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Interpreting cancer genetics through a two-step "evolutionary cascade hypothesis": bridging neutral and selective perspectives

Alessandro Ottaiano[1*], Mariachiara Santorsola[1], Francesco Sabbatino[2], Roberto Sirica[3], Francesco Caraglia[4], Anna Ceccarelli[5], Vincenza Granata[1], Ines Simeone[6], Silvia Zappavigna[4], Massimiliano Berretta[7], Giovanni Savarese[3] and Michele Caraglia[4,8*]

Abstract

Background DNA mutations are the fundamental engines of cancer, driving its initiation and progression. The forces that fuel malignancy are also the architects of evolution, shaping life through genetic variations. Mutations, in fact, can emerge naturally from endogenous processes, such as oxidative DNA damage or errors in replication, as well as induced by external factors, including cosmic radiation and chemical carcinogens.

Main body A key question in cancer research is whether tumor evolution is primarily governed by selective bottlenecks, neutral evolution, or dynamic genetic plasticity. In this work, we examine cancer as a disease driven by evolutionary processes rooted in fundamental biological requirements, including sustained proliferation and nutrient utilization. We hypothesize that the accumulation of mutations activates an evolutionary switch, enabling tumor cells to acquire an enhanced capacity for survival, adaptation, and growth at rates far exceeding typical evolutionary timescales. We propose the "evolutionary cascade hypothesis," a unifying framework that integrates these models into a coherent sequence. At its core lies the failure of DNA repair mechanisms, representing a critical transition in cancer progression. This shift marks the transition from an initial non-Darwinian, neutral phase to a Darwinian, more deterministic phase.

Conclusions As predictive models of tumor evolution advance through genomic big data and artificial intelligence-driven analysis, the future of cancer treatment may extend beyond targeting individual mutations to disrupting the underlying evolutionary mechanisms that sustain malignancy. This paradigm shift could redefine therapeutic strategies and ultimately improve patient outcomes.

Keywords Cancer evolution, Cancer genetics, DNA mutations, Genomic instability, DNA repair

*Correspondence:
Alessandro Ottaiano
a.ottaiano@istitutotumori.na.it
Michele Caraglia
michele.caraglia@unicampania.it

Full list of author information is available at the end of the article

Background

Mutations of DNA occur physiologically, driven by a variety of natural endogenous and exogenous factors. Among the endogenous sources are reactive oxygen species (ROS), bioproducts of cellular metabolism such as superoxide anions, hydrogen peroxide, and hydroxyl radicals, which can damage DNA through oxidative stress [1–3]. Additionally, errors spontaneously occur in DNA replication during cell division and contribute to mutation accumulation [4, 5]. Natural exogenous factors include cosmic radiation (e.g., solar ultraviolet [UV] rays and high-energy particles from space) [6, 7] and chemical carcinogens such as polycyclic aromatic hydrocarbons, which are bioproducts of combustion processes, including the burning of organic matter [8, 9] (Fig. 1). These mutagenic events induce structural alterations in DNA, such as point mutations, insertions, deletions, and chromosomal rearrangements, which may disrupt genomic stability and contribute to carcinogenesis.

Throughout evolution, life has persisted due to a delicate balance between mutagenic pressures and the remarkable DNA repair mechanisms. Enzymatic pathways such as base excision repair, nucleotide excision repair, mismatch repair, and homologous recombination ensure the fidelity of genetic information [10–12]. Without these efficient resilience systems, life as we know would be unsustainable, as genetic instability would preclude organism survival based upon the conservation of the genetic information that is indispensable for the functionality of the cell engine. However, mutations serve as a fundamental driver of evolution by introducing phenotypic plasticity, allowing organisms to adapt to ever-changing environments [13–15]. In this light, mutations are not merely detrimental errors but also essential contributors to biodiversity and survival. Cancer cells exhibit genetic heterogeneity, sometimes in excess, with a strikingly similar adaptive capability [16]. Through the acquisition of mutations, they adapt rapidly and effectively to their microenvironment. This includes responding to hypoxia, evading immune surveillance, and resisting therapeutic interventions.

The progression of cancer from a benign state to a fully malignant phenotype—marked by uncontrolled proliferation, invasion, metastasis, and colonization of distant organs—can be interpreted through an evolutionary point of view [17]. Within this framework, tumorigenesis is driven by the accumulation of genetic mutations, which contribute to the heterogeneity of cancer [18].

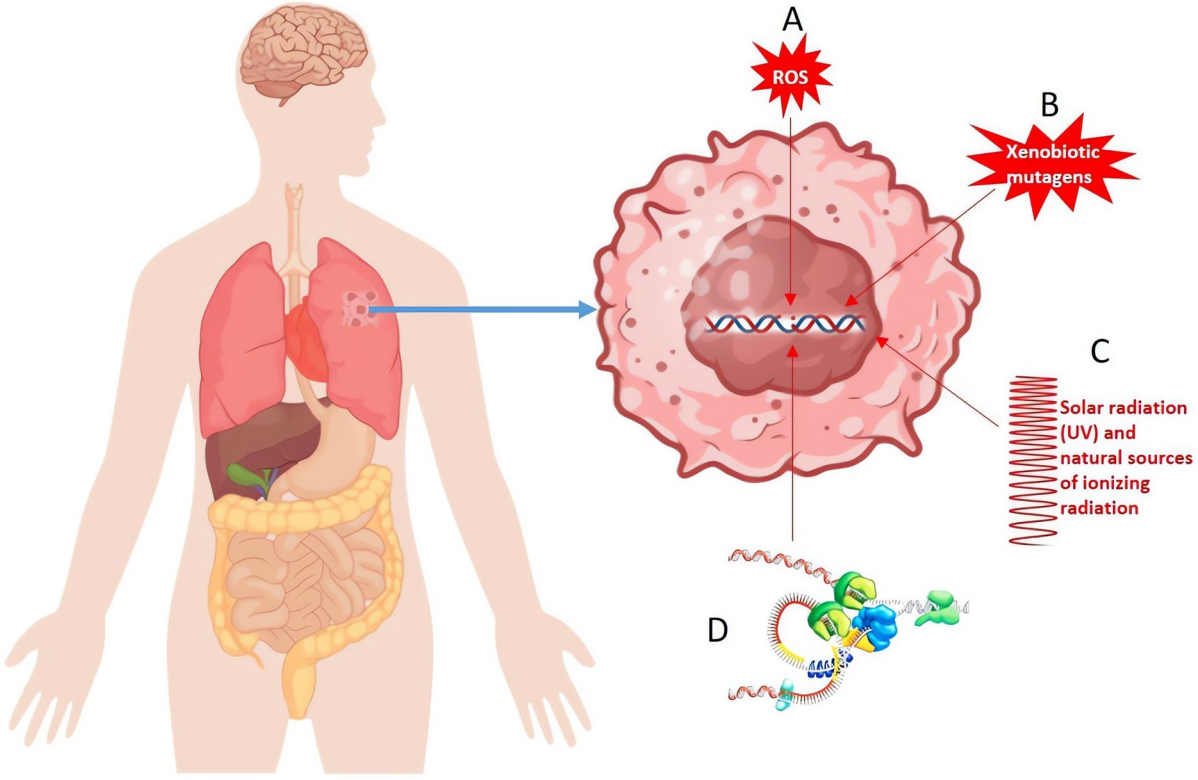

Fig. 1 Sources of endogenous and exogenous DNA damage in multicellular organisms. In a complex multicellular organism, the DNA of any cell can be naturally damaged by: **A**) natural xenobiotic mutagens, **B**) ROS (reactive oxygen species), which can arise from mitochondrial respiration and enzymatic reactions, **C**) solar ultraviolet (UV) radiation and natural ionizing radiation, **D**) spontaneous errors at the replication fork. These factors are not always detrimental but can contribute to adaptive genetic plasticity

These mutations play a pivotal role in fostering cellular diversity, enabling cells to adopt a wide range of phenotypic states. The accumulation of mutations is the result of an elevated mutation rate—typically exceeding 1 mutation per megabase (mut/Mb) in the vast majority of cases, regardless of histological origin [19]—significantly higher than the mutation rate observed in normal eukaryotic cells, which is approximately 0.01 mut/Mb [20, 21].

This review seeks to present an innovative perspective on cancer, framing it not merely as the progression of a disease but as a pathological manifestation intrinsically linked to the fundamental mechanisms of evolution. Without engaging in the longstanding and unresolved debate between "neutralists" and "selectionists" [22] regarding evolutionary models of biological systems, we propose a unifying two-phase model. In this framework, neutral processes predominate during an initial "non-Darwinian" phase, persisting until the activation of an evolutionary engine, after which selective ("Darwinian") forces become the principal drivers of malignant tumor progression.

Genomic stability and the evolutionary switch in cancer

Biological organisms, from the simplest unicellular entities to the most complex multicellular systems, evolve to preserve and optimize two fundamental characteristics: the ability to replicate (i.e., transfer genetic information) and the ability to extract and use nutrients from their surrounding environment [23–25]. These two basic prerogatives constitute what is often described as the evolutionary purpose of biological entities. However, the underlying reasons why living biological systems universally tend to acquire and optimize these two capacities remain elusive [26, 27]. From a scientific perspective, the mechanisms driving replication and nutrient use can be explained through the principles of natural selection and the conservation of energy [28, 29]. Yet, this explanation does not fully address the deeper question of why these two traits emerged as the cornerstones of life. While the deeper organizing principles of life have been widely discussed, from Aristotle to modern studies on multicellularity [30], these remain largely conceptual. Importantly, in multicellular organisms, replication and nutrient acquisition are tightly regulated to ensure cooperation among cells and maintain functional integrity [31, 32]. Since all cells undergo physiological turnover, the control of DNA stability is pivotal for the proper functional expression of these biological characteristics. DNA stability is maintained through a complex molecular machinery. Key players in preserving genomic stability include genes such as *p53*, *BRCA1*, *BRCA2*, *RAD51*, *ATM*, *ATR*, *MLH1*, *MSH2*, *XRCC1*, *WRN*, among others. Together, they form an extensive network of molecular systems, composed of thousands of genes [33–38], which, over evolutionary time, have enabled the remarkable diversity of life on Earth. Table 1 presents a selection of key genes renowned for their roles in DNA repair. In Fig. 2, these genes are organized into functional groups corresponding to distinct DNA repair pathways, illustrating the impact of their dysfunction on genomic integrity.

Cancer, as a biological system, demonstrates a remarkable ability to enhance both replication and nutrient use, often to the detriment of the host organism. By analyzing the evolutionary trajectories that drive these processes in cancer, we can consider the disease as a pathological context in which these fundamental characteristics of life are co-opted and amplified. Cancer is inherently a multigenic disease, and genetic analysis of malignant tumors—particularly metastatic lesions—reveals alterations in numerous genes. Notably, many of these genes are primarily involved in maintaining DNA stability, both at the structural level and through the regulation of gene expression [39–41]. Modern technologies, such as next-generation sequencing (NGS), provide unprecedented insights into the genetic landscape of tumors, revealing a snapshot of their mutational burden. Although the mutational rate physiologically increases with age cancer genomes display a significantly higher mutational burden compared to healthy tissues [42, 43]. This underscores the critical role of genomic instability in cancer development.

In Fig. 3, we present a two-step model of cancer genetic evolution in which the critical event is the activation of an evolutionary driver. In other words, we hypothesize that when the evolutionary switch is ON, cancer cells rapidly acquire traits that enhance their ability to proliferate and optimize nutrient utilization, effectively advancing toward fundamental biological evolutionary goals. These cells may even gain the ability to colonize biologically distinct environments within the same organism, such as distant organs or tissue types, a hallmark of metastasis [44]. This capacity underscores the striking adaptability and plasticity of cancer cells, drawing a parallel to the evolutionary mechanisms observed in natural selection.

Among the most frequently studied and altered genes in cancer, *p53* stands out as a key regulator. P53 is a critical factor in preserving DNA stability by regulating cell cycle arrest, DNA repair, and apoptosis in response to genomic stress, preventing the propagation of damaged or mutated cells [38]. Mutations in *p53* are among the most prevalent across various cancer types, irrespective of histology (an observation that is far from coincidental). Evidence suggests that the alteration of *p53* or other genes essential for DNA repair constitutes a critical disruption of cellular homeostasis [45] and is among the most frequently shared abnormalities between primary tumors and matched metastases [46–50]. These alterations are often a critical event in early tumorigenesis,

Table 1 Molecular landscape of DNA repair: genes and their functions

Gene name	DNA repair group	Specific role
ALKBH2	Direct DNA Repair (DDR)	Repairs alkylated bases in double-stranded DNA, mainly 1-methyladenine (1-meA) and 3-methylcytosine (3-meC).
ALKBH3	DDR	Similar to ALKBH2, but primarily acts on single-stranded DNA and RNA
APEX1	Base Excision Repair (BER)	Cleaves apurinic/apyrimidinic sites.
ATM	Homologous Recombination (HR)	Activates repair proteins through phosphorylation.
ATR	HR	Responds to replication stress and single-strand breaks.
BRCA1	HR	Coordinates HR repair and checkpoint activation.
BRCA2	HR	Mediates RAD51 filament formation for strand invasion.
CSA	Nucleotide Excision Repair (NER)	Coordinates repair in transcription-coupled NER.
CSB	NER	Facilitates lesion recognition in actively transcribed genes.
DNA-PKcs	Non-Homologous End-Joining (NHEJ)	Catalytic subunit, phosphorylates proteins to mediate repair.
ERCC1	NER	Partners with XPF to perform 5′ incision near the damage site.
FEN1	BER	Processes flaps during long-patch BER.
KU70	NHEJ	Recognizes and binds to DNA double-strand break ends.
KU80	NHEJ	Stabilizes the DNA ends and recruits repair machinery.
LIG1	BER/MMR (MisMatch Repair)	Ligates nicks after repair.
LIG4	NHEJ	Ligates the DNA ends in double-strand break repair.
MGMT	DDR	Removes alkyl groups from the O6 position of guanine, preventing O6-methylguanine mispairing with thymine during replication.
MLH1	MMR	Part of the MutL complex, coordinates repair of mismatches.
MRE11	HR	Part of the MRN (MRE11-RAD50-NBS1) complex, processes DNA double-strand breaks.
MSH2	MMR	Recognizes and binds to DNA mismatches.
MSH3	MMR	Partners with MSH2 to repair insertion/deletion loops.
MSH6	MMR	Forms a complex with MSH2 to detect single base mismatches.
MUTYH	BER	Repairs misincorporation of adenine opposite 8-oxoguanine.
NBS1	HR	Recruits ATM to DNA breaks, part of MRN complex.
NTHL1	BER	Removes oxidized pyrimidines like thymine glycol.
OGG1	BER	Removes 8-oxoguanine lesions caused by oxidative stress.
p53	Multiple repair pathways	Acts as a DNA damage sensor; regulates cell cycle checkpoints and apoptosis to maintain genomic stability.
PARP1	BER	Senses single-strand breaks and recruits repair machinery.
PMS2	MMR	Forms a heterodimer with MLH1, initiates excision.
POLD1	BER/NER	Synthesizes DNA during repair synthesis.
POLQ	Alternative End-Joining (alt-EJ)	Fills gaps during repair with low fidelity.
RAD50	HR	Maintains stability of broken DNA ends.
RAD51	HR	Facilitates strand invasion and exchange during repair.
UNG	BER	Excises uracil from DNA caused by deamination of cytosine.
XLF	NHEJ	Stimulates LIG4 activity in end-joining.
XPA	NER	Damage recognition and recruitment of repair factors.
XPB	NER	Helicase activity; unwinds DNA around the lesion.
XPC	NER	Initiates global genomic repair by recognizing DNA damage.
XPD	NER	Helicase activity in transcription-coupled repair.
XPF	NER	Catalyzes 5′ incision with ERCC1.
XRCC4	NHEJ	Stabilizes DNA ligase IV at the repair site.

conferring a significant evolutionary advantage to the nascent tumor cell [51]. While p53 is undoubtedly one of the crucial factors in this process, it remains uncertain which and how many of the DNA repair factors must be altered to surpass a critical threshold, particularly in cases where p53 remains intact. Although definitive scientific evidence regarding the relative importance of each regulatory mechanism is still lacking we can nonetheless measure the consequences of their disruption, namely an increased mutational burden [52]. Ultimately, by disabling the key checkpoints of genomic integrity, the cell circumvents normal regulatory controls and acquires the ability to tolerate additional mutations. This capacity fosters adaptation in the face of escalating genetic instability, thereby propelling the tumor's evolutionary progression.

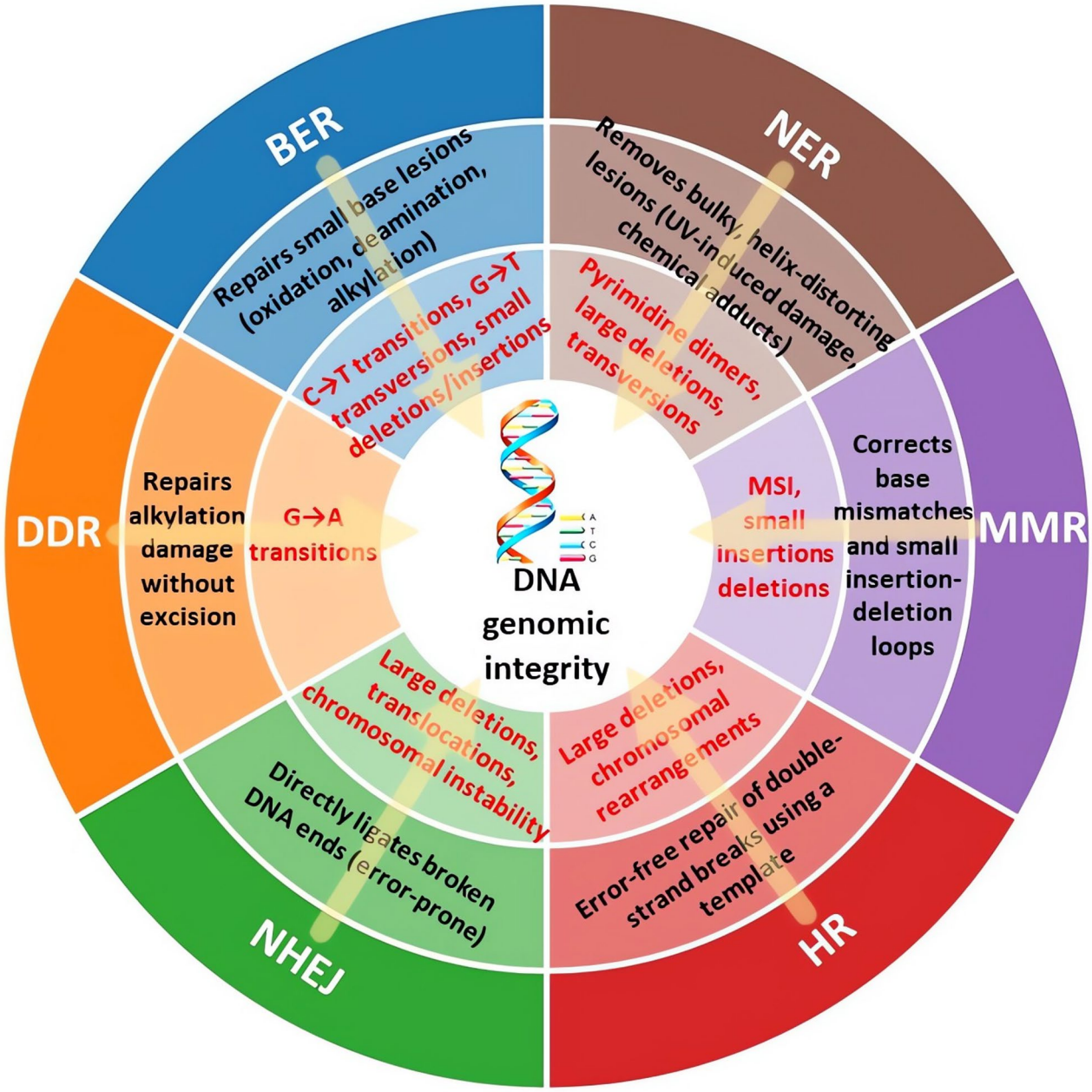

Fig. 2 Overview of genetic consequences associated with defective DNA repair mechanisms. The figure provides a schematic overview of the major DNA repair pathways and the characteristic genetic alterations that arise when these mechanisms are impaired. In each sector, white text denotes the type of DNA repair, black text describes the general function of the pathway, and red text highlights the alterations associated with defective repair. Each DNA repair pathway contributes, through distinct but complementary mechanisms, to the maintenance of DNA genomic integrity, which is represented as the central hub of the figure. The central hub symbolizes the attainment of DNA genomic integrity and contains a simplified representation of a DNA double helix, with the four nucleotide bases indicated using different colors. BER, Base Excision Repair; DDR, Direct DNA Repair; HR, Homologous Recombination; MMR, Mismatch Repair; MSI, Microsatellite Instability; NER, Nucleotide Excision Repair; NHEJ, Non-Homologous End Joining; UV, Ultraviolet

Low- and high-mutation tumors within a unified evolutionary cascade framework

As discussed above, cancer tissue is characterized by increased genetic plasticity and a mutation rate higher than that of normal tissues [20, 21]. From a strict biological standpoint, once the evolutionary engine is activated, all tumors can be regarded as highly evolvable systems. Nonetheless, the oncology community has pragmatically introduced operational thresholds to distinguish "low" versus "high" mutational states, a distinction that has proven clinically meaningful, particularly in the context of responsiveness to immunotherapy [53]. Within this accepted framework, we propose that the evolutionary cascade hypothesis does not qualitatively change

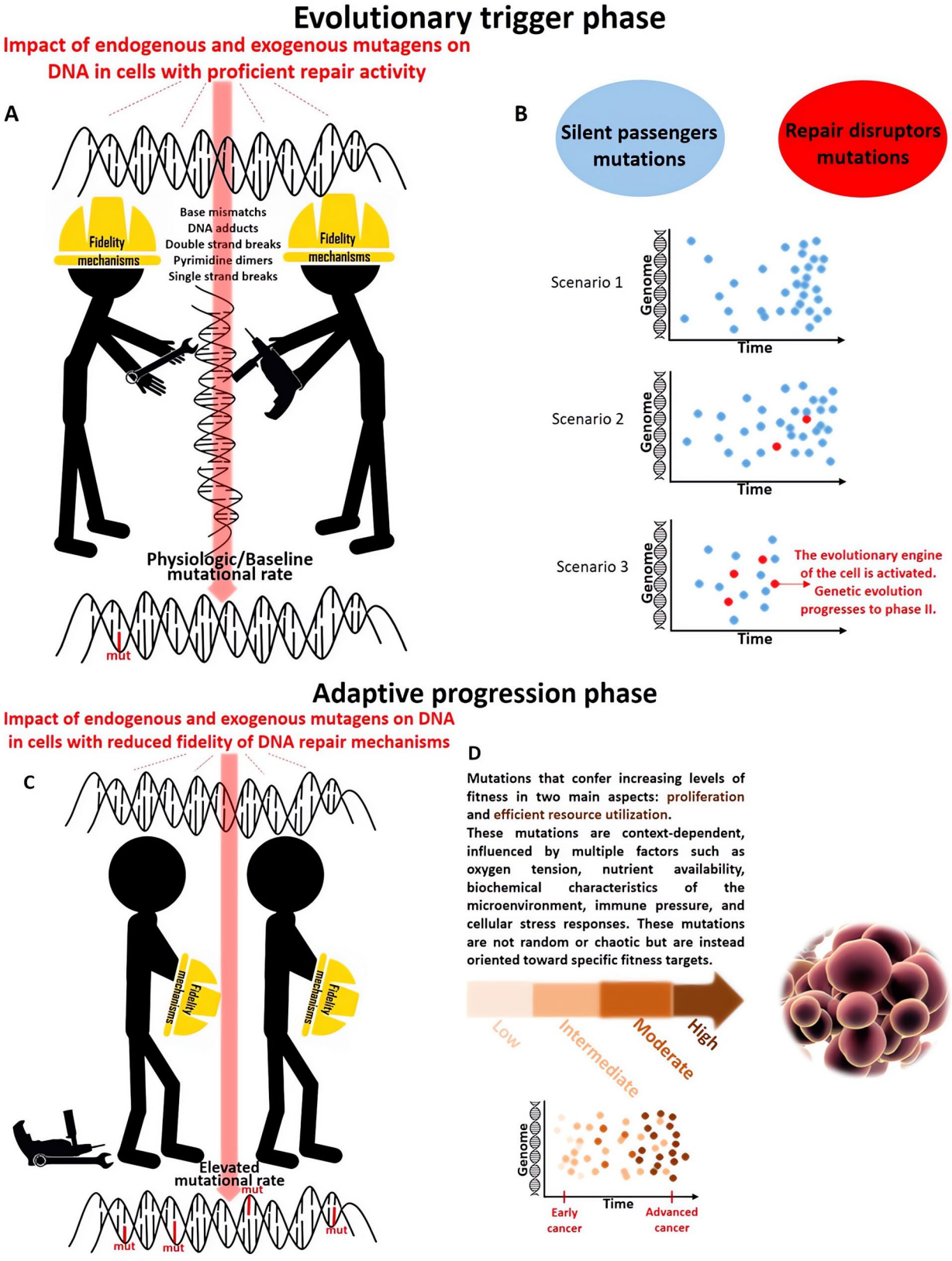

Fig. 3 (See legend on next page.)

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Fig. 3 A two-step theory of carcinogenesis: from DNA repair–constrained stability to evolutionary acceleration. DNA is frequently impacted by both endogenous and exogenous mutagens. As a result, one of the most fundamental, if not the primary, resilience mechanisms that has enabled life to persist is DNA repair. **A**) Genetic information must be stably maintained and faithfully transmitted to progeny. DNA repair mechanisms act as guardians, ensuring that the mutation rate remains reasonably low in normal cells. **B**) Mutations are inevitable and initially occur as stochastic events. The mutational burden of a cell is shaped by two critical variables: the extent of DNA damage incurred and the efficiency of DNA repair. Three distinct scenarios are presented regarding the effects of mutations. Scenario (1) The genome accumulates numerous "silent passenger mutations" that do not significantly impair DNA repair mechanisms. Scenario (2) The genome acquires only two relevant mutations, neither of which substantially compromises DNA repair. Scenario (3) Paradoxically, the genome sustains fewer mutations than in Scenarios 1 and 2, but these affect four key genes involved in fidelity mechanisms. In this last scenario, an "evolutionary engine" is activated. The affected cell acquires such a high degree of genetic plasticity that it rapidly achieves key evolutionary targets, outcompeting other cells in the hierarchy of life. However, this comes at a cost: the cell loses a fundamental rule of multicellular cooperation—the regulation of its own evolutionary potential—threatening its ability to function within a multicellular community. This cell and its progeny transition into a second phase: the adaptive progression phase. **C**) At this stage, DNA repair mechanisms are no longer functional, leading to a significantly elevated mutation rate compared to normal cells. **D**) A Darwinian phase begins, during which the emerging neoplastic cell pursues two fundamental evolutionary objectives: proliferation and optimal resource utilization. This phase is intensely selective, as the cell encounters a multitude of microenvironments, each imposing distinct selective pressures. Consequently, its genome accumulates increasingly refined mutations, enhancing its adaptability to even the most hostile or fluctuating conditions. Evolutionary timescales are profoundly distorted, accelerating at an extraordinary pace

across low- versus high-tumor mutational burden (TMB) tumors, but rather quantitatively and kinetically adapts to these different genomic landscapes. In tumors with low mutation burden and relatively preserved genomic stability, selective pressures act on a limited pool of variants. We hypothesize that the transition point—i.e., activation of the evolutionary engine through failure of DNA maintenance pathways—represents a more gradual inflection. Conversely, in tumors with high mutation burden and pronounced genetic instability—such as mismatch repair–deficient or POLE-mutant cancers [54]—the cascade may unfold through a more pronounced transition between phases, characterized by a steeper increase in mutation rate and a denser mutational background in which selection continuously acts upon a vast repertoire of variants. High-TMB tumors still undergo strong clonal selection despite their apparent genetic chaos. Thus, we suggest that TMB modulates the tempo and resolution of the evolutionary cascade rather than its fundamental logic. We believe this perspective helps reconcile an apparent paradox in cancer biology: tumors with extremely high mutation burdens may appear evolutionarily "disordered," yet they often converge toward reproducible phenotypic solutions (immune escape, metabolic rewiring, therapy resistance). This convergence is precisely what the evolutionary cascade hypothesis predicts once the evolutionary engine is engaged, regardless of the absolute number of accumulated mutations.

The evolutionary engine of cancer: distinguishing drivers from passengers

Once a cell—or a group of cells—escapes the stringent control mechanisms that govern multicellular systems, it acquires a high evolutionary potential. This trait is fundamentally incompatible with the balanced coexistence required within a regulated multicellular context. This transition raises a central and intuitively fascinating scientific question: once a cell gains this evolutionary capacity, which mutations are essential for its progression to a malignant phenotype? This question is central to the pursuit of identifying the so-called "driver mutations" [55]. These pivotal mutations hold substantial therapeutic promise, as they represent actionable targets for medical intervention. At the same time, the intrinsic plasticity of cancer cells enables them to continuously acquire and stabilize mutations that support their core evolutionary objectives, including proliferation and optimized nutrient use. Driver mutations propel cancer progression, whereas irrelevant mutations—termed "passenger mutations"—arise as random byproducts of the tumor's accelerated evolutionary dynamics not significantly affecting tumour properties and thus not working as actionable targets. Although passenger mutations do not directly contribute to malignancy, they can create confusion in the detection of functionally significant genetic alterations, complicating cancer genetic analyses. These mutations result from stochastic events occurring at high frequency, effectively creating a background of genetic noise [56]. Although not necessarily advantageous to the tumor, the increased occurrence of these random events provides the raw material for rapid tumor evolution—a process significantly amplified when the cancer's evolutionary machinery is fully activated. A key driver of this amplification is the loss of p53 function, which disrupts genomic integrity and allows cells to accumulate a cascade of stochastic mutations. A critical challenge in cancer research is deciphering the sequence of events that follow the loss of *p53* as well as other pivotal mutations. These subsequent events are instrumental in driving clinically relevant phenotypes, such as invasion, metastasis, and therapeutic resistance. Gaining a deeper understanding of these dynamics is essential for developing more effective and targeted cancer therapies.

A two-step model of cancer evolution provides a robust framework for understanding why driver mutations can be detected in normal tissues, particularly in the context of aging or environmental stress, without leading to malignancy [57]. We propose a model in which the initial

step involves the activation of an "evolutionary engine" at the culmination of a non-Darwinian phase characterized by the progressive accumulation of defects in DNA repair and genome maintenance mechanisms. We define this activation as the transition point at which failure of genome maintenance enables sustained mutational plasticity. This early phase is hypothesized to generate the genetic diversity required for a subsequent Darwinian phase, in which selective pressures act on this diversity to favor the emergence and fixation of alterations that promote malignant transformation. Within this framework, activation of the evolutionary engine represents a necessary precondition for the transition from normal tissue to malignancy.

Distinguishing between irrelevant genetic events and those that drive tumor progression remains a critical yet challenging task in understanding the mechanisms of cancer development. Once a cancer cell acquires evolutionary potential—marked by heightened mutational plasticity and adaptability—random mutations are subjected to selective pressures. At this juncture, "late" tumor genetics becomes highly context-dependent, influenced by factors such as the tumor microenvironment, hypoxia, nutrient availability, and immune responses. Identifying the relevance of mutations typically involves large-scale genomic studies comparing tumor genomes across patients and cancer stages even in a dynamic fashion during the different treatments to which the patients are subjected. Bioinformatic tools like MutSig and Oncodrive [58] are pivotal in distinguishing driver mutations from passenger mutations by analyzing mutation frequency and their functional impact.

To refine the understanding of mutation relevance, several approaches have been proposed. Mutation frequency analysis suggests that mutations recurring frequently in independent tumors of the same type are more likely non-stochastic, as their recurrence implies selective pressure [59]. Functional impact scoring methods, such as SIFT (Sorting Intolerant From Tolerant), PolyPhen (Polymorphism Phenotyping v2), and CADD (Combined Annotation Dependent Depletion), evaluate the consequences of mutations, with non-stochastic mutations often disrupting critical protein domains or regulatory elements, while stochastic mutations typically show lower functional impact [60, 61]. SIFT predicts whether an amino-acid substitution is likely to affect protein function by evaluating the evolutionary conservation of the affected residue across homologous protein sequences; substitutions at highly conserved positions are more likely to be deleterious. PolyPhen-2 estimates the potential impact of missense variants by integrating sequence conservation, protein structural features, and known functional domains, classifying variants as benign, possibly damaging, or probably damaging. CADD is a genome-wide scoring system that integrates multiple annotations (including evolutionary constraint, regulatory information, and functional genomic data) into a single metric, ranking variants according to their predicted deleteriousness relative to all possible substitutions in the human genome. Clonal dynamics, studied through longitudinal tumor evolution, offer additional insights, as mutations linked to rapid clonal expansions or reduction, the latter due to the different treatments, tend to be non-stochastic, while those persisting without a significant growth advantage may be stochastic [62]. Environmental factors, including hypoxia and immune responses, also play a role, with computational models integrating microenvironmental data to reveal mutations favored under specific conditions [63, 64]. Moreover, advancements in single-cell genomics and lineage tracing allow for the reconstruction of phylogenetic trees, helping to differentiate mutations that arise randomly from those selected for adaptive benefits [65, 66].

Measuring the impact of passenger mutations

In our framework, passenger mutations accumulate predominantly when the "evolutionary engine" is ON, generating a dense background of genetic variation that can act both as (i) phylogenetic markers of clonal history and (ii) a potential collective fitness burden ("deleterious load"). Measuring the impact of these mutations is inherently complex, and no general consensus has yet been reached. To avoid systematic over- or underestimation of their role, the impact of passenger mutations should be assessed by explicitly separating (A) evolutionary inference of neutrality or selection from (B) direct estimation of phenotypic or fitness consequences, while carefully controlling for major confounders such as mutation rate, copy-number alterations, tumor purity, and sampling time.

A first approach consists of adapting cancer-specific dN (nonsynonymous substitutions)/dS (synonymous substitutions) frameworks to quantify selection by comparing nonsynonymous versus synonymous mutation rates while accounting for gene- and context-specific mutational processes [67]. These analyses consistently show that most coding mutations behave close to neutrality at the genome-wide level, with positive selection confined to a limited set of driver genes. Importantly, this approach provides a principled way to avoid overcalling passenger mutations as functional merely because of their numerical abundance. The same class of methods can also detect signals of purifying selection in specific tumor subsets (e.g., those with low mutational burden), thereby preventing the opposite bias of assuming universal neutrality when some passenger mutations may instead be selected against [68].

In addition, neutral evolution in bulk sequencing data can generate characteristic variant allele frequency (VAF) distributions, often approximating a power-law, which have been used to infer whether subclonal mutations are consistent with neutral dynamics [69]. However, such inferences are highly sensitive to tumor purity, copy-number alterations, and subclonal architecture. Consequently, VAF-based neutrality tests should be interpreted only after rigorous correction for these variables, as failure to do so may lead to spurious over- or underestimation of passenger effects driven by technical artifacts [70].

Passenger mutations can also be exploited as lineage markers to characterize clonal dynamics without assuming functional relevance. High-resolution lineage tracing and single-cell sequencing approaches enable the reconstruction of tumor phylogenies in which most passenger mutations serve as temporal markers rather than effectors. This strategy provides an unbiased view of clonal expansions and contractions over time (e.g., during tumor progression or under therapeutic pressure), allowing functional associations with passenger subclasses to be tested only at a subsequent stage [71].

From a phenotypic perspective, a practical way to estimate passenger impact is to quantify the burden of predicted damaging passenger mutations, for example by aggregating deleteriousness scores across the genome [72]. Such measures should be normalized for mutational opportunity and stratified by underlying mutational processes. Both simulations and analyses of human cancer data indicate that passenger mutations can accumulate while partially escaping purifying selection. Crucially, passenger load must be evaluated within mutational-burden strata to avoid a common bias—namely, conflating a high number of mutations with a high number of deleterious mutations [68].

Ultimately, the strongest evidence that passenger mutations can influence tumor evolution comes from experimental perturbation studies in which increasing passenger load is directly introduced and associated changes in proliferative capacity or metastatic potential are measured [73]. Passenger mutations can collectively reduce cellular fitness and slow tumor progression, thereby supporting the concept of a measurable deleterious load and providing a concrete operational definition of passenger impact.

Challenges in studying late-stage cancer evolution: methods and timing

Investigating the events that follow the acquisition of high evolutionary potential in vitro or in experimental animal models presents substantial limitations. In vitro models often fail to replicate the complexity of the human tumor microenvironment, as they cannot accurately mimic dynamic factors such as heterogeneous oxygen tensions, nutrient gradients, and immune surveillance [65]. On the other hand, animal models offer greater physiological relevance, but they fall short in fully recapitulating the evolutionary pressures experienced by human cancers. Discrepancies in immune system composition, metabolism, and lifespan of the animals if compared to humans additionally constrain their translational applicability [74, 75].

Human studies, particularly those documenting molecular progression from normal mucosa to polyps and eventually to cancer, provide more accurate timelines of cancer development [76, 77]. These investigations, typically spanning 5–15 years, effectively capture the early alterations driving cancer initiation. However, they rarely include the critical late-stage events that define the fully malignant phenotype. This later stage is characterized by rapid tumor evolution over a comparatively brief time frame, which has even a large impact on clinical outcomes.

Once cancer cells activate their "evolutionary engine"—a phase of accelerated mutation and adaptation—the tumor reaches a state of high adaptability. This transition likely occurs within a narrow time window. Understanding this phase is challenging because it unfolds quickly, leaving behind a "snapshot" of genetic changes that is difficult to interpret. For instance, the sequence of mutational events following *p53* loss, a pivotal alteration in many cancers, remains poorly understood [78–81]. When the primary tumor becomes clinically evident or, even more, in metastatic patients, cancer is observed at an advanced stage, often resembling "genetic chaos." At this point, the tumor has undergone thousands of environmental interactions, resulting in heterogeneous genetic drift across a myriad of neoplastic subclones. Although identifying driver genes remains relevant in this context, the therapeutic opportunities diminish as tumor biodiversity increases.

It is plausible that the initial phase immediately following the activation of the evolutionary engine exhibits greater homogeneity. This stage may encompass critical events that are mechanistically informative and potentially actionable. Investigating this early post-activation phase could provide valuable insights into the evolutionary trajectory of malignant tumors, potentially uncovering novel therapeutic opportunities.

The context-dependent mutational relevance

The relevance of mutational events is not absolute and is highly context and environment-dependent. A mutation or rearrangement classified as driver in one neoplasm is not necessarily oncogenic in all settings. Some of these alterations, including point mutations and rearrangements, may be present in normal tissue [49], precancerous lesions, or may lack any significant functional

impact [82–86]. In other words, a group of cancer cells exposed to specific environmental pressures—such as hypoxia, glucose deprivation, the presence of particular metabolites, collagen density, or immune surveillance—will selectively fix certain mutations while disregarding others that are irrelevant or even detrimental to survival under those specific conditions. Therefore, the significance of a mutation cannot be evaluated alone but must be contextualized within the constraints of the tumor microenvironment. This perspective highlights a sequential interplay between the non-Darwinian (neutral) and Darwinian (selective) phases of cancer evolution. This sequential progression would justify the increased clonality and mutational status of metastatic lesions compared to primary tumors, suggesting that selection plays a fundamental role in the late stages of tumor evolution [87].

Contextualizing the "evolutionary cascade hypothesis"

Several hypotheses have been proposed to explain tumor evolution. These hypotheses can be broadly categorized into three main groups [88–91]. The first, known as the "selective bottleneck hypothesis," suggests that tumor evolution is characterized by selective bottlenecks. According to this model, environmental or therapeutic pressures dramatically reduce genetic diversity, and the mutations that survive these bottlenecks are likely non-stochastic, reflecting adaptive responses to the changing conditions. However, this hypothesis primarily focuses on selection and does not specify the particular genes or functions involved. The second hypothesis, referred to as the "neutral evolution hypothesis," proposes that tumors may evolve neutrally over extended periods, accumulating stochastic mutations that are later followed by bursts of selection triggered by environmental changes. Although emphasizing a neutral evolutionary perspective, this model also lacks specific identification of the genes or functional processes involved in this evolution. A third hypothesis suggests that tumors exhibit dynamic genetic plasticity, where mutations that initially appear stochastic can later confer a selective advantage under new environmental conditions. This theory, which we associate with the concept of "the animal within", aligns with our perspective, proposing that tumor evolution leverages dynamics similar to natural evolutionary processes but at an immensely accelerated time scale. Despite their differences, all these hypotheses share a unifying concept: the fluid boundary between stochastic and non-stochastic mutations. In this manuscript, we propose an alternative sequence of events, which we term the "evolutionary cascade hypothesis." We suggest that an initial "stochastic" phase, favoured by the lack of DNA integrity machinery, triggers an "evolutionary engine", identifying specific genes that must be altered to activate this engine. This is followed by a "selective" phase, during which selection predominates, and non-stochastic events take over. This "evolutionary cascade hypothesis" can be regarded as a unifying framework that integrates multiple existing models into a cohesive timeline. In fact, not only it does not contradict the dichotomy between neutral and punctuated evolution, but it also aligns with theories regarding the direction of neoplastic subclonal expansion—linear [90, 91], branching [92], convergent [93], and parallel evolution [94]. Rather than opposing these models, it organizes them into a logical sequence, making the mechanism more clear (via DNA repair failure) and emphasizing the explicit role of environmental pressures in shaping tumor evolution. This hypothesis may explain the rapid pace at which tumors evolve toward their evolutionary targets. Our "evolutionary cascade hypothesis" offers an integrated framework to understand cancer as not merely a genetic disease but as a dynamic evolutionary process. It provides insights into the mechanisms driving cancer aggressiveness and clarifies how highly sophisticated regulatory systems, honed over millions of years of evolution, can be bypassed by tumor cells.

Proliferation efficiency and nutrient use efficiency as the theoretical foundations of the "evolutionary cascade hypothesis"

In the context of tumor evolution, proliferation (PE) and nutrient use efficiency (NUE) are pivotal metrics that provide probabilistic insights into cellular fitness and metabolic adaptability over a defined temporal window (ΔT). These parameters, each quantified on a scale from 0 to 1, are not fixed absolute values but probabilistic metrics reflecting the cellular fitness and metabolic adaptability as well as dynamic indicators of cellular behaviour. PE specifically measures the probability of a cell or cell population successfully replicating DNA and completing the cell cycle, with a particular emphasis on the S phase. A PE of 0 indicates complete replication failure, whereas a PE of 1 represents an idealized scenario in which every cell within a population undergoes successful division. This efficiency depends on intrinsic factors such as DNA repair capacity, apoptosis resistance, and resilience against replication stress, as well as extrinsic factors like growth factor availability, nutrient supply, and competition within the microenvironment. Experimentally, PE can be assessed using markers and techniques that quantify DNA replication and cell proliferation, including Ki-67 expression and thymidine analog incorporation assays, such as bromodeoxyuridine (BrdU) or 5-ethynyl-2′-deoxyuridine (EdU) incorporation. In biological systems, PE values vary across different cellular states and tissue contexts. For quiescent cells, which are typically in the G0 phase, PE is effectively zero. These cells can be identified by the absence of Ki67 expression or a lack of BrdU incorporation. Cells undergoing physiological

turnover, such as those in tissues with moderate renewal rates, exhibit PE values in the range of greater than 0 but less than or equal to 0.2. This low efficiency is reflected in moderate Ki67 expression and low rates of EdU incorporation [95, 96]. In tissues with high regenerative capacity, such as the epidermis, PE values increase to a range between 0.2 and 0.4, as evidenced by higher cell cycle activity and elevated mitotic indices [97]. As cellular proliferation becomes dysregulated, benign tumors and hyperplastic lesions demonstrate PE values exceeding 0.4 but remaining below or equal to 0.6. These lesions exhibit heightened levels of cyclins and phosphorylated retinoblastoma protein (phospho-Rb) alongside Ki67 positivity [98, 99]. In pre-malignant and malignant states, PE values frequently surpass 0.6, indicative of aggressive proliferation. This is accompanied by overexpression of markers associated with the S phase, such as PCNA and cyclin E, as well as reduced activity of tumor suppressors like p53 [100–102]. Despite the prominence of high PE values in malignant cells, dormant cancer cells represent a biologically distinct subpopulation with a unique role in tumor progression [103]. These cells enter a quiescent state, effectively reducing their PE to near zero. Dormant cells evade immune surveillance and therapeutic interventions while residing in specialized niches within distant organs, where microenvironmental cues maintain their inactivity. Evolutionarily, dormancy can be viewed as an adaptive strategy enabling survival under adverse conditions, shaped by genetic and phenotypic changes. However, clinically advanced cancers are often dominated by highly proliferative cells with elevated PE values, presenting a dual challenge addressing both dormant cells, which are resistant to conventional therapies, and actively proliferating cells, which drive disease progression. This complexity underscores the need for therapeutic approaches that consider the entire range of tumor cell behaviour.

The NUE is a more complex parameter that evaluates a cell's capacity to metabolize available resources efficiently. It is complex because the environmental and metabolic conditions in cancer are highly variable and depend on multiple biochemical and physical factors [104]. The simplest method to measure NUE for a given cell population is to assess the availability of essential nutrients such as glucose and glutamine and quantify how much of these are effectively converted into biomass [105, 106]. However, validated or proposed indices for measuring this parameter are lacking. At least theoretically, the score we propose aims to provide an intuitive definition of this evolutionary target. Obviously, in a biological system, the amount of biomass produced is fundamentally limited by the amount of available nutrients. This is governed by the law of conservation of mass, which states that mass (or, in this case, nutrients) cannot be created or destroyed, only transformed [107]. Furthermore, NUE = 1 is an ideal value. Indeed, the production of biomass requires nutrients as raw materials (such as carbon, nitrogen, and phosphorus) and energy (often derived from glucose but also from lipids). However, even in the most efficient biological systems, there is always some degree of inefficiency, such as energy losses in metabolism, waste production, and other biological processes. A score of 0 represents metabolic failure, where cells cannot extract or use nutrients. A score close to 1 indicates maximal metabolic adaptability, reflecting the cell's ability to optimally convert nutrients into energy and biomass precursors under environmental constraints. Intermediate scores reflect stages of metabolic evolution. As NUE increases, cells may acquire enhanced abilities to exploit alternative fuels, such as glutamine or lipids, and exhibit metabolic shifts like the Warburg effect—whereby cells preferentially use anaerobic glycolysis even in oxygen-rich conditions, enabling rapid ATP production and biosynthesis [108–110]. Therefore, intermediate scores could be characterized by values indicative of normal cells (low NUE scores) with standard metabolic activity, primarily relying on oxidative phosphorylation (OXPHOS) with limited flexibility to use alternative substrates [111]. Metabolic reprogramming, marked by increased glucose uptake, dependence on anaerobic glycolysis (Warburg effect), and the use of alternative nutrients, becomes predominant during malignant transformation (high NUE scores) [108]. Although these metabolic phases can be assessed using biochemical assays—such as lactate production [112], extracellular acidification rate (ECAR) and oxygen consumption rate (OCR) [113], mitochondrial integrity assays [114], or increased glucose uptake via 18 F-FDG PET imaging [115]—there is currently no comprehensive scoring system to evaluate NUE. Developing such a metric is an intriguing yet challenging goal, requiring a multidisciplinary effort that integrates biochemistry, computational biology, and physics.

In Fig. 4, we have described the trends of PE and NUE, along with the burden of efficacy mutations required for their attainment.

The relationship between these scores and time is critical, as both PE and NUE are dynamic. Over early time points, in a normal tissue environment, PE and NUE may remain relatively low, reflecting homeostasis. A tumor might initially exhibit a modest increase in PE, driven by clonal expansions of cells with enhanced replicative capacity. Simultaneously, NUE may rise more slowly as metabolic pathways adapt, with a sharp increase seen during later stages of tumorigenesis when metabolic plasticity becomes critical for survival and proliferation in nutrient-deprived microenvironments. Both scores increase non-linearly, reflecting the two steps in tumor evolution. Notably, the sharpest increases in PE and NUE coincide with a critical evolutionary transition

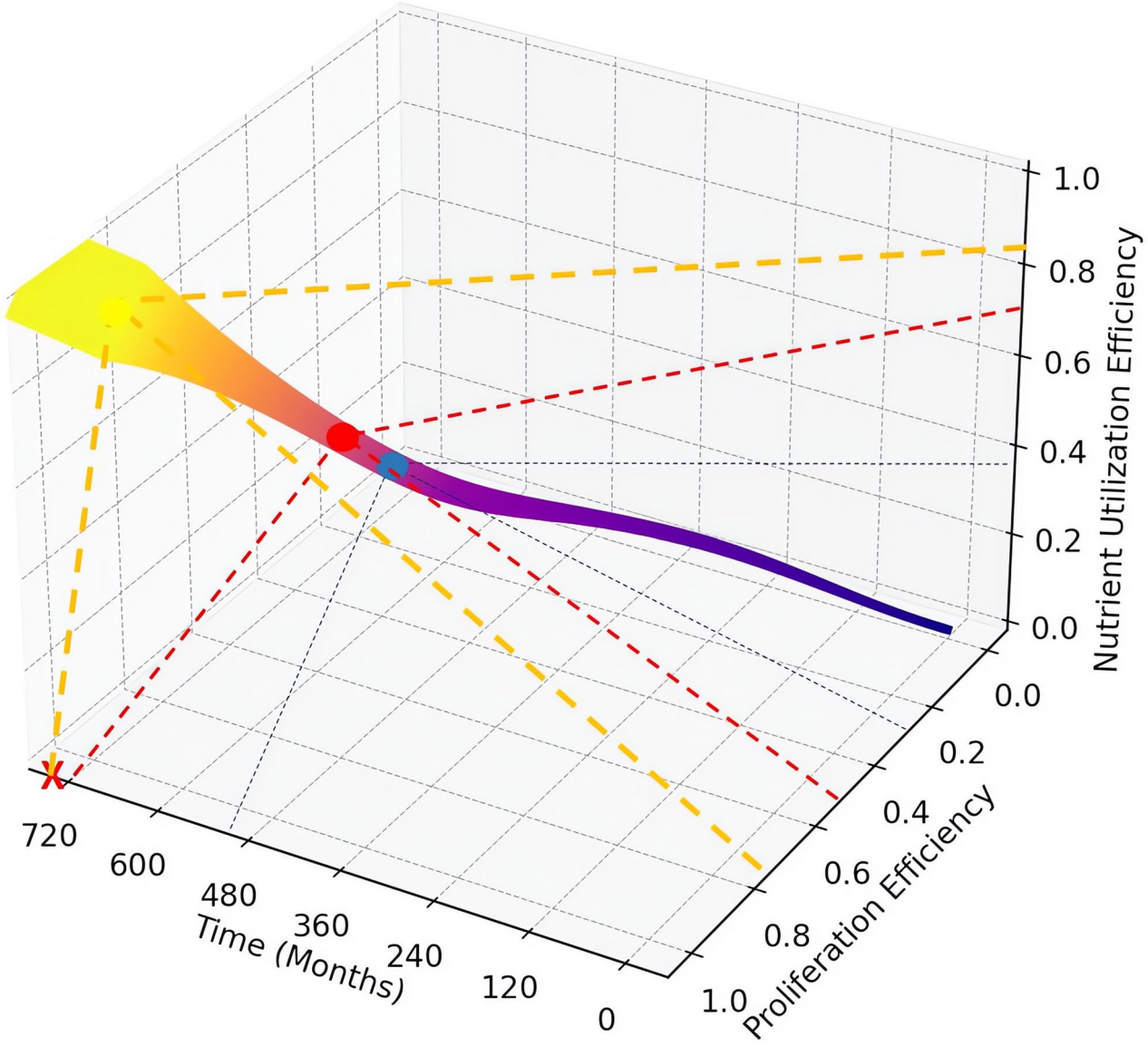

Fig. 4 Temporal dynamics of mutational load and evolutionary adaptation in malignant progression. The graph illustrates the progression of the mutational load (the volume of the curve is proportional to the number of mutations necessary to efficiently achieve evolutionary targets) over time, in relation to the attainment of high levels of Proliferation Efficiency (PE) and Nutrient Utilization Efficacy (NUE). The color gradient, from blue to progressively lighter and brighter hues, culminating in yellow, indicates the increasing level of adaptability. The time axis represents a reasonable simulation of malignant cancer development around the age of 60. At the time points corresponding to the blue and red spheres, cells still retain control over their evolutionary potential. The red sphere represents the activation of the evolutionary engine, with sufficient genetic plasticity to rapidly achieve evolutionary targets, which constitute the ultimate goal of the cancer biological system. Beyond this point, cancer acquires a significant mass, becoming clinically detectable, and evolves into a highly efficient evolutionary machine, capable of responding rapidly to environmental changes. The patient's death is marked by a red X on the time axis. This theoretical graph does not represent tumor mass, which can undergo drastic changes due to dramatic environmental factors such as radio-chemotherapy, surgical interventions, or interactions with the patient's immune system. Instead, it simplifies the cumulative genetic progression concerning mutations that are effective for achieving and maintaining evolutionary targets

(the onset of malignancy), highlighting the interplay between genetic mutations, metabolic reprogramming, and environmental constraints. As mutations accumulate and selective pressures intensify, these scores begin to move and diverge. These changes reflect our hypothesis on cancer evolution. The graph, in fact, exhibits an inflection point, marking a sharp acceleration in cancer progression, which translates into a relatively short clinical trajectory compared to the preceding phase, as observed in clinical practice. By capturing key dynamic processes, these models provide valuable insights into the underlying mechanisms governing cellular behavior. Therefore, even if our primary focus remains on the biological implications, we have included a mathematical

framework to support our analysis. The model underlying this analysis is detailed in Supplementary File 1.

The complexity and nonlinear dynamics of cancer progression: key determinants within the host

Early selective pressures during the latent phase of tumor evolution

In our hypothesis, we state that following a predominantly neutral phase in the genetic evolution leading to cancer, an evolutionary engine is activated, initiating a stage in which both endogenous and exogenous selective pressures shape genotypic and phenotypic trajectories. The selective phase is characterized by non-linear tumor growth, detected as neoplastic mass progression. From a genetic perspective, the information becomes more refined and adapted depending upon environmental needs, while quantitatively, the tumor mass undergoes profound changes. In other words, tumor mass, in addition to being highly variable, does not simply serve as a surrogate for the genetic progression of cancer. A small mass can have a far greater potential for genetic diversification than a larger mass, and consequently, may generate progeny with much higher efficiency of growth and propagation. If we focus on the most significant selective pressures considered according to the timing, among the initial ones are microenvironmental factors (such as nutrient and oxygen availability and interactions with the immune system), while later-stage pressures include therapeutic interventions (such as chemotherapies, radiotherapies, and surgical resections).

The initial selective pressures often act on a much smaller cell population compared to those in later stages. However, a tumor with a diameter of 2 mm, assuming a spherical shape, would contain several millions of tumor cells. It is very difficult to intercept and study the biological and immunological dynamics that precede the establishment of this large number of cells. The first phase is relatively latent and invisible, observable primarily through its reproduction and study in experimental systems.

Hypoxia-driven metabolic and angiogenic adaptation as an initial selective force

Oxygen is fundamental for nutrient use because it serves as the terminal electron acceptor in the mitochondrial electron transport chain, a crucial component of cellular respiration [116]. Through this process, oxygen enables the production of ATP, the primary energy currency of cells, via oxidative phosphorylation. This process generates much more ATP compared to anaerobic metabolism, such as glycolysis, which is essential for sustaining energy-demanding cellular processes, including growth, proliferation, and maintenance [117]. In tissues with high metabolic activity, such as rapidly dividing tumor cells, oxygen is indispensable to support their energetic needs. When oxygen supply becomes limited, such as in growing tumors that outpace their blood supply, cells experience hypoxia, which profoundly impacts their metabolism, survival, and genetic evolution [118]. To study how oxygen levels influence tumor biology, especially during the early stages of cancer, experimental models can simulate these hypoxic conditions and allow researchers to investigate the molecular and cellular responses that occur [119]. The key molecular pathway involved in the cellular response to hypoxia is the activation of hypoxia-inducible factors (HIFs), primarily HIF-1α [120]. Under normal oxygen conditions, HIF-1α is rapidly degraded. However, under low oxygen (hypoxic) conditions, HIF-1α is stabilized and translocates to the nucleus, where it binds to hypoxia-responsive elements in the promoters of various genes. This activation leads to the transcription of genes that help the cell adapt to the hypoxic environment, including those involved in angiogenesis, metabolic reprogramming, cell survival, and apoptosis resistance [121–124]. Experimental models, particularly organoid systems and cell cultures, offer valuable platforms for studying the molecular mechanisms that regulate hypoxia-induced gene expression [125]. In these models, oxygen tension can be precisely controlled, allowing researchers to simulate hypoxic microenvironments similar to those found in early-stage tumors. By exposing cells or organoids to hypoxia, researchers can observe how the stabilization of HIF-1α alters gene expression and impacts cellular behaviors [126]. For example, the activation of the gene for vascular endothelial growth factor (VEGF), a key regulator of angiogenesis, allows tumor cells to induce the formation of new blood vessels, thereby improving their oxygen and nutrient supply. These adaptations are not only beneficial for tumor growth, but contribute to the invasive nature of tumors by facilitating their expansion into surrounding tissues [127].

Nutrient limitation and metabolic plasticity in expanding neoplastic populations

In addition to HIF-1α, hypoxia also affects other signaling pathways involved in nutrient use. A well-studied example is the interplay between oxygen levels and cellular metabolism. Under normoxic conditions, cells predominantly use oxidative phosphorylation in mitochondria to produce ATP. However, when oxygen becomes scarce, cells shift their metabolism toward anaerobic glycolysis, known as the Warburg effect. The latter is a hallmark of cancer cells and allows them to maintain energy production even in hypoxic environments. The Warburg effect is driven, in part, by HIF-1α activation, which upregulates genes involved in glycolysis, such as those encoding hexokinase, phosphofructokinase, and lactate dehydrogenase

[128, 129]. Experimental models, by exposing tumor cells or organoids to different oxygen levels, can study this process through the evaluation of changes in glucose use, lactate production, and activity of key glycolytic enzymes. Such experiments can shed light on how tumor cells adapt their nutrient use in response to hypoxic stress and how these changes contribute to the aggressive nature of cancer [130–132].

As tumors grow, they have not only to counteract the reduced oxygen supply but also the limited access to essential nutrients, such as glucose and amino acids. To do this cancer cells may increase their uptake of glucose through the upregulation of glucose transporters, such as GLUT1, or switch to alternative nutrient sources, such as amino acids, lipids, and fatty acids, to support biosynthetic processes and energy production [133–135]. In experimental systems, nutrient gradients can be established within 3D cultures or organoid models to simulate the limited nutrient conditions found in growing tumors. By measuring cellular uptake of glucose, amino acids, and other essential nutrients, researchers can explore how nutrient deprivation influences cellular metabolism and gene expression. These experiments can reveal key metabolic adaptations that may drive tumor progression and provide novel potential therapeutic targets [136–138].

Immune surveillance as a dynamic selective pressure in early tumor progression

The third selective pressure during this early phase is undoubtedly the immune system. In the initial stages of tumor development, the immune system plays a crucial role in influencing the trajectory of cancer progression. Although the tumor remains small and often clinically undetectable, the immune system is already actively engaged in dynamic interactions with emerging neoplastic cells. In most cases, immune cells, such as T lymphocytes and natural killer (NK) cells, mount an effective response that results in the regression of nascent tumors [139–142]. These immune cells can recognize tumor cells by detecting abnormal antigens on their surface, such as mutated proteins or overexpressed growth factor receptors. Regardless, they exert significant selective pressure on the neoplastic population, which may develop various strategies to escape immune surveillance and continue to grow [143, 144]. Understanding the complex interaction between the immune system and early-stage tumors is essential, as it provides valuable insights into the genetic trajectories of cancer [145]. Experimental models are indispensable for studying these interactions, offering methods to observe and manipulate immune-tumor dynamics that are not directly visible in vivo, especially during the early, small-scale phases of tumor development. One of the most widely used and historically significant experimental approaches to studying immune-tumor interactions is the co-culture assay [146]. In these in vitro models, tumor cells and immune cells are cultured together, enabling direct interactions between the two cell types. Co-culture systems serve as a platform to investigate how immune cells recognize and respond to tumor cells, as well as how tumor cells may modulate immune cell function. For example, co-culture assays can be employed to evaluate the cytotoxic activity of NK cells or T cells against tumor cells, or to assess the effects of immune checkpoint inhibitors (such as anti-PD-1 or anti-CTLA-4 antibodies) on immune responses. These assays can be modified to simulate various aspects of immune-tumor interactions by altering conditions such as nutrient availability, oxygen levels, or the presence of immune-suppressive molecules, offering valuable insights into how the tumor microenvironment influences immune responses during the early stages of cancer.

Another important experimental model for studying immune-tumor interactions is the humanized mouse model [147, 148]. In these models, human immune cells are introduced into immunocompromised mice, creating a system that more closely mimics human immune-tumor dynamics. These models allow researchers to observe immune responses against tumors in a living organism, providing more physiologically relevant data than in vitro systems alone. Humanized mice are especially useful for studying the adaptive immune response, including the behavior of T cells and NK cells, as well as for testing immunotherapies targeting specific immune checkpoints or other immune-modulating pathways. By transplanting small, early-stage tumors into humanized mice, researchers can study how immune cells interact with nascent tumor cells in vivo and assess how different immune components influence tumor growth and regression. Additionally, humanized mouse models offer the advantage of enabling the testing of immune-based therapies, such as monoclonal antibodies or engineered T cells, in a system that closely mimics human immune responses. In addition to co-culture assays and humanized mouse models, single-cell RNA sequencing (scRNA-seq) has emerged as a powerful tool for studying immune-tumor interactions at the single-cell level, thereby replicating conditions that more closely resemble the interactions occurring in this small-scale phase [149]. scRNA-seq enables the analysis of gene expression in individual cells, providing a detailed snapshot of the transcriptional landscape within both tumor and immune cells during their interactions [150]. By using scRNA-seq, researchers can identify subtle changes in gene expression that occur in response to immune pressure, such as the upregulation of immune checkpoints in tumor cells or the activation of immune effector pathways in immune cells.

While these experimental models provide invaluable insights into the interactions between the immune system and early-stage tumors, they have certain limitations. In vitro models, such as co-culture assays, often fail to fully replicate the complexity of the tumor microenvironment, including factors such as extracellular matrix interactions, tissue architecture, and the presence of non-immune cells such as fibroblasts, macrophahes and endothelial cells. Furthermore, while humanized mouse models provide a more physiologically relevant context, they still have limitations in their ability to fully replicate the human immune system, particularly regarding the immune cell differentiation and function. Nonetheless, these experimental systems remain essential for advancing our understanding of immune responses in the early stages of cancer and for identifying potential therapeutic strategies that can enhance immune surveillance and promote tumor elimination.

The second phase, where the tumor is clinically evident, is more accessible for study as more tissue is available for direct human analysis. Genetic analysis of this phase, particularly comparative studies of the primary tumor and some or all metastases, provides intriguing insights. In fact, in colorectal cancer, sequencing the DNA using broad panels to identify shared non-synonymous genetic mutations reveals extremely heterogeneous overlap percentages (ranging from 0% to 100%), clearly indicating the exceptionally high genetic plasticity of cancer compared to normal cells [151–155]. It is crucial to distinguish between studies that compare the genomic evolution of primary tumors and metastases within the same patient and those that do not (e.g., independent cohorts, public datasets). The former are significantly more informative, as they enable a direct assessment of clonal evolution, selection pressures, and treatment-driven genetic divergence at the individual patient level. In contrast, studies comparing separate cohorts of primary and metastatic tumors may introduce confounding factors related to interpatient variability, tumor heterogeneity, and differences in treatment history, potentially limiting conclusions about the effective dynamics of cancer progression.

Nonetheless, in late-stage, clinically evident phases, therapeutic interventions represent a major selective pressure, actively shaping the genetic landscape of cancer. For instance, comparisons between primary tumors and single metachronous metastatic lesions reveal that metastases arising after adjuvant chemotherapy exhibit lower genetic concordance with the primary tumor than those not exposed to chemotherapy [156]. Additionally, the disappearance of driver mutations, such as KRAS, in the absence of therapeutic selective pressure has been observed in patients with better prognoses and indolent disease courses, such as those with oligometastatic disease [157]. This phenomenon likely reflects a selective process driven by the immune system's elimination of clones with high differential metastatic potential [158]. Therefore, the persistence of an alteration classified as a driver is not an absolute rule but rather a context- and microenvironment-dependent phenomenon, as illustrated above.

Therapeutic intervention as a selective perturbation: hypothetical evolutionary scenarios

Within the framework of the "evolutionary cascade hypothesis", therapeutic intervention, including pharmacological and radiotherapeutic treatments, does not represent the trigger of tumor evolution, but rather acts after the activation of the evolutionary engine, when the neoplastic population has already acquired sufficient genetic and phenotypic variability to respond to selective pressures. We propose that, in this second, selection-dominated phase, therapy functions as a major external perturbation factor, reshaping the evolutionary trajectories of the tumor rather than initiating them. A successful therapeutic intervention may occur through at least two evolutionary routes. First, treatment may eradicate all malignant clones, including any pre-existing resistant minority. In this scenario, tumor evolution is effectively halted, as no residual population remains to explore adaptive trajectories. Within the "evolutionary cascade hypothesis", this corresponds to an abrupt termination of the cascade following a sufficiently deep selective bottleneck. Such an outcome is most plausible when (i) baseline intratumoral heterogeneity is limited, (ii) the therapy targets a truncal dependency shared across all clones, and/or (iii) protected niches or sanctuary sites are absent. Second, even without complete eradication, therapy may achieve durable disease control if it sufficiently suppresses PE and/or NUE, thereby preventing tumor expansion while maintaining competitive pressure from therapy-sensitive clones on resistant ones. In evolutionary cascade terms, this situation constrains progression along the second phase of the cascade: the evolutionary engine remains active, but the accessible evolutionary "space" is restricted, preventing resistant clones from gaining a dominant fitness advantage. Conversely, in the case of unsuccessful treatment, therapy preferentially eliminates sensitive clones—often representing the dominant population—while resistant or tolerant subclones survive. After treatment, the ecological landscape is effectively "emptied," enabling resistant clones to undergo competitive release, rapidly expand, and drive relapse or metastatic progression. Within the "evolutionary cascade hypothesis", this represents an acceleration of the second phase, where therapy amplifies selection intensity and promotes clonal sweeps rather than evolutionary stabilization.

Finally, therapy is intrinsically mutagenic, surviving cells may acquire additional mutations characterized by therapy-related mutational signatures. In the evolutionary cascade framework, this does not initiate the evolutionary engine but can further fuel it by increasing genetic diversity during the selection-dominated phase, thereby facilitating additional branching and the emergence of secondary resistance mechanisms.

Proliferation, nutrient efficiency, and immune pressure as determinants of driver selection

Within our evolutionary cascade framework, the transition to an evolutionary engine primarily increases the supply of heritable variation (i.e., mutational diversity), whereas PE, NUE, and immune predation define the selection filter that converts part of this variation into context-specific dependency. In other words, "driver" status is not an intrinsic property of a mutation, but an emergent property of the fitness landscape imposed by microenvironmental constraints [159]. When PE is limiting, selection favors alterations that reduce replication stress, bypass checkpoints, and sustain rapid cycling; when NUE becomes limiting (e.g., hypoxia and nutrient scarcity), selection enriches for mutations and regulatory programs enabling metabolic rewiring and substrate flexibility, including hypoxia-adaptive signaling; when immune pressure is dominant, selection favors genetic/epigenetic changes that blunt antigen presentation or interferon signaling, or otherwise promote immune escape [160]. As selective regimes fluctuate across space and time, variants that were neutral "passengers" in one niche may become conditional "drivers" in another, thereby blurring the driver/passenger dichotomy and explaining why genetic relevance is often stage- and site-dependent. This conceptual separation between "mutation supply" (engine activation) and "fitness filtering" (PE/NUE/immune) is consistent with quantitative and evolutionary models of driver/passenger dynamics and with contemporary evidence that microenvironmental and immune pressures reshape the observable landscape of selected cancer drivers.

Role of epigenetics in the "evolutionary cascade hypothesis"

While our "evolutionary cascade hypothesis" is centered on DNA repair failure as the pivotal event that activates an "evolutionary engine", epigenetic mechanisms provide a conceptually consistent additional layer that can amplify adaptability without requiring immediate changes in DNA sequence. Epigenetic regulation—through DNA methylation, histone modifications, chromatin remodeling, and non-coding RNAs—controls gene expression programs that are heritable across cell divisions yet potentially reversible, enabling rapid transitions among phenotypic states [161]. In cancer, it is increasingly recognized that evolution is driven not only by genetic alterations but also by epigenetic dysregulation, which contributes to tumor initiation, intratumor heterogeneity, and therapy resistance [162, 163]. Within our two-step framework, epigenetics can be interpreted as a "fast-adaptation layer" that operates both before and after the switch. During the initial, predominantly non-Darwinian phase, stochastic epigenetic variability may generate a reservoir of cell states that can persist neutrally or transiently, thereby increasing the phenotypic search space available for subsequent selection [164]. After DNA repair impairment activates the evolutionary engine, genetic–epigenetic coupling becomes stronger: genome instability and chronic stress responses reshape chromatin landscapes, while epigenetic states can in turn modulate DNA damage responses and DNA repair gene expression, creating feedback loops that stabilize adaptive transcriptional programs under hostile microenvironmental conditions [165]. In the Darwinian phase, microenvironmental pressures (e.g., hypoxia, nutrient deprivation, immune surveillance, and therapy) are expected to select not only for advantageous mutations but also for epigenetically encoded transcriptional and metabolic programs that enhance proliferation efficiency and nutrient use efficiency. Therefore, epigenetic heterogeneity and plasticity can be incorporated into the cascade as a dynamic mediator of context-dependent adaptation, acting in synergy with (and sometimes upstream of) genetic fixation [166]. From a methodological standpoint, estimating the relative "weight" of epigenetics in this model is feasible in principle but endpoint-dependent, and would require longitudinal and/or perturbational designs integrating single-cell multi-omics (genome, methylome/chromatin accessibility, transcriptome) to partition phenotypic variance into genetic versus epigenetic components across evolutionary time and selective constraints [167].

Therapeutic implications of understanding cancer genetic evolution

What is the purpose of modeling tumor genetic evolution? It is evident that such modeling is not merely a mathematical or academic endeavor. Developing increasingly reliable and coherent models of tumor genetic evolution holds the potential to predict the genetic trajectories of tumor development. Specifically, it can shed light on the sequence of events required for the acquisition of metastatic capabilities. The variables influencing this process are numerous. In our model, once the evolutionary engine is activated, it follows a "deterministic" path, with cells evolving toward specific evolutionary targets. Achieving these targets requires tumor cells to acquire particular mutations that are uniquely suited to

the microenvironment in which they reside. This microenvironment comprises a variety of factors, including oxygen levels [168], nutrient availability [169], growth factors [170], immune cells [171], protein concentrations that occupy intercellular spaces [172], matrix remodeling enzymes [173], cytokines [174], chemokines [175], pH fluctuations [176, 177], mechanical stress [178], vascular architecture [179], metabolic bioproducts [180], interactions with the microbiota [181], and stromal components such as cancer-associated fibroblasts [182]. This list represents only a subset of the elements shaping the tumor microenvironment, which influence the attainment of evolutionary targets without themselves being direct evolutionary targets.

The growing understanding of these variables, along with their quantification, has the potential to revolutionize models of cancer genetic evolution. In particular, it will be essential to integrate these variables with the genetic characterization of tumors. In this regard, extensive repositories of genetic big data from cancer patients (e.g., TCGA—The Cancer Genome Atlas, CGC—Cancer Genomics Cloud, and ClinVar) already provide a methodological and technological framework for managing such data [183, 184].

Furthermore, artificial intelligence (AI) enables rapid, high-throughput analysis of these complex datasets, identifying patterns and predictive insights with unprecedented computational power [185, 186]. While this scientific horizon may seem daunting or even futuristic, it is increasingly within reach due to continuous technological advancements. These include molecular approaches such as NGS [187], single-cell sequencing [188], and emerging techniques like spatial transcriptomics [189], CRISPR-Cas9 genome editing for functional genomic studies [190], high-resolution proteomics [191], and multi-omics integration platforms [192, 193]. When embedded within mechanistically informed biological models and supported by the rich experimental datasets outlined above, AI-based tools can realistically contribute to anticipating critical inflection points in tumor evolution. One promising strategy is to combine mechanistic tumor models—which explicitly simulate how cancer cells grow, compete, and adapt under gradients of oxygen and nutrients—with AI systems that learn from these simulations and from patient data. For example, agent-based platforms such as PhysiCell allow researchers to model how hypoxia and nutrient limitation shape clonal selection and therapy response; these frameworks already demonstrate how microenvironmental pressures can be translated into measurable evolutionary outcomes [194]. On top of this, machine-learning and generative models trained on longitudinal genomic data (e.g., paired primary and recurrent tumors or serial liquid biopsies) can estimate the rate at which new mutations emerge and detect transition points toward aggressive or therapy-resistant states, as shown in glioma evolution studies [195]. Importantly, hypoxia is not just a passive condition: experimental and computational studies indicate that low oxygen levels can actively increase genetic instability and accelerate tumor evolution, making it feasible to model the "weight" of environmental factors as true drivers rather than background variables [196]. Finally, reinforcement-learning approaches have been proposed to test adaptive treatment strategies in silico, identifying intervention schedules that intentionally reshape evolutionary trajectories instead of reacting to resistance after it occurs [197].

Once again, it is worth emphasizing that increasingly reliable models of genetic evolution provide a solid foundation for data interpretation and analysis.

Beyond theoretical considerations, what are the therapeutic implications of deciphering cancer's genetic evolution? In our view, the first phase of genetic evolution is predominantly neutral—except in clear hereditary cases where a germline mutation predisposes individuals to early destabilization of cell proliferation control mechanisms or even to a more pronounced susceptibility to undergo to DNA mutations due to the lack of DNA maintenance controls [198]—and is therefore largely unpredictable at the individual level with current tools. In other words, in the vast majority of so-called sporadic cases, the initiation of the evolutionary process is driven by stochastic, neutral events: the only viable intervention is primary prevention. This involves mitigating modifiable risk factors and minimizing DNA-damaging exposures. The primary goal of oncological prevention is to reduce the likelihood of mutational events that disrupt genomic integrity, thereby preventing the activation of oncogenic pathways. Key strategies include lifestyle modifications, vaccination (e.g., HPV), smoking cessation, and reducing exposure to environmental carcinogens [199]. However, the second phase of this model, has numerous therapeutic opportunities, as it represents the stage at which selective pressures shape tumor cell evolution, making them dependent on specific pathways for survival and proliferation. If we were able to comprehensively map the genetic responses to all conceivable environmental conditions, it might become possible to predict and categorize cancer progression with high precision, paving the way for a deterministic understanding of cancer biology. However, current knowledge and available technologies remain insufficient to achieve this level of predictive capability. In the (hopefully not-too-distant) future, advanced technologies may enable the prediction of essential driver mutations or mutation patterns based on histological type and tumor microenvironment composition. Targeting these vulnerabilities with precision therapies, immunomodulation, or metabolic inhibitors

could effectively disrupt tumor progression by exploiting the adaptive constraints imposed by the microenvironment and evolutionary dynamics.

Conclusions

Despite remarkable advances in multi-omics and computational modeling, the intricate landscape of cancer genetic evolution remains a formidable challenge. However, adopting an innovative perspective—anchored in a two-step "evolutionary cascade hypothesis"—offers both scientific and conceptual value for translational research. This paradigm provides a forward-looking framework that not only deepens our understanding of tumor progression but also inspires novel investigative strategies, paving the way for future breakthroughs in cancer biology.

Abbreviations

ROS	Reactive oxygen species
UV	Ultraviolet
mut/Mb	mutations per megabase
NGS	Next-generation sequencing
POLE	Polymerase (DNA directed), Epsilon
PE	Proliferation efficiency
NUE	Nutrient use efficiency
ΔT	Defined temporal window
BrdU	Bromodeoxyuridine
EdU	5-ethynyl-2′-deoxyuridine
PCNA	Proliferating cell nuclear antigen
OXPHOS	Oxidative phosphorylation
ECAR	Extracellular acidification rate
OCR	Oxygen consumption rate
18F-FDG PET	Fluorine-18 fluorodeoxyglucose positron emission tomography
ATP	Adenosine triphosphate
HIFs	Hypoxia-inducible factors
HIF-1α	Hypoxia-inducible factor 1 alpha
VEGF	Vascular endothelial growth factor
GLUT1	Glucose transporter 1
NK	Natural killer (cells)
PD-1	Programmed cell death protein 1
CTLA-4	Cytotoxic T-lymphocyte–associated protein 4
scRNA-seq	single-cell RNA sequencing
TCGA	The Cancer Genome Atlas
CGC	Cancer Genomics Cloud
AI	Artificial intelligence
CRISPR-Cas9	Clustered regularly interspaced short palindromic repeats–CRISPR-associated protein 9
HPV	Human papillomavirus

Supplementary Information

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Supplementary Material 1

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Author contribution

A.O.: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft preparation. M.S.: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft preparation. F.S.: Conceptualization, Data curation. R.S.: Methodology, Software, Formal analysis, Data curation, Writing – review & editing. F.C.: Conceptualization, Validation, Data curation. A.C.: Validation. V.G.: Validation. I.S.: Validation, Writing – review & editing. S.Z.: Methodology, Validation. M.B.: Validation. G.S.: Conceptualization, Resources, Writing – review & editing. M.C.: Conceptualization, Resources, Writing – review & editing.

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Data availability

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Declarations

Ethics approval and consent to participate

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Competing interests

The authors declare that this study was conducted without any commercial or financial relationships that could be perceived as potential conflicts of interest.

Author details

[1]Istituto Nazionale Tumori di Napoli, IRCCS "G. Pascale", Via M. Semmola, 80131 Naples, Italy
[2]Medical Oncology, University of Salerno, Via Salvador Allende 43, 84081 Baronissi, Italy
[3]Centro Polidiagnostico Strumentale srl, AMES, Via Padre Carmine Fico 24, 80013 Casalnuovo Di Napoli, Italy
[4]Department of Precision Medicine, University of Campania "Luigi Vanvitelli", Via Luigi De Crecchio 7, 80138 Naples, Italy
[5]Medical Oncology, Fondazione Policlinico Universitario "A. Gemelli" IRCCS, Università Cattolica del Sacro Cuore, Largo Agostino Gemelli 8, 00168 Rome, Italy
[6]University of Naples "Federico II", Via Sergio Pansini 5, 80131 Naples, Italy
[7]Department of Clinical and Experimental Medicine, University of Messina, Via Consolare Valeria 1, 98125 Messina, Italy
[8]Laboratory of Molecular and Precision Oncology, Biogem SCARL, Ariano Irpino (AV), Italy

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