

REVIEW ARTICLE

Reevaluating cancer stem cells and polyploid giant cancer cells from the evolutionary cancer cell biology perspective

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Abstract

According to the principles of Evolutionary Cancer Cell Biology (ECCB), cancer stem cells (CSCs) do not derive from normal stem cells; rather, they originate from a distinct functional phenotype of germline cells characterized by asymmetric cell division (ACD). This phenotype proliferates through ACD, producing self-renewing cells alongside non-proliferating daughter cells with CSC qualities. ECCB posits that CSCs do not proliferate. Similar to protists, there exists a close reciprocal relationship between sister cells that collectively form a germline and stem cells. These sister cells perform complementary roles: proliferating cancer germline cells generate stem cells, whereas the non-proliferating CSCs give rise to progenitor cells for the formation of new germline clones. ECCB distinguishes between primary CSCs, which are associated with carcinogenesis and primary tumors, and secondary CSCs, which are linked to metastases. This unicellular stem cell system is homologous to that of parasitic protists, such as amebae. Both CSCs and amebae stem cells are produced by an oxygen-sensitive germline and are vulnerable to damage when oxygen levels exceed 6.0% (germline hyperoxia), as elevated oxygen concentrations can harm the germline genome. Germline cells that lose their stemness quality continue to cycle through defective symmetric cell divisions (DSCD). However, to restore functionality, the DSCD genome must be repaired through hyperpolyploidization. This process occurs in native polyploid giant cancer cells, which are homologous to the multinucleated genome repair structures found in protists.

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1. Introduction

Contemporary definitions of stem cells and cancer stem cells (CSCs) commonly describe them as either (i) undifferentiated or partially differentiated cells capable of differentiating into various types of cells and cycling indefinitely to produce more stem cells, or (ii) undifferentiated cells that continuously divide to produce progeny, some of which remain as stem cells whereas others are destined to differentiate.¹ However, recent findings indicate that these definitions may be insufficient.

Researchers have long sought a model analogous to the cancer cell system to understand the origins and nature of cancer and CSCs. Such a model could potentially explain

the spread of cancer not only in humans and vertebrates but also in invertebrates and primitive metazoans. In the absence of such a model, cancer has frequently been described as the uncontrolled proliferation of cells driven by a complex interplay of extrinsic and intrinsic factors.² Consequently, the origin of CSCs has been traced back to other stem cells, such as normal adult stem cells (ASCs) or embryonic stem cells (ESCs).

This work explores the origin of CSCs and cancer through the framework of Evolutionary Cancer Cell Biology (ECCB), arguing that current CSC hypotheses are not evolutionarily valid. ECCB uncovers the deep homologous relationship between CSCs and the non-gametogenic oxygen-sensitive germline (Urgermline) of the common ancestor of amoebozoans, metazoans, and fungi (AMF), highlighting the shared characteristics of cancer and CSCs in terms of unicellularity.² However, before delving into this perspective, a brief overview of current CSC research outside the ECCB framework will be presented.

1.1. Current CSC hypotheses

Recent empirical and molecular research on CSCs has yielded significant progress, resulting in a wealth of new data. However, a lack of evolutionary insight has hindered a comprehensive understanding of the origins and development of CSCs.

Several studies conducted over the past 3 – 4 years have underscored the limitations of earlier CSC concepts. Researchers have identified CSCs as a small subset of cells that play a fundamental role in cancer development, progression, metastasis, and treatment resistance.³ The similarities between CSCs and normal stem cells (NSCs) have been well-documented, but the differences remain inadequately explained.

As reported by Rossi *et al.* in 2020,⁴ there is an ongoing controversy regarding the origin of CSCs, particularly whether they derive from NSCs or ESCs. While some researchers support this hypothesis, others propose alternative origins, such as cell-cell fusion, gene transfer, or mutations.⁵ In addition, some findings suggest that CSCs may arise from NSCs that fail to regulate their proliferation under abnormal conditions.^{6,7}

According to Tweedell,⁸ NSC populations typically consist of a mixture of quiescent stem cells, active stem cells, and progenitor cells at various stages of differentiation. A portion of the stem cell progeny is sequestered within tissue niches throughout different stages of organ development and differentiation. This concept has been applied to CSCs and their niche, which regulates both stem and progenitor cells, serving as a specific topographical and

functional site.^{9–11} In summary, the origin of CSCs remains controversial, with ongoing debate regarding whether CSCs arise from NSCs, progenitor cells, or dysfunctional progenitor cells present in tissue.²

1.2. Significant differences between CSCs and NSCs

Significant phenotypic differences challenge the assumption that CSCs originate from functional NSCs, ASCs, or ESCs. CSCs exhibit unlimited proliferation, excessive self-renewal, drug resistance, and the ability to generate heterogeneous populations of progeny (bulk tumor cells) that are incapable of replicating the tumor or forming metastases. These divergent characteristics imply that CSCs possess a genomic makeup distinct from that of NSCs. Most notably, CSCs lack the regulatory systems present in NSCs that prevent uncontrolled proliferation;^{5,12} however, they possess hyperpolyploid genome repair mechanisms that are absent in NSCs.¹³ From an evolutionary perspective, the existing similarities between CSCs and NSCs may reflect their shared ancestry with the common ancestor of AMF and its Urgermline.²

1.3. Requirements for a modern stem cell concept

According to most researchers, further efforts are needed to clarify the origin of CSCs to enhance existing therapies and develop new, clinically relevant cancer treatments. Recently, Loh and Ma¹⁴ emphasized the necessity of reevaluating the origins, hallmarks, and characteristics of CSCs. They argued that substantial evidence suggests cancer cells possess a plastic state influenced by the interplay of stressors and the environment, known as the CSC niche. The researchers propose that the features acquired through dedifferentiation require a reassessment of the basic attributes of the CSC state. Cellular plasticity allows various cancer cells to change into a stem-like phenotype, enabling tumor cells to enhance their malignant properties, including resistance to therapy and metastasis.

The author of the current work also advocates for a reevaluation of the CSC concept concerning germline progenitors and germline plasticity, as proposed by ECCB. From the ECCB perspective, current definitions of CSCs are imprecise and warrant revision. Many inconsistencies could be clarified through this framework. ECCB posits that CSCs are “born” from dysfunctional cells that have lost their ability to perform asymmetric cell division (ACD), with cancer representing a fundamental transition to a lower level of cellular organization of unicellular imprinting. This transition and the subsequent evolution of the cancer cell system are governed by an ancestral gene regulatory network (aGRN). The genomic elements of the ancestral cell system, including aGRN, reside within the ancestral genome compartment of all metazoans and

humans and can be reactivated at any time by both intrinsic and extrinsic factors. These switching mechanisms, which originated during the transition from unicellularity to multicellularity,^{2,13} are crucial for the initiation of cancer.

1.4. Proliferating germline and non-proliferating CSCs

Similar to the ancestral cell system of the common ancestor of AMF, the unicellular model of cancer consists of a germline and a somatic cell lineage.² During cancer evolution, this unicellular system generates heterogeneous germline clones that give rise to various stem cell lineages. All CSCs are germline cells produced by progressively evolved ACD clones, which cycle asymmetrically to yield two unequal daughter cells – one that self-renews and another that exits the cell cycle (Figure 1). The existing cells may differentiate, in an environmentally dependent manner, into either non-committed quiescent cells or committed CSCs for cyst-like amplification, both of which are non-proliferative. Quiescent cells have the potential to revert to a self-renewing state, producing proliferating ACD clones. Under optimal environmental conditions, they can also commit to becoming genuine CSCs.

In summary, the present ECCB paper challenges the current definition of stem cells and CSCs into four key statements: (i) the cells capable of infinite proliferation are not stem cells per se, but cancer germline cells, as long as they remain in the ACD state; (ii) during the ACD state, germline cells differentiate into CSCs; (iii) CSCs are non-proliferating germline stem cells capable of differentiation and the accumulation of germline progenitor cells; and (iv) CSCs and the ACD germline form a reciprocal, inseparable germline-CSC unit. Proliferating germline cells generate CSCs, whereas non-proliferating CSCs may generate proliferating germline clones through cyst-like polyploidization and progenitor amplification. The sister cell community functions only as long as the germline proliferates in its functional ACD state. When the germline transitions to its dysfunctional defective symmetric cell divisions (DSCD) state, CSC production ceases, and CSC depletion occurs. The ACD state governs this community, which is referred to as the “ACD germline and CSC sister cell unit” or “ACD lineage” for short.

2. The unicellular model of cancer and CSCs

As already mentioned, cancer is a complex and highly atypical disease that cannot be fully explained by genetic alterations or the accumulation of mutated genes over a lifetime. Rather, it represents a systemic shift from a multicellular cell system to a more primitive unicellular system, driven by hyperoxic shock above 6.0% oxygen, genome dysfunctionalities, and mechanisms for genomic

repair. In the past, cancer has even been likened to a parasitic multistage disease caused by a highly transformative pathogen.¹⁵

2.1. The germ and soma model of parasitic amebae

ECCB is a new oncological discipline that has emerged from the comparative analysis of the life cycles of cancer and protists, along with their respective stem cells.² This framework reveals that CSCs and stem cells of protists are evolutionary siblings (Figure 1) and that parasitic protists possess a cellular system remarkably similar to the germ and soma cell systems found in cancer. One of the most striking similarities between the two systems is the presence of a cyst-like polyploid stage, followed by a hyperpolyploid stage, which has been observed both in the multinucleated genome repair syncytia (MGRS) of amebae and the polyploid giant cancer cells (PGCC). These unique unicellular features, observed exclusively in cancer and amebae, strongly suggest a common evolutionary origin, indicating that native CSCs arise from the cancer germline and not from ESC or NSC lineages. Both stem cell systems exhibit the inherent mechanisms of the evolutionary Urgermline and its stemness.

The history of the ECCB began with the development of oxygen-consuming culture media for parasitic amebae. Contrary to the prevailing trend of cultivating *Entamoebae* in synthetic media, the author of this work successfully established oxygen-consuming cell cultures using metabolically suppressed bacteria. These oxygen-consuming bacteria (OCB) effectively mimicked the hypoxic conditions found in the intestine, characterized by an oxygen gradient ranging from 0.1 to 5.7%. In 2013, the author reviewed and updated these findings.^{16,17}

The *in vitro* results obtained with OCB media underlines the remarkable plasticity of the Urgermline and reveal that hypoxia is the natural physioxenic environment for all germline and stem cell activities. The hypoxic environment provided by OCB sediment cultures facilitates several key processes, including (i) ACD and the formation of CSCs (Figure 1), (ii) the germ-to-soma transition (GST) and soma-to-germ transition (SGT) processes that generate new germline clones, (iii) cyst-like accumulation of genome copies through cycles of polyploidization and depolyploidization, (iii) the production of fusogens in dysfunctional (DSCD) phenotypes that proliferate through symmetric cell division, (iv) homotypic cell fusion and formation of MGRS, and (v) genome repair through the MGRS repair pathway.²

In contrast, studies using synthetic media with an oxygen content above 6.0% (germline hyperoxia) have shown that: (i) hyperoxic conditions damage the germline

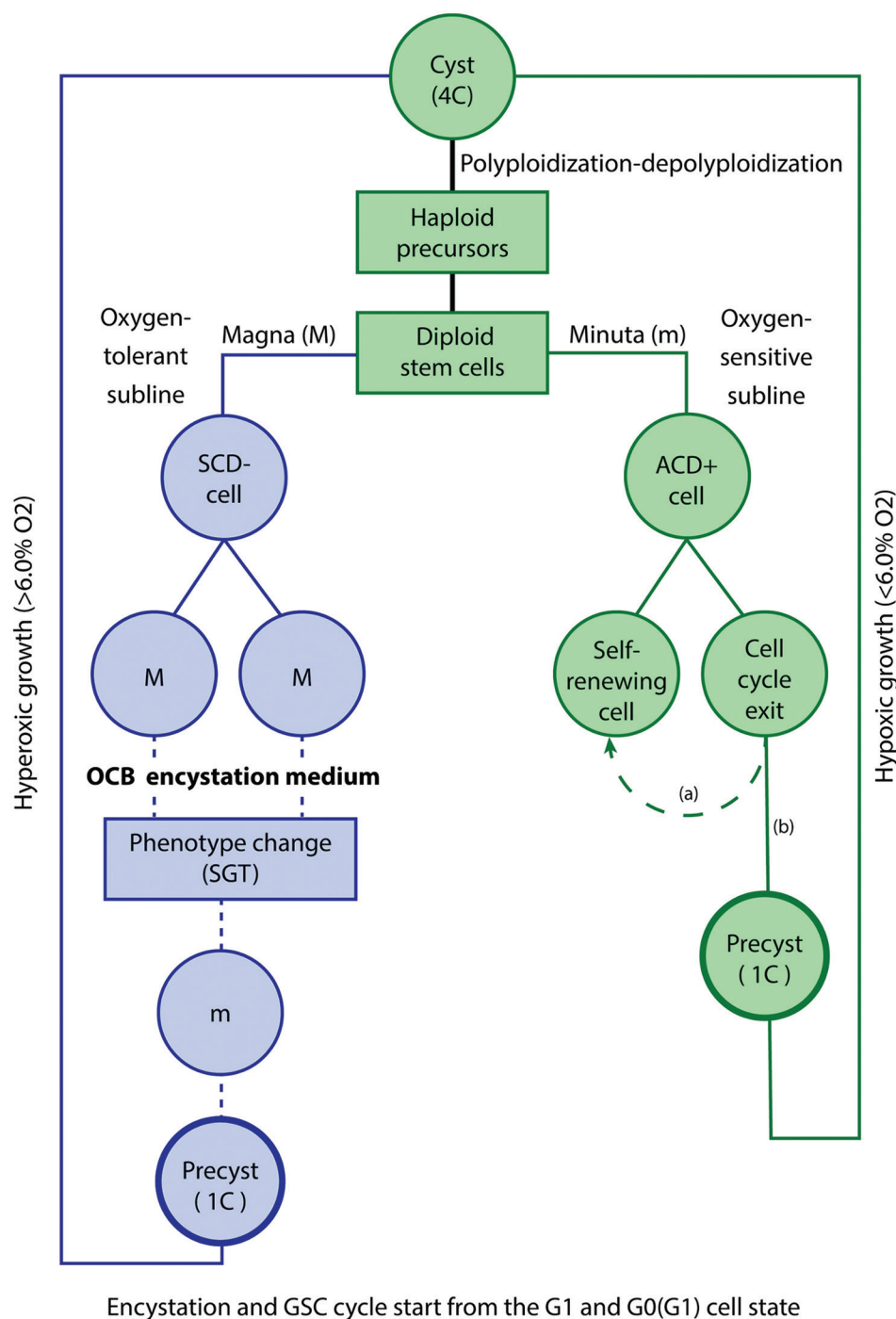


Figure 1. The life cycle of cancer and protists (*Entamoeba*) consists of a germline (green) and a somatic cell line (blue). The oxygen-sensitive germline proliferates through ACD, resulting in unequal daughter cells: one that self-renews and another that exits the cell cycle, giving rise to both committed and uncommitted stem cells (germline stem cells and cancer stem cells). Committed stem cells (referred to as pre-cysts) are capable of differentiation and can form cysts (in the *Entamoeba* context) or cyst-like structures (in the cancer context), amplifying their DNA content (cyst- or cyst-like polyploidization) and form progenitor cells for new germline clones or subclones via reductive nuclear division and cellularization (depolyloidization). During cancer evolution, the unicellular cancer cell system generates clones and subclones through polyploidization–depolyloidization cycles, which give rise to a large number of heterogeneous germline clones. Consequently, these clones give rise to a large number of CSC lineages. All CSCs are germline cells generated by more or less evolved ACD germline clones. Figure created by the author.

Abbreviations: ACD: Asymmetric cell division; GSC: Germline stem cell; G0(G1): Arrest state; G1: Growth state; O₂: Oxygen; SCD: Symmetric cell division; SGT: Soma-to-germ transition.

genome, (ii) the functional ACD germline phenotype, which normally gives rise to germline stem cells (GSCs, also referred to as CSCs), is irreversibly replaced by a DSCD phenotype, (iii) DSCD cells proliferate indefinitely through DSCD, resulting in tetraploidy and aberrant mitosis, and (iv) dysfunctional genomes can only be repaired through homotypic cell fusion and hyperpolyploidy.¹⁸⁻²¹

In 2023, Hazra *et al.*²¹ wrote, “In *Entamoeba*, a protozoan parasite that causes amebic dysentery and liver abscesses in humans, the formation of multinucleated giant cells (MGCs) is a unique phenomenon and has not been reported in any other protozoa. Accordingly, the formation of MGCs in *Entamoeba* is thought to be a survival strategy to cope with adverse conditions. This organism forms MGCs through cell aggregation and fusion in response to osmotic and heat stress. The MGCs in *Entamoeba* are thought to have increased resistance to various stresses and can survive under adverse conditions. The authors hypothesized that the increased survival ability could provide redundancy in case of DNA damage or mutations. In addition, MGCs may play a role in the virulence of *Entamoeba* as they are found in the inflammatory foci of amebic liver abscesses and other infections caused by *Entamoeba*.” However, the researchers do not realize that MGCs also serve to repair the DSCD phenotype that has developed in hyperoxic cultures.

Parallel to advancements in the study of the hypoxic cell biology of parasitic protists^{17,22,23}, cancer researchers have made significant strides in understanding the hypoxic biology of CSC lineages. Nevertheless, the unicellular origin of ACD lineages has not been fully acknowledged due to a lack of evolutionary understanding. Despite this, the phenomena of polyploidy and hyperpolyploidy have been accurately characterized, as has the genotoxic induction of PGCCs following radiation and chemotherapy treatments.²⁴⁻²⁶ In addition, cancer researchers have detailed the various aspects of epithelial-mesenchymal transition (EMT) in cancer,²⁷⁻³¹ and demonstrated how oxygen-sensitive germ cells disseminate into the bloodstream and tissues, forming surviving cell clusters with oxygen-resistant cells.³²⁻³⁷

2.2. Evolutionary origin of the unicellular germ and soma cell system adopted by cancer

According to the ECCB, the evolutionary origin of ACD lineages consisting of self-renewing germline cells and differentiated, non-proliferative CSCs dates back approximately 2,300 million years ago (mya), predating the emergence of metazoans.^{2,38} During this period, the common AMF ancestor evolved the dual life cycle consisting of an Urgermline and a somatic, oxygen-resistant

cell lineage. The non-gametogenic Urgermline, capable of generating unipotent GSCs, serves as an ancestral blueprint for all modern germlines. It also plays a central role in cancer cell biology, largely reflecting that the AMF heritage has also been transferred to the parasitic protists affecting humans and other metazoans.¹⁷ In contrast, somatic cancer cells, which are oxygen-resistant, are not adversely affected by excess oxygen and contribute to the reconstruction of functional germline clones and CSCs, both through SGT and by circulating cancer cell clusters.

2.3. The unicellular germ and soma cell system during the transition period to multicellularity

Approximately 1,750 mya, the emergence of multicellularity imposed evolutionary pressures that led to the suppression of the unicellular life cycle of the AMF ancestor. However, as early multicellular organisms became unstable and dysfunctional, they reverted to the stable AMF life cycle along with its associated MGRS repair mechanisms. From this point forward, all early and later metazoans incorporated the AMF genome into their ancestral genome compartment, preserving it in a latent state that can be activated when necessary.

This evolutionarily conserved strategy of switching between multicellular and phylogenetic genome compartments was a recurring phenomenon during the transition to multicellularity. It allowed early and later metazoans to toggle between different genomic states. Genes associated with this dynamic strategy remained in a constant standby mode, facilitating transitions from multicellularity back to unicellularity even today, particularly when multicellular genomic errors require repair through unicellular MGRS mechanisms, as evidenced in cancer.^{2,13}

In this context, cancer mimics the alternative lifestyles exhibited by organisms during the transitional phase to multicellularity. Just as early multicellular organisms oscillated between impaired multicellular evolution and a stable unicellular lifestyle, the cancer cell system alternates between a restricted DSCD phenotype, which lacks stemness and differentiation capacities, and a functional ACD phenotype, which retains stemness and differentiation potential.

Suppressor and anti-suppressor genes from this transition period have been preserved in the ancestral genome compartments of all metazoans. Over time, these genes evolved into tumor suppressor genes (TSGs) and oncogenes. In addition, genes from early non-viable multicellular organisms that reverted to a unicellular life cycle have been retained as supplementary reservoir genes, which can be repurposed during metazoan evolution.

2.4. Hyperoxic conditions (above 6.0% oxygen) in tissues and their impact on ACD lineages in cancer

All modern germlines, including progenitor and stem cells, trace their origins back to the AMF Urgermline and are predominantly hypoxic (referred to as germline physioxia), where normoxic ranges below 6.0% oxygen. Oxygen levels above 6.0% (germline hyperoxia), which occur in tissues and the bloodstream far from the hypoxic niche, are detrimental to germ and stem cells and cause significant damage across all animal cell systems, including cancer cells. This sensitivity to hyperoxic environment is consistent across humans, mammals, vertebrates, invertebrates, and protists, such as parasitic amoebae. When exposed to hyperoxic conditions, germlines exhibit a reduction in the gene activity associated with homologous recombination (HR) repair, leading to irreparable DNA double-strand breaks (DSBs) and subsequent loss of function.^{2,13}

3. Non-proliferating CSCs and cyst-like amplification cycles

As illustrated in Figure 1, CSCs and the productive germline form a reciprocal, inseparable unit, where proliferating germline cells can generate non-proliferating CSCs that are committed to differentiation. These CSCs undergo amitotic cyst-like polyploidization–depolyloidization cycles, accumulating precursor cells for the subsequent generation of CSCs. Under favorable environmental conditions, such committed stem cells, capable of polyploid amplification, can differentiate into cysts (as seen in *Entamoeba*) or cyst-like structures (in the context of cancer). This amplification process increases their DNA content and produces progenitors for new germline clones. Throughout cancer evolution, this unicellular cancer cell system continuously generates a large number of heterogeneous germline clones and subclones, further contributing to the emergence of new CSC phenotypes and pools. According to the ECCB model, the functional ACD cancer phenotype producing primary CSCs arises from a dysfunctional non-cancerous phenotype, which cycles through unlimited tetraploid cell cycles and DSCD phenotype.^{2,13}

Cyst-like polyploidization and depolyloidization cycles in both cancer cells and protists comprise four distinct phases (Figure 2) – (i) committed genome copying and polyploidization, (ii) reductive nuclear division, (iii) haploidization of daughter nuclei, and (iv) diploidization and cellularization for germline cells. Polyploidization is more efficient than conventional proliferation through slower ACD cell cycling.

In the past, the biology of CSCs assumed that stem cells “proliferate indefinitely” to produce more identical

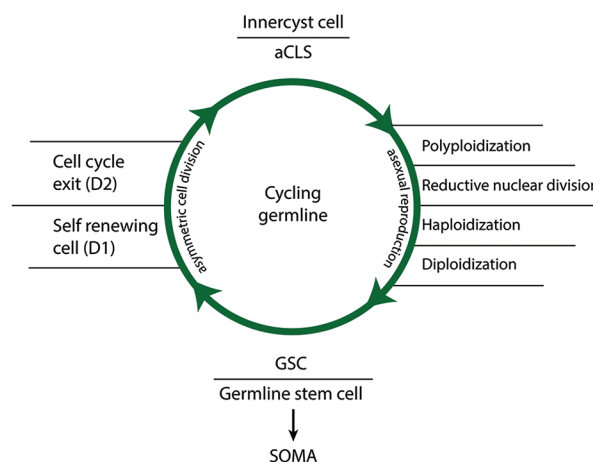


Figure 2. Amplification through cyst-like polyploidization cycles. In both cancer and protists, polyploidization and depolyploidization cycles have four distinct phases: (i) committed genome copying and polyploidization, (ii) reductive nuclear division, (iii) haploidization of the daughter nuclei, and (iv) diploidization to germline cells. Polyploidization is more efficient than propagation by slowly proliferating ACDs. In *Entamoeba histolytica* and *E. invadens*, polyploidization results in four polyploid nuclei, which yield 16 haploid germline cell progenitors during excystation. In *Entamoeba coli*, eight polyploid nuclei are produced, leading to the formation of 32 progenitors. Reprinted with permission of the Chonking Medical University.⁷²

Abbreviations: aCLS: Autonomous cyst-like structure; GSC: Germline stem cell (Figure is adopted from autonomous cyst-like structure; GSC: Germline stem cell).

stem cells. This perspective overlooks the fundamental differences between CSCs of unicellular origin and the more evolved multicellular stem cells found in humans and metazoans. Moreover, current research often neglects critical distinctions, such as the differences between proliferating ACD germline cells and non-proliferating CSCs, as well as between committed and non-committed CSCs. In addition, it frequently conflates CSC amplification through polyploidization and reductive nuclear division with mitotic proliferation. However, it is important to recognize that committed stem cells, such as those found in amoebae and cancer, are not inherently proliferative. Instead, uncommitted, quiescent germline cells can revert to a proliferative, self-renewing state, thereby reinforcing the germline from which they originally originated (Figure 2).

4. Functional and dysfunctional germline states

As previously mentioned, all germlines and clones are sensitive to oxygen levels. Germline hyperoxia, characterized by oxygen concentrations exceeding 6.0% (typically found in blood and tissue), can inflict damage on these cells, leading to irreversible loss of ACD and stemness

potential due to severe DSBs and alterations in HR genes, ultimately resulting in genome dysfunction.² Hyperoxic damage occurs when oxygen-sensitive germline cells and CSCs migrate from hypoxic niches into well-oxygenated tissues through the bloodstream.^{13,24} The elevated oxygen pressure in these tissues and the bloodstream transforms ACD germline cells into an irreparably DSCD cell state, halting the production of CSCs.

4.1. The “life cycle of stemness”

Naturally occurring DSCD phenotypes in cancer and protists, which exhibit loss of function, do not activate apoptosis programs. Instead, they engage in aberrant mitotic cell cycles and DSCD. These DSCD phenotypes are tetraploid and exhibit cytokinetic failure due to the presence of both mature and immature nuclei in the same cell. They undergo depolyploidization and re-polyploidization cycles, alternating between tetraploidy and diploidy ($4n > 2n > 4n$).^{2,13} To restore functionality, these cells require repair through the MGRS/PGCC pathway.

The loss of stemness and irreparable germline dysfunction occurs cyclically in both tumorigenesis and amoebiasis, an infectious disease caused by parasitic amebae. The “life cycle of stemness” (Figure 3) is a constant alternation between three distinct phases – (i) a functional ACD germline phase, from which stem cells arise, (ii) a cyst-free DSCD germline phase, and (iii) an MGRS/PGCC repair phase, which reconstructs the functional germline genome with stemness, ACD potential, and CSC

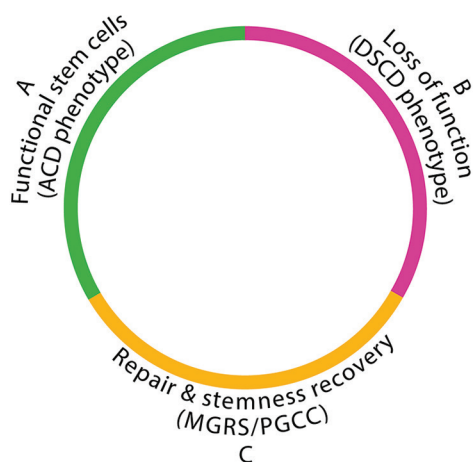


Figure 3. The life cycle of stemness (stemness cycle) in cancer and protists. Asymmetric and dysfunctional symmetric cell division phenotypes alternate and repair through the multinucleated genome repair syncytium/polyploid giant cancer cell pathway. Figure created by the author.

Abbreviations: ACD: Asymmetric cell division; DSCD: Dysfunctional symmetric cell division; MGRS: Multinucleated genome repair syncytium; PGCC: Polyploid giant cancer cells.

production. This “stemness cycle” leads to amoebiasis in both cyst-positive and cyst-negative phases, during which the disease may or may not be detectable via coprological analysis. Similarly, in cancer, an alternation occurs between ACD phenotypes that generate CSCs and DSCD phenotypes that lack stemness.^{2,13}

4.2. Contradictions to the “life cycle of cancer”

The concept of the “life cycle of stemness,” as described by the ECCB, refines the earlier “cancer life cycle” model put forth by genotoxic PGCC research, which interprets cancer as an alternation between somatic cell cycles and polyploidization cycles (Figure 4).

According to the ECCB model, the direct transition from mitotic cell cycles to germline ploidy cycles and hyperpolyploidization (Figure 4) does not occur in somatic cells³⁹ but exclusively in germline cells. This transition may arise either from committed CSCs entering cyst-like polyploidization cycles or from DSCD cells that require genomic repair through the polyploid/hyperpolyploid MGRS/PGCC pathway. It is crucial to note that cells that enter the MGRS/PGCC pathway are already germline DSCD cells, but they are not necessarily senescent or arrested in mitosis, contrary to claims made by genotoxic PGCC research.³⁹

In contrast, the “cancer life cycle” concept suggests that “mitotic cancer cells enter accelerated senescence due to oncogenic, genotoxic, or oxidative stress”⁴⁰ and hypothesizes that “DNA damage associated with senescence activates the DNA damage response (DDR),” subsequently leading to germline-related ploidy cycles. However, ECCB’s perspective diverges from this interpretation. It argues that

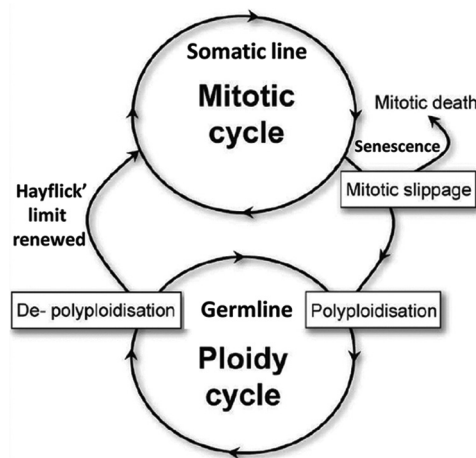


Figure 4. The “cancer life cycle,” suggests a switch from somatic mitotic cell cycles to polyplod amitotic germline cycles via senescence and mitotic slippage and back via Hayflick’s limit reactivation. Adopted from Erenpreisa *et al.*³⁹

DSCD cells requiring MGRS/PGCC repair can proliferate indefinitely without triggering senescence programs. These cells can proliferate indefinitely through symmetric cell cycles and aberrant mitoses, resulting in tetraploidy, binucleation, and immature nuclei. Therefore, DDR-induced MGRS/PGCC repair is more closely associated with the intrinsic potential of DSCD cells that transforms them into fusogenic cells rather than with senescence.

5. Intratumoral heterogeneity: CSCs and germline clones

As previously discussed, ECCB distinguishes between primary CSCs derived from native ACD lineages and secondary CSCs originating from secondary tumors and metastatic ACD lineages.

The term “ACD lineages” replaces “CSC lineages” to more accurately indicate that CSCs originate as non-proliferative products of germline sublines and clones, and are differentiation products of various ACD germline phenotypes present in primary and secondary tumors. This distinction clarifies the differences between the primary (pCSC) phenotype in primary tumors and the secondary (sCSC) phenotypes found in later tumors and metastases.

In contrast to pCSCs originating from the native cancer germline, sCSC phenotypes emerge from the heterotypic fusion of cancer cells with non-cancerous somatic cells, followed by fractal SGT processes, commonly referred to as EMT and formation of new ACD germline sublines (clones) (Table 1), ECCB provides a clearer framework

for understanding CSC heterogeneity and the origins of cancer stem-like cells.

5.1. The primary ACD lineage, primary CSCs, and stem cells from heterotypic cell fusion and fractal EMT

According to ECCB, the germline of cancer originates from a dysfunctional non-cancerous DSCD cell that requires repair through the unicellular MGRS/PGCC pathway. This atypical repair process results in the formation of spores with carcinogenic potential, which act as progenitors for the native ACD lineage, ultimately leading to the development of primary tumors. This primary ACD germline proliferates through ACD cell cycles, generating pCSCs as long as environmental factors support its hypoxic proliferation (Figure 3).

When the primary ACD phenotype becomes dysfunctional and transits to a DSCD phenotype, the depletion of CSCs and associated signaling factors trigger SGT/EMT from the primary somatic cell line. This transition regenerates germline sublines from the oxygen-resistant somatic sister line that has preserved the primary germline genome. Primary SGT processes, which form functional replacement sublines for the now dysfunctional maternal germline, are essential for ensuring the continued production of CSCs.

5.2. Secondary ACD lineages and secondary CSCs

As cancer progresses, primary somatic cells can fuse with non-cancerous host cells, such as macrophages, thereby

Table 1. Functional and dysfunctional germline cells and stem cells in cancer and protists

Characteristics	Germline phenotypes	
	Functional ACD phenotype	Dysfunctional DSCD phenotype
Proliferation	Asymmetric cell division	Dysfunctional symmetric cell division
Progeny	Unequal daughter cells (D1: self-renewing, D2: stem cell)	Equal daughter cells (no stem cells)
Stem cells (non-proliferating)	i. Committed stem cells: Capable of differentiation or/and cell amplification through polyploidization–depolyloidization cycles; form haploid progenitors for new germline clones and stem cells (CSC) ii. Non-committed quiescent stem cells: capable of transforming into self-renewing germline cells, which continue ACD proliferation	
Cell cycle characteristics	No aberrations	Tetraploidy, multinucleation, mature and immature nuclei, cytokinesis failure, and mitotic defects
Cell fusion	No fusion	Homotypic cell fusion forms multinucleated syncytia (MGRS, PGCC) that produce spores, germline clones, and stem cells (CSCs)
Cell conversion (plasticity)	GST, SGT, and EMT; Somatic cells maintain germline genome integrity; SGT generates new functional germline clones and stem cells (CSCs)	The MGRS/PGCC pathway for genome repair and function regain: It generates viable spores that, in turn, form new functional germline clones and new stem cells (CSCs)

Abbreviations: ACD: Asymmetric cell division; CSC: Cancer stem cell; DSCD: Dysfunctional symmetric cell division; EMT: Epithelial-mesenchymal transition; GST: Germ-to-soma transition; MGRS: Multinucleated genome repair syncytium; PGCC: Polyploid giant cancer cells; SGT: Soma-to-germ transition.

enriching the primary germline genome with active multicellular genes (MGs) acquired through heterotypic cell fusion. This fusion process leads to the formation of secondary sublines and clones through multiple SGT/EMT processes. In amoebae, SGT processes occur continuously, whereas, in cancer, EMT can be fractal, generating multiple germline sublines and clones, as well as multiple secondary ACD lineages with distinct secondary CSC profiles.

Alongside the existing primary ACD germline and its sublines, which continue to produce primary CSCs, multiple secondary ACD lineages give rise to numerous secondary CSC fractions and subpopulations with enhanced anti-host capabilities. These alternative mechanisms of CSC formation deepen our understanding of the heterogeneous nature of CSCs and stem-like cell generations, often described as resulting from “somatic cell dedifferentiation”.¹⁴ Each instance of heterotypic cell fusion with non-cancerous host cells and the fractal SGT/EMT processes may further expand the germline genome, increasing CSC heterogeneity, potency, and resistance to anti-tumor therapies.

6. The two phases of the hyperpolyploid repair

As previously described, dysfunctional tetraploid DSCD germlines express stemness and differentiation potential – traits exclusively exhibited by the “healthy” diploid ACD phenotype. In contrast, DSCD cell lines persist in aberrant symmetric cell proliferation, leading to the accumulation of DSCD populations. This symmetrical cell accumulation increases the likelihood of homotypic cell fusion, which triggers the biphasic MGRS/PGCC repair process, resulting in the formation of viable spores that give rise to new functional ACD lineages.^{2,13}

6.1. Phase 1: Multiple defective cyst-like polyploidization–depolyloidization cycles

After cell fusion, each of the dysfunctional DSCD nuclei within the MGRS/PGCC structure undergoes a cyst-like polyploidization–depolyloidization cycle, which serves to increase the DNA content within the MGRS/PGCC structure.^{13,24} In contrast to the functional polyploidization–depolyloidization cycle observed in cysts of *Entamoeba* (Figure 5), which culminates in the generation of 8 – 16 viable daughter nuclei capable of generating new germline clones and stem cells, the daughter nuclei produced in this initial MGRS/PGCC phase remain dysfunctional and require additional repair. Conventional repair mechanisms, such as HR, are insufficient to correct DSB damage present in the embedded DSCD nuclei.^{13,24}

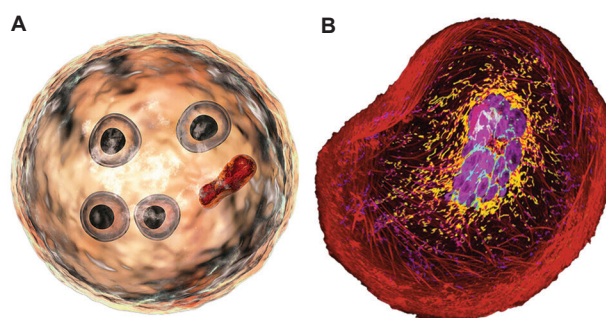


Figure 5. The polyploid PGCC structure of cancer and the polyploid cyst of *Entamoeba*. Both cell structures have a protective envelope and are designed to produce functional progenitor cells capable of stemness and asymmetric cell proliferation. These evolutionarily related cell structures share a thick protective envelope that provides suitable conditions for hypoxic polyploidization (A) A mature, terminal differentiated polyploid cyst of *Entamoeba* with four functional tetraploid nuclei (10 - 20 mm). (B) A multinucleated PGCC structure with several dysfunctional nuclei, which can be up to ten times larger than normal cancer cells, is capable of hyperpolyploidizing up to 380n. PGCC structures undergo two distinct phases of polyploidization and maturation. The first phase is a cyst-like polyploidization in which each embedded dysfunctional nucleus of the fused DSCD cells undergoes a polyploidization and depolyloidization cycle similar to the polyploidization cycles observed in *Entamoeba*, but resulting in dysfunctional daughter nuclei. In the second phase, these dysfunctional daughter nuclei fuse to form a hyperpolyploid giant nucleus that reconstructs the functional genome. The above images are reprinted with permission from StockFood GmbH, Munich, Germany (A) and Atena Editora, Brazil (B).

Abbreviations: PGCC, polyploid giant cancer cell; ACD, asymmetric cell division; DSCD, dysfunctional symmetric cell division.

6.2. Phase 2: Defective nuclear fusion and the formation of hyperpolyploid giant nuclei

During this phase, the dysfunctional nuclear progeny fuse, resulting in the formation of high-grade hyperpolyploid nuclei characterized by a significantly increased DNA mass. These giant nuclei possess the capability to eliminate DSB fragments and reconstitute functional germline genome architecture alongside restoring HR gene functionality. Consequently, the MGRS structures themselves acquire stemness potential, which is subsequently transferred to the viable spores they produce.

7. Native multinucleated genome repair structures

Native PGCCs arising from cell-to-cell fusion are integral to the dynamic life cycle of stemness within tumors (Figure 3). Homologous to the MGRS found in protists, PGCCs are vital for reconstructing the functional genome of the germline and regenerating the ACD germline phenotype, ultimately leading to the generation of CSCs.

Despite their significance, knowledge about native PGCCs – which emerge during malignancy, early

carcinogenesis, and within primary tumors – remains sparse, and they are often challenging to detect until malignancy is suspected. Native PGCCs play a crucial role in continuously repairing newly formed tumor DSCD cells, which are induced by deleterious hyperoxic conditions in blood and tissues.

Historically, the similarities between the MGRS of amoeba and the native PGCC structures in cancer were not well understood. Consequently, insights into the role and function of PGCCs, occasionally observed in tissue and cancer cell cultures, have primarily stemmed from experimental induction through irradiation, chemotherapeutic agents, and stress factors such as cobalt chloride (CoCl_2).^{41–43} Notably, CoCl_2 appears to create hypoxic conditions that prompt oxygen-damaged DSCD cells and force them to enter the MGRS/PGCC pathway.

However, the characteristics and outcome of genotoxically induced mononucleated PGCCs can only be partially extrapolated to native PGCCs. First, the effects of genotoxic treatments are significantly more severe than the “natural” hyperoxic stress resulting from oxygen levels exceeding 6.0% outside of hypoxic niches. Second, native PGCCs remain largely unexplored and inadequately categorized within the cancer development process. These limitations have fostered assumptions that may not always be valid.

Several unresolved questions persist regarding native PGCCs. A critical area of inquiry is whether a causal relationship exists between native PGCCs and aneuploidy, or whether aneuploidy is rather a consequence of apoptosis evasion. While significant evidence suggests a link to PGCC structures, many aspects remain unclear. Key questions include – (i) Are some DSCD cells that have experienced hyperoxic DSB damage predisposed to aneuploidy? (ii) Do certain genomic defects that the PGCC process cannot fully repair lead to the formation of aneuploid-prone spores and clones, thereby perpetuating aneuploid cell cycling? or (iii) could PGCCs themselves generate aneuploidy? Addressing these questions is essential for elucidating the role of native PGCCs in the development of cancer aneuploidy.

Further inquiries include determining whether native PGCCs arise exclusively from *primary* DSCD cell fusions or whether they can also originate from different secondary DSCD phenotypes in later tumors and metastases. Investigating the potential for homotypic and heterotypic cell fusion to contribute to native PGCC formation could offer deeper insights into their role and origin in cancer development. Rigorous testing of these hypotheses using biopsy samples from untreated cancers is warranted.

8. Uninucleated, genotoxic-induced PGCCs

Unlike native PGCCs, which arise naturally during carcinogenesis and cancer progression, genotoxic-induced uninucleated PGCC structures resulting from irradiation and chemotherapeutics require significantly more time to initiate hyperpolyploidization and rely on the support of nursing cells.²⁴

8.1. Consequences of genotoxic damage: A unique amplification cycle and extreme hyperpolyploidization

In contrast to native PGCCs, which consist of multiple DSCD nuclei and that undergo several polyploidization–depolyloidization cycles, genotoxic-induced PGCCs follow a singular, cyst-like polyploidization cycle. By the end of phase 1, the number of daughter nuclei and the overall DNA mass are considerably lower than those in native PGCCs. The daughter nuclei produced at the end of this phase are homogenous to the damaged parent nucleus, thereby retaining the same DNA defects.

In the subsequent phase 2, genotoxic-induced PGCCs achieve extreme levels of hyperpolyploidy (up to 380n), accumulating the critical DNA mass needed for genome repair. However, the outcome of this PGCC repair process remains genomically ambiguous. Numerous studies suggest that the final spore progeny is heterogeneous and may retain persistent genomic damage, such as aneuploidy.

8.2. Accelerated senescence, senescence exit, and hyperpolyploidization

The few cancer cells that survive genotoxic insults appear to undergo a prolonged adaptation, which contrasts with the rapid formation of MGRS observed in amoebae. In amoebae, the MGRS process is initiated by tetraploid or multinucleated DSCD cells cultured in synthetic, nutrient-deficient media.^{19,20} This process can be induced in young and middle-aged DSCD cells but not in senescent DSCDs cells from aging cultures.¹⁸

Conversely, the delayed polyploidization observed in living, genotoxically treated cancer cells has been interpreted by some researchers as a phase of temporary senescence (accelerated senescence) before genotoxically induced polyploidization. This interpretation rests on the assumption that cells capable of genome repair through PGCCs can circumvent senescence programs, suggesting that PGCCs may actually shorten, accelerate, or interrupt senescence processes.^{39,40} However, according to the ECCB framework, this statement is not applicable. Senescence exit precedes the long-term proliferation of DSCD cells, homotypic cell fusion, and MGRS/PGCC formation. The delayed prepolyloidization phase in genotoxically

induced PGCCs more likely reflects the extent of cellular damage that must be resolved before initiating the cyst-like amplification cycle of phase 1.

8.3. Recent statements from genotoxic cancer cell research

Despite the limited understanding of the natural MGRS/PGCC pathway, recent years have witnessed the publication of numerous insightful reviews by Jekaterina Erenpreisa's research group and even a book by Brazilian researchers that comprehensively describes the results of genotoxic-induced PGCC research.⁴⁴

Over the years, investigations into genotoxic effects on cancer cells have revealed that PGCCs are responsible for extensive genomic restructuring, leading to the emergence of tumor-initiating cells in response to stress. In 2016, Niu *et al.*⁴⁵ proposed that the giant cell cycle is a source for mitotically competent tumor-initiating cell production, contributing to genomic instability. By 2018 – 2019, researchers emphasized that giant cancer cells act as unrecognized triggers of tumorigenesis, metastasis, and resistance to therapy.^{46–48}

According to a review by Amend *et al.*,⁴⁶ PGCCs arise in the hypoxic environment of primary tumors as a response to therapeutic interventions. The progeny of these PGCCs exhibit CSC characteristics and have the potential to repopulate the tumor. The proportion of giant cells can significantly increase in response to genotoxic stress, and PGCCs may develop enhanced metastatic and invasive capabilities. The clinicopathological significance of PGCCs has been examined by many researchers,^{49,50} along with their role as circulating cancer cells,⁵¹ which is associated with tumor grading and metastasis.⁴⁷ PGCCs can arise in response to stress,^{41,52} and the phenotypes of PGCCs observed during cancer development are highly diverse.^{53,54}

9. Convergences and controversies between ECCB and current cancer research

It is evident that some recent findings from genotoxic PGCC research align with the principles of the ECCB, whereas others do not. Notably, ECCB's considerations regarding the compartmentalized genome, ancestral genome modules, and regulation by hypoxic/hyperoxic environments are increasingly shared across both research domains. The author of the current paper, who reported on the advancements of the ECCB field in 2018/2019, contributed to this convergence by demonstrating that all eukaryotes, from protists to mammals, retain the genome of their unicellular common AMF ancestor within their ancestral genome.^{55–57} This ancestral genome can be reactivated under adverse environmental conditions, such as oxygenic stress and germline hyperoxia.

The primary controversy between the ECCB, which posits that cancer represents a unicellular cell system, and genotoxic-driven PGCC research revolves around the nature of the cancer cell system itself. Specifically, is the cancer cell system fundamentally unicellular, or is it a manifestation of aberrations within the multicellular cell system? While it is well-established that polyploidy, hyperpolyploidy, and PGCCs are not associated with multicellularity, current PGCC research asserts that CSCs originate from multicellular ASCs or embryonic cell stages. Current opinion attributes CSC formation to epigenetic changes, mutations, DNA damage, and altered MG activity. In contrast, ECCB suggests that the potential unicellular cancer genome is preserved within the ancestral genome compartment of humans and metazoans, existing in a reactivatable silent state. According to this view, the reactivation of this genome originates from non-cancerous DSCD cells.^{57,58}

Emerging hypotheses from genotoxic-induced PGCC research continue to view cancer as a disease within the multicellular framework, suggesting that it does not necessarily involve a complete transition to a unicellular cell system. In contrast, the ECCB relies on extensive evidence and proposes that spontaneous solid tumors result from an irreversible shift to an extensive pre-metazoan cellular system that evolved from the common AMF ancestor. According to the ECCB, this transition to a unicellular lifestyle occurs through the loss of ACD capacity and stemness potential, cessation of CSC production, and activation of a natural DSCD program within the multicellular cell system.

In contrast to the ECCB, the conclusions drawn from current cancer research remain ambiguous. While they describe various evolutionary links between cancer origins and processes such as mammalian embryogenesis, sporulation in protozoa, gametogenesis, and embryogenesis in insects, or PGCC reproduction,³⁹ these connections primarily highlight the deep homology of cancer to a common ancestor and its evolutionary branches. Such homologies evoke distant relatives that have inherited certain traits from a shared ancestor but are no longer closely related.

As a result, current statements regarding cancer origins do not present a viable alternative to the ECCB framework. They fall short of providing a comprehensive understanding of cancer, do not clarify its origins, and fail to illuminate the fundamental nature of cancer itself.

10. Is cancer unicellular or multicellular?

The debate over whether cancer represents a unicellular or multicellular system is fundamental to our understanding

of the disease. The multicellular cancer paradigm interprets carcinogenesis as a series of aberrations within a multicellular framework. This perspective links cancer to processes such as cellular plasticity, epigenomic alterations, disrupted differentiation, and the reconfiguration of gene regulatory networks (GRNs).³⁹ In this context, the multicellular GRN acts as a regulatory mechanism striving for equilibrium with its environment.^{39,59} It can adopt a stable, pre-programmed configuration through a “pre-programmed attractor.” This “attractor” can be likened to a software application, programmed during early phylo-ontogenetic evolution, which can be activated under stress to reprogram the genome. Consequently, cancer can be viewed as an imbalance in MG expression – older genes of unicellular origin are often overexpressed, whereas newer genes from more recent phylostates, which are responsible for multicellular evolution, are underexpressed. These changes in gene expression patterns enable cancers to adopt phenotypes typically seen in unicellular organisms.

This hypothesis is supported by phylostratigraphy and phylogenetic studies that trace the origins of cancer genes back to the transitional period from unicellularity to multicellularity around 1,750 mya. However, critics contend that these later evolutionary stages fail to encompass the true nature of carcinogenesis, tumorigenesis, and metastasis, which dates back to the cell system of the AMF ancestor around 2,300 mya ago.^{60–65} Further concepts emphasize the regulation of gene expression and the self-organization of gene networks during tissue and organ differentiation,^{39,40} whereas a more traditional hypothesis suggests that changes in cell fate, akin to those occurring during the differentiation of highly specialized cell types, can also lead to the formation of dysfunctional tumors under atypical environmental stress.

Recent work by Erenpreisa and Giuliani.⁶⁶ aligns more closely with the ECCB framework, suggesting that “cancer cells can adapt to unforeseen environmental challenges through exploration by trial and error, existing at the edge between order and chaos. When faced with potentially lethal damage, these cells scan their gene networks, revisiting hidden transcriptional configurations preserved in the mammalian genome, reflecting 3.5 billion years of cellular evolution.” This view draws parallels with the ECCB’s approach to carcinogenesis, particularly in the interplay between unicellularity and multicellularity during the evolutionary transition period. Erenpreisa *et al.*⁶⁷ describe this translational phase as involving “vestigial transcriptional programs” or “predetermined chaos,” which may be the most effective strategy for facilitating the survival of “lucky” cells from near-lethal damage. The forward motion is uncertainty, fluctuations, and a duality of opposites engaged in an intensive “dialog with the environment.”

A critical question remains: Does cancer have full access to the unicellularity of the ancestral genome compartment, or is it indicative of an intrinsic flaw within the multicellular system? The ECCB framework presents substantial arguments in favor of a complete transition to a unicellular cell system.^{2,13} It posits that the activation of ancestral unicellular genome modules and their associated repair genes occurred repeatedly during the transition to multicellularity, as primitive multicellular systems could not independently repair stress-induced genomic damage. Similarly, damaged germlines and cancer germline cells, as well as damaged stem cells CSC and DSCD lineages in both cancerous and non-cancerous systems, require the activation of ancestral unicellular genome repair mechanisms, such as MGRS and hyperpolyploidy to regain stemness and ACD potential for generating new stem cells. However, these repair mechanisms ultimately reintroduce MGRS progeny into a unicellular state, as MGRS and hyperpolyploidy drive the transition of defective multicellular germline cell systems back to unicellularity.

In this ongoing debate regarding the alignment of cancer cell systems with unicellularity or multicellularity, Vinogradov and Anatskaya⁶⁸ recently proposed a genomic unicellular attractor (UCA) model. This model integrates key aspects of existing theories, including the “atavistic reversal,” “cancer attractor,” “somatic mutation theory,” “genome chaos,” and others, with “atavistic reversal” as a central concept. The UCA model suggests that the UCA emerges due to a gradual core-to-periphery expansion of cellular networks, resulting in increased protein interaction density and higher global interactome centrality within the unicellular core.

11. Conclusions and perspectives

From an evolutionary standpoint, cancer signifies a transition to a lower level of cellular organization, marking a shift from multicellularity to unicellularity. This transition is thought to occur frequently during the early stages of multicellular evolution, as primitive multicellular organisms oscillated between the two states in response to both intrinsic and extrinsic factors. All multicellular organisms, including humans and mammals, retain a unicellular cell system – derived from the AMF ancestor – within their ancestral genome compartments, in a state of responsiveness. This explains the distinct stem cell biology observed in cancer relative to that of the host organism.

According to the ECCB framework, CSCs are non-proliferative cells arising from the ACD germline phenotype; they are sister cells of self-renewing germline cells.

The ECCB contradicts the widely held belief that CSCs are a small subset of tumor cells characterized by self-

renewal and continuous proliferation. The ECCB recognizes that the ability for self-renewal, previously attributed to the CSC lineage, is exclusive to the cancer germline and its ACD variants. It also determines that CSCs are a differentiation product of the germline, capable of further differentiation and the accumulation of germline progenitors through polyploidization/depolyloidization cycles. According to the ECCB framework, only the germline can proliferate indefinitely, but not the differentiated CSC pool.

ECCB distinguishes between two types of cells that exit the cell cycle: Non-committed quiescent CSCs, which have the potential to revert into self-renewing germline cells, and committed CSCs (true CSCs), capable of amplification through cyst-like polyploidization–depolyloidization cycles (though not through proliferation).

The ECCB conceptualizes self-renewing germline cells and non-proliferating CSCs as interchangeable entities termed the ACD lineage. In this model, self-renewing ACD cells generate CSCs, while committed, non-proliferating CSCs generate progenitor cells for new germline sublines and clones. This dynamic interplay is of fundamental importance for understanding the roles and behaviors of CSCs in cancer cell biology.

The diversity of secondary ACD lineages, including secondary CSCs in older tumors and metastases, offers a more comprehensive explanation of the heterogeneity within the stem cell population than the current notion of CSC lineages. It demonstrates that generating heterogeneous, non-proliferative CSC lineages depends on numerous germline sublines and clones, generated through heterotypic cell-to-cell fusions with non-cancerous somatic cells, followed by fractal SGT/EMT processes.

Stemness is a hallmark of each ACD lineage. It can be lost but also regained through the MGRS/PGCC repair pathway. The DSCD phenotype, which requires repair, does not exhibit stemness. This distinction highlights the unique capacity of the MGRS/PGCC pathway to re-establish stemness.

Multinucleated MGRS and native PGCCs are hyperpolyploid genome repair structures formed through homotypic cell fusion. These structures are distinct from genotoxically induced PGCCs, which arise following irradiation or treatment with chemotherapeutic agents. MGRS was first documented in protists as early as 1906.⁶⁹ Unbeknownst to these early findings, the term “neosis” was introduced in 2008 to describe PGCCs as a novel type of ACD.^{70,71} This designation was a mischaracterization – PGCCs do not proliferate or undergo typical ACD; instead, they produce spores via cyst-like genome accumulation and hyperpolyploidization.

The ECCB framework further distinguishes between primary CSCs and secondary CSCs. Primary CSCs are both carcinogenic and tumorigenic, arising either post-malignancy from the pCSC lineage or through native PGCC processes within tumors. In contrast, secondary ACD lineages, which give rise to secondary CSCs, originated from heterotypic somatic cell fusion and fractal SGT/EMT processes. The resulting secondary CSCs incorporate functional MGs into the unicellular cancer genome, forming new germline clones capable of producing metastatic secondary sCSCs with enhanced invasive potential. This distinction highlights the varying origins and roles of primary and CSCs in cancer development and progression, with secondary CSCs playing a critical role in augmenting the metastatic capabilities of cancer.

In summary, a deeper understanding of CSC biology is crucial for advancing experimental cancer research. The novel insights into the biology of CSCs outlined in this study could reveal new molecular targets in the fight against cancer. This finding is particularly important for elucidating the surface antigenicity of the ancestral cell types and other closely associated cell phenotypes involved in transformation and carcinogenesis, such as non-cancerous DSCD cells and tumor DSCDs. Investigating the ACD-DSCD-MGRS sequence for antigenicity, carcinogenic potential, and surface markers could yield valuable new research data. Ultimately, these findings may contribute to the development of an effective anti-cancer vaccine.

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