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Non-genetic mechanisms of ancestral origin involved in cancer drug resistance

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Abstract

Cancer progression is commonly attributed to the stepwise accumulation of somatic mutations; however, this framework does not fully account for the regulated and recurrent nature of many tumor-associated phenotypes. Here, we advance an evolutionary systems perspective in which cancer reflects the partial reactivation of conserved cellular programs that predate multicellularity. The present paper identifies conceptual and functional parallels between the cancer life cycle and that of protists, particularly parasitic species that inhabit comparable microenvironmental niches within host organisms. These parallels suggest a potential evolutionary continuity linked to a common ancestor of metazoans and amoebozoans. We hypothesize that adaptive traits originating in early historic environments—especially mechanisms conferring tolerance to oxidative stress—have been retained in the genomes of modern metazoans. Reactivation of these traits may contribute to tumor survival and progression under adverse conditions. Importantly, the occurrence of intrinsic and multidrug resistance in untreated tumors indicates that resistance is not exclusively induced by therapeutic exposure. Instead, it may arise from conserved, non-genetic stress-response programs that are activated by the tumor microenvironment. This framework provides a basis for reinterpreting cancer drug resistance as an evolutionarily embedded mechanism rather than a purely treatment-driven outcome.

Keywords: Cancer; Unicellularization; Evolution; Epigenetic protection; Transgenerational; Desensitization

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1. Introduction

Cancer can no longer be regarded merely as a consequence of cell-cycle disturbances and unlimited multicellular proliferation. Rather, it represents a transition into a unicellularized mode of life. Cancer results from a complex unicellularization process that activates a dual germ–soma cell system governed by ancestral organizational principles and evolutionarily conserved regulatory programs.

In recent years, it has become increasingly clear that cancer is not a diseased or dysfunctional multicellular program, but rather a reactivated, unicellularized biological cell system derived from conserved ancestral genome compartments, whose organization, regulation, and evolutionary logic are fundamentally distinct from those of the multicellular host organism.[1-4] This conceptual shift has made it possible to resolve

many previously unanswered questions in cancer biology and to explain the profound differences between the biology of cancer cells and the biology of the multicellular host cell system, including the stem cell complex.

The present work analyzes the current understanding of cancer drug resistance from the perspective of evolutionary cancer cell biology (ECCB) and argues that non-genetic and epigenetic mechanisms involved in cancer drug resistance can be traced back to ancestral-unicellular programs that evolved in the common ancestor of amoebozoans, metazoans, and fungi (AMF) millions of years ago (Mya), during the early oxygen enrichment of the world's ocean.

The ECCB framework emerged from recognition of genomic and epigenomic relationships between unicellularized cancer cell systems and unicellular protist systems, pointing to deep evolutionary homology with the common AMF ancestor. This evolutionary relationship is supported by numerous studies on the biology of both cellular systems published by the present author over the past two decades.[1-4]

However, as long as cancer continues to be viewed primarily as a disease of multicellularity driven by dysfunction and mutation, rather than as a consequence of unicellularization and reactivation of conserved unicellular programs evolved by the common AMF ancestor, conceptual divisions between evolutionary and non-evolutionary perspectives are likely to persist. Such divisions may obscure the causal basis of fundamental cancer processes mediated through genetic, non-genetic, and epigenetic regulation.

Based on the premise that many—if not most—epigenetic mechanisms observed in cancer have an ancestral origin, the present work proposes the hypothesis that cancer cell systems retain genome-protection mechanisms evolved by the common AMF ancestor that are largely absent or less prominent in multicellular systems.

Integrating molecular and evolutionary cancer research is a central challenge for contemporary oncology. Only by integrating evolutionary insights with their historical-ecological drivers will it be possible to bridge these conceptual gaps and achieve a more comprehensive understanding of epigenomically regulated hallmarks, such as reversible germ-to-soma plasticity and drug resistance.

To date, epigenetically regulated non-genetic cancer mechanisms have largely been interpreted as processes operating beyond DNA sequence variation and have frequently been equated with classical forms of transgenerational non-genetic inheritance (NGI) observed in multicellular organisms. However, such an imposed interpretation is neither adequate nor conceptually sufficient for the unicellular-like cancer cell system, which is characterized by interconnected germ–soma plasticity and reversible cell phenotypes. A more complete understanding requires integration of insights derived from evolutionary research.

In this context, Adrian-Kalchhauser *et al.*[5] likewise emphasized that collaboration between molecular and evolutionary specialists is essential for understanding non-genetic cancer mechanisms. Only such interdisciplinary cooperation can bridge evolutionary and molecular knowledge, and contribute to the development of an accurate "eco-evolutionary" model of cancer. To shed light on the origins of cancer epigenetics and the interplay among ecology, epigenetics, and evolution, it is first necessary to better understand the epigenetic mechanisms in unicellular systems such as ciliates and parasitic protists.

2. Epigenetically regulated non-genetic mechanisms of amoebozoans, metazoans, and ancestral origin

Epigenetic mechanisms regulate gene expression by enabling cells to finely adjust messenger RNA and protein production in response to environmental stimuli. Although diverse epigenetic mechanisms exist, all operate without altering the primary DNA sequence.

Several years ago, Weiner and Katz[6] argue that: (i) epigenetic processes that rapidly modify genomes and gene expression states play central roles in phenotypic plasticity, differential adaptation, and ultimately the diversification of protists; and (ii) epigenetic mechanisms regulating genomes and gene expression were already present in the last eukaryotic common ancestor and are widespread across all major eukaryote lineages.

Epigenetic modifications are generally understood to regulate gene expression by activating or silencing specific genes in both unicellular and multicellular systems. As a consequence, affected genes may produce reduced, absent, or, in some cases, increased protein levels relative to their normal expression state. Such regulatory processes are influenced by environmental factors acting on DNA-associated regulatory elements that, although not part of the coding sequence itself, are essential for gene function. Epigenetic mechanisms, therefore, play a central role in adaptation and genome regulation.

2.1. Epigenetics in unicellular systems

In protists, epigenetic regulation is closely associated with the germ-to-soma transition (GST) and soma-to-germ transition (SGT) processes. Among the most

common epigenetic mechanisms are DNA methylation and histone modification. In general, DNA methylation is associated with gene silencing, whereas demethylation promotes gene activation. Environmental conditions can therefore directly influence protein expression patterns by modulating methylation states.

2.1.1. Epigenetics of ciliates

Ciliates exhibit highly sophisticated genome reprogramming programs. A single ciliate cell contains both germline and somatic genomes,[7] organized within distinct intranuclear genomic compartments. These organisms have evolved a specialized form of sequential genomic rearrangement that occurs during meiotic reproduction and nuclear segregation, a process known as conjugation.

During conjugation, small RNA-mediated genome rearrangements occur within the same germline nucleus and contribute to the formation of the new somatic nucleus.[7] Small RNAs and macronuclear RNAs bind to specific genomic regions or direct histone modifications, such as H3K9 methylation, thereby marking genomic regions for either retention or elimination.[8,9]

In addition, proteins involved in the non-homologous end-joining pathway are induced early in meiosis and accumulate in the developing somatic nucleus, with more than 80% of the germline sequences being "filtered out." These epigenetic processes ultimately regulate gene activation and silencing, playing a central role in nuclear differentiation and genome restructuring.

2.1.2. Epigenetics in parasitic protists

As emphasized by Weiner and Katz[6] in 2020, knowledge of epigenetic regulation remains limited for most free-living protists. Parasitic protists, however, represent an important exception. This is particularly evident in parasites such as *Toxoplasma, Entamoeba, Acanthamoeba, Naegleria,* and *Giardia*, in which the epigenome regulates phenotypic switching, cell differentiation, antigenic variation, and cellular plasticity. In these parasites, epigenomic regulation is mediated primarily through histone acetylation and methylation.[10,11] Such mechanisms enable rapid adaptation to changing host environments and environmental stressors.

Epigenetic mechanisms implicated in the phenotypic plasticity of asexual lineages have been considered adaptive responses to environmental fluctuations.[12] In parasitic protists, rapid epigenetically driven plasticity is particularly evident in systems employing antigenic variation. In several parasites, including *Entamoeb*a, epigenetic modifications additionally regulate virulence-associated programs and exoenzyme secretion mechanisms (amoebapores), thereby supporting the increased metabolic and nutritional demands of the somatic, trophic cell state.

Detailed molecular knowledge of epigenetic processes in free-living protists remains comparatively limited. Nevertheless, epigenomic regulation appears to play a central role in genome dynamics and the control of cellular plasticity. Unlike ciliates, rapid GST transitions in asexual protists such as *Entamoeba* are unlikely to involve permanent elimination of germline genome compartments. Instead, rapid GST processes, followed by subsequent SGT, more likely depend on reversible gene silencing, epigenetic inactivation, and the latent preservation of complex genomic compartments.

2.2. Epigenetic changes and transgenerational inheritance

Over the past decade, considerable debate has emerged regarding whether inheritance can extend beyond DNA sequence variation and the extent to which non-genetic factors contribute to heritable phenotypes. Many of these questions, particularly in the context of cancer, remain unresolved because the evolutionary origins and historical development of these mechanisms are still insufficiently understood.

According to the review work of Adrian-Kalchhauser *et al.*,[5] the functionality of NGI ultimately depends on DNA sequence information and therefore cannot be considered entirely independent of the genome. Nevertheless, the ecological and evolutionary significance of NGI likely arises from complex epigenetic mechanisms.

Molecular epigenetic processes—including DNA methylation, histone modifications, and small non-coding RNAs—have been interpreted as carriers of additional phenotypic information distinct from classical DNA sequence transmission.[5,13-15] Many researchers describe transgenerational NGI factors as a network of dynamic and often unpredictable interactions that may persist across multiple generations.[16-20]

As emphasized by Weiner and Katz[6] in 2020, examples of "soft inheritance" mediated through the transgenerational transmission of epigenetic marks have become increasingly numerous in recent years.[18,21] Transgenerational NGI was found among ciliates, in which acquired changes in physiology—including mating types—can be inherited by the progeny without alterations in the underlying nucleotide sequence.[22,23] Furthermore, researchers have shown that environmentally induced epigenetic modifications can produce phenotypic plasticity and differential gene expression. Such epigenetic marks may remain stably inherited across generations—a process referred to as "epigenetic assimilation."

Although many questions remain unresolved, most contemporary studies support the view that transgenerational mechanisms can substantially promote adaptive phenotypes in offspring exposed to environmental conditions similar to those experienced by the parental generation.[5,24-27] Evolutionary biological studies are therefore particularly well suited to elucidating the origins, adaptive significance, ecological context, and evolutionary consequences of NGI mechanisms.

2.3. Epigenomic protection and genomic integrity

As noted by Feng and Riddle[28] in 2020, a large network of interconnected pathways is responsible for maintaining genome integrity in the face of continuous DNA-damaging challenges. These researchers emphasized the central role of epigenetic mechanisms in maintaining genomic stability and demonstrated that epigenetic regulation directly contributes to multiple DNA repair pathways. Numerous histone modifications—including phosphorylation, methylation, acetylation, and ubiquitylation—occur at specific histone residues in response to DNA damage. Such modifications play essential roles in the sensing, processing, and repair of damaged DNA, thereby preserving genomic integrity and cellular homeostasis.[29]

More recently, several researchers have examined how epigenetic alterations and epigenomic heterogeneity contribute to cancer evolution. It has been proposed that disruption of epigenetic processes regulating DNA-based functions and transcription is a hallmark of cancer, enabling the emergence of defective cellular states. In 2025, Laisné *et al.*[30] concluded that epigenomic heterogeneity and plasticity act as reservoirs of cellular states and therefore constitute important drivers of tumor evolution. These authors further suggested that novel therapeutic approaches aimed at restricting epigenomic heterogeneity may help limit cancer development and progression. Their work particularly emphasized questions related to epigenomic reprogramming and long-lasting epigenetic memory.

There is increasing evidence that epigenetic mechanisms mediate cellular plasticity and dynamic responsiveness to environmental signals. Epigenetic regulation appears to govern both critical developmental periods and lifelong cellular plasticity. Environmental perturbations and stress can induce long-lasting epigenetic modifications in key genes involved in stress-response pathways. These modifications lead to broad epigenome-wide alterations. In 2026, Peña *et al.*[31] highlighted the close relationship between the epigenome, environmental stress, sensitive developmental periods, and cellular plasticity.

Plasticity is particularly relevant under fluctuating environmental conditions. As recently noted by Romero-Mujalli *et al.*,[32] despite apparently stochastic epigenetic modifications within genotype–phenotype mapping processes—such as DNA methylation and demethylation—the emergence, evolution, and limits of plasticity remain incompletely understood. The frequency of epigenetic modifications appears to be highly sensitive to organismal stress, yet genetically determined and evolutionarily adaptable. According to these models, the epigenome may improve adaptation, particularly under periodically changing environmental conditions, although always within the constraints imposed by the genotype.

In the present work, the concepts proposed by Adrian-Kalchhauser *et al.*[5] are further developed within the framework of ECCB, with particular emphasis on genomic–epigenomic interactions and the deep homology that exists between cancer cell systems and ancestral unicellularity.

2.4. Epigenetics in cancer

In contrast to protist research, epigenetic cancer research has experienced remarkable expansion in recent years, driven by increasing scientific interest and substantially greater research resources. This development has advanced the broader understanding of epigenetic biology and, indirectly, evolutionary epigenetics as well. Owing to the deep homology and unicellularized nature of the cancer cell system, it is plausible that many fundamental epigenetic mechanisms and their underlying molecular structures originated during the evolution of the common AMF ancestor.

Recently, Galassi *et al.*[33] provided an in-depth analysis of the impact of epigenetic alterations and epigenetic modifiers on cancer stemness, defined as the capacity of a small population of poorly differentiated malignant cells to repopulate the cancer lineage with phenotypes of exceptional plasticity and increased resistance. The authors emphasized the central role of transcription factors and their regulators, which are controlled through reversible modifications of DNA and histones collectively referred to as epigenetic marks. They further pointed out that processes such as methylation, acetylation, and ubiquitination are catalyzed by antagonistic enzyme systems, including methyltransferases and demethylases, acetyltransferases and deacetylases, and ubiquitin ligases and deubiquitinating enzymes.

Most researchers continue to interpret the epigenetic alterations observed in cancer as aberrations of the normal epigenetic state of the multicellular host organism. As recently as 2025, Sherif *et al.*[34] described cancer as a complex and heterogeneous disease characterized by

aberrant proliferation and differentiation and referred to the cancer epigenome as a "labyrinth of molecular modifications and epigenetic dysregulation" capable of silencing tumor suppressors while activating oncogenic programs. According to the researchers, the principal epigenetic mechanisms involved are DNA methylation, histone modification, chromatin remodeling, and regulation by non-coding RNAs. In contrast to the normal multicellular epigenome, the cancer epigenome is characterized by widespread abnormalities, including global hypomethylation and hypermethylation.

Unlike genetic mutations, epigenetic alterations are potentially reversible. Consequently, defining the epigenetic defects associated with malignant transformation has become a major focus of contemporary cancer research, leading to progressive elucidation of mechanisms of epigenetic regulation and dysregulation. Yu *et al.*,[35] for example, reviewed past and current applications of epigenetic inhibitors in cancer diagnosis and therapy, with particular emphasis on inhibitors targeting epigenetic enzymes. Their foundational studies provided evidence that epigenetic regulators are integral components of normal cellular function. They also demonstrated that disruption of epigenetic homeostasis can initiate and promote tumorigenesis in both *in vitro* and animal models.[36]

More recently, Turna and Demokan[37] described the dynamics of epigenetic reprogramming during metastasis. According to these authors, epigenetic mechanisms enable stable yet reversible changes in gene expression that drive cellular plasticity throughout the metastatic cascade, including epithelial–mesenchymal transition (EMT), intravasation, survival of circulating tumor cells, extravasation, dormancy, and metastatic colonization. They further demonstrated that epigenetic regulators interact with major EMT-associated signaling pathways to control phenotypic plasticity and adaptation to metastatic niches. Increasing evidence also supports the clinical relevance of metastasis-associated epigenetic alterations as diagnostic and prognostic biomarkers.

Accordingly, epigenetic regulation operates not only during EMT but throughout the entire metastatic cascade, coordinating dynamic and context-dependent cellular transitions. Consistent with this interpretation, recent updates to the Hallmarks of Cancer framework introduced the terms "unlocking phenotypic plasticity" and "non-mutational epigenetic reprogramming" as hallmarks of cancer progression and metastasis.

Within this context, Marine and Dawson[38] discussed the principal mechanisms of non-genetic therapy resistance in cancer and reviewed emerging clinical evidence concerning adaptive non-genetic resistance pathways. Their work showed that certain molecular drivers enable cancer cells to rapidly adapt to therapeutic pressure, whereas others confer intrinsic cellular plasticity that facilitates lineage switching and evasion of anticancer immune surveillance.

Identifying the origins of non-genetic heterogeneity is therefore crucial for achieving major clinical advances. Increasing evidence suggests that non-genetic heterogeneity plays a central role in therapy resistance and metastasis. Consequently, elucidating both the patterns and evolutionary origins of non-genetic heterogeneity has become a major objective of contemporary cancer research.[39]

2.5. Epigenetics in soma-to-germ transition

Interestingly, Wessel *et al.*[40] pointed out that recent experimental findings in several animal species demonstrate that somatic cells can re-enter the "germline club" by converting into germ cells (SGT) after elimination of the original germline. This apparent "violation" of classical germline restriction received further support from studies showing that induced pluripotent stem cells, experimentally generated from adult somatic cells, can give rise to functional oocytes and spermatozoa *in vitro*. Notably, the genes and molecular pathways used to induce pluripotency are normally present in cells and the *in vitro* conditions that direct germline commitment replicate *in vivo* conditions.

In multicellular organisms, germ cells possess the unique capacity to generate gametes that retain totipotency, whereas unicellular and unicellularized systems are characterized predominantly by unipotent stem/germline states. The formation of germ cells from somatic cells, as well as germ cell differentiation, is generally regulated through complex interactions between genetic circuitry and epigenetic mechanisms.[41-43]

Epigenetic regulation refers to heritable changes in gene expression that occur without alteration of the primary DNA sequence. In this context, Eun *et al.*[44] argued as early as 2010 that germ cells must preserve and transmit relevant epigenetic memory across generations, thereby maintaining continuity of germline identity and function.

3. Cornerstones of evolutionary cancer cell biology

3.1. The ancestral germ and soma concept

Evolutionary cancer cell biology does not support a close relationship between the highly evolved, multipotent stem cells of multicellular organisms, and cancer stem cells (CSCs) of unicellular origin. Instead, CSCs are evolutionarily much closer to the unipotent Ur-germline

(stemgermline) of the common AMF ancestor than to multipotent human stem (HSCs). HSCs are characterized by extensive differentiation capacity and function as central regulators of tissue and organ development. In contrast, CSCs represent primitive, largely unipotent stem cells with little differentiation capacity.[1-4]

In the evolutionarily related cell system of parasitic amoebas, committed stem cells differentiate into cysts capable of polyploidization, genomic amplification, and the generation of stemgermline precursor cells. In cancer, committed cancer stem cells (com-CSCs) give rise to a homologous cellular structure with comparable functional capacity but without cyst wall formation.

Because of its evolutionary origin and role in system control, the stemgermline is a fundamental biological unit critically involved in cancer drug resistance. Similar to the Ur-germline of the AMF ancestor, the cancer stemgermline consists of a strongly hypoxic self-renewing germline with high sensitivity to oxygen excess and tumor-associated stressors. Hyperoxic conditions (about 2–3% O_2) irreversibly damage the oxygen-sensitive parts of the stemgermline genome.[1-4]

Historically, this problem was resolved at the onset of the era of increasing environmental oxygenation, when the AMF cell system evolved a second, oxygen-resistant cell lineage (the soma). This lineage functionally inactivates the sensitive stemgermline genome and protects it through an epigenetic shielding mechanism. From this evolutionary innovation emerged an autonomously developmental program that remains active in dual germ and soma cell systems, such as cancer and protists.

3.2. Oxygen gradients

Tumors consist of a dual-structured ancestral cell system comprising a central, oxygen-sensitive stemgermline that accounts for approximately 1–2% of the total tumor cell population and is localized within the tumor core, and an oxygen-resistant somatic cell population representing roughly 98% of the tumor mass (bulk tumor cells). This somatic compartment surrounds and functionally supports the stemgermline.[1-4] Importantly, bulk tumor cells are not differentiation products of the stemgermline; instead, they represent the somatic lineage of the cancer cell system that arises naturally through a GST/EMT. The bulk tumor mass develops due to its high proliferative capacity rather than through differentiation from self-renewing stemgermline cells.

The somatic compartment protects the oxygen-sensitive stemgermline genome in two complementary ways.[1-4] First, somatic tumor cells actively consume oxygen within the tumor microenvironment, thereby maintaining the hypoxic conditions required for full functionality of the core stemgermline system. Second, somatic cells harbor an epigenetically inactivated stemgermline genome, thereby serving as a reservoir that protects the stemgermline from extrinsic oxygen and intrinsic oxygen-derived stressors.

Schematically, the tumor can be represented as a structure composed of two concentric compartments: A hypoxic core and a multilayered hyperoxic periphery with varying oxygen content (Figure 1). Angiogenic processes establish a dynamic oxygen gradient and promote extensive cellular trouble within the tumor. Because somatic bulk tumor cells cannot consume all available oxygen immediately, excess oxygen diffuses inward from the periphery toward the hypoxic core. This excess oxygen can irreversibly damage the genome of stemgermline cells, leading to dysfunction. In response, somatic bulk tumor cells initiate reverse SGT processes, generating intact replacement stemgermline clones and sublines that restore functionality within the hypoxic core compartment, just as Wessel *et al.* predicted.[40]

Conversely, when somatic bulk tumor cells at the tumor periphery are damaged by chronic intrinsic stressors or intensive chemotherapy, functional stemgermline cells within the inner compartment are rapidly signaled. Upon receiving these signals, they activate an extended GST program that desensitizes and protects all the vulnerable genome sites and generates a more resistant bulk-cell phenotype that is no longer susceptible to environmental stressors or chemotherapeutic agents. These resistant cells migrate as somatic replacement sublines toward the tumor periphery, where they establish a drug- and stressor-resistant boundary.

3.3. Stemgermlines and committed cancer stem cells

Evolutionary cancer cell biology redefines cancer stem cell populations within a system-centered framework. Rather than viewing CSCs as a cell population in which each cell possesses both self-renewal and differentiation capacity, ECCB distinguishes between two fundamentally different functional entities: (i) self-renewing CSC lineages (stemgermlines) that lack differentiation capacity, and (ii) differentiated, com-CSCs that lack proliferative capacity.[1-4]

Stemgermlines and stem–germ clones represent the principal evolutionary drivers of cancer progression. They form self-renewing lineages capable of indefinite propagation through asymmetric cell division (ACD). However, ACD does not expand the stem cell pool; rather, it maintains a constant number of self-renewing stemgermline cells. In contrast, non-proliferating com-CSCs retain the capacity to expand through cyst-like cycles of polyploidization and depolyploidization. This process

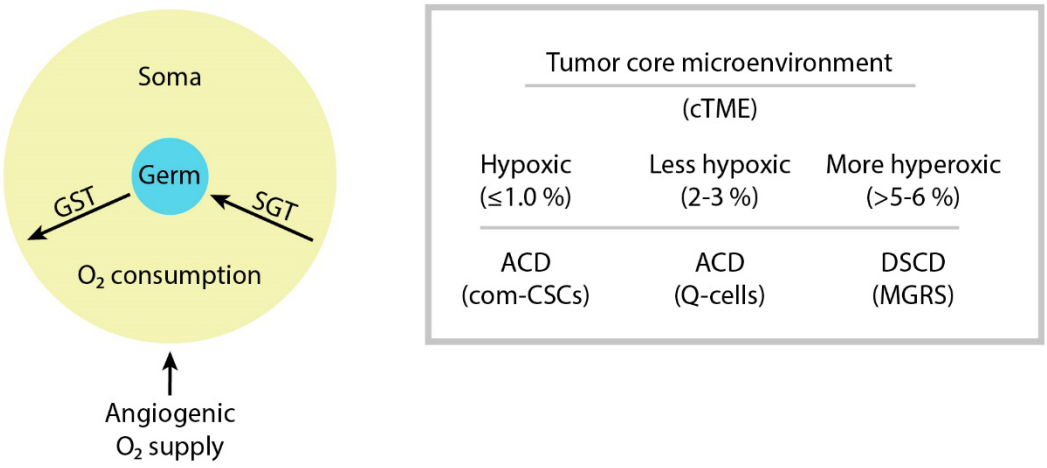

Figure 1. Intratumoral oxygen gradient and hypoxic versus hyperoxic core living conditions. The intratumoral oxygen gradient results from the balance between oxygen supply through angiogenic processes and oxygen consumption by the peripheral somatic bulk tumor layer (yellow). Adequate oxygen consumption maintains hypoxic conditions within tumor niches (core), which support proliferation of the stemgermline (blue) in a self-renewing mode, accompanied by differentiation into committed cancer stem cells (com-CSCs) or by non-differentiation leading to the formation of quiescent (Q) cells. Reduced oxygen consumption leads to hyperoxic conditions in the tumor core. Under these conditions, stemgermline cells proliferate in a DSCD mode and reconstruct dysfunctional genomes by activating DNA damage response circuits and the multinucleated genome-repair syncytium (MGRS).
Abbreviations: ACD: Asymmetric cell division; GST: Germ-to-soma transition; SGT: Soma-to-germ transition (or epithelial-to-mesenchymal transition); DSCD: Defective symmetric cell division, which proliferates through symmetric cell division.

generates progenitors that give rise to new sublines, thereby expanding the pool of self-renewing cells.

The life cycle of the stemgermline comprises multiple phenotypic states[1-4] including: (i) the strongly hypoxic ACD phenotype, which proliferates via asymmetric division and generates com-CSCs under severe hypoxia; (ii) a less hypoxic ACD phenotype, which produces non-committed quiescent (Q) cells; and (iii) the defective symmetric cell division (DSCD) phenotype, which has lost functional stemness and proliferates through aberrant symmetric division.

Dysfunctional stemgermline cells, having lost stemness, can activate a characteristic extended DNA damage response (DDR) program. Multiple DSCD cells may fuse to form a hyperpolyploid multinucleated genome-repair syncytium (MGRS), within which genome reconstruction occurs. The progeny generated from this structure (MGRS-derived buds or spores) give rise to replacement stemgermlines. In this way, stemgermline continuity is restored through regulated repair cycles. The extended DDR, including the hyperpolyploid MGRS pathway, reintroduces germline genome functionality[1-4] (Table 1).

This stemgermline-centered cancer cell system, which assigns the stemgermline a central evolutionary role, contrasts with the prevailing dualistic CSC model. By distinguishing functional stemgermline states from their committed derivatives, ECCB provides a more coherent framework for understanding cancer evolution, plasticity, and therapeutic resistance.

3.4. Lineage plasticity: Germ-to-soma and soma-to-germ dynamics

Tumor organization is characterized by mutual support and reciprocal protection between the inner and outer compartments mediated by complementary GST and SGT programs, in cancer, better known as mesenchymal–epithelial transition (MET) and EMT plasticity. Somatic cell populations, such as bulk tumor cells in cancer and trophozoite populations in parasitic amoebas, do not arise through classical differentiation from self-renewing stemgermline cells, but rather through GST. This process is more accurately defined as cell conversion rather than differentiation.[1-4]

When cells of the hypoxic core become dysfunctional, bulk tumor cells compensate by generating replacement stemgermline clones. In this way, new stemgermline cells and replacement sublines can be generated through reverse SGT/EMT, highlighting the bidirectional plasticity of these cellular systems. Conversely, when peripheral bulk cells

Table 1. The stem cell family concept reflects the architecture of an ancestral unicellular system that evolved approximately one billion years ago in the common fungi ancestor (1,000 Mya)

Stemgermline phenotypes	Description/definition
The productive asymmetric cell division (ACD) phenotype (≤1.0% O_2)	Capable of self-renewal and generation of committed cancer stem cells (com-CSCs) under severe hypoxia. It represents the fundamental state of the stemgermline.
Non-proliferating com-CSCs	Unipotent cells capable of forming polyploid cyst-like structures that accumulate progenitors for new stemgermlines; native com-CSCs retain tissue-specific profiles originating from their defective symmetric cell division (DSCD) precursors.
Quiescent-cells	Intermediate germline phenotypes capable of either differentiating into com-CSCs or reverting to self-renewing ACD states.
DSCD phenotypes	Dysfunctional, stemness-negative phenotypes that proliferate through defective symmetric cell division. They activate DNA damage response (DDR) and genome-reconstruction pathways, forming hyperpolyploid multinucleated genome-repair syncytia (MGRS).
MGRSs	In cancer, stemness-positive giant cells are termed polyploid giant cancer cells (PGCCs). They have hyperpolyploid giant nuclei capable of reconstructing the genome integrity for the stemgermline.
Spores or buds	Stemness-positive progenitors generated via reductive division of giant MGRS nuclei.

Notes: Most of the stemgermline phenotypes represent stages within extended DDR circuits and germline repair cycles. Importantly, oxygen concentration critically regulates germ-to-soma transitions. When angiogenesis modestly increases intratumoral oxygen levels beyond ~2% O_2, cancer stemgermline cells lose their ability to differentiate into com-CSCs. Excess oxygen levels of 5.7–6.0% O_2 irreversibly damage the stem germline, leading to stemness-negative DSCD phenotypes. These, in turn, activate unicellular DDR circuitry and genome-reconstruction programs (MGRS or PGCC pathways), ultimately restoring genomic integrity in replacement stemgermlines, sublines and clones.

are damaged or eliminated, stemgermline cells generate new, increasingly resistant bulk-cell layers (GST/MET) that reinforce the protection of the hypoxic stemgermline genome compartment.

All of these intratumoral dynamics are driven by non-genetic mechanisms inherited from the AMF ancestor and reveal a deep homology between the cancer cell system and its unicellular evolutionary predecessors. In the following sections, the ancestral mechanisms underlying cancer drug resistance are discussed in detail. This paper first reviews contemporary, non-evolutionary cancer research on drug resistance and subsequently reinterprets these findings within an evolutionary framework.

4. Drug resistance in current conventional oncology

4.1. Pre-existing and treatment-induced drug resistance

In contemporary cancer research, the term drug resistance refers to the capacity of malignant cells to become less responsive or refractory to chemotherapeutic agents, a phenomenon observed across most cancer types and a major contributor to treatment failure and poor clinical outcomes. Accordingly, drug resistance arises through multiple mechanisms, including alterations in the tumor microenvironment, genetic mutations, changes in drug targets or receptors, and increased drug efflux capacity.

Pre-existing drug resistance refers to resistant cancer cell states or clones that are present in the tumor before therapy begins. In contrast, treatment-induced drug resistance is considered a dynamic adaptive response to therapeutic stress. Under the selective pressure imposed by chemotherapy, cancer cells undergo extensive epigenetic and transcriptional reprogramming.

From the ECCB perspective, both pre-existing and treatment-induced resistance represent manifestations of conserved ancestral survival programs encoded within the unicellularized cancer cell system. Both programs reflect the capacity of the cancer cell system to activate ancient genome-protection and stress-survival mechanisms in response to environmental pressure.

Drug resistance is generally described as a highly complex, multifactorial process involving numerous interacting determinants that may function as inducers, drivers, or effectors of resistance[45] and lead to desensitization to chemotherapy. According to the researchers, these determinants include: (i) genetic diversity, (ii) mutations of drug targets, (iii) oncogene amplification, (iv) compensatory bypass signaling pathways, (v) epigenetic

alterations that increase intratumoral heterogeneity, (vi) tumor cell plasticity, (vii) activation of the DDR pathways, (viii) EMT, (ix) tumor micro-environmental influences, (x) cellular reprogramming, and (xi) genes directly implicated in the development of drug resistance.[45-51]

More recently, drug resistance research has increasingly focused on identifying and targeting specific molecules and proteins that mediate resistance. Pharmaceutical strategies now emphasize the development of targeted inhibitors designed to counteract drug and multidrug-resistance mechanisms and to overcome intrinsic or therapy-induced resistance to cancer cell death.

Conventional cancer research classifies drug resistance into intrinsic (primary) and acquired (secondary) categories.[45-51] In addition, Bell and Gilan[46] further distinguish between reversible and irreversible drug resistance, as well as non-mitotic drug persistence. Intrinsic or native drug resistance reflects pre-existing stress-response mechanisms in untreated primary tumors, whereby intrinsic stressors exert effects comparable to those induced by endogenous tumor agents. Both stress conditions activate innate cellular defense programs against toxic insults, rendering tumor cells partially or fully refractory to subsequent chemotherapy.

By contrast, acquired drug resistance develops during or after treatment as an adaptive response of the entire tumor cell population to therapeutic pressure therapy. It represents a progressive transition from an initially drug-sensitive state to a therapy-resistant state and is associated with the loss of treatment efficacy and clinical responsiveness.[52-55]

In 2024, Khan *et al.*[48] proposed that cancer therapy resistance arises from a complex interplay between intrinsic (innate) and acquired (extrinsic) resistance. According to this model, intracellular defense pathways acting in concert with oncogenic signaling networks can promote therapeutic desensitization and confer resistance. These coordinated responses may modify drug targets, enhance DNA repair capacity, and activate survival pathways, thereby enabling cancer cells to evade treatment-induced cytotoxicity.[54] Among the mechanisms conferring resistance, the authors highlight multiple oncogenic pathways, including (i) alterations in drug targets, (ii) enhanced DNA repair capacity, and (iii) activation of survival pathways, which collectively enable cancer cells to evade the cytotoxic effects of therapy.

4.2. Desensitization, loss of responsiveness to drugs

Accordingly, reduced drug responsiveness (desensitization)[47] is understood to result from the combined actions of genetic, nongenetic, and environmental factors. Most commonly, resistance is attributed to a conglomerate of genetic diversity, acquired mutations in drug targets, oncogene amplification within compensatory or bypass signaling pathways, and epigenetic modifications. Together, these processes further shape intra-tumoral heterogeneity, tumor cell plasticity, DNA repair capacity, and susceptibility to cell death pathways, ultimately giving rise to multifactorial drug resistance.[56-59]

Extrinsic tumor factors primarily include components of the tumor microenvironment (TME) that actively contribute to cancer cells' ability to evade the cytotoxic effects of anticancer therapies.[60,61] These components encompass a wide range of TME elements, including alterations in the extracellular matrix, tumor-associated stromal cells, growth factors, extracellular vesicles, immune cells, and cancer-associated fibroblasts. Collectively, these factors play critical roles in tumor growth, metastasis, and resistance to cancer therapy by secreting diverse growth- and survival-promoting signals and by modulating therapeutic accessibility and immune surveillance.[62,63]

4.3. Epigenetic regulation and reprogramming

As reported by Li *et al.*,[45] epigenetic regulation and transcriptional reprogramming play central roles in the emergence and modulation of cancer drug resistance.[64–65] Accordingly, drug resistance is closely associated with two interconnected processes: (i) epigenetic regulation, including DNA methylation and histone modification, which alter chromatin accessibility and gene expression, thereby enabling transcriptional plasticity; and (ii) transcriptional reprogramming, defined as the dynamic reorganization of transcription factor activity and regulatory gene networks in response to therapeutic and environmental stress. Together, these mechanisms allow tumor cells to establish stable yet adaptable gene expression programs that sustain drug resistance and promote survival under selective pressure.[66-71]

Histone modifications likewise play a critical role in regulating DNA repair pathways and in the development of acquired cancer resistance. Epigenetic alterations favor the DNA damage repair in cancer cells,[72-74] while DNA hypermethylation has been shown to increase the accumulation of somatic mutations.[75-76] Epigenetically altered gene expression and transcription can persist for multiple cell divisions, eventually leading to the development of nongenetic heterogeneity and drug non-responsiveness.[73]

Consistent with this view, Lei *et al.*[47] proposed that epigenetic regulatory mechanisms can suppress the expression of DNA repair genes, thereby directly contributing to cancer drug resistance. This supports the

idea of a functional interplay between DNA methylation, genome maintenance pathways, and therapeutic resistance, and suggests that targeting aberrant epigenetic regulation—particularly DNA methylation—may represent a promising strategy for overcoming drug resistance in cancer.

4.4. Reversible drug resistance

In a 2020 work, Bell and Gilan[46] described several heterogeneous cancer cell phenotypes with varying resistance potentials, ranging from reversible drug tolerance to stable, irreversible resistance as follows:

4.4.1. Drug-tolerant phenotypes

Drug-tolerant persistent (DTP) cells are genetically indistinguishable from the bulk tumor population, and their resistance is reversible upon drug withdrawal. These cells typically arise at low frequencies within tumor cell populations and are characterized by reduced proliferative capacity and altered metabolic states, which together would confer tolerance to therapeutic stress. Because of these properties, DTP cells are widely considered critical precursors of stable drug resistance.[77-79]

4.4.2. Unstable drug resistance

Beyond transient persistence, cancer cells acquire reversible, epigenetically mediated drug resistance that reverts to drug sensitivity when interrupted.[80,81] This type of phenotypic plasticity reflects dynamic state transitions that depend on the presence or absence of chemotherapeutic agents.

According to these researchers, unstable resistance represents transient states along a resistance continuum. DTP cells and temporarily resistant phenotypes can progress toward stable resistance, thereby serving as reservoirs from which durable genetic or non-genetic resistance mechanisms may emerge.[77,82] In many cases, therapeutic withdrawal leads to partial or complete re-sensitization; however, such reversibility can be suppressed by cyclic or adaptive treatment regimens.[80]

In contrast, sustained therapeutic pressure promotes the emergence of mitotically active, stably resistant phenotypes that persist independently of drug presence and display long-term resistance stability.[83-87] These stable resistance states are typically associated with fixed genetic alterations or heritable epigenetic programs that lock tumor cells into an irreversible drug-refractory phenotype.

According to several researchers, the epigenetic mechanisms underlying drug resistance remain largely unknown and require further investigation.[88,89] There is currently no precise explanation for why reprogramming into resistant phenotypes occurs.[46,84-94] Earlier studies on drug resistance described the stable resistant phenotype as the final stage of an innate chemoresistance program that exists prior to chemotherapy. Subsequent treatment then selects pre-existing stable resistance clones from the heterogeneous intra-tumoral population; these clones survive therapy because they evade apoptosis and cannot be effectively eliminated.[46]

4.4.3. Multidrug resistance

Vaidya *et al.*[72] describe multidrug resistance (MDR) as a complex phenomenon involving multiple intricate pathways through which malignant cells become insensitive to various drugs and acquire the ability to survive repeated exposure to anticancer therapies. This includes the development of non-genetic mechanisms of drug resistance.

According to Alhazza *et al.*,[95] MDR includes non-cellular mechanisms and overactivation of compensatory cellular pathways, ranging from inhibition of apoptosis to DNA damage repair.[96] Such adaptive responses increased DNA damage repair capacity and epigenetic reprogramming.[97]

Persistent activation of DDR pathways plays a pivotal role in MDR by enabling tumor cells to repair therapy-induced DNA damage and survive under selective pressure.[95-98] Consequently, targeting DDR components could represent a promising strategy to overcome MDR.

4.5. Presumed ancestral origin

Gradually but steadily, the balance of evidence is shifting toward evolution and unicellularization. With each new finding, the weight of experimental and theoretical support accumulates on this side of the scale. In contrast, the opposing view—centered primarily on mutation and abnormal cellular proliferation as the fundamental explanation for cancer—appears increasingly insufficient to account for the full complexity of cancer biology and is consequently losing explanatory power. Many of these findings no longer have meaningful correlates within the autochthonous multicellular host system and show little direct or derived relationship to the host organism's tissue, embryonic, or stem-cell structures. Instead, they increasingly point to biological programs and regulatory mechanisms that are distinct from those governing normal multicellular organization.

Recently, several relevant non-evolutionary review articles support the evolutionary perspective, although they do not acknowledge unicellularization or the ancient origin of the cancer cell system.[65,72,99] These authors questioned whether the classical Darwinian model of evolution[91,99-102]—based on rigid, irreversible phenotypes caused by genetic alterations and natural selection—

adequately explains cancer drug resistance. Instead, they proposed that drug-resistant cancer cells frequently exhibit reversible phenotypes and are influenced by non-Darwinian factors that are interconnected with phenotypic plasticity and epigenetic memory. They further emphasized the role of a stressful microenvironment capable of acclimating cancer cells to dynamic adaptive processes.

According to Menon *et al.*,[65] epigenetic regulation modifies gene expression through mechanisms such as DNA methylation and histone modifications. Epigenetic modulation plays a central role in phenotypic plasticity and determines cell fate across multiple transition processes, including germ–soma transitions (evolutionary GST) and the induction and maintenance of senescence. Patterns of gene expression and epigenetic memory contribute to stabilizing cellular states or enabling transitions between them. These mechanisms are also crucial for the development and maintenance of cancer subpopulations with stem-like characteristics, including high tumor growth potential, drug resistance, and intratumoral heterogeneity.[89,103] The researchers argue that epigenetic memory plays an important role in stabilizing cellular states and enabling transitions between them.[104,105] Compared with the bulk tumor population, clones or sublines capable of cell-state transitions are likely to exist in a dynamic state characterized by continuous phenotype switching. This statement aligns well with ECCB statements. It also aligns with the stress-induced premature senescence (SIPS) phenotype,[106-111] which is analogous to the senescent predecessor of DSCD cells.[1-4]

4.5.1. Premature senescence prior to and following cancerous transformation: Common features and differences

Similar to SIPS, the senescent predecessor of DSCD cells, which have suspended their mitotic activity and entered a transformation-competent state, represents a form of stress-induced premature senescence states triggered by intrinsic and extrinsic factors. These include oncogenic stress, ionizing radiation, or hyperoxia, all of which activate the DDR circuitry, enabling genome reconstruction, stemness recovery, and the generation of replacement stemgermline cells.[1-4]

It is proposed that senescence exiters, which leave the premature senescent state as DSCD phenotypes, are already transformed and unicellularized cells. Through DDR-mediated genome reorganization and MGRS-associated genome reconstruction, these cells establish a nascent cancer stemgermline and subsequently generate the hypoxic ACD stemgermline phenotype characteristic of the unicellularized cancer cell system.

Hazardous intrinsic and extrinsic stressors may likewise damage the strongly hypoxic ACD population and induce a premature senescence state analogous to SIPS. These cells also require DDR circuitry, including MGRS/PGCC-mediated genome repair and reorganization, to restore genomic integrity and cancer cell stemness. This dynamic corresponds to the previously described non-genetic cycles of stemness loss and stemness recovery.[1]

The seemingly paradoxical association between senescence and stemness[87] is therefore not contradictory. Therapy-induced senescence can indeed promote cancer stemness through cellular reprogramming, but this occurs within the framework of DDR-mediated genome reconstruction and recovery processes. This phenomenon is not unique to cancer cells. Rather, its evolutionary roots may extend back to the common AMF ancestor and its dual germ–soma life cycle, in which epigenetic modulation emerged as a central mechanism governing phenotypic plasticity, genome protection, and cell-fate transitions.

Finally, in 2021, Shlyakhtina *et al.*[112] proposed that non-genetic heterogeneity plays a critical role in shaping the evolutionary trajectory of cancer cell populations. This view is supported by multiple studies demonstrating that (i) cancers display extensive non-genetic heterogeneity, often manifested as phenotypic variability, and (ii) genetically identical subclones may adopt distinct and relatively stable phenotypic states that respond very differently to environmental stimuli.[113-122]

The author of the present work agrees that stress-induced phenotypic plasticity and epigenetic wrapping are key drivers of cancer drug resistance and significantly contribute to cell reprogramming. Likewise, strategies targeting phenotypic plasticity, epigenetic regulation, and metabolic adaptation represent promising therapeutic approaches.

5. Drug resistance from an evolutionary perspective

Evolutionary oncology provides a broader interpretative framework for previously reported findings on cancer drug resistance and their evolutionary origins, and explains why these ancestral non-genetic mechanisms of cancer drug resistance are retained specifically in dual-patterned cell systems of cancer and parasitic protists.

The present analysis aligns with recent calls within conventional cancer research for a more integrative understanding of the mechanisms underlying cancer drug resistance and their cooperative interaction.[47]

Within the framework of ECCB, therapeutic stress acts as a powerful ecological and evolutionary selective

pressure comparable to historical environmental stressors such as oxygen fluctuations, nutrient deprivation, toxic exposure, or oxidative stress. In response, cancer cells activate conserved adaptive mechanisms including epigenetic reprogramming, phenotypic plasticity, dormancy, senescence-associated reprogramming, polyploidization, genome reconstruction, and irreversible (transgenerational) or reversible drug-tolerant (non-transgenerational) states.

Accordingly, drug resistance is not exclusively a genetically determined phenomenon, but also a dynamic and reversible process regulated through non-genetic and eco-epigenetic mechanisms. These adaptive responses generate heterogeneous cancer cell subpopulations with distinct survival capacities, enabling rapid adaptation and resistance to therapeutic pressure and facilitating tumor persistence, relapse, metastasis, and progression.

From this viewpoint, pre-existing transgenerational resistance and treatment-induced, evolutive drug resistance represent two complementary manifestations of the same ancestral adaptive system: one reflecting the persistence of intratumoral selected resistant cell states, and the other reflecting the inducible reactivation of conserved stress–survival pathways under therapy-induced environmental pressure.

In this context, ECCB[1–4] offers a unifying framework in which cancer drug resistance is understood as the coordinated output of ancestral genome-protection and damage-prevention networks. These systems function to minimize DNA damage, safeguard genomic integrity, and sustain stemness-related capacities.

Moreover, ECCB analysis suggests that the cancer cell system is genomically structured into distinct functional sub-compartments, some of which are selectively shielded by epigenetically regulated protective processes. The activation of these ancestral protection programs is orchestrated through expanded DDR circuitry that integrates hyperpolyploid genome reconstruction and repair with coordinated programs of cellular plasticity and epigenetic reinforcement.[1-4]

5.1. Ancestral eco-dependent mechanisms of cancer cell protection

From an evolutionary perspective, cancer drug resistance is not merely the consequence of random mutations or therapy-induced gene defects, but represents an adaptive survival strategy of the unicellularized cancer cell system. Drug resistance reflects the reactivation of ancient genome-protection and stress-response programs that originated during early evolutionary history and were conserved within ancestral genome compartments.

Cancer drug resistance (desensitization) can be interpreted as an evolutionary extension of ancient genome-protection and damage-prevention networks that originated in the common AMF ancestor and its strong hypoxic Ur-germline. From an evolutionary perspective, two ancient oxygenation events acted as key stressors that triggered the emergence of protective mechanisms, shaping the coordinated genome-protection networks in unicellular and unicellularized cell systems, including cancer.[1-4]

5.1.1. The first historic oxygenation event

Alpha-moment-imposed selective pressure favored the emergence of an oxygen-resistant, somatic–ancestral lineage capable of shielding the hypoxic genome compartment from the damaging effects of elevated oxygen levels. This ancient GST process resulted in a constitutive soma program and generated a dual-cell system in which oxygen-resistant somatic cells shielded an epigenetically inactivated hypoxic genome compartment (the Ur-germline genome) from the deleterious effects of oxygenation.

During the transition from the hypoxic Ur-germline to the oxygen-adapted somatic line, conceptualized as GST, oxygen-sensitive regions of the hypoxic Ur-germline genome became tightly packaged around histone proteins, resulting in epigenetic silencing and inactivation of parts of the hypoxic genome that controlled stemness and self-renewal (Figure 2). In this way, DNA methylation helps preserve fundamental stem cell hallmarks.

Such methylation-dependent mechanisms have also been implicated in therapy resistance across diverse treatment modalities.[76] Hypermethylation-mediated silencing of tumor suppressor genes, together with modulation of DNA damage response pathways, represents a recurrent strategy for cellular adaptation to stress.

This epigenetic inactivation by wrapping, conceptualized as the α-protection shield, protects vulnerable genome loci of the hypoxic stemgermline genome during oxygenic life phases. This protective α-configuration ensured the long-term integrity and function of the hypoxic stemgermline genome.

Such methylation-dependent mechanisms have also been implicated in therapy resistance across diverse treatment modalities.[76] Hypermethylation-mediated silencing of tumor suppressor genes, together with modulation of DNA damage response pathways, represents a recurrent strategy for cellular adaptation to stress.

5.1.2. The second historic oxygenic event

The β-moment is designated, marking a critical evolutionary

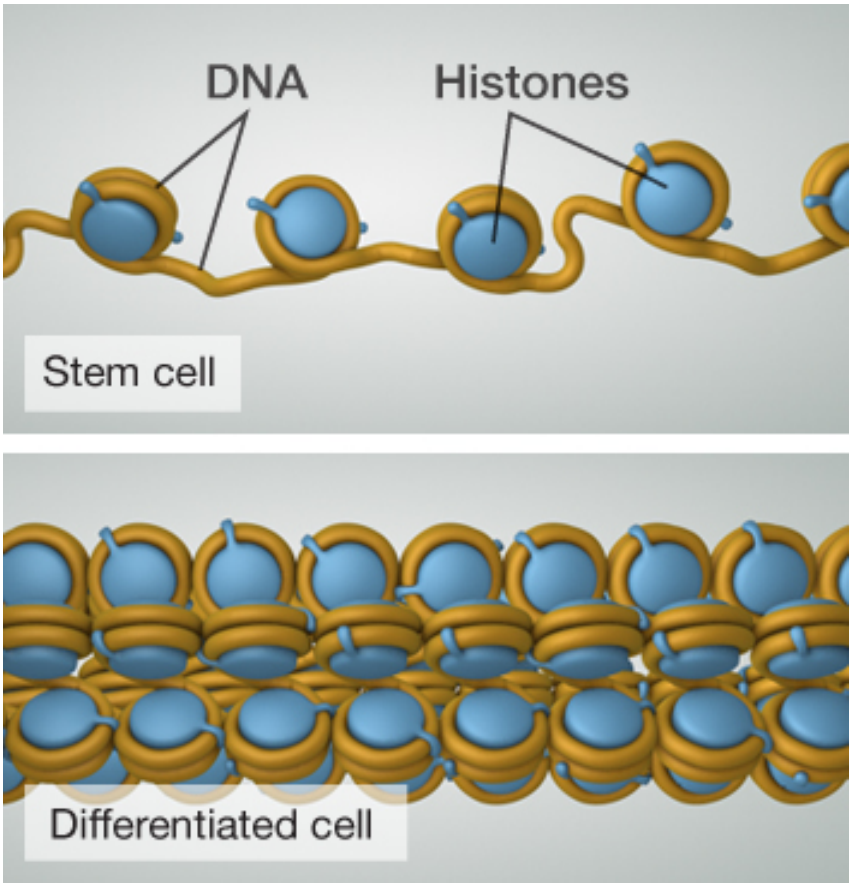

Figure 2. In stemgermline cells that are converted into oxygen-resistant somatic cells during the germ-to-soma transition, DNA (gold) is loosely wrapped around histone proteins (blue). In somatic cells that lack stemness, the stem cell genome compartment is hidden and protected from hyperoxic stress. Reproduced from: https:// learn.genetics.utah.edu/ content/stemcells/ips.

transition in which sustained increases in surrounding oxygen exposed residual vulnerabilities that were not safeguarded by the α-protection shield. These vulnerable sites, evident in the somatic cells exposed to chronic hyperoxia, became targets of intensified oxidative stress. The second oxygenic crisis necessitated expansion of the evolutionary epigenetic system into a more comprehensive β-protection program capable of securing all sensitive genomic loci to β-stressors and apoptotic death.

The cancer cell system appears to have retained both of these ancestral genome-protection programs. In conventional cancer research, activation of the β-protective program is commonly described as pre-treatment resistance or intrinsic MDR—a condition in which tumors exhibit broad refractoriness to various therapeutic agents.

Historically, the roots of the cancer cell plasticity (EMT/MET cycles) lie in the ancestral α- and β-GST cycles that evolved millions of years ago, at the evolutionary turning points marked by the first and second oxygenic events, as the increase in environmental oxygen destabilized the hypoxic stemgermline genome, abolishing differentiation and stemness functions through irreversible genomic damage and dysfunction of DNA repair systems.[1,2]

5.2. Autonomous α-protection programs

The emergence of an additional oxygen-resistant soma lineage provided an adaptive solution to the first evolutionary crisis. This newly evolved soma was capable of shielding the hypoxic Ur-germline genome from irreversible oxygen-induced damage, thereby preventing genomic destabilization. During the ancestral GST process, the hypoxic genome compartment was transferred into the somatic cell line in an epigenetically protected cell state. The protective epigenetic wrap, which originated in the first evolutionary oxygen crisis, is conceptualized here as the α-protection shield.

Soma serves multiple objectives of the dual cancer cell system. First, it enabled more efficient utilization of tumoral resources, thereby supporting sustained symmetric proliferation and population expansion (bulk tumor cell proliferation). Second, it secured the hypoxic genome compartment during the transition to oxygenic life. Third, the oxygen-resistant soma functions as an effectively unlimited reservoir for generating replacement stemgermlines and restoring genomic integrity. And fourthly, when the damaged stemgermline signals irreparable DNA damage, somatic cells are activated to undergo a restorative SGT, generating progenitors for stemgermline replacement. Somatic cancer cells closed the ancestral GST/SGT (MET/EMT) cycle and ensured the evolutionary persistence of functional stemgermlines (Figure 3).

5.3. Soma as a reservoir for replacement germlines

In cancer, the constitutive soma arises from the nascent cancer stemgermline and not from mitotic differentiation. The constitutive GST program establishes a somatic phenotype that safeguards the primordial functions of the stemgermline, stemness and phenotypic ACD potential, through functional inactivation and protection during the oxygenic phase of life.

Both unicellular protists and unicellularized cancer cell systems have evolved a dual strategy to cope with evolutionary α-stressors, enabling the replacement of damaged stem-germline and/or somatic cell populations by newly generated stemgermline or somatic sublines. When stem-germline cells are genomically compromised by α-stressors, specific molecular signaling pathways activate SGT processes. During this process, the intact, intra-somatically protected stemgermline genome is reactivated, generating replacement stemgermline cells capable of self-renewal and polyploid cyst-like differentiation. Conversely, when somatic sublines undergo apoptotic damage due to α-stressor activity, the remaining intact stemgermline cells generate new fully functional somatic clones and replacement sublines through α-GST processes (Figure 3).

In cancer, stemgermline cells located in the vicinity of newly formed capillaries may experience acute oxygenic stress. This hyperoxic exposure can compromise the stemgermline through severe DNA damage. Such angiogenesis-associated damage (Figure 1) may have profound consequences. The stemgermline may become dysfunctional, aborting ACD proliferation and com-CSC differentiation. Under these circumstances, multiple molecular signaling pathways are activated, initiating a dual strategy for the development of replacement stemgermlines, namely DDR and SGT/EMT processes.

5.4. DNA damage response, multinucleated genome repair structures, circuitry, and genome reconstruction

The first DDR pathway represents an attempt by injured stemgermline cells to restore their dysfunctional genome through a hyperpolyploid genome-reconstruction program. Cancer stemgermline cells harboring irreversible DNA damage may resist apoptotic death and continue proliferating as dysfunctional DSCD cells. These cells can fuse to form MGRS'/PGCCs (Figure 3).

Within hyperpolyploid MGRS syncytia, damaged genomic parts are selectively eliminated, reorganized, or reduced, while intact stemgermline genomic configurations are progressively reconstructed. The progeny emerging from these structures give rise to replacement stemgermline sublines. Thus, this first DDR/MGRS pathway functions as a direct regenerative response to damage in the stemgermline compartment.

5.5. Soma-to-germ plasticity is a rapid alternative to the DNA damage response

The second DDR pathway targets somatic tumor cells carrying the epigenetically inactivated stemgermline compartment (Figure 4). A subset of these somatic cells is stimulated to undergo α-SGT processes. During this transition, both the repressive epigenetic configuration and the accumulated somatic mutations are selectively eliminated, and the functional stemgermline genome is reactivated in a state of genomic integrity.

Progenitor cells resulting from DDR pathways generate replacement stemgermlines that assume the functional role of the damaged stemgermline. In this manner, the cancer cell system preserves its regenerative stemgermline core not only through direct genome reconstruction of the injured stemgermline genome, but also through activation of the protected germline genome embedded within somatic cells.

However, this epigenetic α-wrap did not fully desensitize all cancer cells, leaving many somatic sensitive sites unprotected and vulnerable to stressors. It produced merely a state of partial protection; while it effectively safeguarded the core functions of the stemgermline, somatic bulk tumor cells remain sensitive to chronic stress and chemical stressors such as drugs and chemotherapeutic agents.

5.6. Comprehensive transgenerational β-protection mechanisms and full resistance memory

Cancer drug sensitivity arises from multiple sensitive genomic loci that persist in somatic cells derived from

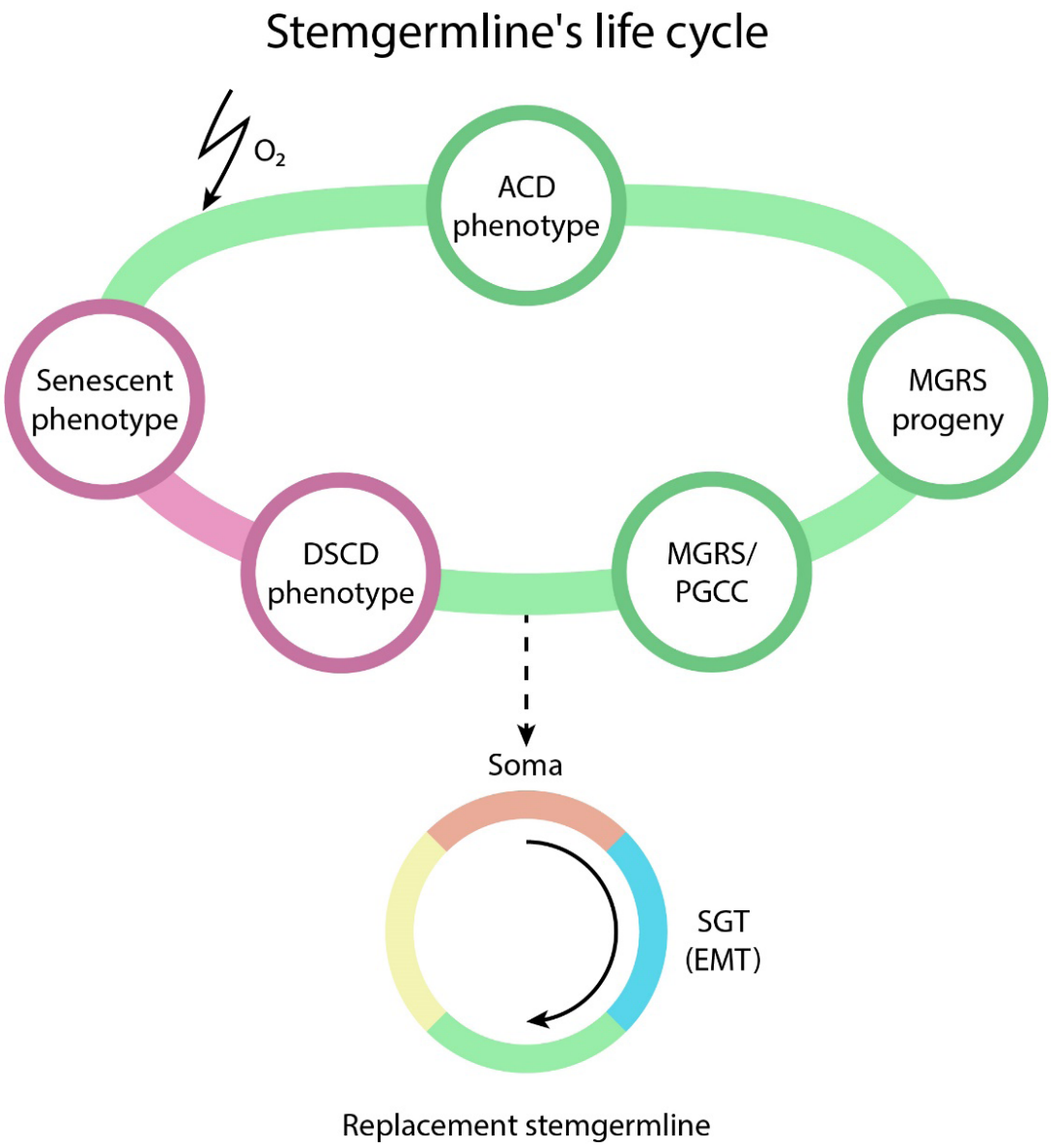

Figure 3. Stemgermline life cycle, genome reconstruction, and generation of replacement clones and sublines. Upper left: Hyperoxia-stressed ACD stemgermline cells undergo severe DNA damage, enter senescence, and evade apoptosis (red). These amitotic, senescent cells persist and give rise to DSCD exiters, which proliferate via defective symmetric cell division (red). Genome repair is mediated through stemness-positive multinucleated genome repair structures (MGRS) and polyploid giant cancer cells (PGCCs), whose progeny serve as precursors for replacement clones and sublines (green). Lower panel: An additional soma-to-germ transition (SGT) pathway contributes to the generation of replacement stemgermlines. At the terminal stage of the SGT process, transiting cells (blue-to-green transition) undergo progenitor accumulation through cyst-like polyploidization, ultimately giving rise to new stemgermline lineages.
Notes: Blue: Soma-to-germ transition; Green: ACD stemgermline phenotypes; Yellow: Germ-to soma transition; Red: Somatic cell phenotypes.
Abbreviations: ACD: Asymmetric cell division; DSCD: Defective symmetric cell division; EMT: Epithelial–mesenchymal transition.

α-GST processes. However, these vulnerabilities can be progressively overcome through recurrent β-GST processes and repeated transgenerational epigenetic shielding (Figure 4), ultimately rendering somatic cells resistant to β-like stressors, including sustained, chronic hyperoxia and therapeutic agents.

In cancer, this evolutionary β-resistance typically becomes detectable during therapy and is accompanied by recurrent GST processes that expand the epigenetic β-protection shield. In some tumors, however, β-resistance may also arise in the absence of therapy through chronic intrinsic stressors.

This evolutionary resistance mechanism, inherited from the common AMF ancestor, suggests that β-resistance is a specialized adaptive feature of early non-genetic evolution that confers a substantial transgenerational survival advantage to the drug-resistant somatic cells. Corresponding β-GST processes enabled the formation of somatic resistant clones and sublines long before cancer appeared as a pathological condition.

6. Reassessment of drug resistance

When the findings of conventional cancer research on reversible and irreversible drug resistance[77–87] are considered in light of the previously analyzed α-protection program, four fundamental principles emerge:

(i) Transgenerational drug desensitization unfolds progressively throughout recurrent β-GST processes;
(ii) The desensitization process is completed only when somatic, β-GST-derived bulk tumor cells reach a transgenerational, irreversible state of drug resistance;
(iii) Complete epigenetic protection against therapeutic agents generally occurs when all evolutionarily sensitive genomic regions are epigenetically silenced through β-wrapping;
(iv) Along the trajectory toward this terminal-resistant transgenerational state, tumor cells pass through several intermediary configurations that represent reversible, non-permanent states of drug resistance.

It becomes evident that tumor cell desensitization comprises a hierarchy of transitional stages. During extrinsically induced recurrent β-GST phenotypes, drug-sensitive genomic loci must be sequentially identified and epigenetically shielded. Only when all stress-sensitive loci are inactivated and epigenetically protected is the transition to a permanently resistant somatic phenotype completed. These fully protected somatic cells correspond to the resistant bulk tumor cell population observed clinically.[77-87]

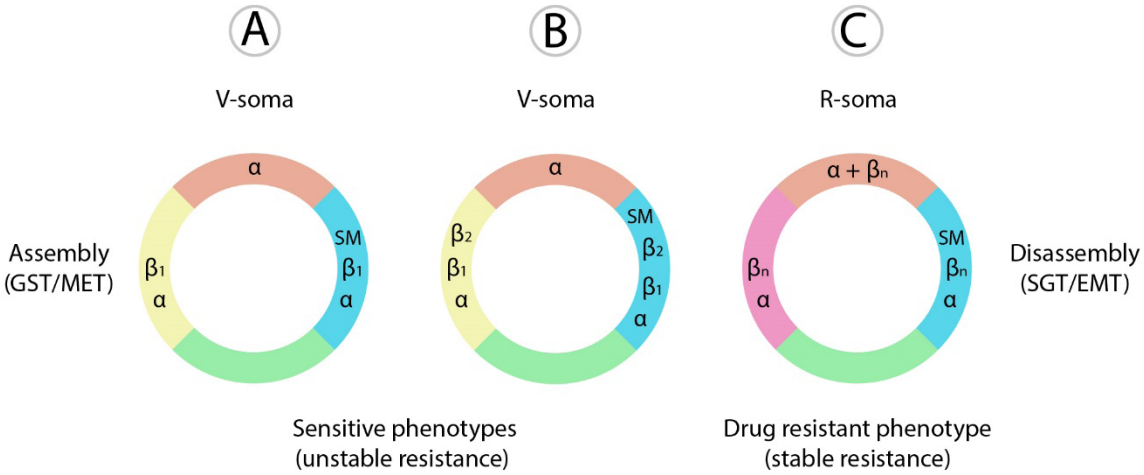

Figure 4. Reversible and irreversible somatic bulk tumor resistance. Panels A and B illustrate reversible resistance phenotypes (non-transgenerational), whereas panel C represents an irreversible resistant (transgenerational) state. V-soma denotes somatic cells with vulnerable genomic sites, while R-soma denotes resistant somatic cells capable of sustained proliferation under drug-resistant conditions. During SGT-mediated disassembly processes, α- and β-wrapping structures, as well as somatic mutations, are progressively removed, enabling phenotypic transitions from resistant somatic cells and new stemgermline clones of genomic integrity. Optionally, following dissolution, an environment-dependent, hidden process of precursor accumulation may occur through cyst-like polyploidization and depolyploidization.
Notes: Blue: Soma-to-germ transition; Green: ACD stemgermline phenotypes; Yellow: Germ-to-soma transition; Red: somatic cell phenotypes.
Abbreviations: ACD: Asymmetric cell division; EMT: Epithelial–mesenchymal transition; GST: Germline-to-soma transition; MET: Mesenchymal–epithelial transition; SGT: Soma-to-germ transition.

If, however, exposure to drugs or chemotherapeutic agents is too brief or insufficiently intense, the eco-epigenetic β--protection remains incomplete and reversible. Under such conditions, intermediary phenotypes (β_1, β_2 … β_n) emerge (Figure 5) that are neither fully desensitized nor permanently resistant. In cases of therapy withdrawal, partially protected β-clones may subsequently lose their epigenetic shielding and revert to drug-sensitive states or regress toward a germline-like condition.

In contrast, fully protected somatic cells that have achieved permanent and irreversible resistance retain this expanded protective configuration during subsequent mitotic proliferation and recurrent plasticity cycles (epigenetic memory). On the other hand, during reverse fractal SGT processes, the protected genome compartments may be progressively dismantled. Fully functional replacement stemgermlines, clones, and sublines preserve the once-activated epigenetic memory. Upon re-entry into a new GST process, this memory facilitates the rapid re-establishment of the complete protective configuration. Consequently, the derived somatic bulk tumor population remains stably resistant.

This comprehensive eco-epigenetic β-protection enabled the emergence of desensitized somatic phenotypes that were resistant to oxygen and chemical stressors, including therapeutic agents. Unlike the restricted α-protection in hypoxic stemness compartments, this β-protection program ensures full-genome protection and prevents genome destabilization, even under chronic, permanent stress.

The unicellularized cancer cell system has adopted both α- and β-plasticity programs (Figure 4). While the first, epigenetic-restricted α-protection program leaves many vulnerable (V-) sites open, the β-protection program closes them all, providing comprehensive epigenetic protection.

This framework indicates that cancer drug resistance is fundamentally rooted in evolutionarily conserved programs designed to preserve the genomic integrity and functional capacity of the cancer stem cell germline. In conventional cancer research, activation of this second ancestral β-plasticity program is commonly described as intrinsic or pre-treatment MDR, a state against which a broad spectrum of therapeutic agents proves ineffective.

Significantly, the comprehensive β-protection program does not prevent somatic mutations. However, such mutations exert limited impact on long-term genomic integrity within the genome-preserving GST/SGT cycles (Figure 4). During SGT processes, the epigenetic wrap structures characteristic of the resistant somatic phenotype are dismantled, and somatically acquired mutations are largely eliminated or rendered functionally irrelevant. The newly generated hypoxic replacement stemgermlines are thus restored to full functionality in stemness, ACD, and differentiation capacity. Subsequent GST processes re-establish the comprehensive epigenetic β-wrap, thereby reinstating the somatic progeny's resistance to environmental stressors and therapeutic agents.

7. α- and β-phenotypes as observed in parasitic amoebas

In earlier years, the present author devoted considerable effort to the study of parasitic amoebas and identified in *Entamoeba* a hypoxia-adapted, hyperoxia-responsive cellular system exposed to microenvironmental conditions similar to those encountered by cancer cells within human and other metazoan hosts. These investigations revealed striking similarities in the common adaptive strategies of *Entamoeba* and cancer, pointing to deep homology between the two parasitic systems and, by implication, to their shared evolutionary origin in the common AMF ancestor.

During this period, numerous publications emerged on the relatively little-known life-cycle biology of *Entamoeba*,[1] including studies on stemness, polyploidization, encystation, cell differentiation, genome reconstruction, and adaptive responses to hypoxic and hyperoxic stress. Collectively, these investigations provided the foundation for recognizing the deep evolutionary parallels between the *Entamoeba* cell system and the unicellularized cancer cell system.

Parasitic amoebas such as *Entamoeba* provide compelling biological evidence that evolutionary α- and β-genome protection programs operate beyond the cancer cell system. The parasitic life cycle of *Entamoeba* encompasses the very same phenotypes as the parasitic system of cancer, both *in vivo* and *in vitro*. The most significant difference between the two life cycles lies in how they form stemgermline precursor cells. In cancer, this occurs through a hidden, cyst-like polyploidization at the end of the SGT process. In contrast, *Entamoeba* exhibits a species-specific polyploid cyst that can interrupt the GST/SGT cycle for an extended period.

Different *Entamoeba* culture conditions characterized by varying oxygen gradients selectively favor either hypoxic stemgermline populations (formerly referred to as "Minuta") or more oxygen-resistant somatic cell types (trophozoites, or "Magna"). Culture growth is regulated by oxygen gradients comparable to those found in host organs, blood, and tumor environments. Hypoxic growth conditions, achieved in sediments containing metabolically

suppressed, oxygen-consuming bacteria (OCB).[122,123] OCB sediment cultures promote the emergence of somatic α-phenotypes. In contrast, synthetic, bacteria-free hyperoxic conditions (axenic hyperoxic cultures) favor the development of somatic hyperoxia-resistant β-phenotypes alongside the formation of defective stemgermline cells (DSCD phenotypes).

Somatic cells arise through the GST and not through differentiation processes from self-renewing stemgermline phenotypes. *In vivo*, somatic cells formed through GST can invade the intestinal mucosa and migrate via the bloodstream to more oxygenic host organs, particularly the liver, where they cause abscesses. During migration, the cells undergo the α-protection program. Hepatic *Entamoeba* cells are somatic cells that protect their hypoxic stemgermline genome with a suitable α-wrap.

Hepatic trophozoites typically represent somatic α-phenotypes that can proliferate extensively until host death. They are deep homologous to unicellularized, drug-sensitive bulk tumor cells that lose stemness and ACD potential, and protect the hypoxic stemgermline through α-wrapping. Although hepatic trophozoites no longer form cysts, they represent an epigenetically stabilized α-phenotype that preserves the capacity to regenerate stemgermline clones and sublines via reverse SGT processes.

Axenic trophozoites arise in synthetic, hyperoxic, bacteria-free cultures originally developed by Diamond.[124] Due to their simplicity and high yield of somatic cells, these cultures became standard reference systems for *Entamoeba*. However, this artificial culture imposes strong oxygenic stress. As a result, both somatic trophozoites and oxygenic-stressed DSCDs fail to form cysts under axenic culture conditions. Encystation can only be induced when cells leave the culture environment and are exposed to hypoosmotic stress and nutrient deprivation.

Axenic trophozoites arise through axenization, defined as the transfer of *Entamoeba* from the microbiota-rich intestinal environment into bacteria-free synthetic culture media. Adaptation to this extreme environmental shift and chronic hyperoxic stress is slow and demanding, typically requiring up to 30 days. This transition is enabled by extensive hyper-polyploidization and the emergence of multiple intermediary cell states.[125,126]

Polyploidization and MGRS-hyperpolyploidization are unicellular hallmarks of both parasitic cell systems in amoebas and cancer. These processes not only facilitate progenitor accumulation during the late stages of reverse SGT processes (Figure 5) but also restructuring and functional restoration of dysfunctional DSCD genomes into a functional stemgermline genome (Figure 3).

Genome reconstruction via MGRS proceeds in two sequential phases.[3,127,128] Following DSCD cell fusion and MGRS formation, each individual MGRS nucleus undergoes cyst-like polyploidization, reaching an amplification ratio of up to 1:16. This genetic amplification process is observed in both protist- and cancer-associated MGRS (PGCC) structures. In the second phase, the amplified but still dysfunctional genome copies are merged and reorganized into one or more hyper-polyploid giant repair nuclei. During genome reconstruction, damaged DNA parts are resolved, and intact genomic regions are selectively assembled to generate a new, functionally competent stemgermline genome. MGRS were described in *Entamoeba* by Craig in 1908.[129]

In long-term axenic cultures exposed to sustained chronic stress, intestinal amoebas progressively convert into a resistant somatic β-phenotype that is homologous to drug-resistant somatic bulk tumor cells. Chronic oxidative stress in primary cultures gradually eliminates genomic vulnerabilities through successive rounds of hyperpolyploidization during serial culture passages,[129–131] ultimately culminating *in vitro* in the establishment of a stable-resistant β-phenotypes.

These observations suggest that chronic and persistent stress may promote the establishment and maintenance of stable transgenerational DSCD-resistance programs in both somatic and stemgermline cell lineages.

As observed in axenic cultures of *Entamoeba*, chronic hyperoxia may interrupt MGRS depolyploidization before complete functional genome restoration has been achieved. Consequently, full genome regeneration may require additional rounds of cystic polyploidization or hyperpolyploidization. Somatic DSCD cells appear to regenerate their genomes primarily through cystic polyploidization (Figure 5), whereas DSCD stemgermline cells require the complete two-phase MGRS repair and reconstruction program, consisting of an initial phase of cystic polyploidization followed by a second phase of hyper- and depolyploidization.

It cannot be excluded that hepatic abscess clones developing *in vivo* may undergo partial conversion toward β-like phenotypes. Notably, different *Entamoeba* strains exhibit substantial variation in pathogenicity and virulence. Whether such differences are related to the progressive reduction of vulnerability (fractal prevention) through β-shielding processes remains to be determined.

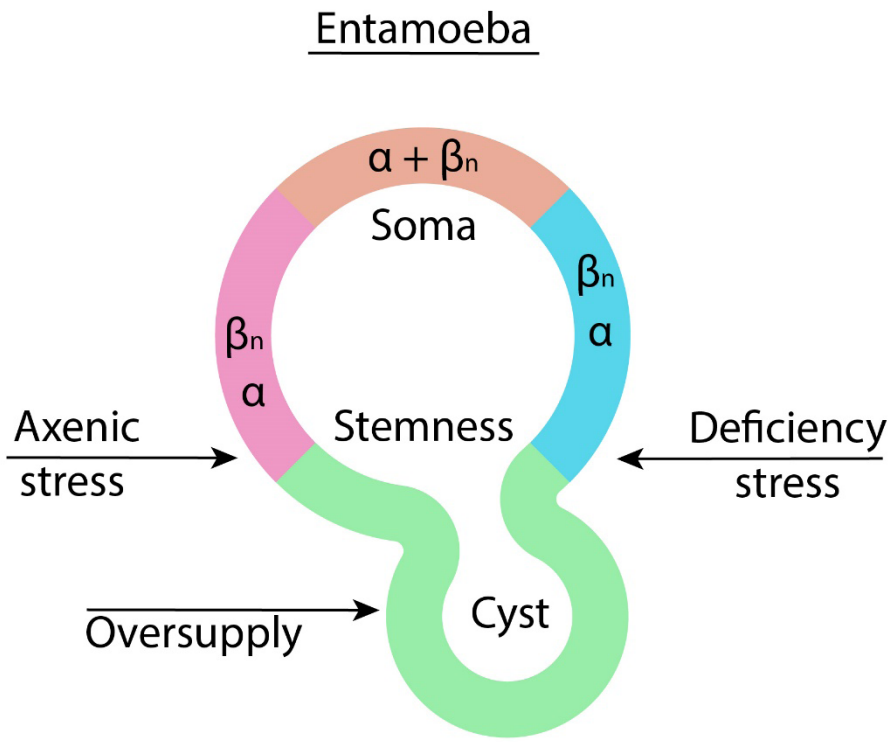

Figure 5. β-plasticity cycles in axenically grown somatic *Entamoeba* (hyperoxic culture media) and after exposure to encystation conditions (encystment media). Upon transfer to encystment media (deficiency stress), chronic stress, transgenerational soma phenotypes derived from axenic cultures interrupt proliferation and initiate soma-to-germ transition, followed by encystation. Cyst formation is particularly essential for species survival and dissemination; following uptake by secondary hosts or exposure to culture media, cysts encounter nutrient-rich conditions (oversupply) that trigger excystation and the release of stemgermline progenitors (amoebulas).[121,122,128,129] These haploid progenitors are capable of re-establishing functional stemgermlines.
Notes: Red: Germ-to-soma transition; Brown: Somatic cell phenotype; Blue: Soma-to-germ transition; Green: Cyst formation (encystment) by the asymmetric cell division stemgermline phenotype and excystment and formation of progenitor cells (amoebulas).

8. Conclusion

An increasing number of researchers are now supporting the unicellularization theory proposed by ECCB and the fundamentally evolutionary nature of cancer. Recently, Ashouri *et al.*[130] argued that cancer development begins when cells transition from a multicellular state toward a condition resembling unicellular organisms. According to these authors, this shift disrupts the regulatory architecture of multicellularity and promotes the emergence of diverse cancer cell lineages that have ancestral characteristics. Such insights are essential for understanding the multiple evolutionary trajectories of cancer and for developing therapies targeting the principal drivers of cancer evolution.

A large body of studies on protists published by the present author over the past two decades further suggests that the cellular organization and stress-response strategies of parasitic amoebas and cancer cells are not merely analogous, but instead reflect deeply conserved evolutionary programs. The α- and β-phenotypes observed in *both systems* represent ancient genome-protection strategies that emerged during the two major evolutionary oxygen transitions in early eukaryotic history.

The ECCB framework proposes that the ancestral α- and β-programs responsible for genome protection, maintenance of genomic integrity, cellular plasticity, and adaptive survival under fluctuating oxygen conditions are executed through distinct germ-to-soma transition pathways that originated during the evolution of the common AMF ancestor. These programs, which combine genome reconstruction with reversible cell state plasticity, may underlie the remarkable resilience of both parasitic amoebas and cancer cells to environmental and therapeutic stress.

This work in evolutionary oncology provides a broader interpretative framework for previously reported findings

on cancer drug resistance and their evolutionary origins, and explains why these non-genetic, ancestral mechanisms of cancer drug resistance are retained specifically in the dual, unicellular patterned cell systems of cancer and parasitic protists. It demonstrates that the majority of non-genetic cancer mechanisms reflect conserved evolutionary programs that emerged in the common AMF ancestor under selective pressures when the Ur-germline of the AMF ancestor was exposed to rising oxygen levels.

Together, multiple reparative plasticity cycles, stemness loss, and recovery dynamics, expanded DDR processes including the MGRS pathway, and polyploidy–hyperpolyploidy cycles constitute a complex and highly resilient lifestyle. This integrated system enables cells to withstand diverse stressors and therapeutic challenges. All of these processes are encoded within conserved compartments of the ancestral human genome.

The evolutionary plasticity programs, which can still be observed in cancer and parasitic protists today, function to protect the hypoxic stemgermline genome of both ancestral cell systems. They ensure that sensitive stemgermline genome compartments are inactivated under hyperoxic stress or drug exposure and preserved as epigenetically protected genomic reserves within the somatic bulk cancer cell population.

These mechanisms comprise: (i) tyhe intrinsic α-GST program generating somatic bulk tumor cells; (ii) the extrinsic, induced β-GST program, which extends α-wrapping and identifies additional sensitive β-genome sites for epigenetic β-wrapping; (iii) the GST/SGT cycles—better known as MET/EMT cycles—where the SGT (EMT) process removes epigenetic wrapping and somatic mutations, thereby reactivating genomically intact replacement stemgermlines; and (iv) epigenetic transgenerational memory mechanisms that maintain the β-wrapping configuration during proliferation of the somatically resistant population. α-GST and β-GST are distinct genome-protection programs that emerged during GST in response to the first (α-) and second (β-) oxygenic crises. In cancer, all of these eco- evolutionary processes are activated by hyperoxic damage to the hypoxic cancer genome compartments.

From an evolutionary standpoint, cancer drug resistance represents a cellular state that arose long before the emergence of cancer in multicellular host organisms. The chemoresistance of dual-cell systems—observed in both protists and cancer—reflects a universal principle of evolutionary cell biology. Both forms of cancer drug resistance, native and acquired drug resistance, described in conventional research as intrinsic and extrinsic, are rooted in ancestral chemoresistance mechanisms established during the second oxygen event (β-crisis). Cancer drug resistance represents a modern manifestation of this ancestral β-resistance program, which inactivates and epigenetically shields sensitive hypoxic genomic regions in the common ancestor, as well as in cancer and protist genomes.

During β-GST/MET processes, drug-triggered sensitive genomic sites are progressively identified, inactivated, and epigenetically protected. This results in a series of intermediate β phenotypes (β_1-, β_2-,… β_n) with variable resistance. Only when β-wrapping is fully completed, and no sensitive genomic sites remain unprotected, is the stable transgenerational phenotype achieved. If exposure to extrinsic stressors or chemotherapeutic agents is insufficient in duration or intensity, epigenetic remodeling remains incomplete, and the intermediary phenotypes are neither fully desensitized nor irreversibly resistant.

In contrast, cells that achieve a fully established epigenomic protective shield (transgenerational β_n-phenotype) retain this configuration during subsequent β_n-SGT cycles and somatic proliferation. They preserve molecular memory of the β-program and autonomously maintain β-wrapping.

Insights from evolutionary oncology indicate that future successful therapies must target the vulnerable nodes of these ancestral mechanisms, including senescent phenotypes, DDR circuitry, and the GST/SGT plasticity cycles. Even more promising may be molecular strategies directed against the hypoxic nascent cancer stemgermline and the unicellularization process of the cell of origin, potentially including vaccine-based approaches. An evolutionary framework may therefore substantially enhance the effectiveness of future molecular cancer therapies.

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