toxin pump activity whereas the shaded cells have higher pump activity. Cells occasionally change between these states. Treatment kills cells with weak toxin pumping but strong pumpers persist. Among the surviving persister cells, some may stochastically flip to low pump activity, which makes them susceptible to the killing action of the treatment. A persisting active pumper may acquire (epi)genetic changes that stabilize the high toxin-pumping trait, changing that cell into a stably heritable progenitor of resistance to treatment. (B) The stress created by the treatment may induce cells to respond in a phenotypically plastic manner. Increasing toxin pump activity is a common cellular response. The treatment kills those cells that respond slowly. If the treatment ends or some cells move into a protected niche that the treatment does not reach, the cells reduce their toxin pump activity. When treatment stress returns, the cells respond again. Eventually, with continued treatment, some of the cells may acquire (epi) genetic changes that stabilize the higher level of toxin pump activity, making these cells stably and heritably resistant to treatment.

triggers may be. In any case, some fraction of the melanoma population transits through a temporary state that confers resistance.

Resistance from temporary persisters

Temporarily resistant cells are often called persisters. That name comes from an analogy with the widely documented persister phenomenon in bacteria [28–30]. When treated with antibiotics, a small fraction of a bacterial population can survive. Initially, bacterial survivors usually do not

transmit a heritable resistance phenotype. Instead, the initial resistance arises from stochastic fluctuations in proteins that control traits such as cell division, general stress resistance, and toxin pumps. Temporarily nondividing cells resist drugs that attack DNA replication. Upregulated toxin pumps clear the drugs from the cells more efficiently.

Nonheritable traits do not provide permanent resistance. They can, however, extend survival time such that heritable changes can subsequently arise to stabilize resistance. For example, melanoma stabilizes initially transient treatment resistance by epigenetic reprogramming [31]. Such reprogramming includes changes in accessible chromatin at TF binding sites and TF occupancy of such sites. Ultimately, the initial nonheritable cellular fluctuations provide the phenotypic variability by which new heritable forms are subsequently created, thus accelerating the rate of adaptation and the rise of resistance. Similarly, phenotypically plastic cellular responses to locally varying conditions or novel stresses create phenotypic variability that often accelerates evolutionary adaptation.

Many recent studies of cancer resistance to treatment emphasize the important role of persister cells. For example, initial resistance of melanoma to immunotherapy may arise by inflammation-induced cellular dedifferentiation [17,19]. Resistance of tissue culture organoids to a variety of drugs can occur through a cellular stress response that suppresses MYC, a master regulator of biosynthesis and metabolism [32]. Suppressed MYC induces an embryonic diapause cell state that arises in development in response to stress. That transient diapause state stops cell division and resists many drugs. Similarly, in colorectal cancer, response to chemotherapy associates with an embryonic diapause state. A small fraction of cells stochastically move into and out of diapause, creating nonheritable variability that primes the evolution of resistance [33].

Carcinogenesis

It is easy to see that drugs provide an extreme challenge. It is perhaps less obvious that the normal daily life of a cell also imposes an extreme evolutionary challenge. Over the short timescale of an individual lifetime, natural selection works powerfully on cells as competitive reproductive entities against other cells [34–37]. By contrast, over the long timescale of reproductive generations, cells within bodies are naturally designed to enhance the reproduction of the multicellular individual rather than their own cellular reproduction. The longer timescale of individual adaptation means that bodies have evolved strong limits on cellular reproduction and survival that mostly repress the opportunity for cellular natural selection. However, the short-term evolutionary force of cellular natural selection is ubiquitous and powerful, favoring cellular evolution that overcomes the body's repressive mechanisms.

From this cellular evolutionary perspective, the body's mechanisms that limit the reproductive rate or survival of cells impose an extreme challenge to short-term cellular evolution. We may not normally think of the physical structure of epithelial tissues as imposing an extreme challenge to cells. However, physical barriers to movement strongly limit the opportunity for a cell to increase its reproductive rate.

Our bodies impose many other limits on cellular proliferation [38]. Programmed cell death limits cellular survival. Immune clearance of cells expressing novel antigens limits the survival of phenotypically variable cells. Checkpoints along the cell cycle limit cellular division.

Thus, we may consider both treatment resistance and carcinogenesis as the evolutionary response to extreme environmental challenge. A previous section outlined the special concepts of evolutionary theory for the origin and spread of phenotypic novelty in response to extreme challenge [1,3]. These concepts provide a unifying theoretical framework for cancer evolution. The examples in this section link carcinogenesis to these broader evolutionary concepts.

Classic cancer genes enhance cellular plasticity

Some genetic mutations commonly associate with particular stages of cancer progression [38–40]. *APC* mutations associate with the earliest steps in colorectal cancer and *RB* mutations initiate retinoblastoma. *KRAS* mutations sometimes occur early in lung and pancreatic cancers. These mutations seem to cause key steps in carcinogenesis. *APC* knockout abrogates normal cell death, whereas *RB* knockout releases checkpoints on the cell cycle. *TP53* knockout stops many checks on cellular integrity, allowing the accumulation of subsequent cellular abnormalities. *KRAS* mutation accelerates cell division (Figure 2).

Recent studies show that classic cancer genes, such as *Kras* and *Tp53*, also enhance cellular plasticity [20,22]. This enhancement of plasticity likely increases evolutionary rate by phenotypes-first processes, driving the adaptation of tumors (Figure 3). The greater evolutionary rate through plasticity may turn out to be the most important role of these mutations in carcinogenesis. A mouse model of lung cancer provides a good example [22]. Mice with an induced *Kras* (K) oncogenic mutation in alveolar type 2 (AT2) cells develop precancerous adenomas. Typically, the precancerous tumors of these type K mice do not progress further. The oncogenic K mutation associates with increased cell division and clonal expansion. However, the size and aggressiveness of the tumor remain limited.

Sometimes the same experimental method for mutation induction causes both a K oncogenic mutation and knockout of *Tp53* (P). The precancerous adenomas in these KP mice progress to advanced adenocarcinomas. In the classical genetic interpretation, the initial K oncogenic mutation induces clonal expansion. Then, within the large cellular target of the expanded clone, knockout of *Tp53* allows a variety of subsequent aberrant genetic changes to accumulate that would normally be removed by the suppressive apoptotic cell death barrier.

This genetic explanation arose from three methods of study. First, bulk DNA sequencing of naturally occurring tumors often linked K mutations to adenomas and linked the combination of KP mutations to full cancer progression. Second, mice engineered with K mutations in lung cells typically limit tumor development to the adenoma stage, whereas mice with KP mutations progress to aggressive cancers. Third, K mutations tend to be point changes that alter protein function, typically associated with increased cell division. *TP53* mutations are knockouts that cause loss of cellular ability to sense aberrant states and induce apoptosis.

New single-cell RNA sequencing studies to measure the diversity of cellular states in the developing tumor have shown that a K mutation increases cellular plasticity, initiating a broad diversity of cellular states in a mouse model of lung cancer [22]. After that initial expansion of cellular diversity, tumors with P mutations continued to broaden the range of cellular states. The expanding range of states are loosely associated with different types of cells in normal development, with different cellular physiologies. That broadening expression of cellular plasticity matches the progression to more aggressive tumors. It seems that *KRAS* and *TP53* mutations drive carcinogenesis by releasing greater phenotypic variety to accelerate the phenotypes-first process of cancer evolution.

Key role of developmental recombination

Evolutionary biology emphasizes that significant phenotypic novelty often originates by developmental recombination, the recombining of parts from different developmental programs to generate a new form [1]. The lung cancer study described in the previous subsection supports this key role of developmental recombination [22]. As lung tumors progressed, a specific pattern of increasing cell-state diversity emerged, consistently repeating across different animals. During the early to middle stages of progression, a transitional and highly plastic cell state

appeared. This highly plastic cell type played a central role in cellular diversification and progression.

Notably, this plastic cell type expressed a cellular program that incorporated features from diverse cell types, including trophoblast stem cells, chondroblasts, and kidney tubular epithelium. This mixture of cell types from different developmental stages and locations supports the key role of developmental recombination and evolutionary biology's broad theoretical framework for the origin of the novel traits that drive cancer progression.

In this lung cancer study, 12 different cell types recurred during tumor progression in different animals. Most of the cellular diversity arose by reversing the normal paths of cellular differentiation during development. Early stages included alternative lung epithelial states. Several states then arose that were similar to primordial gut cells. Toward the end, a state appeared that had mesenchymal features, a form of EMT. The highly plastic central state was a transitional form in the middle of this expanding sequence of cellular phenotypic diversity.

In this sequence, EMT took place late in progression and therefore did not play a key role in generating diversity or driving progression. However, it may be important later in tumor evolution, for example in generating further changes that promote treatment resistance or metastatic spread.

In summary, lung cancer progression follows the phenotypic variety of normal cellular plasticity [22]. That plasticity partially recapitulates normal cellular development or normal cellular responses to altered environments [41]. Recombination of these normal states creates novel forms that play an important role in cancer evolution [22].

Cellular plasticity guides evolutionary trajectories

We have emphasized that classic cancer mutations such as *KRAS* and *TP53* may drive carcinogenesis primarily by promoting cellular dedifferentiation and enhancing phenotypic cellular plasticity. This subsection summarizes another mouse study that links cellular plasticity to lung cancer evolution [21]. In this case, *Tp53* mutations alter normal cellular plasticity during wound healing. These Tp53-induced changes in plasticity guide the evolutionary trajectory of tumors. This trajectory partially follows transitions along the normal route of cellular differentiation in development.

Lung epithelium contains two particularly important cell types. Thin, flat AT1 cells cover much of the alveolar surface, where they facilitate gas exchange. AT2 cells produce surfactant and also act as stem-like cells to repair injury. AT2 cells produce AT1 cells during normal development and during wound healing.

Typically, TP53 promotes AT2 to AT1 differentiation through an intermediate cellular state that resembles a transitional cell type in alveolar injury repair. In a lung cancer model with a *Kras* mutation in AT2 cells, knockout of *Tp53* caused an accumulation of the intermediate repair-like state, increased growth signaling, and divergence in expression from a typical lung cell [21]. This plastic repair-like cell type is a key step along the trajectory toward cancer.

Once again, mutation of a classic cancer gene, *TP53*, alters normal cell-state transitions and cellular plasticity to guide the pathway toward cancer. The repair-like intermediate state may be particularly important because of the intrinsically high plasticity associated with wound healing. Greater plasticity generates more phenotypic diversity and accelerates evolutionary change.

Multicellular plasticity and the tumor microenvironment

Evolving a new tumor microenvironment requires novel interactions between different cell types. Enhanced cellular plasticity of the interacting cells can accelerate the evolution of novel cell–cell interactions. Pancreatic cancer provides an example [20]. Initially, *Kras* mutation causes pancreatic epithelial cells to transit through a variety of cell states. These recurring cell states by themselves do not cause tissues to change from normal appearance or function. However, some of these Kras-induced states play an important role later in carcinogenesis. Other Kras-induced states increase cellular plasticity, triggering the early steps in carcinogenesis.

The chromatin in these initial high-plasticity cells opens up around genes involved in cell–cell communication [20]. With potentially greater expression of ligands and cell-surface receptors, the modified pancreatic epithelial cells may respond plastically to a wide range of signals from other cells. For example, inflammation induces rapid remodeling of interactions between immune cells and the highly plastic epithelial cells. The rapid changes in the cell–cell interactions suggest that the epigenetically defined plasticity in cellular communication guides the evolutionary trajectory of the tissue microenvironment. In this case, inflammation transforms the initially benign *Kras* mutants into precancerous lesions that depend on local interactions with immune cells, creating an early tumor microenvironment.

In the initial Kras-induced repertoire of states of the precancerous tissue, different states express unique sets of ligands and receptors [20]. That variability in cell–cell communication allows exploration of a variety of microenvironmental combinations of interacting cellular states. In other words, the phenotypic variability at the level of aggregate cell–cell interactions in local microenvironments may determine how plasticity guides the evolutionary trajectory of tumors. For example, when inflammation transforms the initial repertoire of Kras-induced epithelial states in premalignant tissue into an early step in cancer progression, positive feedback arises between particular epithelial states and immune cell states.

On the epithelial side, some Kras-induced cell states express the immune signaling molecule IL-33 which triggers T cells to respond by secreting IL-4. The *Kras* mutant epithelial cell states that initially secreted IL-33 also tend to express a receptor for IL-4, implying positive feedback. Several lines of evidence suggest that this positive feedback triggers rapid and repeatable tissue transformation in response to inflammation. Early in the responding inflamed tissue, some of the rare *Kras* mutant epithelial cells that express IL-33 and some of the rare T cells that respond are in close spatial proximity. Subsequently, many T cells that respond to IL-33 also express IL-4, to which the initial IL-33 secreting epithelial cells respond.

In a newly engineered mouse model [20], IL-33 expression could be turned off in the *Kras*-mutant epithelial cells, blocking potential feedback with immune cells and allowing a test of whether that feedback is important. When IL-33 is suppressed, both the epithelial and immune cell states that arose in response to inflammation differed greatly from the cell-state patterns normally observed in the emerging precancerous microenvironment. Thus, the epithelial–immune positive feedback via IL-33 and IL-4 may play a key role in the normally observed response. Overall, plastic cellular variability triggered by *Kras* mutation creates novel cell–cell interactions that drive the evolution of the tumor microenvironment.

Concluding remarks

Cancer evolution follows the general principles of evolutionary biology, as it must. The newly observed empirical detail of cancer studies provides great insight into these broad evolutionary principles.

Outstanding questions

For the first steps in cancer, what types of challenge favor the origin of novel traits by mutation versus cellular plasticity? Do specific carcinogenic challenges such as smoking and UV radiation favor particular generators of novelty?

How often do initiating mutations contribute to cancer evolution by enhancing cellular plasticity, thereby increasing the amount of nonheritable phenotypic variation?

What is the origin of a crucial high-plasticity cell state? Does it arise by de-differentiation toward a cell type that occurs in normal development, or by recombination of different cellular or developmental programs?

Is a high-plasticity type an important early stage in the evolutionary trajectory of a tumor and a burden to its growth later on? Do evolutionary trajectories follow a pathway of early high variability early and later low variability, or does continuing resistance or carcinogenesis require retention of the high-plasticity type in later evolutionary stages – in effect, a necessary stem-like cell type that regenerates a diversity of other essential types? Is metastatic spread distinct from local tumors because highly plastic stem-like cells are needed for dispersal?

Does cellular plasticity trigger novel synergism between cell types in the tumor microenvironment? What stabilizes the cell–cell interactions discovered by highly plastic cell types?

The body has many features designed to suppress cancer. Apoptosis or checkpoints on the cell cycle act as tumor suppressors. To that list, can we add the repression of phenotypic plasticity as a key tumor suppressor? Is plasticity tightly regulated in normal terminally differentiated tissue, and is only relaxed when this becomes necessary? Can we think of plasticity repression as a designed feature of multicellularity? If so, how does plasticity repression vary between tissues and between organisms? In tissues at greater risk of cancer, is plasticity more strongly repressed?

On the cancer side, new technology measures the state of individual cells. We now see that classic cancer gene mutations, such as *KRAS* and *TP53*, greatly increase cellular plasticity. Inflammation or stress can also induce greater cellular plasticity. Variable cells transit between several states, many of which recapitulate the normal cellular plasticity of development. These states guide the evolutionary trajectory of treatment resistance and carcinogenesis.

In cancer evolution, novel traits seem to first emerge through nonheritable phenotypic changes, which are later genetically stabilized. A phenotypes-first process drives the origin of traits, in contrast to the classic genes-first view that new traits first arise by genetic mutations. Importantly, initial mutations in genes such as *KRAS* enhance the overall cellular rate of this phenotypes-first process but often may not themselves directly encode the novel traits that drive cancer.

On the evolution side, recent theory claims that novel traits typically arise by phenotypes-first processes that often follow the contours of normal developmental or phenotypic plasticity. Variants of this theory are old but never gained much traction until West-Eberhard's great reformulation and synthesis, *Developmental Plasticity and Evolution* [1]. The theory remains controversial [4,42]. However, each year provides more supporting evidence. The new cancer studies give the greatest insight.

Based on the recent cancer studies, the updated hallmarks of cancer add the unlocking of phenotypic plasticity as a key step in carcinogenesis [43]. We agree [6] but go further, emphasizing that nonheritable phenotypic variation may be the primary driver of the novel traits that create cancer and promote treatment resistance. Evolutionary theory supports this claim that novelty typically comes from plasticity, a hallmark of evolution [1]. Going forward, we may think of the hallmarks of cancer and the hallmarks of evolution as synergistic conceptual frameworks.

This synergism between cancer studies and evolutionary concepts unifies several ongoing research trends into a broad framework for the origin of novelty in evolution. And it raises several unresolved questions about cancer (see Outstanding questions).

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Declaration of interests

The authors declare no competing interests.

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